Poster 32

Tomato spotted wilt virus genes expressed in antisense orientation and their ability to control virus progression in *Nicotiana benthamiana*

V. Pires[1], S. A. Dandlen[2], G. Nolasco[2], M. R. Félix[2], P. Materatski[2], C. Varanda[2], N. Marques[1]

[1]CEOT Centro de Eletrónica, Optoeletrónica e Telecomunicações, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro.
[2]MED Instituto Mediterrâneo para a Agricultura, Ambiente e Desenvolvimento, Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal.

Email: nmarques@ualg.pt

Tomato spotted wilt virus (TSWV) is a member of the *Tospoviridae* family and is ranked among the top ten economically important viruses in the world. The genome of TSWV consists of three linear negative-sense or ambisense RNA segments, denoted as segments L, M and S. Segment S RNA encodes the silencing suppressor NSs, and the nucleocapsid protein N. Segment M RNA encodes the cell-to-cell movement protein NSm and two glycoproteins (Gn and Gc). The TSWV is mainly transmitted by thrips and can infect a wide range of hosts, including tomatoes, an economically important crop. Thus, control measures need to be implemented to reduce the damage caused by this virus. In the present work, a TSWV isolate from *Nicotiana rustica* was acquired through Leibniz DSMZ Institute. The expression of antisense transcripts of the N, NSs and M viral genes in leaves of *Nicotiana benthamiana* was assayed for its ability to silence virus progression. For this, each construct in the binary vector pK7WG2 was co-agroinfiltrated with pK7WG2-GFP into *N. benthamiana* leaves, followed by inoculation with TSWV after 48h. Inoculated leaves were harvested 5 days after agroinfiltration for RNA extraction. The ability of antisense transcripts, expressed throughout the plant to control TSWV progression was also assayed using the *Tobacco rattle virus* viral vector (pTRV). In this case, partial sequences of the above-mentioned genes cloned into pTRV2 were expressed as antisense transcripts. New leaves were harvested 10 days after agroinfiltration of the pTRV viral vector. TSWV detection and absolute quantification was performed by a TaqMan real-time RT-PCR assay. Inoculated leaves with TSWV alone and new leaves showed a low viral titer, a result that indicates host plant resistance to TSWV infection. In both assays, TSWV accumulation was higher for constructs carrying the N or the NSs sequences than with M sequences. These studies allowed us to conclude that M gene transcripts in the antisense orientation greatly limit virus progression in *N. benthamiana* plants.

This research was financially supported by National Funds through FCT under the projects PTDC/ASP-PLA/28266/2017 and PTDC/ASP-PLA/28263/2017, and from the Investment and Structural European Funds (FEEI), by Portugal 2020 – Alentejo Regional Operational Programme (CRESC 2020) through projects ALT20-03-0145-FEDER-028266 and ALT20-03-0145-FEDER-028263. This study was also supported by FCT through Project UIDB/04326/2020 CEOT BASE.