Development of OMMV as a VIGS vector for plant protection

Varanda, C.M.R.[1], Félix, M.R. [2], Materatski, P.[1], Campos, M.D.[1], Patanita, M.[1], Marques, N.[3], Clara, M.I.[2], Nolasco, G.[4]

[1]MED – Mediterranean Institute for Agriculture, Environment and Development, Instituto de Investigação e Formação Avançada, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [2]MED – Mediterranean Institute for Agriculture, Environment and Development & Departamento de Fitotecnia, Escola de Ciências e Tecnologia, Universidade de Évora, Pólo da Mitra, Ap. 94, 7006-554 Évora, Portugal; [3]Departamento de Ciências Biológicas e Bioengenharia da Universidade do Algarve, Laboratório de Virologia, Faro, Portugal; [4]MeditBio, Universidade do Algarve, Campus de Gambelas 8005-139, Faro, Portugal; Email: carlavaranda@uevora.pt

Background Viruses are responsible for several important plant diseases, however, they have also been used in biotechnology with different purposes. Virus induced gene silencing (VIGS) allows specific silencing of foreign genes that can be inserted in a viral vector and then inoculated in plants. When a sequence of a foreign gene is introduced in a VIGS vector, the plant infected with this vector will be signalized to target that foreign viral RNA and destroy it. In addition, plant defense mechanisms will also target and destroy any homologous RNA, even if it is constitutively expressed by the plant. The VIGS approach can also be used for plant protection purposes; for example, if a fragment of the genome of a pathogenic virus is inserted in a VIGS vector, the plant will destroy it and become protected against a possible further infection of that virus. Several plant viruses have been used as VIGS vectors however, their large genomes, their difficult manipulation and the reduced number of hosts they infect restrain their use as vectors. The Alphanecrovirus *Olive mild mosaic virus* (OMMV) has characteristics that place it as a very promising vector tool. Its small genome makes it easy to manipulate, and it causes only mild systemic symptoms in a wide range of crops, which will facilitate their manipulation into symptomless constructs and allow its application to a high number of plants.

Methods An OMMV-based vector is being developed under the project TOMVIRPROTECT. An infectious OMMV full length clone, available at our laboratory (pUC_OMMVFL5), was manipulated to obtain a symptomless OMMV strain. A single mutation at nt18 (C to A) of the OMMV p6, a silencing suppressor protein, formed a STOP codon at this region, resulting in the gene knockout. OMMVp6mutant was then manipulated to carry the GFP reporter gene in its 5' and 3' ends and in both sense and antisense directions to test silencing efficiency.

Results The new mutated OMMV genome (OMMVp6mutant) was inoculated onto *Nicotiana benthamiana* indicator plants where it caused no visible symptoms but viral accumulation levels similar to OMMV wild type, as well as a systemic presence. Relative GFP mRNA accumulation level in 16 C plants (plants constitutively expressing GFP) was the lowest when GFP was placed in the 3'end of OMMVp6mutant and in antisense direction. A multiple cloning site was introduced in this region to facilitate introduction of further fragments to be silenced.

Conclusions These transformations resulted in obtaining an efficient OMMV VIGS vector, which is intended to become available for the control of many important viral plant diseases.

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