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Oligogenic inheritance for resistance to *Zucchini yellow mosaic virus* in *Cucurbita pepo*

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Summary

'True French' is an open-pollinated cultivar of the Zucchini (Courgette) Group of *Cucurbita pepo* and is susceptible to *Zucchini yellow mosaic virus* (ZYMV). Using *C. moschata* 'Menina' as the source of ZYMV resistance and following six generations of backcrossing, a true-breeding line nearly isogenic to 'True French', designated 381e, was recovered that carried ZYMV resistance, albeit not at as high a level as in 'Menina'. 'True French' and accession 381e were crossed, and their reciprocal F_1, F_2, and backcross progenies were grown in a chamber and inoculated with a highly virulent, non-aphid-transmissible strain of ZYMV. Nearly all F_1 plants and all plants of the backcross to 381e were classified as resistant. Segregation to resistant and susceptible individuals occurred in the backcross to the susceptible parent, in accordance with a 3:5 three-gene ratio of resistant: susceptible. The F_2 segregated in accordance with a ratio of 45 resistant : 19 susceptible, which would be obtained if there was one major gene for resistance, *Zym-1* (*Zym*), and two other genes, herein designated *Zym-2* and *Zym-3*, both of which are complementary to *Zym-1*. The presence of *Zym-1* and either *Zym-2* or *Zym-3* is necessary for resistance to be expressed in young plants, but the presence of all three might be necessary for resistance to continue to be expressed during subsequent development of the plants. Evidently, *Zym-2* and *Zym-3* are ubiquitous in *C. moschata* but their susceptible alleles are much more common in *C. pepo*. As the level of resistance of 381e to ZYMV is not as high as that of *C. moschata* 'Menina', additional, as yet unidentified, genes must be involved in conferring high resistance to this virus.

Key words: Courgette, *Cucurbita pepo*, genetics, pumpkin, squash, virus resistance, zucchini, *Zucchini yellow mosaic virus*

Introduction

Cucurbita pepo L. is one of the most economically important species of vegetable crops, embracing summer squash as well as many pumpkins and some winter squash. The potyvirus, *Zucchini yellow mosaic virus,* (ZYMV) is a devastating pathogen of this species in many regions (Lisa & Lecoq, 1984; Desbiez & Lecoq, 1997). No sources of resistance to this virus have been found which originated within *C. pepo*. Some new cultivars carrying resistance, through transgenic (Clough & Hamm, 1995; Quemada & Groff, 1995) or conventional (Provvidenti, 1997) methods, are now available. The cultivars carrying resistance derived through conventional breeding have as their ultimate source forms of other species of *Cucurbita*.

Resistance to ZYMV has been found in several accessions of two cultivated species, *Cucurbita moschata* Duchesne and *C. ficifolia* Bouché (Provvidenti, Gonsalves & Humaydan, 1984; Paris, Cohen, Burger & Yoseph, 1988) as well as in a wild species, *C. ecuadorensis* Cutler & Whitaker (Herrington, Greber, Brown & Persley, 1988; Robinson, Weeden & Provvidenti, 1988). *C. moschata* is partly cross-compatible with *C. pepo*, producing a few viable seeds in some cross combinations (Whitaker & Davis, 1962).

Resistance to ZYMV in *C. moschata* is conferred by a single dominant gene, *Zym* (Munger & Provvidenti, 1987; Paris *et al.*, 1988; Gilbert-Albertini, Lecoq, Pitrat & Nicolet, 1993). In spite of the simple inheritance, much difficulty has been encountered in attempts to transfer resistance to *C. pepo*, in part due to barriers to interspecific crossing (Whitaker & Davis, 1962). Most of the difficulty experienced in introgressing resistance into *C. pepo* has occurred during initial backcrossing. The high level of resistance

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of the original material can be lost during backcrossing, this being attributed to a change in the mode of inheritance through interspecific transfer (Kyle, 1995).

In the mid-1980s we initiated a breeding programme having the goal of transferring ZYMV resistance to *C. pepo*. 'Menina', a *C. moschata* cultivar from Portugal, was used as the source of resistance. The ultimate goal was to transfer the high level of resistance possessed by 'Menina' to zucchini (courgette) squash, this being by far the economically most important cultivar-group of *C. pepo* (Paris, 1989, 1996). Following six generations of backcrossing resistance to the zucchini squash cultivar True French, a true-breeding ZYMV-resistant line nearly isogenic to this cultivar was established. Although the resistance of this line traces back to 'Menina', the level of resistance recovered in it was not as high. The objective of this work was to study the mode of inheritance of this level of resistance to ZYMV in *C. pepo* zucchini squash 'True French'.

Materials and Methods

Plant material

Seeds of *C. moschata* 'Menina' were obtained as described previously (Paris *et al.*, 1988). This original seed stock contained resistant and susceptible individuals to ZYMV. The resistant individuals exhibited a systemic infection of small (≈1 mm diameter) circular yellow spots on the leaves starting within 7-10 days of inoculation with ZYMV. The susceptible individuals were stunted and exhibited systemic infection with severe leaf distortion and yellowing. A line true-breeding for resistance to ZYMV, designated MEN-4-2-12, was derived through three generations of self-pollination and selection in 'Menina'. MEN-4-2-12 was used as the male parent in crossing with 'Spookie', a cultivar of the Pumpkin Group of *C. pepo* (Paris, 1986), seeds of which were kindly provided by Joseph Harris Co., Rochester, New York. The F_1, designated S36 (Fig. 1), reacted the same way to inoculation with ZYMV as did MEN-4-2-12, showing small yellow spots.

S36 was crossed with an intraspecific F_1 of *C. pepo*, so as to exploit gametic diversity (Wall & York, 1960). The *C. pepo* F_1, which was designated as no. 218, employed 'Vegetable Spaghetti', a cultivar of the Vegetable marrow Group, seeds of which were obtained from Sakata Seeds, Yokohama, Japan, as the female parent, the male being a wild gourd from Texas, seeds of which were kindly provided by H D Wilson of Texas A & M University, College Station, USA. The resulting double-cross F_1 was designated S78 (Fig. 1). Plants of S78 were inoculated with ZYMV (see below). Plants of S78 that did not exhibit severe leaf

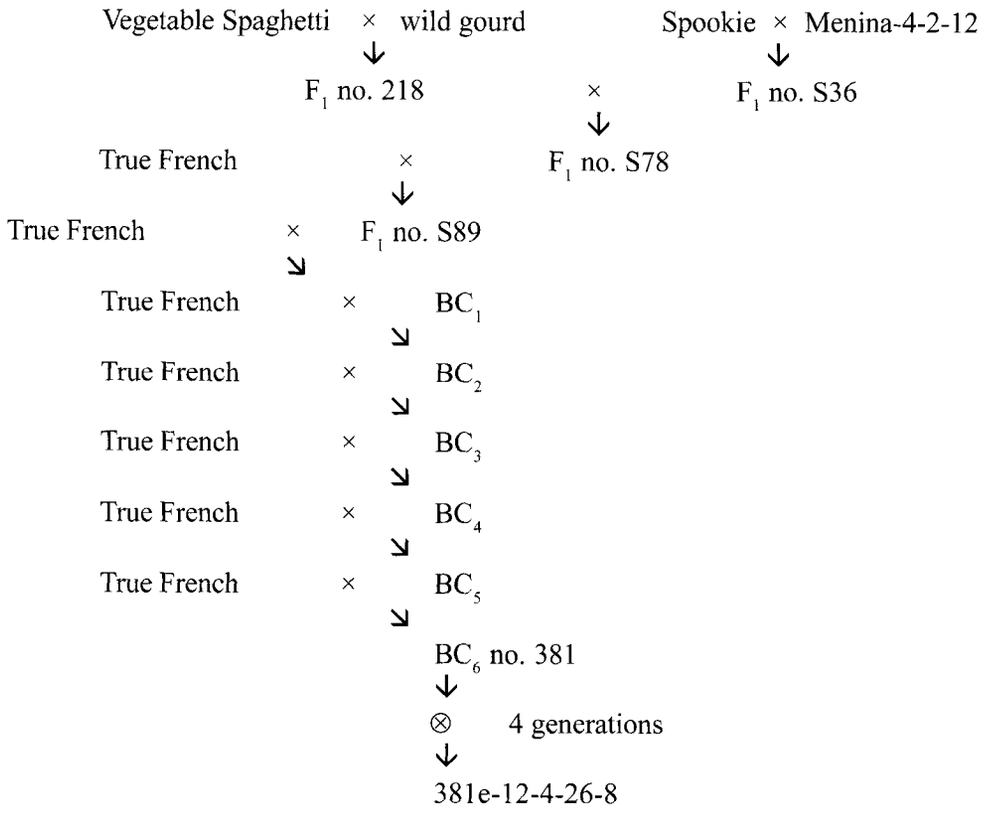

Fig. 1. Derivation of a near-isogenic ZYMV-resistant accession of True French, 381e-12-4-26-8.

yellowing and distortion, and therefore appeared to be resistant, were selected and crossed, as male parents, with plants of 'True French', a cultivar of the Zucchini Group of *C. pepo*, to give F_1 no. S89. Seeds of 'True French' had been obtained from Thompson & Morgan, Ipswich, England, in 1978 and maintained and increased by self-pollination at the Newe Ya'ar Research Center. Six successive backcrosses were then made to 'True French', always using 'True French' as the female parent, with selection for resistance in each generation. The sixth backcross generation was designated no. 381. A resistant plant of this generation, designated 381e-12, was self-pollinated. Plants of four successive generations of self-pollination were selected for resistance to obtain the BC_6F_5 plant 381e-12-4-26-8, which subsequently proved to be true-breeding for resistance to ZYMV. Progenies obtained from this plant after one to three further generations of self-pollination were used for this study. For brevity, these progenies will be referred to simply as 381e throughout the rest of the text. As 381e was obtained after six generations of backcrossing to 'True French', 381e and 'True French' can be considered as nearly isogenic and therefore are ideally suited for an inheritance study. However, adult plants of 381e are phenotypically distinguishable from those of 'True French', even after the six generations of backcrossing, by their silver-mottled leaves and slightly shorter fruit. 'True French' and 381e were crossed reciprocally and several of the resulting F_1 plants were self-pollinated and backcrossed to each of the parents. The parental, filial, and backcross progenies were then grown together in order to study the mode of inheritance of resistance to ZYMV.

Growing conditions

Seeds of plants to be tested were sown, one per pot, in 330 ml pots filled with a 1:1 (v/v) mixture of volcanic tuff and peat moss. The pots were placed in PLEXIGLASS chambers. Growing conditions in the chambers, including temperature, relative humidity, light duration and intensity, irrigation, and fertilisation were as described by Danin-Poleg *et al.* (1997), except that the sources of supplemental lighting were fluorescent and incandescent. There were 12 sowings, one per month, in four consecutive months beginning in May 1996 and March 1997, and one each in February, March, June, and July 1998.

ZYMV inoculum and inoculation procedure

The techniques employed were similar to those of Danin-Poleg *et al.* (1997). The non-aphid-transmissible ZYMV isolate designated ZYMV-NAT (Antignus, Raccah, Gal-On & Cohen, 1989) was maintained in plants of the susceptible cucumber 'Shimshon' (Hazera' Seed Co., Berurim, Israel) in an insect-free greenhouse and in the chamber described above. Virus purity was checked periodically using diagnostic plants (Lisa & Lecoq, 1984).

Seedlings were inoculated with ZYMV-NAT once or twice. The first inoculation was conducted when the first true leaf had unfolded, 9-12 days after sowing. Both the cotyledons and the first true leaf were inoculated. The second inoculation of the cotyledons and the first true leaf was conducted four days after the first. Inoculation was mechanical, using carborundum powder, the infective homogenate being prepared by grinding approximately 5 g of infected cucumber leaves in 10 ml distilled water. In the first year, plants of the first sowing were inoculated twice whereas in the second, third, and fourth sowings, half of the plants were inoculated once and the other half twice. As all inoculated plants developed symptoms of viral infection, even if inoculated only once, and as the number of inoculations was observed to have no consistent effect on the expression of symptoms or the proportion of resistant plants, in the second and third years the plants were inoculated only once, 9-12 days after sowing.

Scoring procedure and statistical analysis

Plants of the parents, the reciprocal F_1s, and the reciprocal F_2s were included in the first sowing, in May 1996, and were identified prior to their being scored as resistant or susceptible. In all subsequent sowings, pots containing seeds of the parents, F_1s, F_2s, and backcrosses were arranged randomly in the chamber in groups of 10 pots (second sowing) or five pots (all subsequent sowings) and coded numerically. The identity of the number codes of each group of pots was revealed only after the scoring had been completed. Five pots containing plants of each of the parents and the reciprocal F_1s were marked and included among the coded groups of pots; they served as controls and aided the scoring of plants in the coded pots as resistant or susceptible.

The results from segregating generations were subjected to χ^2 analysis. The F_2 and backcross data from the various sowing dates were also subjected to χ^2 tests for heterogeneity. As the results of the tests for heterogeneity were statistically non-significant, the data have been pooled for presentation.

Results

Symptoms of systemic virus infection, expressed as a multitude of tiny yellow spots and leaf curling, appeared four days after inoculation in plants of 381e, several days sooner than in plants of 'True French'. By 11 days after inoculation, it was observed that the third true leaf of plants of 381e was quite stunted. Thereafter, the plants of 381e exhibited recovery: the newly developing fourth and fifth leaves were successively less stunted than the third leaf. Only mild symptoms of infection, including yellow spots and mild blistering or distortion of the leaves, occurred

thereafter. Thus, the resistance expressed by 381e was not as high as that of the source, 'Menina'. Although the third leaf of the plants of the susceptible 'True French' was not stunted, by the fifth leaf the 'True French' plants showed stunting, severe mosaic, yellowing, and/or distortion, and had white streaks extending from the leaf margin through one-third or more of the distance to the petiole; this whitening was the most diagnostic symptom of susceptibility. F_1 plants, regardless of the direction of the cross, behaved similarly to 381e. Plants of the parents and of the reciprocal F_1s were most easily and consistently classified as resistant or susceptible at 20-22 days after inoculation (Fig. 2) and, therefore, the data presented herein were taken during this period, at which time all plants had unfolded at least eight true leaves.

Nearly all F_1 plants as well as all plants of the backcross to 381e were resistant (Table 1). Therefore, resistance was dominant to susceptibility. However, the symptoms observed in F_1 plants were not always as mild as those observed in 381e, which is reflected in the fact that a few F_1 plants had been classified as susceptible (Table 1).

The result for the F_2, regardless of the direction of the cross, was approximately twice as many resistant as susceptible plants (Table 1). The result for the backcross to the susceptible parent was the opposite, there being nearly twice as many susceptible plants as resistant ones. These results cannot be accounted for by one gene or two genes: segregation of the backcross

Fig. 2. Zucchini squash plants 20 days after inoculation with zucchini yellow mosaic virus (old, senescing leaves removed). Top left, 381e (resistant); top right, 'True French' (susceptible); bottom left, 381e × 'True French', F_1 (resistant); bottom right, 'True French' × 381e, F_1 (resistant).

Table 1. *Segregation for resistance and susceptibility to ZYMV in the cross of 381e with True French (TRF), 20-22 days after inoculation*

Generation	Description	Number of plants			Expected ratio*	χ^2	*P*
		Total	Resistant	Susceptible			
P_1	381e	60	60	0	—	—	—
P_2	TRF	81	0	81	—	—	—
F_1	$P_1 \times P_2$	122	120	2	—	—	—
F_1	$P_2 \times P_1$	121	120	1	—	—	—
F_1	Total	243	240	3	—	—	—
F_2	$(P_1 \times P_2) \otimes$	486	338	148	45:19	0.136	0.70
F_2	$(P_2 \times P_1) \otimes$	540	360	180	45:19	3.439	0.06
F_2	Total	1026	698	328	45:19	2.558	0.11
BC_1	$P_1 \times F_1$	297	297	0	—	—	—
BC_1	$P_2 \times F_1$	631	221	410	3:5	1.651	0.19

* The 45:19 and 3:5 ratios were derived assuming that all *Zym-1/— Zym-2/ —* and *Zym-1/— Zym-3/—* genotypes are resistant and that all *Zym-1/Zym—1* and *Zym-1/— Zym-2/Zym-2 Zym-3/Zym-3* genotypes are susceptible.

population into resistant and susceptible deviated significantly from the 1:1 one-gene ratio and the 1:3 two-gene ratio ($\chi^2 = 56.61$, $P < 0.001$ and $\chi^2 = 33.81$, $P < 0.001$, respectively). However, the segregation in the backcross population fits a three-gene, 3:5 ratio of resistant to susceptible (Table 1). This ratio would be obtained if one gene of major effect was interacting with two others. A four-gene, 5:11 ratio of resistant to susceptible had a less satisfactory fit to the result ($\chi^2 = 4.183$, $P = 0.03$).

The segregation in the F_2 was in reasonable accord with a ratio of 45 resistant to 19 susceptible (Table 1). The 45:19 ratio is obtained by assuming that there are three complementary dominant genes for resistance. For resistance to be expressed, the first gene plus at least one of the other two must be present in the genotype. This is the simplest hypothesis that fits all of the results presented in Table 1. Four-gene ratios could be thought of that also fit the F_2 data, but the simpler, 3-gene hypothesis fits the backcross data whereas the 4-gene ratio (considered above) deviates significantly.

Discussion

Research groups working in several countries have observed that resistance to ZYMV in *C. moschata* is conferred by a single dominant gene, *Zym* (Gilbert-Albertini *et al.*, 1993; Munger & Provvidenti, 1987; Paris *et al.*, 1988). However, the results of segregation in the F_2 and backcross to *C. pepo* Zucchini Group 'True French' (Table 1) cannot be explained by a one-gene or even a two-gene model. The simplest explanation for these results is that three dominant genes confer resistance to ZYMV in this genetic background. One of these genes, herewith designated *Zym-1* and assumed to be identical to the *Zym* of 'Menina' designated in previous reports (Gilbert-Albertini *et al.*, 1993; Paris *et al.*, 1988), must be present for resistance to be expressed. The other two dominant genes, herewith designated *Zym-2* and *Zym-3*, are complementary to *Zym-1*. Only plants carrying *Zym-1* and *Zym-2* and/or *Zym-3* are resistant.

The source of resistance to ZYMV of 381e is *C. moschata* cv. Menina, which is the same source as was used by Gilbert-Albertini *et al.* (1993) and Paris *et al.* (1988). As only one gene, *Zym-1* (*Zym*), had been observed to segregate when 'Menina' was crossed with ZYMV-susceptible *C. moschata*, the additional two genes for resistance, *Zym-2* and *Zym-3*, must have been present in 'Menina' as well as in the ZYMV-susceptible *C. moschata* accessions used in the earlier experiments. Perhaps these other factors are ubiquitous or at least common in that species, but rare or non-existent in *C. pepo*. Thus, the baffling difference among *Cucurbita* species in mode of inheritance of resistance (Kyle, 1995) can be accounted for quite simply. Moreover, it is well-known that *C. moschata* is less susceptible than *C. pepo* to various pathogens, especially viruses. Although *Zym-2* and *Zym-3* do not confer resistance in the absence of *Zym-1*, it is possible that they do contribute to lowering the degree of susceptibility to the virus in *C. moschata*.

If *Zym-2* and *Zym-3* act merely as duplicate factors in a complementary interaction with *Zym-1* for expression of resistance, then it would be highly unlikely that both would have been retained during the six generations of backcrossing resistance into 'True French'. Much more likely, the presence of both *Zym-2* and *Zym-3* is somehow necessary for resistance to be expressed. During the backcrossing of resistance into 'True French', we observed in each of the successive generations that approximately half of the plants that had been considered to be resistant in the growth chamber, at 20-22 days after inoculation, exhibited severe symptoms within 2 wk after transplanting to larger pots in the greenhouse (H S Paris, unpublished observations). Thus, there may be a simple explanation for the retention of both *Zym-2* and *Zym-3* during backcrossing: both are necessary, together with *Zym-1*, for the continued expression of resistance in plants after transplanting and beyond. Three complementary dominant genes have also been identified as conferring resistance to ZYMV in melons, *Cucumis melo* L. (Danin-Poleg *et al.*, 1997). In this cucurbit species, a dominant gene of major effect confers resistance (Pitrat & Lecoq, 1984) but, depending on the cross, additional dominant genes were observed to be necessary for resistance to be expressed (Danin-Poleg *et al.*, 1997).

The resistant accession, 381e, and several F_1 hybrids obtained by crossing it with susceptible zucchini breeding lines were observed and compared under field conditions with 'True French' and susceptible commercial zucchini hybrids (H S Paris, unpublished). Resistance was expressed as milder symptoms on the foliage and a 7-10 day delay in the appearance of symptoms (distortion) on the fruit. Zucchini and other summer squash are usually picked 2-4 times weekly and the harvest period normally is several weeks long. The extension of the harvest period by a week or more, as observed for zucchini hybrids derived from 381e, would therefore be important for insuring profitability for the grower.

The resistance reaction of 381e differed from that of the source of resistance, 'Menina'. Plants of 381e reacted to virus inoculation within four days, faster than even the susceptible 'True French' plants. Plants of 381e exhibited stunting of some of the early leaves but not thereafter, having only mild leaf yellowing and distortion later on. Thus, the salient characteristics of the resistance exhibited by 381e plants was the quick response to inoculation and the recovery from the stunting caused by virus infection. On the other hand, the resistance possessed by 'Menina' did not

involve stunting and then recovery, only tiny yellow spots were exhibited after inoculation. Often the intensity of a desirable characteristic possessed by the donor parent, such as disease resistance, is lost during backcrossing to the recurrent parent (Allard, 1960). This indeed occurred during the backcrossing of ZYMV resistance from 'Menina' to 'True French'. Moreover, backcrossing is not effective in transferring recessive genes. Presumably, the high-type resistance of 'Menina' is based on the genes for resistance, *Zym-1*, *Zym-2*, and *Zym-3*, combined with a recessive gene or genes that raise the level of resistance but that had been lost during backcrossing. As the interspecific F_1 of 'Spookie' × 'Menina', no. S36, had been observed to be as highly resistant to ZYMV as 'Menina', 'Spookie' apparently carries the putative recessive gene or genes necessary for enhanced resistance.

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