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Closteroviridae: A New Family of Flexous Plant Viruses

I. TÓBIÁS

Plant Protection Institute, Hungarian Academy of Sciences,
H-1525 Budapest, P.O. Box 102, Hungary

Members of the family *Closteroviridae* have been traditionally defined as plant viruses with thread-like particles having messenger-sense single-stranded RNA, the largest genomes among RNA plant viruses. Individual virus species are distributed worldwide and some of them cause devastating crop losses. The natural host range usually narrow. Diseases symptoms are yellowing type or pitting and/or groowing of the woody cylinder. Infection systemic, but usually limited to the floem. Natural vectors are aphids, whiteflies, pseudococcids, coccids and mealybugs. Transmission is semipersistant. Closteroviruses contains 9–13 ORFs flanked by 5'- and 3'- untranslated regions with different length. The genome strategy is based on polyprotein precessing, +1 ribosomal frameshift and formation of subgenomic RNAs. Common features of closteroviruses that encode a homologue of HSP70 molecular chaperones found in all cells (HSP70h) and a duplicate (CPd) of the coat protein gene.

Keywords: *Closteroviridae,* flexous viruses, chaperons, coat proteins.

In 2000 The International Commitee on Taxonomy of Viruses (ICVT) approved the establishment of a new plant virus family named *Closteroviridae* (Regenmortel et al., 2000). Members of the family *Closteroviridae* have been traditionally defined as plant viruses with thread-like (their name coming from κλωστερ Greek word for spindle, thread) particles having messenger-sense single-stranded RNA genomes of up to 20 kb (Bar-Joseph et al., 1979; Dolja et al., 1994; Agranovsky, 1996). The very flexuous filamentous particles about 12 nm in diameter, but their length varies from 1000 nm to 2200 nm according to the genus they belong or the individual species. Individual virus species are distributed worldwide (*Grapevine leafroll-associated virus 1–6,* GLRV-1-6) and some of them (*Citrus tristeza virus,* CTV, *Beet yellows virus,* BYV) cause devastating crop losses. The natural and experimental host ranges vary from narrow to moderate.

Taxonomy of Closteroviridae

The family *Closteroviridae* belongs to order Nidovirales and consists of two genera: members with monopartite genome belong to the genus Closterovirus and those with bipartite genome are in the genus Crinivirus. The members of the family *Closteroviridae* are shown in *Table 1.*

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Table 1

List of species in the family *Closteroviridae*

Genus **Closterovirus**
Type species: *Beet yellows virus* (BYV)
Aphid-transmitted:
- *Beet yellow stunt virus* (BYSV)
- *Beet yellows virus* (BYV)
- *Burdock yellows virus* (BuYV)
- *Carnation necrotic fleck virus* (CNFV)
- *Carrot yellow leaf virus* (CYLV)
- *Citrus tristeza virus* (CTV)
- *Wheat yellow leaf virus* (WYLV)

Whitefly-transmitted:
- *Beet pseudoyellows virus* (BPYV)

Mealybug-transmitted:
- *Grapevine leafroll-associated virus 3* (GLRV-3)
- *Little cherry virus* (LChV)

Vector unknown:
- *Grapevine leafroll-associated virus 2* (GLRV-2)

Tentative species:
Aphid-transmitted:
- Clover yellows virus (CYV)
- Dendrobium vein necrosis virus (DVNV)
- Heracleum virus 6 (HV-6)

Mealybug-transmitted:
- Pineapple mealybug wilt-associated virus 1 (PMWaV-1)
- Pineapple mealybug wilt-associated virus 2 (PMWaV-2)
- Sugarcane mild mosaic virus ((SMMV)

Whitefly-transmitted:
- Cucumber chlorotic spot virus (CCSV)
- Diodea vein chlorosis virus (DVCV)

Vector unknown:
- Alligatorweed stunting virus (AWSV)
- Festuca necrosis virus (FNV)
- Grapevine leafroll-associated virus 1 (GLRV-1)
- Grapevine leafroll-associated virus 4 (GLRV-4)
- Grapevine leafroll-associated virus 5 (GLRV-5)
- Grapevine leafroll-associated virus 6 (GLRV-6)
- Grapevine leafroll-associated virus 7 (GLRV-7)
- Megakepasma mosaic virus (MegMV)

Genus **Crinivirus**
Type species: *Lettuce infectious yellows virus*
- *Abutilon yellows virus* (AbYV)
- *Cucurbit yellow stunting disorder virus* (CYSDV)
- *Lettuce chlorosis virus* (LCV)
- *Lettuce infectious yellows virus* (LIYV)
- *Sweet potato chlorotic stunt virus* (SPCSV)
- (Sweet potato sunken vein virus)
- *Tomato chlorosis virus* (ToCV)
- *Tomato infectious chlorosis virus* (TICV)

Biological properties

A) DISEASES CAUSED IN PLANTS

Diseases symptoms caused by different closteroviruses are very specific and often reflect in their name (*Beet yellows virus, Cucurbit yellow stunting disorder virus,* CYSV, *Grapevine leafroll associated virus, Tomato chlorosis virus,* ToCV, etc.).

Disease symptoms are different type: a) yellowing or reddening (*Beet yellows virus, Grapevine leafroll-associated virus 1–6,* etc.), b) stunting *(Beet yellow stunt virus),* c) rolling *(Grapevine leafroll-associated virus 1–6),* d) small and late ripening fruits (*Little cherry virus,* LChV) and e) pitting and/or grooving of the woody cylinder *(Citrus tristeza virus).*

The observed symptoms are different due to virus strain, host and variety of the plant, season and also to the growing practice.

Mild BYV strain produce mild yellowing on beet plants. Characteristic symptoms of severe strain include obvious vein clearing, veinal necrosis of younger leaves, older leaves become yellow, thickened and usually have numerous small red or brown necrotic "pinpoint" spots giving bronsing effect (Lister and Bar-Joseph, 1981).

Symptoms of BYSV on sugar beet are characterize severe twisting and epinasty. Leaves have shortened internodes, mottled or chlorotic. The plants are severaly stunted and often collapse and die. The same BYSV strain causes mild symptoms on sowthistle *(Sonchus deraceus)* which is the main reservoir of the virus.

Citrus tristeza virus (tristeza Spanish for "sedness") occurs wordwide in citrus crops and causes from "quick decline" to symptomless infection depending on host and variety (sweet orange, mandarin or grapefruit). Disease symptoms also changed after replacing traditional sour orange rootstock by CTV tolerant (mainly citranges *C. sinensis* × *Poncirus trifoliata*) ones (Cambra et al., 2000). Characteristic symptoms of *Grapevine leafroll-associated viruses* appeared by late summer, diseased leaves turn yellowish or reddish, depending on the specific cultivar, and the leaves roll dawnward. Symptoms do not show on all diseased vines, and are not apparent in winter season, spring time and many American rootstocks show no symptoms when infected.

B) TRANSMISSION AND VECTORS

Some closteroviruses are transmissible by mechanical inoculation (BYV, CTV) but most of them are not. In vegetatively propagated crops (*Citrus,* grapes) long distance dissemination is primarily through infected propagating material.

Natural vectors are aphids, whiteflies, pseudococcids, coccids and mealybugs. Transmission is semipersistant – except CTV – regardless of the type of vector. A semi-persistent transmission pattern implies a stricter virus-vector specificity comparing to non-persistent virus transmission. Among aphid species that can transmit BYV *Myzus persicae* is the most efficient vector (Duffus, 1973). Cambra et al. (2000) have observed that CTV become primary importance in Spain when the inefficiant vectors *Toxoptera aurentii* and *Aphis spiraecola* were replaced and became predominant *Aphis gossipii* an efficient vector of CTV. Interaction of the whitefly-transmitted closteroviruses with their vector seem to be even more specific, thus LIYV and SPCSV can only transmitted by

Bemisia tabaci (Cohen et al., 1992), whereas BPYV can only transmitted by *Trialeurodes vaporariorum* (Duffus, 1973). Likewise the mealybug-transmitted GLRV-3 and LChV are specifically transmitted by *Pulvinaria vitis* and *Phenacoccus aceris,* respectively (Raine et al., 1986; Belli et al., 1994).

Transmission through seeds is very rare.

Physical and Physicochemical properties

The elementary properties such as stability *in vitro,* thermal inactivation and dilution end points are rather similar in *Closteroviridae* family. Thermal inactivation point varies around 45–55 ºC, stability *in vitro* 1–6 days, and dilution end point is between 10^{-3} and 10^{-5}. Viruses of both genera usually sedimented as a single band in sucrose or Cs_2SO_4, bouyant density in CsCl is 1.30–1.34 and in Cs_2SO_4 1.24–1.27 g/cm^3 (Lister and Bar-Joseph, 1981). Members of the family have virions containing a single molecule of linear, positive sense, ss RNA, constituting 5–6% of the particle weight. In genus Clostrovirus genome size ranges from 15.5 Kb (BYV) to 19.3 Kb (CTV), the largest genome among positive strand RNA plant viruses. The type member of genus Crinivirus is *Lettuce infectious yellows virus* is 15.3 Kb. The genome size is related to particle length. The 5'end of the genome is likely to be capped, the 3' end is not polyadenylated and does not possess a tRNA-like structure, but may have several hairpins structures.

Virions of all member possess a long body and a short tail that are formed by the CP and divergent CP analogue (CPd), respectively. The CP is ranging 22 to 40 KDa, according to the individual species. Virion proteins are moderately antigenic. Most of the species are serologicaly unrelated or distantly related *(Grapevine leafroll-associated virus 1–6).* Polyclonal and monoclonal antibodies to number of closteroviruses are used for virus diagnosis (Gugerli et al., 1984; Tóbiás et al., 1996; Cambra et al., 2000).

Non-structural proteins common to all members of the family are: 1) a large polypeptide containing the conserved domains of papain-like protease (P-Pro), methyltransferase (MT) and helicase (HEL), 2) protein with sequence motifs of viral RNA dependent RNA polymerase (RdRp), 3) a small hydrophobic protein with membrane binding properties, 4) homologue of the cellular HSP70 heat-shock proteins, that implicated in the cell-to-cell movement of the viruses, 5) 55–64 × 10^3 product with unknown function. Lipids and carbohydrates are not reported in the virion.

Genome organization and replication

Closteroviruses have the largest genome among positive strand ssRNA plant viruses. Members of the genus Closteroviruses have monopartite genome, and in genus Crinivirus have bipartite genome. The genome of both genera is characterized by the presence of unique genes coding for a homologue of the HSP70 proteins and for an analogue of the CP. The genome organization, the number and relative position of ORFs varies with the genus and/or individual virus species. In aphid-transmitted members of the family (BYV, CTV, BYSV), the CPd is upstream of the CP, whereas the reverse is true with whitefly-transmitted (LIYV, SPCSV, CCSV) and mealybug-transmitted (GLRV-3, LChV) viruses.

The ORFs coding for the small hydrophobic protein, the HSP70 homologue, the 55–64 KDa product, the CP and its CPd, form a five-gene module conserved among members of the family. The genome strategy is based on 1) polyprotein precessing encoded by ORF1a, 2) +1 ribosomal frameshift for replication of RdRp coded by ORF1b, 3) expression of the downstream ORFs via the formation of a nested set of 3'-coterminal sgRNAs.

Replication occurs in the cytoplasm, possibly in association with membranous vesicles and vesiculated mitochondria.

The closteroviruses studied so far have apparent similarities in genome organization, which include replication-associated genes that consists MT, HEL and RdRp conserved domain and an unique five-gene array including a small hydrophobic transmembrane protein, HSP70 homologue, HSP90 homologue, the CP and its diverged duplicate. In addition to these genes different closteroviruses acquired different new genes in their evolution.

Generalized genome organization will be shown by prototypic beet yellows virus *(Fig. 1).*

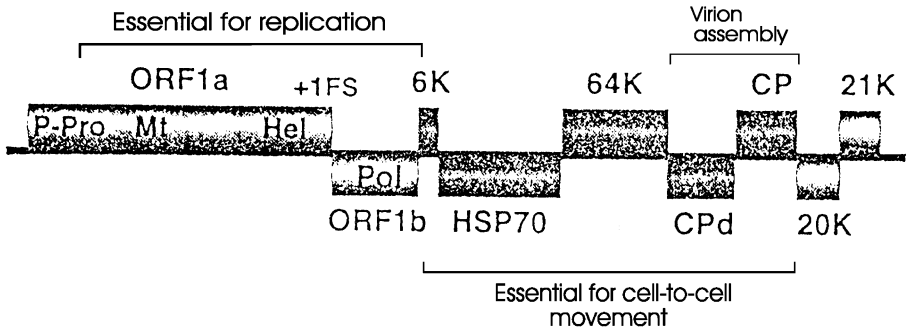

Fig. 1. Genome organization of *Beet yellows virus,* showing the relative position of ORFs and their expression products. The figure is complying with work of Agranovsky et al., 1994 and Alzhanova et al., 2000. Abbreviations: P-Pro – papain-like protease, Mt – methyltransferase, HEL – helicase, Pol – RNA dependent RNA polymerase, HSP70 – heat-shock related protein, CP – coat protein, CPd – coat protein duplicate. The five boxes with the same shadowing represent the five gene blocks conserved among closteroviruses

The 5'-proximal ORF1a codes for the 295 KDa product revealed a putative papain-like leader protease (P-Pro) domain followed by methyltransferase (Mt) and RNA helicase (Hel) sequence motifs. The ORF1b overlaps the last 40 triplets of ORF1a and codes protein showing significant similarity to RNA dependent RNA polymerase (RdRp) domains of positive strand RNA viruses. The next ORF codes 6.4 KDa protein which shows similarity to the small hydrophobic proteins encoded in the "triple gene block" of potex and carlaviruses and has been suggested to involve in virus infection transport (Alzhanova et al., 2000). The 65 KDa protein coded by ORF4 is strikingly similar to the

HSP70 family of the cell heat-shock proteins. Closteroviruses encoded HSP70 homologue protein involved in virion assemble and formation (Satyanarayana et al., 2000) and also in virus translocation (Premyslov et al., 1999). Internal segment in BYV 64 KDa protein shows similarity to domain in the HSP90 heat shock protein and involved in the virus movement process (Agranovsky, 1996; Alzhanova et al., 2000).

ORF5 and ORF6 code for a 24 KDa and 22 KDa capsid proteins of BYV, respectively (Agranovsky et al., 1994) and as a structural proteins are required to potentiate the cell-to-cell movement (Alzhanova et al., 2000).

The remaining 3'-proximal BYV ORFs code for a 20 KDa protein of unknown function, and for a 21 KDa protein required for efficient RNA accumulation (Premyslov et al., 1998). Recently was demonstrated that BYV gene expression is under temporal regulation including early (HSP70, CPd, CP and p21 genes) and late (p64 and p20 genes) phases (Hagiwara et al., 1999).

Phylogenetic analysis of conserved proteins – replication-associated proteins and five-gene module – in closteroviruses suggest common ancestor for each of these conserved genes (Dolja et al., 1994; Klassen et al., 1995). In addition to these conserved genes different closteroviruses acquired different new genes in their evolution, which leads to complex and heterogeneous genome organization *(Fig. 2).* Acquisition of ORF2 (30 KDa protein) by BYV-like genome located between RdRp and 6-KDa protein resulted BYSV-like genome. Further acquisition of two or three 3'-proximal ORFs resulted CTV-like or GLRV-3-like genome (Karasev et al., 1996; Zhu et al., 1998). These acquisition and rearrangements (CTV ORF1a contains duplicated P-Pro domains) of closterovirus genome

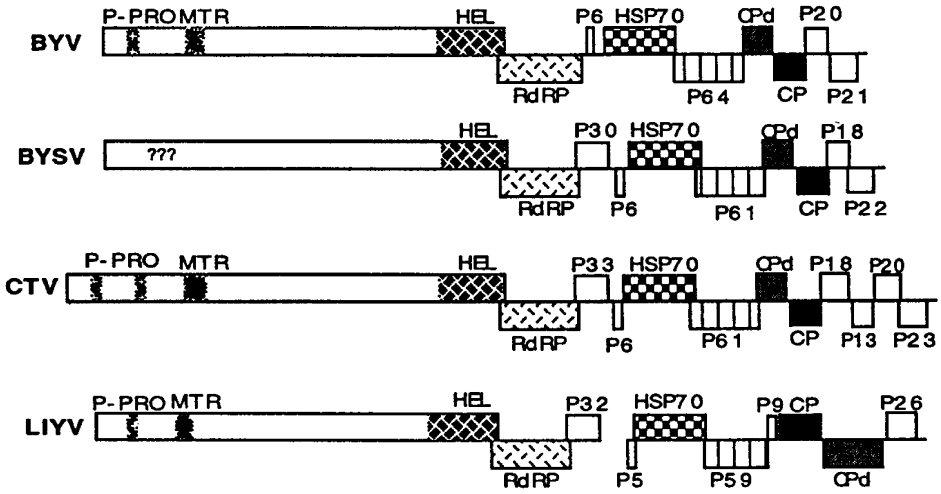

Fig. 2. Comparison of genome organization of BYV, BYSV, CTV and LIYV. ORFs are shown as boxes, with related domains indicated by the same fill-pattern. P-Pro – papain-like proteinase, MTR – methyltransferase, HEL – helicase, RdRP – RNA dependent RNA polymerase, HSP70 – heat-shock related protein, CP – coat protein, CPd – coat protein duplicate

could be mediated by RNA-RNA recombination in the course of the virus infection (Simon and Bujarski, 1994). The findings of multiple defective RNAs provide strong evidence that recombination is common in the course of the closterovirus infection (Mawassi et al., 1995; Bar-Joseph et al., 1997).

Conclusions

Closteroviruses attract interest from an applied standpoint, since the disease they cause in citrus and sugar beet are listed among the most economically important plant virus diseases. On the other hand, closteroviruses have the largest genome of all positive strand RNA viruses and possess unique properties to code homologue of HSP70 molecular chaperones, a duplicate CP gene and those of temporal regulation of gene expression.

The unusual diversity in genome organization among the characterized closteroviruses, which surpasses diversity in any other plant virus group, creates new opportunities for studying mechanism of virus pathogenesis and evolution.

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