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GENOME EDITING APPLIED TO CITRUS TO INDUCE SEEDLESSNESS

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In the framework of "*CITRUS Citrus improvement by sustainable biotechnologies*" project (part of the wider BIOTECH project, funded by MIPAAF), new plant breeding techniques have been applied to citrus in order to improve selected agronomic traits. One of the objectives consists in the use of the genome editing to try to produce seedlessness mandarins, particularly requested by consumers, protecting at the meantime the high quality and the traditional background of local varieties. In particular, a *genome editing* approach is being used to reduce seed presence in model species and in mandarin and mandarin-like varieties. The target gene considered is *iku1*, involved in the regulation of seed size, and for which a mutation, described in *Arabidopsis*, prevents the development of the endosperm at the syncytial stage. The construct *pIKU-editing_GB* has been assembled using GoldenBraid technology and has been validated through enzymatic digestion and sequencing. The plasmid vector contains 2 RNA guides (*sgIKU10 and sgIKU4*), designed in order to create a 350 bp deletion of *iku1* gene and/or deletions at the cut point, and the cassettes for *Cas9* and for *nptII* genes. The plasmid was introduced in EHA105 strain of *Agrobacterium tumefaciens* through electroporation and used for transformation of two model genotypes (Carrizo citrange and Duncan grapefruit). Also, transformation of two mandarin varieties with good fruit quality but high seed content (Tardivo di Ciaculli and Avana) is in progress. Internodes from nucellar seedlings were infected with Agrobacterium and subjected to coculture and cultivation onto selective media, till regeneration. Shoots obtained from Duncan grapefruit explants were micro-grafted *in vitro* onto Troyer citrange seedlings, while the Carrizo ones were self-rooted. Putative transgenic shoots were tested through PCR for the presence of *Cas9* and *nptII* genes. So far, 48 PCR positive Carrizo citrange and 6 Duncan grapefruit shoots have been obtained and recovered. Sequencing analysis of the *iku1* region considered for the guide design is in progress in order to ascertain mutation and/or deletion typology obtained. In addition, a PCR-mutagenesis approach based on the base editing technology is currently applied to the target gene *iku1* to induce a modification of just one base, causing a premature stop codon. The validation of the construct is actually in progress.