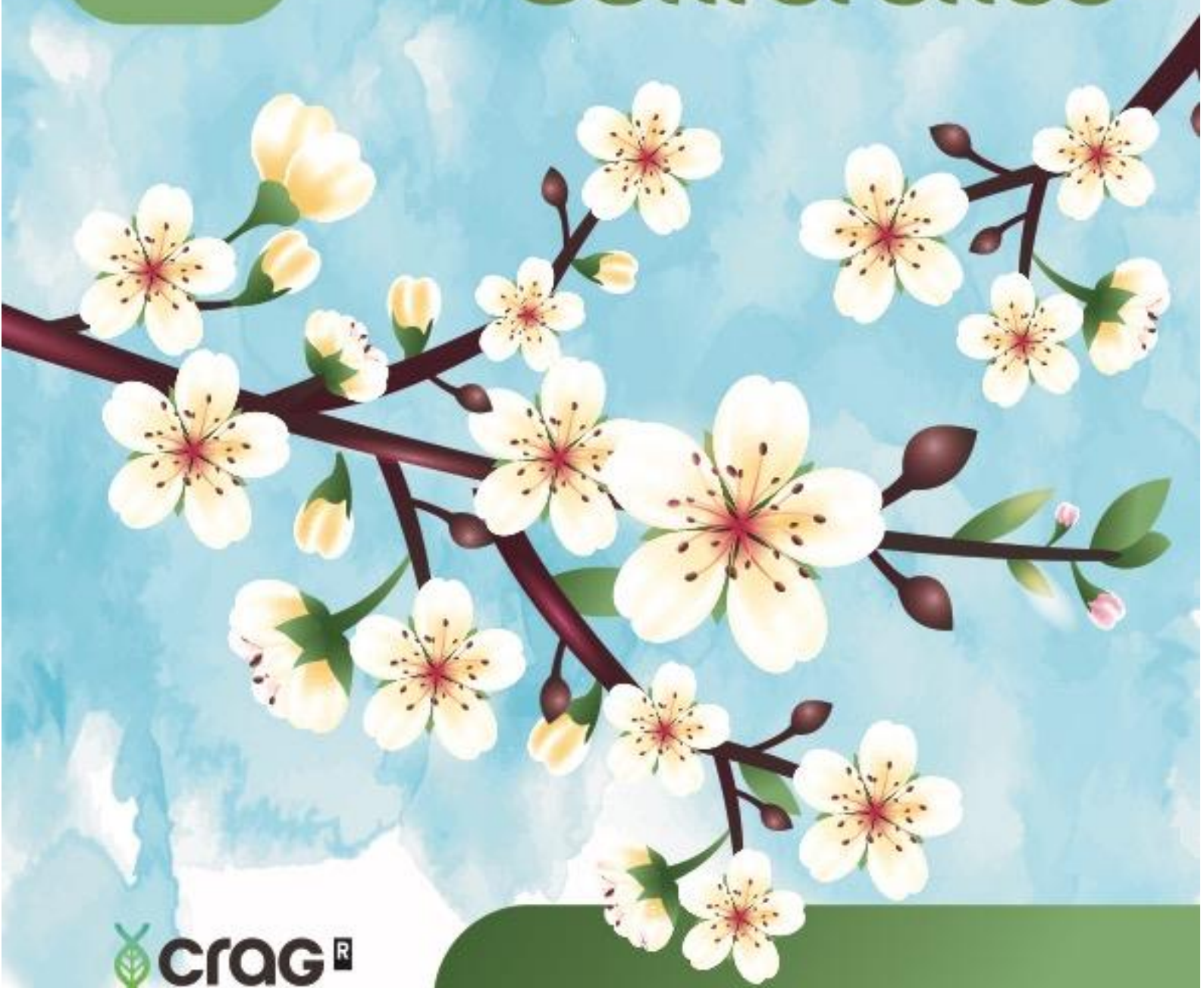


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**DUAL NBT APPROACH IN APPLE: CISGENESIS AND GENE EDITING TO IMPROVE RESISTANCE TO FIRE BLIGHT, APPLE SCAB AND POWDERY MILDEW**

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**Abstract**

Fire blight, caused by the gram-negative bacterium *Erwinia amylovora*, is a destructive necrotic disease, affecting most members of the Rosaceae family, including apple (*Malus x domestica* Borkh). Similarly, apple scab caused by *Venturia inaequalis* and powdery mildew are fungal diseases negatively impacting apple production. No sustainable management strategies are currently available for controlling these pathogens, with intensive antibiotic and fungicide treatments being the main control measures utilized during the growing season. In this context, genetic improvements focusing on susceptibility and resistance genes may offer a more durable alternative to develop cultivars with enhanced disease tolerance. The use of New Breeding Techniques (NBTs), such as CRISPR/Cas9 gene-editing and cisgenesis, hold significant potential to achieve trait improvement, especially in a regulatory landscape nowadays leaning towards exempting them from conventional GMO regulation. Using an excisable cassette, we aimed to produce two commercial apple cultivars gene-edited simultaneously in three fire blight susceptibility genes (*MdDIMP1*, *MdDIMP4a*, *MdHIPM1b*), along with a susceptibility gene for powdery mildew (*MdMLO19*). Additionally, we aimed to introduce *via* cisgenesis an apple scab resistance gene (namely, *Rvi12*). Regenerant lines were obtained for both constructs and analyzed for gene editing profile of target genes, with lines showing mutations ranging from a single to all target susceptibility genes. In addition, we evaluated *Rvi12* gene expression in cisgenic lines, as well as the copy number of inserted cassettes. Based on these results, we selected lines to conduct infection tests for fire blight, apple scab and powdery mildew. We present the transcriptional and phenotypic response to pathogen infection assays, showing that fire blight and apple scab can be noticeably reduced by simultaneously editing all susceptibility genes, together with inserting the resistance *Vb* gene *via* cisgenesis from a wild relative.