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Rice–Potato Rotation Pattern Affects 2-Acetyl-1-Pyrroline Biosynthesis and Productivity in Aromatic Rice Grains

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Abstract: Aromatic rice has gained significant attention due to its high economic and nutritional value. 2-Acetyl-1-pyrroline (2-AP), a key aroma compound in aromatic rice, plays a crucial role in elucidating the aroma characteristics of aromatic rice. However, there is no report on the effect of aromatic rice in rice–potato rotation on aroma characteristics. In order to study the influences of winter-planted potatoes on the yield, quality, and 2-AP biosynthesis of aromatic rice grains, the commonly cultivated aromatic rice variety Meixiangzhan-2 and the potato cultivar Huashu-5 were selected as experimental materials for a three-year consecutive field experiment with different tillage patterns consisting of rice–winter fallow as the control group (CK) and rice–potato rotation as the experimental group (RP). The results indicated that the RP treatment enhanced the soil nutrient content and decreased the bulk density. Compared with CK, RP treatment increased the effective panicle number by 10.88% and grain number per panicle by 8.82%, thereby increasing the yield by 11.99%. Meanwhile, RP treatment improved the brown rice rate by 2.61%, milled rice rate by 4.53%, head milled rice rate by 7.51%, and crude protein content by 6.98%. Regarding 2-AP biosynthesis in grains, in contrast to CK, the RP treatment raised the levels of related precursors (Δ1-pyrroline, Δ1-pyrrolidine-5-carboxylic acid, and proline increased by 8.95%, 18.14%, and 13.75%, respectively) and enzymes (proline dehydrogenase, ornithine transaminase, and diamine oxidase increased by 18.37%, 14.61%, and 11.36%, respectively) in its synthesis pathway, thereby facilitating the accumulation of 2-AP. Furthermore, we also observed a more stable yield and grain 2-AP content in aromatic rice under RP treatment. Overall, with regard to enhancing the aromatic rice yield and aroma, the rice–potato rotation system can be contemplated for vigorous promotion.

Keywords: aromatic rice; rice–potato rotation; 2-acetyl-1-pyrroline; rice quality

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1. Introduction

Rice serves as the primary staple grain for over 62.8% of the global population, thus highlighting its significant role in agricultural production [1]. Aromatic rice is popular with

consumers due to its unique aroma and superior cooking qualities [2], exhibiting a high market value. Aromatic rice contains more than 100 volatile compounds [3–5], of which 2-acetyl-1-pyrroline (2-AP) is the principal compound responsible for rice aroma [6–8]. However, the biosynthesis of 2-AP is greatly affected by production environmental conditions [9–11]. In addition, the grain yield of aromatic rice is usually lower than that of non-aromatic rice [12]. Therefore, the improvement of rice aroma while ensuring yield is a challenging scientific issue.

It is widely acknowledged that the biosynthesis of 2-AP is a very complicated process involving multiple precursors and enzymes. Proline, Δ1-pyrrolidine-5-carboxylic acid (P5C), Δ1-pyrroline, proline dehydrogenase (ProDH), Δ1-pyrroline-5-carboxylate synthetase (P5CS), ornithine aminotransferase (OAT), and diamine oxidase (DAO) have been reported as precursors and enzymes highly related to 2-AP formation [13–15]. The genetic analysis indicated that rice aroma is regulated by a single recessive gene (*fgr*) on chromosome 8, which encodes betaine aldehyde dehydrogenase (BADH2) [14,16,17]. Some previous studies have revealed that an 8-bp deletion and three single nucleotide polymorphisms (SNPs) in exon 7 of the *Badh2* gene result in the loss of function of the encoded protein, thereby promoting the accumulation of 2-AP [18,19].

The impact of rice cropping systems on the biosynthesis of 2-AP has garnered growing research attention in recent years. It has been reported that the grain yield and aroma content of aromatic rice are closely related to the farming system, soil type, and field management practices [20]. In order to increase the 2-AP content in aromatic rice grains, various crop cultivation practices and tillage patterns have been introduced to rice production [20]. For example, Zhao et al. [21] found that continuous plow tillage improved the yield, quality, and aroma of aromatic rice, compared with rotary tillage. Du et al. [22] pointed out that conservation tillage increased the grain yield and the grain 2-AP content of aromatic rice. In addition to various tillage patterns, different cultivation management practices can also affect the biosynthesis of 2-AP. The content of 2-AP in brown rice has also been reported to be significantly enhanced by the interaction between water and nitrogen during the tillering stage [23] and the Mn fertilizer application [24]. Nitrogen fertilizer application can effectively enhance free proline content (2-AP precursor) in rice grains during the ripening stage, thereby increasing 2-AP content [25].

Crop rotation, as an important tillage pattern, is often used for rice cultivation. Through rotation, the cropland can be fully and effectively utilized, meanwhile maintaining soil fertility [26]. China has emerged as a prominent global producer of rice and potato production [27,28], showcasing remarkable accomplishments in ensuring food security. The paddy-upland crop rotation is one of the main cropping systems in the Yangtze River Basin, China [29]. Song et al. [30] rotated rice with four crops (ryegrass, potato, milk vetch, and oilseed rape) and found that the rice–potato rotation produced the highest biomass. Tang et al. [25] reported that among different winter cover crop treatments with straw returning, the practice of returning potato straw to the field exhibited the most significant impact on enhancing soil microbial activity, while also providing a supplementary nitrogen source [31]. Goswami et al. [32] proposed that the aromatic rice–potato rotation could potentially serve as a valuable cropping system from an economic perspective. However, there was a scarcity of relevant reports regarding the potential impact of the rotation between aromatic rice and potato on the aromatic characteristics of rice.

Based on the above findings, we conducted field experiments on the aromatic rice–potato rotation system for three consecutive years. The objective was to assess the influence of rice–potato rotation on the performance of aromatic rice by analyzing the levels of key precursors and related enzymes involved in 2-AP biosynthesis, as well as the grain quality and yield formation.

2. Materials and Methods

2.1. Experimental Materials

In this study, we selected Meixiangzhan-2 as the aromatic rice experimental material, which was bred by Guangdong Academy of Agricultural Sciences. The rice has a strong aroma and a growth period of about 113 days when planted in Guangdong province. The potato variety selected was Huashu-5, which was bred by Huazhong Agricultural University. The Huashu-5 variety is commonly grown in Central China with the characteristics of a high emergence rate, good taste, and a growth period of 78 days.

2.2. Experimental Site

The field experiment was conducted consecutively for three years, from 2020 to 2022, at the Agricultural Science and Technology Demonstration Base (30°21′ N and 112°09′ E) of Yangtze University in Jingzhou city, Hubei Province, China. The experimental site is situated in the Yangtze River Basin and falls under the north subtropical monsoon climate zone, characterized by an average annual temperature of 17.9 °C and an average annual precipitation of 1049 mm. Figure 1 illustrates the monthly rainfall and mean temperatures during the primary rice growing season from 2020 to 2022, data provided by the Hubei Meteorological Service. The experimental soil was sandy loam with 1.86 g/kg total nitrogen content, 13.81 g/kg total phosphorus content, 1.02 g/kg total potassium content, and 21.58 g/kg organic carbon content.

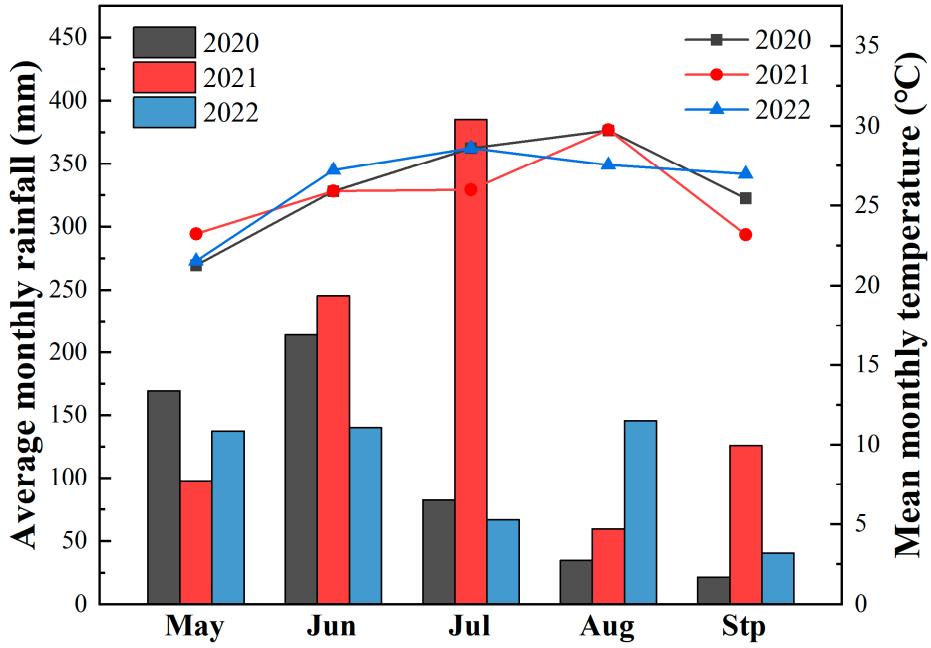

Figure 1. The monthly rainfall and mean monthly temperature in the main growing season of rice from 2020 to 2022 in Jingzhou, Hubei Province, China.

2.3. Experimental Treatments and Design

In the experiment, we employed a single-factor randomized block design. Two tillage patterns were employed: aromatic rice–winter fallow (CK) and aromatic rice–potato rotation (RP), meaning no crops were planted during the winter season in CK while potatoes were planted after the rice harvest in RP. For each tillage pattern, three replicate plots were applied, with each plot having an area of 36 m^2. In two tillage patterns, on day 20 before rice transplanting, paddy fields were submerged in water to a depth of approximately 4–5 cm, and soil plowing occurred on day 15 prior to transplanting while rotary tillage was performed on day 10 beforehand. Rice seedlings aged 18 days old were obtained

from a seedling-raising factory. The annual planting date of seedlings is presented in Table 1. A high-speed ride-type rice transplanter (Jingguan, PZ60ADTLF) was utilized for transplantation with a spacing between plants set at 30 × 14 cm. The compound fertilizer Yaran (N:P:K = 15:15:15) was applied as the base fertilizer before transplanting at a rate of 450 kg ha^{-1} and as a tillering fertilizer in a shallow water layer on day 5 after turning green, with an application rate of 150 kg ha^{-1}. Subsequently, the fields followed routine management practices for the timely prevention and control of diseases, pests, and weeds by manual labor. Table 1 presents the annual planting and harvesting dates for potatoes. Potatoes were planted at a density of 90,000 individuals ha^{-1}. Fertilizer was applied before potato planting at rates of 180 kg N ha^{-1}, 90 kg P_2O_5 ha^{-1}, and 210 kg K_2O ha^{-1} using urea, calcium superphosphate, and potassium sulfate, respectively. The fertilizers were provided by Yuntianhua Co., Ltd. (Kunming, China). After planting the potato seed tubers, the ridges were covered with white plastic film for heat preservation and water retention. Following the potato harvest, all stems and leaves were returned to the field.

Table 1. Planting and harvesting dates of crops.

Tillage Pattern	Year	Crop	Planting Date	Harvesting Date
Aromatic rice–winter fallow (CK)	2020	Meixiangzhan-2	15 May 2020	4 September 2020
	2021	Meixiangzhan-2	13 May 2021	5 September 2021
	2022	Meixiangzhan-2	14 May 2022	4 September 2022
Aromatic rice–potato rotation (RP)	2020	Meixiangzhan-2	15 May 2020	4 September 2020
		Huashu-5	3 December 2020	19 February 2021
	2021	Meixiangzhan-2	13 May 2021	5 September 2021
		Huashu-5	5 December 2021	21 February 2022
	2022	Meixiangzhan-2	14 May 2022	4 September 2022
		Huashu-5	3 December 2022	18 February 2023

2.4. Project Determination

2.4.1. Rice Plant Sampling and Rice Yield Determination

After rice maturity, the five-point sampling method was employed, and 1 m^2 of rice plants was randomly selected in each plot for the measurement of effective panicle number (EPN). Based on the effective panicle number, 6 representative plants were selected from these 30 rice plants for the measurements of grain number per panicle (GNP), seed setting rate (SSR), and 1000-grain weight (TGW). The average grain yield (GY) per unit area of 1 m^2 was measured from three random sampling points within each plot after artificial threshing and sun drying (until reaching a moisture content of 14%) [33]. Additionally, fresh grains isolated from three representative plants selected from each plot were stored at −80 °C for the determination of 2-AP content and its related physiological characteristics.

2.4.2. Collection and Determination of Soil Samples

At the time of potato harvest, the soil bulk density (BD) at 0–20 cm soil layers was measured using a standard cutting ring with a volume of 100 cm^3. The 0–20 cm surface soil was collected according to the previously reported soil sampling method [34] for the measurements of total nitrogen (TN), total phosphorus (TP), total potassium (TK), and soil organic carbon (SOC) on day 7 before the rice harvest. Soil property determination was conducted according to the methods described by Zhang et al. [35]. SOC content was determined via the dichromate oxidation method. Soil samples were digested using potassium dichromate and sulfuric acid, oxidizing organic matter to carbon dioxide while reducing chromium to its trivalent state. The SOC content was calculated based on the extent of dichromate ion reduction. Soil TN was measured via dry combustion method using a C-N analyzer, the soil sample was combusted at high temperature to convert

organic nitrogen into nitrogen gas, and TN content was determined based on the measured volume of nitrogen gas. Soil TP was determined using a digestion solution containing sodium hydroxide. Soil samples were fused with sodium hydroxide at high temperatures. The resulting digestion solution was then reacted with the molybdenum-antimony reagent under acidic conditions. Absorbance was measured at a wavelength of 700 nm, and the TP content in the soil was calculated based on the standard curve. Soil TK was measured with a flame photometer. Soil samples were fused with sodium hydroxide at high temperatures. The resulting fused samples were subsequently dissolved in hydrochloric acid. The potassium ion concentration in the resultant solution was then determined using flame photometry.

2.4.3. Determination of Processing Quality and Nutritional Quality

The rice grains were naturally air-dried following the processes of harvesting and threshing. The brown rice rate (BRR), milled rice rate (MRR), and head milled rice rate (HMRR) were determined using a previously reported method [24]. The crude protein content (PC) was determined using near-infrared spectroscopy (Perkone Scientific PD-9600, Hangzhou Perkone Scientific Co., Ltd., Hangzhou City, China), while amylose content (AC) was measured by spectrophotometry [36].

2.4.4. Determination of 2-AP Content in Grains

A certain quantity of fresh grains was placed in a mortar and treated with liquid nitrogen to maintain enzyme activity at low temperatures, followed by grinding into powder. Subsequently, 1–2 g of the grain powder was transferred into a crimp top vial and mixed thoroughly with 10 mL of chromatographically pure dichloromethane through shaking. The resulting mixture underwent ultrasonic treatment in an ultrasonic cleaner (40 kHz, 40 °C) for 240 min, cooled to room temperature, and then supplemented with an appropriate amount of anhydrous sodium sulfite. Afterwards, the supernatant was immediately extracted using a sterile syringe (1 mL), injected into a sample headspace bottle via an organic needle filter membrane (pore size: 0.22 μm, diameter: 13 mm), and fortified with 0.2 mg kg-1 of 2,4,6-trimethylpyrimidine as the internal standard compound. The content of 2-AP was determined using a GCMS-QP2010 Plus instrument (Shimadzu Corporation, Kyoto, Japan) for three biological replications [9].

2.4.5. Determination of Proline Content in Grains

The proline content in grains was measured according to the previously reported method [37]. A 0.3 g sample was added into 4 mL of 3% sulfosalicylic acid and subjected to a boiling water bath for 10 min. Then, 2 mL of the supernatant was taken, added into 2 mL of glacial acetic acid or 2 mL of ninhydrine reagent, and subjected to a boiling water bath for 30 min. After cooling, the mixture was added with 3 mL of toluene, shaken well, and stood for stratification. The 1.5 mL of the toluene at the upper layer was taken and centrifuged at 4000 rpm for 5 min. The absorbance was recorded at 530 nm for three biological replications.

2.4.6. Determination of Δ1-Pyrrolidine-5-Carboxylic Acid (P5C) in Grains

The P5C concentration was determined in reference to the method reported by Miller et al. [38]. Specifically, 0.50 g of the sample was placed in a centrifuge tube, added with 6 mL of 3% sulfosalicylic acid, and ground into a homogenate in a grinder. After centrifugation, 1.35 mL of the supernatant was taken, added with 1.5 mL of 10% trichloroacetic acid and 0.15 mL of 2-aminobenzaldehyde, successively, left at room temperature for 25 min, and then centrifuged at 10,000 rpm for 10 min. Finally, the absorbance was recorded at 440 nm for three biological replications.

2.4.7. Determination of Δ1-Pyrroline Content in Grains

The Δ1-pyrroline concentration in grains was determined by the previously reported method [32]. Briefly, using a pipette, 0.1 mL of related enzyme extract solution was added into 0.02 M phosphate buffer (pH = 7.0) containing 0.2 mL of 0.01 M 2-aminobenzaldehyde, which was further added with 0.2 mL of 0.2 M phosphate buffer (pH = 7.0), and then added with 0.1 mL of ultrapure water. The reaction was carried out at room temperature for 30 min, and then the absorbance was recorded at 430 nm for three biological replications.

2.4.8. Determination of Proline Dehydrogenase (ProDH) Activity in Grains

The ProDH activity was determined according to the previously reported method [39]. Specifically, 0.3 mL of the reaction solution contained 100 mM potassium phosphate buffer (pH = 7.4), 15 mM L-proline, 0.01 mM cytochrome c, 0.5% (v/v) triton X-100, and 0.1 mL enzyme extract, and the reaction was performed at 37 °C for 30 min. Then, 0.5 mL of trichloroacetic acid (10%) was added to terminate the reaction, and 0.5 mL of 2-aminobenzaldehyde (0.25%) dissolved in ethanol (95%) was added. The mixture was subject to a water bath at 37 °C for 10 min for color development and centrifuged at 8000 rpm for 10 min. Finally, the absorbance was recorded colorimetrically at 440 nm for three biological replications.

2.4.9. Determination of Ornithine Aminotransferase (OAT) Activity in Grains

The ornithine aminotransferase (OAT) activity was measured according to the method reported by Mo et al. [33]. Briefly, 1 mL of reaction solution contained 100 mM potassium phosphate buffer (pH 8.0), 50 mM ornithine, 20 mM α-ketoglutarate, 1 mM pridoxal 5-phosphate, and 0.1 mL of crude enzyme extract, and the reaction was conducted at 37 °C for 30 min. Afterwards, 0.5 mL of trichloroacetic acid (10%) was added to terminate the reaction. Subsequently, 0.5 mL of o-aminobenzaldehyde (0.25%) was added for a 60-min color development. The mixture was centrifuged at 8000 rpm for 10 min, and absorbance was recorded at 440 nm for three biological replications.

2.4.10. Determination of Diamine Oxidase (DAO) Activity in Grains

The DAO activity was detected using the method described by Yang et al. [40]. The 3.0 mL of reaction mixture contained 2.5 mL 0.1 M sodium phosphate buffer (pH = 6.5), 0.1 mL crude enzyme extract, 0.1 mL peroxidase (250 U mL^{-1}), and 0.2 mL 4-aminoantipyrine/N, N-Dimethylaniline. The reaction was initiated with 0.1 mL of 20 mM Put. The absorbance was recorded at 555 nm for three biological replications.

2.4.11. Determination of GABA Content and BADH Activity in Grains

The gamma-aminobutyric acid (GABA) content in grains was determined according to the method reported by Poonlaphdecha et al. [11]. After the reaction, the absorbance was recorded at 645 nm. The betaine aldehyde dehydrogenase (BADH) activity was detected according to the methods described by Hibino et al. [41]. The experiments were repeated three times.

2.5. Statistical Analyses

The average data from the three biological replications were subjected to a one-way analysis of variance (ANOVA) and independent sample *t*-test using SPSS 21.0. Differences in means among groups were determined by using the least significant difference (LSD) test. $p < 0.05$ was considered statistically significant. The diagrams were plotted using Origin Pro 2018 software.

3. Results

3.1. Paddy Soil Properties

The effects of different tillage patterns on paddy soil properties are illustrated in Figure 2. In contrast to CK, three consecutive years of RP significantly increased the contents of soil TN, TP, TK, and SOC by 16.10%, 9.86%, 17.79%, and 21.55%, respectively. Additionally, the soil BD was reduced significantly by 6.83%.

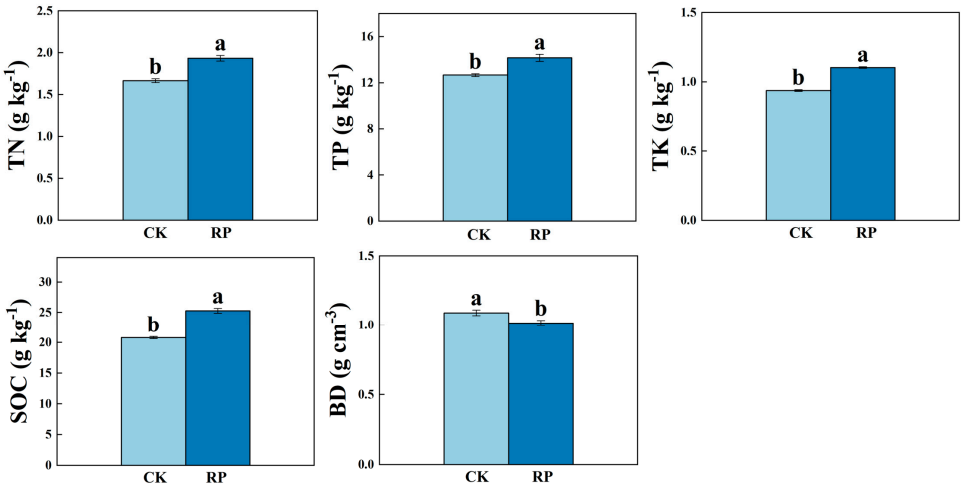

Figure 2. Effects of different rice tillage patterns on soil properties. TN, total nitrogen; TP, total phosphorus; TK, total potassium; SOC, soil organic carbon; BD, bulk density; CK, aromatic rice–winter fallow; RP, aromatic rice–potato rotation. Different letters indicate significant differences between the tillage pattern at $p < 0.05$ according to independent sample *t*-test.

3.2. Yield and 2-AP Content in Grains

Winter planting of potatoes significantly enhanced rice grain yield (Figure 3A). Between tillage patterns, in comparison to CK, RP treatment resulted in a substantial increase in grain yield, with increments of 8.93%, 12.44%, and 14.61% observed in 2020, 2021, and 2022, respectively, averaging a total increase of 11.99%. As the experimental years increased, the yield of the two tillage patterns initially decreased and then increased. However, only the RP pattern was significantly higher in the third year than in the first year (increased by 7.84%).

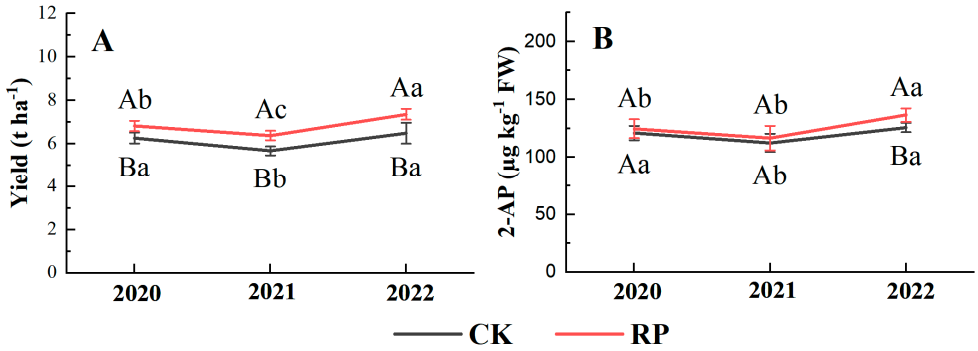

Figure 3. Grain yield (**A**) and 2-AP content (**B**) of rice under different tillage patterns and years. 2-AP, 2-acetyl-1-pyrroline; CK, aromatic rice–winter fallow; RP, aromatic rice–potato rotation, FW, fresh weight. Different uppercase letters indicate significant differences between the tillage patterns within the same year at $p < 0.05$ according to independent sample *t*-test (longitudinal comparison). Different lowercase letters indicate significant differences among the same tillage patterns across different years at $p < 0.05$ according to LSD's multiple range test (transverse comparison).

In terms of grain 2-AP content (Figure 3B), from 2020 to 2022, the RP pattern was 3.11%, 3.69%, and 8.69% higher than CK, respectively, showing a significant difference in the third year. Similarly, the 2-AP content of grain under the two tillage patterns decreased initially and then increased along with the years, and the decrease in the RP pattern was smaller and the increase was larger.

3.3. Yield Component

Different tillage patterns and years on yield components were found to be significant (Figure 4). Compared with CK treatment, the three-year average EPN, GNP, and SSR increased significantly by 10.88%, 8.82%, and 5.52% under RP treatment, respectively. The TGW also showed an upward trend, but there was a significant difference only in 2021 between RP and CK, with a three-year average increase of 3.79%.

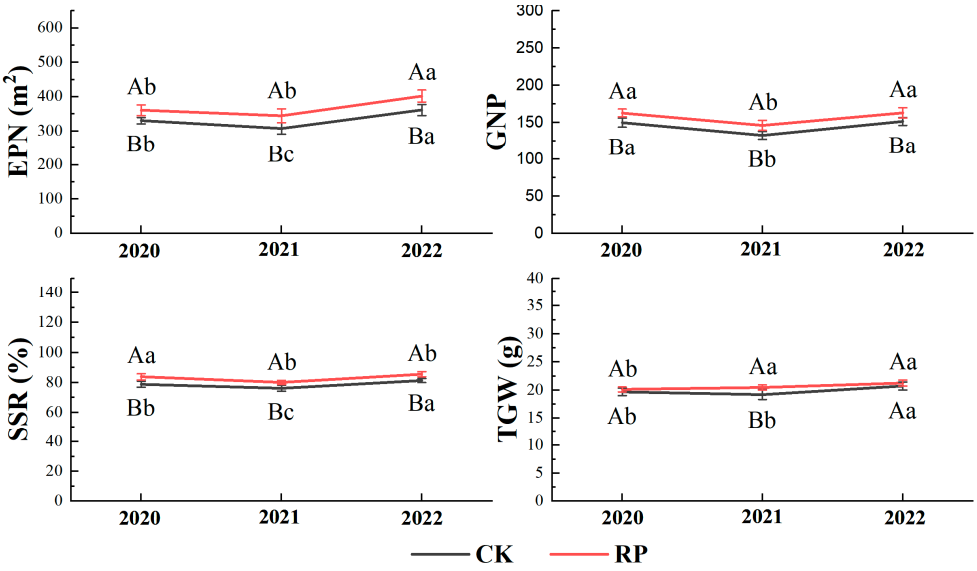

Figure 4. Yield composition of rice under different tillage patterns and years. CK, aromatic rice–winter fallow; RP, aromatic rice–potato rotation; EPN, effective panicles number; GNP, grain number per panicle; SSR, seed setting rate; TGW, 1000-grain weight. Different uppercase letters indicate significant differences between the tillage patterns within the same year at $p < 0.05$ according to independent sample *t*-test (longitudinal comparison). Different lowercase letters indicate significant differences among the same tillage patterns across different years at $p < 0.05$ according to LSD's multiple range test (transverse comparison).

3.4. Rice Grain Quality

The results depicted in Figure 5 illustrate that grain quality exhibits variations across different years and tillage patterns. Regarding rice milling quality, the average data from the past three years indicate that the contents of BRR, MRR, and HMRR in rice grown during the winter after potato cultivation are 2.61%, 4.53%, and 7.51%, respectively, higher than those in rice grown during the winter fallow period. From the temporal perspective, the rice quality of the RP pattern exhibits a gradually ascending trend with the augmentation of rotation years, while fallow manifests a downward trend.

Furthermore, tillage patterns have exerted a considerable influence on the contents of crude PC and AC in fragrant rice. Crude PC content in RP was 6.98% higher than that in CK, while the AC content was 7.36% lower. Generally speaking, rice with a lower AC content has a softer texture and is more popular among people.

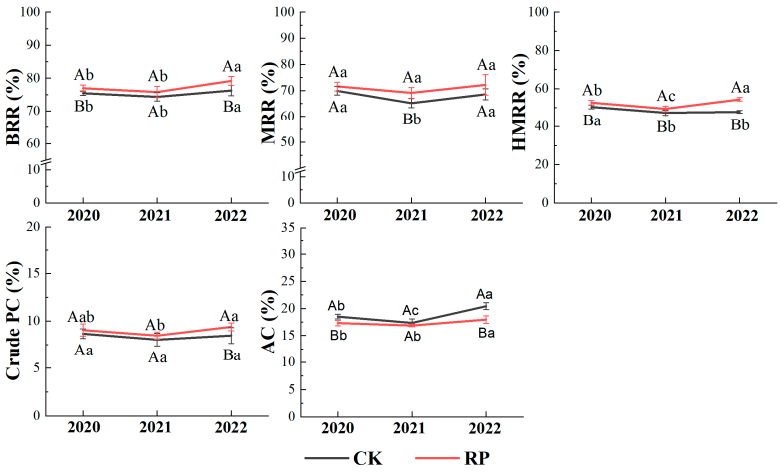

Figure 5. Rice quality of rice under different tillage patterns and years. CK, aromatic rice–winter fallow; RP, aromatic rice–potato rotation; BRR, brown rice rate; MRR, milled rice rate; HMRR, head milled rice rate; Crude PC, crude protein content; AC, amylose content. Different uppercase letters indicate significant differences between the tillage patterns within the same year at $p < 0.05$ according to independent sample *t*-test (longitudinal comparison). Different lowercase letters indicate significant differences among the same tillage patterns across different years at $p < 0.05$ according to LSD's multiple range test (transverse comparison).

3.5. Precursors Involved in 2-AP Biosynthesis

Different tillage patterns exerted significant effects on the associated precursors involved in 2-AP synthesis (Figure 6). Compared with CK, Δ1-pyrroline, P5C, and proline under RP treatment increased by 8.95%, 18.14%, and 13.75%, respectively, while GABA decreased by 3.61%%. It can be observed that the tillage pattern had the greatest effect on P5C, followed by proline, and had the least effect on GABA. In terms of tillage time, Δ1-pyrroline, P5C, and proline under the final RP treatment increased significantly by 7.22%, 14.43%, and 24.01%, respectively (compared with the first year). Meanwhile, the CK treatment also demonstrated varying degrees of increase, but the extent of the increase was lower than that of the RP treatment.

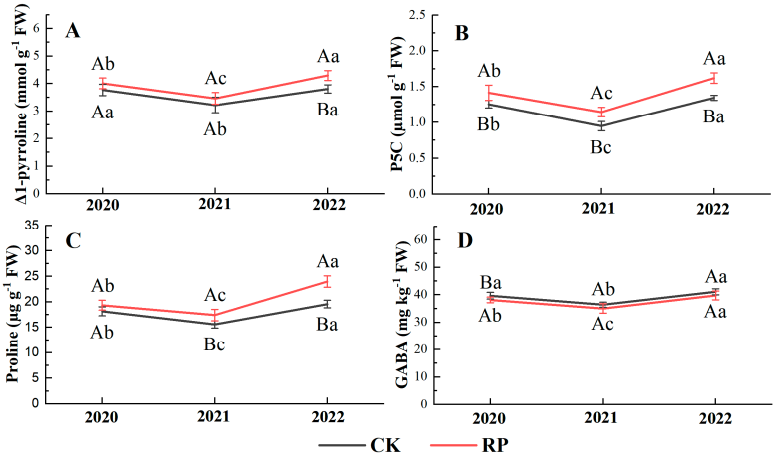

Figure 6. Effects of different tillage patterns and years on Δ1-pyrroline (**A**), P5C (**B**), proline (**C**) and GABA (**D**) levels in grains; CK, aromatic rice–winter fallow; RP, aromatic rice–potato rotation; FW, fresh weight. Different uppercase letters indicate significant differences between the tillage patterns within the same year at $p < 0.05$ according to independent sample *t*-test (longitudinal comparison). Different lowercase letters indicate significant differences among the same tillage patterns across different years at $p < 0.05$ according to LSD's multiple range test (transverse comparison).

3.6. Enzymes Involved in 2-AP Biosynthesis

Different tillage patterns exerted significant effects on the associated enzymes involved in 2-AP synthesis (Figure 7). Among them, regarding ProDH, the activity was significantly elevated by 18.37% (average value over three years) under RP treatment as compared to CK, and a significant difference was observed in all three years. For DAO activity, potato planting in winter significantly increased DAO activity by 10.48%, 15.44%, and 8.17% in 2020–2022, respectively, with an average increase of 11.36%. Regarding OAT activity, different tillage patterns also exerted significant effects on its activity for 3 years, and RP treatment increased by an average of 14.61% compared with the CK treatment. However, the BADH activity of the RP treatment was significantly lower than that of CK (decreased by 7.82%). Additionally, with the increase in cultivation years, the activities of enzymes related to the 2-AP synthesis pathway in the two cultivation modes initially decreased and then increased. Compared with the first year, the OAT activity under the RP treatment showed the highest increase in the third year, amounting to 23.93%, followed by ProDH activity, which increased by 22.70% in the same pattern.

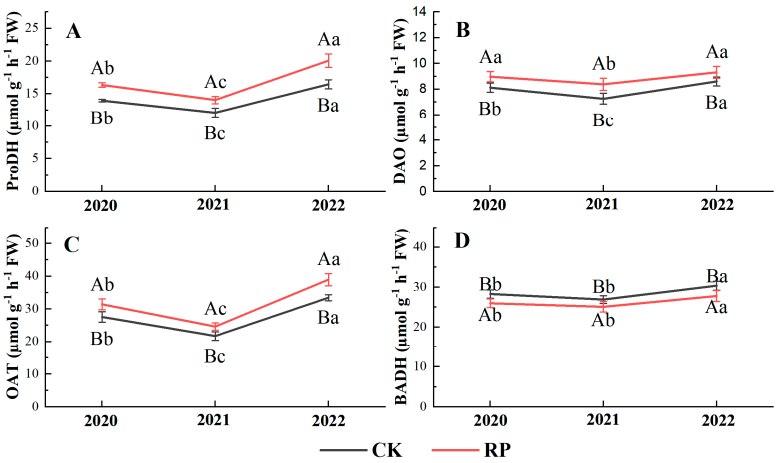

Figure 7. Effects of different tillage patterns and years on ProDH (**A**), DAO (**B**), OAT (**C**), and BADH (**D**) activities in grains; CK, aromatic rice–winter fallow; RP, aromatic rice–potato rotation; FW, fresh weight. Different uppercase letters indicate significant differences between the tillage patterns within the same year at $p < 0.05$ according to independent sample *t*-test (longitudinal comparison). Different lowercase letters indicate significant differences among the same tillage patterns across different years at $p < 0.05$ according to LSD's multiple range test (transverse comparison).

4. Discussion

In this study, we evaluated the impact of winter planting of potatoes on aromatic rice performances through short-term experiments involving rice–potato rotation. This study demonstrated that the winter planting of potatoes substantially enhanced the productivity and quality of aromatic rice, concurrently facilitating the accumulation of 2-AP in grains. The increment of yield was attributed to the improvement of effective panicle number, grain number per panicle, and seed setting rate. Chen et al. [42] found that rice–potato showed the highest yield among five different winter cropping practices. This suggests that winter potato cultivation is more likely to enhance the tillering capacity of plants, facilitate grain filling, and thereby boost the yield. Furthermore, the contents of total N, total P, available K, and SOC in the RP system increased significantly [42], which was similar to our results. The enhancement of soil nutrients should be associated with straw returning. The rotten potato straw stems, leaves, and roots can maintain soil organic activity, increase active organic carbon, and improve the soil microecological environment, thereby improving soil fertility [43]. At present, the role of fallow in the crop rotation system remains controversial. Some scientists contend that fallow treatment can enhance crop production through the

restoration of soil fertility and the sequestration of carbon [44–46]. However, the current evidence indicates that returning winter crop stems and leaves to the soil might be more efficacious than winter fallowing in augmenting soil N supply [47]. Our research findings support the aforementioned conclusions. In the rice–winter fallow treatment, the yield in the third year increased by 2.50% compared to the first year, which may be attributed to improved soil nutrient retention during the fallow period [48]. However, in the rice-potato rotation treatment, the yield in the third year increased by 7.84% relative to the first year, demonstrating a more pronounced yield enhancement effect compared to the winter fallow treatment. The application of straw will result in an increase in pH and a decrease in soil bulk density, thereby facilitating plant growth and increasing grain yield in the paddy field [42]. In addition, this study also found that the performance of yield, quality, and 2-AP content in the second year was poor. The main reason for this phenomenon should be related to the rainfall during the main growing season of rice in the same year (Figure 1). Excessive water levels in paddy fields could induce root hypoxia, thereby affecting tillering and young panicle differentiation during the panicle initiation stage, resulting in decreased yield and quality [49,50]. Simultaneously, elevated water levels during the rice booting stage can impede grain proline oxidase activity and hinder 2-AP synthesis, consequently diminishing grain aroma [51]. However, it should be noted that the yield and 2-AP content of rice in the RP system declined to a lesser extent compared to the CK in the second year, and increased to a greater extent than the CK in the third year. This indicates that under the influence of adverse environments, the yield and quality of aromatic rice in the rice–potato rotation system are more stable and have stronger recovery capability.

Crop management practices and soil characteristics are important factors affecting rice quality [52,53]. Increasing soil fertility can increase the accumulation of protein matrix between endosperm starch, thus reducing grain breakage during milling [54]. This could be the main reason for the increase in grain milling quality (BRR, MRR, HMRR) after potato planting in winter. Additionally, this study also observed lower levels of amylose content and higher crude protein content in RP. The decrease in amylose content might be due to the low content of long-chain branches of amylopectin at a high nitrogen level within the RP system [55]. Simultaneously, the enhancement of soil nitrogen levels facilitated the growth of the above-ground part of rice, thereby influencing the supply of nitrogen and carbon as well as the grain-filling process, and enhancing the quality of rice [50].

The 2-AP content in grains is a crucial parameter for evaluating rice quality. Our data consistently demonstrated higher levels of 2-AP content in grains compared to winter fallow over three consecutive years under the rice–potato rotation system. This increase can primarily be attributed to elevated levels of biosynthesis precursors (P5C, Δ1-pyrroline, and proline) and enzymes (DAO, ProDH, and OAT) involved in 2-AP production. P5C, Δ1-pyrroline, and proline serve as direct precursors for 2-AP biosynthesis [37,56]. OAT and ProDH enzymes facilitate the conversion of ornithine and proline into P5C, respectively. Subsequently, pyrrole-5-carboxylic acid decarboxylase catalyzes P5C to produce Δ1-pyrroline, which is further converted non-enzymatically into 2-AP [57]. Moreover, DAO enzyme converts putrescine into gamma-aminobutyric aldehyde (GAB-ald), while the functional enzyme encoded by BADH2 transforms GAB-ald into GABA thereby inhibiting 2-AP biosynthesis. Conversely, in the absence of the functional BADH2 enzyme, GAB-ald cannot be converted into GABA, resulting in its accumulation. Then, the accumulated GAB-ald directly forms Δ1-pyrroline through spontaneous cycling, eventually leading to increased synthesis of 2-AP content [14,58]. In our study, the RP pattern exhibited significantly lower levels of GABA content and BADH enzyme activity compared to the rice–winter fallow system, indicating that more Δ1-pyrroline was generated from GAB-ald in RP for subsequent biosynthesis of 2-AP. Furthermore, the 2-AP content may be attributed to the soil

nutrient levels in the rice fields. A recent study affirmed by the KEGG functional analysis of soil microorganisms indicated a positive correlation between the increase of 2-AP content and the enhancement of soil quality (particularly an escalation in SOC content) [59]. SOC provides organic matter for maintaining microbial activity, thereby promoting the release and availability of amino acids. Soil carbon metabolism and amino acid metabolism play crucial roles in the production of 2-AP [59]. Elevated nitrogen and phosphorus content in the soil can promote proline synthesis, thereby facilitating the accumulation of 2-AP in grains [60]. Another potential reason for the higher 2-AP content in the grains of RP compared to CK could be the lower soil bulk density in paddy-upland crop rotation, which results in higher soil porosity compared to conventional paddy fields, leading to increased water runoff and, consequently, water loss [52]. Appropriate water deficit stress can induce proline accumulation in plants and promote 2-AP synthesis [51].

5. Conclusions

We found that winter planting of potatoes led to higher plant productivity and better rice quality compared to winter fallowing. Compared with rice–winter fallow, the rice yield in the rice–potato rotation system significantly increased by 11.99%, mainly due to the increase in effective panicle number and grain number per panicle. Following the winter potato planting, the higher total N and SOC content in soil could furnish more nitrogen and carbon sources for the synthesis of proline, a key precursor for 2-AP biosynthesis, thereby promoting the accumulation of rice aroma. Besides proline, the levels of rice Δ1-pyrroline, P5C, ProDH, DAO, and OAT (important precursors and enzymes for 2-AP synthesis) in the RP system were also significantly increased by 8.95%, 18.14%, 18.37%, 11.36%, and 14.61%, respectively. Furthermore, it was discovered that the productivity of the rice–potato rotation system in the extreme environment was more stable. Future research will focus on investigating the effects of rice–potato rotation on soil microbial diversity and soil structure, as well as how these factors influence the biosynthesis of 2-AP in rice grains.

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