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Short communication

Putative RFLP Analysis between HSVd-sb and Closely Related Variant

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Abstract
Sweet lime split bark is a disorder of increasing importance in Fars province. Previously we showed that a new variant of hop stunt viroid (HSVd-sb) was related to the disorder. It had homology with a noncachexia variant of hop stunt viroid (CVd-IIa-117) which causes cracking the bark of trifoliate orange rootstocks. In this study, showed that *M.Ngo*BIX, *Bgl*I and *Bsr*I restriction enzymes can cut HSVd-sb according to putative RFLP analysis using Vector NTI program (version 9.0.0) database but these restriction enzymes can't cut CVd-IIa-117. In other hand some restriction enzymes can cut CVd-IIa-117 but can't cut HSVd-sb. Therefore these two variants can be recognized by these restriction enzymes.

Keywords: HSVd-sb; CVd-IIa-117; Restriction Fragment Length Polymorphism analysis

Introduction

Hop stunt viroid (HSVd) (6) has been identified in a large spectrum of hosts including hop, cucumber, grapevine, citrus, plum, peach, pear (10), apricot and almond (1; 3). In some hosts, like grapevine (10; 7) and apricot (1), the contamination appears to be latent; in others it is related to serious disorder of economic importance, i.e. hop stunt (10), dapple fruit disease of plum and peach (8) and citrus cachexia (4; 9).

Split bark is a disorder that was first reported in sweet lime (*Citrus limettoides*) in 1983 among declining sweet lime trees (5). The disorder is described by cracks in surface of the young bark, which spreads quickly along foliage bark. The barks turned wide and deep. The infected tree shows delayed growth without any signs on their fruits and leaves. Split bark disorder is becoming important sweet lime (*Citrus limettoides*), a commercially important variety in Iran. Previously we reported that a novel variant of HSVd (split bark variant; HSVd-sb) was constantly associated with the disorder. It had only 94.8% homology with a noncachexia variant of *Hop stunt viroid* (CVd-IIa-117) which causes mild bark-cracking symptoms on Pomeroy trifoliate orange rootstocks. According to the performed molecular comparisons, HSVd-sb differed from CVd-IIa-117 in the variable domain in the so-called "cachexia expression motif". The number of nucleotide of CVd-IIa-117 is four nucleotides more than HSVd-sb and so it's molecular weight and probably severe cracks induced by HSVd-sb occurred because of variation in this motif and/or sequences.

Our previous nucleotide sequence data (2) were used to predict differential restriction enzymes between HSVd-sb and CVd-IIa-117 using Vector NTI program (version 9.0.0).

In silico analysis by using Vector NTI program (version 9.0.0) proved that showed that *M.Ngo*BIX, *Bgl*I and *Bsr*I restriction enzymes can cut HSVd-sb according to putative RFLP

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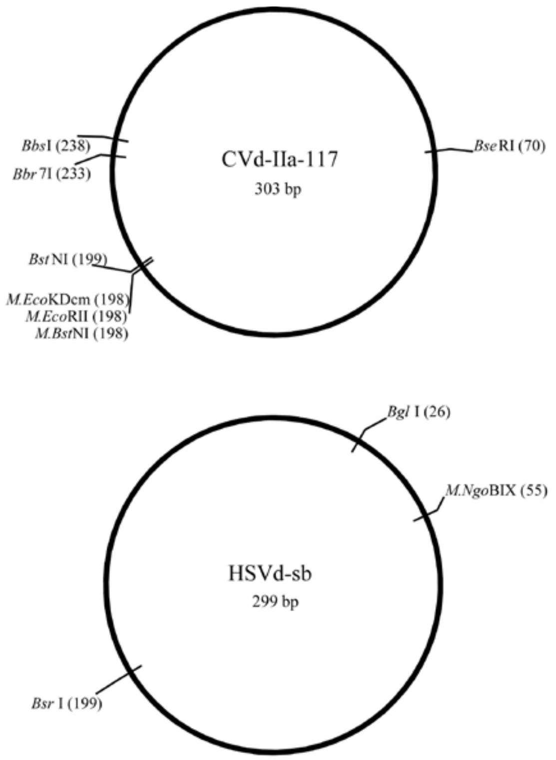

Fig. 1. Putative restriction sites of HSVd-sb vs CVd-IIa-117 using Vector NTI program (version 9.0.0) database.

analysis using Vector NTI program (version 9.0.0) database but these restriction enzymes can't cut CVd-IIa-117. In other hand *Bbr*7I, *Bbs*I, *Bse*RI, *Bst*NI, *M.Bst*NI, *M.Eco*KDcm and *M.Eco*RII restriction enzymes can cut CVd-IIa-117 but these restriction enzymes can't cut HSVd-sb. Therefore these two variants can be recognized by these restriction enzymes (Figure 1). Hence these two variants can be recognized by these restriction enzymes.

Although viroid interactions are reported to alter plant reaction (Verniere *et al.,* 2006), none of the common citrus viroids except HSVd-sb were detected constantly in split bark 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.

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