Title	Postharvest heat and conditioning treatments activate different molecular responses and
	reduce chilling injuries in grapefruit
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Abstract

A combination of hot water (a rinse at 62 °C for 20 s) and conditioning (pre-storage at 16 °C for 7 d) treatments synergistically reduced chilling injury development in grapefruit (Citrus paradisi, cv. 'Star Ruby') during cold storage at 2 °C, suggesting that the treatments may activate different chilling tolerance responses. To study the molecular mechanisms involved, chilling- and conditioning-responsive genes were isolated by polymerase chain reaction (PCR) cDNA subtraction, cDNA libraries were constructed from hot water- and conditioning-treated fruit, and cDNA sequencing was used to identify putative stressresponsive and chilling tolerance genes. PCR cDNA subtraction revealed the identification of 17 chillingresponsive and heat- and conditioning-induced genes, and the expression patterns of 11 additional stressrelated genes, antioxidant defensive genes, and genes encoding enzymes involved in membrane lipid modifications were characterized. It was found that hot water and conditioning treatments had little effect on gene expression by themselves, but rather had a priming effect, and enabled the fruit to activate their defence responses after subsequent exposure to chilling. RNA gel blot hybridizations revealed that the expression patterns of eight genes, including HSP19-I, HSP19-II, dehydrin, universal stress protein (USP), EIN2, 1,3;4-β-D-glucanase, and superoxide dismutase (SOD), were specifically regulated by the heat treatment, and four genes, including fatty acid desaturase2 (FAD2) and lipid transfer protein (LTP), were specifically regulated by the conditioning treatment. Furthermore, four more genes were identified, including a translation initiation factor (SUI1), a chaperonin, and alcohol dehydrogenase (ADH), that were commonly regulated by both heat and conditioning treatments. According to these data, it is suggested that pre-storage heat and conditioning treatments may enhance fruit chilling tolerance by activating different molecular mechanisms. The hot water treatment activates mainly the expression of various stress-related genes, whereas the conditioning treatment activates mainly the expression of lipid membrane modification enzymes.