



Resistance of ten elite African yam bean cultivars to the bean weevil *Callosobruchus maculatus*

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Abstract

The African yam bean *Sphenostylis stenocarpa* is a nutritious under-exploited legume from Africa, with a potential to contribute greatly towards food security. The seed of this crop is attacked by the bean weevil *Callosobruchus maculatus* during storage, which can render them unfit for consumption or replanting. Insect-resistant varieties would be ideal and desired as these will require little or no additional costs for insecticides from farmers and will offer immense and long-term health, economic and environmental benefits to the farmers and consumers. In this study, standard procedures were followed to ascertain the resistance of 10 elite *S. stenocarpa* cultivars to stored product insect pests, *C. maculatus* and the maize weevil *Sitophilus zeamais*. Under conditions of natural infestation, seven (7) tropical *S. stenocarpa* accessions (TSs 9, 10, 33, 60, 93, 126 & 349) were resistant to *C. maculatus* and had no infestation at all while 3 accessions (TSs 23, 111 & 116) were susceptible. When the accessions were subjected to intentional infestation with *C. maculatus*, they showed varying levels of resistance, with TSs 93 as the most resistant accessions and TSs 23 as the least resistant accession. The maize weevil, *S. zeamais* however failed to establish on *S. stenocarpa* seeds. While lectin has been implicated in insect resistance by *S. stenocarpa*, this study found no significant correlation between lectin and the resistance of different accessions. Elite cultivars of the African yam bean *S. stenocarpa* that are resistant to its major insect pest *C. maculatus* have been identified in this study. These resistant cultivars will be of significance to future breeding programmes and food security in developing tropical countries and beyond.

Key words: *Callosobruchus maculatus*, *Sphenostylis stenocarpa*, resistant, infestation, African yam bean, weevils

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INTRODUCTION

African yam bean (AYB), *Sphenostylis stenocarpa*, is one of the most nutritious and promising under-exploited and neglected grain legumes of African origin. Nigeria has huge AYB biodiversity and is reputed as one of the largest producers of the crop in Africa. In both the southern and northern parts of Nigeria, AYB is adaptable to different agro-ecologies and has the potential, with more study, to play a significant role in the sustainability of food and nutrition security (Baiyeri *et al.*, 2022). The crop produces appreciable grain yield under marginal soils and enhances the growth, yield and yield-related traits of other component crops in intercropping systems (Baiyeri *et al.*, 2018), possibly because of its huge nodulation capacities. AYB also produces edible tubers (Baiyeri *et al.*, 2019).

AYB is attacked by insect pests, with the bean weevil *Callosobruchus maculatus* being a major threat to stored AYB seeds resulting in food and economic losses. Due to the fact that they deposit their eggs on seed and pods in the field and hatch during storage, insect pests like *C. maculatus* are challenging to control (Chauhan and Ghaffar, 2002). Under a shorter length of time and in humid circumstances, they reproduce quickly and eat a lot of stored seeds that would have been consumed or planted the next year (Lawrence *et al.*, 2017). Reports on seed saving technologies in AYB production and storage are scanty in the literature when compared to what have been developed and reported for other major grain legumes like cowpea and soybean. One of the low-cost and sustainable strategies for combating insect-infestation challenges in legumes either while pods are on the field or the seeds are in storage is the host-plant resistance, which can be achieved by selecting or breeding varieties that combine good yields with enhanced insect pest resistance and other important farmer-preferred and consumer-preferred traits. Developing improved AYB insect-resistant varieties will require little or no additional costs from farmers for seed protection chemicals from insects and will have immense and long-term food, health, economic and environmental benefits to the AYB farmers, consumers and other end-users.

A large collection of AYB accessions from various locations in Africa exists at the International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria from which breeding programmes could benefit. Despite this, no singular insect-pest resistant improved AYB

variety has been released from any AYB breeding programme in Africa till date to the best of our knowledge. Several studies have characterized different AYB accessions (Popoola *et al.* 2011; Adewale *et al.* 2012; Abdulkareem *et al.* 2015), but these studies have largely investigated morphological diversity and morphology-related intraspecific variabilities. Studies that evaluated AYB accessions for variability with respect to insect-pest resistance are scanty in the literature. Baiyeri *et al.* (2022) evaluated 36 AYB accessions from IITA for yield performance and other yield-related traits, and identified some elite cultivars. This study was initiated to determine the levels of resistance of seeds of those elite AYB accessions to the bean weevil. These insect-pest resistant AYB accessions would be ideal for hybridization in future AYB breeding programmes.

MATERIALS AND METHODS

Study location

This study was carried out in the Department of Animal and Environmental Biology, Federal University, Oye-Ekiti, Nigeria (N07° 46.692', E005° 18.930'). The ambient conditions were daylight to darkness period of 12:12 hours, average temperature of 27.5 ± 8°C and relative humidity of 82.5 ± 5%.

Seed materials

Seeds of the African yam bean (AYB) *S. stenocarpa* used in this study were taken from seeds harvested from AYB accessions that had previously been obtained from the Genetic Resources Center of the International Institute for Tropical Agriculture (IITA), Ibadan Nigeria, and evaluated at the research farm of the Department of Crop Science, Federal University, Oye-Ekiti, Nigeria (Baiyeri *et al.*, 2022). The AYB seeds had been harvested from the field and kept in storage in paper envelopes 3 months prior to this study. The 10 elite AYB cultivars used in this study were the tropical *Sphenostylis stenocarpa* (TSs) 9, 10, 23, 33, 60, 93, 111, 116, 125 and 349.

Insects

Bean weevils

C. maculatus adults were retrieved from infested stored AYB stock. The bean weevils were separated according to their sexes under a stereo microscope depending on the physical appearances of the posterior abdominal plates or segments. Males have a light colored and

blunt-ended posterior, while females have an enlarged and protruding rear end with two distinctive black spots (Beck and Blumer, 2019).

Maize weevils

Sitophilus zeamais adults were obtained from an infested maize stock. They were separated according to their sexes using a stereo microscope based on the physical appearance of the rostrum. Males have a rough and broad rostrum, while females have a shiny and slender rostrum (Ojo and Omoloye, 2012).

AYB susceptibility trials

To determine the susceptibility of the AYB accessions to the bean weevil *C. maculatus*, two different strategies were employed resulting in two different experimental set up. Set-up 1 was a trial based on natural infestation. Here, 30 g of each of the 10 different AYB accessions were taken at random, weighed and kept in a round plastic container (diameter 8.5 cm, height 4.2 cm) covered with a muslin cloth before capping to prevent entry or escape of any insect. Set-up 2 was a trial based on artificial or introduced infestation and performance of the introduced bean weevils. Again, 30 g of each of the 10 different AYB accessions were weighed out and put in a round plastic container. However, the AYB seeds from the 10 different accessions were inspected prior to inclusion in the study and those seeds with eggs deposited on the surface or other signs of prior infestation were excluded. Fifteen (15) males and (15) females respectively were added to each container before covering the container with a muslin cloth and capping as previously described. There were 3 replicate containers for each AYB accession in either of the set-up 1 and 2 (see supplementary information). Because *C. maculatus* has a generation time of about 4-5 weeks at 25°C (Beck and Blumer, 2019), these experimental set-ups were allowed to stay for at least 6 weeks before reassessment was made to allow for emergence of the succeeding weevil generation. During reassessment, the number of live and dead bean weevils in each plastic container was determined by physical counting.

To determine if AYB could be infested by other common stored products pests, the maize weevil *S. zeamais* was introduced into containers with AYB seed (set-up 3). For each of the 10 different AYB accessions, 30 g of seeds devoid of prior infestation were weighed

into a round plastic container as previously described and then 5 males and 5 females of *S. zeamais* were taken at random and added to each container before covering with a muslin cloth and capping. For each AYB accession, 3 replicate containers were established. The containers were reassessed as previously described after 1 week (set-up 3a) and then again after 6 weeks (set-up 3b) (see supplementary information).

Lectin assay

First, 0.20 g of ground AYB sample was weighed into a screw cap centrifuge tube with 10 ml of 0.1M phosphate and 10 ml of 0.85% NaCl was added. The mixture was stirred/shaken at room temperature on a shaker. After 18 hours, the suspension was centrifuged at 1,500 rpm for 15 minutes. The supernatant obtained was transferred into a separate clean screw cap centrifuge tube by decantation. Then the photometric heamagglutinin inhibitor activity as described by Liener (1955) was performed. A set of heamagglutinin standard solutions was prepared by serial dilution of range 0 to 1.0 ml of the stock heamagglutinin to give 0 ppm, 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm and 1.0 ppm of working standard solutions. The AYB sample extracts were pipetted into a triplicate set of test tubes with each set for each level of heamagglutinin. Each sample and working standard solution were treated with 0.9% saline cell suspension solution. The absorbance (readings) of the blank solution, the AYB sample extracts, as well as the working standard solutions were read on a Spectronic 20D Spectrophotometer. The heamagglutinin activity was calculated using the formula: HU/mg or LU/mg = 98.56 (Absorbance of sample – Absorbance of blank); where HU/mg is Heamagglutination unit per milligram and LU/mg is lectin unit per milligram.

Data analysis: Data collected were analysed using the *R* statistical software version 4.1.1. The analysis of variance (ANOVA) was done using the library: Agricolae. The significance of the treatment means was determined by the Tukey's Honest Significant Difference (HSD) test at 5% probability level. The standard deviation from the mean was analysed using the library: Psych. Pearson's correlation analysis was done using the library: Hmisc (correlation analysis was done to understand the strength of relationships that existed among the lectin content and number of bean weevils in the various experimental set-ups in this study using R package).

RESULTS

In general, 7 out of the 10 AYB accessions (70%) utilized in this study had not been naturally infested by the bean weevil, *C. maculatus*. Only TSs 23 (12.33 ± 6.43), TSs 111 (1.00 ± 1.17) and TSs 116 () were infested with *C. maculatus*. The other seven accessions, namely TSs 9, 10, 33, 60, 93, 125 and 349, were not infested. TSs 23 which had the highest level of natural bean weevil infestation, was also significantly ($P < 0.01$) different from the infestation recorded for all the other accessions (Table 1). Beside TSs 23, the natural bean weevil infestation levels in TSs 116 was also significantly ($P < 0.01$) different, while the infestation in TSs 111 was not significantly different from those accessions that were not infested.

Introduction of *C. maculatus* to AYB seeds led to successful colonization of the seeds in each respective container. The infestation of AYB seeds by introduced *C. maculatus* was highest in TSs 23 and this was found to be highly significantly ($P < 0.01$) different from other accessions (Table 2). The lowest infestation due to introduced *C. maculatus* was in TSs 93 and this was also found to be highly significantly ($P < 0.01$) different from other accessions,

except for TSs 125 which had the second lowest infestation by *C. maculatus* (Table 2). TSs 93 and TSs 125 in all the experimental set-ups showed significant levels of resistance to infestation by the bean weevil.

On the assessment of the potential of cross-infestation of AYB cultivars by maize weevils, no maize weevils (*S. zeamais*) could survive beyond 1 week after they were introduced to different experimental containers of AYB accessions (Table 3). However, few individuals of *C. maculatus* were recovered from TSs 23 and TSs 116. When the experimental set-ups were allowed to stay for 6 weeks, no progeny of *S. zeamais* was recovered from any of the accessions. Rather, more individuals of *C. maculatus* were recovered from the experimental set-ups. The infestation of the AYB accessions by *C. maculatus* in the experimental set-ups with introduced *S. zeamais* mirrored that recorded earlier in Table 1 as the same accessions either showed infestation or showed no infestation, except for TSs 60 which showed some low-level infestation (Table 4). This time however, the level of infestation for TSs 23 and TSs 116 were found to be highly significantly ($P < 0.01$) different from each other and from other accessions used in the study (Tables 4).



Figure 1. (A) African yam bean seeds (B) Bean weevil, *Callosobruchus maculatus*, ©Georg Goergen (CABI Compendium) (C) Maize weevil, *Sitophilus zeamais* ©Georg Goergen (CABI Compendium)

Table 1. Performance of introduced *C. maculatus* on the seeds of 10 African yam bean accessions

Accession	Total number of bean weevils (\pm SD)
TSs 9	79.67 \pm 28.11 ^{cd}
TSs 10	105.67 \pm 52.20 ^{bc}
TSs 23	202.67 \pm 18.93 ^a
TSs 33	82.00 \pm 30.79 ^{bcd}
TSs 60	83.67 \pm 16.26 ^{bc}
TSs 93	18.00 \pm 8.66 ^e
TSs 111	92.33 \pm 41.63 ^{bc}
TSs 116	91.33 \pm 25.70 ^{bc}
TSs 125	30.00 \pm 21.17 ^{de}
TSs 349	134.33 \pm 40.87 ^b

Means followed by different letters are significantly different by a Tukey's HSD test at $\alpha = 0.05$. SD = standard deviation.

Table 2. Performance of introduced maize weevils *Sitophilus zeamais* on the seeds of 10 African yam bean accessions

Accession	Number of dead maize weevils (\pm SD)	Number of maize weevils alive (\pm SD)	Number of bean weevils from natural infestation (\pm SD)
TSs 9	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c
TSs 10	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c
TSs 23	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	2.33 \pm 2.08 ^b
TSs 33	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c
TSs 60	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c
TSs 93	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c
TSs 111	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c
TSs 116	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	3.67 \pm 0.58 ^a
TSs 125	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c
TSs 349	10.00 \pm 0.00 ^a	0.00 \pm 0.00 ^a	0.00 \pm 0.00 ^c

Means followed by different letters are significantly different by a Tukey's HSD test at $\alpha = 0.05$. SD = standard deviation.

Table 3. Performance of bean weevils on the seeds of 10 African yam bean accessions previously exposed to maize weevil

Accession	Total number of bean weevils (\pm SD)
TSs 9	0.00 \pm 0.00 ^c
TSs 10	0.00 \pm 0.00 ^c
TSs 23	12.67 \pm 4.16 ^b
TSs 33	0.00 \pm 0.00 ^c
TSs 60	2.67 \pm 4.62 ^c
TSs 93	0.00 \pm 0.00 ^c
TSs 111	1.00 \pm 1.00 ^c
TSs 116	18.00 \pm 5.00 ^a
TSs 125	0.00 \pm 0.00 ^c
TSs 349	0.00 \pm 0.00 ^c

Means followed by different letters are significantly different by a Tukey's HSD test at $\alpha = 0.05$. SD = standard deviation.

The lectin content of the seeds of different AYB accessions was investigated and it was discovered that TSs 33 had the highest lectin concentration of 67.17 LU/mg, while TSs 60 had the lowest lectin content of 62.40 L.U/mg (Table 5). However, there was no significant correlation between the lectin content and the level of infestation of these AYB accessions (Table 6).

Table 4. Lectin concentration (L.U/mg) in the seeds of 10 African yam bean accessions

Accession	Lectin Concentration (L.U/mg) (±SD)
TSs 9	63.86±0.01 ^d
TSs 10	66.30±0.02 ^b
TSs 23	66.08±0.02 ^c
TSs 33	67.17±0.13 ^a
TSs 60	62.40±0.02 ^f
TSs 93	66.03±0.05 ^c
TSs 111	63.51±0.04 ^e
TSs 116	63.91±0.02 ^d
TSs 125	67.15±0.04 ^a
TSs 349	63.87±0.04 ^d

Means followed by different letters are significantly different by a Tukey's HSD test at $\alpha = 0.05$. SD = standard deviation.

Table 5. Correlation coefficients among the number of bean weevil recorded in various set-ups and lectin content (L.U/mg) of the seeds of 10 African yam bean accessions

	Set-up1	Set-up2	Set-up3a	Set-up3b	Lectin
Set-up1	1.00				
Set-up2	0.58*	1.00			
Set-up3a	0.73*	0.38*	1.00		
Set-up3b	0.78*	0.42*	0.93*	1.00	
Lectin	0.02	-0.12	-0.08	-0.15	1.00

* Correlation is significant at the 0.05 level; Set-up1=total number of bean weevils from natural infestation; Set-up3a=total number of bean weevils from natural infestation in set-up 3 after 1 week; Set-up3b=total number of bean weevils in set-up 3 after 6 weeks; Set-up2=total number of bean weevils from introduced infestation after 7 weeks; Lectin = lectin content (L.U/mg). SD = standard deviation.

DISCUSSION

In this study, we have identified AYB cultivars that are resistant to bean weevil infestation under conditions of both natural and introduced infestations respectively. Of these cultivars, TSs 93 and TSs 125 exhibited the best and significant levels of resistance to infestation by the bean weevil, while TSs 23 was the most susceptible. AYB seeds and pods have previously been reported to show some resistance to major cowpea pests such as *C. maculatus* and *Clavigralla tomentosicollis* (Machuka *et al.*, 2000; Okeola *et al.*, 2001). However, these studies did not involve any of the elite AYB cultivars used in this study.

The highest bean weevil-tolerant or resistant accessions, TSs 93 and TSs 125, may possess some genes that confer resistance to bean weevil infestation. These genes might influence some physical and (or) chemical factors that could have affected oviposition, hatching of eggs or the development of *C. maculatus* larvae and pupa in the AYB seeds. Fadelmula and Horber (1984) noted that hardness and resistance to pest insects were closely related, while Ibrahim (2001) reported that a positive correlation exists between the severity of insect damage and the hardness of the seed coat.

Doka and Mohamed (2018) also reported a positive relationship between seed coat hardness of grains and reduced grain infestation by insect pests. In their opinion, the physical and chemical compositions (moisture content, hardness, grain size, digestibility, starch, and protein) greatly influence susceptibility to stored sorghum grain infestation. Antibiotic factors in the endosperm and the difficulty in obtaining the optimal quantity of nutrients needed for growth from a harder endosperm may be primarily responsible for the relative resistance of seeds (Jansen, 1969; Barros and Zucoloto, 1999; Zakka *et al.*, 2013). These in turn could account for why the maize weevil *S. zeamais* could not survive on any cultivar of AYB. Further studies will be required to evaluate the seeds of AYB accessions from this study for potential physical and chemical factors that could be responsible for the observed resistance to insect pests.

In AYB, lectin found in the seeds is thought to be the main cause of resistance (Ojuederie *et al.*, 2016). Crop plants' lectin insecticidal properties have been the subject of several studies (Pratt *et al.*, 1990; Huesing *et al.*, 1991; Powell *et al.*, 1993; Gatehouse *et al.*, 1997), although lectin's mechanism of action is partially understood (Peuman and Van Damme,

1995). Machuka *et al.* (2000) inferred from the results of their study that although the AYB lectin prevents pupation, it does not necessarily prevent adult emergence. Our study has however shown that lectin had no significant correlations with resistance or susceptibility (number of bean weevil) observed in the AYB accessions evaluated in the various experimental set-ups. Susceptible accessions like TSs 23 also recorded significantly higher lectin content than some of the *C. maculatus* resistant/tolerant AYB accessions. This suggests that the resistance to bean weevil infestation expressed by TSs 93, TSs 125 and other *C. maculatus* infestation tolerant accessions like TSs 9, TSs 10 and TSs 33 could have been due to other factors in the accessions other than lectin concentration alone or other factors combined with lectin content. Although Omitogun *et al.* (1999) found that the crude seed lectin extracts of AYB and hyacinth beans were extremely toxic to *C. maculatus*, *Maruca vitrata* and *C. tomentosicollis*, they also noted that AYB has a wide range of resistance which their study did not address. Numerous metabolic products, such as primary (lectin, proteinase, and amylase) inhibitors and secondary (tannins, alkaloids, and rotenoids) metabolites, can play a role in crop plant resistance (Omitogun *et al.*, 1999). It is safe to speculate that other phytochemicals in AYB seed that probably have combined effects with lectin could have been responsible for the resistance to *C. maculatus* infestation observed. Admassu (2008) reported that high phytochemical concentrations especially lectin and saponins led to increased resistance to storage pests. The non-significant correlation between lectin content and the reduced level of insect pest infestation, and the reduced lectin contents of some of the *C. maculatus* moderately resistant/tolerant AYB accessions like TSs 9, TSs 60 and TSs 111 also suggest the possibility of selecting and breeding for *C. maculatus* infestation resistance in AYB seeds without necessarily increasing the lectin content. Lectins are antinutrients and not highly desirable in foods considering the fact that antinutrients limit or alter the hydrolysis and absorption of carbohydrates from the gut by binding to them in food. As a result, they reduce the level of energy for growth and maintaining physiological processes (Pusztai *et al.*, 1990; Machado *et al.*, 2008). Additionally, they encourage the growth of harmful bacteria in the gut (Pusztai *et al.*, 1995). Information on the magnitude of the correlation coefficient, the degree of correlated response as a function of the genetic correlation between the traits, as well as the heritability of the correlated traits will

assist in selecting a breeding strategy that will result in rapid improvement and maximum genetic gains (Falconer and Mackay, 1996; Agaba *et al.*, 2021). For the future, the non-significant correlation between resistance to *C. maculatus* and lectin will also be helpful in developing improved AYB varieties with low lectin contents and resistance to *C. maculatus*. This could result in the breeding of improved AYB varieties with enhanced insect pest resistance, high yield and low lectin content that should enhance the economic returns for AYB seed end-users and farmers that grow this important grain legume. Furthermore, such improved AYB varieties will hugely facilitate the continued production and utilization of a multipurpose and nutrient-dense pulse like AYB that is gradually going into extinction.

CONCLUSION

In conclusion, our study has identified several AYB cultivars that showed resistance to infestation by *C. maculatus*. While TSs 93 and TSs 125 exhibited the highest levels of resistance, some other AYB accessions TSs 60, TSs 93 and TSs 111 showed moderate levels of resistance and are also promising accessions for future research on *C. maculatus* infestation tolerance/resistance. The tolerance or resistance to *C. maculatus* infestation observed in the AYB accessions could be due to physical and (or) chemical factors in the accessions other than lectin concentration alone. The revealed variability with respect to resistance to *C. maculatus* infestation evident in this study could be utilized by future AYB improvement programmes in developing improved AYB varieties.

Conflict of interest

The authors have no conflict of interest to declare.

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The authors received no funding for this study.

Authors' contributions

SB conceived study, contributed materials, performed experiments, analysed data and wrote manuscript. CO designed study, contributed materials, performed and supervised experiments, and wrote the manuscript. Both authors read and approved final draft of the manuscript.

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