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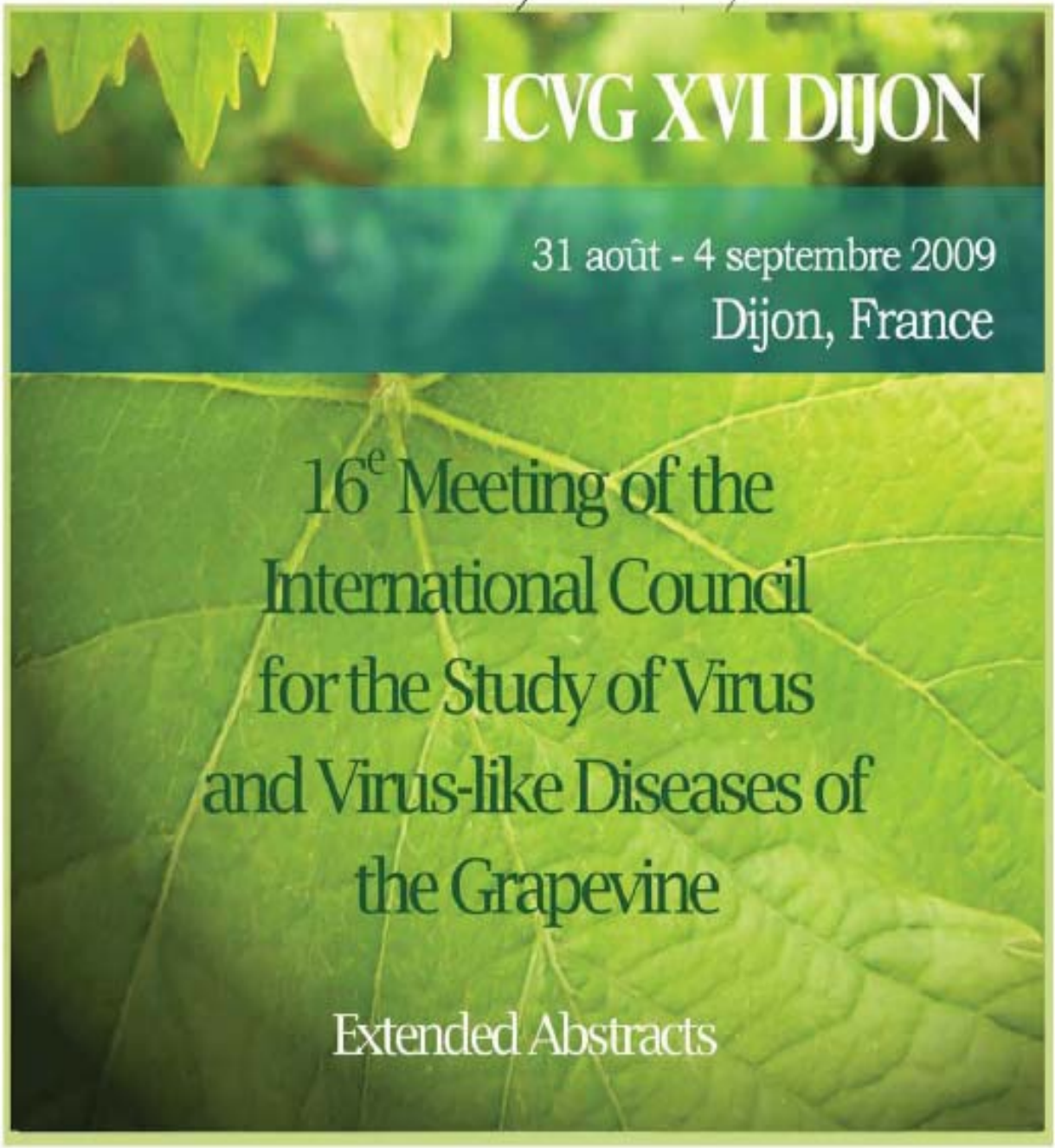

16e Meeting of the International Council for the Study of Virus and Virus-like Diseases of the Grapevine

Extended Abstracts

IDENTIFICATION AND PARTIAL CHARACTERIZATION OF GRAPEVINE VIROIDS IN SOUTHERN IRAN

Mohammad ZAKI-AGHL, Keramatollah IZADPANAH

Plant Virology Research Center, College of Agriculture, Shiraz University, SHIRAZ, Iran
Corresp. Author: K. Izadpanah. izadpana@shirazu.ac.ir

INTRODUCTION

Up to now five viroids have been identified from grapevine (5). They include Grapevine yellow speckle viroid 1 (GYSVd1), Grapevine yellow speckle viroid 2 (GYSVd2), Australian grapevine viroid (AGVd), Hop stunt viroid (HSVd) and Citrus exocortis viroid (CEVd). Although these viroids are reported to have worldwide distribution (Elleuch *et al.*, 2003; Flores *et al.*, 1985; Hadidi *et al.*, 2003; Koltunow & Rezaian, 1988; Koltunow *et al.*, 1989; Koltunow & Rezaian, 1989; Rezaian *et al.*, 1988; Rezaian, 1990; Taylor & Woodham, 1972), little information is available regarding their presence and/or their properties in Iran. This paper reports identification and partial characterization of four viroids in this country.

MATERIALS AND METHODS

Random sampling of grapevine was carried out in Fars province in late June or early September in 2007-08. Total nucleic acid was extracted from mature grapevine leaves using isopropanol (Wan Chow Wah & Symons, 1997) or silicon dioxide (Boom *et al.*, 1990). Purified nucleic acid was stored at -80 °C until used.

cDNA was prepared using specific primers for each viroid (Staub *et al.*, 1995; Wan Chow Wah & Symons, 1997). Total nucleic acid was heated at 70 °C for 10 min. and chilled on ice. Reverse transcription mixture (50 mM Tris-HCl pH8.3, 50 mM KCl, 4 mM MgCl2, 10 mM DTT, 1mM each dNTP) and MMuLV reverse transcriptase (200 units) were added and incubated at 42 °C for 60 min. PCR amplification was carried out using an optimized protocol. DMSO (5%) and glycerol (10%) were added to enhance amplification (Zaki-Aghl & Izadpanah, 2003).

Amplified fragments were inserted in pTZ57R plasmid and sequenced (Sambrook *et al.*, 1989).

Cucumber seedlings were mechanically inoculated with purified nucleic acid preparations from grapevine leaves using 0.07 M Tris-HCl buffer, pH8. The plants were assessed for the presence of viroids by RT-PCR four weeks postinoculation (Zaki-Aghl & Izadpanah, 2003). Infected cucumber leaves were used as the source of some of the viroids.

RESULTS AND DISCUSSION

Using RT-PCR, GYSVd1 (DQ408542, FJ940920), GYSVd2 (FJ940922, FJ940921), AGVd (FJ940923) and HSVd (EU647233) were identified in vineyards of southern Iran (Figure 1).

AGVd and HSVd were also detected in inoculated cucumber seedlings either alone or in mixed infection. No samples were found infected with *Citrus exocortis viroid* in surveyed vineyards.

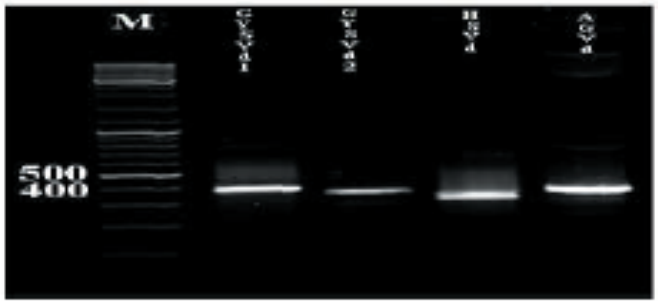

Figure 1. Electrophoretic pattern of grapevine viroids amplified by RT-PCR

GYSVd1 and GYSVd2 were either associated with yellow speckle or with no symptoms. Yellow speckle symptoms were more severe in mixed infection with grapevine fanleaf virus and symptoms changed to yellow vein banding (Szychowski *et al.*, 1995). AGVd and HSVd were associated with no obvious symptoms in grapevine as reported earlier but induced stunting and leaf rugosity in cucumber (Hadidi *et al.*, 2003; Rezaian, 1990).

GYSVds were identified in vineyards more often than other viroids and AGVd had lowest frequency (Figure 2).

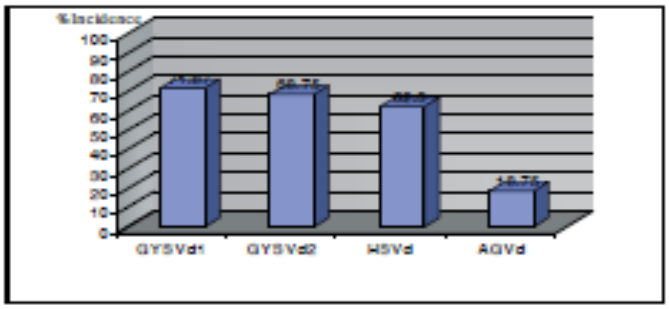

Figure 2. Relative frequency of grapevine viroids in leaf samples from vineyards in southern Iran

Molecular analysis showed some differences between Iranian grapevine isolates and their non-Iranian counterparts. GYSVd1, GYSVd2, HSVd and AGVd had 89-93%, 97-99%, 94-99% and 90-98% homology with the sequences deposited in the GenBank, respectively. Based on whole genome alignment and symptom expression Iranian, isolates of GYSVd are grouped with type II sequences (Szychowski *et al.*, 1995); however they had enough difference to be considered as a new type. More isolates must be sequenced to confirm these results. They

also had similar structure in hairpin I formation as variant IX (Amari *et al.*, 2001; Polivka *et al.*, 1996).

AGVd had two extra nucleotides in the genome located opposite to pathogenicity domain. They caused a difference in secondary structure of the viroid.

Grapevine isolates of HSVd were not homogenous and at least 5 different variants were identified by SSCP or sequencing. All of these isolates were grouped in hop type group of HSVd besides other HSVd-g isolates (Amari *et al.*, 2001, Kofalvi *et al.*, 1997, Polivka *et al.*, 1996).

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