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Impact of Rapeseed Sequential Follow Paddy Crop on Its 2-Acetyl-1-pyrroline Biosynthesis and Economic Yield under the Double-Cropping System

Wentao Yi [1,2,3,†], Haowen Luo [1,2,3,†], Mingliang Zhang [4,†], Zhigui Sun [4,†], Qichang Gu [1,2,3], Sicheng Deng [1,2,3], Yizhu Wu [1,2,3], Yugang Yan [5], Zisheng Chen [4], Jianying Qi [1,2,3], Dongfeng Liu [4,*] and Xiangru Tang [1,2,3,*]

1 State Key Laboratory for Conservation and Utilization of Subtropical Agricultural Bioresources, College of Agriculture, South China Agricultural University, Guangzhou 510642, China; ywt1999@stu.scau.edu.cn (W.Y.); luohaowen@stu.scau.edu.cn (H.L.); guqichang@stu.scau.edu.cn (Q.G.); dengsc@stu.scau.edu.cn (S.D.); awuyizhu@163.com (Y.W.); qi@scau.edu.cn (J.Q.)
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 Shenzhen Agricultural Science and Technology Promotion Center, Nanshan, Shenzhen 518000, China; tcjwzml@163.com (M.Z.); sunzhi123456@foxmail.com (Z.S.); sznczx@mail.amr.sz.gov.cn (Z.C.)
5 College of Agriculture, South China Agricultural University, Guangzhou 510642, China; yanyugang@stu.scau.edu.cn
* Correspondence: ldf@frontier-ag.com (D.L.); tangxr@scau.edu.cn (X.T.)
† These authors contributed equally to this work.

Abstract: Rapeseed–rice rotation is a cropping system that improves the land-use rate. The present study conducted a field experiment with winter planting of rapeseed as treatment (WR) and winter fallow as control (CK) to investigate the effects of winter planting of rapeseed on growth, yield formation, and 2-acetyl-1-pyrroline (2-AP) biosynthesis of fragrant rice in the two subsequent cropping seasons. The results show that WR treatment improved alkali-hydro nitrogen and rapidly available phosphorus contents in soil. Compared with CK, WR treatment significantly increased grain yield and effective panicle number per plant by 21.16–27.26% and 7.33–21.24%, respectively. Higher net photosynthetic rate, leaf area index, and dry matter accumulation of fragrant rice plants were recorded in WR treatment and CK. Furthermore, compared with CK, WR treatment significantly increased grain 2-AP content, which could be explained by increased content of pyrroline-5-carboxylic acid, methylglyoxal, 1-pyrroline, and enhanced activity of proline dehydrogenase. In addition, future studies should be conducted at a molecular level to reveal the regulation mechanism in 2-AP biosynthesis of fragrant rice under conditions of winter planting of rapeseed.

Keywords: fragrant rice; rapeseed–rice rotation; 2-acetyl-1-pyrroline; photosynthesis; yield formation

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1. Introduction

In the early 1980s, the aroma of fragrant rice was first analyzed and defined as "Similar to the smell of popcorn"; it has been reported that 2-acetyl-1-pyrroline (2-AP) is a key volatile in fragrant rice aroma [1]. Fragrant rice, as a high-grade rice variety, is very popular and has a high market share [2].

As an important compound in fragrant rice aroma, 2-AP has a very complicated biosynthesis process, which includes precursors like proline, ornithine, and glutamic acid, pyrroline-5-carboxylic acid (P5C), and enzymes likes proline dehydrogenase (ProDH), pyrroline-5-carboxylic acid synthetase (P5CS) and ornithine aminotransferase (OAT), which eventually form 2-AP [3,4]. Furthermore, various environmental factors and cultivation management measures all affect the 2-AP synthesis. A previous study shows that foliar application of glutamic acid and phenylalanine can promote the yield, quality, and 2-AP content of rice [5]. In a study, the effects of different temperature conditions at the grain-filling stage on the content of 2-AP in fragrant rice were revealed [6]. A previous study demonstrated the effect of the interaction between different irrigation methods and planting density on the aroma of fragrant rice [7]. In addition, a previous study found that the 2-AP content of fragrant rice can be significantly improved by shading treatment at the grain-filling stage [8].

Crop rotation could substantially affect rice yield and quality. The rice yield under previous winter crop rotation was significantly higher than that of fallow–rice rotation [9]. Crop rotation also improves soil fertility, which is conducive to the growth and development of crops [10]. Rapeseed (*Brassica campestris* L.)–rice rotation is a common cropping system in China. Previous studies have shown that rapeseed–rice rotation can significantly increase rice yield compared with wheat rotation in traditional agriculture [11,12]. The reason for this phenomenon may be that after rapeseed is returned to the field, it promotes the recycling of soil nutrients, reduces nutrient loss in the field, and better maintains the soil aggregate structure and reduces crop diseases and pests [13,14]. Therefore, under this rotation system, compared with leaving fields fallow, planting rapeseed in the winter fallow period seems to have potential advantages for post-crop crops, and effectively regulates the growth cycle of field crops through various ways, so as to improve crop yield. However, the application of rapeseed–rice rotation in fragrant rice production and its effects on 2-AP biosynthesis have not been reported.

Considering all these findings, the current study was evaluated with three cropping seasons (a winter season planting with rapeseed and two sequential cropping seasons planting with fragrant rice) to determine the fragrant rice in fallow winter planting of rapeseed, focusing on economic yield and quality traits.

2. Materials and Methods

2.1. Plant Materials and Experimental Details

A field experiment with two cropping seasons was conducted at Teaching and Research Base, South China Agricultural University, Zengcheng District, Guangzhou, (23°24′ N, 113°64′ S, 43.40 m from sea level) China, from November 2022 to November 2023 (November 2022 to March 2023 for rapeseed, March 2023 to July 2023 for early cropping season of fragrant rice, and July 2023 to November 2023 for late cropping season of fragrant rice). The experimental soil was sandy loam with 0.89 g/kg total nitrogen content, 47.71 mg/kg available nitrogen content, 24.03 mg/kg rapidly available phosphorus content, 56.67 mg/kg rapidly available potassium content, 8.97 g/kg organic matter content, and 5.08 pH. Winter planting of rapeseed was taken as treatment (WR) and winter fallow was taken as control (CK). For the planting of rapeseed, a rapeseed cultivar, Qingza-7, which was provided by Qinghai Hufeng Agricultural Science and Technology Group Co., Ltd., Haidong city, China, was sown in November 2022 and harvested in March 2023. Fertilizer dose of N-P_2O_5-K_2O, 15%–4%–6%, was applied at 750 kg/ha. In 2023, two fragrant rice cultivars, 19xiang and Nanjingxiangzhan, provided by Guangdong Xianmei Seed Co., Ltd., were used as the plant materials. For fragrant rice planting, in the early cropping seasons, the seeds (19xiang and Nanjingxiangzhan) were sown on 10 March for nursery raising. Then, the seedlings were transplanted on 29 March. The harvest was carried out on 5 July. In the late cropping seasons, the seeds (19xiang and Nanjingxiangzhan) were sown on 13 July for nursery raising. Then, the seedlings were transplanted on 29 July. The harvest was carried out on 3 November. The treatments were arranged in a split block design with winter planting treatment as the main factor and cultivar as the secondary factor in triplicate with a net plot size of 108.39 m^2. A fertilizer dose of N-P_2O_5-K_2O, 15%–4%–6%, was applied at 900 kg/ha in each plot.

2.2. Determination of Soil Chemical Properties

At the harvest of rapeseed, soil at 0–20 cm depth was collected and air-dried in each plot. Before the test, soil samples were treated with a 4 M HCl solution to eliminate the influence of inorganic carbon on the experimental data, and then the total nitrogen content and organic carbon of the soil were analyzed with an elemental analyzer (Elementar Vario Macro Cube, Germany). In addition, 10.0 g of soil was mixed with 25 mL of ultrapure water and the pH of the soil was measured with a pH meter (TitroLine 5000, SI Analytics, Mainz, Germany). According to the above method, the content of alkali-hydrolyzable nitrogen, available phosphorus, and available potassium contained in the screened soil samples can be determined [15,16]. Total nitrogen and organic carbon were expressed in g/kg; alkali hydrolyzable nitrogen, available phosphorus, and available potassium were all expressed in mg/kg.

2.3. Determination of Grain Yield and Yield-Related Traits

At harvest, the rice grains were harvested from three sampling areas (1.00 m^2) in each plot to measure the grain yield. Ten representative plants in each plot were investigated to calculate and record the effective panicle number per hill. Three representative hills from each plot were sampled to measure grain number per panicle, seed setting rate, and 1000-grain weight [5].

2.4. Measurement of Grain Quality Parameters

During this study, the grain quality traits viz. brown rice rate, milled rice rate, head rice rate, chalky rice rate, and chalkiness degree were estimated [17].

2.5. Measurement of Net Photosynthetic Rate, Leaf Area Index, and Dry Matter Accumulation

At the tillering, booting, full heading, and maturity stages of fragrant rice plants, the net photosynthetic rate was measured with LI-6800XT portable photosynthesis system (Licor, USA). The area for all green leaves was measured with a Li-Cor area meter (Li-Cor Model 3100, Lincoln, NE), and leaf area index was then calculated. Meanwhile, the aboveground parts of five representative plants in each plot were collected and oven-dried at 80 °C until constant weight to measure the dry matter accumulation [7].

2.6. Determination of Grain 2-AP Content

The current study evaluated the 2-AP content from harvested samples [18]. At the harvest of both cropping seasons, the fragrant rice grains were collected. After grounding to powder with liquid nitrogen, the samples were extracted by dichloromethane. Then, the supernatant was transferred to GCMS-QP 2010 Plus (Shimadzu Corporation, Japan) for the 2-AP quantization.

2.7. Determination of Contents of P5C, MG, 1-Pyrroline, and Activity of ProDH

The current study estimated the P5C in µmol/g [19], MG in µmol/g [20,21], 1-pyrroline in mmol/g [22], and ProDH in mol/min/g [23,24].

2.8. Statistical Analysis

Experimental data in the present study were analyzed using statistical software 'Statistix' (Version 8.0, Analytical Software, Tallahassee, FL, USA) using the one-way analysis of variance. The differences among means were separated using the LSD test at the 5% probability level.

3. Results

3.1. Effects of Winter Planting of Rapeseed on Soil Chemical Properties

Winter planting of rapeseed substantially impacted some soil chemical indexes (Figure 1). Compared with CK, WR treatment significantly increased the content of alkali-hydrolyzable nitrogen and available potassium by 19.19% and 32.06%, respectively. Lower content of available phosphorus content was recorded in WR than CK. There was no significant difference between WR treatment and CK in the content of total nitrogen and organic carbon.

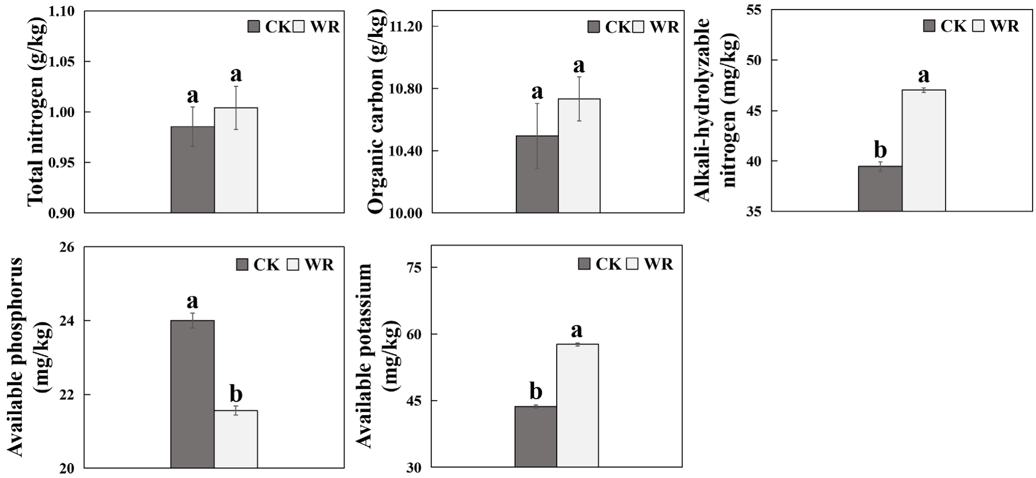

Figure 1. Effects of winter planting of rapeseed on soil chemical properties. Bars sharing a common letter do not differ significantly at ($p \leq 0.05$) in each cropping season according to the LSD test. The same as below.

3.2. Effects of Winter Planting of Rapeseed on Net Photosynthetic Rate

Winter planting of rapeseed substantially impacted the net photosynthetic rate of fragrant rice (Figure 2). In the early cropping season, for 19xiang, compared with CK, WR treatment significantly increased the net photosynthetic rate at the full heading stage and maturity stage by 6.04% and 47.02%, respectively (Figure 2A). For Nanjingxiangzhan, a higher net photosynthetic rate was recorded in WR treatment than CK in the booting stage and maturity stage (Figure 2C). In the late cropping season, for 19xiang, compared with CK, WR treatment significantly increased the net photosynthetic rate at the tillering stage, booting stage, full heading stage, and maturity stage by 16.67%, 16.24%, 11.48%, and 10.63%, respectively (Figure 2B). For Nanjingxiangzhan, a higher net photosynthetic rate was recorded in WR treatment than CK in the booting stage, full heading stage, and maturity stage (Figure 2D).

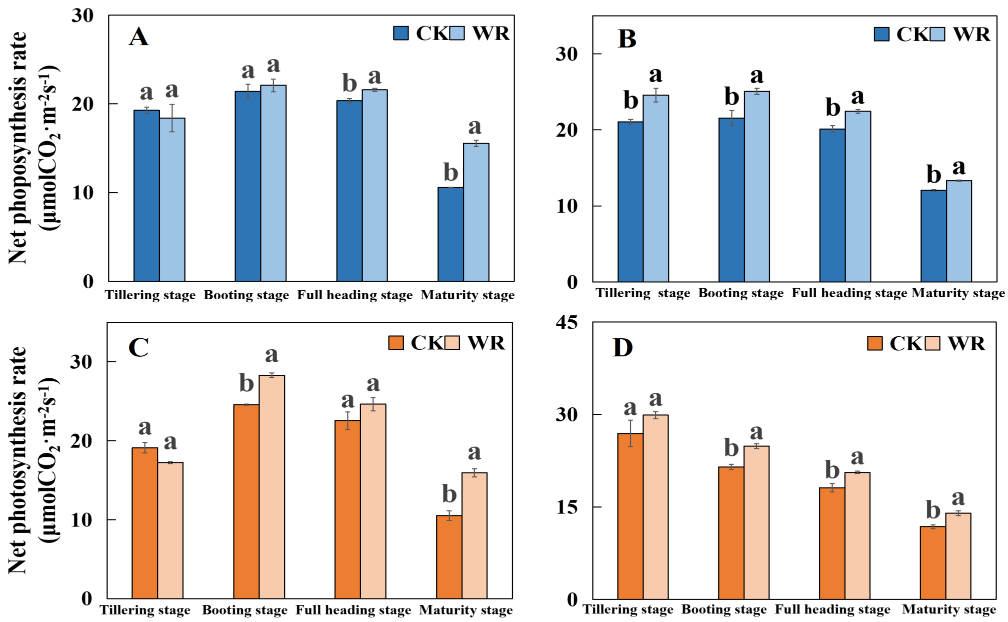

Figure 2. Effects of winter planting of rapeseed on the net photosynthetic rate of fragrant rice. (**A**) For 19xiang in the early cropping season. (**B**) For 19xiang in the late cropping season. (**C**) For Nanjingxiangzhan in the early cropping season. (**D**) For Nanjingxiangzhan in the late cropping season.

3.3. Effects of Winter Planting of Rapeseed on Chlorophyll Content

Winter planting of rapeseed substantially impacted the chlorophyll content of fragrant rice (Figure 3). In the early cropping season, for 19xiang, compared with CK, WR significantly increased the chlorophyll content at the booting stage, full heading stage, and maturity stage by 17.21%, 4.19%, and 27.96%, respectively (Figure 3A). For Nanjingxiangzhan, the chlorophyll value of WR treatment was significantly higher than CK treatment at booting stage, full heading stage, and maturity stage (Figure 3C). In the late cropping season, for 19xiang, compared with CK, WR treatment significantly increased the chlorophyll content from tillering stage to maturity stage by 5.67%, 9.28%, 11.62%, and 7.56%, respectively (Figure 3B). For Nanjingxiangzhan, a higher chlorophyll value was recorded in WR treatment than CK in the booting stage, full heading stage, and maturity stage (Figure 3D).

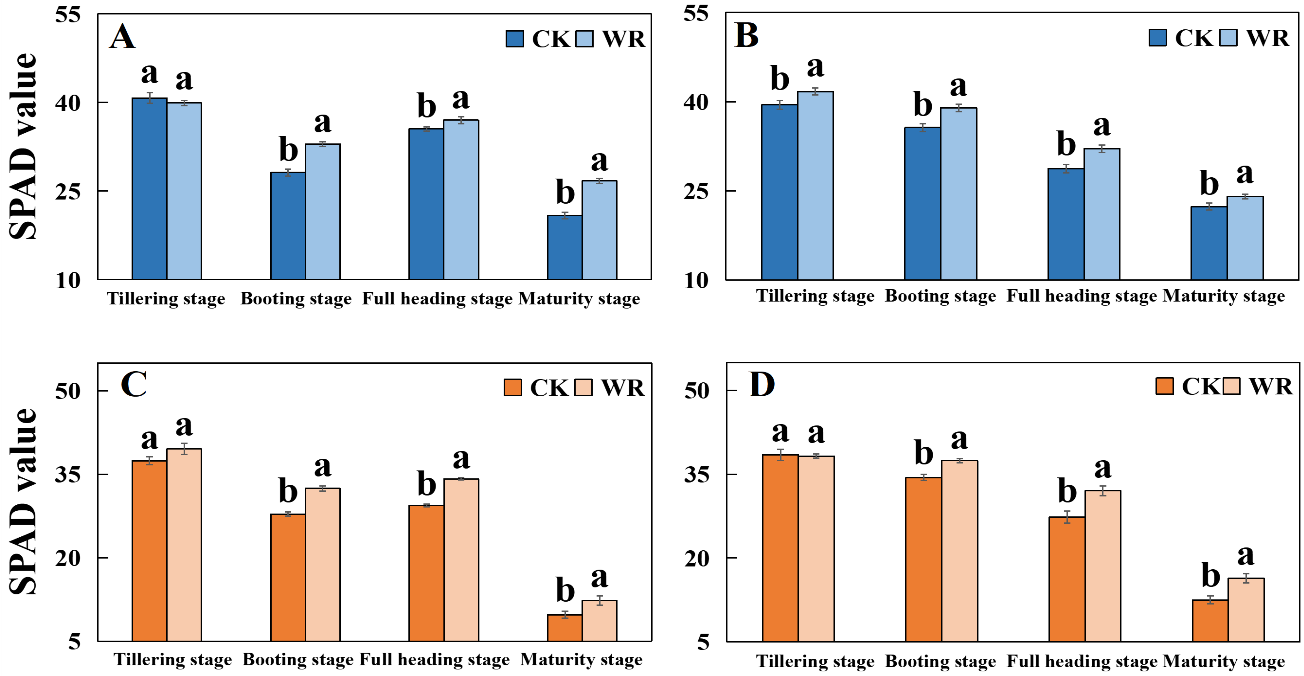

Figure 3. Effects of winter planting of rapeseed on the chlorophyll content of fragrant rice. (**A**) For 19xiang in the early cropping season. (**B**) For 19xiang in the late cropping season. (**C**) For Nanjingxiangzhan in the early cropping season. (**D**) For Nanjingxiangzhan in the late cropping season.

3.4. Effects of Winter Planting of Rapeseed on LAI

Winter planting of rapeseed substantially impacted the LAI of fragrant rice (Figure 4). In the early cropping season, for 19xiang, compared with CK, WR treatment significantly increased the LAI at the full heading stage and maturity stage by 115.33% and 46.52%, respectively (Figure 4A). For Nanjingxiangzhan, a higher LAI was recorded in WR treatment than CK in the full heading stage and maturity stage (Figure 4C). In the late cropping season, for 19xiang, compared with CK, WR treatment significantly increased the LAI at the booting stage, full heading stage, and maturity stage by 42.72%, 61.00%, and 32.29%, respectively (Figure 4B). For Nanjingxiangzhan, LAI was significantly higher in WR treatment than in CK at the booting stage, full heading stage, and maturity stage (Figure 4D).

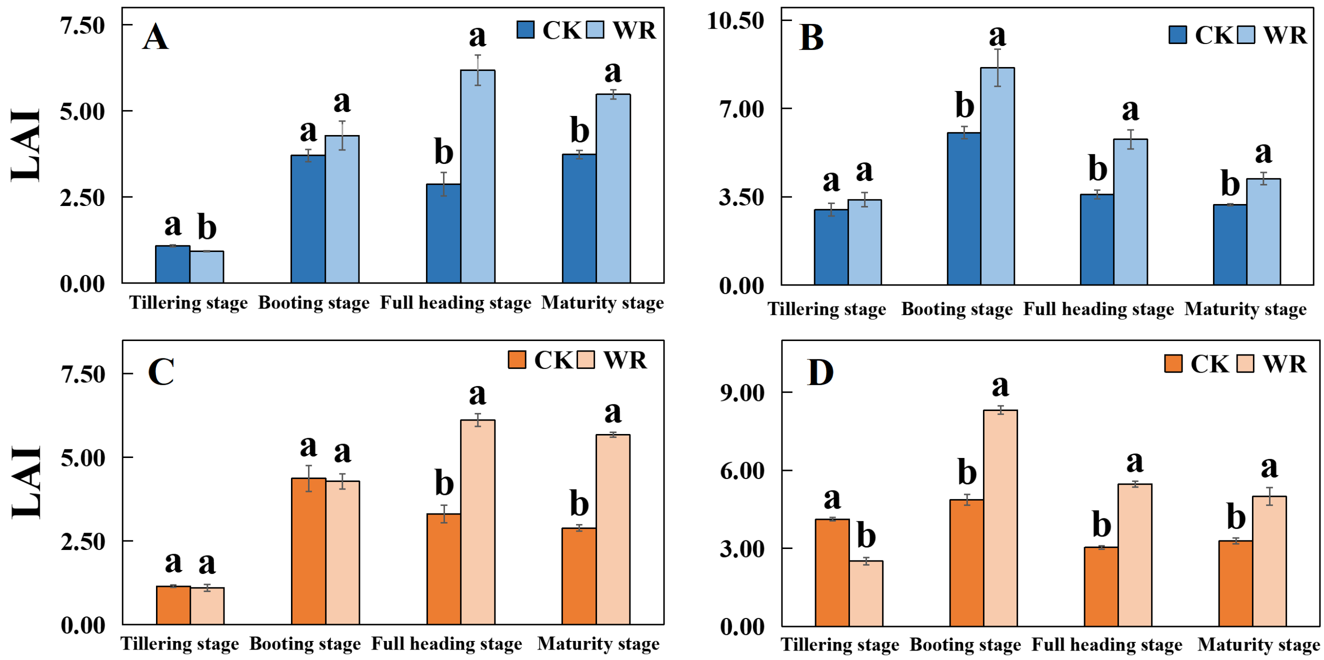

Figure 4. Effects of winter planting of rapeseed on the LAI of fragrant rice. (**A**) For 19xiang in the early cropping season. (**B**) For 19xiang in the late cropping season. (**C**) For Nanjingxiangzhan in the early cropping season. (**D**) For Nanjingxiangzhan in the late cropping season.

3.5. Effects of Winter Planting of Rapeseed on Dry Matter Accumulation

Winter planting of rapeseed substantially affected the dry matter accumulation of fragrant rice (Figure 5). WR treatment significantly increased the dry matter accumulation of 19xiang at the maturity stage by 18.05%, but had no significant effect on the Nanjingxiangzhan (Figure 5A). In the late cropping season, compared with CK, WR treatment significantly increased the dry matter accumulation by 30.61% and 50.19% for 19xiang and Nanjingxiangzhan, respectively (Figure 5B).

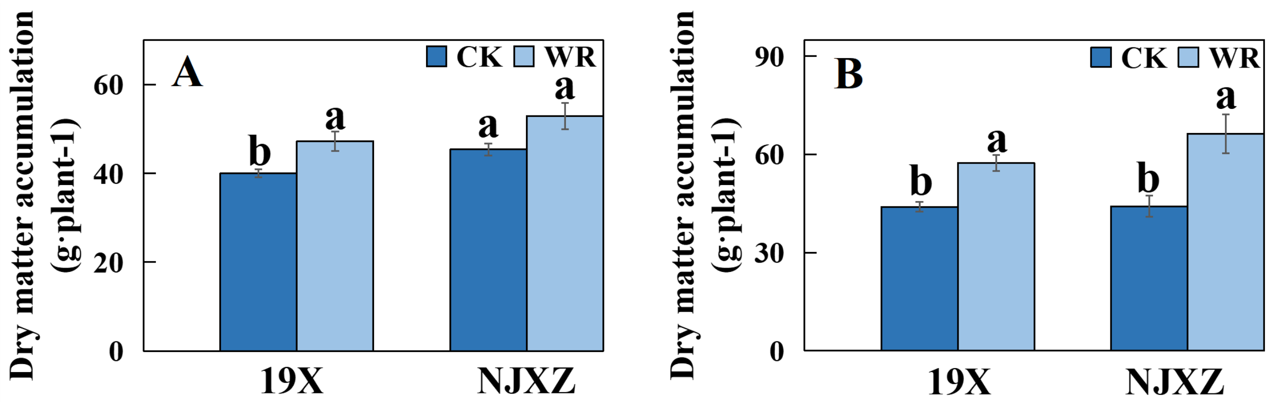

Figure 5. Effects of winter planting of rapeseed on the dry matter accumulation of fragrant rice; 19X for 19xiang, NJXZ for Nanjingxiangzhan. (**A**) For the early cropping season. (**B**) For the late cropping season.

3.6. Effects of Winter Planting of Rapeseed on Grain Yield and Yield-Related Traits

Winter planting of rapeseed substantially affected the yield formation of fragrant rice (Table 1). In the early cropping season, for 19xiang, compared with CK, WR treatment significantly increased the effective panicle number and grain yield by 27.26% and 21.24%, respectively. For Nanjingxiangzhan, compared with CK, WR treatment significantly increased the effective panicle number and grain yield by 24.21% and 16.60%, respectively.

In the late cropping season, for 19xiang, compared with CK, WR treatment significantly increased the effective panicle number and grain yield by 23.46% and 17.51%, respectively. For Nanjingxiangzhan, compared with CK, WR treatment significantly increased the effective panicle number and grain yield by 21.16% and 7.34%, respectively.

Table 1. Effects of winter planting of rapeseed on grain yield and yield-related traits of fragrant rice.

Cropping Season	Cultivar	Treatment	Effective Panicle Number per Plant	Grain Number per Panicle	Seed-Setting Rate (%)	1000-Grain Weight (g)	Yield (t/ha)
Early cropping season							
	19xiang						
		CK	9.39 ± 0.06 b	61.63 ± 0.45 b	143.42 ± 10.20 a	20.86 ± 0.54 a	3.86 ± 0.03 b
		WR	11.95 ± 0.19 a	65.00 ± 0.97 a	133.81 ± 0.32 a	19.55 ± 0.52 a	4.68 ± 0.14 a
	Nanjingxiangzhan						
		CK	10.08 ± 0.30 b	85.60 ± 0.96 a	125.92 ± 1.90 a	22.58 ± 0.76 a	5.18 ± 0.05 b
		WR	12.52 ± 0.04 a	85.68 ± 1.44 a	121.59 ± 8.09 a	21.92 ± 0.14 a	6.04 ± 0.18 a
Late cropping season							
	19xiang						
		CK	11.17 ± 0.66 b	61.89 ± 1.44 a	142.71 ± 7.13 a	20.58 ± 0.51 a	3.54 ± 0.04 b
		WR	13.79 ± 0.39 a	53.21 ± 0.76 b	144.31 ± 15.00 a	19.94 ± 0.08 a	4.16 ± 0.17 a
	Nanjingxiangzhan						
		CK	9.64 ± 0.19 b	83.00 ± 0.57 a	118.74 ± 6.77 a	22.76 ± 0.67 a	4.77 ± 0.11 b
		WR	11.68 ± 0.36 a	83.70 ± 1.97 a	123.17 ± 6.41 a	22.36 ± 0.12 a	5.12 ± 0.04 a

Note: Values sharing a common letter within a column do not differ significantly at ($p \leq 0.05$) in each cropping season according to the LSD test. WR means the winter planting of rapeseed and CK means winter fallow. The same as below.

3.7. *Effects of Winter Planting of Rapeseed on Grain Quality Parameters*

Winter planting of rapeseed impacted some grain quality parameters of fragrant rice (Table 2). Compared with CK, WR treatment significantly decreased the brown rice rate for both cultivars in both cropping seasons. A higher milled rice rate was recorded in WR treatment than CK for 19xiang in both cropping seasons. Compared with CK, WR treatment significantly decreased the head rice rate for Nanjingxiangzhan in both cropping seasons. There was no significant difference between WR treatment and CK in chalky rice rate and chalkiness degree for both cultivars in both cropping seasons.

Table 2. Effects of winter planting of rapeseed on grain quality parameters of fragrant rice.

Cropping Season	Cultivar	Treatment	Brown Rice Rate (%)	Milled Rice Rate (%)	Head Rice Rate (%)	Chalky Rice Rate (%)	Chalkiness Degree (%)
	Early cropping season						
	19xiang						
		CK	75.16 ± 0.85 a	61.38 ± 0.28 b	42.95 ± 0.35 a	0.58 ± 0.19 a	2.33 ± 0.33 a
		WR	71.18 ± 0.18 b	63.89 ± 0.52 a	39.23 ± 0.35 b	0.64 ± 0.34 a	2.33 ± 0.67 a
	Nanjingxiangzhan						
		CK					
		WR	77.31 ± 0.38 a	66.53 ± 0.30 a	42.66 ± 0.10 a	0.41 ± 0.35 a	3.33 ± 1.45 a
	Late cropping season		75.75 ± 0.13 b	67.05 ± 0.03 a	42.97 ± 0.58 a	0.41 ± 0.14 a	3.00 ± 0.58 a
	19xiang						
		CK	74.32 ± 0.22 a	61.60 ± 0.38 b	39.69 ± 0.53 b	0.62 ± 0.2 a	3.33 ± 0.67 a
		WR	72.10 ± 0.26 b	64.03 ± 0.49 a	44.39 ± 0.15 a	0.77 ± 0.31 a	4.00 ± 1.00 a
	Nanjingxiangzhan						
		CK	75.41 ± 0.23 a	66.29 ± 0.19 a	50.10 ± 0.41 a	0.72 ± 0.33 a	2.00 ± 0.58 a
		WR	74.03 ± 0.23 b	65.59 ± 0.21 a	50.28 ± 0.16 a	0.5 ± 0.17 a	3.33 ± 0.33 a

Note: WR means the winter planting of rapeseed and CK means winter fallow.

3.8. *Effects of Winter Planting of Rapeseed on Grain 2-AP Content*

Winter planting of rapeseed substantially affected grain 2-AP content of fragrant rice (Figure 6). In the early cropping season, WR treatment significantly increased grain 2-AP content by 18.49% and 32.23% for 19xiang and Nanjingxiangzhan, respectively (Figure 6A). In the late cropping season, for 19xiang and Nanjingxiangzhan, compared with CK, WR treatment significantly increased grain 2-AP content by 20.41% and 11.51% for 19xiang and Nanjingxiangzhan (Figure 6B).

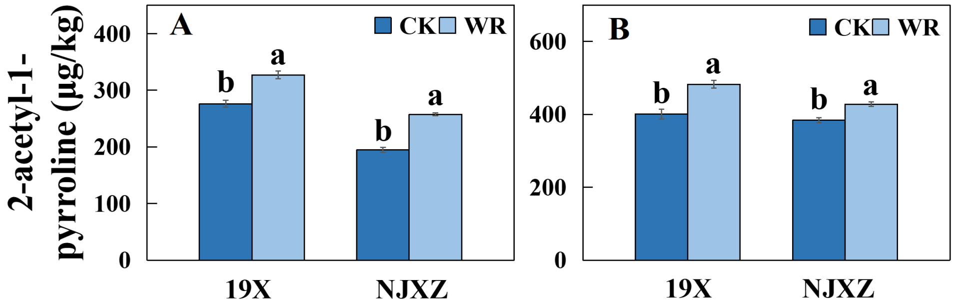

Figure 6. Effects of winter planting of rapeseed on the grain 2-AP content of fragrant rice; 19X for 19xiang, NJXZ for Nanjingxiangzhan. (**A**) For the early cropping season. (**B**) For the late cropping season.

3.9. Effects of Winter Planting of Rapeseed on 2-AP Biosynthesis

Winter planting of rapeseed substantially affected 2-AP biosynthesis in fragrant rice (Figure 7). MG content showed a more upward trend in WR treatment than in CK for both cultivars and in both cropping seasons. In the early season, WR treatment significantly increased the MG content by 235.29% and 73.05% for 19xiang and Nanjingxiangzhan, respectively. In the late cropping season, compared with CK, WR significantly increased the MG content by 88.34% and 85.07% for 19xiang and Nanjingxiangzhan, respectively.

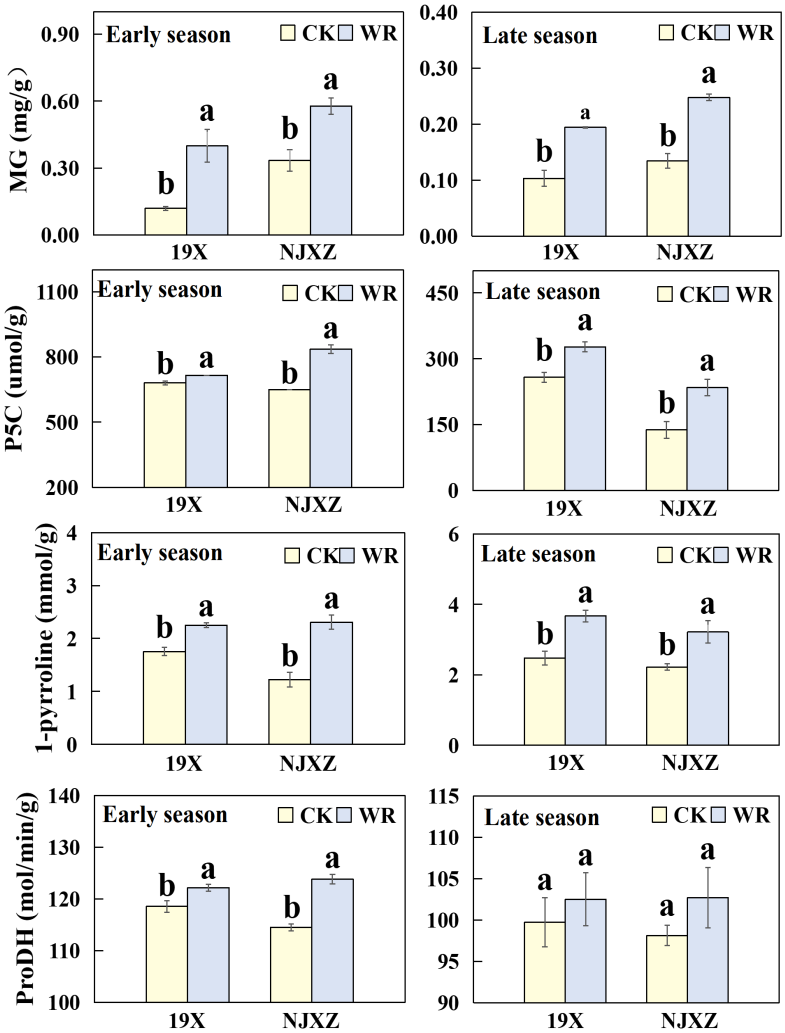

Figure 7. Effects of winter planting of rapeseed on the content of MG, P5C, 1-pyrroline, and activity of ProDH of fragrant rice.

For P5C content, in the early cropping season, WR treatment significantly increased the P5C content by 5.18% and 28.75% for 19xiang and Nanjingxiangzhan, respectively. In the late cropping season, compared with CK, WR treatment significantly increased the P5C content by 27.01% and 69.89% for 19xiang and Nanjingxiangzhan, respectively.

For 19xiang, WR treatment increased 1-pyrroline content by 28.22% and 48.34% compared with CK in the early and late cropping seasons, respectively. For Nanjingxiangzhan, WR treatment increased 1-pyrroline content by 88.96% and 44.91% in the early and late cropping seasons, respectively.

For ProDH activity, in the early cropping season, compared with CK, WR treatment significantly enhanced the ProDH activity by 3.06% and 8.14% for 19xiang and Nanjingxiangzhan, respectively. In the late cropping season, WR treatment resulted in a small increase in ProDH activity compared with CK for both cultivars although the difference did not reach a significant level.

4. Discussion

4.1. Effects of Winter Planting of Rapeseed on Growth and Yield Formation of Fragrant Rice

The present study revealed the effects of winter planting of rapeseed on fragrant rice performances. The current study showed that winter planting of rapeseed enhanced yield formation and 2-AP biosynthesis in fragrant rice, which were similar results to the studies of Fang et al. [25] and Chen et al. [9]. The increment of yield was attributed to the improvement in effective panicle number per plant, which indicated that winter planting of rapeseed might enhanced the tillering capacity or/and percentage of productive tiller. Furthermore, the present study results showed that winter planting of rapeseed substantially improved fragrant rice growth in terms of net photosynthetic rate, chlorophyll content, LAI, and dry matter accumulation, which indicated that fragrant rice performances exhibited a better photosynthesis, i.e., the utilization capacity of light. The yield formation of rice depends on many factors, including environmental factors such as light, temperature, precipitation, soil fertility, etc., and among these, light is one of the most important factors affecting the growth and development of rice [26,27]. The previous study shows that the light-use ability of rice is significantly affected by different tillage patterns [28]. According to Yang et al. [29], the use of a mechanical transplanting method to plant rapeseed could significantly increase the chlorophyll content, LAI, and net photosynthetic rate of rice at all growth stages. In general, within a certain range, an increase in LAI means that the plant is able to absorb more light energy, thereby increasing the net photosynthetic rate. Chlorophyll is also an important pigment for photosynthesis in plants, and having a higher chlorophyll content means a plant can usually absorb more light energy for photosynthesis [30], while the increase in photosynthesis can speed up the synthesis of organic matter in plants [31]. In this study, WR treatment significantly increased the chlorophyll content, LAI, net photosynthetic rate, and dry matter accumulation of fragrant rice plants and, thus, resulted in an increment in grain yield.

4.2. The Possible Relationship between Soil Biochemical Properties and Yield Formation of Fragrant Rice after the Winter Planting of Rapeseed

The enhanced growth of fragrant rice plants after the winter planting of rapeseed might be explained by the increased content of nitrogen and potassium in the soil. In this study, winter planting of rapeseed significantly increased alkali-hydrolyzable nitrogen in the soil, which was more easily absorbed and utilized by roots, and had a more direct impact on the growth and development of crops [9,14]. Moreover, after planting rapeseed in winter, the content of available potassium in the soil increased, and a previous study pointed out that potassium is essential for the growth and development of plants, and potassium ions can regulate stress resistance, photosynthesis, etc. [32]. The study of Maschmann et al. [33] also demonstrates that potassium increases crop yields by promoting the development of plant organs. In addition, an early study shows that the coordinated application of nitrogen and potassium fertilizers can improve the yield and quality of crops [34,35]. Therefore, the appropriate increase in available potassium content in soil is of great significance to ensure the normal growth, high yield, and high quality of rice.

4.3. Effects of Winter Planting of Rapeseed on 2-AP Biosynthesis of Fragrant Rice

It is known that 2-AP is a key flavor component in fragrant rice aroma. This study showed that winter planting of rapeseed substantially enhanced 2-AP biosynthesis and led to the increment in 2-AP content in fragrant rice. The improved 2-AP biosynthesis was explained by increased contents of precursors including P5C, MG, and 1-pyrroline. In this study, winter planting of rapeseed significantly increased the grain 2-AP content. The present study observed that WR treatment significantly increased the contents of P5C, MG, and 1-pyrroline, suggesting that winter planting of rapeseed could increase the content of precursors to enhance the biosynthesis of 2-AP. A previous study shows that 1-pyrroline, and P5C are important precursors for the 2-AP formation in fragrant rice [36]. MG is also considered as a precursor for 2-AP biosynthesis, where it may react directly with P5C to produce 2-AP [37]. Furthermore, ProDH was identified as the key enzyme in 2-AP biosynthesis and the winter planting of rapeseed significantly enhanced ProDH activity in our study. As per a previous study, ProDH catalyzes the P5C biosynthesis and the activity of ProDH is directly related to the biosynthesis of rice 2-AP [6]. Therefore, the increase in ProDH activity is also conducive to 2-AP formation. In addition, other studies also show that medium-term nitrogen fertilization can also increase the content of 2-AP in fragrant rice grains [38,39], which indicates the increment in soil nitrogen content contributes to increased 2-AP content.

5. Conclusions

The current study results showed that winter planting of rapeseed significantly increased the content of alkali-hydrolyzable nitrogen and available potassium in soil and improved fragrant rice growth in terms of net photosynthetic rate, chlorophyll content, LAI, and dry matter accumulation. Higher effective panicle number per plant and grain yield of fragrant rice was observed after the winter planting of rapeseed. The content of 2-AP and its biosynthetic precursors including P5C, MG, and 1-pyrroline, and the activity of ProDH of fragrant rice also improved after the winter planting of rapeseed. In the future, studies should be focused at a molecular characterization to reveal the regulation mechanism in 2-AP biosynthesis of fragrant rice under conditions of winter planting of rapeseed.

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