Avances en poscosecha de frutas y hortalizas

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II. Fisiología y bioquímica de la poscosecha

Molecular analysis of rachis browning in table grapes during storage at 0 ºC: beneficial effect of pretreatments with high levels of CO_2

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ABSTRACT

Low temperature storage is an effective technology to extend table grape postharvest life. However, the length of storage is limited by their high susceptibility to fungal decay and the sensitivity of rachis to desiccation and browning. Previous work demonstrated that pretreatment with high CO_2 levels (20% CO_2 plus 20% O_2) for 3 days was able to control fungal decay and reduce rachis browning in table grapes. To better understand the beneficial effect of this pretreatment on maintaining table grape rachis quality at 0 ºC, we analyzed the expression of genes codifying enzymes related to the oxidation of phenolic compounds (phenylalanine ammonia-lyase, *VcPAL*; and polyphenol oxidase, *GPO1*), the detoxification of reactive oxygen species (catalase, *GCAT*; and ascorbate peroxidase, *VcAPX*) in rachis of treated and non-treated bunches. In addition, and due to their role in senescence, the implication of ethylene and abscisic acid (ABA) in the onset of rachis deterioration was investigated by the expression pattern of key regulatory genes such as ACC synthase (*ACS1*) and oxidase (*ACO1*), *VvNCED1* and 2.

INTRODUCTION

Storage at low temperature, around 0 ºC, is recommended for the maintenance of postharvest quality of non-climateric fruit, such as table grape. However, the length of storage is limited by their high susceptibility to fungal decay and rachis dehydration and enzymatic browning. The application of SO_2 (Nelson 1985) and controlled atmospheres (Retamales et al., 2003), have been used to maintain table grape quality and reduce rachis browning. In this sense, previous works of our group demonstrated the efficacy of a 3-day pretreatment with high CO_2 levels maintaining the quality of table grapes and reducing rachis browning during storage at 0 ºC (Sanchez-Ballesta et al., 2006). However, very little is known about the molecular mechanisms that

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participate in the appearance of rachis browning and the favorable effect of high levels of CO_2 in this process.

The objective of the present work was to investigate the molecular changes occurring at the onset of rachis browning in table grapes and how high levels of CO_2 modulate this response leading to an improvement of rachis quality. In order to do so, changes in the expression of genes that catalyze key enzymes involve in different mechanisms associated to rachis browning were evaluated by quantitative RT-PCR in rachis of table grapes bunches stored at 0 ºC during 3 days and treated or not with 20% CO_2. More specifically the expression of the following genes was examined: a) polyphenol oxidase (*GPO1*) and phenylalanine ammonia-lyase (*VcPAL*) that participates in the oxidation of phenolic compounds; b) catalase (*GCAT*) and ascorbate peroxidase (*VcAPX*) as indicators of the state of the antioxidant mechanism; c) the key enzymes of ethylene and ABA biosynthesis pathways ACC synthase (*ACS1*), ACC oxidase (*ACO1*), 9-cis-epoxycarotenoid dioxygenase (*VvNCED1* and *VvNCED2*).

MATERIALS AND METHODS

Table grape clusters (*V. vinifera* L. cv. Cardinal) were harvested at early-harvesting stage (12.7% total soluble solids; 0.81% tartaric acid). The field-packaged bunches were transported to the lab and immediately forced-air precooled for 14 h at -1 ºC (time 0). After cooling, bunches free from physical and pathological defects were randomly divided into two lots (containing about 3kg table grapes) and stored at 0±0.5 ºC and 95% relative humidity in two sealed neoprene containers of 1 m^3 capacity. One lot was stored under normal atmosphere (non-treated) and the other one under a gas mixture containing 20% CO_2 + 20% O_2 + 60% N_2 (CO_2-treated) for 3 days. Five clusters of grapes (approximately 300 g from each cluster) were sampled and the rachis collected, frozen in liquid nitrogen and stored at - 80 ºC until analysis.

Browning indexes of the rachis was determined for each bunch, using at least 3 replicates per sample and according to the following subjective scale: (0) none, (1) slight, (2) moderate, (3) severe, and (4) extreme.

Quantification of the end product of lipid peroxidation, malondialdehyde (MDA), was assayed using a thiobarbituric acid method (Ederli et al., 1997), by determining absorbance at 532 nm and adjusted for non-specific absorbance at 600 nm. MDA content was estimated by using a molar extinction coefficient of 155 nmol L^{-1} cm^{-1}.

Relative expression of all studied genes was assayed using quantitative RT-PCR (RT-qPCR) with rachis samples from bunches CO_2-treated and non-treated stored for 0 and 3 days at 0 ºC as described in Fernandez-Caballero et al. (2012).

All statistics were performed using Statistical Analysis System for PC (SAS Institute Inc., Cary, NC). Data were analyzed using ANOVA and means were separated by Duncan's multiple-range test ($p \leq 0.05$).

RESULTS AND DISCUSSION

After only 3 days at 0 ºC, non-treated bunches showed a slight browning. CO_2 treatment was effective in preventing the appearance of browning although there was a slight increase compare with time 0 samples (Table 1).

In order to relate the oxidation of phenolic compounds with the onset of rachis browning in *V. vinifera*, we analyzed the expression of *VcPAL* (DQ887093) and *GPO1* (A27657) in Cardinal bunches. It was found that *VcPAL* expression level was significantly lower in rachis of CO_2-treated clusters compared with time 0 and non-treated samples (Fig. 1). Regarding *GPO1*, 3 days of storage at 0 ºC increased significantly the transcript levels in non-treated rachis, while the application of high CO_2 levels during 3 days caused a significant decrease in the accumulation of *GPO1* mRNA in comparison with non-treated rachis. It has been reported (Carvajal-Millan et al., 2001) that rachis browning as well as PPO activity increased during 0 ºC storage in Flame Seedless grapes. It is also known that phenolic compounds serve as substrates for the browning reactions (Tomas-Barberan et al., 1997). Our results also pointed to a relation between rachis browning and *VcPAL* and *GPO1* gene expression, what is lower in CO_2-treated bunches.

The role of oxidative stress in the deterioration of rachis was evaluated by analyzing the formation of MDA and changes in *VcAPX* (DQ887095) and *GCAT* (XM_003695412) gene expression in rachis of CO_2-treated and non-treated bunches. The level of MDA in rachis of non-treated clusters was higher than both rachis at time 0 and CO_2-treated ones (Table 1). In the latter, application of 3-day high CO_2 levels did not change MDA content compared to time 0. Interestingly the lower lipid peroxidation in CO_2-treated rachis coincided with a higher expression of the gene that codifies for catalase (Fig. 2). In the case of *VcAPX* gene expression at 3 days of cold storage did not show any significant change compared with time 0 rachis. In previous work, we have observed that in Cardinal grapes the effectiveness of high CO_2 levels to control fungal decay during low-temperature storage was the result of the ability to prevent the formation of ROS rather than their inactivation once formed (Sanchez-Ballesta et al., 2006). By contrast, in CO_2-treated rachis a lower lipid peroxidation during the first 3 days of cold storage is mediated by activation in the gene expression of the antioxidant enzymes CAT.

To analyze the involvement of ethylene on rachis browning we analyzed changes in the expression pattern of the genes that codify for key regulator enzymes of ethylene biosynthesis *ACS1* (XM_002263552) and *ACO1* (AY211549) in rachis of Cardinal bunches stored at 0 ºC and treated or not with high levels of CO_2. The results revealed a slight but significant increase in *ACS1* gene expression in non-treated bunches compared with time 0 and CO_2-treated samples (Fig. 3). More important was the induction of *ACO1* transcription level that reached 2.5-fold increase in 3 days of cold storage (Fig. 3) and was prevented by high levels of CO_2 in treated rachis.

The expression profile of *VvNCED1* (AY337613) and *VvNCED2* (AY337614) were studied in rachis of CO_2-treated and non-treated table grape bunches to better understand the role of ABA in rachis deterioration. *VvNCED1* gene expression was down-regulated by low temperature, with no effect due to the CO_2 pretreatment (Fig. 3). With regards to *VvNCED2* relative gene expression, there was a slight increase in non-treated rachis although it was not significant compared with time 0 or CO_2-treated rachis (Fig 3). These results revealed that cold storage for 3 days triggered an activation of the biosynthesis pathways of ethylene, and to a lower extend ABA; and that the 3-day CO_2 pretreatment was effective in preventing this activation, what could have an impact in the lower rachis browning of treated samples. In this sense, it has been observed that cold treatment induce *NCED1* expression in skin of table grapes under cold storage (Becatti et al., 2010). Moreover it has been reported that growth regulators that delay senesce such as cytokinins, diminished postharvest browning by reducing PPO activity (Carvajal-Millan et al., 2001). Nonetheless and to the best of our knowledge, this is the first report linking the induction of ethylene biosynthesis to the appearance of rachis browning in table grapes during cold storage.

In conclusion, our results indicate that the beneficial effect of the high levels of CO_2 on the onset of rachis browning could be related to a lower lipid peroxidation and higher expression of the catalase gene, as well with the down-regulation of the phenolic metabolism. Furthermore, our results revealed a differential regulation of ethylene and ABA by low temperature and high levels of CO_2 in rachis, and pointed to a role of ethylene in the appearance of rachis browning.

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TABLE 1. BROWNING INDEX AND MALONDIALDEHYDE (MDA) CONTENT OF RACHIS FROM TABLE GRAPES STORED AT 0 ºC UNDER NORMAL ATMOSPHERE (NON-TREATED) OR TREATED WITH 20% CO_2 (CO_2-TREATED) FOR 3 DAYS

	Rachis browning index[a]		**MDA** (nmol/g FW)	
	Non-treated	CO_2-treated	Non-treated	CO_2-treated
0	0 c		55.60 ± 1.20 b	
3	0.93 ± 0.16 a	0.53 ± 0.21 b	62.78 ± 0.17 a	52.35 ± 0.68 b

a Scale of browning: 0, none; 1, slight; 2, moderate; 3, severe; 4, extreme. Values are the mean of three replicate samples ± SE. Different letters indicate that means are statistically different (Duncan test, $p<0.05$).

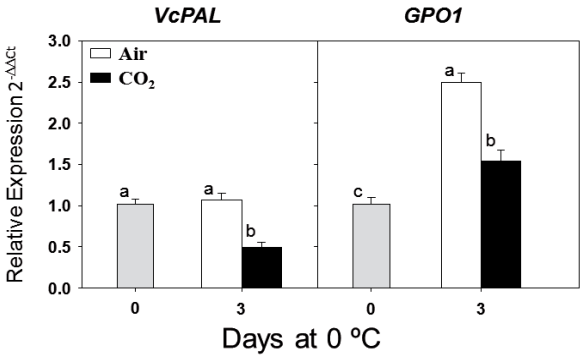

Fig. 1. Relative transcript level by RT-qPCR of VcPAL and GPO1 from rachis of non-treated and CO_2-treated table grapes stored at 0 ºC. Values are the mean ± SD, n=3. Different letters on bars indicate means are statistically different using Duncan's test ($p>0.05$).

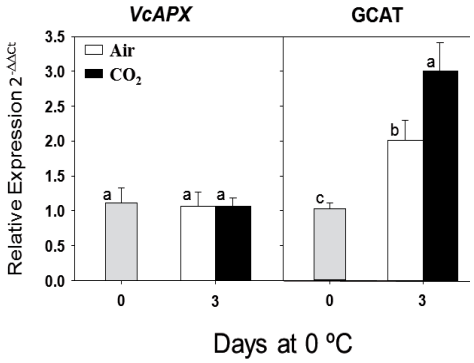

Fig. 2. Relative transcript level by RT-qPCR of VcPX and GCAT from rachis of non-treated and CO_2-treated table grapes stored at 0 ºC. Values are the mean ± SD, n=3. Different letters on bars indicate means are statistically different using Duncan's test (p>0.05).

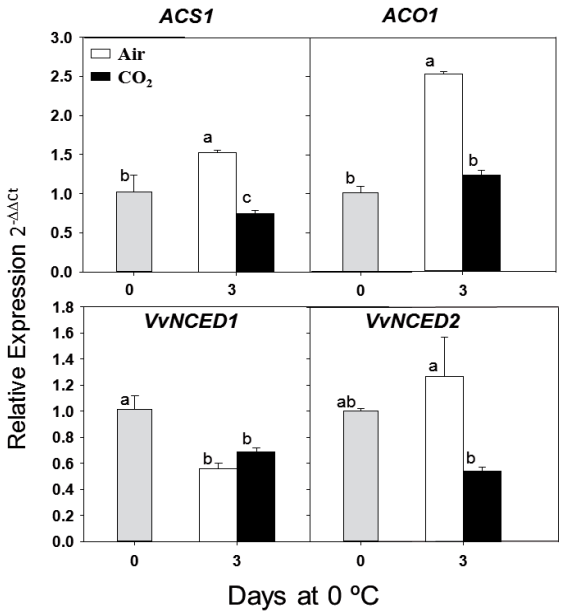

Fig. 3. Relative transcript level by RT-qPCR of *ACS1*, *ACO1* (top row), VvNCED1 and 2 (bottom row) from rachis of non-treated and CO_2-treated table grapes stored at 0 ºC. Values are the mean ± SD, n=3. Different letters on bars indicate means are statistically different using Duncan's test (p>0.05).