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Melatonin limited ethylene production, softening and reduced physiology disorder in pear (*Pyrus communis* L.) fruit during senescence

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ABSTRACT

Some pear (*Pyrus communis* L.) fruit show a certain resistance to ripening. Thus, postharvest cold storage is commonly used to accelerate their ripening process by promoting ethylene production. However, the enhanced ethylene biosynthesis also accelerates pear fruit senescence. Here, the effects of melatonin on the senescence process in pear fruit was investigated. In a rapidly softening pear 'Starkrimson', melatonin delayed the ethylene burst. In 'Abbé Fetel' and 'Red Anjou', normally softening pears, melatonin inhibited ethylene production during the entire senescence process. The limited ethylene production resulted in a lower loss of firmness in melatonin-treated fruit than in water treated fruit. *PcPG*, a major cell wall degradation-related gene, was inhibited by melatonin in all three cultivars. The expressions of ethylene biosynthesis genes *PcACS1* and *PcACO1* are correlated with the senescence process. The former was inhibited by melatonin in 'Starkrimson', both were inhibited in 'Red Anjou', but neither was inhibited in 'Abbé Fetel'. Moreover, the antioxidant systems in 'Starkrimson' and 'Abbé Fetel' were enhanced by melatonin and their fruit did not undergo water soaking or core browning. Thus, melatonin has the potential to retain the commercial value of postharvest pear fruit and delay senescence by limiting ethylene production and the reactive oxygen burst.

1. Introduction

Ripening and senescence are both developmentally programmed processes in most fleshy fruit. While senescence shares some similar characteristics with ripening, such as fruit softening, respiration bursts, enhanced ethylene production, cell wall modifications and color changes, they are still distinct processes (Gapper et al., 2013; Klee and Giovannoni, 2011). Senescence is defined as the final stage of plant development. Programmed cell death marks this final stage and causes a series of irreversible events, such as cellular breakdown and organ death (Humbeck, 2013; Gomez et al., 2014; Tian et al., 2013; Liang et al., 2015). In contrast, fleshy fruit ripening is usually marked by the traits that contribute to the final fruit quality (Sun et al., 2016; Osorio et al., 2013).

Based on the different respiration rates during ripening, fruit is classically defined as climacteric and non-climacteric (Cherian et al., 2014; Pech et al., 2008). An accelerated respiration rate and enhanced ethylene production are commonly observed in climacteric fruit, such as apple, peach and banana, during their ripening processes. Most European pear cultivars are typical climacteric fruit. The ethylene production and respiration rate influence their ripening and senescence (Nashima et al., 2013; Hiwasa et al., 2004). However, unlike other climacteric fruit, most European pear cultivars show a certain resistance to ripening (Villalobos-Acuña and Mitcham, 2008; Murayama et al., 1998). Thus, postharvest cold storage is commonly used to properly ripen pear fruit by inducing ethylene production (Fonseca et al., 2005).

However, the promotion of ethylene biosynthesis also accelerates senescence with excess reactive oxygen species (ROS) production in fruit, shortening its shelf-life and causing physiological disorders, such as water soaking and core browning (Mao et al., 2004; Tian et al., 2013). Over-accumulated ROS in cells may threaten membrane stability by causing lipid peroxidation and form toxic products such as MDA (Matamoros et al., 2010). Antioxidant enzymes are involved in modulating the steady state of ROS concentration in plant cells. Superoxide dismutase (SOD) converts superoxide anion radical (O_2^-) to hydrogen peroxide (H_2O_2), while peroxidase (POD) convert H_2O_2 to water. Ascorbate, as an efficient antioxidant, regenerated by dehydroascorbate

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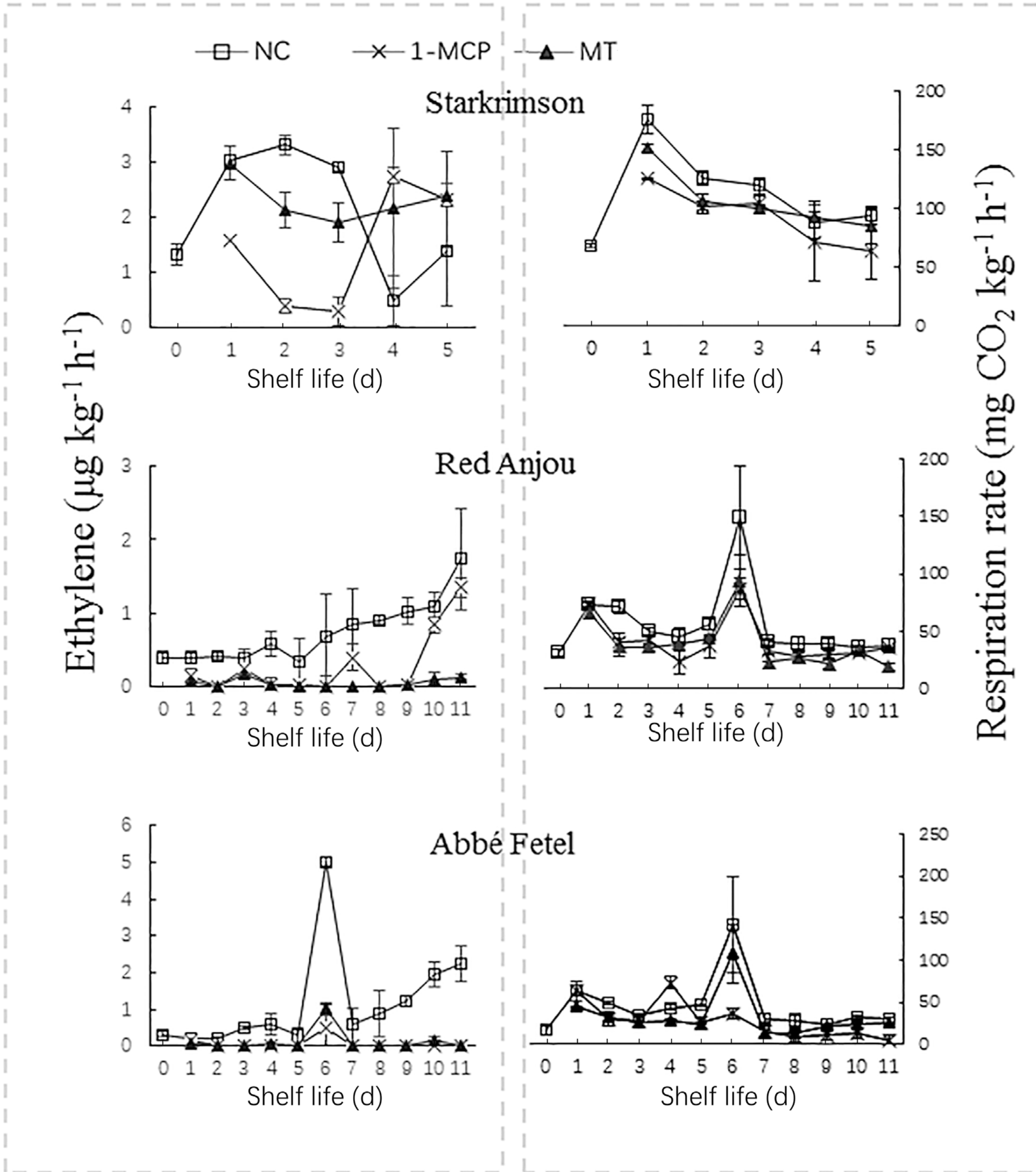

Fig. 1. Ethylene production and respiration rates during shelf life at 26 °C. NC, 1-MCP and MT represent the negative control, 1-MCP treatment and melatonin treatment, respectively. Bars indicate standard deviations from each mean of 5 replications. Tukey's HSD, α = 0.05.

reductase (DHAR) using reduced glutathione (Matamoros et al., 2010). Moreover, as biomarkers of lipid peroxidation, lipoxygenase (LOX) can catalyze oxidation of polyunsaturated fatty acids (Imahori et al., 2016).

Cell wall modification and degradation influence fruit flesh firmness, juiciness and crispness during ripening and senescence (Goulao and Oliveira, 2008; Manrique and Lajolo, 2004; Sun et al., 2013). In plant primary cell walls, cellulose microfibrils are coated with and cross-linked together with hemicellulose, and the spaces in these networks are filled with pectin, which also forms a network (Wang et al., 2013). It is a complicated process in which a wide range of cell wall-modifying and −degrading enzymes and proteins act in an interdependent and correlated manner to modify cell wall polymers (Bashline et al., 2014; Zdunek et al., 2014). Polygalacturonase (PG) and cellulase (Cel) are responsible for the modification of pectin and cellulose, respectively, in pear fruit. The genes encoding these two enzymes have also been isolated (Song et al., 2016). The expression patterns of *PcPG1* were correlated with the loss of firmness in 'Starkrimson' and 'La France' pears during postharvest storage (Hiwasa et al., 2004; Song et al., 2016). *PcCel* regulates the rate of cellulose synthesis (Song et al., 2016).

N-acetyl-5-methoxytryptamine (melatonin) is a pleiotropic molecule that was initially identified as a powerful scavenger of free radicals involved in various plant processes (Posmyk and Janas, 2009; Tan et al., 2011; Arnao and Hernández-Ruiz, 2015; Van Tassel et al., 2001). A browning disorder during plant tissue culturing is inhibited by melatonin (Dan et al., 2014), indicating that melatonin is an effective antioxidant to limit the oxidation of phenolic compounds to quinines. Moreover, melatonin shows a diversity of functions in plant growth regulation and affects the biosynthesis pathways of other plant hormones (Arnao and Hernández-Ruiz, 2014; Pelagio-Flores et al., 2012; Sun et al., 2015; Arnao and Hernández-Ruiz, 2007; Li et al., 2015). Ethylene production is slightly induced by melatonin during tomato ripening, and the ethylene biosynthetic gene 1-aminocyclopropane-1-carboxylic acid synthase 4 (*ACS4*) was also up-regulated by melatonin