Characterization of Resistance to Pepper Huasteco Geminivirus in Chili Peppers from Yucatán, México

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Abstract. Seven pepper (Capsicum chinense Jacq.) populations from the Yucatán Peninsula, México, that were selected from a field screening for viral diseases were tested for pepper huasteco geminivirus (PHV) resistance. Two populations (UX-SMH-1 and UX-SMH-24) displayed <50% of infection with PHV. Four plants did not show viral symptoms 3 months postinoculation using biolistic and grafting methods. When leaf tissue from these individuals was analyzed for PHV using quantitative PCR, it supported PHV replication, thus, the ineffective PHV infection in these symptomless individuals may be a result of restricted viral movement.

Materials and Methods

Plant material. Forty-nine wild populations of C. chinense were collected in several different areas of Yucatán. All these accessions were evaluated in the field for viral diseases and the best seven were selected for this work (Trujillo-Aguirre and Díaz-Plaza, 1995) and designated as UX-SMH-1, UX-SMH-5, UX-SMH-18, UX-SMH-22, UX-SMH-24, UX-SMH-26, and UX-SMH-55. Individual plants within an accession were used as an experimental unit. Capsicum annuum cv. Sonora Anaheim, which is highly susceptible to PHV, was used as the control (Torres-Pacheco, 1997). All the accessions used in this work are available from Instituto Nacional de Investigaciones Forestales y Agropecuarias (INIFAP) (Campo Experimental Bajío, carretera Celaya-San Miguel de Allende Km 6.5, apartado postal 112, código postal 38000, Celaya, Guanajuato, México).

Virus inoculation. Natural inoculum was used in all field experiments (Trujillo-Aguirre and Díaz-Plaza, 1995). For greenhouse experiments, one inoculation method was a biolistic procedure using a particle delivery system (model PDS 1000; DuPont), with 5 μg of DNA from PHV genomic components A and B deposited on the surface of 3 mg of tungsten microparticles (Sylvania No. 10), a gap distance of 1.2 cm and 56.4 kg·cm–2 of pressure for each bombardment (Garzón-Tiznado et al., 1993). Cloned PHV genomic components A and B (Garzón-Tiznado et al., 1993) were excised from the plasmid by digestion with HindIII before inoculation in order to improve the efficiency of plant infection (Bonilla-Ramírez et al., 1997). Twenty plants of each population of C. chinense were inoculated by the biolistic method. Symptomless plants 4 weeks post-bombardment were inoculated via grafting using stem pieces from PHV-infected C. annuum plants, (cv. Sonora Anaheim). During the 3 months of greenhouse incubation, plant symptoms were measured as previously described (Torres-Pacheco et al., 1996).

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1996). A resistant (symptomless) individual showed neither symptoms nor virus detection during 3 months of plant incubation; tolerant individuals were plants displaying delayed symptoms (at ≈70 d) as compared with ‘Sonora Anaheim’.

**Evaluation of viral resistance and virus detection.** During the 3 months of incubation, samples were taken at 14, 25, and 36 d post-inoculation (dpi) from the newly developed upper and lower leaves of biolistically inoculated plants, and at 27 and 44 dpi for grafted plants. The presence of PHV in the samples was detected by PCR analysis using specific primers for PHV (primer 240: 5´GGC TTATTTGTAATAAGAGAGGTGT3´ and primer 241: 5´GAATTAAGGTTACATGGACCTAT 3´) to amplify a 350-bp fragment from the intergenic region of PHV component A (Torres-Pacheco et al., 1996). The procedure of Dellaporta et al. (1983) was used for extraction of DNA for PCR analysis. PCR reactions contained 2.5 mm each dNTP, 1 µL of oligonucleotides (50 ng·µL⁻¹), 1 µL of Taq polymerase (5 U·µL⁻¹), and 250 ng plant DNA in a reaction volume of 50 µL. The PCR conditions were 94 °C for 2 min, 50 °C for 2 min and 72 °C for 2 min, for 35 cycles. The PCR products were analyzed on 0.8% agarose gels.

**Determination of the resistance mechanism.** Leaves from both susceptible and resistant plants were bombarded twice with 5 µg of DNA from PHV genomic component A, using the same bombardment conditions as described above. Before bombardment, leaf tissue was incubated for 4 h at 25 °C on solid Murashige-Skoog medium (Ruiz-Medrano et al., 1999). Tissue samples (0.5 g) were taken at 24 and 72 h after bombardment and total DNA was extracted (Dellaporta et al., 1983). Total DNA was quantified and brought to a concentration of 50 ng·µL⁻¹. An estimation of relative quantities of viral DNA was carried out using a quantitative PCR technique (Sykes et al., 1992). Serial dilutions of each sample and PCR amplifications with specific primers for PHV were carried out as described above. Agarose gels showing amplification products were analyzed using an IS-100 digital Image System (Alpha Innotech Corporation, San Leandro, Calif.), and integrated densitometry values were measured.

**Results and Discussion**

Seven populations of *C. chinense* with the lowest percentage of field-infected plants (Trujillo-Aguirre and Díaz-Plaza, 1995) were selected and inoculated with PHV by the biolistic method. Plants that did not show PHV symptoms 4 weeks postbombardment were inoculated by grafting, in order to determine whether they were resistant to PHV infection or were escapes. The original frequency of escapes using biolistic inoculation was 30% (Garzón-Tiznado et al., 1993); however, our biolistic method had only 5% escapes (Table 1). Moreover, our grafting method yielded no escapes when the scion survived (Table 1). The *C. chinense* accessions initially showed high intrapopulation variation for percentage of PHV infected plants (40% to 90%, Table 2). This work used individual plants within an accession as the experimental unit. Two months after grafting, the UX-SMH-1 and UX-SMH-24 populations had the highest percentage of symptomless plants (Table 2). Although population UX-SMH-24 contained 43% infected plants, it had no symptomless individuals (Table 2). However, UX-SMH-22 had 60% infected plants, with one asymptomatic individual (Table 2). After biolistic and grafting inoculations, several tolerant individuals were detected in almost all populations. Four resistant/symptomless individuals were detected in populations UX-SMH-1 and UX-SMH-22 (Table 2).

Following biolistic inoculation, PHV was detected on upper or lower leaves of susceptible plants with symptoms (Fig. 1A), but was confined to symptomatic zones, suggesting that in *C. chinense* are determined by viral presence. Following biolistic inoculation, symptomless individuals did not possess detectable levels of viral DNA (Fig. 1B). Occasionally, viral DNA was detected in the earlier samplings from resistant plants, perhaps because DNA was introduced into leaf pieces by bombardment (Fig. 1B, lanes 1–4). Analysis by PCR confirmed that some asymptomatic individuals from the biolistic inoculation were effectively resistant to PHV infection; no viral DNA was detected on upper leaves 44 d after inoculation by grafting (Fig. 1C, lanes 7–9). Three resistant individuals were identified in the UX-SMH-1 population and 1 in the UX-SMH-22 population (Table 2).

In susceptible plants, higher dilutions were needed to diminish PHV amplification via PCR at 72 h than at 24 h after bombardment (Table 3). Densitometric analyses of amplified DNA from serial dilutions from the same individuals indicated that samples were significantly stronger at 72 h than at 24 h (Table 3). This suggests that the amount of viral DNA increased between 24 and 72 h postinoculation. The result was the same in both resistant and susceptible plants (Table 3). These results suggested that PHV replication was not affected in resistant plants, and that resistance to PHV may affect viral movement. Resistance to viral infection is commonly the result of restricted viral movement within the plant (Hull, 1991). Several reports indicate that a nonhost virus can multiply in inoculated protoplasts of nonhost species (Fraser, 1990; Hull, 1991; Paje-Manalo and Lommel, 1989). Apparently, immune plants support virus multiplication in a few inoculation methods, on *Capsicum annuum* cv. ‘Sonora Anaheim’.

| Inoculation method | No. of plants | PHV detection |
|--------------------|---------------|---------------|
| Biolistic           | 34            | 32            | 2  |
| Grafting            | 40            | 40            | 0  |

*Viruses detection by PCR, as indicated in materials and methods. In all cases, only symptomatic plants showed the presence of PHV.

These plants were later inoculated by grafting, and are included in the account of total grafted plants.

These plants were those in which the scion survived; originally 43 plants were inoculated by grafting.

**Table 2.** Percentage of infected plants, and severity and number of symptomless plants in *C. chinense* populations after PHV infection using both biolistic and grafting methods.

| Population       | Infected plants (%) | Severity | No. of symptomless plants |
|------------------|---------------------|----------|--------------------------|
| UX-SMH-1         | 40                  | 8.0      | 3                        |
| UX-SMH-5         | 85                  | 8.4      | 0                        |
| UX-SMH-18        | 50                  | 8.0      | 0                        |
| UX-SMH-22        | 60                  | 8.6      | 1                        |
| UX-SMH-24        | 43                  | 8.0      | 0                        |
| UX-SMH-26        | 84                  | 8.4      | 0                        |
| UX-SMH-35        | 90                  | 8.1      | 0                        |

*Severity based on a scale from 1 (no symptoms) to 10 (severe symptoms).

**Table 3.** Relative quantitation of PHV using PCR analysis on serial dilutions from DNA samples extracted from bombarded pepper leaves. (Arithmetic mean = 276, and standard deviation = 184.6).

| Sample          | Time after inoculation | Dilution 1:10 | Dilution 1:100 | Dilution 1:1000 | Dilution 1:10,000 |
|-----------------|------------------------|---------------|---------------|-----------------|-------------------|
| Susceptible     | 25                     | 416           | 363           | 104             | 0                 |
| Immune          | 25                     | 410           | 368           | 106             | 0                 |

*Values are densitometry readings expressed as integrated densitometry values (IDV).
Fig. 1. PCR amplifications of intergenic region of PHV component A from inoculated *C. chinense* plants. (A) Set of four typical PHV-susceptible plants that resulted after biolistic inoculation. Lanes 1–8, 9–16, and 17–24 are amplifications from DNA samples taken 14, 25, or 36 d postinoculation (dpi) respectively. (B) Set of four typical PHV-resistant plants that resulted after biolistic inoculation. Descriptions of lanes, but not the plants, are the same as in (A). (C) PCR amplifications of DNA samples from grafted plants. Lanes 1–6 and 7–12 correspond to samples taken 27 and 44 d after grafting, respectively. In the grafting experiment, only DNA samples from upper leaves were analyzed. The amplification of the intergenic region of PHV component A was primed using oligonucleotides 240/241, which amplify a 350-bp fragment (indicated by arrows on each panel). U = upper leaves, L = lower leaves, C+ = PCR amplification from DNA samples of PHV-infected plant from 'Sonora Anaheim', C– = PCR analysis from noninoculated plant from 'Sonora Anaheim', and M = molecular weight marker (1 Kb).
lated cells (Fraser, 1990; Paje-Manalo and Lommel, 1989).

Capsicum chinense plants immune to PHV infection are excellent resources with which to study the genetics of the resistance and for use in breeding programs.

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