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ARTICLE

Foliar Application of Brassinolide Induced Regulation of Grain Yield and Quality, Antioxidant Responses and Aroma in Fragrant Rice

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ABSTRACT

Brassinolide (BR) is a new green plant growth regulator. The present field study was conducted on two fragrant rice cultivars (i.e., *Meixiangzhan-2* and *Xiangyaxiangzhan*) to study the effects of foliar application of BR on fragrant rice performance. At the heading stage, BR solutions at 0.05, 0.15, 0.25 and 0.50 mg L-1 were sprayed on fragrant rice at 600 liters per hectare; these treatments were named as BR1, BR2, BR3 and BR4, respectively. The treatment sprayed with distilled water was taken as a control (CK). Compared with CK, the BR2 treatment significantly enhanced the chlorophyll concentration and net photosynthetic rate for *Meixiangzhan-2*, and *Xiangyaxiangzhan*. The BR3 and BR4 treatments increased the concentrations of chlorophyll a, chlorophyll b and carotenoid, and also enhanced net photosynthetic rate by 31.91% and 40.43%, respectively. Higher grain yields were recorded in the BR2 treatment than on CK on *Meixiangzhan-2*, while on *Xiangyaxiangzhan*, the BR3 and BR4 treatments increased grain yield compared with CK. In relation to CK, higher head rice rates were recorded in the BR2 treatment for *Meixiangzhan-2*, and in the BR3 and BR4 treatments for *Xiangyaxiangzhan*. BR treatments (BR2 for *Meixiangzhan-2*, BR3 and BR4 for *Xiangyaxiangzhan*) also significantly enhanced the activities of GPX, SOD and CAT by 10.22% to 23.00%, and reduced the malonaldehyde concentration. In addition, we observed that some BR treatments (BR2 for *Meixiangzhan-2*, BR3 and BR4 for *Xiangyaxiangzhan*) decreased the grain 2-acetyl-1-pyrroline concentration of fragrant rice.

KEYWORDS

Brassinolide; chlorophyll; yield formation; fragrant rice; antioxidant enzyme

1 Introduction

As a new green plant growth regulator, brassinolide (BR) can promote crop growth and development, improve crop quality, increase the yield of crops, and make the color of crops bright and the leaves thicker [1]. Many studies have been conducted to investigate the effects of BR on the growth, development, quality and abiotic stress tolerance of crops. For example, an early study revealed that exogenous BR was able to alleviate the negative impacts from water logging stress on maize [2]. The study of Dehghan et al. [3] showed that the application of BR could significantly improve the seed quality characters of wheat under different irrigation systems. Lv et al. [4] demonstrated the BR would alter *Leymus chinensis* performance at both morphology and physiology levels under drought stress. Furthermore, the study of Li et al. [5] showed that BR induced regulation of growth and development on adventitious roots which involved nitric oxide in cucumber.

Rice is the main food crop in China which could be significantly affected by plant growth regulators on grain yield and quality attributes [6]. As a special kind of rice, fragrant rice is well known by people around the world for its good grain quality characters especially the unique aroma with 2-acetyl-1-pyrroline (2-AP) as the main component [7]. A previous study revealed that foliar application of plant growth regulators such as paclobutrazol, indole acetic acid and gibberellic acid had significant effects on fragrant rice growth and development as well as on yield and quality [8]. However, the effects of BR on fragrant rice performances (i.e., yield formation, grain quality attributes, aroma, antioxidant responses, and photosynthesis) have not been reported.

The present study was conducted in the field to study the effects of the foliar application of BR on grain yield, grain quality and rice aroma in fragrant rice cultivars. We hypothesized that foliar application of BR will enhance antioxidant enzymatic activities and improve photosynthesis and grain yield. This study would provide new information about the application of BR in agricultural production.

2 Materials and Methods

2.1 Plant Materials and Growing Conditions

In 2019, a field experiment was conducted at the Experimental Research Farm, College of Agriculture, South China Agricultural University, Zengcheng (23°13′N, 113°81′E, altitude 11 m.a.s.l.), China. The experimental site enjoys a subtropical-monsoon climate. The experimental soil was sandy loam consisting of 14.60 g kg^{-1} organic matter, 1.23 g kg^{-1} total nitrogen, 53.49 mg kg^{-1} available nitrogen, 0.88 g kg^{-1} total phosphorus, 15.95 mg kg^{-1} available phosphorus, 12.02 mg kg^{-1} total potassium, and 122.76 mg kg^{-1} available potassium, and the pH was 6.57. Seeds of two fragrant rice cultivars, "*Meixiangzhan-2*" (*Lemont* × *Fengaozhan*) and "*Xiangyaxiangzhan*" (*Xiangsimiao126* × *Xiangyaruanzhan*), well known and widely cultivated in South China, were provided by the College of Agriculture, South China Agricultural University, Guangzhou, China. Detailed information about the cultivars can be found at https://www.ricedata.cn/. The seeds were sowed in July, transplanted in August, and harvested in November. A special biological organic fertilizer (Jing Lv) (manufactured by Dongguan Foota, Ltd., China; comprised of (N-P_2O_5-K_2O, 15%–4%–6%) was applied at 900 kg ha^{-1}. Sixty percent of it (540 kg ha^{-1}) was applied as a basal dose and 40% (360 kg ha^{-1}) at tillering. Water management practices were followed as adopted by local farmers. After the rice seedlings were transplanted and turned green, the paddy field kept a 3 cm water layer until the end of tillering. Drain water for 7 days at the end of tilling to control the production of non-productive/infertile tillers. At the following stages, a water of 5–7 cm was kept to the grain-filling stage. All other agronomic practices (i.e., pest and diseases management, and weed control) were the same in all treatments and followed the guidelines and standards recommended by the province.

2.2 Treatment Description and Sampling

At the heading stage of fragrant rice plants, 0.05, 0.15, 0.25 and 0.50 mg L^{-1} BR solutions were sprayed on aromatic rice at 600 liters per hectare; treatments were named BR1, BR2, BR3 and BR4, respectively. The foliar application was carried out once during the whole experiment. The treatment sprayed with distilled water was taken as a control (CK). At the grain filling phase (10 days after treatment application), fresh leaves were collected and stored at –80°C for the estimation of chlorophyll and antioxidant responses. Meanwhile, the net photosynthetic rate was measured using a portable photosynthesis system (LI-6400, LI-COR, USA). At harvest, fresh grains were collected and stored at –80°C for estimation of 2-AP.

2.3 Estimation of the Grain 2-AP Concentration

The grain samples were finely ground and used for measurements of 2-AP via the simultaneous distillation-extraction method (SDE) to make a molar solution, which was then analyzed by a GCMS-QP 2010 Plus instrument (Shimadzu Corporation, Japan) following Luo et al. [7].

2.4 Estimation of Net Photosynthetic Rate

At the grain filling stage (10 days after spraying with the BR solution), the net photosynthetic rate was determined with the portable photosynthesis system (LI-6400, LI-COR, USA) with the following adjustments: the photosynthetically active radiation at the leaf surface was between 1100 and 1200 μmol m^{-2} s^{-1}, and the ambient CO_2 concentration was between 385.5 and 399.7 μmol mol^{-1}.

2.5 Estimation of Grain Yield and Related Traits

At the maturity stage, the rice grains were harvested from three sampling areas (1 m^2 each) in each plot, and they were threshed by a machine. The average panicle number per unit surface area (1 m^2) was calculated from the three 1 m^2 replicates at each plot. The effective panicle number of 3 m^2 was manually cleared in each plot. Then six hills of rice plants from each plot were taken to determine the other yield-related traits including seed-setting rate, 1000-grain weight and grain number per panicle.

2.6 Estimation of Photosynthetic Pigments Concentrations

The concentrations of total chlorophyll (total Chl), chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoids were measured according to the methods described by He et al. [9]. A ground leaf sample was placed within 95% absolute ethyl alcohol until the sample turned white. Then, the absorbance was read at 645, 652 and 663 nm, respectively, on an ultraviolet-visible spectrophotometer. The formulas of the calculations were as follows:

$$CT = A652 \times 1000/34.5$$

$$Ca = 12.21 \times A663 - 2.59 \times A646$$

$$Cb = 20.13 \times A646 - 5.03 \times A663$$

$$Cx.c = (1000 \times 470 - 3.27 \times Ca - 104Cb)/229$$

CT: Total chlorophyll concentration; Ca: Chlorophyll a concentration; Cb: Chlorophyll b concentration; Cx.c: Carotenoid concentration.

2.7 Estimation of Antioxidant Enzyme Activities and Malonaldehyde (MDA) Concentration

The activities of the guaiacol peroxidase (GPX, EC 1.11.1.7), superoxide dismutase (SOD, EC 1.15.1.1) and (CAT, EC 1.11.1.6) were estimated following Luo et al. [10] as well as the MDA concentration. The absorbances of GPX and SOD and CAT were read at 470, 560 and 240 nm, MDA concentration was determined after reacting with thiobarbituric acid and the absorbance was read at 532, 600, and 450 nm, respectively, and the activities were expressed as U g^{-1} min^{-1} FW. The concentration of MDA was expressed as μmol g^{-1} FW.

2.8 Estimation of Grain Quality Attributes

After air drying, the brown rice rate was estimated using a rice huller (Jiangsu, China) and milled rice and head rice rate were determined and calculated by using a Jingmi testing rice grader (Zhejiang, China). Grains with chalkiness and chalkiness degree were estimated by using an SDE-A light box (Guangzhou, China) while an Infratec-1241 grain analyzer (FOSS–TECATOR) was used to determine the grain amylose and protein concentrations [11].

2.9 Statistical Analysis

Data were analyzed using Statistix 8.1 (Analytical Software, Tallahassee, FL, USA) while differences among means were separated by using the least significant difference (LSD) test at a 5% probability level. Graphical representation was conducted via Sigma Plot 14.0 (Systat Software Inc., California, USA).

3 Results

3.1 Grain Yield and Yield Related Attributes

Exogenous BR application significantly affected the grain yield of fragrant rice as shown in Table 1. For *Meixiangzhan-2*, BR2 treatment significantly increased grain yield and seed-setting rate by 11.67% and 17.46%, respectively compared with CK whilst there was no significant difference between CK and other BR treatments in grain yield. For *Xiangyaxiangzhan*, compared with CK, both BR1 and BR2 treatment significantly increased grain yield; compared with CK, BR2 treatment significantly increased Seed-setting rate. On the other hand, there were no significant differences among all treatments in panicle number per unit surface area, grain number per panicle and 1000-grain weight.

Table 1: Effects of exogenous BR on fragrant rice yield formation

Cultivar	Treatment	Grain yield (t ha^{-1})	Effective panicle number per m^2	Grain number per panicle	Seed-setting rate (%)	1000-grain weight (g)
Meixiangzhan-2						
	CK	8.80bc	333.67a	147.25a	76.99b	20.91a
	BR1	9.09ab	329.67a	149.03a	81.22ab	21.18a
	BR2	9.83a	329.67a	149.26a	90.44a	20.70a
	BR3	8.51bc	324.33a	151.83a	78.98b	20.91a
	BR4	8.21c	330.33a	147.64a	76.27b	21.10a
Xiangyaxiangzhan						
	CK	6.89b	324.00a	150.86a	61.58b	21.20a
	BR1	7.33b	328.33a	149.42a	65.79b	21.09a
	BR2	7.04b	326.00a	148.42a	64.45b	20.93a
	BR3	9.09a	334.67a	152.20a	81.26a	21.03a
	BR4	9.68a	324.67a	151.61a	89.30a	20.88a

Within each cultivar, means in the same column followed by different lowercase letters differ significantly at $P \leq 0.05$ according to the *t*-test. CK: water was sprayed at 600 liters per hectare. BR1: 0.05 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR2: 0.15 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR3: 0.25 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR4: 0.5 mg L^{-1} BR solution was sprayed at 600 liters per hectare.

3.2 Photosynthetic Pigments

As shown in Fig. 1, exogenous BR application significantly affected the concentrations of total chlorophyll, chorophyll a, chorophyll b and carotenoid. For *Meixiangzhan-2*, the highest photosynthetic pigment concentrations were recorded in the BR2 treatment when compared with the CK and the remaining BR treatments (Fig. 1). The BR2 treatment significantly increased the concentrations of total chlorophyll, chorophyll a, chorophyll b and carotenoids by 13.78, 13.94, 11.98 and 12.26%, respectively. For *Xiangyaxiangzhan*, concentrations of total chlorophyll, chorophyll a, chorophyll b and carotenoids were higher on the BR1, BR2, BR3 and BR4 treatments than on CK. The highest concentrations of total chlorophyll, chorophyll a, chorophyll b and carotenoids were recorded in BR4.

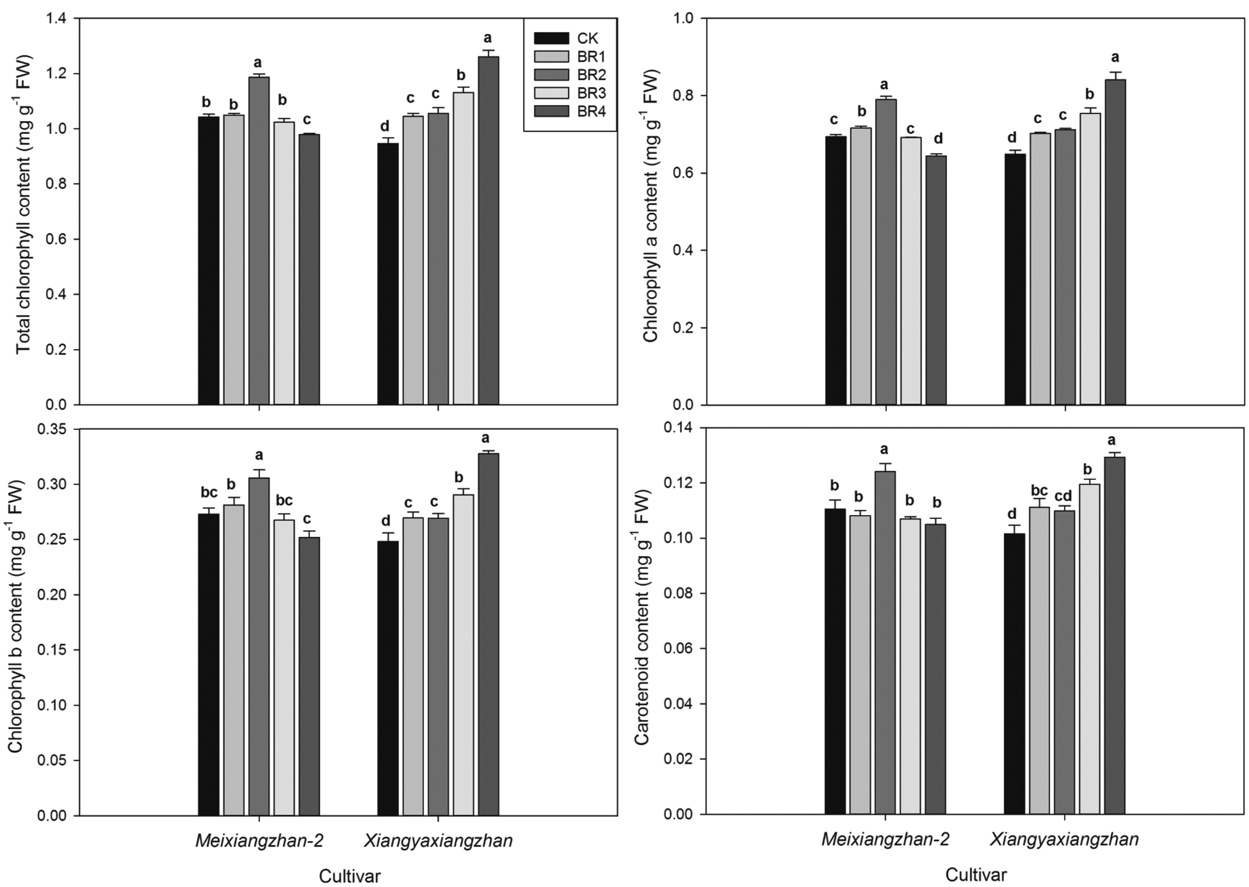

Figure 1: Effects of exogenous BR on the photosynthetic pigment concentrations of fragrant rice. Within each cultivar, means followed by different lowercase letters differ significantly at $P \leq 0.05$ according to the LSD test. CK: water was sprayed at 600 liters per hectare. BR1: 0.05 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR2: 0.15 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR3: 0.25 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR4: 0.5 mg L^{-1} BR solution was sprayed at 600 liters per hectare

3.3 Net Photosynthetic Rate

As shown in Fig. 2, foliar application of BR affected the net photosynthetic rate of fragrant rice. For *Meixiangzhan-2*, there were no statistical differences among the CK, BR1, BR3 and BR4 treatments on the net photosynthetic rate. The highest net photosynthetic rate was recorded on the BR2 treatment.

For *Xiangyaxiangzhan*, compared with CK, the BR3 and BR4 treatments significantly increased the net photosynthetic rate by 31.91% and 40.43%, respectively. Also, there were no significant differences ($P > 0.05$) among the CK, BR1 and BR2 treatments.

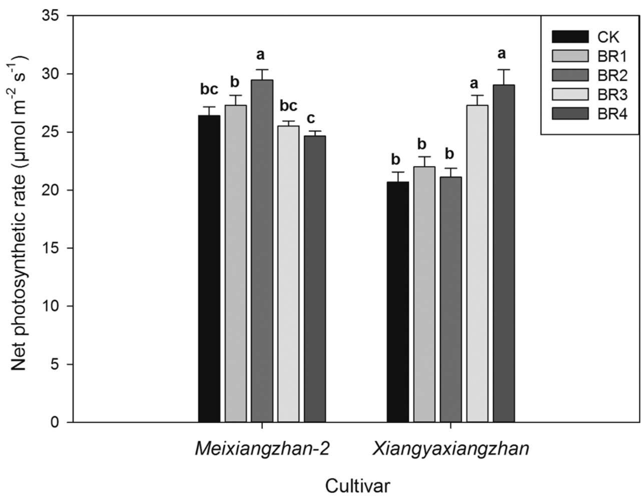

Figure 2: Effects of exogenous BR on the net photosynthetic rate at the grain filling phase of fragrant rice. Means sharing a common letter do not differ significantly at $P \leq 0.05$ according to the LSD test. CK: water was sprayed at 600 liters per hectare. BR1: 0.05 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR2: 0.15 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR3: 0.25 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR4: 0.5 mg L^{-1} BR solution was sprayed at 600 liters per hectare

3.4 Antioxidant Enzymes and MDA Concentration

As shown in Fig. 3, exogenous BR treatments significantly influenced the antioxidant enzymes activities as well as the MDA concentrations. For *Meixiangzhan-2*, the highest activities of SOD, GPX and CAT were all recorded in BR2 treatment. Compared with CK, BR2 treatment significantly enhanced SOD, GPX and CAT activities by 10.22, 12.17 and 24.54% while 23.00% lower MDA concentration was recorded in BR2 than CK; BR4 treatment significantly reduced CAT and GPX activities. BR3 treatment significantly reduced GPX activities. For *Xiangyaxiangzhan*, higher activities of SOD, GPX and CAT and lower MDA concentrations were recorded in BR4. Compared with CK, BR3 treatment significantly enhanced SOD, GPX and CAT activities, significantly reduced MDA concentration. Whilst there was no remarkable difference among CK, BR1 and BR2 (except GPX activity in BR1).

3.5 Grain Quality Attributes

As shown in Table 2, foliar application of BR had no significant influences on fragrant rice grain quality attributes except head rice rate. For *Meixiangzhan-2*, BR2 treatment significantly increased head rice rate by 4.23% compared with CK, whilst there was no remarkable difference among CK, BR1, BR3 and BR4; For *Xiangyaxiangzhan*, BR3 and BR4 treatments significantly increased head rice rate than CK. Whilst there was no remarkable difference among CK, BR1 and BR2.

Within each cultivar, means in the same column followed by different lowercase letters differ significantly at $P \leq 0.05$ according to the LSD-test. Akali: Alkali dissipation value. CK: water was sprayed at 600 liters per hectare. BR1: 0.05 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR2: 0.15 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR3: 0.25 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR4: 0.5 mg L^{-1} BR solution was sprayed at 600 liters per hectare.

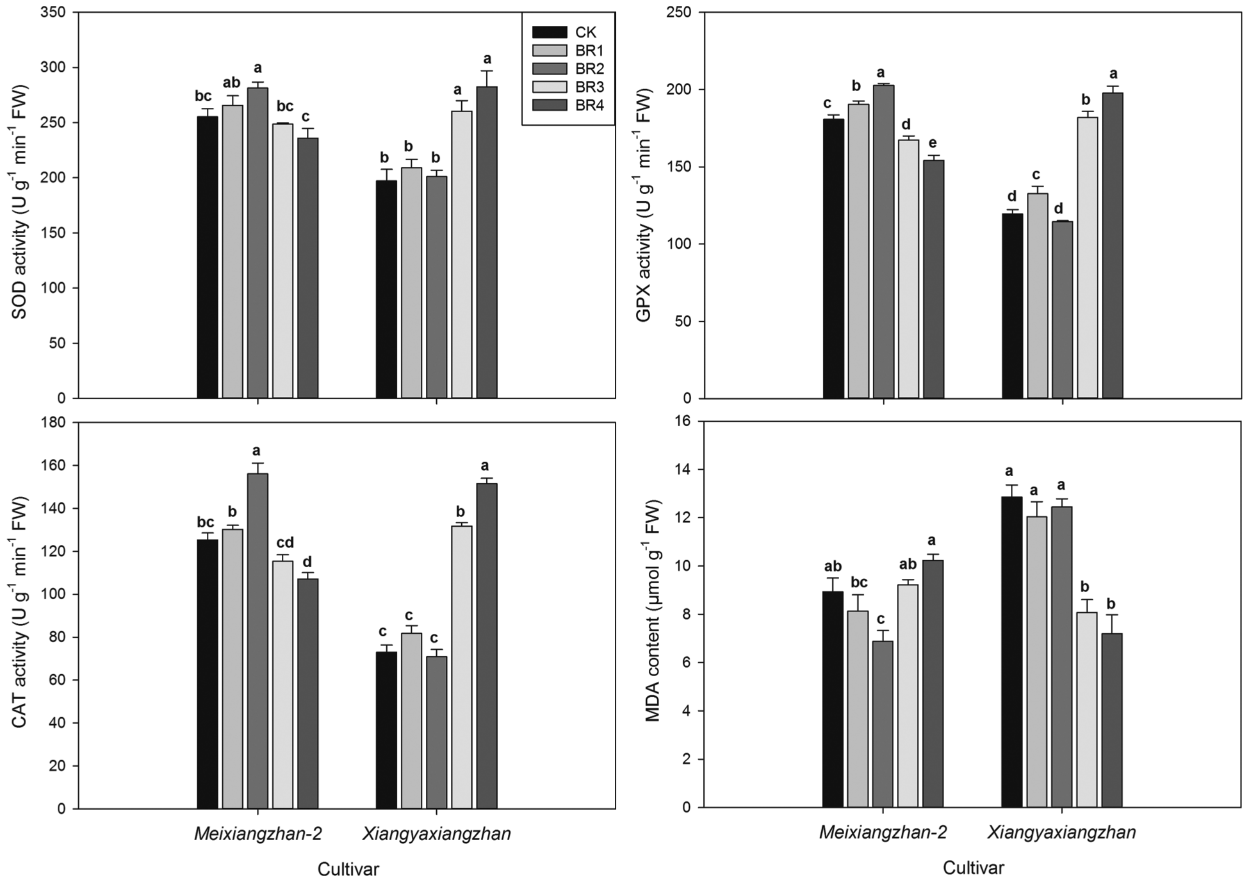

Figure 3: Effects of exogenous BR on antioxidant enzymes activities and MDA concentrations of fragrant rice. Means sharing a common letter do not differ significantly at ($P \leq 0.05$) according to the LSD test. CK: water was sprayed at 600 liters per hectare. BR1: 0.05 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR2: 0.15 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR3: 0.25 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR4: 0.5 mg L^{-1} BR solution was sprayed at 600 liters per hectare

Table 2: Effects of exogenous BR on grain quality attributes of fragrant rice

Cultivar	Treatment	Brown rice rate (%)	Milled rice rate (%)	Head rice rate (%)	Crude protein concentration (%)	Amylose concentration (%)	Akali
Meixiangzhan-2							
	CK	81.36a	71.82a	62.75bc	7.93a	17.97a	6.57a
	BR1	81.78a	71.44a	63.59ab	8.00a	18.00a	6.43a
	BR2	81.30a	71.29a	65.40a	8.03a	17.97a	6.53a
	BR3	82.39a	72.27a	61.64bc	8.03a	18.03a	6.47a
	BR4	82.65a	71.91a	61.06c	7.97a	17.97a	6.53a

(Continued)

Table 2 (continued)

Cultivar	Treatment	Brown rice rate (%)	Milled rice rate (%)	Head rice rate (%)	Crude protein concentration (%)	Amylose concentration (%)	Akali
Xiangyaxiangzhan							
	CK	81.78a	72.23a	57.75b	8.17a	18.07a	6.53a
	BR1	82.26a	72.41a	59.34b	8.17a	18.00a	6.50a
	BR2	81.41a	72.09a	57.91b	8.23a	18.00a	6.53a
	BR3	82.36a	72.14a	64.60a	8.18a	18.03a	6.50a
	BR4	81.72a	71.55a	65.64a	8.23a	17.97a	6.50a

3.6 Grain 2-AP Concentration

As shown in Fig. 4, exogenous BR influenced the grain 2-AP concentration of fragrant rice. For *Meixiangzhan-2*, there were no significant differences among CK, BR1 and BR2, in the 2-AP concentration whilst BR3 and BR4 significantly decreased the 2-AP concentrations by 22.22% and 44.09%, respectively. Compared with CK, for *Xiangyaxiangzhan*, BR1, BR2, BR3 and BR4 showed a similar ($P > 0.05$) 2-AP concentration. Also, the concentration of 2-AP was similar ($P > 0.05$) among CK, BR1 and BR3 (Fig. 4).

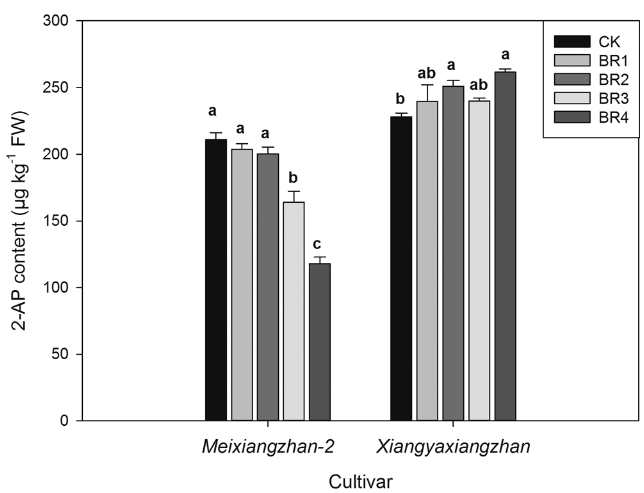

Figure 4: Effects of exogenous BR on grain 2-AP concentration of fragrant rice. Means sharing a common letter do not differ significantly at ($P \leq 0.05$) according to the LSD test. CK: water was sprayed at 600 liters per hectare. BR1: 0.05 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR2: 0.15 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR3: 0.25 mg L^{-1} BR solution was sprayed at 600 liters per hectare. BR4: 0.5 mg L^{-1} BR solution was sprayed at 600 liters per hectare

4 Discussion

The present study revealed the effects of exogenous BR on fragrant rice performances, i.e., yield formation, photosynthesis, antioxidant response and grain quality. Compared with CK, BR treatments (BR2 for *Meixiangzhan-2*, BR3 and BR4 for *Xiangyaxiangzhan*) significantly increased grain yield of fragrant rice, which could be explained by increased seed-setting rate. The improvement in yield formation of fragrant rice cultivars was attributed to the enhancement in photosynthesis including photosynthetic pigments concentrations and net photosynthetic rate. In our study, higher concentrations of chlorophyll a, chlorophyll b and carotenoid were recorded in BR treatments (BR2 for *Meixiangzhan-2*, BR3 and BR4 for *Xiangyaxiangzhan*) than CK. A similar trend was also recorded in the net photosynthetic rate. Chlorophyll concentration is an important factor in affecting fragrant rice photosynthesis which is a key bioprocess for yield formation [12]. A previous study showed that agronomy measures such as water management and microelement were used to increase chlorophyll concentrations and photosynthesis so the goal of higher grain yield would be achieved in fragrant rice production [13,14]. In our study, BR had similar positive effects on the yield formation of fragrant rice and the possible reason might be exogenous BR enhancing biosynthesis of chlorophyll. Moreover, the nitrogen fertilizer was not applied at the later growth stage, which possibly cause premature senility. Our results suggest that BR may be able to reduce the phenomenon of premature aging by improving photosynthesis. In addition, we observed that exogenous BR induced regulations in antioxidant responses in terms of GPX, SOD, CAT and MDA. The activities of GPX, SOD and CAT all be enhanced due to exogenous BR treatments for both *Meixiangzhan-2* and *Xiangyaxiangzhan* (BR2 for *Meixiangzhan-2*, BR3 and BR4 for *Xiangyaxiangzhan*) while BR treatments significantly decreased MDA concentrations. The microclimate and environment of the paddy field are very complex, sometimes it will cause stress (transient extreme temperature, pest, water stress) to the growing rice and induce oxidative damage and membrane peroxidation which could be judged by MDA concentration [14–17]. As the key enzymes on cleaning reactive oxygen, GPX, SOD and CAT play important roles in an antioxidant system in fragrant rice [18]. The enhancement in GPX, SOD and CAT activities indicated that foliar application of BR at a suitable concentration is able to promote the stress resistance of fragrant rice. Our results agreed with the research of Wang et al. [19] who demonstrated BR application was able to improve the resistance of rice at the booting stage to cold stress. The study of Su et al. [20] also showed that BR could mediate various physiological processes and improve crop resistance to abiotic stresses.

Besides grain yield, grain quality attributes such as milling, nutrients, aroma, texture and appearance are the factors that influenced the income of rice production [21–23]. The results of the present study showed that exogenous BR application (BR2 for *Meixiangzhan-2*, BR3 and BR4 for *Xiangyaxiangzhan*) improved the head rice rate of fragrant rice although other quality characters such as amylose concentration and protein concentration had no remarkable response to BR treatments. The biosynthesis and accumulation of 2-AP in aromatic rice is a complicated phenomenon which is affected by many factors [24,25]. In our study, for *Meixiangzhan-2*, BR4 treatment significantly decreased grain 2-AP concentration but other treatments had no significant effect. For *Xiangyaxiangzhan*, BR4 treatment significantly increased grain 2-AP concentration but other treatments had no significant effect. The different results on aroma in two fragrant rice cultivars induced by BR indicated that 2-AP biosynthesis in different fragrant rice cultivars responds differently to exogenous BR, and more studies are required to be conducted to understand the mechanism of the regulation of BR on 2-AP formation in fragrant rice.

In our study, we observed that different fragrant rice cultivars' responses applied concentration of BR differently. For *Meixiangzhan-2*, the optimum applied concentration of BR was 0.15 mg L^{-1} because the highest grain yield was recorded in BR2 treatment while BR3 and BR4 treatments inhibited chlorophyll biosynthesis and net photosynthetic rate, and consequently decreased grain yield. For *Xiangyaxiangzhan*, the highest grain yields were recorded in BR4 treatment, which indicated that the optimum applied concentration of BR was 0.50 mg L^{-1}. Our finding indicated the effects of different applied concentrations of BR on different rice cultivars could vary largely and thus, people should pay more attention to cultivar characteristics when applying BR for rice production.

5 Conclusion

Our study indicated that foliar application has substantial effects on yield formation, grain quality attributes, photosynthesis, antioxidants and 2-AP concentration of fragrant rice. The trend of chlorophyll concentration in two fragrant rice varieties treated with different BR concentrations was not consistent. For *Meixiangzhan-2*, BR2 was the best-applied treatment whilst for *Xiangyaxiangzhan*, BR4 was the best-applied treatment. The application of BR at a suitable concentration could increase chlorophyll concentration, enhance photosynthetic rate, improve antioxidant enzymatic activities, and lead to a higher grain yield of fragrant rice.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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