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Resistance to potato leaf roll virus multiplication in potato is under major gene control

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Abstract The concentration of potato leaf roll virus (PLRV), as measured by a quantitative enzyme-linked immunosorbent assay, in the foliage of potato plants (*Solanum tuberosum*) of cv 'Maris Piper' with secondary infection was 2900 ng/g leaf, whereas in clones G7445(1) and G7032(5) it was 180 ng/g leaf and 120 ng/g leaf, respectively. To examine the genetic control of resistance to PLRV multiplication, reciprocal crosses were made between the susceptible cultivar 'Maris Piper' and the two resistant clones, and the three parents were selfed. Seedling progenies of these families were grown to generate tubers of individual genotypes (clones). Clonally propagated plants were graft-inoculated, and their daughter tubers were collected and used to grow plants with secondary infection in which PLRV concentration was estimated. The expression of resistance to PLRV multiplication had a bimodal distribution in progenies from crosses between 'Maris Piper' and either resistant clone, and also in progeny from selfing the resistant parents, with genotypes segregating into high and low virus titre groups. Only the progeny obtained from selfing 'Maris Piper' did not segregate, all genotypes being susceptible to PLRV multiplication. The pattern of segregation obtained from these progenies fits more closely with the genetical hypothesis that resistance to PLRV multiplication is controlled by two unlinked dominant complementary genes, both of which are required for resistance, than with the simpler hypothesis that resistance is conferred by a single dominant gene, as published previously.

Key words Potato breeding · Potato leaf roll virus
Virus resistance · Major gene resistance · Genetics

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Introduction

Several types of resistance to potato leaf roll virus (PLRV) can be expressed in potato (*Solanum tuberosum*) clones, such as, resistance to: (1) virus accumulation in infected plants; (2) infection by viruliferous aphids; (3) movement of virus from infected foliage to tubers; (4) aphid vectors (Barker 1987; Wilson and Jones 1992, 1993a). The first type is expressed as a severe restriction of the amount of virus that accumulates in infected plants and has been found in a number of potato clones (Barker and Harrison 1985; Gase et al. 1988; Swiezynski et al. 1988; Wilson and Jones 1993b). Infected plants of potato clones with this type of resistance are poor sources of virus for vector aphids (Barker and Harrison 1986). In field trials, the amount of virus spread from infected plants of such clones is considerably less than that from plants of clones susceptible to multiplication (Barker and Woodford 1992).

The phenotypic trait of resistance to PLRV multiplication has evident advantage in a breeding programme and knowledge of its genetic control will be useful in designing hybridization and screening strategies. In preliminary experiments with tetraploid *S. tuberosum*, Barker and Solomon (1990) examined the inheritance of this trait in seedlings obtained from crossing the PLRV-resistant Scottish Crop Research Institute (SCRI) clone G7445(1) and the susceptible cultivar 'Maris Piper'. Evidence was obtained that virus multiplication may be controlled by a major gene or a group of closely linked genes, although minor genes may also affect virus accumulation. Barker and Solomon (1990) suggested two hypotheses to explain these results. Either the resistant parent, G7445(1), has a dominant resistance gene in a simplex state and the susceptible parent, 'Maris Piper', is homozygous recessive, or 'Maris Piper' has a dominant major gene for susceptibility in a simplex state and G7445(1) is homozygous recessive. In the investigation reported here, we have examined progenies obtained from crosses between 'Maris Piper' and the resistant SCRI clones G7445(1) and G7032(5) and from selfing the parents in an attempt to determine more fully the

inheritance of the trait of resistance to PLRV multiplication.

Materials and methods

Test material

Potato plants were grown in soil-less potting compost in an aphid-proof glasshouse at approximately 20 °C. Test material originated from seedling progenies produced from crosses between cv 'Maris Piper' and either SCRI clones G7445(1) or G7032(5), and selfing pollinations of these three parents. The true seeds produced by these crosses were sown and each seedling plant (genotype) was grown to maturity to produce tubers. One of these virus-free tubers per genotype (numbers of genotypes per progeny is shown in Table 1) was planted to produce shoots for graft inoculation in May/June as described by Barker and Harrison (1985). Four or five plants of the three parent clones were also graft-inoculated. Two PLRV-containing daughter tubers from each inoculated plant were retained and used to grow plants with secondary infection in May/June of the following year. The PLRV concentration in the foliage of these plants was estimated by the enzyme-linked immunosorbent assay (ELISA) as described below.

PLRV detection and assay

PLRV concentrations in tissue extracts were assessed by double-antibody sandwich ELISA using a microcomputer-aided technique described by Barker and Solomon (1990). The concentration of PLRV in the samples was estimated by comparison with known concentrations of purified virus particles. In a previous work (Barker and Solomon 1990), we examined PLRV concentration both in upper leaves (the youngest leaf with a terminal leaflet ca. 40 mm long) and lower leaves (fully expanded leaves approximately halfway up the stem) but found that results from the lower leaves gave the best differentiation between susceptible and resistant genotypes. In the work reported here, only lower leaf samples were used for virus titre estimates which are given as the mean of 3 measurements on pooled samples of leaf lamina taken from the 2 plants of each genotype.

Mixture models

In our preliminary analyses of the PLRV titre in progeny from crosses between resistant and susceptible parents we found that although most genotypes could be assigned to either high or low virus titre groups, the PLRV titre in a few genotypes was intermediate between these two groups. Mixture models were used to describe the data more accurately and to determine the division of the progeny into low and high titre groups. Thus, we describe the resistant genotype response by a $N(\mu_1,\sigma_1^2)$ distribution and the susceptible genotype response by a $N(\mu_2,\sigma_2^2)$ distribution, assuming $\mu_2>\mu_1$ The response of a genotype whose resistance/susceptibility status is unknown is described by $f(x)=p\ f_1(x)+(1-p)\ f_2(x)$, where $f_1(x)$ and $f_2(x)$ denote $N(\mu_1,\sigma_1^2)$ and $N(\mu_2,\sigma_2^2)$ distributions, respectively, and p is the (unknown) probability that a genotype is resistant. This model allows us to estimate simultaneously both p, the probability that a genotype is resistant, and the parameters μ and σ^2. The parameters were estimated by the method of maximum likelihood (Whitaker 1992).

The data obtained from the progeny of each cross were analysed by the mixture model and each genotype was allocated to its most likely group (low or high PLRV titre). The observed frequencies (low:high titre genotypes) were compared with those expected (theoretical) from one of two genetical models: the resistant phenotype is controlled by either a single dominant resistance gene (model 1), or by two unlinked dominant complementary genes, both of which are required for resistance (model 2). Details of these models are given in Table 2. The theoretical segregation ratios assume autotetraploid (tetrasomic) segregation with no disturbance of meiosis and no double reduction (Cadman 1942). The observed and theoretical (expected) frequencies were compared by the χ^2 statistic, although in this case the statistic cannot be compared directly with tabulated χ^2 percentage points as observed frequencies are not known with certainty and therefore the distribution of the test statistic is not exactly that of χ^2. However, large values of the calculated statistic still indicate inconsistency between the observed and theoretical frequencies.

Results

Virus concentration in the parent clones

The mean PLRV titre in 'Maris Piper', G7445(1) and G7032(5) was 2900 ng/g, 180 ng/g and 120 ng/g, respectively (Table 1).

Virus concentration in progeny from selfing crosses

All genotypes from the progeny obtained by selfing 'Maris Piper' had a high PLRV titre with a combined mean that was very close to that of the parent 'Maris Piper' (Fig. 1, Table 1). Assessment by a mixture model of the virus concentration data from the selfing cross of G7445(1) suggested that the progeny contained two groups of genotypes, low and high titre (Fig. 1, Table 1). The progeny from selfing G7032(5) also consisted of low and high-titre genotypes (Fig. 2, Table 1) but the separation into two groups was less clear-cut than for G7445(1). The mean of the low and high titre groups was close to the mean PLRV titre of the respective resistant parents and susceptible 'Maris Piper' clones (Table 1).

Virus concentration in progeny from crosses between resistant and susceptible parents

The progeny from crosses between 'Maris Piper' and the resistant parents G7445(1) and G7032(5) also segregated into two groups of either low or high virus titre genotypes. The mean PLRV titres of these two groups were very similar to those obtained from the respective resistant parents and susceptible 'Maris Piper' clones (Table 1). The means of the high titre groups are remarkably similar among the four crosses. The means of the low titre groups are lower for both selfing crosses than for both crosses of G7445(1) or G7032(5) clones by 'Maris Piper'.

Genetical analysis

Although the genotypes from our crosses do not fall into two discrete phenotypic groups, the distributions in Figs 1 and 2 provide strong evidence for the bimodal nature of the trait of resistance to PLRV multiplication in the progenies. The estimated numbers of genotypes in the high and low titre groups (Table 1) fit best with the genetical hy-

Table 1 Mixture model parameter estimates based on $\log_e$ of PLRV titres in leaves (*NA* not applicable)

	Sample size	Estimated proportion belonging to low titre group (p)	Low titre group		High titre group	
			Mean[a]	SD	Mean[a]	SD
Parent clones						
Maris piper	19	NA	–	–	7.97 (2900)[b]	0.23
G7445(1)	7	NA	5.17 (180)[b]	0.23	–	–
G7032(5)	12	NA	4.70 (120)[b]	0.55	–	–
Crosses						
Female×male						
Maris Piper ×self	21	NA	–	–	7.66 (2100)[b]	0.36
G7445(1)×self	34	0.73	4.72 (110)	0.48	7.51 (1800)	0.45
G7032(5)×self	31	0.47	4.90 (130)	0.78	7.56 (1900)	0.45
G7445(1)×Maris Piper	69	0.62	5.58 (260)	0.75	7.63 (2000)	0.38
Maris Piper×G7445(1)	29	0.69	5.37 (210)	0.53	7.53 (1900)	0.38
Combined reciprocal crosses	98	0.62	5.46 (230)	0.65	7.57 (1900)	0.41
G7032(5)×Maris Piper	62	0.30	5.28 (200)	0.49	7.69 (2200)	0.38
Maris Piper ×G7032(5)	74	0.42	5.89 (360)	0.42	7.98 (2900)	0.30
Combined reciprocal crosses	136	0.39	5.75 (310)	0.63	7.87 (2600)	0.32

[a] Data are means of $\log_e$ of virus titres. Figures in parentheses are untransformed means in ng PLRV/g leaf (fresh wt.)
[b] Means not derived from mixture model

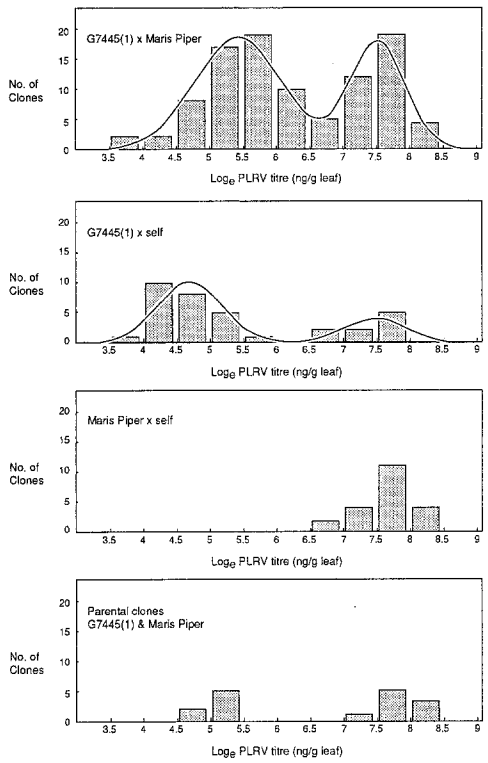

Fig. 1 Histograms and fitted mixture densities (*curves*) of $\log_e$ PLRV titres (ng PLRV/g leaf) of parent clones of G7445(1) and cv 'Maris Piper' and the genotypes from progenies obtained from crosses using these parents. All titre values are the means of three virus concentration estimates

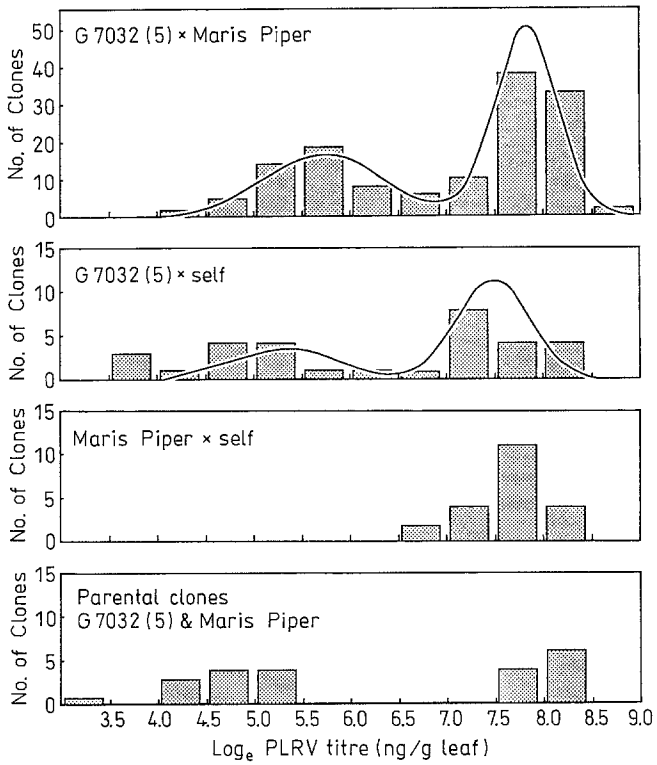

Fig. 2 Histograms and fitted mixture densities (*curves*) of $\log_e$ PLRV titres (ng PLRV/g leaf) of parent clones of G7032(5) and cv 'Maris Piper' and the genotypes from progenies obtained from crosses using these parents. All titre values are the means of three virus concentration estimates

pothesis that restricted PLRV multiplication in G7445(1) and G7032(5) is controlled by two unlinked dominant complementary genes, both of which are required for resistance (model 2 in Table 2). Furthermore, our data suggest that G7445(1) is duplex for one of these genes and simplex for the other, whereas G7032(5) is simplex for both and 'Maris Piper' is simplex for one of them. Theoretical ratios for other genetical models (e.g. Model 1 in Table 2) were also compared with the observed ratios but were found to provide a poorer fit.

Table 2 A comparison of theoretical and observed segregation ratios using two models of inheritance of resistance to PLRV multiplication

Cross Female×male	Genotype	Theoretical ratio of genotypes in titre groups low:high	Observed ratio of genotypes in titre groups low:high	χ^2 [a]
Model 1[b]				
Maris Piper×self	*rrrr*×*rrrr*	0:∞	0:21	–
G7445(1)×self	*Rrrr*×*Rrrr*	3:1	25:9	0.04
G7032(5)×self	*Rrrr*×*Rrrr*	3:1	15:16	11.71
G7445(1)× Maris Piper	*Rrrr*×*rrrr*	1:1	43:26	4.19
Maris Piper ×G7445(1)	*rrrr*×*Rrrr*	1:1	20:9	4.17
Combined reciprocal crosses		1:1	61:37 [d]	5.88
G7032(5)× Maris Piper	*Rrrr*×*rrrr*	1:1	19:43	9.29
Maris Piper × G7032(5)	*rrrr*×*Rrrr*	1:1	31:43	1.95
Combined reciprocal crosses		1:1	53:83 [d]	6.62
Model 2[c]				
Maris Piper ×self	*aaaaBbbb*×*aaaaBbbb*	0:∞	0:21	–
G7445(1)×self	*AAaaBbbb*×*AAaaBbbb*	105:39	25:9	0.01
G7032(5)×self	*AaaaBbbb*×*AaaaBbbb*	9:7	15:16	0.78
G7445(1)× Maris Piper	*AAaaBbbb*×*aaaaBbbb*	15:9	43:26	0.001
Maris Piper × G7445(1)	*aaaaBbbb*×*AAaaBbbb*	15:9	20:9	0.52
Combined reciprocal crosses		15:9	61:37 [d]	0.003
G7032(5)× Maris Piper	*AaaaBbbb*×*aaaaBbbb*	3:5	19:43	1.24
Maris Piper × G7032(5)	*aaaaBbbb*×*AaaaBbbb*	3:5	31:43	0.61
Combined reciprocal crosses		3.5	53:83 [d]	0.13

[a] The upper 5% point of a χ^2 (1 df) is 3.84
[b] Model 1: resistance determined by a single dominant gene *R*
[c] Model 2: resistance determined by unlinked dominant complementary genes *A* and *B*
[d] Two/three genotypes were classified differently by the mixture model when the reciprocal crosses were combined

Discussion

In most studies on the inheritance of virus resistance, the classification of genotypes into resistant or susceptible groups is made on the basis of a readily recognized qualitative response, e.g. the appearance of necrotic lesions on inoculated leaves or a hypersensitive reaction. Such a response does not occur when plants become infected with PLRV because infection does not induce a severe necrotic pathogenic response and the expression of resistance to viral multiplication is quantitative (Barker and Harrison 1985). Despite the difficulty in analysing the quantitative trait of resistance to PLRV multiplication, our data provide good evidence that in clones G7445(1) and G7032(5) resistance is controlled by two unlinked dominant complementary genes, both of which are required for resistance (model 2). A simpler genetical explanation would be that there is a single major gene with dominance for resistance (model 1), although in this case the observed segregation ratios do not fit well with the theoretical ratios (Table 2). In a previous work (Barker and Solomon 1990) that was much more limited in scope than the present study, the combined segregation ratio obtained from reciprocal crosses between 'Maris Piper' and G7445(1) gave a better fit to model 1. However, only 40 genotypes were assessed in comparison with 98 from the crosses between 'Maris Piper' and G7445(1) reported here. Furthermore, in our previous work (Barker and Solomon 1990) we may have selected a somewhat biased population because there were reciprocal differences between the two crosses, whereas these did not occur in the present study. The verification of the correct genetical model will require further substantial and long-term tests, which are now in progress. For example, backcrosses to 'Maris Piper' of susceptible genotypes, from progeny of resistant clones G7445(1) and G7032(5) should result in the appearance of low titre genotypes in some progeny if resistance is controlled by two unlinked dominant complementary genes but not if resistance is controlled by a single dominant gene. There are many cases of single dominant major genes that control resistance to viruses in potato (Cockerham 1970; Ross 1986). Reports of virus resistance controlled by complementary genes are much less common, but Swiezynski et al. (1990) reported that one possible explanation of their studies on the inheritance of resistance to PLRV in diploid potatoes was that more than two genes were responsible for resistance.

Provisionally, we propose that these major genes for resistance to PLRV should be called *Rl* with the nomenclature Rl_1 and Rl_2 if it is confirmed that resistance is controlled by a complementary pair of dominant genes, both of which are required for resistance. There are other potato clones with levels of resistance to PLRV multiplication that are similar to those in G7445(1) and G7032(5) (Barker and Harrison 1985; Solomon-Blackburn and

Barker 1993). It is possible that many of these other clones will also contain the same or similar gene(s). Indeed, preliminary evidence (H. Barker and R.M. Solomon-Blackburn, unpublished results) suggests that PLRV multiplication resistance in cvs 'Pentland Crown' and 'Kingston' (a selection from the cross 'Pentland Crown'×'Maris Piper') may also be under major gene control.

It is noticeable that within the high and low titre groups from the crosses there was a range of response to PLRV multiplication (Figs. 1 and 2). This is likely to be caused, at least in part, by environmental variation but may also have a genetical basis. For example, it is probable that minor genes present in resistant and susceptible parents also affect virus accumulation, and varying numbers of these will be inherited by genotypes in the progenies. It is also relevant in this context that a proportion of the genotypes from the crosses (particularly the selfs) will contain more than one copy of the major genes and PLRV multiplication may be affected by gene dose. Indeed, it is noteworthy that the means of the low titre groups are lower for both selfing crosses than for both crosses of G7445(1) or G7032(5) clones by 'Maris Piper' (Table 1).

Potato contains several major resistance genes, such as the *Rx* and *Ry* genes that give resistance to potato virus X and potato virus Y, respectively. The provenance of these genes is often well known (Cockerham 1970). However, the source of resistance to PLRV is less well known, probably because several wild potato species have been used during the SCRI breeding programme (Davidson 1980). Another source of resistance to PLRV multiplication has been found in *Solanum brevidens* (Jones 1979). Transfer of this resistance from *S. brevidens* into a *S. tuberosum* background by somatic hybridization has been reported (Austin et al. 1985; Pehu et al. 1990). Pehu et al. (1990) observed that of their somatic hybrids, those with a morphology resembling *S. brevidens* were the more resistant. Such hybrids are likely to require a considerable programme of backcrossing to eliminate undesirable genes. The germ plasm sources we have used are conventional tetraploid *S. tuberosum* clones. The advantage of the major-gene resistance to PLRV multiplication that we have identified in *S. tuberosum*, is that the transfer of this trait in a breeding programme should be much easier than with sources of resistance from wild *Solanum* species such as *S. brevidens*. Furthermore, in SCRI germ plasm, the effectiveness of resistance to PLRV multiplication in greatly diminishing virus spread has been established in field tests (Barker and Woodford 1992). The combination of gene(s) *Rl* with other forms of resistance, such as resistance to infection with vector aphids (Solomon-Blackburn and Barker 1993) will produce germ plasm with enhanced resistance. Different forms of resistance may prove to be more durable in combination than when one form alone is used. We think that conventional breeding programmes using gene(s) *Rl* in clones of *S. tuberosum* are more likely to facilitate the rapid production of PLRV-resistant cultivars than using sources of resistance from wild species.

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