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Foveavirus, a new plant virus genus

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Summary. *Foveavirus* is a novel genus of plant viruses with helically constructed filamentous particles ca. 800 nm long, typified by apple stem pitting virus (ASPV). Virions do not contain lipids or carbohydrates, have a positive sense, single-stranded, polyadenylated RNA genome 8.4 to 9.3 kb in size, and a single type of coat protein with a size of 28 to 44 kDa. The genome of definitive viral species is made up of five ORFs encoding respectively, the replication-related proteins (ORF 1), the putative movement proteins (ORF 2 to 4, constituting the triple block gene), and the coat protein (ORF 5). Virions accumulate in the cytoplasm, where replication is likely to occur with a strategy comparable to that of potexviruses, based on direct expression of the 5'-proximal ORF, and expression of downstream ORFs through subgenomic RNAs. No vector is known. Virus transmission is by grafting, and dispersal is through infected propagating material. The genome structure and organization (i.e. number and order of genes) closely resembles that of the genera *Potexvirus*, *Carlavirus* and *Allexvirus*, but ORF 1 and the coat protein cistron (ASPV only) are significantly larger.

Introduction

Apple stem pitting virus (ASPV) is widespread in commercial apple cultivars which are symptomlessly infected, unless they are grafted on sensitive rootstocks [18]. Symptoms consisting of pitting of the woody cylinder, or epinasty and decline, develop in some ornamental *Malus* species and in Virigina Crab and Spy 222 indicators. Moreover, a close relationship was reported between ASPV and the causal agent of necrotic spot and vein yellows diseases of pear [5, 7, 8, 17, 18]. The identity of the two agents was proven by back transmissions to apple and pear [10] and by comparative characterization of a large number of viral isolates from Europe [15].

A virus with filamentous flexuous particles ca. 800 nm long was transmitted by inoculation of sap from stem pitting-diseased apples to *Nicotiana occidentalis*, and partially characterized [9]. This virus appeared to differ from other known plant viruses and was tentatively assigned to subgroup A of the then extant Closterovirus group [1]. However the revision of the plant virus taxonomic system [11] led to the establishment of the genus

Closterovirus (now family *Closteroviridae*), from which viruses of subgroup A were separated to be assigned to either of two to new genera, *Trichovirus* and *Vitivirus* [12, 14]. ASPV was not assigned to any of these genera and was excluded from the 6th Report of the ICTV [14].

Meanwhile, molecular studies had provided detailed information on the ASPV genome [4, 5], disclosing wide enough differences with other sequenced filamentous plant viruses, so as to support the likelihood that ASPV was the representative of a novel taxon. More recently, two additional viruses with molecular properties comparable to those of ASPV were described, grapevine rupestris stem pitting-associated virus (GRSPaV) [13, 22] and cherry green ring mottle virus (CGRMV) [21].

The establishment of a new viral genus denoted *Foveavirus* (from "*fovea*", Latin for pit, hole) having ASPV as the type species was therefore proposed, and approved by the Executive Committee of ICTV at its 1998 mid-term meeting in La Jolla, California, USA.

Taxonomic structure of the genus

Foveavirus has the following taxonomic structure:

Type species:	Apple stem pitting virus (= Pear vein yellowing virus)
Species in the genus:	Grapevine rupestris stem pitting-associated virus
Tentative species in the genus:	Sour cherry green ring mottle virus

Biological properties

The natural host range of individual species is restricted to a single (GRSPaV) or a few hosts (ASPV, CGRMV). ASPV infects primarily pome fruits, causing diseases of apple (top-working disease) when grafted on the susceptible rootstocks *Malus prunifolia* var. *ringo*, *M. sieboldii*, and *M. sieboldii* var. *arborescens*, and of pear (vein yellows and necrotic spot) [5], whereas GRSPaV is pathogenic to grapevines [3, 16]. Stone fruits (sweet, sour and flowering cherries, peach, and apricot) are the natural hosts of CGRMV [21]. Interestingly, all three viruses induce wood pitting in some of the natural hosts and/or indicators [3, 5, 20]. The experimental host range is restricted, but all virus species have a rather wide geographical distribution.

No vector is known for any of the viruses but all are transmitted by grafting and persist in the host propagative material. ASPV is mechanically transmissible, with some difficulty, to *Nicotiana occidentalis* and its subspecies *obliqua* [9] and to *Chenopodium murale*, in which it elicits localized infections [2]. CGRMV was transmitted by sap inoculation from cherry to cherry but not to a range of herbaceous hosts [19], whereas GRSPV is not mechanically transmissible.

ASPV elicits a severe derangement of the cytology of infected cells but no specific cytopathic structures or inclusion bodies. Virus particles accumulate in bundles in the cytoplasm [2, 9]. Massive accumulations of virus-like particles were also observed in mesophyll cells of CGRMV-infected Kwazan flowering cherry [19].

Morphological and physicochemical properties

Virions are very flexuous filaments with a length varying from about 800 nm (ASPV) to 1000–2000 nm (CGRMV), have helical symmetry and exhibit a surface pattern with cross-

banding and longitudinal lines. ASPV particles show the tendency to aggregate end-to-end, a condition that may also occur with CGRMV, thus accounting for the very high measurement values reported for its particles which, based on genome size [21], should have a length in the range of 750–800 nm.

ASPV particles sediment as two or three bands in sucrose density gradients but yield a single band at equilibrium in Omnipaque 350 density gradients, resist moderately high temperatures (thermal inactivation is around 60°C) but not organic solvents, and are unstable in cesium chloride and sulphate [9]. CGRMV particles resist organic solvents and have buoyant density of 1.24–1.25 g/cm^3 in cesium sulphate gradients [19].

Virions contain a single molecule of single-stranded positive-sense RNA, polyadenylated at the 3' terminus, with a size ranging from 8.4 to 9.3 kb according to the virus species. dsRNA extraction from infected host tissues yields a prominent band of ca. 10 kbp and at least four bands with lower molecular weight for ASPV [5], and major bands of ca. 8.7 kbp for GRSPaV [13], and of ca. 9 kbp for CGRMV [20, 21].

The viral capsid of ASPV is a single polypeptide of 44 kDa, whereas the capsid proteins of GRSPaV and CGRMV are 28 and 30 kDa in size, respectively. Non-structural proteins of all viruses consist of a large polypeptide 230–247 kDa in size containing the viral RNA-dependent RNA polymerase (RdRp), helicase and methyltransferase signatures, a 24–25 kDa polypeptide with the NTP-binding helicase domain, and two small polypeptides (13 kDa and 7 kDa) with membrane-binding functions. Virions apparently do not contain lipids or carbohydrates.

Chimeric fusion coat proteins expressed in *Escherichia coli* have been used to raise specific antisera to ASPV [6] and CGRMV [20].

Molecular properties

The complete nucleotide sequences (EMBL/GenBank accession number in parentheses) of ASPV (D21829, D21828), GRSPaV (AF026268), and CGRMV (AF017780) are available. The ASPV genome contains five ORFs, the 5' region initiating with a non-coding sequence of 59 nucleotides (nt). ORF 1 codes for the replication-related proteins with conserved motifs of the “Alpha-like” supergroup of positive-strand ssRNA viruses (i.e. helicase, methyltransferase, and RdRp). ORF 2 to 4 constitute the triple gene block thought to be involved in cell-to-cell spread of the virus and ORF 5 is the coat protein cistron. A non coding sequence of 135 nt followed by a poly(A) tail terminates the sequence [4].

A GRSPaV isolate from New York State posesses an identical genome structure and organization, expressing products of similar size as those of ASPV, except for the CP, which is smaller (28 kDa versus 44 kDa). The non-coding regions at the 5' and 3' end encompass 61 and 176 nt, respectively [13]. Californian isolates of the same virus have a

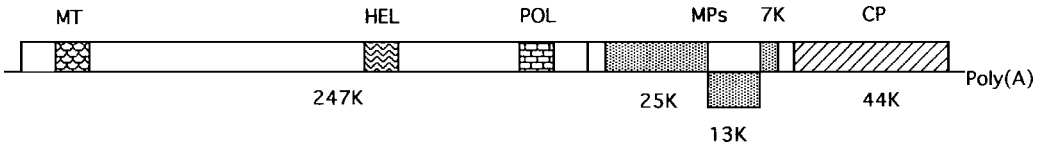

Fig. 1. Genome organisation of ASPV showing the relative position of the ORFs and their expression products. *MT* Methyltransferase; *HEL* helicase; *POL* polymerase; *MPs* putative movement proteins constituting the triple gene block; *CP* coat protein

6th ORF at the 3' end, encoding a product of 14 kDa whose function is not known. The 5' and 3' end non-coding regions are 60 and 140 nt in length, respectively [22].

The 5' end of CGRMV is capped and the viral genome sequence contains two additional ORFs (ORF 2a and 5a) nested in ORF 2 and ORF 5, respectively, potentially encoding polypeptides 14 kDa and 18 kDa in size, for which no similarity was found with other proteins in databases [21]. ASPV virions accumulate in the cytoplasm of infected cells where replication is likely to occur with a strategy comparable to that of potexviruses, based on direct expression of the 5'-proximal ORF, and expression of downstream ORFs through subgenomic RNAs [4].

Discriminating characters for species demarcation are differences in: (i) natural host range; (ii) mechanical transmissibility; (iii) serological specificity (species are serologically unrelated to one another); (iv) coat protein size; (v) amino acid sequence of any gene product greater than 10%.

Similarity with other taxa

Virions have particle morphology similar to that of viral species in the genera *Closterovirus*, *Crinivirus*, *Trichovirus*, *Vitivirus*, *Capillovirus* and *Allexivirus*. The structure and organization of the viral genome closely resembles that of the genera *Potexvirus*, *Carlavirus* and *Allexivirus* in the number and order of genes, but ORF 1 and, in ASPV only, the coat protein cistron are significantly larger. Replication-related proteins encoded by ORF 1 contain signature sequences homologous to those found in other members of the "Alpha-like" supergroup of ssRNA positive-sense viruses, especially those of the genera *Potexvirus*, *Carlavirus* and *Allexivirus*. Comparable similarities are found between the expression products of the triple gene block which is also present in the above genera. Coat proteins of all species share homologies with those of potex-, carla-, and allexiviruses, the closest being potexviruses, as shown by phylogenetic analysis of the coat protein sequences of the above three genera [5].

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