

Comparative Transcriptomic Analysis of Plum Fruit Treated with 1-MCP

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Abstract

Microarray technology has allowed the large scale transcriptomic analysis of fruit ripening. The μ PEACH1.0 microarray containing 4,806 probes corresponding to genes expressed in peach fruit tissues has been used in a heterologous fashion in two studies of plums ripening behavior. Gene expression of different cultivars of plums treated with the ethylene antagonist, 1-methylcyclopropene (1-MCP) and stored for short periods at room temperature or for longer periods of cold storage was examined. In the first study, mature fruit of a suppressed ethylene climacteric cultivar 'Shiro' and a cultivar characterized by a typical increase of ethylene production during ripening ('Santa Rosa') were harvested and incubated for 24h in air (control) or 1-MCP and allowed to ripen at room temperature. Different levels of transcripts of genes implicated in cell wall metabolism, hormone (ethylene and auxin) regulation, stress and defense, and in the transcription/translation machinery, as well as others involved with ripening were identified. In the second study, the effects of 1-MCP on gene expression in relation to the development of chilling injury (CI) in the climacteric cultivars 'Ruby Red' (RR) and 'October Sun' (OS) and 'Zee Lady' peaches (ZP) were analyzed. The fruit were treated for 24h at room temperature with 1-MCP prior to storage at 0°C. For RR, there was no significant effect of 1-MCP on the level of CI symptoms, while 1-MCP significantly reduced CI symptoms in OS fruit and an increase of CI in treated ZP fruit. Microarray analysis showed that immediately following treatment, 186, 134 and 56 genes were differentially expressed between the control and 1-MCP-treated fruit of these cultivars, respectively: after 4 weeks cold storage, 311, 52 and 224 genes for RR, OS and ZP, respectively, were differentially expressed between control and treated fruit. Thus, for OS, the number of differentially expressed genes reduced during storage while the number increased in RR and ZP. Comparisons of the data suggest that the transcript profile is altered by 1-MCP more in plums than peaches. These studies, carried out within an international collaborative network, will increase our understanding of the regulation of pathways involved in plum fruit ripening and in metabolic processes related to storage and shelf life.

INTRODUCTION

1-methylcyclopropene (1-MCP), is an influential chemical compound both in technological (applied) aspects for maintaining fruit quality and in order to elucidate the role of ethylene in fruit ripening and other developmental processes (Watkins, 2006).

Plum fruit is a highly perishable commodity with a relatively short shelf life, however, differences in terms of ripening properties exist and plum cultivars are categorized as suppressed-climacteric and climacteric cultivars (Abdi et al., 1998). In addition, plum fruit are susceptible to the incidence of chilling injury (CI) symptoms after removal from cold storage. Although these constraints represent a strong limitation to market life, research has been focused on the identification of the symptoms and strategies to alleviate CI (Crisosto et al., 2004; Manganaris et al., 2008), while no information at molecular level is available. Application of 1-MCP in plums showed that its postharvest life can be extended after harvest or after removal from cold storage due to both a delay of ripening processes and, in some cases, a reduced incidence of CI disorders (Martinez-Romero et al., 2003; Manganaris et al., 2007; Khan and Singh, 2008, 2009). However, in peach, a related species, 1-MCP has been shown to have limited effect in maintaining fruit quality and increased incidence of CI following treatment and cold storage (Girardi et al., 2005; Dal Cin et al., 2006; Ziliotto et al., 2008).

In the current study the effect of 1-MCP alone or combined with cold storage on plum fruit with diverse ripening properties was studied at a transcriptome level. For transcriptomic studies the first available peach microarray (μ PEACH1.0) (Trainotti et al., 2006) was used in a heterologous and comparative fashion.

MATERIALS AND METHODS

Mature fruit of 'Shiro' plums (SH, suppressed ethylene climacteric) and 'Santa Rosa' (SR, climacteric) were harvested and incubated for 24h in air (control) or 1-MCP ($1 \mu\text{l L}^{-1}$). After treatment fruit were allowed to ripen at room temperature for 3 days. Data obtained from earlier studies (Ziliotto et al., 2008) in nectarine fruit ('Fantasia') were used in order to define plum-specific, peach-specific or genes that showed a similar expression pattern in both species during postharvest ripening.

In a different experiment, the effects of 1-MCP on gene expression in relation to the development of CI in the climacteric cultivars 'Ruby Red' (RR) and 'October Sun' (OS) were analyzed also in comparison with the peach 'Zee Lady' (ZP). Fruits were treated for 24h at room temperature with 1-MCP (625 nl L^{-1}) prior to cold storage at 0°C for 4 weeks.

RNA was isolated according to Bonghi et al. (1998). In order to remove contaminant DNA from the RNA samples, the nucleic acid extract was treated with DNase (RQ1, Promega), according to the manufacturer's instructions. Absorbance at 260 nm was used to measure the concentration of RNA and its integrity was checked on agarose gel. RNA conversion into target cDNA, microarray hybridization, and data analysis were carried out as described by Trainotti et al. (2007). Genes showing \log_2 ratio either >1 (up-regulated) or <-1 (down-regulated) were annotated following the Gene Ontology categories (GO) developed by TAIR.

RESULTS AND DISCUSSION

Effects of 1-MCP after Treatment

At the end of the treatment, no differentially expressed genes were identified in common to the two plum cultivars SH and SR, and between SH and F nectarine (Fig. 1). Considering SR and F fruit, 10 and 15 genes induced or repressed by 1-MCP at the end of treatment, respectively, were common to both cultivars (Fig. 1). After treatment, the inhibitory effect of 1-MCP was maintained for SR fruit, while in 'Fantasia' a marked recovery of transcription was observed for many genes including some involved in the ethylene perception, auxin-action and cell wall metabolism. These genes could be responsible for the different effects of 1-MCP observed in climacteric peach and plum

cultivars.

Effects of 1-MCP after Removal from Cold Storage

For RR, there was no significant effect of 1-MCP on the level of chilling injury while for OS 1-MCP significantly reduced CI. Microarray analysis showed that immediately following treatment, among genes identified after SAM analysis (186 and 134 for control and 1-MCP-treated fruit of RR and OS, respectively) those already identified by the first approach (Table 1) were also present. After 4 weeks of cold storage, the number of genes in OS decreased, while they increased in RR. Comparison of these microarray data with those obtained from peaches (ZP) suggests that the transcript profile is altered in plums by 1-MCP more than in peaches. Among the genes showing up-regulation after 4 weeks, some were involved in temperature-stress responses in all genotypes, in gamma-aminobutyrate acid (GABA) metabolism (*γ-aminobutyrate transaminase*) only in RR and OS and in some components of a redox-system responsible for cold-resistance. This latter gene, in addition to others already identified with roles in cell wall metabolism, membrane permeability, endomembrane trafficking, could be involved in the development of resistance to CI symptoms.

CONCLUSIONS

Overall, this preliminary transcriptomic approach suggests that the peach microarray can be employed in a heterologous fashion for plum fruit where relatively few data regarding gene expression during fruit ripening exist. Further experiments should be carried out in order to shed light on plum ripening after harvest or after removal from cold storage. The existence of plum cultivars with different ripening properties in terms of ethylene production, along with the available knowledge in peach fruit ripening can be used to define common or divergent mechanisms between the two related, yet phenotypically distinct species.

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Tables

Table 1. Number of genes differentially expressed by comparing untreated and treated fruit at time 0 (T0) and after four weeks.

Variety	T0	4 weeks storage (0°C)
‘October Sun’ plums (OS)	134	52
‘Ruby Red’ plums (RR)	186	311
‘Zee Lady’ peaches (ZP)	56	224

Figures

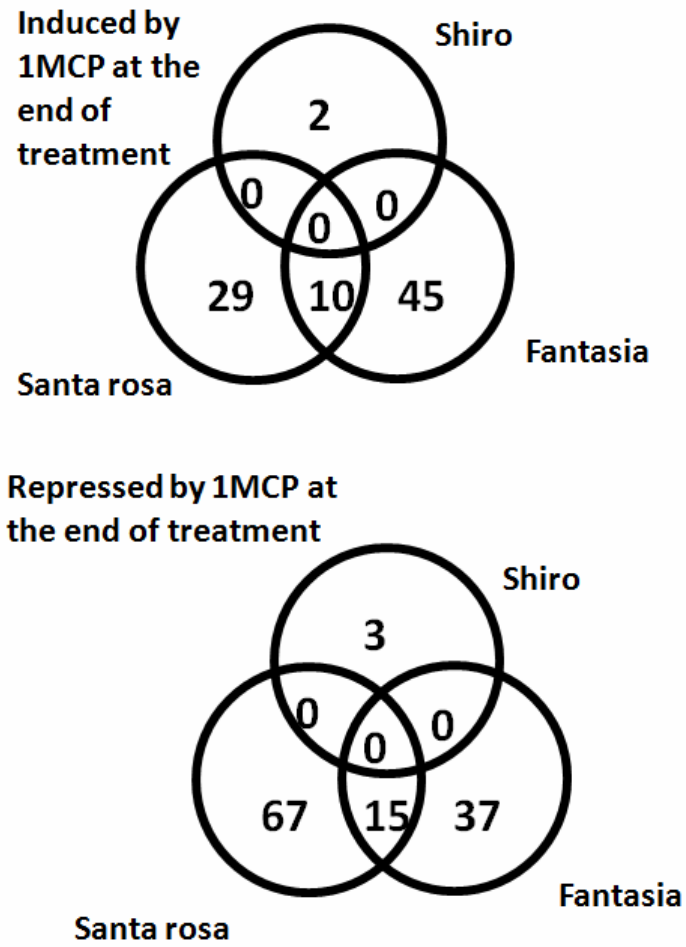


Fig. 1. The Venn diagrams show the numbers of differentially expressed genes (induced, top and repressed, bottom) at the end of the 1-MCP treatment in 'Shiro', 'Santa Rosa' and 'Fantasia'.

