Research Article

Effects of Seed Physical Characteristics of Benin Soybean Germplasm on their Resistance to *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae)

Yêyinou Laura Estelle Loko[1*], Joelle Toffa[1], Azize Orobiyi[1], Gbèblonoudo Anicet Dassou[2], Rolande Okpeicha[1], Dieudonné Gavoedo[1] and Alexandre Dansi[2]

[1]Laboratory of Applied Entomology (LEnA), Ecole Nationale Supérieure des Biosciences et Biotechnologies Appliquées (ENSBBA), Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), BP 14 Dassa-Zoumé, Benin; [2]Laboratory of Biotechnology, Genetic Resources and Plant and Animal Breeding (BIORAVE), ENSBBA, UNSTIM, BP 14 Dassa-Zoumé, Benin.

Abstract | The pulse beetle *Callosobruchus maculatus* Fabricius (Coleoptera: Bruchidae) is an important pest of stored soybean grains. It is imperative to screen out the prevailing genotypes of Beninese soybean cropping systems in order to find out the resistant ones against *C. maculatus* infestations. The effect of seven grain physical traits (testa thickness, colour, texture, hardness, length, breadth, and 100-grain mass) on the susceptibility of eight soybean varieties to *C. maculatus* were evaluated in the laboratory. The correlations and contributions of the studied traits were evaluated using correlation path coefficient analysis. The tested soybean varieties showed a variation in physical seed characteristics. A differential susceptibility of soybean varieties to *C. maculatus* was observed, with the white seeded variety Whéwhé having longest and largest grains was the most susceptible. Based on the Dobie susceptibility index, the Yovoton variety was proved to be resistant to *C. maculatus* attacks. While, Kecheke, Houeton, Adjaton and Vovoh varieties were classified as moderately resistant to *C. maculatus*. The correlation analysis indicated that 100-seed weight had significant positive correlation with F1 progeny (r = 0.439), seed consumption (r = 0.467), number of eggs laid (r = 0.295) and susceptibility index (r = 0.453). Path coefficient analysis showed that each seed physical character and its interactions with the others characters influenced soybean grains susceptibility to *C. maculatus*. Soybean seed thickness showed the higher direct positive effect on soybean susceptibility to *C. maculatus* indicating that breeding should be done based on this trait to improve soybean seed resistance.

Received | June 24, 2021; **Accepted** | July 26, 2022; **Published** | October 18, 2022
***Correspondence** | Yêyinou Laura Estelle Loko, Laboratory of Applied Entomology (LEnA), Ecole Nationale Supérieure des Biosciences et Biotechnologies Appliquées (ENSBBA), Université Nationale des Sciences, Technologies, Ingénierie et Mathématiques (UNSTIM), BP 14 Dassa-Zoumé, Benin; **Email:** lokoestelle@yahoo.fr
Citation | Loko, Y.L.E., J. Toffa, A. Orobiyi, G.A. Dassou, R. Okpeicha, D. Gavoedo and A. Dansi. 2022. Effects of seed physical characteristics of benin soybean germplasm on their resistance to *Callosobruchus maculatus* fabricius (Coleoptera: Bruchidae). *Sarhad Journal of Agriculture,* 38(4): 1468-1477.
DOI | https://dx.doi.org/10.17582/journal.sja/2022/38.4.1468.1477
Keywords | *Glycine max*, Path analysis, Mediation analysis, Bruchids, Varietal resistance

Introduction

The soybean (*Glycine max* (L.) Merr.) is a food crop of economic importance in Republic of Benin with an estimated production of 230000 tons in 2019 (FAO, 2019). This legume seed, with its high protein value, is an important pillar in the fight against malnutrition in rural areas and is mainly used in infant food (Wendland and Sills, 2008; Chadare *et al*., 2018). Beninese population consume soybean under various forms (flour, milk, cheese, etc.), and use it in animal feed due to its low cost and availability (Ayenan *et al*., 2017; Hounhouigan *et al*., 2020; Idrissou *et al*., 2020). However, soybean seeds are subject to enormous postharvest losses due to storage insect attacks with an estimated losses average 10% of produced soybean (Chelladurai *et al*., 2014). In addition, these storage insects lead to a rapid degradation of the soybean grain quality and a loss of germination viability (Ulemu *et al*., 2016).

The beetle *Callosobruchus maculatus* (Fabricius) (Coleoptera: Bruchidae) was found to be the main pest of stored soybean grains in the Republic of Benin (Loko *et al*., 2021). Farmers to protect stored soybeans from bruchid attacks mainly use synthetic chemical insecticides (Loko *et al*., 2021). While, the use of synthetic insecticides such as imidacloprid insecticide, not only have undesirable effects on human health, they also reduce the germination capacity (up to 22.6%) of soybean seeds (Pereira *et al*., 2020). Among the alternative control methods against bruchid pests, the use of resistant varieties is one of the cheapest control methods that seems to be more easily adopted by farmers (Msiska *et al*., 2018). Although varietal resistance of soybean to *C. maculatus* attacks has been demonstrated by several studies (Allotey *et al*., 2004; Sharma and Thakur, 2014a; Ulemu *et al*., 2016), no information is available on the resistance of Benin soybean germplasm to bruchid infestations. Whereas, the identification of soybean varieties resistant to *C. maculatus* could be directly useful for scientific research (varietal development and improvement) and development (varietal introduction and exchange).

A great diversity of soybean varieties is found in Beninese agriculture (Loko *et al*., 2021). However, it is known that physical characteristics of soybean seeds influence their resistance to bruchid pests (Ulemu *et al*., 2016). Therefore, it is important to identify the physical traits responsible of soybean resistance to *C. maculatus*. Indeed, a good knowledge of soybean resistance factors to bruchid infestations is necessary for the breeding of resistant varieties (Msiska *et al*., 2018). The objective of this study was to: (i) assess the resistance level of soybean genotypes grown in Republic of Benin against *C. maculatus* attacks; (ii) assess the influence of soybean physical grain characteristics on their susceptibility to *C. maculatus*.

Materials and Methods

Plant material

Eight soybean varieties presenting different morphological characteristics grown in the south and centre Benin were used for experiments (Loko *et al*., 2021). The soybean seeds were obtained from farmers in eight villages in the southern Benin (Figure 1). The soybean seeds were sorted using a binocular microscope to ensure that they were not damaged or infested. Sterilization of soybean seeds was done by drying them in an oven at a temperature of 30°C for 24 h (Msiska *et al*., 2018). Healthy seeds were conditioned at room temperature (25±2°C) and relative humidity of 65±5% in the laboratory for 2 weeks.

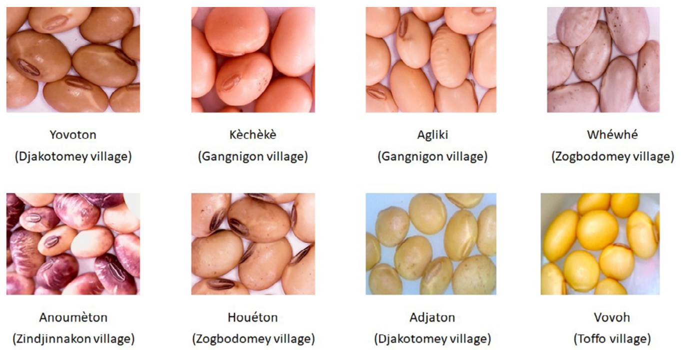

Figure 1: *Seeds of soybean cultivars along with their collection site names.*

Physical characteristics of soybean varieties

Seven grain physical traits (testa thickness, colour, texture, hardness, length, breadth and 100-seed weight) were evaluated using various tools. Ten seeds of each soybean variety were randomly chosen to measure their testa thickness and size (length and width) using a Marathon electronic micrometer (measuring range 0-25 mm). The pigmentation and texture of the grains of the different soybean varieties were analysed by observation under a stereoscopic microscope coupled with a digital video camera. While the seed hardness was measured with a "Shore A" hardness tester and the one hundred seed weight was measured with an electronic scale. The data were taken from 10 randomly selected seeds. The seed moisture content was measured using a digital moisture meter lds-1g analyser with a Kohstar micro-control computer.

Bruchid rearing

Bruchids were collected from infested soybeans obtained from farmers in the village of Gangnigon in the district of Kpankou. These bruchids were kept in the laboratory of entomology of the International Institute of Tropical Agriculture (IITA-Benin). For this purpose, 50 *C. maculatus* adults (unsexed) were putted in plastic boxes (6 cm × 10 cm) containing 300 g of soybeans from a mixture of seeds of the different soybean varieties, previously sorted and sterilised. These boxes were covered with a muslin cloth to allow ventilation of the conservation medium and avoid insect escape. The plastic boxes were kept on shelves under laboratory conditions (70 ± 5% RH and 26±2°C). The adult insects were removed of experimental boxes after seven days of oviposition, and the boxes were kept until adult emergence. Progeny were used for experiments.

Screening of soybean varieties for resistance to Callosobruchus maculatus

The resistance of eight soybean varieties to *C. maculatus* was tested using the methodology described by Sharma and Thakur (2014a). The trials were conducted on soybeans containing 12-13% relative humidity (Table 1). For this purpose, 50 seeds of each previously sterilised soybean genotype were weighed using an electronic scale and placed in plastic boxes (3 cm × 4 cm). Four (2 males and 2 females) *C. maculatus* adults (1-3 days old) were placed in each plastic box. The sexing of *C. maculatus* was done base on the shape and size of the abdomens as described by Bandara and Saxena (1995). The experiments were conducted under laboratory conditions (24 ± 2°C, 75 ± 5%, and 12 h / 12 h) and deposited in a randomized block design with 4 replicates. Daily, dead insects were removed from the experimental boxes and replaced by live ones (Oigiangbe and Onigbinde, 1996). After seven days, the adult bruchids were removed from the soybean samples and the number of eggs laid on seeds of each variety was recorded. After 25 days, the experimental boxes were observed daily to count the number of adult insect emergence (Adebayo *et al.*, 2016). The emerged adults were removed from the boxes after counting. Daily counting was stopped when no emergence was observed after 5 consecutive days (Lephale *et al.*, 2012). The percentage of adults emerged was calculated following the formula (Sharma and Thakur, 2014a):

$$\text{Emerging adults (\%)} = \frac{\text{Number of emerged adults}}{\text{Total number of eggs laid}} * 100$$

After the period of observation of adult emergence, the number of attacked grains (based on the emergence holes) and the final weight of the seeds in each experimental box were determined according to the formulas (Sharma and Thakur, 2014a):

$$\text{Attacked soybeans (\%)} = \frac{\text{Number of damaged seeds}}{\text{Total number of seeds}}$$

$$\text{Weight loss (\%)} = \frac{\text{Initial grain weight} - \text{Final grain weight}}{\text{Initial grain weight}} * 100$$

The Dobie susceptibility index was calculated according to the formula (Dobie, 1977):

$$\text{Dobie index} = \frac{\text{Log total number of emerged adults}}{\text{Mean development time}}$$

Where the mean development time is the time (days) from the middle of the oviposition period to the emergence of fifty percent of the F1 progeny (Dobie, 1977). The soybean varieties were classified using the following sensitive scale: 0-3 = resistant, 4-7 = moderately resistant, 8-10 = susceptible, and ≥ 11 = highly susceptible (Dobie, 1974).

Data analysis

The data expressed in percentage (mortality, weight loss and reproductive inhibition), and the number of F1 offspring emerged were arcsine (arscine$\sqrt{x}$) and log (log (x)) transformed respectively to homogenise their variance before subjected to ANOVA. The Student–Newman–Keuls (SNK) test with a probability of 5% was performed using IBM SPSS software version 25 to identify significant differences between the means. The correlation between the physical characteristics of soybeans and susceptibility to attack by *C. maculatus* was calculated using Pearson's coefficient using Minitab 17 software. To identify the direct and indirect effects of the correlation coefficients, the path coefficient analysis and mediation analysis was done using SPSS AMOS software version 21 (Deway and Lu, 1959) with Dobie susceptibility index taken as the dependent variable while the seed physical characteristics were considered as the independent variables.

Table 1: *Physical characteristics of seeds of the soybean cultivars from southern Benin screened for resistance to Callosobruchus maculatus.*

Soybean varieties	Seed coat features			Seed dimensions (mm)		Seed characteristics		
	Colour	Texture	Thickness (mm)	Length	Width	Hardness (Shore A)	Moisture content (%)	Weight of 100 seeds (g)
Adjaton	Yellowish white	Smooth	0.16±0.02abc	5.37 ± 0.43a	4.68 ± 0.33a	95.70 ± 7.62ab	12.41 ± 0.30a	7.59 ± 0.32a
Agliki	Buff	Smooth	0.15 ± 0.09ab	6.36 ± 0.79b	5.42 ± 0.50b	101.15±5.63bc	12.60 ± 0.27a	10.52 ± 0.29b
Anoumèton	Reddish brown	Rough	0.12 ± 0.01a	7.34 ± 0.38cd	5.32 ± 0.29b	96.25 ± 4.43ab	13.04 ± 0.41a	10.85 ± 0.39b
Houéton	Buff	Smooth	0.15 ± 0.02ab	7.35 ± 0.41cd	5.32 ± 0.37b	89.95 ± 5.13a	12.53 ± 0.32a	10.61 ± 0.54b
Kèchèkè	Buff	Smooth	0.21 ± 0.05c	6.70 ± 0.88bc	5.63 ± 0.55bc	97.25 ± 9.28ab	12.39 ± 0.27a	10.97 ± 0.45b
Vovoh	Yellow	Smooth	0.13 ± 0.03a	6.64 ± 0.70bc	5.49 ± 0.53bc	104.20 ± 1.73c	12.31 ± 0.34a	10.60 ± 0.20b
Whéwhé	White	Smooth	0.19 ± 0.03bc	7.74 ± 0.57d	5.92 ± 0.36c	95.30 ± 3.37ab	12.73 ± 0.31a	14.24 ± 0.90d
Yovoton	Buff	Smooth	0.14 ± 0.03ab	6.80 ± 0.49bc	5.70 ± 0.38bc	102.5 ± 6.06bc	12.56 ± 0.34a	12.32 ± 0.69c

Mean in a column followed by the same letter(s) do not differ significantly at the 5% level by SNK test.

Table 2: *Mean number of eggs laid, percent of adults emergence, number of adult progeny, median development time of Callosobruchus maculatus, seed damage, weight loss, and Dobie index susceptibility.*

Soybean genotype	Mean number of eggs laid	Number of adult progeny	Percent of adults emerged	Median of development time (days)	Seed damage (%)	Weight loss (%)	Dobie susceptibility index	Resistance category
Adjaton	14.50±4.20a	1.75±1.25a	12.62 ± 9.33a	35.87±5.07a	3.50 ± 2.51a	2.04 ± 1.40abc	5.42	Moderately resistant
Agliki	27.50±17.07a	6.00±3.16ab	29.41±21.32ab	29.83±1.76a	12.00±6.32ab	2.38 ± 0.88bc	10.66	Susceptible
Anoumèton	18.00±9.62a	7.75±9.50ab	37.79±26.61ab	33.50±1.69a	15.50±19.00ab	3.25 ± 1.58c	10.24	Susceptible
Houéton	14.00±4.32a	3.75±1.70ab	27.79±12.67ab	34.25±3.52a	7.50 ± 3.41a	0.91 ± 0.48ab	7.91	Moderately resistant
Kèchèkè	21.50±15.52a	3.00±1.15a	21.59±16.81a	31.75±1.30a	6.00 ± 2.30a	0.72 ± 0.29ab	7.82	Moderately resistant
Vovoh	14.25±3.77a	1.25±1.50a	7.28±8.46a	35.12±5.26a	2.50 ± 3.00a	0.37 ± 0.15a	4.58	Moderately resistant
Whéwhé	27.00±23.50a	14.50±9.46b	75.29±47.27b	30.79±0.48a	29.00 ± 18.93b	7.76 ± 2.54d	13.18	Very susceptible
Yovoton	20.00±14.14a	1.01±0.81a	5.00 ± 4.08a	35.33±5.43a	2.00 ± 1.63a	0.31 ± 0.01a	3.92	Resistant

Mean values ± standard error in a column followed by the same letter(s) do not differ significantly at the 5% level by SNK test.

Results and Discussion

Physical characteristics of the soybean seeds

The soybeans tested had a colour diversity and only the Anoumèton variety showed a rough texture (Table 1). The seed coat thickness of the different varieties varied between 0.01 to 0.21 mm. The Kèchèkè variety showed significantly (F = 4.760, df = 79, P ≤0.000) the thickest seed coat. While, the Adjaton variety significantly exhibited the shortest grain (F = 18.845, df = 79, P ≤ 0.000) and the narrowest (F = 7.692, df = 79, P ≤ 0.000). The hardness of the seeds varied from 89.95 to 104.2 Shore A. The Houeton variety exhibited significantly (F = 6.123, df = 79, P ≤ 0.000) the softer seeds. The moisture of the seeds of the different varieties varied between 12.31 and 13.04% (Table 2). The Whéwhé variety exhibited significantly the highest 100 seed weight (F = 153.363, df = 79, P ≤ 0.000) (Table 1).

Resistance of soybean varieties to C. maculatus attacks

The average number of eggs laid by *C. maculatus* on the different soybean varieties varied from 14.00 ± 4.32 (Houeton) to 27.00 ± 23.50 (Whèwhè) (Table 2). However, this difference was not significant (F = 0.481, df = 31, P = 0.839) between the different varieties. The number of hatched eggs varied from 1.01 ± 0.81 (Yovoton) to 14.50 ± 9.46 (Whéwhé). The number of eggs hatched on the Whéwhé variety was significantly (F = 5.627, df = 31, P = 0.001) different from that of the other varieties (Table 3). The percentage of emerged adults ranged from 5 to 75.29%. The lowest percentage of emerged adults was observed on the Yovoton variety (5.00 ± 4.08%) which differed significantly (F = 4.326, df = 31, P = 0.003) from those of the Vovoh (8.46 ± 7.28%), Adjaton (12.62 ± 9.33%) and Kèchèkè (21.59 ± 16.81%) varieties. The development time of *C. maculatus* on the different

Table 3: *Correlation coefficients between seed physical characters of eight soybean cultivars and their susceptibility parameters to Callosobruchus maculatus.*

Variables	COL	TEX	THI	LEN	WID	HAR	MOC	WEI	SI	NEL	PRO	CON
COL	-											
TEX	0.192	-										
THI	-0.208	-0.267*	-									
LEN	-0.263*	0.234*	0.074	-								
WID	-0.281*	-0.082*	0.251	0.578***	-							
HAR	0.165	-0.083	-0.018	-0.064	0.181	-						
MOC	-0.129	0.444***	-0.004	0.448***	0.164	0.080*	-					
WEI	-0.396***	-0.022	0.115	0.620***	0.597***	0.052	0.337**	-				
SI	-0.318**	0.283*	0.159	0.418***	0.220*	-0.249*	0.671***	0.453***	-			
NEL	-0.222*	-0.034	-0.058	0.089	0.296**	0.191	0.096	0.295**	0.321**	-		
PRO	-0.116	0.208	-0.030	0.373***	0.241*	-0.097	0.372***	0.439***	0.654***	0.561***	-	
CON	-0.017	0.194	0.091	0.353***	0.220	-0.188	0.357***	0.467***	0.765***	0.288**	0.761***	-

*Colour (COL), texture (TEX), thickness (THI), length (LEN), width (WID), hardness (HAR), moisture content (MOC) and weight of 100 seeds (WEI), susceptibility index (SI), number of eggs laid (NEL), F1 progeny (PRO), and seed consumption (CON). Significant correlations at *p < 0.05, **p < 0.01, ***p < 0.001; ns: not significant.*

varieties was extended from 29 (Agliki) to 35 (Adjaton) days. There was no significant difference (F = 1.843, df = 31, P = 0.125) in the development time of *C. maculatus* on the different varieties tested. The percentage of damaged seed varied from 2.00 ± 1.63 (Yovoton) to 29 ± 18.93% (Whéwhé). A significant difference (F = 4.619, df = 31, P = 0.002) was observed between the different varieties in terms of damage. The weight loss of the different varieties due to the consumption of *C. maculatus* varied from 0.31 ± 0.01% (Yovoton) to 7.76 ± 2.54% (Whéwhé). The maximum weight loss by the Whéwhé variety was significantly (F = 19.244, df = 31, P = 0.000) different from the other varieties. The susceptibility index ranged from 3.92 to 13.18 with the variety Yovoton classified as resistant to attack by *C. maculatus*.

Correlation between the seed physical characteristics and the different observations

The correlation analysis showed a positive and significant correlation between 100-seed weight and F1 progeny (r= 0.439), seed consumption (r = 0.467), and number of eggs laid (r= 0.295), respectively (Table 3). A positive and significant correlation also exist between seed length and F1 progeny (r = 0.373), seed consumption (r= 0.353), respectively. It is the same with seed moisture content and F1 progeny (r = 0.372), seed consumption (r = 0.357), respectively. While, seed width was positively correlated with number of eggs laid (r = 0.296) and F1 progeny (r = 0.241), respectively. Only seed coat colour showed a significant and negative correlation (r = -0.222) with number of eggs laid by *C. maculatus* females.

The correlation analysis between the physical characteristics of soybeans and their resistance to attack by *C. maculatus* showed that there was a significant negative correlation (r = -0.32) between the colour of the seeds and the susceptibility index. Likewise, a significant and negative correlation (r = -0.25) was observed between the seed hardness and the susceptibility index. However, a significant positive correlation (r = 0.28) was noted between the seed texture and the susceptibility index. Seed measurements (length and width), moisture and 100-seed weight showed a significant positive correlation with the resistance of the tested soybean varieties to *C. maculatus* (Table 3).

Direct and indirect effects of the physical characteristics of soybeans on resistance to attack by C. maculatus

Direct and indirect effects of the seed physical characteristics on the resistance to *C. maculatus* attacks were evaluated (Figure 2). The root mean square error of approximation (RMSEA) was inferior to 0.05 indicating the good fit of the used model. The high value of the determination coefficient of path analysis estimated at 0.64, and the low effect of the residual variable (5.45) showed a strong relationship between the susceptibility to *C. maculatus* attacks and analysed variables. The seed coat thickness showed the highest positive direct effect (10.54) on soybean susceptibility to *C. maculatus*. Direct path coefficient values on soybean susceptibility to *C. maculatus* were also found for seed coat texture (3.02), 100-grain mass (0.65), and seed length (0.28). Seed width (-0.50), seed colour (-0.33), and seed hardness (-0.08) had negative direct effects on soybean susceptibility to *C. maculatus*. The interrelation between the evaluated seed physical characteristics also showed that each variable influenced the soybean susceptibility to *C. maculatus* by acting with the others variables (Table 4). The highest positive indirect effects of 3.982 on soybean susceptibility to *C. maculatus* was induced by seed coat thickness through 100 grain mass (Table 4). The indirect effect of seed colour and seed width via seed hardness were more important and masked its direct effect on susceptibility index of soybean to *C. maculatus*.

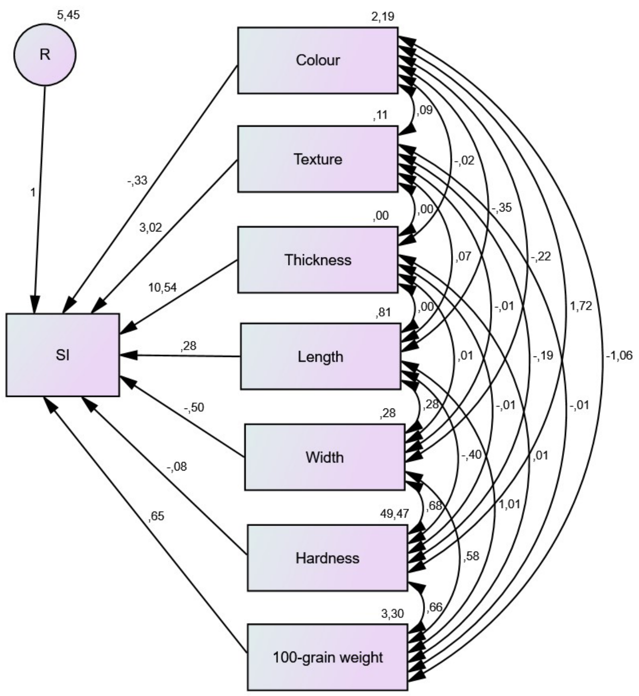

Figure 2: *Path diagram and coefficients showing causal relationship between Dobie susceptibility index (SI) and physical characteristics of soybean seeds from Benin. While the residual (R) shows the undetermined traits, the single and double arrowed lines illustrate a mutual association and direct influence, respectively.*

Among the alternative control methods against stored grain pests, the use of resistant varieties is one of the most economical means of control and the adoption of which by producers seems to be easier. Our study revealed a significant variability in the physical characteristics of Beninese soybean seeds and a differential susceptibility to infestation by *C. maculatus*. Indeed, several authors have demonstrated

Table 4: *Estimates of direct and indirect effects of the seed physical characteristics on the susceptibility index of soybean genotypes from southern Benin to Callosobruchus maculatus.*

Pathway	Path analysis
Seed colour and susceptibility index	
Direct effect	-0.331
Indirect effect via seed length	-0.160
Indirect effect via seed width	-0.101
Indirect effect via seed coat thickness	-0.007
Indirect effect via seed hardness	0.786
Indirect effect via seed texture	0.043
Indirect effect via 100 grain mass	-0.486
Seed texture and susceptibility index	
Direct effect	3.018
Indirect effect via seed length	0.636
Indirect effect via seed width	-0.132
Indirect effect via seed coat thickness	-0.042
Indirect effect via seed hardness	-1.764
Indirect effect via seed colour	0.857
Indirect effect via 100 grain mass	-0.123
Seed hardness and susceptibility index	
Direct effect	-0.082
Indirect effect via seed length	-0.008
Indirect effect via seed width	0.014
Indirect effect via seed coat thickness	0.000
Indirect effect via seed colour	0.035
Indirect effect via seed texture	-0.004
Indirect effect via 100 grain mass	0.013
Seed length and susceptibility index	
Direct effect	0.280
Indirect effect via seed colour	-0.432
Indirect effect via seed width	0.382
Indirect effect via seed coat thickness	0.004
Indirect effect via seed hardness	-0.498
Indirect effect via seed texture	0.086
Indirect effect via 100 grain mass	1.252
Seed width and susceptibility index	
Direct effect	-0.500
Indirect effect via seed length	0.977
Indirect effect via seed colour	-0.782
Indirect effect via seed coat thickness	0.025
Indirect effect via seed hardness	2.398
Indirect effect via seed texture	-0.051
Indirect effect via 100 grain mass	2.039
Seed thickness and susceptibility index	
Direct effect	10.539
Indirect effect via seed length	1.275

Pathway	Path analysis
Indirect effect via seed width	2.560
Indirect effect via seed colour	-5.895
Indirect effect via seed hardness	-2.448
Indirect effect via seed texture	-1.688
Indirect effect via 100 grain mass	3.982
100-grain mass and susceptibility index	
Direct effect	0.646
Indirect effect via seed length	0.307
Indirect effect via seed width	0.175
Indirect effect via seed coat thickness	0.003
Indirect effect via seed hardness	0.200
Indirect effect via seed texture	-0.004
Indirect effect via seed colour	-0.322

the varietal resistance of soybean to attacks by *C. maculatus* (Allotey *et al*., 2004; Ulemu *et al*., 2016). The white seeded variety Whéwhé with significantly the longest and largest grain was most susceptible to attack by *C. maculatus*. This is not surprising because it is known that the small seed size is among the physical characteristics of tolerant soybean varieties to *C. maculatus* (Sharma and Thakur, 2014b) because larval growth is limited by low food availability and space. Indeed, the Adjaton variety with yellow white seed and having the smallest measurements was found to be moderately resistant to *C. maculatus*. However, the big size of soybean seed is among the varietal preferential criteria of Beninese farmers (Loko *et al*., 2021). Therefore, it is important to make in place a national breeding program involving Whéwhé variety and Yovoton variety as progenitors to meet farmer's needs. Indeed, Yovoton variety, which exhibited average seed physical characteristics, was found to be resistant to *C. maculatus* attacks. The resistance of this variety may relate to the low adult bruchid emergence, low seed damage, and low seed weight loss due to the existence of physical and or chemical barriers in the seeds thus affecting larval penetration. Therefore, the Yovoton variety must be popularized and integrated into varietal creation programs in order to minimize losses recorded during soybean storage. However, the identification of the genes and biochemicals that are responsible of Yovoton variety resistance should be determine and taken into account in research programs.

Our results revealed that soybean seed physical characteristics did not influence the oviposition of *C. maculatus* females. Indeed, Sekender *et al*. (2020) reported that *C. maculatus* is able to lay eggs on any seed, even if the seed is not suitable for larval development. However, the number of eggs laid by *C. maculatus* females was negatively correlated to seed coat colour. This is in accordance with Baidoo *et al*. (2015), which showed that *C. maculatus* uses less Bambara groundnut-coloured seeds as oviposition site. In addition, Chen *et al*. (2019) demonstrated that the oviposition of *C. maculatus* females at high densities is affected by the seed coat colour. The fact that the oviposition of *C. maculatus* females, F1 progeny and seed consumption were positively correlated to soybean seed size is not surprising because it is known that larger seeds provide more surface area and nutrients for developing bruchids (Nwanze and Horber, 1975). Likewise, similarly to Kaur and Ramzan (2001), we reported the negative correlation between seed hardness and the susceptibility index of soybean variety to *C. maculatus*. Indeed, the seed hardness is known as a factor limiting the penetration of *C. maculatus* larvae through the soybean seed coat (Kosini and Nukenine, 2019). This could be explained the fact that Vovoh variety with the highest seed hardness was found to be moderately resistant. The significant positive correlation of seed texture, seed length, width, moisture content and 100-seed weight with the susceptibility index of soybean variety indicated that these characters are efficient in seed resistance determination to *C. maculatus*.

The path coefficient analysis revealed that the seed coat thickness exhibited the strongest positive effect on susceptibility index of soybean to *C. maculatus*. This indicated that seed coat thickness is a good predictor of soybean resistance to *C. maculatus* and must be taken in account in soybean resistance breeding to bruchid pests. Indeed, soybean seed coat acts as a physical (Msiska *et al*., 2018), and biochemical (Sharma and Thakur, 2004c; Silva *et al*., 2018) barrier against penetration by *C. maculatus*. The positive direct effects of seed coat texture, 100-grain mass, and seed length were expected because these seed physical characteristics were previously reported to be positively correlated to susceptibility of some pulses to *C. maculatus* (Dasbak *et al*., 2009; Tripathi *et al*., 2020). Therefore, soybean seed resistance to *C. maculatus* could be improved by selecting for seed coat thickness, seed coat texture, 100-grain mass and seed length. The negative direct effects of seed width, seed colour, and seed hardness on susceptibility index of soybean to *C. maculatus* suggest that only their positive indirect effects on other traits influence the seed resistance. Therefore, breeders should take into account the direct and indirect effects of the physical characteristics of the soybean seed on their susceptibility to *C. maculatus* for the breeding of resistant soybean varieties.

Conclusions and Recommendations

There is a large diversity of seed physical characteristics among the eight soybean varieties cultivated in the Republic of Benin. Only Yovoton variety was resistant to *C. maculatus* and could serve as progenitor in soybean breeding programs. Seed texture, seed length, seed width, seed moisture content and 100-seed weight showed a significant positive correlation with the resistance of the tested soybean varieties to *C. maculatus*. Positive direct effects of seed coat thickness, seed coat texture, 100-grain mass, and seed length on soybean resistance to *C. maculatus* suggest that their integration in a breeding program could improve soybean seed resistance against this pest.

Novelty Statement

Our study assessed for the first time, the susceptibility of soybean seeds of diverse varieties cultivated in south and central Benin to *C. maculatus* and identified the contribution of diffident physical seed characters to their resistance.

Author's Contribution

Yêyinou Laura Estelle Loko: Designed the experimentation, analysed the data, and wrote the first draft.
Azize Orobiyi, Rolande Okpeicha and Dieudonné Gavoedo: Performed the entomological essays.
Joelle Toffa, Gbèblonoudo Anicet Dassou and Alexandre Dansi: Corrected the first draft of the manuscript.
All authors read and approved the final manuscript.

Conflict of interest
The authors have declared no conflict of interest.

References

Adebayo, A.M., S.A. Babarinde, G.O. Adesina and S.O. Oladoye. 2016. Evaluation of selected cowpea lines and cultivars for inherent resistance against cowpea seed beetle, *Callosobruchus maculatus* Fabricius (Coleoptera: Chrysomelidae: Bruchinae). J. Nat. Sci. Res., 6(10): 6-10.

Allotey, J. and E.O. Oyewo. 2004. Some aspects of the biology and control of *Callosobruchus maculatus* (F.) on some stored soyabean *Glycine max* (L.) Merr varieties. Afr. J. Food Agric. Nutr. Dev., 4(2): 1-11. https://doi.org/10.4314/ajfand.v4i2.19160

Ayenan, M.A.T., P.L. Sèwadé and S.M. Agboton. 2017. Towards effective soybean seed systems in Benin: Current situation and prospects for production and delivery of good quality seed. J. Crop Improv., 31(3): 379-399. https://doi.org/10.1080/15427528.2017.1304479

Baidoo, P., N. Kwansa and C. Annin. 2015. The role of seed coat and its pigmentation on the acceptance of Bambara groundnut (*Vigna subterranea* L. Verdc.) variety by the cowpea beetle, *Callosobruchus maculatus* (F.). Adv. Entomol., 3: 125-131. https://doi.org/10.4236/ae.2015.34015

Bandara, K.A.N.P. and R.C. Saxena. 1995. A technique for handling and sexing *Callosobruchus maculatus* (F.) adults (Coleoptera: Bruchidae). J. Stored Prod. Res., 31(1): 97–100. https://doi.org/10.1016/0022-474X(94)00030-W

Chadare, F.J., Y.E. Madode, N. Fanou-Fogny, J.M. Kindossi, J.O.G. Ayosso, S.H. Honfo, A.P.P. Kayodé, A.R. Linnemann and D.J. Hounhouigan. 2018. Indigenous food ingredients for complementary food formulations to combat infant malnutrition in Benin: A review. J. Sci. Food Agric., 98(2): 439-455. https://doi.org/10.1002/jsfa.8568

Chelladurai, V., K. Karuppiah, D.S. Jayas, P.G. Fields and N.D.G. White. 2014. Detection of *Callosobruchus maculatus* (F.) infestation in soybean using soft X-ray and NIR hyperspectral imaging techniques. J. Stored Prod. Res., 57: 43–48. https://doi.org/10.1016/j.jspr.2013.12.005

Chen, Q., J. Ma, H. Yang, J. Gong, X. Gong, and Q. Weng. 2019. Seed-coat colour affects oviposition in the bean beetle, *Callosobruchus maculatus* (Coleoptera: Chrysomelidae). Ann. Zool. Fenn., 56(1-6): 199-205. https://doi.org/10.5735/086.056.0116

Dasbak, M.A., B.C. Echezona and J.E. Asiegbu. 2009. Pigeon pea grain physical characteristics and resistance to attack by the bruchid storage

pest. Int. Agrophys., 23: 19-26.
Dewey, D.R. and N.H. Lu. 1959. A correlation and path-coefficient analysis of components of crested wheat grass seed production. Agron. J., 51: 515-518. https://doi.org/10.2134/agronj1959.00021962005100090002x
Dobie, P., 1974. The laboratory assessment of the inherent susceptibility of maize varieties to post-harvest infestations by *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). J. Stored Prod. Res., 10: 183-197. https://doi.org/10.1016/0022-474X(74)90006-X
Dobie, P., 1977. The contributions of tropical stored products centre to the study of insect resistance in stored maize. Trop. Stored Prod. Info., 3: 4-7.
FAO (Food and Agricultural Organization). 2019. Food and Agricultural Organization of the United Nations. http://www.faostat.org
Hounhouigan, M.H., K.M. Kounouewa, C. Ayesiga, and P. Ingenbleek. 2020. Sojagnon: shaping the Beninese soy system to meet the challenges of an emerging market. Int. Food Agribus. Manag. Rev., 23: 143-156. https://doi.org/10.22434/IFAMR2019.0026
Idrissou, Y., W.H.S. Sanni, A.A. Seidou, J.A. Ayena, B.G.C. Assogba and T.I. Alkoiret. 2020. Cotton seed cake replacement by soybean pulp in the diet of West African dwarf lambs in Benin: Zootechnical and economic performances. Rev. Elev. Med. Vet. Pays Trop., 73(2): 107-111. https://doi.org/10.19182/remvt.31875
Kaur, H. and M. Ramzan. 2001. Effect of physical characters of soybean on oviposition and development of *Callosobruchus maculatus* (Fabricius). J. Res., 38 (3-4): 207-211.
Kosini, D. and E. Nukenine. 2017. Susceptibility of three legume species to *Callosobruchus maculatus* (Coleoptera: Chrysomelidae) attack and impact of rearing medium on female oviposition host preference. Agric. Res. Tech. Open Access J., 8(1): 555728. https://doi.org/10.19080/ARTOAJ.2017.08.555728
Lephale, S., A. Addo-Bediako and V. Ayodele. 2012. Susceptibility of seven cowpea cultivars (*Vigna unguiculatus*) to cowpea beetle (*Callosobruchus maculates*). Agric. Sci. Res. J., 2(2): 65-69.
Loko, Y.L.E., M. Zandjanakou-Tachin, D. Montcho, J. Toffa, A. Agolo, R. Okpeicha, A. Orobiyi, D. Gavoedo and A. Dansi. 2021. On-farm management of soybean (*Glycine max* [L.] Merr.) varietal diversity in southern and central regions of Benin republic. Agric. Res., https://doi.org/10.1007/s40003-021-00576-6
Msiska, U.M., B. Miesho, M. Hailay, S. Kyamanywa, P.R. Rubaihayo, T. Odong, P. Tukamuhabw, E. Nuwamanya and D.L. Nabirye. 2018. Biochemicals associated with *Callosobruchus chinensis* resistance in soybean. Afr. J. Rural Dev., 3(3): 859-868.
Nwanze, K.F. and E. Horber. 1975. How seed size affects the occurrence of "active" and "miniature" forms of *Callosobruchus maculatus* in laboratory populations 1. Environ. Entomol., 4(5): 729–732. https://doi.org/10.1093/ee/4.5.729
Oigiangbe, N.O. and A.O. Onigbinde. 1996. The association between some physico-chemical characteristics and susceptibility of cowpea (*Vigna unguiculata* (L.) Walp) to *Callosobruchus maculatus* (F.). J. Stored Prod. Res., 32(1): 7-11. https://doi.org/10.1016/0022-474X(96)00001-X.
Pereira, R.C., M.F. Pelloso, L.V. Correia, T.C. Matera, R.F. dos Santos, A.L. Braccini, G.G. De Bastiani, C. Coppo and B.G. da Silva. 2020. Physiological quality of soybean seeds treated with imidacloprid before and after storage. Plant Soil Environ., 66: 513–518. https://doi.org/10.17221/364/2020-PSE
Sekender, S., S. Sultana, T. Akter and S. Begum. 2020. Susceptibility of different stored pulses infested by pulse beetle, *Callosobruchus chinensis* (Lin.). Dhaka Univ. J. Biol. Sci., 29(1): 19-25. https://doi.org/10.3329/dujbs.v29i1.46527
Sharma, S. and D.R. Thakur. 2014c. Biochemical basis for bruchid resistance in cowpea, chickpea and soybean genotypes. Am. J. Food Technol., 9(6): 318-324. https://doi.org/10.3923/ajft.2014.318.324
Sharma, S. and D.R. Thakur. 2014a. Comparative developmental compatibility of *Callosobruchus maculatus* on cowpea, chickpea and soybean genotypes. Asian J. Biol. Sci., 7: 270-276. https://doi.org/10.3923/ajbs.2014.270.276
Sharma, S. and D.R. Thakur. 2014b. Studies on the varietal preference of *Callosobruchus maculatus* on soybean genotypes. Asian J. Biol. Sci., 7: 233-237. https://doi.org/10.3923/ajbs.2014.233.237
Silva, N.C., J.G. Conceição, K.E. Ventury, L.F. De Sá, E.A. Oliveira, I.S. Santos, V.M. Gomes, M.N. Costa, A.T. Ferreira, J. Perales, J. Xavier-Filho, K.V. Fernandes and A.E. Oliveira. 2018.

Soybean seed coat chitinase as a defense protein against the stored product pest *Callosobruchus maculatus*. Pest Manag. Sci., 74: 1449–1456. https://doi.org/10.1002/ps.4832

Tripathi, K., T.V. Prasad, R. Bhardwaj, S.K. Jha, D.P. Semwal, P.G. Gore and S. Bhalla. 2020. Evaluation of diverse germplasm of cowpea [*Vigna unguiculata* (L.) Walp.] against bruchid [*Callosobruchus maculatus* (Fab.)] and correlation with physical and biochemical parameters of seed. Plant Genet. Resour., pp. 1–10. https://doi.org/10.1017/S1479262120000180

Ulemu, M.M., S. Kyamanywa and P. Tukamuhabwa. 2016. Genetic sources of bruchid resistance in soybean: A review. Ruforum Work. Doc. Ser., 14 (3): 151-159.

Wendland, K.J. and O.E. Sills. 2008. Dissemination of food crops with nutritional benefits: Adoption and disadoption of soybeans in Togo and Benin. Nat. Resour. Forum, 32: 39–52. https://doi.org/10.1111/j.1477-8947.2008.00169.x