# The dual role of Plant Viruses in CRISPR

C. Varanda[1*], M.R. Félix[2], M. D. Campos[1], M. Patanita[1], P. Materatski[1]

[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

*Email: carlavaranda@uevora.pt

Plant viruses cause devastating diseases in many agriculture systems, being a serious threat for the provision of adequate nourishment to a continuous growing population. At the present there are no chemical products that directly target the viruses, and their control rely mainly on preventive sanitary measures to reduce viral infections that, although important, have proved to be far from enough. The current most effective and sustainable solution is the use of virus-resistant varieties, which require too much work and time to obtain. In the recent years, the versatile gene editing technology known as CRISPR/Cas has simplified the engineering of crops and has successfully been used for the development of viral resistant plants. CRISPR stands for Clustered regularly interspaced short palindromic repeats and CRISPR-associated (Cas) proteins, and is based on a natural adaptive immune system that most archaeal and some bacterial species present to defend themselves against invading bacteriophages.

Plant viral resistance using CRISPR/Cas technology has been achieved either through manipulation of plant genome (plant-mediated resistance), by mutating host factors required for viral infection, or through manipulation of virus genome (virus-mediated resistance), for which CRISPR/Cas systems must specifically target and cleave viral DNA or RNA.

Viruses present an efficient machinery and comprehensive genome structure and, in a different perspective, they have been used as biotechnological tools in several areas such as medicine, materials industry and agriculture with several purposes. Due to all this potential, it is not surprising that viruses have also been used as vectors for CRISPR technology, namely to deliver CRISPR components into plants, a crucial step for the success of CRISPR technology.

Here we discuss the basic principles of CRISPR/Cas technology, with a special focus on the advances of CRISPR/Cas to engineer plant resistance against DNA and RNA viruses. We also describe several strategies for the delivery of these systems into plant cells, focusing on the advantages and disadvantages of the use of plant viruses as vectors. We conclude by discussing the constrains faced by the application of CRISPR/Cas technology in agriculture and future prospects.

This work is funded by the project "Control of olive anthracnose through gene silencing and gene expression using a plant virus vector" with the references ALT20-03-0145-FEDER-028263 and PTDC/ASP-PLA/28263/2017 and the project "Development of a new virus-based vector to control TSWV in tomato plants" with the references ALT20-03-0145-FEDER-028266 and PTDC/ASP-PLA/28266/2017, co-financed by the European Union through the European Regional Development Fund, under the ALENTEJO 2020 (Regional Operational Program of the Alentejo), ALGARVE 2020 (Regional Operational Program of the Algarve) and through the Foundation for Science and Technology, in its national component. M. Patanita is funded by Portuguese National Funds through FCT under a PhD Scholarship (SFRH/BD/145321/2019).