agriculture

Article

A New Organic-Inorganic Compound Fertilizer for Improving Growth, Yield, and 2-Acetyl-1-Pyrroline Biosynthesis of Fragrant Rice

Haowen Luo [1,2,3,†], Meiyang Duan [1,2,3,†], Longxin He [1,2,3], Shuying Yang [4], Yingbin Zou [4] and Xiangru Tang [1,2,3,*]

1 State Key Laboratory for Conservation and Utilization of Subtropical Agricultural Bioresources, South China Agricultural University, Guangzhou 510642, China; luohaowen@stu.scau.edu.cn (H.L.); scdmy213@163.com (M.D.); helx@stu.scau.edu.cn (L.H.)
2 Scientific Observing and Experimental Station of Crop Cultivation in South China, Ministry of Agriculture, Guangzhou 510642, China
3 Guangzhou Key Laboratory for Science and Technology of Aromatic Rice, Guangzhou 510642, China
4 College of Agronomy, Hunan Agricultural University, Changsha 410128, China; ysyalxh@126.com (S.Y.); ybzou123@126.com (Y.Z.)
* Correspondence: tangxr@scau.edu.cn
† These authors contributed equally to the work.

Citation: Luo, H.; Duan, M.; He, L.; Yang, S.; Zou, Y.; Tang, X. A New Organic-Inorganic Compound Fertilizer for Improving Growth, Yield, and 2-Acetyl-1-Pyrroline Biosynthesis of Fragrant Rice. *Agriculture* **2021**, *11*, 1121. https://doi.org/10.3390/agriculture11111121

Academic Editor: Jose L. Gabriel

Received: 22 October 2021
Accepted: 8 November 2021
Published: 10 November 2021

Abstract: Fragrant rice (*Oryza sativa* L.) is a high-valued rice type and possesses a unique aroma with 2-acetyl-1-pyrroline (2-AP) as the critical component. However, the cultivation measures in fragrant rice production are far from perfect. In this study, a new organic-inorganic compound fertilizer was made with organic matter, urea, superphosphate, potassium chloride, zinc sulfate, and lanthanum chloride. A four-year field experiment was conducted to investigate its effects on fragrant rice growth, yield formation, and 2-acetyl-1-pyrroline biosynthesis. Three treatments, i.e., (CK) no fertilizer was applied, (IF) the urea, superphosphate, and potassium chloride were applied at 234 kg ha^{-1}, 450 kg ha^{-1} and 108 kg ha^{-1}, and (OICF) this new fertilizer composed of 10% organic matter, 26% urea, 50% superphosphate, 12% potassium chloride, 1.9% zinc sulfate, and 0.1% lanthanum chloride, was applied at 900 kg ha^{-1}, were adopted in the present study. Across four experimental years, the results showed that the grain yield in OICF treatment ranged between 5.86–8.29 t ha^{-1}, and was significantly ($p < 0.05$) higher than that in IF treatment and CK. The improvement in grain yield due to OICF treatment was explained by increased effective panicle number per m^2 and seed-setting rate. The highest or equally highest chlorophyll content and the net photosynthetic rate at 20, 40, 60, and 80 days after transplanting were recorded in OICF treatment among three treatments. OICF treatment also increased the aboveground biomass of fragrant rice compared with IF treatment and CK. Moreover, compared with CK and IF treatment, OICF treatment significantly ($p < 0.05$) increased grain 2-AP content by 30–38% and 10–21%, respectively. The contents of 2-AP related precursors, including proline and 1-pyrroline, also increased due to OICF treatment. This study provided a new organic-inorganic compound fertilizer and suggested that it could be used to achieve the goals of high yield and high grain 2-AP content in fragrant rice production.

Keywords: fertilizer; fragrant rice; photosynthesis; yield formation; 2-acetyl-1-pyrroline

1. Introduction

Rice is a staple food and consumed by more than 65% of the population in China [1]. Among all rice types, fragrant rice is high-valued and famous for its better cooking qualities and pop-corn aroma [2]. The study by Pan et al. [3] showed that demand for fragrant rice has been increasing in the international market despite the premium price in the last decade. In recent years, it has been established that 2-acetyl-1-pyrroline (2-AP) is the essential compound of the unique aroma in fragrant rice [4–6], thus improving grain yield and 2-AP content in fragrant rice have been the main goal for the fragrant rice breeders and cultivators.

2-AP biosynthesis in fragrant rice is very complicated and includes multiple substances and biochemical reactions [4,7]. A previous study revealed that in fragrant rice tissues, proline is a vital precursor and nitrogen source of 2-AP [8]. Poonlaphdecha et al. [6] demonstrated that 1-pyrroline is a limiting substrate in 2-AP biosynthesis in fragrant rice. The study by Chen et al. [9] discovered that the expression of gene *BADH2*, which encodes betaine aldehyde dehydrogenase (BADH) to inhibit 2-AP formation by transforming γ-amino butyraldehyde to γ-aminobutyric (GABA) rather than 1-pyrroline, is essential in controlling the 2-AP content in fragrant rice. Moreover, the study by Mo et al. [10] indicated activity of proline dehydrogenase (PDH) in fragrant rice affected 2-AP by balancing proline content. It is widely believed that there are two pathways to synthesize 2-AP. One is PDH catalyzes the degradation of proline to produce 1-pyrroline and then to form 2-AP. Another is γ-amino butyraldehyde converts into 1-pyrroline and forms 2-AP, while BADH inhibits the second pathway.

Previous attempts have been made to understand the effects of different agronomic practices on 2-AP production in fragrant rice [11,12]. The study by Imran et al. [13] indicated that molybdenum improved nitrogen uptake and utilization to increase 2-AP content under cadmium toxicity. Xie et al. [14] demonstrated that foliar application of GABA in fragrant rice production substantially increased grain 2-AP content. Mo et al. [15] revealed drought conditions remarkably increased grain 2-AP content in fragrant rice. Bao et al. [16] indicated that alternate wetting and drying was an excellent strategy to improve fragrant rice's 2-AP content and productivity. In addition, several studies found that high input of nitrogen fertilizer effectively improved both grain yield and 2-AP content [10,17,18].

The information mentioned above depicted that 2-AP content in fragrant rice could be sensitive to agronomic practices such as fertilizer and water management. It is meaningful to agglomerate those findings into systematic cultivation for fragrant rice production. Fertilization is a critical part of achieving the goals of high grain yield in rice production, as reported by numerous studies [19,20]. Previous studies found that the application of zinc and lanthanum in fragrant rice production could enhance 2-AP production and promote fragrant rice productivity [21,22]. In the present study, we mixed organic matter (to be more eco-friendly), urea, superphosphate, potassium chloride, zinc sulfate, and lanthanum chloride and placed them into a pelletizer to synthesize a new compound granular fertilizer, and conducted a field experiment across four years to investigate its roles in improving fragrant rice yield and 2-AP content as an alternative fertilizer, along with the underlying mechanisms. Our findings also provide more information about fragrant rice production and the mechanism of 2-AP biosynthesis in fragrant rice.

2. Materials and Methods

2.1. Plant Materials and Growing Conditions

A four-year field experiment was carried out at the Experimental Research Farm, South China Agricultural University, Ningxi county (23°16′ N, 113°22′ E and 11 m from the mean sea level), Guangdong Province, China, in 2017–2020. This site enjoys a subtropical monsoon climate. A fragrant rice cultivar, Meixiangzhan-2 (Lemont × Fengaozhan), as the main fragrant rice cultivars and widely planted in South China, was used as plant material in the present study. The soil properties were determined according to the protocols of Mo et al. [15] before the start of field experiment and showed as: pH = 5.89, organic matter = 14.08 g kg^{-1}, total n = 0.57 g kg^{-1}, available n = 44.05 mg kg^{-1}, total p = 0.31 g kg^{-1}, available p = 11.55 mg kg^{-1}, total K = 16.04 g kg^{-1}, available K = 47.60 mg kg^{-1}. Available nutrients refer to the sum of water-soluble nutrients and exchangeable nutrients in soil. In each experimental year, pre-germinated seeds were sown in plastic trays for nursery raising on 15 July. Then, sixteen-day-old seedlings were mechanically transplanted into a paddy field at a hill spacing of 30 × 15 cm with five seedlings per hill. The fragrant rice was harvested on 10 November.

2.2. Experimental Details and Sampling

Three treatments were adopted in the present experiment, i.e., (CK) no fertilizer was applied; (IF) urea, superphosphate, and potassium chloride were applied at 234, 450, and 108 kg ha^{-1}, respectively, with 60% as basal dose and 40% at the tillering stage; (OICF) a new organic-inorganic compound fertilizer composed of 10% organic matter, 26% urea, 50% superphosphate, 12% potassium chloride, 1.9% zinc sulfate, and 0.1% lanthanum chloride, was applied at 900 kg ha^{-1} with 60% as basal dose and 40% at three days after transplanting. This new organic-inorganic compound fertilizer was made by mixing organic matter, urea, superphosphate, potassium chloride, zinc sulfate, and lanthanum chloride at corresponding proportion and then placed into pelletizer to make 3mm diameter granular fertilizer. The organic matter used to make this fertilizer was purchased from Foota Biotechnology Co., Ltd. (Dongguan City, Guangdong Province, China). The treatments were arranged in a randomized complete block design (RCBD) in triplicate with a net plot size of 9 $\times$ 4 m. Water management, pathogens, insects, and weeds in all plots were the same and intensively controlled using agrochemicals by following the guidelines and standards recommended by the province. Fresh grains from each plot were collected and stored at -80 °C for biochemical analysis at the grain-filling stage and harvest.

2.3. Determination of Chlorophyll Content, Net Photosynthetic Rate, and Aboveground Biomass

At 20, 40, 60, 80, and 100 days after transplanting, the net photosynthetic rate of fragrant rice was determined with the portable photosynthesis system (LI-6400, LI-COR, Lincoln, NE, USA), while leaf chlorophyll content was determined using SPAD meter 'SPAD-502' (Konica Minolta, Japan). After determining chlorophyll content and net photosynthetic rate, nine fragrant plants were randomly selected in each plot to record aboveground biomass after oven-dried at 80 °C till constant weight.

2.4. Measurement of Grain Yield and Yield-Related Traits

At harvest, the effective panicle number was counted from a1–m^2 area in each plot. Panicles from four fragrant rice plants in each plot were collected to determine grain number per panicle, seed-setting rate, and 1000-grain weight. Grain yield was measured from a4–m^2 area in each plot and adjusted to a moisture content of 14%.

2.5. Determination of Grain 2-AP Content at Harvest

The content of 2-AP in harvest grain of fragrant rice was determined with the methods by Xie et al. [23]. Briefly, Grain samples (10 g) were ground into powder and then extracted with dichloromethane. The 2-AP concentration was quantized using the simultaneous distillation-extraction method (SDE) and analyzed by a GCMS-QP 2010 Plus (Shimadzu Corporation, Kyoto, Japan). The grain 2-AP content was expressed as ng g^{-1}.

2.6. Determination of Contents of Proline, 1-Pyrroline, and γ-Aminobutyric Acid (GABA) in Grains at the Grain-Filling Stage

The proline content was determined using the methods by Mo et al. [24]. Briefly, proline in grains was extracted by sulfosalicylic acid and reacted with ninhydrin reagent. The absorbance was read at 520 nm. The 1-pyrroline was determined using the methods by Hill et al. [25]. Samples (0.5 mL) of the reaction mixtures were mixed with 0.01 M o-amino benzaldehyde, 0.2 M phosphate buffer, and water. The absorbance was read at 430 nm, and molar absorptivity was 1860 cm^{-1}. The GABA content was determined according to the methods of [16]. The absorbance was read at 645 nm, and the content was expressed as mg g^{-1}.

2.7. Determination of PDH and BADH in Grains at the Grain-Filling Stage

Enzymatic samples were extracted with 1.0 M K-phosphate buffer (pH 8.0). The activity of PDH was determined according to the methods by Tateishi et al. [26]. Briefly, the reaction mixture contained 15 mM proline, 0.5% Triton X-100, and 0.01 mM cytochrome c and enzyme extract. The absorbance was read at 510 nm, and the activity was expressed by the change of absorbance value per hour (U g^{-1} h^{-1}, U refers to the absorbance value of 1.0). The activity of BADH was determined using the methods of Holmstrom et al. [27]. The reaction mixture contained 1.0 M dithiothreitol, 0.10 M NAD, and enzyme extract. The absorbance was read at 340 nm, and activity was also expressed by the change of absorbance value per hour (U g^{-1} h^{-1}).

2.8. Statistical Analysis

All experimental data were subjected to an analysis of variance (ANOVA) using Statistix 8.0 (Analytical Software, Tallahassee, FL, USA). The statistical model of the ANOVA included replication (experimental plot), year, treatment, and the interaction between year and treatment; the significance level was set at $p < 0.05$. Graphical representation was represented using Sigma Plot 9.0 (Systat Software Inc., San Jose, CA, USA). Statistix 8.1 was also used to perform correlation analysis, and the heatmap for the investigated parameters was established with Microsoft Excel, according to the Mo et al. [10].

3. Results

3.1. Grain Yield and Yield-Related Traits

In general, OICF treatment significantly ($p < 0.05$) increased fragrant rice yield compared with both IF treatment and CK across four experimental years (Table 1). Analysis of variance showed that year had significant ($p < 0.05$) effects on grain yield, effective panicle number per m^2, grain number per panicle, seed setting rate, and 1000-grain weight. Treatment had significant ($p < 0.05$) effects on grain yield, effective panicle number per m^2, seed setting rate, and 1000-grain weight. The responses of grain number per panicle to treatment were not significant ($p < 0.05$). The highest grain yield was recorded in OICF treatment among all treatments in four experimental years. Compared with CK, IF and OICF treatments significantly increased grain yield by 23–56% and 46–67%, respectively. Higher effective panicle number per m^2 was recorded in IF and OICF treatments across four experimental years. In 2018, the effective panicle number per m^2 in OICF treatment was significantly ($p < 0.05$) higher than in IF treatment. The highest or equally highest seed setting rate was recorded in OICF treatment in four experimental years. Compared with CK, IF and OICF treatments significantly ($p < 0.05$) increased seed setting rate by 5–7% and 9–12%, respectively. There was no significant ($p < 0.05$) difference among three treatments in 1000-grain weight each experimental year.

3.2. Net Photosynthetic Rate

The net photosynthetic rates of fragrant rice at different growing periods are presented in Figure 1. The highest or equally highest net photosynthetic rate was recorded in OICF treatment at 20, 40, 60, and 80 days after transplanting across four experimental years. There was no significant ($p \leq 0.05$) difference in net photosynthetic rate among all treatments 100 days after transplanting. For IF treatment, 14–28%, 10–30%, 6–37%, and 8–16% higher net photosynthetic rates were observed compared with CK at 20, 40, 60, and 80 days after transplanting, respectively. For OICF treatment, 25–39%, 14–33%, 19–32%, and 10–30% higher net photosynthetic rates were observed compared with CK at 20, 40, 60, and 80 days after transplanting, respectively.

3.3. Chlorophyll Content

SPAD values of fragrant rice at different growing periods are presented in Figure 2. The highest or equally highest SPAD value was recorded in OICF treatment at 20, 40, 60, and 80 days after transplanting. There was no significant ($p \leq 0.05$) difference in SPAD value among all treatments 100 days after transplanting. For IF treatment, 2–29%, 13–27%, 12–23%, and 2–8% higher SPAD values were observed compared with CK at 20, 40, 60, and 80 days after transplanting, respectively. For OICF treatment, 11–38%, 18–30%, 8–24%, and 3–18% higher SPAD values were observed compared with CK at 20, 40, 60, and 80 days after transplanting, respectively.

Table 1. Effects of new organic-inorganic compound fertilizer on grain yield and yield-related traits of fragrant rice.

Year	Treatment	Grain Yield (t ha^{-1})	Effective Panicle Number per m^2	Grain Number per Panicle	Seed Setting Rate (%)	1000-Grain Weight (g)
2017						
	CK	5.67 ± 0.21 c	276.33 ± 17.04 b	143.73 ± 9.81 a	72.05 ± 0.74 c	20.17 ± 0.14 a
	IF	7.01 ± 0.48 b	356.00 ± 15.10 a	130.88 ± 16.74 a	77.50 ± 2.22 b	19.90 ± 0.36 a
	OICF	8.29 ± 0.20 a	385.00 ± 12.29 a	145.36 ± 6.68 a	81.32 ± 1.11 a	20.20 ± 0.27 a
2018						
	CK	3.92 ± 0.21 c	224.00 ± 3.46 c	124.04 ± 9.37 a	70.65 ± 2.07 b	20.52 ± 0.05 a
	IF	6.15 ± 0.06 b	316.67 ± 7.76 b	130.40 ± 1.41 a	74.27 ± 1.14 a	20.54 ± 0.24 a
	OICF	6.55 ± 0.09 a	355.00 ± 14.80 a	116.99 ± 14.80 a	78.14 ± 2.58 a	20.85 ± 0.35 a
2019						
	CK	4.49 ± 0.29 c	245.67 ± 4.73 b	127.81 ± 4.87 a	73.19 ± 0.29 b	19.88 ± 0.18 a
	IF	6.60 ± 0.44 b	343.00 ± 16.70 a	127.66 ± 7.86 a	77.40 ± 0.48 a	19.89 ± 0.52 a
	OICF	7.45 ± 0.28 a	362.33 ± 2.08 a	131.25 ± 7.73 a	79.87 ± 2.77 a	20.25 ± 0.11 a
2020						
	CK	3.89 ± 0.21 c	281.67 ± 12.50 b	121.76 ± 8.27 a	70.71 ± 1.51 c	19.94 ± 0.30 a
	IF	5.17 ± 0.23 b	372.00 ± 11.27 a	114.79 ± 3.80 a	75.24 ± 1.13 b	19.93 ± 0.38 a
	OICF	5.86 ± 0.38 a	366.67 ± 10.69 a	114.32 ± 7.39 a	79.16 ± 1.33 a	19.80 ± 0.58 a
Analysis of variance (*F* value)						
Year (Y)		75.04 **	28.70 **	8.82 *	5.97 *	9.15 *
Treatment (T)		239.36 **	268.66 **	0.49 ns	62.56 **	7.20 *
T * Y		3.26 *	2.53 ns	1.57 ns	0.39 ns	6.00 **

Seed setting rate is the ratio of the number of full filling grains to the total number of grains. Values sharing a common letter within a column do not differ significantly ($p \leq 0.05$) according to the least significant difference (LSD) test. ns denotes non-significance at the 0.05 probability level; * and ** denote significance at the 0.05 and 0.01 probability levels, respectively.

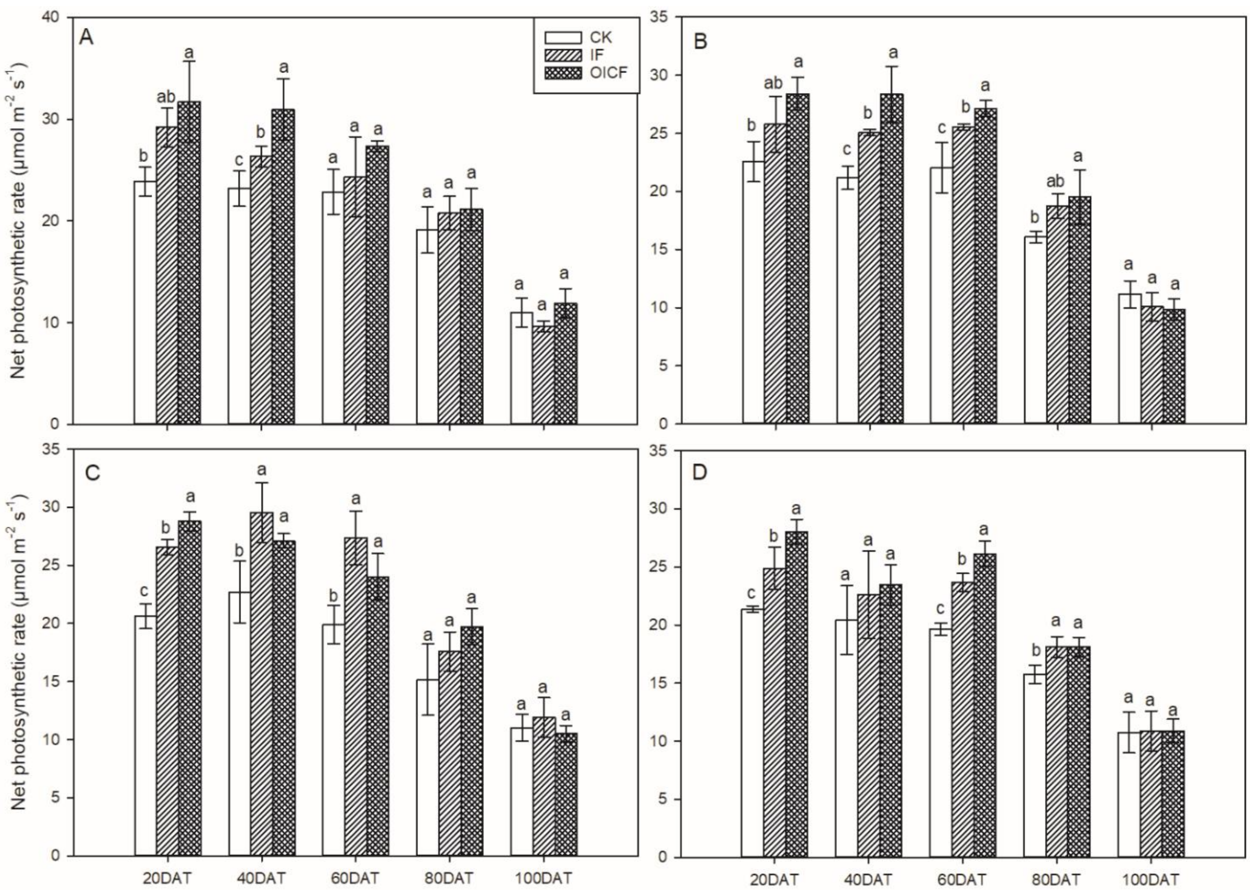

Figure 1. Effects of new organic-norganic compound fertilizer on the net photosynthetic rate of fragrant rice in 2017 (**A**), 2018 (**B**), 2019 (**C**), and 2020 (**D**). Bars sharing a common letter do not differ significantly at $p \leq 0.05$.

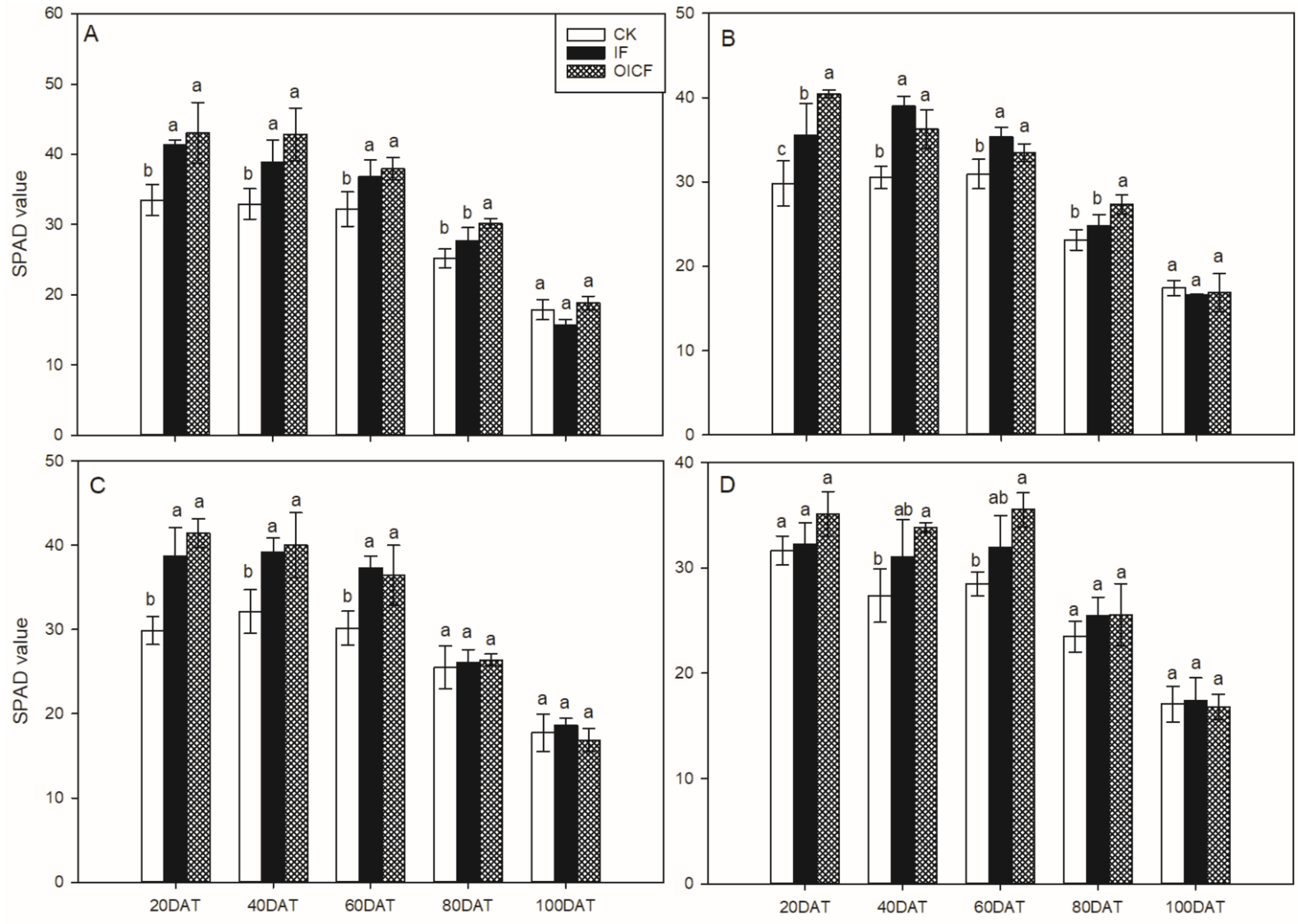

Figure 2. Effects of new organic-inorganic compound fertilizer on SPAD value of fragrant rice in 2017 (**A**), 2018 (**B**), 2019 (**C**), and 2020 (**D**). Bars sharing a common letter do not differ significantly at $p \leq 0.05$.

3.4. Aboveground Biomass

As shown in Figure 3, OOCF treatment substantially enhanced dry matter accumulation of fragrant rice plants. At 20 days after transplanting, compared with CK, IF treatment and OICF treatments significantly ($p < 0.05$) increased aboveground biomass by 19–44% and 34–60%, respectively. At 40 days after transplanting, 20–24% and 26–40% higher aboveground biomasses were recorded in IF treatment and OICF treatments than that in CK, respectively. At 60 days after transplanting, 19–45% and 40–61% higher aboveground biomasses were recorded in IF treatment and OICF treatments than that in CK, respectively. At 80 days after transplanting, compared with CK, IF treatment and OICF treatments significantly ($p < 0.05$) increased aboveground biomass by 21–35% and 37–51%, respectively. At 100 days after transplanting, compared with CK, IF treatment and OICF treatments significantly ($p < 0.05$) increased aboveground biomass by 25–42% and 39–56%, respectively.

3.5. Grain 2-AP Content at Harvest

OICF treatment remarkably increased grain 2-AP content of fragrant rice, and the highest grain 2-AP content was recorded in OICF treatment among all treatments in four experimental years (Figure 4). Compared with CK, OICF treatment significantly ($p < 0.05$) increased grain 2-AP content at harvest by 38, 37, 33 and 31% in 2017, 2018, 2019, and 2020, respectively. Compared with IF treatment, OICF treatment significantly ($p < 0.05$) increased grain 2-AP content at harvest by 17, 10, 22 and 14% in 2017, 2018, 2019, and 2020, respectively.

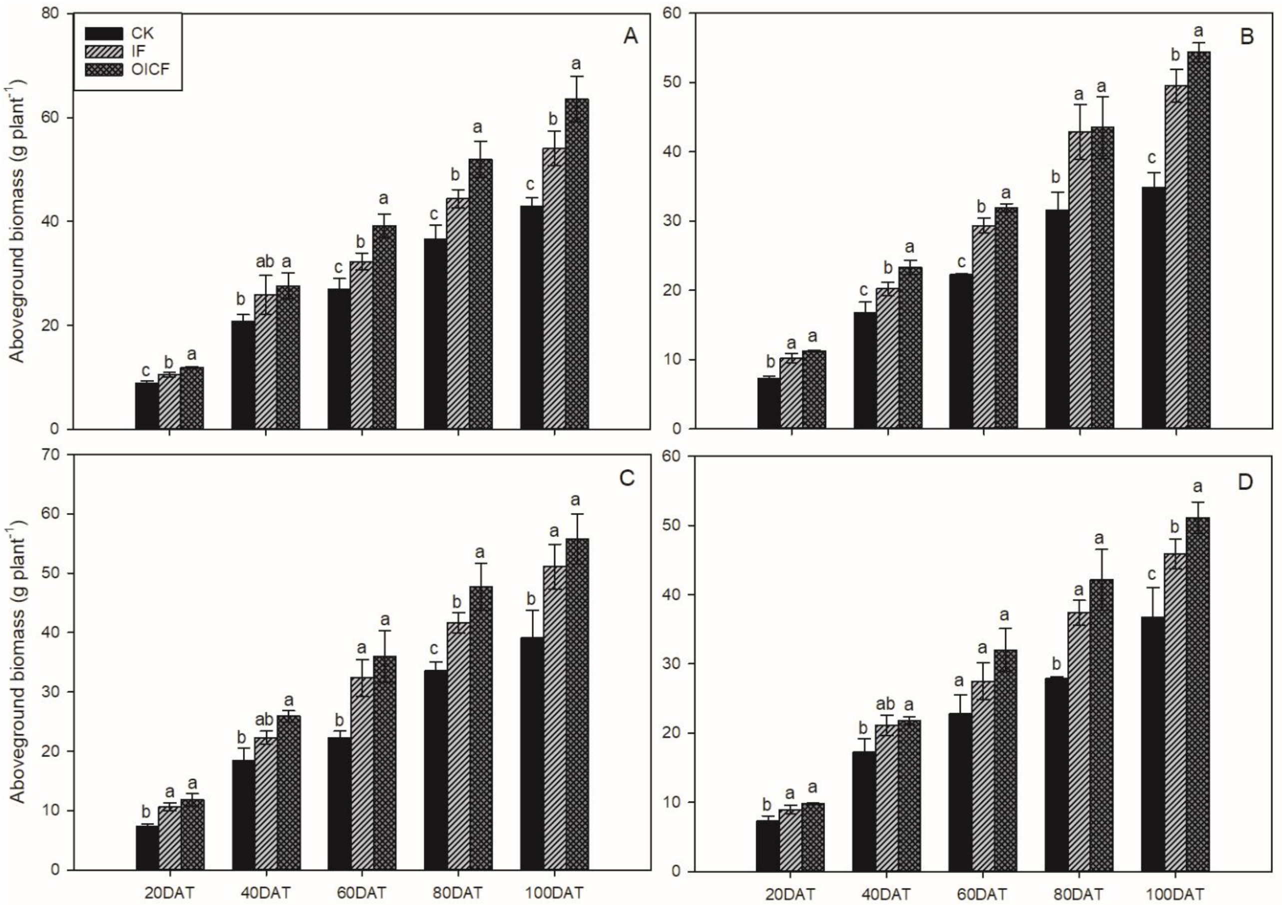

Figure 3. Effects of new organic-inorganic compound fertilizer on aboveground biomass of fragrant rice in 2017 (**A**), 2018 (**B**), 2019 (**C**), and 2020 (**D**). Bars sharing a common letter do not differ significantly at $p \leq 0.05$.

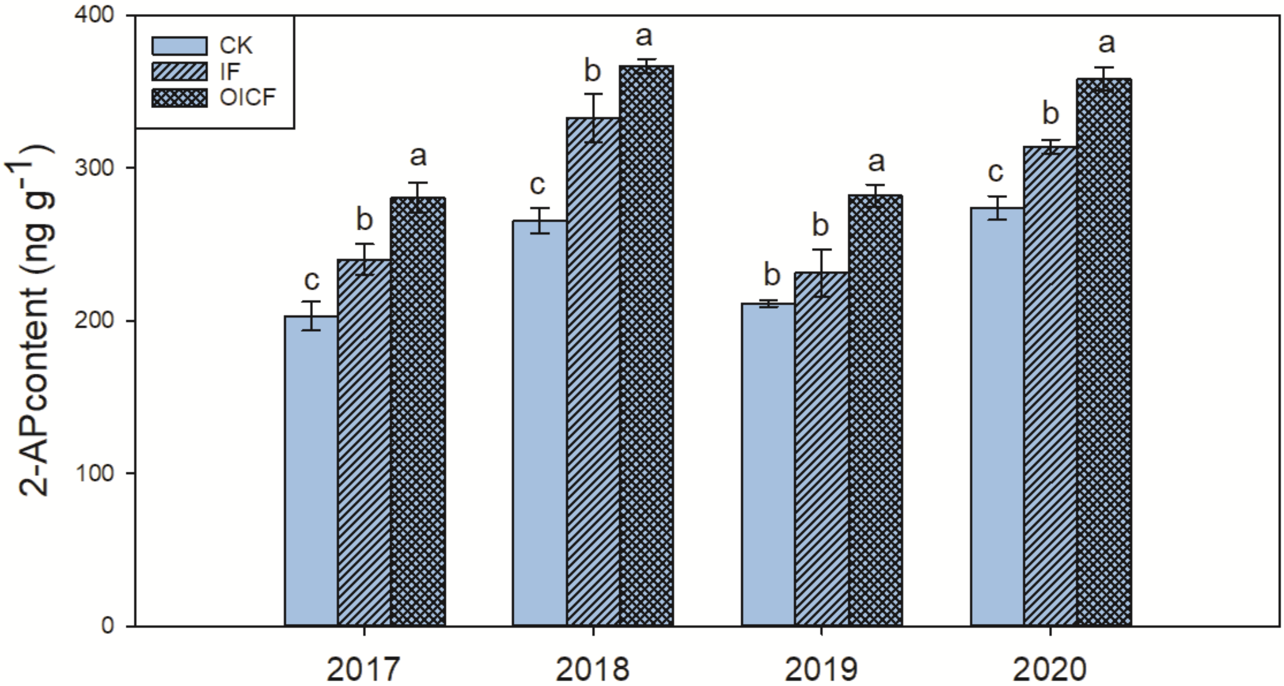

Figure 4. Effects of new organic-inorganic compound fertilizer on grain 2-AP content of fragrant rice. Bars sharing a common letter do not differ significantly at $p \leq 0.05$.

3.6. Contents of Proline, 1-Pyrroline, and GABA

As shown in Figure 5, different treatments affected the contents of proline, 1-pyrroline, and GABA in fragrant rice differently. The highest or equal highest grain proline content was recorded in OICF treatment in four experimental years for proline. Compared with CK, IF treatment and OICF treatment increased grain proline content by 6–24% and 13–31%, respectively. For 1-pyrroline, compared with CK, IF treatment and OICF treatment increased grain proline content by 6–18% and 10–37%, respectively. For GABA content, 5–10% and 9–17% lower grain GABA contents were recorded in IF treatment and OICF treatment than that in CK, respectively.

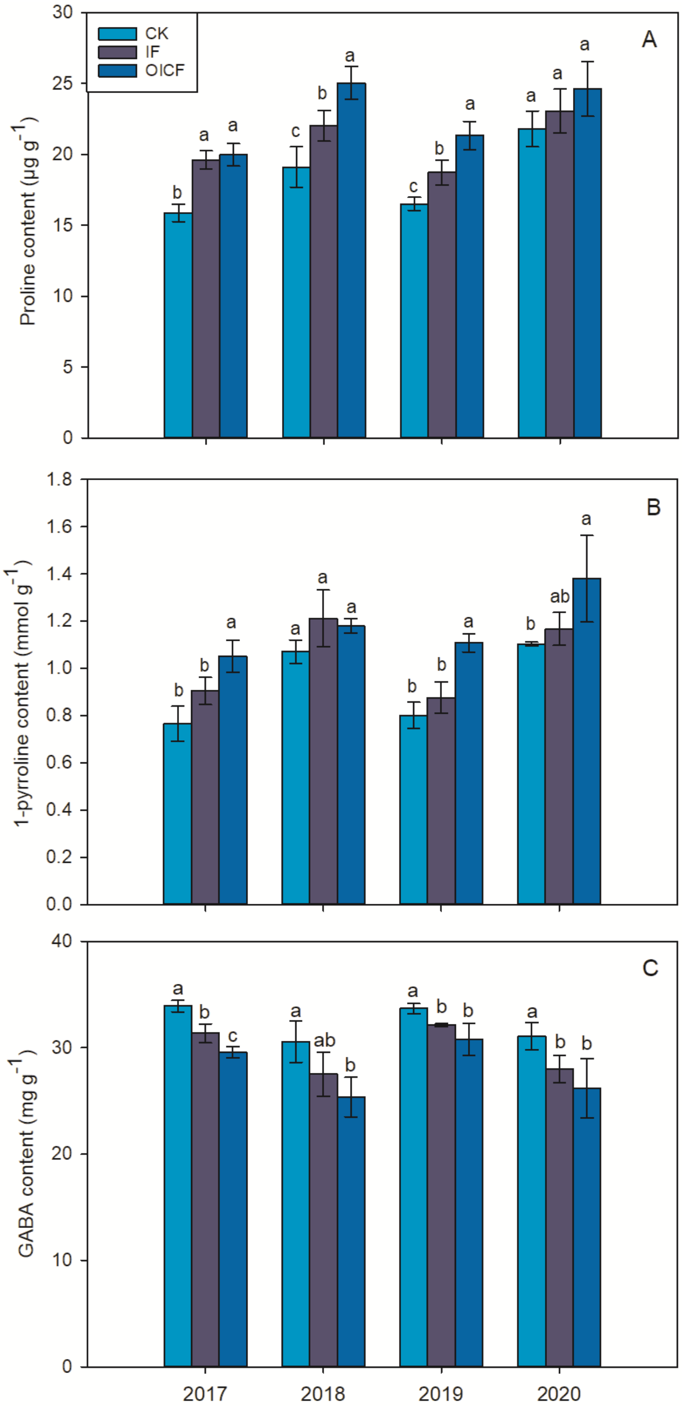

Figure 5. Effects of new organic-inorganic compound fertilizer on the content of proline (**A**), 1-pyrroline (**B**), GABA (**C**) in fragrant rice. Bars sharing a common letter do not differ significantly at $p \leq 0.05$.

3.7. Activities of PDH and BADH

Activities of PDH and BADH in grains at the grain filling stage are presented in Figure 6. In four experimental years, the highest or equal highest activity of PDH was recorded in OICF treatment and compared with CK, IF treatment, and OICF treatment enhanced PDH activity by 7–18% and 17–27%, respectively. In 2017, there was no significant difference between CK and IF treatment in BADH activity, while OICF treatment reduced BADH activity by 15%. From 2018–2020, 11–17% and 9–19% lower BADH activities were recorded in IF and OICF treatments than CK, respectively.

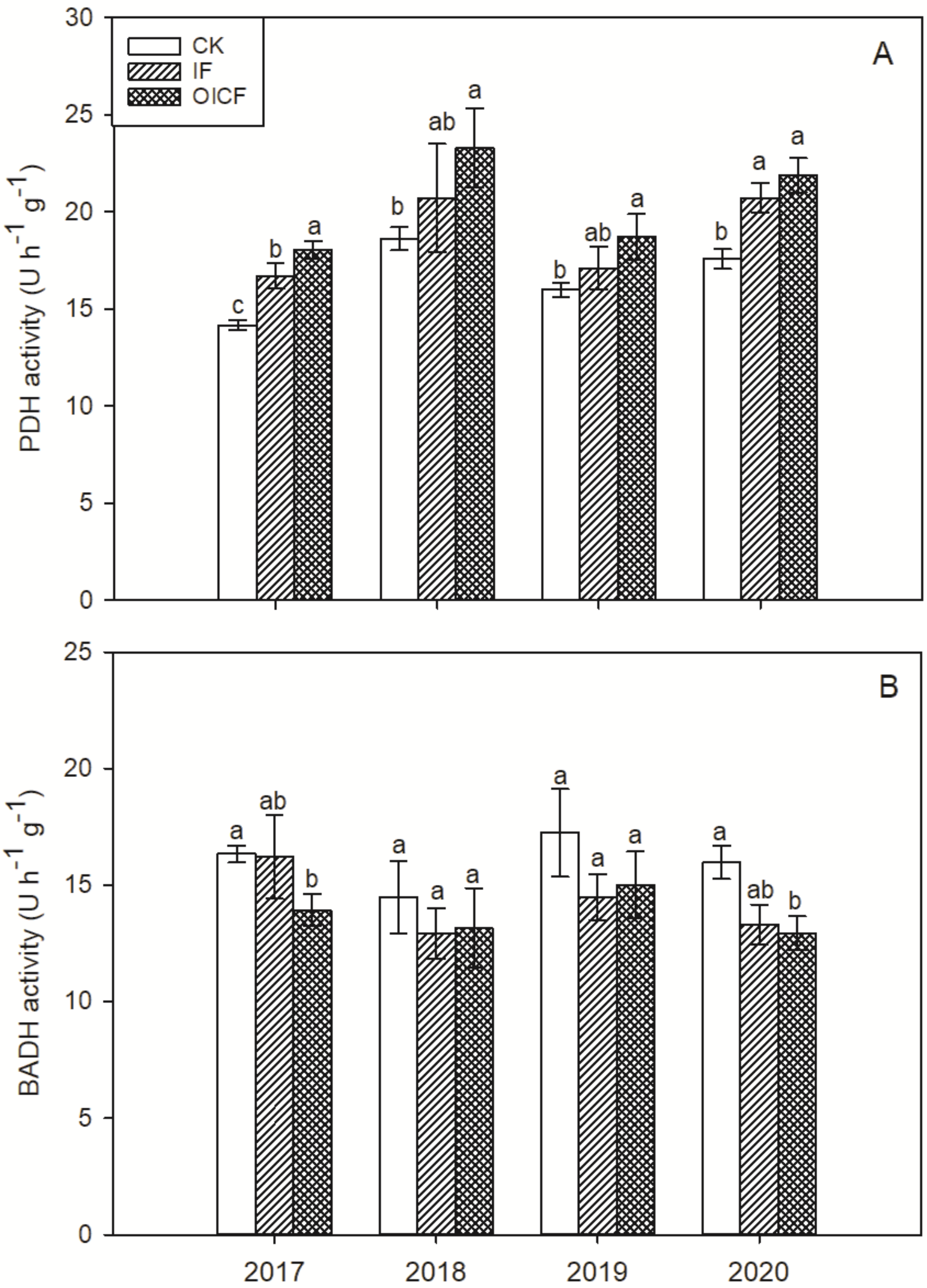

Figure 6. Effects of new organic-inorganic compound fertilizer on activities of PDH (**A**) and BADH (**B**) in fragrant rice. Bars sharing a common letter do not differ significantly at $p \leq 0.05$.

3.8. Correlation among Grain Yield, Yield-Related Traits, SPAD Value, and Net Photosynthetic Rate

The heat map was estimated to discover the relationship between grain yield further and investigated agronomic traits (Figure 7). It showed that the grain yield of fragrant rice was significantly and positively related to effective panicle number per m^2, grain number per panicle, and setting rate. Positive correlations were also observed among grain yield, SPAD values, and net photosynthetic rates at 20, 40, 60, 80 days after transplanting and aboveground biomass at all growing periods.

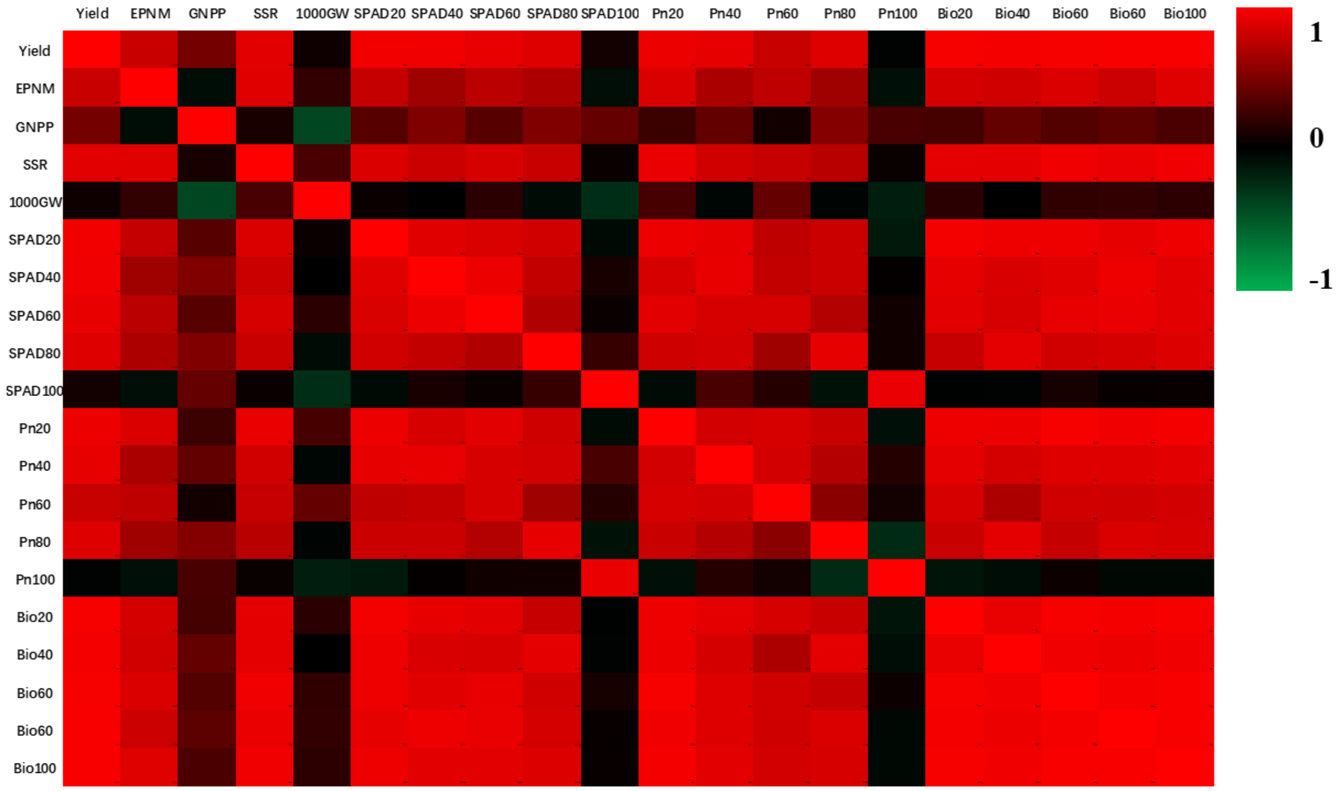

Figure 7. The heat map for grain yield, yield-related traits, SPAD value, and net photosynthetic rate. EPNM: Effectively panicle number per m^2; GNPP: Grain number per panicle; SSR: Seed setting rate; 1000GW: 1000-grain weight; SPAD20: SPAD value at 20 days after transplanting; SPAD40: SPAD value at 40 days after transplanting; SPAD60: SPAD value at 60 days after transplanting; SPAD80: SPAD value at 80 days after transplanting; SPAD100: SPAD value at 100 days after transplanting; Pn20: Net photosynthetic rate at 20 days after transplanting; Pn40: Net photosynthetic rate at 40 days after transplanting; Pn60: Net photosynthetic rate at 60 days after transplanting; Pn80: Net photosynthetic rate at 80 days after transplanting; Pn100: Net photosynthetic rate at 100 days after transplanting; Bio20: Aboveground biomass at 20 days after transplanting; Bio40: Aboveground biomass at 40 days after transplanting; Bio60: Aboveground biomass at 60 days after transplanting; Bio80: Aboveground biomass at 80 days after transplanting; Bio100: Aboveground biomass at 100 days after transplanting.

3.9. Correlation among 2-AP, Proline, 1-Pyrroline, GABA, PDH, and BADH

The relationships among 2-AP content, proline content, 1-pyrroline content, GABA content, PDH activity, and BADH activity are present in Figure 8. It revealed the significant and positive correlations between 2-AP content and both proline and 1-pyrroline contents and the negative correlation between 2-AP content and GABA content. There exited a significant and positive correlation between 2-AP content and PDH activity. There was also a significant and negative correlation between 2-AP content and GABA activity.

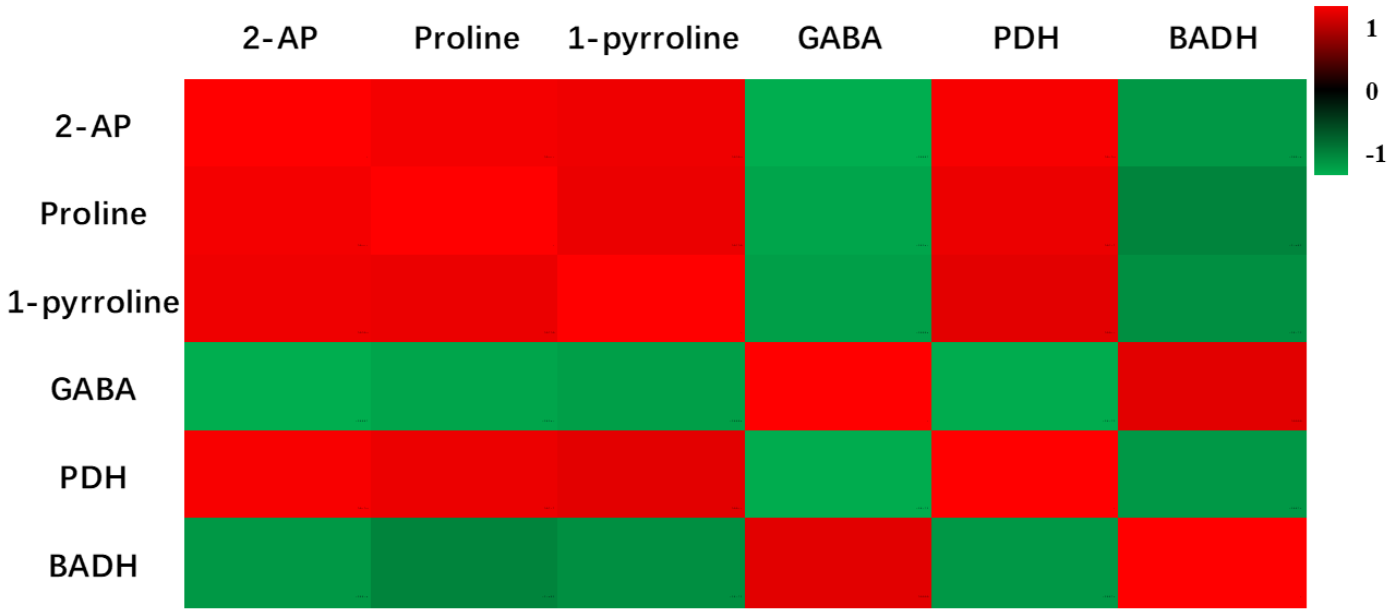

Figure 8. The heat map for 2-AP content, proline content, 1-pyrroline content, GABA content, PDH activity, and BADH activity.

4. Discussion

With the growing demand for fragrant rice in the international market, developing new techniques and products is important to increase fragrant rice yield and aroma. The present study provided a new organic-inorganic compound fertilizer to fit the fragrant rice production. The OICF treatment exerted the optimal performances in increasing grain 2-AP content and grain yield of fragrant rice across four experimental years. The improvement in grain yield was attributed to the high effective panicle number and seed setting rate. In our study, a higher effective panicle number per m^2 and seed setting rate was recorded in OICF treatment than both IF treatment and CK in four experimental years (except effective panicle number per m^2 in 2020), while analysis of variance showed that grain yield was significantly and positively correlated to both effective panicle number per m^2 and seed setting rate. This new organic-inorganic compound fertilizer contains extra zinc, lanthanum, and organic matter compared with conventional chemical fertilizer. Our previous study has revealed the benefits of lanthanum on growth, 2-AP biosynthesis, and yield formation of fragrant rice [22]. The research by Saha et al. [28] showed the basal application of zinc at 4.5–5.0 kg ha^{-1} substantially improved rice yield. The results of our study also were consistent with the study by Farooq et al. [29], who demonstrated that the productivity of fragrant rice grown in both dry seeded and flooded transplanted cultivation systems could be enhanced by the application of zinc. In addition, the purpose of adding organic matter into the new organic-inorganic compound fertilizer was to make it more eco-friendly. However, the effects of organic matter on growth and 2-AP production in fragrant rice have not yet been reported. Thus, it isn't easy to discuss the roles of organic matter in fragrant rice performances in the present study.

The enhancement in yield formation of fragrant rice due to application of the new organic-inorganic compound fertilizer was explained by improvement in chlorophyll content, net photosynthetic rate, and biomass accumulation. Previous studies indicated that rice grain yield is composed of translocation of accumulated biomass before heading stage to panicles and biomass production during grain-filling stage [1,30]. In our study, OICF treatment increased SPAD value and improved the net photosynthetic rate at 20, 40, 60, and 80 days after transplanting compared with CK and IF treatment. However, the differences were not always significant. There was no remarkable difference among all treatments in both SPAD value and the net photosynthetic rate 100 days after transplanting. This period was near maturity, and the leaves of fragrant rice began to age. The biomass accumulation of rice plants depended on the intensity of photosynthesis [31,32]. Our results show that the enhancement in photosynthesis due to IOCF treatment led to the higher biomass at each growing stage. The increment in dry matter accumulation before the heading stage means more matter could be translocated to panicles [33]. The present study suggested that this new organic-inorganic compound fertilizer provided significant positive effects on photosynthesis which led to enhanced dry matter accumulation and consequently resulted in improvement in yield formation of fragrant rice.

2-AP is the key compound of fragrant rice aroma and the characteristic substance that distinguishes fragrant rice from other rice [7,34]. Compared with the conventional chemical fertilizer and control, the new organic-inorganic compound fertilizer provided a notable increment in grain 2-AP content across four experimental years. OICF treatment substantially increased the content of 1-pyrroline, the limiting precursors in 2-AP biosynthesis [6]. The increased 2-AP content was also attributed to the increased proline content and enhanced PDH activity, which was consistent with the studies by Mo et al. [10] and Yoshihashi et al. [8]. Huang et al. [35] demonstrated that PDH catalyzes the decomposition of proline and biosynthesis of Δ1-pyrroline-5-carboxylic acid to form the 2-AP. The research by Li et al. [36] showed significant and positive correlations among 2-AP content, proline content, and PDH activity. In addition, the present study found that 2-AP was negatively related to both GABA content and BADH activity, which agreed with the study by Bao et al. [16]. Wongpanya et al. [37] demonstrated BADH is involved in the production of 2-AP in fragrant rice, and the insertion of Y420 to gene BADH2 might lead to 2-AP accumulation by lowering BADH activity. The increased grain 2-AP content highlights the benefits of applying this new organic-inorganic compound fertilizer in fragrant rice production.

In addition, although this new organic-inorganic compound fertilizer contains metallic elements including zinc and lanthanum, the contents of two elements in paddy field soil did not substantially increase, while the contents of two elements in grains did not exceed the maximum levels of contaminants in foods according to National food safety standard, GB 2762-2017 (the data not shown). Therefore, there was no health safety problem on applying this fertilizer. According to our results, the recommended application of the new fertilizer was at 900 kg ha^{-1} with 60% as basal dose and 40% at three days after transplanting for fragrant rice production. This fertilizer has been commercial in China and is available to rice producers.

5. Conclusions

This study provided a new organic-inorganic compound fertilizer and identified its feasibility in fragrant rice production. Namely, compared with control and conventional chemical fertilizer, applying this new organic-inorganic compound fertilizer improved photosynthesis, dry matter accumulation and consequently promoted yield formation of fragrant rice. Applying this new fertilizer also regulated the precursors' accumulation and enzymes activities to increase the grain 2-AP content of fragrant rice significantly. Up-regulation of proline, 1-pyrroline, PDH, and down-regulation of GABA and BADH were observed in OICF treatment.

Author Contributions: Conceptualization, X.T. and H.L.; methodology, H.L. and M.D.; validation, X.T.; investigation, H.L., M.D. and L.H.; writing—original draft preparation, H.L.; writing—review and editing, H.L.; supervision, X.T. and M.D.; project administration, X.T., S.Y. and Y.Z.; funding acquisition, X.T. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by National Natural Science Foundation of China (31971843), The Technology System of Modern Agricultural Industry in Guangdong (2020KJ105) and Guangzhou Science and Technology Project (202103000075).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Huang, M.; Tao, Z.; Lei, T.; Cao, F.; Chen, J.; Yin, X.; Zou, Y.; Liang, T. Improving lodging resistance while maintaining high grain yield by promoting pre-heading growth in rice. *Field Crops Res.* **2021**, *270*, 108212. [CrossRef]
2. Li, M.; Ashraf, U.; Tian, H.; Mo, Z.; Pan, S.; Anjum, S.A.; Duan, M.; Tang, X. Manganese-induced regulations in growth, yield formation, quality characters, rice aroma and enzyme involved in 2-acetyl-1-pyrroline biosynthesis in fragrant rice. *Plant Physiol. Biochem.* **2016**, *103*, 167–175. [CrossRef] [PubMed]
3. Giraud, G. The world market of fragrant rice, main issues and perspectives. *Int. Food Agribus. Man.* **2013**, *16*, 1–20.
4. Hashemi, F.S.G.; Rafii, M.; Ismail, M.R.; Mahmud, T.M.M.; Rahim, H.A.; Asfaliza, R.; Malek, M.A.; Latif, M.A. Biochemical, Genetic and Molecular Advances of Fragrance Characteristics in Rice. *Crit. Rev. Plant Sci.* **2013**, *32*, 445–457. [CrossRef]
5. Luo, H.; He, L.; Du, B.; Pan, S.; Mo, Z.; Duan, M.; Tian, H.; Tang, X. Biofortification with chelating selenium in fragrant rice: Effects on photosynthetic rates, aroma, grain quality and yield formation. *Field Crops Res.* **2020**, *255*, 107909. [CrossRef]
6. Poonlaphdecha, J.; Gantet, P.; Maraval, I.; Sauvage, F.; Menut, C.; Morère, A.; Boulanger, R.; Wüst, M.; Gunata, Z. Biosynthesis of 2-acetyl-1-pyrroline in rice calli cultures: Demonstration of 1-pyrroline as a limiting substrate. *Food Chem.* **2016**, *197*, 965–971. [CrossRef]
7. Wakte, K.; Zanan, R.; Hinge, V.; Khandagale, K.; Nadaf, A.; Henry, R. Thirty-three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (*Oryza sativa* L.): A status review. *J. Sci. Food Agric.* **2016**, *97*, 384–395. [CrossRef]
8. Yoshihashi, T.; Huong, N.; Inatomi, H. Precursors of 2-acetyl-1-pyrroline, a potent flavor compound of an aromatic rice variety. *J. Agric. Food Chem.* **2002**, *50*, 2001–2004. [CrossRef]
9. Chen, S.; Yang, Y.; Shi, W.; Ji, Q.; He, F.; Zhang, Z.; Cheng, Z.; Liu, X.; Xu, M. Badh2, Encoding Betaine Aldehyde Dehydrogenase, Inhibits the Biosynthesis of 2-Acetyl-1-Pyrroline, a Major Component in Rice Fragrance. *Plant Cell* **2008**, *20*, 1850–1861. [CrossRef]
10. Mo, Z.; Li, Y.; Nie, J.; He, L.; Pan, S.; Duan, M.; Tian, H.; Xiao, L.; Zhong, K.; Tang, X. Nitrogen application and different water regimes at booting stage improved yield and 2-acetyl-1-pyrroline (2AP) formation in fragrant rice. *Rice* **2019**, *12*, 1–16.
11. Liu, X.; Huang, Z.; Li, Y.; Xie, W.; Li, W.; Tang, X.; Ashraf, U.; Kong, L.; Wu, L.; Wang, S.; et al. Selenium-silicon (Se-Si) induced modulations in physio-biochemical responses, grain yield, quality, aroma formation and lodging in fragrant rice. *Ecotox. Environ. Saf.* **2020**, *196*, 110525. [CrossRef]
12. Okpala, N.E.; Mo, Z.; Duan, M.; Tang, X. The genetics and biosynthesis of 2-acetyl-1-pyrroline in fragrant rice. *Plant Physiol. Biochem.* **2019**, *135*, 272–276. [CrossRef]
13. Imran, M.; Hussain, S.; Rana, M.S.; Saleem, M.H.; Rasul, F.; Ali, K.H.; Potcho, M.P.; Pan, S.; Duan, M.; Tang, X. Molybdenum improves 2-acetyl-1-pyrroline, grain quality traits and yield attributes in fragrant rice through efficient nitrogen assimilation under cadmium toxicity. *Ecotox. Environ. Saf.* **2021**, *211*, 111911. [CrossRef] [PubMed]
14. Xie, W.; Kong, L.; Ma, L.; Ashraf, U.; Pan, S.; Duan, M.; Tian, H.; Wu, L.; Tang, X.; Mo, Z. Enhancement of 2-acetyl-1-pyrroline (2AP) concentration, total yield, and quality in fragrant rice through exogenous γ-aminobutyric acid (GABA) application. *J. Cereal Sci.* **2020**, *91*, 102900. [CrossRef]
15. Mo, Z.; Tang, Y.; Ashraf, U.; Pan, S.; Duan, M.; Tian, H.; Wang, S.; Tang, X. Regulations in 2-acetyl-1-pyrroline contents in fragrant rice are associated with water-nitrogen dynamics and plant nutrient contents. *J. Cereal Sci.* **2019**, *88*, 96–102. [CrossRef]
16. Bao, G.; Ashraf, U.; Wang, C.; He, L.; Wei, X.; Zheng, A.; Mo, Z.; Tang, X. Molecular basis for increased 2-acetyl-1-pyrroline contents under alternate wetting and drying (AWD) conditions in fragrant rice. *Plant Physiol. Biochem.* **2018**, *133*, 149–157. [CrossRef] [PubMed]
17. Ren, Y. Irrigation and Nitrogen Management Practices Affect Grain Yield and 2-Acetyl-1-Pyrroline Content in Aromatic Rice. *Appl. Ecol. Environ. Res.* **2017**, *15*, 1447–1460. [CrossRef]
18. Li, Y.; Mo, Z.; Li, Y.; Nie, J.; Kong, L.; Ashraf, U.; Pan, S.; Duan, M.; Tian, H.; Tang, X. Additional Nitrogen Application Under Different Water Regimes at Tillering Stage Enhanced Rice Yield and 2-Acetyl-1-Pyrroline (2AP) Content in Fragrant Rice. *J. Plant Growth Regul.* **2021**, 1–11. [CrossRef]
19. Chen, J.; Cao, F.; Xiong, H.; Huang, M.; Zou, Y.; Xiong, Y. Effects of single basal application of coated compound fertilizer on yield and nitrogen use efficiency in double-cropped rice. *Crop J.* **2017**, *5*, 265–270. [CrossRef]
20. Mi, W.; Zheng, S.; Yang, X.; Wu, L.; Liu, Y.; Chen, J. Comparison of yield and nitrogen use efficiency of different types of nitrogen fertilizers for different rice cropping systems under subtropical monsoon climate in China. *Eur. J. Agron.* **2017**, *90*, 78–86. [CrossRef]
21. Luo, H.; Du, B.; He, L.; He, J.; Hu, L.; Pan, S.; Tang, X. Exogenous application of zinc (Zn) at the heading stage regulates 2-acetyl-1-pyrroline (2-AP) biosynthesis in different fragrant rice genotypes. *Sci. Rep.* **2019**, *9*, 1–10. [CrossRef] [PubMed]
22. Luo, H.; Chen, Y.; He, L.; Tang, X. Lanthanum (La) improves growth, yield formation and 2-acetyl-1-pyrroline biosynthesis in aromatic rice (*Oryza sativa* L.). *BMC Plant Biol.* **2021**, *21*, 1–13. [CrossRef]
23. Xie, W.; Ashraf, U.; Zhong, D.; Lin, R.; Xian, P.; Zhao, T.; Feng, H.; Wang, S.; Duan, M.; Tang, X.; et al. Application of γ-aminobutyric acid (GABA) and nitrogen regulates aroma biochemistry in fragrant rice. *Food Sci. Nutr.* **2019**, *7*, 3784–3796. [CrossRef]
24. Mo, Z.; Li, W.; Pan, S.; Fitzgerald, T.L.; Xiao, F.; Tang, Y.; Wang, Y.; Duan, M.; Tian, H.; Tang, X. Shading during the grain filling period increases 2-acetyl-1-pyrroline content in fragrant rice. *Rice* **2015**, *8*, 1–10. [CrossRef] [PubMed]
25. Hill, J.M. The inactivation of pea-seedling diamine oxidase by peroxidase and 1,5-diaminopentane. *Biochem. J.* **1967**, *104*, 1048–1055. [CrossRef]

26. Tateishi, Y.; Nakagawa, T.; Esaka, M. Osmotolerance and growth stimulation of transgenic tobacco cells accumulating free proline by silencing proline dehydrogenase expression with double-stranded RNA interference technique. *Physiol. Plant.* **2005**, *125*, 224–234. [CrossRef]
27. Holmstrom, K.; Welin, B.; Mandal, A.; Kristiansdottir, I.; Teeri, T.H.; Lamark, T.; Strom, A.R.; Palva, E.T. Production of the *Escherichia coli* betaine-aldehyde dehydrogenase, an enzyme required for the synthesis of the osmoprotectant glycine betaine, in transgenic plants. *Plant J.* **1994**, *6*, 749–758. [CrossRef] [PubMed]
28. Saha, S.; Chakraborty, M.; Padhan, D.; Saha, B.; Murmu, S.; Batabyal, K.; Seth, A.; Hazra, G.C.; Mandal, B.; Bell, R.W. Agronomic biofortification of zinc in rice: Influence of cultivars and zinc application methods on grain yield and zinc bioavailability. *Field Crops Res.* **2017**, *210*, 52–60. [CrossRef]
29. Farooq, M.; Ullah, A.; Rehman, A.; Nawaz, A.; Nadeem, A.; Wakeel, A.; Nadeem, F.; Siddique, K. Application of zinc improves the productivity and biofortification of fine grain aromatic rice grown in dry seeded and puddled transplanted production systems. *Field Crops Res.* **2018**, *216*, 53–62. [CrossRef]
30. Yang, W.; Peng, S.; Laza, R.C.; Visperas, R.M.; Dionisio-Sese, M.L. Yield Gap Analysis between Dry and Wet Season Rice Crop Grown under High-Yielding Management Conditions. *Agron. J.* **2008**, *100*, 1390–1395. [CrossRef]
31. Xu, G.; Jiang, M.; Lu, D.; Wang, H.; Chen, M. Nitrogen forms affect the root characteristic, photosynthesis, grain yield, and nitrogen use efficiency of rice under different irrigation regimes. *Crop Sci.* **2020**, *60*, 2594–2610. [CrossRef]
32. Peng, S.B.; Biswas, J.C.; Ladha, J.K.; Gyaneshwar, P.; Chen, Y.Z. Influence of rhizobial inoculation on photosynthesis and grain yield of rice. *Agron. J.* **2002**, *94*, 925–929. [CrossRef]
33. Liu, K.; Yang, R.; Lu, J.; Wang, X.; Lu, B.; Tian, X.; Zhang, Y. Radiation Use Efficiency and Source-Sink Changes of Super Hybrid Rice under Shade Stress during Grain-Filling Stage. *Agron. J.* **2019**, *111*, 1788–1798. [CrossRef]
34. Wei, X.; Sun, Q.; Methven, L.; Elmore, J.S. Comparison of the sensory properties of fragrant and non-fragrant rice (*Oryza sativa*), focusing on the role of the popcorn-like aroma compound 2-acetyl-1-pyrroline. *Food Chem.* **2021**, *339*, 128077. [CrossRef]
35. Huang, T.; Huang, Y.; Hung, H.; Ho, C.; Wu, M. Δ1-Pyrroline-5-carboxylic Acid Formed by Proline Dehydrogenase from the *Bacillus subtilis* ssp. Natto Expressed inEscherichia coli as a Precursor for 2-Acetyl-1-pyrroline. *J. Agric. Food Chem.* **2007**, *55*, 5097–5102. [CrossRef] [PubMed]
36. Li, M.; Li, R.; Liu, S.; Zhang, J.; Luo, H.; Qiu, S. Rice-duck co-culture benefits grain 2-acetyl-1-pyrroline accumulation and quality and yield enhancement of fragrant rice. *Crop J.* **2019**, *7*, 419–430. [CrossRef]
37. Wongpanya, R.; Boonyalai, N.; Thammachuchourat, N.; Horata, N.; Arikit, S.; Myint, K.M.; Vanavichit, A.; Choowongkomon, K. Biochemical and Enzymatic Study of Rice BADH Wild-Type and Mutants: An Insight into Fragrance in Rice. *Protein J.* **2011**, *30*, 529–538. [CrossRef]