18th Congress of the International Council for the Study of Virus and Virus-Like Diseases of the Grapevine (ICVG)

PROCEEDINGS
September 7,11, 2015 Ankara - Turkey
INTRODUCTION

Despite their small size, viroids have a complex genome in which the mechanism of pathogenicity and symptom induction remains unclear (Owens and Hammond, 2009). Species of the family Pospiviroidae have a rod-like secondary structure with five structural-functional domains: P (pathogenicity), C (central), V (variable), TL (terminal left) and TR (terminal right) (Keese and Symons, 1985).

Complex interactions between structural domains are determinant of symptom development and pathogenicity in Pospiviroidae. Studies with artificial chimeras derived from the viroids (Góra et al., 1996; Visvader and Symons, 1986) have provided information on the relationship between specific regions of the viroid molecule and symptom expression particularly in the members of the genus Pospiviroid (Góra et al., 1996; Visvader and Symons, 1996; Sano et al., 1992). Members of the genus Apscaviroid are restricted to woody plants and because of this, studies on the relationship between their RNA secondary structural domains and their biological properties are very limited. Australian grapevine viroid (AGVd), a member of the genus Apscaviroid, is symptomless in grapevine, but induces stunting and mottling on tomato plants. Grapevine yellow speckle viroid 1 (GYSVd1), on the other hand, induces yellow speckle symptoms in developed leaves of grapevine under favorable conditions. It is naturally restricted to grapevine (Hadidi et al., 2003).

The aim of this study was to evaluate the effect of exchanging distinct domains of AGVd with their corresponding parts from GYSVd1 on symptom expression and identifying the pathogenicity determinants in a member of the genus Apscaviroid.

MATERIALS AND METHODS

The compositions of four chimeric molecules designed to exchange the structural domains of the secondary structure of AGVd and GYSVd1 are shown in Fig. 1A. The segments were replaced so to preserve the CCR and the secondary structure of AGVd. Constructs designated AGYSd-TL, AGYSd-P, AGYSd-V and AGYSd-TR contained replacements of TL, P, V, or TR, respectively, from GYSV into the corresponding regions of AGVd genome. The constructs were put under control of the 35s promoter and agroinoculated to tomato and cucumber plants. Monomeric AGVd and GYSVd1 DNA in the same vector were inoculated as positive and negative controls, respectively. Three weeks post-inoculation, RNA was extracted from newly grown leaves of inoculated plants and RT-PCR was carried out using AGV-H/C primer pair corresponding to CCR domain of AGVd (Wan Chow Wah and Symons, 1997).

RESULTS

RT-PCR analysis and amplicon sequencing confirmed production of de novo populations of AGVd-GYSVd1 chimeras in the inoculated plants. This meant that, all chimeras could replicate in the inoculated plants. Sequencing of PCR fragments from infected plants showed that the resulting progeny is identical to the original sequence. GYSYd1 did not replicate in the inoculated plants.

Symptoms of AGVd wild type in tomato plants were stunting, mottling and leaflet deformation. But, tomato plants inoculated with AGYS-TL showed rugosity, severe leaf deformation, leaf curl and severe narrowing of apical leaves; AGYS-P induced rugosity, leaf curl and mild yellowing on new leaves of tomato. AGYS-V caused severe deformation and twisting of leaves in infected plants. AGYS-TR developed only stunting and mottling (Fig. 1B). All chimeric molecules were similar to AGVd wild type in inducing stunting in tomato. Stunting was the only symptom generated by replication of the chimeras in cucumber plants.

DISCUSSION

The mechanisms of symptom induction by viroids are poorly understood (Owens and Hammond 2009). In the present study, replacement of the TL and P domains of AGVd by those of GYSVd1 altered the severity of symptoms in tomato. But
exchange of V domain led to change in the symptom types. TR domain had no obvious effect on the symptoms. Symptom development and pathogenicity are complex aspects of viroid biology. Disease is not generated only by a single domain of the viroids. Rather, interactions of different viroid domains as well as interaction of the latter with host factors play important roles in disease development (Gomez et al. 2008).

In chimera construct of the genus Pospiviroid severity of symptoms is determined by TL and P domains. However V and TR domains also interact with those domains in symptom induction (Góra et al., 1996, Owens and Hammond 2009).

In PSTVd, severity of symptoms and symptom type are highly dependent on the concentration of the viroid generated siRNA in the tissues (Góra et al., 1996; Owens and Hammond, 2009; Sano et al., 1992). Enhancement of symptom severity by the replacement of the TL and P domains may be due to changes in affinity of the viroid RNA for host factors (Itaya et al., 2002), or provide the generation of certain new specific viroid-derived siRNAs (Carrington and Ambros, 2003).

**Figure 1.** A, Schematic representation of AGVd-GYSVd 1 chimeras used in the present study. Hatched areas represent parts of GYSVd 1 in corresponding regions of AGVd genome. B, Symptoms induced in tomato by AGVd (mottling), AGYS-TL (severe leaflet deformation), AGYS-P (rugosity and mild yellowing), AGYS-V (twisting and severe malformation of leaves), and AGYS-TR (mottling and leaf curl) compared to healthy control (H).

**ACKNOWLEDGMENTS**

This study was supported in part by funds from the Iranian Council of Centers of Excellence and National Foundation of Elites.

**REFERENCES**


