start codon into the MP region by inverse PCR. Plasmid constructs were agroinfiltrated in N. benthamiana, pepper and broad bean plants. Infectivity and BBWV-1 titre was estimated by molecular hybridization and RT-qPCR, respectively. The role of MP2 as pathogenesis determinant was evaluated by transient expression assays in N. benthamiana plants by cloning the MP2 gene in a viral expression vector based on Potato Virus X (PVX). The ability of MP2 to suppress the PTGS was evaluated with two assays based on transient expression in N. benthamiana 16C plants and complementation with Turnip Crinkle Virus (TCV-sGFP).
Results. P35S-wt-BBWV-1 induced typical symptoms of viral infection such as stunting, severe mosaic and leaf necrosis in N. benthamiana and severe or mild mosaic in broad bean and pepper plants, respectively, whereas P35S-ΔMP2-BBWV-1 induced only slight stunting and mosaic symptoms. This reduction of plant symptom severity was not correlated with virus titre. MP2 expression through PVX induced systemic necrosis in N. benthamiana plants whereas TCV-GFP complementation and MP2 transient expression in N. benthamiana 16c plants showed that this viral protein had PTGS suppressor activity.
Conclusions. Results presented here evidenced that the MP2 protein is a pathogenesis determinant and a PTGS suppressor and suggested that MP2 had not effect in on the virus movement or replication.

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MIXED INFECTION OFWATERMELON MOSAIC VIRUS AND CUCURBIT YELLOW STUNTING DISORDER VIRUS IN MELON PLANTS: EFFECTS ON VIRAL LOAD DYNAMICS

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Viral diseases in plants can be caused by single infections, or by combinations of viruses that occasionally result in synergistic interactions. A classical and well studiedexample is the sweet potato virus disease, caused by the mixed infection of a crinivirus and a potyvirus, in which the titersof the second partner are being boosted by the presence of the first. On the contrary, a similar combination of the crinivirusCucurbit yellow stunting disease virus (CYSDV) with different potyvirusesin cucurbitaceous hosts have been described to result in increased viral load of the crinivirus partner. This variability in responses might derive from the different host plants, or from peculiarities of the viruses involved, or from combined effects in each one of the pathosystems.
To characterize in depth the mixed infections by crinivirus and potyvirus in cucurbits, we focused in the pathosystem of CYSDV and the potyvirusWatermelon mosaic virus (WMV) infecting susceptible melon plants (Cucumismelo L., cv. "Piel de sapo").As a first objective, we decided to determine the dynamics of co-infection under controlled conditions.Viral loads were quantified in infected tissues for each one the viruses throughqRT-PCR analysis at different time points, sampling the plants up to 60 days after infection (dpi).In our plant growth conditions, the double infected plants presented reduced levels of WMV compared to the individually infected controls. The viral load of CYSDV showed a remarkable but transient increase in the co-infected plants, peaking at 24 dpi, and going down later, although maintaining levels above those reached in the case of single infections. Furthermore, super-infected plants in which the viruses were inoculated sequentially are being analysed.Preliminary results suggested that late arrival of CYSDV caused less alterations of the WMV levels that the simultaneous co-infection. In the case ofthe superinfection by WMV, a transient rise of CYSDV accumulation was also observed. To complete the work, analysis of the gene products with presumed RNA silencing suppression activity, HCPro in the case of WMV, and P25 in the case of CYSDV,are being performed by standard in vivo assays, comparing their activities alone or in combination with other gene products of the heterologous partner.

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FAST DETECTION OF SOUTHERN TOMATO VIRUS BY ONE-STEP TRANSCRIPTION LOOP-MEDIATED ISOTHERMAL AMPLIFICATION (RT-LAMP)

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Interest and Objective. Southern tomato virus (STV) is a member of the genus Amalgavirus (family Amalgaviridae) with a double stranded RNA genome (dsRNA) of about 3.5 kb containing two open reading frames (ORFs) which are partially overlapped. The 5'-proximal ORF1 encodes for a putative coat protein (CP) and the ORF2 contains typical motifs of an RNA-dependant RNA-polymerase (RdRp). STV has been detected in different tomato (Solanum lycopersicum) varieties showing symptoms of stunting, fruit discoloration, and reduced fruit size from different production areas (USA, Mexico, China, Bangladesh, Italy, France and Spain). The role played by STV on development of symptoms remains unclear since STV is frequently detected on mixed infections with other viruses such as Pepino mosaic virus (PepMoV) and Tomato mosaic virus (ToMV) and sometimes in asymptomatic tomato plants. STV is seed transmitted at rates higher than 70% but "horizontal" transmission by vectors is unknown. A specific and sensitive method for STV detection is essential to improve our understanding on virus symptoms development and transmission, as well as implement sanitation programs.
Material and Methods: One-step reverse-transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed for the detection of STV from total RNA or sap extracts (obtained just by grinding in buffer) by using a set of three primers pairs corresponding to the STV putative CP gene. Amplification products were visualized by gel electrophoresis or direct staining in the tube.
Results: The sensitivity of RT-LAMP was identical to that of the conventional RT-PCR but less affected by the presence of polymerase inhibitors. RT-LAMP detected STV from RNA or sap extracts obtained from field tomato plants whereas conventional RT-PCR only detected STV in RNA extracts. RT-LAMP detected STV in different tomato tissues, i.e. leaves, roots, fruits and seeds.
Conclusion: Results of this work show that RT-LAMP is a specific, rapid and cheap procedure to detect STV and it could be implemented on field surveys and sanitation programs.