In vitro cultures of grape tissues: new possibilities to study grape berry physiology

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Grape berries suffer important morphological, biochemical and physiological changes during its development and maturation. It is known that photoassimilates translocated from leaves serve as the major source of carbon and energy to support fruit needs, but recent findings revealed that, at least in the green phase, grape berries show high photosynthetic activity especially in the exocarp. The contribution of fruit photosynthesis for fruit growth and production of organic compounds is far from being understood. In this study photomixotrophic cell suspensions were established as an *in vitro* model to complement the study grape berry photosynthesis. *Calli* of CSB (Cabernet Sauvignon Berry) cells derived from the inner tissues of the grape berry were sub-cultured in liquid modified MS medium supplemented with 2% sucrose and different hormonal combinations, one auxin (NAA) and three cytokinins (BAP, ZEA and KIN) at two different final concentrations (0.5 and 1 $\mu g\ mL^{-1}$). Two different growth light intensities (45- 60 and 80-105 $\mu mol\ m^{-1}\ s^{-1}$) were also tested. Chlorophyll fluorescence PAM fluorometry was used to evaluate the photochemical efficiency (F_v/F_m) of all suspensions and chlorophyll content was also determined. Results showed that the cytokinin type was crucial to induce the photosynthetic phenotype, but F_v/F_m was low when compared to the value exhibited by grape berry skin. To further study the photoautrophy of the grape berry tissues new *callus* cultures were established from the exocarp tissues of the fruit, harvested at the green stage (Alvarinho cv). Portions of detached exocarp were cultured on B5 solid medium supplemented with different hormonal combinations (NAA or 2,4-D with BAP or ZEA) at different concentrations (0.1 or 0.2 $\mu g\ mL^{-1}$ for auxins and 0.2 or 0.4 $\mu g\ mL^{-1}$ for cytokinins). Only two combinations were responsive but both induced pale green *calli* with higher growth rates. To our knowledge, this is a pioneer study on *calli* production from the exocarp of the grape berry from the Portuguese variety Alvarinho. This approach opens good perspectives to study in more detail the physiology of these cells, namely the role of photosynthesis on cell growth and

metabolite production. Moreover, it will allow to investigate the impact of several environmental factors on fruit photosynthesis, such as temperature, light and water potential.

Keyword: *Vitis vinifera*, cell cultures, exocarp, phytohormones, PAM-fluorometry.