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Jasmonic acid biosynthetic inhibitor ibuprofen inhibits the accumulation of ascorbic acid in strawberry fruit induced by lanthanum nitrate

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Abstract: By using jasmonic acid (JA) biosynthetic inhibitor ibuprofen (IBU), we investigated the roles of JA in the process of lanthanum nitrate ($La(NO_3)_3$)-regulated ascorbic acid (AsA) content and metabolic enzymes responsible for AsA metabolism in strawberry fruit. Findings demonstrated that $La(NO_3)_3$ markedly improved AsA content by enhancing the activities and transcript levels of glutathione reductase (GR), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR) and L-galactono-1,4-lactone dehydrogenase (GalLDH), and inhibiting the activities and transcript levels of ascorbate peroxidase (APX) and ascorbic acid oxidase (AAO). In comparison with $La(NO_3)_3$ alone, all the concentrations of IBU plus $La(NO_3)_3$ markedly inhibited the activities and transcript levels of DHAR, MDHAR, GalLDH and AAO and improved the activities and transcript levels of GR and APX, which further reduced AsA content. Besides, $La(NO_3)_3$ increased JA content, and IBU decreased JA content induced by $La(NO_3)_3$. Meanwhile, the results of Pearson correlation analysis showed that JA content had significant correlations with the activities and transcript levels of DHAR, MDHAR and GalLDH. Above findings implied that $La(NO_3)_3$ induced JA production, which further increased AsA content in fruits by mainly up-regulating the activities and transcript levels of DHAR, MDHAR and GalLDH.

Keywords: hormone; antioxidant enzyme; ascorbate; rare earth element; *Fragaria* × *ananassa*

The strawberry is one of the most prevalent fruit due to its high economic and nutritional value (Sun et al. 2020). In plants, much evidence implied that many exogenous substances can regulate ascorbic acid (AsA) content, including phytohormones, rare earth elements (REEs) and trace elements (Shan et al. 2018a, Zhang et al. 2020, Aksakal 2022). Lanthanum (La) belongs to the REE group, which is constituted of 17 elements due to their physical and chemical similarity. For strawberries, it has been documented that lanthanum nitrate ($La(NO_3)_3$) improved the AsA content in fruit (Shan et al. 2017, 2018a). Meanwhile, Shan et al. (2017) also found that $La(NO_3)_3$ improved AsA content by enhancing the activities and transcript levels of glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and L-galactono-1,4-lactone dehydrogenase (GalLDH), and down-regulating the activities and transcript levels of ascorbate peroxidase (APX) and ascorbic acid oxidase (AAO). While the deep mechanism of $La(NO_3)_3$ in improving AsA content in fruit is still not fully elucidated.

Phytohormones have important roles in regulating fruit growth and development (Khew et al. 2020, Ali et al. 2022, Fan et al. 2022). In plants, there are many important hormones, such as jasmonic acid (JA) and abscisic acid (ABA). JA plays a considerable role in regulating plant growth and development and

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the responses of plants to various stresses (Deepika and Singh 2021, Li et al. 2021). Many studies have shown that JA is vital in regulating fruit development and ripening (Feng et al. 2017, Asghari et al. 2019). Garrido-Bigotes et al. (2018) demonstrated that JA regulated fruit development and ripening for strawberries. Kumar et al. (2016) showed that the redox state is important in regulating fruit ripening. Through transcriptome analysis, previous studies demonstrated that AsA regulated the process of fruit ripening (dos Santos et al. 2019). Thus, AsA is not only an important quality and nutritional component in fruit but also an important antioxidant in regulating the redox state of fruits during ripening. We previously found that JA regulated AsA biosynthesis and regeneration in *Arabidopsis thaliana* L., which further increased AsA content and maintained the redox state of plants (Shan et al. 2018b). Besides, Zhou et al. (2012) showed that La induced JA accumulation in *Scutellaria baicalensis* Georgi. While whether JA is involved in the process of $La(NO_3)_3$-regulated AsA content in strawberry fruit is still unclear. Therefore, it will be very interesting to investigate the roles of JA in the regulation of AsA content by $La(NO_3)_3$, which can provide a deep understanding of the improvement of AsA content by $La(NO_3)_3$ in strawberry fruit.

In the current study, we used a pharmacological method to study the roles of JA in the regulation of AsA content and the enzymes responsible for AsA regeneration, biosynthesis and degradation in strawberry fruit by $La(NO_3)_3$ at different stages. The purpose of the current study was to clarify the roles of JA in the process of $La(NO_3)_3$-regulated AsA content in fruit, which will uncover more knowledge for AsA accumulation induced by $La(NO_3)_3$ and provide a more theoretical basis for the application of $La(NO_3)_3$ in improving fruit quality of strawberry in the production and cultivation.

MATERIAL AND METHODS

Plant material and treatment. We used strawberry (*Fragaria* × *ananassa* Duch.) cv. Sweet Charlie is the material. The seedlings of strawberries with five fully expanded leaves were supplied by Zhengzhou Fruit Research Institute, Chinese Academy of Agricultural Sciences. The seedlings with similar growth status were selected to be cultured in plastic pots containing 3.5 kg substrate, a mixture of 70% peat and 30% cinnamon soil. The water content of the above substrate was 70% field water-holding capacity. The pH and the contents of organic carbon, available nitrogen, available phosphorus and available potassium of the above substrate were 6.9, 11.3 g C/kg, 105.0 mg/kg, 99 mg/kg and 123 mg/kg, respectively. Above plants were placed in the growth chamber with a day/night temperature of 25/15 °C and relative humidity of 70%. The active photosynthetic radiation was 600 μmol/m^2/s, and the photoperiod was 12 h. Plants were divided into five groups at the budding stage, with 6 pots per group. Each pot had two seedlings. One group of seedlings was first sprayed with distilled water through foliar spraying and then subjected to 10 μmol/L $La(NO_3)_3$ through foliar spraying. In the above group, the total application dose of distilled water and 10 μmol/L $La(NO_3)_3$ was 50 mL per pot. Ibuprofen (IBU) is an inhibitor of lipoxygenase (LOX), a key enzyme for JA biosynthesis. Thus, IBU can inhibit JA biosynthesis and reduce JA content. To study whether IBU can reverse the effect of $La(NO_3)_3$ on AsA accumulation in strawberry fruit, three groups of seedlings were first sprayed with 1, 3 and 5 mmol/L IBU, respectively. The above three groups were all subjected to 10 μmol/L $La(NO_3)_3$ through foliar spraying. In the above three groups, the total application dose of IBU and 10 μmol/L $La(NO_3)_3$ was 50 mL per pot. The control group of seedlings was treated with distilled water alone through foliar spraying. In the control group, the total application dose of distilled water was 100 mL per pot. Each treatment was repeated 6 times. Above foliar spraying for different treatments was done every 6 days. The fruits with similar growth status were sampled at three stages, including the large green fruit period (LGFP), pink fruit period (PFP) and red fruit period (RFP), respectively. The above samples were used to measure AsA content and the activities and transcript levels of enzymes responsible for AsA metabolism.

Measurement of JA content. JA content was quantified through a gas chromatograph, according to Shan and Liang (2010). Fresh fruit samples were ground to fine powder and extracted using 80% methanol solution with dihydro jasmonic acid (dhJA) as the internal standard at 4 °C for one day. The above samples were centrifuged, and the supernatant was evaporated and then dissolved by phosphate buffer (pH 8.2). Polyvinylpyrrolidone was added to the above solution and mixed. After the mixture, the solution was centrifuged, and then ether was used to extract the supernatant three times. The water phase was extracted by hexane and then concentrated to 1 mL. The above solution was dried with nitro-

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gen and then dissolved in the solution containing benzene-petroleum ether and KOH-methanol. After reacting at 25 °C for 20 min, the above solution was extracted with 8 mL distilled water. The upper solution was dried with nitrogen, redissolved in 200 mL ethanol, and then injected into a gas chromatograph to quantify JA content.

Analysis of the activities of enzymes in the AsA-GSH cycle. Ascorbate peroxidase (APX, EC 1.11.1.11), glutathione reductase (GR, EC 1.6.4.2), monodehydroascorbate reductase (MDHAR, EC 1.6.5.4) and dehydroascorbate reductase (DHAR, EC 1.8.5.1) were extracted and analysed as described by Shan and Liang (2010). One unit of APX activity was defined as the oxidation of 1 μmol ascorbate per minute. One unit of GR activity was defined as the reduction of 1 μmol NADPH per minute. One unit of DHAR activity was defined as the production of 1 μmol AsA per minute. One unit of MDHAR activity was defined as the oxidation of 1 μmol NADH per minute. The specific enzyme activity for the above enzymes was expressed as units/g FW (fresh weight).

Analysis of the activities of GalLDH and AAO. L-galactono-1,4-lactone dehydrogenase (GalLDH, EC 1.3.2.3) was extracted and determined at 550 nm, as described by Shan and Liang (2010). Ascorbic acid oxidase (AAO, EC 1.10.3.3) was extracted and monitored as described by Arrigoni et al. (1992). One unit of GalLDH and AAO was all defined and expressed in a previous study (Shan et al. 2018a).

Quantitative real-time PCR (qRT-PCR) of APX, GR, DHAR, MDHAR and GalLDH. 0.1 g of pulp tissue was used for RNA extraction for each treatment. Total RNA was isolated from the fruit by using RNA easy kit (Qiagen, Dusseldorf, Germany) according to the instruction supplied by the manufacturer. An amount of 2.5 μg RNA from each sample was treated with DNase I and then was used to synthesise cDNA by using Quant One Step qRT-PCR Kit (TIAN-GEN, Beijing, China). Above cDNAs were used to determine the expression levels of *APX*, *GR*, *DHAR*, *MDHAR*, *GalLDH* and *AAO* with qRT-PCR. The StepOne Plus Real-Time PCR system (Applied Biosystems, Waltham, USA) was used for analyses. The gene-specific primers and conditions for qRT-PCR were as the previous study (Shan et al. 2017). *GAPDH* was used as a reference gene to normalise expression values. All experiments were repeated six times, and the results were analysed using the delta-delta threshold cycle method.

Measurement of ascorbic acid (AsA) content. Fresh pulp samples (0.5 g) were ground in 5 % (*w*/*v*) metaphosphoric acid at 2 °C and then centrifuged at 13 000 *g* for 20 min. Then, the supernatant was used to determine AsA content, according to Hodges et al. (1996). A standard curve prepared by using AsA was used to calculate AsA content.

Statistical analysis. Data in all figures were the mean of 6 replications. All the means were compared by one-way analysis of variance and Duncan's multiple range test at a 5% significance level. The one-way and Pearson correlation analyses were done using SPSS Statistics 25 (Chicago, USA).

RESULTS

Effects of $La(NO_3)_3$ and IBU on JA content. In comparison with the control, $La(NO_3)_3$ improved JA content at different stages (Figure 1). Compared with $La(NO_3)_3$ alone, different concentrations of IBU plus $La(NO_3)_3$ significantly reduced JA content at LGFP, PFP and RFP, especially for 3 and 5 mmol/L IBU. At LGFP, PFP and RFP, 3 mmol/L IBU plus $La(NO_3)_3$ decreased JA content in La-treated plants by 48.4, 48.4 and 50.0%, respectively. The application of 5 mmol/L IBU plus $La(NO_3)_3$ decreased JA content by 67.7, 64.5 and 62.9%, respectively. Above findings uncovered that $La(NO_3)_3$ induced JA accumulation, and IBU inhibited JA accumulation at different stages.

Effects of $La(NO_3)_3$ and IBU on the activities of enzymes in the AsA-GSH cycle. In comparison with the control, $La(NO_3)_3$ reduced APX activity and enhanced the activities of GR, DHAR and MDHAR at different stages (Figure 2). However, different concentrations of IBU plus $La(NO_3)_3$ significantly enhanced the activities of APX and GR and reduced the activities of DHAR and MDHAR in La-treated plants at different stages, especially for 3 and 5 mmol/L IBU. At LGFP, 3 mmol/L IBU plus $La(NO_3)_3$ respectively increased the activities of APX and GR in La-treated plants by 126.5% and 51.3%, and respectively decreased the activities of DHAR and MDHAR by 38.2% and 42.9%. The application of 5 mmol/L IBU plus $La(NO_3)_3$ respectively increased the activities of APX and GR by 85.7% and 29.1%, and respectively decreased the activities of DHAR and MDHAR by 43.4% and 71.4%. At PFP, 3 mmol/L IBU plus $La(NO_3)_3$ respectively increased the activities of APX and GR by 96.8% and 56.8%, and respectively decreased the activities of DHAR and MDHAR by 28.6% and 30.8%. The application of 5 mmol/L IBU plus $La(NO_3)_3$ respectively increased the activities of APX and GR by 151.6% and 51.4%, and respectively decreased the activities of DHAR and

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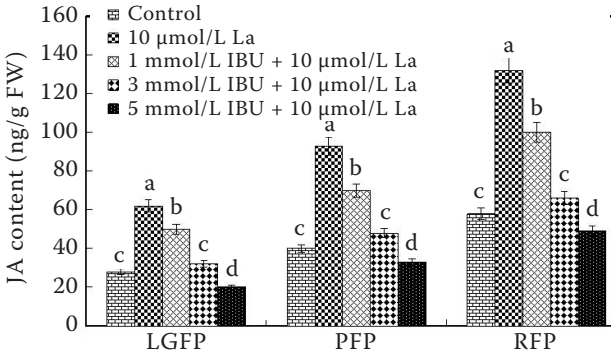

Figure 1. Effects of $La(NO_3)_3$ and ibuprofen (IBU) on jasmonic acid (JA) content in fruit at different stages. The plants were treated as below: Control – distilled water; 10 μmol/L La – 10 μmol/L $La(NO_3)_3$; 1 mmol/L IBU + 10 μmol/L La – 1 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 3 mmol/L IBU + 10 μmol/L La – 3 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 5 mmol/L IBU + 10 μmol/L La – 5 mmol/L IBU + 10 μmol/L $La(NO_3)_3$. Values represent mean ± standard deviation (n = 6), and small letters indicate statistical difference at $P < 0.05$ at the same stage. LGFP – large green fruit period; PFP – pink fruit period; RFP – ripen fruit period; FW – fresh weight

MDHAR by 29.3% and 34.6%. At RFP, 3 mmol/L IBU plus $La(NO_3)_3$ respectively increased the activities of APX and GR by 92.3% and 38.3%, and respectively decreased the activities of DHAR and MDHAR by 47.4% and 34.3%. The application of 5 mmol/L IBU plus $La(NO_3)_3$ respectively increased the activities of APX and GR by 103.8% and 33.3%, and respectively decreased the activities of DHAR and MDHAR by 54.4% and 40.0%. Above findings implied that IBU reversed the effects of La on the activities of enzymes in the AsA-GSH cycle except for GR.

Effects of $La(NO_3)_3$ and IBU on the expression of APX, GR, DHAR and MDHAR at the transcript level. In comparison with the control, $La(NO_3)_3$ down-regulated APX expression and up-regulated the expression of DHAR, GR and MDHAR at the transcript level in fruit at different stages (Figure 3). Compared with $La(NO_3)_3$ alone, different concentrations of IBU plus $La(NO_3)_3$ significantly up-regulated the expression of APX and GR and down-regulated the expression of DHAR and MDHAR at different stages, especially for 3 and 5 mmol/L IBU. At LGFP, 3 mmol/L IBU plus $La(NO_3)_3$ respectively up-regu-

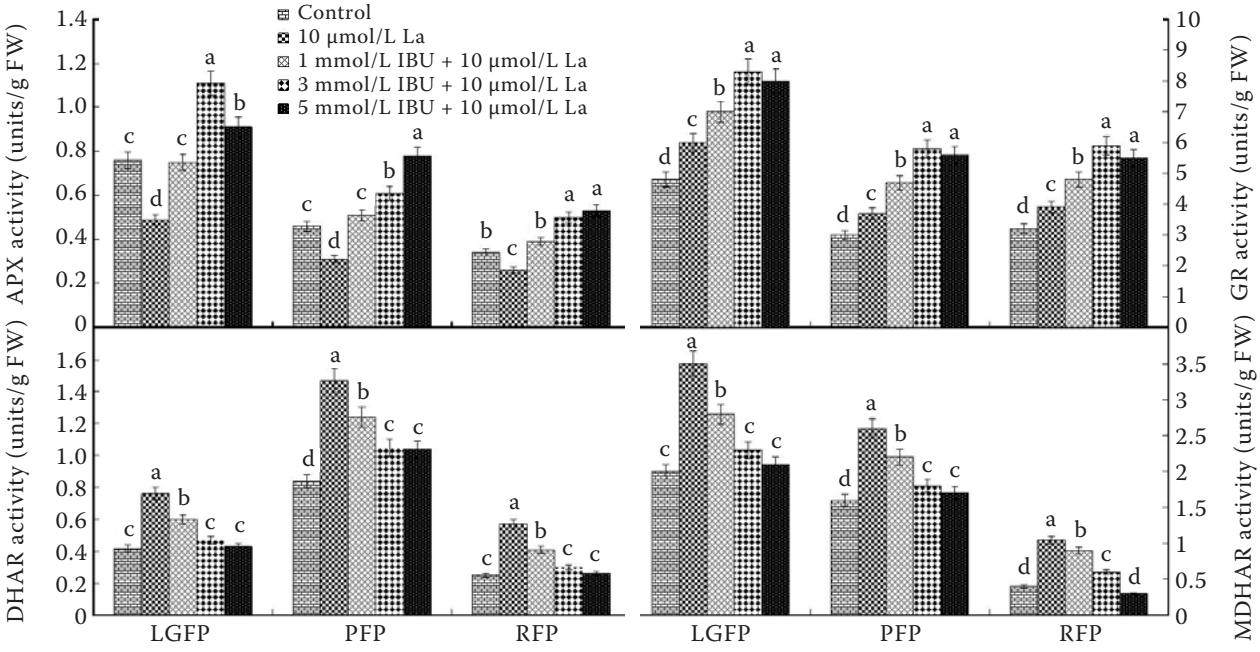

Figure 2. Effects of $La(NO_3)_3$ and ibuprofen (IBU) on the activities of enzymes in the ascorbate-glutathione (AsA-GSH) cycle in fruit at different stages. The plants were treated as below: Control – distilled water; 10 μmol/L La – 10 μmol/L $La(NO_3)_3$; 1 mmol/L IBU + 10 μmol/L La – 1 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 3 mmol/L IBU + 10 μmol/L La – 3 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 5 mmol/L IBU + 10 μmol/L La – 5 mmol/L IBU + 10 μmol/L $La(NO_3)_3$. Values represent mean ± standard deviation (n = 6), and small letters indicate statistical difference at $P < 0.05$ at the same stage. LGFP – large green fruit period; PFP – pink fruit period; RFP – ripen fruit period; APX – ascorbate peroxidase; GR – glutathione reductase; DHAR – dehydroascorbate reductase; MDHAR – monodehydroascorbate reductase; FW – fresh weight

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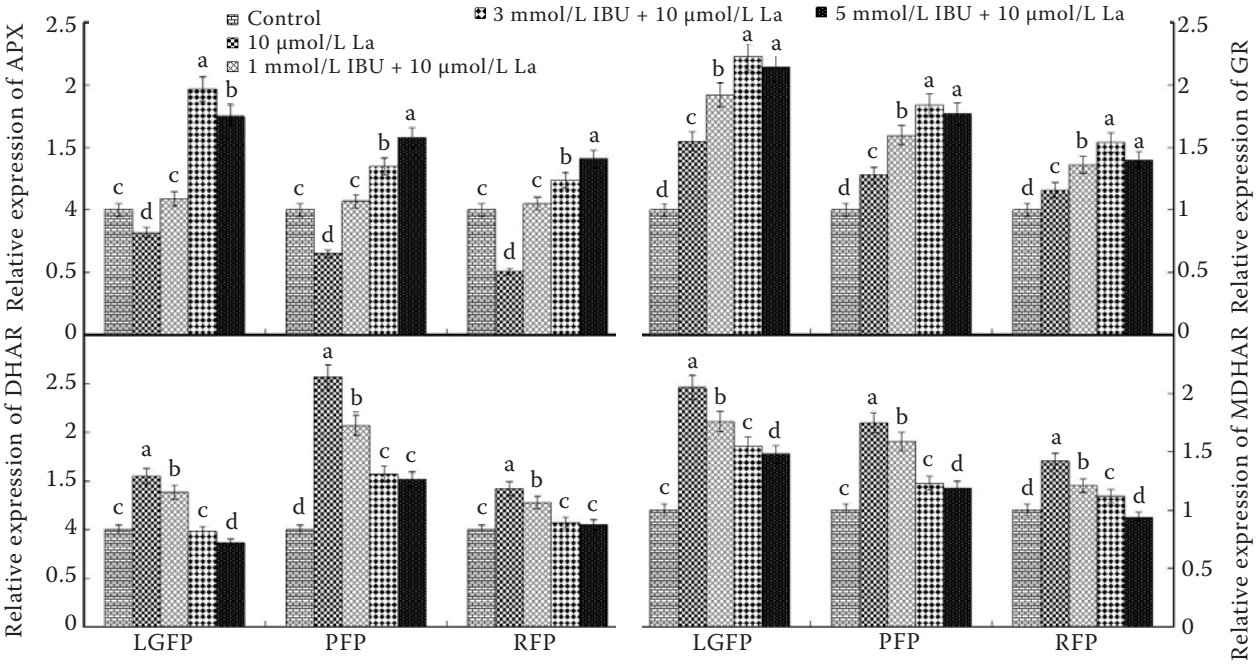

Figure 3. Effects of $La(NO_3)_3$ and ibuprofen (IBU) on the transcript levels of enzymes in the ascorbate-glutathione (AsA-GSH) cycle in fruit at different stages. The plants were treated as below: Control – distilled water; 10 μmol/L La – 10 μmol/L $La(NO_3)_3$; 1 mmol/L IBU + 10 μmol/L La – 1 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 3 mmol/L IBU + 10 μmol/L La – 3 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 5 mmol/L IBU + 10 μmol/L La – 5 mmol/L IBU + 10 μmol/L $La(NO_3)_3$. Values represent mean ± standard deviation (n = 6), and small letters indicate statistical difference at $P < 0.05$ at the same stage. LGFP – large green fruit period; PFP – pink fruit period; RFP – ripen fruit period; APX – ascorbate peroxidase; GR – glutathione reductase; DHAR – dehydroascorbate reductase; MDHAR – monodehydroascorbate reductase

lated the expression of APX and GR by 140.2% and 32.8%, and respectively down-regulated the expression of DHAR and MDHAR by 36.8% and 21.1%. The application of 5 mmol/L IBU plus $La(NO_3)_3$ respectively up-regulated the expression of APX and GR by 113.4% and 20.7%, and respectively down-regulated the expression of DHAR and MDHAR by 44.5% and 33.8%. At PFP, 3 mmol/L IBU plus $La(NO_3)_3$ respectively up-regulated the expression of APX and GR by 107.7% and 43.8%, and respectively down-regulated the expression of DHAR and MDHAR by 38.9% and 29.7%. The application of 5 mmol/L IBU plus $La(NO_3)_3$ respectively up-regulated the expression of APX and GR by 143.1% and 38.3%, and respectively down-regulated the expression of DHAR and MDHAR by 40.9% and 32.0%. At RFP, 3 mmol/L IBU plus $La(NO_3)_3$ respectively up-regulated the expression of APX and GR by 143.1% and 43.9%, and respectively down-regulated the expression of DHAR and MDHAR by 24.6% and 24.4%. The application of 5 mmol/L IBU plus $La(NO_3)_3$ respectively up-regulated the expression of APX and GR by 176.5% and 38.1%, and respectively down-regulated the expression of DHAR and MDHAR by 26.1% and 27.8%. Above findings suggested that IBU reversed the effects of La on the expression of genes coding enzymes in the AsA-GSH cycle at the transcript level except for GR.

Effects of $La(NO_3)_3$ and IBU on the activities of GalLDH and AAO. In comparison with the control, $La(NO_3)_3$ increased GalLDH activity and reduced AAO activity at different stages (Figure 4). Compared with $La(NO_3)_3$ alone, different concentrations of IBU plus $La(NO_3)_3$ significantly decreased the activities of GalLDH and AAO at different stages. At LGFP, 3 mmol/L IBU plus $La(NO_3)_3$, respectively, decreased the activities of GalLDH and AAO by 40.0% and 36.8%. The application of 5 mmol/L IBU plus $La(NO_3)_3$, respectively, decreased the activities of GalLDH and AAO by 42.5% and 38.2%. At PFP, 3 mmol/L IBU plus $La(NO_3)_3$, respectively, decreased the activities of GalLDH and AAO by 30.0% and 51.1%. The application of 5 mmol/L IBU plus $La(NO_3)_3$, respectively, decreased the activi-

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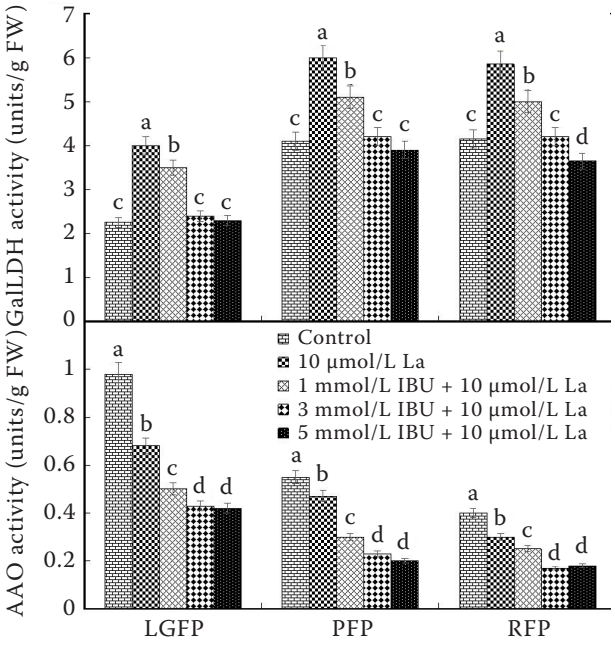

Figure 4. Effects of $La(NO_3)_3$ and ibuprofen (IBU) on the activities of L-galactono-1,4-lactone dehydrogenase (GalLDH) and ascorbate oxidase (AAO) in fruit at different stages. The plants were treated as below: Control – distilled water; 10 μmol/L La – 10 μmol/L $La(NO_3)_3$; 1 mmol/L IBU + 10 μmol/L La – 1 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 3 mmol/L IBU + 10 μmol/L La – 3 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 5 mmol/L IBU + 10 μmol/L La – 5 mmol/L IBU + 10 μmol/L $La(NO_3)_3$. Values represent mean ± standard deviation (n = 6), and small letters indicate statistical difference at $P < 0.05$ at the same stage. LGFP – large green fruit period; PFP – pink fruit period; RFP – ripen fruit period

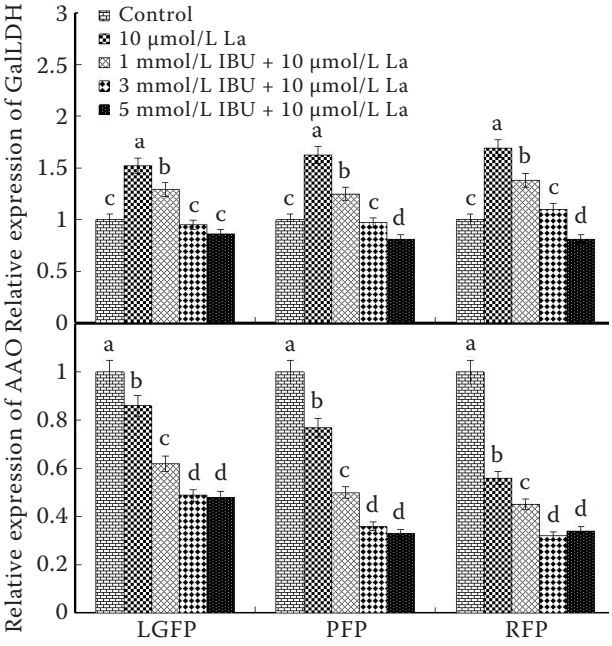

Figure 5. Effects of $La(NO_3)_3$ and ibuprofen (IBU) on the transcript levels of L-galactono-1,4-lactone dehydrogenase (GalLDH) and ascorbate oxidase (AAO) in fruit at different stages. The plants were treated as below: Control – distilled water; 10 μmol/L La – 10 μmol/L $La(NO_3)_3$; 1 mmol/L IBU + 10 μmol/L La – 1 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 3 mmol/L IBU + 10 μmol/L La – 3 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 5 mmol/L IBU + 10 μmol/L La – 5 mmol/L IBU + 10 μmol/L $La(NO_3)_3$. Values represent mean ± standard deviation (n = 6); different letters indicate statistical difference at $P < 0.05$ at the same stage. LGFP – large green fruit period; PFP – pink fruit period; RFP – ripen fruit period

ties of GalLDH and AAO by 35.0% and 57.4%. At RFP, 3 mmol/L IBU plus $La(NO_3)_3$, respectively, decreased the activities of GalLDH and AAO by 28.2% and 43.3%. The application of 5 mmol/L IBU plus $La(NO_3)_3$, respectively, decreased the activities of GalLDH and AAO by 37.6% and 40.0%. Above findings suggested that IBU reversed the effect of La on GalLDH activity and enhanced the effect of La on AAO activity.

Effects of $La(NO_3)_3$ and IBU on the expression of GalLDH and AAO at the transcript level. Compared with the control, $La(NO_3)_3$ up-regulated GalLDH expression and decreased AAO expression in fruit at different stages (Figure 5). All the concentrations of IBU plus $La(NO_3)_3$ significantly down-regulated the transcript levels of GalLDH and AAO in La-treated plants at different stages. At LGFP, 3 mmol/L IBU plus $La(NO_3)_3$, respectively, down-regulated the expression of GalLDH and AAO by 37.5% and 43.3%. The application of 5 mmol/L IBU plus $La(NO_3)_3$, respectively, down-regulated the expression of GalLDH and AAO by 43.4% and 44.2%. At PFP, 3 mmol/L IBU plus $La(NO_3)_3$, respectively, down-regulated the expression of GalLDH and AAO by 40.3% and 53.2%. The application of 5 mmol/L IBU plus $La(NO_3)_3$, respectively, down-regulated the expression of GalLDH and AAO by 50.2% and 57.1%. At RFP, 3 mmol/L IBU plus $La(NO_3)_3$ respectively down-regulated the expression of GalLDH and AAO by 34.9% and 42.9%. The application of 5 mmol/L IBU plus $La(NO_3)_3$, respectively, down-regulated the expression of GalLDH and AAO by 52.1% and 39.3%. Above findings suggested that IBU reversed the effect of La on GalLDH expression and enhanced the effect of La on AAO expression.

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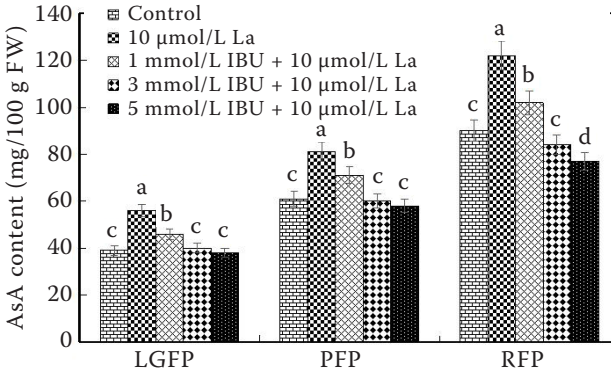

Figure 6. Effects of $La(NO_3)_3$ and ibuprofen (IBU) on ascorbic acid (AsA) content in fruit at different stages. The plants were treated as below: Control – distilled water; 10 μmol/L La – 10 μmol/L $La(NO_3)_3$; 1 mmol/L IBU + 10 μmol/L La – 1 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 3 mmol/L IBU + 10 μmol/L La – 3 mmol/L IBU + 10 μmol/L $La(NO_3)_3$; 5 mmol/L IBU + 10 μmol/L La – 5 mmol/L IBU + 10 μmol/L $La(NO_3)_3$. Values represent mean ± standard deviation (n = 6), and small letters indicate statistical difference at $P < 0.05$ at the same stage. LGFP – large green fruit period; PFP – pink fruit period; RFP – ripen fruit period; FW – fresh weight

Effects of $La(NO_3)_3$ and IBU on AsA content. In comparison with the control, $La(NO_3)_3$ improved AsA content at different stages (Figure 6). All the concentrations of IBU plus $La(NO_3)_3$ significantly reduced AsA content in La-treated plants at different stages. At LGFP, PFP and RFP, 3 mmol/L IBU plus $La(NO_3)_3$ decreased AsA content in La-treated plants by 28.6, 25.9 and 31.1%, respectively. The application of 5 mmol/L IBU plus $La(NO_3)_3$ decreased AsA content by 32.1, 28.4 and 36.9%, respectively. Above findings suggested that IBU reversed the effect of La on AsA content in fruit.

Correlation analysis between JA content and other indicators in fruit. Through Pearson correlation analysis, we found that JA content was positively correlated with the activities and transcript levels of DHAR, MDHAR and GalLDH at different stages (Table 1). Especially for PFP and RFP, JA content had significant correlations with the activities and transcript levels of DHAR, MDHAR and GalLDH. Besides, JA content was negatively correlated with the activities and transcript levels of APX and GR at different stages. However, there was no significant correlation between JA content and the activities and transcript levels of APX and GR at every stage. Meanwhile, we found that JA content had a significant correlation with AsA content at different stages. Above results of the correlation analysis indicated that JA mainly participated in La-regulated AsA content in fruit by enhancing the activities and transcript levels of DHAR, MDHAR and GalLDH.

DISCUSSION

JA is an important phytohormone in regulating plant physiological and biochemical processes and stress resistance (Wu et al. 2022, Yuan et al. 2022). It has been documented that La induced JA production in *Scutellaria baicalensis* Georgi seedlings (Zhou et al. 2012). In this study, we found that La could also induce JA production in strawberry fruit. The results of previous and current studies indicated that La could induce the accumulation of JA in plants. Besides, our results showed that JA biosynthetic inhibitor IBU reversed the effect of La on JA content. IBU is an inhibitor of lipoxygenase (LOX), a key enzyme for JA biosynthesis. Thus, the current results indicated that La enhanced JA production through LOX.

The biosynthesis of AsA is closely related to the activity of GalLDH, a key enzyme responsible for

Table 1. Correlation analysis between jasmonic acid (JA) content and other indicators

Indicator		LGFP	PFP	RFP
AsA content		0.960**	0.985**	0.973**
Activity	APX	−0.733	−0.797	−0.753
	GR	−0.276	−0.327	−0.313
	DHAR	0.969**	0.920*	0.988**
	MDHAR	0.963**	0.982**	0.972**
	GalLDH	0.973**	0.994**	0.993**
	AAO	0.073	0.329	0.195
Expression	APX	−0.672	−0.823	−0.873
	GR	−0.15	−0.221	−0.202
	DHAR	0.991**	0.915*	0.979**
	MDHAR	0.799	0.938*	0.980**
	GalLDH	0.986**	0.984**	0.991**
	AAO	0.323	0.236	−0.041

**at the level of 0.01 (two-tailed), the correlation was significant; *at the level of 0.05 (two-tailed), the correlation was significant; LGFP – large green fruit period; PFP – pink fruit period; RFP – ripen fruit period; AsA – ascorbic acid; APX – ascorbate peroxidase; GR – glutathione reductase; DHAR – dehydroascorbate reductase; MDHAR – monodehydroascorbate reductase; GalLDH – L-galactono-1,4-lactone dehydrogenase; AAO – ascorbate oxidase

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AsA biosynthesis in the L-galactose pathway in plants (Liu et al. 2020). Previous reports demonstrated that $La(NO_3)_3$ regulated AsA content in strawberry fruit through GalLDH (Shan et al. 2017, 2018a). This study obtained the same result as previous studies (Shan et al. 2017, 2018a). In addition, the findings displayed that $La(NO_3)_3$ promoted JA accumulation, which further regulated GalLDH activity and transcript level in fruit. However, IBU reversed the above effects of $La(NO_3)_3$. Meanwhile, the results of Pearson correlation analysis showed that JA content had significant correlations with AsA content and the activity and transcript level of GalLDH. These findings suggested that $La(NO_3)_3$ could increase AsA content by enhancing the activity and transcript level of GalLDH through the JA pathway.

AsA-GSH cycle is responsible for AsA regeneration in plants, including four enzymes APX, GR, DHAR and MDHAR (Alsahli et al. 2021, Shad et al. 2022). We previously found that $La(NO_3)_3$ reduced APX activity in strawberry fruit at low concentrations (Shan et al. 2017, 2018a). Current findings showed the same result as the previous study (Shan et al. 2017). However, some researches showed that JA increased APX activity in maize and soybean, which did not agree with current results. It was probably related to the difference in species (Mir et al. 2018). In addition, we found that IBU reversed the effects of $La(NO_3)_3$ on the activity and transcript level of APX, which suggested that La-induced JA enhanced the activity and transcript level of APX. In a previous study, we found that La increased GR activity in strawberry fruit. The current study showed the same result as previous studies (Shan et al. 2017, 2018a). However, current results showed that IBU further improved the activity and transcript level of GR in La-treated fruit. Meanwhile, the results of Pearson correlation analysis showed that JA content had no significant correlations with the activity and transcript level of GR. These results indicated that JA did not play an important role in the regulation of GR activity and transcript level by La. Thus, it will be interesting to further investigate the mechanism for the regulation of GR activity and transcript level by La. For DHAR and MDHAR, current findings displayed that IBU reversed the effects of $La(NO_3)_3$ on their activities, which suggested that JA played a positive role in the regulation of DHAR and MDHAR activities by La. Previous studies also demonstrated that JA increased the activities of DHAR and MDHAR in *Arabidopsis thaliana* (Shan et al. 2018b), which was consistent with our current results in strawberry fruit. Besides, we found that IBU also reversed the effects of $La(NO_3)_3$ on the transcript levels of DHAR and MDHAR in strawberry fruit, which indicated that JA improved their activities by up-regulating their expression. Meanwhile, the results of Pearson correlation analysis showed that JA content had significant correlations with AsA content and the activities and transcript levels of DHAR and MDHAR. Above findings suggested that $La(NO_3)_3$ mainly increased AsA content by enhancing the activities and transcript levels of DHAR and MDHAR through the JA pathway.

AAO is responsible for AsA degradation. Thus, high AAO activity could decrease AsA content. In this study, the findings displayed that $La(NO_3)_3$ markedly reduced AAO activity, which agreed with our previous studies (Shan et al. 2017, 2018a). However, we found that IBU further reduced the activity and transcript level of AAO in La-treated fruit. Meanwhile, the results of Pearson correlation analysis showed that JA content had no significant correlations with the activity and transcript level of AAO. These results indicated that JA did not play an important role in the regulation of AAO activity and transcript level by La. Thus, it will also be interesting to further investigate the mechanism for the regulation of AAO activity and transcript level by La.

In the current study, we found that IBU had negative effects on the activities and transcript levels of GalLDH, DHAR and MDHAR and had positive effects on the activity and transcript level of APX, which indirectly suggested that JA positively regulated GalLDH, DHAR and MDHAR, and negatively regulated APX. In the previous study, we showed that ABA had positive effects on the activities of GalLDH and MDHAR and had negative effects on APX activity in the process of $La(NO_3)_3$-regulated AsA content (Shan et al. 2018a). Combined previous results with current results, we found that JA played the same roles as ABA in regulating the activities of GalLDH, APX and MDHAR in $La(NO_3)_3$-treated fruit. It has been documented that there were cross-talks between ABA and JA in regulating many physiological and biochemical processes in plants (Wang et al. 2021). For strawberries, Garrido-Bigotes et al. (2018) showed that there were cross-talks between ABA and JA in regulating fruit development and ripening of strawberries. While whether there are cross-talks between ABA and JA in regulating GalLDH, APX and MDHAR induced by $La(NO_3)_3$ is unclear. Thus, it is important to further study the

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cross-talk between JA and ABA in the regulation of AsAcontent by $La(NO_3)_3$.

In conclusion, our results showed that JA participated in $La(NO_3)_3$-regulated AsA content in strawberry fruit by mainly regulating the activities and transcript levels of GalLDH, DHAR and MDHAR. These results provide a more theoretical basis for applying $La(NO_3)_3$ in improving the fruit quality of strawberries in production and cultivation.

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REFERENCES

Aksakal Ö. (2022): Silicone alleviates palladium toxicity by modifying palladium uptake, ascorbate-glutathione cycle, and glyoxalase system in maize seedlings. Journal of Plant Growth Regulation. https://doi.org/10.1007/s00344-022-10828-6

Ali M.M., Anwar R., Malik A.U., Khan A.S., Ahmad S., Hussain Z., Hasan M., Nasir M., Chen F. (2022): Plant growth and fruit quality response of strawberry is improved after exogenous application of 24-epibrassinolide. Journal of Plant Growth Regulation, 41: 1786–1799.

Alsahli A.A., Bhat J.A., Alyemeni M.N., Ashraf M., Ahmad P. (2021): Hydrogen sulfide (H_2S) mitigates arsenic (As)-induced toxicity in pea (*Pisum sativum* L.) plants by regulating osmoregulation, antioxidant defense system, ascorbate glutathione cycle and glyoxalase system. Journal of Plant Growth Regulation, 40: 2515–2531.

Arrigoni O., De Gara L., Tommasi F., Liso R. (1992): Changes in the ascorbate system during seed development of *Vicia faba* L. Plant Physiology, 99: 235–238.

Asghari M., Merrikhi M., Kavoosi B. (2019): Methyl jasmonate foliar spray substantially enhances the productivity, quality and phytochemical contents of pomegranate fruit. Journal of Plant Growth Regulation. https://doi.org/10.1007/s00344-019-10050-x

Deepika, Singh A. (2021): Expression dynamics indicate the role of jasmonic acid biosynthesis pathway in regulating macronutrient (N, P and K^+) deficiency tolerance in rice (*Oryza sativa* L.). Plant Cell Reports, 40: 1495–1512.

Dos Santos C.P., Batista M.C., da Cruz Saraiva K.D., Maia Roque A.L., de Souza Miranda R., e Silva L.M.A., Herbster Moura C.F., Alves Filho E.G., Canuto K.M., Costa J.H. (2019): Transcriptome analysis of acerola fruit ripening: insights into ascorbate, ethylene, respiration, and softening metabolisms. Plant Molecular Biology, 101: 269–296.

Fan D., Yang L., Jin J., Hao Q., Abudoukayoumu A., Zhao J., Liao K. (2022): Hormonal and transcriptomic analysis reveal gibberellin-induced fruit set in jujube (*Ziziphus jujuba* Mill.). Plant Molecular Biology Reporter, 40: 163–175.

Feng S., Sun J., Sun S., Wang Y., Tian C., Sun Q., Chen X. (2017): Transcriptional profiles underlying the effects of methyl jasmonate on apple ripening. Journal of Plant Growth Regulation, 36: 271–280.

Garrido-Bigotes A., Figueroa P.M., Figueroa C.R. (2018): Jasmonate metabolism and its relationship with abscisic acid during strawberry fruit development and ripening. Journal of Plant Growth Regulation, 37: 705–706.

Hodges D.M., Andrews C.J., Johnson D.A., Hamilton R.I. (1996): Antioxidant compound responses to chilling stress in differentially sensitive inbred maize lines. Plant Physiology, 98: 685–692.

Khew C.Y., Mori I.C., Matsuura T., Hirayama T., Harikrishna J.A., Lau E.T., Augustine Mercer Z.J., Hwang S.S. (2020): Hormonal and transcriptional analyses of fruit development and ripening in different varieties of black pepper (*Piper nigrum*). Journal of Plant Research, 133: 73–94.

Kumar V., Irfan M., Ghosh S., Chakraborty N., Chakraborty S., Datta A. (2016): Fruit ripening mutants reveal cell metabolism and redox state during ripening. Protoplasma, 253: 581–594.

Li C., Kong X., Luo Z., Li W., Tang W., Zhang D., Ma C., Dong H. (2021): Exogenous application of acetic acid improves the survival rate of cotton by increasing abscisic acid and jasmonic acid contents under drought stress. Acta Physiologia Plantarum, 43: 32.

Liu J.X., Feng K., Wang G.L., Wu X.J., Duan A.Q., Yin L., Shen D., Xu Z.S., Xiong A.S. (2020): Effect of elevated CO_2 on ascorbate accumulation and the expression levels of genes involved in ascorbate metabolism in celery. Journal of Plant Growth Regulation, 39: 1046–1060.

Mir M.A., Sirhindi G., Alyemeni M.N., Alam P., Ahmad P. (2018): Jasmonic acid improves growth performance of soybean under nickel toxicity by regulating nickel uptake, redox balance, and oxidative stress metabolism. Journal of Plant Growth Regulation, 37: 1195–1209.

Shad M.I., Ashraf M.A., Rasheed R., Hussain I., Ali S. (2022): Exogenous coumarin decreases phytotoxic effects of manganese by regulating ascorbate-glutathione cycle and glyoxalase system to improve photosynthesis and nutrient acquisition in sesame (*Sesamum indicum* L.). Journal of Soil Science and Plant Nutrition, https://doi.org/10.1007/s42729-022-00983-w

Shan C., Liang Z. (2010): Jasmonic acid regulates ascorbate and glutathione metabolism in *Agropyron cristatum* leaves under water stress. Plant Science, 178: 130–139.

Shan C., Zhang H., Zhang Y., Zhou H. (2017): Lanthanum nitrate regulates the content of vitamin C through its biosynthesis, regeneration and degradation in the fruit of strawberry. Scientia Horticulturae, 224: 102–108.

Shan C., Zhang Y., Zhang H. (2018a): ABA participates in the regulation of vitamin C content in the fruit of strawberry using lanthanum nitrate. Scientia Horticulturae, 233: 455–459.

Shan C., Wang T., Zhou Y., Wang W. (2018b): Hydrogen sulfide is involved in the regulation of ascorbate and glutathione metabo-

https://doi.org/10.17221/384/2022-PSE

lism by jasmonic acid in *Arabidopsis thaliana*. Biologia Plantarum, 62: 188–193.

Sun Y., Asghari M., Zahedipour-Sheshgelani P. (2020): Foliar spray with 24-epibrassinolide enhanced strawberry fruit quality, phytochemical content, and postharvest life. Journal of Plant Growth Regulation, 39: 920–929.

Wang X., Li Q., Xie J., Huang M., Cai J., Zhou Q., Dai T., Jiang D. (2021): Abscisic acid and jasmonic acid are involved in drought priming-induced tolerance to drought in wheat. The Crop Journal, 9: 120–132.

Wu B., Zeng Z., Wu X., Li Y., Wang F., Yang J., Li X. (2022): Jasmonic acid negatively regulation of root growth in Japonica rice (*Oryza sativa* L.) under cadmium treatment. Plant Growth Regulation, 98: 651–667.

Yuan J., Cheng L., Li H., An C., Wang Y., Zhang F. (2022): Physiological and protein profiling analysis provides insight into the underlying molecular mechanism of potato tuber development regulated by jasmonic acid *in vitro*. BMC Plant Biology, 22: 481.

Zhang N., Li L., Zhang L., Li J., Fang Y., Zhao L., Ren Y., Chen F. (2020): Abscisic acid enhances tolerance to spring freeze stress and regulates the expression of ascorbate-glutathione biosynthesis-related genes and stress-responsive genes in common wheat. Molecular Breeding, 40: 108.

Zhou J., Fang L., Li X., Guo L., Huang L. (2012): Jasmonic acid (JA) acts as a signal molecule in $LaCl_3$-induced Baicalin synthesis in *Scutellaria baicalensis* seedlings. Biological Trace Element Research, 148: 392–395.

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