Selection of a Mild Sub-isolate of *Citrus tristeza virus* for Preimmunization of Pera Sweet Orange

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ABSTRACT. *Citrus tristeza virus* is endemic in Brazil and its major destructive effect is stem pitting. Pera sweet orange, the most important Brazilian variety, requires preimmunization with protective mild isolates of the virus to be successfully grown. Since the 1970s, over 50 million trees of this variety have been propagated from plants infected with the protective IAC mild isolate. Recently, the citrus industry has expanded to the cooler southwest areas, where development of stem pitting proved to be more severe in Pera and other sweet orange varieties. Sub-isolates of IAC were therefore selected from trees with superior field performance, and one protective sub-isolate, named Citrovita, showed efficient protection when challenged by severe strains both in greenhouse and field tests. Pera sweet orange trees infected with this sub-isolate are showing excellent field performance, with little or no stem pitting, high productivity, and large fruit size. Single-strand conformation polymorphism analysis of the viral coat protein gene revealed differences between this sub-isolate and the original IAC protective isolate.

The use of scion/rootstock combinations tolerant to *Citrus tristeza virus* (CTV) was critical for post-war revival of the Brazilian citrus industry, but soon growers discovered that severe CTV stem pitting (SP) was affecting trees of many varieties, mainly sweet orange, grapefruit and Mexican lime, reducing yield, fruit size and life expectancy of the orchards (3, 4, 5, 10). SP in small or moderate amounts also occurs in most other citrus varieties, but without significant economic effects (9).

Pera sweet orange was abandoned by most growers for over 10 yr (1961-1975) but regained popularity in the mid 1970s when cross-protected budwood from the variety Pera IAC (6) became available. Selections of Pera sweet orange, to be grown commercially on the São Paulo plateau (500 to 1,000 m above sea level), need to be preimmunized with mild protective CTV isolates. Over 25 yr, Pera sweet orange preimmunized with the IAC isolate has shown little or no breakdown in the warmer areas, but new orchards in the cooler south and southwest are damaged by mild to severe SP affecting Pera trees of several non-preimmunized lines, and to a smaller extent, also Pera IAC, suggesting an effect of temperature similar to that reported elsewhere (2, 14, 15).

Segregation of various strains within the IAC isolate may have been induced by environmental conditions (2, 12), and may have led to the occasional presence of field trees of high performance. Other Pera nucellar lines naturally infected with mild CTV strains have also been found in the field and selected for commercial planting, for example Bianchi and Ipiguá-2 (13).

To control SP in these cooler areas, several CTV isolates were selected and evaluated for their cross protection ability. Here we report on a new superior sub-isolate for cooler areas, named Citrovita, selected from plants originally preimmunized with the protective IAC isolate.

Visual surveys in commercial orchards of Pera sweet orange cross protected with the mild IAC isolate revealed some trees outstanding in vigor, productivity and fruit quality. Budwood was collected from 100 such trees, grafted on Rangpur lime, and planted in an experimental field in the cool southwest. The selected

plants were indexed for CTV on Mexican lime. SP in the shoots was recorded as mild to severe (level 0 to 4) over 10 yr.

Selected trees containing three sub-isolates named UNESP-1, UNESP-2, and Citrovita, plus the original IAC isolate as control were indexed under greenhouse conditions for CTV, expression of seedling yellows (SY), psorosis, vein enation, exocortis, and cachexia according to Roistacher et al. (7). Mexican lime and shoot-tip grafted Pera sweet orange, which are sensitive to SP, were used to assess the severity of the four strains. Sour orange was used for indexing SY. All indexing was repeated five times.

Challenge of trees containing the isolates was carried out by super-inoculation, both via aphids and infected budwood, with a severe stem pitting isolate in Hamlin sweet orange. Evidence that these inoculations were effective was determined by ELISA and presence of CTV dsRNA (16).

Field experiments were established to evaluate growth, occurrence of SP and productivity. Three thousand plants of a virus-free (shoot-tip grafted) old line of Pera Bianchi sweet orange were bud-inoculated separately with the four isolates. The controls were the original old line Pera Bianchi sweet oranges, and Pera IAC. The rootstock was Rangpur lime. SP rating, plant vigor (visual evaluation), fruit productivity and fruit size were recorded over 10 yr.

Leaf tissues were used to determine differences in banding patterns using SSCP analysis. Leaf midribs were frozen in liquid nitrogen and pulverized. Double-stranded RNA was isolated using CF 11 cellulose chromatography (16). The coat protein (CP) gene was amplified by one-step RT-PCR. SSCP analysis was performed directly on the PCR products obtained according to Rubio et al. (8). Gels were silver stained (1).

The three cross-protecting isolates selected for further study performed excellently in the field. Indexing for CTV showed that all trees were infected with the mild isolates. SP in the original trees changed from 0 (no stem pitting) to 2 (moderate). In the same orchard, non-preimmunized trees of Pera sweet orange developed mild to severe SP, rated 2 to 4.

Indexing results showed that trees carrying the four mild isolates were free of other agents indexed, except, but this did not appear to compromise the performance of the trees. The Pera Bianchi sweet orange tree was infected with a severe CTV complex plus an SY component and psorosis, but not vein enation.

In sour orange there were no differences in growth of trees infected with IAC or the three new sub-isolates. However, all three new sub-isolates were less virulent than IAC, and did not induce SP in sour orange. Sour orange seedlings inoculated with Bianchi Pera induced severe SY. The SY component present in the Bianchi field tree probably represented a normal situation for non-preimmunized trees in commercial orchards of this variety.

The three new sub-isolates and IAC gave different degrees of cross protection against SP (though all were effective), and no breakdown of protection or further segregation of strains was observed in the five years following inoculation. Trees of Pera sweet orange carrying the sub-isolates had SP ratings of 0.3 (Citrovita), 0.5 (UNESP-2), and 0.8 (UNESP-1), whereas Pera sweet orange protected with IAC rated 0.8 to 1.4. In contrast, Pera sweet orange IAC grown under the warmer conditions of the north and northeast areas of the plateau rarely exhibits any stem pitting.

Trees of virus-free Bianchi Pera sweet orange inoculated with Citrovita performed well in field conditions. The trees were 35% larger

than the other selections of Pera sweet orange, with faster growth, and 20-30% higher productivity (data not shown). Production of large fruit for the fresh fruit market is important in this variety. This selection has thus become one the most prominent varieties for the cooler south and southwest.

After reverse transcription and amplification (RT-PCR), all the isolates showed a band corresponding to the CP gene (11) (data not shown). SSCP revealed differences between the CP genes of IAC and Citrovita (Fig. 1).

In conclusion, preimmunization with the new Citrovita sub-isolate appears to have resolved the problem of increased SP severity in Pera sweet orange grown in the cooler south-west parts of the São Paulo plateau.

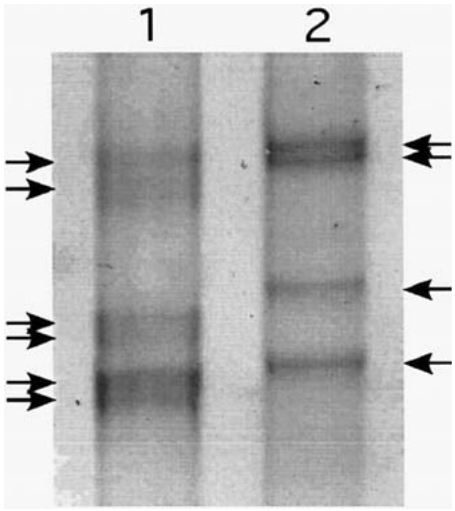

Fig. 1. Single-strand conformation polymorphism patterns of the coat protein gene of *Citrus tristeza virus* isolates: 1, IAC; 2, Citrovita. The differences observed are indicated by arrows.

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