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Poster Communication Abstract – 7.31

ADVANCEMENTS IN THE USE OF NEW BREEDING TECHNOLOGIES TO IMPROVE CITRUS FRUIT QUALITY, REDUCE THE FLOWERING PERIOD AND INTRODUCE THE RESISTANCE TO PLANT DISEASES

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New Breeding Technologies (NBTs) represent an efficient alternative to conventional breeding, although several drawbacks can limit its use mainly in tree fruit crops. The principle obstacles are represented by the poor knowledge on gene controlling the character to improve, the capacity to regenerate the variety of interest, the long juvenility that in citrus takes around ten years to produce fruits. Furthermore, the selection of modified shoots, generally performed through the use of the antibiotic kanamycin, causes a lot of limitations in the framework of current regulation 2001/18/CE which recognizes NBTs products as genetically modified organisms.

At CREA Research Centre for Olive Fruit and Citrus Crops of Acireale (Italy) many efforts have been dedicated to optimize and adapt the regeneration protocols to several anthocyanin- and lycopene-rich citrus varieties (never evaluated before) belonging to sweet orange (*Citrus sinensis* (L.) Osbeck) and grapefruit (*C. paradisi*) species, in addition to seedy mandarins and other citrus varieties, starting from different explant types (i.e. internodes, cotyledons, callus from non-developed seeds). The optimization of regeneration and transformation protocols in term of medium composition, explants to transform and mode have been considered essential and strategic to apply the cisgenesis (of *Ruby*, the causative elements for the anthocyanin accumulation in citrus fruits) and the genome editing (of *b-LCY2*, converting the lycopene in b-carotene; of *Iku1* reducing the seed dimensions) with the final aim to improve, for the first time, citrus fruit

quality. This is because we were interested to conjugate anthocyanins and lycopene in a single fruit and to produce citrus seedless varieties. Moreover, we developed and optimized two different strategies to produce marker-free plants: the first uses the FRT/Flp recombinase system which heat-shock activates the excision of the cassette containing the *nptII*; the second replaces the kanamycin resistance with *VvMYBA1* as visual marker for anthocyanins accumulation, allowing to individuate mutated explants precociously, to separate chimeric shoots, and to observe the purple pigmentation in the leaves longly. Finally, a CRISPR/Cas9 was also used to speed up the flowering using a unique single guide RNA able to intercept two different *Centroradialis-like* genes simultaneously, accelerating as much as possible the production of fruits with improved traits; the phenotyping of early flowering plants is ongoing.

The expertise developed in optimizing NBTs to improve fruit quality traits represented an essential prerequisite that is allowing us to take advantage of genomes availability (such as that of *Eremocitrus glauca*, a resistant species to Huanglongbing, HLB) and knowledge of candidate genes/loci to dedicate our energy to introduce the resistance to some of the most important worldwide and local citrus diseases, such as HLB and Alternaria rot.