SHORT COMMUNICATIONS

Differential Genomic Regions Among Citrus Tristeza Virus Isolates

S. Gago-Zachert, N. Costa, L. Semorile, and O. Grau

ABSTRACT. As a first step to study the molecular basis of citrus tristeza virus (CTV) biological variability, we obtained a cDNA library from random-primed dsRNA extracted from a field isolate, C268-2, from Concordia, Entre Rios, Argentina. Southern blot analyses was performed with the clones after digestion with restriction enzymes, using cDNA probes derived from seven CTV isolates. Fifty-four percent of the restriction fragments from the cDNA library showed a differential hybridization pattern using probes from the seven CTV isolates. From differential hybridization patterns, 11 genomic regions were detected that might be associated with pathogenic characteristics: protease, methyltransferase, methyltransferase-helicase interdomain, helicase, 5' and 3' regions of p65, p27, p25 to p18 intergenic region, p13, p20 and p23.

Citrus tristeza virus (CTV) isolates display a large array of biological variability evidenced in the severity of symptoms induced. The two most important symptoms are decline of citrus on sour orange rootstock and stem pitting of many cultivars regardless of the rootstock. Biological properties may depend on the composition of the viral RNA populations and, therefore, identifying the nature of the sequence variants present is essential for understanding pathogenicity (6). To date, glasshouse indexing is the only method available to determine the biological properties of a given CTV field isolate (3). However, it is time consuming, labor intensive and there may be strains that are "hidden" in the isolate through "cross-protection" or by interference mechanisms (1, 8).

At the molecular level, several studies based on capsid protein gene (CPG) sequence have grouped isolates having either common biological activity or geographical origin (4, 5, 7, 11). In a number of cases, the biological data show that there is a genetic variation based on CPG sequence within CTV groups (5). The sequence of other regions of the CTV genome have also been used to discriminate isolates. The variability in the 5' untranslated region (UTR) allowed a classification into three groups (6). Single-strand conformation polymorphism (SSCP) patterns of p25 (9), p20 (10) and p27 (2) genes also have been used to differentiate CTV isolates from the five different biogroups from various geographical origins (9). No common marker characteristic of severe or mild isolates could be identified, but this method is useful for the analysis of viral population variability in CTV isolates.

These studies have been based on single sequences for each isolate. Genomic determinants responsible for the different pathogenic activities of CTV are still unknown. Therefore, the simultaneous analysis of several genomic regions in the whole viral population of a CTV isolate may result in a more realistic approach for understanding the biological traits of the isolate. In a previous work (12), we obtained a cDNA library from dsRNA extracted from a field CTV isolate, C268-2, and screened for clones showing differential hybridization between mild (T312 from Spain, biogroup II) and severe (T387 from Japan, biogroup V) CTV isolates (12). We have

now extended the Southern blot hybridization analyses to include five additional CTV isolates: T385 from Spain, biogroup I; VT from Israel and Pera GS from Brazil, biogroup III; Barão B from Brazil, biogroup IV; and T388 from Spain, biogroup V. The objective was to select a panel of isolate-discriminating cDNA probes.

To perform Southern blot analyses, all cDNA clones were digested with either *Bam*HI + *Xho*I + *Hin*f1 or with *Bam*HI + *Xho*I + *Dde*I, then electrophoresed on 2% agarose gels and then transferred by capillary blotting onto Z-probe nylon membranes (BioRad). The blots were hybridized under stringent conditions (6 × SSC, 68°C) with short-copy cDNA probes reverse transcribed from dsRNA from each CTV isolate.

The following panel of cDNA clones were used in the hybridization assays: seven clones (21 restriction fragments) comprising segments of the polyprotein gene region, 10 clones (35 restriction fragments) including the triple gene block region (p6, p65 and p61) and 13 clones (40 restriction fragments) containing the 3' viral genome region (p27, p25, p18, p13, p20 and p23). Five typical hybridizations patterns were observed: i) fragments that hybridized with similar signal intensity with the seven CTV short-copy cDNA probes regardless the biogroup; ii) fragments that showed an increased hybridization signal with the increase in the biological severity of the isolate (biogroups I to V); iii) fragments that showed a decrease of hybridization signal with the increase in the biological severity of the isolate, iv) fragments that gave a variable hybridization signal with no correlation with the biological characteristics of the CTV isolate; and v) fragments that hybridized with only with one CTV isolate (VT, biogroup III).

Fifty-four percent of the restriction fragments from the C268-2 cDNA library exhibited differential hybridization among the seven CTV isolates tested. The VT and Pera GS CTV isolates belong to biogroup III, but they showed different hybridization patterns. On the other hand, hybridization results indicated that C268-2 cDNA library contains sequences which hybridize to more than one CTV biogroup.

Eleven genomic regions were identified, based on hybridization patterns, that may be associated with pathogenic characteristics: protease, methyltransferase, methyltransferase-helicase interdomain, helicase, 5' and 3' regions of p65, p27, p25-p18 intergenic region, p13, p20 and p23. These probes will be used in dot blot assays to look for discrimination among biologically different CTV isolates.

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