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Evidence of simple genetic control in potato of ability to restrict potato leaf roll virus concentration in leaves

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Summary. The concentration of potato leaf roll virus (PLRV), measured by quantitative enzyme-linked immunosorbent assay, in foliage of plants of cv Maris Piper and clone G7445(1) with secondary infection was 2,700 ng/g leaf and 120 ng/g leaf, respectively. In experiments to examine the genetic control of their ability to restrict the multiplication of PLRV, reciprocal crosses were made between these two clones. Among 40 genotypes from the progeny of the crosses, about half had a low PLRV concentration in plants with secondary infection and the other half had a high concentration. The possibility of monogenic control of the character that restricts PLRV multiplication in such clones of *Solanum tuberosum* is discussed.

Key words: Potato breeding – Potato leaf roll virus – Virus resistance – Enzyme-linked immunosorbent assay

Introduction

Some potato cultivars react to infection with potato leafroll virus (PLRV) by developing systemic necrosis, which is controlled by a dominant major gene and modifying minor genes (Ross 1986). However, the kinds of resistance to PLRV that are used most frequently in breeding programs are not believed to be controlled by major genes. Cockerham (1945) concluded that several genes control resistance and Ross (1958) described the genetic system as polygenic, a view which was supported by Dziewońska and Pochitonow (1971), Davidson (1973) and Hamann and Möller (1979). This type of resistance to PLRV is expressed as a quantitative resistance to infection that is difficult to measure, although resistance ratings of cultivars can be derived from the results of field exposure trials Davidson 1973). Selection for a polygenically controlled character in a breeding program is difficult and the pattern of inheritance of all characters in *Solanum tuberosum* is further complicated because it is a tetraploid species. Improved techniques to assess and screen for resistance to PLRV and to study the inheritance of resistance are therefore highly desirable.

In recent work, a more precise analysis of resistance to PLRV was attempted and three components of resistance, which can be found in some clones and cultivars, were identified (Barker and Harrison 1985, 1986; Barker 1987). These components are: (1) restriction of virus multiplication, (2) resistance to infection and (3) inhibition of virus movement from foliage to tubers. Of these components the first, which is expressed as a severe restriction on the amount of virus that accumulates in infected plants (Barker and Harrison 1985), may be the most important because infected plants of potato clones with this type of resistance are poor sources of virus for vector aphids (Barker and Harrison 1986). If breeding programs could systematically incorporate resistance to virus multiplication, the products would be cultivars in which the spread of PLRV is greatly minimised. This type of resistance is well expressed in infected plants of clone G7445(1), in which the concentration of PLRV was found to be 60 ng/g leaf compared with 1,200 ng/g leaf in cv Maris Piper (Barker and Harrison 1985). The work reported in this paper has made a preliminary assessment of the nature of inheritance of the ability to restrict PLRV multiplication by seedlings (genotypes) obtained by crossing the resistant clone G7445(1) and the susceptible cultivar Maris Piper.

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Table 1. Concentration of PLRV in leaves of genotypes and parents from cross G7445(1) (female) × Maris Piper (male)

Source of leaves		Upper leaves		Lower leaves	
		Mean PLRV concentration (ng/g leaf)	Mean $\log_e$ of PLRV concentration	Mean PLRV concentration (ng/g leaf)	Mean $\log_e$ of PLRV concentration
Genotypes[a]					
1	(L)	44	3.48	44	3.73
21	(H)	430	5.97	3,000	7.88
24	(H)	810	6.63	2,100	7.61
29	(H)	550	6.21	2,300	7.72
35	(H)	560	6.08	2,200	7.68
44	(H)	990	6.86	2,200	7.63
57	(L)	140	4.84	200	5.29
71	(H)	660	6.15	1,500	7.25
73	(L)	67	3.15	59	3.91
78	(H)	810	6.66	1,700	7.34
89	(L)	67	4.05	70	4.22
100	(L)	89	4.32	130	4.74
101	(H)	800	6.52	2,700	7.83
113	(H)	750	6.55	1,100	6.97
118	(H)	1,400	7.11	1,600	7.37
125	(H)	1,700	7.35	1,900	7.54
126	(L)	180	5.06	98	4.58
Parents[a]					
M Piper	(H)	2,200	7.65	2,600	7.82
M Piper	(H)	1,400	7.22	2,000	7.59
G7445(1)	(L)	65	3.95	140	4.88
G7445(1)	(L)	71	3.99	150	4.99
LSD (5%)		410	0.85	950	0.61

[a] Letter in parentheses in this column represents the arbitrary status of virus concentration (H = high, L = low) based on measurements in lower leaves. Log_e of virus concentration of genotypes in low group: lower leaves 3.73–5.29, upper leaves 3.48–5.06; in high group: lower leaves 6.97–7.88, upper leaves 5.97–7.35

Materials and methods

Plant material and virus inoculation

Potato plants were grown in soilless potting compost in an aphid-proof glasshouse kept at about 20 °C. Supplementary illumination (16 h/day) was provided in winter months by high-pressure mercury vapour lamps. Test material was made up of 40 genotypes of two seedling progenies produced from reciprocal crosses between cv Maris Piper and clone G7445(1). The true seeds produced by these crosses were sown and each seedling plant (genotype) was grown to produce tubers. Plants grown from a single tuber of each genotype and from two tubers of each parent were tested.

Virus-free plants to be tested were graft-inoculated with scions from PLRV-infected plants of cv Maris Piper, as described by Barker and Harrison (1985). Two PLRV-containing daughter tubers from each inoculated plant were retained and used to grow plants with secondary infection. The PLRV concentration in the foliage of these plants was estimated by enzyme-linked immunosorbent assay (ELISA).

PLRV detection and assay

PLRV concentration in tissue extracts was estimated by a microcomputer-aided ELISA technique that can make accurate measurements of PLRV concentration in large numbers of samples (up to 100/day), essentially as described by Barker and Harrison (1985). The technique uses randomisation of sample position in the microtitre plates and other procedures to improve the accuracy of measurement of virus concentration. Wells of microtitre plates (Nunc Immuno Plate 1, Gibco) were coated with PLRV-G (Tamada and Harrison 1980) antiserum γ-globulin at 1 μg/ml. Leaf samples of either the youngest leaves, with a terminal leaflet ca. 4 cm long (upper leaf), or fully expanded leaves approximately halfway up the stem (lower leaf) were disrupted in a Pollahne roller press (1 g leaf/25 ml buffer). Samples of each diluted tissue extract were tested in three wells, and other wells in each test contained a range of known concentrations of purified PLRV particles (6 wells per concentration). Samples were kept in the wells overnight at 4 °C. Phosphatase-conjugated antiserum γ-globulin (1/1000 dilution) was allowed to react for 4 h at 37 °C. Substrate was allowed to react for up to 3 h at 20 °C and then for about 16 h at 4 °C. Absorbance ($A_{405\,nm}$) values were determined by a Titertek Multiskan photometer (Flow Laboratories). Measurements made after 3 h incubation with substrate were used for estimating virus concentration in samples with large PLRV contents, and measurements made after a further 16-h incubation were used for estimations on samples with low PLRV contents. The microcomputer program (copyright SCRI) was used to generate a randomised design for samples in the microtitre plate wells and aid the placement of samples in wells. Absorbance data from the Titertek Multiskan

Table 2. Concentration of PLRV in leaves of genotypes and parents from cross Maris Piper (female) × G7445(1) (male)

Source of leaves	Upper leaves		Lower leaves	
	Mean PLRV concentration (ng/g leaf)	Mean $\log_e$ of PLRV concentration	Mean PLRV concentration (ng/g leaf)	Mean $\log_e$ of PLRV concentration
Genotypes[a]				
3 (L)	36	3.57	160	5.05
6 (L)	87	4.46	160	5.06
9 (H)	590	6.36	2,500	7.82
17 (L)	130	4.82	120	4.70
23 (H)	380	5.84	1,000	6.86
27 (H)	1,300	7.15	2,300	7.70
30 (H)	810	6.61	2,300	7.69
33 (L)	84	4.36	150	4.97
43 (H)	1,000	6.87	1,900	7.50
46 (L)	210	5.33	260	5.51
50 (L)	81	4.37	120	4.82
51 (L)	250	5.44	260	5.42
56 (L)	160	5.05	120	4.76
60 (L)	160	5.00	270	5.58
65 (L)	170	5.10	130	4.87
81 (L)	390	5.94	360	5.86
86 (H)	280	5.60	2,100	7.67
92 (L)	150	4.95	220	5.30
97 (L)	280	5.62	140	4.86
103 (L)	150	4.97	94	4.54
108 (L)	45	3.76	180	5.11
116 (H)	200	5.24	1,700	7.37
122 (H)	350	5.80	2,200	7.65
Parents[a]				
M Piper (H)	1,200	7.02	2,600	7.84
M Piper (H)	1,200	7.07	3,500	8.15
G7445(1) (L)	70	4.25	85	4.44
G7445(1) (L)	66	4.12	91	4.47
LSD (5%)	310	0.57	630	0.55

[a] Letter in parentheses in this column represents the arbitrary status of virus concentration (H = high, L = low) based on measurements in lower leaves. Log_e of virus concentration of genotypes in low group: lower leaves 4.54–5.86, upper leaves 3.57–5.94; in high group: lower leaves 6.86–7.82, upper leaves 5.24–7.15

was received directly by the microcomputer and processed to derive the virus concentration in ng/g of leaf. This included calculation of the mean A_{405} values from samples, producing a standard curve from absorbance values given by known concentrations of purified virus and interpolation of absorbance values of samples onto the standard curve to derive virus concentration.

Results

Virus concentration in 40 genotypes from the progenies of reciprocal crosses between Maris Piper and G7445(1) was estimated in extracts of both upper and lower leaves on several occasions (between 3 and 5) during the growth of the plants. A preliminary, and subjective, assessment of the data suggested that the variation in virus concentration of the genotypes was of a discontinuous rather than a continuous nature. The genotypes appeared to form two distinct groups, with either low or high virus titre on the basis of measurements on extracts of lower leaves (Tables 1 and 2). An analysis of variance, using a natural log transformation of the data, showed that these two groups were significantly different from each other. This is consistent with the strong suggestion, by examination of the distribution of the data (Fig. 1 b), that there are two distinct groups. The $\log_e$ of the virus titre in the genotypes in the low-titre group ranged from 3.73 to 5.86 and from 6.86 to 7.88 in the genotypes of the high-titre group. However, differences between these two groups were much less obvious when measurements made on extracts of upper leaves were considered (Fig. 1 a, Tables 1 and 2).

The mean $\log_e$ of the virus concentration in the upper and lower leaves of genotypes from both progenies and

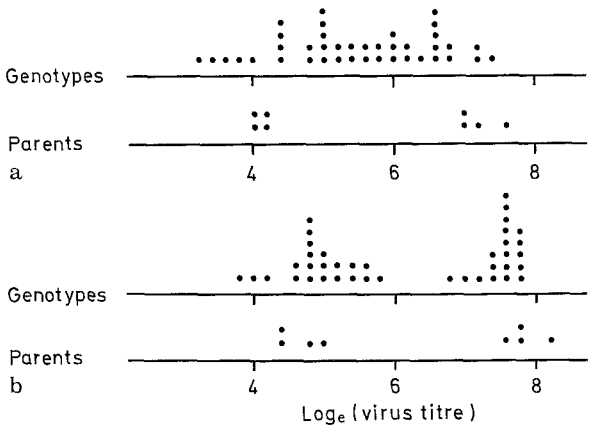

Fig. 1 a and b. Histogram of mean Log$_e$ of virus titre measurements in leaves of parents Maris Piper and G7445(1) and 40 genotypes from reciprocal crosses between them: measurements from upper leaves (**a**) and from lower leaves (**b**). Each dot represents a different genotype or parental plant

Table 3. Partitioning the genotypes of progenies from crosses between Maris Piper and G7445(1) into high and low virus titre groups

Cross		Virus concentration of genotypes	
Female parent	Male parent	High titre	Low titre
Maris Piper	× G7445(1)	8	15
G7445(1)	× Maris Piper	11	6
Total		19	21

from their parents is presented as a histogram (Fig. 1). The data from measurements on lower leaves of the genotypes appears to have a distinct bimodal distribution. The mean virus concentration of all the low-titre genotypes (based on measurements in lower leaves) is 159 ng/g leaf (SE ± 17) and 2,008 ng/g leaf (SE ± 116) in high titre genotypes in comparison to 116 ng/g leaf (SE ± 16) and 2,664 ng/g leaf (SE ± 320) for parents G7445(1) and Maris Piper, respectively.

When the results from both crosses were combined, there are 19 genotypes with high virus concentration and 21 with low virus concentration (Table 3). If there is a major gene in potato that controls multiplication of PLRV, there are several possible theoretical distributions of the two phenotypic groups within the progenies, depending on whether the gene is present in a simplex, duplex, triplex or quadruplex state (Williams 1964). Our observed ratio of 19:21 is closest to a theoretical 1:1 ratio, which would occur if a major gene was present in a simplex state. Assuming that a 1:1 ratio is correct, a χ^2 test on our data gives a $P = 0.9-0.5$, implying a very close fit with this hypothesis.

Discussion

The results of our tests with progenies from crosses between parents Maris Piper and G7445(1) indicate that the genotypes can be tentatively assigned to two phenotypic classes based on virus content of lower leaves. It is not clear why virus content measurements in upper leaves provide a less certain distinction between these two phenotypic classes. However, the lower leaves used for our tests appeared to be representative of the majority of leaves on the plant and are probably the most useful material for tests on the inheritance of the ability to restrict virus replication. The division of genotypes into two phenotypic groups of equal size suggests that virus multiplication is likely to be controlled by a major gene or a group of closely linked genes. However, although a major gene may be operating, this may not account for all the observed variation, and it is probable that minor genes also affect virus accumulation. Indeed, the tendency of the mean virus content of the two phenotypic classes not to match exactly that of the parents, and the lack of a distinct bimodal distribution of measurements in upper leaves (Fig. 1 a) are two features that may be influenced by minor genes. It is also possible that nonheritable variance is greater in upper than in lower leaves, and that it may cause differences in distribution of the data.

There are two main alternative explanations of the 1:1 segregation ratio for the two phenotypic groups. 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. Further tests will be required to determine whether either of these possibilities is correct and to find whether this type of resistance is inherited similarly in other clones. Barker and Harrison (1986) suggested that decreased PLRV accumulation in infected plants of particular cultivars may occur because of the inhibition of virus spread within the phloem system. Results of the work reported here suggest that intercellular spread of PLRV is under simple genetic control by the host and, therefore, that one or a few host proteins play a crucial role in the processes involved in spread.

Resistance to PLRV multiplication is reported to occur in several wild non-tuber-bearing diploid *Solanum* species such as *S. brevidens* and *S. etuberosum* of the *Etuberosa* series (Jones 1979; Rizvi 1983). Such resistance in *S. etuberosum* has been transferred into the tuber-bearing *Solanum* species, *S. acaule,* by bridging crosses with *S. pinnatisectum* and embryo rescue (Chavez et al. 1988). Segregation of resistance to PLRV multiplication in selfed progeny of the hybrids produced by Chavez et al. (1988) suggests that this character is controlled oligogenically. Our studies suggest that similar forms of resistance

to PLRV also occur frequently in *S. tuberosum* and, like that in *S. etuberosum,* are under simple genetic control. It should be possible to breed new cultivars containing this form of resistance more rapidly than by utilising sources of resistance from wild species.

It is well known that the phenotypic resistance of potato clones to PLRV, as measured in field exposure trials, varies continuously (Ross 1958; Davidson 1973; our unpublished data). The established view is that the quantitative nature of such resistance results from a combination of heritable variance and nonheritable environmental variance, and that control of the heritable component is polygenic (Ross 1958). However, Vanderplank (1984) has suggested, somewhat controversially, that resistance used by plant breeders is seldom, if ever, polygenic and that continuously variable phenotypes can accompany monogenic resistance if nonheritable variance exceeds heritable variance. In our experiments we have examined one component of resistance to PLRV in *S. tuberosum* that may be under major gene control. There are other components of virus resistance (Barker 1987) whose inheritance has yet to be determined. In the light of our present results we cannot be certain that resistance to PLRV is controlled polygenically. We think that more closely controlled experiments, such as are reported here, should be done to ascertain whether control of the other components of resistance to PLRV is also oligogenic. Nevertheless, the complete plattern of inheritance of the several components of resistance to PLRV, and of resistance to vector aphids, is likely to be complex and difficult to analyse. Irrespective of whether resistance to PLRV is inherited polygenically or oligogenically, it may be profitable for breeders to concentrate more effort on those components of resistance to PLRV that are controlled in a simple manner.

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