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A new simple and effective method for PLRV infection to screen for virus resistance in potato

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ABSTRACT

Effective screening of plant germplasm collections for resistance to plant viruses requires that there is a rapid and efficient system in place to challenge individual plants with the virus. Potato leafroll virus (PLRV), a commercially important pathogen of potato, is able naturally to infect only the phloem-associated tissue of plants and is delivered to this tissue by feeding aphids. Mechanical (non-vector-mediated) infection by PLRV does not occur thus screening for PLRV resistance is currently laborious and time consuming. We constructed an infectious cDNA clone of a new (Hutton) isolate of PLRV in the binary vector pDIVA and transformed it into *Agrobacterium tumefaciens* strain LBA4404. Infiltration of this culture into leaves of *Nicotiana benthamiana*, a highly susceptible model plant, produced a systemic infection with PLRV, although this approach was not successful for potato. However, a very efficient and reproducible systemic infection of potato was achieved when we submerged cut stems of the plant into the agrobacterium cell suspension and then transplanted the stems into compost to grow roots and new apical leaves. Using a standardised protocol developed for this new PLRV inoculation method we have confirmed the previously described resistance to the virus in the JHI breeding line G8107(1) and identified 62 plant accessions from the Commonwealth Potato Collection in which no PLRV infection was detected.

Potato leafroll virus (PLRV) is a member of the genus *Polerovirus* (family: *Solemoviridae*) and causes significant yield (up to 90%) and quality losses to potato production worldwide (Kumari et al., 2020). PLRV is naturally transmitted in a circulative, non-propagative manner by *Myzus persicae* and several other species of aphid (Herrbach, 1999), meaning that after being ingested by a feeding aphid the virus must circulate through the aphid gut, move into the haemocoel, and then pass back into the aphid salivary gland before it can be transmitted, during a subsequent feed, into a new plant. This process, known as the latent period, takes a minimum of 1 h during which time the aphid is unable to transmit PLRV (Taliansky et al., 2003) unlike some other potato viruses, including potato virus Y (PVY), that do not circulate through the body of the vector aphid but become attached to the aphid stylet and so are immediately available to be transmitted between plants during aphid feeding. The latent period for PLRV transmission provides an opportunity to control the spread of the virus by the use of pesticides against vector aphids. However, pesticide use is becoming more tightly regulated because of concerns about environmental toxicity and development of insecticide resistance (Singh et al., 2021). Aphid numbers are expected to rise, and their geographical distribution expand as a result of climate change (Norse and Gommes, 2003) with epidemics of aphid-borne viruses, including PLRV, becoming increasingly likely (Jones, 2009).

Natural resistance to viruses occurs in many plants. This can result from the plant lacking or having mutations in a gene that is essential for some part of the virus infection cycle (Truniger and Aranda, 2009). Natural resistance also can be provided by genes that encode proteins which specifically recognise and supress infection by different viruses (Akhter et al., 2021; Tomita et al., 2019). Genetic resistance resulting in reduced levels of PLRV is present in some potato (*Solanum tuberosum*) cultivars, including the breeding line G8107(1) (Solomon-Blackburn and Barker, 1993), but no cultivar is immune to infection and, at present, there are no known major genes for PLRV resistance (Solomon-Blackburn and Barker, 2001). The Commonwealth Potato Collection (CPC; https://ics.hutton.ac.uk/germinate-cpc/#/home) comprises around 1500 accessions of about 80 wild and cultivated potato species which possess many previously unexplored resistances to important pests and diseases. Germplasm from this collection has been used to identify new resistances to PVY (Torrance et al., 2020), potato cyst nematode (Varypatakis et al., 2020) and might also contain

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Table 1
Primers used in this study.

Name of primer	Sequence (5′−3′)
For construction of pCB-DIVA-PLRV	
PLRV-REV	ACTACACAACCCTGTAAGAGGATCC
PLRV-FOR	ACAAAAGAATACCAGGAGGAATTGC
PLRV-F2	GGAAGTTCATTTCATTTGGAGAGGACAAAAGAATACCAGGAGGAATTGC
PLRV-R2	TGGAGATGCCATGCCGACCCACTACACAACCCTGTAAGAGGATCC
pDIVA-F	CCTCTCCAAATGAAATGAACTTCC
pDIVA-R	GGGTCGGCATGGCATCTCCA
For RT-PCR detection of PLRV	
PLRV-CP-REV	AACTTCCTTGGGAGTTGCCC
PLRV-CP-FOR	TTCAGAAATCCGACCCTCGC

Table 2
ELISA values of potato cv. Desiree leaves following agro-inoculation of cut stems indicating systemic PLRV infection.

	ELISA value after 1 h o/n		PLRV infection[a]
Plant 1	0.09	0.12	-
2	0.33	1.95	+
3	0.07	0.10	-
4	0.07	0.10	-
5	0.01	0.43	+
6	0.11	0.42	+
7	0.22	1.24	+
Healthy plant	0.08	0.14	-
PLRV-infected	0.52	3.13	+

[a] Positive result (PLRV infection) denotes ELISA value twice or more than that of healthy plant value. These results from a single inoculation experiment.

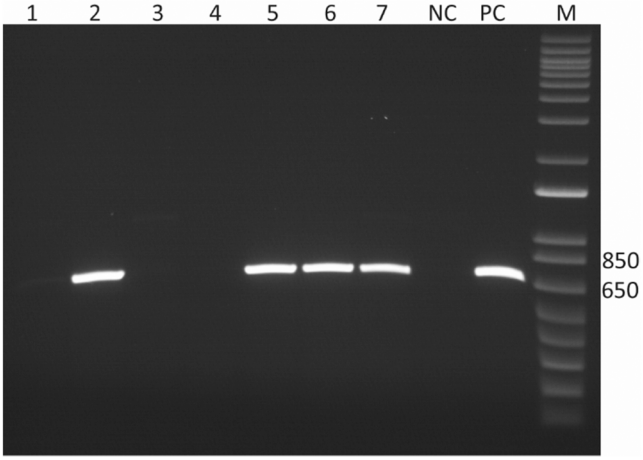

Fig. 1. RT-PCR detection of PLRV in systemic leaves of Desiree potato following agro-inoculation of cut stems at 28 dpi. NC; non-inoculated, negative control. PC; PLRV agro-infected *N. benthamiana*. M; DNA molecular mass marker (ThermoFisher Scientific, Loughborough, UK), numbers to the right indicate size of selected marker fragments.

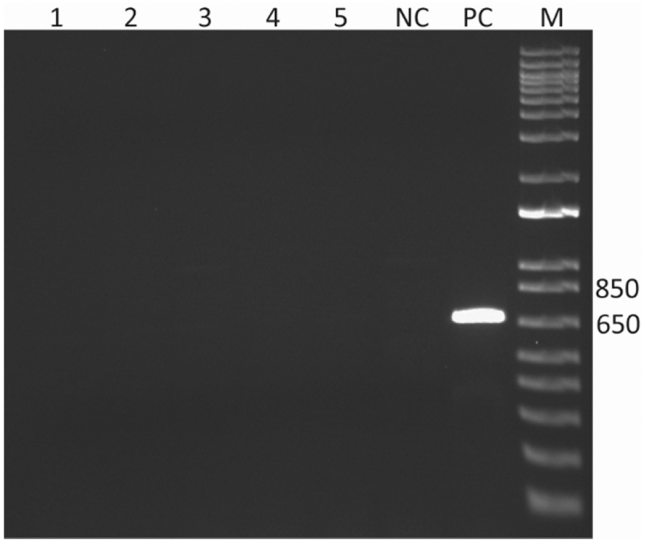

Fig. 2. Demonstration of PLRV resistance in G8107(1) following agro-inoculation of cut stems. Lanes 1–5: PLRV challenged G8107(1) at 32 dpi. NC; non-inoculated, negative control. PC; PLRV agro-infected *N. benthamiana*. M; DNA molecular mass marker (ThermoFisher Scientific), numbers to the right indicate size of selected marker fragments.

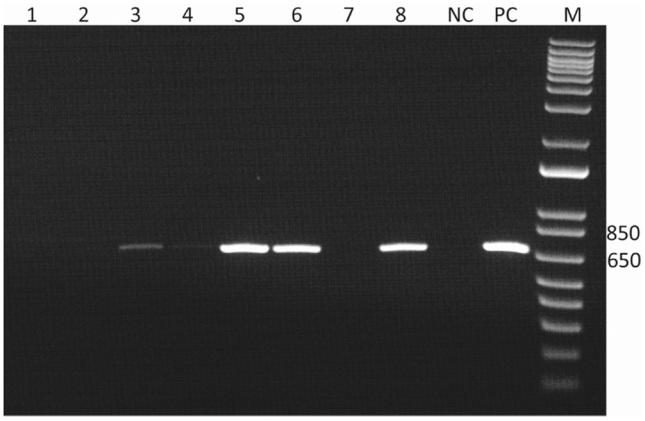

Fig. 3. RT-PCR detection of PLRV in agro-inoculated CPC accessions at 28 dpi. Lanes 1–8 accessions 4566, 4549, 4581, 315, 508, 258, 4777 and 318, respectively. NC; non-inoculated, negative control. PC; PLRV agro-infected *N. benthamiana* plant. M; DNA molecular mass marker (ThermoFisher Scientific), numbers to the right indicate size of selected marker fragments.

resistances to PLRV. Although they did not select plants from the CPC, Barker and Waterhouse (1999) reported that several diploid *Solanum* species do contain resistance to PLRV, including *S. brevidens*, *S. etuberosum*, *S. chacoense* and *S. raphanifolium* that have very high levels of resistance.

However, screening a potato germplasm collection for PLRV resistance requires there to be a robust and reproducible method in place to inoculate plants with the virus. PLRV, like all luteoviruses, is phloem-limited, meaning that it infects and moves only in phloem-associated cells (phloem parenchyma, phloem companion cells and phloem sieve elements) (Taliansky et al., 2003). Natural transmission of PLRV by its vector aphid occurs only to and from the phloem and, with the exception of some highly virus-susceptible *Nicotiana* species, mechanical inoculation of the virus to plant leaves does not produce a spreading infection. Previously, testing whether a potato plant carried any form of resistance or tolerance to PLRV would require the virus to be delivered to the phloem either using viruliferous aphids or by grafting with a scion taken

Table 3
List of Solanum species and PLRV resistance/susceptibility in CPC accessions.

Resistant	
Species*	**CPC #**
ACL	7098
ADG	189, 257, 303, 509, 2805, 2988, 3063, 3073, 3077, 3062 A, 3065 A, 3072 A, 3072B
CHC	5851, 5853
DMS	7524
HMP	7813
HOU	2045
IFD	3276
JAM	7656
KTZ	7056
MCD	7160, 7163
OPL	7184
PHU	4549, 4560, 4562, 4566, 4570, 4582, 4547, 4549, 4560, 4562, 4566
PLT	7075
PNT	3863, 7659, 7664, 7191
PTA	7078, 7079
SCR	7682, 7633
SEM	7331
SIC	7105
SND	6027
SNK	7033, 7164
SPG	7195, 7767
SPL	7694, 7696
STN	4777, 4809
TOR	7119
TRF	7125
VER	7776
VIO	7128
Susceptible	
Species*	**CPC #**
ADG	258, 315, 318, 508, 3076
BLB	7650
CAN	7615
DMS	1342
FEN	3206
MCD	3766
NRS	7287
PHU	4552, 4581
PLD	7787, 3501
SCT	6269
STO	7197, 7198
VRN	4075

* ACL, acaule; ADG, andigena; BLB, bulbocastanum; CAN, canasense; CHC, chacoense; DMS, demissum; FEN, fendleri; HMP, humectophilum; HOU, hougasii; IFD, infundibuliforme; JAM, jamesii; KTZ, kurtzianum; MCD, microdontum; NRS, neorossii; OPL, oplocense; PHU, phureja; PLD, polyadenium; PLT, polytrichon; PNT, pinnatisectum; PTA, papita; SCR, sucrense; SCT, sanctaerosae; SEM, semidemissum; SIC, sicuanum; SND, sandemanii; SNK, schenckii; SPG, spegazzinni; SPL, sparsipilum; STN, stenotomum; STO, stoloniferum; TOR, toralapanum; TRF, trifidum; VER, verrucosum; VIO, violaceimarmoratum; VRN, vernei.

from an infected potato plant. These are laborious and time-consuming approaches, and for the aphid transmission test requires the use of specialised facilities and expertise.

A newer approach is to use *Agrobacterium tumefaciens* to deliver a cloned copy of the virus genome into the plant cell nucleus, which is transcribed into a replicating viral RNA that infects the whole plant (Peyret and Lomonossoff, 2015). The usual method, that works well with mechanically-transmissible viruses, is to infiltrate the agrobacterium suspension into air spaces within the leaf, so that infection of leaf mesophyll cells can occur. However, PLRV infection occurs in phloem-associated cells which, in plants such as potato, are not accessible to agrobacterium via this route. Nevertheless, transgenic potato plants carrying a full-length cDNA clone of PLRV did become infected with the virus but died within 6 weeks of removal from tissue culture (Prüfer et al., 1997). Similarly, Franco-Lara et al., (1999) could not generate viable cultivar (cv.) Maris Piper (PLRV susceptible) potato plants carrying the PLRV genome but were able to produce viable PLRV-containing transgenic G8107(1) plants that were, presumably, protected from the more severe effects of the virus. Nurkiyanova et al., (2000) were able to produce a systemic infection of *N. benthamiana* and *N. clevelandii* plants using an agrobacterium-delivered PLRV infectious clone. There is a single report (Lee et al., 2005) of infection of potato, by either potato phloem injection or by leaf infiltration, with agrobacterium carrying a PLRV cDNA clone. The leaf infiltration method resulted in an infection that was confined to the infiltrated patch of cells, whereas the phloem injection method, using a hypodermic needle, did lead to a systemic PLRV infection. Subsequent studies showed that higher levels of PLRV infection of mesophyll cells could be achieved if PLRV was co-infiltrated with a second virus, the umbravirus pea enation mosaic virus 2 (PEMV2) (Mayo et al., 2000). To overcome these difficulties we devised a novel, rapid and robust PLRV inoculation method that results in reproducibly high infection rates in susceptible potato plants and we have used this new method to identify previously unknown resistances to PLRV in plants curated within the CPC.

To produce a full-length, infectious cDNA clone of PLRV we isolated total RNA from PLRV-infected cv. Maris Piper plants (obtained from JHI; Farm Code No: 123/0028, FID NO/29947/32976) and used a 3′ co-terminal, minus-strand primer (PLRV-REV) designed from GenBank PLRV sequences (Table 1) and the Lunascript RT Supermix Kit (New England Biolabs, Ipswich, MA, USA) to synthesise cDNA. The cDNA was used as template in PCR using PLRV-FOR (Table 1; 5′ terminus PLRV plus-strand) and PLRV-REV primers. The PCR conditions were an initial denaturation at 98 °C for 30 s, then 30 cycles of 98 °C 10 s, 64 °C 30 s, 72 °C 3 min, followed by incubation at 72 °C for 10 min, using the proofreading Phusion™ High Fidelity DNA polymerase (New England Biolabs). To facilitate cloning into the low copy number, binary vector pDIVA (Laufer et al., 2018) the product of this initial PCR was used as a template with primers PLRV-F2 and PLRV-R2, carrying PLRV-terminal sequences and either pDIVA-derived CaMV 35 S promoter or HDV ribozyme sequences for re-amplification (Table 1). The pDIVA vector was amplified by inverse PCR using primers pDIVA-F and pDIVA-R (Table 1). pDIVA vector and PLRV genome amplicons were ligated together using the NEBuilder HiFi DNA reagent (New England Biolabs) to create the full-length PLRV clone pDIVA-PLRV-Hutton. The cloned PLRV-Hutton isolate was sequenced using a Sanger-based approach and the data deposited in GenBank (Accession number OK245432). pDIVA-PLRV-Hutton was transformed into *Agrobacterium tumefaciens* strain LB4404, cells grown in liquid cultures were pelleted, resuspended in infiltration buffer (10 mM $MgCl_2$, 10 mM MES pH 5.7, 150 μM acetosyringone) at an OD_{600} of 0.25 and incubated in the dark for 2 h at room temperature before use.

Initial experiments used a syringe without a hypodermic needle to infiltrate the agrobacterium suspension carrying the PLRV cDNA clone directly into the lower epidermis of leaves of different plants. In these preliminary experiments we adopted an enzyme-linked immunosorbent assay (ELISA) method to detect assembled PLRV particles as described previously by Torrance (1992) using the PLRV-double antibody sandwich alkaline phosphatase (AP)-conjugated ELISA reagents supplied by SASA (Edinburgh, UK). AP substrate absorbance values (A405 nm) were recorded using a Titertek Multiskan PLUS Photometer (Titertek, Huntsville, AL, USA) after incubation with the substrate for 1 h at RT, and also, when assessing weakly-infected plants, after an overnight (12 h) incubation at 4 °C. ELISA (A405 nm) values were considered positive if they were more than twice those of the mean control extracts from uninoculated leaves. Infiltration of *N. benthamiana* leaves produced a systemic PLRV infection in each of five inoculated plants as detected by ELISA after 1 h substrate incubation with a mean A405 nm value of 1.38 (healthy control plant value was 0.07). In potato, infection by PLRV could be detected in the infiltrated leaves in each of three plants of the known PLRV-susceptible cv. Desiree by ELISA after 1 h substrate incubation with a mean A405 nm value of 2.57 (healthy control plant value was 0.07). No systemic PLRV infection was detected in any of these plants (mean A405 nm value of 0.06). Attempts to initiate systemic

Fig. 4. Typical potato stem cutting (10 – 14 cm) in length (A) as used for immersion in agrobacterium suspension (B) and example of a test plant 4 weeks post challenge (C).

infection in cv. Desiree potato following the method of Lee et al., (2005) by agro-infiltrating the PLRV cDNA clone by hypodermic needle into mid-ribs of either leaves or petioles were also not successful (data not shown). Alternative experiments, in which PLRV was co-infiltrated with an infectious clone of PEMV-2 (S. MacFarlane, unpublished) enhanced PLRV infection in *N. clevelandii and N. benthamiana,* similar to results obtained previously (Mayo et al., 2000; Ryabov et al., 2001) but PEMV2 was not able to infect potato and, thus, promote PLRV infection (data not shown).

Our new approach to inoculate potato plants with the virus was to take stem cuttings (of approximately 10–14 cm in length and with 3–4 leaves (Fig. 4A) from *S. tuberosum* and diploid *Solanum* species plants and place the freshly cut ends into the pDIVA-PLRV-Hutton agrobacterium suspension (Fig. 4B) for 2 h in the dark. The cuttings were then planted directly into plastic pots containing a standard compost mixture (85% (v/v) Irish moss peat, 7% (v/v) sand, 0.2% (w/v) magnesium limestone, 0.2% (w/v) calcium limestone, 0.15% (w/v) Osmocote Start controlled release fertiliser (Everris, ICL, UK), 7% (v/v) Perlite (Sinclair Pro, UK), 0.05% (w/v) Celcote wetting agent (Certis, Abington, UK) and 0.3% Osmocote Exact Standard fertilizer (Everris, ICL, UK) presoaked with water. The potted stems were placed in a temperature-controlled glasshouse (constant 20°C and 16 h daylength) for 4 weeks during which time they developed roots and additional apical shoots and became established as growing plants (Fig. 4C).

At various times post inoculation with the agrobacterium suspension, a leaflet was collected from each of four different upper leaves of each plant, the samples were combined and total RNA was isolated using the NucleoSpin RNA Plant Kit (Macherey-Nagel, Dueren, Germany). cDNA was prepared using the Lunascript RT Supermix Kit (New England Biolabs) supplemented with primer PLRV-CP-REV followed by PCR amplification using Phusion DNA polymerase (New England Biolabs) with primers PLRV-CP-FOR and PLRV-CP-REV (Table 1). The PCR conditions were an initial denaturation at 98 °C for 30 s, then 30 cycles of 98 °C 10 s, 65 °C 30 s, 72 °C 30 s, followed by incubation at 72 °C for 5 min. The amplified PLRV CP fragment (649nt) was resolved by electrophoresis in a 0.8% agarose gel using Tris-borate buffer. PLRV was detected in the upper leaves of *N. benthamiana* by 10 days post inoculation (dpi), whereas for potato, systemic infection was reproducibly detected by 28 dpi, at which time each cutting had produced a significant root system and expanding apical leaves suitable for virus testing. Thereafter, the standard PLRV infection method used a 4-week post-inoculation propagation period before virus detection.

Using the stem immersion method we were able to achieve a systemic PLRV infection that could be detected by ELISA (Table 2) and RT-PCR (Fig. 1) in agrobacterium-treated cv. Desiree potato plants. Experiments were done to optimise and assess the reproducibility of this infection method, enabling us to design a standardised experimental plan in which 4 – 6 potato stem cuttings from a CPC accession were inoculated together with a single *N. benthamiana* plant, for rapid (within 7–10 days) confirmation of the infectivity of the PLRV treatment, that was inoculated by leaf infiltration. A single, un-inoculated potato cutting was also included as a healthy control. The potato cuttings were grown for 4 weeks before being assessed for PLRV infection by RT-PCR of upper leaflets.

To compare the results from our new PLRV inoculation method with previous resistance screening experiments we challenged the JHI potato breeding line G8107(1) that had been identified by field exposure trials as being resistant to PLRV (Solomon-Blackburn and Barker, 1993). The earlier work showed that this line could become infected by graft inoculation using PLRV-infected scions but that the level of virus in G8107(1) remained extremely low. Subsequent work showed this line to be resistant to aphid inoculation of PLRV and concluded that virus movement within or from leaves was strongly inhibited in G8107(1) (Solomon-Blackburn et al., 2008; Nikan and Barker, 2012). When stem cuttings of this line were agro-inoculated with the PLRV-Hutton cDNA clone no PLRV infection could be detected in any of the plants (two independent experiments using a total of sixteen cuttings. Fig. 2 shows RT-PCR testing of 5 inoculated plants.

To investigate whether sources of genetic resistance to PLRV might be found in the CPC we randomly selected 86 of the approximately 1500 accessions curated in the collection for testing. Using the stem immersion method described above we found, by RT-PCR analysis, that the

majority (62) of the tested accessions were not infected by PLRV, whereas 19 selections were susceptible to PLRV (Fig. 3; Table 3). Two of the CPC accessions that we were able to infect with our PLRV clone, namely, CPC 318 and CPC 4075, were reported to be PLRV-susceptible in the CPC Germplasm Attribute Data. PLRV resistance has not been documented in this data, and so we are not able to cross-correlate our identification of resistant accessions with pre-existing results. In this small test population, both resistant and susceptible accessions were found for S. *tuberosum* Group Andigena, *S. tuberosum* Group Phureja and *S. microdontum* subsp. Gigantophyllum, pointing to a spread of genetic diversity existing between different accessions of the same species that have been collected at different geographical locations.(Fig. 4).

The apparent resistances to PLRV shown by the various CPC accessions here could have many different underlying mechanisms, including not only specific antiviral pathways but also, potentially, blocks in translocation and expression of the virus from the agrobacterium cells. If any of these "PLRV resistant" accessions are to be used in breeding efforts it will be necessary to confirm their virus resistance by the conventional methods of graft inoculation or aphid transmission. Nevertheless, our new approach for PLRV inoculation will be extremely useful for rapid, initial screening of the entire CPC for PLRV resistance and phenotyping progeny of crossing experiments to understand and map the genes involved in this resistance. This method may also be applicable for resistance testing of a wider range of commercially-relevant plants to other phloem-limited viruses.

CRediT authorship contribution statement

Graham Cowan: Investigation, Methodology, Writing – original draft. **Stuart MacFarlane:** Methodology, Writing – original draft. **Lesley Torrance:** Project Conception, Manuscript Reviewing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Akhter, M.S., Nakahara, K.S., Masuta, C., 2021. Resistance induction based on the understanding of molecular interactions between plant viruses and host plants. Virol. J. 18, 176. https://doi.org/10.1186/s12985-021-01647-4.

Barker, H., Waterhouse, P.M., 1999. The Luteoviridae/Eds. In: Smith, H.G., Barker., H. (Eds.), CAB International. Wallingford, UK, pp. 169–210. ISBN 08519 9324 9.

Franco-Lara, l , McGeachy, K.D. , Commandeur, U. , Martin, R.R. , Mayo, M.A. , Barker, H. , 1999. https://doi.org/10.1099/0022–1317-80–11-2813.

Herrbach, E., 1999. Virus–vector interactions. In The Luteoviridae (Smith, H.G. and Barker, H., eds). CAB International,, Wallingford, UK, pp. 85–146.

Jones, R., 2009. Plant virus emergence and evolution: origins, new encounter scenarios, factors driving emergence, effects of changing world conditions, and prospects for control. Virus Res. 141, 113–130. https://doi.org/10.1016/j.virusres.2008.07.028.

Kumari, P., Kumar, J., Kumar, R.R., Ansar, M., Rajani, K., Kumar, S., Ranjan, T., 2020. Inhibition of potato leafroll virus multiplication and systemic translocation by siRNA constructs against putative ATPase fold of movement protein. Sci. Rep. Dec. 16 10 (1), 22016. https://doi.org/10.1038/s41598-020-78791-4.

Laufer, M., Mohammad, H., Maiss, E., Richert-Pöggeler, K., Dall'Ara, M., Ratti, C., Gilmer, D., Liebe, S., Varrelmann, M., 2018. Biological properties of Beet soil-borne mosaic virus and Beet nectrotic yellow vein virus cDNA clones produced by isothermal in vitro recombination: Insights for reassortment appearance. Virology 518, 25–33. https://doi.org/10.1016/j.virol.2018.01.029.

Lee, L., Kaplan, I.B., Ripoli, D.R., Liang, D., Palukiatis, P., Gray, S., 2005. A surface loop of the potato leafroll virus coat protein is involved in virion assembly, systemic movement, and aphid transmission. J. Virol. 79 (Issue 2), 1207–1214 https://doi.org/10.1128/JVI.79.2.1207-1214.2005.

Mayo, M., Ryabov, E., Fraser, G., Taliansky, M., 2000. Mechanical transmission of Potato leafroll virus. J. Gen. Virol. Nov;81 (Pt 11), 2791–2795. https://doi.org/10.1099/0022-1317-81-11-2791.

Nikan, J., Barker, H., 2012. Study of resistance in potato clone G8107(1) to potato leafroll virus infection. World Appl. Sci. J. 20 (10), 1347–1353.

Norse, D., Gommes, R., 2003. Climate change and agriculture: physical and human dimensions. In: Bruinsma, J. (Ed.), World Agriculture Towards 2015/2030: An FAO Perspective. Earthscan Publications Lts., London, UK, pp. 357–372.

Nurkiyanova, K.M., Ryabov, E.V., Commandeur, U., Duncan, G.H., Canto, T., Gray, S.M., Mayo, M.A., Taliansky, M.E., 2000. Tagging potato leafroll virus with the jellyfish green fluorescent protein gene. J. Gen. Virol. Mar;81 (Pt 3), 617–626. https://doi.org/10.1099/0022-1317-81-3-617.

Peyret, H., Lomonossoff, G.P., 2015. When plant virology met Agrobacterium: the rise of the deconstructed clones. Plant Biotechnol. J. 13, 1121–1135. https://doi.org/10.1111/pbi.12412.

Prüfer, D., Schmitz, J., Tacke, E., et al., 1997. In vivo expression of a full-length cDNA copy of potato leafroll virus (PLRV) in protoplasts and transgenic plants. Mol Gen Genet 253, 609–614. https://doi.org/10.1007/s004380050363.

Ryabov, E.V., Fraser, G., Mayo, M.A., Barker, H., Taliansky, M., 2001. Umbravirus gene expression helps potato leafroll virus to invade mesophyll tissues and to be transmitted mechanically between plants. Virology 286 (2), 363–372. https://doi.org/10.1006/viro.2001.0982.

Singh, K.S., Cordeiro, E.M.G., Troczka, B.J., Pym, A., Mackisack, J., Mathers, T.C., Duarte, A., Legeai, F., Robin, S., Bielza, P., Burrack, H.J., Charaabi, K., Denholm, I., Figueroa, C.C., Ffrench-Constant, R.H., Jander, G., Margaritopoulos, J.T., Mazzoni, E., Nauen, R., Ramírez, C.C., Ren, G., Stepanyan, I., Umina, P.A., Voronova, N.V., Vontas, J., Williamson, M.S., Wilson, A.C.C., Xi-Wu, G., Youn, Y.N., Zimmer, C.T., Simon, J.C., Hayward, A., Bass, C., 2021. Global patterns in genomic diversity underpinning the evolution of insecticide resistance in the aphid crop pest Myzus persicae. Commun. Biol. Jul. 7 4 (1), 847. https://doi.org/10.1038/s42003-021-02373-x.

Solomon-Blackburn, R.M., Barker, H., 1993. Resistance to potato leafroll luteovirus can be greatly improved by combining two independent types of heritable resistance. Ann. Appl. Biol. 122, 329–336. https://doi.org/10.1111/j.1744-7348.1993.tb04038.x.

Solomon-Blackburn, R.M., Nikan, J., Barker, H., 2008. Mechanism of strong resistance to Potato leafroll virus infection in a clone of potato (*Solanum tuberosum*). Ann. Appl. Biol. 125 (3), 339–347. https://doi.org/10.1111/j.1744-7348.2008.00223.x.

Solomon-Blackburn RM, Barker H. Breeding virus resistant potatoes (Solanum tuberosum): a review of traditional and molecular approaches. Heredity (Edinb). 2001 Jan;86(Pt 1):17-35. doi: 10.1046/j.1365-2540.2001.00799.x. PMID: 11298812.

Taliansky, M., Mayo, M.A., Barker, H., 2003. Potato leafroll virus: a classic pathogen shows some new tricks. Mol. Plant Pathol. Mar. 1 4 (2), 81–89. https://doi.org/10.1046/j.1364-3703.2003.00153.x.

Tomita, R., Sekine, K.T., Tateda, C., Kobayashi, K., 2019. Identification and functional analysis of NB-LRR-type virus resistance genes: Overview and functional analysis of candidate genes. In Antiviral resistance in plants: Methods and Protocols. In: Kobayashi, K., Nishiguchi, M. (Eds.), Methods Mol Biol, vol 2028. Humana Press, pp. 1–10. https://doi.org/10.1007/978-1-4939-9635-3_1.

Torrance, L., 1992. Serological methods to detect plant viruses: production and use of monoclonal antibodies. In: Duncan, J.M., Torrance, L. (Eds.), Techniques for the Rapid Detection of Plant Pathogens, Blackwell Scientific Publications. Oxford, United Kingdom, pp. 27–33. ISBN 9780632030668.

Torrance, L., Cowan, G.H., McLean, K., MacFarlane, S., Al-Abedy, A.N., Armstrong, M., Lim, T.Y., Hein, I., Bryan, G.J., 2020. Natural resistance to Potato virus Y in Solanum tuberosum Group Phureja. Theor. Appl. Genet. Mar. 133 (3), 967–980. https://doi.org/10.1007/s00122-019-03521-y.

Truniger, V., Aranda, M.A., 2009. Chapter 4 - Recessive Resistance to Plant Viruses. In: Gad Loebenstein John P. Carr, Advances in Virus Research, Volume 75. Academic Press, pp. 119–231. https://doi.org/10.1016/S0065-3527(09)07504-6.

Varypatakis, K., Véronneau, P.-Y., Thorpe, P., Cock, P.J.A., Lim, J.T.-Y., Armstrong, M.R., Janakowski, S., Sobczak, M., Hein, I., Mimee, B., Jones, J.T., Blok, V.C., 2020. The genomic impact of selection for virulence against resistance in the Potato Cyst Nematode, *Globodera pallida*. Genes 11, 1429. https://doi.org/10.3390/genes11121429.