Preliminary Observations on the Phytosanitary Status of the Croatian Satsuma Mandarin (*Citrus unshiu* Marc.) Collection

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ABSTRACT. The presence of graft-transmissible pathogens in Croatia, especially *Citrus tristeza virus* (CTV) had been previously confirmed in citrus field trees during preliminary surveys. The highest occurrence of CTV was recorded in satsuma mandarin (*Citrus unshiu* Marc.), commercially the most important citrus in the country. With the aim of assessing the phytosanitary status of the established satsuma collection, biological indexing of graft-transmissible pathogens was performed, followed by serological assay of CTV and *Citrus psorosis virus* (CPsV). Results of 18 tested clones from the collection orchard showed that only two clones were CTV-negative, while they were all CPsV-negative, both in biological and laboratory tests. Furthermore, severe CTV strains displaying stem pitting and seedling yellows in grapefruit and sweet oranges were found in four tested clones. No biological indication of other disease presence in the collection has been found so far. Further testing of the satsuma collection is underway, as well as shoot-tip grafting and thermotherapy of the most important cultivars.

Preliminary surveys of citrus field trees in Croatia showed their unsatisfactory sanitary status especially with regard to the presence of *Citrus tristeza virus* (CTV) even though symptoms in the field are mostly absent. The highest CTV infection rate was recorded in satsuma mandarin (*Citrus unshiu* Marc.), commercially the most important citrus in the country (2, 5). The citrus germplasm collection had been lost during the time of war (1991-1995) in ex-Yugoslavia. In 2005 the satsuma collection orchard was re-established, comprising 12 mandarin cultivars and 51 clones collected from different field sources.

Eighteen clones of the 12 satsuma cultivars were tested for the presence of major graft-transmissible pathogens. The presence of CTV and *Citrus psorosis virus* (CPsV) was tested by serological and biological assays, while the presence of *Citrus variegation virus* (CVV), *Satsuma dwarf virus* (SDV), Citrus tatter leaf virus (CTLV), and oak leaf pattern disease agents were tested only by biological assays.

Indicator plants of seven citrus species were used for bioassays. Indexing was done by grafting, following procedure described by Roistacher (4). Mexican lime (*Citrus aurantifolia* Swing.), Madame Vinous and Pineapple sweet oranges (*C. sinensis* (L.) Osbeck), Sicillian sour orange (*C. aurantium* L.) and CRC grapefruit (*C. paradisi* Macf.) were used primarily as indicators for different CTV strains. In addition, sweet oranges were used for CPsV and CVV detection, as well as for concave gum, impietratura and cristacortis which may induce oak leaf patterns symptoms. Troyer and Carizzo citrange (*C. sinensis* (L.) Osbeck *x Poncirus trifoliata* (L.) Raf.) were used for CTLV detection. Herbaceous hosts (white sesame, red kidney bean, and *Chenopodium quinoa*) were also utilized for SDV, CVV and CTLV detection.

The appearance of major disease symptoms after bud grafting of the main citrus indicator plants, in comparison with positive and negative control plants, was monitored. Serological tests for CTV and CPsV detection were performed using DAS-ELISA (1) and DTBIA (4) directly on 18 selected clones from the collection orchard. In addition, Mexican lime and Madame Vinous sweet orange indicator plants grafted with the same 18 clones were also tested.

CPsV was not detected either biologically or serologically, while only two clones (Chahara 1/10-01 and Wakiyama 7/11-01) were CTV-negative in both assays. Symptoms of leaf cupping, vein clearing, stem pitting (SP), seedling yellows (SY) and retarded growth were observed in CTV-infected Mexican lime seedlings (Table 1). The severe CTV strains were found in four tested clones (Ichimaru 2/13-01 and 3/32-01; Aoshima 3/9-01 and 3/27-01). Besides intense symptom appearance on lime indicator plants, SP and SY were also displayed in grapefruit and sweet oranges, but only CTV strains found in Aoshima 3/27-01 induced SP symptoms in sour orange indicators.

TABLE 1
THE PRESENCE AND INTENSITY* OF SYMPTOMS ON MEXICAN LIME PLANTS AFTER GRAFTING WITH BUDS FROM SATSUMA MANDARIN COLLECTION SOURCES

Satsuma clone	Leaf cupping	Vein clearing	Stem pitting	Chlorotic blotching	Retarded growth
Aoshima Bud3/9-01	3	3	+	-	+
Aoshima 3/27-01	3	3	+	+	+
Chahara 1/25-01	2	-	-	-	-
Chahara 1/10-01	-	-	-	-	-
Ichimaru 2/13-01	3	3	+	+	+
Ichimaru 3/32-01	3	2	+	+	+
Kuno Vu2/9-98	2	2	+	-	-
Miho 9/5-01	-	-	+/-	-	-
Okitsu 7/9-01	-	-	-	-	-
Ootsu 1/5-01	3	3	+	+	+
Owari 145	-	-	-	-	-
Saigon 9/5-01	-	-	-	-	-
Seto Vu3/9-98	1	2	+/-	-	-
Wakiyama 7/11-01	-	-	-	-	-
Wakiyama 7/27-01	3	1	-	-	-
Zorica Rana 3/21-01	2	1	+	-	-
Zorica Rana 5/19-01	3	1	+	+	-
Zorica rana 9/19-01	3	2	+	+	+

*Symptoms presence was classified as: symptom clearly present (+); not present (-); doubtful (+/-). For leaf cupping and vein clearing symptoms, positive results were classified using a scale of three severity classes (3-the most severe symptoms).

ELISA and DTBIA results for CTV detection are in agreement with biological indexing, except in the cases of inefficient virus transmission during the grafting procedure (Okitsu 7/9-01, Saigon 9/5-01 and Owari 145). No biological indication of other pathogen presence has been found so far, but further testing is under way, as well as the thermotherapy and shoot tip grafting of the most important Satsuma cultivars.

For the first time, the biological characterization of principal virus diseases was attempted in the Croatian satsuma mandarin collection. Biological and serological assays showed extremely high CTV infection rate in the collection. Results obtained here indicate higher CTV infection rate than suspected. The appearance of different symptoms suggests biological diversity among detected CTV isolates and confirms the existence of severe CTV strains in the country (2). So far, the presence of other graft-transmissible pathogen has not been confirmed.

LITERATURE CITED

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