Lanthanum (La) improves growth, yield formation and 2-acetyl-1-pyrroline biosynthesis in aromatic rice (Oryza sativa L.)

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Abstract
Background: Lanthanum (La) is a rare earth element that can influence plant growth and development. However, the effect of La on growth, yield formation and 2-acetyl-1-pyrroline (2-AP, a key compound responsible for the aroma of rice) biosynthesis in aromatic rice (Oryza sativa L. subsp. japonica Kato) has not been reported. Therefore, the present study investigated the effects of La on growth, photosynthesis, yield formation and 2-AP biosynthesis in aromatic rice through three experiments.

Results: Two pot experiments and a two-year field trial were conducted with different rates of La application (20–120 LaCl₃ mg kg⁻¹ and 12 kg ha⁻¹ LaCl₃), and treatments without La application were used as controls. The results showed that the application of LaCl₃ at 80 and 100 mg kg⁻¹ and at 12 kg ha⁻¹ greatly increased the 2-AP content (by 6.45–43.03%) in aromatic rice seedlings and mature grains compared with the control. The La treatments also increased the chlorophyll content, net photosynthetic rate and total aboveground biomass of rice seedlings. Higher antioxidant enzyme (superoxide, peroxidase, and catalase) activity was detected in the La treatments than in the control. The La treatments also increased the grain yield, grain number per panicle and seed-setting rate of aromatic rice relative to the control. Moreover, the grain proline and γ-aminobutyric acid contents and the activity of betaine aldehyde dehydrogenase significantly decreased under the La treatment. The application of La to soil enhanced the activity of proline dehydrogenase by 20.62–56.95%.

Conclusions: La improved the growth, yield formation and 2-AP content of aromatic rice and enhanced 2-AP biosynthesis by increasing the conversion of proline to 2-AP and decreasing the conversion of GABald to GABA.

Keywords: 2-acetyl-1-pyrroline, Aromatic rice, Lanthanum, Photosynthesis, Proline, Yield formation

Background
Aromatic rice (Oryza sativa L.) is well known worldwide for its characteristic aroma and is also highly desired by consumers, attracts premium prices in many international markets [1–4]. The aromatic compounds of aromatic rice are very complicated, and more than 200 volatile substances were detected in aromatic rice in previous studies [5]. In recent years, it has been clearly established that 2-acetyl-1-pyrroline (2-AP) is the key flavor compound in aromatic rice that imparts its characteristic aroma [6, 7].

The biosynthesis of 2-AP in aromatic rice is a relatively clear process. Previous studies discovered that 2-AP formation is closely related to proline and γ-aminobutyric acid (GABA). In 2002, Yoshihashi et al. [8] revealed that proline is the nitrogen source for 2-AP through an isotope tracing test. Mo et al. [4] showed that 2-AP content is positively correlated with both the proline and GABA in aromatic rice. Chen et al. [9] demonstrated that 2-AP
biosynthesis in aromatic rice is inhibited by the expression of the BADH2 gene; this gene encodes betaine aldehyde dehydrogenase (BADH) which catalyzes the conversion of γ-aminobutyl aldehyde (GABald) into GABA instead of 2-AP. Furthermore, the study of Mo et al. [10] indicated that the 2-AP in aromatic rice was transformed mainly from proline catalyzed by proline dehydrogenase (PDH).

Lanthanum (La) is one of the rare earth elements, a group that includes 17 elements with similar physical and chemical properties [11]. An earlier study showed that exogenous La significantly ameliorated copper toxicity in rice by reducing oxidative stress and increasing the chlorophyll content [12]. Liang et al. [13] discovered that La application remarkably enhanced plasma membrane H⁺-ATPase activity in rice under acid rain stress. Liu et al. [14] indicated that exogenous La³⁺ induced regulation by the antioxidant system and affected the concentrations of hydrogen peroxide, superoxide anion, and malondialdehyde in rice roots. Wang et al. [15] also demonstrated that the combination of LaCl₃ and acid rain substantially increased the net photosynthetic rate, stomatal conductance, Hill reaction activity and carboxylation efficiency of rice plants. Hence, it can be concluded that La has multiple effects on rice growth and development.

In 2016, Mo et al. [10] showed that supplementation with La in basic culture medium enhanced the activity of PDH and increased the 2-AP content in detached aromatic rice panicles in Vitro. La might have the ability to enhance 2-AP biosynthesis and the potential to be used in aromatic rice production to cultivate highly aromatic rice. However, no additional studies of the effects of La on aromatic rice growth performance have been published. Furthermore, the mechanism underlying the regulation of 2-AP formation under exogenous La application remains unexplored.

Therefore, the present study was conducted with the hypothesis that La could increase grain yield and 2-AP content in aromatic rice and with the objective of studying the mechanism underlying the effects of La on 2-AP biosynthesis in aromatic rice.

### Results

#### Effects of La application on the performance of aromatic rice seedlings

The application of La to soil significantly influenced the 2-AP content of the aromatic rice seedlings (Fig. 1). Compared with CK, the La treatments (La2, La3 and La4 treatments) notably improved the 2-AP content, by 11.10–41.01% and 11.10–23.38% for Meixiangzhan-2 and Xiangyaxiangzhan, respectively and the highest contents were recorded in the La3 and La4 treatments. In addition, we observed that the La5 treatments suddenly decreased 2-AP content for Meixiangzhan-2 and Xiangyaxiangzhan.
**Xiangyaxiangzhan** compared with that in the La3 and La4 treatments. This may have occurred because the content of La in soil in the La5 treatment exceeded the suitable range for fragrant rice and caused metal toxicity. Regarding seedling quality was concerned, the aromatic rice seedlings grown in La containing soils exhibited significant improvements in fresh weight, dry weight and stem diameter compared to those grown in soil without La (Table 1). In comparison with CK, La3 and La4 treatments exhibited significantly higher fresh weight (by 6.00–13.68%), dry weight (by 5.98–19.45%) and stem diameter by (5.43–9.74%).

**Effects of La application on yield and yield related traits of aromatic rice**

The yield and yield-related traits of aromatic rice were affected by La application in both the second and third experiments (Table 2 & Table 3). For Meixiangzhan-2 in the second experiment, compared with CK, the La80 treatment significantly increased the grain yield (by 12.22%) while the La80 and La100 treatments significantly increased the grain number per panicle, seed-setting rates and total yield. For Xiangyaxiangzhan in the second experiment, compared with CK, the La80 and La100 treatments significantly increased the grain yield and total yield by 9.33–10.34% and 15.16–17.19%, respectively. Compared with CK, the La100 and La80 treatments also significantly increased the grain number per panicle, by 3.84–12.22% while the La80 and La100 treatments significantly increased the grain number per panicle, seed-setting rates and total yield. For Meixiangzhan-2 in the third experiment, compared with CK, the La80 and La100 treatments increased the SPAD value by 7.45–13.57% compared with CK, and at the maturity stage, slightly higher SPAD values were recorded in the La treatment than in the CK treatment. At the booting stage, heading stage and grain-filling stage, the La80 and La100 treatments increased the SPAD value by 4.32–18.95% higher SPAD values were recorded in the La80 and La100 treatment than CK. In the third experiment, 4.32–18.95% higher SPAD values were recorded in the La treatment than in the CK treatment at the booting stage, heading stage and grain-filling stage. Compared with CK, the La treatment also slightly increased the SPAD value at the maturity stage.

**Effects of La application on the chlorophyll content of aromatic rice**

La application substantially increased the chlorophyll content of aromatic rice (Fig. 2 & Fig. 3). In second experiment, there were no significant differences in SPAD values among all the treatments including CK at the tillering stage. At the booting stage, heading stage and grain-filling stage, the La80 and La100 treatments increased the SPAD value by 7.45–13.57% compared with CK, and at the maturity stage, slightly higher SPAD values were recorded in the La80 and La100 treatment than CK. In the third experiment, 4.32–18.95% higher SPAD values were recorded in the La treatment than in the CK treatment.

**Effects of La application on the net photosynthetic rate of aromatic rice**

La application substantially improved the net photosynthetic rate of aromatic rice (Fig. 4 & Fig. 5). In the second experiment, there were no significant differences in net photosynthetic rate among all the treatments including CK at the tillering stage. At the booting stage, heading stage and grain-filling stage, the La80 and

| Cultivar          | Treatment | Fresh weight (mg) | Dry weight (mg) | Plant Height (cm) | Stem diameter (mm) |
|-------------------|-----------|-------------------|-----------------|-------------------|-------------------|
| **Meixiangzhan-2** |           |                   |                 |                   |                   |
| CK                | 168.37 ± 5.61b | 27.63 ± 5.94b | 20.68 ± 0.94b  | 2.20 ± 0.34bc     |
| La1               | 164.23 ± 1.74b | 27.13 ± 6.33b | 21.52 ± 0.13b  | 2.14 ± 1.33bc     |
| La2               | 168.60 ± 2.64b | 27.14 ± 4.53b | 21.45 ± 0.87b  | 2.11 ± 0.68bc     |
| La3               | 178.47 ± 3.52ab | 33.00 ± 2.40a | 26.29 ± 0.46a  | 2.39 ± 1.17a      |
| La4               | 189.87 ± 3.08a | 31.37 ± 4.43a | 25.15 ± 0.78a  | 2.32 ± 0.98a      |
| La5               | 169.00 ± 2.22b | 27.55 ± 4.72b | 21.48 ± 1.32b  | 2.22 ± 0.58b      |
| **Xiangyaxiangzhan** |           |                   |                 |                   |                   |
| CK                | 137.20 ± 1.95b | 25.02 ± 2.21cd | 24.54 ± 0.98ab | 1.85 ± 0.80c      |
| La1               | 135.80 ± 4.43b | 24.29 ± 6.81d  | 23.55 ± 0.11b  | 1.97 ± 1.33ab     |
| La2               | 136.33 ± 2.48b | 26.67 ± 1.63b  | 24.51 ± 0.91ab | 1.90 ± 1.12bc     |
| La3               | 154.73 ± 6.12a | 29.67 ± 1.69a  | 25.35 ± 0.16ab | 2.03 ± 0.04a      |
| La4               | 155.97 ± 1.87a | 26.52 ± 4.44bc | 26.98 ± 0.30a  | 2.03 ± 1.02a      |
| La5               | 137.10 ± 5.25b | 25.16 ± 6.43bcd | 25.41 ± 0.11ab | 1.95 ± 0.34abc    |

Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)
La100 treatments improved the net photosynthetic rate by 7.45–14.08% compared with that in CK, and at the maturity stage, slightly higher net photosynthetic rates were recorded in the La80 and La100 treatments than in CK. In the third experiment, 4.98–18.41% higher net photosynthetic rates were recorded in the La treatment than in CK at the booting stage, heading stage and grain-filling stage. Compared with CK, the La treatment also slightly improved net photosynthetic rate at the maturity stage.

**Effects of La application on dry matter accumulation in aromatic rice**

La application remarkably enhanced the dry matter accumulation in aromatic rice in the field experiment (Fig. 6). For Meixiangzhan-2 in 2018, 14.33, 18.41, 17.69 and 16.83% higher total aboveground biomasses were recorded in the La treatment than in CK at the booting stage, heading stage, grain-filling stage and maturity stage, respectively. For Xiangyaxiangzhan in 2018, 8.74, 11.76, 10.79 and 11.66% higher total aboveground biomasses were recorded in the La treatment than in CK at the booting stage, heading stage, grain-filling stage and maturity stage, respectively. For Meixiangzhan-2 in 2019, compared with CK, the La treatment significantly increased the total aboveground biomass by 11.69, 18.15, 21.15 and 22.14% in the four growth stages, respectively; for Xiangyaxiangzhan in 2019, compared with CK, the La treatment significantly increased the total aboveground biomass by 18.12, 20.53, 20.96 and 19.40% in the four growth stages, respectively.

**Table 2** Effects of La application on grain yield, effective panicle number, grain number per panicle, seed-setting rate, 1000-grain weight and total yield of aromatic rice in the second experiment

| Cultivar          | Treatment | Grain yield (g pot⁻¹) | Effective panicle number per pot | Grain number per panicle (%) | Seed-setting rate (%) | 1000-grain weight (g) | Total yield (g pot⁻¹) |
|-------------------|-----------|-----------------------|---------------------------------|-----------------------------|-----------------------|-----------------------|-----------------------|
| Meixiangzhan-2    | CK        | 53.46 ± 2.18b         | 28.67 ± 1.03a                   | 133.12 ± 0.39c              | 71.31 ± 0.21b         | 19.92 ± 0.03a         | 19.68 ± 0.04b         |
|                   | La40      | 56.32 ± 1.01ab        | 27.00 ± 2.84a                   | 130.23 ± 0.50bc             | 75.46 ± 0.56a         | 20.08 ± 0.02a         | 18.63 ± 0.02b         |
|                   | La80      | 59.99 ± 1.30a         | 27.67 ± 0.81a                   | 138.81 ± 1.13ab             | 75.33 ± 0.44a         | 19.86 ± 0.03a         | 22.63 ± 0.06a         |
|                   | La100     | 58.08 ± 0.65ab        | 29.67 ± 1.93a                   | 141.83 ± 0.85a              | 72.24 ± 0.22b         | 20.18 ± 0.03a         | 22.31 ± 0.04a         |
| Xiangyaxiangzhan  | CK        | 53.94 ± 1.74bc        | 28.33 ± 1.69a                   | 129.83 ± 0.36b              | 71.40 ± 0.39b         | 20.47 ± 0.05a         | 21.35 ± 0.07b         |
|                   | La40      | 53.17 ± 0.38c         | 26.67 ± 2.44a                   | 137.03 ± 0.44ab             | 72.67 ± 0.36ab        | 20.10 ± 0.03a         | 22.09 ± 0.02b         |
|                   | La80      | 59.52 ± 1.71a         | 28.67 ± 2.16a                   | 135.95 ± 0.42ab             | 75.04 ± 0.28a         | 20.08 ± 0.04a         | 24.89 ± 0.02a         |
|                   | La100     | 58.97 ± 1.35ab        | 28.33 ± 2.59a                   | 140.75 ± 0.97a              | 73.49 ± 0.73ab        | 20.07 ± 0.01a         | 25.36 ± 0.04a         |

Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test).

**Table 3** Effects of La application on grain yield, effective panicle number, grain number per panicle, seed-setting rate and 1000-grain weight of aromatic rice in the third experiment

| Year   | Cultivar          | Treatment | Grain yield (g pot⁻¹) | Effective panicle number per pot | Grain number per panicle (%) | Seed-setting rate (%) | 1000-grain weight (g) |
|--------|-------------------|-----------|-----------------------|---------------------------------|-----------------------------|-----------------------|-----------------------|
| 2018   | Meixiangzhan-2    | CK        | 5.14 ± 0.09b          | 260.94 ± 21.33a                 | 132.91 ± 7.69b              | 77.82 ± 3.68a         | 20.61 ± 0.05a         |
|        |                   | La        | 5.54 ± 0.10a          | 295.59 ± 26.74a                 | 158.80 ± 5.16a              | 76.89 ± 4.19a         | 20.69 ± 0.04a         |
|        | Xiangyaxiangzhan  | CK        | 4.95 ± 0.18b          | 257.04 ± 1.00a                  | 141.05 ± 4.92b              | 77.96 ± 1.25a         | 19.96 ± 0.40a         |
|        |                   | La        | 5.14 ± 0.17a          | 269.80 ± 7.77a                  | 154.14 ± 7.45a              | 77.02 ± 2.18a         | 20.54 ± 0.15a         |
| 2019   | Meixiangzhan-2    | CK        | 5.21 ± 0.08b          | 260.24 ± 5.13a                  | 143.40 ± 2.38b              | 77.12 ± 0.89a         | 20.14 ± 0.11a         |
|        |                   | La        | 5.69 ± 0.07a          | 287.22 ± 13.00a                 | 158.88 ± 3.06a              | 78.31 ± 1.92a         | 20.39 ± 0.46a         |
|        | Xiangyaxiangzhan  | CK        | 4.69 ± 0.18b          | 267.97 ± 22.61a                 | 138.61 ± 8.06a              | 78.46 ± 1.47a         | 20.00 ± 0.44a         |
|        |                   | La        | 5.19 ± 0.06a          | 288.07 ± 11.59a                 | 153.06 ± 7.72a              | 77.24 ± 1.07a         | 19.69 ± 0.06a         |

Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test).
Effects of La application on the activities of SOD, POD, and CAT and content of MDA in aromatic rice

As shown in Fig. 7, La application affected the MDA content and induced regulation in antioxidative enzyme (SOD, POD and CAT) activity. There was no significant difference between CK and the La treatment in the activities of SOD, POD, and CAT or the content of MDA at the tillering stage. However, compared with CK, the La treatment increased the activities of SOD, POD and CAT by 10.86–23.08%, 8.89–24.44% and 7.14–36.37%, respectively, from the booting stage to the maturity stage. The MDA contents were 8.26–24.83% lower than in the La treatment than CK from the booting stage to the maturity stage.

Fig. 2 Effects of different La treatments on the SPAD values of aromatic rice in the second experiment. TS: tillering stage; BS: booting stage; HS: heading stage; GFS: grain-filling stage; MS: Maturity stage. Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)

Fig. 3 Effects of different La treatments on the SPAD values of aromatic rice in the third experiment. Meixiangzhan in 2018; Xiangyaxiangzhan in 2018; Meixiangzhan in 2019; Xiangyaxiangzhan in 2019. TS: tillering stage; BS: booting stage; HS: heading stage; GFS: grain-filling stage; MS: Maturity stage. Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)
Effects of La application on 2-AP content and 2-AP biosynthesis in aromatic rice

In the second experiment, aromatic rice plants grown in La containing soil showed higher grain 2-AP contents (Fig. 8, Fig. 9, Fig. 10 and Table 4). For Meixiangzhan-2, the La40, La80, and La100 treatments substantially increased the grain 2-AP contents by 12.61, 27.53, 26.72% compared with those in CK; For Xiangyaxiangzhan, 28.36, 37.62, and 43.03% higher 2-AP contents were recorded in the La40, La80, and La100 treatments than CK. As shown in Fig. 10, La application regulated 2-AP biosynthesis in terms of the proline, and GABA contents and, PDH and BADH activity. In comparison with CK, La80 and La100 treatments significantly reduced the

Fig. 4 Effects of different La treatments on the net photosynthetic rate of aromatic rice in the second experiment. TS: tillering stage; BS: booting stage; HS: heading stage; GFS: grain-filling stage; MS: Maturity stage. Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)

Fig. 5 Effects of different La treatments on the net photosynthetic rate of aromatic rice in the third experiment. a Meixiangzhan in 2018; b Xiangyaxiangzhan in 2018; c Meixiangzhan in 2019; d Xiangyaxiangzhan in 2019. TS: tillering stage; BS: booting stage; HS: heading stage; GFS: grain-filling stage; MS: maturity stage. Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)
grain contents of proline and GABA. Higher PDH activity was recorded in the La80 and La100 treatments than in CK. Lower BADH activity was recorded in the La80 and La100 treatments than in CK.

In the field production (third Experiment), La application to the soil markedly increased the 2-AP content in aromatic rice grains (Fig. 9). In 2018, compared with CK, the La treatment significantly increased the grain 2-AP content by 31.36 and 32.85% in Meixiangzhan-2 and Xiangyxiaxiangzhan, respectively. In 2019, in comparison with CK, the La treatment significantly increased the grain 2-AP content by 28.55 and 23.08% in Meixiangzhan-2 and Xiangyxiaxiangzhan, respectively. As shown in Table 4, compared with CK, the La treatment remarkably reduced the contents of proline and GABA, and the activity of BADH. Higher PDH activity was recorded in the La treatment than in CK.

**Discussion**

Crop growth and development are substantially affected by La, this has been previously reported in several plant species, such as wheat (*Triticum aestivum* L.), maize (*Zea mays* L.) and soybean (*Glycine max* (Linn.) Merr.) [16–18]. The current study demonstrated the modulatory effects of La application to soil on 2-AP biosynthesis in aromatic rice. In the first experiment, we observed that aromatic rice seedlings grown in La containing soil (80 and 100 LaCl₃ mg kg⁻¹) not only had higher fresh weight, dry weight, plant height and stem length, but also had higher 2-AP contents than those grown in soil without La. In both the second and third experiments, the La treatments also substantially increased the grain yield of the aromatic rice cultivars. The increment in grain yield due to La application was attributed to improvement in grain number per panicle and seed-setting rate. Our results were consistent with the study of [19] who demonstrated that exogenous La significantly increased rice yield. As the method of dry matter accumulation in plants, photosynthesis was significantly influenced by La application in terms of the chlorophyll content and net photosynthetic rate in aromatic rice. The results of the present study showed that La treatments remarkably increased SPAD values and enhanced the net photosynthetic rate of the aromatic rice cultivars, Meixiangzhan-2 and Xiangyxiaxiangzhan at the booting stage, heading stage, grain-filling stage and maturity stage. The total aboveground biomass at the booting stage, heading stage, grain-filling stage and maturity stage (total yield) also increased due to La application. Therefore, we deduced that La enhanced yield formation in aromatic rice by regulating photosynthesis and dry matter accumulation.

In our study, higher activities of antioxidant enzymes (SOD, POD, CAT) and lower MDA content were observed in aromatic rice under the La treatments. Previous studies indicated the important roles of SOD, POD...
and CAT play in maintaining cellular structures and functions, and quenching reactive oxygen species in plant tissues [20, 21]. These enhancements in antioxidant activity indicated that La might improve ability of aromatic rice to resist different abiotic stresses. In the field, variations in climate especially the sudden occurrence of short-term extreme weather, have substantial effects on rice yield [22]. Moreover, the microclimate in paddy fields which is complicated and difficult to measure, also influences yield formation of rice [23]. A stronger antioxidant system in rice ensures stable rice production stabilization. Furthermore, chlorophyll biosynthesis and gas exchange for photosynthesis are affected by the environment [20, 24] while higher antioxidant enzyme activity can better maintain the stability of cells and ensure the progress of various physiological activities [25, 26]. Therefore, we deduced that the application of La might protect chlorophyll biosynthesis in aromatic rice by increasing the activities of antioxidant enzymes and that this would enhance photosynthesis and dry matter accumulation and finally increase grain yield.

Regarding 2-AP, a key component of the aroma of aromatic rice, the La treatments increased the grain 2-AP content and decreased the grain proline content in the second experiment. A similar result was also observed in the La treatment in the third experiment. Furthermore, the results of both the second and third experiments showed that the activity of PDH was significantly enhanced in the La treatments; this result is similar to the research of Mo et al. [10], who indicated that the additional La in culture medium increased the activity of PDH and the 2-AP content in aromatic rice panicles in vitro. The function of PDH is to catalyze the oxidation reaction of proline to convert △1-pyrroline-5-carboxylic acid, which is an important precursor for 2-AP biosynthesis [27, 28]. Therefore, the increase in grain 2-AP content in aromatic rice could be attributed to exogenous La enhancing PDH activity and thus leading to the promotion of the conversion from proline to 2-AP.
In addition, the present study revealed that lower grain proline and GABA contents were recorded in the La treatments than in CK in both the second and third experiments. In 2-AP biosynthesis in aromatic rice, a competitive relationship exists between GABA and 2-AP and the conversion from GABald to Δ1-pyrroline (a limited substrate in 2-AP formation [6]) or GABA depends on the reaction catalyzed by the enzyme BADH [9, 29, 30]. The results of the second and third experiments showed that the activity of BADH declined under the La treatments. Therefore, we deduced that exogenous La also increases the conversion from GABald to Δ1-pyrroline by inhibiting GABA formation, thus increasing the 2-AP content in aromatic rice.

![Fig. 8](image)

**Fig. 8** Effects of different La treatments on the 2-AP content of aromatic rice in the second experiment. Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)

![Fig. 9](image)

**Fig. 9** Effects of La application on the 2-AP content of aromatic rice grains in the third experiment. Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)
possible mechanism for the regulation of grain 2-AP biosynthesis in aromatic rice by exogenous La is shown in Fig. 11.

**Conclusion**
The application of La to soil increased the chlorophyll content and antioxidant enzyme activities of aromatic rice. Increases in the net photosynthetic rate, dry matter accumulation and yield formation were observed in the La treatments. La application also improved the conversion from proline to 2-AP by upregulating the activity of the enzyme PDH and thus increased the grain 2-AP content. In addition, La application inhibited BADH activity and thus reduced the conversion of GABald to GABA during 2-AP biosynthesis in aromatic rice.

**Table 4** Proline content, GABA content, PDH activity and BADH activity of aromatic rice under La application in the field in the third experiment

| Year       | Cultivar            | Treatment | Proline (μg g⁻¹ FW) | GABA (μg g⁻¹ FW) | PDH activity (U L⁻¹ FW) | BADH activity (U L⁻¹ FW) |
|------------|---------------------|-----------|---------------------|------------------|-------------------------|--------------------------|
| 2018       | Meixiangzhan-2      | CK        | 43.14 ± 1.37a       | 32.19 ± 1.52a    | 13.45 ± 0.67b          | 22.48 ± 0.35a            |
|            |                     | La        | 34.12 ± 0.43b       | 25.63 ± 1.10b    | 17.58 ± 0.72a          | 18.54 ± 0.43b            |
|            | Xiangyaxiangzhan    | CK        | 40.59 ± 0.62a       | 35.73 ± 1.15a    | 13.46 ± 1.15b          | 20.01 ± 0.45a            |
|            |                     | La        | 31.19 ± 0.53b       | 28.08 ± 1.71b    | 18.27 ± 1.07a          | 17.13 ± 0.84b            |
| 2019       | Meixiangzhan-2      | CK        | 41.98 ± 0.84a       | 34.51 ± 0.23a    | 12.04 ± 0.91b          | 21.51 ± 0.39a            |
|            |                     | La        | 33.21 ± 1.20b       | 24.12 ± 1.57b    | 18.89 ± 1.27a          | 17.39 ± 0.59b            |
|            | Xiangyaxiangzhan    | CK        | 43.31 ± 2.67a       | 39.16 ± 1.28a    | 13.23 ± 1.09b          | 20.99 ± 0.28a            |
|            |                     | La        | 32.77 ± 1.71b       | 25.89 ± 2.54b    | 16.40 ± 0.32a          | 18.59 ± 0.25b            |

Values (means ± SEs) of each treatment were obtained from three independent replications (n = 3). Different letters indicate significant differences among the treatments (P < 0.05, least significant difference test)
Methods
Experimental details and plant materials
To study the effect of La on 2-AP biosynthesis in aromatic rice, three experiments (two pot experiments and a two-year field experiment) were conducted with two aromatic rice cultivars, *Meixiangzhan-2* (*Lemont × Fengaozhan*) and *Xiangyaxiangzhan* (*Xiangsimiao126 × Xiangyaruanzhan*). These cultivars are widely planted in South China, and were provided by the College of Agriculture, South China Agricultural University, Guangzhou, China. Detailed information on the varieties could be found at [https://ricedata.cn/](https://ricedata.cn/). The three experiments were performed as described below:

The first experiment was conducted at the College of Agriculture, South China Agricultural University, Guangzhou, China between October and December 2017. After soaking and germination, the seeds of aromatic rice were sown in plastic pots (14.5 cm upper diameter, 10.5 cm lower diameter and 7.5 cm height) filled with paddy soil. There were five La treatments i.e., La1: 20 mg kg⁻¹ LaCl₃; La2: 40 mg kg⁻¹ LaCl₃; La3: 80 mg kg⁻¹ LaCl₃; La4: 100 mg kg⁻¹ LaCl₃; and La5: 120 mg kg⁻¹ LaCl₃. Pots without applied La were used as controls (CK). Twenty days after sowing, the seedlings were collected and the fresh weight, dry weight, plant height, stem diameter and 2-AP content were determined.

The second experiment was conducted in the greenhouse of the Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou, China (23°09'N, 113°22'E and 11 m above sea level) between March and July 2018. The germinated seeds of aromatic rice were sown in soils contained in plastic nursery trays. When the seedlings were twenty days old, they were transplanted into pots with five hills per pot (32 cm upper diameter, 21 cm lower diameter and 23 cm height) and four seedlings per hill. According to the results of the first experiment, three La treatments were implemented, i.e., La40: 40 mg kg⁻¹ LaCl₃; La80: 80 mg kg⁻¹ LaCl₃; and La100: 100 mg kg⁻¹ LaCl₃. Pots without La application were used as the controls (CK). At the grain-filling and maturity stages, fresh panicles were collected and stored at -80 °C for biochemical and molecular analysis. The grain yield and yield-related traits of aromatic rice were also estimated at the maturity stage.

The third experiment was conducted at the Experimental Research Farm, South China Agricultural University, Ningxi County (23°16'N, 113°22'E and 11 m above sea level), Guangdong Province, China between July and November in 2018 and again in 2019. After germination in the nursery, fifteen-day-old seedlings were mechanically transplanted into paddy fields at a planting distance of 30 cm × 16 cm. According to the results from both the first experiment and the second experiment, the La treatment was set as 12 kg ha⁻¹ LaCl₃, and a treatment without La application was used as a control (CK). The treatments were arranged in a randomized complete block design (RCBD) in triplicate with a total plot size of 10 m × 3 m. At the tillering stage (20 days after transplanting), booting stage (40 days after transplanting), heading stage (60 days after transplanting), grain-filling stage (75 days after transplanting) and maturity stage (90 days after transplanting, also 1 day before harvesting), fresh flag leaves from each plot were collected and stored at -80°C. At the grain-filling stage and maturity stage, fresh panicles were also collected and stored at -80°C for biochemical analysis. The grain yield and yield-related traits of aromatic rice were also estimated at the maturity stage.

![Fig. 11](https://ricedata.cn/) The potential roles of exogenous La in regulation of 2-AP biosynthesis in aromatic rice plants
Determination of net photosynthetic rate, chlorophyll content and dry matter accumulation
At the tillering stage (20 days after transplanting), booting stage (40 days after transplanting), heading stage (60 days after transplanting), grain-filling stage (75 days after transplanting) and maturity stage (90 days after transplanting, also 1 day before harvesting) in the second and third experiments, the net photosynthetic rate was determined with the portable photosynthesis system (LI-6400, LI-COR, USA) according to the method described by Luo et al. (2019). At the same time, a ‘SPAD-502’ SPAD meter (Konica Minolta, Japan) was used to perform a precise, rapid and nondestructive estimation of the leaf chlorophyll contents. In the second experiment, the total above-ground tissue of aromatic rice from three random pots in each treatment was collected at the maturity stage and oven-dried at 80 °C for the determination of the total yield. In the third experiment, plants from 9 randomly selected hills were collected at the tillering stage, booting stage, heading stage, grain-filling stage and maturity stage and oven-dried at 80 °C to a constant weight for the determination of the total above-ground biomass.

Determination of activities of superoxide (SOD), peroxidase (POD), catalase (CAT) and contents of malondialdehyde (MDA)
The MDA content and activities of antioxidant enzymes in the leaves of aromatic rice were determined according to the methods described by Kong et al. [20]. The MDA content was detected after a reaction with thiobarbituric acid while the absorbance was read at 532 nm, 600 nm and 450 nm. The activities of SOD, POD and CAT were determined and expressed as U L⁻¹ FW.

Determination of 2-AP content
The 2-AP contents in the grain samples collected at maturity and the seedling samples were determined using the simultaneous distillation–extraction method (SDE) and analyzed by a GCMS-QP 2010 Plus (Shimadzu Corporation, Japan) according to the methods of [31].

Determination of the contents of proline and GABA, and activities of PDH and BADH
The contents of proline and GABA in the grain samples collected at the grain-filling stage were determined according to the methods described by Mo et al. [4]. The proline content was determined after reacting with sulfosalicylic acid and the absorbance was measured at 520 nm. The GABA in the samples was extracted using 60% ethanol, and the absorbance was read at 645 nm. The GABA content was expressed as µg g⁻¹ FW. The activities of PDH and BADH in grains at the grain-filling stage were estimated according to the methods of Luo et al. [2]. For BADH activity, the absorbance was read at 450 nm and expressed as U L⁻¹. For PDH activity, the absorbance was read at 440 nm and expressed as U L⁻¹.

Statistical analyses
All data were analyzed statistically using Statistix 8 (Analytical software, Tallahassee, Florida, USA) with one-way analysis of variance. The differences among means were separated by using the least significant difference (LSD) test at the 5% significance level.

Abbreviations
2-AP: 2-Acetyl-γ-L-pyrroline; BADH: Betaine aldehyde dehydrogenase; CAT: Catalase; GABA: γ-Aminobutyric acid; La: Lanthanum; MDA: Malondialdehyde; PDH: Proline dehydrogenase; POD: Peroxidase; SOD: Superoxide dismutase.

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Authors’ contributions
HL and XT planned and designed the research; YC and LH performed the experiments; HL wrote the first version of manuscript. XT provided the guidance during the experiment and paper writing. All authors have read and approved the final manuscript.

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