Twenty-Three Citrus Psorosis Virus Isolates of Different Origin Detected by RT-PCR Using Primers Designed from Sequences of the Isolate CRSV-4 from Florida

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ABSTRACT. The citrus ringspot virus isolate CRSV-4 of citrus psorosis virus (CPsV) possesses three types of particles which encapsidate RNAs 1, 2 and 3, respectively. These RNAs were purified, reverse transcribed, cloned and partially sequenced. Based on these results, two sets of primers for RNAs 1 and 2, respectively, were designed and used for RT-PCR diagnosis of psorosis. Total nucleic acids extracted from young symptomatic leaves were used for diagnosis. Six isolates from Argentina, seven from the USA, nine from Spain and one from Italy were detected with primers designed from RNA 2, and some of the isolates were detected also with primers designed from RNA 1.

Citrus psorosis virus (CPsV) causes a damaging disease of citrus in many parts of the world including South America and the Mediterranean basin (9). In Argentina, the disease is epidemic and appears to be spread by an unknown vector (3). Spread of CPsV through nursery material is controllable by budwood certification using biological indexing for diagnosis. This procedure requires special skills and good plant-growing facilities and is slow and costly. ELISA using polyclonal and monoclonal antibodies raised against CPsV has also been used for diagnosis of psorosis infection in the greenhouse and field (1, 5, 6).

In Dr. Derrick's laboratory (2), the CPsV RNA encoding the coat protein was sequenced, and primers were designed for diagnosis using RT-PCR and tested on a limited number of isolates from the USA. We previously reported that CPsV has three RNAs (8). These RNAs were reverse transcribed, cloned, partially sequenced and a set of primers from RNA 1 were used for detection of CPsV (6). In those experiments, only a few isolates were tested and in some cases, reproducibility was weak. Therefore, to test the possibilities of psorosis diagnosis by PCR, 24 isolates from different regions (Table 1) were tested and the results are reported here.

The CPsV isolate CRSV-4 from Florida (7) was used as the positive control. All isolates were maintained in sweet orange in the greenhouse. Crude extracts were prepared from symptomatic young shoots or from healthy shoots using 10 ml of TACM buffer (4) per g of tissue. Total RNA was extracted as previously described (6) using proteinase K treatment before phenol extraction. RNA was reverse transcribed with primers A and B from RNA 2, and some with primers 1 and 7 from RNA 1.

Twenty-three of the 24 isolates were detected with primers A-B, and nine of the isolates tested with primers 1-7 also gave positive amplification (Table 1). The RT-PCR products were hybridized in Southern blot analyses using probes from the CRSV clones in order to confirm the identity of the amplicons. Variations in the intensity of the bands were attributed to different virus concentrations in the tissue, however, since CRSV always yielded an intense DNA band, these variations could also be due to sequence differences among the isolates.

Isolate CPsV Italia1 was not detected by primers A-B (Table 1)

TABLE 1
RT-PCR RESULTS ON TWENTY THREE PSOROSIS ISOLATES OF DIFFERENT ORIGIN

Isolate	Origin	RT-PCR[z]	
		Primers A-B	Primers 1-7[y]
CRSV-4 (+ control)	USA	+	+
CPsV 243-44	"	+	+
CPsV 200	"	+	NT
CPsV 201	"	+	NT
CPsV 215	"	+	NT
CPsV B108	"	+	NT
CPsV 173-6	"	+	+
CPsV 173-22	Argentina	+	+
CPsV 90-1-1	"	+	+
CPsV 189-34	"	+	+
CPsV 504-5	"	+	+
CPsV 100-40-1	"	+	+
CPsV 100-16-5	"	+	+
CPsV 121	Spain	+	NT
CPsV 126	"	+	NT
CPsV A H	"	+	NT
CPsV 129	"	+	NT
CPsV SR	"	+	NT
CPsV 101	"	+	NT
CPsV SOR	"	+	NT
Spagna 1	"	+	NT
Spagna 2	"	+	NT
Italia 1	Italy	—	NT
Italia 3	"	+	NT
Healthy	"	—	—

[z]A clear band seen after electrophoresis and ethidium bromide staining was taken as positive.
[y]NT = not tested.

nor with several other primers (data not shown). Symptom expression of this isolate was also atypical, and it was doubtful that it really was CPsV.

The Argentinian isolates were detected with primers from RNA1 and RNA2, indicating that they have sequence similarity with the CRSV-4 in both parts of the genome. The amplification products probably corresponded to the fraction of the population which is similar to CRSV-4, but we do not know how representative this fraction is within the actual RNA population of each isolate. All that we can say is that CRSV-4-type sequences are present in the RNA population of all isolates.

Djelouah et al. (5) found a close relationship among isolates taken from the same geographical region using monoclonal antibodies against the coat protein of the Italian isolate, IAM-191Xa. It will be interesting to check if the same is true for Argentine isolates and whether other regions of the genome are also conserved among geographically related isolates.

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Part of these results have been published elsewhere.

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