Oral Session V

Peach latent mosaic viroid: further dissection of the molecular determinant inducing peach calico disease

Navarro, Beatriz[1], Delgado, Sonia[2], Rodio, Maria Elena[1], Flores, Ricardo[2], Di Serio, Francesco[1]
[1]Consiglio Nazionale delle Ricerche, Istituto di Virologia Vegetale, 70126 Bari, Italy,
[2]Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, 46022 Valencia, Spain
Contact: f.diserio@ba.ivv.cnr.it

Peach calico (PC) disease is a severe chlorosis induced by Peach latent mosaic viroid (PLMVd) (1). This symptomatology is exclusively elicited by PLMVd variants containing a specific insertion of 12-13 nt that, in the proposed secondary structure of the viroid RNA, folds into a hairpin composed by a short stem of four base pairs capped by a U-rich loop. In previous works, we showed that both the loop and the stem of this structural element play critical pathogenic roles (2-4). Here we have further dissected this structural element by sitedirected mutagenesis, bioassay of the mutated variants and molecular analysis of the progenies, confirming that not only the size but also nucleotide composition of the stem strongly affect the pathogenic properties. We have also observed an uneven distribution of PLMVd variants in the chlorotic and adjacent green leaf sectors of GF-305 seedlings inoculated with in vitro dimeric transcripts of PC-inducing variants: new variants, most likely non-pathogenic and with a modified stem of five base pair capped by a U-rich loop, were generated during the infection and accumulated preferentially in the green sectors. Our data support the use of PLMVd as model system for studying the pathogenesis and evolution of non-coding RNAs.

1. Hernández and Flores (1992). PNAS USA 89: 3711-3715; 2. Malfitano et al. (2003). Virology 313: 492-501;
3. Rodio et al. (2006). J. Gen. Virol. 87: 231-240; 4. Rodio et al. (2007). Plant Cell 19: 3610-3626.

Towards dissecting the structural determinant of peach latent mosaic viroid inducing mosaic symptoms

Delgado, Sonia[1], Navarro, Beatriz[2], Gentit, Pascal[3], Di Serio, Francesco[2], Flores, Ricardo[1]
[1]Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, 46022 Valencia
[2]Istituto di Virologia Vegetale del CNR, 70126 Bari, Italy
[3]Centre Technique Interprofessionel de Fruits et Légumes de Lanxade, 24130 Prigonrieux, France
Contact: rflores@ibmcp.upv.es

Most isolates of *Peach latent mosaic viroid* (PLMVd) (1,2) do not incite foliar symptoms, but a few number cause peach mosaic (PM) or peach calico (PC), an extreme albino phenotype. The structural determinant of PC maps at an insertion of 12-13 nt folding into a hairpin capped by a U-rich loop (3,4). However, the molecular determinant of PM remains unidentified because, not being associated with a specific insertion, it could reside in one or more domains of the branched PLMVd conformation. Moreover, cloning of latent and PM-inducing PLMVd isolates has revealed that they are formed by complex populations of variants, and bioassays on GF-305 peach seedlings of individual variants have shown that the biological properties of PLMVd isolates depend on the complexity of their populations and on the presence of specific variants. From the variants recovered from a typical PM isolate (GDS), we have selected for further dissection one (gds6) that is very infectious and elicits consistently a characteristic PM. We have initially focused on G339, a position that appears associated with PM in multiple alignments with other variants. To determine the role of G339 in infectivity and symptoms, blocks of GF-305 seedlings were inoculated with in vitro transcripts of recombinant plasmids containing dimeric tandem inserts of PLMVd-cDNAs with all possible changes at this position introduced by site-directed mutagenesis. Deletion of G339 reduced the number of plants showing symptoms or their severity, while substitutions to A, U or C had moderate effects. Sequencing of the resulting progenies should provide hints on the feasibility of this approach for identifying the PM determinant.

1. Hernández and Flores (1992). PNAS USA 89: 3711-3715; 2. Flores et al. (2006). Mol. Plant Pathol. 7: 209-221 ;
3. Malfitano et al. (2003). Virology 313: 492-501 ; 4. Rodio et al. (2007). Plant Cell 19: 3610-3626.

Deep sequencing of the viroid-derived small RNAs accumulating in peach infected by two symptomatic variants of peach latent mosaic viroid

Di Serio, Francesco[1], Giesel, Andreas[2], Navarro, Beatriz[1], Delgado, Sonia[3], Martínez de Alba, Angel-Emilio[3], Donvito, Giacinto[4], Flores, Ricardo[3]
[1]Istituto di Virologia Vegetale del CNR, 70126 Bari, Italy
[2]Istituto di Tecnologie Biomediche del CNR
[3]Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC)
[4]Istituto di Nazionale di Fisica Nucleare, 70126 Bari, Italy
Contact: rflores@ibmcp.upv.es

Northern-blot hybridization of nucleic acid preparations from plants infected by representative members of both viroid families has revealed the presence of 21- 24 nt viroid-derived small RNAs (vd-sRNAs) with the structural properties of the small interfering RNAs (siRNAs), the characteristic effectors of RNA silencing (1). These results strongly support that viroids are elicitors and targets of the RNA silencing machinery of their hosts. Moreover, conventional sequencing of the vdsRNAs from two members of the family Pospiviroidae (2) and one of the *Avsunviroidae* (3) has confirmed that they are predominantly of 21 and 22 nt and (+) polarity. This approach, however, generates incomplete datasets and, therefore, the ensuing conclusions may be biased. To overcome this limitation we have subjected to deep sequencing the vd-sRNAs accumulating in GF-305 peach seedlings infected by two molecular variants of Peach latent mosaic viroid (PLMVd), one causing peach mosaic and the other peach calico (4). The analysis of the resulting PLMVd-sRNAs permits to draw inferences about the dicer-like enzymes catalyzing their genesis and the RNA substrates upon which they act, as well as to assess whether some specific PLMVd-sRNAs containing the pathogenicity determinant of peach calico may be involved in symptom development.

1. Flores et al., (2005). Annu. Rev. Phytopathol. 43: 117-139 ; 2. Itaya et al., (2007). J. Virol. 81: 2980-2994 ;
3. St-Pierre et al., (2009). Virology 383: 178-182 ; 4. Flores et al. (2006). Mol. Plant Pathol. 7: 209-221.

Molecular characterization and variability analysis of Apple scar skin viroid

Walia, Yashika, Kumar, Yogesh, Rana, Tanuja, Hallan, Vipin, Ram, Raja, Zaidi, A.A
Plant Virology Lab, Institute of Himalayan Bioresource Technology, CSIR, Palampur, 176061, Himachal Pradesh, India
Contact: yashikawalia@gmail.com

Apple scar skin viroid (ASSVd) infection is a major limitation to apple fruit quality and causes huge economic losses. In orchard surveys of apple growing regions of Himachal Pradesh (H.P), ASSVd was detected and molecularly characterized from apple plants showing dappling symptoms. Ten clones were sequenced out of which seven new sequence variables of ASSVd were found. The clones showed significant sequence variability (94-100%) with each other. Variability was more common in the pathogenic domain of the viroid genome. Four of the clones were 330 nucleotides (nt) long and the other six that of 331 nt. Multiple alignment and phylogenetic analysis of the present isolates was carried out taking different ASSVd sequences available in EMBL database reported from different countries. In phylogenetic study, the present clones showed close affinity with some Chinese and Korean isolates. The study reports seven new variables of ASSVd and also gives a first molecular evidence of a viroid infection (ASSVd) in apple from India. Infectious clones of the ASSVd were constructed for in vitro mutagenic studies.

The molecular characterization of hop stunt viroid isolates associated to dapple fruit and fruit rugosity in plum seedlings suggests a possible role of the breeding in viroid dissemination

Luigi, Marta[1], Faggioli, Francesco[1], Barba, Marina[1], Giunchedi, Luciano[2]
[1]CRA-Centro di Ricerca per la Patologia Vegetale, 00156 Rome, Italy
[2]Dipartimento di Scienze e Tecnologie Agroambientali - Università di Bologna
Contact: francesco.faggioli@entecra.it

Hop stunt viroid (HSVd) has been found in a wide range of hosts where the infection appears to be latent, whereas in others (hop, cucumber, citrus, Japanese plum, peach) it is frequently pathogenic. In this work, the presence of HSVd has been found to be associated to symptoms of dapple fruit and occasionally fruit rugosity in plum seedlings 4 years old obtained from cross breeding. In particular, the plum samples have been analyzed for the presence of viroids (HSVd and PLMVd) and viruses (PPV, ACLSV, ApLV, PDV, PNRSV, ApMV). HSVd was found in all symptomatic samples, whereas no other viruses or viroids were found in the analyzed samples with the exception of ACLSV, rarely and sporadically detected. All the HSVd isolates have been cloned and sequenced and a high percentage of homology (98-100% with the exception of one clone obtained from the isolate F20) has been obtained among them, making it possible to hypothesize a potential unique origin of the infection. For this purpose, the 'Black Sunrise' and 'Black Glow' plum trees, the cultivars used in the breeding as pollen donors, have been analyzed. The results showed the presence of HSVd in parental plants and the obtained sequences resulted similar (95-100 %) to the isolates found in the seedlings, suggesting a possible role of the breeding in the dissemination of the viroid. In the next months, pollen from infected parental plants will be collect and analyzed to confirm the possible presence of HSVd. Moreover, these results confirm the hypothesis that HSVd could be the cause of the dapple fruit and fruit rugosity diseases.

The value technical assistance of M. Mantovani and of L. Martini is gratefully Acknowledged.

Two novel variants of hop stunt viroid associated with yellow corky vein disease of sweet orange and split bark disorder of sweet lime in Iran

Bagherian, Seyed Ali Akbar, Izadpanah, K.
College of Agriculture, Shiraz Univ., Shiraz, Iran
Contact: izadpana@shirazu.ac.ir

Yellow corky vein was reported as a graft-transmissible disease of lime in India. It was attributed to infection by Hop stunt viroid (HSVd)and citrus exocortis viroid (CEVd). Recently similar symptoms have been observed in Washington navel orange in Jahrom and Darab in the Fars province of Iran. It is characterized by yellowing and suberization of veins followed by tree decline. In the case of sweet lime, split bark is a disorder of increasing importance in the Fars province. It is characterized by cracks in the bark of the main stem which may spread to branches of the tree. Since these symptoms resembled those of certain viroids, a study was undertaken to determine possible association of viroids with the disorders. Reverse transcription polymerase chain reaction (RT-PCR) followed by cloning and sequencing of PCR products and dot-blot hybridization were used to identify the viroids associated with the diseases. Comparison of molecular properties (nucleotide composition, primary and secondary structures, molecular weights, phylogenetic relationships, percent nucleotide similarity and difference and restriction sites) of viroid variants were carried out. It was found that, a novel variant of hop stunt viroid (HSVd-sycv) was associated with yellow corky vein disease of Washington navel and another new variant (HSVd-sb) with split bark disorder of sweet lime. No other viroids were constantly detected. HSVdsycv was closely related to noncachexia variant of hop stunt viroid (HSVd-cit) but only with 93.7% homology with HSVd-lycv. It differed in a single nucleotide from HSVd-cit, in the variable domain in the so-called "cachexia expression motif". HSVd-sb had only 94.8% homology with a noncachexia variant of hop stunt viroid (CVd-IIa-117) which causes mild bark-cracking symptoms on St. Michael orange-Wakayama rootstocks. According to the performed molecular comparisons, HSVd-sb differed from CVd-IIa-117 in "cachexia expression motif" and probably severe cracks induced by HSVd-sb occurred because of variation in this motif.