Transmission of *Little cherry virus* -1 (LChV-1) by *Cuscuta europea* to herbaceous host plants

Jelkmann, W., Hergenhahn, F., Berwarth, C.
Julius Kühn Institute (JKI), Federal Research Centre for Cultivated Plants, Institute for Plant Protection in Fruit Crops and Viticulture, Schwabenheimer Str. 101, 69221 Dossenheim, Germany. Email: Wilhelm.Jelkmann@jki.bund.de

Abstract

Little cherry virus -1 (LChV-1) was transmitted from infected *Prunus avium* F12 rootstocks by *Cuscuta europea* to *Nicotiana occidentalis* '37B'. Transmission of the virus was confirmed by RT-PCR analysis of total nucleic acid extracts from dodder and *N. occidentalis*. Symptoms consisted of curled leaves, reddening of leaf margins and veins, and plant decline. In parallel attempts virus transmission was not successful for LChV-2. Propagation of LChV-1 by mechanical transmission on *N. occidentalis* failed, however the virus was transferred serially by grafting.

Introduction

Little cherry disease has been associated with two different long flexuous filamentous viruses of the family Closteroviridae. *Little cherry virus* -1 (LChV-1) is an unassigned member in the family while *Little cherry virus* -2 (LChV-2) has been assigned to the genus *Ampelovirus*. Both viruses have been characterized at the molecular level (Rott and Jelkmann 2005;Jelkmann, Fechtner et al. 1997).

The viruses can be found individually and in mixed infections. The disease is distributed worldwide in ornamental and sweet cherry and has a great impact on fruit quality of infected trees. Symptoms of infection consist of small angular and pointed fruit that do not ripen fully and are imperfectly coloured. Fruit have reduced sweetness and are unsuitable for consumption. The disease is readily graft–transmissible from cherry to cherry. There is no known vector associated with LChV-1, however, LChV-2 is transmitted by the apple mealybug (*Phenacoccus aceris*). Both viruses can be detected by RT-PCR and woody indexing on sensitive indicator plants (Jelkmann and Eastwell 2010). The host range of the viruses is limited to species within the genus *Prunus*. In addition to cherry, LChV-1 was identified in plum, peach and almond (Matic, Minafra et al. 2009b).

A recent report described the transmission of *Grapevine leafroll associated virus* -7 (GLRaV-7) from a grapevine accession to *Tetragonia expansa* and to *Nicotiana occidentalis* by different *Cuscuta* species (Mikona and Jelkmann 2010). Although this method had been used for many virus transmission experiments in past decades (Hosford 1967) no reports of closterovirus transmission from woody plants to herbaceous hosts were found in the literature. In order to evaluate potential herbaceous host plants for LChV-1 and LChV-2 a similar experimental setup was investigated as used for GLRaV-7 transmission.

Materials and methods

Transmission experiments. *Cuscuta europea* L. (greater dodder) seeds were germinated on moist filter paper or soil and transferred to healthy *N. occidentalis*. After three weeks of growth on healthy tobacco the dodder shoots were connected to *Prunus avium* F12 rootstock plants infected with LChV-1 or LChV-2. Experiments were done in an insect-proof greenhouse at temperatures between 20° and 30°C. After three months the parasite was removed completely from the virus donor and acceptor and put on young tobacco plants for maintenance. During this time the first virus detections were carried out. Virus detection. Total plant nucleic acid was extracted from cherry and tobacco leaves using a silica capture protocol (Rott and Jelkmann 2001). LChV-1 and -2 detection by RT-PCR was done as previously described (Jelkmann, Leible et al. 2008).

Results and discussion

In order to identify alternative hosts the use of *C. europea* as a vector was investigated in transmission trials (Fig. 1, 2). LChV-1 and -2 were graft inoculated onto *Prunus avium* F12 rootstocks and parasited by *C. europea*. Healthy *N. occidentalis* '37B' served as receptor host plant and could be infected systemically with LChV-1. Transmissions were done in the greenhouse over a period of up to 6 month. Virus detection in *Cuscuta* and *N. occidentalis* tissue was confirmed by RT-PCR. Attempts at propagation of LChV-1 by mechanical transmission to *N. occidentalis* failed, however the virus was serially transferred by grafting. This result is in accordance with our observations for GLRaV-7,

the most closely related virus of LChV-1 (Mikona and Jelkmann 2010). Virus transmission was not successful for LChV-2 under the same circumstances.

Fig. 1 and 2 Transmission experiment with *Little cherry virus* -1 (LChV-1) infected *Prunus avium* F12 rootstock plants, parasitic *Cusucta europea* acting as a vector, and healthy *Nicotiana occidentalis* 37B.

Symptoms on *N. occidentalis* '37B' consisted of curled leaves, reddening of leaf margins and veins, and decline. The severity of reduction in vegetative growth, and the survival of plants up to 6 months depended on the virus isolate.

After our recent reports for GLRaV-7 (Mikona and Jelkmann 2010), this is the second time that a virus in the family *Closteroviridae* was successfully transferred to a herbaceous plant by dodder. Transmissions of members of the family *Closteroviridae* by dodder have been reported earlier, however with the natural host as acceptor plant (Nariani and Raychaudhuri 1970;Fuchs and Reiss 1954). These results will allow attempts at virus purification from a non-woody host for the purpose of producing antibodies suitable for sensitive virus detection in ELISA. Antisera for LChV-1 produced from purified virus preparations, bacterially expressed protein, or a DNA vector that expressed the cloned coat protein (CP) gene *in vivo* have been described (Matic, Minafra et al. 2009a;KeimKonrad and Jelkmann 1996), however, the sensitivity was not suitable for virus detection in ELISA. Attempts will be continued to identify a herbaceous host for LChV-2.

Literature

Fuchs, W. and Reiss, U., 1954: Transmission of the Beet yellows virus (Corium betae) with the aid of Cuscuta. Naturwissenschaften **41**, 506.

Hosford, R. M., 1967: Transmission of Plant Viruses by Dodder. Bot.Rev. **33**, 387-406.

Jelkmann, W. and Eastwell, K. C., 2010: Little cherry virus -1 and -2. In: A. Hadidi, M. Barba, T. Candresse, and W. Jelkmann (eds.), Virus and Virus-Like Diseases of Pome and Stone Fruits, (in press). APS Press, St. Paul, MN 55121, USA.

Jelkmann, W., Fechtner, B., and Agranovsky, A. A., 1997: Complete genome structure and phylogenetic analysis of little cherry virus, a mealybug transmissible closterovirus. J.Gen.Virol. **78** Part 8, 2067-2071.

Jelkmann, W., Leible, S., and Rott, M. E., 2008: Little cherry closteroviruses -1 and -2, their genetic variability and detection by Real-Time-PCR. Acta Hortic. **781**, 321-329.

KeimKonrad, R. and Jelkmann, W., 1996: Genome analysis of the 3'-terminal part of the little cherry disease associated dsRNA reveals a monopartite clostero-like virus. Arch.Virol. **141**, 1437-1451.

Matic, S., Minafra, A., Boscia, D., da Cunha, A. T. P., and Martelli, G. P., 2009a: Production of antibodies to Little cherry virus 1 coat protein by DNA prime and protein boost immunization. J.Virol.Methods **155**, 72-76.

Matic, S., Minafra, A., Sanchez-Navarro, J. A., Pallas, V., Myrta, A., and Martelli, G. P., 2009b: 'Kwanzan Stunting' syndrome: Detection and molecular characterization of an Italian isolate of Little cherry virus 1. Virus Res. **143**, 61-67.

Mikona, C. and Jelkmann, W., 2010: Cuscuta species replicate Grapevine leafroll-associated virus -7 (GLRaV-7) and allow transmission to herbaceous plants . Plant Dis. (in press).

Nariani, T. and Raychaudhuri, S., 1970: Transmission of Citrus tristeza virus by Dodder, Cuscuta reflexa Roxb. Ann.phytopath.Soc.Japan **36**, 289-290.

Rott, M. E. and Jelkmann, W., 2001: Characterization and detection of several filamentous viruses of cherry: adaptation of an alternative cloning method (DOP-PCR), and modification of an RNA extraction protocol. Eur.J.Plant Pathol. **107**, 411-420.

Rott, M. E. and Jelkmann, W., 2005: Little cherry virus-2: Sequence and genomic organization of an unusual member of the Closteroviridae. Arch.Virol. **150**, 107-123.