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Sequencing studies for the identification and characterization of new and old *Rubus* viruses

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Abstract

In Europe, raspberry plants are commonly infected with a complex of aphid-transmitted viruses that together cause raspberry mosaic disease (RMD). During the previous 30 years, by grafting and vector transmission to a range of red and black raspberry cultivars, these viruses have been loosely characterized and identified as Raspberry leaf spot virus (RLSV), Raspberry leaf mottle Virus (RLMV), Black raspberry necrosis virus (BRNV) and Rubus yellow net virus. An additional, very widespread virus, Raspberry vein chlorosis virus (RVCV), is spread by a different aphid vector. Recently some sequence data have been obtained for RYNV, BRNV and Raspberry mottle virus (RMoV), a virus found in plants showing RMD symptoms. We have carried out sequencing studies using random amplification and mass analysis approaches and will present information on the relationship between RMoV, RLSV and RLMV, as well as the first data for RVCV and a novel, possibly segmented minus-strand RNA virus infecting raspberry.

Keywords: raspberry viruses, RLMV, RLSV, RVCV, RLBV

Soft fruit virology at SCRI

The purpose of soft fruit virus research at SCRI is to identify and characterize viruses causing disease in the soft fruit industry in Scotland and elsewhere, to obtain sequence information from these viruses enabling us to design diagnostic tools for testing of UK Soft Fruit Nuclear Stock material (maintained as a collection at SCRI), and to use this new knowledge ultimately to identify new or improved sources of virus resistance in raspberry and blackcurrant germplasm that can be used by fruit breeders at SCRI. Our current work looks at a range of different viruses, some that have been known for a long time and others are newly identified. For example, two of the most well understood viruses we study are Raspberry bushy dwarf virus (RBDV), a pollen transmitted virus for which an effective antibody test is available, and Blackcurrant reversion virus (BRV), currently the most important virus affecting blackcurrant which has been addressed by breeding for resistance to its vector, the gall mite *Cecidophyopsis ribis*. Viruses that are known to cause disease in raspberry but for which little or no sequence information is available include Raspberry leaf spot virus (RLSV), Raspberry leaf mottle virus (RLMV) and Raspberry vein chlorosis virus (RVCV). In addition, our work has identified two new viruses, Rubus chlorotic mottle virus (RuCMV) and Raspberry leaf blotch virus (RLBV), which are now undergoing more extensive study.

Clarification of relationship between Raspberry leaf spot virus (RLSV), Raspberry leaf mottle virus (RLMV) and Raspberry mottle virus (RMoV): Leaf spotting is a common disease symptom in raspberries, attributed in Europe to two viruses, RLMV (Cadman, 1951) and RLSV (Cadman, 1952). These viruses have the same aphid vector, the large raspberry aphid *Amphorophora idaei*, and are similarly heat-labile but are differentiated by host reaction – RLMV causes chlorotic spots on the red raspberry cultivars Malling Delight, Malling Landmark and St. Walfried, whereas, RLSV causes chlorotic spots on the cultivars Burnetholm, Glen Clova and Norfolk Giant. Recently a new closterovirus, RMoV, was isolated from an asymptomatic red raspberry plant in Washington state, USA and the complete sequence of the virus was determined (Tzanetakakis et al., 2007). As part of this study, material containing RLSV from Scotland was tested by RT-PCR and found to contain the same closterovirus. To examine the possibility that these three viruses are strains of the same closterovirus we have carried out a sequencing study comparing RLSV and RLMV from the SCRI virus collection, together with plant samples showing leaf spot symptoms newly collected at various farms in the UK. Using PCR primers based on the RMoV sequence we amplified and sequenced from RLMV and RLSV a 3kb region including the genes encoding the coat protein homologue (CPh), minor coat protein (CPm) and major coat protein (CP). In addition the same region was sequenced from a new RLMV isolate (PM1) that was collected from the field.

The results of this study showed that the RLMV and RLSV RNAs are 99 % identical over the 3kb region, and both are 97 % identical to RMoV. In addition, one of the field isolates, PM1, was found to be only 75 % identical to the other isolates in this region. Isolate PM1 was found in all locations that were sampled, RLMV was found in fewer locations and samples from some locations contained both isolate types. Furthermore, the CP amino acid sequences are 98 % identical between RLMV, RLSV and RMoV but there was only 78% CP sequence identity between PM1 and the three other viruses. Our conclusions from this work are that RLMV, RLSV and RMoV are all isolates of the same virus, and we propose all should now be referred to as RLMV as this was the earliest name to be given in the literature. The

taxonomic situation of isolate PM1 is less clear. Current ICTV guidelines suggest closterovirus species are demarcated by a CP amino acid sequence difference of 10 % or more which would mean that PM1 should be considered as a different virus to RLMV.

Design of a RT-PCR diagnostic for Raspberry vein chlorosis virus (RVCV): RVCV was first described in 1952 in Scotland, is extremely common in Europe and Russia, and also is present in Canada, and New Zealand. It is transmitted by the small raspberry aphid *Aphis idaei*, causing loss of vigour, fruit yield and fruit set in some cultivars. Large bacilliform particles found in infected plants and in sections of vector aphid (Jones *et al.*, 1974; Murant and Roberts, 1980), suggested RVCV might be a rhabdovirus, an enveloped, negative-strand RNA virus, able to replicate in both insect and plant hosts.

Several approaches were taken in order to obtain some sequence information for RVCV. We were unable to clone the virus using RNA extracted from material collected from virus-infected plants using polyethylene precipitation or gradient centrifugation, or by isolating dsRNA from infected plants or by RT-PCR amplification with rhabdovirus-specific degenerate primers of total RNA isolated from infected plants. Subsequently, we used rhabdovirus-specific degenerate primer RT-PCR of total RNA extracted from small raspberry aphids reared on RVCV-infected plants. This approach was successful, and RVCV-specific primers designed from the cDNA cloned obtained in these experiments are able to prime amplification of the virus both from infected plants and aphids. Phylogenetic analysis confirms that RVCV is a rhabdovirus that is most closely related to Strawberry crinkle virus (SCV).

A newly discovered virus of raspberry – Raspberry leaf blotch virus (RLBV): Leaf samples were collected from red raspberry plants growing at several farms in Scotland and showing strong symptoms of leaf malformation, leaf necrosis and blotchy discolouration that was most apparent on the underside of affected leaves.

Double-stranded RNA was extracted from these leaves and cloned by semi-random primer amplification. In addition, extracts of these leaves were inoculated to a range of herbaceous plants. Inoculation to *Nicotiana benthamiana* resulted in obvious symptoms of a systemic yellow-green mosaic or blotching. The infection could be passaged a limited number of times from *N. benthamiana* to *N. benthamiana*, and did not survive freezing. DsRNA cloning identified one small (200nt) cDNA with sequence related to wheat mosaic virus (previously High Plains Virus). This is a new group of viruses with a multipartite, negative-strand RNA genome that also includes pigeonpea sterility mosaic virus (PPSMV), fig mosaic virus (FMV) and European mountain ash ringspot-associated virus (EMARAV).

We have named this new virus raspberry leaf blotch virus (RLBV), have completed the sequencing of the RLBV nucleocapsid RNA, have raised an antibody to the nucleocapsid protein, and have designed a RT-PCR diagnostic that detects the virus in infected plants. The symptoms seen on infected raspberry plants were previously associated with an eriophyid mite, raspberry leaf and bud mite (*Phyllocoptes gracilis*). WMV, FMV and PPSMV are also associated with eriophyid mites, and experiments to show whether *P. gracilis* is responsible for transmission of RLBV are underway.

Literature

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