Virus Genes (2010) 41:295–297
DOI 10.1007/s11262-010-0510-7

Presence of *rose spring dwarf-associated virus* in Chile: partial genome sequence and detection in roses and their colonizing aphids

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Received: 20 May 2010 / Accepted: 24 June 2010 / Published online: 7 July 2010

Abstract Rose is one of the most important cut flowers produced in the world. It is also grown in landscape plantings and public gardens for ornamental purposes. However, there is no detailed information available about viruses infecting roses in Chile. In order to gain insight about the viruses that could be present, a plant showing yellow vein chlorosis in its leaves was collected from a garden in Santiago. Double-stranded RNA (dsRNA) was isolated and after a random primed RT-PCR amplification procedure followed by sequencing, *Rose spring dwarf-associated virus* (RSDaV) presence was established. In order to widen the survey, several additional symptomatic and asymptomatic plants as well as aphids were screened by RT-PCR using two different pairs of virus-specific primers. RSDaV was detected in 24% of the analyzed samples. To our knowledge, this is the first report of RSDaV in Chilean rose plants and *Rhodobium porosum* (Sanderson) aphids.

Keywords RSDaV · *Luteovirus* · Rose · Aphid

At least, nine viral species belonging to the genera *Nepovirus* and *Ilarvirus* have been described to infect roses. These include *Tomato ringspot virus* (ToRsV), *Tobacco ringspot virus* (TRSV), *Strawberry latent ringspot virus* (SLRSV), *Arabis mosaic virus* (ArMV), *Blackberry chlorotic ringspot virus* (BCRV), *Tobacco streak virus* (TSV), *Prunus necrotic ringspot virus* (PNRSV), and *Apple mosaic virus* (ApMV). In addition, *Rose spring dwarf-associated virus* (RSDaV) was recently described in the United States, and the authors suggested a correlation with Rose spring dwarf disease (RSD) [1]. RSDaV genome contains 5,808 nucleotides and codes for five major and three minor open reading frames (ORF). It belongs to the family *Luteoviridae* and the *Luteovirus* genus. This family includes features such as persistent circulative aphid transmission and phloem restriction [2].

Previous studies have determined a 14% loss in salable blooms due to virus-infected roses grown outdoors and in greenhouses [3]. Rose plants are often carriers of viruses without showing symptoms; thus, viruses are easily spread during budding and grafting procedures. Logically, efficient control relies on nurseries and commercial growers. In Chile as elsewhere, screening of the viral species present in roses may contribute to establish future virus-tested plant certification programs.

In order to identify virus species infecting roses in Chile, a rose sample exhibiting leaves with yellow vein chlorosis resembling a viral infection (sample Cl-R01) was collected from a private garden (Fig. 1), and dsRNA was extracted as described elsewhere [4]. In brief, 2 g of bark scrapings was powdered in liquid nitrogen followed by column chromatography in CF-11 (Sigma). Reverse transcription was done

The nucleotide sequence data reported in this article have been submitted to the GenBank nucleotide sequence database and have been assigned the accession numbers HM236354, HM236355, HM236356, HM236357, HM236358, HM236359, HM236360, HM236361, HM236362, HM236363, HM236364, HM236365, HM236366, and HM236367.

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Fig. 1 Photograph of sample Cl-R01 showing leaves with yellow vein chlorosis. The presence of *Rose spring dwarf-associated virus* was confirmed by RT-PCR and sequencing of two different viral regions

as previously described [5] using primer EErnd 5′-GTA AGGTGCACGTAGTTGNNNNNNNNN-3′. This was followed by 40 cycles of PCR with primer EEadp 5′-GTAAG GTGCACGTAGTTG-3′ to complete the random primed RT-PCR amplification strategy. We have previously demonstrated that this approach can be used to enrich samples in viral templates, overcoming problems related to low viral titers or limited amount of plant tissue [6]. The amplified sample was fluorescently labeled with Cy3 (GE Healthcare) and screened with an oligonucleotide microarray able to simultaneously detect 44 plant (mainly grapevine) viruses [5], but no conclusive results were obtained. In order to further investigate the putative viral agent present in the plant, the amplified sample was cloned into pGEMT-easy vector (Promega) and transformed into *E. coli*. One of the 30 clones sequenced (GenBank accession HM236354) contained a fragment of 377 bp corresponding to a partial region of the coat protein gene of RSDaV (genome position 3235-3611). The sequence shared 99% of nucleotide and 100% of amino acid identity with a recently reported RSDaV isolate (GenBank accession EU024678). Clearly, the microarray was unable to detect RSDaV because probes for the *Luteoviridae* family were not considered among the 570 unique oligonucleotides printed.

In order to determine the presence of RSDaV in additional rose plants and aphids, samples collected from nurseries, public, and private gardens were submitted to RT-PCR analysis. For this purpose, a pair of primers spanning the 377-bp region initially identified in sample Cl-R01, RSDVFor 5′-TCACAATCAACAACCTTAAGGC-3′, and RSDVRev 5′-CGAGCTGGGTCCATTTCCTG-3′ was used. We were able to detect RSDaV using these pair of primers in 6 of the 25 samples (24%) collected (GenBank accessions HM236355, HM236356, HM236357, HM236358, HM236359, and HM236360). Sequence comparison of each of the positive RT-PCR products with the reference genome (GenBank accession EU024678) showed between 98 and 100% of nucleotide identity. The translated sequences showed between 96 and 100% of amino acid identity when compared to either the viral aphid transmission or coat proteins if frame +1 was considered. When frame +3 was compared with the reference RSDaV genome, between 96 and 100% of identity at the amino acid level was obtained with the viral movement protein.

In order to further confirm the presence of RSDaV in all the samples, a second pair of primers LutFor 5′-TGGCGC CACCTGGAATGTAGG-3′ and LutRev 5′-GGGACGTC GCTACCTGCTGGAAAT-3′ that amplified a 417-bp fragment corresponding to a partial region of viral ORF 3 was also used (RSDaV genome position 4058–4474) [1]. Again, the same six positive plants described above (GenBank accessions HM236361, HM236362, HM236363, HM236364, HM236365, and HM236366) shared between 93.8 and 99.8% nucleotide identity and between 97 and 100% amino acid identity with the reference RSDaV genome (GenBank accession EU024678).

In summary, a total of 25 rose samples collected from different geographic locations in Santiago, Chile considering nurseries, public, and private gardens were screened. We were able to detect RSDaV in six (24%) of the samples analyzed.

In order to determine, whether the same screening approach was able to detect RSDaV in aphids, four specimens that corresponded to *Rhodobium porosum* (Sanderson), which have been recently described as efficient vectors of RSDaV with a 37% of infection rate [1], were collected from sample Cl-R01 and then analyzed all together. Total RNA was extracted from the aphids as described elsewhere [7, 8] followed by RT-PCR. RSDaV was efficiently detected using the same primers described above (GenBank accession HM236367).

In this study, we report the initial detection of RSDaV in Chile. The methodology used allowed us to an unequivocal determination of the virus in rose plants since two different pairs of primers spanning different viral regions were used on each case. The same experimental approach was useful for virus screening in aphids, the natural vector of several plant viruses including RSDaV. Our preliminary findings suggest that RSDaV is widely spread; therefore, viral certification of rose plants should be established in the near future in Chile. It is important to mention that RSDaV has been only recently reported in California, USA [1]. To our knowledge, this is the first report of RSDaV in roses and their aphids in Chile. Further studies to determine the incidence, genome variability, and effects of this virus are pending.

Acknowledgments This study was partially supported by the CCTE/PFB-16 program from CONICYT (Chile) and Universidad Andrés Bello Young Scientist Award to EAE. Authors thank Dr. Pablo D.T. Valenzuela, Dr. Mario Rosemblatt, and Dr. Erwin Krauskopf for support and comments on this manuscript.

References

1. N. Salem, D.A. Golino, B.W. Falk, A. Rowhani, Plant Dis. **92**, 508–512 (2008)
2. R.R. Martin, P.K. Keese, M.J. Young, P.M. Waterhouse, W.L. Gerlach, Annu. Rev. Phytopathol. **28**, 341–363 (1990)
3. N.R. Pataky, *Virus and Virus-Like Diseases of Roses* (University of Illinois, Urbana-Champaign, 1988), pp. 1–5
4. R.A. Valverde, S.T. Nameth, R.L. Jordan, Plant Dis. **74**, 255–258 (1990)
5. E.A. Engel, P.F. Escobar, L.A. Rojas, P.A. Rivera, N. Fiore, P.D. Valenzuela, J. Virol. Methods **163**, 445–451 (2010)
6. D. Wang, L. Coscoy, M. Zylberberg, P.C. Avila, H.A. Boushey, D. Ganem, J.L. DeRisi, Proc. Natl. Acad. Sci. USA **99**, 15687–15692 (2002)
7. S. Chang, J. Puryear, J. Cairney, Plant Mol. Biol. Rep. **11**, 113–116 (1993)
8. E.A. Engel, C. Girardi, P.F. Escobar, V. Arredondo, C. Dominguez, T. Perez-Acle, P.D. Valenzuela, Virus Genes **37**, 110–118 (2008)