Journal of Cell and Molecular Research (2016) 8 (1), 35-36

Research Commentary

Putative RFLP Analysis Between HSVd-sycv and Closely Related Variants

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Received 23 October 2015 Accepted 19 November 2015

Abstract

Yellow corky vein is a prevalent disease among navel oranges in the Fars province of Iran. Previously we showed that a variant of Hop stunt viroid (HSVd-sycv) was associated with the disease. It was closely related to citrus variant of HSVd from Japan (HSVd-cit8) and with 93.7% homology with lime yellow corky vein variant of HSVd (HSVd–lycv). In this study, putative RFLP (Restriction Fragment Length Polymorphism) analysis using Vector NTI program (version 9.0.0) showed that some restriction enzymes could cut HSVd-sycv but not HSVd-lycv. On the other hand, *M.Ngo* BIX and *Fau*I which could cut HSVd-lycv at positions of nt 53 and 97, respectively, could not cut HSVd-sycv. Likewise, some restriction enzymes could cut HSVd-cit8 but could not cut HSVd-sycv. Therefore these variants can be recognized by these restriction enzymes.

***Keywords*:** HSVd-sycv, HSVd-lycv, Restriction Fragment Length Polymorphism analysis

Hop stunt viroid (HSVd), associated with serious disorders of economic importance, i.e. hop stunt, dapple fruit disease of plum and peach, apricot and jujube disease and citrus cachexia (Amari et al., 2000; Avina-Padilla et al., 2015; Gorsane et al., 2010). HSVd is one of the most widespread and economically important viroid of citrus plants (Su et al., 2015) and citrus strains of this viroid, have been implicated in yellow corky vein disease of Kagzi lime (*Citrus aurantifolia*), reported from India (Anirban Roy and Ramachandran, 2003).

The disease was characterized by yellow spots on leaf lamina, and rapid spread along the mid and lateral veins. The veins became rough on the lower surface of their leaves and developed corky tissues. A yield loss of 51.3–60.4% was reported from Assam (Azad, 1993). An isolate of HSVd (lime yellow corky vein variant; HSVd–lycv) was associated with the disease (Roy and Ramachandran, 2006). In recent years, a disease with specific signs of yellow corky vein has appeared in navel oranges in the Fars province of Iran. Previously we reported that a novel variant of HSVd (sweet orange yellow corky vein variant; HSVd-sycv) is constantly associated with the disease. The full sequence of the viroid was composed of 302 nucleotides. It was closely related to citrus variant of HSVd from Japan and with 93.7% homology with lime yellow corky vein variant of HSVd–lycv. It was different in a single nucleotide from a noncachexia variant of HSVd from Japan (Bagherian and Izadpanah, 2010). In this research, putative Restriction Fragment Length Polymorphism (RFLP) analysis (Jamshidi et al., 2014) using Vector NTI program (version 9.0.0) showed that these related variants of HSVd could be recognized by some restriction enzymes.

Our previous nucleotide sequence data (Bagherian and Izadpanah, 2010) were used to predict mutual differential restriction enzymes between HSVd-sycv and closely related variants using Vector NTI program (version 9.0.0). *In silico* analysis by using Vector NTI program (version 9.0.0) proved that *Bbr*7I, *Bbs*I, *Bse*RI, *Cvi*AII, *Fat*I, *Hpy*CH4I, *M.Cvi*AII, *M.Cvi*QVII, *M.Cvi*SII and *M.Esa*BS1I restriction enzymes could cut HSVd-sycv but not HSVd-lycv. On the other hand, *M.Ngo*BIX and *Fau*I which could cut HSVd-lycv at positions of nt 53 and 97, respectively, could not cut HSVd-sycv (Figure 1). In the same manner, *M.Eco*RII, *M.Bst*NI, *M.Eco*KDcm and *Bst* NI could cut HSVd-cit8 but could not cut HSVd-sycv (Figure 2). Although viroid interactions are reported to alter plant reaction (Katsarou et al., 2015), none

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of the common citrus viroids except HSVd-sycv were detected constantly in yellow corky vein affected plants. Point mutation experiments and testing the variants on the same host must be carried out to verify the role of single nucleotide change in production of specific symptoms.

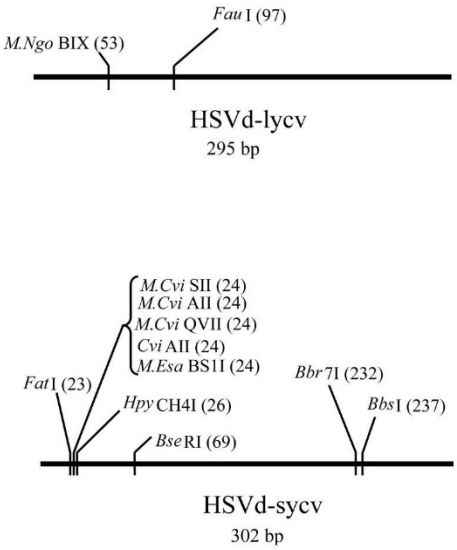

Figure 1. Putative restriction sites of HSVd-lycv vs HSVd-sycv using Vector NTI program (version 9.0.0) database.

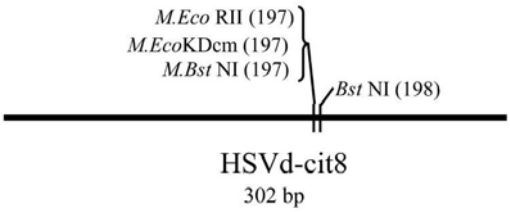

Figure 2. Restriction enzymes able to cut HSVd-cit8 but not HSVd-sycv according to *in silico* analysis using Vector NTI program (version 9.0.0) database.

Acknowledgments

The author would like to thank the Iran's National Foundation of Elites and Center of Excellence in Plant Virology for supporting this research.

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