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Short communication

New host for raspberry bushy dwarf virus: arctic bramble (*Rubus arcticus*)

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Abstract

Raspberry bushy dwarf virus (RBDV) was detected in three new host plants in *Rubus* species, *i.e.*, arctic bramble (*R. arcticus* ssp. *arcticus*), Alaskan arctic bramble (*R. arcticus* ssp. *stellatus*) and their hybrid (*R. arcticus* L. nothosubsp. *stellarcticus* G. Larsson). The virus was identified as RBDV by the symptoms elicited in the test plants *Chenopodium quinoa* and *C. amaranticolor*, by sedimentation profile in sucrose density gradient, by RNA banding pattern in agarose gel electrophoresis, by protein analysis of the purified viruses in SDS-polyacrylamide gel electrophoresis, and by Western blotting. There was a high incidence of RBDV-infected plants in the experimental plots. The presence of the virus in arctic bramble did not always induce foliar symptoms. However, yellowing of the leaves around central and lateral veins was quite frequently observed, especially in spring and autumn.

Raspberry bushy dwarf virus (RBDV) is an RNA virus with a bipartite genome (Ziegler et al., 1993). RBDV differs from other plant viruses in its overall genome strategy, and it has been proposed that it should be placed in a distinct virus genus, idaeoviruses (Natsuaki et al., 1991; Ziegler et al., 1992). The biological and molecular similarities suggest that idaeoviruses should be included in the family *Bromoviridae* (Ziegler et al., 1993).

RBDV is seed-transmitted, but in established plants spread occurs through pollen, and both the maternal parent and seeds may become infected. No vector is known (Murant, 1976). RBDV is mechanically transmissible to a range of herbaceous host plants, but it has been found naturally only in *Rubus* species: red raspberry (*Rubus idaeus*), black raspberry (*R. occidentalis*), loganberry (*R. ursinus* × *R. idaeus*) and boysenberry [(*R. ursinus* × *R. idaeus*) × (*R. baileyanus* × *R. argutus*)] (Legg, 1960; Barnett and Murant, 1970; Converse, 1973).

Arctic bramble (*Rubus arcticus* ssp. *arcticus*), also called arctic raspberry, nectarberry, or arctic ruby, is a small fruit crop. It crosses with its closest relative Alaskan arctic bramble (*R. arcticus* ssp. *stellatus*), resulting in fully fertile arctic bramble hybrids (*R. arcticus* L. nothosubsp. *stellarcticus* G. Larsson). Because of its delicious berries, arctic bramble is a desired raw material for liqueur and food industry. It is also used in some countries in breeding programs for developing primocane fruiting raspberry cultivars or more aromatic berries. During the past few years, the cultivation of arctic bramble has markedly increased in Finland. One problem in the cultivation is that the annual yield fluctuates considerably and often is low. Occasionally, there are also symptoms like yellowing of the foliage, or interruption of fruit development and drying of the raw berries.

We have initiated research aimed at finding causes for the yield fluctuation and yellowing symptoms. This paper reports on the properties of a sap-transmissible virus isolated from raspberry, arctic bramble and arctic bramble hybrid in Finland and identified as raspberry bushy dwarf virus.

The virus transmission to the test plants *Chenopodium quinoa* and *C. amaranticolor* was first achieved in 1989 from young, vigorous shoots of the

raspberry breeding line (Malling Promise × Merva) × (Malling Promise × Merva) which showed a slight down-curling of the upper leaves; the raspberry leaf tissue was ground in 2% (v/v) nicotine solution (pH 9.8) and the carborundum-dusted leaves of the test plants were inoculated with the extract. Subsequent transfers from one *C. quinoa* to another were done by using 0.05 M Na-K-phosphate buffer (pH 7.0). The virus was subsequently isolated from one cultivated (cv. 'Asker') and two wild raspberries showing yellow foliage, a symptom also commonly occurring in RBDV-infected raspberries. In 1993, the virus was transmitted to *C. quinoa* from the leaves of two Finnish arctic bramble cultivars, 'Mespi' and 'Pima' (Ryynänen, 1972; Ryynänen and Dalman, 1983), as well as from seven clones of arctic bramble (Tammisola, 1988) and from the arctic bramble hybrid cultivar 'Sofia (Larsson, 1969), some of which showed yellowing of a marked proportion of the leaves around central and lateral veins. In the transmission, 0.05 M Na-K-phosphate buffer (pH 7.0), 2% polyvinylpyrrolidone (PVP, K30) was used.

The first systemic symptoms were observed in *C. quinoa* five to seven days after inoculation. At first, yellow or yellow-green small spots appeared on young, expanding leaves. Later, chlorotic rings and line patterns were observed. No obvious local lesions were detected. Also the infected *C. amaranticolor* test plants developed systemic mottle and ringspot symptoms on the youngest leaves 6 to 8 days after inoculation. All isolates derived from raspberry, arctic bramble or arctic bramble hybrid caused similar symptoms. The only exception was the appearance of small necrotic local lesions 5 days after inoculation, when the virus isolated from arctic bramble was repeatedly inoculated from one *C. quinoa* to another.

In the test plants which developed symptoms, the presence of RBDV was analyzed by gel double-diffusion test. Leaf extracts from *C. quinoa* infected with a virus isolate from the raspberry breeding line or from wild raspberry, or partially purified virus preparations were used as antigens. Plant sap was used undiluted or diluted 1:10. Reaction with an antiserum (diluted 1:20) raised against the Scottish RBDV strain D200 (Barnett and Murant, 1970) resulted in a sharp, single precipitin line without spur formation.

Four RBDV isolates were purified from 14- and 20-days-infected *C. quinoa*. Using the method described by Murant (1976) virus yields ranged from 0.2 to 3 mg of protein per 100 g leaves with an average of 1 mg and with the highest yield at 14 days.

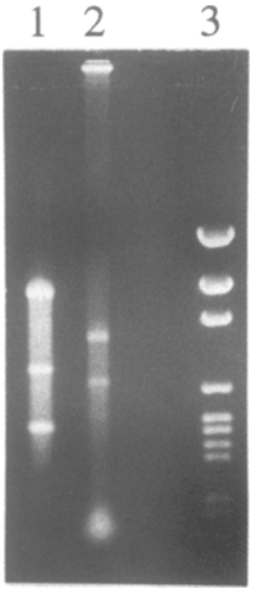

Figure 1. Agarose gel electrophoresis of RNA isolated from a purified RBDV preparation. The gel concentration was 0.8%. Lane 1, 5 μg of viral RNA; Lane 2, 5 μg of total RNA from healthy arctic bramble; Lane 3, pGEM molecular weight markers.

The sedimentation profiles of different virus isolates in sucrose density gradients were very similar, with one sharp absorbance peak. The only exception was an isolate from wild raspberry where a second small peak of a lower sedimentation coefficient appeared. The purified virus particles stained with 1% uranyl acetate, pH 4.2, were quasi-isometric in the electron microscope.

The purified virus particles were lysed as described by Murant et al. (1986). RNA was extracted with phenol/chloroform (Ausubel et al., 1988) and analyzed in agarose gel electrophoresis. Three RNA species with estimated sizes of about 1 kb, 2.2 kb and 6 kb were detected (Figure 1).

In SDS-polyacrylamide gel electrophoresis of total viral protein, a major polypeptide of about 30 kDa was detected by Coomassie brilliant blue staining. Some minor bands were present in preparations of raspberry or arctic bramble hybrid isolates but not in those of arctic bramble.

Western blot analysis was modified from Kokko and Kärenlampi (1992). The proteins from SDS-PAGE gel were transferred to activated PVDF membrane by electro-blotting in 10 mM CAPS [3-(cyclohexylamino)-1-propanesulfonic acid], 10% methanol for 30 min at 100 V. The membrane was blocked with PM-TBS (2% non-fat powdered milk, 10 mM Tris-

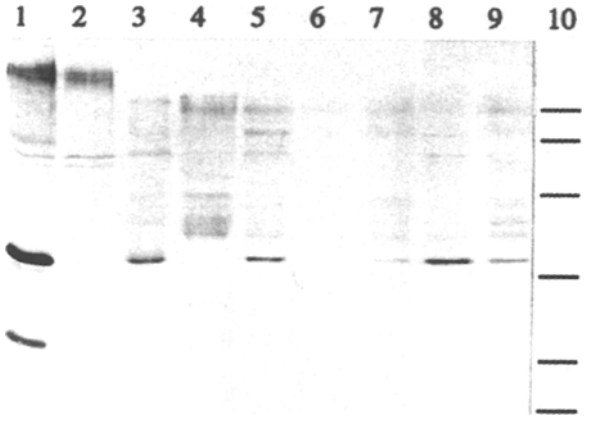

Figure 2. Western blot analysis of healthy and infected plants using anti-RBDV antibody. Antigens: 1, Infected *C. quinoa* leaves; 2, Healthy *C. quinoa* leaves; 3, Infected wild raspberry leaves; 4, Healthy raspberry leaves (Malling Landmark); 5, Infected leaves of arctic bramble; 6, Flowers of healthy arctic bramble; 7, Petals of infected arctic bramble; 8, Stamens of infected arctic bramble; 9, Flowers of infected arctic bramble; 10, Molecular weight markers: 14.4, 20.1, 30, 43, 67 and 94 kDa.

HCl, 140 mM NaCl, pH 7.4) for 30 min. The Scottish anti-RBDV antibody (1:1000) was used as the primary antibody and AP (alkaline phosphatase)-goat anti-rabbit antibody (1:500, Zymed, San Francisco, CA) as the second antibody (Harlow and Lane, 1988). The 30 kDa band was detected immunologically in leaf samples of RBDV-infected but not in uninfested *C. quinoa*, raspberry and arctic bramble plants (Figure 2). In arctic bramble, RBDV was also found in flowers and stamens, the latter containing much higher amounts of virus than petals. This agrees well with the observation that RBDV is pollen-transmitted (Cadman, 1965; Murant et al., 1974). In infected plant material, cross-reactions with proteins below c. 30 kD were shown only in *C. quinoa* and not in *Rubus* samples. This is not unexpected, since the viruses for both Western analysis and antibody production were isolated from *C. quinoa* by a method which obviously does not remove all host plant proteins. Thus, during the immunisation, antibodies have been raised also against *C. quinoa* proteins present as impurities in the virus preparations.

Distribution of RBDV was studied in the South Savo Research Station of the Agricultural Research Centre, Mikkeli, and Muuruvesi experimental field. In Mikkeli, 81 arctic bramble clones, two cultivars of arctic bramble, five arctic bramble hybrids and an Alaskan arctic bramble grow in individual test plots. The wild arctic bramble clones have been collected from various locations in Finland (Tammisola, 1988). The arctic bramble hybrid cultivars 'Sofia', 'Anna' and 'Linda' originate from Dr. Gunny Larsson, Swedish Agricultural University, Röbäcksdalen, Sweden (Larsson, 1969). The arctic bramble hybrid cultivars 'Aura' and 'Astra' have been described by Hiirsalmi et al. (1987). In the Muuruvesi experimental field planted in 1990, there were 70 randomly located test plots, 2 to 4 plots of each clone or cultivar: 'Mespi', 'Pima', 17 arctic bramble clones and all five arctic bramble hybrid cultivars. The plants were grown in 10 m rows with alternation of different genotypes to ensure pollination. Samples consisting of five leaves and five to ten flowers were collected from each plot.

RBDV was analyzed with DAS-ELISA (double-antibody sandwich). First, antibodies against RBDV (chicken-anti-RBDV) were raised in a chicken according to Kokko et al. (1994). The hens were immunised with an RBDV isolate (raspberry breeding line) purified from infected *C. quinoa*. Antibodies were separated from eggs as described by Kokko and Kärenlampi (1992). HRP-antibody (horseradish peroxidase) conjugate (chicken-anti-RBDV-HRP) of the crude IgY (immunoglobulin yolk) preparation was made according to the procedure described in the manual of Liddell and Cryer (1991).

Leaf and flower samples from healthy and infected plants were ground using a sap extractor (Erich Pollähne, Germany). The samples were diluted 1:10 in sample buffer (PM-TBS, 2% PVP). The wells of U-bottom Costar (PVC) microplates were coated with the chicken-anti-RBDV antibody. After incubation with the samples, the bound viruses were detected with chicken-anti-RBDV-HRP using ABTS [2,2′-azino-di(3-ethylbenzothiazoline) sulfonic acid] chromogen (Zymed, San Francisco, CA). Wells containing only the substrate buffer were used as blanks. The samples were analyzed in duplicate and healthy controls in triplicate. The samples were considered infected when their absorbance values exceeded two times the mean value plus two standard deviations of the mean values for healthy control samples.

RBDV was found in 1993 both in Mikkeli and in Muuruvesi (Table 1). Based on the leaf analyses from Muuruvesi, over 60% of all the plots were recorded RBDV positive. When flower samples were analyzed, over 80% of all the plots were recorded positive. Ten arctic bramble clones and the arctic bramble hybrid 'Aura' have not so far been recorded positive in any of the samples tested.

From all the studies performed – transmission to test plants, purification and sedimentation analysis,

Table 1. RBDV in leaf samples of *Rubus arcticus*. The clones/cultivars are grouped (healthy/infected) based on the ELISA values (A405). The healthy control plants ('Mespi' and 'Pima') were produced through meristem culture and subsequently grown in a greenhouse. There were 89 test plots with 89 clones/cultivars in Mikkeli, and 70 test plots with 24 clones/cultivars in Muuruvesi

		Number of clones/ cultivars	RBDV (A405) Mean ± SD[1]	Range[2]
Mikkeli				
R. a.	Control		0.084 ± 0.021	0.068 – 0.116
	Healthy	12	0.079 ± 0.019	0.059 – 0.114
	Infected	71	0.225 ± 0.068	0.149 – 0.436
R. s.	Infected	1	0.307 ± 0.033	0.284 - 0.330
Hybrid	Healthy	2	0.060 ± 0.000	0.055 – 0.065
	Infected	3	0.286 ± 0.009	0.277 – 0.296
Muuruvesi				
R. a.	Control		0.077 ± 0.014	0.067 – 0.096
	Healthy	2	0.107 ± 0.019	0.091 – 0.158
	Infected	17	0.477 ± 0.090	0.201 – 0.617
Hybrid	Healthy	1	0.096 ± 0.002	0.094 – 0.097
	Infected	4	0.560 ± 0.099	0.306 – 0.637

[1] Means and standard deviations calculated from the means of duplicate assays for each clone/cultivar;
[2] Minimum and maximum values for each group;
R. a., arctic bramble; *R. s.*, Alaskan arctic bramble

electron microscopy, immunological and RNA analyses – it is evident that the virus isolated in Finland first in 1989 from raspberry and later in 1993 from the new hosts arctic bramble and its hybrid with Alaskan arctic bramble, is RBDV. In SDS-polyacrylamide gel electrophoresis of the purified virus, one major polypeptide was observed, as detected earlier in raspberry (Murant et al., 1986). In agarose gel electrophoresis, RNA extracted from the virus particles showed three bands of sizes corresponding with the published sequences for these species (Mayo et al., 1991; Natsuaki et al., 1991, Ziegler et al., 1992).

RBDV has been shown to cause stunted growth and significant decreases in fruit size and yield (Daubeny et al., 1978). Infection with this virus has been associated with fruit abortion or 'crumbly fruit' (Murant et al., 1974; Daubeny et al., 1978) and the virus has been implicated as a causal agent of the 'yellows' disease in raspberry (Jones et al., 1982). Interruption of the druplet development is seen occasionally in the Finnish arctic bramble cultivations, leading to an almost complete loss of crop in the affected fields. Yellowing of the leaves is also common in arctic bramble, particularly in spring and autumn. No association has so far been observed between the presence of RBDV and the symptoms developed in arctic bramble or its hybrid. In fact, the virus can often be detected by ELISA in plants showing no symptoms of disease. In accordance, although RBDV commonly occurs in raspberry in North America and New Zealand, bushy dwarf disease is not reported (Jones and Wood, 1979). Yellowing is a common symptom but consistent leaf yellowing of RBDV has been found by Wood (1995) in one red raspberry cultivar ('Autumn Britten') in New Zealand. Blackberry proved unreliable, and leaf symptoms or growth degeneration were not found on boysenberry or loganberry graft-inoculated with RBDV. Further clarification of the correlation between symptoms and disease in Rubus species would benefit from more accurate molecular biological methods.

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