

## *Short Report*

# **SIMULTANEOUS ASSOCIATION OF SEVERAL CITRUS VIROIDS WITH PSOROSIS AND RING PATTERN SYMPTOMS IN CITRUS TREES IN MAZANDARAN PROVINCE**

**S. M. ALAVI<sup>1</sup> and H. RAHIMIAN<sup>2</sup>**

1. Genetic and Agricultural Biotechnology Institute of Tabarestan

2. Department of Plant Pathology Sari Agricultural Sciences and Natural Resources University

### **Abstract**

Citrus is one of the most important fruit crops in Iran. Among the citriculture areas of Iran, Mazandaran province has the highest acreage (about 100,000 ha), production and number of varieties nationwide. Unauthorized importation of citrus species and cultivars and propagation by growers and nurserymen have led to perpetuation and spread of virus, viroid and other graft-transmissible pathogens of citrus in Mazandaran. Nonetheless, the putative agents of several of such graft-transmissible diseases including citrus ring pattern (CRP) have remained undetermined and the use of certified disease-free propagative stocks has not gained widespread attention. With the onset of the aphid transmission of *Citrus tristeza virus* (CTV) in the area, trend has shifted toward the use of CTV-tolerant rootstocks, mainly trifoliolate orange (*Poncirus trifoliata*) and its hybrids, citranges (*P. trifoliata* × *Citrus sinensis*) and citrumelos (*P. trifoliata* × *C. paradisi*) with concurrent use of disease free (mostly CTV-free) budwoods and budlings. Some nurserymen and growers are becoming keen about the impact of several viroid species which have, herebefore, been regarded as harmless to orange and mandarins produced on sour orange (*C. aurantium*) rootstock but would adversely affect vigor and yield of trees on trifoliolate orange and its hybrids. Preliminary attempts to detect viruses causing citrus psorosis and CRP diseases in symptomatic orchard trees and in plants graft-inoculated with the infected sources in the greenhouse, have remained unsuccessful and no amplicon specific for *Citrus psorosis virus* has been obtained in PCR trials thus far.

In the present study, bark patches from twigs of Satsuma mandarin (*C. unshiu*) trees showing psorosis young leaf symptoms and those displaying CRP symptoms on mature leaves were budded onto one-year-old seedlings of Etrog citron (*C. medica*, Arizona 861) and sour orange and 2-year-old Washington navel sweet orange on sour orange rootstock. The budded plants were kept in the greenhouse at 22-30°C, and observed periodically for the appearance of symptoms. Leaf flecking appeared on all citrus plants inoculated with buds from the psorosis-infected trees within one to three months. Midvein browning and leaf epinasty developed on Etrog citron seedlings budded with bark strips from several of the CRP-infected trees. Sweet orange budlings started to show some ring and line patterns in three to four years after inoculation with two CRP sources. Sour orange seedlings remained symptomless up to the termination of the experiment (year 6). Ribonucleic

acid(RNA) was extracted from both symptomatic sweet orange and Etrog citron plants with TRIzol reagent(Invitrogen, Paisley, UK) and subjected to Reverse transcription-polymerase chain reaction(RT-PCR) using primers specific for CPV and citrus viroids in single PCR. The PCR products were electrophoresed on 1.5% agarose gels and stained with ethidium bromide. Bands representing those of citrus viroids including citrus bent leaf, hop stunt, citrus dwarfing, citrus bark cracking and citrus viroid VI were present in the Etrog citron and sweet orange plants inoculated with several sources of CRP and CP-like symptomatic Satsuma mandarin trees. *Citrus exocortis viroid* was not detectable in any sample tested. This is the first report on the simultaneous presence of multiple citrus viroids in citrus trees showing symptoms of psorosis and ring pattern diseases in Iran.