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Short Article

STUDY OF THE MECHANISM OF RESISTANCE TO POTATO LEAFROLL VIRUS (PLRV) ACCUMULATION IN POTATO CLONE G8107(1)*

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Abstract

Post-transcriptional gene silencing (PTGS) has been proposed as a natural mechanism by which plants recognize and degrade foreign nucleic acids, such as virus genomes. It was thought that such a mechanism might underlie the resistance of potato clone G8107(1) to PLRV accumulation. The idea was examined by graft transmission experiment in which, stem segments of potato clone G8107(1) as test plant and those of the cultivar Maris Piper (MP) (susceptible to PLRV) as control, were grafted on top of the stems of a PLRV-infected root sock plant of the cultivar MP. The virus-free scions of the cultivar MP (as receptors) were also grafted on top of both test and control grafts. The results indicated that the amounts of PLRV antigen in the receptor scions grafted on top of either test or control intermediate grafts were not substantially different, indicating that the presence of stem segments of the clone G8107(1) as intermediate graft has not affected the accumulation of PLRV in the receptor scions, grafted on top of them. Apparently, no silencing factor capable of degrading PLRV-RNA has been transmitted from clone G8107(1) to the cultivar MP. Therefore, the putative silencing factors are either not present in this clone or if there are, they are not transmitted. However, because it has been demonstrated that silencing is transmissible, it is less likely that the virus RNA degradation due to a gene silencing mechanism is involved in the resistance of this potato clone to PLRV. It seems more plausible that the inhibition of PLRV replication underlies the resistance rather than virus degradation.

Keywords: PTGS, Gene-silencing, Graft-transmission, RNA-degradation.

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Introduction

Like in other viruses, the most economic and environmentally safest strategy in controlling *Potato leafroll virus* (PLRV), is the use of resistant potato genotypes (Barker, 1992; Wilson & Jones, 1993; Stevenson *et al*., 2001; Solomon-Blackburn &Barker, 1993). The potato clone G8107(1), obtained from the James Hutton Research Institute (JHRI) potato breeding program, has been identified as resistant to both PLRV infection and accumulation (Solomon-Blackburn & Barker, 1993; Solomon-Blackburn *et al*., 2008; Nikan & Barker, 2012). In three field exposure trials, no plants of G8107(1) became infected. Moreover, this clone was the most resistant potato genotype to PLRV multiplication in the experiments in which the same potato breeding lines and cultivars were graft-inoculated (Solomon-Blackburn & Barker, 1993). The potato clone G8107(1) can be infected by PLRV following graft inoculation, but is completely resistant following aphid inoculation in field conditions. However, the level of virus accumulation (particularly in leaves) in graft-inoculated plants is very low (Solomon-Blackburn & Barker, 1993). Barker and Woodford (1992) showed that the spread of PLRV within potato crops is significantly limited from clones in which the virus multiplication 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. Resistance to virus accumulation is very useful in situations where most PLRV inoculum originates from sources within the crops. It has been suggested that the resistance of potato clone G8107(1) to PLRV infection might be due to PLRV replication failure in the tissues of this potato clone or inability of the virus to transport from initially infected cells or, somehow, degradation of the virus particles after inoculation (Nikan and Barker 2012). Various observations that viruses are potentially both the initiators and the targets of post-transcriptional gene silencing (PTGS) (Ratcliff *et al*. 1997) has led to the idea that this phenomenon is a natural mechanism by which plants recognize and combat foreign nucleic acids, such as virus genomes (Baulcombe, 1996; Ratcliff *et al*., 1997; 1999). Barker *et al*. (2001) suggested that an RNA-mediated form of PTGS-like resistance operates in non-vascular cells which might be part of the mechanism that restricts PLRV to vascular tissues. Some plant viruses such as potyviruses can suppress PTGS (Brigneti *et al*., 1998; Kasschau & Carrington, 1998). Voinnet *et al*. (1999) concluded that the PTGS suppression is a property of many plant viruses with a range of spatial pattern and degree suggesting the involvement of different mechanisms. Multiplication of PLRV in the phloem of graft inoculated transgenic *Nicotiana benthamiana* expressing the potyviral helper-component proteinase (HC-Pro) gene, was enhanced (Savenkov & Valkonen, 2001). Another silencing suppressor, the 2b protein encoded by *Cucumber mosaic virus* (CMV), facilitated multiplication of PLRV in mesophyll tissues (Ryabov *et al*., 2001). Moreover, it has been demonstrated that silencing is transmitted efficiently from silenced to non-silenced plants by grafting (Palauqui *et al*., 1997). It was thought that such a PTGS-like mechanism might be involved in the resistance of the potato clone G8107(1) to PLRV accumulation. Therefore, an experiment was designed in which the transfer of inducing factors of the hypothesized gene silencing from this clone to the susceptible cultivar by grafting, could result in the induction of gene silencing and consequently resistance to PLRV accumulation in the latter.

Materials and Methods

The PLRV isolate used in the experiments was PLRV-C, a standard culture maintained in potato cv. Maris Piper at JHRI. In this experiment four glasshouse grown PLRV-infected potato plants of the susceptible cv. Maris Piper (tested by ELISA) each with four or five stems were used as root stock plants. A ‘double-graft sandwich’ technique (Wilson & Jones, 1992) was used in which virus free stem segments of potato clone G8107(1) or cv. Maris Piper were grafted between a PLRV-infected root sock plant and a virus-free scions of cv. Maris Piper (Fig.1). To make the grafts, the shoot tip of each stem of the root stock plants was removed and a scion taken from either virus-free plants of clone G8107(1) or from virus-free cv. Maris Piper (as a control) was cleft-grafted onto the top of each stem. The graft union was wrapped with plastic film to prevent water loss. To minimize the transpiration of the grafted stems, they were enclosed in polythene

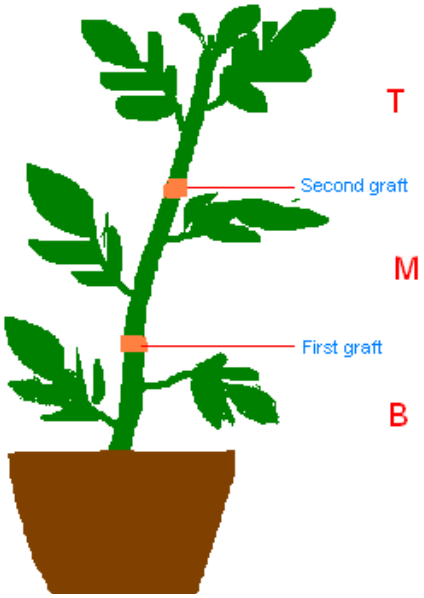

Fig.1. Grafting procedure in the experiment related to graft transmission of the putative silencing factors from resistant potato clone into susceptible cultivar, T: initially virus-free Maris Piper receptor scion; M: test stem section, virus-free stem segment of Maris Piper or clone G8107(1); B: PLRV-infected Maris Piper rootstock

bags for 5 days. Three pots containing overall 11 stems and two pots containing overall 8 stems of root stock plants were grafted with virus-free scions of clone G8107(1) and cv. Maris Piper, respectively. After recovering the functional phloem connections and when the grafted scions had grown enough (c.10 cm long and developed at least two leaves) the second grafting was made with scions taken from virus-free plants of the cv. Maris Piper by the same method. Three weeks after the second grafting, each part of the grafted stems (i.e. the root stock part, the intermediate part and the top part) was sampled separately and tested by ELISA for detecting PLRV. The second ELISA test was performed nearly one month after the first one.

For detecting PLRV antigens in test plants, the triple antibody sandwich enzyme linked immunosorbent assay (TAS-ELISA) method (Torrance 1992) was used. The coating antibody used was PLRV-G polyclonal antibody (Tamada & Harrison 1980), the second antibody was PLRV monoclonal antibody (SCR3) (Massalski & Harrison 1987) and the conjugated antibody was alkaline phosphatase conjugated anti-mouse antibody (Sigma Chemical Co).

Results and discussion

These results of the absorbance values (A_{405}) of the samples in ELISA tests made on leaf tissues from different parts of the grafted stems have been summarized in Table 1. The difference between the average absorbance value for the receptor scions grafted on stem segments of G8107(1) and those grafted on stem segments of cv. Maris Piper was not substantial. The results indicated that the presence of the stem sections of clone G8107(1) as intermediate graft has not affected the multiplication of PLRV in the susceptible receptor scions, grafted on top of them.

The idea of PLRV-RNA degradation due to gene silencing in the potato clone G8107(1) was examined by the graft transmission experiment. The results of this experiment showed that the absorbance values in ELISA tests varied for leaves from different parts of the grafted stems, particularly of those with stem segments of clone G8107(1) as their intermediate grafts. Compared to the leaf samples taken from stems of PLRV infected root stock plants, the ELISA absorbance values in samples taken from the stem segments of clone G8107(1) dropped dramatically, indicating a lower concentration of PLRV antigen in this clone. This confirmed the resistance of the clone G8107(1) to PLRV accumulation (Solomon-Blackburn &Barker 1993, Solomon-Blackburn *et al*., 2008, Nikan &Barker 2012). The amounts of PLRV antigen (estimated by ELISA) in the receptor scions grafted on top of the stem sections of

Table.1 The mean absorbance values of ELISA tests for PLRV accumulation in leaf samples taken from different parts of the grafted stems.

Intermediate Graft	Number of stems tested	First ELISA	Second ELISA				
		Part B[1]	Part M[2]	Part T[3]	Part B[1]	Part M[2]	Part T[3]
G8107(1)	11	2.02	0.77	1.88	1.89	0.27	0.78
Maris Piper	8	2.06	1.58	1.68	2.00	1.62	1.11

[1].The bottom part, PLRV-infected Maris Piper root stock
[2].The middle part, initially virus –free intermediate graft (test stem section)
[3].The top part, initially virus-free Maris Piper receptor scion

G8107(1) and those grafted on top of Maris Piper stem sections were nearly the same, indicating that no silencing factor was transmitted from clone G8107(1) to the cv. Maris Piper. According to these results, the putative factors capable of degrading PLRV-RNA are either not present in clone G8107(1) or if there are, they are not transmissible. However, because it has been demonstrated that silencing is transmitted efficiently from silenced to non-silenced plants by grafting (Palauqui *et al*. 1997), it is unlikely that the virus RNA degradation due to a gene silencing mechanism is involved in the resistance of this potato clone to PLRV. It seems therefore, that the resistance of clone G8107(1) to PLRV accumulation, manifested by low concentration of the virus after graft inoculation, is probably the result of the virus replication inhibition rather than degradation of the virus due to mechanism of gene silencing. For instance some components required for the virus replication may not be provided at an optimum level by the host. It also seems that resistance to PLRV accumulation in potato clone G8107(1) could be due at least partly to the same mechanism underlying its resistance to PLRV infection (Solomon-Blackburn *et al*. 2008). Resistance to long-distance movement of PLRV particles in the vascular tissues of stems, stolons or both tissue types in Potato clone M62759 was reported by Syller (2003). The lack of a substantial difference between the ELISA absorbance values of the scions grafted on the stem segments of clone G8107(1) and of those grafted on the stem segments of cv. Maris Piper can be considered as an implication of the lack of resistance to PLRV long-distance movement in clone G8107(1) as reported by Derrick and Barker (1992). This can also be deduced from the fact that when this clone becomes infected with PLRV (for example after graft inoculation) it produces infected tubers. It can also be deduced from these results that the mechanism of resistance to PLRV in G8107(1) operates at the level of virus movement to the vascular tissues. Similar results, resistance to the movement of PLRV within or from the leaves of this clone, have been reported by Solomon-Blackburn *et al*. (2008). If resistance to PLRV occurred in clone G8107(1) is due to virus transport failure from the initially infected cells, it could be examined by co-infection of clone G8107(1) with PLRV and an *Umbravirus* such as *Pea enation mosaic virus-2* (PEMV-2) or *Groundnut rosette virus* (GRV) or by transformation of this clone with the open reading frame-4 (OFR4) movement protein of these viruses. It has been demonstrated that umbraviral OFR4 movement protein complemented PLRV spread within the inoculated plant and its mechanical transmission (Ryabov *et al*. 2001). Even though the involvement of a type of gene silencing in the resistance of clone G8107(1) to PLRV could not be established by these preliminary experiments, the hypothesis cannot be ruled out completely and further investigations are needed to reveal the mechanism(s) involved. For instance, in graft-transmission experiments, grafting stem segments of both test and control plants on the same virus-infected root stock plant could give more reliable results and are recommended for future experiments.

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