European Journal of Plant Pathology **105:** 191–199, 1999.

Rupestris stem pitting associated virus-1 is consistently detected in grapevines that are infected with rupestris stem pitting

Baozhong Meng[1], Ray Johnson[3], Silvano Peressini[4], Philip L. Forsline[2] and Dennis Gonsalves[1]
[1]*Department of Plant Pathology, and* [2]*USDA Plant Genetic Resources Unit, Cornell University, Geneva, NY 14456, USA (Fax: 315 787 2389; E-mail: bm10@cornell.edu);* [3]*Center for Plant Health, Canadian Food Inspection Agency, 8801 East Saanich Road, Sidney, B.C., V8L 1H3, Canada;* [4]*Servizio Chimico-Agrario e della Certificazione, 33050 Pozzuolo del Friuli, UD, Italy*

Accepted 28 December 1998

Key words: RT-PCR, rugose wood (RW) complex, *Vitis rupestris* Scheele St. George (Synonym: Du Lot), woody indicator indexing

Abstract

Rupestris stem pitting (RSP) appears to be the most widespread disease of the rugose wood complex of grapevines. In previous work we showed that rupestris stem pitting associated virus-1 (RSPaV-1), an agent that has similarities in genome structure to apple stem pitting virus is associated with RSP (Meng et al., 1997, 1998). To validate the association of RSPaV-1 with RSP, grapevines that had been indexed on the RSP indicator St. George (*Vitis rupestris* Scheele) were collected from four major grape-growing countries and tested for RSPaV-1 by reverse transcription-polymerase chain reaction (RT-PCR) assay. Depending on the sets of primers used, we detected RSPaV-1-specific sequences from 85% to nearly 100% of the grapevines that had indexed positive for RSP. These data further demonstrate that RSPaV-1 is closely associated with, and likely to be the causal agent of RSP. We have also shown that RT-PCR is a fast and reliable alternative to biological indexing for the diagnosis of RSP.

Abbreviation: nt – nucleotide; RSP – rupestris stem pitting; RSPaV-1 – RSP associated virus-1; RT-PCR – reverse transcription-polymerase chain reaction.

Introduction

Rugose wood (RW) is a term used to describe a complex of graft-transmissible diseases of grapevines characterized by pitting, grooving, and other distortions on the woody cylinder (Martelli, 1993). RW is found world-wide, occurring in many countries where grapevines are grown (Bovey et al., 1980; Prudencio, 1985; Goheen, 1988; Azzam and Gonsalves, 1988; Engelbrecht et al., 1991; Martelli, 1993; Borgo and Bonotto, 1993; Garau et al., 1994; Chevalier et al., 1995; Credi, 1997) and may cause severe decline and even death to grapevines (Savino et al., 1985; Credi et al., 1991; Credi and Babini, 1996). RW can be differentiated into four distinct diseases when Kober 5BB (*Vitis berlandieri* Planch. X *V. riparia* Michx.), LN-33 (Couderc 1613 X Thompson Seedless), and St. George (synonym: Du Lot, *V. rupestris* Scheele) are used as woody indicators for indexing. These diseases are Kober stem grooving (KSG), LN-33 stem grooving (LNSG), grapevine corky bark (GCB), and rupestris stem pitting (RSP) (Savino et al., 1989; Garau et al., 1989; Martelli, 1993).

Rupestris stem pitting was discovered in California in the 1970s among grapevine selections introduced from Western Europe. It is defined as a disease that induces on St. George indicator a strip of small pits on the woody cylinder only below the site where the inoculum is grafted (Prudencio, 1985; Goheen, 1988). RSP appears to be the most widespread disease of the RW complex. Records of indicator indexing in California over a 23 year period indicated that 30.5% of the 6,482 grapevine selections introduced from throughout the world were infected with RSP (Golino and Butler, 1990). A preliminary field survey in New York for 257 grapevines with suspicious stem pitting symptoms showed that 66% of the grapevines were positive for RSP when indexed by St. George (Azzam

and Gonsalves, 1988). The occurrence of RSP is also the most frequent among the RW diseases in Europe (Borgo and Bonotto, 1993; Garau et al., 1993; Credi, 1997).

The etiology of RSP has not been conclusively determined. A major double-stranded RNA (dsRNA) of ca. 8.0–8.3 kbp was initially isolated from grapevines that had been indexed positive for RSP (Azzam et al., 1991; Walter and Cameron, 1991). We recently cloned and sequenced a dsRNA molecule of 8.7 kbp that is consistently detected in grapevines that had been indexed positive for RSP (Meng and Gonsalves, 1997; Meng et al., 1997, 1998). This dsRNA was apparently the equivalent of the 8.0–8.3 kbp dsRNA reported by Azzam et al. and Walter and Cameron. Sequence analysis of the cDNA clones derived from the dsRNA revealed a viral genome structure that was similar to apple stem pitting foveavirus (ASPV) (Martelli and Jelkmann, 1998) and potato virus M carlavirus (PVM). Minafra et al. (1997) identified a cDNA clone which has sequence similarity with the replicases of ASPV and blueberry scorch carlavirus. RT-PCR using primers derived from the clone detected DNA of expected size in 86% of the grapevines that were indexed positive for RSP on St. George. Zhang et al. (1998) also recently cloned and sequenced a dsRNA associated with RSP, which has 98% nucleotide sequence identity to RSPaV-1. Taking together the information generated, it seems reasonable to conclude that a putative virus, designated rupestris stem pitting associated virus-1 (RSPaV-1), is involved in RSP.

In order to further validate the relationship of RSPaV-1 with RSP and to develop rapid methods for RSP diagnosis, primers were designed from the nucleotide (nt) sequence of RSPaV-1 and used in reverse transcription-polymerase chain reaction (RT-PCR) assays to test a large number of grapevines that had indexed for RSP by St. George. We report here that RSPaV-1 is consistently detected in grapevines that had been indexed positive for RSP. We also show that RT-PCR is a fast and sensitive alternative to diagnose RSP as compared to graft-inoculating St. George. Preliminary results have been reported by Meng et al. (1997).

Materials and methods

Grapevine materials

Dormant cuttings of 129 grapevine accessions were collected from the United States (US), Canada and Italy in three consecutive years starting from 1994 and from Portugal in 1996. Among the samples collected, 67 were *V. vinifera* L., 34 were French-American hybrids, one was *V. riparia* Michx., and the genotypes of the others were not known. A large number (115) of grapevines had been previously indexed for RSP on St. George. Pinot Noir (1186-9A2) from Center for Plant Health, Canadian Food Inspection Agency (Sidney, B.C.) and Thompson Seedless (RSP105, Golino, 1992) from University of California (Davis) were used as RSP positive standards. Sauvignon Blanc, which was generated from meristem tissue culture and tested free of viruses and viroids, was provided by Dr. J. Semancik, University of California (Riverside) and used as an RSP negative standard. The chip-bud grafting method (Martelli et al., 1993) was used for the indexing of all grapevines except that the Italian samples were inoculated onto St. George by the top-grafting method (Martelli et al., 1993). Grafted St. George plants were examined for wood symptoms two to three years after graft-inoculation.

Oligonucleotide primers

Primers used in RT-PCR and their positions on the genome of RSPaV-1 (Meng et al., 1998) are indicated in Table 1. Sequence analysis indicated that on the genome of RSPaV-1 the 3′ end region of ORF1 and the 5′ end region of ORF2 were conserved in nt sequence when compared to ASPV and PVM (Meng et al., 1998). Thus, two sets of primers were designed in these regions of RSPaV-1. Primers 9 and 10 were from near the 3′ end of ORF1 and near the 5′ end of ORF2 respectively and generated a cDNA fragment of 498 bp. Primers 11 and 12 were from near the 3′ end of ORF1 and produced a cDNA fragment of 486 bp. Primers 13 and 14 were generated from sequences of six cDNA clones which may represent different sequence variants of RSPaV-1.

Reverse transcription-polymerase chain reaction (RT-PCR)

dsRNAs were isolated from the phloem tissue (average 10 g) of dormant canes by the method described by Hu et al. (1990). The dsRNAs were denatured with 10 mM of methylmercuric hydroxide (CH_3HgOH) for 10 min at room temperature before 20 mM of mercaptoethanol was added. One µl of the denatured dsRNA was taken and added to 19 µl of reverse transcription mix which

Table 1. Primers used in RT-PCR to detect the genome sequences of RSPaV-1 in grapevines

Primers	Orientation	Position	Sequences	Amplified cDNA (bp)
Primer 9[a]	sense	6244–6260	5′-GGCCAAGGTTCAGTTTG-3′	498
Primer 10[a]	antisense	6741–6725	5′-ACACCTGCTGTGAAAGC-3′	
Primer 11[a]	sense	5980–5995	5′-GGGGTTGCCTGAAGAT-3′	486
Primer 12[a]	antisense	6465–6449	5′-TGGGCCTCCACTTCTTC-3′	
Primer 13	sense	4373–4392	5′-GATGAGGTCCAGTTGTTTCC-3′	339
Primer 14	antisense	4711–4692	5′-ATCCAAAGGACCTTTTGACC-3′	
Primer 16	sense	4102–4118	5′-GCCAAGGGAATTGACTG-3′	265
Primer 2	antisense	4366–4350	5′-CACACAGTGGCCAGCCT-3′	

[a] In Meng et al. (1997), primers 9, 10, 11, and 12 were designated as RSP149R1, RSP149F1, RSP95R1, and RSP95F1, respectively.

contained 4 µl of H_2O, 4 µl of 5X reverse transcription buffer, 8.0 µl of 10 mM dNTPs, 1 µl each of sense and antisense primers (100 ng/µl), 0.5 µl of RNasin inhibitor (40 units/µl, Promega), and 0.5 µl of moloney murine leukemia virus (MMLV) or avian myeloblastosis virus (AMV) reverse transcriptase (200 units/µl, Promega) at 42 °C for 1–3 h. Five µl of the RT reactions were added to a 40 µl of PCR mix which contains 29 µl of H2O, 4 µl of 10X Taq DNA polymerase amplification buffer, 2 µl of 25 mM MgCl, 3.2 µl of 10 mM dNTPs, 0.8 µl each of the primers (100 ng/µl), and 5 units of Taq DNA polymerase (Promega). Amplification was carried out in a thermal cycler (HYBAID OmniGene, National Labnet Company) using the following parameters: initial denaturation at 94 °C for 5 min, 35–40 cycles of amplification at 94 °C for 45 s, 55 °C for 1 min, 72 °C for 1 min, and a final extension at 72 °C for 10 min. Products of the PCR amplification were electrophoresed on 1% agarose gels containing ethidium bromide. *Hae* III digested ØX174 fragments were used as molecular weight markers.

DNA probes and Southern blot

Probe 1 was produced by ^{32}P random labeling of PCR-amplified DNA fragments from cDNA clone RSP149 using primers 9 and 10. Similarly, probe 2 and probe 3 were generated by ^{32}P labeling of PCR-amplified DNA fragments from plasmid RSP94 with primers 16 and 2 and primers 13 and 14, respectively. Probe 2 was used in plaque hybridization (Stratagene) to identify cDNA clones from which primers 13 and 14 were derived. Products of RT-PCR from selected grapevine samples were electrophoresed on 0.8% agarose gels, transferred to nylon membranes, and hybridized with probe 1 or probe 3 at 65 °C following the manufacturer's instructions (Du Pont).

Results

Indexing on St. George reveals 'specific' and 'non-specific' symptoms of rupestris stem pitting

Among the 129 grapevine accessions included in this study, 91 were indexed positive and 24 were indexed negative for RSP, and 14 were not indexed (Table 2). Symptoms expressed on the wood of St. George depended on the grafting methods used. All the grapevines that were top-grafted onto St. George induced pits and grooves around and just below the graft union on the woody cylinder. In contrast, grapevines that were inoculated onto St. George using the chip-bud method elicited 'specific' or 'non-specific' symptoms. Inoculated indicators with specific symptoms of RSP developed pits and/or grooves on the wood of St. George only below the inoculation site. In contrast, inoculated indicator with non-specific symptoms developed pits and/or grooves that were not confined to the region below the inoculation site. For example, among the 51 Canadian grapevine accessions that had indexed positive for RSP, 37 (74.5%) had the 'specific' symptoms, while 13 (25.5%) had the 'non-specific' symptoms.

RT-PCR effectively amplifies expected cDNA fragments from grapevines

A summary of the RT-PCR test results is presented in Table 2. In order to avoid bias in judging results from the RT-PCR, blind tests were conducted for samples collected from Canada in 1995 and 1996. The indexing results of the samples were made known only after the RT-PCR tests had been completed. In general, RSPaV-1 nt sequences were detected in grapevines regardless of the genotypes (*V. vinifera* L., *V. riparia*

Table 2. Detection of RSPaV–1 by RT-PCR in grapevines collected from Italy, USA, Canada, and Portugal

RSP status by St. George indexing	RT-PCR[a]		
	No. tested	No. negative	No. positive
Positive	91	14 (15.4%)	77 (84.6%)
Negative	24	12 (50%)	12 (50%)
Not indexed	14	2 (14.3%)	12 (85.7%)

[a]Phloem tissue of grapevine dormant canes from 67 *Vitis vinifera* L., one *V. riparia* Michx., 34 French-American hybrids and 27 of unknown genotype were used to isolate dsRNA which was used as template in RT-PCR tests. RT-PCR was conducted using primers 9 and 10, and 11 and 12 (Table 1), with the former pair being used in most cases. Sauvignon Blanc, generated from meristem tissue culture and tested free of viruses and viroids, was used as a negative control. Pinot Noir (1186-9A2) and Thompson Seedless (RSP105) were used as positive controls (Golino, 1992).

Michx., or French-American hybrids). Among the 91 grapevines that had indexed positive for RSP, 77 (84.6%) produced cDNA fragments of expected sizes in repeated RT-PCR tests using primers 9 and 10 and/or primers 11 and 12, while the other 14 did not (Figure 1A and B, Table 2). Furthermore, 12 of the 14 grapevine accessions (85.7%) that had not been indexed for RSP were also positive in RT-PCR (Figure 1B lanes 12 and 13, Table 2). Surprisingly, 12 of the 24 grapevines that indexed negative for RSP produced cDNAs of expected size in RT-PCR (Figure 1B lanes 1, 3, 4, and 5, Table 2).

In most cases, the bands of expected sizes were the only or the predominant ones observed on the agarose gels. However, the intensity of the expected cDNA bands amplified by RT-PCR varied among the grapevine accessions (Figure 1A and B). Samples that produced weak bands were tested 2–3 times. Similar results were obtained in all cases. As expected, the healthy control, Sauvignon Blanc, did not produce cDNA fragments in repeated RT-PCR (data not shown).

RT-PCR-amplified cDNA fragments hybridize with a probe derived from RSPaV-1

To confirm that the RT-PCR products were specific to RSPaV-1, selected samples that were positive in RT-PCR were tested in Southern blot with probe 1 (see Materials and methods) under high stringency conditions. The RT-PCR-amplified cDNAs from all the samples selected hybridized with the probe (Figure 1C).

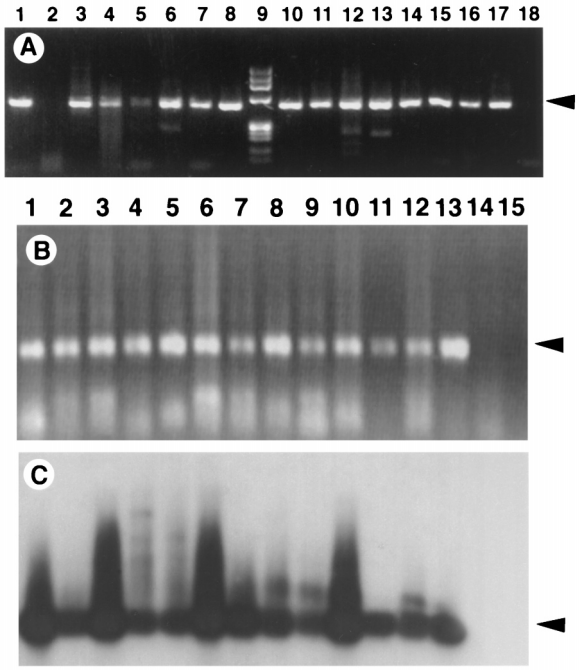

Figure 1. Detection of RSPaV-1 in representative grapevines by reverse transcription-polymerase chain reaction (RT-PCR) using primers 11 and 12 (A) or primers 9 and 10 (B) and Southern blot of cDNA fragments amplified by RT-PCR (C). RSP positive samples included in (A): lane 1, Bertille Seyve 5563; lane 2, Canandaigua; lane 3, Colobel 257; lane 4, Couderc 28-112; lane 6, Grande Glabre; lane 7, Ill 344-1; lane 8, Joffre; lane 11, Ravat 34; lane 12, Seyval; lane 13, Seyve Villard 14-287; lane 14, Seyve Villard 3160; lane 15, Verdelet (PI 186260); lane 16, Pinot Noir (1186-9A2) (positive standard for RSP); lane 17, Thompson Seedless (RSP105, Golino 1992) (positive standard). Freedom (lane 5) was indexed negative for RSP on St. George. The molecular weight markers used were *Hae* III-digested Ø X174 (lane 9). Plasmid RSP95 was used as a control for PCR reaction (lane 10). Water was used as a blank control (lane 18). Grapevines included in (B) and (C) were: lane 1, Ehrenfelser PM1; lane 2, Cabernet Franc 147A; lane 3, Chardonnay 80A; lane 4, Refosco 181A; lane 5, Touriga Francesa 313; lane 6, 3309 C; lane 7, 420 A P 653; lane 8, Chardonnay 83A; lane 9, Malsavia 153A; lane 10, Aragonez 350; lane 11, Aminia; lane 12, Chardonnay 127; lane 13, Kober 5BB 100; lane 14, Verduzzo 233A (RSP-negative control); lane 15, H_2O. Note that in (A) and (B) the intensities of the RT-PCR products varied among grapevine accessions. Arrows show the positions of the 486 bp (A) and the 498 bp (B and C) long PCR-amplified cDNA fragments. Probe 1 was derived from plasmid RSP149 by PCR with primers 9 and 10, radio-labeled with ^{32}P and used in Southern blot. See Table 2 for the indexing results by St. George indicator.

However, in some samples the intensities of the cDNA bands from the RT-PCR amplification did not match with those of the hybridization signals from Southern blot (Figure 1B and C). For example, Cabernet Franc 147A (lane 2), Refosco 181A (lane 4), Touriga Francesa 313 (lane 5), Chardonnay 83A (lane 8), and Kober 5BB 100 (lane 13) had strong bands from RT-PCR (Figure 1B) but their hybridization signals were relatively weak (Figure 1C).

Use of primers 13 and 14 increases the efficiency of RT-PCR to detect RSPaV-1

As shown above, primers 9 and 10, and primers 11 and 12 failed to amplify DNA fragments from 14 of the 91 grapevine accessions that had indexed positive for RSP on St. George (Table 2). The discrepancy between the RT-PCR and the indicator indexing could have been due to the existence in grapevines of different sequence variants and/or strains of RSPaV–1 (Meng et al., 1998). This idea was supported by the discovery that several cDNA clones that were derived from a dsRNA preparation had nucleotide sequence identities of 88–98% to RSPaV-1. Upon alignment of the sequences from these clones, primers 13 and 14 were designed so that the primers could anneal to and amplify DNA fragments from all the clones (Figure 2). The primers were positioned in the beginning part of Region II of RSPaV-1 ORF1 (Meng et al., 1998).

Primers 13 and 14 were used to re-test 12 of the 14 grapevines that had indexed positive for RSP but were negative when tested by primers 9 and 10, and 11 and 12 (Table 2). All 12 samples produced cDNA fragments in RT-PCR (Table 3). DNA fragments from 8 of the 9 tested grapevine accessions hybridized with probe 3 (Table 3).

Discussion

Our results clearly show that RSPaV-1 is closely associated with RSP, providing more evidence that RSPaV-1 is the causal agent of the disease. We obtained 85% to nearly perfect correlation between the results of RT-PCR and St. George indexing, depending on the sets of primers that were used. The fact that nearly 100% detection was achieved with primers 13 and 14 further supports the contention that more than one sequence variants of RSPaV-1 is involved in RSP (Meng et al., 1998). We conclude that the RT-PCR technique developed in this study can be used as an alternative to the classical indicator indexing for diagnosing RSP. The RT-PCR technique has the advantages of being quick, simple, sensitive, and inexpensive.

Our data have also shown that RSPaV-1 frequently occurs in grapevines. Among the 14 grapevines of unknown infection status for RSP, 85.7% were positive by RT-PCR (Table 2). The existence of RSPaV-1 in grapevines seems to be independent of the source and of the genotype. RSPaV-1 was equally detected from grapevines collected from Italy, Portugal, Canada, and the US. Furthermore, these grapevines represent *V. vinifera* L., French-American hybrids, and a *V. riparia* Michx. This is in good agreement with the reports of the biological indexing conducted in different laboratories throughout the world (Golino and Butler, 1990; Azzam and Gonsalves, 1988; Borgo and Bonotto, 1993; Garau et al., 1993; Credi, 1997). Although the world-wide exchange of grapevine germplasms and grafting among different scions and rootstocks of various origins may play a major role in disease spread, the possibility that RSP may be disseminated by other means such as vectors and seeds needs to be investigated.

Surprisingly, we detected RSPaV-1 sequences from 50% of the grapevines that had been indexed negative for RSP on St. George. Several possibilities may account for the discrepancy between the RT-PCR and the St. George indexing. First, our recent findings suggest that a number of sequence variants of RSPaV-1 are associated with RSP (Meng et al., 1998; unpublished results). Although the biological impact of these sequence variants is unclear at this moment, it is possible that some sequence variants induce severe symptoms on the woody cylinder of St. George, while others elicit only mild or no symptoms at all. However, this hypothesis needs to be tested by further experiments. Second, RT-PCR may be much more sensitive than the St. George indicator indexing method. Thus, inoculum buds with extremely low concentration of virus particles may not induce symptoms on St. George within the duration of indexing, while RT-PCR could detect low titers of RSPaV-1. Third, uneven distribution of RSPaV-1 within grapevines could lead to negative results in indexing if the inocula are taken from places where the virus does not exist. In contrast, the RT-PCR test developed here has a better coverage of detecting the virus because we used 5–10 canes with three or more buds to isolate the template dsRNA. Finally, judging indexing results can, in some cases, be subjective. For example, it is very difficult to reach a conclusion

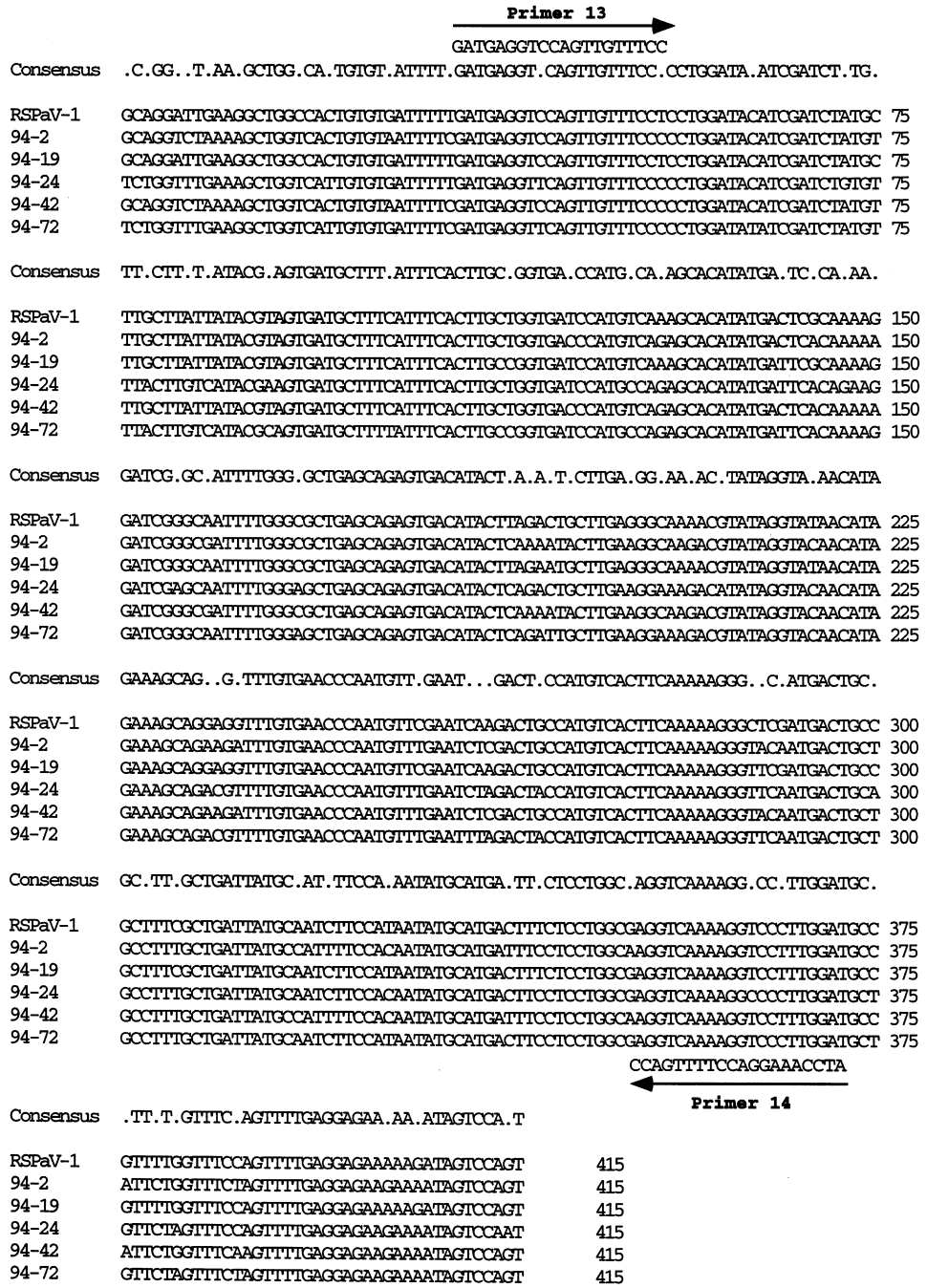

Figure 2. Primers 13 and 14 derived from the alignment of the nucleotide sequences of cDNA clones that were identified by plaque hybridization. Probe 2, corresponding to nucleotide positions of 4102–4366 of RSPaV-1 genome, was generated by ^{32}P labeling of PCR fragments of plasmid RSP94 using primers 16 and 2 and used in Southern blot. The positions and orientations of the primers are indicated by arrows.

Table 3. Results of RT-PCR using primers 13 and 14 to retest grapevines that indexed positive for RSP on St. George but were negative by RT-PCR (Table 2)[a] using primers 9 and 10 and/or primers 11 and 12

Grapevine accessions	Identification numbers	RT-PCR	Southern blot[b]
Aligote	637-2B2	+	−
Aragonez	232	+	+
Canandaigua	PI 597225	+	+
GM 7746-6	1362-6A1	+	+
Gravesac cl 264	1551-3A1	+	+
Fercal cl 242	1551–4A1	+	+
Kee-Wah-Din	1278-1A1	+	+
Periquita	72	+	+
Verdelet	1170-3D2-2A1	+	+
Kober 5BB cl 114	1236–2A1	+	not tested
Honey Red	1339-6A1	+	not tested
Thurling	1047-4A2-1A2	+	not tested

[a]Two of the 14 samples were not retested because material was no longer available.
[b]The probe (probe 3), was generated from plasmid RSP94 by PCR using the primers 13 and 14 (Table 1) and labeled with ^{32}P.

that a grapevine is infected with RSP when only one or a few small pits are present on the woody cylinder of St. George.

According to Goheen (1988), RSP is a disease which induces, after graft-inoculation with a chip-bud from an infected grapevine, a row of small pits on the woody cylinder of St. George below the point of inoculation. Our indexing records indicated that two types of stem pitting (specific vs. non-specific) were observed on the woody cylinder of St. George. For example, 74.5% of the 51 Canadian grapevines that had indexed positive for RSP had the specific symptoms, while the other 25.5% developed non-specific symptoms. Credi (1997) also observed these types of stem pitting symptoms in his indexing with the indicator St. George. Since we detected the nucleotide sequences of RSPaV-1 by RT-PCR in grapevines showing both types of symptoms on St. George, it is reasonable to suggest that the definition of RSP should be revised to include specific and non-specific symptoms.

The economic impact of RSP on grapevine industry is not well documented and consequently often subject to debate. Goheen (1988) observed a slow decline in the growth of *V. vinifera* L. cultivars. He also indicated that RSP and leafroll had similar effect on fruit quality and yield. Prudencio (1985) graft-inoculated 68 grapevine germplasm lines of various genotypes and concluded that RSP had no significant effect on vine growth by the 18th month after inoculation. More recently, Reynolds et al. (1997) inoculated Kerner (*V. vinifera* L.), Michurinetz (*V. amurensis* X *V. vinifera* L.), Okanagan Riesling (*V. labruscana* Bailey), Madeleine Sylvaner (*V. vinifera* L.), and Ortega (*V. vinifera* L.) and compared the performance of graft-inoculated vs. non-inoculated grapevines over a period of 9 years. They concluded that RSP had no major impact on the growth and yield of grapevines. However, RSP seemed to advance berry maturity in terms of titratable acidity and pH (Reynolds et al., 1997).

Unfortunately, information on the sequence of RSPaV-1 was not known when the above studies were done. The development of a reliable RT-PCR detection method allows a fast and precise monitoring of test vines for RSPaV-1 infection. This will allow investigators to critically evaluate the effect of RSP on growth and yield of grapevines. In addition, the rapid diagnosis of RSP can be further simplified through the use of antibodies to the coat protein of RSPaV-1. We have expressed the coat protein of RSPaV-1 in bacteria and generated polyclonal antibodies against it. The usefulness of the antibodies in detecting RSPaV-1 from RSP-infected grapevines is being investigated.

In conclusion, RSPaV-1 was consistently detected by RT-PCR in RSP-infected grapevines of diverse genotypes and from various geographic regions. Our data clearly suggest that RSPaV-1 is closely associated with

and likely to be the causal agent of RSP. The RT-PCR technique we have developed should be a useful alternative to the biological indexing procedure for diagnosing RSP in grapevines.

Acknowledgements

This research is supported in part by USDA/ARS cooperative agreement #58-1908-4-023. We thank G.M. Mittak, A. Gubba, M. Carvalho, and T. Momol for reviewing the manuscript. We thank H.Y. Zhu for testing some of the samples.

References

Azzam OI and Gonsalves D (1988) Survey of grapevine stem-pitting in New York and isolation of dsRNA from a grapevine selection infected with stem pitting. Phytopathology 78: 1568 (abstr)

Azzam OI, Gonsalves D and Golino DA (1991) Detection of dsRNA in grapevines showing symptoms of *Rupestris* stem pitting disease and the variabilities encountered. Plant Disease 75: 960–964

Borgo M and Bonotto A (1993) Rugose wood complex of grapevine in Northeastern Italy: Occurrence of *Rupestris* stem pitting and Kober stem grooving. In: Extended abstracts of the 11th Meeting of the International Council for the Study of Viruses and Virus Diseases of the Grapevine (ICVG) (pp 61–62). Sept. 6–9, 1993, Montreux, Switzerland

Bovey R, Gärtel W, Hewitt WB, Martelli GP and Vuittenez A (1980) Virus and virus-like diseases of grapevines. Payot Lausanne, La Maison Rustique Paris, and Verlag Eugen Ulmer Stuttgart. 181 p

Chevalier S, Creif C, Bass P and Walter B (1995) Investigations on the aetiology of Kober stem grooving. Journal of Phytopathology 143: 369–373

Credi R (1997) Characterization of grapevine rugose wood disease sources from Italy. Plant Disease 81: 1288–1292

Credi R and Babini AR (1996) Effect of virus and virus-like infections on the growth of grapevine rootstocks. Advances in Horticultural Science 10: 95–98

Credi R, Silvestroni A and Canova A (1991) Variation in grapevine rootstock susceptibility to the rugose wood disease 'lengno riccio'. Phytopathologia Mediterranea 30: 41–46

Engelbrecht DJ, Kasdorf GGF and Maré FA (1991) Field spread of stem-grooving diseases in South Africa grapevines. Phytophylactica 23: 239–240

Garau R, Prota U and Cugusi M (1989) Investigations on wood disorders (stem pitting and/or stem grooving) of grapevine in Sardinia. In: Proceedings of the 9th Meeting of the ICVG (pp 135–141) Kiryat Anavim, Israel, 1987

Garau R, Prota VA, Piredda R, Boscia D and Porta U (1994) Kober stem grooving and grapevine virus A: a possible relationship. Vitis 33: 161–163

Goheen AC (1988) *Rupestris* stem pitting. In: Pearson RC and Goheen AC (eds.) Compendium of grape diseases (p 53). American Phytopathological Society Press, St. Paul, Minnesota

Golino DA (1992) The Davis grapevine virus collection. American Journal of Enology and Viticulture 43: 200–205

Golino DA and Butler V (1990) A preliminary analysis of grapevine indexing records at Davis, California, USA. In: Proceedings of the 10th Meeting of the ICVG (pp 369–372). Ores Publishing, Volos, Greece, 1991

Hu JS, Gonsalves D and Teliz D (1990) Characterization of closterovirus-like particles associated with grapevine leafroll disease. Journal of Phytopathology 128: 1–14

Martelli GP (1993) Rugose wood complex. In: Martelli GP (ed.) Graft-transmissible diseases of grapevines, handbook for detection and diagnosis (pp 45–54). Food and Agriculture Organization of the United Nations, Rome, Italy

Martelli GP and Jelkmann W (1998) Foveavirus, a new plant virus genus. Archives of Virology 143: 1245–1249

Martelli GP, Savino V and Walter B (1993) Indexing on *Vitis* indicators. In: Martelli GP (ed.) Graft-transmissible diseases of grapevines, handbook for detection and diagnosis (pp 137–155). Food and Agriculture Organization of the United Nations, Rome, Italy

Meng B, Forsline P and Gonsalves D (1997) Rupestris stem pitting of grapevines: nucleotide sequence, RT-PCR detection, and viral origin of associated dsRNA. In: Extended Abstracts of the 12th Meeting of the ICVG (pp 35–36). Lisbon, Portugal

Meng. B. and Gonsalves, D. (1997). Nucleotide sequence and genome organization of grapevine rupestris stem pitting-associated virus and its detection by RT-PCR. Phytopathology 87, S65.

Meng B, Pang S-Z, Forsline PL, McFerson JR and Gonsalves D (1998) Nucleotide sequence and genome structure of grapevine rupestris stem pitting associated virus-1 reveal similarities to apple stem pitting virus. Journal of General Virology 79: 2059–2069

Minafra A, Mackenzie DJ, Casati P, Bianco PA, Saldarelli P and Martelli GP (1997) Detection of an unusual RNA in grapevines indexing positive for rupestris stem pitting. In: Extended Abstracts of the 12th Meeting of the ICVG (pp 43). Lisbon, Portugal

Prudencio S (1985) Comparative effects of corky bark and rupestris stem pitting diseases on selected germplasm lines of grapes. MS Thesis. University of California, Davis, California, 36 p

Reynolds AG, Lanterman WW and Wardle DA (1997) Yield and berry composition of five *Vitis* cultivars as affected by rupestris stem pitting virus. American Journal of Enology and Viticulture 48: 449–458

Savino V, Boscia D, Musci D and Martelli GP (1985) Effect of legno riccio (stem pitting) on 'Italia' vines grafted onto rootstocks of different origin. Phytopathologia Mediterranea 24: 68–72

Savino V, Boscia D and Martelli GP (1989) Rugose wood complex of grapevine: can grafting to *Vitis* indicators discriminate

between diseases? In: Proceedings of the 9th Meeting of the ICVG (pp 91–94). Kiryat Anavim, Israel, 1987

Walter MH and Cameron HR (1991) Double-stranded RNA isolated from grapevines affected by rupestris stem pitting disease. American Journal of Enology and Viticulture 42: 175–179

Zhang Y-P, Uyemoto JK, Golino DA and Rowhani A (1998) Nucleotide sequence and RT-PCR detection of a virus associated with grapevine rupestris stem-pitting disease. Phytopathology 88: 1231–1237