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THE TRANSCRIPTOME OF CITRUS AURANTIUM SEQUENCED AND ASSEMBLED BY USING A HYBRID APPROACH: A NOVEL BASIS TO INVESTIGATE THE CROSS-PROTECTION MECHANISM OF CITRUS TRISTEZA VIRUS

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Citrus tristeza virus (CTV) is a phloematic and aphid-transmitted virus, responsible for tremendous economic losses of citrus grafted on sour orange (*Citrus aurantium*). In the last 20 years, CTV has caused serious epidemics in Southern Italy leading the use of tolerant rootstocks, alternative to sour orange. In Sicily, severe seedling yellows CTV isolates belonging to the VT strain are the most diffused along with a smaller percentage of mild T30 isolates. The understanding of pathogen infection processes is of great importance to discern host-pathogen interactions and to explore new control strategies. Among those, the use of cross-protection, considered to prevent secondary infections of genetically homologous aggressive isolates, is under evaluation in many countries to reintroduce the use of sour orange as rootstock.

For the purpose we have investigated by a transcriptomic approach the differential response of sour orange seedlings when infected with the aggressive SG29 CTV isolate or the homologous asymptomatic and protective M39 variant. The genome of *C. aurantium* (a complex interspecific

pummelo–mandarin hybrid) is missing, even though it represents one of the most important rootstocks, particularly appreciated for essential oils of peel and fruits, used to prepare marmalade. The study of the transcriptome without the availability of a reference genome is quite ambitious. To bypass the absence of a reference genome for *C. aurantium*, we sequenced and assembled *de novo* its transcriptome, exploiting a novel hybrid assembly approach, combining long (Oxford Nanopore) and short reads (Illumina) data. Sequencing was conducted on five tissues, namely peel, bark, leaves, petal, whole plant from *in vitro* culture, and also juice. The assembled transcriptome is 56.9 Mb long, consists of 26,994 transcripts, with the longest of 29,456 bp. The 96.1% of BUSCO completeness supports the high quality of transcriptome reconstruction. Three levels of confidence have been used to make the functional annotation as complete as possible, integrating orthologous and protein domain search with the propagation of Gene Ontology (GO) and KEGG terms. In the final transcriptome 97.2% transcripts have been annotated and 95.1% is associated to a GO/KEGG term.

Analysis of differentially expressed transcripts (DETs) of Illumina RNAseq data, performed to compare the transcriptome profile, showed 2,869 DETs in plants inoculated with SG29 versus uninoculated plants, and only 16 DETs in plants inoculated with M39, whereas the comparison between SG29 and M39 revealed 2,256 DETs (padj<0.01, log2FC>|1|). Among DETs commonly shared in plants inoculated with SG29, we selected 7 to be validated through qRT-PCR analysis, confirming the up/down regulation for 6 out of 7 (r=0.89***). Preliminary data suggest that the protective strain reacts likely to uninoculated plants as supported also by phenotypic observations.