Molecular Detection of *Rupestris stem pitting-associated virus* in Grapevines in Brazil

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RESUMO

Detecção por métodos moleculares do *Rupestris stem pitting-associated virus* em videiras no Brasil

RT-PCR foi utilizada para amplificar parte do gene da replicase do *Rupestris stem pitting-associated virus* (RSPaV) a partir de videiras (*Vitis* spp.) no Brasil. O segmento amplificado foi clonado e seqüenciado e, por comparação da seqüência de nucleotídeos e dos aminoácidos deduzidos, verificou-se que correspondiam, respectivamente, em 88% e 94% com as de isolados do RSPaV de outros países.

Rupestris stem pitting (RSP), a component of the rugose wood complex, is one of the most widespread graft-transmissible grapevines (*Vitis spp*.) virus. It was detected in Brazil late in the 60's (Kuniyuki. Fitopatol. Bras. 5:137, 1972). The RSP is characterized by the presence of small pits in the woody cylinder below the point of inoculation by chip budding on *Vitis rupestris* Scheele cv. St. George ('du Lot') (Goheen. Compendium of Grape Diseases. 1988. p.53). A virus named *Ruspestris stem pitting-associated virus* (RSPaV), *Foveavirus* genus, has been associated with the disease (Zhang *et al*. Phytopathology 88:1231, 1998; Meng *et al*. Eur. J. Plant Pathol. 105:191, 1999). This work reports the molecular detection of RSPaV in grapevines by RT/PCR, nucleotide sequence analysis and a non-isotopic cDNA probe specific to RSPaV. The RNA was extracted from petioles and used in the RT-PCR reaction with RSPaV-specific primers (Zhang *et al*. Phytopathology 88:1231, 1998). The amplified fragment was cloned and sequenced. The nucleotide (88%) and deduced amino acid (94%) sequences of the gene fragment (831 pb; Figure 1A) showed high homologies with those of two other RSPaV isolates (GenBank AF6278; AF057136). A digoxigenin-labeled cDNA probe was generated by PCR and used to positively detect RSPaV in RNA extracted from varieties of *V. vinifera* L. (Figure 1B). These results confirm the presence of RSPaV in grapevines that had indexed positive for RSP on *V. rupestris* 'St. George' in Brazil. The RT-PCR technique together with the cDNA probe will be a useful procedure for rapidly detecting the RSP disease in grapevines.

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          10        20        30        40        50        60
001  AGCATGCTCTTGGCAACTGCAGTGATTCCCCCAGAAGTGCTTGTTGGCTCTCCTGAAAGT
      S  M  L  L  A  T  A  V  I  P  P  E  V  L  V  G  S  P  E  S 020
061  CTAAATCCTTGGGCCTACCAGTACAGAATTAGTGATAATCAACTGCTCTTCGCACCTGAT
      L  N  P  W  A  Y  Q  Y  R  I  S  D  N  Q  L  L  F  A  P  D 040
121  GGTAATTGGAGTGAAATGTATTCACAGCCTTTGTCATGCAGATACCTACTTAAGGCTAGA
      G  N  W  S  E  M  Y  S  Q  P  L  S  C  R  Y  L  L  K  A  R 060
181  TCCGTTGTTTTGCCTGATGGTTCACGCTATTCAGTTGACATCATTCATTCAAAATTTAGC
      S  V  V  L  P  D  G  S  R  Y  S  V  D  I  I  H  S  K  F  S 080
241  CACCACTTGCTTAGCTTCACCCCCATGGGCAATCTTTTAGCTTCAAACATGAGGTGCTTC
      H  H  L  L  S  F  T  P  M  G  N  L  L  A  S  N  M  R  C  F 100
301  TCTGGCTTTGATGCAATAGGCATAAAGGATCTTGAACCTCTAAGCCGTGGCATGCACAGT
      S  G  F  D  A  I  G  I  K  D  L  E  P  L  S  R  G  M  H  S 120
361  TGTTTTCCAGTGCATCATGATGTTGTTACCAAAATATATCTTTACTTGAGGACCCTCAAA
      C  F  P  V  H  H  D  V  V  T  K  I  Y  L  Y  L  R  T  L  K 140
421  AAGCCAGACAAGGAGTCTGCAGAGGCAAAGCTTCGTCAACTCATTGATAAGCCCACAGGG
      K  P  D  K  E  S  A  E  A  K  L  R  Q  L  I  D  K  P  T  G 160
481  AGGGAGATAAAATTCATTGAAGATTTTTCCTCACTAGTTATAAGTTGTGGAAGGAGTGGT
      R  E  I  K  F  I  E  D  F  S  S  L  V  I  S  C  G  R  S  G 180
541  TCTTTGCTTATGCCCAACATTTCTAAGTTGGTAATATCATTCTTTTGCCGAATGATGCCA
      S  L  L  M  P  N  I  S  K  L  V  I  S  F  F  C  R  M  M  P 200
601  AATGCACTTGCTAGGCTTTCTTCCAATTTTCGGGAGTGCTCACTGGATTCATTTGTGTAT
      N  A  L  A  R  L  S  S  N  F  R  E  C  S  L  D  S  F  V  Y 220
661  TCACTTGAGCCTTTCAATTTTTCAATTAATTTGGTGGATATCACTCCCGATTTCTTTGAG
      S  L  E  P  F  N  F  S  I  N  L  V  D  I  T  P  D  F  F  E 240
721  CATTTATTTCTTTTCTCTTGTCTCAATGAGTTAATCGAGGAGAATGTTGAAGAGGTCATG
      H  L  F  L  F  S  C  L  N  E  L  I  E  E  N  V  E  E  V  M 260
781  GACAACTCTTGGTTTGGACTCGGGGATTTGCAATTCAATCGCCAGAGGG
      D  N  S  W  F  G  L  G  D  L  Q  F  N  R  Q  R           276
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B

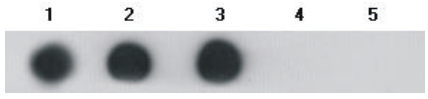

FIG. 1 - (A) Partial nucleotide (above) and deduced amino acid (below) sequences of *Rupestris stem pitting-associated virus* (RSPaV). (B) Dot-blot hybridization with RSPaV-specific probe. RNA extracted from infected plants of *Vitis vinifera* cultivars: (1) Itália (Biritiba Mirim, SP); (2) Itália (São Miguel Arcanjo, SP); and (3) Benitaka (Londrina, PR). RNA extracted from healthy plants of 'Itália' (4) and 'Benitaka' (5).

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