CHAPTER 29

Cherry virus A

A. Marais, T. Candresse, L. Svanella-Dumas, and W. Jelkmann

Introduction

Cherry virus A (CVA) was first described in Germany in a sample of sweet cherry (*Prunus avium*) presenting typical symptoms of little cherry disease and was discovered during the cloning of *Little cherry virus*-1 (Jelkmann, 1995). The molecular characterization of CVA revealed that its genome organization resembles that of *Apple stem grooving virus* (ASGV), the type species of the genus *Capillovirus* in the family *Betaflexiviridae,* thus, CVA was classified as a Capillovirus. Further efforts showed that the virus is frequent in sweet and sour cherry and that it could have a worldwide distribution in these hosts. CVA was later identified in natural infection in other *Prunus* hosts (apricot, peach, plum, Japanese apricot) but seems to be less frequent in these hosts. So far, CVA has not been associated with any specific disease or symptoms in any of its various hosts.

Taxonomic Position and Nucleotide Sequence

The complete nucleotide sequence of the genomic RNA of CVA has been determined from cDNA clones generated from double stranded RNAs extracted from plant tissue exhibiting symptoms of little cherry disease (Jelkmann, 1995). The genome is 7,383 nucleotides long, excluding the poly(A) tail, and the genomic organization resembles that of ASGV, the type species of genus *Capillovirus* in the family *Betaflexiviridae*. The genomic RNA harbors two ORFs. ORF1 (nt 55-7081) encodes a putative 266 kDa protein. It is followed by a 3′ non-transcribed region (NTR) of 303 nucleotides, upstream of the 3′-poly (A) tail. ORF2 (nt positions 5,400–6,790) is nested within ORF1 and encodes, in a different reading frame, a putative 52 kDa protein. In the ORF1-encoded protein, typical motifs for viral RNA replicases (RNA-dependant RNA polymerase) are found. In the C-terminal part of this protein, significant identity is observed with the capsid proteins of other members of the family *Betaflexiviridae,* raising the possibility that the CP is expressed as a fusion to the viral replicase and later matured through proteolytic cleavage of the replicase-CP precursor protein. The ORF2 protein presents significant similarity with the putative movement protein of ASGV and of other *Betaflexiviridae* members characterized by a single MP.

In the study of Foissac et al. (2005), the analysis of the genomic diversity of CVA in a short fragment of the viral RNA-dependant RNA polymerase showed that ASGV and CVA did not cluster together, raising the possibility that despite their common genomic organization, the genus *Capillovirus* could be polyphyletic. Using the same genomic region, Marais et al. (2008b) analyzed the diversity of 69 CVA isolates. The results showed a substantial level of variability, with an average level of nucleotide divergence between isolates reaching 9%. The majority of isolates analyzed clustered together with the CVA reference isolate. Four additional groups of isolates could be identified, particularly a cluster regrouping together all the CVA isolates from non-cherry hosts, including the CVA isolate identified in plum by Svanella-Dumas et al. (2005), in apricot and plum by Barone et al. (2006), and two of the isolates from *Prunus mume* (Marais et al., 2008a) (Fig. 29.1). This finding suggests the common origin of the non-cherry isolates of CVA and very limited movement potential of CVA between cherry and non-cherry hosts.

Economic Impact and Disease Symptoms

Even if CVA was discovered initially in *Prunus avium* displaying typical symptoms of little cherry disease (Welsh and Cheney, 1976), it was never assumed that CVA was associated with this disease, which is caused by two related Closteroviridae members, *Little cherry virus*-1 (LChV-1) and *Little cherry virus*-2 (LChV-2) (see chapter 31 in this book). In fact, the available data about the symptomatology of CVA infection is very poor and uncertain, as is the information about its potential economic impact.

In the original study on CVA by Jelkmann (1995), the virus was identified in a little cherry diseased source but also in two other sources without any little cherry symptoms, so that the connection between CVA and this disease seemed unlikely. The same type of analysis led to the conclusion that CVA is also unrelated to the rusty mottle, necrotic rusty mottle, and "Shirofugen" diseases. Eastwell and Bernardy (1998) confirmed that there was no correlation between the expression of the little cherry disease symptoms and the presence of CVA. During a survey in commercial orchards in California, CVA was identified in Bing cherry with leaf vein necrosis, in Kwanzan flowering cherry with leaf necrotic lesions, and in Colt cherry with leaf chlorotic rings. The first two hosts were also infected by *Prune dwarf virus* (PDV), the last one by *Plum bark necrosis and stem pitting associated virus* (PBNSPaV) (Sabanadzovic et al., 2005). Thus, even if the authors suggested that an association of CVA with the leaf necrosis symptoms observed should not be excluded, complementary experiments are clearly needed to ascertain the role of each of the viruses in the observed symptomatology. More recently, the potential role of CVA in a new decline disease of sweet cherry in Southern France was investigated (Marais et al., 2008b). The symptoms were distinct from those described in known cherry diseases and consist of reddish spots that become brown and in some

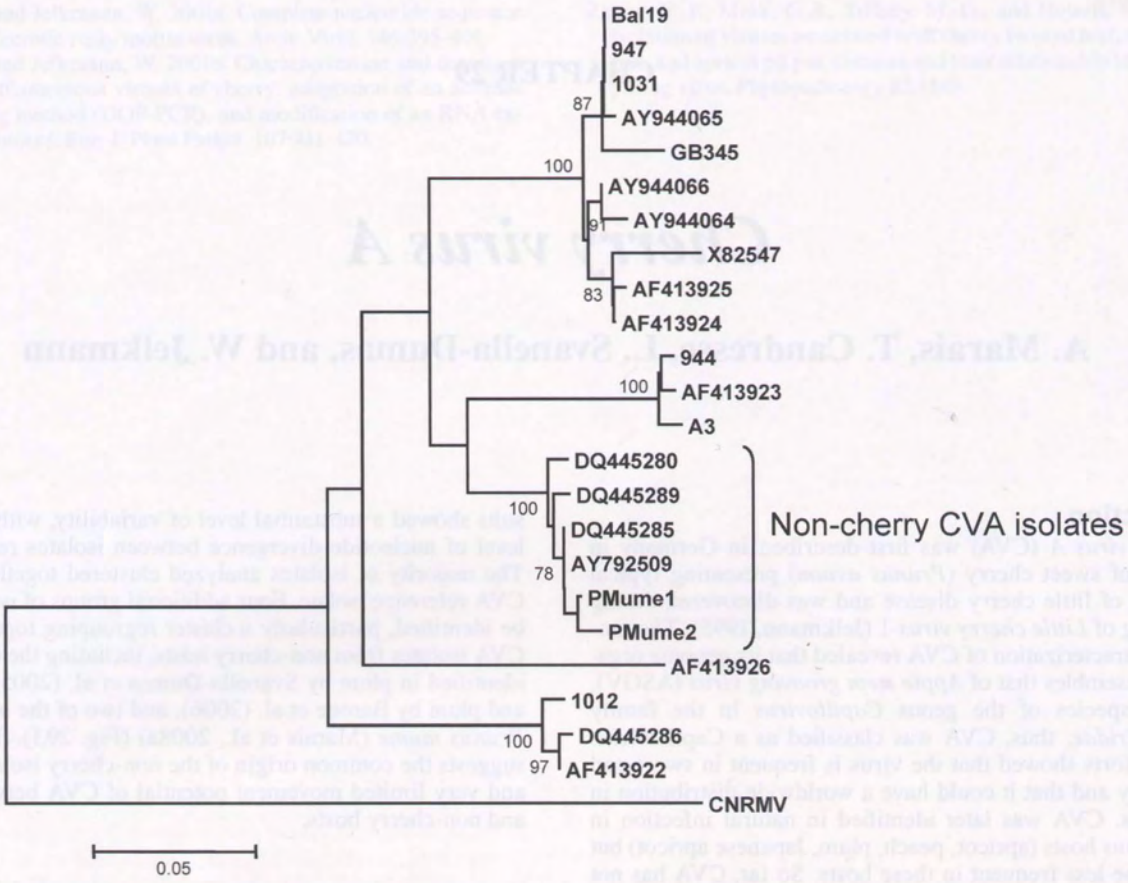

Fig. 29.1. Unrooted phylogenetic tree reconstructed using partial nucleotide sequences (258 bp) from a fragment of the RNA-dependant RNA polymerase (PDO fragment) of CVA isolates. The tree was reconstructed using the neighbor-joining method. Only bootstrap values > 70% (from 1000 performed bootstrap trials) are indicated. The scale represents 0.05 substitutions per site. The *Cherry necrotic rusty mottle virus* isolate (AF237816) was used as outgroup. GenBank accession numbers are indicated.

cases necrotic accompanied by tree decline. The decline of the affected trees is not systematic, but it seems that all factors weakening the tree may trigger the decline process in trees showing the foliar symptoms (Gentit et al., 2006). Using a polyvalent molecular test allowing the detection of Trichoviruses, Foveaviruses, and Capilloviruses (Foissac et al., 2005), symptomatic and asymptomatic trees were tested for the presence of filamentous viruses in these genera. CVA was detected in about 75% of the samples irrespective of their disease status, thus ruling out the involvement of CVA in this new decline disease.

In a number of cases, CVA has been observed in mixed infection with other common fruit tree infecting viruses, further complicating the analysis of CVA-induced symptomatology. For example, Svanella-Dumas et al. (2005) reported mixed infection of *P. domestica* with CVA and PDV, so that the observed symptoms (rosetting, severe leaf deformation, and superficial fruit blotching) could not easily be correlated with the presence of CVA. In the survey of Barone et al. (2006) in the Campania region of Italy, all identified isolates of CVA were found in mixed infections with *Apple chlorotic leaf spot virus* (ACLSV), *Apricot pseudo chlorotic leaf spot virus* (APCLSV), or *Cherry green ring mottle virus* (CGRMV). Mandic et al. (2007) reported CVA in Serbia in mixed infections with PNRSV, PDV, ACLSV, CGRMV, and *Cherry necrotic rusty mottle virus* (CNRMV), but also in single infection in two asymptomatic trees. In survey of viruses in sweet cherry trees with symptoms of bud blight disease in Japan, Isoagai et al. (2004) detected CVA in mixed infections with LChV-1, LChV-2, CGRMV, and CNRMV. In symptomless cherry samples, CVA was identified in 10 to 12 samples, alone or in combination with LChV-2.

Given the currently available information, CVA is generally considered to be a latent virus in all of its hosts. However, even if CVA alone does not cause symptoms, there remains the possibility that it could affect the severity of symptoms caused by other viruses when occurring in mixed infections. This hypothesis will be difficult to test given the problems encountered to separate different viruses in mixed infections. It should be kept in mind that potentially damaging reactions could also occur when new combinations of rootstocks and cultivars are assembled. In field trials, a graft-transmissible agent has been observed in sources of some sweet cherry cultivars, which results in decline when grafted onto dwarfing rootstocks. The agent is currently unknown (W. Jelkmann, *unpublished results*).

Host Range

CVA was first reported in 1995 in sweet cherry (*P. avium*) (Jelkmann, 1995) and was later observed in apricot and peach (James and Jelkmann, 1998) and in *P. serrulata* 'Kwanzan' (Eastwell and Bernardy, 1998). It has been detected at high frequency in sweet cherry (*P. avium*) during several surveys (Kirby and Adams, 2001; Isogai et al., 2004; Sabanadzovic et al., 2005; Eastwell and Bernardy, 1998; Rao et al., 2009). The

works of Svanella-Dumas et al. (2005) and Barone et al. (2006) extended the host range of CVA to plum and that of Marais et al. (2008a) to japanese apricot (*P. mume*). Infection in non-cherry hosts is, however, less frequent than infection in sour and sweet cherry.

Experimentally, the virus has been transmitted by grafting to susceptible *Prunus* hosts (*P. armeniaca* cv 'Priana' and *P. domestica* cv. 'Prune d'Ente' for example) (Svanella-Dumas et al., 2005). So far, no herbaceous host has been identified to permit the propagation of CVA, but considering the difficulty to find sources infected only with CVA, the identification of a specific host, which could be used as a biological "filter," would represent a major advance.

Transmission

Like ASGV, the type species of genus *Capillovirus,* CVA is transmissible by grafting or other vegetative propagation techniques. Despite the wide distribution of CVA and its high prevalence, at least in its cherry hosts, there is no information to date about the possible existence of (a) potential vector(s).

Geographical Distribution and Epidemiology

CVA seems to be very widely distributed, possibly worldwide, wherever suitable *Prunus* hosts are grown. Given the paucity of symptoms associated with CVA infection, its presence is generally largely overlooked, unless surveys specifically targeting it are carried out. CVA has been reported from several countries in Europe (Germany, France, Italy, the United Kingdom, Poland, and Serbia) (James and Jelkmann, 1998; Kirby and Adams, 2001; Komorowska and Cieslinska, 2004; Svanella-Dumas et al., 2005; Barone et al., 2006; Mandic et al., 2007), in North America (British Columbia in Canada, California in the United States) (James and Jelkmann, 1998; Eastwell and Bernardy, 1998; Sabanadzovic et al., 2005), and also in Asia (Japan and China) (Isogai et al., 2004; Marais et al., 2008a; Rao et al., 2009). Several surveys indicated that its prevalence can be very high. In Canada and Germany, James and Jelkmann (1998) reported that nearly 40% of the samples investigated in the study (*P. avium,* peach, or apricot with different status of disease) were found to be infected by CVA. Other studies reported even higher infection levels of cherry trees (up to 90% in Japan, Great Britain, and Serbia). The study of Barone et al. (2004) performed on collections of ancient varieties from the Campania region of Italy confirmed the ability of CVA to naturally infect non-cherry *Prunus* species hosts, with relatively high prevalence in apricot (43%) and lower prevalence in plum (17%).

Detection

Due to the likely absence of CVA symptoms in woody hosts and the lack of susceptible herbaceous hosts, the detection of CVA by visual inspection of symptoms or by biological indexing cannot be used for this agent. Likewise, only limited data are available concerning the use of immunological techniques for the detection of CVA. An antiserum has been raised against the capsid protein of CVA expressed as a fusion protein in *Escherichia coli*. The reactivity of the antiserum allowed its use in immunosorbent electron microscopy (ISEM) for virus characterization, but the antiserum was not suitable for virus detection in different ELISA assays (Jelkmann, 1995).

CVA can be detected by molecular hybridization (James and Jelkmann, 1998). Three probes, located in three different regions of the genome, were described by these authors and used in a survey. As a member of the genus *Capillovirus,* CVA can be detected using the polyvalent nested RT-PCR assay (PDO) developed by Foissac et al. (2005), but the identification of the viral species present requires the sequencing of the amplified fragment. Several publications have described the development and use of CVA-specific PCR primers: James and Jelkmann (1998) used primers located in the ORF1 and the overlapping ORF2, allowing the amplification of a fragment spanning from nucleotide 4,621 to nucleotide 5,454 of the genome. The other pairs of primers described for the specific detection of CVA are located in the same region of the genome (Eastwell and Bernardy, 1998; Mandic et al., 2007). More recently, Marais et al. (2008b) analyzed the genetic diversity of CVA isolates in the 360 bp region of the ORF1 amplified by the PDO RT-PCR developed by Foissac et al. (2005). By this method, they revealed an unexpectedly large molecular variability and identified five divergent phylogenetic groups of CVA isolates with up to 19% nucleotide divergence between isolates belonging to the different groups (Fig. 29.1). When evaluating the ability of available detection techniques to detect members of these 5 lineages of CVA, Marais et al. (2008b) surprisingly observed that only 69% of the isolates tested were detected either by molecular hybridization or using CVA-specific RT-PCR described by James and Jelkmann (1998). Moreover, the percentage of detected isolates was largely dependent of the phylogenetic group considered. For example, in the group containing the non-cherry CVA isolates, the RT-PCR assay was unable to detect any of the isolates tested. This analysis suggested that except for the polyvalent PDO RT-PCR, none of the available detection assays is able to detect all CVA isolates, making it likely that even surveys specifically targeting CVA could under-report its presence of its prevalence. Partial genomic sequencing of divergent CVA isolates has allowed the identification of conserved genomic regions and the development of new, more polyvalent primers that allow the detection of all isolates of CVA by RT-PCR (Marais et al., *manuscript in preparation*).

Control

Similar to other viruses for which no vector has been identified so far, the control measures are limited to the detection of CVA and to the use of certified virus-free planting material.

REFERENCES

Barone, M., Alioto, M., Marais, A., Candresse, T., and Ragozzino, A. 2006. First report and high prevalence in noncherry hosts of *Cherry virus A* in Italy. Plant Dis. 90:1459.

Eastwell, K. C., and Bernardy, M. G. 1998. Relationship of *Cherry virus A* to little cherry disease in British Columbia. Acta Hortic. 472:305-313.

Foissac, X., Svanella-Dumas, L., Gentit, P., Dulucq, M. J., Marais, A., and Candresse, T. 2005. Polyvalent degenerate oligonucleotides reverse transcription-polymerase reaction: A polyvalent detection and characterization tool for Trichoviruses, Capilloviruses, and Foveaviruses. Phytopathology 95:617-625.

Gentit, P., Marais, A., Charlot, G., and Millan, M. 2006. Cherry decline: CVA a suspect virus? Current status and results for 2005. Infos-CTIFL 223:35-40.

Isogai, M., Aoyagi, J., Nakagawa, M., Kubodera, Y., Satoh, K., Katoh, T., Inamori, M., Yamashita, K., and Yoshikawa, N. 2004. Molecular detection of five cherry viruses from sweet cherry trees in Japan. J. Gen. Plant Pathol. 70:288-291.

James, D., and Jelkmann, W. 1998. Detection of *Cherry virus A* in Canada and Germany. Acta Hortic. 472:299-303.

Jelkmann, W. 1995. *Cherry virus A:* cDNA cloning of dsRNA, nucleotide sequence analysis and serology reveal a new plant capillovirus in sweet cherry. J. Gen. Virol. 76:2015-2024.

Kirby, M. J., and Adams, A. N. 2001. Occurrence of *Cherry virus A* in the UK. Plant Pathol. 50:801.

Komorowska, B., and Cieslinska, M. 2004. First report of *Cherry virus A* and *Little cherry virus*-1 in Poland. Plant Dis. 88:909.

Mandic, B., Matic, S., Al Rwahnih, M., Jelkmann, W., and Myrta, A. 2007. Viruses of sweet and sour cherry in Serbia. J. Plant Pathol. 89:103-108.

Marais, A., Faure, C., Svanella-Dumas, L., and Candresse, T. 2008a. First report of *Cherry virus A* (CVA) infection in Japanese apricot (*Prunus mume*) in China. Plant Dis. 92:1589.

Marais, A., Svanella-Dumas, L., Barone, M., Ragozzino, A., Gentit, P., and Candresse, T. 2008b. Genomic diversity of *Cherry capillovirus A* (CVA) and suitability of various assays for its detection. Acta Hortic. 781:37-45.

Rao, W. L., Zhang, Z. K., and Li, R. 2009. First report of Cherry virus A in sweet cherry trees in China. Plant Dis. 93:425.

Sabanadzovic, S., Abou-Ghanem-Sabanadzovic, N., Rowhani, A., Grant, J. A., and Uyemoto, J. K. 2005. Detection of *Cherry virus A, Cherry necrotic rusty mottle virus,* and *Little cherry virus 1* in California orchards. J. Plant Pathol. 87:173-177.

Svanella-Dumas, L., Marais, A., Gentit, P., Lamorte, J., and Candresse, T. 2005. First report on the natural occurrence of *Cherry virus A* in Mirabelle plum (*Prunus domestica* var. *insititia*). Plant Dis. 89:443.

Welsh, M. and Cheney, P. W. 1976. Little cherry. Pages 231-237 in: Virus Diseases and Non Infectious Disorders of Stone Fruits in North America. R. M. Gilmer, J. D. Moore, G. Nyland, M. F. Welsh, and T. S. Pine, eds. Agricultural Reaserch Service, USDA Agriculture Handbook, Washington, DC.