

Genome editing approach for silencing FLS in *Petunia × hybrida* cultivar Sophistica Blackberry

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The flavonoid pathway plays a main role in pigment formation in petunia flowers. *Petunia × hybrida* Sophistica Blackberry (cv. Blackberry) is a black cultivar with alluring flowers. It has been shown that obtaining such dark colors flavonols, with anthocyanins, play a major role and serve as co-pigments. Flavonols form stable complexes with anthocyanins, leading to more intense color. Flavonol synthase (FLS), an enzyme that converts dihydroflavonols to flavonols, is pivotal to flavonol synthesis. Dihydroflavonols also serve as substrate for dihydroflavonol 4-reductase (DFR), one of the key enzymes in the anthocyanin biosynthesis pathway. Enzyme balance is a deciding factor in color formation. To evaluate the impact of flavonols on pigment formation in cv. Blackberry, the FLS gene was silenced using CRISPR/Cas9. Transgenic plants were created by *Agrobacterium* mediated transformation. Regenerated shoots were screened by PCR for nptII and Cas9 genes. In total 12 transgenic lines were obtained. Mutation success was confirmed by cloning and sequencing of the FLS fragment. Also, very low levels of FLS gene expression was detected in comparison to wild types. After flower development there was, however, no discernable change in the flower color in transgenic lines. Obtained results suggest that silencing of the FLS gene was successful, but the impact of this gene on flower color in this species is slight. Lack of visible change in flower color could also be explained by a higher availability of the substrate for DFR, which can result in higher production of anthocyanins. Further analysis of pigment composition in transgenic flowers is needed to confirm this. The research performed represents one of the first successful attempts at genome editing on petunia. The fact that it was performed on non-standard, commercial line, adds commercial relevance to this work.