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Rhizospheric application with 5-aminolevulinic acid improves coloration and quality in 'Fuji' apples

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ABSTRACT

5-Aminolevulinic acid (5-ALA), an eco-friendly plant growth regulator, has been increasingly applied to crop and fruit production. In the actual production, many fruits are often covered by plastic bags which will not be removed until the fruits are harvested. Therefore, although 5-ALA shows promotive effect on fruit quality, fruit spray cannot be implemented in the orchards using plastic bags. In this study, rhizospheric application of 5-ALA increased the endogenous 5-ALA content in the leaves and fruits, suggesting that 5-ALA is transported from roots to aboveground. Using a multiple plant efficiency analyzer (M-PEA), we also found that rhizospheric application of 5-ALA significantly improved PS II and PS I photosynthetic activities of apple leaves. It means that 5-ALA-treated apple trees might accumulate more assimilates for fruits to improve their quality. Meanwhile, rhizospheric application of 5-ALA obviously increased fruit weight, the content of flavonoids, total soluble solids and ascorbic acid, as well as activities of antioxidant enzymes. Most importantly, rhizospheric irrigation of 5-ALA also significantly improved fruit coloration. Using calli induced from the flesh of 'Fuji' fruits, we found that 5-ALA up-regulated the expressions of many genes involved in flavonoid anabolism. These results suggest that rhizospheric application of 5-ALA solution is effective to improve external and interior qualities of apple fruits although 5-ALA was not directly applied to fruit surface. Taken together, we conclude that rhizospheric application of 5-ALA can be transported to the leaves and fruits of apple trees, where it enhances leaf photosynthetic capacity and improves fruit coloration and interior qualities simultaneously. This finding provides an effective and handy method to improve apple quality in modern orchards.

1. Introduction

Red apple fruit coloration is mainly attributed to anthocyanin accumulation (Ban et al., 2007). All anthocyanins in apple are derivatives of cyanidin, and the principal constituent is cyanidin-3-galactoside (Jakopic et al., 2007). They are biosynthesized from phenylalanine through the phenylpropanoid pathway (Vogt, 2010), which are controlled by the function genes and regulatory genes (Xie et al., 2013). As natural pigments and important secondary metabolites, anthocyanins are not only involved in multiple physiological functions of fruits (Zhang and Chen, 2007), but also very helpful for human health (Cos et al., 2004; Clifford, 2000; Castañeda-Ovando et al., 2009; Wang et al., 2016). Therefore, revealing the methods improving anthocyanin content is very important for fruits.

Many methods improving fruit coloration have been reported, such as laying reflective film (Layne et al., 2001; Jakopic et al., 2007), and spraying plant regulation substances (e.g. B_9, paclobutrazol, and ethylene) (Belakbir et al., 1998; Mohamed and Abugoukh, 2003). However, reflective film may bring garbage pollution in orchards, and artificial synthetic regulators may affect other quality of fruits or cause food safety problems (Lairon, 2010). In addition, bagging young fruit with paper or plastic bags can reduce disease (Moreramontoya et al., 2010) and physical damage as well as improve anthocyanin synthesis (Huang et al., 2009). This approach has been widely practiced in apple cultivation to produce high quality and unblemished fruit, and to control fruit coloration in China (Liu et al., 2013).

5-Aminolevulinic acid (5-ALA), an essential biosynthetic precursor of all tetrapyrrole compounds such as chlorophyll, heme and vitamin B_{12}, has been proved to be an eco-friendly natural plant growth regulator (Wang et al., 2003; Jahn and Heinz, 2009; Akram and Ashraf, 2013). Wang et al. (2004b) proposed that spraying 5-ALA on the fruit surface of 'Fuji' apples before harvest increased the anthocyanin content in apple peel. Afterwards, Watanabe et al. (2006) reported that 5-ALA promoted grape anthocyanin accumulation. Lately, several groups of researchers demonstrated that 5-ALA also promoted fruit coloration of pear (Xiao et al., 2012), peach (Guo et al., 2013), sour cherry (Kurlus

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and Lysiak, 2014) and litchi (Feng et al., 2015). These results indicate that 5-ALA can significantly promote coloration of many species of fruits. However in the actual production, many fruits are often bagged by plastic bags which are not removed until fruits have been harvested (Huang et al., 2009). In this cultivation system, therefore, 5-ALA solution cannot be directly sprayed to fruits to promote coloring. Finding some other ways of 5-ALA application is important for its use in improving fruit coloration. Wang et al. (2004a) reported that rhizospheric irrigation of 5-ALA significantly improved the chilling tolerance of melon leaves, suggesting that 5-ALA can be transported from roots to shoots to regulate the physiological functions of whole plants. Therefore, we hypothesized that 5-ALA can be applied through roots to improve coloration in fruits. If rhizospheric application is effective, 5-ALA can be widely applied in modern orchards which using plastic bags to improve fruit coloration.

To test our hypothesis, in this study, we investigated the effect of rhizospheric irrigation of 5-ALA solution on 'Fuji' apple fruit coloration and found that rhizospherically irrigated 5-ALA significantly stimulated anthocyanin accumulation in apple skin. Then, using apple fruit callus, we investigated the effect of 5-ALA on the expressions of flavonoid biosynthetic genes to explore the molecular mechanisms behind 5-ALA-induced anthocyanin accumulation. Meanwhile, we detected the effect of rhizospherric application of 5-ALA on leaf photosynthetic capacity and fruit interior nutritional quality of apples. Our results provide theoretical support for directly rhizospherric application of 5-ALA to promote anthocyanin accumulation and improves quality in plastic bagged cultivation.

2. Materials and methods

2.1. Plants and treatments

'Fuji' apple cultivar (*Malus domestica* Borkh) was used in this study. The trees were about 20 years old and grown in apple production area, Xiayi County (34.24° N, 116.13° E; elevation 42 m), Henan Province, China. The trees are planted in 4 × 5 m density with *Malus robusta* Rehd. as rootstocks. Field experiments were conducted from 26 Sept. to 24 Oct. in 2015. These trees were rhizospherically irrigated with five liters of 0, 20, 40, 80, and 160 mg L^{-1} 5-ALA, respectively. Three replications were carried out for each treatment and each replication includes four trees. One month later, 20 fruits from each tree were harvested and immediately transported to the lab for analysis.

2.2. Measurement of endogenous 5-ALA levels

The endogenous 5-ALA content in apple leaves, peels and fleshes was measured according to An (2016a). About 0.1 g apple tissue was homogenized in 200 mM acetic acid buffer (pH 4.6), and the homogenate was centrifuged at 5000 × g for 15 min. One milliliter of supernatant were added to 0.5 mL acetylacetone, and boiled for 10 min. After cooling, 0.5 mL Ehrlich's reagent was added. The absorbance was recorded at 553 nm after static hierarchy for 7 min by spectrophotometer.

2.3. Measurement of prompt fluorescence, modulated light reflection at 820 nm and OJIP test

Prompt fluorescence and modulated light reflection at 820 nm were measured simultaneously by a Multiple Plant Efficiency Analyzer (M-PEA) (Hansatech, UK) according to Strasser et al. (2010). The leaves used were from the trees rhizospheric treated by 5-ALA. When the fruits were harvested, the leaf PS II and PS I activities were measured by the M-PEA. After dark adapted for 20 min, the leaves were immediately exposed to a saturating light pulse (3000 μmol m^{-2} s^{-1} PFD) for 1 s. Then the prompt fluorescence characteristics and modulated light reflection at 820 nm were analyzed according to Zhang et al. (2013). All treatments were repeatedly measured eight times.

2.4. Measurement of fruit quality

Ten fruits from each treatment were selected for quality evaluation. Fruit weight was measured by an electronic balance (Sartorius Germany). Fruit length and diameter were detected by a vernier caliper (Forgestar China). Fruit firmness was evaluated by compression test using a Fruit hardness tester (model GY-1, Shenzhen Handsome Technology Co., Ltd; 0–15 kg) fitted with a 10 mm diameter plunger.

Ascorbic acid content was measured by oxidation of ascorbic acid with 2, 6-dichlorophenol endophenol dye and expressed as mg 100 g^{-1} tissues. Soluble protein content was detected as coomassie brilliant blue G250 staining (Bradford, 1976). Total soluble solid (TSS) content was measured by Brix percentage in fruit juice using a PAL-1 digital refractometer (Atago, Japan). The content of soluble sugar was tested by anthrone-H_2SO_4 colorimetry. Titratable acidity (TA) was determined by titrating method (Shrestha et al., 2012). Mean values were obtained from at least three independent replicates.

Total flavonoids were isolated according to the methods by Nie et al. (2010) with slight modifications. Apple flesh powder (about 0.1 g) was added to 2 mL 80% ethyl alcohol and assisted by ultrasonic and then centrifugation, the supernatant was combined to a final volume of 5 mL. Five milliliter of deionized water, 0.3 mL $NaNO_2$ (5%), 0.5 mL 10% aqueous $AlCl_3$ were added to one milliliter of the extracts, respectively. After 5 min, 2 mL of 1 mol L^{-1} NaOH was added and the final volume was made up to 10 mL with deionized water. The samples were put in a refrigerator at 4 °C for 15 min of color-developing. The absorbance was measured against the blank at 500 nm. Total flavonoids of samples were expressed on the fresh weight basis as mg catechinic acid equivalent 100 g^{-1}. Mean values were obtained from three independent replicates.

The activities of superoxide dismutase (SOD) (EC 1.15.1.1) and peroxidase (POD) (EC 1.11.1.7) were detected according to An et al. (2016b). Mean values were obtained from four independent replicates.

2.5. Measurement of fruit coloration

Total anthocyanin content of apple peel was measured according to Xie et al. (2013) with slight modifications. Apple peel was collected by a hole-puncher with 0.65 cm radius and extracted with 1% (v/v) HCl-methanol for 16 h at room temperature in the dark. After centrifugation at 8000 × g for 15 min, the absorbance of supernatants was measured at 530, 620, 650 nm with a spectrophotometer. The content of anthocyanin was expressed as nmol of cyanidin-3-galactoside in one gram of fresh sample using a molar extinction coefficient of 3.43 × 10^4 (Ubi et al., 2006). Mean values were obtained from five independent replicates.

The compositions of anthocyanins and flavonols were analyzed with high performance liquid chromatography (HPLC). Four standards including cyanidin-3-galactoside, proanthocyanidin B2, quercetin and kaempferol were purchased from Aladdin, China, prepared in methanol and stored at −40 °C before utilization. Extraction procedure referred to Wojdyło et al. (2008) with slight modifications. Fruit peel sample was ultrasonically vibrated in 1% (v/v) HCl-methanol for 30 min, and extracted at 4 °C for 16 h, and then centrifuged at 5000 × g at 4 °C for 15 min. The sample solution was filtered through a 0.45 μm filter before utilization. They were measured using an Agilent 1200 HPLC (USA) equipped with a reversed phase C_{18} column (250 × 4.6 mm i.d.). The injection volume was 20 μL. The mobile phase was composed of (A) acetonitrile and (B) 0.1% phosphoric acid (aqueous). The gradient elution was performed as follows: 0 min, (A) 25%; 2.5 min, (A) 75%; 8 min, (A) 90%, 10 min, (A) 25%. The flow rate was 1 mL min^{-1}, and the column temperature was 35 °C. The UV absorbance at 280 nm for flavonoid was measured. Mean values were obtained from three independent replicates.