Effect Of conservation tillage practices on aroma, yield and quality of mechanical-transplanting fragrant rice

Yong Ren, a,d*, Siren Cheng, a,d*, Shenggang Pan, a,b,c, Hua Tian, a,b,c, Meiyang Duan, a,b,c, Shuli Wang, a,b,c and Xiangru Tang a,b,c

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ABSTRACT
In our study, two popular fragrant rice cultivars (Meixiangzhan 2 and Xiangyaxiangzhan) were conducted by three treatments (CK: conventional tillage; T1: minimum tillage; T2: no-tillage) to assess the effects of conservation tillage on the aroma, yield and quality of mechanical-transplanting fragrant rice. Results depicted that the 2-acetyl-1-pyrroline (2-AP) content in mature grains of mechanical-transplanting fragrant rice remained high level under T1 and T2 treatments. 2-AP accumulation in mature grains was significant associated with the 2-AP, proline and pyrroline-5-carboxylic acid content, proline dehydrogenase and ornithine aminotransferase activity in grains and leaves during the grain-filling period. T1 and T2 treatments improved yield components, increased the photosynthetic matter accumulation, and increased yields. T1 and T2 treatments also resulted in a higher head milled rice rate and significant changes in protein content of mechanical-transplanting fragrant rice. Overall, conservation tillage practices were beneficial to increase the 2-AP content, quality and yield of mechanical-transplanting fragrant rice.

1. Introduction

Agriculture has undergone significant supply-side structural reform in recent years in China. With respect to rice production, there has been a shift from maximizing yield to achieving high yields without compromising quality. Fragrant rice is the treasure of rice, rich in special aroma and nutrition, for their ability to promote green and sustainable development. Previous studies have reported that a truncated version of the betaine aldehyde dehydrogenase (BADH2) gene located on chromosome 8 is responsible for aroma production in fragrant rice (Bradbury et al. 2005). In addition, Kamaraj and Purohit (2013) through in-silico analysis pointed out that the mutant BADH2 N162A may be the most fragrant form for the 2-AP accumulation. Singh (2021) suggested that agrochemicals could affect metabolic pathway of flavour development in rice by binding though in silico molecular docking of fragrance protein BADH2 with pesticides. So that various breeding and cultivation studies have been conducted to improve the aroma (Buttery et al. 1983; Sakthivel et al. 2009; Hashemi et al. 2013). Important precursor substances in the synthesis pathway of 2-AP include proline and pyrroline-5-carboxylic acid (P5C); proline dehydrogenase (PDH) and ornithine aminotransaminase (OAT) are important enzymes that regulate 2-AP formation (Li et al. 2016; Mo et al. 2017a; Mo et al. 2018). Various studies relating to culture and environmental conditions, including investigations of the effect of water (Bao et al. 2018), nutrient elements (Yang et al. 2012; Mo et al. 2017; Mo et al. 2018b), temperature (Kong et al. 2017), growth-regulating substances (Goufo et al. 2011; Xiao 2017).
et al. 2020), and interactions among these factors (Xie et al. 2019; Mo et al. 2019b), have been conducted. These work has suggested that reduced water irrigation, an appropriate amount of nitrogen and silicon addition, reduced light, low temperature, and the spraying of exogenous regulators can effectively promote the synthesis of 2-AP. In fact, the use of an appropriate tillage method (e.g. conservation tillage) can improve soil properties, increase crop yields, and improve crop quality (Liu et al. 2007; Liu et al. 2009; Huang et al. 2015; Yadav et al. 2019; Page et al. 2020). Previous studies have shown that tillage practices can affect the aroma formation of direct-seeded fragrant rice (Du et al. 2019).

Tillage methods affect the farmland soil environment, rice population, yield, and quality vary (Huang et al. 2015; Yadav et al. 2019; Page et al. 2020). However, few studies have explored the impact of tillage practices on the aroma, quality, and yield of mechanically transplanted fragrant rice. There is also a lack of research on the effects of conservation tillage on aroma formation as well as the physiology of mechanically transplanted aromatic rice.

In this study, two Oryza sativa ssp. indica fragrant rice cultivars, ‘Meixiangzhan 2’ and ‘Xiangyaxiangzhan’, were used to evaluate the effects of tillage practices on the aroma, yield, and quality of machine-transplanted fragrant rice with the objectives to provide new insights and scientific basis that could aid the sustainable production of fragrant rice with high quality and yield.

2. Materials and methods

2.1. Experimental site and plant materials

The experiment was conducted in 2016 and 2017 early season at the Research Farm of the College of Agriculture, South China Agricultural University, Guangzhou, China. Meixiangzhan 2 and Xiangyaxiangzhan, which are popular and widely cultivated in south china, were provided by the Rice Research Office of South China Agricultural University as the experimental materials (Tong et al. 2020). The physical and chemical properties of soil in the experimental field were as follows: pH, 5.10; organic matter, 37.64 g·kg⁻¹; total nitrogen, 1.87 g·kg⁻¹; total phosphorus, 1.25 g·kg⁻¹; total potassium, 21.69 g·kg⁻¹; available nitrogen, 132.73 mg·kg⁻¹; available phosphorus, 34.37 mg·kg⁻¹; and available potassium, 132.48 mg·kg⁻¹.

2.2. Experimental design and field management

Rice seedlings were cultivated uniformly with a special substrate for carpet-seedlings. Three tillage methods were applied before transplanting rice: (1) conventional tillage (CK), wherein the traditional pattern of plowing tillage rotated with rotary tillage was applied; (2) minimum tillage (T1), wherein rotatory land tillage was applied one time; and (3) no-tillage (T2), wherein the soil was not disturbed from harvest to sowing, and herbicide (Roundup) was used to eliminate rice stubble and weeds. In all treatments, the previous crop is fragrant rice and the seedlings were carpet-seedling transplanted.

The experimental plot area was 30 m², and there were four replications for each treatment. The planting distance was 30×14 cm. The seeds were sown on March 12, transplanted on April 4, and harvested on July 8 in 2016. In 2017, the seeds were planted on March 10, transplanted on March 30, and harvested on July 8. A total of 675 kg of compound fertilizer/ha (basal fertilizer) was applied by manual broadcasting in 2016; deep basal fertilizer was applied by machine in 2017. Other management measures were carried out per the cultivation regulations of fragrant rice.

2.3. Sampling and measurements

2.3.1. Determination of 2-AP content and physiological characteristics of 2-AP synthesis

Fresh leaves and grains of plants showing consistent growth were taken to the laboratory at the full heading stage (FHS), 7 d after full heading (d AFH), 14 d AFH, 21 d AFH, and the mature stage (MS). Fresh samples were stored in a refrigerator at −20°C to determine the 2-AP content in leaves and grains. The other fresh samples were fixed with liquid nitrogen and stored at −80°C to determine the physiological characteristics of 2-AP synthesis, such as the content of proline and pyrroline-5-carboxylic acid (P5C) and the activity of proline dehydrogenase (PDH) and ornithine aminotransferase (OAT), in leaves and grains.

2-AP content was measured using the synchronization distillation and extraction method combined with the GCMS-QP 2010 Plus (Shimadzu Corporation, Japan) method following Huang et al. (2012) and Hinge et al. (2016), which required grinding 6.0-g samples to powder. The content was expressed as μg·g⁻¹·fresh weight (FW). The proline, P5C, PDH and OAT were measured according to the methods of Li et al. (2016) and Mo et al. (2019b), which were expressed as μg·g⁻¹·FW, μmol·g⁻¹·FW, μmol·g⁻¹·h⁻¹·FW and μmol·g⁻¹·h⁻¹·FW.

2.3.2. Determination of grain yield and yield composition

At the MS, 30 representative fragrant rice plants were selected from each plot to investigate the number of effective panicles. Nine hills of representative plants were selected from each plot to investigate the number of effective panicles, and the yield composition was determined in the laboratory. The actual yield was determined by mechanical harvesting.

2.3.3. Determination of dry weight and leaf area index (LAI)

The plant samples were separated into leaves, stems, and panicles and oven-dried at 80°C to a constant weight for detecting the dry weight at the tillering stage (TS), booting stage (BS), FHS, and MS. The LAI was measured using the coefficient of length and width, which was 0.75.

2.3.4. Determination of grain quality

The harvested rice was stored at room temperature for 3 months and used to determine rice quality. The brown rice rate, milled rice rate, and whole milled rice rate were determined according to the National standard GB/T17891-2017 Quality Rice of the People’s Republic of China. The protein content and amylose content of rice were automatically determined by a FOSS InfratecSM 1241 Grain Analyzer.
2.4. Statistical analysis

The experimental data were processed using Microsoft Excel 2016 and Statistix 8 (Analytical Software, and Tallahassee, Florida, USA) and analyzed using an analysis of variance. Multiple comparisons were performed using the least significant difference method.

3. Results

3.1. 2-AP content in leaves and grains

Compared with CK, the 2-AP content was higher in mature Meixiangzhan 2 grains in T1 and T2. The 2-AP content in T2 in 2016 and 2017 was increased by 21.30% and 92.01%, respectively, and T1 in 2017 by 91.11% relative to CK (Figure 1A, B). No significant differences in the 2-AP content were observed in mature Xiangyaxiangzhan in T1 and T2 relative to CK. In T1, the 2-AP content of Meixiangzhan 2 and Xiangyaxiangzhan in 2016 was significantly increased at 7 d AFH; that of Meixiangzhan 2 in 2016 and 2017 was significantly decreased at 21 d AFH and 14 d AFH, respectively; and that of Xiangyaxiang in 2016 was decreased at 21 d AFH relative to CK. In T2, the 2-AP content of Xiangyaxiangzhan in 2016 was significantly increased at 7 d AFH and 21 d AFH, and that of Meixiangzhan 2 in 2016 was significantly increased at 21 d AFH relative to CK. Compared with CK, the 2-AP content in grains of Meixiangzhan 2 in 2016 at 7 d AFH and in 2017 at 14 d AFH was significantly decreased, and that of Xiangyaxiangzhan in 2017 at 7 d AFH was also significantly decreased.

The 2-AP content in leaves was increased at the FHS in T1 and T2 compared with CK; the exception was Xiangyaxiangzhan in 2017 (Figure 1B,D). Compared with CK, the 2-AP content in the leaves of Xiangyaxiangzhan at 7 d AFH in 2016 was significantly increased, and that in the leaves of Meixiangzhan 2 at 7 d AFH in 2016 was significantly decreased in T1 and T2. In T2, the 2-AP content in Xiangyaxiangzhan leaves was significantly increased relative to CK at 7 d AFH in 2017. The 2-AP content in Meixiangzhan 2 leaves at 21 d AFH was significantly increased relative to CK in T1 and T2. The 2-AP content in MS leaves of Meixiangzhan 2 in 2017 was significantly reduced relative to CK in T1 and T2. The 2-AP content in MS leaves of Meixiangzhan 2 in 2016 was significantly increased, and that in MS leaves of Xiangyaxiangzhan in 2017 was significantly decreased in T2 relative to CK. There were no significant differences in the 2-AP content in other years, varieties, or periods.

3.2. Physiological basis of aroma synthesis in grains and leaves

3.2.1. Proline content in leaves and grains

Compared with CK, the proline content in Xiangyaxiangzhan was significantly increased in 2016 at 7 d AFH (Figure 2(a)) in T1, and that of Meixiangzhan 2 was significantly decreased in 2017 at 7 d AFH in T1 and T2 (Figure 2B). The grain proline content of Meixiangzhan 2 and Xiangyaxiangzhan was significantly decreased in 2016 at 14 d AFH in T1 and T2 relative to CK (Figure 2A). The grain proline content of Meixiangzhan 2 was significantly increased in 2017 at 14 d AFH in T1 and T2 compared with CK. In 2016, the grain proline content of Meixiangzhan 2 was significantly increased in T1 and T2 at 21 d AFH compared with CK. However, the grain proline content in 2017 was significantly decreased for Xiangyaxiangzhan in 2016 and the two varieties at 21 d AFH compared with CK. The grain proline content at the MS of Meixiangzhan 2 in 2016 was significantly decreased, and that of Xiangyaxiangzhan was significantly decreased in 2017, in T1 and T2 relative to CK. There were no significant differences in grain proline content in other years, varieties, or periods.

In 2016, proline content was significantly higher in Meixiangzhan 2 leaves in T1 and T2 from the FHS to 21 d AFH compared with CK (Figure 2C). The proline content in MS leaves of Meixiangzhan 2 was significantly decreased in T2 compared with CK. The proline content in the MS leaves of Xiangyaxiangzhan was significantly increased in T1 compared with CK. The proline content in Xiangyaxiangzhan leaves at 14 d AFH was significantly increased in T2 but significantly decreased at 7 d AFH and the MS compared with CK. In 2017, the proline content in Meixiangzhan leaves at 2 d AFH was significantly increased in T1 and significantly decreased at the FHS in T2 relative to CK. The leaf proline content in Xiangyaxiangzhan was significantly decreased at the FHS and 7 d AFH but significantly increased at 14 d AFH in T1 and T2 compared with CK. At the MS, the proline content in Xiangyaxiangzhan leaves was significantly increased in T1 and significantly decreased in T2 compared with CK.

3.2.2. P5c content in grains and leaves

The P5C content in the grains of the two fragrant rice varieties was higher in T1 and T2 than in CK; the exception was the reduced P5C content in grains at 14 d AFH in T2 compared with CK (Figure 3A). The P5C content of the two fragrant rice cultivars was maintained at a high level in T1 and T2 in 2017; however, the P5C content in grains was significantly reduced at 14 d AFH and the MS in T1 and at the MS in T2 compared with CK (Figure 3B).

In 2016, P5C content was higher in Meixiangzhan 2 leaves in T1 and T2 compared with CK (Figure 3C). Furthermore, the P5C content in leaves was lower in T1 than in CK from the FHS to 21 d AFH and was significantly decreased in T2 compared with CK. In 2017, the P5C content of the two fragrant rice cultivars at the FHS was significantly increased in T1 compared with CK, and that of Meixiangzhan 2 at 14 d AFH and Xiangyaxiangzhan at 7 d AFH was significantly decreased in T1 compared with CK. The P5C content in Meixiangzhan 2 leaves was maintained at a high level at various stages in T2; P5C content was significantly increased in Xiangyaxiangzhan leaves at the FHS and significantly decreased at 7 d AFH and the MS compared with CK (Figure 3D).

3.2.3. PDH activity in grains and leaves

In 2016, PDH activity was increased in Meixiangzhan 2 grains at 7 d AFH but decreased in Meixiangzhan 2 and Xiangyaxiangzhan grains at the MS in T1 compared with CK (Figure 4A). The PDH activity of Meixiangzhan 2 was significantly increased at 14 d AFH and at the MS but decreased at 21 d AFH in T2 compared with CK. In 2017, the PDH activity in Meixiangzhan 2 grains was decreased at the MS in T1 compared with CK; the PDH activity in Xiangyaxiangzhan grains was decreased at 7 d AFH, 21 d AFH, and MS and increased at 14 d AFH in T1 relative to
Figure 1. Effect of different tillage methods on 2-AP content of mechanical-transplanting fragrant rice (A and B in grains; C and D in leaves). Bars represent the mean ± SE of three different biological replicates. For each bar, different lowercase letters indicate significant differences ($p < 0.05$). 2-AP: 2-acetyl-1-pyrroline; CK: conventional tillage; T1: Minimum tillage; T2: No-tillage; FHS: full heading stage; AFH: after full heading; MS: mature stage.

Figure 2. Effect of different tillage methods on the proline content of mechanically transplanted fragrant rice grains (A and B) and leaves (C and D). Bars represent the mean ± SE of three different biological replicates. For each bar, different lowercase letters indicate significant differences ($p < 0.05$). CK: conventional tillage; T1: Minimum tillage; T2: No-tillage; FHS: full heading stage; AFH: after full heading; MS: mature stage.
The PDH activity in Meixiangzhan 2 gains was increased at 7 d AFH in T2 compared with CK, and that of Xiangyaxiangzhan grains was increased at 14 d AFH and the MS in T2 relative to CK. However, the PDH activity in Meixiangzhan 2 and Xiangyaxiangzhan grains was significantly decreased at 14 d AFH and 7 d AFH, respectively, in T1 relative to CK.

In 2016, the PDH activity in Meixiangzhan 2 leaves at the FHS and 7 d AFH was significantly decreased in T1 relative to CK, and that at 14 d AFH and the MS was significantly increased in T1 relative to CK (Figure 4C). The PDH activity in Xiangyaxiangzhan leaves was increased at 7 d AFH. PDH activity was significantly decreased in Xiangyaxiangzhan leaves at the FHS in T2 compared with CK; PDH activity was significantly increased in Meixiangzhan 2 leaves at the MS and in Xiangyaxiangzhan leaves at 14 d AFH in T2 compared with CK. The PDH activity of leaves was decreased at 21 d AFH in both T1 and T2 compared with CK. In 2017, the PDH activity in the leaves of the two fragrant rice varieties was decreased at 7 d AFH in T1 and T2 compared with CK. The OAT activity in grains at the MS was decreased in T1 compared with CK; the exception was Xiangyaxiangzhan in 2016. In 2017, the OAT activity in grains was significantly increased at 14 d AFH, but the OAT activity in grains of the two fragrant rice varieties was decreased at 7 d AFH. The OAT activity in grains was decreased at 7 d and 14 d AFH in T2.

In 2016, OAT activity was decreased in Meixiangzhan 2 leaves from the FHS to 21 d AFH and increased at the MS in T1 compared with CK. The OAT activity in Xiangyaxiangzhan leaves was increased at 7 d and 14 d AFH and decreased at 21 d AFH in T1 compared with CK. The OAT activity in Meixiangzhan 2 leaves was decreased at the FHS but increased at the MS in T2 compared with CK. The OAT activity in Xiangyaxiangzhan leaves was increased at 14 d AFH and at the MS but decreased at 21 d AFH in T2 compared with CK. In 2017, OAT activity was significantly increased in Xiangyaxiangzhan leaves at 7 d and 14 d AFH, significantly decreased in both cultivars at 21 d AFH, and significantly decreased in Meixiangzhan 2 at the MS in T1 and T2 compared with CK. OAT activity was increased in Meixiangzhan 2 leaves at the FHS but decreased in Xiangyaxiangzhan leaves at the MS in T1 compared with CK. Compared with CK, OAT activity was increased in Meixiangzhan 2 at 7 d AFH and in Xiangyaxiangzhan at the MS but decreased in Xiangyaxiangzhan at the FHS in T2.

### 3.2.4. OAT activity in grains and leaves

The OAT activity in Meixiangzhan 2 grains was increased at 7 d and 14 d AFH in T2 compared with CK (Figure 5A, B). The OAT activity in the grains of the fragrant rice varieties was decreased at 21 d AFH in T1 and T2 compared with CK. The OAT activity in grains at the MS was decreased in T1 compared with CK; the exception was Xiangyaxiangzhan in 2016. In 2017, the OAT activity in grains was significantly increased at 14 d AFH, but the OAT activity in grains of the two fragrant rice varieties was decreased at 7 d AFH. The OAT activity in grains was decreased at 7 d and 14 d AFH in T2.

### 3.3. Grain yield and yield components

Grain yield increased in response to T1 and T2 (Table 1). The effect of tillage treatment on the seed-setting rate and 1000-
Figure 4. Effect of different tillage methods on the PDH activity of mechanically transplanted fragrant rice grains (A and B) and leaves (C and D). Bars represent the mean ± SE of three different biological replicates. For each bar, different lowercase letters indicate significant differences (p < 0.05). PDH: proline dehydrogenase; CK: conventional tillage; T1: Minimum tillage; T2: No-tillage; FHS: full heading stage; AFH: after full heading; MS: mature stage.

Figure 5. Effect of different tillage methods on the OAT activity of mechanically transplanted fragrant rice grains (A and B) and leaves (C and D). Bars represent the mean ± SE of three different biological replicates. For each bar, different lowercase letters indicate significant differences (p < 0.05). OAT: ornithine transaminase; CK: conventional tillage; T1: Minimum tillage; T2: No-tillage; FHS: full heading stage; AFH: after full heading; MS: mature stage.
in LAI among T1, T2, and CK were observed in the other stages (Figure 6C, D).

### 3.6. Grain quality

As shown in Table 3, in 2016, the amylose content of Meixiangzhan 2 and the brown rice rate and protein content of Xiangyaxiangzhan were significantly increased, and the protein content of Meixiangzhan 2 was significantly decreased in T1 compared with CK. The protein content of the two fragrant rice varieties was significantly decreased in T2 compared with CK. In 2017, the brown rice rate and milled rice rate of Meixiangzhan 2 were significantly decreased, and the protein content of Meixiangzhan 2 and the milled rice rate and protein content of Xiangyaxiangzhan were significantly increased in T1 compared with CK. The milled rice rate and protein content of the two fragrant rice varieties were significantly increased in T2 compared with CK.

### 3.7. Correlation analysis

The 2-AP content in mature grains was significantly positively correlated with the 2-AP content in grains at 14 d AFH, the 2-AP content in leaves at 7 d AFH, the proline content in leaves at the MS, the P5C content in grains at 21 d AFH, and the PDH activity in grains at 14 d AFH (Table 4). There was a significant negative correlation between the 2-AP content in mature grains and the OAT activity in leaves at 14 d AFH.

Yield was significantly positively correlated with the seed-setting rate, 1000-grain weight, dry matter accumulation at each stage, and LAI at the FHS and significantly negatively correlated with LAI at the TS (Table 5).

### Table 1. Effect of different tillage methods on the yield and yield components of mechanically transplanted fragrant rice.

| Time   | Cultivar | Treatment | Panicle number (×10⁴ ha⁻¹) | Grains number per panicle | Seed-setting rate (%) | 1000-grain weight (g) | Yield (t ha⁻¹) |
|--------|----------|-----------|-----------------------------|---------------------------|----------------------|-----------------------|--------------|
| 2016   | Meixiangzhan 2 | CK        | 299.68 ± 17.41a             | 158.70 ± 9.23a            | 71.96 ± 0.97a         | 19.16 ± 0.44a         | 5.65 ± 0.19a  |
|        |          | T1        | 312.70 ± 9.94a             | 145.80 ± 8.53a            | 76.18 ± 5.94a         | 19.49 ± 0.15a         | 5.98 ± 0.21a  |
|        |          | T2        | 306.83 ± 8.05a             | 141.16 ± 8.86a            | 81.19 ± 0.66a         | 19.70 ± 0.26a         | 5.88 ± 0.07a  |
|        | Xiangyaxiangzhan | CK     | 279.37 ± 4.20              | 161.00 ± 12.06ab          | 73.36 ± 4.23a         | 18.20 ± 0.22a         | 4.89 ± 0.37b  |
|        |          | T1        | 286.19 ± 13.75b            | 154.46 ± 8.09b            | 75.73 ± 2.86a         | 18.01 ± 0.15a         | 5.12 ± 0.32ab |
|        |          | T2        | 308.92 ± 15.93a            | 173.96 ± 11.18a           | 75.95 ± 0.33a         | 18.52 ± 0.22a         | 5.31 ± 0.19a  |
| 2017   | Meixiangzhan 2 | CK        | 311.11 ± 27.68ab           | 140.86 ± 6.59a            | 83.36 ± 1.42a         | 22.40 ± 0.08a         | 7.67 ± 0.46a  |
|        |          | T1        | 334.92 ± 18.31a            | 151.42 ± 8.96a            | 85.73 ± 1.16a         | 22.33 ± 0.21a         | 7.87 ± 0.22b  |
|        |          | T2        | 295.97 ± 11.95b            | 143.57 ± 8.39a            | 85.79 ± 1.94a         | 22.55 ± 0.20a         | 7.75 ± 0.04a  |
|        | Xiangyaxiangzhan | CK     | 298.73 ± 4.393b            | 139.36 ± 5.31b            | 87.65 ± 0.79a         | 19.38 ± 0.05a         | 7.03 ± 0.15a  |
|        |          | T1        | 323.81 ± 12.60a            | 141.93 ± 5.26b            | 84.04 ± 2.93a         | 19.13 ± 0.33a         | 7.36 ± 0.46a  |
|        |          | T2        | 304.13 ± 11.3ab           | 170.21 ± 6.66a            | 87.61 ± 1.32a         | 19.33 ± 0.31a         | 7.62 ± 0.58a  |

A lower-case letter illustrates comparisons among the treatment by LSD test at p = 0.05. Three biological replicates were used for statistical analyses. CK: Conventional tillage; T1: Minimum tillage; T2: No-tillage.

### Table 2. Effect of different tillage methods on the aboveground dry matter accumulation of mechanically transplanted fragrant rice (g·m⁻²).

| Time   | Cultivar | Treatment | TS | BS | FHS | MS |
|--------|----------|-----------|----|----|-----|----|
| 2016   | Meixiangzhan 2 | CK        | 132.55 ± 2.27a         | 688.37 ± 5.04a            | 936.51 ± 10.42c       | 1846.90 ± 6.77b       |
|        |          | T1        | 139.45 ± 3.06a         | 741.80 ± 28.38a           | 1112.50 ± 11.79b      | 2024.60 ± 37.04a      |
|        |          | T2        | 130.30 ± 4.05a         | 676.61 ± 17.41a           | 1213.20 ± 2.77a       | 1987.90 ± 43.40a      |
|        | Xiangyaxiangzhan | CK     | 97.09 ± 3.51b          | 526.05 ± 23.64c           | 903.40 ± 21.09b       | 1102.30 ± 38.12c      |
|        |          | T1        | 126.53 ± 7.89a         | 596.97 ± 10.42c           | 693.28 ± 16.65b       | 1629.80 ± 18.55b      |
|        |          | T2        | 143.32 ± 11.16a        | 676.61 ± 17.41a           | 1044.30 ± 26.87a      | 1915.20 ± 20.77a      |
| 2017   | Meixiangzhan 2 | CK        | 181.17 ± 6.76a         | 805.22 ± 60.01a           | 1462.53 ± 54.04b      | 2389.6 ± 293.84a      |
|        |          | T1        | 182.65 ± 6.44a         | 809.15 ± 26.41a           | 1794.35 ± 53.32a      | 2623.74 ± 156.72a     |
|        |          | T2        | 179.09 ± 5.75a         | 817.14 ± 25.46a           | 1721.91 ± 100.51a     | 2694.28 ± 143.11a     |
|        | Xiangyaxiangzhan | CK     | 157.27 ± 12.58a        | 754.6 ± 35.07a            | 1525.74 ± 47.11a      | 1691.37 ± 10.99a      |
|        |          | T1        | 157.24 ± 10.85a        | 721.55 ± 17.62a           | 1530.32 ± 115.09a     | 1735.06 ± 69.17a      |
|        |          | T2        | 166.22 ± 2.08a         | 781.11 ± 16.67a           | 1589.63 ± 25.61a      | 1775.83 ± 100.38a     |

A lower-case letter illustrates comparisons among the treatment by LSD test at p = 0.05. Three biological replicates were used for statistical analyses. CK: Conventional tillage; T1: Minimum tillage; T2: No-tillage; TS: tillering stage; BS: booting stage; FHS: full heading stage; MS: mature stage.
4. Discussion

4.1. Effects of conservation tillage on the aroma of mechanically transplanted fragrant rice and its physiological basis

The accumulation of 2-AP in fragrant rice is affected by various environmental conditions, such as light, temperature, water, and nutrition (Mo et al. 2015; Kong et al. 2017; Mo et al. 2017; Bao et al. 2018; Mo et al. 2019a, 2019b). Previous researches have shown that the 2-AP content in fragrant rice increases by 14.97–31.52% under the no-tillage method of direct-seeded fragrant rice (Du et al. 2019). In this study, conservation tillage practices (T1: minimum tillage and T2: no-tillage) increased the 2-AP content of Meixiangzhan 2 at the MS by 4.81–92.01% compared with conventional tillage (CK). The difference in the 2-AP content between T2 and CK was significant in 2016 and 2017; however, the difference in the 2-AP content between T1 and CK only reached significance in 2017. No significant differences in the 2-AP content at the MS of Xiangyaxiangzhan were detected between any of the tillage treatments. The formation of 2-AP is physiologically complex. Previous studies have indicated that the physiological basis of 2-AP accumulation under tillage treatment

Table 3. Effects of different tillage methods on the grain quality of mechanically transplanted fragrant rice.

| Time   | Cultivar         | Treatment | Brown rice rate (%) | Milled rice rate (%) | Whole milled rice rate (%) | Protein content (%) | Amylose content (%) |
|--------|------------------|-----------|---------------------|----------------------|---------------------------|---------------------|---------------------|
| 2016   | Meixiangzhan 2   | CK        | 79.88 ± 0.51a       | 86.09 ± 1.43a        | 73.81 ± 5.56a             | 8.53 ± 0.03a        | 18.03 ± 0.12b       |
|        |                  | T1        | 80.04 ± 0.27a       | 86.47 ± 0.08a        | 77.20 ± 3.2a              | 7.77 ± 0.03b        | 19.20 ± 0.25a       |
|        |                  | T2        | 79.65 ± 0.30a       | 85.79 ± 0.27a        | 74.42 ± 0.49a             | 7.27 ± 0.03c        | 18.37 ± 0.37ab      |
|        | Xiangyaxiangzhan | CK        | 77.44 ± 0.56b       | 83.15 ± 0.41a        | 60.07 ± 0.37ab            | 8.17 ± 0.03b        | 18.97 ± 0.34a       |
|        |                  | T1        | 80.88 ± 1.10a       | 81.64 ± 0.76a        | 57.84 ± 1.77b             | 8.50 ± 0.00a        | 19.00 ± 0.25a       |
|        |                  | T2        | 77.45 ± 0.32b       | 83.51 ± 0.36a        | 63.19 ± 1.22a             | 8.10 ± 0.00c        | 19.23 ± 0.38a       |
| 2017   | Meixiangzhan 2   | CK        | 78.76 ± 0.45a       | 68.24 ± 0.36a        | 55.51 ± 2.11b             | 7.43 ± 0.03c        | 18.43 ± 0.07a       |
|        |                  | T1        | 74.32 ± 0.39b       | 65.07 ± 0.46b        | 55.15 ± 2.69b             | 8.03 ± 0.03a        | 17.93 ± 0.15a       |
|        |                  | T2        | 78.86 ± 0.38a       | 68.56 ± 0.35a        | 63.45 ± 0.63a             | 7.53 ± 0.03b        | 18.33 ± 0.60a       |
|        | Xiangyaxiangzhan | CK        | 76.81 ± 0.26a       | 66.90 ± 0.54a        | 52.97 ± 1.22b             | 8.47 ± 0.03c        | 17.67 ± 0.09a       |
|        |                  | T1        | 77.18 ± 0.07a       | 68.4 ± 0.09a         | 58.79 ± 1.69a             | 8.97 ± 0.03a        | 17.20 ± 0.06a       |
|        |                  | T2        | 77.52 ± 0.11a       | 67.1 ± 0.87a         | 59.03 ± 0.38a             | 8.77 ± 0.03b        | 17.50 ± 0.32a       |

A lower-case letter illustrates comparisons among the treatment by LSD test at $p = 0.05$. Three biological replicates were used for statistical analyses. CK: Conventional tillage; T1: Minimum tillage; T2: No-tillage.
is closely related to the activity of OAT and PDH and P5CS in grains (Du et al. 2019). Under water and nitrogen treatment, the accumulation of 2-AP and P5C in leaves and other parts of grains before fragrant rice reaches maturity is closely related to the 2-AP content in mature grains (Mo et al. 2019a). The results of our study indicate that conservation tillage practices affected the physiology underlying 2-AP accumulation in machine-transplanted fragrant rice. The accumulation of 2-AP in mature grains was closely related to the 2-AP content in grains at 14 d AFH, the 2-AP content in leaves from 7 d AFH to the MS, the proline content in leaves at the MS, the P5C content in grains at 21 d AFH, the PDH activity in grains from 14 d AFH to the MS, and the OAT activity in leaves at 14 d AFH. Previous studies have shown that the total nitrogen content of the surface soil increases under no-tillage because of the presence of stubble (Limousin and Tessier 2007; López-Fando and Pardo 2009). The soil nutrient content is higher under no-tillage than under traditional tillage (Guo et al. 2015; Huang et al. 2015; Page et al. 2020). The aroma of fragrant rice is closely related to the soil nitrogen content (Yang et al. 2012), and nitrogen fertilizer has been reported to regulate the accumulation of 2-AP in fragrant rice and its physiological basis (Mo et al. 2018; Mo et al. 2019a). In conclusion, the effect of conservation tillage practices on the accumulation of 2-AP and its physiological basis of mechanically transplanted fragrant rice may be related to the effect of conservation tillage practices on soil physical and chemical properties.

### 4.2. Effects of conservation tillage on the yield formation and grain quality of mechanically transplanted fragrant rice

Previous studies showed that the conservation tillage practices alters the physical, chemical, and biological properties of soil and increases crop yield (Sen and Sharma 2002; Gan et al. 2008; Yaduvanshi and Sharma 2008; Zhang et al. 2011). Nandan et al. (2018) reported that no-tillage can increase rice yield by improving the LAI and population quality of rice and increasing the effective panicle number. Wu et al. (2017) showed that there was no significant difference in rice yield between traditional and rotary tillage methods. Du et al. (2019) noted that the yield of direct-seeded fragrant rice is significantly reduced under no-tillage. In our study, conservation tillage practices (T1 and T2) did not lead to decreases in yield compared with conventional tillage (CK), and there was no significant difference in the seed-setting rate and 1000-grain weight among tillage treatments. In 2016, the yield of Xiangyaxiangzhan was significantly increased in T2 by 8.59% compared with CK, which was attributed to the significant increase in the number of effective panicles. In 2017, the number of effective panicles and the number of grains per panicle were significantly increased in T1 and T2, respectively, compared with CK. The effect of tillage methods on yield is related to multiple factors, including tillage years, planting methods, and fertilization methods (Feng et al. 2006; Wu et al. 2010; Zhu et al. 2011; Zhang et al. 2013; Tang et al. 2014). Our study shows that the dry matter accumulation and LAI of mechanically transplanted fragrant rice were improved under conservation tillage (T1 and T2) compared with CK. Correlation analysis indicated that the yield, seed-setting rate, 1000-grain weight, and dry matter accumulation of each period and LAI at the FHS were significantly positively related. Therefore, greater photosynthetic matter accumulation in mechanically transplanted fragrant rice under conservation tillage promoted higher yields.

Liu et al. (2007) noted that no-tillage interplanting and straw returning can increase the protein content and reduce the amylose content of rice. In our study, the whole milled rice rate of fragrant rice was higher under conservation tillage treatment compared with CK; the differences in the brown rice rate, milled rice rate, and amylose content were comparatively less pronounced. The effect of conservation tillage practices on the protein content of fragrant rice was significant. This may stem from the effect of conservation tillage practices on the physical and chemical properties of the soil. However, additional work is needed to clarify the underlying mechanisms.

### 5. Conclusion

Conservation tillage practices (minimum tillage and no-tillage) increased the 2-acetyl-1-pyrroline content in machine-transplanted fragrant rice. The accumulation of 2-acetyl-1-pyrroline in grains at the mature stage was significantly correlated with the 2-acetyl-1-pyrroline content in

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Table 4. Correlation analysis between the 2-AP content in grains at maturity and other 2-AP-related physiological parameters.

| Parameter          | Position | FHS 7 d AFH | 14 d AFH | 21 d AFH | MS          |
|--------------------|----------|-------------|----------|----------|-------------|
| 2-AP content       | Grain    | −0.0185ns   | 0.9671** | −0.384ns |            |
|                    | Leaf     | −0.0530     | 0.8401** | 0.9665** | 0.7293**    | 0.9687**    |
| Proline content    | Grain    | −0.5753ns   | −0.3984ns| 0.5431ns | −0.2272ns   |
|                    | Leaf     | 0.0477ns    | −0.1569ns| 0.2704ns | 0.8553**    |
| P5C content        | Grain    | −0.5616ns   | −0.5507ns| −0.4472ns| −0.3834ns   |
|                    | Leaf     | 0.4842ns    | 0.8303** | 0.7487** | 0.5955**    |
| PDH activity       | Grain    | 0.3695ns    | 0.1765ns | 0.5296ns | 0.5174ns    |
|                    | Leaf     | 0.5671ns    | 0.4734ns | −0.4225ns| −0.4379ns   |
| OAT activity       | Grain    | 0.5676ns    | −0.4468ns| −0.6868**| −0.3752ns   |
|                    | Leaf     | −0.0885ns   | 0.4292ns | 0.3984ns |

Three biological replicates were used for statistical analyses. FHS: full heading stage; AFH: after full heading; MS: mature stage; 2-AP: 2-acetyl-1-pyrroline; P5C: pyrroline-5-carboxylic acid; PDH: proline dehydrogenase; OAT: ornithine aminotransferase; *: significant at p < 0.05 level; **: significant at p < 0.01 level; ns: no significant.

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Table 5. Correlation analysis between grain yield and yield components, dry weight, and LAI.

| Yield component       | Stage | Dry weight | LAI   |
|-----------------------|-------|------------|-------|
| Panicle number        | −0.5414ns | TS | 0.9022** | −0.6164* |
| Grains number per panicle | −0.2699ns | BS | 0.8864** | 0.4823ns |
| Seed setting rate     | 0.8388** | FHS | 0.9574** | 0.7256** |
| 1000-grain weight     | 0.8339** | MS | 0.7298** | −0.0472ns |

Three biological replicates were used for statistical analyses. TS: tillering stage; BS: booting stage; FHS: full heading stage; MS: maturity stage; *: significant at p < 0.05 level; **: significant at p < 0.01 level; ns: no significant.
grains at 14 d after full heading stage, the 2-acetyl-1-pyrroline content in leaves from 7 d after full heading stage to the mature stage, the proline content in leaves at the mature stage, the pyroline-5-carboxylic acid content in grains at 21 d after full heading stage, the proline dehydrogenase activity in grains from 14 d after full heading stage to the mature stage, and the ornithine transaminase activity in leaves at 14 d after full heading stage. The yield of fragrant rice under conservation tillage practices (minimum tillage and no-tillage) was higher because of the improved yield composition and the increase in photosynthetic matter accumulation. Overall, this study will support future assessment of utilizing conservation tillage practices to regulate the yield formation and aroma accumulation of fragrant rice. Further studies are needed to explore the molecular basis with optimize tillage cultivation methods.

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Notes on contributors
Yong Ren is a faculty member of the College of Biology and Pharmacy, Yulin Normal University. His main works include the research on crop production and physiology of fragrant rice.
Siren Cheng is a faculty member of the College of Biology and Pharmacy, Yulin Normal University. Her main works include the research on crop production and physiology of fragrant rice.
Shenggang Pan is a faculty member of the College of Agriculture, South China Agricultural University. His main works include the research on crop production and physiology of fragrant rice.
Hua Tian is a faculty member of the College of Agriculture, South China Agricultural University. Her main works include the research on crop production and physiology of fragrant rice and super rice.
Meiyang Duan is a faculty member of the College of Agriculture, South China Agricultural University. Her main works include the research on crop production and physiology of fragrant rice.
Shuli Wang is a faculty member of the College of Agriculture, South China Agricultural University. Her main works include the research on crop production and physiology of fragrant rice and super rice.
Xiangru Tang is a faculty member of the College of Agriculture, South China Agricultural University. His main works include the research on crop production and physiology of fragrant rice and super rice.

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