Maculavirus, a new genus of plant viruses

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Summary. *Maculavirus* is a new genus of plant viruses typified by *Grapevine fleck virus* (GFkV). A possible second member is Grapevine redglobe virus (GRGV). Maculaviruses are phloem-limited non-mechanically transmissible viruses with isometric particles *c*. 30 nm in diameter that have a rounded contour and prominent surface structure. Vectors, if any, are unknown. GFkV preparations contain two centrifugal components, T made up of empty protein shells and B, which contains 35% RNA. The coat protein (CP) has a molecular mass of 24 kDa. The genome is a single-stranded RNA that has *c.* 50% cytosine residues. It is 7564 nt in size, excluding the poly(A) tail and contains four putative open reading frames (ORF) that encode a 215.4 kDa polypeptide with the conserved motifs of replication-associated proteins of positive-strand RNA viruses (ORF1), the CP (ORF2), and one (GRGV) or two (GFkV) proline-rich polyproteins of 31.4 kDa (ORF3) and 15.9 kDa (ORF4), respectively, with unknown function. Replication-associated proteins and CP are phylogenetically related to those of members of the genera *Tymovirus* and *Marafivirus*. GFkV-infected grapevine cells contain vesiculated mitochondria, the possible site of RNA replication. In the natural host, GFkV particles accumulate in great quantity, sometimes in crystalline arrays in phloem cells.

Introduction

Fleck, a widespread disease of grapevines (*Vitis* spp.), is latent in European grapevine varieties (*Vitis vinifera*) and in most American rootstocks [15]. The disease agent is *Grapevine fleck virus* (GFkV), a non-mechanically transmissible phloem-limited RNA-containing virus with isometric particles *c.* 30 nm in diameter that have a rounded contour and a prominent surface structure. GFkV is not assigned to any of the currently established taxonomic groups of plant viruses [5].

Recent investigations have shown that grapevines host a family of GFkV-like viruses and for two, Grapevine asteroid mosaic-associated virus (GAMaV) and Grapevine redglobe virus (GRGV), some molecular characters and the ultrastructural effects of infection are known [19].

Similarities in particle morphology and physico-chemical properties exist between GFkV and members of the genera *Tymovirus* [9] and *Marafivirus* [10], but wide differences at the biological, ultrastructural, and molecular level [1, 3, 6, 19, 20] suggested that GFkV might represent a new taxon. The establishment of a novel viral genus denoted *Maculavirus* (from "*macula*" Latin for fleck) having GFkV as the type species, was therefore proposed and approved by the ICTV in June 2002.

GRGV is phylogenetically closely related to GFkV and has a similar genome organization [19 and unpublished information]. Thus it seems to qualify as an additional possible member of the genus *Maculavirus*. By contrast, GAMaV is phylogenetically more closely related to the marafivirus *Oat blue dwarf virus* (OBDV), with which shows a virtual identity in genome structure and organization [19 and unpublished information].

Taxonomic structure of the genus

Maculavirus has the following taxonomic structure:

Type species:	*Grapevine fleck virus*	(GFkV)
Tentative species:	Grapevine red globe virus	(GRGV)

Biological properties

The natural and artificial host range of GFkV and GRGV is restricted to European and American *Vitis* species. Whereas GRGV infections are apparently symptomless in all hosts, in *Vitis rupestris* GFkV elicits clearing of the veins of the third and fourth order, which results in localized translucent spots. Leaves with intense flecking are wrinkled, twisted and may curl upwards. Severe strains also induce varying degrees of stunting [17]. Neither virus is transmitted by mechanical inoculation of sap, but both are readily transmitted by grafting. Although there is circumstantial evidence that GFkV spreads naturally in the field [12, 13], no vector has been identified. Long distance dissemination of fleck disease is primarily through infected propagative material, which accounts for its worldwide distribution. GFkV is not seed-transmissible [14] but its transmission through dodder has been reported [22].

GFkV-infected grapevine cells contain cytopathic structures, called "multivesiculate bodies" derived from severely deranged mitochondria that have undergone peripheral vesiculation following invagination of both lamellae of the organelle's limiting membrane [7]. GFkV particles accumulate in great quantity, sometimes in crystalline arrays, in differentiating sieve tubes and companion cells of naturally infected vines [6].

Morphological and physico-chemical properties

Virions are isometric *c*. 30 nm in diameter, have a rounded contour and a surface structure like that of particles of tymoviruses and marafiviruses, suggesting clustering of coat protein (CP) subunits into pentamers and hexamers [1, 3] (Fig. 1). The viral genome is a capped, positive-sense single-stranded RNA that is characterized by a very high cytosine content (*c.* 50%). GFkV genome is 7564 nt in size and the CP consists of a single type of subunit with a molecular mass of *c.* 28 kDa, as estimated by electrophoretic migration, and of 24.3 kDa, based on sequence data. When centrifuged in density gradients, virus prepara-

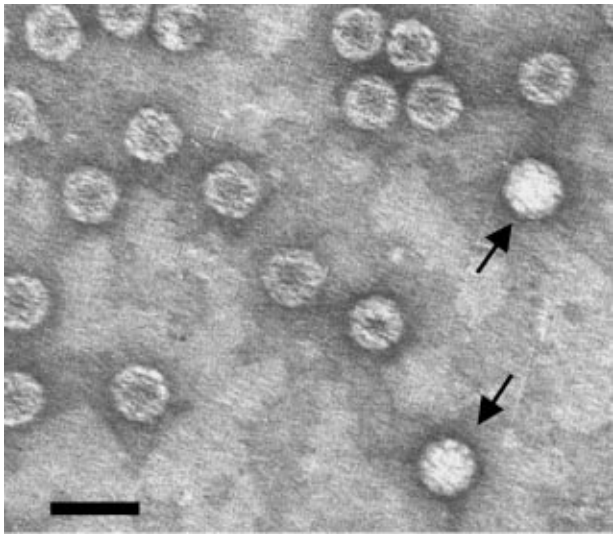


Fig. 1. Negative contrast electron micrograph of *Grapevine fleck virus* (GFkV) particles. Arrows point to intact virions showing a surface structure suggestive of clustering of coat protein subunits in pentamers and hexamers. Bar = 50 nm

tions sediment as two components, T, made up of apparently empy protein shells and B, that contains 35% RNA [3]. T component particles seem to contain two subgenomic RNAs with estimated sizes of *c*. 1000 and 1300 nt, respectively [20].

GFkV virions are efficient immunogens. Polyclonal antisera and monoclonal antibodies have been raised to purified GFkV particles and these are widely used for diagnosis [2, 18, 21]. GFkV and GRGV are serologically unrelated [19].

The complete nucleotide sequence (EMBL accession number in parentheses) of the viral genome of GFkV (AI3090229) and more than one third of the GRGV genomic sequence (AF521977), have been determined [19, 20 and unpublished information]. The GFkV genome (Fig. 2) is 7564 nt in size, excluding the 3' terminal poly(A) tail, and contains four putative open reading frames (ORF) and untranslated regions of 291 and 35 nt at the 5' and 3' end, respectively. ORF1 encodes a 215.4 kDa polypeptide (p 215), which has the conserved motifs of replication-associated proteins of positive-strand RNA viruses and a papain-like protease domain. ORF1 of GFkV and GRGV lacks the highly conserved 16 nt long subgenomic RNA promoter referred to as “tymobox” or “marafibox” located near the end of the viral replicase of all sequenced tymoviruses [8] and marafiviruses [4, 11, 16]. ORF2 encodes a 24.3 kDa polypeptide (p24) identified as the coat protein (CP). ORFs 3 and 4 are located at the extreme 3' end of the viral genome and encode proline-rich proteins of 31.4 kDa (p31) and 15.9 kDa (p16), respectively, with unknown functions. The GRGV

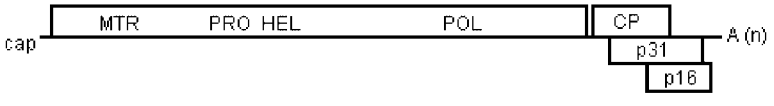


Fig. 2. Genome organization of *Grapevine fleck virus* (GFkV) showing the relative position of the ORFs and their expression products. *MTR*, methyltranferase; *PRO*, papain-like protease; *HEL*, helicase, *POL* polymerase (RdRp); *CP*, coat protein. *p31* and *p16* are proline-rich proteins

genome apparently lacks one of the 3' most ORFs. The coat protein, methyltransferase (MTR) and RNA-dependent RNA polymerase (RdRp) of both viruses are phylogenetically related to those of members of the genera *Tymovirus* and *Marafivirus* [19, 20] (Fig. 3). Replication is likely to occur in the cytoplasm, possibly in association with the vesicles in mitochondria. The replication strategy probably involves autoproteolytic cleavage of the 215 kDa polypeptide by the papain-like protease encoded by ORF1 and the production of subgenomic RNA.

Species demarcation criteria in the genus

The criteria demarcating species in the genus are:

– Overall sequence identity of less than 70%
– Capsid protein sequences less than 85%
– Serological specificity
– Reaction of the indicator *Vitis rupestris*

Similarities and differences with other taxa

Virions have the same morphology, structural organization, and hydrodynamic behaviour as particles of species of the genera *Tymovirus* and *Marafivirus*. There are also phylogenetic relationships with members of both taxa (Fig. 3) and similarities in molecular traits (very high cytosine content, genomic organisation). However, GFkV and GRGV are separated from tymoviruses and marafiviruses by distinct differences:

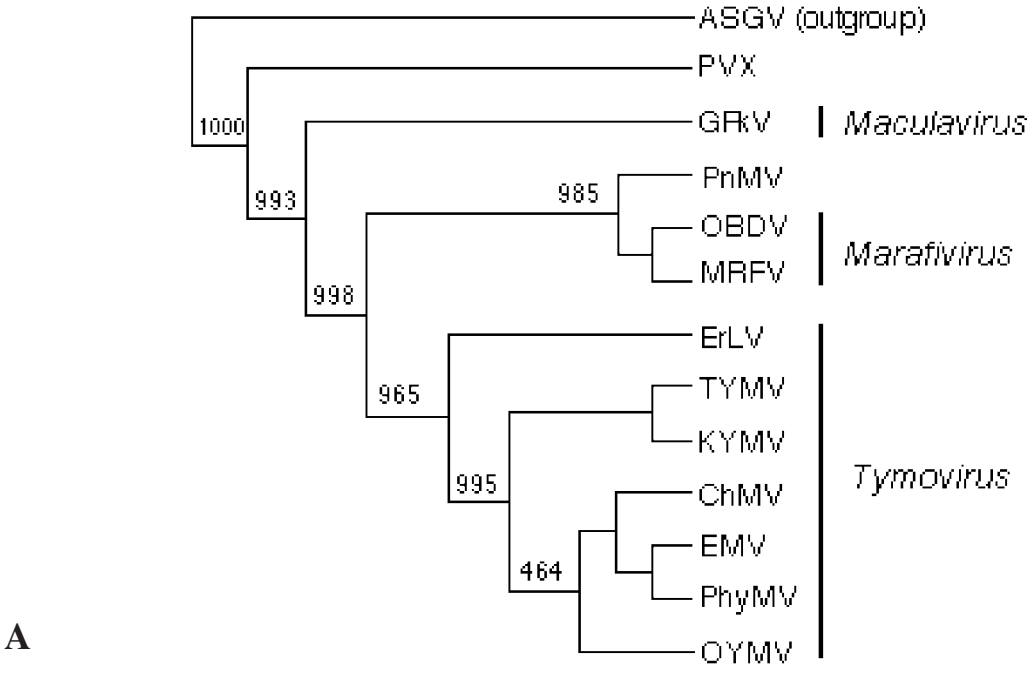
(i) Biological. Tymoviruses invade parechyma tissues and are mechanically transmissible, maculaviruses are not and are phloem-limited. Marafiviruses infect preferentially monocotyledonous plants.

(ii) Epidemiological. Tymoviruses are transmitted by beetles and several marafiviruses are transmitted by leafhoppers, whereas maculaviruses have no known vector.

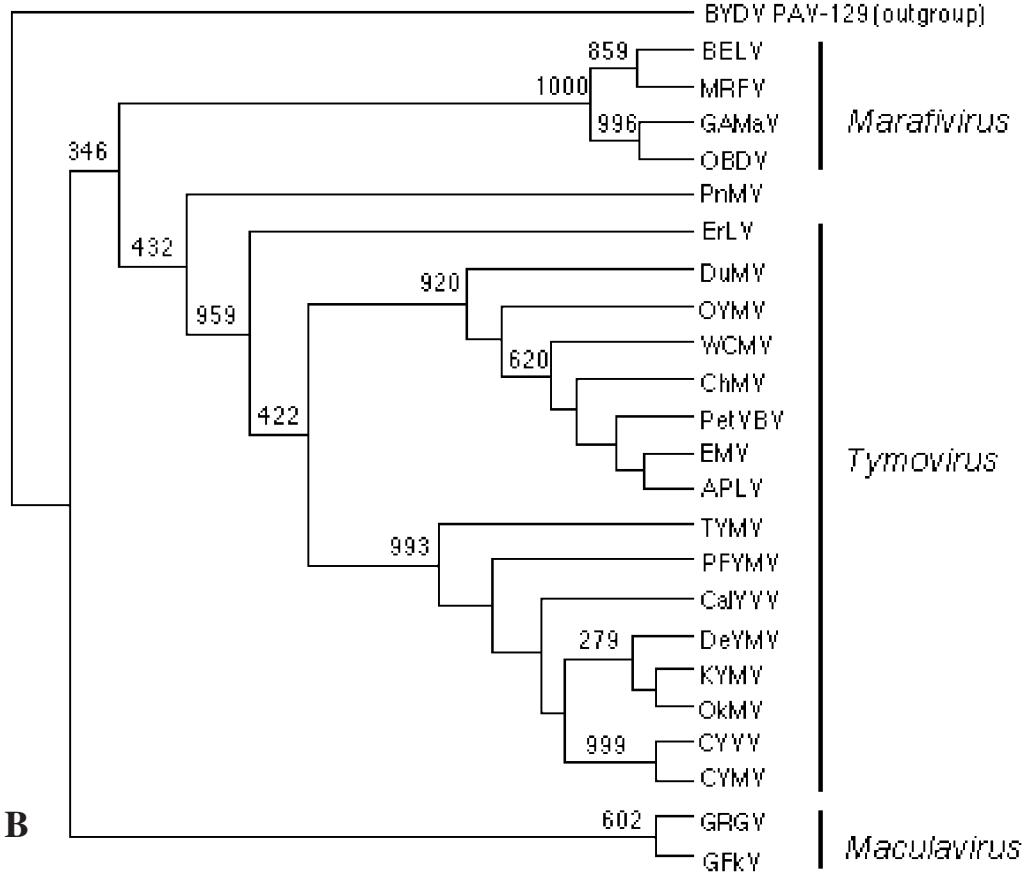
(iii) Physico-chemical. Compared with maculaviruses, tymoviruses have smaller genomes (6.0 versus 7.5 kb) and coat proteins (20 kDa versus 24 kDa), and marafiviruses have smaller genomes (6.0–6.5 kb versus 7.5 kb) and two coat protein subunits.

Fig. 3. Phylogenetic analyses of polymerase (**A**) and capsid protein (**B**) sequences of members of the genera *Maculavirus, Tymovirus,* and *Marafivirus.*. Amino acid sequences were aligned and the trees were constructed by using CLUSTAL W. Numerical values at each branch indicate the bootstrap test value for each node. EMBL or GenBank accession numbers of viral sequences used in phylogenetic analysis are: *Grapevine fleck virus* (GFkV, AJ309022) ; Grapevine redglobe virus (GRGV, AF521977) ; *Eggplant mosaic virus* (EMV, J04374); *Erysimum latent virus* (ErLV, AF098523); *Kennedya yellow mosaic virus* (KYMV, D00637); *Ononis yellow mosaic virus* (OYMV, J04375); *Turnip yellow mosaic* (TYMV (X16378); *Physalis mottle virus* (PhyMV, S97776); *Clitoria yellow vein virus* (CYVV, AF035200); *Cacao yellow mosaic virus* (CYMV, X54354); *Chayote mosaic virus* (ChMV, AF195000); *Calopogonium yellow vein virus* (CalYVV, U91413); *Dulcamara mottle virus* (DuMV, AF035634); *Okra mosaic virus* (OkMV, AF035202); *Wild cucumber mosaic virus* (WCMV, AF035633); *Passion fruit yellow mosaic virus* (PFYMV, AF467107); *Petunia vein banding virus* (PetVBV, AF210709); *Bermuda grass etched-line virus* (BELV, AY040531).

Oat blue dwarf virus (OBDV, U87832); *Poinsettia mosaic virus* (PnMV, AJ271595); *Maize rayado fino virus* (MRFV, AF265566); *Potato virus X* (PVX, X55802). *Barley yellow dwarf virus*, isolate PAV-129 (BYDV, AF218798) and *Apple stem grooving virus* (ASGV, D14995) were used as outgroups.


ASGV (outgroup)
PVX
GRkV
Maculavirus
1000
PnMV
993
985
OBDV
MRFV
Marafivirus
998
ErLV
TYMV
965
KYMV
995
ChMV
Tymovirus
EMV
464
PhyMV
OYMV

A


BYDV PAV-129 (outgroup)
859
BELV
1000
MRFV
996
GAMaV
Marafivirus
346
OBDV
PnMV
ErLV
432
DuMV
920
OYMV
959
WCMV
620
ChMV
PetVBV
422
EMV
APLV
Tymovirus
TYMV
993
PFYMV
CalYVV
279
DeYMV
KYMV
OkMV
999
CYYV
CYMV
602
GRGV
GFkV
Maculavirus

B

(iv) Molecular. Tymoviral genomes have three ORFs, a structural organization different from that of maculaviruses, and a tRNA-like structure at the 3' teminus. The genome of most marafiviruses has a single large ORFs encoding a polyprotein. The genome of maculaviruses has three or four ORFs, the CP cistron is separared from ORF1, the 3' proximal ORF(s) encodes proline-rich protein(s) and the genome RNA is polyadenylated. Maculavirus RNA lacks the conserved nucleotide sequence known as the tymobox or marafibox.

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