

## **A TRANSCRIPTOMIC APPROACH ON A HETEROZYGOUS REFERENCE GENOME UNRAVELS THE APTITUDE OF CITRUS FRUITS FOR IV GAMMA USES**

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Ready-to-eat (RTE) products are gaining a large food market in Italy, where they are commercialized as “IV Gamma”. Several factors concur in the success of these products and the customer’s choice. Citrus is a natural source of several compounds essential for human health; most of these are strongly coloured by carotenoids and anthocyanins, which consumers recognize as anti-ageing factors. Adding these fruits, such as anthocyanins-rich sweet oranges and pigmented mandarin-like hybrids, to an RTE salad could enhance its nutraceutical quality, making it more appreciated by consumers. The accessions belonging to the abovementioned varietal groups are indirect complex interspecific hybrids among two ancestral species, pummelo and wild mandarin. Thanks to the long-read sequencing technology more precise and reliable genomes are released, resolving the genome to a haplotype level, permitting it to obtain maternal and paternal assembly. Recently the first heterozygous genome of sweet orange consisting of two assemblies (pummelo and mandarin) has been released (Wu et al., 2023) solving the bias of haploid genomes. For optimal functionality, it is crucial to integrate both assemblies.

In our study 12 accessions belonging to early, medium and late maturity time Tarocco orange clones and four pigmented mandarin-like were evaluated through the use of pomological and rheological parameters, allowing the separation of citrus accessions in “suitable” and “unsuitable” to RTE products. Several studies are already conducted in other species applying genomics and transcriptomics to leverage the comprehension of mechanisms intrinsic in RTE propension; it could reflect the expression of specific

genes or gene variants. We performed RNAseq on the juice to compare the Tarocco clone suitable ('Amantea') versus that unsuitable ('Rosso'); the same approach was performed for two pigmented mandarin-like, 'Early Sicily' and 'Galatea'. The RNA was sequenced by Illumina platform. The sweet orange combined heterozygous genome assembly was used as reference. The genes from maternal and paternal haplotypes were treated as ortho-group instead of alleles, reducing the effect caused by the different genetic backgrounds of the selected accessions. In the two comparisons, about one thousand differentially expressed genes are in common ( $p_{adj} \leq 0.05$ ,  $|\log_2FC| \geq 1$ ). As an overview of the pathways involved in RTE aptitude, the KEGG Orthology and the Gene Ontology enrichments were performed. Being the aptitude for RTE a vast concept, a clear common functional trend in gene expression between Tarocco and mandarin-like hybrids is delineated. Although preliminary, the results demonstrated that the use of the heterozygous genome is applicable to transcriptomic studies, and it is more accurate for highly heterozygous accessions, especially in interspecific hybrids, as in this context. Moreover, developing new pipelines is essential for the correct and routinary application of this approach.