# Enhancement of the flower longevity of petunia by CRISPR/Cas9-mediated targeted editing of ethylene biosynthesis genes

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## Abstract

The transcriptional activation of genes that encode the ethylene biosynthesis enzyme 1-aminocyclopropane-1-carboxylate oxidase (*PhACO3* and *PhACO4*) during petunia flower senescence has been reported. However, no studies have elaborately investigated their specific roles in ethylene production and flower longevity using genetic manipulation. Hence, we used the CRISPR/Cas9 system to edit the genes (*PhACO3* and/or *PhACO4*) involved in ethylene production and flower longevity in petunia cv. Mirage Rose. The use of the CRISPR/Cas9 system with a sgRNA, which was designed from exon 2 of *PhACO3*, allows for the specific editing of the genes *PhACO3* and/or *PhACO4* with high mutation frequency, consequently producing different types of zygotes. The *PhACO3* and *PhACO4*-edited lines 8 and 9 showed remarkably reduced ethylene production (approximately 2.8- to 3.0-fold in corollas and 1.5-fold in pistils) during flowering and extended flower longevity (approximately 9.5 d), while the *PhACO3*-edited bi-allelic and *PhACO4*-edited homozygous $T_0$ mutant lines (14 and 23) showed enhanced flower longevity (approximately 8.0 d) compared with 6.0 d for the WT line. This was associated with reduction of PhACO4 protein levels in *PhACO4*-edited lines, which was confirmed using Western blot analysis and Image J software. Moreover, there was no undesirable editing effect on the *PhACO1* gene. The transmission of the edited alleles to the $T_1$ generation was also observed, and ethylene production and flower longevity were identical to those of the $T_0$ mutant lines. Taken together, this study demonstrated not only the single and combined role of *PhACO3* and *PhACO4* in ethylene production in petunia flowers but also reports improvements in flower longevity by editing of the aforementioned genes using the CRISPR/Cas9 system. Therefore, our study can pave the way for the editing of homologous genes in other ornamental plants using the CRISPR/Cas9 system with a common sgRNA, thus allowing for a time- and cost-effective approach to advancing plant biology and the floricultural industry.