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Gibberellic acid enhances postharvest toon sprout tolerance to chilling stress by increasing the antioxidant capacity during the short-term cold storage

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ABSTRACT

Gibberellic acid (GA_3) has been known as an important phytohormone signal in plants. Here, we investigated the physiology responses underlying GA_3-induced postharvest toon sprout tolerance to chilling stress for 5 d. Results showed that exogenous application of GA_3 remarkably decreased the browning and decay index of toon sprout. GA_3 treatment prevented anthocyanin breakdown and inhibited the decreases of the total flavonoid, Vitamin C and titratable acidity in toon sprout during postharvest cold storage. In comparison to distilled water treatment, exogenous GA_3 application maintained significantly higher levels of reducing sugar, soluble sugar and proline in toon sprout. Meanwhile, GA_3 significantly reduced the accumulation of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in toon sprout. Furthemore, GA_3 enhanced the activities of antioxidant enzymes catalase (CAT) and superoxide dismutase (SOD) and reduced those of peroxidase (POD) and polyphenol oxidase (PPO). Taken together, our results suggested that exogenous application of GA_3 effectively enhanced postharvest toon sprout tolerance to chilling stress by regulating antioxidant enzymes and weakening lipid peroxidation.

1. Introduction

Toon sprout (*Toona sinensis*), which is also called Chinese "Xiangchun" sprout, is one of the most economically important woody vegetable in China, especially in Taihe county of Anhui province (Zhao et al., 2017). It is also cultivated commercially in many other parts of China including Henan, Shandong, Yunnan, and Sichuan province (Wang et al., 2008; Zhou et al., 2010; Liu et al., 2012). The commercial value of toon sprout has increased steadily due to its unique flavor and abundant nutrition, its market price is RMB 60 yuan per kilogram (Mu et al., 2007; Xia et al., 2015). Multi-functional properties of toon sprout are mainly involved in its antioxidant, anti-aging, and antimicrobial activities (Huang et al., 2012; Kakumu et al., 2014; Su et al., 2015). However, toon sprout has only half month growth period and very short shelf-life at ambient temperature due to loss of flesh, rapid browning and sprout decay after postharvest (Zhou et al., 2011; Wang and Liu, 2015). Postharvest preservation of toon sprout becomes the major constraint which greatly reduces its market value.

Several methods such as modified storage atmosphere, membrane packaging, low temperature storage, postharvest treatment with exogenous polyphenol, chitosan, antioxidants, herb extracts and other preservatives have been used for postharvest preservation of toon sprout (Zhang et al., 2009; Zhu et al., 2014; Chen et al., 2015; Diao and Gao, 2016). Among these methods, low temperature storage is commonly used to prevent toon sprout senescence and extend its shelf life. However, low temperature storage leads to symptoms of chilling injury such as watery lesions and browning of internal tissue (Zhou et al., 2011). Therefore, it has been a long-term aim for people to extend shelf-life of postharvest toon sprouts to alleviate or avoid chilling injury under low temperature storage.

Gibberellic acid (GA_3), an important phytohormone, has multi-functional physiological activities in plant, such as breaking seed dormancy, promoting flower bud differentiation and stem elongation, and delaying the senescence of plant organs (Achard et al., 2009; Seo et al., 2009; Sun, 2010). Recently, applications of exogenous gibberellic acid (GA_3) to delay ripening and to hold good quality of pre and postharvest crops have been monitored carefully (Steffens et al., 2011; Krisha et al., 2012; Huang et al., 2014; Souza et al., 2016;). Moreover, the treatment of horticultural crops and fruits with GA_3 could alleviate their chilling injury symptoms under low temperature storage (Tian et al., 2014; Harman and Sen, 2016; Zhu et al., 2016). Therefore, based on literature reports, applications of exogenous GA_3 may be a promising approach to withstand chilling stress on toon sprout during cold storage. However, there is scarcely relevant reference available on postharvest physiology

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and quality of toon sprout responses on GA_3 treatment. The aim of this study was to estimate the effects of exogenous GA_3 on postharvest physiological characteristics of toon sprout to alleviate chilling injury and to maintain quality of toon sprout.

2. Materials and methods

2.1. Plant material and treatment

Toon sprouts were collected in April 2017 from a greenhouse in the *T. sinensis* industry demonstration zone in Taihe County, Anhui, China, and delivered promptly to the laboratory after plucking. Robust sprouts with uniform size, color, and free from blights and insect pests were selected. Toon sprouts were sterilized with sodium hypochlorite 1% (v/v) for 2 min, rinsed and air-dried. After disinfection, the toon sprouts were soaked in 100 mg l^{-1} GA_3 solution for 20 min (the concentration of GA_3 was determined according to the preliminary experiment). The toon sprouts soaked in distilled water were used as the control group. All samples from control and treatment with GA_3 were stored at 4 ± 1 °C refrigerator with relative humidity 80 to 90% for 5 d. After 0, 3, and 5 d of cold storage, the toon sprouts from the control and treatment were taken out randomly for sprout browning evaluation and postharvest physiological and quality indices determination. All measured data were analyzed in triplicate with 10 toon sprouts per replicate.

2.2. Toon sprout appearance

Toon sprout appearance evaluation was based on changes in sprout color, taste and odor according to Wu et al. (2015). Decay index (DI) was calculated following formula as described by Han et al. (2015), $DI = (\Sigma D \times Q)/(4 \times N)$, where D represents the decay scale in toon sprout, Q and N represent its number of corresponding scale and total number in control and GA_3 treatment.

2.3. Total flavonoid and anthocyanin determination

The total flavonoid content was determined using colorimetric analysis as described by Feng et al. (2014) with certain improvement. A calibration curve was drawn using rutin as the standard. In brief, all samples were dried at 60 °C until constant weight and ground prior to further test. 1.0 *g* dried powder were immersed in 30 ml 70% ethanol and incubated for 24 h at 70 °C, and ultrasonic wave – assisted extraction for 30 min at 50 °C. The extract was filtered and tested at 510 nm using a spectrophotometer for the total flavonoid content. The total volume of colorimetric reaction mixture was 10 ml containing extract 1.0 ml, $NaNO_2$ solution 0.4 ml (5%, w/v), Al $(NO_3)_3$ solution 0.4 ml (10%, w/v), NaOH solution 4.0 ml (5%, w/v), and distilled water 5.2 ml. The total flavonoid content was calculated based on the calibration curve. Water content (WC) was calculated based on the following formula, WC = (FW-DW)/FW × 100%; where FW = fresh weight, DW = dry weight, the ultimate results were expressed as mg rutin per 100 *g* fresh weight according to WC. The quantification of anthocyanin was determined following the protocol of our previous work (Zhao et al., 2017). 0.3 *g* samples were immersed in HCl methanol solution 1 m l (1%, v/v) and incubated for 18 h at 21 °C. After centrifugation, supernatant was drawn, diluted and assayed at 530 and 657 nm using spectrophotometer. The anthocyanin content was calculated using the method described by Mano et al. (2007).

2.4. Total soluble sugars, reducing sugars and proline determination

50 mg of dried powder were immersed in 4 ml 80% ethanol and incubated for 40 min at 80 °C. Samples were centrifuged, and supernatant was collected and sediment was extracted repeatedly using 80% ethanol for two times. All supernatant was combined and set the total volume to 10 ml. After filtration, supernatant was drawn for determining the content of total soluble sugars and reducing sugars. The content of total soluble sugars was quantified using anthrone colorimetric method (Fairbairn, 1953). 1 ml sample solution was mixed with 5 ml anthrone reagent. The mixture was boiled for 10 min and cooled to ambient temperature immediately. The mixture was assayed through absorbance at 625 nm. The content of reducing sugars was determined according to 3, 5-dinitro salicylic acid (DNS) method (Miller, 1959). 1 m l DNS reagent could be used instead of anthrone for determining the content reducing sugars at spectrophotometrically 540 nm. Glucose as standard was used to make the calibration curve, and results were expressed mg glucose per 100 *g* fresh weight. Proline content was measured by the protocol as described by Bates et al. (1973) with minor modifications. In brief, 0.5 *g* of toon sprouts were ground in 5 ml of 3% sulphosalicylic acid and homogenate was boiled for 10 min and centrifuged at 3000 rpm for 10 min after cooling. The reaction mixture containing 2 ml of supernatant, 2 ml of glacial acetic acid, and 2 ml of ninhydrin reagent was incubated at boiled water bath for 1 h. The mixture was extracted from the organic phase using toluene, and proline was quantified through absorbance at 520 nm.

2.5. Vitamin C and titratable acidity determination

Vitamin C content in toon sprout was determined using 2, 6-dichlorophenolindophenol titration method as described by Nath et al. (2011). 2.0 g of toon sprouts were ground in 10 ml of 4% oxalic acid and centrifugated at 5000 rpm for 15 min at 4 °C. The supernatant was collected and adjusted to 20 ml with oxalic acid solution, 2, 6-dichlorophenolindophenol reagent was used to titrate the extact to endpoint (color change from achromaticity to a pink color). The results were expressed as mg ascorbic acid per 100 g fresh weight. The titratable acidity of toon sprout was assayed by titrating 10 ml extract with 0.1 mM NaOH solution to endpoint (pH 8). The results were expressed in malic acid equivalent as g 100 g^{-1}.

2.6. Measurement of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) contents

MDA content was measured according to the protocol of Hodges et al. (1999). 5 *g* of toon sprouts were ground in 20 ml 50 *g* l^{-1} trichloroacetic acid. The homogenate was heated at 95 °C for 15 min and quickly cooled on ice. After centrifuging at 12 000 *g* for 15 min, the supernatant was assayed at spectrophotometrically 532 nm. For correcting the reading values from the interference of soluble sugars and proteins in measurement, the absorbances at 440 nm and 600 nm were recorded and subtracted. MDA content was calculated using an extinction coefficient of 155 mM^{-1} $cm^{-1}$and the result was expressed as nmol g^{-1} FW. For measurement of H_2O_2, 5 *g* of toon sprouts were ground and extracted with 3 ml of re-cold acetone. The homogenate was centrifuged at 12 000 *g* for 30 min at 4 °C. 1 ml of supernatant was drawn and mixed with 0.1 ml of 5% titanic sulfate (w/v) and strong ammonia water (17 mol l^{-1}). The mixture was centrifuged at 12 000 *g* for 10 min at 4 °C. The supernatant was discarded and the precipitate was washed repeatedly with acetone until the pigment was removed. Subsequently, the precipitate was dissolved in 5 ml of 2 mol l^{-1}sulfuric acid. According to a standard curve, H_2O_2 content was measured at 410 nm, and indicated as μmol g^{-1}.

2.7. Assay of peroxidase (POD), catalase (CAT), superoxide dismutase (SOD) and polyphenol oxidase (PPO) activities

All operation of enzyme extraction was carried out at 4 °C. For enzyme extraction, 1.0 *g* toon sprouts were ground in 10 ml of 0.1 mol l^{-1} sodium phosphate buffer containing 2% (w/v) polyvinylpolypyrrolidone (PVPP). For assay of POD, CAT, SOD, and PPO, the phosphate buffer pH was 6.4, 7.0, 7.8, and 6.8, respectively. The