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Response of progeny from the cross *Cucurbita moschata* × *C. ecuadorensis* to infection with papaya ringspot virus type W and watermelon mosaic virus type 2

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Abstract

C. moschata cv. Butternut was compared with eleven selected lines produced through three backcrosses from cv. Butternut × *C. ecuadorensis*. The comparisons involved pruned or not, and naturally infected or mechanically inoculated with papaya ringspot virus type W (PRV-W) plus watermelon mosaic virus type 2 (WMV-2). The objectives were to determine suitable evaluation procedures and the extent of genetic variation for resistance in the populations. All plants expressed moderately severe leaf symptoms which were only poorly correlated with fruit symptoms (r = 0.46) and yield (r = -0.56). Fruit distortion, which was greatest on manually inoculated and on pruned plants, differed among genotypes being highest in cv. Butternut. Fruit number was highest (5.5) on naturally infected unpruned plants and lowest (3.3) on mechanically inoculated pruned plants. The effects of pruning and method of inoculation on yield (kg) varied with genotype.

Estimates of broad sense heritability on a plot mean basis were high (0.8) for yield and moderate (0.4) for fruit symptoms. It was concluded that sufficient variation existed to improve yield and reduce severity of fruit symptoms but not to decrease leaf symptoms. In our population yield and severity of fruit symptoms on manually early-inoculated unpruned plants should be the basis of selection.

Development of techniques to produce large populations of early-backcross plants would be useful.

INTRODUCTION

In Queensland, papaya ringspot virus type W (PRV-W) and watermelon mosaic virus type 2 (WMV-2) are major diseases of butternut pumpkin, *Cucurbita moschata* Duch. (Greber 1978). Resistance to both these viruses occurs in *C. ecuadorensis* Cutler and Whitaker (Provvidenti *et al.* 1978), a wild species with few other desirable horticultural characteristics. The F_1 hybrid, *C. moschata* × *C. ecuadorensis*, can be produced (Cutler and Whitaker 1969). Greber and Herrington (1980) found that following cotyledonary inoculation of F_1 plants with either PRV-W or WMV-2 the next 2 to 5 leaves expressed mild symptoms but later leaves were symptomless and virus was not reisolated from them. The partial dominance of resistance in the F_1 suggested backcrossing as an appropriate strategy to transfer resistance from *C. ecuadorensis* or *C. moschata*, so a breeding programme was commenced with this objective.

In this programme, despite the use of embryo culture (after Wall and York 1960), only a total of 24 plants was established in the first backcross generation. Twenty-one plants were mechanically inoculated and showed symptoms, however, three remaining plants expressed no symptoms during natural epidemics in which inoculum of both PRV-W andWMV-2 was present. These three plants were used as parents in a continued backcrossing programme.

Selection in other progeny was based on the severity of leaf symptoms following manual inoculation. However, the relationship between yield and the intensity of symptoms on leaves was not known. Plants of *Cucumis sativus* L. infected with cucumber green mottle mosaic virus have a reduced yield even in the absence of leaf symptoms (Kooistra 1968). We therefore investigated the relationship between leaf symptoms, fruit symptoms and yield in our population.

In advanced backcross generations derived from *C. moschata* × *C. ecuadorensis* no manually inoculated plants had exhibited tolerance as high as that of naturally infected plants of the first backcross generation (R. S. Greber and M. E. Herrington unpub. data). Resistance to natural infection in advanced progeny would be useful in controlling epidemics, so we investigated the possible occurrence of this form of resistance in our advanced population.

The rapid growth of butternut pumpkin plants can make identification of individual plants difficult when they have intergrown extensively. Pruning of inoculated plants followed by the evaluation of symptoms in the regrowth was investigated as a means of comparing the reactions of individual plants to viral infection.

The maintenance of genetic variability is essential if continued selection for a character is to be effective (Simmonds 1979). Selection of small populations often reduces genetic variability. Selection intensities of less than 10% were practised on the already small populations in the early generations (M. E. Herrington and R. S. Greber unpub. data). We thereforedetermined the extent to which this had limited the genetic variability in our population derived from *C. moschata* × *C. ecuadorensis*. We also determined the progress made through selection by comparison of the performance of selected lines with the standard cv. Butternut.

MATERIALS AND METHODS

Seed of the F_1, *C. moschata* × *C. ecuadorensis*, was produced using *C. moschata* cv. Butternut (Waltham strain), hereafter referred to as cv. Butternut WS, as the female plant. In backcross generations cv. Butternut WS was the male parent. Pollination was controlled by enclosing unopened flowers in paper packets. In the first and second cycle of back-crossing, plants were produced through *in vitro* embryo culture (Wall and York 1960). Isolates of PRV-W and WMV-2 were obtained from pumpkin (*Cucurbita maxima* Duch.), identified by host range and serology (Greber 1978) and maintained in cucumber.

Plants of the second backcross and subsequent generations were selected for combined resistance to PRV-W and WMV-2. Carborundum dusted young leaves were rub-inoculated with PRV-W and WMV-2 infective sap when plants had two or three expanding leaves. Alternatively, plants were grown to flowering, pruned to the crown and the new growth inoculated about two weeks later. Plants showing mild symptoms were selected and backcrossed to cv. Butternut WS or open-pollinated, sib-pollinated, or self-pollinated.

The major experiment, which compared pruning and inoculation methods and determined the advance of selected lines over cv. Butternut (large strain) hereafter referred to as cv. Butternut LS had a factorial design. There were two replicates each of ten plant plots of each of 12 lines (11 experimental lines plus cv. Butternut LS), × mechanical inoculation (PRV-W plus WMV-2) or natural infection, × pruned or unpruned plants. The 11 experimental lines had been selected through three generations of back-crossing, one generation of self-pollination or sib-pollination and one of mass selection with open-pollination. Appropriate plants were rub-inoculated at 22 days and pruned at 62 days, symptoms on the youngest mature leaf were evaluated at 59 and 95 days and fruit harvested at maturity, namely 106 days for unpruned plots and 138 days for pruned plots after incubation of seed on 8 September, 1981.

Severity of viral symptoms was rated always by the same operator using a 0 to 5 scale. With leaf assessments the scale units corresponded to no symptoms, minor chlorotic lesions, more chlorosis, significant chlorosis and some distortion, moderate distortion, substantial distortion and stunting of plant, respectively. Fruit symptoms were rated on a scale similar to that used for *C. maxima* (Herrington 1987) where 0 (no symptoms), 1 (minor distortion), 2 or 3 (substantial distortion but marketable depending on severity), 4 (severe distortion and unmarketable), 5 (very severely affected). The significance of differences between means was tested using the protected *t*-test. The estimate of error was provided by the mean square of the combined factor × replicate interaction.

Heritability was calculated by partitioning variances on a plot mean basis using data from the unpruned, manually inoculated plots. This treatment was thought most likely to approximate normal field conditions when inoculum and vectors are abundant. Correlations were determined on a line mean basis after adjusting for treatment effects, or on unadjusted values of individual plants.

RESULTS AND DISCUSSION

All manually or naturally inoculated plants in advanced selected lines expressed symptoms indicating that resistance to infection was low. Severity of symptoms on the leaf at 95 days was correlated with the severity of symptoms on fruit ($r = 0.46$; df 44, $P<0.01$) and negatively correlated with yield ($r = -0.56$; df 44, $P<0.01$). However, the relatively low proportion of variation accounted for by these correlations indicated that selection on the basis of severity of leaf symptoms alone was not an acceptable method in the range of severities studied. Yield and severity of symptoms on fruit needed to be assessed, or an alternative rating system devised.

Fruit on pruned plants were more distorted than those on unpruned plants, rating 1.6 and 1.0 respectively, (LSD ($P = 0.05$) = 0.2). Similarly, fruit on manually inoculated plants were more distorted than those on naturally infected plants, rating 1.4 and 1.2 respectively (LSD ($P = 0.05$) = 0.2). Because both PRV-W and WMV-2 are readily transmitted by the same vectors it seems unlikely that natural infection would have transmitted WMV-2 only with a concomitant reduction in fruit symptoms. Severity of symptoms on the fruit (Table 1) differed with genotype but there was no interaction (*F*-test not significant at $P = 0.05$) of genotype and pruning method. These results probably reflect differences in growth rates and time of infection relative to the time of fruit initiation and development. Virus is more likely to be present at initiation in pruned and/or inoculated plants than in unpruned and/or naturally infected plants, where fruit may develop before infection.

The number of fruit was reduced ($P<0.05$) by pruning in naturally infected plants but not significantly reduced in manually inoculated plants (Table 2). Inoculated plants already have low yield and this may reduce the potential for a decreased yield resulting from pruning. The effect was consistent across lines. By contrast the effect of pruning and method of inoculation on total yield (kg) varied with genotype (Table 3) and this indicated differences among lines in tolerance to virus and pruning. Line 10 had consistently high yields across all management regimes (Table 3) and this response suggested high tolerance. cv. Butternut LS had lower yield (Table 3).

Table 1. Mean severity of fruit and leaf symptoms, and mean number of fruit on plants following infection with PRV-W and WMV-2*

Selected lines	Mean severity of fruit symptoms †	Mean severity of leaf symptoms at 95 days †	Mean yield number of fruit/plant
1	0.5	3.0	4.7
2	0.9	2.9	4.3
3	1.0	3.0	5.3
4	1.1	3.1	4.2
5	1.2	3.2	4.1
6	1.2	3.5	3.7
7	1.4	3.2	3.7
8	1.5	3.3	4.9
9	1.5	3.4	4.2
10	1.6	3.2	4.2
11	1.8	3.6	4.1
Butternut LS	2.1	3.5	4.1
LSD $P = 0.05$	0.4	0.7	0.5

* Seed incubated September 8; plants inoculated with PRV-W and WMV-2 at 22 days and pruned at 62 days. Unpruned plants and pruned plants harvested at 106 and 138 days, respectively.

† 0 = no symptoms, 5 = very severe symptoms.

Table 2. Yield (no. of fruit per plant) as affected by pruning and method of inoculation with PRV-W and WMV-2

Pruning regime	Method of Inoculation		
	Manual*	Natural	Mean
unpruned	3.7	5.5	4.6
prune †	3.3	4.2	3.7
mean	3.5	4.9	
LSD ($P = 0.05$) = 0.4			

* Young leaves manually inoculated with PRV-W and WMV-2 22 days after incubation of seed.

† Plants pruned to the crown 62 days after incubation of seed.

Plants of cv. Butternut LS had severe leaf symptoms at 95 days, and fewer fruit, which were also more severely affected, than those of most other lines (Table 1). This indicates progress has been made in incorporating tolerance into the *C. moschata* phenotype. However, selection has been based on leaf symptoms and there is only a moderate correlation of yield and fruit symptoms with leaf symptoms. This probably contributes to an inability to accurately predict progeny reaction from the parent, as shown by the non-significant correlations of leaf virus rating of parent with the mean of progeny derived by open-pollination (r = 0.40; df. 10, $P<0.05$).

Broad sense heritabilities were high for yield (0.84) and moderate (0.4) for severity of fruit symptoms in unpruned, manually inoculated plots. These values indicate that a large proportion of the variation observed was a result of genetic differences. Genetic coefficients of variation, which give an indication of the extent of genetic variation relative to the mean, were 26% and 37% for fruit weight and severity of fruit symptoms respectively. These results suggest that variation for tolerance of viral infection has been maintained in the population in respect of these characteristics. Improvement could therefore be expected by selection. By contrast there were no differences (at $P = 0.05$) among selected lines in the severity of leaf symptoms at 95 days, or in the number of fruit following manual inoculation without pruning and no gain could be expected.

Table 3. Yield (kg/plant) of genotypes under two regimes of pruning and inoculation with PRV-W and WMV-2 *

	Infection method			
	Manual inoculation		Natural infection	
Line†	Unpruned	Pruned	Unpruned	Pruned
1	2.3	2.6	3.0	3.3
2	3.0	4.4	5.3	3.2
3	3.0	3.1	4.1	4.2
4	3.0	3.1	4.6	4.6
5	3.2	2.2	3.6	3.8
6	2.2	2.0	4.0	2.3
7	1.5	2.2	3.8	3.4
8	1.9	2.6	3.7	5.2
9	1.9	2.4	3.9	3.4
10	3.8	4.2	4.0	3.8
11	2.5	1.5	3.9	2.6
Butternut LS	2.6	2.0	2.4	2.4
LSD (P = 0.05) = 1.2				

* Seed incubated September 8; plants inoculated with PRV-W and WMV-2 at 22 days, and pruned at 62 days. Unpruned plants and pruned plants harvested at 106 and 138 days, respectively.

† Each line, except cv. Butternut, was derived as open-pollinated seed from single plant selections following 3 backcrosses to cv. Butternut from cv. Butternut × *C. ecuadorensis*.

Progress has been made in the selection of PRV-W and WMV-2 tolerant *C. moschata* phenotypes from the cross *C. moschata* × *C. ecuadorensis* but a high level of resistance is lacking. Sufficient genetic variation exists in the breeding population to continue selection for yield and reduced severity of symptoms on the fruit. However, in our population, severity of leaf symptoms alone does not provide an adequate description on which to base selection for tolerance. This criterion must be supplemented with assessment of yield and severity of fruit symptoms. Similarly, pruning increased the severity of symptoms on fruit and may be useful in discriminating among genotypes for this characteristic. As the effect of pruning on total yield varied with genotype, evaluation strategies based on pruning and subsequent evaluation of yield must be applied cautiously.

Yield and severity of symptoms on fruit following early manual inoculation offer the most reliable means to evaluate tolerance. This suggests slow progress in a breeding programme as only one to one and a half generations per year will be possible. Higher levels of resistance–tolerance exist in *C. ecuadorensis*, but these are not present in our population. Development of improved techniques to establish large populations of backcross plants and to evaluate resistance more critically on young plants would likely lead to more efficient transfer of resistance from *C. ecuadorensis* into *C. moschata.*

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