SP
/10391

Mittes Lima

15th Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine (ICVG)

3-7 April 2006

Stellenbosch, South Africa

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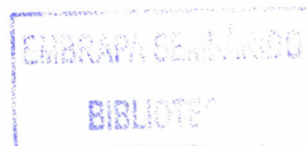
ISBN 1-86849-318-0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Acknowledgements: Prof P.G. Goussard, Mr Abraham Vermeulen and Mrs R. Carstens photo's on cover page

Detection of Rupestris stem
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DETECTION OF RUPESTRIS STEM PITTING ASSOCIATED VIRUS IN SEEDLINGS OF VIRUS-INFECTED MATERNAL GRAPEVINE PLANTS

M.F. Lima, C. Rosa, D.A. Golino and **A. Rowhani***

Department of Plant Pathology, University of California, Davis, CA 95616, USA
*-Corresponding author: tel: (1)530-752-5401, fax: (1)530-752-2132, e-mail: akrowhani@ucdavis.edu

Introduction

Rupestris stem pitting associated virus (RSPaV) is a species in the genus *Foveavirus* (Martelli and Jelkman, 1998) and the family Flexiviridae. The virion has a positive sense, single stranded, polyadenylated RNA genome of 8.7kb in size and a coat protein of 28kD (Martelli and Jelkman, 1998). The virus has been reported to be present in pollen (Rowhani et al., 2000) and seeds (Stewart and Nassuth, 2001), however, it has not been proved to be seed-transmitted. In our investigation reported here we have proven that RSPaV transmits by seed from RSPaV-infected mother plants to their siblings.

Methods

Seeds were collected from RSPaV-infected grapevine cultivars Pinot Noir, Muscadelle and Cabernet Sauvignon. Before stratification, some seeds from each grape cultivar were tested by RT-PCR for the presence of RSPaV. For the test, the seed coat surface was cleaned and disinfected in a solution of 3.5% sodium hypochlorite for 5 min, then each individual seed was ground and total RNA was extracted using RNeasy column (Qiagene Inc) followed the manufacturer's instruction and used as a template for RT-PCR tests.

For germination, seeds were treated with 1.5% hydrogen peroxide and 350 ppm gibberelic acid solutions for 24 hr and then stored at about 4-6 C for stratification. After stratification, they were germinated on wet sterile filter paper in sterile petri dishes in the growth chamber. Afterwards, part of the germinated seeds were selected, their seed coat were removed and stored at -80 C for further analyses. The remaining germinated seeds were transplanted into trays with individual cells containing sterile soil and maintained in the growth chamber for about two weeks to grow. The seedlings were then transferred into 5x5 cm pots and transferred to a greenhouse. To test the germinated seeds kept in the freezer, total RNA was extracted from individual small plantlets using RNeasy extraction kit (Qiagene) and tested by RT-PCR. For the seedlings grown in the greenhouse, petiole samples from each 5 plants were combined and total RNA was extracted as described above and tested. The one step RT-PCR as described by Rowhani et al (2000) using RSPaV 48/49 primers (Zhang et al, 1998) was used to test the samples. For confirmation, purified RT-PCR products from positive seedlings were cloned, sequenced and the sequences were compared with those present in the GenBank.

Results and Discussion

RSPaV was detected in total RNA obtained from seeds originated from infected plants (Table 1) indicating the presence of virus in the seed. Some of the seedlings grown from seeds of infected plants were also tested positive for the virus (Table 1). The percentage of infection varied from 0.4% (seedlings grown in the greenhouse) and 6.2-14.3% (small plantlets stored in the freezer). Clones obtained from the RSPaV-positive seedlings were compared to sequences reported in the database. Comparative analysis of amino acid sequences of 22 clones revealed percent identity varying from 94% to 100% to RSPaV. As a conclusion, the data indicated that RSPaV is present in the seed and could tramsmit from infected mother plants to their siblings.

Cultivar	Seed	Small Plantlets	Seedlings
Cab. Sauv.	100%	14.3%	0.4%
Muscadelle	100%	6.2%	0%
Pinot Noir	80%	0%	0%
Pinot Noir (healthy control)	0%	0%	0%

Table 1. Percent of RSPaV-infection in seeds, and its transmission to plantlets and seedlings.

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