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Partial genome sequence of an apricot isolate of *Cherry green ring mottle virus* (CGRMV)

Annotated Sequence Record

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Provenance of the virus material

The ARPox1 isolate of *Cherry green ring mottle virus* (CGRMV) was tentatively identified in an apricot source from the Campania region of southern Italy which displayed symptoms reminiscent of those described for the apricot ringpox disease [2, 3]. Following propagation by grafting on GF305 peach seedlings, the partial genomic sequence of the ARPox1 isolate was determined from cDNA clones obtained by random PCR [2] or by sequence-specific PCR amplification from purified viral double-stranded RNAs (dsRNAs). The sequence obtained has been deposited in the EMBL database under accession number AY172334.

Sequence properties

Comparison of ARPox1 sequence with the complete CGRMV sequences [CGRMV-NA, AF017780; CGRMV-P1A, AJ291761] revealed a similar genetic organisation with the TGB3 (5′-truncated in the ARPox1 cDNA), CP and 5a ORFs in similar positions and 3′ non coding regions (3′NCRs) of almost identical size (189 nt for ARPox1 versus 192 and 191 nt for CGRMV-NA and CGRMV-P1A, respectively) (see Fig. 1). The overall nucleotide sequence identity between ARPox1 and CGRMV was 81.8% and reached 89% when considering the 3′-NCR alone.

The partial TGB3 ORF of ARPox1 (nt 1 to 66) encodes a 21 aa fragment 96% identical to the C-terminal portion of CGRMV-NA TGB3. The CP gene (nt 116 to 922) encodes a 268 aa long polypeptide (29.7 kDa) with 89.2% aa

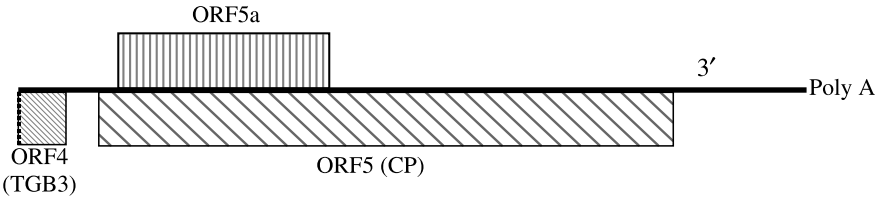

Fig. 1. Schematic representation of the partial genomic organisation of the ARPox1 apricot isolate of Cherry green ring mottle virus

sequence identity with the CP of CGRMV-NA but only 73.1% identity with the CP of the closely related *Cherry necrotic rusty mottle virus* (CNRMV, AF237816). The putative ORF 5a (nt 141 to 539) nested within the CP ORF, potentially encodes a polypeptide of 132 aa (14.8 kDa) which shows an identity level of 51.5% with CGRMV-NA. The initiating ATG and stop codons of ORF 5a are, however, not conserved, leading to an ARPox1 protein lacking the first 6 N-terminal and last 25 C-terminal amino acids as compared to other CGRMV isolates. The existence of this polypeptide and its potential role are still a matter of speculation but the presence and overall size of ORF 5a are conserved for all CGRMV isolates known to date and for the closely related CNRMV. This potential protein appears, however, to be the least conserved in the genome of these viruses.

Given the new species demarcating criteria for the family *Flexiviridae* [1] of ca. 72% nucleotide and 80% amino acid sequence identity in the CP gene, the values obtained here for the ARPox1 sequence [82.3% (nt) and 89.2% (aa)] clearly indicate that this isolate belongs to the CGRMV species, thus providing the first instance of natural CGRMV infection in apricot. The position of this isolate within the known variability of CGRMV was evaluated by comparing it with the CP sequences of all cherry isolates analyzed so far [5] and calculating the average intra-group and inter-group nucleotide and amino acid distances (Table 1). As a comparison, the intra-specific average distances were also calculated for the CP gene of *Apple stem pitting virus* (ASPV), the type member of the genus *Foveavirus*, for which a rather large intra-specific diversity has been reported [4]. At 7% and 2.8% for nucleotide and amino acid sequences respectively, the values

Table 1. Average intra- and interspecific divergence levels for selected coat protein genes. Pairwise comparisons of nucleotide and amino acid sequences were performed to calculate the percentage of divergence before calculating intra-group and inter-group average values and standard deviation. When applicable, extreme values are given in parentheses. Calculations were performed using all relevant complete CP gene sequences available in the databanks

	Nucleotide sequence	Amino-acid sequence
Between ASPV isolates	19.2% ± 0.8%	14.8% ± 1.2%
Between cherry CGRMV isolates	7.0% ± 0.6%	2.8% ± 0.5%
Between apricot and cherry CGRMV isolates	18.1% ± 0.6% (*extremes: 17.3–19.2%*)	11.7% ± 0.7% (*extremes: 10.4–12.7%*)

obtained for CGRMV when considering only the cherry isolates were much lower than those obtained for ASPV (Table 1). Comparisons between the apricot ARPox1 isolate and the cherry isolates, however, reached values in the same range as those observed for ASPV [18.1% (nt) and 11.7% (aa), respectively]. These results indicate that the divergence of the apricot ARPox1 isolate is more than two-fold (nt) or more than four-fold (aa) the previously known variability of cherry isolates of CGRMV. However, even including the ARPox1 data, the overall variability of CGRMV still remains within the range of the type *Foveavirus*, ASPV (Table 1).

The sequence reported here demonstrate that the ARPox1 isolate belongs to the CGRMV species but that it is significantly removed from all cherry isolates of this virus. On this basis, it is possible to propose the separation of CGRMV isolates into two strains, a cherry strain which can be typified by CGRMV-NA and which includes all cherry isolates analyzed to date and a new apricot strain, typified by the ARPox1 isolate described here. It should be stressed that this structuration of the CGRMV diversity by the host plant is probably not related to the ability to infect one or the other hosts since it has been known for a long time that cherry isolates of CGRMV can be propagated experimentally in other *Prunus* species, including apricot, without causing symptoms.

The discovery of the CGRMV apricot strain raises questions about the suitability of existing CGRMV detection techniques for of isolates belonging to this new strain. The PCR primers NCPh and NCPc described by Zhang [5] have five (including one on the second 3′ base of the primer) and one mismatches, respectively, with the ARPox1 sequence making it unlikely that they would be suitable for the detection of the apricot strain of CGRMV.

Finally, the role of the ARPox1 isolate in the apricot ringpox-like symptoms observed in the original apricot material [2, 3] cannot be evaluated simply because this material was found to be simultaneously infected by CGRMV, by *Apple chlorotic leaf spot Trichovirus* and by a second, previously uncharacterized *Trichovirus*. Therefore, the individual contribution of these agents cannot readily be identified.

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