Queensland Journal of Agricultural and Animal Sciences Vol. 45 (2), 145-149 (1988)
Published by the Queensland Department of Primary Industries

Inheritance of resistance to zucchini yellow mosaic virus in *Cucurbita maxima* cv. Queensland Blue × *C. ecuadorensis*

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Abstract

Inheritance of resistance to a Queensland isolate of ZYMV in *Cucurbita ecuadorensis* was investigated in a glasshouse using *C. maxima* as susceptible parent (P_1), *C. ecuadorensis* as resistant parent (P_2), F_1, F_2, backcross and self-pollinated backcross populations. Major gene models did not fit all the data well but estimates of heritability were high (h^2_B = 97%; h^2_N = 90%), implying that selection of *C. maxima* genotypes with resistance to ZYMV should be efficient.

INTRODUCTION

In Queensland where pumpkin (*Cucurbita maxima* Duch.) is an important culinary vegetable approximately 38 000 tonnes, valued at $8 million are produced annually (Hassall and Peachey 1985).

The major constraints on production of pumpkins are viral diseases incited by the three potyviruses, papaya ringspot virus type W (PRV-W), watermelon mosaic virus type 2 (WMV-2) and zucchini yellow mosaic virus(ZYMV), (Greber 1978; Greber *et al.* 1987). ZYMV although only recently described has attained world-wide importance as a disease of *Cucurbita* spp. (Lisa *et al.* 1981; Lisa and Lecoq 1984; Nameth *et al.* 1986; Davis and Mizuki 1986). ZYMV has recently been found in all regions of Queensland where pumpkin is a major crop (Greber *et al.* 1987). Crops infected with ZYMV appear to suffer even greater damage than those infected with PRV-W or WMV-2. Consequently, cultivars resistant to ZYMV would be useful to growers.

Resistance to ZYMV has not been found in *C. maxima*, but occurs in *C. ecuadorensis* Cutler and Whitaker (Provvidenti *et al.* 1984).

C. ecuadorensis is considered compatible with *C. maxima* (Cutler and Whitaker 1969).

Both *C. ecuadorensis* and *C. maxima* are considered to be allotetraploids but functionally diploid, with 2n = 40 (Weeden and Robinson 1986). Fertility, determined using the stained pollen method, is reduced in the F_1 (55–57%), F_2 (30%) and backcross generations (61%) (Wall and Whitaker 1971; M. Herrington, unpub. data 1987). This reduced fertility is considered due to genetic factors spread throughout the genome and not due to structural differences between chromosomes (Weeden and Robinson 1986). Although random and inconsistent deviations from Mendelian segregation ratios may occur in the cross *C. maxima* cv. Pink Banana × *C. ecuadorensis* the recombination frequencies among linked loci and the frequencies of homozygous and heterozygous genotypes in backcross progeny (Weeden and Robinson 1986) suggest that meiosis in this cross could be considered

normal. Similarly in the F_1 of *C. maxima* cv. Queensland Blue × *C. ecuadorensis*, 92 ± 2.3% of tetrads contained four daughter cells of approximately equal size (M. Herrington, unpub. data 1988) and in subsequent generations segregation patterns for fruit and leaf characteristics were consistent with normal meiosis of a functional diploid (Herrington and Brown 1988).

Although *C. ecuadorensis* is highly resistant to ZYMV, inheritance of this resistance is not known. Our study was undertaken to determine the inheritance of resistance to ZYMV in *C. ecuadorensis* using *C. maxima* cv. Queensland Blue as the susceptible parent.

MATERIALS AND METHODS

Plants of *C. maxima* cv. Queensland Blue 'Selected Strain' originally from Standards Branch of the Queensland Department of Primary Industries, and *C. ecuadorensis* were self-pollinated for two or three generations before use as parents (P_1 and P_2 respectively) to produce F_1, F_2, BC_1P_1 and BC_1P_2 generations. A random sample of 24 BC_1P_1 plants were self-pollinated to produce $BC_1P_1S_1$ families, of which 17 with adequate germination were used for genetical analysis.

The ZYMV isolate used in inoculations was the single lesion Queensland isolate described by Greber *et al.* (1987). It was originally recovered from *C. maxima* at Gatton, 100 km west of Brisbane, and confirmed as ZYMV using differential hosts and serology (Greber *et al.* 1987). Expanded cotyledons dusted with carborundum [22μm (600 mesh)] were rub-inoculated with infective sap extracts. These extracts were prepared from the terminal two or three leaves of plants of *C. maxima* cv. Queensland Blue, whose cotyledons had been inoculated with ZYMV about 14 days previously. Leaf tissue was ground with 10 parts of 0.1M sodium potassium phosphate buffer (pH 7.0) containing 0.1% sodium sulphite. Plants were grown, inoculated and evaluated in an aphid-free glasshouse at 22° to 27°C. Plants of the first six generations were grown together in December 1986. In May 1988 plants of the $BC_1P_1S_1$ generation were similarly grown with plants of P_1 and P_2. Uninoculated plants, of each generation in the first experiment and of P_1 and P_2 in the $BC_1P_1S_1$ experiment, served as controls.

The severity of disease was assessed by recording the chlorotic and/or distorted percentage (x) of the youngest expanded leaf of each plant. Each score was transformed to a logarithmic scale, namely $\log_{10}(x + 1)$ for analysis. Preliminary observations indicated that symptoms were well developed at the stage chosen for evaluation, namely 17 to 20 days after cotyledonary inoculation when plants had 6.2 ± 0.4 and 6.2 ± 0.2 expanded leaves on 1 December 1986 and 9 May 1988 respectively. Leaves were classified as expanded when both the length and breadth of their lamina were greater than 30 mm.

In order to compute segregation ratios frequency distributions were sectioned into intervals of 0.22 and 0.5 on the logarithmic scale of disease severity. In separate monogenic analyses distributions were also sectioned so that plants in a resistant class had either disease severities less than 0.66 (similar to the F_1) or less than 1.1 on the log scale. Various other combinations of classes were also used in digenic models. The numbers of resistant and susceptible plants were compared with those expected in mono and digenic models for major genes and goodness of fit tested using Chi-square (Srb *et al.* 1965). The models tested included resistance being conferred by; a single dominant gene; either or both of two dominant genes; a dominant gene epistatic to a second locus; and a recessive gene epistatic to a second locus. These models give F_2 segeration ratios of 3:1, 9:3:3:1, 12:3:1 and 9:3:4 respectively.

Preliminary observations suggested that the difference in reaction of P_1 and P_2 to infection by ZYMV was immense. Therefore only small populations of plants of P_1 (9 plants), P_2 (4 plants) and F_1 (10 plants) were used. Population size of F_2, BC_1P_1 and BC_1P_2 was 103, 39 and 35 respectively. There were 17 to 32 plants in each $BC_1P_1S_1$ family. Plants, eleven in the F_2, and two in the BC_1P_2 population, which had a genetic virus-like-syndrome [VLSmax] (Whitaker and Bemis 1964; T. W. Whitaker, pers. comm. 1985) were not included in the analysis.

Plants with VLSmax are readily distinguishable six days after emergence and four days prior to symptom development on inoculated plants. Gene(s) responsible for VLSmax was assumed to be independent of those conferring reaction to virus. While reisolation was not attempted from any plant in the 1986 experiment, in the later $BC_1P_1S_1$ experiment the association of symptoms and the presence of virus was assessed by reisolation from 22 plants each to 5 to 10 seedlings of *C. pepo* L. cv. Small Sugar.

Narrow-sense and broad-sense heritabilities were computed using the variance component method of Mather and Jinks (1971). These computations involved the calculations:

$$D/2 = V_{F2} - (V_{B1} + V_{B2})$$

$$H/4 = V_{F2} - D/2 - E$$

$$E = (V_{F1} + V_{P1} + V_{P2}) / 3$$

in which V = variance, and subscripts denote population; B1 = BC_1P_1 and B2 = BC_1P_2;

$$D/2 = \text{additive variance;}$$

$$H/4 = \text{dominance variance;}$$

$$E = \text{environmental variance and;}$$

$$\text{Narrow-sense heritability } (h^2_N) = (D/2)/V_{F2} \text{ and;}$$

$$\text{Broad-sense heritability } (h^2_B) = (D/2 + H/4)/V_{F2}.$$

Heritability expresses the degree to which a particular characteristic of a plant is transmitted to its progeny.

RESULTS AND DISCUSSION

The plants of the F_1 generation were substantially more resistant than those of the susceptible parent, *C. maxima*, but not as resistant as *C. ecuadorensis* (Tables 1 and 2). The distributions of the reactions of plants in F_2, BC_1P_1 and BC_1P_2 populations were essentially continuous and did not readily separate into classes of resistance (Table 1). Major gene models did not fit all the data well; goodness of fit was dependent on where sectoring occurred (Table 1). Similarly, only 5 and 3 of 17 $BC_1P_1S_1$ families could be classified as derived from the susceptible genotype when the F_1, and a more susceptible reaction, respectively, were used as the boundary between resistant and susceptible reactions. Only 1 and 3 families presumed derived from the F_1 genotype occurred in $BC_1P_1S_1$ at these boundaries of resistance. The distribution of the pooled data for reaction of plants in $BC_1P_1S_1$ families was consistent with an expected segregation pattern of

resistance only in the mongenic model when the boundary for sectioning the distribution was more susceptible than the F_1 reaction, and then the F_2 distribution was not consistent with the model.

Table 1. Segregation of populations of *C. maxima* × *C. ecuadorensis* for reaction 17 to 20 days after inoculation with ZYMV

Population	Number of plants									
	Total no.	No. in severity of disease classes 1–9*								
		1	2	3	4	5	6	7	8	9
(a)† *C. maxima* (P_1)	9	0	0	0	0	0	0	0	0	9
C. ecuadorensis (P_2)	4	4	0	0	0	0	0	0	0	0
F_1	10	0	6	4	0	0	0	0	0	0
F_2	103	35	32	8	8	7	5	2	3	3
BC_1P_1	39	1	2	2	3	8	3	5	4	11
BC_1P_2	35	32	3	0	0	0	0	0	0	0
(b) $BC_1P_1S_1$	478	52	33	38	32	36	57	35	36	159
C. maxima	12	0	0	0	0	0	0	0	1	11
C. ecuadorensis	6	6	0	0	0	0	0	0	0	0

* Categories 1 to 9 represent equal sectors on log scale of visually estimated percentage of the area of youngest expanded leaf which expressed symptoms 17 to 20 days after inoculation correspond approximately to 0.5, 2, 4, 7, 12, 20, 35, 57 and 100% of leaf area respectively.

† Inoculated (a) December 1986, (b) May 1988.

Table 2. Population means and variances for six genetic populations in *C. maxima* × *C. ecuadorensis*.

Population	Number of plants	Mean severity of disease*	Variance (V)
(*C. maxima*) P_1	9	1.9786	0.0013
(*C. ecuadorensis*) P_2	4	0.0200	0.0005
F_1	10	0.3660	0.0252
F_2	103	0.4786	0.2637
BC_1P_1	39	1.3390	0.2817
BC_1P_2	35	0.0674	0.0090

* Before computation each datum was transormed to log (x + 1) where × was the percentage of area of youngest expanded leaf affected by disease.

There was no association of pollen fertility (30–92%) of parental BC_1P_1 plants and the mean reaction of a $BC_1P_1S_1$ family to ZYMV (R^2 = 0.01). Virus was recovered from infected plants but reisolation was low, 1 to 2 plants infected/10 inoculated, when symptom severity was low; for example, 0.1 to 0.2% of leaf area infected. Virus was not recovered from *C. ecuadorensis*.

Weeden and Robinson (1986) found inconsistent deviations from Mendelian segregation ratios scattered among 14% of the data sets for individual loci, with no single locus consistently displaying skewed ratios. In our experiment similar random deviations were possible but we considered this an unlikely cause of the failure of data to adequately fit the Mendelian ratios. The continuous distribution of the data suggested polygenic inheritance was more likely.

Estimates of both narrow-sense and broad-sense heritability were high (h^2_N = 90%; h^2_B = 97%). The high heritabilities indicated that the variation of reaction among plants was primarily due to genetic rather than environmental causes. It was concluded that resistance of the population could be improved through selection. In particular, as progress in selection depends primarily on additive gene action, the high narrow-sense heritability (h^2_N) indicated high additive variance and that improvement by selection could be efficient.

The quantitative inheritance of resistance to ZYMV in *C. ecuadorensis* contrasts with the single dominant gene conferring resistance to pathotype O of ZYMV in *Cucumis melo* L. (Pitrat and Lecoq 1984). *C. ecuadorensis* is resistant to isolates of ZYMV in the United States and Queensland. This, together with its quantitative inheritance suggested that resistance may be broadly based and result from disruption to more than one component of the viral infection and multiplication cycles. Such quantitative resistances are generally considered to be more durable than resistances conferred by singlegenes (Browning 1979).

Our results confirm that *C. ecuadorensis* would be a usable source of ZYMV resistance for *C. maxima*. In fact selection for ZYMV resistance in *C. maxima* phenotypes derived from *C. ecuadorensis* is progressing well in our breeding programme.

ACKNOWLEDGEMENTS

We thank Professor H. Munger, Cornell University, New York for the original seed of *C. ecuadorensis*. This study was conducted with financial support from the Committee of Direction of Fruit Marketing and Australian Government (ASRRF) through the 'Breeding for resistance to zucchini yellow mosaic in cucurbits' project.

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(Accepted for publication 14 September 1988)