



# Article Preliminary Application of Vermicompost in Rice Production: Effects of Nursery Raising with Vermicompost on Fragrant Rice Performances

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Abstract: Vermicompost is an excellent organic fertilizer, but the application of vermicompost in fragrant rice production has not yet been reported. Seedling nursery is an important component of rice production. The present study firstly applied vermicompost in fragrant rice production through nursery raising. The seedlings of three fragrant rice cultivars were raised in matrix with different rations of vermicompost (the treatment without vermicompost was taken as the control), and the growth parameters and physiological characters of seedlings were investigated. The results showed that, compared with the control, the application of vermicompost significantly (p < 0.05) increased the plant height, stem diameter, fresh weight, and dry weight of fragrant rice seedlings by 11.22–24.73%, 38.34-65.87%, 16.74-30.46%, and 16.61-35.16%, respectively. Nursery raising with vermicompost significantly (p < 0.05) enhanced the net photosynthetic rate by 5.99–12.93%, relative to the control. Higher contents of chlorophyll a, chlorophyll b, carotenoids, and total chlorophyll were recorded in vermicompost treatments than in the control. Compared with the control, vermicompost treatments also increased root length, surface area, mean diameter, root volume, root tip number, and root activity of fragrant rice seedlings by 12.42–27.82%, 15.04–38.65%, 12.64–23.12%, 42.41–63.58%, 18.62–24.95%, and 12.01-26.29%, respectively. Moreover, nursery raising with vermicompost enhanced the activities of antioxidant enzymes including superoxide, peroxidase, and catalase by 7.97-24.21%, 17.11-44.99%, and 7.37–15.95%, respectively, relative to the control. Moreover, 7.92–29.40% lower malondialdehyde contents were recorded in vermicompost treatments compared with the control. Therefore, nursery raising with vermicompost could be a good agronomy practice in machine-transplanted fragrant rice.

Keywords: fragrant rice; photosynthesis; root morphology; root activity; vermicompost

## 1. Introduction

Rice is the main staple food in Asia [1]. In order to feed the growing population, the productivity of rice must exceed current levels [2]. In recent years, because of rapid economic growth, the labor availability for agricultural production has declined [3], thus traditional rice cultivation with manual transplanting is not suitable for China and many other regions anymore because of the high labor consumption [4]. Mechanical transplanting is a technology that is simple and incurs less labor costs in rice production. Briefly, rice seeds are raised in nursery trays filled with matrix, and then the seedlings are transplanted into a paddy field by compatible machinery [5]. "Pot-raised seedling" is a type of seedling raising in machine-transplanted rice production, which sows rice seeds in a bowl-shaped tray, and the holes on the tray ensure the relatively independent growth space of the



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**Copyright:** © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). seedlings, which makes the seedlings grow more vigorously and cause less root damage during mechanical transplanting [5].

Vermicompost is a product transformed by organic residues using earthworms [6]. A previous study showed that vermicompost is an excellent organic fertilizer, with physical, chemical, and biological properties that could improve soil fertility and control crop diseases [7]. The study of Fernandaez et al. [8] showed that vermicompost has high microbial functional diversity and the potential to be used for the treatment of pesticide pollution in agricultural production. Jahanbakhshi et al. [9] demonstrated that vermicompost was a good organic fertilizer with an appropriate carbon and nitrogen ratio, acidity, as well as salinity. Al Jaonui et al. [10] also indicated that vermicompost can improve the chemical composition of jujube coconut fruit and increase the nutritional and medicinal value. Nursery seedlings is an important component in the production of mechanical transplanting rice, and the seedling matrix can substantially affect the growth of rice seedlings and, consequently, influence the rice productivity [5]. In consideration of the multiple benefits of vermicompost into the matrix can promote the rice seedlings' performance in mechanized transplanting rice production.

Fragrant rice is a special rice that is highly popular in the world for the good grain quality and 'nutty-like' aroma [11]. In present study, we used three fragrant rice cultivars as plant materials and carried out an experiment using matrix with different ratios of vermicompost to raise seedlings in bowl-shaped tray in order to explore the effects of the incorporation of vermicompost into the matrix on fragrant rice seedlings' performances.

## 2. Materials and Methods

#### 2.1. Plant Materials and Experimental Design

Seeds of three fragrant rice cultivars, 'Meixiangzhan-2', 'Xiangyaxiangzhan', and '19-xiang', which are widely planted in South China, were used as plant materials. After soaking in tap water for 24 h, the seeds were germinated under 30 °C for 12 h. Then, the germinated seeds were sewn in a bowl-shaped tray, with one seed for each hole. The plastic tray is 580 mm in length and 310 mm in width. Each tray contains 448 holes, while each hole is 26 mm in height and 16.5 mm in diameter. The experiment was conducted in College of Agriculture, South China Agricultural University, Guangzhou, China and the fragrant rice seedlings were grown under controlled conditions (temperature:  $25 \pm 2$  °C, relative humidity: 60–70%). Before sowing, the trays were firstly filled with matrix contain different ratios of vermicompost, and four treatments were adopted in the present study and are described below:

(CK) The trays were filled with matrix without vermicompost;

(V1) The trays were filled with matrix containing 25.00% volume of vermicompost;

(V2) The trays were filled with matrix containing 33.33% volume of vermicompost;

(V3) The trays were filled with matrix containing 50.00% volume of vermicompost.

Each tray was filled with about 1500 cm<sup>3</sup> matrix and there were three independent trays for each treatment. "Special matrix for rice seedling" was used in the present study. This matrix was produced by Guangzhou Shengsheng Agriculture Co., Ltd. (www.ssagr. com, accessed on 10 January 2021), and is widely used in rice cultivation in Guangdong Province, China. The matrix is composed of peat, vermiculite, coconut bran, and clay, and the main technical characteristics were as follows: pH 4.5–5.5, EC value 0.1–0.5, organic matter (45%), unit weight (320–350 g m<sup>-3</sup>), total nutrient (2–4%), and moisture (30%). Before sowing, the matrix was mixed with vermicompost at responding ratios in each treatment and then the nursery trays were filled. The analyses of vermicompost manufactured by Dongguan Foota Biotechnology Co., Ltd. China, were as follows: organic matter (34.9%), total nitrogen (1.48%), available phosphorus (1.21%), available potassium (0.90%), iron (0.31%), manganese (0.03%), copper (0.01%), and magnesium (0.84%); pH 7.60. The experiment was repeated thrice for individual parameters, and three independent

replications of each treatment were considered for assessing different parameters following the same experimental conditions.

#### 2.2. Measurements of Agronomic Traits

Twenty days after sowing, the net photosynthetic rate of fragrant rice seedlings in each treatment was measured with a LI-6400XT Portable Photosynthesis System (LI-COR, Inc., Lincoln, NE, USA) at 09:00–10:30, according the method of Kong et al. [12] with the following adjustments: photosynthetically active radiation at leaf surface was 1100–1200 µmol m<sup>-2</sup> s<sup>-1</sup>, ambient CO<sub>2</sub> concentration was 385.5–399.7 µmol mol<sup>-1</sup>, and 60–80% relative humidity. Meanwhile, fragrant rice seedlings from each treatment were collected and the plant height, stem diameter, and fresh weight were measured. The morphological characteristics of the root of seedlings were measured using a root analysis instrument, WinRhizo-LA1600 (Regeng Instuments Inc., Quebec, QC, Canada). The dry weight of seedlings was measured after being oven-dried at 70 °C for 48 h.

## 2.3. Physio-Biochemical Analysis

Twenty days after sowing, the fresh leaves and roots of 100 seedlings from each treatment were collected and stored at -80 °C for physiological and biochemical analysis. The contents of chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll in leaves were determined according to the methods described by Luo et al. [13]. About 0.10 g samples were extracted by 10 mL 95% alcohol for six hours. The absorbance was read at 665, 649, 652, and 470 nm. The concentrations of photosynthetic pigments were expressed as mg g<sup>-1</sup> FW. The malondialdehyde (MDA) concentration and antioxidant enzymes' (superoxide (SOD, EC 1.15.1.1), peroxidase (POD EC1.11.1.7), and catalase (CAT, EC 1.11.1.6)) activities were determined according to the methods of Huang et al. [14]. MDA concentration was determined after reacting with thiobarbituric acid and the absorbance was read at 532, 600, and 450 nm. The final result was expressed as  $\mu$ mol g<sup>-1</sup> FW. SOD activity was determined after the reaction with nitro blue tetrazolium. The reaction mixture contained 1.75 mL of sodium phosphate buffer (pH 7.8), 0.3 mL of 130 mM  $L^{-1}$  methionine buffer, 0.3 mL of 750  $\mu$ mol L<sup>-1</sup> NBT buffer, 0.3 mL of 100  $\mu$ mol L<sup>-1</sup> EDTA-Na2 buffer, 0.3 mL of 20  $\mu$ mol L<sup>-1</sup> lactoflavin, and 0.05 ml of enzyme extract. After the reaction, the change in color was measured at 560 nm. The activities of POD and CAT were determined after reacting with H<sub>2</sub>O<sub>2</sub> and absorbances were read at 470 and 240 nm, respectively, and the activities were both expressed as U  $L^{-1}$ . Root activity was measured by the triphenyl tetrazolium chloride (TTC) method according to Yuan et al. [15]. The absorbances were read at 485 nm and activity was expressed as  $\mu g g^{-1} h^{-1}$  FW.

## 2.4. Statistical Analyses

All the experimental data were subjected to a one-way analysis of variance (ANOVA) with software Statistix 8.1 (Analytical Software, Tallahassee, FL, USA). Differences among means were separated using the least significant difference (LSD) test at a 5% probability level. Graphical representation was conducted via the software Sigma Plot 14.0 (Systat Software Inc., San Jose, CA, USA).

#### 3. Results

#### 3.1. Growth Parameters of Fragrant Rice Seedlings

The plant height, stem diameter, fresh weight, and dry weight of fragrant rice seedlings are presented in Table 1. In comparison with CK, V2 and V3 treatments significantly (p < 0.05) increased plant height by 11.22–17.98% and 19.63–24.73%, respectively. Moreover, 38.35–59.28% and 36.94–65.87% higher stem diameters were recorded in V2 and V3 treatments, respectively, than CK. The application of vermicompost also increased the biomass of fragrant rice seedlings. For fresh weight, compared with CK, V2 and V3 treatments significantly (p < 0.05) increased the fresh weight of fragrant rice seedlings by 16.74–22.91% and 30.26–32.27%, respectively. For dry weight, compared with CK, V2 and V3 treatments

significantly (p < 0.05) increased the dry weight of fragrant rice seedlings by 16.61–24.63% and 30.36–35.16%, respectively.

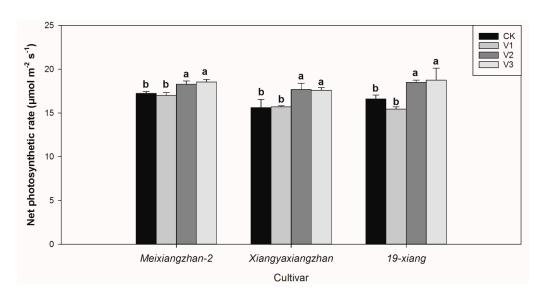
Cultivar	Treatment	Plant Height	Stem Diameter	Fresh Weight Dry Weig	
		cm	mm	mg	mg
Meixia	Meixiangzhan-2				
	CK	25.43c	2.71b	90.82c	11.65d
	V1	27.18b	3.41a	97.88c	12.95c
	V2	29.02a	3.75a	106.02b	14.52b
	V3	30.43a	3.89a	118.49a	15.75a
Xiangya	Xiangyaxiangzhan				
	CK	25.22c	2.59b	81.93d	11.18d
	V1	26.19c	2.60b	88.64c	12.12c
	V2	28.05a	3.59a	100.670b	13.66b
	V3	30.45a	3.55a	108.36a	14.64a
19-xiang					
	CK	24.27c	2.22c	85.64c	11.63c
	V1	27.54b	3.17b	89.33c	11.94c
	V2	28.63ab	3.53ab	100.09b	13.56b
	V3	30.27a	3.68a	111.55a	15.16a

Table 1. Effects of vermicompost incorporation on growth parameters of fragrant rice seedling.

Values sharing a common letter within a column do not differ significantly (p < 0.05) according to the least significant difference (LSD) test for three fragrant rice cultivars.

## 3.2. Net Photosynthetic Rate

The net photosynthetic rate of fragrant rice seedlings was enhanced by vermicompost treatments (Figure 1). Compared with CK, V2 treatment significantly (p < 0.05) increased the net photosynthetic rate by 5.99%, 13.17%, and 11.41% for 'Meixiangzhan-2', 'Xiangyaxiangzhan', and '19-xiang', respectively. Further, 7.48%, 12.59%, and 12.93% higher net photosynthetic rates were also recorded in V3 treatment than CK for 'Meixiangzhan-2', 'Xiangyaxiangzhan', and '19-xiang', respectively.



**Figure 1.** Effects of vermicompost incorporation on the net photosynthetic rate of fragrant rice seedlings; those sharing a common letter do not differ significantly at p < 0.05 according to the least significant difference (LSD) test for three fragrant rice cultivars.

## 3.3. Chlorophyll Content and Carotenoid Content

The contents of chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll in the leaves of fragrant rice seedlings increased in V2 and V3 treatments (Figure 2). For chlorophyll a, compared with CK, V2 and V3 treatments significantly (p < 0.05) increased the chlorophyll a content by 12.23–17.12% and 16.67–28.82%, respectively. For chlorophyll b, 4.95–11.41% and 8.62–16.52% higher chlorophyll b contents were recorded in V2 and V3 treatments, respectively, than CK. For carotenoid, compared with CK, V2 and V3 treatments increased the carotenoid content by 13.09–20.17% and 22.46–41.72%, respectively. For total chlorophyll, compared with CK, V2 and V3 treatments significantly (p < 0.05) increased the total chlorophyll content by 11.96–12.53% and 18.29–18.67%, respectively.

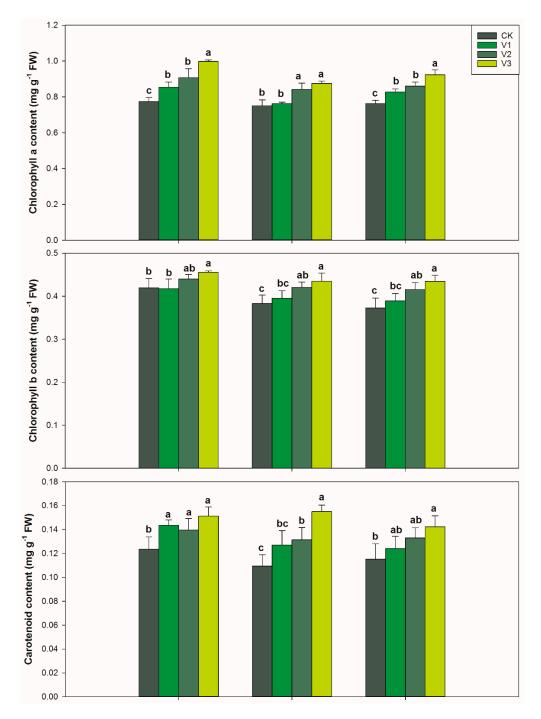
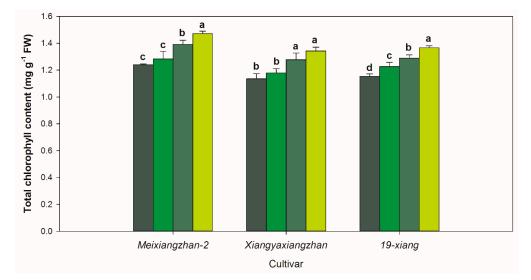


Figure 2. Cont.



**Figure 2.** Effects of vermicompost incorporation on contents of chlorophyll a, chlorophyll b, carotenoid, and total chlorophyll of fragrant rice seedlings; those sharing a common letter do not differ significantly at p < 0.05 according to the least significant difference (LSD) test for three fragrant rice cultivars.

## 3.4. Root Morphology

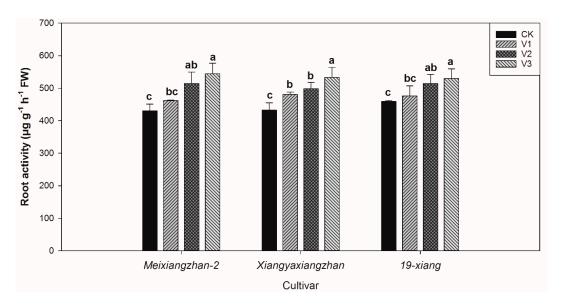
The root length, root surface area, mean diameter, root volume, and root tip number of fragrant rice seedlings are presented in Table 2. Compared with CK, V2 and V3 treatments significantly (p < 0.05) increased root length by 12.42–21.52% and 18.41–21.95%, respectively. Moreover, 15.05–21.94% and 29.46–38.65% higher root surface areas were recorded in V2 and V3 treatments, respectively, than CK. Relative to CK, V2 and V3 treatments significantly (p < 0.05) increased the mean diameter of root by 12.64–16.00% and 20.27–23.12%, respectively. Vermicompost treatments (V1, V2, and V3) also significantly (p < 0.05) increased root volume by 13.26–66.63% in comparison with CK. Moreover, compared with CK, V2 and V3 treatments significantly (p < 0.05) increased root tip number by 18.62–19.12% and 19.37–25.58%, respectively.

Table 2. Effects of vermicompost incorporation on root characteristics of fragrant rice seedlings.

Cultivar	Treatment	Root Length	Surface Area	Mean Diameter	Root Volume	Root Tip Number
		cm	cm <sup>2</sup>	mm	cm <sup>3</sup>	nr
Meixiar	ngzhan-2					
	CK	115.59c	10.52c	0.28c	0.09d	625.83c
	V1	131.81b	12.15b	0.30bc	0.10c	709.94b
	V2	140.47a	12.83b	0.32ab	0.12b	742.36ab
	V3	147.75a	14.59a	0.35a	0.14a	785.93a
Xiangyax	kiangzhan					
0,	CK	127.87b	11.06c	0.28c	0.08d	651.27b
	V1	128.01b	11.75c	0.30c	0.10c	674.46b
	V2	143.76a	12.73b	0.32b	0.12b	775.80a
	V3	151.42a	14.32a	0.35a	0.14a	777.45a
19-xiang						
_	CK	123.27b	10.78c	0.27b	0.08c	633.11b
	V1	131.09b	11.59bc	0.30ab	0.10b	671.88b
	V2	142.77a	12.73ab	0.32a	0.12ab	753.66a
	V3	150.32a	13.97a	0.33a	0.14a	791.09a

Values sharing a common letter within a column do not differ significantly (p < 0.05) according to the least significant difference (LSD) test for three fragrant rice cultivars.

The root activity of fragrant rice seedling was enhanced by vermicompost treatments (Figure 3). Compared with CK, V2 treatment significantly (p < 0.05) increased root activity by 19.46%, 15.30%, and 12.01% for 'Meixiangzhan-2', 'Xiangyaxiangzhan', and '19-xiang', respectively. Moreover, 26.29, 23.35, and 15.36% higher root activities were recorded in V3 treatment than CK for 'Meixiangzhan-2', 'Xiangyaxiangzhan', and '19-xiang', respectively.



**Figure 3.** Effects of vermicompost incorporation on root activity of fragrant rice seedlings; those sharing a common letter do not differ significantly at p < 0.05 according to the least significant difference (LSD) test for three fragrant rice cultivars.

#### 3.6. Antioxidant Responses

Vermicompost treatments induced regulation in the antioxidant system in fragrant rice seedlings (Figure 4). Compared with CK, V2 and V3 treatments improved POD activity by 7.97–21.25% and 19.20–24.21%, respectively. Compared with CK, V2 and V3 treatments improved CAT activity by 19.93–27.37% and 17.11–44.99%, respectively. Further, 7.37–15.45% and 11.08–15.95% higher SOD activities were recorded in V2 and V3 treatments, respectively, than CK. Moreover, vermicompost treatments (V1, V2, and V3) significantly (p < 0.05) reduced MDA content by 7.92–29.40%.

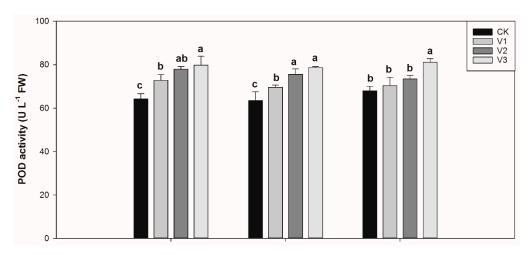
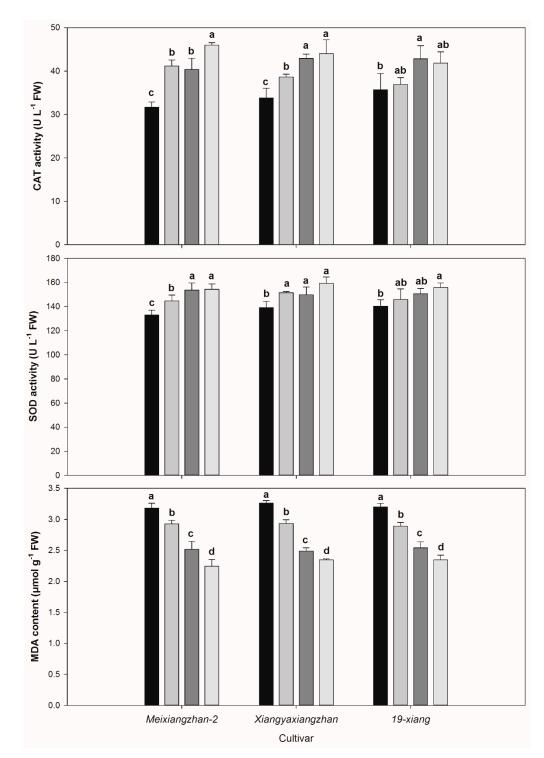


Figure 4. Cont.



**Figure 4.** Effects of vermicompost incorporation on activities of POD, SOD, and CAT, as well as content of MDA, of fragrant rice seedlings; those sharing a common letter do not differ significantly at p < 0.05 according to the least significant difference (LSD) test for three fragrant rice cultivars.

## 4. Discussion

The present study firstly applied vermicompost in fragrant rice production through seedling nursery. The results of our study showed that fragrant rice seedlings raised in matrix containing vermicompost exhibited better growth in terms of higher plant height, bigger stem diameter, and increased biomass. These results were similar to those of the study of Blouin et al. [6], who indicated that application of vermicompost enhanced growth and development of crops. The enhanced growth of fragrant rice seedlings was attributed

to the improvement in photosynthesis. Carbon assimilation is the major way to accumulate biomass of plants [13]. The present study showed that vermicompost application substantially increased the contents of photosynthetic pigments including chlorophyll a, chlorophyll b, and carotenoids, and improved the net photosynthetic rate. Considering the multiple nutrients in vermicompost, we deduced that fragrant rice seedlings uptake the those nutrients such as nitrogen, phosphorus, potassium, iron, manganese, copper, and magnesium from vermicompost, and consequently enhance the photosynthesis. The effects of the nutrients on rice performances have been reported by many researchers. For

effects of the nutrients on rice performances have been reported by many researchers. For example, a previous study showed that both iron and magnesium play important roles in the biosynthesis of photosynthetic pigments in plants [16]. The research of Pan et al. [3] indicated that nitrogen application increased the chlorophyll contents and improved the net photosynthetic rate of rice. Ding et al. [17] demonstrated that vermicompost as an organic fertilizer could improve soil quality and enhance crop productivity. Therefore, we believe that the improvement in growth of fragrant rice was explained by the increased availability of plant nutrients in the matrix owing to vermicompost incorporation.

Moreover, we observed that application of vermicompost improved root growth in terms of root length, surface area, mean diameter, root volume, and root tip number. Furthermore, the root activity was also enhanced owing to vermicompost treatments. The increased nutrients' availability in the matrix as a result of vermicompost incorporation might be the main reason for the enhanced growth of root. As previous study reported, vermicompost is an excellent organic fertilizer with multiple mineral elements [7]. Our results were consistent with the study of Mandal et al. [18], which showed that integrated use of mineral fertilizers and farmyard or green manure could markedly improve the growth and development of roots of crops. In addition, the root system is the organ that plants use to absorb water and nutrients from soil, and its morphological characteristics and activity can significantly influence the growth and development of plants themselves [19]. A better root system would help contribute to the growth and development as well as biomass accumulation of rice in growing time after the transplanting. An earlier study also indicated that the root characteristics have substantial influences on yield formation of rice by affecting the nitrogen recovery efficiency [20]. In our study, fragrant rice seedlings grown in matrix containing vermicompost exhibited better root performances, which indicated that the application of vermicompost in a seedling nursery has benefits in the early establishment of fragrant rice after the transplanting and might improve the yield formation of fragrant rice. However, more studies should be carried out in field traits to confirm this hypothesis.

In addition, we found that the fragrant rice seedlings grown in matrix containing vermicompost displayed higher activities of antioxidant enzymes including POD, CAT, and SOD, as well as a lower content of MDA. A previous study revealed that MDA content is an important index of oxidative stress that imparts the characteristics of inter- and intracellular membranes, and hence results in increased ion leakage through cell membranes [12], while POD, CAT, and SOD are important parts in plants' anti-oxidative defense responses for quenching the reactive oxygen species [21]. Therefore, we deduced that the application of vermicompost might improve the stress resistance of fragrant rice seedlings by promoting the antioxidant system, but more studies should be performed at the physiological level to prove this hypothesis.

#### 5. Conclusions

The present study involved the preliminary application of vermicompost in fragrant rice production. Incorporation of vermicompost into the seedling matrix for nursery raising substantially increased the photosynthetic pigment contents, enhanced net photosynthetic, and substantially improved the growth of fragrant rice cultivars during nursery raising. The increased root length, surface area, mean diameter, root volume, and root tip number, as well as enhanced root activity of fragrant rice seedlings, were observed due to vermicompost application. Moreover, fragrant rice seedlings grown in matrix containing

vermicompost exhibited higher activities of antioxidant enzymes including POD, CAT, and SOD, as well as a lower content of MDA. The results of the present study indicated that nursery raising with vermicompost could be a good agronomy practice in fragrant rice production.

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