



The Use of Modern Biotechnology Approaches to Improve Citrus Fruit Quality

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Body

Additional Categories: Plants: Trees|forestry, Technology: Sequencing, Plants and Animals: Others, such as Functional Genomics, Plants and Animals: Population, Conservation and Ecological Genomics

Fruit species are suffering of the difficulties related to classical breeding techniques, in terms of financial commitment, land resources availability and long generation times. The most recent 'new genomic techniques' (NGTs) reduce the time required to obtain new varieties as well as to precisely target specific DNA sequences without altering any other trait.

We used the NGTs to improve two important traits for consumer needs consisting in the combination of anthocyanins and lycopene in a unique fruit, and in reducing seeds content in mandarin and mandarin-like varieties. For the first aim, we introduced *Ruby* (the Myb-like transcription factor known for regulating the anthocyanin synthesis in citrus fruits) as a cisgene in lycopene-rich accessions, using a vector containing a FLP/FRT system to obtaining marker-free cisgenic plants. We are optimizing the heat shock treatment so to excise the entire cassette containing the kanamycin resistance. On the other hand, we performed the knockout of β -LCY2, through a dual-single guide (sgRNA) approach using the GoldenBraid 3.0 technology, to produce loss-of-function mutants able to induce lycopene accumulation in anthocyanin-rich sweet oranges. The amplicons sequencing and the CRISPResso tool confirmed the presence of several editing events.

On the second aim, seedlessness in citrus is a complex trait under the control of several biological processes. So far, any candidate genes that can be managed to produce seedless varieties. Therefore, we decided to edit *Iku1*, the homologous of *Arabidopsis*, which loss of function mutations cause a decrease in seed size. Two sgRNAs were designed to knock-out *Iku1* in 'Carrizo' citrange and in sweet orange. Several plantlets with different editing events have been produced.

With the aim to speed-up the fruits production so to see the effects of the cisgenic and genome editing strategies we approached, we also produced a vector containing the Cas9 and the sgRNA in order to induce the knockout of *TFL1*, the gene responsible of the precocious flowering. Our final purpose is to producing, in the next future, early flowering citrus plants producing highly healthy fruits.

Our work represents the unique examples in which NGTs, so far, were used to improve the citrus fruits quality.

Sessions



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Saturday, Jan 14 10:19 AM

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