Investigations into the Molecular and Physiological Factors Influencing Low Temperature Breakdown in Stonefruit

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Abstract

Cold storage is essential for the successful distribution of stonefruit to distant markets; however most cultivars suffer chilling injury (CI), commonly referred to as low temperature breakdown (LTB). LTB is a significant problem for industry and the genetic factors responsible for its onset are not understood. Treatment of stonefruit with the ethylene antagonist, 1-methylcyclopropene (1-MCP) before cold storage has been shown to differentially affect the development of CI in peaches and plums. 1-MCP treatment increases the incidence of LTB in peaches but reduces it in plums (Fernández-Trujillo and Artés, 1997; Fan et al., 2002). These observations were confirmed and preliminary research into the effects of 1-MCP and cold storage on gene expression is reported herein.

INTRODUCTION

Stonefruit can be successfully exported by air freight but it is uneconomical. The alternative transport by sea is cheaper but takes longer. Sea freight is limited to voyages of about three weeks at 0°C because most cultivars suffer chilling injury (CI), commonly referred to as low temperature breakdown (LTB). Symptoms include mealiness or woolliness, bleeding of pigments into the flesh from the stone and skin in peaches, flesh browning and gel breakdown in plums (Lurie and Crisosto, 2005).

Treatment of stonefruit with the ethylene antagonist, 1-methylcyclopropene (1-MCP) before cold storage has given variable results. LTB is increased in peaches and nectarines treated with 1-MCP prior to cool storage but reduced in Japanese-type plums (Fernández-Trujillo and Artés, 1997; Fan et al., 2002). Late season plums frequently have a longer cold storage life than peaches and nectarines and early season plums. The differential responses of peaches and plums to 1-MCP and cold storage offer a tool that could assist the identification of genes that control development of LTB. Identification of these genes could enable the development of markers that will assist plant breeders to select cultivars with reduced susceptibility to LTB. Interactions between 1-MCP and cool storage at the gene expression levels are observed in 'Zee Lady' peach (ZP), 'Ruby Red' (RR) and 'October Sun' (OS) plums.

MATERIALS AND METHODS

ZP, OS and RR plums were obtained at commercial maturity from commercial

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orchards in Eastern Australia in 2008 and treated within 48 hours after harvest with 1-MCP (625 ηL.L^{-1}) for 24 hours at 20°C. The fruit were stored at 0 and 5°C and transferred to 20°C at set intervals to assess the development of LTB. Symptoms of LTB were scored visually in fruit halves, cut at the equatorial plane. The fruit were assessed on a scale of 0 to 4 based on area affected by LTB. 0 = no LTB, 1 = less than 1/4 LTB, 2 = 1/4 to 1/2 LTB, 3 = 1/2 to 3/4 LTB and 4 = more than 3/4 LTB. The index is calculated where N1, N2, N3, N4 correspond to the number of fruit at the relevant LTB scores (1, 2, 3 and 4, respectively), and N is the total fruit number, ((1* N1) + (2* N2) + (3* N3) + (4* N4))*(100/(4*N)). Juice recovery was used as a measure of mealiness in peaches (Fan et al., 2002). RNA was extracted from fruit samples before cold storage and immediately after removal from four weeks of cold storage at 0°C. Gene expression was assessed using comparative analysis between 1-MCP treated and control fruit using the μ-peach 1.0 (Ziliotto et al., 2008). Significantly expressed genes were then matched to their relative ontologies using the Arabidopsis genome as a reference base by using MAPMAN platform (http://mapman.mpimp-golm.mpg.de) as described by Thimm et al. (2004).

RESULTS AND DISCUSSION

Cold Storage Responses

The severity of LTB in ZP stored at 5°C was greater following treatment with 1-MCP (Fig. 1). No significant differences in the incidence of LTB following treatment with 1-MCP were detected by subjective assessment of RR (data not shown) but LTB was significantly lower in OS following treatment with 1-MCP (Fig. 2). The interaction of the differences in the responses of ZP and OS was significant ($p \leq 0.05$).

Comparative Expression Analysis

A large number of genes were differentially expressed by the three cultivars following treatment with 1-MCP and cold storage at 0°C (ZP 181, RR 264 and OS 38). Thirty-nine expressed genes were common to both ZP and RR, of these genes only 9 were induced or suppressed by 1-MCP. Similar comparative data were obtained for OS and RR; however, a smaller number of genes were found to be in common (Fig. 3). Categorization of the differentially expressed genes, based on the ontology of corresponding Arabidopsis genes, resulted in identifying the following BINs; metabolism of membranes, cell walls, lipids and stress enzymes. Genes involved in signalling and stress were also identified (Fig. 4).

This preliminary study enabled the identification of genes that are differentially expressed in cultivars of peach and plums in response to treatment with1-MCP and cold storage. The storage experiments were repeated in 2009 and further RNA extracts was prepared for μ-array analysis. Quantitative PCR will be performed to evaluate the exact expression levels of genes of interest, previously identified in comparative μ-array analysis. Most of the genes that are differentially expressed and common between the cultivars have opposite trend of expression (i.e., up regulated in peach and down regulated in plum). Some of the genes that were differentially expressed relate to membrane structure which may be affected by chilling in sensitive plant species.

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Figures

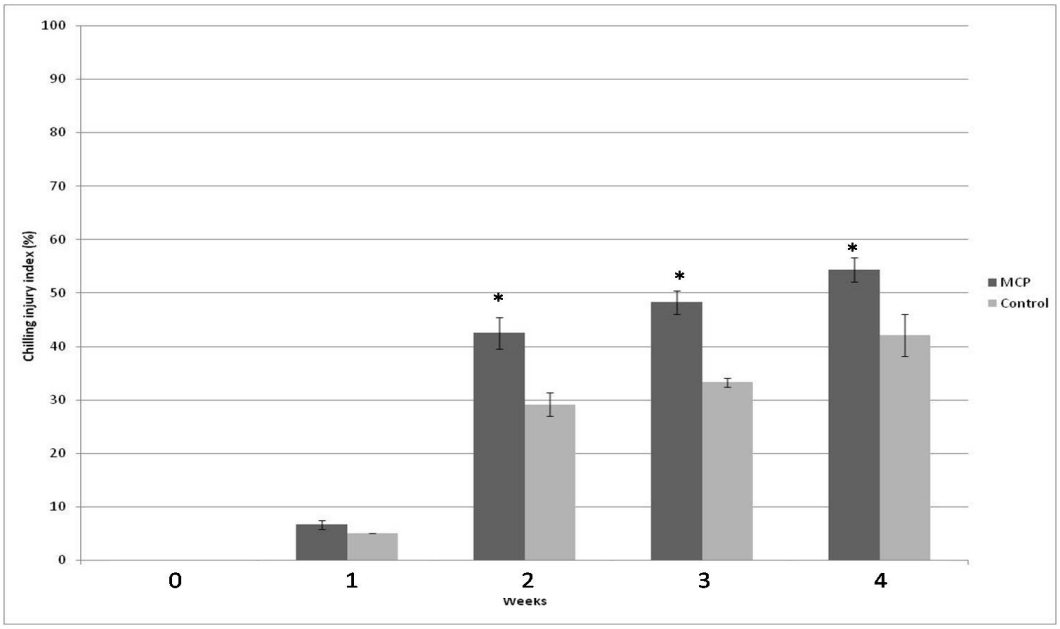


Fig. 1. Index of LTB in ZP after 1-MCP treatment and storage at 5°C. *Indicates significant difference ($p \leq 0.05$). Error bars represent standard errors of means (n=30).

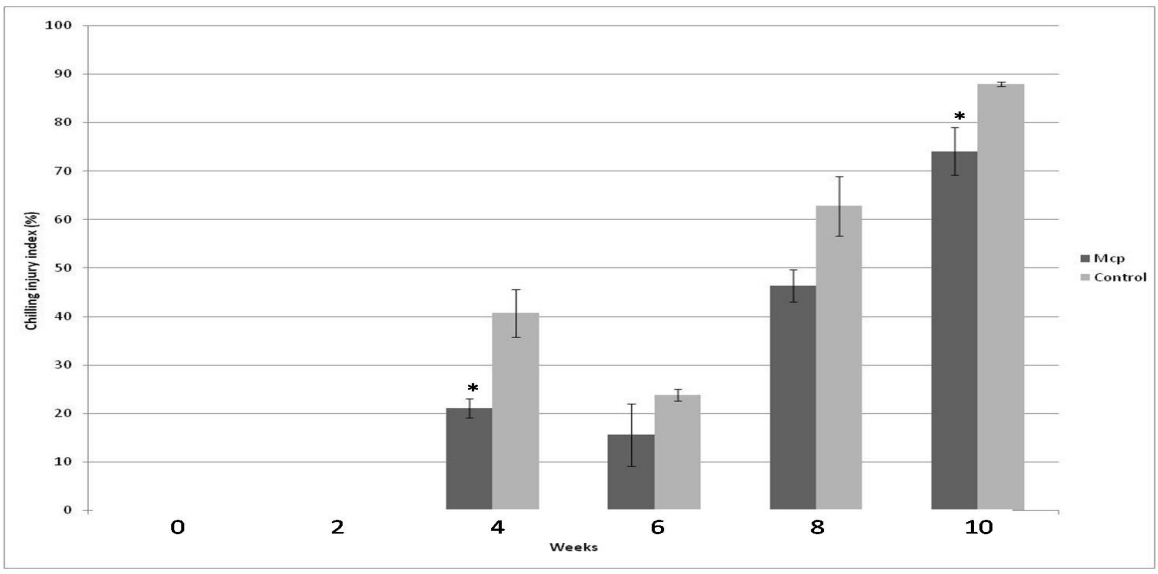


Fig. 2. Index of LTB in OS after 1-MCP treatment and storage at 5°C. *Indicates significant difference ($p \leq 0.05$). Error bars represent standard errors of means (n=30).

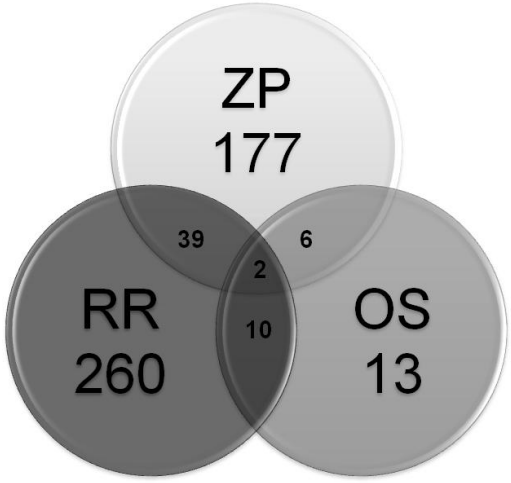
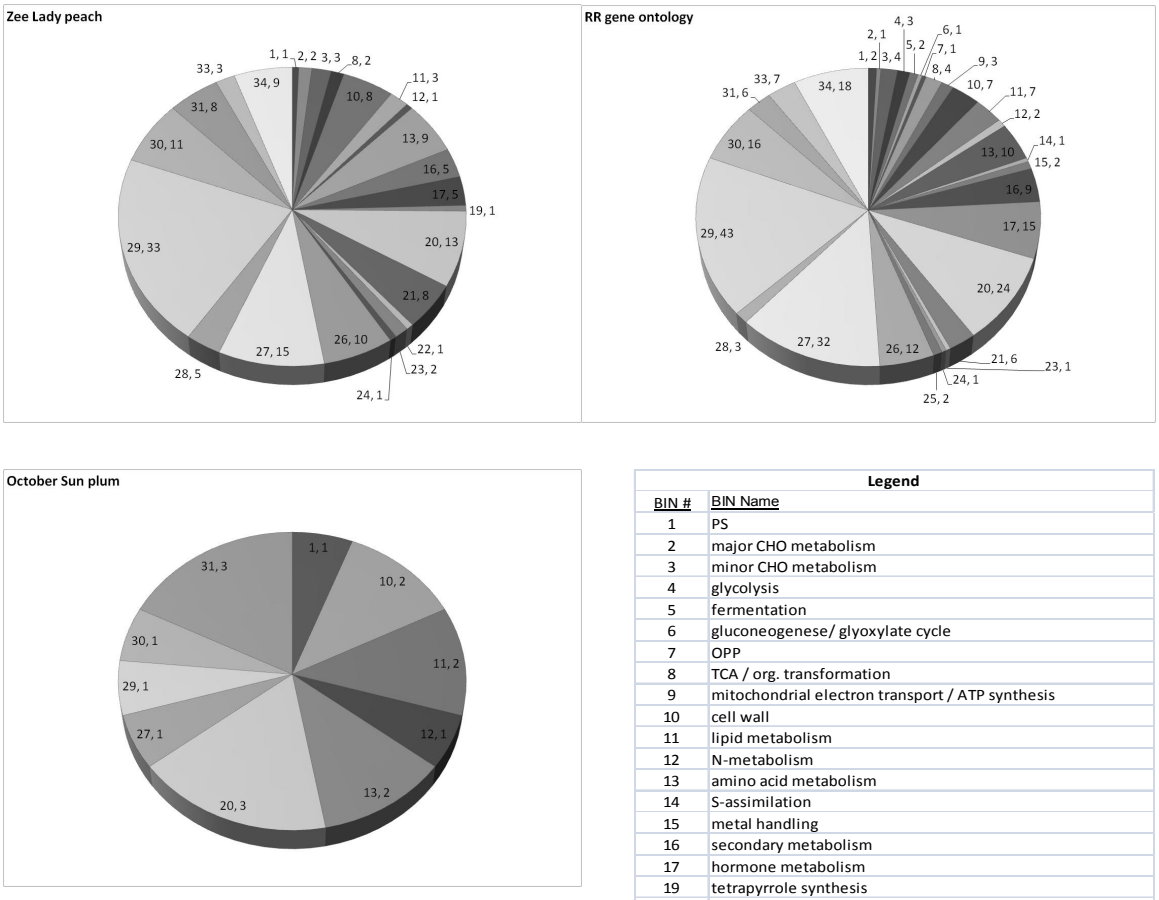


Fig. 3. Venn diagram showing the number of common (in overlapped region) and specific genes significantly affected by 1-MCP treatments in three cultivars after 4 weeks of storage at 0°C.



Legend	
BIN #	BIN Name
1	PS
2	major CHO metabolism
3	minor CHO metabolism
4	glycolysis
5	fermentation
6	gluconeogenese/ glyoxylate cycle
7	OPP
8	TCA / org. transformation
9	mitochondrial electron transport / ATP synthesis
10	cell wall
11	lipid metabolism
12	N-metabolism
13	amino acid metabolism
14	S-assimilation
15	metal handling
16	secondary metabolism
17	hormone metabolism
19	tetrapyrrole synthesis
20	stress
21	redox.regulation
22	polyamine metabolism
23	nucleotide metabolism
24	Biodegradation of Xenobiotics
25	C1-metabolism
26	misc-pectin methyl esterases inhibitor family, protease inhibitor and other enzymes
27	RNA
28	DNA
29	protein
30	signalling
31	cell
33	development
34	transport

Fig. 4. Distribution of differentially expressed genes after 1-MCP treatment and 4 weeks of storage at 0°C in according the Mapman platform. BINs were assigned on the basis of homology to Arabidopsis sequences. First digit is the BIN number which corresponds to the annotated BIN name (legend), second digit is the number of genes significantly expressed at this BIN.