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Study of Resistance in Potato Clone G8107(1) to *Potato leafroll virus* Infection

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Abstract: The potato clone G8107(1) has been identified as resistant to both PLRV infection and multiplication. In this study, attempts were made to reveal the mechanism(s) involved in the resistance of this clone to PLRV infection. Considering the existence of a rigid cell wall as a passive resistance barrier or mature-plant resistance as barrier to virus infection, experiments were conducted in which softer tissues of this potato clone (sprouts and *in vitro* propagated plantlets) were aphid inoculated with PLRV. The results indicated that aphid inoculation of both tissue types of this potato clone with PLRV did not lead in breakdown of its resistance to virus infection. Therefore, it was concluded that the resistance of this clone to PLRV infection *via* aphid inoculation is not associated with the existence of a rigid cell wall or mature-plant resistance. It is suggested that the observed resistance might be due to the PLRV replication failure in the tissues of this potato clone, or inability of the virus to transport from the initially infected cells and or, somehow, degradation of the virus particles after replication. However, it seems that this very strong resistant potato clone is an excellent choice to be exploited in PLRV resistance breeding programs.

Key words: Mechanism · Resistance · PLRV · Aphid inoculation · Potato clone G8107(1)

INTRODUCTION

Potato leafroll disease, caused by *Potato leafroll virus* (PLRV), is the most widespread and economically important viral disease of potato and occurs in most places where potatoes are grown [1]. Infection with *Potato leafroll virus* can cause losses of both total and marketable yield of potato. It has been considered that the yield loss due to infection with this virus to range from 10% in the most tolerant cultivars to about 95% in the most sensitive ones [2]. Crop yield losses attributed to PLRV worldwide have been estimated at 20 million tons per year [3]. The annual value of yield loss resulting from infection with PLRV has been estimated up to £3-5x10^7 [4]. An additional problem caused by PLRV infection, is that *Potato spindle tuber viroid* (PSTVd) which normally is not aphid-transmissible, can be transmitted by aphids from plants co-infected with PLRV and PSTVd [5]. Like other viruses, the most economic and environmentally safest strategy in controlling PLRV is the use of resistant potato genotypes [6-9].

The clone G8107(1), a potato genotype that has been obtained from the JHRI* potato breeding program, was identified as resistant to PLRV (resistant to both infection and multiplication) by Solomon-Blackburn and Barker [7]. They found that this clone was highly resistant to PLRV infection in three field exposure trials in which resistance of 12 potato clones to PLRV infection compared to the standard cultivars Maris Piper (susceptible) and Pentland Crown (resistant) was evaluated and no plants of G8107(1) became infected. Moreover, it was found that this clone was the most resistant potato genotype to PLRV multiplication in the experiments in which the glasshouse-grown plants of the same potato breeding lines and cultivars were graft-inoculated and their resistance to virus accumulation was assessed by quantitative ELISA [7].

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The potato clone G8107(1) can be infected with PLRV following graft inoculation, but is completely resistant following aphid inoculation in field conditions. However, once plants become infected by graft inoculation, the level of virus accumulation (particularly in leaves) is very low [7]. Barker and Woodford [10] showed that the spread of PLRV within potato crops is significantly limited from clones in which the virus accumulation is restricted, irrespective of their degree of resistance to infection. They reported that the spread of PLRV from infected plants of the resistant clone G8107(1) was approximately 40-fold less than from infected plants of the susceptible cv. Maris Piper which contained 20-fold more virus than plants of G8107(1) [10]. This kind of resistance (resistance to virus accumulation) is very useful in situations where most PLRV inoculum originates from sources within the crops. In environments in which the PLRV inoculum pressure is higher and those in which substantial spread may also occur from sources outside the crops, a combination of the two types of resistance (resistance to both virus infection and accumulation) is of great importance. For example, in Scotland in 1976, where there was a considerable PLRV spread in seed growing areas, a very small proportion of the acreage (1.5%) of the cv. Pentland Crown that has both these types of resistance, was rejected from the Scottish Seed Potato Classification Scheme, whereas 24.4% of Maris Piper crops were rejected for virus infection [11]. In the expectation that greater knowledge of the underlying resistance mechanism(s) would lead to improved breeding strategies, we attempted to study the resistance mechanism(s) of the potato clone G8107(1) to PLRV infection by trying to overcome its resistance. The hypothesis arose from the fact that this potato clone becomes infected with PLRV following graft inoculation but it is completely resistant to infection when inoculated by viruliferous aphids [7]. Therefore, somehow the introduction of the virus by aphid vectors into the plant is prevented.

Considering the existence of natural passive resistance to plant viruses like the presence of a rigid cell wall [12] and the fact that the older potato plants do not become infected by PLRV as easily as young plants [13, 14], the tissues of the mature plants were considered to act as a barrier to aphid introduction of the virus and be responsible for the resistance to infection. This could be tested by breaking the resistance through aphid inoculation of very young tissues. Thus, experiments were designed in which the softer tissues of this potato clone rather than mature plants would be inoculated with viruliferous aphids. Here the results of these experiments are reported.

MATERIALS AND METHODS

The Virus Isolate and Aphid Vector: The PLRV isolate used for inoculation was PLRV-C, a standard culture maintained in potato cv. Maris Piper at JHRI. Inoculation was by aphids. The aphids used were *M. persicae* genotypes J or C [15]. The genotype J was used for most tests and latterly genotype C which in recent virus transmission experiments proved to be more efficient vector of PLRV than genotype J [16] was also employed. Viruliferous aphids were obtained from the aphid cultures raised on PLRV-infected potato plants of cv. Maris Piper or *Physalis floridana*. The aphid cultures were maintained on plants kept at 18°C with a 16 h photoperiod in sealed cages with controlled conditions.

Plant Culture for Glasshouse Experiments: Seed tubers of potato clone G8107(1) (resistant to PLRV infection) and different potato cultivars including: Cara, Saturna and Maris Piper (susceptible to PLRV infection) were allowed to produce sprouts. All sprouts emerged on the tubers, except one of about 5-7 cm long, were removed and the remaining single sprout on each tuber was used for aphid inoculation. *In vitro* propagated plants were maintained and multiplied using standard tissue culture techniques on Murashige & Skoog basal medium [17] with sucrose (3%, w/v). Cultures were grown at 20 ± 2°C with a 1000 lux photoperiod of 16 h.

ELISA Test: For detecting PLRV antigens in test plants, the triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) method [18] was used. Microtitre plates (Nunc Immuno plate Maxisorb, Denmark) were coated with 100 µl per well of PLRV-G polyclonal antibody [19] at 1 µg ml^{-1} in coating buffer (0.015 M sodium bi-carbonate, 0.035 M sodium carbonate, pH 9.6) and incubated at 37°C for 3 h. Antigens were extracted in extraction buffer (2% PVP in PBS-T) at the ratio of 1:10 leaf tissue in extraction buffer and added to the wells in 100 µl aliquots. The plates were then incubated at 4°C overnight. The PLRV monoclonal antibody (SCR3) [20] was added to the wells (100 µl per well) at 1 µg ml^{-1} in PBS-T containing 2% non-fat dried milk, incubated at 37°C for 3 h. Alkaline phosphatase conjugated anti-mouse antibody (Sigma Chemical Co, Cat no: A5153) was added to the wells (100 µl per well) at the ratio of 1: 2000 in PBS-T containing 2% non-fat dried milk and incubated at 37°C for 2 h. The substrate, 4-nitrophenyl phosphate (Roche) was added at the ratio of 1 mg ml^{-1} in substrate buffer (10% v/v diethanolamine pH 9.6) in 100 µl aliquots and incubated at 37°C for 1 h.

Plates were washed three times for 3 min with PBS-T between each of the above steps. After 1 h incubation at 37°C, the absorbance at 405 nm (A_{405}) was measured with a Titertek Multiskan Plus MK[22] colorimeter (Flow laboratories). The plates were then left at 4°C over night and the absorbance measurement repeated the following day. Samples in which the average absorbance value was at least twice that of the negative control were regarded as infected.

Aphid Inoculation of Potato Sprouted Tubers with PLRV: Nine sprouted tubers of the clone G8107(1) were each inoculated with 25 viruliferous aphids and two groups of three tubers were each inoculated with either 50 or 100 aphids per tuber. Five sprouted tubers from each of the potato cvs Cara, Saturna and Maris Piper were also aphid inoculated (25 aphids per tuber). The fourth instar nymphs or young adults from the aphid colonies formed on the infected plants were used for inoculation tests. The acquisition access period (AAP) was 7 days or more at 18°C with 16 h photoperiod. The inoculation access period (IAP) was 7 days with the same temperature and light conditions as for the AAP. During the first three days of the IAP, some extra aphids were added to replace those that had walked off. One week after inoculation the aphids were killed by nicotine fumigation. Each inoculated tuber was then planted in a pot containing soil-less potting compost in an aphid-proof glasshouse with temperature of about 20°C. The plants grown from these tubers were tested three times post-inoculation by ELISA test for detecting primary infection with PLRV. The first test was done 30 days after inoculation and the second and third each 10 days after the previous test. The daughter tubers produced by sprout inoculated plants were collected and stored at 4°C. Seven months later the harvested tubers were planted and tested for detecting secondary infection with PLRV.

Aphid Inoculation of the *In Vitro* Propagated Potato Plantlets with PLRV: The *in vitro* propagated plantlets (7-10 cm tall) of the potato cv. Maris Piper and clone G8107(1) were transferred into small plastic pots containing insecticide-free soil-less potting compost and kept in a humid plant propagator. After 24 h, plantlets were inoculated with viruliferous aphids raised on PLRV-infected potato or *Physalis floridana* plants. A group of 5 plantlets of clone G8107(1) were each inoculated with 5 viruliferous aphids and a group of 4 plantlets were each inoculated with 15 viruliferous aphids. Individuals of a group of 2 and a group of 5 plantlets of cv. Maris Piper were each inoculated with 5 and 15 viruliferous aphids, respectively. Three days after inoculation, the aphids were killed by spraying with insecticide Pirimicarb (0.5 g $Litre^{-1}$). The plants were then tested by ELISA for detecting PLRV, three times after inoculation at intervals of about three weeks.

Inoculation of Potato Plant Materials with PLRV Using Genotype C of Scottish *M. Persicae*: As mentioned, in the previous tests the aphid genotype used was the standard laboratory genotype of *M. persicae* (genotype J) that has been used for many years at JHRI for virus transmission tests. However, in recent studies it was found that other genotypes of *M. persicae* are more efficient vectors of PLRV [16]. One of the most effective vector genotypes was genotype C which infected approximately 3 times more receptor plants than the standard genotype (genotype J) in tests in which single viruliferous aphids were used as sources for transmission. This genotype was selected for another series of aphid inoculation tests. Two groups of three sprouted tubers of clone G8107(1) were each inoculated with 20 or 50 viruliferous aphids. Groups of three *in vitro* propagated plantlets of this clone were each inoculated with groups of either five or 25 aphids per plantlet. Also, as a control three sprouted tubers and two plantlets of cv. Maris Piper were each inoculated with 20 aphids. The resultant plants were then tested by ELISA for detecting PLRV, 30 days after inoculation.

RESULTS AND DISCUSSION

The results of the study on aphid inoculation of sprouted tubers of G8107(1) to examine the resistance to PLRV infection are presented in Table 1. No PLRV infected plants developed from aphid inoculated sprouted tubers of clone G8107(1), whereas most of the plants grown from the tubers of control cvs Saturna, Cara and Maris Piper treated with the same way became infected with PLRV. Of 63 plants grown from the daughter tubers harvested from the sprout inoculated mother plants of clone G8107(1), only three were secondarily infected with PLRV. These originated from one mother plant that grew from a tuber that had been inoculated with 25 aphids. Almost all plants (14 of 17) grown from the daughter tubers harvested from the sprout inoculated susceptible cultivars were infected with PLRV.

The results of the resistance study using *in vitro* propagated plantlets of clone G8107(1) to aphid inoculation, are presented in Table 2. None of the aphid

Table 1: PLRV infection[a] in plants grown from aphid inoculated sprouted tubers

		Primary infection		Secondary infection	
Potato genotype	Number of aphids[b] used for inoculation	Number of inoculated plants	Number of infected plants	Number of progeny plants tested	Number of progeny plants infected
G8107(1)	25	9	0	21	3[c]
G8107(1)	50	3	0	21	0
G8107(1)	100	3	0	21	0
Maris Piper	25	8	6	14	12
Saturna	25	5	3	3	2
Cara	25	4	4	-	-

[a]Infection determined by ELISA

[b]Inoculation was done using genotype J of Scottish *M. persicae*

[c]These originated from one mother plant that grew from a tuber that had been inoculated with 25 aphids

-Not tested

Table 2: PLRV infection[a] in aphid-inoculated *in vitro* propagated potato plantlets

Potato genotype	Number of aphids[b] used for inoculation	Number of inoculated plants	Number of infected plants
G8107(1)	5	5	0
G8107(1)	15	4	0
Maris Piper	5	3	1
Maris Piper	15	5	5

[a]Infection determined by ELISA

[b]Inoculation was done using genotype J of Scottish *M. persicae*

Table 3: PLRV infection[a] in aphid-inoculated *in vitro* propagated plantlets or plants grown from aphid inoculated sprouted tubers using genotype C of Scottish *M. persicae*

Type of inoculated tissue and potato genotype	Number of Ap aphids used for Inoculation	Number of inoculated plants	Number of infected plants
S-t[b] G8107(1)	20	3	0
S-t G8107(1)	50	3	0
S-t Maris Piper	20	3	2
I-P[c] G8107(1)	5	3	0
I-P G8107(1)	25	3	0
I-P Maris Piper	20	2	2

[a]Infection determined by ELISA

[b]Sprouted-tuber

[c]*In vitro* propagated plantlet

inoculated plantlets of clone G8107(1) became infected with PLRV, whereas most of the plantlets of control cv. Maris Piper treated with the same way were infected with PLRV (six out of eight became infected).

The resistance of potato clone G8107(1) to PLRV infection persisted, even when genotype C of Scottish *M. persicae,* a more efficient vector genotype than the JHRI standard genotype J, was used to transmit the virus (Table 3). Four of five similarly treated tubers or plantlets of the control cv. Maris Piper became infected (Table 3).

Severe phloem necrosis associated with PLRV infection in potato cvs Bismark [21] and Apta [22] have been reported and it has been suggested that this might be a resistance mechanism [23].

In contrast, in many studies, the clone G8107(1) did not develop phloem necrosis when infected with PLRV[24]. Unlike G8107(1), cv. Bismark is not resistant to PLRV accumulation [23]. In G8107(1) and other JHRI clones with resistance to PLRV accumulation, PLRV is restricted largely to internal phloem bundles, whereas in susceptible clones it also occurs in external phloem bundles [25]. Potato clone M62759, like G8107(1), has resistance to PLRV infection and accumulation and, unlike G8107(1), it also, has resistance to infection by grafting [26]. Syller [26] reported strong inhibition of PLRV translocation to the tubers of M62759, whether plants were inoculated by grafting or by aphids. Syller [26] deduced that resistance to long-distance movement of PLRV particles occurs in the vascular tissues of stem, stolons or both. This type of resistance was demonstrated by Wilson and Jones [23] in cv. Bismark, by grafting lengths of stem from Bismark and other potato cvs between a susceptible healthy stock plant and a PLRV-infected scion.

G8107(1) lacks this kind of resistance to long-distance movement [27]. PLRV movement along the phloem in stems of grafted plants is unimpeded. It is easily infected by graft inoculation and secondary infection takes place, although in both primary and secondary infection the titre of virus is very low. It is, however, highly resistant to infection by aphids, as demonstrated here, local primary infection occurs but is rarely followed by secondary infection. This appears to due to the lack of virus movement within the leaf or from leaf to petiole, not to impeded movement along the stem [24].

Ross [28] referred to mature plant resistance, whereby plants could escape infection because they matured early. It is well known that when sprouting tubers become infested with aphids in store prior to planting, PLRV can be spread readily from infected tubers to virus-free tubers. However, the resistance to infection in G8107(1) was not overcome when sprouting tubers were inoculated with viruliferous aphids; no primary infection was detected and secondary infection was rare. Therefore, the resistance of G8107(1) to infection with PLRV through aphid inoculation is not linked to mature plant resistance [13, 14, 28].

Most of the aphid-inoculation tests in the present study were performed using the genotype J of *M. persicae* [15]. This is characteristic of Scottish clones that can be found in the field and has been used for many years at JHRI for virus transmission tests. However, in recent studies, it was found that other clones of *M. persicae* are even more efficient vectors of PLRV. The most effective, clone C, infected approximately three times more receptor plants than Clone J in tests where single viruliferous aphids were used as sources for transmission [16]. When clone C was used in the present study for another series of transmission tests, where tuber sprouts and *in vitro* propagated plantlets were inoculated, no infections were found in G8107(1). Thus, resistance to aphid-borne PLRV infection in G8107(1) is effective even with the more efficient vector aphids and furthermore seems to be expressed from the earliest stages of plant growth.

Solomon-Blackburn, *et al*. [24] considered the possibility that the extremely low rates of PLRV infection in G8107(1) might be due to the inability of *M. persicae* to feed on the phloem tissue and hence transmit virus. However, Barker and Woodford [10] found that in field conditions, aphids accumulated on G8107(1) plants to levels that were not greatly different from other potato clones. Also, during transmission tests, it was realized that *M. persicae* behaved similarly on plants of Maris Piper and G8107(1). Moreover, it was shown that aphids can acquire and transmit PLRV from secondarily infected plants of G8107(1), although at a lower success rate than from the susceptible cv. Maris Piper which can be explained by the low titer of PLRV in G8107(1) [24].

Potato leafroll virus was detected in several individual inoculated leaves of heavily aphid-inoculated G8107(1). However, PLRV could, rarely, move into other parts of aphid-inoculated G8107(1) plants and it was not possible to detect PLRV in their petiole or stem tissues [24].

We detected secondary infection in only two tuber progenies of 63 tested (one from a plant inoculated with 100 aphids and one from a sprouted tuber inoculated with 25 aphids). This is in contrast with the tests on graft-inoculated G8107(1) plants, in which virus was detected at the stages of primary and secondary infection, although the levels of virus accumulated were very low [24]. It was, therefore, suggested that an important element in the very strong resistance of this clone to PLRV is resistance to virus movement within or from the leaves [24].

As discussed by Solomon-Blackburn, *et al*. [24], the resistance to infection in G8107(1) is different from the resistance to PLRV movement described previously [29, 23]. Unlike clone M62759 [26], clone G8107(1) is not resistant to infection by graft inoculation although, it is highly resistant to infection by aphids and to PLRV accumulation following either infection method.

Our results, gave no support to the idea that the resistance of potato clone G8107(1) to PLRV infection is based on a natural passive resistance i.e the existence of a physical barrier. The exposure of the very young, delicate and, under normal circumstances, susceptible tissues (sprouts and *in vitro* propagated plantlets) of this potato clone to viruliferous aphids carrying PLRV did not result in breakdown of resistance. Based on the results obtained, it was concluded that the resistance of this clone to PLRV infection *via* aphid inoculation is not associated with the existence of a rigid cell wall as a resistance barrier [12] or mature-plant resistance[13,14] that operates from an early stage of growth in this clone.

CONCLUSIONS

As mentioned the results obtained from these studies, were not in agreement with the idea of resistance to PLRV infection based on a natural passive resistance. Thus, the following possibilities for the observed resistance are suggested: a) replication of PLRV is not supported by the tissues of this potato clone; b) replication happens but the virus cannot be transported from the initially infected cells and c) the virus is able to

multiply and be transported but later is, somehow, degraded for instance by a gene silencing mechanism. Whatever, the mechanism is, the resistance of potato clone G8107(1) to infection with PLRV through aphid inoculation is so strong that could not be overcame even after such a heavy inoculation pressure. Considering these results, it seems that this very strong resistant potato clone is an excellent choice to be exploited in PLRV resistance breeding programs.

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