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RESEARCH PAPER

New sources of cowpea genotype resistance to cowpea bruchid *Callosobruchus maculatus* (F.) in Uganda

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Abstract

Cowpea bruchid *Callosobruchus maculatus* (F.) is a major constraint to cowpea production throughout sub-saharan Africa. The identification of sources of *C. maculatus* resistance and their incorporation into breeding programs would be a beneficial strategy to combat the devastation caused by the bruchid in stored cowpea. We evaluated 145 cowpea genotypes from Uganda and introductions from Kenya and Nigeria for resistance to bruchids. The mean number of eggs and number of holes, percentage pest tolerance, percentage weight loss, bruchid developmental period, bruchid growth and Dobie susceptibility index were significantly different among the 145 genotypes. Based on Dobie susceptibility index value, there were 18 resistant, 114 moderately resistant and 13 susceptible genotypes. Dobie's susceptibility index correlated negatively with insect development period and percentage pest tolerance, and positively with number of eggs, growth index, number of holes and weight loss. The study identified new sources of cowpea from the studied genotypes that could be used by cowpea breeders to develop cultivars with relatively high resistance to cowpea bruchid. However, further investigations and identifcation of biochemicals that are responsible for cowpea seed resistance to bruchid are recommended.

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Introduction

Cowpea (*Vigna unguiculata* (L.) Walp.) is an important indigenous legume providing dietary protein, minerals, carbohydrates, fats, vitamins and income to many poor people in Africa, Asia, and central and South America (Enwere *et al.,* 1998; Popelka *et al.,* 2004; Langyintuo *et al.,* 2005; Agbogidi, 2010). Its protein content ranges from 24.7–33.1% with low anti-nutritional factors (Nielsen *et al.,* 1994; Rangel *et al.,* 2003). Globally, more than 12.32 million hectares of cowpea are harvested, 98.1% being from Africa (FAO, 2016). However, cowpea production in these producing countries is limited by insect pest attacks (Beck and Blumer, 2007).

In storage, cowpea weevil *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) is the most destructive pest (Deshpande *et al.*, 2011). The insect females deposit their eggs on seed coat, and embryogenesis is completed after 3 to 5 days (Beck and Blumer, 2007). After eclosion, the larvae penetrate the cotyledons where they develop by consuming the energy reserves of cotyledons, reducing both the quantity and quality of seeds, making them unfit for planting, marketing and human consumption (Ali *et al.,* 2004). Adult emergence occurs after 25-30 days (Oliveria *et al.*, 2009). The loss in quality is due to contamination with insect exudate, eggs, dead insects and holes, conversion of seed contents (Ali *et al.*, 2004). The loss in quantity is attributed to seed weight loss (Maina *et al.*, 2012).

In Sub-Saharan Africa, chemical control using insecticdes is a common practice used by the majority of farmers to minimize losses due to bruchid infestations (Olakojo *et al.,* 2007). However, the method is expensive, pose health hazards to farmers and consumers and their continuous use can lead to development of insecticide resistant bruchids (Boyer *et al.,* 2012). The use of resistant genotypes offers a promising alternative control method to the hazardous pesticides for the management of *C. maculatus*, especially where huge quantities of grains are involved (Cruz *et al.,* 2015). Several studies have assessed the performance of *C. maculatus* infesting different genotypes (Singh *et al.,* 1985; Shade *et al.,* 1999).

In Nigeria, for example, out of the 8000 germplasm lines screened, only three *C. maculatus* resistant lines (TVu-2027, TVu 11952 and TVu 11953) were identified by the International Institute for Tropical Agriculture (IITA), and *C. maculatus* showed decreased survival and increased developmental times during infestation of those seeds. However, the use of resistant genotypes is affected by the durability of resistance (Appleby and Credland, 2004), which is rapidly being overcome by changes in pest populations (Keneni *et al.,* 2011) and by lack of high-resistance sources (Leach *et al.,* 2001). A study in Nigeria, for example, showed that the already identified bruchid resistance genotype, TVu-2027 has been overcomes by the pest population (Shade et al., 1999). Such breakdown of genetic resistance of improved cowpea genotypes to bruchids highlight the need to search for new sources of resistance from different cultivated varieties and wild species. In Uganda, information on sources of local and improved cowpea bruchid resistant genotypes is scarce. Therefore, in this study, we investigated the susceptibility and resistance of 145 *V. unguiculata* genotypes to infestation and damage by *C. maculatus*. The aim was to identify new sources of cowpea genotypes resistant to bruchid in Uganda for the improvement of the breeding programme.

Materials and methods

Sources of cowpea genotypes

Seeds of 145 cowpea genotypes (130 Ugandan, one Kenyan and 14 genotypes from IITA Nigeria) were used for the study (Table 1). To generate sufficient seeds for laboratory testing, each of the genotypes were grown at the Makerere University Agricultural Research Institute Kabanyolo (MUARIK) (0°28'N and 32°37'E, approximately 1200 m asl), between May and December 2015.

Bruchid laboratory culture

Adult *C. maculatus* (F.) were obtained from the National Agricultural Research Laboratory, Kawanda. A permanent laboratory culture of the insect was established at MUARIK by allowing the insects to lay eggs on a susceptible inbred line IT71. Insects were reared on 12 kg seeds kept in four transparent plastic buckets of five liter capacity whose tops were covered with muslin cloth to provide aeration and prevent the insects from escaping. The insects were allowed to oviposit and their progeny maintained by regularly replacing the infested seeds with fresh seeds.

Table 1. Cowpea genotypes evaluated for bruchid resistance.

Genotype	Cultivar type	source	Genotype	Cultivar type	source	Genotype	Cultivar type	source
182	Landrace	Uganda	MU9	Landrace	Uganda	5T - 3B	Inbred line	Uganda
2282	Landrace	Uganda	NE13	Landrace	Uganda	5T × Acc12	Inbred line	Uganda
2309	Landrace	Uganda	NE15	Landrace	Uganda	5T×4W	Inbred line	Uganda
2392	Landrace	Uganda	NE19	Landrace	Uganda	ACC12 × 3B	Inbred line	Uganda
2419	Landrace	Uganda	NE23	Landrace	Uganda	ACC12 × 2W	Inbred line	Uganda
2434	Landrace	Uganda	NE30	Landrace	Uganda	ACC2× ACC12	Inbred line	Uganda
3306	Landrace	Uganda	NE37	Landrace	Uganda	ACC2 × IT	Inbred line	Uganda
IT109	Improved	IITA	NE39	Landrace	Uganda	ACC23 × 4W	Inbred line	Uganda
IT97	Landrace	IITA	NE39 × SEC2	Inbred line	Uganda	ACC25	Landrace	Uganda
KVU-27-1	Improved	Kenya	NE39 × SEC4	Inbred line	Uganda	ACC26 x ACC2	Inbred line	Uganda
NE20	Landrace	Uganda	NE4	Landrace	Uganda	ALEGI x 4W	Inbred line	Uganda
NE51	Landrace	Uganda	NE40	Landrace	Uganda	ALEGI	Local	Uganda
3B x 2W	Inbred line	Uganda	NE44	Landrace	Uganda	ALEGI×3B	Inbred line	Uganda
ACC12 x 5T	Inbred line	Uganda	NE48	Landrace	Uganda	ALEGI×5T	Inbred line	Uganda
ACC23 x 3B	Inbred line	Uganda	NE5	Landrace	Uganda	ALEGI × ACC2	Inbred line	Uganda
ACC26 * IT	Inbred line	Uganda	NE51 × SEC3	Inbred line	Uganda	CIG	Inbred line	Uganda
EX-1Seke	Landrace	Uganda	NE51 × SEC4	Inbred line	Uganda	EBELAT×NE39	Inbred line	Uganda
IT × ACC23	Inbred line	Uganda	NE55	Landrace	Uganda	EBELAT×NE51	Inbred line	Uganda
IT ×ALEGI	Inbred line	Uganda	NE67	Landrace	Uganda	WC32 × SEC5	Inbred line	Uganda
IT2841 x BROWN	Inbred line	Uganda	NE70	Landrace	Uganda	IT71	Inbred line	IITA
MU17	Landrace	Uganda	NYBOLA	Landrace	Uganda	IT84	Improved	IITA
MU20B	Landrace	Uganda	OBONQ1	Landrace	Uganda	IT889	Improved	IITA
MU24C	Landrace	Uganda	SEC1 × SEC4	Inbred line	Uganda	MU15	Landrace	Uganda
NE21	Landrace	Uganda	SEC5 × SEC2	Inbred line	Uganda	WC5	Landrace	Uganda
NE31	Landrace	Uganda	SEC5 × NE39	Inbred line	Uganda	WC55	Landrace	Uganda
NE32	Landrace	Uganda	SECOW2W	Improved	Uganda	WC60	Landrace	Uganda
NE36	Landrace	Uganda	SECOW5T	Improved	Uganda	WC44	Landrace	Uganda
NE41	Landrace	Uganda	UW × 5T	Inbred line	Uganda	WC46	Landrace	Uganda
NE45	Landrace	Uganda	2W×Acc2	Inbred line	Uganda	WC62	Landrace	Uganda
NE46	Landrace	Uganda	4W × 5T	Inbred line	Uganda	WC63	Landrace	Uganda
NE49	Landrace	Uganda	W10	Landrace	Uganda	WC64	Landrace	Uganda
NE50	Landrace	Uganda	W32	Landrace	Uganda	WC67	Landrace	Uganda
NE53	Landrace	Uganda	WC10	Landrace	Uganda	WC674	Landrace	Uganda
NE6	Landrace	Uganda	WC13	Landrace	Uganda	WC67B	Landrace	Uganda
NE71	Landrace	Uganda	WC15	Landrace	Uganda	WC68	Landrace	Uganda
SEC1×SEC3	Inbred line	Uganda	WC16	Landrace	Uganda	WC684	Landrace	Uganda
SEC5× SEC1	Inbred line	Uganda	WC17	Landrace	Uganda	IT82D - 716	Improved	IITA
WC2	Landrace	Uganda	WC18	Landrace	Uganda	IT84s-2246	Improved	IITA
WC29	Landrace	Uganda	WC19	Landrace	Uganda	IT97K-499-35	Improved	IITA
WC35C	Landrace	Uganda	WC21	Landrace	Uganda	TVu-2027	Improved	IITA
WC42	Landrace	Uganda	WC26	Landrace	Uganda	IT90K-277-2	Improved	IITA
WC52	Landrace	Uganda	WC27	Landrace	Uganda	IT90K-76	Improved	IITA
WC58	Landrace	Uganda	WC30	Landrace	Uganda	IT95K-207-15	Improved	IITA
WC69	Landrace	Uganda	WC32A	Landrace	Uganda	IT98K-205-8	Improved	IITA
WC7	Landrace	Uganda	WC35A	Landrace	Uganda	IT99K-1399	Improved	IITA
WC8	Landrace	Uganda	WC35D	Landrace	Uganda			
WC41	Landrace	Uganda	WC36	Landrace	Uganda			
2W x IT	Inbred line	Uganda	WC37	Landrace	Uganda			
SEC5 x SEC2	Inbred line	Uganda	WC48	Landrace	Uganda			
SEC5 x NE39	Inbred line	Uganda	WC48A	Landrace	Uganda			

Infestation and data collection

Seeds of each of the 145 cowpea genotypes were dried in an oven at 40°C for 24 hours to eliminate any bruchid infestation coming from the field and to keep moisture level of the seeds uniform (Amusa *et al.*, 2014). Ten randomly selected seeds from each genotype were initially weighed and put into a petri-dish of 90 × 15 mm.

Each petri-dish was infested with two pairs of newly emerged male and female adult bruchid and covered to prevent the insects from escaping. The insects were left undisturbed in the petri-dishes for three days to allow for mating and oviposition, after which they were removed (Amusa *et al.,* 2013). The experiment was laid in a completely randomized design with three replications per genotype. Data on number of eggs, number of exit holes, number of damaged and undamaged seeds, initial seed weight (g), residual seed weight (g), were recorded for 44 days and percentage weight loss and percentage pest tolerance were computed using the method of Amusa *et al.* (2014). The number of emerged adult bruchids was recorded daily until no more adults emerged for five days.

Insect growth index and Bruchid resistance rating

Insect growth index (GI) (Badii *et al.,* 2013) was calculated by combining the data on the number of eggs, percentage adult bruchid emergence and the median development period (Sharma and Thakur, 2014) for each genotype using the formula;

$$Adult\ emergence\ (\%) = \frac{Number\ of\ adults\ emerged}{Total\ number\ of\ eggs\ laid} x100$$

$$GI = \frac{Adult\ emergence\ (\%)}{Median\ deelopment\ period}$$

At the end of the experiment, Dobie Susceptibility Index (DSI) was calculated for each genotype using the data on total number of adult bruchid that emerged on each genotype and their median development period (i.e. the time from the middle of oviposition to the emergence of 50% of adult bruchids) using the formula of Dobie (1974);

$$DSI = \frac{\text{Loge F1} \times 100}{MDP}$$

F1– total number of emerging adults and

MDP –median developmental period (days).

The susceptibility index ranging from 0 to 11 was used to categorize the cowpea genotypes; where; 0-3 = resistant, 4-7 = moderately resistant, 8-10 = susceptible and ≥10 = highly susceptible (Dobie, 1974).

Statistical analysis

One-way analysis of variance (ANOVA) was used to examine differences in the performance of different cowpea genotypes for resistance to bruchid and Fisher's LSD test was used to separate the means. Pearson correlation was used to examine the association among resistance parameters including the DSI for the genotypes. Multiple linear regression analysis was used to identify which traits (number of eggs, number of holes, seed weight loss and pest tolerance) were better predictors of resistance (DSI). All analyses were conducted using GenStat Discovery, 16.1th Edition statistical package.

Results

Performance of cowpea genotype resistance to bruchids

The results of performance of cowpea genotype resistance to bruchid are presented in Table 2. Significant differences (P< 0.001) were found in the number of eggs laid (NE) by *C. maculatus,* median time to adult bruchid emergence (MDP), insect growth index (GI), average number of holes (ANH), percentage weight loss (PWL), percentage pest tolerance (PPT) and Dobie susceptibility index (DSI) amongst the 145 cowpea genotypes.

Table 2. Mean squares for the performance of cowpea genotypes to *callusbrocus maculatus* infestation.

Source of variation	Variables							
	df	NE	GI	MDP	ANH	PWL	PPT	DSI
Genotype	144	2302.92	2.089	27.66	13.18	196.88	1718	8.23
Residual	290	51.65	0.12	0.87	0.15	2.96	64.83	0.05

NE= Number of eggs; GI=Growth index; MDP= Median development period; ANH= Average number of holes; PWL= percentage weight loss; PPT= percentage pest tolerance and DSI= Dobie susceptibility index. For all variables P<0.001.

Effects of V. unguiculata genotypes on growth performance of adult C. macculatus

The studied cowpea genotypes showed significant (P< 0.001) impacts on all bruchid growth parameters (Table 3). Result showed that mean number of eggs laid by bruchid ranged from 0-147.7. The top four genotypes in terms of mean number of eggs laid were NE32 (147.7), WC19 (141), WC69 (141) and EBERAT×NE51 (137.7). There was a significant (P<0.001) reduction in the oviposition on genotypes IT84s-2246, IT95K-207-15 and TVu-2027. The median development period to adult emergence of all the genotypes ranged from 20.8 to 44 days. The shortest period was recorded from genotypes, IT889 (20.8 days) and SECOW5T (21.1 days) while the longest was from genotype IT84s-2246 (44 days). The highest bruchid growth index was recorded from genotypes SECOW2W (3.92), MU9 (3.82), WC67B (3.69), IT889 (3.67), IT71 (3.56) and SECOW5T (3.5) whereas genotypes 2419 (0.03), WC42 (0.23), IT97K-499-35 (0.23), TVu-2027 (0.37), IT84s-2246 (0.38), ACC23×3B (0.46) and WC16 (0.51) showed least growth index values.

Table 3. Means of genotypic performance under bruchid infestation.

Genotype	NE/10 seeds	MDP (days)	GI	ANH/seed	PWL	PPT (%)	DSI
IT109	124.0	21.5	2.92	7.8	27.6	0.0	8.8
SECOW2W	87.3	22.8	3.92	7.7	24.2	0.0	8.3
WC19	141.0	23.0	2.42	7.8	22.3	0.0	8.2
WC69	141.0	23.0	2.42	7.7	35.9	0.0	8.2
IT71	87.0	22.8	3.56	6.9	44.7	0.0	8.1
MU9	75.7	22.5	3.82	6.5	16.7	0.0	8.1
SECOW5T	68.3	21.2	3.50	5.1	27.8	0.0	8.1
IT889	61.0	20.8	3.67	4.5	15.5	3.3	8
SEC5×NE39	80.7	24.0	3.40	6.5	19.6	0.0	7.6
IT84	86.0	24.5	3.30	6.9	13.8	10.0	7.5
2282	88.3	25.2	3.19	7.0	16.7	3.3	7.3
OBONQ1	122.7	25.5	2.22	6.9	7.0	26.7	7.2
IT97	67.3	24.5	0.49	5.7	9.9	0.0	7.2
WC10	83.0	26.2	3.02	6.5	10.0	23.3	6.9
ALEGI	103.7	24.8	1.69	4.3	10.0	36.7	6.6
NE15	71.0	24.2	2.16	3.6	14.8	33.3	6.5
WC36	67.7	27.5	3.21	5.9	10.9	26.7	6.5
2W×ACC2	53.3	25.3	3.13	4.2	10.9	33.3	6.4
WC64	74.7	26.5	2.53	4.9	21.6	3.3	6.4
WC26	55.7	24.2	2.63	3.5	6.5	46.7	6.4
EX-1Seke	95.3	28.2	2.37	6.2	12.1	20	6.4
EBERAT×NE39	94.0	28.0	2.30	5.9	14.0	6.7	6.4
NE20	95.7	28.5	2.27	6.1	28.9	3.3	6.3
NE48	61.3	27.3	3.11	5.2	13.4	3.3	6.3
NE5	119.3	29.5	1.88	6.6	22.3	3.3	6.1
WC62	91.3	27.5	1.92	4.7	13.1	23.3	6.1
WC21	45.7	26.2	3.13	3.7	8.7	13.3	6.0
WC32A	96.3	29.5	1.87	5.3	7.6	26.7	5.8
3306	72.7	27.0	1.87	3.7	17.0	3.3	5.8
SEC5×NE51	98.0	29.7	1.69	4.9	14.8	10.0	5.7
NE55	66.3	28.0	2.09	3.8	13.2	20.0	5.7
NE13	82.0	29.5	1.83	4.3	12.9	10.0	5.6
WC37	56.3	27.0	2.10	3.2	20.2	13.3	5.6
ACC25	57.0	28.7	2.29	3.7	7.0	20.0	5.5

Genotype	NE/10 seeds	MDP (days)	GI	ANH/seed	PWL	PPT (%)	DSI
ACC2 × IT	67.0	30.0	2.12	4.1	8.6	13.3	5.4
EBERAT×NE51	137.7	31.3	1.16	2.1	26.6	10.0	5.4
NE21	72.0	32.8	2.46	5.7	18.9	3.3	5.4
WC46	53.0	27.7	2.09	3.0	7.3	16.7	5.4
NE30	73.3	31.8	2.09	4.7	19.9	10.0	5.3
WC18	64.0	31.2	2.28	4.4	12.3	10.0	5.3
WC63	49.3	29.7	2.53	3.7	10.2	23.3	5.3
SEC5×SEC1	76.0	29.8	1.65	3.6	10.9	13.3	5.3
WC684	64.7	29.8	1.93	3.6	20.7	30.0	5.3
NE32	147.7	33.8	1.83	9.2	25.6	0.0	5.2
NE36	55.7	29.3	2.28	3.4	14.7	20.0	5.2
NE46	68.0	28.5	1.63	3.1	14.2	40.0	5.2
MU15	47.7	29.7	2.55	3.5	18.9	30.0	5.2
NE6	72.7	29.5	1.64	3.5	9.7	30.0	5.2
ALEGI×4W	47.7	25.8	1.82	2.3	5.7	56.7	5.2
ITxALEGI	53.7	30.2	2.30	3.5	18.1	16.7	5.2
5T×Acc12	45.7	29.7	2.57	3.4	6.6	36.7	5.2
WC30	50.0	29.0	2.22	3.2	6.1	36.7	5.2
WC17	75.3	30.2	1.62	3.6	41.8	10.0	5.2
NE67	39.3	27.5	2.50	2.6	14.8	10.0	5.2
WC2	78.3	29.5	1.44	3.2	19.7	20.0	5.2
NE71	53.0	29.7	2.11	3.3	11.9	30.0	5.1
NE51×SEC4	58.3	31.2	2.13	3.8	6.9	26.7	5.1
UW×5T	51.3	28.3	1.89	2.6	6.9	36.7	5.1
NE45	39.7	28.8	2.53	2.7	7.7	30.0	5.0
WC44	63.0	29.8	1.73	3.1	9.2	50.0	5.0
NE23	50.0	29.5	2.09	3.0	10.8	30.0	5.0
5T×4W	37.0	30.2	2.96	3.2	7.7	10.0	5.0
NE31	50.3	28.2	1.81	2.6	12.7	16.7	5.0
W32	55.7	30.8	1.98	3.3	10.9	6.7	5.0
ACC26×IT	38.7	29.5	2.60	2.8	23.5	10.0	5.0
MU24C	51.3	29.0	1.80	2.7	5.1	60.0	4.9
NE39 × SEC2	30.7	29.3	3.00	2.6	11.7	23.3	4.9
NE50	48.7	29.3	1.85	2.5	9.6	10	4.8
W10	63.0	30.7	1.59	3.0	7.8	46.7	4.8
WC15	44.3	31.0	2.32	3.0	13.7	50.0	4.8
WC29	54.0	29.0	1.54	2.3	13.8	10.0	4.8
IT82D-716	33.0	30.8	2.80	2.6	14.4	0.0	4.7
NE51	49.3	28.8	1.60	2.2	10.9	36.7	4.7
ACC12×5T	83.0	34.5	1.40	4.0	37.6	6.7	4.6
MU17	35.3	29.5	2.25	2.3	10.2	10.0	4.6
NE37	38.7	29.7	2.08	2.4	7.5	50	4.6
SEC1×SEC3	48.7	28.8	1.59	2.1	15.7	10.0	4.6
IT90K-277-2	26.3	28.8	2.98	2.1	5.6	13.3	4.6
SEC5×SEC2	57.3	28.5	1.27	2.0	15.9	10.0	4.6
WC35C	54.7	28.5	1.33	2.0	14.3	20.0	4.6
WC67B	44.7	29.8	3.69	2.3	19.5	26.7	4.6
ACC26×ACC2	39.0	26.2	1.54	1.5	12.6	50.0	4.5
NE70	44.0	29.0	1.60	2.0	10	40.0	4.5
NE40	35.0	27.8	1.81	1.7	16.9	20.0	4.5

Genotype	NE/10 seeds	MDP (days)	GI	ANH/seed	PWL	PPT (%)	DSI
WC35A	52.0	29.0	1.32	1.9	16.5	0.0	4.5
NE19	75.0	29.7	0.96	2.1	25.2	26.7	4.4
IT2841×BROWN	57.3	33.2	1.56	2.9	13.7	20	4.4
NE49	38.0	29.8	1.80	2.0	22.6	3.3	4.4
WC55	45.3	31.0	1.59	2.2	16.1	36.7	4.4
2309	41.3	30.0	1.56	1.9	22	16.7	4.3
ACC12 × 2W	46.7	31.5	1.52	2.2	21.2	36.5	4.3
WC60	52.7	29.7	1.19	1.7	18.1	26.7	4.3
KVU-271	46.0	30.0	1.40	1.9	8.5	53.3	4.2
WC674	38.0	33.8	2.05	2.5	9.7	20.0	4.2
NE18	50.3	29.5	1.15	1.7	12.5	10.0	4.2
WC7	50.7	29.2	1.09	1.5	11.2	23.3	4.1
WC68	48.0	29.0	1.11	1.5	8.9	30.0	4.1
NE44	52.0	30.5	1.10	1.7	10.4	30.0	4.1
5T×3B	33.0	29.7	1.62	1.6	12.2	30.0	4.0
WC48A	40.3	30.5	1.38	1.7	12.7	13.3	4.0
NYBOLA	29.7	30.5	1.85	1.6	8.6	20.0	4.0
NE51×SEC3	45.7	31.8	1.35	1.8	17.7	30.0	4.0
IT99K-1399	56.0	32.0	1.10	1.9	9.1	33.3	4.0
WC27	18.7	27.2	2.39	1.2	10.0	50.0	4.0
NE41	37.3	29.2	1.32	1.4	10.3	20.0	4.0
ACC12×3B	56.0	29.8	0.91	1.4	10.5	53.3	3.9
WC5	43.0	29.5	1.10	1.4	11.5	26.7	3.9
IT×ACC23	29.7	29.5	1.57	1.4	10.9	46.7	3.8
ACC23x4W	20.3	29.8	2.33	1.3	8.9	36.7	3.8
NE39	22.7	29.2	2.01	1.3	9.8	20.0	3.8
MU20B	27.0	31.3	1.84	1.5	22.7	30.0	3.8
WC58	39.3	32.2	1.28	1.6	1.7	43.3	3.7
WC35D	54.7	30.0	0.80	1.3	4.8	30.0	3.7
WC8	43.7	29.3	0.96	1.2	15.0	40.0	3.7
2392	48.0	30.2	0.85	1.2	12.3	40.0	3.6
ALEGI×3B	54.7	33.2	0.87	1.5	12.2	20.0	3.6
WC32×SEC5	36.0	29.0	1.08	1.0	1.3	66.7	3.6
NE53	47.0	28.3	0.78	1.0	5.7	46.7	3.6
IT98K-205-8	39.7	28.3	0.9	1.0	3.9	53.3	3.5
2434	33.0	30.0	1.15	1.1	10.3	46.7	3.5
4W × 5T	21.3	29.0	1.63	1.0	6.4	33.3	3.4
WC13	23.7	29.0	1.41	1.0	3.5	70.0	3.4
CIG	49.3	29.2	0.61	0.9	13.9	40.0	3.2
2W×IT	38.0	30.0	0.76	0.9	4.4	46.7	3.1
ALEGI×ACC2	39.7	30.5	0.74	0.9	2.2	56.7	3.1
SEC1×SEC4	14.0	25.0	1.62	0.6	1.3	76.7	3.0
3B×2W	28.0	30.0	0.95	0.8	3.4	66.7	3.0
WC48	12.0	25.2	1.88	0.6	6.0	53.3	3.0
WC67	18.3	28.8	1.19	0.6	3.4	60	2.7
ACC2×ACC12	54.7	32.0	0.41	0.7	1.6	70	2.6
ALEGI×5T	77.3	29.3	0.25	0.6	1.0	70	2.6
NE4	17.0	29.3	1.13	0.5	2.5	50	2.5
WC16	36.3	32.3	0.51	0.6	2.2	60	2.4
NE39×SEC4	13.7	25.0	1.16	0.4	2.7	70	2.4

Genotype	NE/10 seeds	MDP (days)	GI	ANH/seed	PWL	PPT (%)	DSI
IT90K-76	12.7	29.2	1.34	0.4	0.7	80	2.3
182	23.3	29.2	3.43	0.4	1.0	80	2.2
ACC23 × 3B	31.7	29.8	0.46	0.4	10.7	66.7	2.1
IT95K-207-15	6.0	28.3	1.41	0.2	1.7	86.7	1.3
IT97K-499-35	19.7	29.2	0.23	0.1	3.7	90	0.3
WC42	17.3	32.0	0.23	0.1	0.5	90	0.3
TVu-2027	7.0	42.0	0.37	0.1	0.0	93.3	0.2
2419	39.7	42.0	0.03	0.0	0.0	96.7	0.0
IT84s-2246	0.7	44.0	0.38	0.0	0.2	96.7	0.0
LSD	11.4	1.5	0.55	0.5	2.7	12.9	0.4

ACC = Accession; NE = Northern and Eastern; WC = Western and Central; Inbred lines at F7 generation; MU=Makerere University and IT = International Institute of Agricultural Research

Effect of bruchid attack on seeds of cowpea genotypes

Bruchid attack caused significant (P< 0.001) effects on seeds of cowpea genotypes (Table 3). The lowest mean number of holes and the highest percentage pest tolerance were observed on four cowpea genotypes including genotype 2419 (0 and 96.7%), IT84s-2246 (0 and 96.7%), TVu-2027 (0.1 and 93.3 %) and WC42 (0.1 and 90%). Meanwhile, the highest number of holes and lowest percentage pest tolerance was recorded on genotype NE32. The weight loss in different genotypes ranged from zero to 44.7 percent. The highest weight loss was recorded on genotype IT71 (44.7%) followed by WC69 (35.9%) while the lowest was recorded from genotype 2419 and TVu-2027 (0.0%), IT84s-2246 (0.2%) and WC42 (0.5%) (Table 3). Based on the Dobie susceptibility index, genotypes IT84s-2246, 2419, TVu-2027, WC42, IT97K-499-35, IT95K-207-15, ACC23 × 3B, 182, IT90K-76, NE39 × SEC4, WC16, NE4, ALEGI × 5T, ACC2×ACC12, WC67, WC48, 3B × 2W and SEC1× SEC4 were considered resistant, whereas IT109, SECOW2W, WC19, WC69, IT71, MU9, SECOW5T and IT889 were susceptible to the pest (Table 3).

Frequency distribution of the 145 genotypes based on the DSI, showed that 12% were resistant, 79.3% moderately resistant and 8.7% susceptible (Table 4).

Table 4. Classification of mean values of genotypes based on Dobie susceptibility index.

Class	Resistance class	No. Of Genotypes	NE/10 seeds	GI	MDP (days)	ANH/seed	PWL (%)	PPT (%)	DSI
1	Resistance	18	0.7-77.7	0.03-3.43	25-44	0.0-0.8	0.0-3.7	50-96.7	0.0-3.0
2	Moderately resistance	114	22.7-147	0.74-3.69	24.2-34.5	0.9-6.6	1.3-28.9	0.0-66.7	3.1-6.9
3	Susceptible	13	61-141	0.49-3.82	20.8-25.5	4.5-7.8	7-44.7	0.0-26.7	7.2-8.8

NE= Number of eggs; GI=Growth index; MDP= Median development period; ANH= Average number of holes; PWL= percentage weight loss; PPT= percentage pest tolerance and DSI= Dobie susceptibility index.

Correlation and regression analysis

The correlation coefficients (r) of cowpea resistance parameters screened are presented in Table 5. The percentage grain weight loss was significantly (P<0.001) positively correlated with the number of eggs (r = 0.55) and number of holes (0.54). Pest tolerance showed significant (P<0.001) negative correlations with number of eggs (-0.56), insect growth index (-0.50), number of holes (-0.66) and seed weight loss (-0.66). Dobie Susceptibility index showed significant (P<0.001) and negative correlations with insect development period (-0.63) and pest tolerance (-0.75); and positively correlated with number of eggs (0.72), growth index (0.7), number of holes (0.88) and weight loss (0. 57). Dobie Susceptibility index was predicted by a multiple linear regression analysis which was performed with number of eggs, number of holes, seed weight loss and pest tolerance as predictor variables.

The results of analysis indicated that these variables accounted for 82.3 % of the total variability among the genotypes for their resistance to bruchid (Table 6), but the best and only significant (P<.001) predictor of DSI was number of holes and pest tolerance (Table 6).

Table 5. Correlation coefficients (r) for cowpea genotype under *Callosobruchus maculatus* artificial infestation.

	NE	GI	MDP	ANH	PWL	PPT	DSI
NE	1						
GI	0.21	1					
MDP	-0.30	-0.48	1				
ANH	0.81	0.61	-0.42	1			
PWL	0.55	0.34	-0.20	0.54	1		
PPT	-0.56	-0.50	0.29	-0.66	-0.66	1	
DSI	0.72	0.70	-0.63	0.88	0.57	-0.75	1

NE= Number of eggs; GI=Growth index; MDP= Median development period; ANH= Average number of holes; PWL= percentage weight loss; PPT= percentage pest tolerance and DSI= Dobie susceptibility index. All correlations are significant *(P<0.001).*

Table 6. The results of multiple regression analysis for cowpea genotypes under *Callosobruchus maculatus* artificial infestation.

Parameter	Regression coefficient (b)	Adjusted R-square	P-value
Regresie (Dobie susceptibility index)	3.778***		.001
NE	0.000ns		.661
ANH	0.543***	82.32	.001
PWL	0.002ns		.672
PPT	-0.021***		.001

***= significant at P< 0.001 level, ns=non-significant; NE= Number of eggs; GI=Growth index; MDP= Median development period; ANH= Average number of holes; PWL= percentage weight loss; PPT= percentage pest toleranc and DSI= Dobie susceptibility index.

Discussion

The study demonstrate the existence of new sources of cowpea resistance to bruchid which could be used to introgress resistance into farmers' preferred but susceptible cowpea cultivars. Substantial variations were observed among the tested cowpea genotypes on their bruchid resistance parameters (Table 2) such as DSI (Dobie, 1974). According to Dobie (1974), the susceptibility index is linearly correlated with the intrinsic rate of increase and the logarithm of the number of insects that emerge over a given time period hence it provides a reliable estimate of resistance levels. Several studies have used Dobie susceptibility index as a measure of resistance to cowpea bruchid (Singh *et al.,* 1985, 2002; Singh, 2005). Genotypes that were identified as resistant based on DSI included IT97K-499-35 (Singh, 2005); IT84S-2246, IT90K-76 and IT95K-207-15 (Singh *et al.,* 2002); and TVu-2027 (Singh *et al.,* 1985). However, IT98K-205-8 and IT82D-716, introduced from IITA, Nigeria as resistance sources were found moderately resistant to the bruchid attack, suggesting the existence of bruchid biotypes which could break resistance of earlier reported resistant genotypes (Shade *et al.,* 1999).

Evidence of the resistance of cowpea genotypes to *C. maculatus* was clearly confirmed by reduced rate of oviposition in the resistance cowpea genotypes. Earlier work (Tripathi, 2012) showed a negative relationship between the number of eggs laid by bruchids and the level of resistance to bruchid, suggesting the existence of physical and/or biochemical factors which could either limit the insect from accessing the grain or make the seeds difficult for eggs to adhere to it. Sharma and Thakur (2014) also reported similar findings on the role of physical and biochemical factors of seed of resistant varieties in reducing oviposition rate.

Amusa *et al.* (2014) also reported significant reduction in oviposition of bruchid on resistant cowpea genotypes.

Differences between the genotypes were apparent with the days to adult emergence. The resistant genotypes were characterized by extended adult emergence period while adult emergence in susceptible lines was rapid. In case of resistant genotypes, the time to adult emergence was long for example 44 days in case of IT84s-2246 compared to 20.8 days, for the susceptible line IT889. This was accompanied by lower growth index values observed on resistant genotypes compared to susceptible ones (Table 3) with the insect progeny development taking a longer time in a resistant than in susceptible genotypes (Jackai and Asante, 2003; Amusa *et al.,* 2014). This significant delay in development of *C. maculatus* on the resistant genotypes could suggest the difficulty the insect was facing to infest the seeds and to cause damage. Badii *et al.* (2013) recorded extended adult emergence and low growth index value from the resistant cowpea genotypes and reported that growth index was the most reliable indicator of resistance of cowpea to bruchid.

Number of holes as an indicator of the innate potential of a genotype to overcome bruchid attack is known to affect the resistance of a particular cowpea genotype by causing a reduction in the rate of oviposition. High number of holes per seed was recorded from susceptible genotypes (ranging 4.5-7.8/seed) compared to the resistant genotypes (0-0.8/seed) (Table 4). This could suggest the existence of physical barrier in the seeds of resistant genotypes which could affect larval penetration (Laphale *et al.,* 2012) resulting in lowered number of holes. Similar results were reported by Appleby and Credland (2003) who observed reduced number of holes in resistant cowpea genotypes. This could also be related to the seed's biochemical compounds and its antixenosis nature (Sales *et al.,* 2005). Oviposition cues utilized by female bruchids may be more related to the presence or absence of certain chemical factors in the seed coats of these resistant cultivars (Epino and Rejesus, 1983). As shown by Sharma and Nwanze (1997) and Afzal *et al.* (2009) the presence or absence of certain plant biochemicals are involved in feeding and oviposition stimulation and deterrency which renders the seed undesirable to be bad host for rather an easy invasion to the insect (Dhaliwal and Arora, 2003). It is possible that the genotypes identified as resistant in this study may have an elevated level of certain chemical deterrents or a reduced level of certain oviposition stimulants in their seed coats than the susceptible genotypes.

Our result also showed wide variability among the cowpea genotypes with respect to seed weight loss (0.0% for the resistant to 44.70% for the susceptible) (Table 3). Low reduction in seed weight by the bruchid could be attributed to low insect growth index and seed damage. It was observed that, genotypes that had low weight loss generally had fewer eggs, low growth index, reduced number of holes and increased percentage pest tolerance. It has been reported that variables such as weight loss, number of holes and growth index are the most reliable indicators for resistance of cowpea to damage by C. maculatus (Jackai and Asante, 2003). Our study indicated that the genotypes which were least preferred by the *C. maculatus* for oviposition recorded less per cent weight loss (0-3.7%) compared to the highly preferred genotypes (16.7-44.7%) (Table 4). Similar reports were given by Jackai and Asante (2003) and Badii *et al.* (2013).

Correlation and regression studies

The extent to which the studied traits contributed to increase bruchid resistance was given by information obtained through correlation studies supplemented by multiple regression analysis. The results of correlation analysis between growth parameters of *C. maculatus* and DSI in the different cowpea genotypes indicated that weight loss was positive and significantly ($P<0.001$) correlated with the number of eggs laid, average number of holes and DSI but correlated negatively with percentage pest tolerance. This suggests that seeds permitting higher number of holes leading to higher weight loss and Dobie's susceptibility value. Similar correlation results were reported by Shade *et al.* (1999).

The results also indicated that number of eggs and number of holes and weight loss could be used as reliable indicators for identifying cowpea genotypes resistant to bruchid damage. Dobie susceptibility index showed significant ($P<0.001$) positive correlation with average number of holes but negatively correlated with percentage pest tolerance and median development period. This indicates that the longer the insect development period, the lesser the seed weight loss during storage due to low rate of insect multiplication as confirmed by a lower number of holes compared to susceptible genotypes. Similar results were reported by Shade *et al.* (1999), Lephale *et al.* (2012); Tripathi (2012) and Amusa et al. (2014) on cowpea and Mwila (2013) on common beans.

The results of multiple regression analysis indicated that number of holes and pest tolerance were major contributors for genotypic variation. The positive correlation relationship between number of holes and number of eggs indicated that these two traits could be controlled by similar, overlapping, linked genetic loci (Acquaah, 2012). This information could guide breeders on how to improve resistance in cowpea genotypes by focusing on reducing number of holes and eggs. The regression and correlation results also indicated that the number of holes and pest tolerance could be considered essential while selecting bruchid resistant genotypes, because they had strong correlations and higher contributions to variation of genotypes for their resistance to bruchid attack.

Conclusions

Results of the study showed the existence of genetic variability among the studied genotypes for resistance to bruchid. We identified new sources of resistance from the studied genotypes and recommend further investigations and identification of biochemicals that are responsible for cowpea seed resistance to bruchid. In addition, genetic studies of resistance to bruchid should be carried to help the incorporation of these factors into developing new resistant cowpea varieties. Among the tested genotypes for resistance against C. maculatus, landraces; 2419, 182, WC42, WC16, NE4, WC67 and WC48, inbred lines; ACC23 × 3B, NE39 × SEC4, ALEGI×5T, ACC2 × ACC12, 3B × 2W and SEC1 × SEC4 and IITA supplied genotypes; IT84s-2246 , TVu-2027, IT97K-499-35, IT95K-207-15 and IT90K-76 were found to be resistant to bruchid damage and therefore are recommended as promising donor source/parent for cowpea resistance to bruchid breeding programmes.

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