Light quality during booting stage modulates fragrance, grain yield and quality in fragrant rice

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ABSTRACT

2-acetyl-1-pyrroline (2AP) is recognized as the key aromatic compound in fragrant rice, however, the effect of light quality on rice aroma is not fully understood. In this study, two fragrant rice varieties (Xiangyaxiangzhan and Yuxiangyouzhan) were grown under four light quality treatments (CK: natural light, L1: red light, L2: blue light and L3: combined light). Results depicted that L1, L2 and L3 treatments enhanced the grain yield by 14.24–35.36%, compared to CK whilst L2 and L3 treatments reduced the 2AP content in grains by 17.18–28.68%. Moreover, L1, L2 and L3 treatments enhanced the grain yield owing to improved filled grain percentage and regulation in antioxidant enzyme activities. On the other hand, L2 and L3 reduced the 2AP content in grains by decreasing pyrrole-5-carboxylic acid content and modulating proline and γ-aminobutyric acid content. Overall, this study revealed that light quality substantially affects the grain yield and quality characters of fragrant rice.

1. Introduction

Rice (Oryza sativa L.) is widely consumed cereal crop worldwide (Ainsworth 2008; Mo et al. 2020), whereas fragrant rice with its better palatability, taste and flavor characters is highly liked by the consumers and well recognized over the globe (Feng et al. 2020; Shan et al. 2015). Nevertheless, fragrant rice comparatively produces less grain yield than non-aromatic rice and found quite sensitive to external plant factors, e.g., limited water application, nutrition deprivation, temperature variations, and solar radiations (Eiji et al. 2009; Mo et al. 2015; Mo et al. 2019). Generally, alteration in weather conditions with changing light intensity affects yield and quality characters of fragrant rice, however, regulations in rice yield, quality attributes, and aroma contents owing to changes in light quality needs further investigations.

Shading and/or reduction in solar intensity generally caused adverse effects on rice yield (Demao et al. 2001; Mo et al. 2015). Demao et al. (2001) revealed that filled grain percentage and grain yield were decreased with increase in shading days at booting stage. Malformed grains and shortened rice hulls were proved to be induced by shading before heading time (Eiji et al. 2009). Moreover, shading treatment reduced chlorophyll contents which led to reduction in photosynthesis and hence dry matter accumulation (Khairunnisa et al. 2019). Likewise, shading on different growth stages caused adverse effects on rice yield, for example, shading at tillering, booting, and filling stage substantially affect tillering ability, grain development, and grain filling percentage whereas the most apparent reduction in yield was observed due to shading at filling stage (Deng et al. 2009). Moreover, the effect of red light was observed to be similar to normal light on total dry matter and 1000-grain weight of winter rice in panicle initiating and flowering stage (Barmudoi et al. 2016). Yield-related attributes under red light treatment were found higher than low solar intensity treatment (Barmudoi et al. 2016).

In addition, shading could inhibit quality and resistant ability of rice (Liu et al. 2013; Liu et al. 2014). Liu et al. (2014) described that low solar intensity from transplanting to booting stage increased head rice yield and amylose content in rice and decreased chalkiness characters. However, Mo et al. (2015) revealed that milled rice rate, head rice rate decreased and chalkiness traits increased under shading treatment at the early filling stage of fragrant rice. Besides, Liu et al. (2013) reported that the malondialdehyde (MDA) content increased and the activities of superoxide dismutase (SOD) and catalase (CAT) were reduced with increased under prolonged shading, i.e. from initial heading to maturity stage. In contrast, a decline in MDA and suable sugar content was observed in shade-resistant rice varieties (Liu et al. 2014).

In general, fragrant rice is featured with aroma due to presence of volatile compounds among rice varieties, whereas 2-acetyl-1-pyrroline (2AP) was found to be the key aromatic compound (Bryant et al. 2010). On the other hand, proline (Pro) was proved to be one of the precursors of 2AP, while γ-aminobutyraldehyde (GABA) and 5-pyrrole-5-carboxylate (PSC) were possibly to be the direct precursors of 2AP in fragrant rice (Yoshihashi et al. 2002). Furthermore, it is observed to be sensitive to light quality changes, which substantially affects the yield and quality of fragrant rice.
speculated that Badh2 encoded an aminoaldehyde dehydrogenase called BADH2 to catalyze the transformation of GABAld to γ-aminobutyric acid (GABA), inhibiting 2AP formation in fragrant rice. However, non-functional of Badh2 might promote cyclization of GABAld to \(^\Delta\)1-pyrroline and synthetize 2AP (Bradbury et al. 2008; Chen et al. 2008). Besides, GABA also serves as a growth promoter/stimulator in plants under stress conditions (Kindersley et al. 2000). Recently, Mo et al. (2015) elucidated that shading during filling stage induced the accumulation of 2AP and GABA, with a positive correlation between each other. Therefore, the fluctuation and correlation between GABA and 2AP under shading stress and studies concentrating on the effect of light quality on fragrant rice during booting stage are yet to be investigated. Therefore, the present study was conducted to assess the regulations in grain yield, quality characters, and aroma contents in fragrant rice under different light quality treatments at booting stage.

2. Materials and methods

2.1 Plant materials and experimental conditions

Seeds of two fragrant rice cultivars, i.e. Xiangyaxiangzhan and Yuxiangyouzhan, were obtained from College of Agriculture, South China Agricultural University. Xiangyaxiangzhan (Xiangsimiao 126 × Xiangyaruazhan) is a thermal conventional indica rice cultivar characterized with superior rice flavor and aroma quality whereas Yuxiangyouzhan (TY36/IR100 × IR100) is a thermal conventional super rice cultivar and characterized with vigorous growth and resistant against various environmental stresses. Both cultivars are widely grown as fragrant rice types in South China (Liu et al. 2020).

The pot experiment was conducted in a greenhouse at Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou, China, from March to July 2018. The climatic type of the region is humid subtropical monsoon with 24.4°C average air temperature and 79.3% average humidity during growth period. The experimental soil was sandy loam containing 36.95 g kg\(^{-1}\) of organic matter, 1.94 g kg\(^{-1}\) of total nitrogen, 1.32 g kg\(^{-1}\) of total phosphorus and 22.96 g kg\(^{-1}\) of total potassium and 5.25 soil pH.

2.2 Experimental treatments

The pot experiment was arranged in a completely randomized design (CRD) with three pots for each treatment. Each pot had a diameter of 32 cm and a height of 24 cm, containing 12 kg air-dried soil. Meanwhile, N\(_2\), P\(_2\)O\(_5\) and K\(_2\)O applied at 1.05, 0.525, and 0.875 g pot\(^{-1}\) were applied as basal fertilizer. Seedlings were transplanted into each pot with 3 seedlings per hill and 5 hills per pot whereas other management practices were carried out according to the provincial crop management guidelines.

At booting stage (the rice growth stage R1–R3 described by Counce et al. (2000)), different light quality treatments were applied to the plants as an additional light supplement, including additionally supplying red light (L1), blue light (L2) and combined light (L3, photon flux ratios of red light: blue light = 1:1:1). Meanwhile, the control (CK) was treated in a natural light condition. From 7:30 a.m. to 7:30 p.m., the light-emitting diode (LED) tubes were utilized to fill the light supplement for 12 h. The peak of red light was 660 nm whereas the peaks of blue light were 439 nm and 455 nm. The additional photosynthetic photon flux density of each light quality treatment was approximately 60 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) at the top of the plants during the 12 h photoperiod.

2.3 Sampling and measurements

The plants were sampled at 15 d after treatments beginning (15d ATB) and at maturity stage (MS), respectively. At 15d ATB and MS, flag leaves and fresh panicles were sampled from representative plants for each treatment. Then half of sampled panicles were stored at -20°C for the determination of 2AP content whereas another half of sampled panicles and all the sampled flag leaves were immediately frozen by liquid N\(_2\) and stored at -80°C for the determination of γ-aminobutyric acid (GABA) content, delta-1-pyrroline-5-carboxylate (PSC) content, proline content, antioxidant enzyme activity and MDA content.

2.3.1 Determination of plant dry weight, yield and yield-related traits

At MS, three representative plants from each treatment were harvested and washed with distilled water, separated into leaves, stems and panicles, and then were dried in an oven at 80°C to constant weight for the determination of dry weight. For the determination of panicle number per pot, grain number per panicle was counted, then harvested and threshed manually, and the grains were sun-dried for approximately 5 days and the moisture content were adjusted to 14% for determination of the grain yield. The grains per panicle, filled grain percentage and 1000-grain weight were recorded.

2.3.2 Determination of 2AP content in grains

The grain 2AP content was determined as described by Huang et al. (2012) and Mo et al. (2015). In brief, grain samples were ground into powder with liquid N\(_2\). Powder sample (2 g) for each treatment in triplicate was added into 10 mL dichloromethane and ultrasonically extracted (40°C, 800W) for 240 min and then add an appropriate amount of anhydrous sodium sulfate to the mixtures until the water is completely absorbed. The supernatant was filtered by using a 0.22 μm filter membrane and collected for determination by using GCMS-QP 2010 Plus (Shimadzu Corporation, Japan). The 2AP content was expressed as μg g\(^{-1}\) fresh weight (FW).

2.3.3 Determination of GABA, PSC and Proline contents in leaves and grains

The GABA content in leaves and grain was determined according to Zhao et al. (2009). In brief, fresh grain samples (0.15 g) were homogenized in 2.5 mL of 60% (v/v) ethanol, treated for 4 h in an oscillations instrument (HZS-H, China) using a frequency of 200 oscillations per minute, then the mixture was added with 1 mL of 60 mmol L\(^{-1}\) lanthanum chloride, oscillated for 15 min and centrifugated for 5 min at 2000xg. The 0.4 mL of supernatant was then transferred to a 2 mL centrifuge tube, mixed with 0.12 mL of 0.2 mol L\(^{-1}\) (pH 10.0) sodium tetraborate, 0.4 mL of 6% (v/v) phenol, and 80 μL of 10% (w/v) sodium hypochlorite, then cooled in an ice bath for 5 min after heating at 100°C in a water bath for 10 min. Then 0.4 mL of 60% (v/v) alcohol
was added into the mixture for chromogenic reaction, the absorbance of the reaction solution was measured at 645 nm and the amount of GABA was determined by comparison with a standard curve and expressed as mg g⁻¹ FW.

The P5C content in leaves and grains was determined according to Miller et al. (2009). Briefly, fresh grain sample (0.5 g) was homogenized in 6 mL sulfo salicylic acid and centrifuged for 15 min at 6000×g. Then 1.35 mL of supernatant was transferred to a 2 mL centrifuge tube, mixed with 1.5 mL of 10% (w/v) trichloroacetic acid and 0.15 mL of 2-amino benzaldehyde. After resting for 25 min at room temperature, the mixture was then centrifuged for 10 min at 6000×g and the absorbance under 440 nm was detected. According to the description of Mezl et al. (1976), molar extinction coefficient of P5C (2.58 mmol cm⁻¹) was used to calculate the P5C content, which was expressed as μmol g⁻¹ FW.

The proline content was determined according to Bates et al. (1973) with modifications. In brief, fresh grain sample (0.3 g) was homogenized in 5 mL of 3% (w/v) sulfo salicylic acid, then cooled and filtrated after heating at a boiling water bath for 10 min. Later on, 1 mL of the filtrate was mixed with 1 mL of glacial acetic acid and 1 mL of ninhydrin reagent (1.25 g ninhydrin in 30 mL glacial acetic acid and 20 mL of 6 mol L⁻¹ phosphoric acid), successively. The reaction mixture was then heated at boiling water bath for 30 min and placed in an ice bath for 20 min before being extracted with 2 mL of toluene. The toluene extraction was then centrifuged at 2000×g for 5 min. The absorbance of the red chromophore in the toluene fraction was measured at 520 nm and the amount of proline was determined by comparison with a standard curve and expressed as μg g⁻¹ FW.

### 2.3.4 Determination of antioxidant enzyme activity and MDA content

Antioxidant enzyme activity and MDA content were determined using the method of Li et al. (2019). For extraction of antioxidant enzymes and MDA, fresh samples (0.3 g) were homogenized with 3 mL of 100 mmol L⁻¹ sodium phosphate buffer, centrifuged at 7500×g at 4°C for 15 min, the supernatant was used to detect the activities of antioxidant enzymes.

Superoxide dismutase (SOD, E.C. 1.15.1.1) activity was measured by the nitro blue tetrazolium (NBT) method and the 50% reduction in the absorbance at 560 nm was used to represent one unit of SOD activity and represented as U g⁻¹ FW.

Peroxidase (POD, EC 1.11.1.7) activity was determined according to Li et al. (2019) with some modifications. Briefly, reactions mixture was comprised of 1 mL of sodium phosphate buffer (pH 7.0), 1 mL of 0.3% H₂O₂, 0.2% of 0.95 mL guaiacol, and 0.05 mL aliquot of enzyme extract. The absorbance of the reaction mixture was read at 470 nm and recorded till 5 readings with an interval of 30 s. One unit of POD activity was defined as the absorbance increase because of guaiacol oxidation by 0.01(U) per min, the expressed as U g⁻¹ min⁻¹ FW.

Catalase (CAT, E.C.1.11.1.6) activity was estimated with method reported below. Briefly, a reaction solution was comprised of 1.95 mL of ultrapure water, 1 mL of 0.3% (v/v) H₂O₂ solution and 0.05 mL of crude extracted enzyme. The absorbance of the reaction mixture was measured at every 30s at 450 nm in 2 min with 3 mL ultrapure water as a control. One CAT unit (U) of enzyme activity was defined as a reduction of absorbance every minute by 0.01 and expressed as U g⁻¹ min⁻¹ FW.

The malondialdehyde (MDA) content was measured by adding 0.5% (w/v) thiobarbituric acid (TBA) in crude enzyme extract. The reaction mixture was cooled after heating in a water bath for 30 min and centrifuged at 500×g for 15 min and the absorbance was read at 532, 600 and 450 nm, respectively. The MDA content in different plant tissues was calculated as MDA content = 6.45(OD₅₃₂–OD₆₀₀)–0.599OD₁₅₀ and expressed as μmol g⁻¹ FW.

### 2.3.5 Determination of grain quality attributes

For determining grain quality attributes, grains from each treatment were stored at room temperature for 4 months, then divided into 3 replications. Brown rice rate, milled rice rate and head rice rate were determined according to the method of Mo et al. (2015), using rice huller (Jiangsu, China) and Jingmi testing rice grader (Zhejiang, China), chalkiness rice rate and chalkiness degree were detected using an SDE-A lightbox (Guangzhou, China). Protein content and amylose content were determined using an Infratec 1241 grain analyzer (Foss Tecator Co., Ltd. Hillerod, Denmark).

### 2.4 Data analysis

Data were analyzed by statistical software Statistix version 8.0 (Analytical Software, Tallahassee, FL, USA), while differences amongst means were separated by using the least significant difference (LSD) test at 5% probability level.

### 3. Results

#### 3.1 2AP content

Both rice cultivars were varied significantly regarding grain 2AP content at 15d ATB and MS (Table 1). The grain 2AP content of Yuxiangyouzhan was significantly higher than Xiangyaxiangzhan at 15d ATB while lower at MS. At 15d ATB, compared with CK, the grain 2AP content in Yuxiangyouzhan was significantly lower in the L2 and L3 treatments. At MS, compared with CK, the grain 2AP content of Xiangyaxiangzhan was decreased by 20.86%–22.30% under L2 and L3 treatments, respectively (Figure 1).

#### 3.2 Proline, P5C and GABA content

Different light bands significantly affected the proline content in leaves and grains at both sampling stages (Table 1). For example, at 15d ATB, compared with CK, the proline content in grains of Xiangyaxiangzhan was significantly increased under L1 but decreased under L2 and L3; meanwhile, proline content in grains of Yuxiangyouzhan was substantially improved under L2 and L3. At MS, compared with CK, proline content in grains of Xiangyaxiangzhan was significantly increased under L3, but decreased under L1 and L2; meanwhile, proline content in grains of Yuxiangyouzhan was significantly increased under L1, but decreased under L2 and L3. Compared with CK, the proline content in leaves of Xiangyaxiangzhan at 15d ATB was significantly lower in L1 and L2 treatments, whereas the proline content in leaves of Yuxiangyouzhan at 15d ATB was significantly increased by all light quality treatment. At MS, the L1 and L2 treatments...
The ANOVA analysis of the investigated parameters.

| Parameters                        | Variety | Treatment | Variety × Treatment |
|----------------------------------|---------|-----------|---------------------|
| Panicle number per pot           | *       | ns        | ns                  |
| Grains per panicle               | ns      | ns        | ns                  |
| Filled grain percentage          | *       | **        | ns                  |
| 1000-grain weight                | **      | ns        | ns                  |
| Grain yield                      | *       | *         | ns                  |
| Total dry weight                 | ns      | ns        | ns                  |
| Harvest index                    | **      | ns        | ns                  |
| Brown rice rate                  | **      | **        | **                  |
| Milled rice rate                 | *       | ns        | ns                  |
| Head rice rate                   | *       | *         | ns                  |
| Chalkiness rate                   | **      | **        | ns                  |
| Length-width ratio               | **      | **        | **                  |
| Protein content                  | *       | **        | **                  |
| Amylose content                  |        | **        | **                  |
| 2AP content in grains at 15d ATB | **      | **        | ns                  |
| 2AP content in grains at MS      | **      | **        | **                  |
| Proline content in grains at 15d ATB | ns | **        | **                  |
| Proline content in grains at MS  | ns      | ns        | ns                  |
| Proline in leaves at 15d ATB     | *       | **        | ns                  |
| Proline in leaves at MS          | **      | **        | ns                  |
| P5C content in grains at 15d ATB | **      | **        | ns                  |
| P5C content in grains at MS      | **      | **        | ns                  |
| P5C content in leaves at 15d ATB | ns      | ns        | ns                  |
| P5C content in leaves at MS      | **      | **        | **                  |
| GABA content in grains at 15d ATB| ns      | ns        | **                  |
| GABA content in grains at MS     | ns      | **        | ns                  |
| GABA content in leaves at 15d ATB| **      | **        | ns                  |
| SOD activity in leaves at MS     |        | ns        | ns                  |
| POD activity in leaves at 15d ATB| **      | **        | **                  |
| POD activity in leaves at MS     | **      | **        | **                  |
| CAT activity in leaves at 15d ATB| ns      | **        | ns                  |
| CAT activity in leaves at MS     | ns      | **        | ns                  |
| MDA content in leaves at 15d ATB | **      | ns        | ns                  |
| MDA content in leaves at MS      |        | ns        | ns                  |

A lower-case letter illustrates comparisons among the treatment by LSD test at \( P = 0.05 \). Three biological replicates were used for statistical analyses. CK: natural light condition; L1: red light; L2: blue light; L3: combined light (photon flux ratios of red light: blue light: white light = 1:1:1); ATB: after treatment beginning; MS: maturity stage; 2AP: 2-acetyl-1-pyrroline; P5C: 1-pyrroline-5-carboxylate; GABA: γ-aminobutyric acid; SOD: superoxide dismutase; POD: peroxidase; CAT: catalase; MDA: malondialdehyde; ns: not significant; *: significant at \( P < 0.05 \) level; **: significant at \( P < 0.01 \) level.

The 3.3 Yield, yield-related traits and plant biomass

Rice cultivars were varied significantly regarding panicle number per pot, filled grain percentage, 1000-grain weight, grain yield and harvest index, which were significantly higher in Yuxiangyouzhan than Xiangyaxiangzhan. Moreover, the significant effects of light quality treatments on filled grain percentage and grain yield were detected. For Xiangyaxiangzhan, all light quality treatment significantly increased the filled grain percentage, whereas L1 and L3 significantly increased grain yield by 35.36% and 34.45%, respectively. For Yuxiangyouzhan, L3 substantially improved the filled grain percentage and grain yield by 29.51% and 34.53%, respectively (Table 2) whilst the grain yield negatively correlated with grain number per panicle but positively correlated with filled grain percentage as well as harvest index (Figure 6).

The 3.4 Antioxidant enzyme activity and MDA content in leaves

Compared with CK, the SOD activity of Xiangyaxiangzhan at MS and the SOD activity of Yuxiangyouzhan at 15d ATB significantly increased the proline content in leaves of Xiangyaxiangzhan. By contrast, the L1, L2 and L3 significantly reduced proline content in leaves of Yuxiangyouzhan, compared with CK (Figure 2).

Both cultivars varied significantly regarding grain P5C content at both sampling stages and in leaves at MS (Table 1). The P5C content in grains was higher in Xiangyaxiangzhan at both stages, while the P5C content in leaves was higher in Yuxiangyouzhan at MS. For Xiangyaxiangzhan, compared with CK, the L1 treatment significantly improved the P5C content in grains in both stages, but L3 treatment resulted in a significant reduction at MS. Moreover, the P5C content in leaves was significantly decreased by L3 and L1 treatment at 15d ATB and MS, respectively (Figure 2). Grain 2AP content at MS negatively correlated with grain 2AP content at 15d ATB but positively correlated with P5C content in grains at both sampling stages (Figure 5).

Both cultivars were found statistically similar regarding GABA content in grains and leaves at both sampling stages (Table 1). For Xiangyaxiangzhan, compared with CK, the GABA content in grains was significantly reduced under L2 at 15d ATB, as well as under L2 and L3 at MS. No significant effect of light quality treatments on GABA content in leaves was observed at 15d ATB, meanwhile, the GABA content in leaves was significantly reduced by L2 at MS. For Yuxiangyouzhan, all light quality treatment significantly increased the GABA content in grains at 15d ATB, while GABA content in grains at MS was significantly increased under L2. The GABA content in leaves was significantly reduced by L1 at 15d ATB, however, no significant effect of light supplement treatment on GABA content in leaves was detected at MS (Figure 3).
were significantly reduced under L2 treatment whereas the SOD activity of Xiangyaxiangzhan at MS was significantly increased under L2 treatment.

The L1 treatment significantly increased the POD activity of Xiangyaxiangzhan at 15d ATB and Xiangyaxiangzhan at MS compared with CK, whereas L2 treatment significantly decreased the POD activity of Xiangyaxiangzhan at both stages, but increased the POD activity of Yuxiangyouzhan at MS. Moreover, the L3 treatment significantly increased the POD activity of Xiangyaxiangzhan and Yuxiangyouzhan at 15d ATB and MS, respectively.

Compared with CK, the L1 treatment significantly increased the CAT activity of Xiangyaxiangzhan at MS but decreased significantly in Xiangyaxiangzhan and Yuxiangyouzhan at 15d ATB and at both sampling stages, respectively. Moreover, the L3 treatment significantly increased the CAT activity of Xiangyaxiangzhan at 15d ATB, but CAT activity was decreased for both rice cultivars at MS.

The MDA content of Xiangyaxiangzhan was significantly increased under L3 treatment at MS, meanwhile, the MDA content of Yuxiangyouzhan was significantly reduced under L1 and L2 at 15d ATB, as well as L1 at MS (Figure 4).

### 3.5 Grain quality

Both rice cultivars varied significantly regarding grain quality traits. For Xiangyaxiangzhan, compared with CK, the brown
rice rate was significantly increased under L1 and L3 treatments whereas chalkiness rate and chalkiness degrees were both significantly increased under L2 and L3 treatments. Length–width ratio and protein content were both significantly decreased by all light quality treatments. Meanwhile, amylose content was significantly increased by all light quality treatments. For Yuxiangyouzhan, compared with CK, the brown rice rate was significantly increased under L1 but decreased under L2 and L3. The head rice rate was significantly decreased under L3 whereas the chalkiness rice rate was significantly enhanced under L2. Moreover, the length–width ratio was significantly increased under L3 whilst the amylose content was substantially enhanced by L1 (Table 3).

4. Discussion

Rice quality characters as well as aroma biosynthesis are largely affected by various internal and external plant factors (Fu et al. 2020; Li et al. 2020; Xie et al. 2020) whereas different-colored-light induced variations in aroma and flavor were recently reported by Peng et al. (2020) in strawberry plants where red light stimulated the gene expression regarding the emission of different aromatic compounds. However, the effect of light quality on the aroma of fragrant rice was never reported. In the present study, 2AP content in grains of Yuxiangyouzhan and Xiangyaxiangzhan was significantly higher at 15 d ATB and at MS, respectively. The 2AP content in grains of Yuxiangyouzhan significantly decreased under L2 and L3 treatments at 15 d ATB compared to CK. Likewise, 2AP content in grains of Xiangyaxiangzhan significantly decreased under L2 and L3 treatments at MS compared to CK (Figure 1). Overall, it is observed that blue light and its combination to red and white light adversely affected the 2AP accumulation in fragrant rice. Our findings corroborate with the recent findings of Peng et al. (2020) who reported that blue light had inhibitory effects on aroma volatiles and/or fragrance in strawberry plants. Additional light management seems to be of more significant adverse effect on 2AP accumulation in Xiangyaxiangzhan after harvest than Yuxiangyouzhan. Besides, it is documented that shading treatment substantially improved the 2AP and GABA accumulation in grains whereas a positive correlation between these compounds was also previously reported by Mo et al. (2015). However, Bradbury et al. (2008) and Chen et al. (2008) illustrated that GABA was generated by GABald via BADH2 and the absence of BADH2 could promote 2AP formation with GABald. These opposite results ascertained the extra approach of GABA accumulation as a shading stress response (Mo et al. 2015). In the present

Table 2. Yield, yield-related traits, and biomass.

| Variety         | Treatment | Panicle number per pot | Grains per panicle | Filled grain percentage (%) | 1000-grain weight (g) | Grain yield (g/pot) | Total dry weight (g/pot) | Harvest index |
|-----------------|-----------|------------------------|--------------------|----------------------------|----------------------|---------------------|-------------------------|--------------|
| Xiangyaxiangzhan| CK        | 34.80 ± 2.01*          | 148.30 ± 6.87*     | 56.85 ± 4.19*              | 15.50 ± 0.17*        | 45.95 ± 4.30*       | 139.58 ± 9.23*          | 0.33 ± 0.02*   |
|                 | L1        | 34.40 ± 1.78*          | 134.24 ± 7.23*     | 80.93 ± 6.59*              | 16.38 ± 0.22*        | 62.20 ± 6.15*       | 156.40 ± 4.43*          | 0.39 ± 0.03*   |
|                 | L2        | 34.00 ± 1.00*          | 133.66 ± 10.04*    | 79.47 ± 4.37*              | 16.14 ± 0.35*        | 59.07 ± 2.37*       | 145.79 ± 4.28*          | 0.41 ± 0.02*   |
|                 | L3        | 34.80 ± 1.62*          | 136.33 ± 5.05*     | 80.93 ± 3.21*              | 16.06 ± 0.71*        | 61.78 ± 3.52*       | 155.84 ± 2.53*          | 0.40 ± 0.02*   |
| Yuxiangyouzhan  | CK        | 30.60 ± 2.06*          | 147.55 ± 4.04*     | 51.04 ± 4.61*              | 17.96 ± 0.35*        | 40.17 ± 2.74*       | 148.61 ± 7.82*          | 0.27 ± 0.03*   |
|                 | L1        | 29.20 ± 1.20*          | 150.26 ± 1.90*     | 59.07 ± 3.93*              | 18.30 ± 0.43*        | 48.45 ± 2.33*       | 152.37 ± 6.27*          | 0.32 ± 0.01*   |
|                 | L2        | 30.80 ± 2.39*          | 136.60 ± 3.50*     | 63.02 ± 4.61*              | 17.65 ± 0.35*        | 45.89 ± 4.23*       | 147.22 ± 3.49*          | 0.31 ± 0.03*   |
|                 | L3        | 30.20 ± 1.93*          | 141.76 ± 6.98*     | 66.10 ± 2.54*              | 19.24 ± 0.59*        | 54.04 ± 3.61*       | 164.33 ± 10.76*         | 0.33 ± 0.03*   |

A lower-case letter illustrates comparisons among the treatment by LSD test at p = 0.05. Three biological replicates were used for statistical analyses. CK: natural light condition; L1: red light; L2: blue light; L3: combined light (photon flux ratios of red light: blue light: white light = 1:1:1).
study, the effect of blue light and its combination with red and white light was observed to inhibit the 2AP accumulation at 15 d ATB and MS (Figure 1), which is contrary to Mo et al. (2015) which suggest that shading treatment decreased the content of blue light to alleviate its adverse effects on 2AP generation. In addition, correlation analysis showed a positive correlation between 2AP and P5C content in grains, validating the hypothesis of Yoshihashi et al. (2002) that P5C appeared to be the direct precursor of 2AP (Figure 5). Our result also depicted the alteration of proline and GABA content, but showed no significant correlation between 2AP, proline and GABA (Figures 1–3). Therefore, it is revealed that the adverse effect of red and blue light was not realized in such unitary ways as inhibiting a single precursor compound. Moreover, opposite trends regarding proline and GABA content under different light quality treatment was observed in both rice cultivars, indicating different response mechanisms of rice variety under different light quality treatments (Figures 2 and 3).

Light quality affects the seedling growth and physiology of rice (Chen et al. 2014; Lal 2018) whereas red light promoted root and shoot elongation and biomass production while blue light restrained biomass production (Lal 2018). Blue light decreased shoot length, and combination of red
Table 3. Grain quality parameters.

| Variety | Treatment | Brown rice | Rice rate (%) | Milled rice | Head rice | Chalkiness | Length-width ratio | Protein content (%) | Amylose content (%) |
|---------|-----------|------------|---------------|-------------|-----------|------------|-------------------|---------------------|---------------------|
|         |           | rate (%)   | Chowder (%)   | rate (%)  | Rice (%)  | degree (%) |                  |                     |                     |
|         |           | 76.72 ± 0.25 | 67.61 ± 1.98  | 57.93 ± 2.13 | 7.30 ± 0.43 | 4.09 ± 0.12 | 3.07 ± 0.02 | 9.20 ± 0.06  | 15.93 ± 0.09  |
|         |           | L1         | 77.77 ± 0.06 | 65.61 ± 1.72 | 56.16 ± 2.82 | 8.00 ± 0.32 | 4.31 ± 0.31 | 2.97 ± 0.02 | 8.50 ± 0.00 |
|         |           | L2         | 76.49 ± 0.43 | 66.66 ± 1.43 | 60.75 ± 0.59 | 12.08 ± 0.88 | 5.97 ± 0.12 | 2.96 ± 0.03 | 8.67 ± 0.03 |
|         |           | L3         | 78.09 ± 0.08 | 66.99 ± 0.34 | 58.89 ± 0.67 | 16.12 ± 0.77 | 7.15 ± 0.47 | 2.95 ± 0.02 | 8.70 ± 0.06 |
|         |           | Yuxiangyouzhan | 80.66 ± 0.08 | 72.32 ± 0.59 | 65.64 ± 0.65 | 28.68 ± 1.58 | 13.16 ± 0.78 | 2.44 ± 0.01 | 9.10 ± 0.00 |
|         |           | L1         | 81.19 ± 0.12 | 70.46 ± 1.24 | 62.56 ± 1.59 | 25.98 ± 1.17 | 11.52 ± 0.64 | 2.43 ± 0.01 | 9.20 ± 0.06 |
|         |           | L2         | 80.03 ± 0.21 | 71.89 ± 0.68 | 64.73 ± 0.82 | 35.40 ± 1.54 | 15.56 ± 0.28 | 2.50 ± 0.04 | 9.20 ± 0.12 |
|         |           | L3         | 79.96 ± 0.23 | 71.93 ± 0.46 | 61.35 ± 1.17 | 32.00 ± 2.28 | 14.86 ± 1.32 | 2.61 ± 0.01 | 8.90 ± 0.00 |

A lower-case letter illustrates comparisons among the treatment by LSD test at = 0.05. Three biological replicates were used for statistical analyses. CK: natural light condition; L1: red light; L2: blue light; L3: combined light (photon flux ratios of red light: blue light: white light = 1:1:1).

Deng et al. (2009) reported that shading at booting stage yield accumulation at different growth stages of rice, revealing the importance of thorough investigation into the effect of light quality on yield during different growth stages of rice, suggesting that light quality treatment might enhance grain yield by promoting grain filling percentage (Figure 6). Hence, light quality management is a promising approach to compensate and/or improve yield-limited attributes of fragrant rice. On the other hand, Chen et al. (2014) and Lal (2018) revealed that the effect of light quality on yield accumulation varied in different growth stages of rice, revealing the importance of thorough investigation into the effect of light quality on yield accumulation at different growth stages. Previously, Deng et al. (2009) reported that shading at booting stage inhibited growth and yields formation in rice owing to abnormal kernel growth. In sum, shading reduced the content of red, blue and white light and hence restrained growth and yield formation in fragrant rice, thus adopting combined light with photon flux ratios of red light: blue light: white light = 1:1:1 is a promising approach to alleviate the adverse effect of shading condition. Nonetheless, further study about the effect of other light quality or different light combinations on yield and quality of fragrant rice is necessary to be investigated.

Plants are equipped with enzymatic defense system to scavenge stress-induced over-production of reactive oxygen species (ROS) (Ashraf et al. 2015; Ashraf et al. 2017; Huang et al. 2020). It is elucidated that higher antioxidant activity indicated higher resistance of plant against stress conditions (Hasanuzzaman et al. 2012). Additionally, lipid peroxidation, one of the indicators of oxidative stress, generally results in the elevation of MDA content in plants (Shah et al. 2001). Yu et al. (2016) reported that red light could reduce ROS production and MDA content and blue light could promote antioxidant enzyme activities i.e., SOD, POD and CAT in Camptotheca acuminate seedlings.
Nonetheless, studies related to the effects of light quality on antioxidant enzyme activities in rice are very few. In this study, L1 treatment substantially improved the POD, and CAT activity in leaves of Xiangyaxiangzhan while decreased CAT activity and MDA content in leaves of Yuxiangyouzhan, indicating that red light treatment might promote antioxidant enzyme activities in fragrant rice, but this effect varies for different rice varieties (Figure 4). Besides, L2 treatment significantly decreased the SOD and POD activity at MS but increased CAT activity at MS in leaves of Xiangyaxiangzhan, while for Yuxiangyouzhan, the L2 treatment significantly increased SOD and POD activity at MS but decreased CAT activity at MS in leaves (Figure 4). Our results suggest that blue light inhibits antioxidant enzyme activities in fragrant rice to some extent. Moreover, no significant effect was noted for L3 treatment regarding SOD activity of both rice cultivars, however, L3 treatment significantly increased the POD activity while decreased CAT activity in both rice varieties at MS (Figure 4). Substantial effect of L3 treatment than L2 on antioxidant ability is possibly due to the involvement of red light. Furthermore, it is found that resistance ability varied in rice varieties under shading, wherein shade-tolerant cultivars showed higher antioxidant enzyme abilities than shade-sensitive cultivars (Liu et al. 2014).

As per our knowledge, very few reports are available regarding effects of light quality on grain quality attributes of fragrant rice. Recently, Chen et al. (2019) reported a decreased chalkiness rate under shading treatment with a black nylon net during heading stage. It is elaborated that a high proportion of blue light under shading treatment with black nylon net enhanced photosynthesis and hence boosted amylose accumulation in grains, with less amylpectin which results in chalkiness in rice grains. Mo et al. (2015) reported that milled rice rate and head rice rate decreased, and chalkiness traits and amylose content increased under black net shading treatment at the early filling stage of fragrant rice, while the effect of shading varied regarding different shade duration. In this study, all light quality treatments significantly reduced protein content in fragrant rice, thus hampering rice nutrient status and/or grain protein contents (Table 3). It is also observed that L2 and L3 treatments significantly enhanced the chalkiness characters, whereas L2 treatment significantly decreased the brown rice rate, hence, providing evidence to the hypothesis that blue light might retard rice quality attributes (Table 3). On the other hand, all light quality treatment significantly decreased the length–width ratio and increased amylose content, thus promoting rice quality (Table 3). The result of additional blue light treatment on chalkiness characters and amylose content in this study is similar to the study of Mo et al. (2015) but in contrast to the Chen et al. (2019). Therefore, it is speculated that the relationship between chalkiness characters and amylose content varied in different growth stages due to differential distribution of photo-assimilates. Furthermore, it is revealed that rice quality of Xiangyaxiangzhan is more sensitive than Yuxiangyouzhan under light quality management in terms of brown rice rate, chalkiness rice rate, length–width ratio and amylose content, however, further investigation about the light-induced regulations in rice quality characters are needed.

5. Conclusion

In sum, light treatments, i.e. L2 and L3 adversely effected the 2AP accumulation in fragrant rice. The 2AP content possessed a positive correlation with P5C content, validating the hypothesis that P5C was the possibly direct precursor of 2AP. Moreover, all light quality treatments significantly improved the filled grain percentage and grain yield whilst L3 treatment found the most efficient in this regard. However, all light quality treatments restrained rice quality traits by reducing brown rice rate and enhancing chalkiness characters. Overall, present study provides a theoretical
foundation of yield-promoting agronomic tools by light quality management in order to regulate yield, quality, and aroma characteristics in fragrant rice.

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