RESEARCH

# Different seedling managements and fertilizer rates at tillering stage influences the yield formation and aroma content in fragrant rice

**Jinhai Liu[1,2], Longxin He[1,2], Haowen Luo[1,2], Tiaoyan Wu[1,2], Tantan Zhang[1,2], Runhao Wen[1], Hua Tian[1,2], and Xiangru Tang[1,2*]**

[1]South China Agricultural University, College of Agriculture, State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, Guangzhou, 510642, PR China.
[2]Scientific Observing and Experimental Station of Crop Cultivation in South China, Ministry of Agriculture, Guangzhou, 510642, PR China. [*]Corresponding author (tangxr@scau.edu.cn).

Received: 29 October 2020; Accepted: 28 December 2020; doi:10.4067/S0718-58392021000200128

## ABSTRACT

In fragrant rice (*Oryza sativa* L. subsp. *japonica* Kato) production, seedling management and fertilization both are important parts. However, the effects of different seedling managements and fertilizer rates on grain yield and 2-acetyl-1-pyrroline (2-AP, main and key compound of fragrant rice aroma) content of fragrant rice are still unknown. In present study, four seedling managements (mechanized transplanting (MT), mechanized direct-seeding (MDS), seedling throwing (ST) and seeds broadcasting (BS)) combined with three fertilizer rates (450 (Fer1), 600 (Fer2) and 750 (Fer3) kg $ha^{-1}$) at tillering stage were applied in a 2-yr field experiment. The results showed that the grain yield and 2-AP content differed in different seedling management and fertilizer rate treatments. The highest grain yield and 2-AP content were recorded in MTFer3 treatment which were 4.81-4.86 t $ha^{-1}$ and 307.68-390.99 μg $kg^{-1}$ FW, respectively, while the lowest were recorded in BSFer1 treatment which were 3.96-4.09 t $ha^{-1}$ and 62.92-165.09 μg $kg^{-1}$ FW, respectively. Furthermore, the lowest expression of gene *BADH2* related to 2-AP biosynthesis was recorded in MTFer3 treatment. Under the same fertilizer rate treatment, MT treatment produced the highest grain yield and 2-AP content which ranged between 4.49-4.86 t $ha^{-1}$ and 222.16-390.99 μg $kg^{-1}$ FW, respectively. Under the same seedling management treatment, the highest or equally highest grain yield and 2-AP content were recorded in Fer3 treatment.

**Key words:** 2-Acetyl-1-pyrroline, fertilizer, fragrant rice, grain yield, *Oryza sativa* subsp. *japonica*, seedling.

## INTRODUCTION

Fragrant rice (*Oryza sativa* L. subsp. *japonica* Kato) is a globally appreciated rice species among consumers and in high demand because of the special aromatic flavor with the 2-acetyl-1-pyrroline (2-AP) as the main and key compound (Mo et al., 2018). It is now clearly established that *BADH2* is the key gene controlling 2-AP biosynthesis by encoding betaine aldehyde dehydrogenase (Chen et al., 2008; Bao et al., 2018). In fragrant rice production, getting high grain yield and improving the aroma intensity are always the main goals of farmers and/or investors (Deng et al., 2018; Mo et al., 2019a). They are also the objectives of the studies which related to fragrant rice cultivars. The study of Li et al. (2016) showed that the application of Mn fertilizer significantly increased grain yield and grain 2-AP concentration while regulating the grain quality attributes of fragrant rice. The research of Xie et al. (2020) revealed that foliar fertilization with γ-aminobutyric acid (GABA) at heading stage improved the 2-AP content as well as total yield of fragrant rice. Mo et al. (2017) demonstrated that Si fertilizer substantially enhanced the yield formation and 2-AP biosynthesis in fragrant rice. Few studies also discovered that the application of N fertilizer and water management had great effects on yield formation and 2-AP biosynthesis in fragrant rice (Ren et al., 2017; Deng et al., 2018; Mo et al., 2019b).

Cultivation and breeding are always the main methods to achieve the goals of getting high yield in crop production. As far as rice production is concerned, the crop managements at different growing period are much different. At seedling stage, for instance, there are a few ways to cultivate the rice seedling such as sowing in trays for raising in the nursery and then transplanting in paddy field or just direct-seeding in the paddy field (Pan et al., 2017; Zhao et al., 2020). At the seedling stage, different managements affect growth and development of rice seedlings by changing the growing environment and it also induce differences in production input (Pan et al., 2017). The study of Cheng et al. (2018) showed that different raising methods had substantial impacts on rice seedling quality attributes including DM accumulation, plant height and antioxidant responses. Hu et al. (2019) also demonstrated that different cultivation methods at seedling stage combined with tillage ways would significantly affect grain yield and quality of rice. However, the effects of different crop managements at seedling stage on fragrant rice performance has never been reported.

Besides crop management, the fertilization is also a crucial part in rice production. The study of Huang et al. (2018b) revealed that both application number and rate of fertilizer had great effects on grain yield and yield related traits of rice under zero tillage system. Zhou et al. (2018) demonstrated that high N fertilizer rate applied was able to reduce yield loss caused by cold stress in rice production. An earlier study also discovered that application of different types of fertilizer substantially affected eating quality and nutritional quality of rice (Liu et al., 2019). As far as fragrant rice was concerned, previous studies showed that application of N fertilizer at both booting stage and grain filling stage substantially affected yield formation and aroma production (Deng et al., 2018; Mo et al., 2019a). But the effects of fertilizer application at tillering stage on fragrant rice performances were rarely reported.

Therefore, present study was conducted in Guangdong province (major rice producing province in South China) with a 2-yr field experiment in order to explore the effects of different crop managements at seedling stage and fertilizer rate at tillering stage on grain yield and aroma content of fragrant rice.

## MATERIALS AND METHODS

### Growing conditions and field experiment details

The field experiment between March to July in 2018 was conducted at Experimental Research Farm, College of Agriculture, South China Agricultural University, Guangzhou (23°09′ N, 113°22′ E; 11 m a.s.l.), China, and repeated at the Experimental Research Farm, South China Agricultural University, Ningxi county (23°16′ N, 113°22′ E; 11 m a.s.l.), Guangdong Province, China, between March to July in 2019. Both experimental sites have a subtropical monsoon climate and sandy loam. Commercial compound fertilizer ($N:P_2O_5:K_2O \approx 15\%:6\%:6\%$; Dao Feng Xiang, Foota Biotech, Dongguang city, Guangdong Province, China) was applied at 450 kg $ha^{-1}$ right after the tillage. Fragrant rice (*Oryza sativa* L. subsp. *japonica* Kato) 'Meixiangzhan-2' and 'Xiangyaxiangzhan' were used in present experiment. Before the experiment, the seeds were sterilized with sodium hypochlorite solution and then soak in water for 24 h and then allowed to germinate for 12 h.

Four crop managements at seedling stage were applied in present study. Mechanized transplanting (MT): Germinated seeds were sown in PVC trays filled with paddy soil for nursery raising in greenhouse and 15 d after transplanting, seedlings were mechanically transplanted into paddy field at a space of 30 × 17 cm while each hill was planted with 4-6 seedlings. Mechanized direct-seeding (MDS): Germinated seeds were hill-seeded with direct-seeded machine at a space of 25 × 20 cm while each hill was planted with 4-6 seeds. Seedling throwing (ST): Germinated seeds were sown in PVC trays filled with paddy soil for nursery in greenhouse and 15 d after the transplanting, seedlings were manually throw into paddy field with the density of $1.96 \times 10^5$ seedlings $ha^{-1}$. Seeds broadcasting (BS): Germinated seeds were manually broadcast sown in paddy field with density of 20.01 kg seeds $ha^{-1}$.

Five days after transplanting or 15 d after paddy field sowing, same fertilizer as above was applied at 450, 600, 750 kg $ha^{-1}$ and named as Fer1, Fer2 and Fer3 treatments, respectively. After the fertilization, other agronomic practices, i.e., water management, pest and diseases management, and weed control, were followed as adopted by local farmers. The treatments were arranged in a randomized complete block design (RCBD) in triplicate with a net plot size of 5 m × 7 m.

**Determination of yield and yield related traits**

At physiological maturity, the effective panicle number was calculated from three random areas (1.0 $m^2$) in each plot. Then, rice grains were harvested from five sampling areas (1.0 $m^2$) in each plot, and then threshed by machine and the harvested grains were sun-dried and weighed in order to determinate grain yield. Six random hills of rice plants were collected from each plot for the estimation of grain number per panicle, seed-setting rate and 1000-grain yield.

**Determination of grain 2-AP content**

At the physiological maturity, fresh grains were harvested and stored at -80 °C. The estimation of grain 2-acetyl-1-pyrroline (2-AP) content followed the methods described by Luo et al. (2020) using synchronization distillation and extraction (SDE) method combined with gas chromatograph mass spectrometer (GCMS-QP 2010 Plus, Shimadzu Corporation, Kyoto, Japan). The 2-AP content was expressed as μg $kg^{-1}$ FW.

**Determination of transcript level of gene *BADH2***

The real-time quantitative RT-PCR was carried out after total RNA was extracted using HiPure Plant RNA Mini Kit (Magen, Guangzhou, China). The sequences of primer which designed using software tool primer 5 used for qRT-PCR was F 5′-GGTTGGTCTTCCTTCAGGTGTGC-3′, R 5′-CATCAACATCATCAAACACCACTAT-3′.

**Statistical analyses**

Experimental data were analyzed using statistical software Statistix 8.1 (Analytical Software, Tallahassee, Florida, USA) while differences among means were separated using the least significant difference (LSD) test at the 5% probability level. Correlation analyses were computed and represented using SigmaPlot 9.0 (Systat Software Inc., San Jose, California, USA).

## RESULTS

**Grain yield and yield related traits**

The crop managements at seedling stage and fertilization at tillering stage remarkably influenced the yield formation of fragrant rice (Tables 1, 2, 3, 4). For 'Meixiangzhan-2' in 2018, the highest or equally highest grain yields were recorded in MTFer3 and MDSFer3 treatments while the lowest grain yields were recorded in BSFer1 treatment. ANOVA showed that both seedling management and fertilizer rate had significant influence on effective panicle number, grain number per panicle as well as grain yield. The seedling management also had a significant effect on seed-setting rate whilst fertilizer rate did not. There was nonsignificant difference among all treatments in 1000-grain weight.

For 'Xiangyaxiangzhan' in 2018, ANOVA showed that seedling management and fertilizer rate had significant influences on effective panicle number, grain number per panicle and grain yield but there was nonsignificant interaction effect between two factors observed. We also found that both seedling management and fertilizer rate had nonsignificant effect on 1000-grain weight. The highest or equally highest grain yields were recorded in MTFer2, MTFer3 and MDSFer3 treatments. The lowest grain yields were recorded in BSFer1 and BSFer2 treatments.

For 'Meixiangzhan-2' in 2019, MTFer3 treatment produced higher grain yield than other treatments. MTFer1, MTFer2, MTFer3, MDSFer1, MDSFer2 and MDSFer3 treatments had higher effective panicle number among all treatments. The highest or equally highest grain number per panicle were recorded in MTFer2, MTFer3, MDSFer2 and MDSFer3 treatments. Higher seed-setting rates were recorded in MT and MDS treatments than ST treatments and BS treatments. There was nonsignificant difference among all treatments in 1000-grain weight.

For 'Xiangyaxiangzhan' in 2019, the highest or equally highest grain yields were recorded in MTFer2, MTFer3 and MDSFer3 treatments while the lowest grain yields were recorded in BSFer1 treatment. The results of ANOVA showed that seedling management significantly affected the effective panicle number, grain number per panicle, seed setting rate and grain yield. The fertilizer rate at tillering stage had significant influence on panicle number, grain number per panicle and grain yield. The interaction between seedling management and fertilizer rate had nonsignificant effect on grain yield and yield related traits.

**Table 1. Effects of different seedling managements and fertilizer rates at tillering stage on grain yield and yield traits of 'Meixiangzhan-2' fragrant rice in 2018.**

| Treatment | Effective panicle number | Grain number per panicle | Seed-setting rate | 1000-grain weight | Grain yield |
|---|---|---|---|---|---|
| | nr | nr | % | g | t $ha^{-1}$ |
| MTFer1 | 221.21cd | 128.27bc | 75.54a | 19.42a | 4.53c |
| MTFer2 | 232.43ab | 132.17ab | 74.89a | 19.59a | 4.71ab |
| MTFer3 | 233.84ab | 135.48a | 75.75a | 19.61a | 4.81a |
| MDSFer1 | 217.79de | 126.90bcde | 74.84a | 19.65a | 4.50cd |
| MDSFer2 | 238.88a | 134.78a | 75.01a | 19.50a | 4.60bc |
| MDSFer3 | 228.57bc | 132.13ab | 74.78a | 19.50a | 4.78a |
| STFer1 | 205.06gh | 120.65ef | 72.86bc | 19.55a | 4.25f |
| STFer2 | 222.11cd | 130.73abc | 73.19b | 19.46a | 4.40de |
| STFer3 | 215.64def | 126.79bcde | 73.03b | 19.44a | 4.49cd |
| BSFer1 | 198.66h | 117.75f | 72.38bcd | 19.73a | 3.96g |
| BSFer2 | 207.25fgh | 123.48cdef | 71.61cd | 19.37a | 4.24f |
| BSFer3 | 211.22efg | 122.59def | 71.53d | 19.63a | 4.37e |
| ANOVA | | | | | |
| Management (M) | ** | ** | ** | ns | ** |
| Fertilizer (F) | ** | ** | ns | ns | ** |
| M×F | ns | ns | ns | ns | ns |

Significance levels: **P < 0.01, *P < 0.05, ns P ≥ 0.05.
Values sharing a common letter within a column do not differ significantly (P < 0.05) according to the least significant difference (LSD) test.
MT: Mechanized transplanting; MDS: mechanized direct-seeding; ST: seedling throwing; BS: seeds broadcasting; Fer1: 450 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer2: 600 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer1: 750 kg compound fertilizer $ha^{-1}$ at tillering stage.

**Table 2. Effects of seedling managements and fertilizer rates at tillering stage on grain yield and yield traits of 'Xiangyaxiangzhan' fragrant rice in 2018.**

| Treatment | Effective panicle number | Grain number per panicle | Seed-setting rate | 1000-grain weight | Grain yield |
|---|---|---|---|---|---|
| | nr | nr | % | g | t $ha^{-1}$ |
| MTFer1 | 215.42cd | 128.40bc | 75.67a | 20.01a | 4.49c |
| MTFer2 | 222.92bc | 131.76ab | 75.71a | 19.91a | 4.75a |
| MTFer3 | 234.612a | 132.13ab | 74.61ab | 19.77a | 4.81a |
| MDSFer1 | 220.23bcd | 127.27bcd | 74.49ab | 20.00a | 4.40cd |
| MDSFer2 | 227.86ab | 135.74a | 75.64a | 19.91a | 4.62b |
| MDSFer3 | 222.71bc | 132.37ab | 75.05a | 19.75a | 4.77a |
| STFer1 | 204.94ef | 124.55cd | 73.69b | 19.71a | 4.21ef |
| STFer2 | 216.69cd | 126.88bcd | 73.71b | 19.72a | 4.44cd |
| STFer3 | 217.98cd | 126.82bcd | 73.59bc | 19.87a | 4.50bc |
| BSFer1 | 199.07f | 114.51e | 71.78d | 19.82a | 4.09f |
| BSFer2 | 213.76cde | 122.93cd | 72.31cd | 19.89a | 4.20f |
| BSFer3 | 212.76de | 121.62cd | 72.07d | 19.77a | 4.33de |
| ANOVA | | | | | |
| Management (M) | ** | ** | ** | ns | ** |
| Fertilizer (F) | ** | * | ns | ns | ** |
| M×F | ns | ns | ns | ns | ns |

Significance levels: **P < 0.01, *P < 0.05, ns P ≥ 0.05.
Values sharing a common letter within a column do not differ significantly (P < 0.05) according to the least significant difference (LSD) test.
MT: Mechanized transplanting; MDS: mechanized direct-seeding; ST: seedling throwing; BS: seeds broadcasting; Fer1: 450 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer2: 600 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer1: 750 kg compound fertilizer $ha^{-1}$ at tillering stage.

**Table 3. Effects of seedling managements and fertilizer rates at tillering stage on grain yield and yield traits of 'Meixiangzhan-2' fragrant rice in 2019.**

| Treatment | Effective panicle number | Grain number per panicle | Seed-setting rate | 1000-grain weight | Grain yield |
|---|---|---|---|---|---|
| | nr | nr | % | g | t ha$^{-1}$ |
| MTFer1 | 220.44abc | 125.28de | 75.29abc | 19.53a | 4.54d |
| MTFer2 | 231.90a | 134.90ab | 76.36a | 19.39a | 4.74b |
| MTFer3 | 230.97a | 131.87abc | 75.58ab | 19.61a | 4.86a |
| MDSFer1 | 221.63ab | 128.57bcd | 74.73bc | 19.49a | 4.38e |
| MDSFer2 | 229.66a | 135.91a | 74.58bcd | 19.46a | 4.62cd |
| MDSFer3 | 226.28ab | 136.18a | 75.78ab | 19.53a | 4.67bc |
| STFer1 | 204.72de | 119.44ef | 73.96cde | 19.72a | 4.19f |
| STFer2 | 216.09bcd | 126.21cd | 72.96ef | 19.60a | 4.33e |
| STFer3 | 220.29abc | 127.46cd | 73.31de | 19.58a | 4.55d |
| BSFer1 | 201.12e | 116.94f | 71.48g | 19.50a | 3.96g |
| BSFer2 | 209.87cde | 117.65f | 71.82fg | 19.60a | 4.30e |
| BSFer3 | 205.21de | 124.05de | 70.87g | 19.61a | 4.33e |
| ANOVA | | | | | |
| Management (M) | ** | ** | ** | ns | ** |
| Fertilizer (F) | * | ** | ns | ns | ** |
| M×F | ns | ns | ns | ns | ns |

Significance levels: **P < 0.01, *P < 0.05, ns P ≥ 0.05.
Values sharing a common letter within a column do not differ significantly (P < 0.05) according to the least significant difference (LSD) test.
MT: Mechanized transplanting; MDS: mechanized direct-seeding; ST: seedling throwing; BS: seeds broadcasting; Fer1: 450 kg compound fertilizer ha$^{-1}$ at tillering stage; Fer2: 600 kg compound fertilizer ha$^{-1}$ at tillering stage; Fer1: 750 kg compound fertilizer ha$^{-1}$ at tillering stage.

**Table 4. Effects of seedling managements and fertilizer rates at tillering stage on grain yield and yield traits of 'Xiangyaxiangzhan' fragrant rice in 2019.**

| Treatment | Effective panicle number | Grain number per panicle | Seed-setting rate | 1000-grain weight | Grain yield |
|---|---|---|---|---|---|
| | nr | nr | % | g | t ha$^{-1}$ |
| MTFer1 | 218.61cd | 126.75de | 74.88ab | 19.80a | 4.49c |
| MTFer2 | 230.09ab | 138.08a | 75.72a | 19.70a | 4.74ab |
| MTFer3 | 228.94ab | 135.15ab | 75.13a | 19.94a | 4.81a |
| MDSFer1 | 221.27bc | 127.42cde | 75.23a | 19.85a | 4.45c |
| MDSFer2 | 232.16a | 129.65bcd | 75.90a | 19.80a | 4.66b |
| MDSFer3 | 231.48a | 133.12abc | 75.52a | 19.93a | 4.67ab |
| STFer1 | 204.03ef | 121.28ef | 73.76bc | 19.78a | 4.21d |
| STFer2 | 218.64cd | 127.15cde | 73.46c | 20.02a | 4.40c |
| STFer3 | 220.48bc | 125.45de | 73.64bc | 19.91a | 4.51c |
| BSFer1 | 195.81f | 116.75f | 71.44d | 19.88a | 4.01e |
| BSFer2 | 210.10de | 121.20ef | 71.15d | 19.98a | 4.22d |
| BSFer3 | 208.97de | 121.80ef | 71.80d | 19.96a | 4.38c |
| ANOVA | | | | | |
| Management (M) | ** | ** | ** | ns | ** |
| Fertilizer (F) | ** | ** | ns | ns | ** |
| M×F | ns | ns | ns | ns | ns |

Significance levels: **P < 0.01, *P < 0.05, ns P ≥ 0.05.
Values sharing a common letter within a column do not differ significantly (P < 0.05) according to the least significant difference (LSD) test.
MT: Mechanized transplanting; MDS: mechanized direct-seeding; ST: seedling throwing; BS: seeds broadcasting; Fer1: 450 kg compound fertilizer ha$^{-1}$ at tillering stage; Fer2: 600 kg compound fertilizer ha$^{-1}$ at tillering stage; Fer1: 750 kg compound fertilizer ha$^{-1}$ at tillering stage.

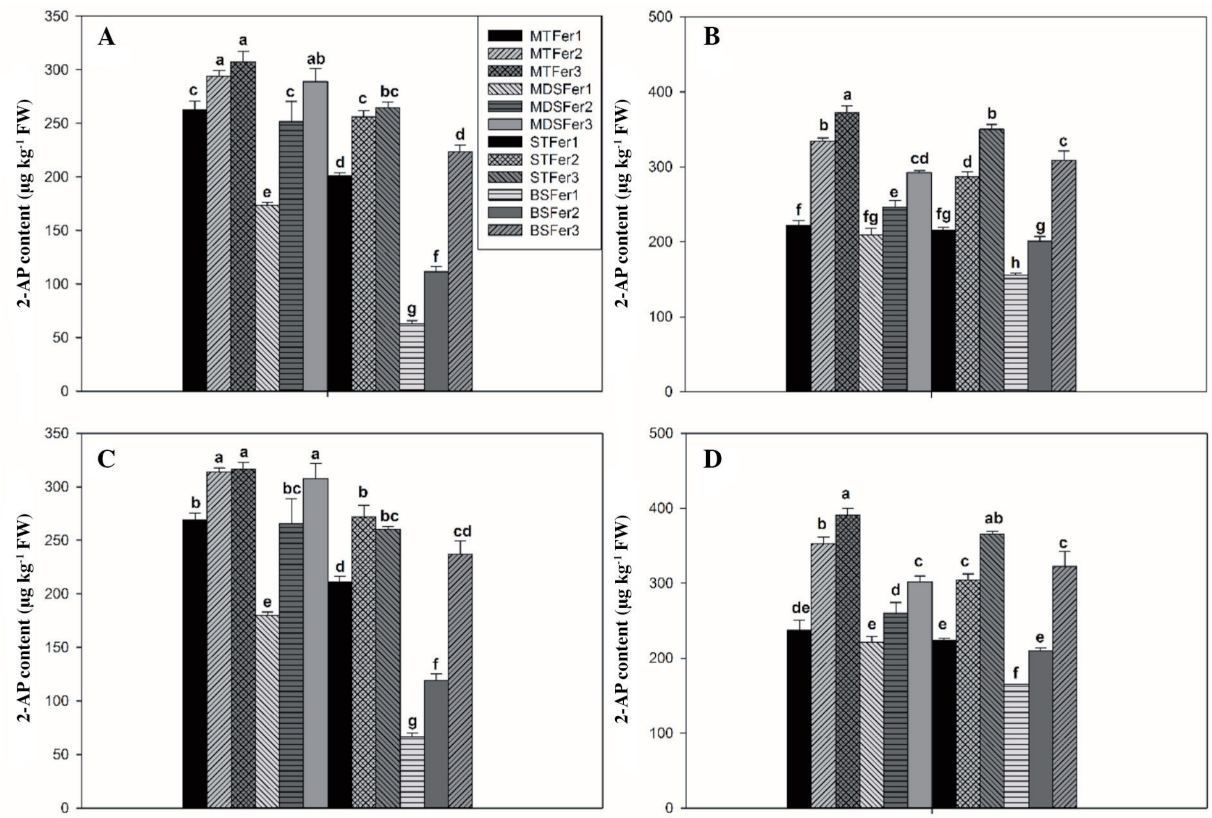
## Grain 2-AP content

As shown in Figure 1, there were some difference among different seedling managements and different fertilizer rates on grain 2-AP content of fragrant rice. The highest grain 2-AP content was recorded in MTFer3 treatment and the lowest grain 2-AP content was recorded in BSFer1 treatment. We observed that the 2-AP content increased with the increment in fertilizer rate at tillering stage under same crop management although for ‘Meixiangzhan-2’ in 2018 and 2019, the difference between MTFer2 and MTFer3 treatments did not reach the significant level. Moreover, under the same fertilizer rate treatment, the grain 2-AP content in BS treatment was always lower than other treatments.

## Expression of gene *BADH2*

As shown in Figure 2, there were some differences among different seedling managements and different fertilizer rates on transcript level of gene *BADH2* in fragrant rice. We observed that the expression of gene *BADH2* decreased with the increment of fertilizer rate under same seedling management although for ‘Meixiangzhan-2’ in 2018 and 2019, the difference between MTFer2 and MTFer3 treatments did not reach the significant level. Under the same fertilizer rate treatment, the transcript level of *BADH2* in BS treatment was always higher than other treatments while the lowest expression was recorded in MT treatment. Moreover, the lowest or equally lowest transcript level was recorded in MTFer3 treatment and the highest transcript level was recorded in BSFer1 treatment.

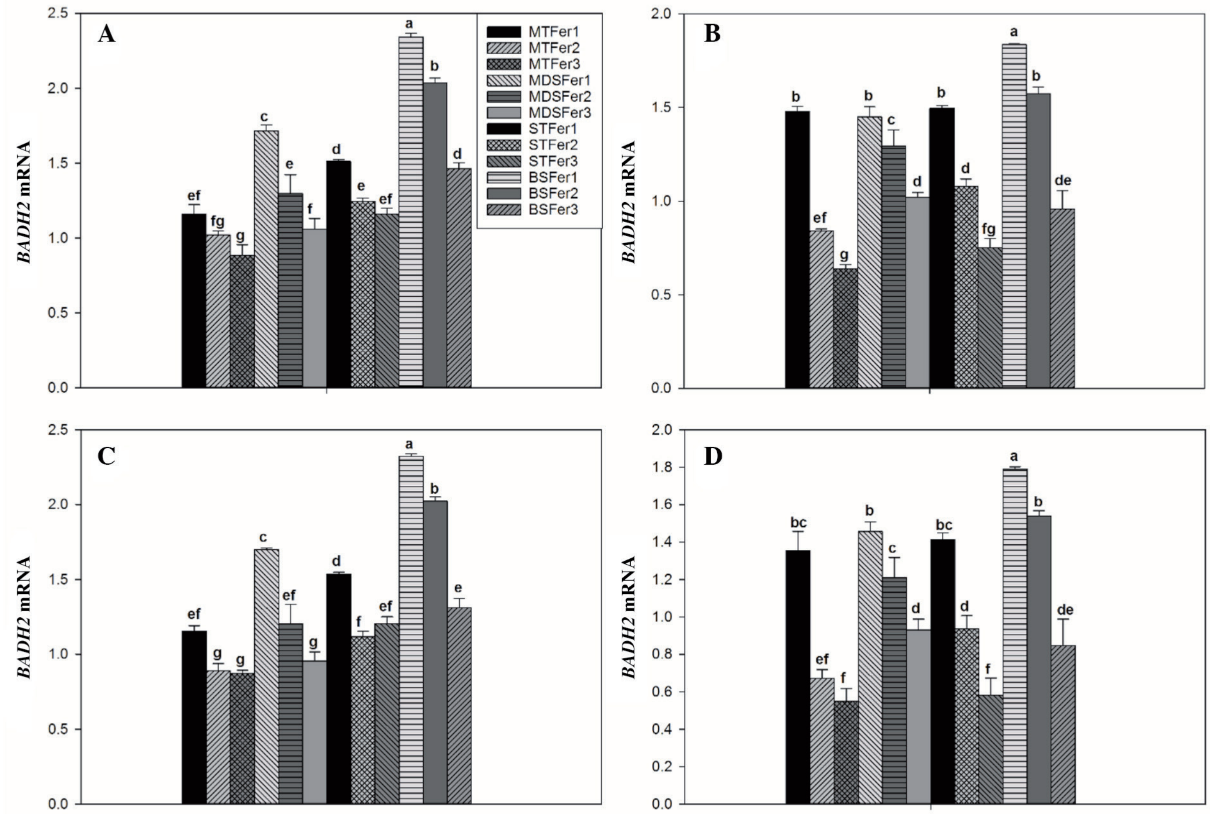
**Figure 1. Effects of seedling managements and fertilizer rates at tillering stage on grain 2-acetyl-1-pyrroline (2-AP) concentration of fragrant rice for ‘Meixiangzhan-2’ in 2018 (A), ‘Xiangyaxiangzhan’ in 2018 (B), ‘Meixiangzhan-2’ in 2019 (C), and ‘Xiangyaxiangzhan’ in 2019 (D).**




Means sharing a common letter do not differ significantly at (P ≤ 0.05) according to the least significant difference (LSD) test for both cultivars.

MT: Mechanized transplanting; MDS: mechanized direct-seeding; ST: seedling throwing; BS: seeds broadcasting; Fer1: 450 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer2: 600 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer1: 750 kg compound fertilizer $ha^{-1}$ at tillering stage.

**Figure 2. Effects of seedling managements and fertilizer rates at tillering stage on transcript level of gene *BADH2* in fragrant rice for 'Meixiangzhan-2' in 2018 (A), 'Xiangyaxiangzhan' in 2018 (B), 'Meixiangzhan-2' in 2019 (C), and 'Xiangyaxiangzhan' in 2019 (D).**




Means sharing a common letter do not differ significantly at (P ≤ 0.05) according to the least significant difference (LSD) test for both cultivars.
MT: Mechanized transplanting; MDS: mechanized direct-seeding; ST: seedling throwing; BS: seeds broadcasting; Fer1: 450 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer2: 600 kg compound fertilizer $ha^{-1}$ at tillering stage; Fer1: 750 kg compound fertilizer $ha^{-1}$ at tillering stage.

## DISCUSSION

Present study explored the effects of different seedling management and fertilizer rate at tillering stage on yield formation and aroma of fragrant rice. The results of field experiment showed that there were significant differences on grain yield and grain 2-AP content of fragrant rice caused by seedling management and fertilization while no interaction effect between seedling management and fertilization was observed. As far as seedling management was concerned, the results of ANOVA showed that seedling management significantly affected the grain yield by affecting the effective panicle number per area, grain number per panicle and seed-setting rate. Under the same fertilizer rate treatment, the highest or equally highest grain yields were recorded in MT and MDS treatments (except 'Meixiangzhan-2' in 2019) followed by ST treatment while BS treatment produced the lowest grain yield. These differences in yield formation could be caused by the differences in population structure. Crop production is group production. The spatial structure of crop population refers to the spatial configuration of crop population in the field, which includes horizontal structure and vertical structure. Proper treatment of the competition between rice individuals for nutrients and light has a substantial effect on yield formation of rice. Previous studies normally regulated the population structure by controlling the plant density. For example, the research of Duan et al. (2019) showed that transplant density significantly affected yield formation of rice while the high transplant density caused yield loss because of the severe intraspecific competition. Huang et al. (2018a) demonstrated that increasing hill density was able to compensate for yield loss caused by lower input N fertilizer in mechanized transplant rice system. In our study, the plants of fragrant rice were planted at the certain row spacing and line spacing in MT and MDS treatments which meant that the horizontal distribution of fragrant rice plants was uniform. Whilst in ST and BS treatments, seedlings or seeds were manually thrown or broadcasted to paddy field which were not able to achieve the precise and uniform planting as the agricultural machine. Furthermore, under machine-transplanted and machine-direct seeded rice system, rice is distributed in rows in the field with the same plant spacing and thus, the ventilation and lighting will be good (Pan et al., 2017; Huang et al., 2018b). Better ventilation and lighting conditions would lead to the better gas exchange as well as higher net photosynthetic rate of rice and increase DM accumulation and enhance yield formation (Luo et al., 2019). Therefore, we deduced that uniform horizontal distribution of fragrant rice in paddy field under MT and MDS treatments enhanced the canopy apparent photosynthesis and led to the improvement in DM accumulation and yield formation. Moreover, in related to MDS treatments, the fragrant rice seedlings were raised in PVC trays in green house in MT treatments which meant seedlings grew in the warmer and stabler environment and it might help to early establishment of fragrant rice.

As far as fertilizer rate was concerned, we observed that the grain yield increased with the increment of fertilizer rate at tillering stage although the differences between Fer2 and Fer3 treatments were not always significant. The difference in grain yield among different fertilizer rate treatments could be explained by effective panicle number and grain number per panicle. Our results agreed with the study of Pan et al. (2016), who demonstrated that rice yield increased with N fertilizer rate. The research of Hirzel et al. (2020) discovered positive response of rice yield to increased N rates and P rates whilst no response to K rates in directed-seed rice system. The investigation of Zhang et al. (2019) also showed that without excessive fertilization, yield of rice will increase with the increase of fertilizer rate. Pan et al. (2017) indicated that fertilization substantially influenced the photosynthetic rate and yield formation of rice. In agricultural production, crop yield mainly depended on application of fertilizer especially N fertilizer because growth and development of crops require a mass of nutrient elements while timely and abundant replenishment of nutrient not only ensures basal crop establishment, but also improves the photosynthesis and resulted increment in grain yield (Ren et al., 2017; Deng et al., 2018). The results from our study confirmed the roles of fertilization in fragrant rice production and present reasonable schemes for application of compound fertilizer (Fer2 and Fer3 treatments) but more studies in the future are required to investigate the roles of K and P fertilizer in fragrant rice performances.

2-AP is the key compound of fragrant rice aroma. In present study, grain 2-AP content differed with the different seedling managements and fertilizer rates. The synthesis of 2-AP is a very complicated bioprocess in fragrant rice. The highest 2-AP content was recorded in MTFer3 treatment for both cultivars in both years. Among four seedling management treatments, the highest or equally highest 2-AP contents were recorded in MT treatment. Opposite trend was recorded in transcript level of gene *BADH2*. Previous study revealed that gene *BADH2* inhibited 2-AP production by encoding betaine aldehyde dehydrogenase to transform γ-amino butyl aldehyde to GABA rather than 1-pyrroline (Chen et al., 2008). Our results agreed with the study of Bao et al. (2018), which also found contradictory trends between *BADH2* expression and 2-AP content. The enhancement in 2-AP content in MT treatment maybe be attributed to the better population structure and the nursery raising as it mentioned above. In other words, better growing condition and early establishment might induce regulation in gene expression and thus benefit 2-AP biosynthesis in fragrant rice. But more studies are required to confirm this deduction in the future. As far as fertilization was concerned, we observed that the 2-AP content increased with the increase of fertilizer rate at tillering stage although the differences between Fer2 and Fer3 treatments were not always significant. Meanwhile, transcript level of gene *BADH2* decreased with the increase of fertilizer rate. The compound fertilizer used in present consist of N, P and K. The study of Ren et al. (2017) and Deng et al. (2018) revealed that different rate of N fertilizer had substantial effects on 2-AP biosynthesis in fragrant rice. Mo et al. (2019a) demonstrated that application of N fertilizer significantly increased grain-2AP content of fragrant rice. An earlier study also revealed that N fertilizer affected 2-AP content in fragrant rice through regulation in plant nutrient condition (Mo et al., 2019b). Therefore, difference in grain 2-AP content is attributed to different applied N amounts due to fertilizer treatments. On the other hand, different fertilizer treatments also caused different input of K and P and it might also contribute to different grain 2-AP content. Up to now, no studies were ever reported about the effects of K or P on 2-AP formation in fragrant rice and more studies should be conducted in order to investigate the effects of K and P on 2-AP biosynthesis in fragrant rice in the future.

## CONCLUSIONS

Different seedling managements and fertilizer rates substantially influenced yield information and 2-acetyl-1-pyrroline (2-AP) content in fragrant rice. In comparison with mechanized direct-seeding, seedling throwing and seeds broadcasting, mechanized transplanting (MT) is the best seedling management because of higher grain yield and 2-AP content. With increment of fertilizer rate, grain yield and 2-AP content also exhibited increased trends under same seedling management. Moreover, opposite trends of 2-AP content and *BADH2* expression were discovered among different seedling management and fertilizer rate treatments. The highest grain yield and 2-AP content were recorded with the treatment MT plus 750 kg ha$^{-1}$ fertilizer rate in the present study.

## ACKNOWLEDGEMENTS

This study was supported by National Natural Science Foundation of China (31971843), Special fund for Rural Revitalization Strategy in Guangdong Province, Technology System of Modern Agricultural Industry in Guangdong (2019KJ105) and World Bank Loan Agricultural Pollution Control Project in Guangdong (0724-1510A08N3684).

## REFERENCES

Bao, G., Ashraf, U., Wang, C., He, L., Wei, X., Zheng, A., et al. 2018. Molecular basis for increased 2-acetyl-1-pyrroline contents under alternate wetting and drying (AWD) conditions in fragrant rice. Plant Physiology and Biochemistry 133:149-157.

Chen, S., Yang, Y., Shi, W., Ji, Q., He, F., Zhang, Z., et al. 2008. *Badh2*, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. The Plant Cell 20(7):1850-1861.

Cheng, S.R., Ashraf, U., Zhang, T.T., Mo, Z.W., Kong, L.L., Mai, Y.X., et al. 2018. Different seedling raising methods affect characteristics of machine-transplanted rice seedlings. Applied Ecology and Environmental Research 16(2):1399-1412.

Deng, Q.Q., Ashraf, U., Cheng, S.R., Sabir, S.R., Mo, Z.W., Pan, S.G., et al. 2018. Mild drought in interaction with additional nitrogen dose at grain filling stage modulates 2-acetyl-1-pyrroline biosynthesis and grain yield in fragrant rice. Applied Ecology and Environmental Research 16(6):7741-7758.

Duan, M.Y., Luo, H.W., Pan, S.G., Mo, Z.M., Yang, X.J., Tian, J.Y., et al. 2019. High transplant density cause loss yield and quality decrement by affecting photosynthesis, dry matter accumulation and transportation in super rice. Applied Ecology and Environmental Research 17(3):6069-6079.

Hirzel, J., Paredes, M., Becerra, V., and Donoso, G. 2020. Response of direct seeded rice to increasing rates of nitrogen, phosphorus, and potassium in two paddy rice soils. Chilean Journal of Agricultural Research 80:263-273.

Hu, L., Du, P., Luo, H.W., Cheng, S.R., Wu, T.Y., He, J., et al. 2019. The effect of different cultivation methods on rice growth and development. Applied Ecology and Environmental Research 17(2):3867-3875.

Huang, M., Chen, J., Cao, F., and Zou, Y. 2018a. Increased hill density can compensate for yield loss from reduced nitrogen input in machine-transplanted double-cropped rice. Field Crops Research 221:333-338.

Huang, M., Zhou, X., and Zou, Y. 2018b. Improving nitrogen management for zero-tillage rice in China. The Crop Journal 6(4):406-412.

Li, M., Ashraf, U., Tian, H., Mo, Z., Pan, S., Anjum, S.A., et al. 2016. Manganese-induced regulations in growth, yield formation, quality characters, rice aroma and enzyme involved in 2-acetyl-1-pyrroline biosynthesis in fragrant rice. Plant Physiology and Biochemistry 103:167-175.

Liu, Q., Ma, H., Lin, X., Zhou, X., and Zhao, Q. 2019. Effects of different types of fertilizers application on rice grain quality. Chilean Journal of Agricultural Research 79:202-209.

Luo, H., He, L., Du, B., Pan, S., Mo, Z., Duan, M., et al. 2020. Biofortification with chelating selenium in fragrant rice: Effects on photosynthetic rates, aroma, grain quality and yield formation. Field Crops Research 255:107909.

Luo, H.W., He, L.X., Du, B., Wang, Z.M., Zheng, A.X., Lai, R.F., et al. 2019. Foliar application of selenium (Se) at heading stage induces regulation of photosynthesis, yield formation, and quality characteristics in fragrant rice. Photosynthetica 57(4):1007-1014.

Mo, Z., Ashraf, U., Tang, Y., Li, W., Pan, S., Duan, M., et al. 2018. Nitrogen application at the booting stage affects 2-acetyl-1-pyrroline, proline, and total nitrogen contents in aromatic rice. Chilean Journal of Agricultural Research 78:165-172.

Mo, Z., Lei, S., Ashraf, U., Khan, I., Li, Y., Pan, S., et al. 2017. Silicon fertilization modulates 2-acetyl-1-pyrroline content, yield formation and grain quality of aromatic rice. Journal of Cereal Science 75:17-24.

Mo, Z., Li, Y., Nie, J., He, L., Pan, S., Duan, M., et al. 2019a. Nitrogen application and different water regimes at booting stage improved yield and 2-acetyl-1-pyrroline (2AP) formation in fragrant rice. Rice 12:74.

Mo, Z., Tang, Y., Ashraf, U., Pan, S., Duan, M., Tian, H., et al. 2019b. Regulations in 2-acetyl-1-pyrroline contents in fragrant rice are associated with water-nitrogen dynamics and plant nutrient contents. Journal of Cereal Science 88:96-102.

Pan, S., Liu, H., Mo, Z., Patterson, B., Duan, M., Tian, H., et al. 2016. Effects of nitrogen and shading on root morphologies, nutrient accumulation, and photosynthetic parameters in different rice genotypes. Scientific Reports 6:32148.

Pan, S., Wen, X., Wang, Z., Ashraf, U., Tian, H., Duan, M., et al. 2017. Benefits of mechanized deep placement of nitrogen fertilizer in direct-seeded rice in South China. Field Crops Research 203:139-149.

Ren, Y., Ashraf, U., He, L.X., Mo, Z.W., Wang, F., Wan, X.C., et al. 2017. Irrigation and nitrogen management practices affect grain yield and 2-acetyl-1-pyrroline content in aromatic rice. Applied Ecology and Environmental Research 15(4):1447-1460.

Xie, W., Kong, L., Ma, L., Ashraf, U., Pan, S., Duan, M., et al. 2020. Enhancement of 2-acetyl-1-pyrroline (2AP) concentration, total yield, and quality in fragrant rice through exogenous γ-aminobutyric acid (GABA) application. Journal of Cereal Science 91:102900.

Zhang, T.T., Li, Y.Z., Xie, W.J., Du, P., Lu, R.H., Chen, Y.L., et al. 2019. Excessive fertilization resulted in decreased antioxidant performance of three varieties of super rice. Applied Ecology and Environmental Research 17(2):1889-1898.

Zhao, R., Luo, H., Wang, Z., and Hu, L. 2020. Benefits of continuous plow tillage to fragrant rice performance. Agronomy Journal 112:4171-4181.

Zhou, Y., Li, X., Cao, J., Li, Y., Huang, J., and Peng, S. 2018. High nitrogen input reduces yield loss from low temperature during the seedling stage in early-season rice. Field Crops Research 228:68-75.