KEY GENES IN ROTUNDONE BIOSYNTHESIS ARE AFFECTED BY TEMPERATURE, LIGHT, WATER SUPPLY, AND NITROGEN UPTAKE

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Abstract:

Context and purpose of the study - Rotundone accumulation and biosynthesis is a complicated process. Previous research highlighted that these phenomenons were affected under ecophysiological conditions by viticultural practices (e.g. defoliation or irrigation). Individually, these practices often impact several abiotic factors that are difficult to separate such as temperature, water or nitrogen status, or radiation. Such dissociation can be achieved under controlled environmental conditions using potted vines. Additionally, the expression of 3 major genes identified in rotundone biosynthesis namely *Vitis vinifera terpene synthase 24* (*VvTPS24*), *Vitis vinifera sesquiterpene oxidase 2* (*VvSTO2*) and *farnesyl pyrophosphate synthase* (*FPPS*) displayed correlation with rotundone accumulation. The objectives of this work were to assess i) the correlation between the expression of *VvTPS24*, *VvSTO2* and *FPPS* in berry and leaf, and ii) the impact of abiotic factors on the expression of these three candidate genes in leaves of non-bearing fruit grapevine cuttings grown under controlled environmental conditions

Material and methods - Cuttings from *Vitis vinifera* L. cv Syrah and Tardif were produced in greenhouses until 16 leaves high. Cuttings were then put for 10 days for acclimatization in chambers with fully controlled atmosphere at 20 °C, 40 % relative humidity and 100 mL water supply per day. With the exception of the control treatment that was kept under these conditions, the other cuttings were then subjected to 4 different treatments, with 5 replicate cuttings per condition: a first batch was placed at 28°C, a second one was given 200 mL of water per day, a third one was put under a blackout net hiding 50 % of incoming light, and finally a fourth one was sprayed on each leaf with a nitrogen solution containing 22 g/L of urea. Another control batch was left in the same original conditions and sprayed with water. Leaves from the cuttings were sampled at 6, 24, 72 and 120 h. Cuttings being exempt of berries, berries and leaves from Syrah and Tardif grown in a neighboring commercial vineyard were sampled in parallel every 7 days from veraison until 49 days post veraison to investigate the correlation between the gene expression in these two organs. Every sample was stored at -80 °C until analysis. Total RNA from berries and leaves samples was extracted and every sample was subjected to RT-qPCR.

Results - *VvTPS24*, *VvSTO2* and *FPPS* expression was directly influenced by all abiotic factors applied and apart for *VvSTO2*, their expression in leaves/berries and Syrah/Tardif could be correlated. This means that their expression in cuttings leaves is likely to be extrapolated to berries, and therefore might reflect rotundone biosynthesis and accumulation. Temperature above 25°C while known to lower rotundone concentration first reduced by a 2 to 10 factor and then after 72 h enhanced expression by 10 fold in every investigated genes. Water intake sequencially activated all genes suggesting a direct impact on biosynthesis which is consistent with previous research highlighting a stimulating effect of irrigation on rotundone accumulation. On the other hand, light regulation and nitrogen spraying did not have a conclusive influence on gene expressions having opposed effects on *VvTPS24* and *VvSTO2*.. If the impact of nitrogen had never been previously investigated, it was recently proposed that radiations could stimulate rotundone production under field conditions.

Keywords: Rotundone, Biosynthesis, Abiotic factors, RT-qPCR, GC-MS, Fruiting cuttings.