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ARTICLE

Enhancement of Ultrasonic Seed Treatment on Yield, Grain Quality Characters, and 2-Acetyl-1-Pyrroline Biosynthesis in Different Fragrant Rice Genotypes

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ABSTRACT

Fragrant rice is popular for the good grain quality and special aroma. The present study conducted a field experiment to investigate the effects of ultrasonic seed treatment on grain yield, quality characters, physiological properties and aroma biosynthesis of different fragrant rice genotypes. The seeds of three fragrant rice genotypes were exposed to 1 min of ultrasonic vibration and then cultivated in paddy field. The results of present study showed that ultrasonic seed treatment increased grain yield of all fragrant rice genotypes but the responses of yield formation to ultrasonic were varied with different genotypes. Compared with control, ultrasonic seed treatment increased grain 2-acetyl-1-pyrroline (2-AP, the key component of fragrant rice aroma) content by 13.40%–44.88%. Ultrasonic seed treatment also reduced the crude protein contents in grains. The head rice rate, rice length, chalky rice rate, and chalkiness degree were influenced by ultrasonic for one or two fragrant rice genotypes. The activities of peroxidase and superoxide dismutase were also enhanced due to ultrasonic seed treatment. In conclusion, ultrasonic seed treatment increased grain, regulated grain aroma and quality, and improved stress resistance of fragrant rice varieties.

KEYWORDS

Fragrant rice; yield formation; crop growth; grain quality; 2-acetyl-1-pyrroline

1 Introduction

Fragrant rice (*Oryza sativa* L.) is a series of rice varieties famous worldwide for its good quality characters and 'popcorn-like' aroma [1]. Compared with non-fragrant rice, fragrant rice is more attractive to consumers, although with a much higher price in markets [2,3]. In recent years, more and more agronomists and scientists have begun to pay attention to improving the productivity and quality of fragrant rice because of the increased global demand [4,5].

The characteristic aroma is the most valuable trait of fragrant rice, and there are more than 200 volatile compounds were detected in fragrant rice aroma [6]. Many scientists believe that 2-acetyl-1-pyrroline (2-AP) is the key flavor compound of that aroma, and it is found that 2-AP biosynthesis is a very complicated process in fragrant rice tissue [7,8]. The study by Yoshihashi et al. [9] showed that supplementation of proline increased 2-AP content in fragrant rice plants and indicated that proline is one of the precursors of 2-AP. The study by Poonlaphdecha et al. [10] showed that 1-pyrroline is a limiting substrate of 2-AP. Chen et al. [11] discovered that the expression of a gene, BADH2, inhibited that 2-AP formation in fragrant rice.

Recently, many studies showed that grain 2-AP content and yield formation of fragrant rice are substantially affected by some cultivation measures. For example, the study by Luo et al. [12] revealed that foliar application of selenium not only promoted biofortification but also substantially increased 2-AP content of fragrant rice. The research by Mo et al. [7] revealed that more input of nitrogen during the growth duration remarkably increased yield and 2-AP content. Bao et al. [13] demonstrated that alternate wetting and drying water management could remarkably increase grain yield and 2-AP content. The study by Li et al. [14] revealed that rice-duck co-culture facilitated the yield formation and 2-AP biosynthesis of fragrant rice. Moreover, it is found that the different planting seasons substantially affected the grain 2-AP content by varying the climates during the growth duration [15]. It seems that the productivity and grain quality of fragrant rice could be influenced by many factors such as fertilization, water management, and climate condition.

Ultrasonic treatment, which utilizes low to medium-frequency waves (20–100 kHz), is an affordable, simple, safe, and eco-friendly method to improve seed germination and crop growth [16]. The study by Liu et al. [17] showed that ultrasound at 20 kHz improved the growth of the solid-cultured aloe callus and indicated that it was attributed to the mechanical stress and microstreaming by acoustic cavitation. Our previous studies revealed that ultrasonic seed treatment not only enhanced the cadmium tolerance of *Brassica napus* L. but also promoted the growth and productivity of rice under lead stress [18,19]. The study by Huang et al. [20] revealed that ultrasonic seed treatment reduced the transportation of cadmium to grain in rice plants. In 2014, we conducted a single-season field experiment and discovered that ultrasonic seed treatment enhanced the net photosynthetic rate and copper uptake of fragrant rice [21].

Besides grain yield, quality is an important factor in determining rice producers' income and commercial value of rice [22]. However, the effects of ultrasonic seed treatment on aroma and other quality characters of fragrant rice remained largely unexplored. Moreover, there need more field experiments with more fragrant rice varieties before the large-scale popularization of ultrasonic technology in agriculture. Hence, we conducted a field experiment in two cropping seasons and three fragrant rice genotypes to investigate the effects of ultrasonic seed treatment on growth, yield formation, grain quality characters, and 2-AP content of fragrant rice.

2 Materials and Methods

2.1 Plant Materials, Growth Conditions, and Experiment Design

A field experiment was conducted in Huangjiashan village (22.62°N, 111.57°E), Luoping Town, Yunfu City, Guangdong Province, China, during two rice growing seasons (early season and late season) of 2021. The site enjoys a subtropical monsoon climate. The experimental soil was sandy loam consisting of 16.54 g kg^{-1} organic matter, 1.26 g kg^{-1} total nitrogen, 1.47 g kg^{-1} total phosphorus, and 10.87 mg kg^{-1} total potassium with a pH of 5.90. Seeds of three fragrant rice genotypes, *Xiangyaxiangzhan* (*Xiangsimiao126* × *Xiangyaruanzhan*, bred by Taishan Institute of Agricultural Sciences), *Meixiangzhan-2* (*Lemont* × *Fengaozhan*, bred by Rice Research Institute, Guangdong Academy of Agricultural Sciences), *19xiang* (*Guguangzhan* × *Xiangyaxiangzhan*, by Rice Research Institute, Guangdong Academy of Agricultural Sciences), were used as plant materials in the present study. All varieties are conventional rice and widely cultivated in South China for fragrant rice production. Their information could be found in China Rice Data Center (https://www.ricedata.cn/variety/). The three varieties were chosen as plant materials because they are the main fragrant rice varieties planted in South China. Such choice in the present study would benefit the popularization of the application of ultrasonic seed treatment in fragrant rice production. In the early season, pre-germinated seeds were sown into seedbeds on March 02, the seedlings were transplanted into the paddy field on April 04, and the harvest was on July 09. In the late season, pre-germinated seeds were sown into seedbeds on July 19, the seedlings were transplanted into the paddy field on August 04, and the harvest was on November 09. Experiments were arranged in a split-plot design with rice varieties as the main plot, and ultrasonic treatment (CK: all seeds were without ultrasonic seed treatment; UT: before germination, the seeds were put into tunnel ultrasonic processor (5ZCG-T6, Golden Rice Agricultural Science & Technology Co., Ltd., Guangzhou, China) in a stainless-steel plate, treated at 50 kHz frequency for 1.0 min at room temperature.) as the subplot with four replicates. The plot size was 24 m^2 (4 m × 6 m).

2.2 Crop Management and Plant Sampling

Commercial compound fertilizer (manufactured by Zhongxiang Phosphorus Fertilizer Company, China, total nitrogen contents = 15%, N:P_2O_5:KCl = 15%:15%:15%) was applied at the same amount of 900 kg ha^{-1} in each plot. The water management was carried out according to the methods of Pan et al. [23]. Weeds, diseases, and insects were strictly controlled throughout two cropping seasons. At the heading stage (about 60 days after transplanting), fresh leaves were separated from the main plants and stored at −80°C for physio-biochemical analysis. At the harvest, fresh grains were also collected and stored at −80°C for biochemical analysis and the determination of 2-AP and volatile compounds.

2.3 Determination of Yield and Yield Components

At harvest, the effective panicle number of ten representative plants was recorded in each plot, and rice grains were harvested from three sampling areas (1.00 m^2) in each plot and then manually threshed to determine the grain yield with the standard grain moisture content adjusted to 14%. Six rice plants in each plot were also collected to measure the 1000-grain weight, seed-setting rate and grain number per panicle according to the methods of Yang et al. [22].

2.4 Determination of the Milling, Nutrient, and Appearance Quality

Before grain quality evaluation, the grain samples were air-dried to 12%–13% of moisture content and stored for at least three months. 210 g rice grains from each treatment were taken from storage, and the brown rice rate was measured and calculated with a rice huller (Jiangsu, China). The milled rice and head rice rates were measured and calculated using a Jingmi testing rice grader (Zhejiang, China). The length, width, chalky rice rate, and chalkiness degree of head rice were estimated by scanner (MRS-9600TFUL, Shanghai Zhongjing Technology Co., Ltd., Shanghai, China) and rice appearance quality analysis and detection system (Hangzhou Wanshen Testing Technology Co., Ltd., Hangzhou, Zhejiang, China). The crude protein and amylose content were determined using the Infratec-1241 grain analyzer (FOSS-TECATOR, HOGANAS, Sweden).

2.5 Determination of 2-AP and Other Volatile Components Contents

The grain 2-AP content was determined according to the methods of Mo et al. [2] by synchronization distillation and extraction method (SDE) combined with GCMS-QP 2010 Plus (Shimadzu Corporation, Japan) and expressed as μg kg^{-1}.

2.6 Determination of Proline, 1-Pyrroline, and Methylglyoxal Contents

The determination of proline was carried out according to the methods of Mostofa et al. [24] with sulfosalicylic for extraction and acidic-ninhydrin for chromogenic reaction. The absorbance was read at

520 nm, and proline content was expressed as μg g^{-1}. The determination of methylglyoxal content was carried out according to the methods of Banu et al. [25]. In a total volume of 1 mL, 250 μL of 7.2 mM 1,2-diaminobenzene, 100 μL of 5 M perchloric acid, and 650 μL of the neutralized supernatant were added in that order. The absorbance of the derivative was read at 336 nm. The determination of 1-pyrroline content was carried out according to the methods of Luo et al. [8]. Samples (0.5 ml) of the reaction mixtures were mixed with 1 ml of 0.01 M o-amino benzaldehyde (in 0.02 M phosphate buffer, pH 7.0), 1 ml of 0.2 M phosphate buffer, pH7.0, and water to give a total volume of 3 ml. The mixtures were left at room temperature for 30 min, and the 1-pyrroline content was calculated from the E430, assuming $\varepsilon = 1860\ cm^{-1}$.

2.7 Determination of Peroxidase (POD EC 1.11.1.7), Catalase (CAT, EC 1.11.1.6), Superoxide (SOD, EC 1.15.1.1) Activities, and Malondialdehyde (MDA) Contents

The POD activity was determined according to the methods of Kong et al. [26]. The Enzyme extract was reacted with 0.3% H_2O_2% and 0.2% guaiacol in phosphate buffer (pH 7.0). The absorbance was read at 470 nm. One POD unit of enzyme activity was defined as the absorbance increase because of guaiacol oxidation. The determination of SOD and CAT activities was carried out according to the methods of Mostofa et al. [24] and expressed as U g^{-1} min^{-1}. The determination of MDA content was carried out according to the methods of Yiğit et al. [27]. After reacting with thiobarbituric acid at a boiling water bath for 20 min, the absorbance of the supernatant was read at 532 nm, 600 nm, and 450 nm. The final result of MDA was expressed as μmol g^{-1}.

2.8 Data Analysis

Analysis of variance was performed with Statistix 8.1 (Analytical Software, Tallahassee, FL, USA), and the means of treatments were compared based on the least significant difference (LSD) test at the 0.05 probability level. The figures were made using SigmaPlot 12.5 (Systat Software Inc., California, USA).

3 Results

3.1 Grain Yield and Yield Components

Table 1 shows different fragrant rice genotypes' grain yield and yield components in two cropping seasons. In the early season, compared with CK, UT treatment increased grain yield by 20.30%, 6.41%, and 8.98% for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang*, respectively, although the difference for Meixiangzhan-2 was insignificant. In the late season, compared with CK, UT treatment increased grain yield by 42.83%, 8.18%, and 4.76% for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang*, respectively, although the difference for *19xiang* was insignificant. The higher grain number per panicle was recorded in UT treatment than CK except for *19xiang* in the late season. Compared with CK, UT treatment also significantly increased 1000-grain weight for *Xiangyaxiangzhan* and *19xiang* in the late season.

3.2 Grain Milling and Nutrient Quality

Table 2 shows different fragrant rice genotypes' grain milling and nutrient quality in two cropping seasons. Compared with CK, UT treatment significantly increased the head rice rate for *Xiangyaxiangzhan* in the early and late seasons. 13.92%, 13.28%, and 15.44% lower crude protein contents were recorded in UT treatment than CK for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang*, respectively in the early season, and 12.08%, 1.94%, and 4.91% lower crude protein contents were recorded in UT treatment than CK for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang*, respectively in late season. Moreover, compared with CK, UT treatment slightly and insignificantly increased amylose content for three fragrant rice varieties in both seasons.

Table 1: Effects of ultrasonic seed treatment on grain yield and yield components of different fragrant rice genotypes

Season	Variety	Treatment	Grain yield (t ha^{-1})	Effective panicle number ($plant^{-1}$)	Grain number per panicle	Seed setting rate (%)	1000-grain weight (g)
Early season	*Xiangyaxiangzhan*						
		CK	7.44 ± 0.58b	20.40 ± 2.95b	118.00 ± 3.61a	78.46 ± 1.91a	21.00 ± 0.46a
		UT	8.95 ± 0.88a	27.10 ± 2.85a	127.67 ± 8.33a	78.13 ± 1.67a	20.70 ± 0.20a
	Meixiangzhan-2						
		CK	7.18 ± 0.39a	18.70 ± 3.92a	145.33 ± 10.50a	78.34 ± 1.16a	20.93 ± 0.23a
		UT	7.64 ± 0.50a	19.00 ± 3.30a	153.00 ± 1.00a	77.98 ± 0.88a	21.17 ± 0.25a
	19xiang						
		CK	7.35 ± 0.17b	16.80 ± 2.35a	154.33 ± 1.15b	80.53 ± 0.86b	23.00 ± 0.20a
		UT	8.01 ± 0.16a	16.70 ± 2.50a	162.67 ± 2.89a	84.61 ± 1.73a	23.13 ± 0.25a
Late season	*Xiangyaxiangzhan*						
		CK	4.81 ± 0.26b	28.00 ± 3.89a	100.67 ± 8.39b	70.32 ± 1.55b	17.09 ± 0.20b
		UT	6.87 ± 0.82a	26.80 ± 3.36a	119.33 ± 7.37a	76.40 ± 1.41a	17.95 ± 0.17a
	Meixiangzhan-2						
		CK	5.38 ± 0.13b	19.90 ± 3.41a	140.33 ± 3.51a	68.01 ± 3.46b	20.03 ± 0.72a
		UT	5.82 ± 0.20a	20.00 ± 2.91a	152.00 ± 6.43a	81.47 ± 4.28a	19.91 ± 0.47a
	19xiang						
		CK	7.35 ± 0.41a	19.10 ± 3.90a	179.00 ± 7.00a	69.05 ± 6.40a	19.54 ± 0.24b
		UT	7.70 ± 0.22a	17.30 ± 1.25a	178.00 ± 13.23a	67.95 ± 5.86a	20.57 ± 0.33a

Note: Within a column for each cropping season and fragrant rice variety, means followed by different letters are significantly different according to the LSD test (0.05), respectively.

3.3 Grain Appearance Quality

Table 3 shows different fragrant rice genotypes' grain milling and nutrient quality in two cropping seasons. Compared with CK, UT treatment significantly decreased chalky rice rate and chalkiness degree for *19xiang* in the late season. Higher rice length was recorded in UT treatment than CK for *Xiangyaxiangzhan* and *19xiang*, while lower rice length was recorded in UT treatment than CK for *Meixiangzhan-2* in early and late seasons, although the differences were insignificant. There was no substantial difference between UT treatment and CK in rice width for three fragrant rice genotypes in both seasons.

3.4 2-AP Content

Fig. 1 shows the grain 2-AP content of different fragrant rice genotypes in two cropping seasons. In the early season, compared with CK, UT treatment significantly increased 2-AP content by 21.26%, 13.40%, and 44.88% for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang*, respectively. In the late season, compared with CK, UT treatment significantly increased 2-AP content by 36.74%, 44.39%, and 30.45% for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang*, respectively.

Table 2: Effects of ultrasonic seed treatment on grain milling and nutrient quality of different fragrant rice genotypes

Season	Variety	Treatment	Brown rice rate (%)	Milled rice rate (%)	Head rice rate (%)	Crude protein (%)	Amylose (%)	Akali
Early season	*Xiangyaxiangzhan*							
		CK	79.16 ± 0.04a	69.14 ± 0.87a	57.07 ± 0.49b	9.10 ± 0.17a	17.40 ± 0.10a	6.53 ± 0.15a
		UT	78.62 ± 0.33a	70.78 ± 0.43a	60.02 ± 0.86a	7.83 ± 0.06b	17.53 ± 0.06a	6.60 ± 0.10a
	Meixiangzhan-2							
		CK	78.63 ± 0.36a	71.60 ± 0.56a	60.63 ± 0.76a	9.03 ± 0.06a	17.30 ± 0.36a	6.67 ± 0.06a
		UT	78.31 ± 0.90a	70.88 ± 0.74a	59.11 ± 1.53a	7.83 ± 0.06b	17.80 ± 0.00a	6.60 ± 0.10a
	19xiang							
		CK	78.99 ± 0.34a	70.89 ± 0.53a	58.50 ± 0.64a	8.63 ± 0.06a	16.90 ± 0.00a	6.53 ± 0.06a
		UT	78.22 ± 0.48a	69.90 ± 0.30a	59.12 ± 1.58a	7.30 ± 0.10b	17.03 ± 0.06a	6.33 ± 0.12a
Late season	*Xiangyaxiangzhan*							
		CK	74.79 ± 0.90a	55.64 ± 1.34b	39.98 ± 1.47b	9.93 ± 0.12a	17.00 ± 0.10a	7.23 ± 0.06a
		UT	76.31 ± 0.10a	60.54 ± 0.61a	51.31 ± 0.16a	8.73 ± 0.06b	17.40 ± 0.10a	7.27 ± 0.06a
	Meixiangzhan-2							
		CK	79.72 ± 0.67a	65.28 ± 0.09a	54.29 ± 1.23a	8.60 ± 0.00a	17.60 ± 0.17a	7.30 ± 0.00a
		UT	78.83 ± 0.50a	66.36 ± 0.79a	56.84 ± 0.98a	8.43 ± 0.06b	17.97 ± 0.06a	7.17 ± 0.06a
	19xiang							
		CK	78.67 ± 0.34b	62.81 ± 0.55a	51.87 ± 0.46a	8.83 ± 0.06a	17.33 ± 0.15a	7.17 ± 0.12a
		UT	80.00 ± 0.33a	62.30 ± 0.95a	50.66 ± 0.59a	8.40 ± 0.00b	17.57 ± 0.23a	7.07 ± 0.06a

Note: Within a column for each cropping season and fragrant rice variety, means followed by different letters are significantly different according to the LSD test (0.05), respectively.

3.5 Proline, 1-Pyrroline, and Methylglyoxal Contents

Fig. 2 shows the grain 2-AP content of different fragrant rice genotypes in two cropping seasons. Compared with CK, UT treatment significantly reduced grain proline content by 33.35%, 12.49%, and 21.05% for *Xiangyaxiangzhan*, *Meixiangzhan-2,* and *19xiang* in the early season, and by 18.61%, 42.13%, and 30.68% for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang* in late season, respectively. Higher 1-pyrroline content was recorded in UT treatment than CK for three fragrant rice varieties in both seasons, although the differences for *19xiang* in the early season were insignificant. There was no significant difference between UT treatment and CK in methylglyoxal content.

3.6 Antioxidant Enzymes Activities and MDA Content

Fig. 3 shows the activities of POD, CAT, SOD, and MDA content of different fragrant rice genotypes in two cropping seasons. Higher POD activity was recorded in UT treatment than CK for three fragrant rice genotypes in early and late seasons, although the difference for *19xiang* in the late season was insignificant. Compared with CK, UT treatment significantly enhanced SOD activity by 13.20%, 41.56%, and 7.35% for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang* in the early season, and by 14.81%, 12.68%, and 29.30% for *Xiangyaxiangzhan*, *Meixiangzhan-2*, and *19xiang* in late season, respectively.

There was no substantial difference between UT treatment and CK in CAT activity. Lower MDA content was recorded in UT treatment than CK for *Xiangyaxiangzhan* and *Meixiangzhan-2* in both seasons.

Table 3: Effects of ultrasonic seed treatment on grain appearance quality of different fragrant rice genotypes

Season	Variety	Treatment	Chalky rice rate (%)	Chalkiness degree	Length/width	Length (mm)	Width (mm)
Early season							
	Xiangyaxiangzhan						
		CK	8.33 ± 2.31a	2.07 ± 0.90a	3.55 ± 0.07a	5.71 ± 0.10a	1.61 ± 0.00a
		UT	11.33 ± 1.15a	2.39 ± 0.34a	3.52 ± 0.07a	5.80 ± 0.07a	1.65 ± 0.01a
	Meixiangzhan-2						
		CK	11.33 ± 2.08a	3.02 ± 0.55a	3.41 ± 0.03a	5.66 ± 0.06a	1.66 ± 0.02a
		UT	9.00 ± 2.65a	1.53 ± 0.29a	3.26 ± 0.06a	5.43 ± 0.03b	1.66 ± 0.03a
	19xiang						
		CK	8.33 ± 4.93a	2.38 ± 1.84a	3.72 ± 0.03a	6.05 ± 0.01a	1.63 ± 0.01a
		UT	9.67 ± 4.16a	2.38 ± 1.63a	3.85 ± 0.11a	6.17 ± 0.06a	1.60 ± 0.05a
Late season							
	Xiangyaxiangzhan						
		CK	5.33 ± 4.16a	0.95 ± 0.38a	3.97 ± 0.10a	5.69 ± 0.04b	1.43 ± 0.03a
		UT	5.33 ± 1.53a	1.18 ± 0.53a	4.11 ± 0.07a	5.86 ± 0.06a	1.43 ± 0.02a
	Meixiangzhan-2						
		CK	3.33 ± 0.58a	0.91 ± 0.36a	3.39 ± 0.02a	5.37 ± 0.08a	1.58 ± 0.02a
		UT	4.00 ± 1.73a	0.95 ± 0.21a	3.41 ± 0.03a	5.24 ± 0.05a	1.54 ± 0.03a
	19xiang						
		CK	4.67 ± 0.58a	0.94 ± 0.12a	3.89 ± 0.06a	5.63 ± 0.06b	1.45 ± 0.01a
		UT	0.67 ± 0.58b	0.33 ± 0.30b	4.09 ± 0.13a	5.98 ± 0.11a	1.46 ± 0.03a

Notes: Within a column for each cropping season and fragrant rice variety, means followed by different letters are significantly different according to the LSD test (0.05), respectively.

4 Discussion

The present study revealed the application potential of ultrasonic in fragrant rice production by showing the benefits of ultrasonic seed treatment on yield formation and grain quality characters of different fragrant rice genotypes. Compared with CK, UT treatment increased the grain yield of three fragrant rice varieties by 4.76%–42.83% across two cropping seasons. Our results were consistent with the study by Mo et al. [21]. The improvement in yield was attributed to the increased grain number per panicle for three fragrant rice varieties. However, we observed that the roles of ultrasonic seed treatment on yield formation of fragrant rice were affected by cropping seasons and varied with genotypes. For example, ultrasonic seed treatment significantly increased seed-setting rate and 1000-grain weight for *Xiangyaxiangzhan* in the late season but had no significant effect in the early season. The differences in the improvement of ultrasonic seed treatment on yield formation might be affected by the climate conditions. The growth period of fragrant rice varieties in the present study was about 120 days, and the microclimate of the paddy field could be complicitly varied across days and months [28]. The study by Yang et al. [29] showed that the alteration of microclimate had substantial effects on rice agronomic performance. The responses of yield formation of different fragrant rice genotypes to ultrasonic seed treatment also were slightly different. Overall, ultrasonic seed treatment had substantial effects on promoting the yield formation of fragrant rice varieties.

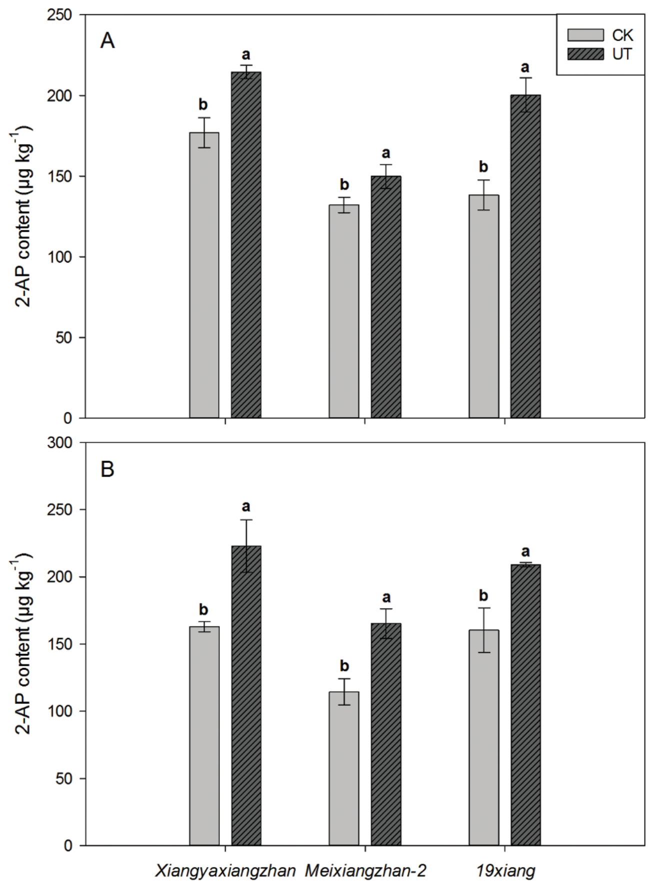

Figure 1: Effects of ultrasonic seed treatment on grain proline content of different fragrant rice genotypes. A for the early season; B for the late season; Means sharing a common letter do not differ significantly at $P \leq 0.05$ according to the least significant difference (LSD) test

In the present experiment, we observed that ultrasonic seed treatment significantly reduced the grain protein content of three fragrant rice varieties in both cropping seasons. Our results were inconsistent with the study of Mo et al. [21], which showed that ultrasonic seed treatment has no effect on protein content. The differences might be attributed to the ultrasonic frequency and treating time. Normally, rice grains are composed of 80%–85% starch, 4%–10% protein, 1% lipid, and 10% moisture. Many factors contribute to rice grain quality, including the content of starch, the fine structure of amylopectin, and the interactions of starch with proteins, lipids, and polysaccharides that do not originate from starch [30]. The study by Zhang et al. [31] showed that high amylose content would cause a higher hardness of cooked rice. Lyon et al. [32] indicated that the harder cooked rice was attributed to high protein content. The research by Tsukaguchi et al. [33] also showed that protein content influences the texture of cooked rice, increases hardness, and reduces stickiness. Our findings showed that ultrasonic seed treatment substantially reduced grain protein content of three fragrant rice genotypes without affecting the amylose content, which indicated that ultrasonic seed treatment could make better texture with the lower hardness of cooked fragrant rice.

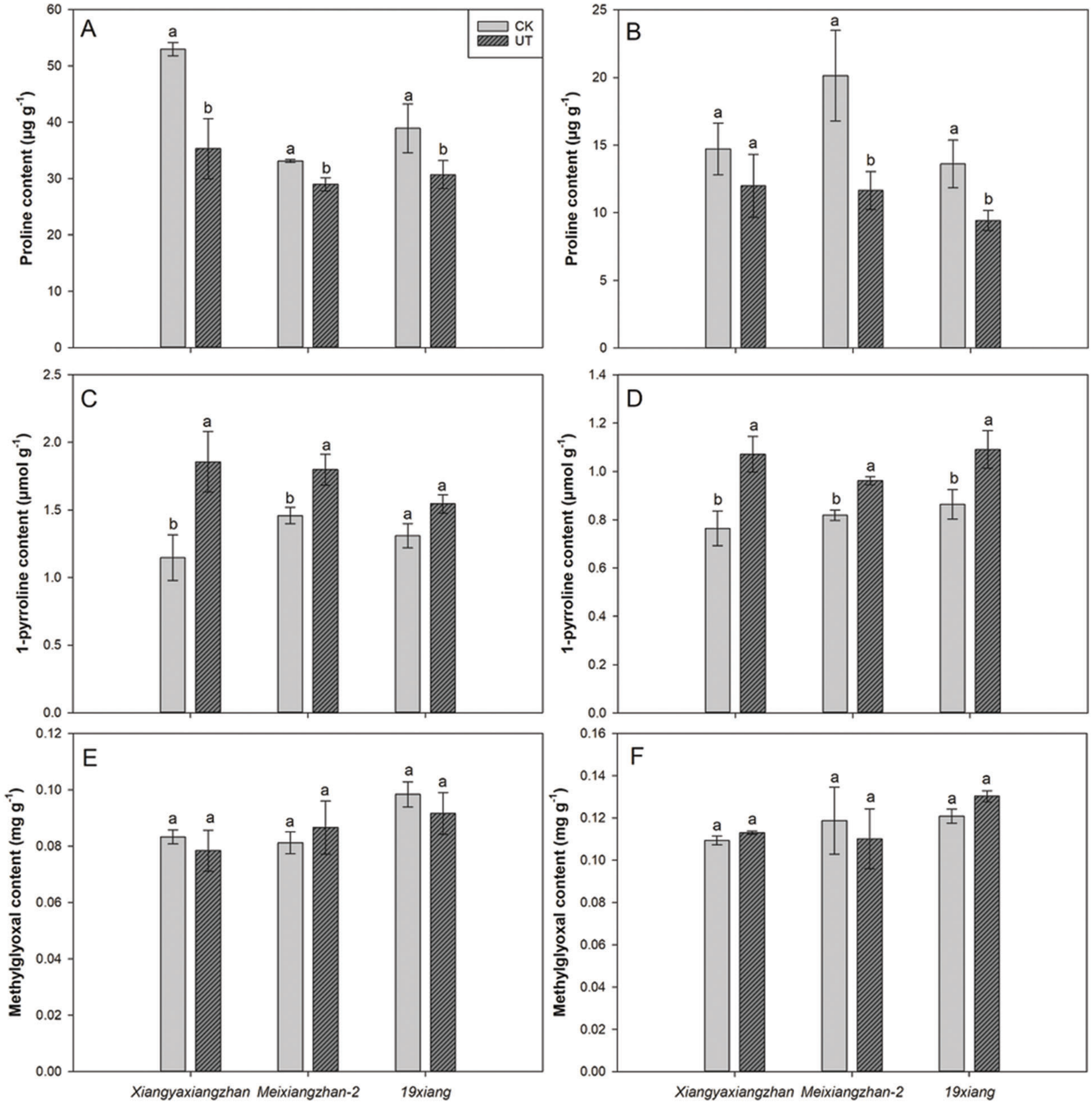

Figure 2: Effects of ultrasonic seed treatment on grain contents of proline, 1-pyrroline, and methylglyoxal of different fragrant rice genotypes. A, C, and E for the early season; B, D, and F for the late season; Means sharing a common letter do not differ significantly at $P \leq 0.05$ according to the least significant difference (LSD) test

2-AP is the key component of fragrant rice aroma. In the present experiment, we observed that ultrasonic seed treatment substantially increased grain 2-AP content of three fragrant rice genotypes. Our results also showed that reduced the proline content and increased 1-pyrroline content, which indicated that ultrasonic seed treatment enhanced 2-AP biosynthesis by promoting the conversion from proline to 1-pyrroline in fragrant rice grains.

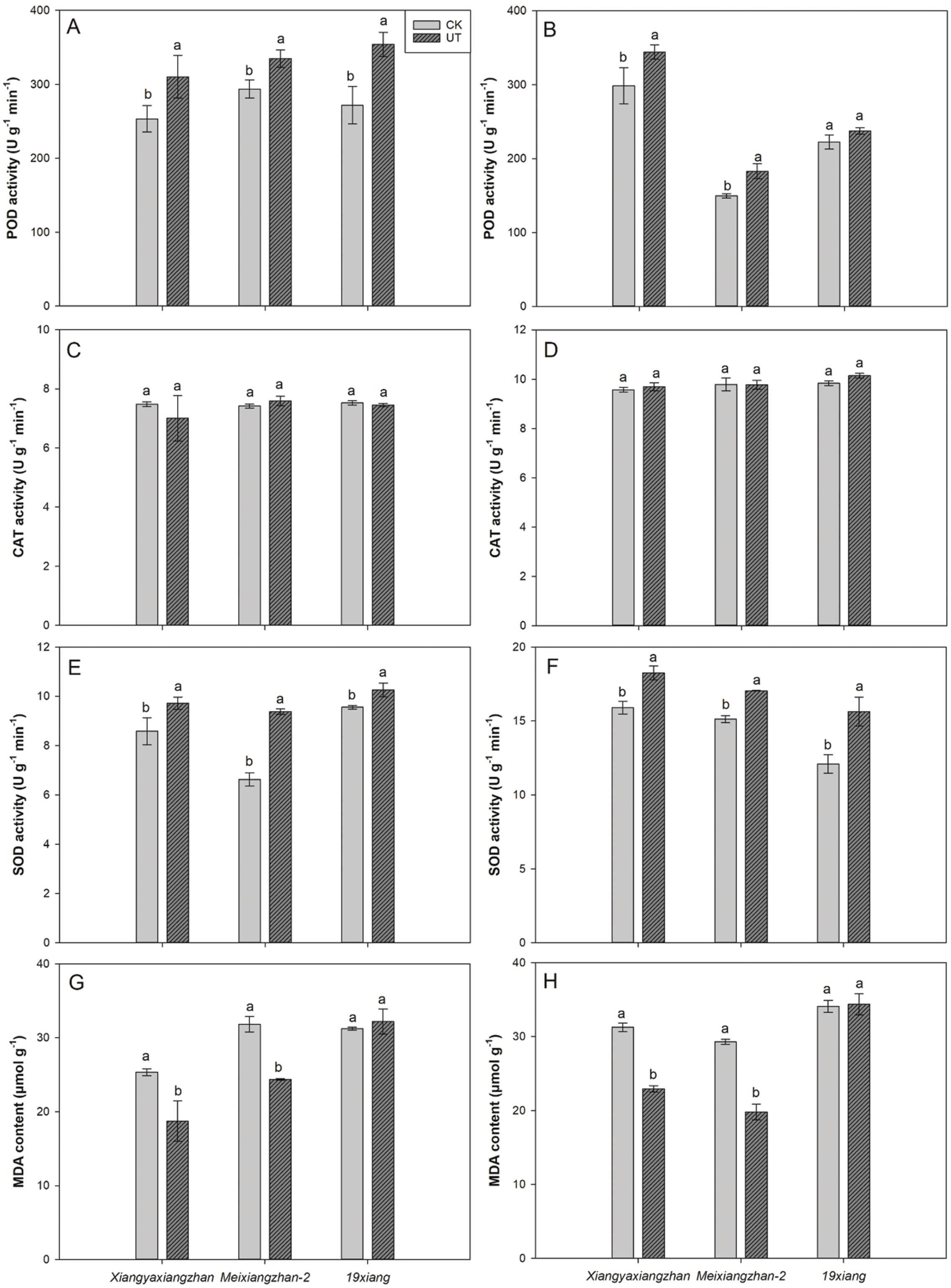

Figure 3: Effects of ultrasonic seed treatment on activities of POD, CAT, SOD, and MDA content of different fragrant rice genotypes. A, C, E, and G for the early season; B, D, F, and H for the late season; Means sharing a common letter do not differ significantly at $P \leq 0.05$ according to the least significant difference (LSD) test

In addition, we observed that ultrasonic seed treatment not only enhanced the activities of enzymes, including POD and SOD, but also reduced MDA content (except for *19xiang*) in fragrant rice leaves. It is thought that MDA production is a sign of oxidative stress, which affects the structure and function of intercellular and intracellular membranes, resulting in increased ion leakage through cell membranes [18,19]. The decreased MDA content indicated that ultrasonic seed treatment improved the conditions of intercellular and intracellular membranes, and it might be attributed to the enhancement of POD and SOD activities. POD and SOD are the key enzymes in eliminating reactive oxygen and alleviating oxidative damage in plant tissue [34]. Our results were consistent with the study by Mo et al. [21]. As mentioned above, the microclimate conditions in paddy fields are complicated, and the rice plants would face irregular stress such as transient extreme temperature, strong wind, and so on. Our results agreed with our previous studies and indicated that ultrasonic seed treatment could enhance the stress resistance of rice plants [19,20].

In general, ultrasonic seed treatment substantially affects fragrant rice varieties' growth, yield formation, grain quality, and physiological properties. The study by Huang et al. [20] indicated that mechanical agitation and physical effects were generated from acoustic cavitation led to ultrasound waves inducing bioeffects on plant cells. In the present study, we observed that ultrasonic seed treatment altered the activities of POD and SOD while the enhancement of 2-AP content also indicated the possible changes of related enzymes. Such regulations might be attributed to the changes of gene expression or/and enzyme structure. In order to reveal the mechanism of ultrasonic application of fragrant rice performances, more studies need to be conducted on multiple levels.

5 Conclusion

Ultrasonic seed treatment increased grain yield of all fragrant rice genotypes, but the responses of yield formation to ultrasonic were varied with different genotypes. Ultrasonic seed treatment reduced the crude protein content and increased the 2-AP content. The increment in 2-AP content was attributed to the conversion form proline. The activities of POD and SOD were also enhanced due to ultrasonic seed treatment. Overall, ultrasonic seed treatment could enhance productivity and stress resistance, increase 2-AP content, and regulate the grain quality of fragrant rice varieties.

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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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