Stimulation of anthocyanin pigmentation in *Solanum lycopersicum* and *Nicotiana tabacum* using CRISPR/Cas9 technology

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Key message. The study is aimed at developing methods for creating plants rich in anthocyanins using the CRISPR/Cas9 genome editing technology to improve consumer and agricultural properties.

Keywords: Plant genome editing, CRISPR/Cas9, *Agrobacterium*, anthocyanins

Anthocyanin pigments are flavonoids that have a wide spectrum of biological activity. There is evidence that plants rich in anthocyanins have an advantage in the context of agriculture and human nutrition. In addition, anthocyanin pigmentation can be used as a selective trait for genetically engineered plants, since a change in the color of tissues can be observed with the naked eye. The aim of the study was to modify the plant genome to assess the possibility to create varieties with a high content of anthocyanins using the CRISPR/Cas9 method. The genetic construct pTC223 [1] was intended to introduce a strong constitutive 35S promoter in front of the *ANT1* gene of *Solanum lycopersicum*, which encodes one of the MYB transcription factors. Nevertheless, since tobacco and tomato are related plants, we suggested that the well-known model plant *Nicotiana tabacum* can be transformed with the same construct. An analysis of the literature describing the principles of guide RNA selection [2] and bioinformatic search suggested that the tobacco genome also has a target site in the region of the *CHS6* chalcone synthase gene, which also plays an important role in flavonoid biosynthesis. The plasmid was delivered to tomato plants (Micro-Tom cultivar) and tobacco (Petit Havana cultivar) by *in vitro* and *in planta* agrobacterial transformation [3] with *A. rhizogenes* and *A. tumefaciens*. The number of anthocyanins was determined by pH differential spectrophotometry. After *in vitro* transformation of tomato using *A. tumefaciens*, only a single plant regenerated. It was extremely rich with anthocyanins, as described in original work [1]. During the transformation of tobacco by the same method, brown and pink callus tissues were formed, but all the regenerants did not have anthocyanin pigmentation. As a result of *in vitro* transformation using *A. rhizogenes*, 10 lines of hairy roots of tomato and 28 lines of hairy roots of tobacco were obtained. 73% of the lines contained the target insert (kanamycin resistance gene and 35S promoter), with no agrobacterial contamination. In three lines of tobacco roots, the mass concentration of anthocyanins was 91.8, 5 and 4.2 g/100 g. The remaining tobacco root lines, although brown in color, appeared to accumulate other polyphenolic compounds. Hairy roots of tomato did not differ in color from control and did not contain more anthocyanins. Seedlings from the plants that were transformed *in planta* [3], did not demonstrate any difference in pigmentation from control plants. Thus, genomic editing can be used primarily to study the role of various plant genes, which can presumably affect the economically valuable traits of plants.

The research was carried out according to the state assignment (topic No. AAAA-A16-116020350028-4).

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