Light and water treatment during the early grain filling stage regulates yield and aroma formation in aromatic rice

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The effect of light and water on aromatic rice remain largely unclear. A pot experiment was conducted to investigate the influences of light-water treatments (CK: natural light and well-watered conditions, WS: natural light and water-stressed conditions, LL: low light and well-watered conditions, LL-WS: low light and water-stressed treatment) on yield and 2-acetyl-1-pyrroline (2AP) formation in aromatic rice. Compared with CK, the light-water treatments decreased grain yield (10.32–39.19%) due to reductions in the filled grain percentage and total dry weight, in the regulation of biomass distribution, and in the attributes of gas exchange and antioxidant response parameters. The 2AP content in grains increased in the LL treatment (5.08–16.32%) but decreased in the WS treatment compared with that in CK. The changes in 2AP were associated with changes in 2AP formation-related traits and element content. Low light and water stress led to yield declines in aromatic rice, but low light alleviated the decrease in 2AP content caused by water stress.

Rice is one of the most important food crops worldwide. Aromatic rice has a higher grain quality than non-aromatic rice, and consumers prefer aromatic rice due to its pleasant smell1,2. Aromatic rice plays a significant role in international rice markets3. The global demand for aromatic rice is increasing4.

Many volatile compounds have been detected in aromatic rice5–7, of these, 2-acetyl-1-proline (2AP) is a determinant of the aromatic properties of aromatic rice8,9. Previous studies have suggested that proline is an important precursor for 2AP formation10,11. In addition, the ornithine, glutamate, γ-aminobutyric acid (GABA), Δ1-pyrroline-5-carboxylate (P5C), Δ1-pyrroline-5-carboxylate synthetase (P5CS), ornithine aminotransferase (OAT) and proline dehydrogenase (PDH) are highly related to the biosynthesis of 2AP12–16. Moreover, some studies have reported that micronutrients such as Mn and Zn contribute to the synthesis of 2AP in aromatic rice14,17.

In addition to the effects of genotype, environmental factors and cultivation practices affect the accumulation of 2AP in aromatic rice6. A previous study reported that the 2AP content was negatively correlated with sunshine hours18. Shading promoted the accumulation of 2AP in aromatic rice19,20. However, shading can lead to the inhibition of the transportation of photosynthetic products which ultimately causes yield decline21. In addition, shading resulted in changes in the antioxidant defence of rice plants22. In previous studies, comparative transcriptome profiling was performed, and certain genes in rice that are expressed under low light were identified23.

Irrigation is important for crop production. Water stress reduces the photosynthesis rate, growth, and biomass production and thereby decreases grain yield24–26. In addition, water stress leads to increases in the production of reactive oxygen species (ROS) and changes in antioxidant parameters27,28, and the expression of a series of genes in response to drought stress has been assessed29,30. However, the accumulation of 2AP in aromatic rice is affected by irrigation practices31. The 2AP content in aromatic rice can be increased with alternate wetting and drying conditions32. Drought stress during the grain filling stage can enhance the accumulation of 2AP in aromatic rice33.
A previous study reported that the synthesis of 2AP was highly related to abiotic stresses. Low light or water stress could lead to improved 2AP accumulation. However, the effects of light-water on aromatic rice remain largely unknown. In this study, two elite Chinese aromatic rice varieties, Xiangyaxiangzhan and Yuxiangyouzhan, were grown under four light-water treatments to explore how light and water regulate yield and 2AP formation in aromatic rice.

**Results**

**Effects of the light-water treatments on yield and yield-related traits.** Compared with CK, LL and LL-WS significantly decreased the grain yield in Xiangyaxiangzhan by 39.19% and 34.64%, respectively. WS, LL, and LL-WS significantly decreased the grain yield in Yuxiangyouzhan by 25.44%, 30.79%, and 29.42%, respectively, when compared to those under CK. The light-water treatments (WS, LL, and LL-WS) decreased the filled grain percentage, and a significant decrease compared to CK was detected under LL and LL-WS. The light-water treatments had no notable effect on the effective panicles or the 1,000-grain weight in either variety (Table 1).

**Effect of the light-water treatments on organ dry weight.** Compared with CK, WS, LL, and LL-WS resulted in reductions in total dry weight due to reductions in the dry weight of the stem sheath, panicle, and leaf, except for the dry weight of the Xiangyaxiangzhan leaves (Table 2).

| Treatment | Effective panicles per pot | Filled grain percentage (%) | 1,000-grain weight (g) | Yield (g pot⁻¹) |
|-----------|-----------------------------|-----------------------------|------------------------|---------------|
| Xiangyaxiangzhan | | | | |
| CK | 24.00a | 59.32a | 16.38a | 19.57a |
| WS | 23.50a | 53.74ab | 15.98a | 17.55a |
| LL | 23.33a | 46.28c | 16.14a | 11.90b |
| LL-WS | 23.33a | 48.06bc | 15.62a | 12.79b |
| Mean | 23.54 | 51.85 | 16.03 | 15.45 |
| Yuxiangyouzhan | | | | |
| CK | 20.33a | 45.92a | 18.82a | 21.11a |
| WS | 18.25a | 40.26ab | 18.44a | 15.74b |
| LL | 19.00a | 38.39b | 17.87a | 14.61b |
| LL-WS | 19.50a | 29.28c | 18.72a | 14.90b |
| Mean | 19.27 | 38.46 | 18.46 | 16.59 |

Table 1. Effect of light-water treatment on rice yield and yield-related traits. Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment.

| Treatment | Stem sheath dry weight | Leaf dry weight | Panicle dry weight | Total dry weight |
|-----------|------------------------|-----------------|-------------------|-----------------|
| AS | MS | AS | MS | AS | MS | AS | MS |
| Xiangyaxiangzhan | | | | | | | |
| CK | 45.62a | 42.40a | 8.39a | 5.38b | 21.34a | 26.74a | 75.35a | 74.52a |
| WS | 38.21ab | 38.66a | 8.04a | 5.84ab | 19.29a | 25.35a | 65.53ab | 69.65ab |
| LL | 31.94b | 37.28ab | 9.18a | 6.51a | 15.28b | 17.79b | 56.40b | 61.59bc |
| LL-WS | 34.52b | 30.86b | 8.68a | 6.26ab | 15.07b | 19.27b | 58.27b | 56.38c |
| Mean | 37.57 | 37.30 | 8.57 | 6.00 | 17.74 | 22.29 | 63.89 | 65.59 |
| Yuxiangyouzhan | | | | | | | |
| CK | 55.33a | 57.00a | 11.25a | 6.14a | 19.86a | 33.58a | 86.44a | 96.73a |
| WS | 49.89ab | 49.83ab | 6.40c | 5.03b | 15.91b | 23.33b | 72.19b | 78.18b |
| LL | 43.41b | 45.11b | 9.30b | 5.18ab | 15.81b | 26.05b | 68.52b | 76.34b |
| LL-WS | 54.41a | 46.02b | 6.34c | 2.81c | 17.41b | 22.65b | 78.16ab | 71.47b |
| Mean | 50.76 | 49.49 | 8.32 | 4.79 | 17.25 | 26.40 | 76.33 | 80.68 |

Table 2. Effect of light-water treatment on plant dry weight (g pot⁻¹). Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment.
Compared with CK, WS significantly increased the P5C content in leaves at AS for Xiangyaxiangzhan. Effects of the light-water treatments on P5C content, proline content, and GABA content.

Effect of the light-water treatment on gas exchange parameters and SPAD value. Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment; AS, After shading; MS, Maturity stage.

Table 3. Effect of light-water treatment on gas exchange parameters and SPAD value. Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment; AS, After shading; MS, Maturity stage.

Effects of the light-water treatments on gas exchange parameters and SPAD value. Compared with CK, WS and LL-WS significantly decreased Pn in Xiangyaxiangzhan at AS (after shading). The LL and LL-WS significantly decreased Pn in Yuxiangyouzhan at AS. There was no significant difference in Pn among the treatments in either variety at MS (maturity stage). For Xiangyaxiangzhan, the Tr decreased substantially in response to light-water treatment at AS. For Yuxiangyouzhan, the WS and LL-WS caused significant reductions in the Tr at AS and MS, respectively. LL significantly improved the Tr in Yuxiangyouzhan at MS, and the Gs in Xiangyaxiangzhan was significantly decreased under the light-water treatments at AS. WS and LL-WS resulted in a marked reduction in Gs in Yuxiangyouzhan at AS and MS, while LL significantly increased Gs. For Xiangyaxiangzhan, the Ci showed a significant reduction under WS and LL-WS compared with that under CK at AS and MS, respectively. For Yuxiangyouzhan, LL and LL-WS significantly increased the Ci at AS compared with that under CK. WS and LL-WS substantially reduced the Ci, but LL significantly increased the Ci in Yuxiangyouzhan at MS. For Xiangyaxiangzhan, significant increase in the SPAD values at AS and MS compared with that under CK were observed in response to the light-water treatments. For Yuxiangyouzhan, WS significantly reduced the SPAD value at AS, while LL and LL-WS significantly increased the SPAD value at MS (Table 3).

Effect of the light-water treatments on antioxidant response and MDA content. Compared with CK, LL and LL-WS significantly increased SOD activity at AS, while the light-water treatments substantially decreased SOD activity at MS in Xiangyaxiangzhan. For Yuxiangyouzhan, WS and LL significantly increased SOD activity at AS and MS compared to that under CK. The Tr decreased substantially under WS and LL-WS compared with that under CK at MS, respectively. For Yuxiangyouzhan, LL and LL-WS significantly increased the CAT activity at MS compared with that under CK. LL and LL-WS substantially reduced the POD activity at MS. For Xiangyaxiangzhan, the POD activity at MS was reduced significantly under WS and LL-WS compared with that under CK. LL and LL-WS significantly increased the POD activity at MS.

Effect of the light-water treatments on the 2AP content. Higher 2AP content in the grains was observed under LL and LL-WS than under CK. LL and LL-WS significantly increased the 2AP content in Yuxiangyouzhan by 18.67% and 16.32%, respectively, compared with that under CK. The WS significantly decreased the 2AP content in grains of Xiangyaxiangzhan and Yuxiangyouzhan by 24.44% and 7.19%, respectively, compared with that under CK (Table 4).

Effects of the light-water treatments on P5C content, proline content, and GABA content. Compared with CK, WS significantly increased the P5C content in grains at AS for Xiangyaxiangzhan, but significantly increased the P5C content in grains at MS for Yuxiangyouzhan. For Xiangyaxiangzhan, LL significantly decreased the P5C content in grains at AS, and LL and LL-WS significantly increased the P5C content in grains at MS. WS significantly decreased the P5C content in grains at AS for Xiangyaxiangzhan, but significantly increased the P5C content in grains at AS for Yuxiangyouzhan compared with those in the control. For Xiangyaxiangzhan, WS and LL-WS significantly increased the P5C content in grains at AS but significantly reduced the P5C content in grains at MS (Table 6).
| Treatment | SOD activity (U g⁻¹ FW) | POD activity (U g⁻¹ FW) | CAT activity (U g⁻¹ FW) | MDA Content (μmol g⁻¹ FW) |
|-----------|-------------------------|-------------------------|-------------------------|---------------------------|
|           | AS MS                   | AS MS                   | AS MS                   | AS MS                     |
| Xiangyaxiangzhan |                       |                        |                        |                           |
| CK        | 141.60c                 | 256.94a                 | 86.26a                  | 41.36b                    |
| WS        | 139.14c                 | 176.14b                 | 66.95bc                 | 50.22b                    |
| LL        | 172.32b                 | 167.27b                 | 60.65c                  | 20.77b                    |
| LL-WS     | 214.04a                 | 138.94c                 | 74.99b                  | 84.82a                    |
| Mean      | 166.78                  | 184.82                  | 72.21                   | 63.86                     |
| Yuxiangyouzhan |                     |                        |                        |                           |
| CK        | 123.76b                 | 194.19b                 | 61.26b                  | 37.14b                    |
| WS        | 186.47a                 | 206.48b                 | 70.77a                  | 54.61a                    |
| LL        | 179.54a                 | 172.72c                 | 62.32b                  | 49.82a                    |
| LL-WS     | 113.91b                 | 239.50a                 | 75.29a                  | 53.25a                    |
| Mean      | 150.92                  | 203.22                  | 67.41                   | 48.71                     |

Table 4. Effect of light-water treatment on antioxidant response and MDA content in leaves. Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment; AS, After shading; MS, Maturity stage.

| Treatment | 2AP Content (μg g⁻¹) |
|-----------|----------------------|
|           | Xiangyaxiangzhan     | Yuxiangyouzhan        |
| CK        | 7.08a                | 7.23b                 |
| WS        | 5.35b                | 6.71c                 |
| LL        | 7.50a                | 8.58a                 |
| LL-WS     | 7.44a                | 8.41a                 |
| Mean      | 6.84                 | 7.73                  |

Table 5. Effect of light-water treatment on 2AP content in grains. Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment.

| Treatment | P5C content (μmol g⁻¹) | Proline content (μg g⁻¹) | GABA content (mg g⁻¹ FW) | Soluble protein content (μg g⁻¹ FW) |
|-----------|------------------------|--------------------------|--------------------------|-----------------------------------|
|           |                        |                         |                         |                                   |
|           | Leaves | Grains | Leaves | Grains | Leaves | Grains | Leaves | Grains | Leaves | Grains |
| Xiangyaxiangzhan |       |         |        |        |        |        |        |        |        |        |
| CK        | 1.19b | 1.90c  | 1.60b  | 0.37a  | 48.06d | 60.68b | 20.14b | 7.07b  | 2.12a  | 1.48b  | 0.92b  | 0.71b  | 7.51b  | 7.86b  | 7.36a  | 7.14a  |
| WS        | 1.35a | 2.03bc | 1.15c  | 0.42a  | 70.07b | 72.20a | 18.75ab| 11.84a | 1.77b  | 1.59b  | 1.07a  | 0.84ab | 7.65ab | 7.87b  | 7.32a  | 7.13a  |
| LL        | 1.05c | 2.15b  | 2.12a  | 0.21c  | 58.14c | 62.57b | 17.64b | 8.69b  | 1.58b  | 2.14a  | 1.12a  | 0.87a  | 7.61a  | 8.08a  | 7.34a  | 7.20a  |
| LL-WS     | 1.27ab| 2.47a  | 2.28a  | 0.29b  | 106.08a| 72.61a | 21.06a | 11.43a | 2.09a  | 1.32b  | 1.16a  | 0.80ab | 7.61a  | 7.90b  | 7.35a  | 7.19a  |
| Mean      | 1.22  | 2.14   | 1.79   | 0.32   | 70.58  | 67.02  | 19.40  | 9.76   | 1.89   | 1.63   | 1.07   | 0.81   | 7.57   | 7.93   | 7.34   | 7.17   |
| Yuxiangyouzhan |       |         |        |        |        |        |        |        |        |        |        |        |        |        |        |        |
| CK        | 1.55a | 2.39b  | 1.23b  | 0.19a  | 34.79b | 33.10b | 16.88c | 10.14a | 1.27b  | 1.33ab | 0.64ab | 0.54ab | 7.64a  | 7.75b  | 7.29a  | 7.05a  |
| WS        | 1.52a | 2.80a  | 1.59a  | 0.21a  | 40.16b | 45.36a | 27.80b | 12.01a | 1.48b  | 1.22bc | 1.01a  | 0.67a  | 7.64a  | 7.81b  | 7.28a  | 7.14a  |
| LL        | 1.57a | 2.48b  | 1.13b  | 0.19a  | 38.94b | 34.61b | 32.47a | 9.98a  | 1.49b  | 0.98c  | 1.18a  | 0.59a  | 7.62a  | 7.79b  | 7.32a  | 7.04a  |
| LL-WS     | 1.62a | 2.50b  | 1.41ab | 0.20a  | 54.51a | 45.08a | 29.36ab| 10.08a | 2.11a  | 1.50a  | 1.03a  | 0.71a  | 7.64a  | 8.02a  | 7.44a  | 7.02a  |
| Mean      | 1.57  | 2.54   | 1.34   | 0.19   | 42.1   | 39.54  | 26.63  | 10.55  | 1.59   | 1.26   | 0.96   | 0.63   | 7.64   | 7.84   | 7.33   | 7.06   |

Table 6. Effect of light-water treatment on P5C content, proline content and GABA content, soluble protein content in leaves and grains. Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment; AS, After shading; MS, Maturity stage.
### Table 7. Effect of light-water treatment on P5CS activity, PDH activity, OAT activity and DAO activity in leaves and grains.

| Treatment         | P5CS activity (U g\(^{-1}\) FW) | PDH activity (U g\(^{-1}\) FW) | OAT activity (U g\(^{-1}\) FW) | DAO activity (U g\(^{-1}\) FW) |
|-------------------|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
|                   | Leaves                          | Grains                          | Leaves                          | Grains                          | Leaves                          | Grains                          | Leaves                          | Grains                          | Leaves                          | Grains                          |
|                   | AS     | MS   | AS     | MS   | AS     | MS   | AS     | MS   | AS     | MS   | AS     | MS   | AS     | MS   |
| Xiangyaxiangzhan  |       |      |        |      |        |      |        |      |        |      |        |      |        |      |
| CK                | 30.15c| 51.31bc| 4.03ab| 2.71b| 23.18a| 32.26b| 38.92ab| 34.51b| 6.04a  | 7.61b| 6.13a  | 5.56a|        |      |
| WS                | 44.01a| 58.41a| 3.69b  | 3.44a| 18.4b  | 21.76a| 47.85a  | 36.19a| 10.95c | 16.95b| 35.86a| 6.72a | 8.20ab| 6.48a| 6.14a |
| LL                | 30.15c| 55.52ab| 4.60a  | 2.33b| 17.56b| 18.27c| 33.02b  | 33.68ab| 9.41c  | 19.27a| 39.54a| 35.63ab| 5.41a | 7.23b | 6.26a| 6.17a |
| LL-WS             | 35.28b| 48.24c| 3.73b  | 3.41a| 15.85b| 21.76bc| 35.75b  | 34.74ab| 14.46b | 17.06b| 35.75b| 37.66a| 6.29a | 9.64a | 6.26a| 5.68a |
| Mean              | 34.90 | 53.37 | 4.01   | 2.97 | 18.75 | 23.04 | 37.63   | 34.22  | 13.95  | 16.81 | 38.94 | 35.92 | 6.11  | 8.17 | 6.28 | 5.89 |
| Yuxiangyouzhan    |       |      |        |      |        |      |        |      |        |      |        |      |        |      |
| CK                | 39.27b| 45.25c| 2.32c  | 2.00b| 20.27a| 28.24a| 32.61a  | 31.68b| 21.93b | 17.28a| 38.60b| 33.95a| 6.89a | 10.09a| 6.26b| 5.52ab|
| WS                | 49.14a| 53.02a| 2.86b  | 1.75b| 20.22a| 27.57a| 31.89a  | 32.90ab| 17.57c | 18.77a| 44.85a| 33.23a| 8.54a | 9.47bc| 6.58a| 5.48b |
| LL                | 37.77b| 45.03c| 2.38bc | 2.85a| 20.43a| 26.78a| 31.39a  | 33.76a| 26.50a | 20.16a| 39.60b| 27.12b| 8.31ab| 8.23c| 5.94c| 5.39b |
| LL-WS             | 40.60b| 49.31b| 3.61a  | 2.84a| 18.17a| 32.33a| 29.43a  | 35.49a| 11.44d | 20.14a| 37.20b| 31.92a| 8.92a | 11.14a| 6.48ab| 5.92a |
| Mean              | 41.70 | 48.15 | 2.79   | 2.36 | 19.77 | 28.73 | 30.33   | 32.96  | 19.36  | 19.09 | 40.06 | 31.55 | 8.16  | 9.73 | 6.31 | 5.58 |

The light-water treatments significantly increased the proline content in leaves at AS in Xiangyaxiangzhan compared with that in CK. WS and LL-WS significantly increased the proline content in leaves at AS in Yuxiangyouzhan compared with that under CK. The light-water treatments significantly increased the proline content in grains at AS in Yuxiangyouzhan. The WS and LL-WS significantly increased the proline content in grains at MS in Xiangyaxiangzhan compared with that under CK (Table 6).

Compared with CK, WS and LL significantly reduced the GABA content in leaves, while LL significantly increased the GABA content in leaves at MS in Xiangyaxiangzhan. For Yuxiangyouzhan, LL-WS significantly increased the GABA content in leaves at AS but LL significantly decreased the GABA content in leaves at MS. The GABA content in Xiangyaxiangzhan and Yuxiangyouzhan grains at AS was noticeably increased by the light-water treatments. LL significantly increased the GABA content in grains at MS compared with that in CK. The WS and LL-WS significantly increased the GABA content in grains at MS in Yuxiangyouzhan as compared with that under CK (Table 6).

Compared with CK, LL-WS and LL significantly increased the soluble protein content in leaves at AS and MS in Xiangyaxiangzhan. LL-WS significantly increased the soluble protein content in grains at MS in Yuxiangyouzhan. The soluble protein content in grains was not noticeably affected by the light-water treatments at AS or MS. (Table 6).

### Effect of the light-water treatments on P5CS, PDH, OAT, and DAO activity.

Compared with CK, WS significantly increased the P5CS activity in leaves at AS and MS in Xiangyaxiangzhan and Yuxiangyouzhan. LL-WS resulted in a significant increase in P5CS activity in leaves at AS in Xiangyaxiangzhan and at MS in Yuxiangyouzhan. WS and LL-WS significantly increased the P5CS activity in grains at AS in Yuxiangyouzhan and at MS in Xiangyaxiangzhan. Compared with CK, LL and LL-WS significantly increased the P5CS activity in grains at MS in Yuxiangyouzhan (Table 7).

The PDH activity in leaves at AS in Xiangyaxiangzhan was significantly decreased under the light-water treatments compared to that under CK. LL significantly reduced the PDH activity in leaves at MS in Xiangyaxiangzhan. The PDH activity in leaves at AS and MS in Yuxiangyouzhan was not significantly affected by the light-water treatments. The WS significantly increased the PDH activity in grains at MS in Yuxiangyouzhan, while LL and LL-WS significantly increased the PDH activity in grains at MS in Yuxiangyouzhan compared with that under CK (Table 7).

Compared with CK, the light-water treatments significantly decreased the OAT activity in leaves at AS but significantly increased the OAT activity in leaves at MS in Yuxiangyouzhan. For Yuxiangyouzhan, WS and LL-WS significantly reduced the OAT activity in leaves at AS but LL significantly increased the OAT activity in leaves at MS. The OAT activity in grains at MS was significantly increased under LL-WS in Xiangyaxiangzhan. The WS resulted in a significant increase in the OAT activity in grains at AS, but LL significantly decreased the OAT activity in grains at MS in Yuxiangyouzhan compared with that under CK (Table 7).

Compared with CK, LL-WS significantly increased the DAO activity in leaves at MS in Xiangyaxiangzhan. For Yuxiangyouzhan, WS and LL-WS significantly increased the DAO activity in leaves at AS, while LL significantly decreased the DAO activity in grains at AS in Yuxiangyouzhan compared with that under CK. The DAO activity in grains at AS and MS in Xiangyaxiangzhan was not significantly affected by the light-water treatments (Table 7).
Table 8. Effect of shading and water stress on Na, Mg, Mn, Fe content in leaves and grains. Within a column for each cultivar, means followed by different letters are significantly different according to LSD (0.05). CK, natural light and well-watered treatment; WS, natural light and water-stressed treatment; LL, low light and well-watered treatment; LL-WS, low light and water-stressed treatment; AS, After shading; MS, Maturity stage.

| Treatment            | Na content (mg kg⁻¹) | Mg content (ug kg⁻¹) | Mn content (mg kg⁻¹) | Fe content (mg kg⁻¹) |
|----------------------|----------------------|----------------------|----------------------|----------------------|
|                      | Leaves | Grains | Leaves | Grains | Leaves | Grains | Leaves | Grains | Leaves | Grains | Leaves | Grains |
| Xiangyaxiangzhan     |        |        |        |        |        |        |        |        |        |        |        |        |
| CK                   | 486.02c | 409.43c | 388.50a | 197.99a | 199.79a | 160.04a | 160.72b | 146.32a | 591.68c | 576.59c | 56.10c | 83.58c |
| WS                   | 435.10c | 435.10c | 439.10c | 205.10c | 173.10c | 169.49a | 148.27a | 205.94a | 924.72a | 90.79a | 132.98a | 213.78a |
| LL                   | 521.49a | 456.01a | 374.00a | 198.00a | 207.86a | 158.28b | 147.03a | 599.84a | 563.71c | 49.64a | 90.51c | 194.71b |
| LL-WS                | 533.46a | 431.34b | 342.67c | 175.62b | 205.54a | 170.78a | 163.71a | 144.84a | 649.45b | 739.79b | 68.04b | 114.19b |
| Mean                 | 502.92  | 431.15  | 368.87  | 187.77  | 204.57  | 170.88  | 163.05  | 146.61  | 624.24  | 701.20  | 66.15  | 105.31  |
|                       |         |         |         |         |         |         |         |         |         |         |         |         |
| Yuxiangyouzhan       | 486.02c | 409.43c | 388.50a | 197.99a | 199.79a | 160.04a | 160.72b | 146.32a | 591.68c | 576.59c | 56.10c | 83.58c |
| CK                   | 524.10b | 430.22b | 400.40a | 188.90a | 210.49a | 172.98b | 166.99b | 147.07a | 526.64c | 534.70d | 57.67a | 48.10c |
| WS                   | 478.14c | 450.23b | 395.09a | 178.06b | 212.97a | 160.84b | 160.72b | 147.03a | 599.84a | 563.71c | 49.64a | 90.51c |
| LL                   | 548.12a | 444.25b | 411.01a | 174.90a | 209.60a | 168.16c | 168.38a | 148.54a | 566.37b | 583.50b | 19.50d | 21.82b |
| LL-WS                | 477.21c | 474.82a | 401.94c | 175.90c | 170.78a | 163.71a | 144.84a | 649.45b | 739.79b | 68.04b | 114.19b |
| Mean                 | 506.90  | 449.88  | 402.11  | 175.90  | 209.75  | 176.88  | 164.92  | 147.97  | 568.83  | 594.31  | 39.56  | 61.35  |

Effects of the light-water treatments on Na, Mg, Mn, and Fe contents. Compared with CK, WS significantly reduced the Na content in leaves at AS while LL and LL-WS significantly increased the Na content in leaves at AS in Xiangyaxiangzhan. The Na content in Xiangyaxiangzhan leaves at MS was significantly increased under the light-water treatments. For Yuxiangyouzhan, WS and LL-WS significantly decreased the Na content in leaves at AS, while LL and LL-WS significantly increased the Na content in leaves at AS and MS, respectively. The Na content in grains at AS and MS was significantly reduced under WS and LL-WS in Xiangyaxiangzhan while LL-WS significantly reduced the Na content in grains at MS in Yuxiangyouzhan compared with that under CK (Table 8).

The Mg content in Xiangyaxiangzhan leaves at AS and MS was not significantly affected by the light-water treatments. For Yuxiangyouzhan, WS significantly increased the Mg content in leaves at MS compared to that under CK. WS significantly increased the Mg content in Xiangyaxiangzhan grains at AS. The light-water treatments did not significantly affect the Mg content in Yuxiangyouzhan grains (Table 8).

Compared with CK, WS and LL-WS significantly increased the Mn content in leaves and grains at AS and MS but LL significantly decreased the Mn content in leaves and grains at AS in Xiangyaxiangzhan. For Yuxiangyouzhan, the light-water treatments significantly increased the Mn content in leaves at MS and AS, and the Mn content in grains at AS was significantly decreased under the light-water treatments compared with that in the control. WS and LL-WS significantly increased the Mn content in grains at MS, but LL significantly decreased the Mn content in grains at AS (Table 8).

Correlation analysis. There was a significant positive correlation between the grain yield and the panicle dry weight and the total dry weight at AS and MS (Fig. 1). The 2AP content in grains was significantly negatively correlated with the P5C content in grains, P5CS activity in leaves at MS, and PDH activity in grains at AS. The Mn content in leaves at MS and in grains at AS and MS showed a significant positive correlation with the 2AP content in grains (Fig. 2).

Discussion
The effects of low light and water stress on grain yield in rice have been reported. Shading and water stress have a negative significant effect on the total dry weight of rice. In this study, we confirmed that low light reduced the yield of rice mainly by reducing the filled grain percentage and the total dry weight (Tables 1 and 2). This study found a significant positive correlation between grain yield and the dry weight of the panicle and total dry weight (Fig. 1). The light-water treatments had no significant effect on the panicle number or the extent of the reduction varies depending on the treatment period. A significant reduction
in effective panicles could be observed at the tillering stage\textsuperscript{36}. Many studies have shown that water stress resulted in a significant reduction in the filled grain percentage towards the mid-tillering, booting and flowering stages\textsuperscript{37,38}.

In this study, the light-water stress treatment reduced the grain yield and the filled grain percentage (Table 1). Studies have reported that shading significantly increased the total chlorophyll content of plants\textsuperscript{39,40}. In this study, a significant increase in the SPAD value in response to light-water treatments was observed in Xiangxiangzhan. For Yuxiangyouzhan, WS significantly decreased the SPAD value, while LL and LL-WS significantly increased the SPAD value at MS (Table 3). Leaf gas exchange is important for plants in response to abiotic stress\textsuperscript{41}. Studies have reported that low light and water deficits caused a change in Pn, Tr, Gs, and Ci\textsuperscript{22,42–47}. In this study, the light-water treatments affected the gas exchange parameters after the shading treatment, and at the maturity stage, the effect varied between varieties (Table 3). The differences in the changes in SPAD and gas exchange parameters were mainly due to the time and degree of the shading and water stress treatments.

Shading and water stress both result in the accumulation of reactive oxygen species (ROS) and cause damage to proteins and lipids\textsuperscript{48–50}. Superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) are key enzymes used for scavenging reactive oxygen species; MDA is the product of lipid peroxidation in cells and reflects the extent of cell membrane damage under stressful conditions\textsuperscript{48,51,52}. Shading significantly reduced SOD activity and increased MDA content during the grain filling stage\textsuperscript{22}. Shade tolerant varieties maintain a lower MDA content and higher SOD, POD, and CAT activity and soluble protein content\textsuperscript{50}. Moreover, the MDA content was significantly increased and the activities of SOD and CAT were significantly reduced after a PEG treatment\textsuperscript{49}, which may have been due to the drought-induced accumulation of H\textsubscript{2}O\textsubscript{2} in the guard cells\textsuperscript{53}. In this study, the low light treatments significantly increased the SOD activity and MDA content in both rice varieties. However, different changes in CAT activity and POD activity after shading were observed in the two varieties. At the maturity stage, the shading treatment resulted in a significant reduction in SOD activity and increased POD and CAT activity in Yuxiangyouzhan rice, while the MDA content was significantly increased (Table 4). The light-water

Figure 1. Correlation analyses between grain yield and plant dry weight. * and **, significant at the 0.05 and 0.01 probability levels, respectively.
Figure 2. Correlation analysis 2AP content and physiological parameters and element content. * and **, significant at the 0.05 and 0.01 probability levels, respectively.
treatments had a regulatory effect on the antioxidant response parameters. Further studies are needed to evaluate the molecular basis of the complex responses of rice plants to abiotic stress, i.e., light-water treatments.

Many previous studies have reported that abiotic stresses increase the content of 2AP in grains. Lower levels of water irrigation affected 2AP accumulation in aromatic rice. The 2AP content in grains increased significantly after shading during the grain filling period. In this study, low light treatments increased the 2AP content in grains of both varieties, but WS significantly decreased the content of 2AP in grains (Table 5). The responses of different genotypes to the levels of water stress may explain the difference in the changes in 2AP accumulation in this study and in previous studies.

Shading significantly increased the GABA content in Yuxiangyouzhan and Nongxiang18 and increased the proline content in Xiangyaxiangzhan grains. Different water regimes coupled with nitrogen affect the biosynthesis of 2AP by regulating physiological and biochemical parameters such as the P5C, proline, and GABA content and the activity of P5CS, PDH, OAT, and DAO. In this study, the light-water treatments regulated the P5C, proline, and GABA content in leaves and grains as well as the P5CS, PDH, OAT, and DAO activity in leaves and grains (Tables 6 and 7). The relationship of the 2AP content in grain to the studied physiological parameters was assessed (Fig. 2a–c). The relationship between the 2AP content and the 2AP-related physiological and biochemical parameters differed among experimental treatments and genotypes. Moreover, the light-water treatments regulated the dynamics of the element content in leaves and grains (Table 8), and the relationship between the 2AP in grains and the element content was also assessed (Fig. 2d–f). Inconsistent results were obtained for the relationship between the 2AP content and the element content of different elements among different experimental treatments. Moreover, element levels in plants and the deficits or excess elements such as iron in plants are related to oxidative stress in plants. Therefore, element absorption regulated by the light-water treatments further influenced oxidative stress in the rice plants, which resulted in more complex changes in the metabolic physiology of the plants. Further studies on the molecular basis of 2AP biosynthesis regulation in aromatic rice under light-water treatments should be conducted.

Overall, light-water treatments during the early grain filling stage regulate yield and 2AP formation, which results from biomass accumulation, photosynthesis, antioxidant responses, 2AP formation related physiological attributes, and element absorption in the plant.

Conclusion
Light-water treatments during the early grain filling stage regulates yield by affecting the plant dry weight, gas exchange parameters, and antioxidant responses. However, these treatments also influence 2AP accumulation by regulating 2AP formation-related physiological parameters and elemental levels. Further study is needed to balance yield with 2AP accumulation under light-water treatments.

Methods
Experimentation and treatments. A pot experiment was conducted at the Experimental Farm, South China Agricultural University, Guangzhou, China during July–November 2017. This region is favourable for the growth of aromatic rice due to its humid subtropical monsoon climate. Two aromatic rice varieties, Yuxiangyouzhan and Xiangyaxiangzhan, were used in this study. The two varieties are popular aromatic rice cultivars in South China. The soil used for the experiment was collected from paddy fields.

Two light levels (natural light and low light) were employed in this study. The low light treatment was implemented with a black netting layer and was equivalent to a 67% reduction in the full natural light level. Two water treatments, well-watered and water-stressed, were conducted in this study (Fig. 3). The water stress treatment was conducted according to the method described in a previous study. The well-watered treatment was flooded to a depth of 1–2 cm by manually adding tap water. Four light-water treatments (CK: natural light and well-watered

Figure 3. Soil water potential for the WS treatment.
treatment, WS: natural light and water-stressed treatment, LL: low light and well-watered treatment, LL-WS: low light and water-stressed treatment) were conducted during the early grain filling stage. The treatments lasted for 15 days, from September 26th to October 10th.

Seeds of the two aromatic rice varieties were sown on July 15th and 15-day-old seedlings were transplanted into pots with four seedlings per hill and five hills per pot. A compound fertilizer (15:15:15) was applied basally in the amount of 5.5 g per pot. The rice plants were harvested on November 6th. Except during the water treatment period, the irrigation was carried out according to routine management practices: a 2–4 cm water layer was maintained from transplanting to 7 days before harvest, and then the soil was allowed to dry out naturally. Other managements practices were the same in all treatments and followed local recommendations.

Sampling and measurement. Determination of yield and yield-related traits and dry matter weight. The determination of yield and yield-related traits was performed according to a previously reported method. At the maturity stage (MS), four pots were randomly harvested from each treatment. The grains were sun-dried to a moisture content of 14%. The effective panicles per pot were determined by counting the panicle numbers in four pots from each treatment. The grain number per panicle and the filled grain number were counted in the same four pots, and the filled grain percentage was calculated. The 1,000-grain weight was measured by weighing 1,000 grains from four random samples. Six representative plants were selected randomly and taken to the laboratory. The plants were separated into their panicles, leaves, and stem sheaths and then dried at 80 °C to a constant weight.

Determination of gas exchange parameters and SPAD value. The net photosynthetic rate (Pn), transpiration rate (Tr), stomatal conductance (Cond) and intercellular CO₂ concentration (Ci) of the leaf blades were determined with an LI-6400XT portable photosynthesis system (LI-COR, Inc., USA) after shading and at maturity from 9:00 am to 11:00 am on sunny days, and four measurements were taken for each treatment. Meanwhile, the SPAD value was measured by a SPAD meter ‘SPAD-502’ (Konica Minolta, Japan), with four replications for each treatment.

Determination of malondialdehyde (MDA) and antioxidant activities. The malondialdehyde (MDA) and antioxidant activities were measured as described method. MDA was reacted with thiobarbituric acid (TBA), and the absorbance of the reaction solutions was recorded at 532 nm, 600 nm, and 450 nm. The MDA content was expressed as μmol g⁻¹ FW. The superoxide (SOD, EC 1.15.1.1) activity was measured by using the nitro-blue tetrazolium (NBT) method. The reaction mixture contained 1.75 ml of sodium phosphate buffer (pH 7.8), 0.3 ml of 130 mM methionine buffer, 0.3 ml of 750 μmol NBT buffer, 0.3 ml of 100 μmol EDTA-Na₂ buffer, 0.3 ml of 20 μmol lactoflavin and 0.05 ml of enzyme extract. After the reaction, the change in colour was measured at 560 nm. The SOD activity was expressed as U g⁻¹ FW. For peroxidase (POD EC1.11.1.7) activity, the enzyme extract (50 μl) was added to the reaction solution containing 1 ml of 0.3% H₂O₂, 0.95 ml of 0.2% guaiacol, and 1 ml of 50 mM sodium phosphate buffer (pH 7.0). The absorbance was read at 470 nm. The POD activity was expressed as U g⁻¹ FW. For the catalase (CAT, EC 1.11.1.6) activity, an aliquot of enzyme extract (50 μl) was added to the reaction solution containing 1 ml of 0.3% H₂O₂ and 1.95 ml of sodium phosphate buffer, and the absorbance was recorded at 240 nm. The CAT activity was expressed as U g⁻¹ FW.

Determination of 2AP concentration. The 2AP concentration in the grains was measured using a previously described procedure that used the synchronization, distillation and extraction method (SDE) combined with GC–MS–QP 2010 Plus system (Shimadzu Corporation, Japan).

Determination of 2AP formation related to physiological traits. Fresh samples of grains and flag leaves were collected from each plot and immediately stored at ~ 80 °C until the determination of the 1-pyrrylone-5-carboxylic acid (P5C) content, proline content, soluble protein content, γ-aminobutyric acid (GABA) content, proline dehydrogenase (PDH) activity, pyrroline-5-carboxylic acid synthases (P5CS) activity, ornithine aminotransferase (OAT) activity, and diamine oxidase (DAO) activity.

The P5C concentration was determined according to a previously described method. The reaction mixture consisted of 0.2 ml of enzyme extraction supernatant, 0.5 ml of 10% trichloroacetic acid (TCA), and 0.2 ml of 40 mM 2-aminobenzaldehyde. The absorbance was measured at 440 nm after the reaction, and the P5C concentration was expressed as μmol g⁻¹ FW. The proline content was analyzed by using a previously reported method. The proline content was expressed as μg g⁻¹ FW. The soluble protein content was determined according to a previously reported method with G=250. The soluble protein content was expressed as μg g⁻¹ FW. The GABA content was measured according to previously described methods. Plant tissue (0.50 g) was homogenized with 60% ethanol (5 ml) and then oscillated for 4 h in an oscillations instrument (HZS-H, China) at 200 oscillations per minute. Then, the supernatant was centrifuged at 8,000 rpm for 3 min. The reaction mixture in a 10 ml test tube consisted of 1 ml of the supernatant, 0.6 ml of 0.2 M (pH 9.0) sodium tetraborate, 2 ml of 5% tolueene and 1 ml of 7% sodium hypochlorite. The prepared mixture was heated in a boiling water bath for 5 min and then cooled. The absorbance of the reaction mixture was measured at 645 nm. The GABA content was expressed as μg g⁻¹ FW.

The activity of PDH was measured by following a previously described method. After the reaction, the absorbance was recorded at 440 nm, and the PDH activity was expressed as U g⁻¹ FW. The P5CS activity was determined according to a reported method. The reaction solutions contained 10 mM ATP, 20.0 mM MgCl₂, 50 mM Tris–HCl buffer, 50 mM sodium glutamate, 100 mM hydroxamate–HCl and 0.5 ml of enzyme extract. The prepared mixture was kept in a 37°C water bath for 5 min, and then the reaction was terminated by the addition of 0.5 ml of a stop buffer (2.5% FeCl₃ and 6% TCA, dissolved in 100 ml of 2.5 M HCl). The P5CS activity was measured at 560 nm. The PDH activity was expressed as U g⁻¹ FW.
expressed as U g⁻¹ FW. The activity of OAT was assayed by using a previously described method. The absorbance of the supernatant fraction was read at 440 nm. The OAT activity was expressed as U g⁻¹ FW. The DAO activity was measured according to previously reported methods. The DAO activity was expressed as U g⁻¹ FW.

**Determination of the Na, Mg, Mn, and Fe contents in leaves and grains.** Briefly, the plant tissue (leaves and grains) was oven-dried and ground into a fine powder. Then 0.30 g of the plant tissue sample was digested with a 10 ml diacidic mixture of HNO₃:HClO₄ (4:1 v/v), after which the resultant solutions were diluted to 25 ml. The Na, Mg, Mn, and Fe contents in leaves and grains were estimated by using an atomic absorption spectrophotometer (AA6300C, Shimadzu, Japan).

**Statistics.** Analysis of variance (ANOVA) and correlation coefficients were performed using Statistix version 8 (Analytical, Tallahassee, Florida, USA). The differences amongst means separated by using the least significant difference (LSD) test at 5% significance level.

**Data availability**

All data generated or analyzed during this study are included in the article.

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Author contributions
Z.M. designed the experiments; Y.L., L.L., X.F., and Z.G. investigated the traits; Y.L. and L.L. analyzed the data and wrote the manuscript; Z.M., H.L., J.T., M.P.P., X.T., S.P., H.T., M.D. revised and edited the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

Additional information
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