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Effect of 1-methylcyclopropene on senescence and sugar metabolism in harvested broccoli florets

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ABSTRACT

The effect of 1-methylcyclopropene (1-MCP) treatment on superficial color, sugar content and the activities of sugar metabolism enzymes, the expression of sucrose transporters and carbohydrate metabolizing enzymes in broccoli florets were investigated. Broccoli florets treated with 2.5 μL L^{-1} of 1-methylcyclopropene (1-MCP) inhibited the increase of lightness (L^*) value and retarded the decrease in hue angle (H) value and chlorophyll content. This treatment maintained higher levels of sugars comparing with control florets. In general, the activities of sucrose synthase-synthesis direction (SS-S) were enhanced in florets treated with 1-MCP, whereas the activities of sucrose synthase-cleavage direction (SS-C), glucokinase (GK) and UDP-glucose pyrophosphorylase (UGPase) were reduced. In addition, the expression of genes encoding sucrose transporters (*BoSUC1* and *BoSUC2*) and carbohydrate metabolizing enzymes (*BoINV1*, *BoHK1* and *BoHK2*) was induced upon 1-MCP treatment. These results indicated that 1-MCP can delay senescence of broccoli florets in the present study may be attributed to maintaining higher sugar content through regulation of sugar metabolism.

1. Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) is harvested at the immature stage and its florets senesces quickly after harvest at ambient temperatures. During broccoli senescence, a loss of the green color is observed due to the degradation of chlorophyll in florets, which reduces the commercial acceptance of this product. Various postharvest techniques such as controlled atmosphere storage (Izumi et al., 1996) and application of ethanol (Han et al., 2006) or 6-BA treatment (Xu et al., 2012) have been demonstrated to delay postharvest yellowing, improve visual quality and extend shelf life of broccoli.

In addition to act as energy substances, sugars have pivotal hormone-like functions in sensing and signaling mechanisms as signaling molecules and have been implicated in plant development and senescence (Rolland et al., 2002). It has been found that a fast accumulation of soluble sugars in banana peel led to repression of chlorophyll catabolism and the development of stay-green ripe fruit (Yang et al., 2009). A change in the sugar concentration was demonstrated to be closely associated with epicarp degreening and regreening of certain citrus fruit (Huff, 1984). In leaves of roquette, the extension in postharvest shelf life was related to higher sucrose level (Clarkson et al., 2005). In broccoli florets, a rapid loss of sucrose was observed during postharvest storage (Downs and Somerfield, 1997). It has been shown that exogenous sucrose supply (Irving and Joyce, 1995), visible light treatment (Büchert et al., 2011) or harvested at sunset time (Hasperué et al., 2011) can delay postharvest senescence and increase the shelf life of broccoli, possibly by maintaining higher endogenous soluble sugar levels. In broccoli tissues treated with cytokinin, the concentrations of sucrose, glucose and fructose remain higher than in controls (Downs et al., 1997). Meanwhile, it is generally accepted that the senescence of broccoli florets is closely associated with endogenous ethylene. The ethylene action inhibitor 1-MCP, has been found effective in delaying senescence and increasing storage life of broccoli (Ku and Wills, 1999; Yuan et al., 2010). However, it is still unclear whether sugars are involved in mechanisms by which 1-MCP delaying senescence in broccoli florets. The objectives of this study were to investigate the effect of 1-MCP on superficial color, chlorophyll and sugar contents, and activities of sugar metabolism enzymes, the expression profiles of genes associated with carbohydrate transport and metabolism

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after harvest in broccoli florets and to evaluate the possible role of sugars in delaying senescence of broccoli florets.

2. Materials and methods

2.1. Plant material and 1-MCP treatment

Broccoli (*Brassica oleracea* L. var. *italica*, cv. Chaoda No 1) heads were obtained from local producers (Nanjing, Province of Jiangsu, China), top iced and immediately transported to the laboratory within 2 h of harvest. Broccoli heads were randomly divided into two groups, untreated (control) and 2.5 $\mu L\,L^{-1}$ 1-MCP for 6 h at 20 °C. This specific 1-MCP treatment condition was chosen as optimal for extending the shelf life of broccoli according to our previous study (Xu et al., 2013). 1-MCP was released from a commercial Smart Fresh™ powder (a.i. 0.14%, Agrofresh Ltd., Philadelphia, PA, USA) by adding distilled water according to the manufacturer's instructions. After treatment, the broccoli heads were stored in the dark at 15 °C and 95% relative humidity for 4 days. There are three replicates of 30 heads each per treatment, and the whole experiment was conducted twice. Florets of three heads from each replicate were excised every day during storage, frozen with liquid nitrogen and stored at −80 °C for enzyme assay and RNA extraction.

2.2. Superficial color and chlorophyll content determination

The superficial color parameters were evaluated with a colorimeter (Minolta, Model CR-400, Osaka, Japan) by measuring the lightness (L^*) and hue angle (H) values. Chlorophyll concentrations were determined as described by Lichtenthaler (1987) with slight modifications. Frozen broccoli florets (0.16 g) were crushed by a mill in 10 mL of 95% ethanol, stirred and centrifuged at 12,000 × g for 10 min, and then the residue was removed. Chlorophyll quantification was performed spectrophotometrically at 665 and 649 nm and the chlorophyll content was expressed as chlorophyll mass on a fresh weight basis (mg/g).

2.3. Sugar analysis

Broccoli samples (2 g) was mixed with 10 mL distilled water and centrifuged at 12,000 × g for 10 min, and then the residue was removed. Sucrose, glucose and fructose were determined by high performance liquid chromatography (HPLC) using a Sugar-D column (250 × 4.6 mm) at a flow rate of 1.0 mL min^{-1}. Sucrose, glucose and fructose were identified by their retention times and were quantified according to standards.

2.4. Enzyme assay

For glucokinase (GK), UDP-glucose pyrophosphorylase (UGPase), soluble acid invertase (SAI) and sucrose-phosphate synthase (SPS) and sucrose synthase (SS), broccoli sample was extracted using a buffer containing 400 mM Tris–HCl (pH 8.5), 5 mM EDTA, 10 mM $MgCl_2$, 10 mM β-mercaptoethanol, 20% (v/v) glycerol, 1 mM phenylmethylsulphonyl fluoride, 10 mM ascorbic acid, 1% (v/v) Triton X-100, 10 mg L^{-1} of leupeptin, and 10 mg L^{-1} of chymostatin. In the case of the crude extract prepared for invertase measurements, 400 mM HEPES–NaOH (pH 8.8) was used instead of Tris–HCl (pH 8.5). In all cases, the samples were completely ground in a cold mortar in the presence of insoluble polyvinyl polypyrrolidone and centrifuged at 10,000 × g for 15 min at 4 °C. These crude extracts were used for enzyme activity measurements.

GK was measured according to the method of Mustroph and Albrecht (2003), the reaction mixtures was 0.1 M Tris–HCl, pH 8.5, with 5 mM $MgCl_2$, 0.5 mM NAD, 10 mM glucose, 2 mM ATP and 2 U of G6PDH. The reaction was started with ATP. UGPase activity was determined in a reaction mixture containing 80 mM Hepes, pH 7.8, 5 mM $MgCl_2$, 0.6 mM NAD, 1 mM UDP-glucose, 0.5 mM Ppi, 1 U of G6PDH, and 1 U of phosphogluconmutase (Sowokinos et al., 1997). SAI and SPS were assayed according to the methods of Hubbard et al. (1989) and Vargas et al. (2008) with some modifications. SAI activity at 37 °C was assayed by adding 100 mM sodium acetate (pH 4.8) and 100 mM sucrose solution. Reaction was stopped at 30 min by adding 1.5 mL DNS solution. The assay solution of SPS contained 50 mM Tris–HCl (pH 7.5), 15 mM $MgCl_2$, 1 mM EDTA, 1 mM fructose-6-P, 2 mM UDP-glucose and 500 μL of sample. Sucrose produced by these reactions was assayed using the anthrone assay (Van Handel, 1968). The SS activities were determined in the synthesis (SS-S) and cleavage (SS-C) direction. The procedure for the sucrose synthesis direction was identical to that of SPS except the reaction mixtures contained 10 mM fructose and did not contain fructose 6-P or glucose 6-P. The SS cleavage activity determination was the same as for SAI except the reaction mixtures contained 1 mM UDP.

2.5. Analysis of sucrose transport and carbohydrate metabolic genes

Reverse transcription PCR (RT-PCR) was used to analyze the expression pattern of the sucrose transporter and carbohydrate metabolic genes Broccoli sucrose transporter 1 (*BoSUC1*), Broccoli sucrose transporter 2 (*BoSUC2*), Broccoli acid invertase 1 (*BoINV1*), Broccoli hexokinase 1 (*BoHK1*) and Broccoli hexokinase 2 (*BoHK2*) in broccoli florets. Total RNA was extracted by the method of Chang et al. (1993). Reverse transcription was conducted according to the manufacturer's instructions of PrimeScriptTM 16 1st Strand cDNA Synthesis Kit (TaKaRa, Japan). The RT-PCR was performed with a total volume of 25 μL which contained aliquots of cDNA samples, 1 μL forward primer and reverse primer (20 μM), 10 μL of 10 × PCR buffer (Mg^{2+} free), 1.5 μL $MgCl_2$ (25 mM), 15.7 μL ddH_2O, 2 μL deoxy-ribonucleoside triphosphate (dNTP) (2.5 mM), 0.3 μL Taq polymerase (5 U μL^{-1}). 18S was used as an internal control. Primers specific to the following genes were used: *BoSUC1* (accession no. AY065840), forward: 5′-CCCGTTCGCAATGACCAA-3′, reverse: 5′-CTCCCACTTTCTTACCAATCCA-3′; *BoSUC2* (accession no. AY065839), forward: 5′-CTCGACGTGGCGAACAAC-3′, reverse: 5′-ACGGCCCATCCAATCAGT-3′; *BoHK1* (accession no. AF454961), forward: 5′-CAGCGGGTAGACAAAGGG-3′, reverse: 5′-GGAGTCGC-CAAAGAAAGC-3′, *BoHK2* (accession no. AF454962), forward: 5′-TTGTCGCCGCTGTTATTC-3′, reverse: 5′-CCTCCACGCTGTCTGATG-3′ *BoINV1* (accession no. AF274298), forward: 5′-AGGACCTCAAC-GACCCAC-3′, reverse: 5′-CCTCAGCCTCAACCACCA-3′; *18S* (accession no. AF513990), forward: 5′-CTTAGTTGGTGGAGCGATTT-3′, reverse: 5′-AAGGGCAGGGACGTAGTC-3′. Each measurement was performed in triplicate.

2.6. Statistical analyses

All statistical analyses were processed using SPSS package program version 16.0 (SPSS Inc., Chicago, IL, USA). All data were expressed as means ± the standard error (SE). The data were analyzed by one-way analysis of variance (ANOVA). Mean separations were performed by Duncan's multiple-range tests, and the differences at $p < 0.05$ were considered to be significant.

3. Results and discussion

Florets attached to broccoli heads began to degreen immediately after harvest, and changes in superficial color during broccoli senescence was evaluated through the color parameters hue angle (H) and lightness (L^*). 1-MCP treatment significantly ($p < 0.05$) inhibited the decrease of H value and the increase of L^* value