PREMIO SCARAMUZZI

MOLECULAR MECHANISMS INVOLVED IN THE PATHOGENESIS OF BEET SOIL-BORNE VIRUSES

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The genus *Benyvirus* includes the most important and widespread sugar beet viruses transmitted through the soil by the plasmodiophorid *Polymyxa betae*. *Beet necrotic yellow vein virus* (BNYVV) causes abnormal rootlet proliferation known as Rhizomania and has a worldwide distribution, whereas *Beet soil-borne mosaic virus* (BSBMV) is present only in the USA. Sequence analysis has shown that these viruses have a similar genomic organization but are sufficiently different at the molecular level to be classified as separate species.

Field isolates of benyviruses usually possess four RNA species. RNA-1 contains a single long ORF encoding the RNA-dependent RNA polymerase (RdRp) and helicase. RNA-2 contains six ORFs coding for the capsid protein (CP), a readthrough protein (RT), the triple gene block proteins (TGB) required for cell-to-cell movement and for a small 14 kDa protein (p14). RNA-3 is involved in disease symptom induction and long distance movement, whereas RNA-4 is essential for transmission by the vector *P. betae*.

Experimentally, BSBMV RNA-3 and -4 can be trans-replicated and transcapsidated by a BNYVV helper strain (RNA-1 and -2), allowing long distance movement within the host and transmission by the vector. However, although in the USA these two viruses frequently coexist in the same plant, no natural chimeric forms have been detected so far.

The main objectives of this study were: (i) develop agroinfectious clones of BNYVV and BSBMV; (ii) test the viability and behaviour of chimeric isolates obtained by exchanging RNA-1 and 2; (iii) characterize Benyvirus p14.

Full-length cDNA clones of BNYVV and BSBMV RNAs as well as viral replicons expressing different proteins were introduced in the pJL89 binary vector downstream of the *Cauliflower mosaic virus* 35S promoter. These plasmids were transferred by electroporation into *A. tumefaciens* and agroclones were used to agroinfect *Nicotiana benthamiana* and *Beta macrocarpa* plants, in which they induced local and systemic symptoms. Viral RNAs and proteins were detected by Northern and Western blot in agroinfected plants, in which the presence of rod-shaped particles was also observed. The capability of our clones to generate viral RNAs able to complete the viral cycle in *B. macrocarpa*, from replication to transmission through the vector, was thus demonstrated.

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Agroclones, as well as infectious transcripts, were used to investigate viral chimeras. To this aim, *Chenopodium quinoa* and *N. benthamiana* plants were inoculated with different combinations denoted Stras12 (BNYVV RNA-1 and -2, control), Bo12 (BSBMV RNA-1 and -2, control), BoStras12 (BSBMV RNA-1 and BNYVV RNA-2) and StrasBo12 (BNYVV RNA-1 and BSBMV RNA-2). Stras12, Bo12 and StrasBo12 induced chlorotic lesions, while the lesions elicited by BoStras12 were necrotic, consequent to the hypersensitive response of the hosts. The necrosis disappeared when a replicon expressing BSBMV p14 was added to the inoculum. The possible interaction between BSBMV p14 and RNA-1 suggested by these results requires further investigations.

A study of the properties of BNYVV and BSBMV p14s started testing their ability to suppress the PTGS through agroinfiltration of *N. benthamiana* plants (line 16C) constitutively expressing the Green Fluorescent Protein (GFP) transgene. *N. benthamiana* 16C plants challenged with the GFP silencing trigger and Benyvirus p14s retained the fluorescence in the infiltrated leaves whereas fluorescence disappeared in the controls. Tissue content analyses evidenced the presence of GFP mRNA and strong reduction of siRNAs, the hallmark of the RNA silencing pathway. Taken together, these results demonstrate that Benyvirus p14s are efficient silencing suppressor proteins (SSPs).

Agroinfiltration of *N. benthamiana* wild type plants with different constructs encoding the GFP target, a hairpin GFFG trigger and SSPs have been carried out in order to investigate at which level the Benyviruses p14s interfere in the post-transcriptional gene silencing pathway. Results showed a normal amount of primary siRNA and a reduced amount of secondary siRNA suggesting that the p14s act downstream of the Dicer proteins without interfering with the transitivity.

Site-directed mutagenesis demonstrated that both zinc-finger and NoLS basic residues, but not a nucleolar localization, are necessary for the silencing suppression activity. Moreover, p14s localize in the nucleolus and cytoplasm and are essential for viral long-distance movement.

Finally, the role of a non coding (nc) BNYVV RNA-3, a cleavage product of RNA-3, and its relationship with p14s, will be the subject of further studies since overproduction of ncRNAs could be a way to saturate the silencing machinery of the host, as proposed for human Adenovirus and Flavivirus.