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Genetics of resistance to red pumpkin beetle (*Aulacophora foveicollis*) in summer squash (*Cucurbita pepo* L.)

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Summary. Resistance to red pumpkin beetle in summer squash was found to be controlled by polygenes. Diallel and Triple test cross analysis revealed the preponderance of non-additive and additive gene effects for resistance respectively. Absence of epistasis for resistance was indicated by both tests.

Key words: *Cucurbita pepo* – *Aulacophora foveicollis* – Diallel analysis – Triple test cross – Additive and non-additive gene effects

Introduction

Red pumpkin beetle (*Aulacophora foveicollis*) is one of the most serious insect-pests of cucurbits and causes severe damage during the early stages of plant growth. This beetle can be controlled with insecticides but it would be ecologically safer and more economical if the genetics of host-parasite resistance could be understood and identified genes for resistance incorporated into adapted cultivars. Even is resistance alone could not give absolute control of the pest, its use in combination with chemical control might prove more useful than resistance or chemicals alone.

In the recent past, work on insect resistance on cucurbits has resulted in numerous significant contributions to the identification, utilization and understanding of host-plant resistance (Kennedy 1978). Nath et al. (1968) discovered that resistance to red pumpkin beetle in summer squash was available in present day cultivars, but the inheritance pattern of resistance to this beetle was not known and it was not clear whether it was high enough to be useful.

The present report will be the first on the genetics of resistance to this insect in summer squash.

Material and methods

This research was done using field screening tests in addition to controlled feeding tests in cages. Ten inbred lines of summer squash were scored for leaf injury using the method given by Grewal and Sandhu (1983):

Score	Approximate leaf area damaged
1	Healthy leaf
2	Approximately 1/4th leaf damaged
3	Approximately 1/2 leaf damaged
4	Approximately 3/4 leaf damaged
5	More than 3/4 leaf damaged

Insect damage was recorded at the two true leaf stage of the plants. Line 1-1-1-2-5 and 15-2-6, which were highly resistant and highly susceptible respectively, were crossed to raise F_1 and F_2 generations. Inbreds 1 to 8 (Table 1) were also crossed in all possible combinations excluding reciprocals. Further, two extreme testers 14-6-7 (highly resistant) and Australian Green (highly susceptible) and their F_1 (third tester) were crossed to all the inbreds 1 to 8, using testers as female parents.

All the inbreds (20 plants) and the F_1 (20 plants) and F_2 (300 plants) generations of the cross 1-1-1-2-5 × 15-2-6 were screened in the field as well as in cages. Cages of wooden frames with wire gauge (170 × 70 × 30 cm) were used. The adult beetles were collected by hand from the unsprayed field in the river bed. These beetles were released into the field cage through a round hole (2.5 cm diameter) plugged with cotton located in the centre of the top surface. The beetles were released thrice at cotyledonary, first leaf and second leaf stages, maintaining the population of 2, 4 and 6 beetles per plant, respectively. These population limits were used so as to simulate maximum field infestation conditions and were based on the maximum field population at each stage. All plants were scored at the 2nd leaf stage.

In addition, progenies of the diallel and triple test cross were sown in a randomized complete block design with two replications. The single plant randomization with 5 plants of each entry in each replication was followed so that each progeny was represented by 10 individually randomized plants. The rows were kept 1.5 m apart with a plant to plant spacing of 40 cm. Border rows were provided with the highly susceptible line Australian Green.

Means and standard errors of P_1, P_2, F_1 and F_2 generations were computed and data plotted graphically. Haymans' diallel analysis procedure (1954) was used for genetic component and variance-covariance analysis. The analysis of variance of general and specific combining ability was done according to Griffing (1956) Method 2, Model I. Triple test cross (TTC) analysis was computed in two parts: (i) test for epistasis was done using method of Kearsey and Jinks (1968) and (ii) the analysis of additive and dominance effects was done as described by Comstock and Robinson (1952) i.e. by obtaining the variances of sums and differences of L_{1i} and L_{2i} except that σ^2ml has been redefined and is equal to $1/2\,\sigma^2$ml of Comstock and Robinson. This change was made simply to facilitate the comparison between additive and dominance components. The additive component of variation (D) was estimated from the estimate of σ_s^2 by relationship $D = 4\sigma_s^2$ and the dominance component (H) was computed from the relationship $H = 4\sigma_d^2$.

Table 1. Rating of the lines for leaf injury caused by red pumpkin beetle

Lines		Score under field conditions $\bar{X} \pm$ S.E.	Rating Field	Rating Cages	Score in cages $\bar{X} \pm$ S.E.
1-1-1	(1)	1.55 ± 0.06	S	S	1.60 ± 0.04
6-5-3-1-5	(2)	1.51 ± 0.02	S	S	1.60 ± 0.02
8-6-8-1-5	(3)	1.15 ± 0.05	MR	MR	1.10 ± 0.01
15-4-1	(4)	1.65 ± 0.02	S	S	1.55 ± 0.02
1-1-1-2-5	(5)	1.00 ± 0.07	HR	HR	1.00 ± 0.01
15-2-6	(6)	3.00 ± 0.06	HS	HS	3.20 ± 0.05
21-4-1	(7)	1.60 ± 0.04	S	S	1.55 ± 0.05
10-1-2-4	(8)	1.60 ± 0.15	S	MR	1.10 ± 0.06
14-6-7	(9)[a]	1.00 ± 0.01	HR	HR	1.00 ± 0.02
Australian Green	(10)[a]	3.15 ± 0.07	HS	HS	4.40 ± 0.06

[a] These two lines and their F_1 served as three testers in Triple test cross analysis

HR = highly resistant (score 1.00); MR = moderately resistant (score 1.1–1.50); S = susceptible (score 1.51–2.00); HS = highly susceptible (score 2.1–5.0)

Results and discussion

Frequency distribution for generations

The pest injury score (in the field as well as in cages) of the F_1 generation corresponds to the score of the resistant parent (P_1), thus indicating a dominance for resistance and all the five injury scores were scored in the segregating (F_2) generation (Table 2). The frequency distribution of F_2 plants (in field and in cages) for injury score showed the pattern of continuous distribution, indicating that the resistance was controlled by polygenes (Fig. 1).

Combining ability analysis

The analysis of variance showed highly significant differences among parents of diallel set for resistance to the insect (Table 3).

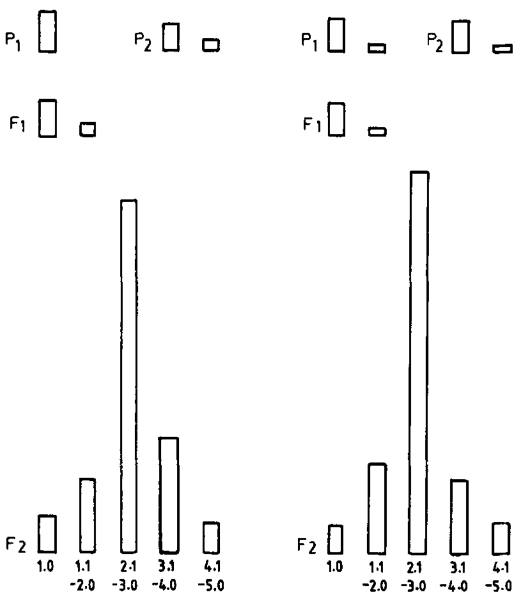

Fig. 1. Histogram of pest injury score on various generations (P_1, P_2, F_1, F_2) in 1-1-1-2-5 x 15-2-6 cross under field conditions (left) and in cages (right)

Table 2. Pest injury score of parents, F_1 and F_2 generations under field conditions and in cages (parentheses) for leaf injury caused by red pumpkin beetle

Generation	Plants scored	No. of plants with score				
		1.0	1.1 – 2.0	2.1 – 3.0	3.1 – 4.0	4.1 – 5.0
(P_1) 1-1-1-2-5	20 (20)	20 (18)	– (2)	– –	– –	– –
(P_2) 15-2-6	20 (20)	– –	– –	– –	14 (16)	6 (4)
F_1	20 (20)	17 (15)	3 (5)	– –	– –	– –
F_2	300 (300)	18 (14)	39 (45)	170 (185)	58 (37)	15 (19)

Table 3. Analysis of variance for experimental design and combining ability for field resistance to red pumpkin beetle

Source of variation	d.f.	MS
Blocks	1	0.317
Genotypes	35	0.418 **
Genotypes × Blocks	35	0.040 **
g.c.a.	7	0.422 **
s.c.a.	28	0.156 **
Between families	35	0.209
Within families	288	0.003
Components:		
$\varepsilon_i\, g_i^2$		0.021
$\varepsilon_{ij}\, s_{ij}^2$		0.058

** Significant at $P = 0.01$

The analysis of variance for combining ability revealed significant differences among gca and sca effects. $\varepsilon_{ij}\, s_{ij}^2/\varepsilon_i\, g_i^2 = 2.76$ showed that non-additive gene effects were more important than additive gene effects.

The estimates of general and specific combining ability effects and the mean performance of the parents and F_1s are presented in Table 4. Parents 8-6-8-1-5 (3), 1-1-1-2-5 (5) and 10-1-2-4 (8) recorded significant general combining ability effects (high negative gca estimates) and were good combiners for imparting resistance to the insect. The mean performance of these first two parents was also low (lowest score) but the mean performance of third one (10-1-2-4) was low in cages (MR) and high in field (S).

Relying more on cage results, particularly in the case of parent 10-1-2-4 (8), it is safe to conclude that sca effects did show consistancy with the respective mean performance or gca effects (Table 4). Crosses showing significant negative estimates of sca effects [6-5-3-1-5 (2) × 10-1-2-4 (8), 15-4-1 (4) × 10-1-2-4 (8) and 15-2-6 (6) × 10-1-2-4 (8)] also had low mean injury scores and these three crosses all involve line 10-1-2-4 (8) as one of the parent which had significant negative gca (−0.122) effects as well as low mean injury score in the cage studies (1.10). Therefore, line 10-1-2-4 (8) can be used as one of the parent in evolving hybrids resistant to this insect-pest.

Variance covariance analysis

The regression coefficient b (0.897 ± 0.136) differed significantly from zero and not from unity, indicating that the additive-dominance model was adequate. From the position of array points it was clear that parents 8-6-8-1-5 (3), 15-2-6 (6) and 10-1-2-4 (8) carried most of the dominant alleles for resistance; lines 6-5-3-1-5 (2) carried most of the recessive alleles and 1-1-1 (1) and 21-4-1 (7) carried an almost equal proportion of dominant and recessive alleles. An interception of regression line indicated the presence of overdominance (Fig. 2).

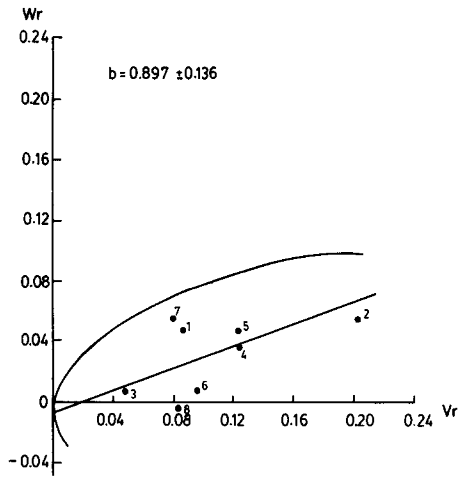

Fig. 2. Vr, Wr graph of the eight-parent diallel analysis for field resistance to red pumpkin beetle

Component analysis in diallel cross

The D, H_1, H_2 components of genetic variance were significantly different from zero, implying that additive as well as non-additive genetic variance are important in the inheritance of resistance to this insect (Table 5). However, non-additive gene action was predominant. The ratio $(H_1/D)^{1/2}$ was greater than unity (1.98) indicating overdominance. Non-significant F value indicated ambidirectional dominance.

Triple test cross analysis (TTC)

The diallel mating design for the estimation of gene effects assumes the absence of non-allelic interaction which, however, is known to be a usual occurrence in almost all crop plants. The diallel cross analysis provides a test of epistasis by the regression of Wr on Vr, consistancy of Wr-Vr over the arrays and by t^2-test but none of these is exclusively meant for epistasis. On the other hand, the TTC analysis of Kearsey and Jinks (1968) provides an unambiguous test of epistasis and in the absence of epistasis this test provides estimates of additive and dominance components of genetic variance with equal precision. Both of these mating designs, i.e. diallel and TTC were applied in the present study so that a reliable information about the presence or absence of epistasis could be obtained.

Analysis of variance for epistasis in TTC indicated the absence of epistasis (Table 6). After detecting the significant mean squares due to sums $(\bar{L}_{1i} + \bar{L}_{2i})$ and differences $(\bar{L}_{1i} - \bar{L}_{2i})$, the estimates of D, H and F were computed (Table 7). The additive component was

Table 4. Mean performance of parents (bottom) and F_1 (lower left) and estimate of general combining ability effects (diagonal) and specific combining ability effects (upper right) for field resistance to red pumpkin beetle

Parents	1	2	3	4	5	6	7	8
1	−0.002	0.270	−0.271	−0.100	−0.182	−0.020	−0.024	0.230
2	2.050	0.238**	−0.160	0.463*	0.482**	0.344	0.193	−0.612**
3	1.102	1.450	−0.172**	−0.081	0.290	0.154	−0.252	0.350**
4	1.503	2.310	1.350	0.063	0.100	0.020	−0.032	−0.383*
5	1.204	2.110	1.501	1.550	−0.157**	0.042	−0.264	−0.060
6	1.650	2.250	1.652	1.752	1.550	0.128*	0.051	−0.450*
7	1.550	2.200	1.150	1.604	1.152	1.750	0.028	0.250
8	1.652	1.050	1.600	1.100	1.210	1.100	1.710	−0.122*
Mean	1.550	1.506	1.154	1.650	1.002	3.004	1.601	1.604

$S.E_{gi} = \pm 0.059$; $S.E_{sij} = 0.183$

* Significant at $P = 0.05$; ** Significant at $P = 0.01$

Table 5. Components of genetic variance in diallel analysis for field resistance to red pumpkin beetle

$D = 0.044^{*} \pm 0.001$	$F = -0.056 \pm 0.021$
$H_1 = 0.174^{**} \pm 0.010$	$E = 0.020 \pm 0.009$
$H_2 = 0.248^{**} \pm 0.046$	$(H_1/D)^{1/2} = 1.98$

* Significant at $P = 0.05$; ** Significant at $P = 0.01$

Table 6. Mean squares from the analysis of variance for epistasis $(\bar{L}_{1i} + \bar{L}_{2i} - 2\bar{L}_{3i})$, sums $(\bar{L}_{1i} + \bar{L}_{2i})$ and difference $(\bar{L}_{1i} - \bar{L}_{2i})$, in a triple test cross, for field resistance to red pumpkin beetle

Item	d.f.	Mean squares
Epistasis	8	0.066
(i) epistasis	1	0.003
(j × l) epistasis	7	0.075
Epistasis × block	8	0.043
(j × l) epistasis × block	7	0.046
Within families	192	0.061
Sums	7	0.195**
Sums × blocks	7	0.072
Within families	128	0.061
Differences	7	0.141**
Differences × blocks	7	0.070
Within families	128	0.061

* Significant at $P = 0.05$; ** Significant at $P = 0.01$

Table 7. Estimates of D, H and F components and degree of dominance as determined by the Comstock and Robinson (1952) analysis for field resistance to red pumpkin beetle

$D = 0.134$	$H = 0.080$
$F = 0.131$	$(H/D)^{1/2} = 0.770$

found to be predominant and the degree of dominance was in the range of partial dominance. The F value was non-significant indicating no dominance or ambidirectional dominance.

The results of combining ability, variance-covariance and component of genetic variation are complementary to each other. The absence of epistasis was indicated in the diallel as well as in the TTC analysis. Field resistance to red pumpkin beetle is under the control of non-additive gene effects as revealed by combining ability and D, H components. Over-dominance for resistance to the insect was revealed by combining ability analysis, variance-covariance graph and the ratio of H_1 and D components, but partial dominance was indicated in the TTC analysis.

In summer squash, resistance to striped cucumber beetle and squash bug has been also reported to be governed by polygenes of additive nature (Nath and Hall 1963; Benepal and Hall 1967).

Many experimental factors can influence the assessment of resistance (Dickson and Eckenrode 1975). To minimise the influence of these factors in this research, firstly, the source and genetic pattern of resistance was assessed in both field grown plants as well as from those grown in cages. Secondly the insect used to study resistance was representative of the natural population in the area (river bed) where the crop is intensively grown. Lastly, experimental efficiency was enhanced by selecting the discriminating experimental (single plant randomisation) and mating designs (qualitative and quantitative studies – frequency distribution of generations, diallel and TTC). The resistance identified is referred to as 'field resistance' (Senson Federation of British Plant Pathologists 1973) since it has primarily been observed under field conditions.

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