

Some properties of the genome of *Raspberry bushy dwarf virus (RBDV)*

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Background

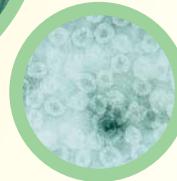
RBDV, genus *Idaeovirus*, occurs in wild and cultivated *Rubus* species and is an increasing problem in many raspberry-producing areas throughout the world. In nature, RBDV is transmitted via pollen and may induce yellows disease and/or crumbly fruit in infectible cultivars, which can affect fruit quality. RBDV particles are quasi-isometric with a diameter of about 33nm.



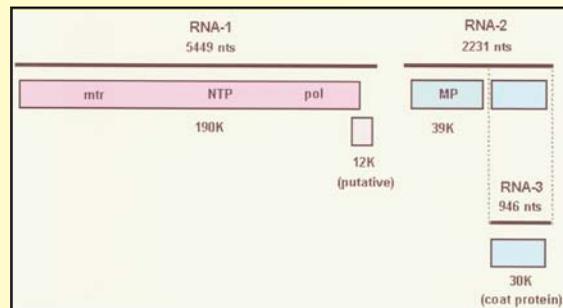
Crumbly Fruit



Yellows Disease



RBDV particles



Genome of RBDV based on RNA sequences

The bipartite genome is positive sense ssRNA. RNA-1 (5.4kbp) contains a large ORF encoding the proteins necessary for virus replication. At the 3' end of this ORF we recently identified a second, overlapping, out-of-frame ORF that encodes a putative 12K polypeptide. The position of this gene corresponds to that of the 2b gene in the genome of *Cucumber mosaic virus*. All RBDV isolates sequenced to date contain the ORF in this 3' region.

We report attempts to detect the gene transcript and its putative translation product in plants and to assess the possible role(s) of this putative 12K protein by developing infectious RNA clones and a 12K deletion mutant.

Results and Conclusions

i. Infectivity of RNA transcripts. In *in vitro* transcription studies, cloned full-length cDNA of all 3 RBDV RNAs under the control of the T7 polymerase promoter yielded full-length transcripts. When manually inoculated to herbaceous test plants, these transcripts were infective as assessed by ELISA.

iv. Effect of 12K deletion on infectivity. Plants were inoculated with transcripts either of RNA-1+2+3 or, RNA-1# (RNA-1 mutated to knock out the putative 12K gene)+2+3. ELISA of inoculated plants showed that the putative 12K gene is not essential for infectivity in herbaceous plants (Table 2).

Table 2

	Inoculated leaf
RNA 1+2+3	2.38* ± 0.55
RNA 1#+2+3	2.66* ± 0.14

*ELISA values for leaves harvested 14 dpi

ii. Effect of RNA-3 on infectivity. Plants inoculated with transcript RNA either as, RNA-1+2+3 or, RNA-1+2 (10µg per plant of each RNA species), indicated that the presence of RNA-3 significantly increased ELISA values in inoculated leaves, and the likelihood of establishing systemic infection (Table1).

Table 1

	Inoculated leaf	Uninoculated leaf
RNA-1+2+3	2.41* ± 0.44	0.30* ± 0.18
RNA-1+2	0.44* ± 0.23	0.20* ± 0.05

*Mean ELISA values from 2 expts of leaves assayed 14 dpi



iii. Attempts to detect the 12K message and protein in plants. To detect the 12K mRNA in RBDV-infected plants, Northern blots of RNA extracted at various times after inoculation were probed with cDNA to the 12K sequence; no transcript of the putative gene was detected. Similarly, Western blots of protein extracted from infected plants, were probed with antibody to a synthetic polypeptide derived from the 12K sequence; no protein was detected.

v. Attempts to detect silencing suppressor activity of 12K. To determine if the putative 12K gene resembles the CMV 2b gene in suppressing post-transcriptional gene silencing, cDNA to the 12K gene was used in 3 different assays for such activity. To date, the results have been negative or inconclusive.