European Journal of Plant Pathology **106:** 623–632, 2000.

Comparisons of some properties of two laboratory variants of *Raspberry bushy dwarf virus* (RBDV) with those of three previously characterised RBDV isolates

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Accepted 30 May 2000

Key words: monoclonal antibody, nucleotide sequence, RT-PCR, RNA

Abstract

The properties of two laboratory variants of *Raspberry bushy dwarf virus* (RBDV), genus *Idaeovirus*, were compared with those of their parental sources and with two naturally occurring variants. Isolate RB is a natural variant able to overcome the resistance to RBDV present in some red raspberry cultivars. Isolate M is a serological variant from black raspberry. Laboratory variant D1, was derived from the Scottish type isolate (D200) by continuous sub-culture in *Chenopodium quinoa*. Laboratory variant Can-S was derived from an isolate infecting Canby red raspberry in Canada (Can) after passage through *Nicotiana benthamiana*. All isolates reacted with a polyclonal antiserum to isolate D200 in agarose gel double-diffusion tests but, whereas isolates D200, RB, Can and Can-S were serologically indistinguishable, the precipitin lines formed by these isolates each spurred over those formed by isolates D1 and M. All six isolates reacted strongly with the polyclonal antiserum in double antibody sandwich and plate-trapped antigen (PTA) forms of ELISA and in Western blotting (WB) and when each of four monoclonal antibodies (Mabs) to an unnamed red raspberry isolate from Canada was used to detect antigen trapped by the polyclonal antiserum. However, the virus isolates differed in their reactions to these four Mabs in PTA-ELISA and in WB. Isolates RB, Can and Can-S behaved similarly in these tests as did isolates D200 and D1, but isolate M was distinct. In herbaceous test plants, variants D1 and Can-S were readily distinguished from their parental sources and from the other two isolates by producing either no symptoms (D1) or very severe symptoms (Can-S) in hosts. Unlike all other isolates studied world-wide, Can-S failed to infect *C. quinoa* systemically but induced severe necrotic local lesions in this and other hosts.

Reverse transcription-polymerase chain reaction was used to amplify the gene encoding the coat protein (CP) in RNA-2, and a region of the gene encoding the polymerase in RNA-1. The nucleotide sequences of the CP genes of the six isolates were > 96% identical but isolate Can-S was the most distinctive. However, the similarity between Can-S and its parent isolate (Can) was no greater than the similarity between Can-S and the other isolates, suggesting that Can-S may not have arisen as the result of a mutation from isolate Can. Sequence comparisons of parts of the polymerase gene of isolates R15, D1, D200 and Can-S showed that they were 95–98% identical.

Introduction

Raspberry bushy dwarf virus (RBDV), genus *Idaeovirus*, occurs in *Rubus* species worldwide (Jones et al., 1996). In some red raspberry cultivars (*R. idaeus* var. *idaeus* L., *R. idaeus* var. *strigosus* Maxim.), it induces yellows disease and/or crumbly fruit and/or degeneration in vigour (Jones et al. 1982; 1996). Most studied isolates of RBDV have similar properties although Jones et al. (1996) categorised

known isolates into three groups. These are

— S isolates, those similar to the Scottish type isolate, especially in their *Rubus* host range; most isolates studied world-wide fall into this group;
— RB isolates, those similar in serological properties and symptomatology in herbaceous test plants to S isolates but distinguishable from them by infecting *Rubus* species and cultivars immune, or very resistant, to S isolates;
— B isolates, Murant and Jones (1976) found that RBDV isolates obtained from American black raspberry (*R. occidentalis*) were serologically distinguishable from those of S and RB isolates and had a lower specific infectivity. However, as only 3 such isolates from *R. occidentalis* L. have been studied in any detail it is not known if these are features common to all isolates from this host species (Jones et al., 1996).

Apart from one or two isolates from each of these groups (Barnett and Murant, 1970; Converse, 1973; Murant et al., 1982), few isolates have been studied in any detail. In this paper, some biological and molecular features of two group S isolates that developed variants during laboratory culture are reported. One, derived from the Scottish type isolate, D200, produced a serologically distinct form with greatly attenuated symptoms in herbaceous test plants. The other, derived from an isolate from the red raspberry cv. Canby from Canada, after passage through *Nicotiana benthamiana* Domin., produced a variant (Can-S) that induced more severe symptoms in herbaceous test plants than its parental culture. For comparison, the characteristics of a group RB isolate from red raspberry and a group B isolate from black raspberry are also described.

Materials and methods

RBDV isolates. The parental cultures of group S RBDV isolates from which the variants were obtained are: isolate D200, the type culture of RBDV described by Barnett and Murant (1970), and isolate Can, obtained by AF Murant from Canby red raspberry in Canada. For comparison, isolate RB, a group RB isolate obtained from the red raspberry selection, EM 4427 from Horticulture Research International, East Malling, England, and isolate M, a group B isolate from Munger black raspberry in North America, were also used.

Mechanical inoculation of plants. Plants were grown in an aphid-proof glasshouse kept at 20 °C. Serial transmission of virus in herbaceous plants was by mechanical inoculation of sap extracts (usually to *Chenopodium quinoa* Willd.) in water and the application of the extract with a finger to corundum-dusted leaves of test plants. Assessments of viral host range and the induction of symptoms in plants, were made on several occasions throughout the year and the results presented are the composite picture obtained from these different tests.

Serological tests. Gel double-diffusion tests were in 0.7% agarose in saline containing 0.02% sodium azide. Antigens, in sap of *C. quinoa*, were tested against a rabbit polyclonal antiserum to isolate D200 (Barnett and Murant, 1970). For double antibody sandwich (DAS)-ELISA, wells were coated with the rabbit polyclonal antiserum to isolate D200 to trap virus antigen and detection of this antigen was by a monoclonal antibody (Mab, M4) to a Canadian isolate of RBDV from red raspberry (Martin, 1984); this Mab was detected using an alkaline phosphatase-labelled antimouse antibody (Sigma). For plate-trapped antigen (PTA)-ELISA, virus antigen was trapped directly onto ELISA plates and the trapped antigen detected by the polyclonal antibody or each of the four Mabs to the Canadian RBDV isolate (Martin, 1984). In all tests, a positive reaction was recorded when the $A_{405\,nm}$ values were more than twice that of healthy controls but in practice, assays of all positive samples typically produced $A_{405\,nm}$ values more than 3× those given by healthy controls.

Immunoblotting. Immunoblotting for RBDV coat protein (CP) was done using extracts of infected *C. quinoa*. Extracts for each RBDV isolate were made in parallel for each experiment. Partially clarified sap extracts were boiled for 5 min in SDS buffer before electrophoresis in a 10% (w/v) polyacrylamide gel for 5 h at 120 V. Following electrophoresis, proteins were transferred to nitrocellulose membrane in a Bio-Rad Trans Blot Cell at 30 V overnight in 190 mM glycine, 25 mM Tris, 20% (v/v) methanol, pH 7.0. After transfer, the nitrocellulose was blocked by incubation at room temperature with 5% (w/v) dried skimmed milk (Marvel: Cadbury Schweppes) in TBS buffer, pH 7.4 (10 mM Tris, 15 mM NaCl, 0.05% (v/v) Tween 20). The nitrocellulose was incubated for 2 h at room temperature with antiserum diluted 1 : 1000 in extracts of healthy *C. quinoa* plants in TBS buffer containing

5% (w/v) dried skimmed milk. After washing 3× in TBS, the membranes were incubated for 2 h with a mouse anti-rabbit antibody conjugated with alkaline phosphatase. Membranes were then washed 3× in TBS buffer before incubating with 5-bromo-4-chloro-3-indolyl-phosphate *p*-toluene salt (15 mg/ml in dimethyl formamide) and *p*-nitroblue tetrazolium chloride (30 mg/ml in 70% (v/v) dimethyl formamide) in 100 mM Tris, 100 mM NaCl, 10 mM $MgCl_2$. The reactions were stopped by rinsing the membranes in water.

Primers for polymerase chain reaction (PCR). Based on the nucleotide sequences determined for RNAs of isolate R15, a sub-culture of isolate RB (Mayo et al., 1991; Natsuaki et al., 1991; Ziegler et al., 1992), primers were designed to amplify specific regions of the RBDV genome by using the PCR. Primers corresponded to, (1) nucleotides 5036-5054 and complementary nucleotides 5447-5427 either side of a putative gene on RNA-1 that codes for a 12 K protein (Jones et al., 1996), (2) to nucleotides 1240-1255 and complementary to nucleotides 2228-2214, either side of the CP gene on RNA-2 (Mayo et al., 1991; Natsuaki et al., 1991), (3) to nucleotides 2419-2438 and complementary to nucleotides 5447-5429 of the 3′ region of RNA-1 (Ziegler et al., 1992).

PCR analysis. Nucleic acid, extracted from RBDV-infected leaves of *C. quinoa* using the Qiagen RNeasy RNA extraction kit, was used as template for reverse transcription (RT). The RT reaction mixture consisted of, 2 µl target RNA, 2 µl 10× PCR buffer (Boehringer Mannheim), 2 µl dNTPs (10 mM), 1 µl of downstream primer (100 $\mu g.ml^{-1}$), 1 µl $MgCl_2$ (50 mM), 0.25 µl RNasin (Promega), 0.25 µl MuMLV reverse transcriptase (Promega), and was made up to 20 µl with sterile distilled water. All 20 µl of the cDNA from the RT reaction was used in the PCR mixture which consisted of 0.5 µl *Taq* DNA polymerase (Boehringer Mannheim), 1 µl second primer, 60 µl 1× PCR buffer (Boehringer Mannheim). The PCR conditions in a Hybaid Omnigene machine were, 5 min at 94 °C, followed by 30 cycles of 1 min at 94 °C, 1 min at 55 °C, 2 min at 72 °C, and then 10 min at 72 °C. PCR products were analysed by electrophoresis in 10% polyacrylamide gels for 5 h at 160 V. A 1 kb molecular weight marker (Gibco) was used.

Cloning and sequencing. In Scotland, cDNA fragments were prepared by RT-PCR using primers for the CP gene or primers for the 3′ region of RNA-1 and cloned in pGEM-T (Promega). Purified plasmid DNA was sequenced using Applied Biosystems (ABI) dideoxy terminator cycle sequencing according to the manufacturer's protocol. The forward and reverse universal sequencing primers were used and the sequence was obtained for nucleotides within about 500 nucleotides of the primer binding site. Sequence was determined using an ABI 373A DNA sequencer. Sequences were analysed, aligned and compared using programs in the GCG Package (Devereux et al., 1984).

In Finland, the solid-phase sequencing method was used for the analysis of the RNAs encoding the CP from the IC-RT-PCR amplified samples as described by Kokko and Kärenlampi (1998).

The complete or near complete CP gene sequences have database accession numbers of, AF259794 (M), AF259795 (D200), AF259796 (D1), AF 259797 (Can), and AF259798 (Can-S).

Results

Initial identification of the laboratory variants of RBDV

After many years of passaging in *C. quinoa*, a sub-culture of isolate D200, termed D1 (Murant et al., 1986), was found that induced greatly attenuated symptoms in this and other herbaceous host plants. By contrast, following passage of an RBDV isolate (Can) from Canby red raspberry, in *N. benthamiana*, a culture was obtained that induced chlorotic/necrotic local lesions followed by systemic chlorotic rings, symptoms that had not been evident previously in this host species during several previous passages. Such diseased plants were found to contain only RBDV and, as the properties of this new sub-culture were consistently different from its parent culture and from other RBDV isolates, this variant, termed Can-S (severe), was studied further.

Properties of isolates D1 and Can-S and their comparison with other RBDV isolates

Herbaceous host range and symptomatology. Table 1 gives the host range and symptomatology in some commonly used herbaceous test plants of five of the RBDV isolates studied. It shows that, with few exceptions, isolates D200, RB and Can were very similar in their behaviour whereas the two variant isolates (D1, Can-S) differed greatly in their behaviour, both from these

Table 1. Herbaceous host range and symptomatology of five RBDV isolates*

Plant species	D200	D1	RB	Can	Can-S
C. amaranticolor	S, lcl, sm	S	S, lcl, scr	S, cll, scr	I
C. murale	·	·	·	I,lnl	I
C. quinoa	S, lcl, sm	S	S, lcl, scr	S, cll, scr	I, lnl
Cucumis sativus	·	·	·	S	S, lcl
Datura stramonium	·	·	·	I, lcl	I
Lycopersicon esculentum	O	O	O	O	O
N. benthamiana	S	S	S	S	S, lnl, scr
N. clevelandii	S	S	S	S	S,lnl,sn
N. debnyii	O	S	S	O	I?, lcl
N. glutinosa	O	O	O	O	I, lcl
N. megalosiphon	S	S/O	S, lcr	·	·
N. occidentalis 37B	S	O	S	S	S, nll
N. occidentalis P1	S, lcr	I	S, lcr	S	S, lcl, sns
N. tabacum cv. Japan X	·	·	·	I	I, lcl
N. tabacum cv. Samsun-nn	S	S	S	O	I, lcl
N. tabacum cv. White Burley	·	·	·	·	S, lnr
N. tabacum cv. Xanthi-nc	·	·	·	·	I, lcr
P. hybrida	I, Lnl	O	I, lpnl	O	O
P. nana compacta	I, Lnl	I, lnl	O	·	·
P. hybrida cv. Rose	I, lnl	O	I, lnl	·	·
P. hybrida cv. Red	O	O	I, lpnl	·	·
P. hybrida cv. Blue	I, Lnl	O	I, Lnl	·	·
Spinacia oleracea	·	·	·	I	S, lcl

*The behaviour of isolate M is very similar to that of isolate D200 (data not shown). I = only inoculated leaves infected; S = inoculated and uninoculated leaves infected; O = not infected; lcl = local chlorotic lesions; sm = systemic mosaic; sn = systemic necrosis; lcr = local chlorotic rings; Lnl = large necrotic local lesions; lnl = local necrotic lesions; lnr = local necrotic rings; lpnl = local pin-point necrotic lesions; scr = systemic chlorotic ringspots; sns = systemic necrotic spots; · = not tested.

three isolates and from each other. Characteristically, isolate D1 failed to induce noticeable symptoms in any host except for occasional local lesions in *Petunia nana compacta* (Table 1; Figure (1A,B)), although at certain times of the year and under ill-defined conditions, it very occasionally induced a very weak systemic chlorotic mottling in *C. quinoa*. By contrast, its parental source, D200, and isolates RB and Can, reliably induced obvious symptoms throughout the year that were typical of RBDV in species of *Chenopodium* (Figure 1(A)) and *Petunia* (Figure 1(B)). In earlier studies, isolate M was found to be similar to isolate D200 in host range and symptomatology (AT Jones and AF Murant, unpublished data).

In contrast to the four other RBDV isolates studied, and indeed to any of the many RBDV isolates studied at SCRI over many years, isolate Can-S consistently induced much more severe symptoms in infected plants (Table 1). These severe symptoms were most noticeable in species of *Chenopodium* (Figure 2(A)) and *Nicotiana* (Figures 2(B–D)). Furthermore, it infected *N. glutinosa* L., which was immune to the other isolates tested (Table 1). Unexpectedly, and unlike all other RBDV isolates studied world-wide, Can-S was detectable by ELISA or infectivity assays in only the inoculated leaves of *C. quinoa* (Table 1) and was unable to spread systemically in this plant species.

Serological tests. In agarose gel double-diffusion serological tests using polyclonal antiserum to isolate D200 and antigens in sap of *C. quinoa*, all RBDV isolates produced a distinct precipitin line. However, the precipitin line formed against isolate D200 spurred over that produced by isolates D1 (Figure 3(A)) and M (Figure 3(B)), and, in these tests, isolate M appeared to be serologically indistinguishable from isolate D1 (Figure 3(B)). In similar tests, isolates D200, RB, Can and Can-S were serologically indistinguishable (Figure 3(A,C)). Indeed, in similar tests at SCRI over many years, with the exception of isolate D1 and group B isolates (black raspberry isolates) (Murant and Jones, 1976; Jones et al., 1996), all of many isolates of RBDV

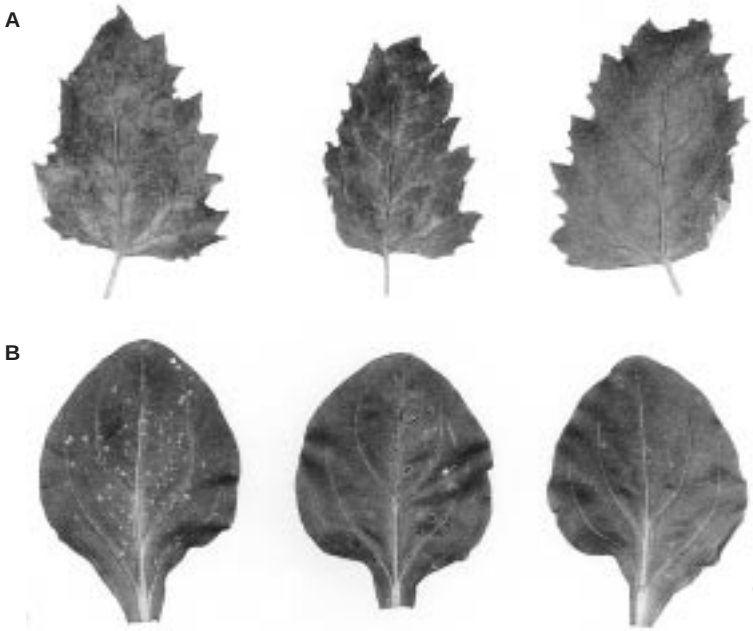

Figure 1. Comparison of the reactions to inoculation with RBDV isolates RB (left), D200 (centre) and D1 (right) in herbaceous test plants. (A) Systemic chlorotic line-patterns and ringspots induced by isolates RB and D200 and symptomless infection by isolate D1 in *C. quinoa*. (B) Necrotic lesions induced by isolates RB and D200 and symptomless infection by isolate D1 in inoculated leaves of *P. nana compacta* cv.'Blue'.

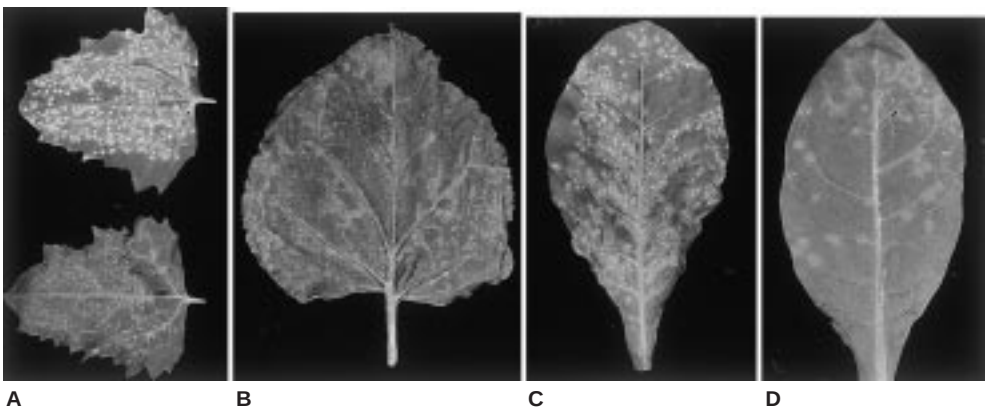

Figure 2. Symptoms induced by RBDV isolate Can-S in some herbaceous test plants. (A) Chlorotic/necrotic lesions in a leaf of *C. quinoa*, four (bottom) and seven (top) days after inoculation. (B) Systemic chlorotic line-patterns and rings in *N. benthamiana*. (C) Necrotic local lesions in a leaf of *N. occidentalis* accession 37B. (D) Chlorotic/necrotic local lesions in a leaf of *N. tabacum* cv. Xanthi-nc.

from several different countries and *Rubus* species were found to be serologically indistinguishable from isolate D200 (Murant et al., 1982; Jones et al., 1996; AT Jones, unpublished information).

In further tests to identify possible serological differences amongst the six RBDV isolates, each was assessed for its reaction in ELISA with four different Mabs prepared to a Canadian isolate of RBDV

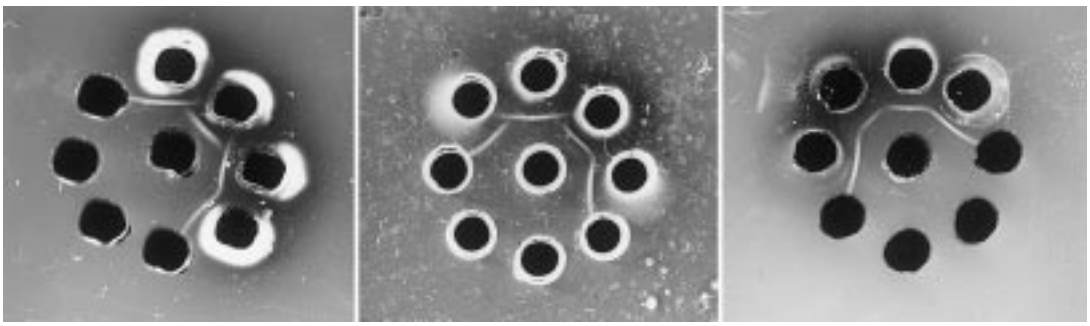

Figure 3. Agarose gel double-diffusion serological tests of RBDV antigens in sap of *C. quinoa* and RBDV polyclonal antiserum to isolate D200 diluted 1 : 20. (A) Precipitin lines formed by isolates D200 and RB spur over those formed by isolate D1. In this test isolates D200 and RB are indistinguishable. (B) Precipitin line formed by isolate D200 spurs over that formed by isolates D1 and the black raspberry isolate, M. In this test isolates D1 and M are indistinguishable. (C) Confluent precipitin lines formed by isolates Can, Can-S and D200, showing that in this test these isolates are indistinguishable.

Table 2. Reactions of the six RBDV isolates in DAS-ELISA, PTA-ELISA and in WB with a polyclonal antiserum to isolate D200 (Poly) and to four Mabs to a Canadian isolate of RBDV (M1, M2, M3, M4)

Isolates	Poly			M1			M2			M3			M4		
	DAS	PTA	W	DAS	PTA	W	DAS	PTA	W	DAS	PTA	W	DAS	PTA	W
M	·	++	++	++	++	+	+++	++	+/−	+++	++	+	+++	++	+
D1	·	++	+++	++	+/−	+++	+++	++	+	+++	++	++	+++	++	+++
D200	·	++	+++	++	−	+	+++	++	+/−	+++	++	−	+++	++	+
RB	·	++	+++	++	−	++	+++	+/−	+	+++	+/−	+++	+++	+/−	+++
Can-S	·	++	+++	++	−	++	+++	+/−	+++	+++	+/−	++	+++	+/−	++
Can	·	++	+++	++	−	++	+++	+/−	++	+++	+/−	++	+++	+/−	+

In each instance, '−' represents no detectable reaction; +/− represents borderline reactions; the numbers of '+' represent the relative strength of reactions ($A_{405\,nm}$ values for DAS- and PTA-ELISA, the strength of the staining for WB – see Figure 4) where '+' is the weakest reaction and '+++' the strongest; and · represents not tested.

from raspberry (Martin, 1984). In DAS-ELISA, using RBDV isolates in infected sap of *C. quinoa*, all four Mabs reacted to each virus isolate and, although the $A_{405\,nm}$ values obtained using Mab1 were consistently lower than those obtained using other Mabs, no obvious or consistent differences were detected between the reactions of any of the isolates (Table 2). In PTA-ELISA using the polyclonal antiserum, all virus isolates were detected readily, whereas using the Mabs only isolate M was detected reliably by Mab1, and only isolates M, D200 and D1 by the other three Mabs (Table 2).

In Western blot (WB) analysis using the polyclonal antiserum prepared against isolate D200, all six RBDV isolates in sap of *C. quinoa*, produced a distinct band of CP with a M_r *c.* 30 000, as expected for RBDV virus particle protein (Figure 4) (Jones et al., 1996). However, in several different experiments, the mobility of the CP of isolates RB and Can was consistently slower than those of the other isolates (Figure 4). Nevertheless, the sequence of the CP genes (see below) showed no evidence of differences in M_r among the CPs. In similar tests with the same protein samples and each of the four Mabs to RBDV, the virus isolates differed in the intensity of their reactions. Isolates M and D200 reacted only very weakly or not at all with Mab2 and only weakly with the other Mabs; Mab3 failed to detect isolate D200 (Figure 4; Table 2). However, for isolate M, the weak reactions may possibly be due to the apparently lower concentration of virus protein in plants, as assessed with the polyclonal antiserum (Figure 4(A)). All other isolates reacted with all four Mabs but, whereas isolates Can and Can-S showed a similar intensity of reaction with each of the four Mabs,

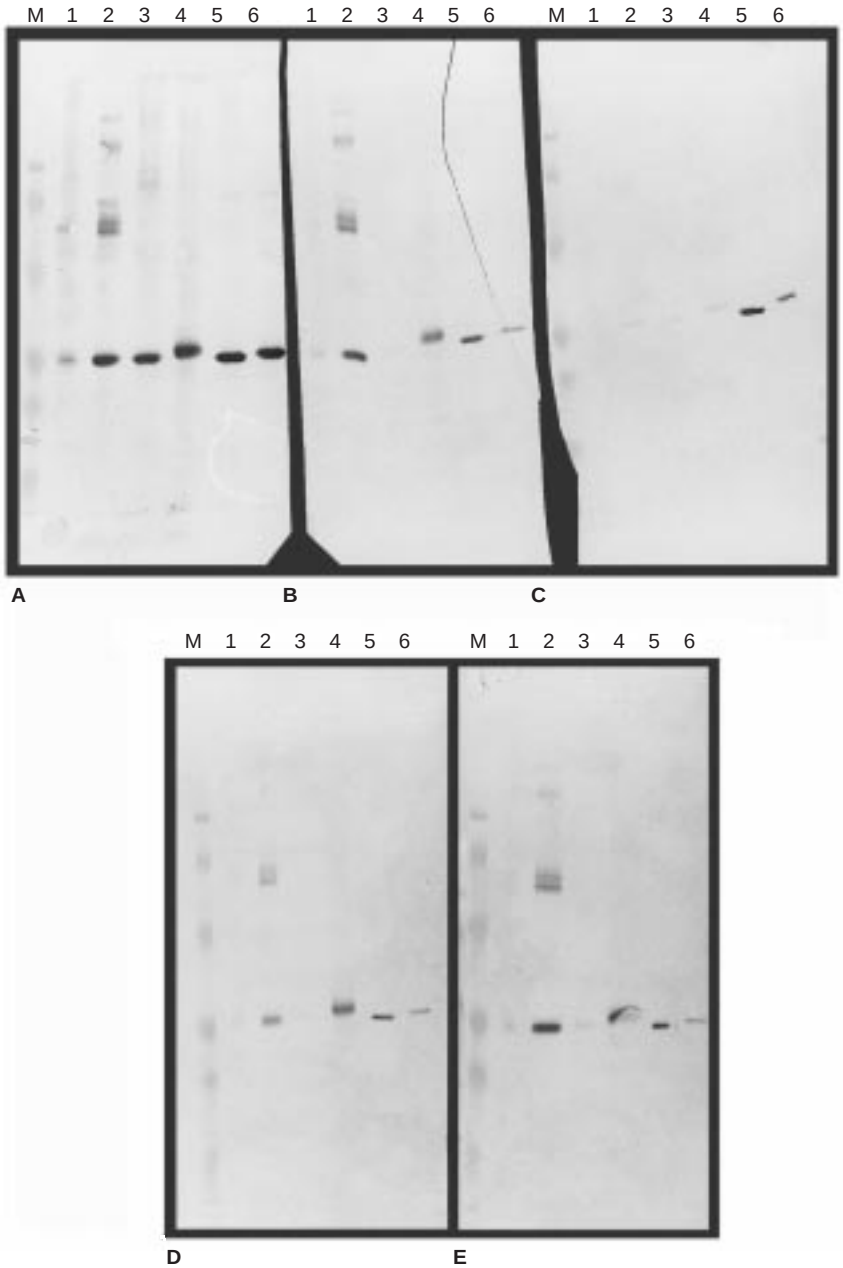

Figure 4. WB analysis of RBDV antigens in sap of *C. quinoa* detected with a polyclonal antiserum to isolate D200 of RBDV (A) and four Mabs (1–4) to a Canadian isolate of RBDV (B–E). It shows the detection of the 30 K CP of RBDV isolates M (lane 1), D1 (lane 2), D200 (lane 3), RB (lane 4), Can-S (lane 5) and Can (lane 6). Lanes marked 'M' contain pre-stained molecular weight markers. Note the slightly slower mobility of the CP band in isolates RB (lane 4) and Can (lane 6) in all blots and the different reactions of the RBDV isolates to the four Mabs.

isolates RB and D1 gave a much weaker reaction with Mab2 than with the other Mabs (Figure 4; Table 2).

The behaviour of the different RBDV isolates in these tests indicate that isolates RB, Can and Can-S are very similar and, in PTA-ELISA, failed to react with, or reacted only very weakly to, the four Mabs. Isolates D1 and M were similar in their behaviour to each other in that they reacted with all antibodies in all

tests; isolate M, although similar in some reactions to isolate D1, was distinguishable by its strong reaction in PTA-ELISA to Mab1 and in reacting only very weakly to all Mabs in WB (Table 2; Figure 4).

Variation in the sequence of the CP genes. Comparisons among the nucleotide sequences of the CP genes of isolates R15 (Mayo et al., 1991), D1, D200, Can, Can-S and M (determined in this study), showed between 14 and 37 differences and these differences resulted in differences among the amino acid sequences of the CPs of between one and eight residues (Table 3).

Variation in the sequence of parts of the polymerase genes. In comparisons among the nucleotide sequences of parts of the polymerase genes of isolates R15, D1, D200 and Can-S, the amount of nucleotide sequence determined differed among the isolates between 326 and 1062 nucleotides (Table 4). Nevertheless, comparisons among those sequences that were obtained showed between 95–98% identity. The nucleotide changes resulted in between one and six differences in amino acid sequence (Table 4).

Discussion

Isolates of RBDV have been assigned to three distinct categories based largely on *Rubus* host range and serology (Jones et al., 1996). However, the work reported here indicates that within these categories there is potentially a wide range of variants. A few of the possibly many variable characters in category S isolates of this virus were analysed in detail. The properties of one of the variant isolates, D1, indicate that it is a variant of isolate D200, derived by passaging in *C. quinoa* for many years. It differed serologically from its parent culture, in having greatly attenuated symptoms in herbaceous plants, and in its nucleotide sequence (Tables 1,3 and 4; Figures 1,3 and 4). By contrast, Can-S, derived from isolate Can, was serologically indistinguishable in gel double-diffusion tests from its parental culture and other category S isolates (Figure 3(a); AT Jones, unpublished data). However, it was distinct from isolate Can and all other isolates of RBDV, by the severity of the symptoms it induced in herbaceous plants, and by its failure to infect *C. quinoa* systemically and by differences in its nucleotide sequence (Tables 1 and 3; Figures 2 and 3).

Table 3. Pairwise comparisons and identity (%) of the sequences of the CP genes of six RBDV isolates. The sequences compared were the 822 nucleotides of the complete gene or, in comparisons involving isolate Can, the sequence between nucleotides 37 and 764 or, for isolate M from nucleotide 17 to the end of the gene. Values are the numbers of nucleotide changes in each comparison and, in parenthesis, the numbers of these changes that result in changes in the amino acid sequences of the encoded CP

Isolate	D1		D200		Can-S		Can		M	
	Changes	%	Changes	%	Changes	%	Changes	%	Changes	%
R15	4 (2)	99.5	34 (4)	95.9	36 (6)	95.6	23 (2)	96.8	26 (3)	96.8
D1			34 (7)	95.9	37 (8)	95.5	21 (4)	97.1	27 (5)	96.6
D200					35 (6)	95.7	6 (2)	99.2	23 (3)	97.1
Can-S							25 (4)	96.6	17 (3)	97.9
Can									14 (1)	98.1

Table 4. Pairwise comparisons and percent identity of the nucleotide sequence of part of the polymerase genes of four isolates of RBDV. The number of nucleotide changes are shown as the numerator and the lengths of the sequence compared as the denominator. Values in parenthesis are the numbers of changes that cause the amino acid sequences of the encoded protein to differ between any pair

Isolate	D1		D200		Can-S	
	Changes	% identity	Changes	% identity	Changes	% identity
R15	24/721 (2)	96.7	43/1140 (6)	96.2	45/1062 (3)	96.0
D1			7/398 (1)	98.2	15/326 (1)	95.4
D200					38/1062 (4)	96.4

The identification of this symptom variant has proved useful for assessing the efficacy of transgenic resistance to RBDV using gene sequences from RBDV introduced into *Nicotiana tabacum* (Angel-Diaz et al., 1997), a plant species that rarely develops symptoms on infection with RBDV and that is generally a poor host of the virus (Barnett and Murant, 1970; Jones and Mayo, 1998).

The finding that isolate D1 was distinguishable serologically from most other isolates in gel double-diffusion tests, is only the second report of a serological variant of RBDV. Other serologically distinct isolates have been reported for only category B isolates from black raspberry (isolate M) and it was postulated that, in nature, this is because such isolates are confined to the black raspberry (*R. occidentalis*) host because one way incompatibility prevents fertilisation between this species and the red raspberry (*R. idaeus* var. *idaeus*, *R. idaeus* var. *strigosus*) (Murant and Jones, 1976; Jones et al., 1996). The paucity of serological variability in RBDV isolates stands in contrast to the extensive variability in this character found for most other pollen-borne viruses, such as *Tobacco streak virus* (Jones and Mayo, 1975), *Apple mosaic virus* and *Prunus necrotic ringspot virus*, each in the genus *Ilarvirus* (Casper, 1973) and *Cherry leaf roll virus*, genus *Nepovirus* (Jones, 1986). However, this may only reflect the fact that RBDV is confined to *Rubus* species in nature (Jones et al., 1996).

Although only two RBDV isolates (D1, M) were found to differ serologically from other isolates based on gel double-diffusion tests, results of WB and ELISA using the four Mabs (Table 2) suggest that other variations in the CP of these, and of other (Jones et al., 1998), isolates occurs. Our evidence for variability in this part of the viral genome supports earlier work based on restriction enzyme digests of PCR products derived from the CP region (Barbara et al., 1995). The basis for the differences between RBDV isolates we report here and elsewhere (Jones et al., 1998) using various serological tests, is presumably a reflection of their differing amino acid sequence (Table 3; Jones et al., 1998). However, at present, the precise molecular basis for these differences is unclear from the sequence data as no simple link between sequence changes and serological distinctiveness was detectable.

Determining the molecular basis for the variations in host range and symptomatology is made difficult because it is not known on which RNA species such determinants are located or if determinants on both RNA species are involved, as is known for some viruses (Harrison et al., 1974; Jones and Duncan, 1980). Production of pseudo-recombinants between contrasting virus isolates may help to identify the role(s) of the two RNA genome pieces of RBDV in host range and symptomatology and isolates Can and Can-S may prove to be ideal for this purpose.

Acknowledgements

We thank A.F. Murant for the gift of the original Canby RBDV isolate and R.R. Martin for the four monoclonal antibodies to RBDV. This work was funded by the Scottish Executive Rural Affairs Department (SERAD), the Rural Business District of Kuopio, and by a grant to J.E. Angel-Diaz from Colciencias (Colombia) and Instituto Colombiano Agropecuario. The work with non-indigenous organisms was done under the conditions of a licence from SERAD.

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