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HIGHLY EFFICIENT CRISPR/CAS9 MEDIATED GENE EDITING IN OCIMUM BASILICUM CV. FT ITALIKO TO INDUCE RESISTANCE TO PERONOSPORA BELBAHRII

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Sweet basil (*Ocimum basilicum* L.) is an important culinary herb and source of aromatic essential oils, widely cultivated around the world. *O. basilicum* is susceptible to downy mildew (DM) disease, which is caused by the obligate oomycete pathogen *Peronospora belbahrii*, that appeared in Europe in 2001 (Belbahri et al., 2005) and quickly widespread greatly reducing basil quality and market value. The short cultivation cycle and the few authorized pesticides on sweet basil made difficult the defence of the crop by chemical means. Identification of host susceptibility (S) genes provides a foundation for developing disease resistant plants through genome editing (Low et al., 2020). The S gene *DMR6* (*Downy Mildew Resistance 6*) was discovered and characterized in *Arabidopsis* (Van Damme et al., 2008): interestingly, its mutation has been shown to confer resistance to oomycetes, in *A. thaliana* (Zeilmaker et al., 2015), in *Solanum lycopersicum* (Thomazella et al., 2016), potato (Sun et al., 2016), barley (Low et al., 2020) and in sweet basil cv. Genoveser (Hasley et al., 2021) and can provide broad-spectrum resistance to different pathogens (bacteria, fusarium). This work describes a highly efficient CRISPR/Cas9 mediated gene editing system for targeted mutagenesis of *ObDMR6* gene (1260bp) for sweet basil cv. FT Italiko (the elite cultivar used to produce “Pesto Genovese DOP”) using *Agrobacterium* transformation. For this purpose, the binary vector pDirect_22c (Cermark et al., 2017) was used, optimized to create single or multiple genetic knockouts. Two target sites (gRNA) on *ObDMR6* exon 2 were identified, and two single guides were designed, to obtain targeted mutations in two points of the gene. The obtained construct was used in genetic transformation experiments mediated by *Agrobacterium rhizogenes* and *tumefaciens*. To evaluate the efficiency of the CRISPR/Cas9 system in sweet basil, genetic transformation experiment mediated by *A. rhizogenes* was performed, considering the high induction of hairy roots (HR) from all the explants tested (hypocotyls, cotyledons and leaves). 29 out of 30 HR were positive to the analysis, confirming a high rate of co-transformation (introduction of the T-DNA genes of the Ri plasmid and the pDirect 22C plasmid). Through an efficient *A. tumefaciens* mediated genetic transformation, 130 kanamycin resistant regenerated plants, from 150 cotyledonary nodes (CN) as starting explants, were obtained (Khan et al., 2015), yielding a transformation efficiency of 82%. 22 out of 26 kanamycin resistant plants were tested positive for Cas9 transgene integration (84,6%), reaching 82,3% edited plants. Experiments on evaluating *in vitro* and *in vivo* resistance to *P. belbahrii* of the edited clones are undergoing at CREA-OF laboratories.