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Short communication

Prediction of Secondary Structure of Citrus Viroids Reported from Southern Iran

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Abstract

Viroids are smallest, single-stranded, circular, highly structured plant pathogenic RNAs that do not code for any protein. Viroids belong to two families, the *Avsunviroidae* and the *Pospiviroidae*. Members of the *Pospiviroidae* family adopt a rod-like secondary structure. In this study the most stable secondary structures of citrus viroid variants that reported from Fars province were drawn. The most stable secondary structures of these viroid variants were a classical rod-like structure and adopted cruciform structure including various additional small hairpins. Comparison of secondary structures of these viroid variants with other viroid variants indicates their highly similarities in the rod-like structures, number of loops and free energies and it's obvious to result these closest variants of the *Pospiviroidae* family. HSVd-cit1 and CVd-III-1 differed from under study variants in the stability and number of secondary structure branches. Because of relationship between secondary structure and pathogenicity of viroids, it is supposed that these two variants possibly will have high risk for citrus cultivations.

Keywords: Citrus viroids, secondary structures, computational analysis

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Introduction

Viroids are the smallest plant pathogenic RNAs with a length of 246 to 401 nucleotides that do not encode any protein (1-15). Nucleotide sequencing of viroids revealed that they are single-standard RNAs with the molecules connecting at both ends to make circular a shape (5). Therefore, secondary structures can be predicted for them in this case while the number of base pairings would become maximum. In this rod-like model a regular arrangement of double-stranded parts and small internal loops is observed. This secondary structure was furthermore proposed for the *Pospiviroidae* family members. However, the viroids of *Avsunviroidae* family have a branched or rod-like secondary structure (3, 6). For the structure of the *Pospiviroidae* family five structural and functional domains have been defined including: Central domain (C) consisting of central conserved domain (CCR), pathogenicity domain, variable domain, terminal right domain and left terminal domain (7, 9).

The *Pospiviroidae* family consists of five genus; *Pospiviroid*, *Apscaviroid*, *Coleviroid*, *Hostuviroid*, *Cocaviroid*. Most viroids belong to this family and the discrimination of these five viroid genus is originated from the nucleotide sequence of central conserved domain (2, 6, 10). Heretofore there are reports of several citrus viroids all of which belong to the *Pospiviroidae* family and are sorted into

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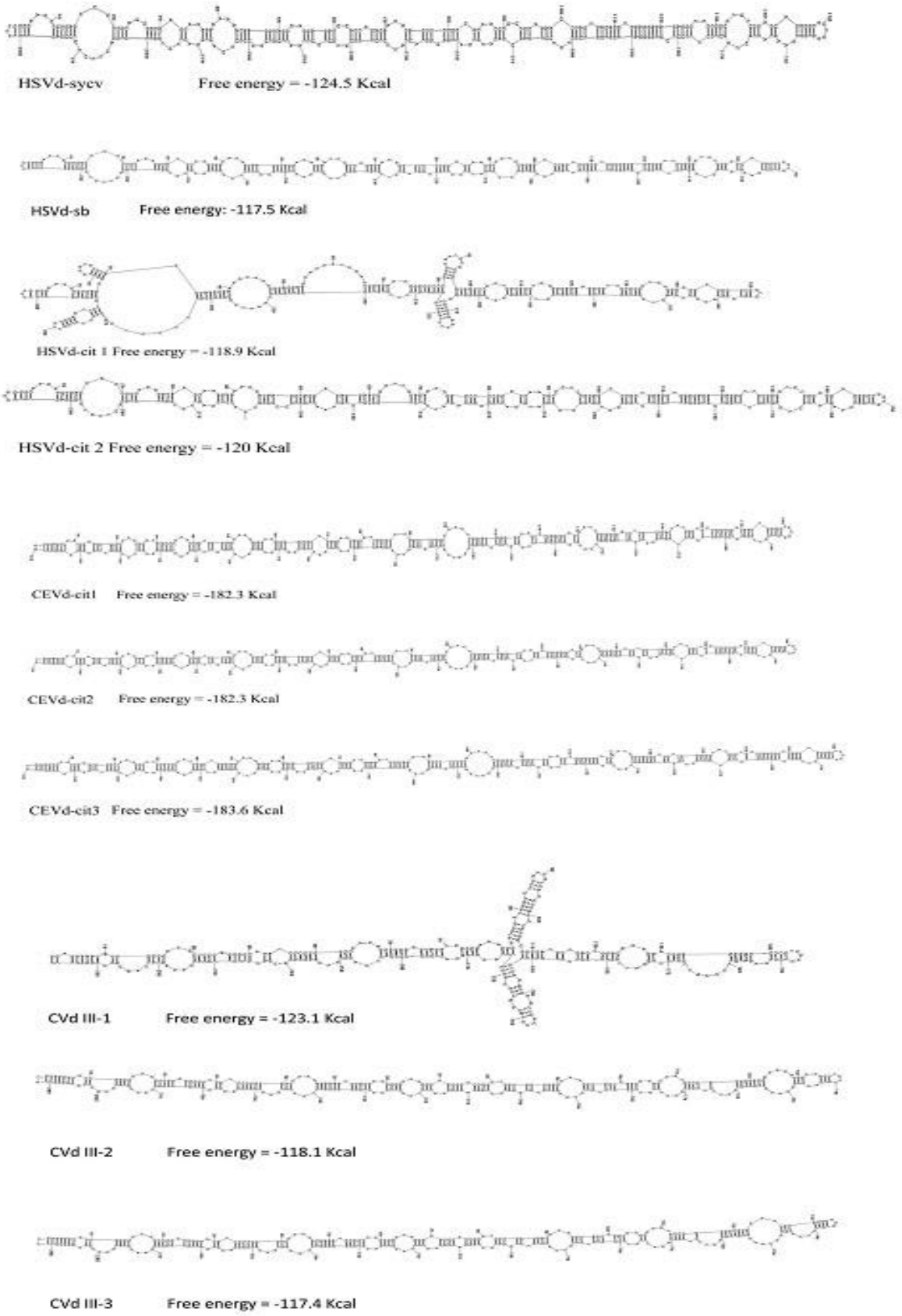

Fig. 1. Secondary structures and minimum free energies of *HSVd, CEVd* and *CVd-III* from Fars province.

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Table 1: Characteristics of viroid isolates used in this study.

Isolate	Accession no.	Host	Origin	Number of nucleotides
HSVd-sycv	FJ465506	Sweet orange	Fars	302
HSVd-sb	FJ465507	Sweet lime	Fars	299
HSVd-cit1	FJ626867	Sweet lime	Fars	302
HSVd-cit2	FJ626868	Lime	Fars	298
CEVd-1	FJ626865	Sweet lime	Fars	370
CEVd-2	FJ626866	Sweet lime	Fars	370
CEVd-3	FJ626864	Sweet lime	Fars	370
CBLVd	GQ166528	Sweet lime	Fars	230
CVd-III-1	GQ166531	Sweet lime	Fars	297
CVd-III-2	GQ166529	Sweet orange	Fars	293
CVd-III-3	GQ166530	Sweet orange	Fars	293

four genus including *Pospiviroid*, *Apscaviroid*, *Hostuviroid*, *Cocaviroid* (1). In recent years, *Citrus exocortis viroid* (*CEVd*), *Hop stunt viroid* (*HSVd*), *Citrus bent leaf viroid* (*CBLVd*) and *Citrus viroid-III* (*CVd-III*) variants of citrus in southern Iran have been reported that the two first belong to the *Hostuviroid* and *Pospiviroid*, respectively and the other two variants are of the *Apscaviroid* genus (Bagherian and Izadpanah unpublished). In this study the most possible stable secondary structure for the citrus viroids reported from Fars province is depicted and analyzed.

Different *HSVd*, *CEVd*, *CBLVd* and *CVd-III* variants sequence reported from Fars citrus (Table 1) was obtained from NCBI database and their secondary structures were depicted by the RNAstructure software (version 4.6) and their free energy levels were compared with each other. Since the designed primer for *CBLVd* was not able to reproduce the entire genome of this viroid (Bagherian and Izadpanah unpublished) available information in GenBank is not sufficient to obtain the secondary structure for this viroid.

Not only nucleotide sequences but also secondary structures are closely related to the functions of non-coding RNAs, so secondary structures are conserved during evolution. Determining the viroid secondary structure has gained significant consideration of the investigators, as it is one of the key subjects in understanding subjected diseases. Secondary structure can be clarified by nuclear magnetic resonance spectroscopy and x-ray crystallography. Techniques involving small-angle x-ray solution scattering, hydroxyl radical probing, in-line probing, and modification of bases by selective 2'-hydroxyl acylation analyzed by primer extension, dimethyl sulfate, l-cyclohexyl-3-[2-morpholinoethyl] carbodiimide metho-p-toluene sulfonate, 1,1-dihydroxy-3-ethoxy-2-butanone, nucleases, diethyl pyrocarbonate, and ethylnitrosourea are also used to determine RNA secondary structures. Overall, these processes are slow, expensive, and difficult. That is why using computational methods and data analysis tools to forecast the secondary structure of RNA is essential (12). Hence, the prediction of these conserved secondary structures is among the most important tasks in RNA bioinformatics, because it provides useful information for further functional analysis (8).

Drawn secondary structures of understudy viroid variants are rod-like and adopt numerous small loop structures like other viroid variants of the *Pospiviroidae* family. Comparison of secondary structures of

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different viroids isolates; *HSVd*, *CEVd*, *CBLVd* and *CVd-III* from Fars province indicated high similarities and this implies great affinity of these variants with each other. Secondary structures of *HSVd-cit1* and *CVd-III-1*, determined the difference in stability and lateral branches with other variants (Fig. 1). Great discrepancy in nucleotide sequence, nucleotide combination and phylogenetic grouping of these two variants with other variants (Bagherian and Izadpanah unpublished) also confirm this diversity.

It has been well accepted that the RNA secondary structures of viroids are closely related to their functions and are conserved during evolution (5, 13). Due to the importance of the viroids, investigating these pathogenic factors specifically HSVd-cit1 and CVd-III-1 throughout their infections clones, performing pathogenicity tests, observing the symptoms and conception of the effect of different secondary structures or their pathogenicity are requisite as it is assumed that variants separating from other variants perhaps will have high risk for the citrus in the future.

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