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Activity of Callosobruchus maculatus (F.) (Coleoptera: Bruchidae) on selected bambara groundnut (Vigna subterranea L. Verdc.) landraces and breeding lines

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Abstract

Bambara groundnut, an indigenous African legume crop, is cultivated as a subsistence crop by resource poor farmers. In storage, yield losses are compounded through damage by insect pests, with *Callosobruchus maculatus*, being a leading one. The development of a variety with minimum susceptibility to this insect is thus desirable for the improvement of this nutritious crop. The study was carried out to determine the susceptibility of three bambara groundnut breeding lines (SSD5, SSD8, SSD9) and three landraces (Uniswa red, AS17, OM1) to attack by the cowpea weevil, *Callosobruchus maculatus* and their germination success after damage. 20g of seeds of each variety, replicated four times, were used in the experiment. Physical characteristics of the seeds were noted and each replicate was infested with five pairs of *C. maculatus* and kept in a breeding chamber at 30°C. After an oviposition period of 7 days, the adult pairs were removed and number of eggs laid was counted. Subsequently, the developmental pattern of the insects, the amount of damage caused by the insects as well as susceptibility of the seeds to the insect were determined. The breeding lines were significantly larger and heavier than the landraces ($p<0.05$). The number of *C. maculatus* eggs laid were significantly different between the treatments ($p=0.0012$), with SSD8 and OM1 having significantly higher numbers laid. While % adult emergence was lower in SSD5, this was not significantly different between the varieties ($p=0.1416$). The susceptibility index was significantly different between varieties ($p=0.0192$) as well as between landraces and breeding lines ($p=0.0255$). On average, the landraces had higher SI (17.928 ± 2.4523) than the breeding lines (13.448 ± 5.9939). Germination success of damaged seeds was significantly higher in the landraces than the breeding lines (48.333 ± 18.007%). Results indicated that SSD5, SSD9 and AS17 were the most resistant to *C. maculatus* attack, while SSD8 and OM1 were the most susceptible. However, due to reduced germination success after damage, the breeding lines (SSD5 and SSD9) were not suitable for planting after storage while the landrace (AS17) was the most suitable due to its higher viability after *C. maculatus* damage.The results indicate that there is variability in resistance of the bambara groundnut varieties against the cowpea weevil. The use of resistant varieties could offer the simplest and cheapest way of improving bambara groundnuts production, especially if these maintain their viability after insect damage. The variability also emphasises the need for the maintenance of genetic diversity when selecting for desired traits.

Keywords: C. maculatus, Vigna subterranea, bambara groundnut, susceptibility, landraces, breeding lines

1. Introduction

Bambara groundnut (Vigna subterranea L. Verdc.) is an indigenous African legume crop which is cultivated throughout sub-Saharan Africa, especially in the drier parts of the continent. It is produced mainly as a subsistence crop, usually by small-scale female farmers. They are a rich source of minerals, energy and protein, with as much as 25.2% protein, 65% carbohydrates and 6% lipid, on a dry weight basis. Its tolerance to drought and poor soils makes it ideally suited to production in marginal areas where low-input arable agriculture is the norm (Doku, 1996; Maina et al., 2006; Amarteifo et al., 2006). In most African countries, its importance comes after cowpeas and groundnuts (Doku, 1996; Sesay et al., 1999).

In tropical subsistence agriculture, several leguminous crops in storage are prone to attack by insect pests, with bruchids (Coleoptera: Bruchidae) being the key pests (Sallam, M. H., 1999; Yakubu et al., 2012). In bambara groundnut, the cowpea weevil, i.e. Callosobruchus maculatus (F.), causes major losses in storage (Keals et al., 1997; Lale and Kolo, 1998; Rees, 2004). It is a field-to-store pest, ranked as the principal postharvest pest of stored pulses, with a cosmopolitan distribution. The cowpea weevil can cause as much as 99% yield loss in susceptible grain legumes. Damage includes reduction in kernel weight, caused by the burrowing larvae as they feed, and diminished market value due to the presence of insects inside the kernels. Bruchid infestation also decreases the germination potential of the kernel (Munthali and Sondashi, 2004; Maina et al., 2006; Rees, 2004). As with the majority of unimproved African crops, bambara groundnut is still cultivated using landraces, with farmers selecting seeds of landraces that are suitable for their local environment and possessing desirable traits such good taste, short cooking time, tolerance to drought and diseases (Lawn, 1989; Khonga et al., 2004; Sesay et al., 1999). The study was thus carried out to evaluate the susceptibility of 3 landraces and 3 breeding lines of local Bambara groundnuts to infestation by Callosobruchus maculatus as well to evaluate viability of bambara groundnut seeds that have been damaged by Callosobruchus maculatus.

2. Material and methods

2.1. Sources of seed and preparation of culture

All seed used was obtained under the auspices of the BAMFOOD project. Three pure breeding lines (SSD5, SSD8 and SSD9) were produced by a combination of artificial hybridization and selection using the single-seed descent (SSD) (Sesay et al., 2004a). These, in addition to 3 landraces (AS17, OM1 and Uniswa red), constitute the basis for bambara groundnut improvement in Swaziland. All varieties used were selected for high yield per plant and large pod size.

2.2 Determination of the physical characteristics of Bambara groundnuts

The diameter of ten kernels was measured from each variety using venier calipers and the average diameter calculated for each variety to obtain the seed size. Additionally, another 10 seeds from each variety were weighed and the average of the seed mass was calculated to obtain the individual seed mass. 10g bambara seeds from each variety were then placed in a petri-dish, dried in an oven at 130° C for 16 hours. They were then removed and re-weighed, to obtain the percentage moisture content. Testa and eye colour were determined by observation of the physical appearance of all the bambara groundnut varieties.

An insect culture was established by infesting clean bambara groundnuts seeds, in 750ml glass jars, with C. maculatus. They were then placed in a breeding chamber at 30°C.After 1 week, the adult weevils were removed and the seeds left for a further 3weeks. Newly emerged adult weevils, of uniform age, were then used for the experiment.

2.3 Infestation of seeds

20g of seeds of each variety were weighed and counted into 100ml glass jars, with each variety replicated four times. 5 pairs of newly emerged weevils were introduced to each jar and then stored in a breeding chamber at 30°C. After 1week, the adults were removed and the number of eggs laid in each jar recorded. Observation of adult emerging was initiated after one week and continued on a daily basis until no adults emerged. Newly emerged adults were removed. Additionally, seeds with emergence holes were counted and used to calculate the severity of damage in each replicate. This was calculated as:

Severity of damage = No. of adult progenies ÷ No. of damaged seeds

Additionally, the susceptibility index (SI) was calculated as follows:

SI=LogeF1 x 100/D

where F1 was the total number of emerging adults and D was the median developmental period, which was calculated as the time from mid-ovipositon period to 50% emergence of F1 generation (Maina, 2006).

2.4 Effect on Germination

Ten seeds, damaged by C. maculatus, of each variety were planted and monitored in flower pots. Another set of undamaged seeds was also planted, with each variety replicated 4 times. Germination success was determined after two weeks and expressed as a percentage of the seeds planted.

3. Result

The physical parameters and lineage of the landraces and breeding lines used are shown in Table 1. SSD5 had the highest weight and this differed significantly from SSD8, OM1 and Uniswa red (p=0.0002). Kernel size also differed significantly, with SSD5 again having the significantly larger kernel size (p=0.0000). Analysis of means revealed that Uniswa red, AS17, OM1 and SSD8 differed significantly from SSD5. Uniswa red also differed significantly from OM1. On average, the landraces had significantly lower weights (p = 0.0000) and size (p = 0.0006) compared to the breeding lines. Moisture content was not significantly different between the varieties assessed, with an average of 9.5296 ± 0.7718% (p = 0.0633).

There were significant differences in the number of eggs laid between the varieties (p = 0.0012) (Table 2). The highest number of eggs were laid on SSD8 (96.3 ± 15.1) and this was significantly higher than those laid on SSD9 (24.5 ± 13.1) and SSD5 (16.0 ± 10.8). OM1, with 73.0 ± 27.7 eggs, also differed significantly from SSD5. Adult emergence was also not uniform between the varieties assessed, with significant differences observed between them ($p = 0.135$). Further comparison of means indicated that SSD8 had a significantly higher number of adults emerging. However, these differences were not significant when comparison of percent emergence was carried out ($p = 0.1416$). Percent seed damaged was also not significantly different between the varieties ($p = 0.1767$) nor between landraces and breeding lines ($p = 0.1015$). The susceptibility index (SI) was significantly different between the varieties ($p = 0.0192$). Further analysis indicated that the SI for OM1 was significantly higher than that for SSD5. The SI also differed significantly between the landraces and breeding lines ($p = 0.0255$), with the land races having higher indices on average (mean = 17.928 ± 2.4523) than the breeding lines (mean = 13.448 ± 5.9939). However, the severity of damages was significantly different between the varieties ($p = 0.0162$). SSD8 suffered significantly higher levels of damage compared to SSD9, AS17 and SSD5. There were no significant differences in severity of damage between the landraces and breeding lines ($p = 0.6611$). There was a significant correlation between the number of eggs laid and the severity of damage ($\sigma = 0.6852$, $p = 0.0003$) while none was observed between the severity of damage and SI ($\sigma = 0.1133$, $p = 0.6069$).

In the undamaged seed, germination success was significantly different between treatments ($p=0.0002$), with further analysis of means indicating that Uniswa red had significantly higher germination success than SSD8 (Fig. 1). However, there were no significant differences in germination success between the breeding lines (85.714 ± 13.469%) and landraces (92.462 ± 8.7235%) ($p=0.1522$). Assessment of germination success of the damaged seeds indicated that there were also significant differences between the varieties assessed ($p = 0.0214$; Fig. 2). Further comparison of means indicated that SSD5 had a significantly lower germination success than AS17. There were significant differences between the landraces and breeding lines ($p = 0.0037$), with the landraces having notably higher germination success of the insect damaged seeds than the breeding lines. Damaged seeds had significantly lower germination success than undamaged seed ($p=0.0002$), with 35.355 ± 23.447% success compared to 89.088 ± 11.621% in the latter.

4. Discussion

The breeding lines had larger seeds compared to the landraces, as seen by the bigger and heavier seeds. These characteristics were considered as desirable by local farmers interviewed during a survey (Sesay et al. 2004b). Seed sizes of at least 1.69cm in diameter were considered as large, e.g. SSD5 and SSD9 in this experiment. However, earlier studies have indicated that differences in physical characteristics such as testa thickness, seed mass and seed size, did not play a major role in conferring resistance to seeds of different cereal and legume cultivars to attack by insect pests (Maina et al., 2006**).** This was confirmed by the non-significant differences in the physical parameters of the seed assessed. Differences may therefore be attributed to their biochemical properties (Munthali and Sondashi, 2004). Seed colour can influence resistance of bambara groundnut varieties to infestation by C. maculatus. Red coloured seeds, e.g. SSD5, SSD9 and Uniswa red, tend to have higher iron content which contributes to their increased resistance while resistant cream coloured seeds, such as AS17, also had lower phosphorus and copper levels than susceptible kernels of the same colour, e.g. OM1 (Munthali and Sondashi, 2004). Lower levels of phosphorus and copper could result in poor development of the weevil.

Adult emergence ranged from 87.56 to 54.23%. A number of factors could have contributed to the less than 100% emergence in all the seeds. Grain legumes contain toxic chemical factors in their seed coat, which act as a basis for resistance against bruchid attack. In bambara groundnuts, there is a trypsin inhibitor known to occur at higher levels in raw bambara groundnuts. This trypsin inhibitor may contribute to high oviposition deterrence and high larval mortality culminating in reduced progeny development (Maina et al., 2006). SSD5 and SSD9, with the lowest number of eggs laid and adult emergence, may have higher levels of chemical deterrents which resulted in reduced progeny development for the cowpea weevil. Other factors contributing to reduced developmental success include bruchins (Doss et al., 2000), larval competition and resource suitability, e.g. SSD8, Uniswa red and OM1, which despite high number of eggs laid, had lower % adult emergence than AS17. Resource limitations within the seed would result in fewer progeny surviving to adulthood, e.g. OM1 and SSD8.

A resistant variety is expected to have relatively lower adult progenies emerging from the seeds or lower number of eggs laid on the seeds than a susceptible variety (Munthali and Ramoranthudi, 2004). From the results obtained, SSD5 and SSD9 had the lowest number of C. maculatus eggs laid as well as % adult emergence, indicating that these two breeding lines were less susceptible to C. maculatus attack. This was confirmed by these two breeding lines having the lowest SI. SSD5 also had the lowest severity of damage observed. On the other hand, SSD8 and OM1 are more susceptible and less resistant to C. maculatus attack because they had a higher number eggs laid per seed, high adult emergence as well as severity of damage than the other four Bambara groundnut varieties. Susceptibility, however, does not translate to actual damage as observed in AS17 which had the second lowest severity of damage, suggesting that this landrace was resistant to C. maculatus damage despite its high susceptibility index.

Infestation and damage of Bambara groundnuts by C. maculatus was expected to have an effect on the germination potential of the seeds and this was confirmed by the results obtained. In the damages seeds, only AS17 seeds had a germination percentage above 50%. Internally feeding larvae damage essential parts such as the plumule thus impeding germination. Results indicated that the landraces had a higher germination percentage than the breeding lines after C. maculatus damage, while no such difference was observed in undamaged seeds. Landraces have a mixture of genotypes with high diversity both between and within populations while breeding lines are selected for a particular attribute, which in this study was yield. Individuals within a landrace population therefore vary in their environmental responses and are more robust to adversity than breeding lines, thus the higher performance after insect damage. This is an advantageous attribute since the landraces were observed to be more susceptible to C. maculatus attack than the breeding lines.

The significant difference in susceptibility indices and severity of damage of the six bambara groundnuts to infestation to C. maculatus shows that there is variability in resistance of the bambara groundnut varieties used. The use of resistant varieties such as SSD5 could offer the simplest and cheapest way of improving bambara groundnuts for resource poor subsistence farmers. Based on results of this study, AS17, SSD5 and SSD9 were the most resistant to damage by the cowpea weevil. These had the fewer eggs laid on them and the lowest severity of damage scores. The breeding lines (SSD5 and SSD9), however, had reduced viability due to this damage and may not be suitable for planting after storage and C. maculatus damage, while the land race (AS17) proved to have high germination success even after insect damage. This emphasises the need for the maintenance of genetic diversity when selecting for desired traits.

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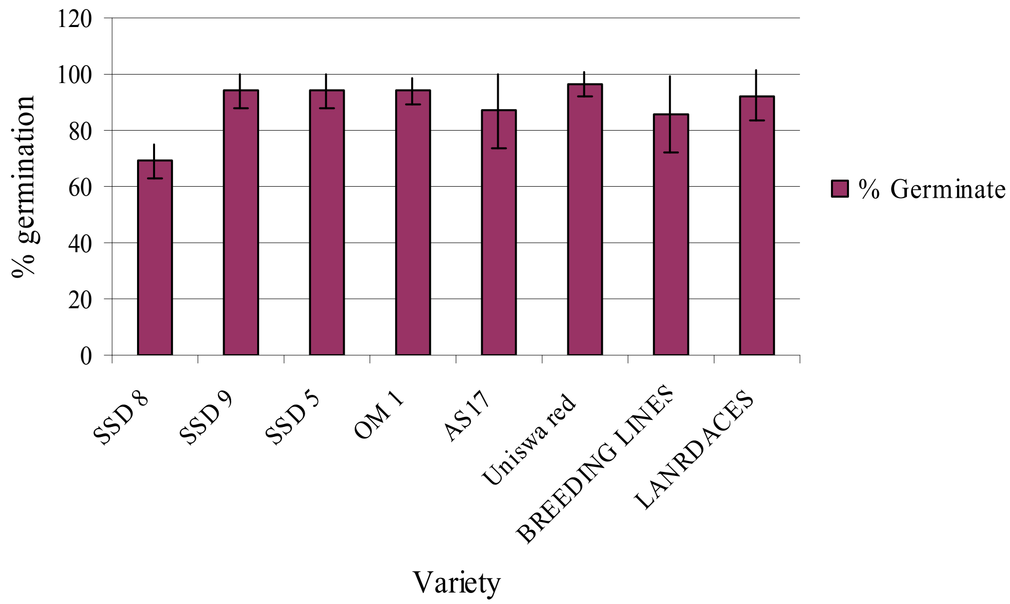

Figure 1. Germination success of undamaged kernels

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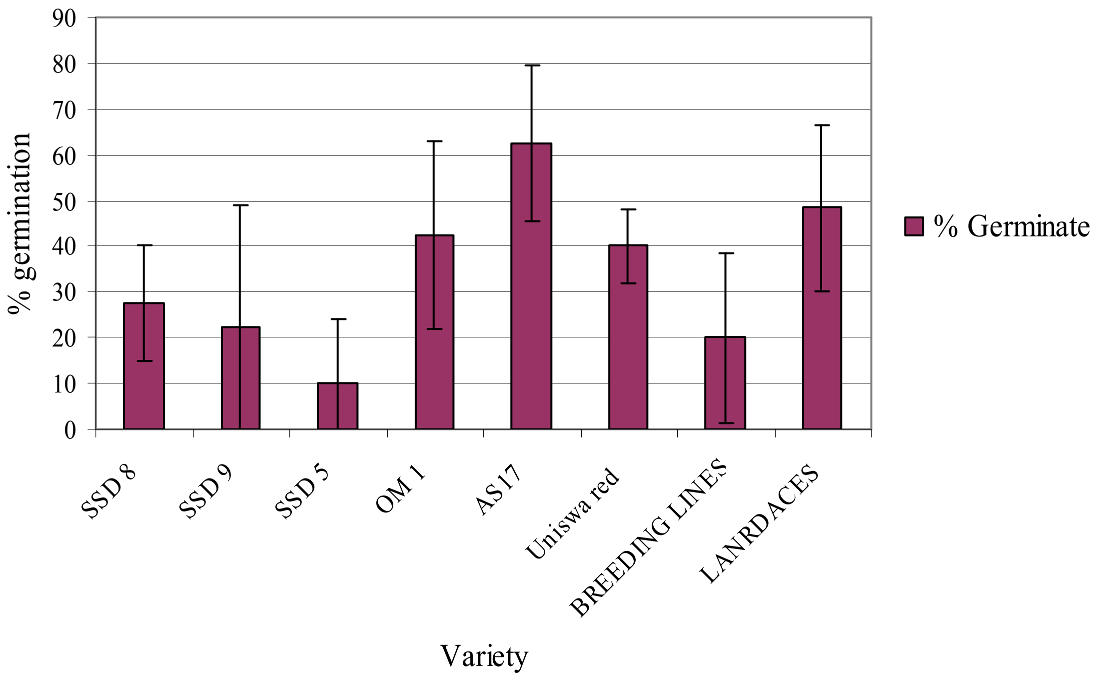

Figure 2. Germination success of kernels damaged by *Callosobruchus maculatus*

Table 1. Physical characteristics of the breeding lines and landraces used

Breeding line/Landraces	Seed weight (g)	Seed size (mm)	Number of seeds	% Moisture content	Seed description	Pedigree
SSD 8	0.7200 ± 0.0919	9.4430 ± 0.5580	32.6 ± 3.79	10.200 ± 0.6160	Cream testa, red butterfly-like eye	OM 1
SSD 9	0.8600 ± 0.1776	11.1670 ± 1.3738	27.7 ± 1.26	9.0976 ± 0.6971	Red, no eye	NTSR
SSD 5	0.9100 ± 0.1197	12.0970	28.0 ± 1.41	9.7690 ± 0.9285	Red, no eye	NTSR
OM 1	0.7200 ± 0.0919	8.6890 ± 0.6186	35.2 ± 1.71	10.1660 ± 0.6277	Cream testa, black butterfly-like eye	
AS17	0.7200 ± 0.1033	9.3620 ± 0.5813	35.5 ± 2.38	9.1774 ± 0.4685	Cream testa with black stripes	
Uniswa red	0.6900 ± 0.738	9.7620 ± 0.5153	33.7 ± 0.96	8.7675 ± 0.1311	Dark red, no eye	
Breeding lines	0.8300 ± 0.1535	10.9020 ± 1.5385	30.417 ± 4.0104	9.6888 ± 0.8145		
Landraces	0.7100 ± 0.0885	9.2710 ± 0.7133	34.833 ± 1.8007	9.3704 ± 0.7386		

Table 2. Susceptibility parameters of the breeding lines and landraces used

Breeding line/Landrace	No. of seeds damaged	No. of eggs laid	No. of adults emerged	% Emergence	Susceptibility Index	Severity of damage
SSD 8	22.500 ± 1.0000^{2}	96.250 ± 15.130^{1}	60.250 ± 4.5735	64.195 ± 13.780^{3}	17.616 ± 3.6251^{3}	2.6765 ± 0.1336^{1}
SSD 9	11.250 ± 8.4607^{5}	24.500 ± 13.178^{5}	17.500 ± 12.124	62.273 ± 24.3480^{5}	12.880 ± 5.0870^{6}	1.6906 ± 0.2405^{4}
SSD 5	7.7500 ± 7.3201^{6}	16.000 ± 10.801^{6}	11.250 ± 10.340	54.2300±38.3260^{6}	13.131 ± 3.6210^{5}	1.1310 ± 0.7645^{6}
OM 1	25.00 ± 6.7330^{1}	73.00 ± 27.797^{2}	50.250 ± 17.689	71.217 ± 17.954^{2}	19.473 ± 1.6541^{1}	1.9887 ± 0.2195^{2}
AS17	20 ± 6.4807^{3}	37.5 ± 13.5280^{4}	32.750 ±12.5270	87.5660 ± 11.070^{1}	19.234 ± 1.4057^{2}	1.6165 ± 0.1096^{5}
Uniswa red	19.000± 10.9540^{4}	61.500 ± 46.744^{3}	36.250 ± 23.114	63.168 ± 12.447^{4}	15.077 ±1.0204^{4}	1.9361 ± 0.4528^{3}
Breeding lines	13.833 ± 8.8094	45.583 ± 39.433	29.667 ± 24.336	60.232 ± 25.187	13.448 ± 5.9939	1.8327 ± 0.7908
Landraces	21.333 ± 8.0038	57.333 ± 33.093	39.750 ± 18.336	73.984 ± 16.613	17.928 ± 2.4523	1.8471 ± 0.3191