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Postharvest dehydration induces variable changes in the primary metabolism of grape berries

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ABSTRACT

Postharvest dehydration causes changes in texture, color, taste and nutritional value of food due to the high temperatures and long drying times required. In grape berries, a gradual dehydration process is normally utilized for raisin production and for making special wines. Here we applied a raisin industry-mimicking dehydration process for eleven days at 50 °C to intact berry clusters from cv. Sémillon plants, and a set of molecular, cellular and biochemical analyses were performed to study the impact of postharvest dehydration in the primary metabolism. Transcriptional analyses by real time qPCR showed that several aquaporins (*VvTIP1;2 and VvSIP1*) and sugar transporters (*VvHT1, VvSWEET11, VvSWEET15, VvTMT1, VvSUC12*) genes were strongly upregulated. Moreover, the study of key enzymes of osmolytes metabolism, including mannitol dehydrogenase (VvMTD) and sorbitol dehydrogenase (VvSDH), at gene expression and protein activity level, together with the transcriptional analysis of the polyol transporter gene *VvPLT1,* showed an enhanced polyol biosynthesis capacity, which was supported by the detection of sorbitol in dehydrated grapes only. The metabolism of organic acids was also modulated, by the induction of transcriptional and biochemical activity modifications in malate dehydrogenases and malic enzymes that led to organic acid degradation, as demonstrated by HPLC analysis. Taken together, this study showed that primary metabolism of harvested berries was severely influenced in response to dehydration treatments towards lower organic acid and higher sorbitol concentrations, while sugar transporter and aquaporin genes were significantly upregulated.

1. Introduction

A gradual dehydration process (either by open sun, shade or mechanical drying) is normally utilized to produce raisin and wine with particular and differentiated characteristics, such as sweet and fortified wines. Raisins are rich in nutritional content and its production is presently a growing export business in many countries. According to the United States Department of Agriculture, the world raisin production is approximately of 1.3 million ton and wines from dehydrated grapes is trending up, particularly in Italy, composing a novel niche in the winemaking industry (Adiletta, Russo, Senadeera, Di Matteo, 2016; Noguerol-Pato, González-Álvarez, González-Barreiro, Cancho-Grande, & Simal-Gándara, 2012; Pangavhane & Sawhney, 2002; Wang et al., 2015).

In general, applied postharvest dehydration causes changes in texture, color, taste and nutritional value of food due to the combination of high temperatures and water loss caused by the long drying times required in the process. These changes suggest that an applied post-harvest dehydration process might strongly influence important primary and secondary metabolic pathways of grape berry cells, such as sugar post-phloem transport and metabolism, organic acids metabolism and phenolics biosynthesis and/or degradation, which are all key metabolic pathways strongly associated with the quality of berries (Costantini, Bellincontro, De Santis, Botondi, & Mencarelli, 2006; Rizzini, Bonghi, & Tonutti, 2009; Schreiner & Huyskens-Keil, 2006). However, concrete information on how the molecular mechanisms involved in these specific metabolic pathways are changed in whole berries during postharvest dehydration similar to the industrially applied in raisin

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production is somewhat scarce. Molecular analysis by amplified fragment length polymorphism (AFLP)-transcriptional profiling in Corvina cv. berries subjected to withering previously revealed, however, some evidence for increased sugar transport capacity, glycolysis and respiration as a consequence of the treatment (Zamboni et al., 2008). An integration of Microarray, LC-MS and GC-MS analyses have also recently unveiled a vast number of molecular responses to postharvest berry withering, mostly of secondary metabolic pathways, that were strongly genotype-dependent (Zenoni et al., 2016).

Still, postharvest withering is somewhat different to postharvest dehydration of berries involving temperatures above 40 °C that are common in raisin production. Transcriptional changes in the skin of postharvest withered Raboso Piave cv. berries were also observed by Rizzini et al. (2009) in a wide variety of biological and metabolic processes, including energetics, information pathways, protein stability and modification, primary and secondary metabolism and developmental processes. Costantini et al. (2006) showed that Malvasia grapes had generally increased abscisic acid (ABA) and proline concentrations in the middle or final stages of the dehydration process of harvested berry clusters, observations interpreted as molecular and cellular responses to the progressive water deficit. They also unequivocally demonstrated that the respiration rate increased up to middle of the process and then was still higher in the final stage of postharvest dehydration when compared to the initial stage. Moreover, the rate of water loss may also induce cell wall enzyme activity, ethylene production, and loss of volatiles (Bellincontro, De Santis, Botondi, Villa, & Mencarelli, 2004; Costantini et al., 2006; Hsiao, 1973).

Sugars (mostly sucrose and monosaccharides) are key for grape berry development and quality due to their role as primary carbon and energy source (Agasse et al., 2009; Conde et al., 2007), and, sometimes, as osmotic and signaling molecules (Afoufa-Bastien et al., 2010) important for responses to biotic or/and abiotic factors (Lemoine et al., 2013). Sugar transport is thus a fundamental process for berry growth and development (Afoufa-Bastien et al., 2010). Also importantly, polyol metabolism has been demonstrated to have an important role in cellular homeostasis during water deficit stress conditions in the berry (Conde et al., 2015), while the metabolism of organic acids is usually affected by peaks of and/or prolonged elevated temperatures (Sweetman, Deluc, Cramer, Ford, & Soole, 2009; Sweetman, Sadras, Hancock, Soole, & Ford, 2014) in a way that results in typically decrease in their concentration. Still, unlike preharvest water stress, limited information is available on the specific processes triggered by typically industrial postharvest dehydration of grape berries and the regulatory mechanisms involved in these changes. In fact, sugar transport mechanisms, sugar and polyol metabolism, and organic acid metabolism are molecular processes for which detailed information regarding the effect of postharvest dehydration is absent. Thus, the purpose of this study was to analyze particular changes in the primary metabolism during the dehydration process using molecular and biochemical analyses to understand the role of sugar transporters, which are known to be (or putatively, in some cases) involved in sugar allocation from berry apoplast into the cells as well as post-phloem transport in the berry milieu, polyol transporters, aquaporins, and the role of the metabolism of organic acids and polyols, due to their involvement in berry/wine flavor and in water deficit stress tolerance.

2. Material and methods

2.1. Grapevine field conditions and sampling

Clusters of *Vitis vinifera* cv. Sémillon, a golden-skinned grape used to make dry and sweet white wines, mostly in France and Australia, but also raisins, were harvested from a particular vineyard in Fafe, north of Portugal. In this region the climate is typically Mediterranean, with a warm temperate climate, dry and hot summers, and with higher precipitation during autumn and winter (Kottek, Grieser, Beck, Rudolf, & Rubel, 2006). Vineyard was managed without irrigation and grown following standard cultural practices applied in commercial farms.

The mature grape clusters (sound berries and uniform size) were randomly, carefully and representatively harvested and, subsequently, a set of grape clusters were placed in small perforated boxes where they were subjected in laboratorial conditions to a raisin production industry-mimicking dehydration process at 50 °C for eleven days, while other set was immediately frozen in liquid nitrogen (control). Sampling was performed after five days and eleven days of dehydration, by collecting randomly and representatively berries from the dehydrated clusters and immediately freezing them in liquid nitrogen. None of the clusters or individual berries presented signs of fungal contamination. Whole berries were deseeded and ground to a fine powder under liquid nitrogen refrigeration and stored in − 80 °C for posterior studies. Dry weight was always used for normalization purposes to avoid biases introduced by water content and berry weight variations.

2.2. RNA extraction

A total of 200 mg of whole grape berry tissue previously grounded in liquid nitrogen was used for total RNA extraction following the protocol by Reid, Olsson, Schlosser, Peng, and Lund (2006) in combination with purification with RNeasy Plant Mini Kit (Qiagen). After treatment with DNase I (Qiagen), RNA integrity was confirmed running the samples in 1% agarose gel stained with SYBR Safe (InvitrogenTM, Life Technologies). cDNA was synthesized from 1 μg of total RNA using Omniscript Reverse Transcription Kit (Qiagen).

2.3. Transcriptional analyses by real-time qPCR

Real-time PCR analysis was performed with QuantiTect SYBR Green PCR Kit (Qiagen) using 1 μL cDNA (diluted 1:10 in ultra-pure distilled water) in a final reaction volume of 10 μL per well. For reference genes, *VvACT1* (actin 1) and *VvGAPDH* (glyceraldehyde-3-phosphate dehydrogenase) were used, as these genes were proven to be very stable and ideal for qPCR normalization purposes in grapevine (Reid et al., 2006). Specific primer pairs used for each target or reference gene are listed on Supplementary Table 1. QuantPrime software (Arvidsson, Kwasniewski, Riaño-Pachón, & Mueller-Roeber, 2008) was used for primer design for amplification on some genes, as referred in the Supplementary Table 1. Melting curve analysis was performed for specific gene amplification confirmation. Stability of the reference genes was confirmed by the automatic M-value analysis performed by the Bio-Rad® CFX Manager 2.0 Software. For each gene, the relative gene expression values were obtained after normalization with the average of the expression of the reference genes as described by Pfaffl (2001). For all experimental conditions tested, two independent runs with triplicates were performed.

2.4. Major sugars and organic acids extraction and quantification by HPLC analyses

The extraction of sugars and organic acids from grape berry samples was adapted from a method described by Eyéghé-Bickong, Alexandersson, Gouws, Young, and Vivier (2012). Extracts were obtained adding 800 μL of dH_2O and 5% (w/v) insoluble PVPP to 80 mg of grape berry frozen powder and by vigorously vortexing. An equal volume of chloroform (800 μL) was added to the mixture and the biphasic solvent was vortexed for 5 min to mix and incubated at 50 °C for 30 min with continuous shaking. After incubation, the samples were centrifuged at 17500 × *g* for 10 min at room temperature to recover the upper aqueous phase containing the sugars and organic acids. The aqueous phase was re-centrifuged (as above) to remove any residual cell debris. The supernatant was transferred to HPLC vials, after filtration, and crimp-sealed for HPLC analysis. Each grapevine sample was extracted in triplicate before HPLC analysis. Chromatographic analyses