## LETTER TO THE EDITOR

## TRANSMISSION OF RASPBERRY BUSHY DWARF VIRUS BY SEED OF CHENOPODIUM QUINOA

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Raspberry bushy dwarf virus (RBDV), the only member of the genus *Idaeovirus*, is a naturally occurring virus that infects susceptible raspberry and blackberry, and is worldwide distributed on *Rubus* species (1). RBDV causes significant yield losses, cane vigour decline and drupelet abortion, and spreads by pollen and seed through field (2). Two serologically indistinguishable strains have been described. Common or Scottish (S) strain infects only a restricted range of red raspberry cultivars and *Rubus* species whereas the resistance-breaking (RB) strain infects nearly all red raspberry cultivars and *Rubus* species tested (10). In the Czech Republic, RBDV was found for the first time in cultivated red raspberry in 1994 (3). Later, frequent RBDV infections were detected in germplasm collections, propagative material and field plantings of raspberry (4).

In this letter, we present results on transmission of two isolates of RBDV by seed of *Chenopodium quinoa*. This is the first report on RBDV transmission by seed of nonrosaceous host plant.

Isolates of VLBR and VLG were obtained from infected raspberry cultivars Bulharský rubín and Gatineau, respectively, originating from the Breeding Station at Velké

Losiny, Northern Moravia. The VLBR isolate belongs to the RB strain of RBDV (4), and according to our recent results from grafting experiments, the VLG isolate is supposedly the S strain of the virus. Both isolates were maintained on *C. quinoa*.

Virus inoculum was prepared by homogenisation of 1 g of *C. quinoa* leaves with symptoms of infection in 2% nicotine solution in water. Fifteen plants of *C. quinoa* originating from Wageningen, the Netherlands, were mechanically inoculated with each isolate using carborundum powder as abrasive. Control plants of *C. quinoa* were inoculated with 2% nicotine solution in water. Plants were kept in an insect-proof greenhouse at 18 – 25°C and virus infection was verified by an enzyme-linked immunosorbent assay (ELISA). Seed was harvested from ELISA-positive plants and stored at 4°C for 2 years.

A hundred seeds of C. quinoa each infected with VLBR and VLG isolate and of healthy control plants were sown in 2 x 2 cm areas of sterilised soil in a greenhouse. Germination of the seed was observed and the seedlings were tested by double antibody sandwich ELISA (5) at four-leaf stage using the RBDV Diagnostic Kit of Loewe Biochemica (Germany). Leaf samples were homogenised and diluted 1:20 (w/v) in phosphate-buffered saline (PBS) buffer pH 7.4 containing 2% polyvinylpyrrolidone and 0.2% bovine serum albumin. Samples with  $A_{405}$  higher than  $x \pm 3$  SD, where x = x value of healthy control and SD = standard deviation, were scored as positive (6).

**Abbreviations:** ELISA = enzyme-linked immunosorbent assay; PBS = phosphate-buffered saline; RBDV = raspberry bushy dwarf virus

Freeze-dried leaves of *C. quinoa* infected with VLBR and VLG isolate were used as positive controls.

All plants of *C. quinoa* inoculated with VLBR and VLG isolates were positive in ELISA yielding A<sub>405</sub> values over 1.8 and showing symptoms of mottling and leaf deformation. The virus was not detected in control plants of *C. quinoa*. Germination of the seed infected with VLBR and VLG isolates was 76% and 75 %, respectively, whereas germination of the healthy control seed was 91%. Some seedlings revealed faint symptoms of leaf mosaic, which were more pronounced with ageing of the plants. Results on the virus transmission by seed are summarised in the table.

RBDV isolate	VLBR	VLG
No. of seedlings tested	76	75
No. (%) of seedlings infected	28 (37%)	51 (68%)
A <sub>405</sub> , healthy control <sup>a</sup>	$0.048 \pm 0.010$	$0.048 \pm 0.010$
A <sub>405</sub> , RBDV-positive control <sup>a</sup>	$0.572 \pm 0.081$	$0.273 \pm 0.070$
A <sub>405</sub> , infected seedlings <sup>a</sup>	$0.183 \pm 0.100$	$0.196 \pm 0.102$

<sup>&</sup>lt;sup>a</sup>The data represent mean ± 3SD.

Transmission of RBDV in plants of the family Rosaceae by seed was discovered by Cadman (7) who found that 30 – 40% of the seedlings from RBDV-infected Lloyd George raspberry were infected. Barnett and Murant (8) confirmed that RBDV was transmitted by seed and identified the virus serologically in 26% of progeny seedlings from raspberry infected with D200 isolate belonging to the common strain of RBDV. Jones et al. (9) demonstrated 14% RBDV transmission by seed of R. idaeus, R. sachalinensis and R. vulgatus buschii. Seed transmission of RB isolate of RBDV by seed was demonstrated by Barbara et al. (10) with seed of cv. Carnival imported from USSR. In contrast to the results with raspberry, infected Fragaria vesca plants yielded only 2 infected seedlings out of 64 tested (11).

Barnett and Murant (8) transmitted RBDV by inoculation of sap to 55 species of 12 families of flowering plants and infected most of them symptomlessly. RBDV caused

systemic symptoms in some species of *Amaranthaceae*, *Chenopodiaceae* and *Cucurbitaceae*, but seed transmission of the virus was not tested, although some of those species are currently used as assay hosts for viruses infecting small fruits and fruit trees.

According to our best knowledge, the only experiment with RBDV transmission by seed of *C. quinoa*, a nonrosaceous host, was conducted by Murant *et al.* (11). They found no infection in 100 seedlings from seed set by RBDV infected plants. The high percentage transmission of two isolates of RBDV in our experiments supported by ELISA data clearly demonstrated a possibility of RBDV transmission by seed of *C. quinoa*, a non-rosaceous host.

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## **ERRATUM**

The title of the article by M. Chen, M.Y. Fan, D.Z. Bi, J.Z. Zhang and X.R. Chen that appeared in Acta virologica 42 (No. 2), 91–93 (1998) should read "SEQUENCE ANALYSIS OF A FRAGMENT OF rOmpA GENE OF SEVERAL ISOLATES OF SPOTTED FEVER GROUP RICKETTSIAE FROM CHINA".