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Genetics of bruchid (*Callosobruchus maculatus* Fab.) resistance in cowpea (*Vigna unguiculata* (L.) Walp.)



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ABSTRACT

Bruchid resistance has been measured using a variety of techniques. Mean development period (MDP) and percentage adult emergence (PAE) are two of the more important traits measuring bruchid (*Callosobruchus maculatus* Fab.) resistance in cowpea. The objective of the study was to evaluate the genetic relationship between MDP and PAE in *C. maculatus* resistant cowpea. Seventy-two F₂ population plants were developed from the cross between *C. maculatus* resistant TVu-11953 (with delayed MDP and low PAE) and *C. maculatus* susceptible Ife Brown (with early MDP and high PAE) which were evaluated for resistance to cowpea *C. maculatus*. MDP at 45 days after infestation (DAI) and PAE at 60 DAI were taken as analysed measures for resistance to *C. maculatus* infestation. Data generated were subjected to chi-square analysis. Varied resistant response to *C. maculatus* infestation was observed among the F₂ generation plants but their responses showed inclination to the susceptible parent. A 15:1 ratio for susceptible to resistant genotypes was observed with MDP among the F₂ genotypes evaluated which is an indication of the presence of two recessive genes that control of MDP. The segregation of PAE also showed a 15:1 ratio goodness of fit ($P > 0.05$) for susceptible to resistant genotypes. Test of independence between MDP and PAE was not significant ($\chi^2 = 2.19$, $P = 0.19$). This study revealed that the recessive genes controlling these two traits (MDP and PAE) were independently assorted and showed no linkage. This was also evident in the observed F₂ genotypes with *C. maculatus* resistance expressing either delayed MDP or low PAE and not both in the study.

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1. Introduction

The cowpea bruchid, *Callosobruchus maculatus*, has been a major threat to stored cowpeas. Grain loss in storage by this beetle is estimated up to 100% due to perforations from insect emergence, thus reducing the degree of seed quality and quantity (Ali et al., 2004; Umeozor, 2005). Identifying *C. maculatus* resistant genotypes among the vast cowpea germplasm has been difficult as only three cowpea accessions, TVu-2027, TVu-11952 and TVu-11953, were identified as *C. maculatus* resistant among 8000 accessions evaluated (Singh et al., 1985). Although TVu-2027 had been fully incorporated into breeding *C. maculatus* resistant cowpeas, efforts are still being made to identify new sources of resistance to this

insect.

Different methods have been developed measuring several traits associated with *C. maculatus* resistance in cowpea among which include percentage of adult emergence over a given period, the mean date of emergence of adults, the natural rate of increase in *C. maculatus* per generation, the mean number of emergence holes per seed, the percentage of infested seed with no emergence holes, and the percentage weight loss per seed, due to *C. maculatus* damage (Redden and McGuire, 1983; Jackai and Asante, 2003; Amusa et al., 2014; Lephale et al., 2012). However, studies have shown that mean adult insect emergence period (MDP) and percentage adult insect emergence (PAE) were the most sensitive parameters to measure *C. maculatus* resistance in cowpea (Redden and McGuire, 1983; Jackai and Asante, 2003). A *C. maculatus* resistant genotype show be able to reduce the percentage of insects emerging as well as delay their development. There is however little in the scientific literature regarding the genetic relationship of

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MDP and PAE in cowpea. Hence, the objective of the study was to evaluate the linkage relationship between MDP and PAE in cowpea.

2. Materials and methods

TVu-11953 (*C. maculatus* resistance) and Ife Brown (*C. maculatus* susceptible) from previous *C. maculatus* resistant assays conducted on Nigerian cowpea landrace samples from the Institute of Agricultural Research and Training (IAR&T) and elite *C. maculatus* resistant breeding lines from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria were selected as parent materials for the study (Jackai and Asante, 2003; Amusa et al., 2014). These parents were planted in pots at the screen house of IAR&T, Ibadan and reciprocal crosses were made between the selected parent cowpea plants, each parent being used as recipient plants are emasculated and pollinated by the other used as donor parent and vice versa. Hybrid seeds from F₁ pods were harvested and bulked into two according to recipient plants and replanted consecutively to obtain the F₂ segregating generation plants. Seeds from individual F₂ progeny plant was then evaluated for *C. maculatus* resistance.

C. maculatus resistant evaluation was done on both selected parents and seeds from 72 F₂ populations using methodology described by Lephale et al. (2012) with modifications. A pair of newly emerged adult insects were introduced on 10 seeds per F₂ individual plant in 4 replicates for 24 h. This was to allow for mating and oviposition. Total number of eggs laid and hatched were counted 5 days after insect infestation. Adult insects emerged were recorded daily and removed. Parent seeds were infested also and evaluated as controls.

To analyse for *C. maculatus* resistance in cowpea, mean development period (MDP) and percentage adult emergence (PAE) were used as independent phenotype to measure the level of *C. maculatus* resistance (Redden and McGuire, 1983; Jackai and Asante, 2003).

$$MDP = \frac{x_1 + x_2 + x_3 + x_4}{4}$$

Where MDP is Mean development period (days), x is the average development period for cumulative adult insects in each experimental replicate setup and PAE is percentage adult emergence

$$PAE = \frac{\text{Number of Emerged adult insects}}{\text{Number of eggs laid}} * 100$$

Data collection was terminated 60 days after insect infestation (DAI) to avoid counting the second-generation of emerging insects. Data from the replicates were pooled together and variables statistically tested for independent assortment using chi-square test of independence and significant difference was considered at 5% probability level.

3. Results

The study shows MDPs of adult insect emergence in F₂ progeny population for the duration of the experimental setup between 30 and 60 DAI (Table 1). The resistant parent (TVu-11953) showed a MDP of 44 days which did not change all through the study after 45 DAI duration of the assay while the susceptible (Ife Brown) parent had 24 days at all DAIs examined. MDPs for 72 F₂ progeny population was observed to show an inclination towards the susceptible parent all through the DAI evaluated. Variation was highest at 30 DAI among the F₂ progenies evaluated (CV = 16.25) and was least at 45 DAI (CV = 12.63). There was a gradual increase in MDP for adult insect emergence in the F₂ population up to 56 DAI compared to the either parents which showed no difference in MDP at 45 DAI (Table 1).

Percentage adult insect emergence performance in F₂ progeny population was evaluated in comparison with both extreme parents' performance (Table 2). There was a gradual increase in PAE in the resistant parent up to 56 DAI (PAE₅₆ = 44.22%) while the susceptible parent attained a 100% insect emergence by 40 DAI. Response for PAE from the 72 F₂ individuals evaluated varied respectively. Variation was highest at 30 DAI (CV = 42.29) among the evaluated F₂ population with a PAE of 48.48%. There was no difference in the range of PAE between 50 and 60 DAI within the F₂ progeny evaluated. At 40 DAI, 57.59% of the insect were emerged from seeds of F₂ progenies compared to 100% adult insect emergence observed in the susceptible parent and 14.44% adult insect emergence in the tolerant parent. At the end of the *C. maculatus* resistant assay, 78.98% of the insect have already emerged from F₂ seeds evaluated compared to 44.22% and 100% insect emergence from tolerant and susceptible parents respectively (Table 2).

MDP from seeds was taken 45 DAI in both F₁ and F₂ generation. All sampled F₁ genotypes evaluated showed MDP similar to the susceptible parent regardless of mother plant line. However, four out of all the F₂ plants evaluated showed seed resistance similar to *C. maculatus* resistant parent. An expression of a goodness of fit to 1:15 ratio for *C. maculatus* resistant to susceptible ($\chi^2 = 0.59$, $P = 0.81$) indicating two recessive epistasis genes involved in *C. maculatus* resistant control as measured by MDP (Table 3). Similarly, all sampled F₁ genotypes showed performance similar to the susceptible parent in PAE. However, pooled sample result from the F₂ population plants evaluated showed that PAE of five genotypes response were similar to the resistant parent while the rest showed similar response close to the susceptible parent. Chi-square analysis showed a goodness of fit to expected 1:15 ratio of *C. maculatus* tolerant to susceptible ($\chi^2 = 0.59$, $P = 0.81$) indicating two recessive epistasis genes control (Table 3).

MDP was found to have a significant positive correlation with PAE ($r = 0.30$, $P = 0.04$). Comparing resistant genotypes in the 72 F₂ individual population showed that only one genotype possessed both MDP and PAE similar to the resistant parent while the other

Table 1
Mean development period (MDP) for parents and F₂ generation.

DAI	Parents		F ₂ generation			
	TVu-11953	Ife Brown	Mean	Min – Max	Std Dev	CV
30	30	24	25	0–30	4.11	16.25
40	39	24	27	21–45	3.69	13.70
45	44	24	30	21–45	3.76	12.63
50	44	24	31	21–46	4.18	13.62
56	44	24	32	21–47	4.72	14.95
60	44	24	32	21–47	4.62	14.63

DAI: days after infestation; Std Dev: standard deviation; CV: coefficient of variation.

Table 2
Percentage adult emergence (PAE) for parents and F₂ generation.

DAI	Parents		F ₂ generation			
	TVu-11953	Ife Brown	Mean	Min – Max	Std Dev	CV
30	1.11	96.15	48.48	0.00–85.71	20.50	42.29
40	14.44	100.00	57.59	12.50–91.49	17.00	29.52
45	39.89	100.00	70.03	13.00–95.77	18.32	26.16
50	43.67	100.00	75.45	13.00–100.00	20.58	27.27
56	44.22	100.00	78.27	13.00–100.00	21.77	27.81
60	44.22	100.00	78.98	13.00–100.00	21.74	27.52

All values are represented in percentage (%); DAI: days after infestation; Std Dev: standard deviation; CV: coefficient of variation.

Table 3
Summary of segregation of mean development period in F₂ progenies.

Trait	Crosses (♀ + ♂)	F ₁ Plants		F ₂ Plants		Expected Ratio	χ ²
		Resistant	Susceptible	Resistant	Susceptible		
MDP	P ₁ × P ₂	0	24	1	23	—	—
	P ₂ × P ₁	0	53	3	45	—	—
	Total	0	77	4	68	1:15	0.59
PAE	P ₁ × P ₂	0	24	1	23	—	—
	P ₂ × P ₁	0	53	4	44	—	—
	Total	0	77	5	67	1:15	0.59

♀: mother (recipient) plant; ♂: father (donor) plant; P₁: TVu-11953; P₂: Ife Brown; MDP: mean development period; PAE: percentage adult emergence; χ²: Chi square.

different F₂ resistant genotypes showed either MDP or PAE similar to the resistant and not both traits. However, chi-square value for test of independent assortment between the two traits at one degree of freedom was not significant, showing that the segregation of MDP and PAE was independent of each other (χ² = 2.19, P = 0.25).

4. Discussion

There was a clear distinction between the two parent genotypes both in MDP and PAE traits. This study showed no significant difference on susceptible parent between 40 and 60 DAI in both MDP and PAE of insects. Redden (1983) had earlier reported 45 DAI infestation to significantly differentiate *C. maculatus* resistant from *C. maculatus* susceptible. However, MDP was not stable until 56 DAI in the F₂ progenies evaluated. Variations in MDPs among F₂ population showed 45 DAI to have the least variation corroborating the report of Redden and McGuire (1983). Records for PAE at 40 DAI showed that while 14.44% of adult insect emerged from the resistant parent, the susceptible parent showed a 100% insect emergence. 100% insect emergence were observed in most of the F₂ population genotypes evaluated, an inclination toward the susceptible parent.

The genetics of MDP and PAE were evaluated independently among the F₂ progeny population from the cross between TVu-11953 and Ife Brown. The absence of any resistant genotypes among the F₁ plants evaluated in terms of reduced MDP and low PAE gives an indication that *C. maculatus* resistant in cowpea is controlled by recessive genes with no maternal effects. However, in the F₂ generation plants, both MDP and PAE were each observed to be controlled by two recessive genes respectively. Results from this study is similar to the work of Rusoke and Fatunla (1987) who earlier reported a ratio of 15:1 for susceptible to resistant in TVu-2027 and TVu-11952 using seed damage as a measure of *C. maculatus* resistance in cowpea. This result did not corroborate with the four QTLs reported to control *C. maculatus* resistance in cowpea by Srinives et al. (2007). Both Redden (1983) and Adjadi et al. (1985) have also reported maternal effects on *C. maculatus* resistance however, the presence of more genotypes from the susceptible parent lines in this study did not indicate any maternal influence on the trait.

The significant positive correlation between MDP and PAE observed in this study does not corroborate with the work of Redden and McGuire (1983) who reported a negative significant relationship between MDP and PAE. Our result may be because most of the F₂ genotypes evaluated inclination to the susceptible parents. Therefore, the insect emergence tends to build up as DAI increased. Further, the test for independence assortment showed that MDP and PAE traits segregated independently of each other

suggesting the genes controlling these traits were not linked. The presence of *C. maculatus* resistant F₂ genotypes expressing either delayed MDP or low PAE and not both in the study also suggest no association between genes controlling MDP and PAE. Either the genes for MDP and PAE were located wide apart on the same chromosome or on different chromosomes.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at <https://doi.org/10.1016/j.jspr.2017.11.004>.

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