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Full Length Research Paper

Induced tolerance of cowpea mutants to *Maruca vitrata* (Fabricius) (Lepidoptera: Pyralidae)

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A study was conducted to screen some cowpea mutants for resistance to *Maruca vitrata*. Fifteen selected cowpea mutants, the parent 'IT84S2246 D' as a susceptible check and a resistant check 'Tvu 946' were used in an intensive free choice test. Potted plant samples were randomly placed in a large screen cage and infested with adult females of *Maruca* for seven days. The results showed that there was a significant variation in the level of resistance among plant types. Mutant 4 with a yield loss of 46.1% as compared with 75.1% of the parent was found to be moderately tolerant while others were susceptible to *Maruca* infestation. Seed yield was found to be significantly and negatively correlated with percent pod damage. Hence, percent pod damage could be used as an index of plant tolerance on the field.

Key words: Cowpea, mutants, *Maruca* pod borer, tolerance, choice test, screening.

INTRODUCTION

In recent times, the difficulties faced by many peasant farmers in obtaining chemicals and the need to curb environmental pollution due to the use of insecticides have been a great concern to farmers, environmentalists and researchers working on cowpea improvement. These had prompted the need to develop a sustainable pest management strategy capable of minimizing pre-harvest loss, enhance production and consequently improve the diets of the people. The development of host resistance is particularly appropriate for the resource poor peasant farmers who prefer growing traditional varieties in inter-crop rather than the improved varieties to avoid the use of insecticides (Smith, 1989).

However, breeding for *Maruca* resistance in cowpea has been very difficult, because effective sources of resistance to *Maruca vitrata* are yet to be found among the cultivated cowpea species (IITA, 1989). Several crosses of cultivated lines with wild cowpea cultivars such as 'Tvu 946', identified to be moderately resistant to *M. vitrata*, had produced offsprings with unacceptable agronomic characters and inadequate level of resistance as compared with the wild parent (Singh, 1995). The use of genetic modification technology, as a means of transferring resistance genes into the cultivated cowpea, is confronted with intense vocal and well-meaning opposition from many organizations in different parts of the world (Murdock et al., 2002). However, success achieved with the genetic transformation of cowpea has been very limited (Matchuka et al., 2002).

In view of serious setbacks in the strategy of developing resistant cowpea in a bid to solve the most serious pest problem of cowpea, it becomes imperative for breeders to look for other avenues of generating new germplasm. The use of induced mutations, had long ago, been recognized as a rapid source of producing genetic variability in crops (Harris, 1979). Mutation breeding offers the possibilities of recovering some of the lost but useful variability in cowpea, especially improved yield and disease/pest resistance. Many mutants have made transnational impact on increasing yield and quality of several seed propagated crops (Ahloowalia et al., 2004).

Though limited work has been done on the use of mutation induction in developing insect resistance in cowpea, Pathak (1988) reported the development of aphid resistant cowpea 'ICV11' and 'ICV12', through induced mutation. By inducing mutations in cowpea, it may be possible to develop new sources of resistant germplasm, which could be used either, directly or in hybridization to produce improved *Maruca* resistant/ tolerant mutant lines. Such cowpea cultivars will make significant contribution in reducing the need for insectici-

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dal protection, promote higher yield per unit area and increase area of cultivation to wetter agro-ecological zones of southern Nigeria where the insect is most ravaging. This study is thus carried out with a view to evaluate level of resistance of mutants to *Maruca* pod borer and to identify mechanism(s) of resistance of mutants to *Maruca*.

MATERIALS AND METHODS

Plant material

Five mutants that were rated below 2 for pod damage due to *Maruca* (Jackai and Singh, 1988) and selected from previous field screenings together with ten selected for changes in agronomic traits were used(Adekola, 2005). In addition, a susceptible check- the non-irradiated parent 'IT 84S 2246D' and a resistant check- 'Tvu 946' were included in the tests. These plants were raised in 20 L plastic pots filled with sterilized topsoil in the screen house.

Maruca pod borer culture

Regular supply of adult *Maruca vitrata* used for these studies was maintained by mass rearing on artificial diet as described by Jackai and Raulston (1982 and 1988). The culture was established from egg infested diets collected from the Entomology laboratory of IITA, Ibadan. Newly emerged adults male and female *Maruca* were caged in 30 x 30 x 30 cm wire mesh cages with cloth sleeves at the rate of 200 adults per cage. They were allowed to mate for five nights (Jackai, et al., 1990) in the cage, after which the mature females were collected from the cages cupped in small plastic containers and transferred into the screened cages containing the potted plants. Only the egg-laden females were used for infestation as identified at Entomology unit, IITA, Ibadan.

Free choice test

At flower bud stage, two plants from each of the fifteen (15) selected mutants and the checks were placed randomly within a large screen cage (2.0 x 1.5 x 1.5 m). Ten adult females ready to oviposit were selected from the numerous females in the insect rearing cages and released per every seven plants (Echendu, 1985; Echendu and Akingbohungbe, 1990). Four replicates were established including a control cage where no insect was released. Plants were thoroughly watered through out the experiment and other insects beside *Maruca* were controlled using monocrotophos (20EC) at 200 g a.i/ha once at vegetative stage and once at 500 g a.i/ha at flower bud stage just before the plants were placed in the screen house. Cages were examined daily for dead moths, which were removed and replaced with live ones. Infestation was sustained for seven days consecutively, after which all moths dead or living were removed from the cages. The plants were left to mature in the cages after which pots were removed from the cages and plants were assessed as described below:

Total number of larvae recovered per plant: The total number of larvae recovered from the pods, flowers, flower buds, peduncles and stem of each plant were pooled for each mutant to give the number of larvae recovered per mutant.

Percentage pod infestation: The number of pods of each mutant showing damage due to *Maruca* was expressed as a percentage of the total number of pods produced per mutant.

Percentage seed damage: The number of seeds of each mutant showing damage by *Maruca* was expressed as a percentage of the total number of seeds produced per mutant.

Seed yield: Fresh weight of seeds from each mutant was taken after extracting all seeds from all the pods produced and the yield loss expressed as [(Yield of the control (uninfested) – Yield of infested plants) / Yield of the control] X 100

Statistical analysis

Data were subjected to Analysis of Variance and significant means separated by Duncan's New Multiple range test. Susceptibility indices and resistance ratings were computed according to Echendu and Akingbohungbe (1990) and interpreted as follows: Mutants with susceptibility index of less than 1 was regarded as tolerant (T); mutants with susceptibility index of between 1 and 1.99 were regarded as moderately tolerant (MT); mutants with susceptibility index of between 2 and 2.99 were regarded as Moderately susceptible (MS); mutants with susceptibility index of between 3 and 4.00 were regarded as Susceptible (S); mutants with susceptibility index above 4 were regarded as Highly Susceptible (HS).

Data were also subjected to correlation analysis to test for significant associations between parameters evaluated.

RESULTS

Table 1 shows the mean values of parameters evaluated on gamma induced mutants infested with adult females of *M. vitrata*. Mean squares from analysis of variance for parameters evaluated on gamma induced mutants and checks infested with *Maruca* are presented in Table 2. The susceptibility indices and resistance ratings obtained for the various mutants relative to the parent, as a fully susceptible standard are presented in Table 3. Mean number of larvae recovered among the mutants was between 7.0 and 19.3. The highest number of *M. vitrata* larvae/mutant (19.3) was recovered from mutant 6 and the least (7.0) was obtained from mutant 4, while the resistant check-Tvu 946 had an average of 5.7 larvae. The parent had 15.7 (Table 1). This is probably an indication of varied preference for different oviposition site. Significant differences were observed among the mutants for this trait at p = 0.05 (Table 2).

The mean number of pods per plant ranged between 4.3 and 21.7 among the mutants while the resistant check had 12.7 pods per plant. The parent had 11.3. The highest number of pods per mutant (21.7) was recorded for mutant 6. This is probably responsible for the high number of larvae found on it, as there was likely to be more abundant food source for the larva. Significant differences were found among the mutants and the checks for this trait at p = 0.05 as shown on Table 2. Number of damaged pods ranged from 4.3 to 18.0 while percent pod damage was between 55.9 and 100%. Highest number of damaged pods (18.0) was found in mutant 6 while the least (4.3) was found in mutant 4. However, percent pod damage was highest for mutants 3 and 10 (100%) which was not significantly different from

Table 1. Means of various parameters evaluated on gamma induced mutants Infested with adult females of *Maruca vitrata* in a free choice test.

Plant Type	No of larvae Recovered /plant	No of Pods/plant	No of damaged Pods/plant	% Pod Damage	No of seed/plant	No of damaged seed/plant	Seed yield/ plant (g)	% Yield loss	% Seed damage
Mutant 1	9.3*^{bcd}	11.7^{cdef}	7.7^{cd}	50.6^{de}	75.0^{cde}	14.3^{bcd}	14.4^{ab}	39.2^{efg}	21.2^{cd}
Mutant 2	9.7^{bcd}	12.0^{cdef}	7.7^{cd}	72.0^{abcde}	74.0^{cde}	11.7^{bcd}	13.3^{abcd}	19.7^{g}	14.9^{d}
Mutant 3	10.3^{bcd}	4.3^{f}	4.3^{d}	100.0^{a}	20.7^{fg}	4.7^{cd}	1.1^{f}	78.5^{abc}	22.6^{cd}
Mutant 4	7.0^{cd}	13.3^{bcdef}	7.3^{cd}	55.9^{cde}	98.7^{abcd}	8.7^{bcd}	15.6^{a}	46.1^{cdefg}	7.8^{d}
Mutant 5	9.3 ^{bcd}	15.7^{bcde}	11.0^{abcd}	69.2^{abcde}	87.3^{bcd}	10.7^{bcd}	15.8^{a}	54.4^{cdef}	12.2^{d}
Mutant 6	19.3 ^{a}	21.7^{ab}	18.0^{a}	86.8^{ab}	96.3^{abcd}	17.3^{ab}	9.6^{abcd}	72.3^{abcde}	19.2^{cd}
Mutant 7	12.7 ^{bc}	12.3^{bcdef}	8.7^{bcd}	77.3^{abcd}	103.3^{abc}	17.7^{ab}	13.3^{abcd}	47.1^{cdefg}	17.4^{cd}
Mutant 8	12.7 ^{bc}	19.3^{abc}	15.7^{ab}	80.3^{abc}	130.7^{ab}	25.7^{a}	13.3^{abcd}	47.3^{cdefg}	19.9^{cd}
Mutant 9	12.0b ^{cd}	18.7^{bcde}	11.3^{abcd}	61.1^{bcde}	89.7^{bcd}	15.3^{bc}	10.8^{abcd}	42.7^{defg}	18.6^{cd}
Mutant 10	11.7 ^{bcd}	9.0^{def}	9.0^{bcd}	100.0^{a}	53.7^{cdefg}	10.0^{bcd}	5.8^{def}	57.9^{bcdef}	20.2^{cd}
Mutant 11	11.7 ^{bcd}	5.0^{f}	4.7^{d}	91.7^{a}	5.0^{g}	3.67^{d}	0.7^{f}	88.5^{ab}	91.1^{a}
Mutant 12	12.7 ^{bc}	6.7^{ef}	5.7^{cd}	90.0^{ab}	11.3^{fg}	5.0^{cd}	1.3^{f}	90.8^{ab}	57.9^{b}
Mutant 13	8.0 ^{cd}	8.3^{ef}	7.7^{cd}	96.0^{a}	55.0^{cdefg}	11.3^{bcd}	1.0^{ef}	95.4^{a}	47.1^{bc}
Mutant 14	11.7 ^{bcd}	12.7^{bcdef}	12.0^{abcd}	95.6^{a}	63.0^{cdef}	9.3^{bcd}	7.6^{cde}	24.6^{fg}	14.9^{d}
Mutant 15	8.0 ^{cd}	15.3^{bcdef}	12.7^{abc}	84.3^{abc}	23.7^{efg}	5.0^{cd}	3.6^{f}	71.6^{abcde}	20.5^{cd}
16 (parent)	15.7 ^{ab}	11.3^{cdef}	10.7^{abcd}	94.3^{a}	46.7^{defg}	14.0^{bcd}	5.4^{def}	75.1^{abcd}	32.1^{bcd}
(Tvu 946) 17	5.7 ^{d}	12.7^{bcdef}	5.5^{d}	44.3^{e}	144.0^{a}	7.7^{bcd}	8.5^{bcde}	37.1^{fg}	5.3^{d}
S.E	2.0	2.9	2.3	9.1	16.1	3.3	2.0	10.3	9.2

*Means followed by different letter(s) in the same column are significantly different at probability of 0.05.

Table 2. Mean Squares (MS) from analysis of variance for parameters evaluated on gamma induced putative mutants infested with *M. vitrata* in a free choice test.

Source of variation	Degree of freedom	MS No. of larvae recovered	MS No. of Pods/plant	MS No. of damaged Pods/plant	Ms No. of Seeds/plant	MS No. of Damaged Seeds/plant	MS % Pod damage	MS %Seed damage	MS %Yield loss	MS Seed Yield/plant
Plant type	16	32.27*	100.32*	40.81*	4844.08*	98.25*	949.49*	1359.23*	1594.25*	90.6*
Replication	2	22.73	9.55	0.31	364.47	15.24	386.25	273.07	1019.9	53.24
Error	32	11.6	25.5	15.4	837.1	32.0	244.6	255.9	317.8	11.6
Total	51									

*Significant at 0.05 level of probability.

that of mutant 6, while the least (50.6%) were found in mutant 4. The resistant check (17) had 5.5 pods damaged per plant, which was 44.3% of the total pods produced. Both number of damaged pods and percent pod damage were found to differ significantly among the mutants and the checks.

Mean number of seeds per plant ranged from 5.0 to 130.7, while number of damaged seeds was between 3.7 and 25.7 (Table 1). Mutant 8 which produced the highest number of seeds (130.7) also had the highest number of

Table 3. Susceptibility indices and resistance ratings of Maruca infested gamma induced mutants in a free choice test.

Plant type	Larval recovery Index	Pod damage Index	Yield loss Index	Seed damage Index	Overall susceptibility Index	Tolerance** Rating
Mutant 1	0.59	0.54	0.52	0.66	2.31	MS
Mutant 2	0.62	0.76	0.26	0.46	2.10	MS
Mutant 3	0.66	1.06	1.05	0.70	3.47	S
Mutant 4	0.45	0.59	0.16	0.24	1.89	**MT**
Mutant 5	0.59	0.73	0.72	0.38	2.42	MS
Mutant 6	1.23	0.92	0.96	0.60	3.71	S
Mutant 7	0.81	0.82	0.63	0.54	2.80	MS
Mutant 8	0.81	0.85	0.62	0.62	2.90	MS
Mutant 9	0.76	0.65	0.57	0.58	2.56	MS
Mutant 10	0.75	1.06	0.77	0.63	3.21	S
Mutant 11	0.75	0.97	1.18	2.84	5.74	HS
Mutant 12	0.81	0.95	1.21	1.80	4.77	HS
Mutant 13	0.51	1.02	1.27	1.47	4.27	HS
Mutant 14	0.75	1.01	0.33	0.46	2.55	**MS**
Mutant 15	0.51	0.89	0.95	0.65	3.00	S
(parent)16	1.00	1.00	1.00	1.00	4.00	S
(Tvu946)17	0.36	0.47	0.49	0.17	1.49	**MT**

Table 4. Correlation between parameters evaluated on gamma induced mutants in a free choice test.

	No. of larvae recovered	No. of pods/ plant	No. of damaged pods	Percent pod damage	No. of seeds/ plant	No. of damaged seeds	Percent seed damage	Seed yield
No. of larvae recovered	1	-0.02	0.26	0.38**	0.09	0.33	0.36**	-0.07
Number of pods/plant		1	0.82**	0.51**	0.80**	0.44**	-0.18	0.55**
No. of damaged pods			1	-0.01	0.56**	0.55**	0.002	0.36**
Percent pod damage				1	0.55**	0.09	0.31*	-0.59**
No. of seeds/plant					1	0.62**	-0.27	0.75**
No. of damaged seeds						1	0.19	0.45**
Percent seed damage							1	0.27
Seed yield								1

*Correlation significant at p = 0.05 ; **correlation significant at p = 0.01.

damaged seeds (25.7). While mutant 11 recorded the least number of seeds per plant (5.0) and it incurred the least seed damage (3.7). Percent seed damage ranged from 7.8% in mutant 4 to 91.1% in mutant 11. The parent had 32.1%.

Seed yield per plant and percent yield loss differ significantly among the plant types at p = 0.05. Seed yield per plant was between 0.7 and 15.8 g as observed in mutants 11 and 5 respectively. Percent yield loss was between 19.7 and 95.4%. The highest yield loss (95.4%) was found in mutant 13 which was not significantly different from that of mutants 11 (88.5%) and 12 (90.8%). The least was observed in mutant 2.

Table 3 shows the susceptibility indices and resistance ratings of *Maruca* infested mutants in a free choice test. Susceptibility indices tend to compare the level of *Maruca* infestation in mutants with that of the parent. The larval recovery index was between 0.45 and 1.23; these values were obtained in mutants 4 and 6 respectively. Pod damage index ranged between 0.59 and 1.06. Mutant 4 had the least while mutants 10 and 3 had the highest. Yield loss index ranged from 0.16 to 1.27. The highest was recorded in mutant 13 while the least was recorded in mutant 4. The highest seed damage index (1.8) was found in mutant 12 while the least (0.24) was found in mutant 4. Based on their overall susceptibility indices, the mutants were grouped into different tolerance ratings. Mutant (4) was classified moderately tolerant, while seven mutants were classified moderately susceptible (1, 2, 5, 7, 8, 9 and 14), five mutants were ranked susceptible (3, 6, 10 and 15) while the three others (mutants 11, 12 and 13) were classified as highly susceptible.

Correlation co-efficients of parameters evaluated on gamma induced mutants in the free choice test is presented in Table 4. Significant positive correlations were observed between number of damaged pods and number of pods per plant (r = 0.82**) and number of seeds per plant (r = 0.56**). Number of larvae recovered were positively correlated with percent pod damage (r = 0.38**) and percent seed damage (r = 0.80**). Seed yield was negatively correlated with percent pod damage (r = -0.59**) but positively correlated with number of seed per plant (r = 0.75**). The number of pods per plant was positively correlated with number of seeds (r = 0.80**) and seed yield (r = 0.55**). However, percent pod damage appeared to be the most reliable indices for evaluating seed yield because of the high correlation co-efficient found between pod damage and seed yield (Table 3). Plants with low percent pod damage could be selected for tolerance to *Maruca* and high seed yield.

DISCUSSION

Significant differences were observed among mutants for all parameters used for evaluating their susceptibility to *M. vitrata* as oviposition site, and to the developing larvae as source of food and survival. This is probably an indication of host plant preference. Some mutants are less preferred for feeding and as site of oviposition than others. Jackai et al. (1996) observed similar findings in their study of resistance to the legume pod borer in some wild *Vigna* under laboratory feeding bioassays. They observed significant varietal differences in their susceptibility to *Maruca* pod borer.

Results of larval count tend to be a reflection of the distribution of adult moth in the screen house. Mutant 6 must have attracted the highest population of the adult moth due to availability of high number of flowers. This is because highest number of larvae was recovered from it. Mutant 6 also has purple flowers which are more conspicuous and therefore more attractive to foraging by adult *Maruca*. However, mutant 4, where the least number of larvae was recovered, had cream yellow flowers that are probably less attractive to the adult moth. Similar findings have been observed by Ntonifor et al. (1996) between cowpea with purple flowers and soybean with small size yellow flowers.

Jackai (1991) also reported such variations in susceptibility among cowpea genotypes using dual-purpose test. Echendu and Akingbohungbe (1990) in a free choice test identified Tvu 1896 and H51-1 as moderately tolerant to *Maruca* using similar ratings. Oghiakhe (1995) observed high level of resistance to *Maruca* in some wild cowpea cultivars 'Tvu N72' and 'TvuN73' using a preference ratio. The use of susceptibility indices as a basis of evaluating resistance mechanisms showed that mutant 4 tolerated *Maruca* damage most, when compared with others (Table 3). This mutant could therefore, be considered good parent materials for further work on cowpea improvement as regards tolerance to *Maruca*, more so that it recorded the least yield loss index of 0.16 as compared with 1.0 of the parent (Table 3).

Conclusion

Significant genetic differences observed among mutants in the cage screening for resistance to *Maruca* suggest possibilities for further improvement of cowpea through induced mutation.

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