Abstract. ‘Chandler’ pummelo [Citrus maxima (Burm.) Merrill] was found to be citrus tristeza virus (CTV)-resistant. The inheritance of this resistance in 84 progeny of two crosses derived from ‘Chandler’ pummelo and trifoliate orange [Poncirus trifoliata (L.) Raf.] was controlled by a single dominant gene designated Ctv2. Progeny analysis of four molecular markers closely linked to the CTV gene, which confers resistance to CTV in trifoliate orange, demonstrated that Ctv2 was an independently assorting gene from Ctv.

Although many CTV-resistant hybrids of citrus and trifoliate orange have been widely used as rootstocks for citrus trees, breeding a marketable CTV-resistant scion cultivar by crossing trifoliate orange with citrus has not been possible because undesirable traits of trifoliate orange remain after several generations of backcrossing with citrus. To overcome this problem, research toward isolating this resistance gene and then transforming it into CTV-susceptible citrus varieties is in progress in our laboratory and in Florida (F.G. Gmitter, personal communication). No genetic analysis has been reported for the CTV resistance in Atlanticia ceylanica, Fortunella crassifolia, or Severinia buxifolia.

Recently, Garnsey et al. (1997) reported that some pummelo [Citrus maxima (Burm.) Merrill] cultivars were resistant to certain CTV isolates. This finding opens a possibility to breed CTV-resistant citrus scion cultivars by sexual hybridization.

To identify molecular markers linked to Ctv in trifoliate orange (Fang et al., 1998), we used two populations of ‘Chandler’ pummelo by trifoliate orange, and ‘Chandler’ pummelo was found to be resistant to the CTV strain used. Because we developed many dominant and codominant molecular markers tightly linked to Ctv from trifoliate orange, we were able to study the inheritance of the CTV resistance present in ‘Chandler’ pummelo and to determine whether this resistance is controlled by a gene that is allelic with Ctv. In this paper, we report our results.

Materials and Methods

Plant materials. Two populations segregating for CTV resistance, C. maxima cv. Chandler × P. trifoliata cv. Rubidoux, and C. maxima cv. Chandler × P. trifoliata cv. Webber Fawcett, were used. These populations had 58 and 26 progeny individuals, respectively. ‘Rubidoux’ is a small-flowered trifoliate orange, while ‘Webber Fawcett’ is a large-flowered one.

Determination of CTV resistance. Four buds from each progeny tree and their parent cultivars were grafted onto ‘Pineapple’ sweet orange rootstocks that were infected with CTV strain T-514 (a moderate strain in California). Three months after the buds grew, leaves from progeny shoots were harvested and their CTV resistance was evaluated by enzyme-linked immunosorbent assay (ELISA) according to Mathews et al. (1997). Leaves from the ELISA-negative progeny and those that gave ambiguous ELISA results were harvested again for ELISA tests after 6, 12, and 15 months. The absorbance at 405 nm was measured after 30, 60, and 90 min of substrate development at room temperature using an Emax plate reader and SoftMax software (Molecular Devices Corp., Menlo Park, Calif.). The sample was scored as susceptible to CTV if the absorbance value was 2.5 times that of the healthy control or higher. The CTV resistance of the parent cultivars was also evaluated by reverse transcriptase polymerase chain reaction (RT-PCR) according to Mathews et al. (1997).

DNA extraction and polymerase chain reaction (PCR) amplification. Total DNA was extracted from leaves using 1.5% hexadecyltrimethylammonium bromide (CTAB) according to Fang et al. (1997). Random amplified polymorphic DNA (RAPD)-PCR amplification was carried out using reaction mixtures and temperature profiles described by Cheng and Roose (1995). Decamer primers were purchased from Operon Technologies, Alameda, Calif. Amplification products were resolved by electrophoresis through 1.8% agarose gel in 1× TBE buffer (89 mM Tris-borate plus 2 mM EDTA, pH 8.2). The RAPD marker OpAD081100 was an 1100-bp product generated by primer OpAD08.
experiment (Table 1). These results suggested that ‘Chandler’ pummelo was also heterozygous for CTV resistance, and that this resistance was controlled by a single dominant gene designated as Ctv2.

Determination of the novelty of Ctv2. In order to determine whether Ctv2 was allelic to Ctv, we proposed the following hypothesis. If these two genes were at the same locus, the markers closely linked to Ctv should be linked to Ctv2 as well. However, if they were different genes, linkage between Ctv2 and Ctv-linked markers should not be observed. We used the data of four marker loci linked to Ctv to test this hypothesis (Table 2). OpAD08_1100 co-segregated with Ctv in a 65-progeny population (Deng et al., 1997). Our recent work (Fang et al., 1998) showed that this RAPD marker and RFLP markers RfC19, RfE20 and RfZ16 were within 2 cM of Ctv in populations of 465 progeny. RfZ16 co-segregated with Ctv, and no sequence homology for it was found in Citrus. This marker existed only in P. trifoliata and its hybrids, in which two codominant alleles were found. The other markers, RfC19 and OpAD08_1100, flanked Ctv at distances of 0.5 and 0.8 cM, respectively. The RAPD marker band and the progenitor bands of RFLP markers were amplified from trifoliate oranges. Since RfZ16 was the marker closest to Ctv, we first used the genotype at this locus to predict the CTV resistance of progeny. All 42 progeny predicted to be CTV resistant by RfZ16 were resistant (Table 2). The other markers also correctly predicted resistant progeny, except for two progeny (type III) that were recombinants between RfC19 or RfE20 and RfZ16 or OpAD08_1100. However, of the 42 progeny predicted to be CTV-susceptible by the markers, only 20 were indeed susceptible. The remaining 22 progeny were CTV-resistant. ‘Chandler’ pummelo alleles at marker loci linked to Ctv were not associated with resistance in progeny that received the susceptible allele of Ctv. These results clearly suggested that Ctv2 in ‘Chandler’ pummelo was not allelic with Ctv in trifoliate orange, although both were dominant genes. The genotypes at Ctv and Ctv2 for the 20 CTV-susceptible progeny were rr R2r2; those for the 22 CTV-resistant progeny that were predicted to be susceptible by the markers should be rr R2r2. For the 42 CTV-resistant progeny that had been predicted to be resistant by the markers, half should be Rr R2r2, and the other half rr R2r2. Since we have not developed any markers linked to Ctv2, we were unable to differentiate between these two genotypes.

Garnsey et al. (1997) reported that the CTV resistance of some pummelo accessions depended on the CTV isolates tested. We do not know whether Ctv2 in ‘Chandler’ pummelo will provide durable resistance to a broad range of CTV strains. The next step of this research would test the resistance of Ctv2 against more CTV isolates, especially severe ones. Moreover, development of molecular markers linked to Ctv2 would allow us to select hybrids carrying the resistant allele of Ctv2 at the seedling stage, and to clone this gene using a map-based cloning strategy. Comparison of the sequences of two CTV resistance genes, Ctv and Ctv2, should provide a better understanding of the mechanisms of CTV resistance in citrus.

Literature Cited


