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ARTICLE

Effects of Different Vermicompost Rates on Growth, 2-Acetyl-1-Pyrroline, Photosynthesis and Antioxidant Responses of Fragrant Rice (*Oryza sativa* L.) Seedling

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ABSTRACT

Vermicompost is an organic fertilizer contains multiple nutrient elements. However, the application of vermicompost in fragrant rice production is rarely reported. In order to study the effects of vermicompost application on fragrant rice (*Oryza sativa* L.) seedling performances, present study was conducted with two fragrant rice cultivars and four vermicompost rate treatments (0 (CK), 2.5 (Wo1), 5.0 (Wo2) and 10.0 (Wo3) g kg^{-1}). The results showed that vermicompost treatments significantly increased dry weight of fragrant rice seedling by 8.31–32.56% compared with CK. 21.10–59.13% higher net photosynthetic rates and 10.66–59.16% higher chlorophyll contents (chlorophyll a, chlorophyll b and total chlorophyll) were recorded in vermicompost treatments than CK. Application of vermicompost also significantly increased 2-acetyl-1-pyrroline (2-AP, the key compound of fragrant rice aroma) content and reduced the transcript level of gene *BADH2* which related to 2-AP biosynthesis in fragrant rice seedling. Moreover, compared with CK, vermicompost treatments enhanced activities of superoxide dismutase, peroxidase, catalase by 24.42–28.66%, 24.98–25.73% and 22.45–23.57%, respectively. 11.54–40.53% lower malonaldehyde contents were recorded in vermicompost treatments in related to CK. In conclusion, vermicompost improved growth, increased 2-AP content and might enhance stress resistant of fragrant rice seedling.

KEYWORDS

2-acetyl-1-pyrroline; fragrant rice; gene expression; photosynthesis; vermicompost

1 Introduction

Earthworm (*Pheretima*) is a low-grade animal which can be used as high protein feed additive. In the process of cultivating earthworm, earthworm can digest organic matter such as garbage and straw, and product vermicompost by mixing soil. Vermicompost is a kind of good organic fertilizer which can promote the growth and development of crops [1,2]. Numerous studies have been conducted to investigate the roles of vermicompost in crop growth and development. For example, the study of Muhie et al. [3] showed that vermicompost priming improved the seed germination and seedling growth of onion (*Allium cepa* L.) under abiotic stresses. An earlier study discovered that application of vermicompost as nitrogen source was able to increase yield of soybean (*Glycine max* L. Merr) and indicated that application of vermicompost could enhance soybean response to rhizobia inoculation as nodulation. Feizabadi et al. [4] demonstrated that vermicompost application significantly influenced the growth and fatty acid profile in rapeseed (*Brassica napus* L.) under drought stress. An earlier study also discovered that application of vermicompost incorporation with deep tillage was able to improve yield formation of wheat and soil quality in saline-sodic soil [5].

Fragrant rice is well-known for a 'popcorn-like' aroma and Thai 'jasmine' and Indian 'bamati' are the most well-known fragrant rice cultivars around the world [6]. The components of fragrant rice aroma are very complicated. An early study found more than 200 volatile compounds in aroma of fragrant rice [7]. In recent years, it is wide-recognized that 2-acetyl-1-pyrroline (2-AP) is the key contributors to the aroma [8,9] and the study of Chen et al. [10] found that *BADH2* is the key gene influenced the biosynthesis of 2-AP in fragrant rice.

Many scientists have made attempts to enhance productivity and 2-AP concentration of fragrant rice. For example, the study of Luo et al. [11] revealed that chelated selenium not only increased grain yield and grain 2-AP content, but also provided the biofortification effects in fragrant rice. Foliar application of selenate at heading stage also enhanced photosynthesis and thus led to the improvement in fragrant rice productivity [12]. The research of Xie et al. [13] showed that exogenous γ-aminobutyric acid substantially increased 2-AP concentration and total yield of fragrant rice cultivars. Moreover, the investigation of Mo et al. [14] revealed that application of nitrogen fertilizer at booting stage increased grain 2-AP content in fragrant rice. Despises of so many researches about fragrant rice were conducted, there is no any study about the application of vermicompost to fragrant rice was ever reported.

Therefore, in order to provide the more database for fragrant rice cultivation and facilitate the application of vermicompost in fragrant rice production, present study was conducted with the objective to study the effects of different vermicompost rates on performances of fragrant rice seedling.

2 Materials and Methods

2.1 Plant Materials, Growing Conditions and Treatment Description

Seeds of Two fragrant rice varieties, '*Meixiangzhan-2*' (*Lemont* × *Fengaozhan*) and '*Xiangyaxiangzhan*' (*Xiangsimiao126* × *Xiangyaruanzhan*), which were widely planted in South China and have been used many times for the studies about fragrant rice [11,12], were used as plant materials in present study. The rice seedlings were grown in soiled filled plastic pot under controlled conditions (temperature: 25 ± 2°C, relative humidity: 60–70%) in College of Agriculture, South China Agricultural University. Before sowing, four vermicompost rate were applied as 0, 2.5, 5.0 and 10 g kg^{-1} to soil and named as CK, Wo1, Wo2 and Wo3 treatments, respectively. The analyses of vermicompost 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%), magnesium (0.84%), pH 7.60. The experimental soil was sandy loam soil and contained containing of soil organic matter 14.95 g kg^{-1}, total nitrogen 0.86 g kg^{-1}, total phosphorus 0.77 g kg^{-1}, total potassium 22.84 g kg^{-1}, available nitrogen 97.63 mg kg^{-1}, available phosphorus 31.74 mg kg^{-1}, available potassium 178.66 mg kg^{-1}, and pH 6.44. The treated 20-day-old seedlings were freshly harvested and stored at 80°C for determination of physiological and biochemical parameters as well as gene expression analysis. The experiment was repeated thrice for individual parameter, and three independent replications of each treatment were considered for assessing different parameters following the same experimental conditions.

2.2 Determination of Fragrant Rice Seedling Quality

The net photosynthetic rate of 19-day-old seedlings were measured with a LI-6400XT Portable Photosynthesis System (LI-COR Inc., USA) in at 09:00–10:30 a.m. according the method of Luo et al. [12]. Photosynthetically active radiation at leaf surface was 1100 and 1200 μmol m^{-2} s^{-1} provided with a 6400-2B LED light source. Ambient CO_2 concentration was 385.5 to 399.7 μmol mol^{-1}. 20-day-old seedlings were collected and immediately determined the fresh weight and plant height. The samples were oven-dried at 70°C for 48 h to record dry weight.

2.3 Determination of 2-AP Concentration and Transcript Level of Gene BADH2

The determination of 2-AP was performed according to methods of Luo et al. [11]. The 2-AP concentration was determined by the synchronization distillation and extraction (SDE) method combined with a GCMS-QP 2010 Plus (Shimadzu Corporation, Japan) and expressed as μg kg^{-1} FW. 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'.

2.4 Determination of Chlorophyll Concentration

The concentration of chlorophyll a, chlorophyll b and total chlorophyll in fragrant rice seedling were determined using the methods described by Luo et al. [12]. About 0.10 g samples were extracted by 10 ml 95% alcohol for six hours. The absorbance was read at 665,649 and 652 nm. The chlorophyll concentrations were expressed as mg g^{-1} FW.

2.5 Determination of Malondialdehyde (MDA) and Antioxidants Responses

The 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 methods described by Kong et al. [15]. MDA concentration was determined after reacted with thiobarbituric acid and absorbance was read at 532, 600 and 450 nm. Final result was expressed as μmol g^{-1} FW. SOD activity was determined after the reaction with nitro blue tetrazolium and expressed as U L^{-1}. The activities of POD and CAT were determined after reacting with H_2O_2 and absorbances were read at 470 and 240 nm, respectively, and the activities were both expressed as U L^{-1}.

2.6 Statistical Analyses

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

3 Results

3.1 Seedling Quality

Different vermicompost rates substantially influenced the growth of fragrant rice seedling (Fig. 1). In comparison with CK, Wo1, Wo2 and Wo3 treatments did not significantly affected the fresh weight of *Meixiangzhan-2* whilst for *Xiangyaxiangzhan*, Wo1 and Wo2 treatments significantly increased fresh weight by 25.84 and 13.83%, respectively. Compared with CK, Wo1 treatment significantly increased dry weight of fragrant rice seedling by 8.31 and 32.56% for *Meixiangzhan-2* and *Xiangyaxiangzhan*, respectively. There was no significant difference among all treatments in plant height of fragrant rice seedling.

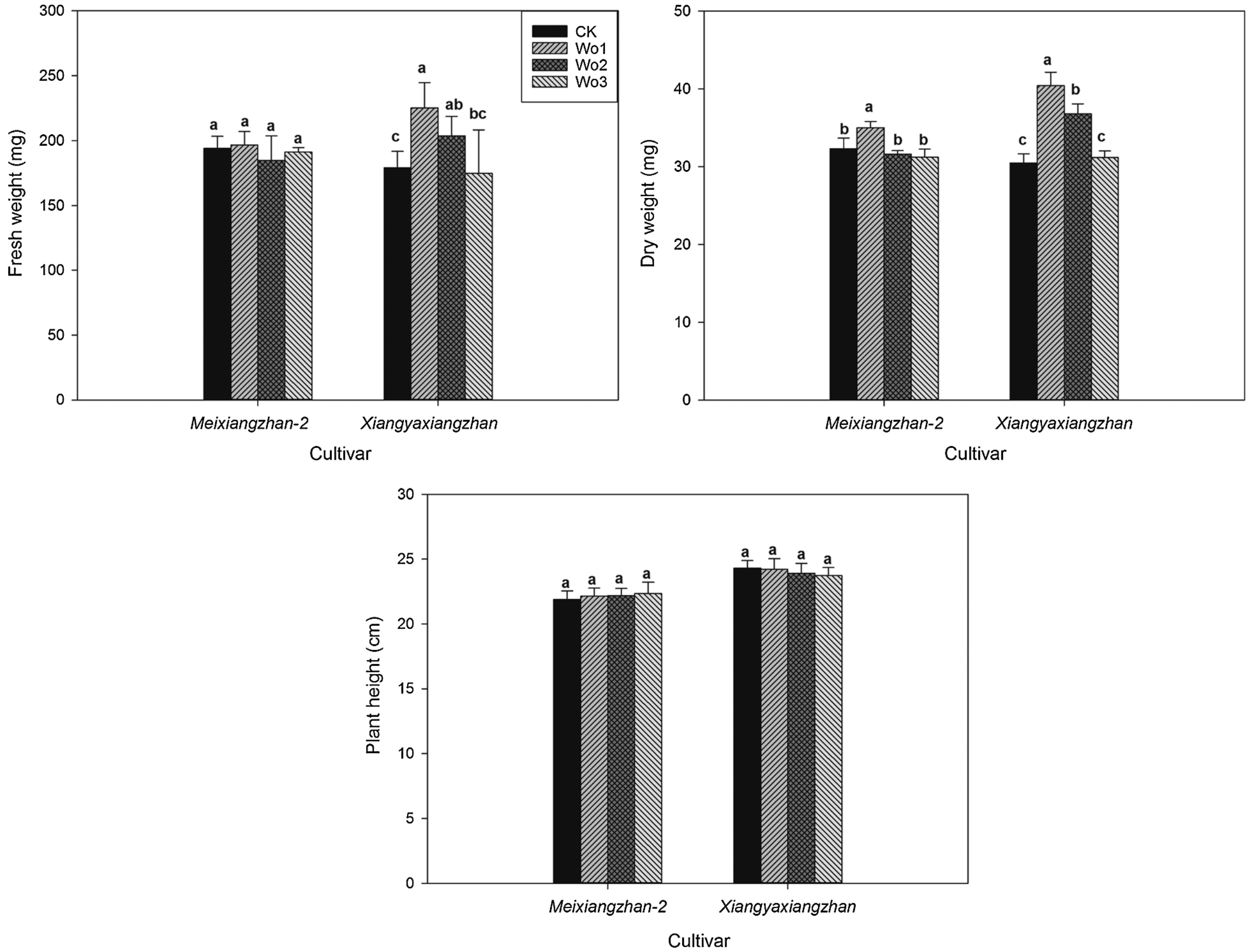

Figure 1: Effects of different vermicompost rates on fresh weight, dry weight and plant height of fragrant rice seedling. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $p \leq 0.05$, according to least significant difference (LSD) test

3.2 Net Photosynthetic Rate

There were some differences among different vermicompost rate treatments in net photosynthetic rate of fragrant rice seedling (Fig. 2). Compared with CK, Wo1 and Wo2 treatments significantly increased net photosynthetic rate by 28.95, 59.13% for *Meixiangzhan-2*, and 21.10, 42.30% for *Xiangyaxiangzhan*. However, there was no remarkable difference in net photosynthetic rate between CK and Wo3 treatments for both fragrant rice varieties.

3.3 Chlorophyll Contents

As shown in Fig. 3, application of vermicompost had impacts on chlorophyll contents of fragrant rice seedling. In comparison with CK, Wo1 treatment increased chlorophyll a, chlorophyll b and total chlorophyll contents by 31.48, 19.92, 22.08% for *Meixiangzhan-2*, and 52.46, 45.54, 59.16% for *Xiangyaxiangzhan*. Wo2 treatment also increased contents of chlorophyll a, chlorophyll b and total chlorophyll by 13.45, 10.66, 14.62% for *Meixiangzhan-2*, and 37.55, 25.18, 42.32% for *Xiangyaxiangzhan*, compared with CK.

3.4 Antioxidant Responses

Application of vermicompost affected the antioxidant system of fragrant rice seedling in terms of SOD, POD, CAT activities and MDA content (Fig. 4). For SOD activity, Wo1 treatment significantly enhanced

SOD activity by 24.42 and 28.66% for *Meixiangzhan-2* and *Xiangyaxiangzhan*, respectively and there was no significant difference among CK, Wo2 and Wo3 treatments. For POD activity, 24.98 and 25.73% higher activities were recorded in Wo1 treatment than CK for *Meixiangzhan-2* and *Xiangyaxiangzhan*, and there was no significant difference among CK, Wo2 and Wo3 treatments. For CAT activity, 22.45 and 23.57% higher activities were recorded in Wo1 treatment than CK for *Meixiangzhan-2* and *Xiangyaxiangzhan*, respectively. Moreover, Wo1 and Wo2 treatments reduced MDA content by 11.54–40.53% in comparison with CK.

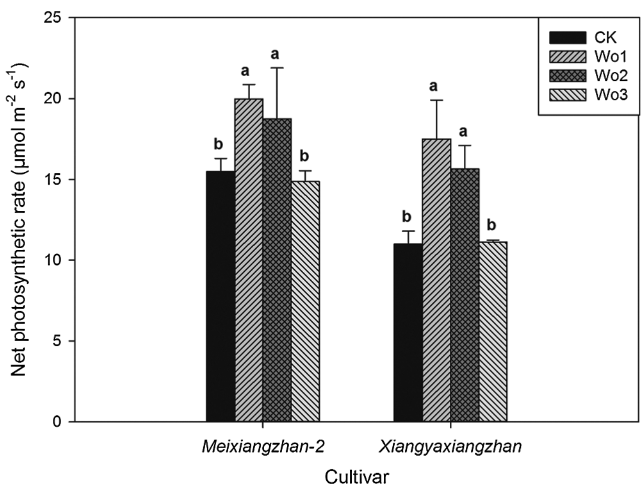

Figure 2: Effects of different vermicompost rates on net photosynthetic rate of fragrant rice seedling. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $p \leq 0.05$, according to least significant difference (LSD) test

3.5 2-AP Concentration

There were some differences among different vermicompost treatments in 2-AP concentration of fragrant rice seedling (Fig. 5). Compared with CK, Wo1 and Wo2 treatments significantly increased 2-AP concentration by 60.89, 48.10% for *Meixiangzhan-2* and 21.89, 55.26% for *Xiangyaxiangzhan*. There was no remarkable difference in 2-AP concentration between CK and Wo3 treatments for *Meixiangzhan-2* whilst for *Xiangyaxiangzhan*, 18.57% higher 2-AP concentration was recorded in Wo3 treatment than CK.

3.6 Expression of Gene BADH2

As the key gene controlled 2-AP production in fragrant rice, *BADH2* had different transcript levels under different vermicompost rate treatments (Fig. 6). In comparison with CK, Wo1 and Wo2 treatments significantly reduced transcript level of gene *BADH2* by 24.10, 34.17% for *Meixiangzhan-2* and 20.51, 34.42% for *Xiangyaxiangzhan*. There was no remarkable difference in transcript level of *BADH2* between CK and Wo3 treatments for *Meixiangzhan-2* whilst for *Xiangyaxiangzhan*, 18.63% lower transcript level of *BADH2* was recorded in Wo3 treatment than CK.

3.7 Correlation Analysis between 2-AP Content and BADH2 Expression

As shown in Fig. 7, transcript level of gene *BADH2* was negatively correlated with 2-AP content. For *Meixiangzhan-2*, 2-AP content was negatively correlated with *BADH2* mRNA although the correlation didn't reach the significant level. For *Xiangyaxiangzhan*, there exited a negative and significant ($p < 0.05$) correlation between 2-AP content and *BADH2* mRNA.

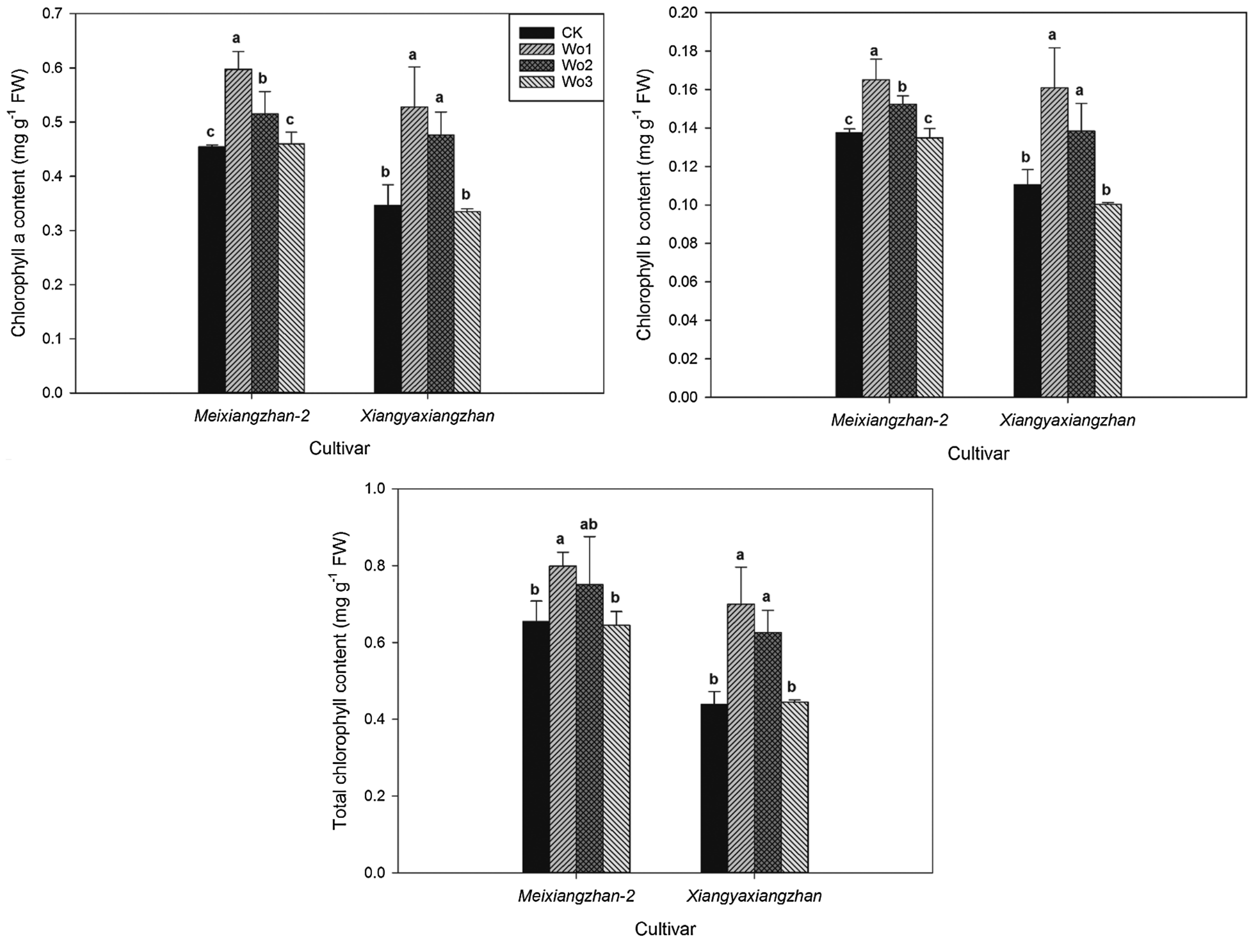

Figure 3: Effects of different vermicompost rates on chlorophyll a content of fragrant rice seedling. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $p \leq 0.05$, according to least significant difference (LSD) test

4 Discussion

Present study revealed the effects of different vermicompost rates on performances of fragrant rice seedling. As an organic fertilizer, vermicompost not only could be used for enhancing crop productivity, but also could be used for improving soil quality [5,16]. In our study, application of vermicompost especially Wo1 treatment substantially enhanced growth of fragrant rice seedling. The results are consistent with the study of Blouin et al. [1] who demonstrated that application of vermicompost was able to enhance growth and development of crops. In comparison with CK, application of vermicompost significantly increased dry matter weight of fragrant rice seedling, in other words, vermicompost enhanced the biomass accumulation of fragrant rice and it might be attributed to improvement in photosynthesis. In related to CK, increments in chlorophyll contents as well as net photosynthetic rate were observed in vermicompost treatments. As the way to accumulate carbohydrate for plant, photosynthesis plays crucial role in plant growth and development while it mainly depends on chlorophyll concentration to some extent [12]. Vermicompost consists of many nutrient elements including nitrogen, phosphorus, potassium, iron, manganese and zinc. Previous study revealed that nitrogen was able to enhance chlorophyll biosynthesis and improve net photosynthetic rate [17]. The study of Luo et al. [18] showed that zinc application improved net photosynthetic rate and increased grain yield of fragrant rice. An early study indicated that manganese improves crop yield and quality characters by improving plant nutritional status and photosynthetic efficiency [19]. Doe et al. [20] also demonstrated that iron has an important role in photosynthetic pigments and chlorophyll fluorescence in soybean. Therefore, we deduced that the improvement in seedling growth was the result of the interaction of various elements in vermicompost.

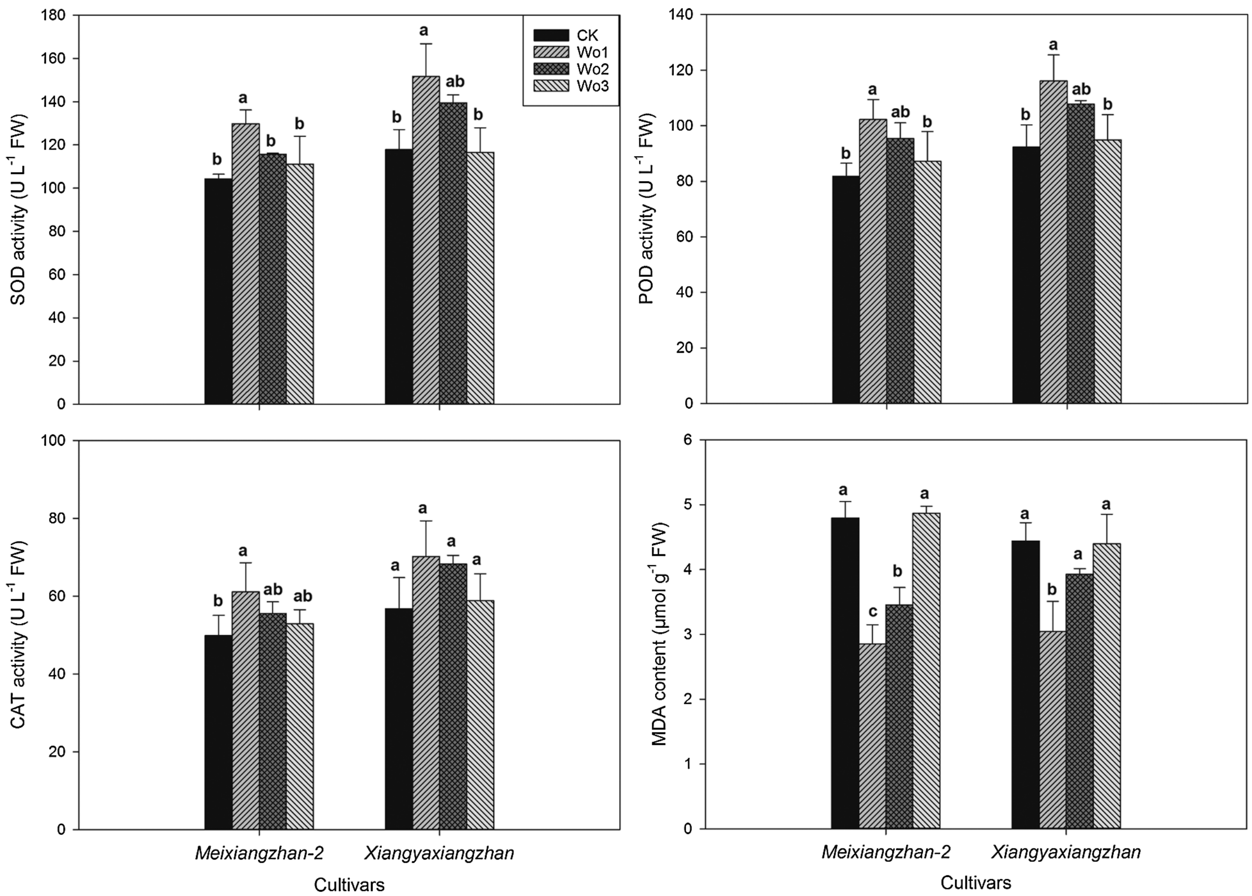

Figure 4: Effects of different vermicompost rates on antioxidant responses in fragrant rice seedling. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $p \leq 0.05$, according to least significant difference (LSD) test

As the key compound of fragrant rice special aroma, 2-AP has a complex biosynthesis process in fragrant rice tissues [21]. Present study showed an improvement of 2-AP concentration in fragrant rice seedling under vermicompost treatments and it might be attributed to the reduction in expression of gene *BADH2* which encoding betaine aldehyde dehydrogenase to transform γ-aminobutyl aldehyde into γ-aminobutyl acid rather than 1-pyrroline [8,10]. The study of Shi et al. [22] showed that two alleles (*BADH2-E7* and *BADH2-E2*) of *BADH2* were responsible for fragrance in fragrant rice. The correlation analysis also showed negative correlations between 2-AP content and transcript level of gene *BADH2*. As above mentioned, vermicompost includes multiple nutrient elements and many of them have been reported to have roles in enhancing biosynthesis of 2-AP in fragrant rice. For example, Li et al. [23] demonstrated manganese application significantly increased 2-AP content in fragrant rice. The study of Luo et al. [18] showed that exogenous zinc induced regulation in 2-AP biosynthesis in fragrant rice.

An earlier study also showed that nitrogen plays an important role in 2-AP formation in fragrant rice [24]. Thus, the increment in 2-AP content might also be the result of the interaction of various elements in vermicompost.

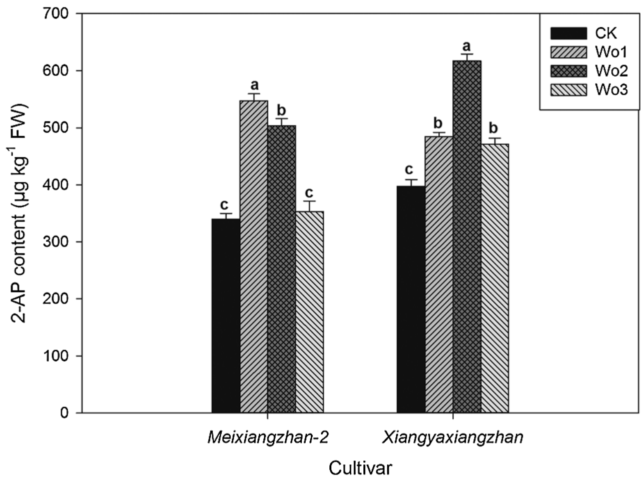

Figure 5: Effects of different vermicompost rates on 2-AP concentration in fragrant rice seedling. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $p \leq 0.05$, according to least significant difference (LSD) test

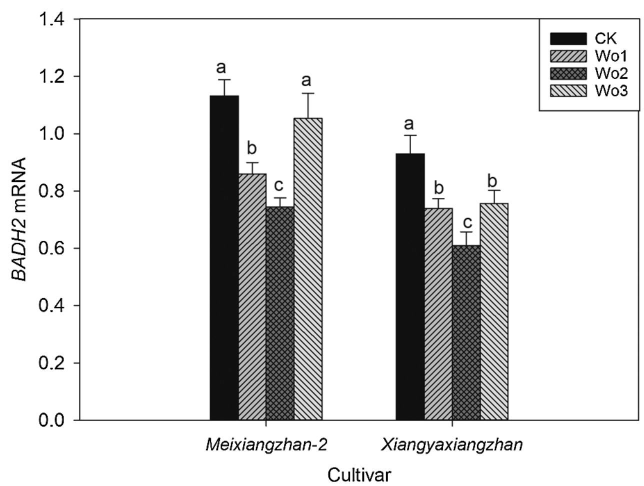

Figure 6: Effects of different vermicompost rates on transcript level of gene *BADH2* in fragrant rice seedling. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at $p \leq 0.05$, according to least significant difference (LSD) test

Interestingly, we observed that application vermicompost regulated the antioxidative system in fragrant rice seedlings in terms of POD, SOD and CAT which have significant roles in maintain cellular structures and quenching reactive oxygen species [25,26]. The enhancement of antioxidant enzymes activities implied vermicompost application could improve resistant of fragrant rice to abiotic stresses but more studies are need to be conducted to confirm this hypothesis.

In present study, we discovered application of vermicompost improved fragrant rice seedlings performances in terms of growth, 2-AP and antioxidative enzymatic activities. These results implied the application prospect of vermicompost in fragrant rice production, which agreed with study of Mathenge et al. [27] who used vermicompost as nitrogen fertilizer in soybean production. Therefore, in fragrant rice production, vermicompost could be used to replace the traditional chemical fertilizer or in combination with chemical fertilizer, so as to reduce the use of chemical fertilizer. Moreover, vermicompost even could be added to seedlings matrix for raising seedling.

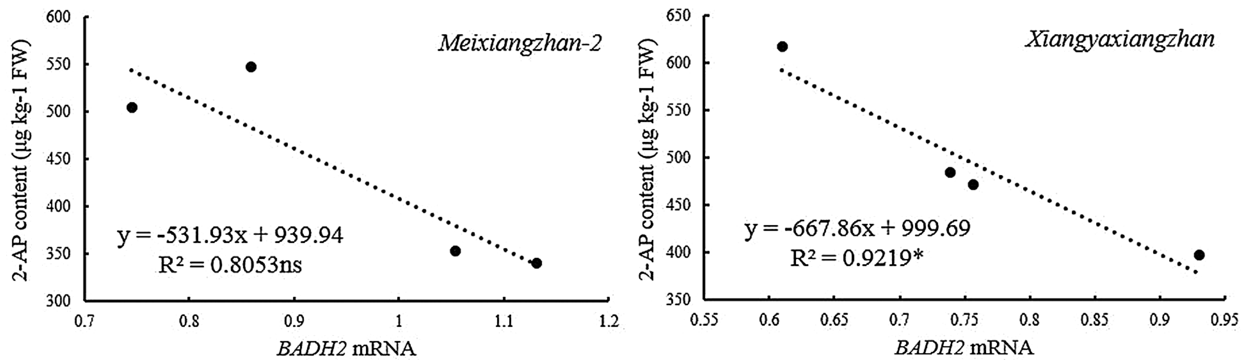

Figure 7: Relationship between 2-AP content and *BADH2* expression. *Significant at $p < 0.05$, **Significant at $p < 0.05$, ns, non-significant

5 Conclusion

Application of vermicompost significantly increased the chlorophyll (chlorophyll a, chlorophyll b and total chlorophyll) contents, net photosynthetic rate and dry weight of fragrant rice seedling. 2-AP content increased and the transcript level of gene *BADH2* decreased due to vermicompost treatments. Moreover, Application of vermicompost also enhanced anti-oxidative enzymatic activities in terms of SOD, POD, CAT and reduced MDA content in fragrant rice seedling.

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Conflicts of Interest: The authors declare that they have no conflicts of interest to report regarding the present study.

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