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Short communication

ABA participates in the regulation of vitamin C content in the fruit of strawberry using lanthanum nitrate

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ABSTRACT

By pot experiment method, this study investigated the role of plant hormone abscisic acid (ABA) in the foliar application of 10 μM lanthanum nitrate ($La(NO_3)_3$)-regulated the biosynthesis, regeneration and degradation of vitamin C (Vc) and the content of Vc in the fruit of strawberry at different periods of growth and development. The results showed that $La(NO_3)_3$ significantly increased the content of Vc by increasingthe activities of recycling enzymes glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and biosynthetic enzyme L-galactono-1,4-lactone dehydrogenase (GalLDH), and decreasing the activities of recycling enzyme APX and degrading enzyme ascorbic acid oxidase (AAO). Compared with $La(NO_3)_3$ alone, applications of different ABA biosynthesis inhibitor sodium tungstate (Tu) all markedly decreased the activities of GR, MDHAR and GalLDH, and increased the activities of DHAR and APX, which resulted in the reduction in the content of Vc. Meanwhile, $La(NO_3)_3$ induced the production of ABA and Tu reversed the effect of $La(NO_3)_3$ on ABA content. Our results suggested that $La(NO_3)_3$-induced ABA participated in the regulation of Vc content in the fruit of strawberry.

1. Introduction

Strawberry is known as the queen of fruit, which has a high nutritional value and therapeutic value. Vc is one of the important nutrients in strawberry fruit. The amount of Vc content is an important factor to evaluate the quality of strawberry (Bona et al., 2015). Meanwhile, many studies showed that the content of Vc in plants can be regulated by many exogenous substances, including plant hormones, plant growth regulators and rare earth elements (Dai et al., 2015; Kang et al., 2013; Shan and Zhao, 2014). In our previous study, we found the primary mechanism for improving Vc of strawberry fruit by rare earth element lanthanum (La) at physiological level (Shan et al., 2017a, 2017b). Our previous findings implied that low concentration of La $(NO_3)_3$ could improve Vc content of strawberry fruit by increasing the activities and the transcript levels of recycling enzymes GR, DHAR, MDHAR and biosynthetic enzyme GalLDH, and decreasing the activity and the transcript level of degrading enzyme AAO (Shan et al., 2017a, 2017b). However, the deep mechanism for improving Vc of strawberry fruit by La is still unknown.

ABA is an important plant hormone in plants. It has many roles in regulating plant growth and development, physiological and biochemical metabolism process and the responses of plants to stresses (Garrido-Bigotes et al., 2017; Xing et al., 2016). It has been documented ABA could regulate Vc content through AsA-GSH cycle in maize and wheat crops (Jiang and Zhang, 2002; Shan et al., 2017a, 2017b). It has been documented that ABA played an important role in the regulation of strawberry fruit ripening (Jia et al., 2011). In strawberry, ABA also regulated the biosynthesis pathway in lots of bioactive compounds in its fruits, including sugars, phenolic compounds and anthocyanin, etc. (Ayub et al., 2016; Kadomura-Ishikawa et al., 2015). However, there is still no report about whether and how ABA regulates the content of Vc in the fruit of strawberry. It has been reported that ABA could be involved in the regulation of root growth by rare earth element La (Liu et al., 2008). However, it is still unclear for whether and how ABA participates in the regulation of Vc content by La in the fruit of strawberry. So, to further investigate the role of ABA in La-regulated the content of Vc in the fruit of strawberry is very important to elucidate the regulatory mechanism of La in regulating the content of Vc in the fruit of strawberry.

In this study, we investigated the effects of ABA biosynthesis inhibitor sodium tungstate (Tu) on La-regulated the activities of enzymes in the regeneration, biosynthesis and degradation of Vc and the contents of Vc in the fruit of strawberry at three different stages of fruit development. The aim of this study was to elucidate the role of ABA in

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La-regulated the content of Vc in the fruit of strawberry.

2. Materials and methods

2.1. Plant material and treatments

Strawberry variety 'Sweet Charlie' was supplied by Zhengzhou Fruit Research Institute. Seedlings substituted with Daughter plants of strawberry with four fully expanded leaves were transferred into plastic pots (diameter of 15 cm) which were filled with 3 kg of culture soil composed of 70% peat and 30% garden soil. The water content of the culture soil was 70% field water-holding capacity. The analysis of main nutrient elements in culture soil was as follows. The contents of organic matter, total nitrogen, total phosphorus and total potassium were 19.5 g kg^{-1}, 1.31 g kg^{-1}, 0.62 g kg^{-1} and 1.23 g kg^{-1}, respectively. The La content in the culture soil was 0.6 mg kg^{-1}. The La content in water was 28 ng L^{-1}. The pH of water was 7.0. Then, the potted plants were placed in artificial climate chamber under following growth conditions. The day/night temperature is 25/15 °C. The photosynthetic active radiation during the day is 600 μmol m^{-2} s^{-1}. The photosynthetic active radiation during the night is 0 μmol m^{-2} s^{-1}. The photoperiod is 12-h. From the beginning of the budding stage, strawberry plants were divided into five groups with 10 pots per group. First group was used as control and only treated by spraying 50 ml distilled water on the leaves of strawberry every 6 days. Second group was treated by spraying 50 ml 10 μM lanthanum nitrate ($La(NO_3)_3$) on the leaves of strawberry every 6 days. Other groups were respectively pretreated by 1, 3 and 5 mM Tu, and then treated by 10 μM $La(NO_3)_3$ on the leaves of strawberry every 6 days. The fruits with similar size and weight at Large green fruit period (LGFP), Pink fruit period (PFP) and Red fruit period (RFP) were sampled and used to determine the activities of enzymes in the metabolism of Vc and the content of Vc in the fruits of strawberry.

2.2. Determination of ABA content

ABA content was determined by gas chromatography–mass spectroscopy according to the method of Jia et al. (2011). One gram per fruit sample was weighed and used to determine the content of ABA. The extraction of ABA were done by centrifugation method. Then the separation of ABA was done on a small C_{18} column after dissolving with 0.1 mM acetic acid. The eluate was collected and ammonia water were added. Then the mixture was dried at 40 °C and diazomethane was added for esterification. Finally, samples were dissolved with acetic acid ethyl ester and concentrated to determine the ABA content by gas chromatography–mass spectroscopy.

2.3. Analysis of APX, GR, DHAR and MDHAR

Enzymes were extracted according to Grace and Logan (1996) with some modifications. Each frozen sample (0.5 g) was ground into a fine powder in liquid N_2 with a mortar and pestle. Fine powder was homogenized in 6 ml 50 mM KH_2PO_4 (pH 7.5) containing 0.1 mM ethylenediaminetetraacetic acid, 0.3% (v/v) Triton X-100, and 1% (w/v) soluble polyvinylpolypyrrolidone, with the addition of 1 mM AsA in the case of the APX assay. The extract was immediately centrifuged at 13,000 × *g* for 15 min at 2 °C. The supernatant was then used immediately for measuring the following enzymes.

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was measured by monitoring the decrease in absorbance at 290 nm (Nakano and Asada 1981). One unit of enzyme was defined as the amount of APX catalyzing the oxidation of 1 μmol ascorbate per minute. Glutathione reductase (GR, EC 1.6.4.2) activity was monitored at 340 nm for 3 min (Grace and Logan 1996). One unit of GR activity was defined as the reduction of 1 μmol NADPH per minute. Dehydroascorbate reductase (DHAR, EC 1.8.5.1) activity was measured at 265 nm for 3 min (Dalton et al., 1986). One unit of DHAR activity was defined as the amount of enzyme that produces 1 μmol AsA per minute. MDHAR was measured according to Miyake and Asada (1992) by following the decrease in absorbance at 340 nm. One unit of MDHAR activity was defined as the amount of enzyme that oxidizes 1 μmol NADH per minute. The specific enzyme activity for all the above enzymes was expressed as Units g^{-1} FW.

2.4. Analysis of GalLDH and AAO

L-galactono-1,4-lactone dehydrogenase (GalLDH, EC 1.3.2.3) was extracted and measured by the method of Tabata et al. (2001). One unit of GalLDH activity is defined as the amount of extract required to oxidize 1 nmol of L-Gal. Ascorbic acid oxidase (AAO, EC 1.10.3.3) activity was determined by measuring the decrease in absorbance at 265 nm reflecting the ascorbate oxidation (Arrigoni et al., 1992). One unit of AAO activity was defined as the amount of AAO required to catalyse the oxidation of 1 μmol of L-AA to DHA per minute. The specific enzyme activity for AAO was expressed as Units g^{-1} FW.

2.5. Analysis of Vc content

The content of Vc was measured according to Hodges et al. (Hodges et al., 1996). The content of Vc was expressed in mg 100 g^{-1} FW.

2.6. Statistical analysis

The whole experiment was repeated 5 times with 6 replicates each time. The results presented were the mean of 5 times. Means were compared by one-way analysis of variance and Duncan's multiple range test at the 5% level of significance.

3. Results

3.1. Effects of $La(NO_3)_3$ and Tu on the content of ABA in the fruits of strawberry at different periods of growth and development

As shown in Fig. 1, different concentrations of Tu had same effects on the contents of ABA regulated by $La(NO_3)_3$ in the fruits of strawberry at different periods of growth and development. Compared with control, $La(NO_3)_3$ markedly increased ABA content in the fruit of strawberry at Large green fruit period (LGFP), Pink fruit period (PFP) and Red fruit period (RFP). Compared with $La(NO_3)_3$ alone, different concentrations of Tu all markedly decreased ABA content in the fruit of strawberry at different periods. These results indicated that $La(NO_3)_3$ could induce the production of ABA in the fruit of strawberry through its biosynthesis pathway.

3.2. Effects of $La(NO_3)_3$ and Tu on the activities of APX, GR, DHAR and MDHAR in the fruits of strawberry at different periods of growth and development

As shown in Fig. 2, different concentrations of Tu had the same effects on the activities of APX, GR, DHAR and MDHAR regulated by $La(NO_3)_3$ in the fruits of strawberry at different periods of growth and development. Compared with control, $La(NO_3)_3$ markedly decreased APX activity and increased the activities of GR, DHAR and MDHAR in the fruit of strawberry at Large green fruit period (LGFP), Pink fruit period (PFP) and Red fruit period (RFP). Compared with $La(NO_3)_3$ alone, different concentrations of Tu all markedly increased the activities of APX and DHAR and decreased the activities of GR and MDHAR in the fruit of strawberry at different periods. These results indicated that La-induced ABA participated in the regulation of Vc content through AsA-GSH cycle in the fruit of strawberry.