

## **COLOURIMETRIC, GENETIC, TRANSCRIPTOMIC AND METABOLOMIC APPROACHES ON FOUR LYCOPENE-RICH CITRUS VARIETIES FOR BROAD APPLICATIONS IN BIOTECHNOLOGY**

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*Citrus* is one of the most important fruit crops, whose fruits are rich in antioxidant compounds. Among them, carotenoids (lycopene included) and flavonoids (such as anthocyanins) are essential for human health because they protect against many diseases; they prevent ROS accumulation and may be toxic to cancer cells. Carotenoids are yellow, orange and pink-red pigments characterizing fruits and vegetables. In citrus, their composition varies in species and fruit tissues. The human body cannot synthesize carotenoids and flavonoids, therefore, they must be consumed through diet. Even though carotenoids are essential for human health, very few chemical, genetic and nutraceutical studies have been performed on citrus. We are characterizing lycopene-rich varieties belonging to sweet orange, grapefruit and pummelo, focusing on pulp, albedo and peel of fruits collected in seven samplings during the developmental stage until maturity. The colourimetric analysis of citrus fruits showed differences, so we expect an additional amount and type of carotenoids/lycopene. We are deepening this statement by chemical analysis through HPLC-PDA on the three main samplings chosen basing on the colourimetric data. We performed the RNAseq for three varieties ('Vaniglia Biondo' and 'Nucellar Cara Cara' sweet oranges, and 'Duncan' grapefruit) and the respective lycopene-rich mutants ('Vaniglia Sanguigno' and 'Cara Cara', and 'Star Ruby'). The RNA

extracted from fruit flesh at the breaking stage was sequenced by the Novaseq Illumina platform. The sweet orange genome v3.0 was used as reference; 47 genes resulting from differential expression analysis are shared among the three comparisons ('Vaniglia Biondo' vs. 'Vaniglia Sanguigno', 'Nucellar Cara Cara' vs. 'Cara Cara', 'Duncan' vs. 'Star Ruby'). The variant discovery analysis was carried out on transcriptomic data, showing mutations with a high impact in all the mutants. The grapefruits turned out the most mutated, which was foreseeable by the 129 years of clonal selection separating 'Duncan' from its mutant 'Star Ruby'. The differentially expressed genes deduced after the RNA sequencing will be evaluated by Real-time PCR in pulp, albedo and peel tissues of all the samplings for the varieties described above, plus 'Webber' (white) and 'Da Seme' (pink) pummelo varieties. Moreover, the mutations detected in the transcriptome of all the varieties will be evaluated by Sanger sequencing. This is the first time that these citrus lycopene-rich and related white mutants are deeply characterized. The discovery of genes differentiating mutants represents a prerequisite to the use of genome editing and cisgenesis aimed at conjugating anthocyanins and lycopene into a unique healthy fruit. Evaluating carotenoids/lycopene in albedo (a waste tissue) could lead to that in the pharmaceutical industry. In fact, it could allow many easy extracts to be evaluated on human cell lines or animal tests for further pharmaceutical applications.