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Effect of 1-methylcyclopropene on phenylpropanoid metabolism, the accumulation of phenolic compounds, and browning of whole and fresh-cut 'iceberg' lettuce

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Abstract

Exposing whole heads or leaves of iceberg lettuce (*Lactuca sativa* L., iceberg) to 1-methylcyclopropene (1-MCP) significantly decreased the accumulation of phenolic compounds (absorbance of methanol tissue extract at 320 nm) and subsequent tissue discoloration induced by exposure to 1.0 $\mu L\,L^{-1}$ ethylene in air at 5 °C. The 0.5 $\mu L\,L^{-1}$ concentration of 1-MCP was just as effective as 1.0 $\mu L\,L^{-1}$, and a 3-h exposure was just as effective as a 24-h exposure at 5 °C. In contrast, exposure to 1-MCP either before or after excision of mid-rib tissue did not interfere with the wound-induced increase in phenolic content of the tissue. It appears that wounding and ethylene act independently in the induction of phenylpropanoid metabolism and the accumulation of those phenolic compounds that contribute to browning of mechanically injured (e.g., fresh-cut) iceberg lettuce.

Keywords: Ethylene; PAL; Phenylalanine ammonia-lyase; Pink rib; Russet spotting; Rusty brown discoloration; Wound signal

1. Introduction

Physiological disorders of lettuce (*Lactuca sativa* L., iceberg) that involve tissue discoloration (e.g. russet spotting, rusty brown discoloration, pink rib, etc.) continue to significantly reduce quality in whole and fresh-cut lettuce. These physiological disorders share common metabolic pathways that involve the production and perception of the plant hormone ethylene, and the induction of increased phenylpropanoid metabolism. Lettuce grown with a minimum of abiotic and biotic stress has low levels of phenylalanine ammonia-lyase (PAL, EC 4.3.1.5) activity, and low levels of the phenolic compounds (e.g., chlorogenic and caffeoltartic acid) that accumulate and contribute to tissue discoloration (Tomás-Barberán et al., 1997). The synthesis and accumulation of stress-induced phenolic compounds in lettuce is dependent upon the de novo synthesis of PAL (Peiser et al., 1998), the first committed step in the synthesis of phenylpropanoid compounds (Hahlbrock and Sheel, 1989).

Wounds incurred during processing of fresh-cut lettuce produce a signal that induces the synthesis of PAL protein, increases PAL activity and stimulates the accumulation of those phenolic compounds that contribute to tissue discoloration in injured and adjacent tissue (Ke and Saltveit, 1989; Campos-Vargas et al.,

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2004). Wounded lettuce tissue is therefore far more prone to develop discoloration than non-wounded tissue because of the elevated phenolic content and activity of associated enzymes induced by wounding (e.g., PPO and POD) (Brecht, 1995; Saltveit, 1997). Wounding also stimulates ethylene synthesis that further increases phenolic metabolism in many plant tissues (Abeles et al., 1992; Saltveit, 1999). However, wound-induced rates of ethylene production from lettuce are very low compared with other species, and they return to non-stressed control levels within 24 h (Ke and Saltveit, 1989). The synergistic action of wounding and exposure to hormonal levels of ethylene can drastically reduce the quality and shelf life by inducing tissue browning (López-Gálvez et al., 1996; Saltveit, 1997). However, wound-induced ethylene has a negligible effect on the increase in phenolic metabolism induced by wounding of lettuce leaf tissue (Ke and Saltveit, 1989; Campos-Vargas and Saltveit, 2002).

Ethylene perception and action are reduced by prior exposure of tissue to 1-methylcyclopropane (1-MCP) (Serek et al., 1995). The development of russet spotting (RS) was delayed in iceberg lettuce leaves exposed to 0.9 $\mu L\,L^{-1}$ 1-MCP for 4 h at 6 °C (Fan and Mattheis, 2000). RS is a postharvest physiological disorder of lettuce induced by ethylene stimulation of phenylpropanoid metabolism (Ke and Saltveit, 1988). Wills et al. (2002) reported that exposure to 0.1 $\mu L\,L^{-1}$ of 1-MCP for 1 h at 5 °C was optimal at increasing the storage life of shredded iceberg lettuce. Tomás-Barberán et al. (1997) showed that both ethylene (10 $\mu L\,L^{-1}$) and wounding induce elevated activity of phenylpropanoid metabolism, and the accumulation of those phenolic compounds that contribute to tissue discoloration in butter leaf, iceberg and Romaine lettuce. In every case, wounding induced greater phenolic accumulation than did ethylene.

Application of 1-MCP to non-wounded lettuce prior to ethylene exposure should interfere with its induction of phenylpropanoid metabolism and reduce ethylene-induced disorders, but application prior to wounding should not disrupt wound-induced increases in phenylpropanoid metabolism and tissue browning if wounding acts independent of ethylene. The object of the research reported here was to determine the effectiveness of 1-MCP treatments in reducing wound- and ethylene-induced phenolic metabolism, accumulation of phenolic compounds and tissue browning induced by wounding during the simulated preparation of fresh-cut lettuce.

2. Materials and methods

2.1. Plant material

Mature heads of lettuce (*L. sativa* L., iceberg) were obtained from local commercial sources and stored at 2.5 °C until used. Outer damaged or wilted leaves were discarded and the whole heads, individual leaves, or excised 2 cm × 0.5 cm mid-rib tissue pieces were used for experimentation. When whole heads or individual leaves were treated with 1-MCP and ethylene, mid-rib tissue was excised from the same general location after treatment. Since wounding induces PAL activity in tissue up to 2.5 cm away from the site of wounding (Ke and Saltveit, 1989), only whole leaves free of injury were used. They were cut at their bases and carefully separated from the head so that wounding (e.g. cracks, rips, etc.) did not occur within at least 3 cm of the tissue to be used for experimentation. Seven grams of excised mid-rib tissue was put into each 25 mm × 100 mm plastic Petri dish. The dishes were placed in a 4-L plastic tub lined with wet paper towels and loosely covered with aluminum foil. The tubs were held at 5 °C for 24 or 48 h after wounding.

2.2. Treatments with 1-MCP

Whole trimmed heads, or an uncovered 4-L tub containing dishes of freshly excised mid-rib pieces were put into a 117-L opaque plastic container. A concentration of 0.5–1.0 $\mu L\,L^{-1}$ 1-MCP (SmartFresh^{TM}) was established in the container following instructions for use of the SmartFresh^{TM} tablets provided by AgroFresh, Inc. (Spring House, PA, USA).

2.3. Treatment with ethylene

Whole heads or excised tissues that had been treated with 1-MCP were enclosed in 20-L glass containers. Sufficient ethylene was injected to produce a 1.0 $\mu L\,L^{-1}$ atmosphere. An open Petri dish containing one layer of KOH pellets was included in the jar to absorb carbon dioxide. Head space gas samples (1 ml) were periodically taken for carbon dioxide and

ethylene analyses. An infrared analyzer (PIR 2000, Horiba) was used to measure carbon dioxide (Saltveit and Strike, 1989), while a gas chromatograph (Model 8000, Carle Instruments) equipped with alumina column and a flame ionization detector was used to measure ethylene (Saltveit and Yang, 1987).

2.4. Measure of phenolic content

Tissue from each Petri dish was cut into small pieces (ca. 1 mm × 2 mm) and put into a 50-mL plastic centrifuge tube along with 20 mL of methanol. The tissue was ground, and a 2-mL aliquot was centrifuged at 10,000 × *g* to produce a clarified solution (Ke and Saltveit, 1989; Campos-Vargas and Saltveit, 2002). The absorbance of the clarified methanol extract was read at 320 nm (Loaiza-Velarde et al., 1997) and expressed as absorbance per gram fresh weight.

2.5. Measure of PAL activity

Phenylalanine ammonia-lyase activity was measured as previously described by Ke and Saltveit (1989), with slight modifications (Campos-Vargas and Saltveit, 2002).

2.6. Statistical analysis

Each experiment had at least three replicates of each treatment and all experiments were run at least twice with similar results. Measurements from all the replicates were combined and treatment effects subjected to ANOVA, and 5% LSD values calculated when significant treatment differences were detected.

3. Results and discussion

3.1. Wound-induced PAL activity and phenolic accumulation

Wounds incurred during the excision of mid-rib tissue were sufficient to induce elevated levels of PAL activity and the accumulation of phenolic compounds (absorbance of a methanol extract at 320 nm) (Fig. 1). PAL activity increased significantly after 12 h at 5 °C, while the phenolic content did not show a significant increase until 24 h after excision. The correlation between PAL activity and phenolic content of excised mid-rib tissue using data from zero to 36 h was very strong ($R^2 = 0.99$), and is given by the equation: Absorbance at 320 nm = (1.0 × PALactivity) + 0.22 (Fig. 1). Browning of the edges of the excised tissue became evident 24 h after excision and was sufficient to decrease visual quality by 36 h (data not shown). In this study, visual quality was significantly reduced when the phenolic content of the tissue exceeded about 0.6. These results confirm previous reports that excision of mid-rib tissue induces elevated levels of PAL activity, and the subsequent accumulation of phenolic compounds and tissue browning (Campos-Vargas and Saltveit, 2002; Ke and Saltveit, 1989; Tomás-Barberán et al., 1997).

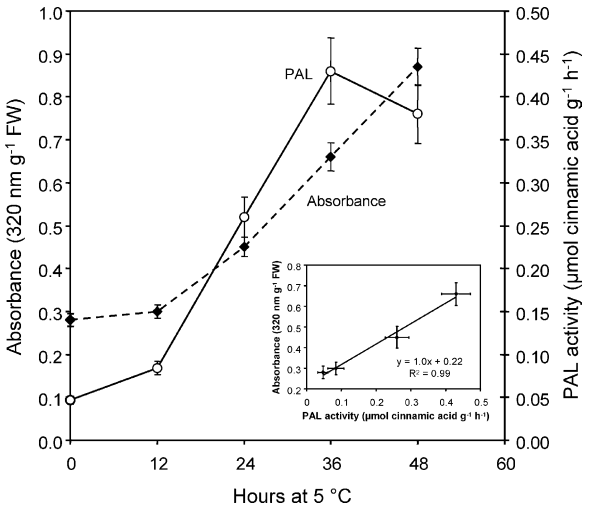

Fig. 1. Increases in PAL activity and phenolic content of excised mid-rib iceberg lettuce tissue. Measurement of PAL activity and phenolic content are described in Section 2. Inset shows relation between PAL activity and phenolic content. Vertical lines represent the standard error about individual means.

3.2. Effect of 1-MCP on ethylene-induced phenylpropanoid metabolism

While there was a slight increase (ca. 30%) in the phenolic content of mid-rib tissue of whole heads of iceberg lettuce during 48 h storage at 5 °C, the increase induced by exposure to 1.0 μL L^{-1} ethylene in air was significantly greater (ca. 300%) (Fig. 2). Exposure to 1.0 μL L^{-1} 1-MCP for 6 h prior to exposure

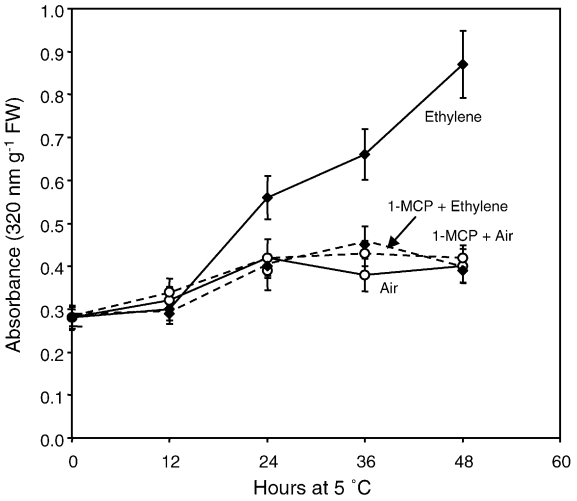

Fig. 2. Increase in phenolic concentration in mid-rib tissue of whole heads of iceberg lettuce exposed to air or 1.0 μL L^{-1} 1-MCP for 6 h at 5 °C before exposure to air or 1.0 μL L^{-1} ethylene for 48 h at 5 °C. Absorbance of a clarified methanol extract was read at 320 nm. Vertical lines represent the standard error about individual means.

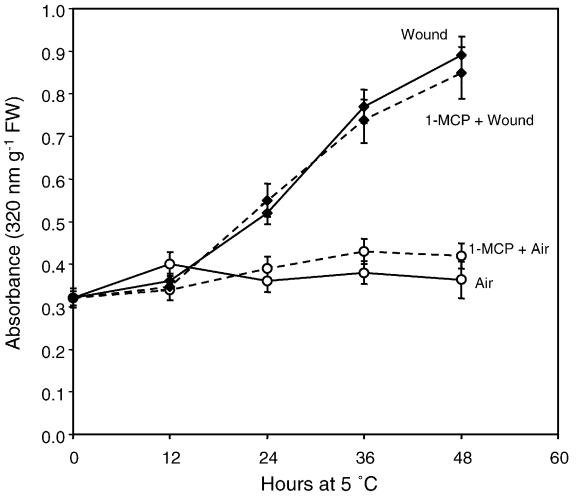

Fig. 3. Increase in phenolic concentration in mid-rib tissue of whole heads of iceberg lettuce exposed to air or 1.0 μL L^{-1} 1-MCP for 6 h at 5 °C before excision of the mid-rib tissue and holding the excised tissue for 48 h at 5 °C in an ethylene-free atmosphere. Absorbance of a clarified methanol extract was read at 320 nm. Vertical lines represent the standard error about individual means.

to air or ethylene did not significantly alter the pattern of phenolic concentration from that seen in the air-treated control tissue. Similar results were obtained from exposing whole heads or individual leaves to 0.5–1.0 μL L^{-1} 1-MCP for 3–24 h before exposure to ethylene (data not shown). These results confirm the ability of ethylene to promote phenolic accumulation in the mid-rib area of lettuce leaves (Ke and Saltveit, 1988; Tomás-Barberán et al., 1997; Campos-Vargas and Saltveit, 2002), and for 1-MCP to inhibit these inductive effects of ethylene (Serek et al., 1995; Wills et al., 2002).

3.3. Effect of 1-MCP on wound-induced phenylpropanoid metabolism

In contrast to its effectiveness in inhibiting ethylene-induced increases in the phenolic content of mid-rib tissue, prior exposure of whole heads of lettuce to 1.0 μL L^{-1} 1-MCP at 5 °C for 6 h before excision did not inhibit the wound-induced accumulation of phenolic compounds in treated tissue excised and held at 5 °C for 0–48 h (Fig. 3). Similar results were obtained with 1-MCP exposure to both whole heads and leaves, and exposure to 0.5 or 1.0 μL L^{-1} 1-MCP for 3–24 h at 5 °C before wounding (data not shown). The wound signal responsible for increased phenylpropanoid metabolism in fresh-cut lettuce was not identified as one of the compounds (i.e., abscisic acid, ethylene, jasmonic acid, methyl jasmonate, salicylic acid) previously reported as a wound signal in other plant tissues (Campos-Vargas and Saltveit, 2002). Wound-induced increases in PAL activity, phenolic accumulation or subsequent tissue browning do not appear to act through the wound induction of ethylene production and the subsequent induction of phenylpropanoid metabolism by increased levels of ethylene (Ke and Saltveit, 1989; Campos-Vargas and Saltveit, 2002). The results with 1-MCP support these previous reports.

3.4. Effect of 1-MCP on phenolic content and quality

Control (non-wounded, no ethylene) and 1-MCP-treated whole heads had low levels of phenolic compounds, as did 1-MCP tissue exposed to ethylene (Fig. 4). Exposure to ethylene in the absence of prior

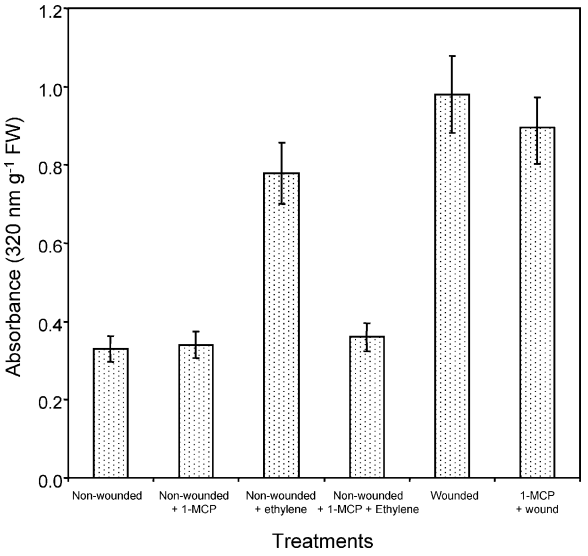

Fig. 4. Phenolic content of mid-rib tissue excised from iceberg lettuce. Whole heads were either exposed to air (non-wounded) or 1.0 μL L^{-1} 1-MCP (+1-MCP) for 6 h at 5 °C either before excision, exposure to 1.0 μL L^{-1} ethylene (+ethylene), or wounding (+wound). The whole heads or excised tissue were held at 5 °C for an additional 48 h before the phenolic content of the mid-rib tissue was determined as the absorbance of a clarified methanol extract at 320 nm. The vertical line atop each bar represents the standard error about that mean.

1-MCP treatment, or to wounding either alone or in tissue previously treated with 1-MCP, led to an increase in phenolic compounds that produced severe edge browning. The accumulation of wound- and ethylene-induced phenolic compounds reached a level sufficiently high (absorbance greater than 0.6) after 48 h at 5 °C to produce enough edge browning to reduce visual quality.

Both wounding (as during the preparation of fresh-cut salads) and exposure to ethylene (as during storage with ethylene-producing commodities or in polluted atmospheres) induced increased phenylpropanoid metabolism which resulted in the synthesis and accumulation of phenolic compounds that contributed to tissue discoloration (Tomás-Barberán et al., 1997). It appears that the effectiveness of 1-MCP in inhibiting ethylene-induced accumulation of phenolic compounds in mid-rib lettuce tissue does not extent to the wound-induced accumulation of phenolic compounds in similar tissue.

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