

VITAMIN D BIOFORTIFICATION IN SOLANACEOUS SPECIES BY USING CRISPR/CAS9-MEDIATED GENOME EDITING

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Vitamin D is a secosteroid biologically converted in human body to products with steroid hormone bioactivities, which function in signaling in many organs. Consequently, deficiencies in vitamin D impact immune function and inflammation and are associated with increased risk of micronutrient deficiencies, altered bone metabolism and development, neurocognitive decline, Parkinson's disease, depression, and dementia. Approximately one billion people worldwide suffer from vitamin D insufficiency, and about 40% of European population has been reported with an estimated average vitamin D intake below the RDA (Recommended Dietary Allowance). Vitamin D can be synthesized by humans from 7-dehydrocholesterol (7-DHC), also known as provitamin D₃, following exposure of skin to ultraviolet B (UVB) light, or by dietary intake, mostly deriving from animal sources, such as fatty fish, cod liver oil, eggs and dairy products.

We have previously reported about a biofortified tomato line by using clustered regularly interspaced short palindromic repeats–CRISPR-associated protein 9 (CRISPR–Cas9) genome editing to knock-out the 7-dehydrocholesterol reductase (*SL7-DR2*), a gene involved in the cholesterol and steroidal glycoalkaloid (SGA) biosynthesis in tomato to increase 7-DHC levels. We recovered homozygous-knockout lines lacking the transfer DNA (T-DNA) carrying the Cas9 gene, and fruit and leaves were analysed for 7-DHC content as well as levels of other phytosterols, cholesterol and SGAs using

liquid chromatography–mass spectrometry (LC–MS).

Compared to the wild-type plants, 7-DHC levels in leaves and fruit resulted increased in the new tomato lines (from 0.3 $\mu\text{g g}^{-1}$ dry weight to 0.2 $\mu\text{g g}^{-1}$ dry weight in green and red fruit, respectively) reflecting the reduced content of 7-DHC in the fruit compared with leaves. In this way, we estimated that a single medium size tomato from the edited line could provide approximately 30% if green or 20% if red of the recommended daily allowance (RDA).

More recently, we extended our work by applying CRISPR/Cas9-mediated genome editing for vitamin D biofortification to other Solanaceous species, such as pepper. Specific sgRNAs on the 7-dehydrocholesterol reductase 2 gene were validated in a pepper hairy roots transient expression system. About the 30% of samples collected from hairy roots expression system were correctly edited with a precise deletion within the *7-DR2* gene. These results indicate that the CRISPR/Cas9 system was effective to target the pepper *7-DR2* gene sequence and provide a new perspective for stable transformation to generate and select new edited pepper lines.