Phyton-
International Journal of
Experimental Botany

Tech Science Press

DOI: 10.32604/phyton.2021.012903

ARTICLE

Effects of Exogenous α-Ketoglutaric Acid on 2-Acetyl-1-Pyrroline, Yield Formation and Grain Quality Characters of Aromatic Rice

Bin Du[1], Qixia Wu[1], Shuochen Jiang[1], Dingyue Zhang[1], Yue Qiao[1], Yimei Xie[2], Jianlong Xu[3,*] and Jianqiang Zhu[1,*]

[1]College of Agriculture, Yangtze University, Jingzhou, 434023, China

[2]National Selenium-Rich Product Quality Supervision and Inspection Center, Enshi, 434023, China

[3]Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing, 100081, China

[*]Corresponding Authors: Jianlong Xu. Email: xujianlong@caas.cn; Jianqiang Zhu. Email: 200572@yangtzeu.edu.cn

Received: 17 July 2020 Accepted: 25 August 2020

ABSTRACT

The improvement of grain quality in aromatic rice is very important for farmer to increase their income. Present study was conducted with a two-year field experiment and three aromatic rice cultivars in order to study the effects of exogenous α-ketoglutaric acid on yield formation, grain quality characters and the biosynthesis of 2-acetyl-1-pyrroline (2-AP, key component of aromatic rice's fragrance) in aromatic rice. At heading stage, 0.50 mmol L^{-1} (T1) and 1.00 mmol L^{-1} (T2) α-ketoglutaric acid solutions were overhead sprinkle to aromatic rice plants, respectively while the treatment which was overhead sprinkled with distilled water was set as control (CK). The results showed that 17.34%–33.04% and 21.39%–34.74% higher grain 2-AP contents were recorded in T1 and T2 treatments, respectively. Compared with CK, T1 and T2 treatments significantly reduced the transcript level of gene *BADH2* which is related to the 2-AP biosynthesis in aromatic rice. 3.86%–7.51% higher grain protein contents and 1.15%–3.37% higher head rice rates were also recorded in α-ketoglutaric acid treatments than CK. Moreover, T1 and T2 treatments remarkably decreased the chalky rice rate, chalkiness and grain amylose content. However, there was no remarkable difference in grain yield and related trails (effective panicle number, grain number per panicle, seed-setting rate and 1000-grain weight) among CK, T1 and T2 treatments. In conclusion, application of exogenous α-ketoglutaric acid enhanced 2-AP biosynthesis and improved grain quality of aromatic rice.

KEYWORDS

α-ketoglutaric acid; aromatic rice; chalkiness; protein; 2-acetyl-1-pyrroline

1 Introduction

As a special type of rice for special aroma and great quality, aromatic rice is popular by people around the world. The compounds of the aroma in aromatic rice are very complicated and many studies have been conducted to identify them in the past. For example, Maraval et al. [1] determinated more than 100 volatile compounds in the aroma of aromatic rice. The research of Yang et al. [2] indicated that there might be 13 volatile compounds which contribute to the differences in aromatic rice aroma. In recent years, it has been established that 2-acetyl-1-pyrroline (2-AP) is the main flavour compound in aromatic rice cultivars which endows the characteristic roasted popcorn-like flavour [3].

The grain quality characters including milling, appearance, nutrient, smell and taste are the points get attention in aromatic rice production because they directly influence the price, popularity and economy. Therefore, Numerous studies were conducted to explore how to improve the grain quality of aromatic rice. Recently, Luo et al. [4] showed that the foliar application of selenite in aromatic rice production was able to increase the grain protein content and decreased both chalky rice rate and chalkiness of aromatic rice. The study of Mo et al. [5] revealed that interaction between water management and nitrogen application would induce the regulation in both yield formation and grain 2-AP biosynthesis in aromatic rice. The research of Li et al. [6] also showed that exogenous manganese significantly affected the yield formation, grain quality characters and rice aroma of aromatic rice.

α-ketoglutaric acid is one of two keto derivatives of glutaric acid while it is also an important intermediate product in plant tricarboxylic acid cycle and a key node connecting carbon and nitrogen metabolism in cells, and the position of *α*-ketoglutaric acid in the tricarboxylic acid cycle is after isocitrate and before succinyl coenzyme A [7]. Although *α*-ketoglutaric acid has potential to be a plant regulator in rice production, there was no more study about the effect of exogenous *α*-ketoglutaric acid on the growth and development of rice especially aromatic rice.

Hence, present study was conducted with a two-year field experiment in order to investigate the effects of exogenous *α*-ketoglutaric acid on 2-acetyl-1-pyrroline, yield formation and grain quality characters of aromatic rice cultivars.

2 Materials and Methods

2.1 Experiment Details

Field experiment was conducted at Experimental Research Farm, Feng village, Zengcheng (23°13′N, 113°81′E, altitude 11 m), China between July and November in 2018 and repeated in 2019. The experimental site enjoys a subtropical-monsoon climate. Three aromatic rice cultivars, "*Guangliangxiangyou-66*" (*GLXY-66*), "*Exiang-1*" (*EX-1*) and "*Wangeng-505*" (*WG-505*), were used in present study. At heading stage of each aromatic rice cultivar, 0.50 mmol L^{-1} and 1.00 mmol L^{-1} *α*-ketoglutaric acid solution were overhead sprinkled respectively and those treatments were named as T1 and T2. Another treatment which was overhead sprinkled with distilled water was set as control (CK). The treatments were arranged in randomized complete block design (RCBD) in triplicate in each year with net plot size of 20 m^2. At grain filling stage (ten days of receiving *α*-ketoglutaric acid treatment), fresh leaves from each treatment were separated from the main plant and stored at −80°C till physio-biochemical analysis. At maturity, fresh grains from each treatment were collected and stored at −80°C till physio-biochemical analysis.

2.2 Estimation of Yield and Related Attributes

At the maturity stage, the rice grains were harvested from six-unit sampling area (1 m^2) in each plot and threshed by machine. Then after sun drying, the grain yield was determined on basis of the dry weight. Average effective panicle number per area (1 m^2) was calculated form six-unit sampling area (1 m^2) in each plot. Then six representative hills of rice plants from each plot were taken to determine the other yield- related traits including seed-setting rate, 1000-grain weight and grain number per panicle.

2.3 Estimation of Grain 2-AP Content

The determination of 2AP content in grains was determinated based on the method of synchronization distillation and extraction method (SDE) combined with GCMS-QP 2010 Plus (Shimadzu Corporation) [8].

2.4 Estimation of Grain Quality

The dried grains of each aromatic rice cultivar from each treatment was taken from storage and then brown rice rate was estimated using a rice huller (Jiangsu, China) while milled rice and head rice recovery rates were calculated by using a Jingmi testing rice grader (Zhejiang, China). Grains with chalkiness and chalkiness degree were estimated by using an SDE-A light box (Guangzhou, China) while an Infratec-1241 grain analyzer (FOSS-TECATOR) was used to determine the grain amylose and protein contents.

2.5 Estimation of Malondiadehyde (MDA) Content and Antioxidant Enzymes Activities

The content of MDA and activities of antioxidant enzymes including superoxide (SOD, EC 1.15.1.1), peroxidase (POD EC 1.11.1.7) and catalase (CAT, EC 1.11.1.6) were determined according to the methods described by Kong et al. [9]. After reacting with thiobarbituric acid, the absorbance was read at the 532, 600 and 450 nm while the MDA content was expressed as μmol g^{-1} FW. POD (EC 1.11.1.7) activity was estimated after the reaction in the solution including enzyme extract, H_2O_2, guaiacol and sodium phosphate buffer (SPB, pH 7.0). One POD unit of enzyme activity was expressed as the absorbance increase by 0.01 (U g^{-1} FW) due to guaiacol oxidation. SOD (EC 1.15.1.1) activity was measured by using nitro blue tetrazolium (NBT). In brief, 0.05 ml of an enzyme extract was added into the reaction mixture which contained SPB (pH 7.8), methionine buffer, NBT buffer, ethylene diamine tetraacetic acid (EDTA)-2Na buffer and lactoflavin. After the reaction, the absorbance was recorded at 560 nm. One unit of SOD activity was equal to the volume of the extract needed to cause 50% inhibition of the color reaction. CAT (EC 1.11.1.6) activity was estimated by adding an aliquot of enzyme extract to the reaction solution containing 0.3% H_2O_2 and SPB and then the absorbance was read at 240 nm. One CAT unit of enzyme activity was defined as the absorbance decrease by 0.01 (U g^{-1} FW).

2.6 Statistical Analysis

The experiment data was analyzed using the statistical software 'Statistix 8.1' (Analytical Software, Tallahassee, FL, USA) and differences among means were separated by using the least significant difference (LSD) test at the 5% probability level. Graphical representation was performed via Sigma Plot 14.0 (Systat Software Inc., California, USA).

3 Result

3.1 Grain 2-AP Content

As shown in Fig. 1, exogenous *α*-ketoglutaric acid treatments (T1 and T2) significantly increased the grain 2-AP content compared with CK. There was no significant difference between two applied concentrations of *α*-ketoglutaric acid. For *GLXY-66*, compared with CK, *α*-ketoglutaric acid treatments significantly increased grain 2-AP content by 23.14%–32.22% in 2018 and by 34.74%–26.58% in 2019; For *EX-1*, T1 and T2 treatments significantly increased 2-AP content by 33.04%, 28.38% in 2018 and 29.12%, 23.60% in 2019 compared with CK respectively; For *WG-505*, compared with CK, T1 and T2 treatments significantly increased 2-AP content by 18.01%, 21.39% in 2018 and 17.34%, 24.49% in 2019, respectively.

3.2 Transcript Level of Gene BADH2

As shown in Fig. 2, lower transcript levels of gene *BADH2* were recorded in T1 and T2 treatments than CK. For *GLXY-66*, compared with CK, T1 and T2 treatments reduced the transcript level of *BADH2* by 17.82%, 24.05% in 2018 and 23.07%, 31.90% in 2019, respectively; For *EX-1*, compared with CK, T1 and T2 treatments decreased the transcript level of *BADH2* by 42.13%, 33.58% in 2018 and 24.08%, 22.31% in 2019, respectively; For *WG-505*, compared with CK, T1 and T2 treatments reduced the transcript level of *BADH2* by 19.29%, 22.08% in 2018 and 29.61%, 31.26% in 2019, respectively.

Further, we observed that there was a significant and positive correlation between grain 2-AP content and transcript level of gene *BADH2* (Fig. 3).

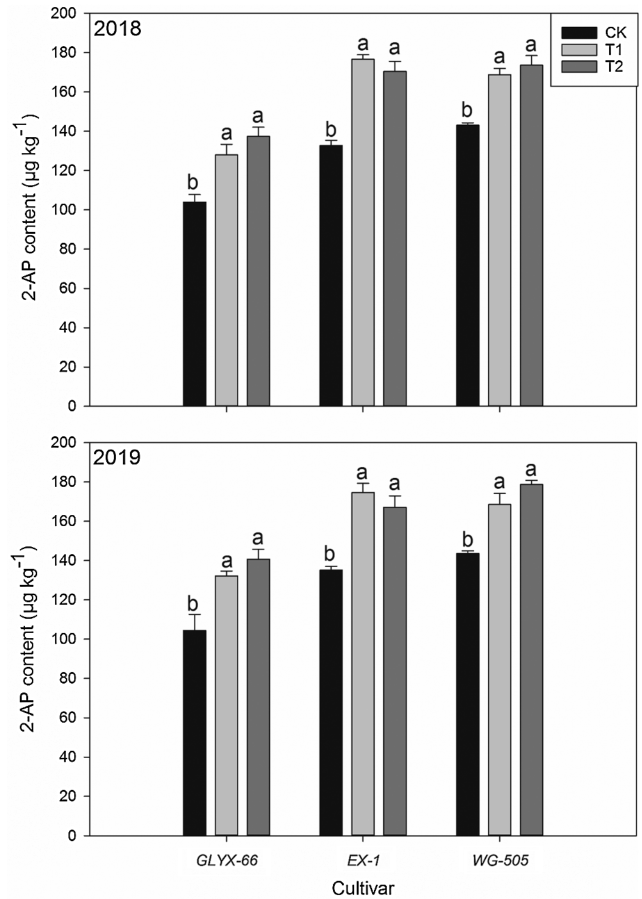

Figure 1: Effects of exogenous α-ketoglutaric acid on grain 2-AP content of aromatic rice. Capped bars represent S.E. of three replicates. Means sharing a common letter do not differ significantly at ($P \leq 0.05$) according to least significant difference (LSD) test for both the years. The same as below

3.3 Grain Protein and Amylose Contents

As shown in Fig. 4, exogenous α-ketoglutaric acid significantly increased the grain protein content and decreased grain amylose content of aromatic rice cultivars. In 2018, compared with CK, 1.17%–4.22% lower grain amylose contents and 4.27%–7.44% higher grain protein contents were recorded in α-ketoglutaric acid treatments; In 2019, 1.94%–5.25% lower grain amylose contents and 3.86%–7.51% higher grain protein contents were recorded in α-ketoglutaric acid treatments than CK.

3.4 Grain Milling and Appearance Quality

Exogenous α-ketoglutaric acid treatments significantly influenced the grain milling and appearance quality characters of aromatic rice (Tab. 1). Compared with CK, both T1 and T2 treatments significantly increased the head rice rate for *GLYX-66*, *EX-1*, *WG-505* in both years but there was no significant difference among CK, T1 and T2 treatments in brown rice rate and milled rice rate for three aromatic rice cultivars. On the other hand, lower chalk rice rates and chalkiness were both recorded in T1 and T2 than CK for all aromatic rice cultivars in 2018 while the similar trends were also observed in 2019.

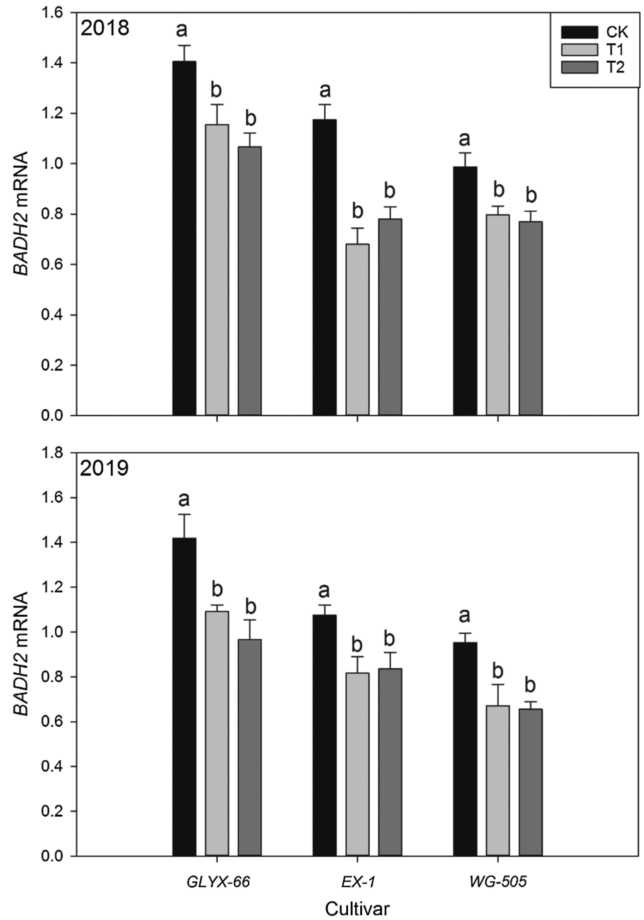

Figure 2: Effects of exogenous α-ketoglutaric acid on transcript level of gene *BADH2* in aromatic rice

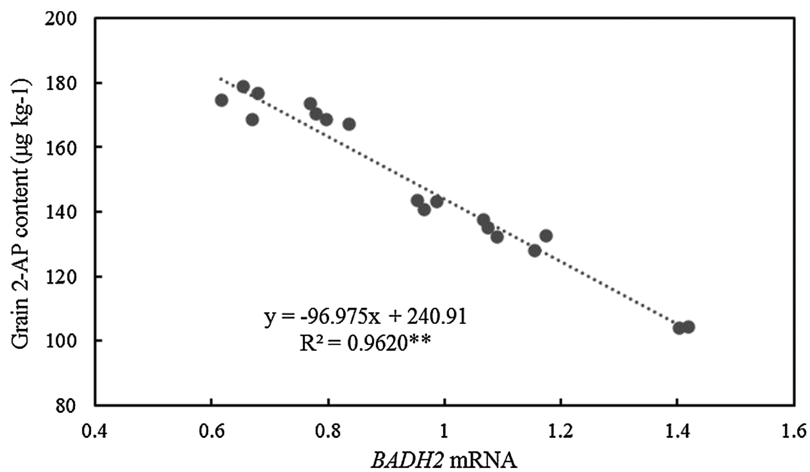

Figure 3: Correlation analyses between grain 2-AP content and transcript level of gene *BADH2*. Significant correlations at $^*P < 0.05$ and $^{**}P < 0.01$

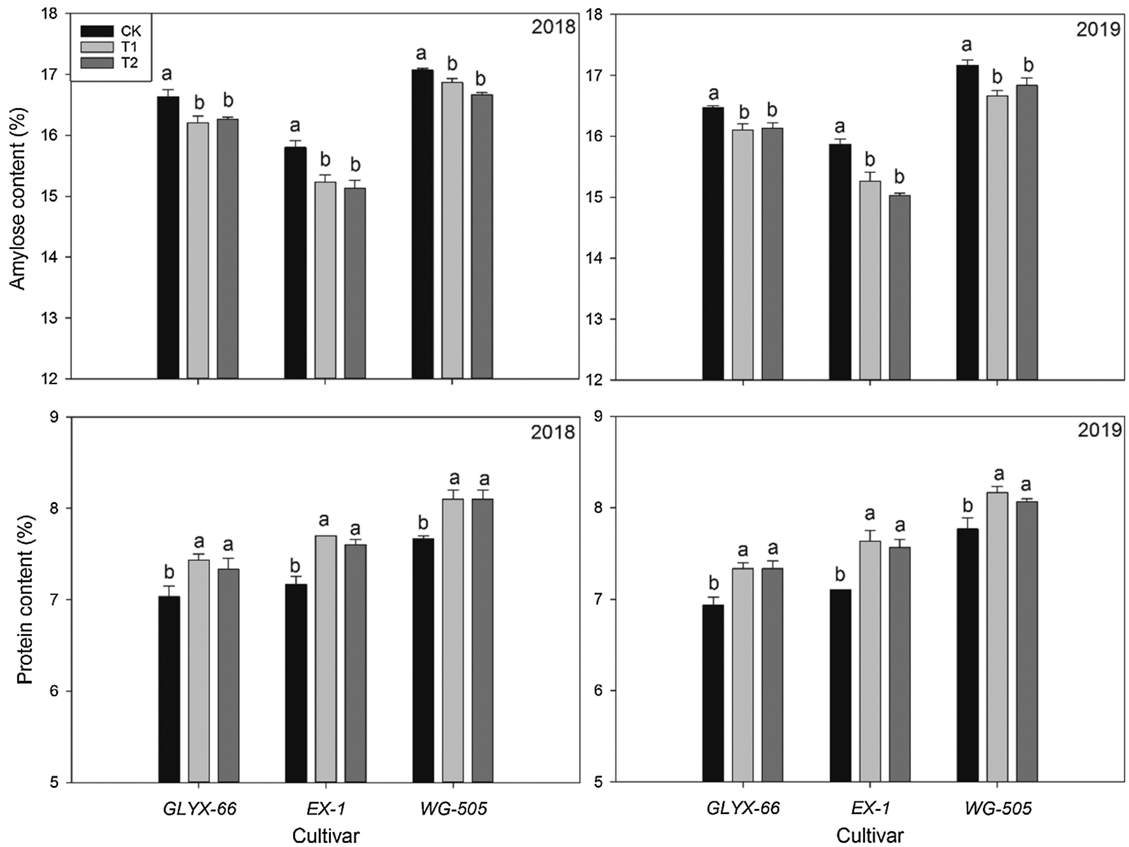

Figure 4: Effects of exogenous α-ketoglutaric acid on grain protein and amylose content of aromatic rice

Table 1: Effects of exogenous α-ketoglutaric acid on grain milling and appearance quality of aromatic rice

Year	Cultivar	Treatment	Brown rice rate (%)	Milled rice rate (%)	Head rice rate (%)	Chalk rice rate (%)	Chalkiness
2018	*GLYX-66*	CK	80.56a	73.83a	65.33b	20.47a	3.39a
		T1	80.71a	74.69a	66.68a	16.33b	2.70b
		T2	79.97a	74.68a	66.89a	16.49b	2.17b
	EX-1	CK	80.58a	70.91a	57.50b	5.53a	1.06a
		T1	80.04a	70.67a	59.40a	4.56b	0.69b
		T2	80.53a	70.79a	59.40a	4.39b	0.69b
	WG-505	CK	83.51a	76.67a	71.71b	27.13a	1.65a
		T1	83.23a	75.90a	74.13a	24.19b	1.30b
		T2	83.17a	76.06a	73.80a	24.01b	1.20b
2019	*GLYX-66*	CK	80.97a	74.36a	65.99b	19.97a	3.64a
		T1	80.83a	74.16a	66.94a	15.75b	2.66b
		T2	80.58a	73.94a	66.76a	15.61b	2.52b

Table 1 (continued).

Year	Cultivar	Treatment	Brown rice rate (%)	Milled rice rate (%)	Head rice rate (%)	Chalk rice rate (%)	Chalkiness
	EX-1	CK	79.76a	71.36a	57.62b	6.08a	1.32a
		T1	80.39a	71.20a	59.24a	4.54b	0.81b
		T2	80.66a	70.72a	58.37a	4.28b	0.79b
	WG-505	CK	83.60a	76.19a	72.22b	27.02a	1.74a
		T1	83.25a	75.98a	74.00a	23.25b	1.24b
		T2	83.09a	76.01a	73.91a	24.52b	1.28b

Values sharing a common letter within a column do not differ significantly at ($P \leq 0.05$) according to least significant difference (LSD) test for both the years. The same as below.

3.5 MDA Contents and Antioxidant Responses

Exogenousα-ketoglutaric acid regulated the antioxidative enzymatic activities in terms of SOD, POD and CAT and lowered lipid peroxidation (MDA production) as shown in Fig. 5. Compared with CK, T1 and T2 treatments significantly enhanced the POD activities for all aromatic rice cultivars in both years. Higher SOD and CAT activities were also recorded in T1 and T2 treatments than CK. There was no remarkable difference between T1 and T2 in POD, SOD and CAT activities. Meanwhile, MDA contents significantly decreased under T1 and T2 treatments compared with CK.

3.6 Yield and Related Attributes

As shown in Tab. 2, exogenous α-ketoglutaric acid did not have significant effects on yield and related attributes of aromatic rice cultivars. There was no remarkable difference among CK, T1 and T2 treatments in grain yield for three aromatic rice cultivars in both 2018 and 2019. Similar trends were also observed in effective panicle number, grain number per panicle and seed-setting rate as well as 1000-grain weight.

4 Discussion

Present study revealed that the effects of exogenous α-ketoglutaric acid on yield formation, grain quality and grain 2-AP content of three aromatic rice cultivars. According to the results, the application of α-ketoglutaric acid improved the appearance, nutrient, taste quality as well as aroma although didn't have significant influences on the grain yield of aromatic rice. As far as the aroma of aromatic rice was concerned, 2-AP is the key compound and the level of 2-AP represents the intensity of the aroma which would significant affected the price of aromatic rice in international market [10]. In our study, grain 2-AP contents of three aromatic rice cultivars all increased under exogenous α-ketoglutaric acid treatments. The biosynthesis of 2-AP in aromatic rice is a very complicated phenomenon which involves many enzymes and substances whilst in recent years, it has been identified that the 2-AP production in aromatic rice is mainly controlled by the expression of gene *BADH2* for encoding the betaine aldehyde dehydrogenase to inhibit the 2-AP biosynthesis [11]. The results showed that application of α-ketoglutaric acid at heading stage substantially reduced the transcript level of *BADH2* while the grain 2-AP concentration was significantly increased. Our results agreed with the study of Bao et al. [11] which showed that lower expression of *BADH2* led to the higher content of 2-AP in aromatic rice.

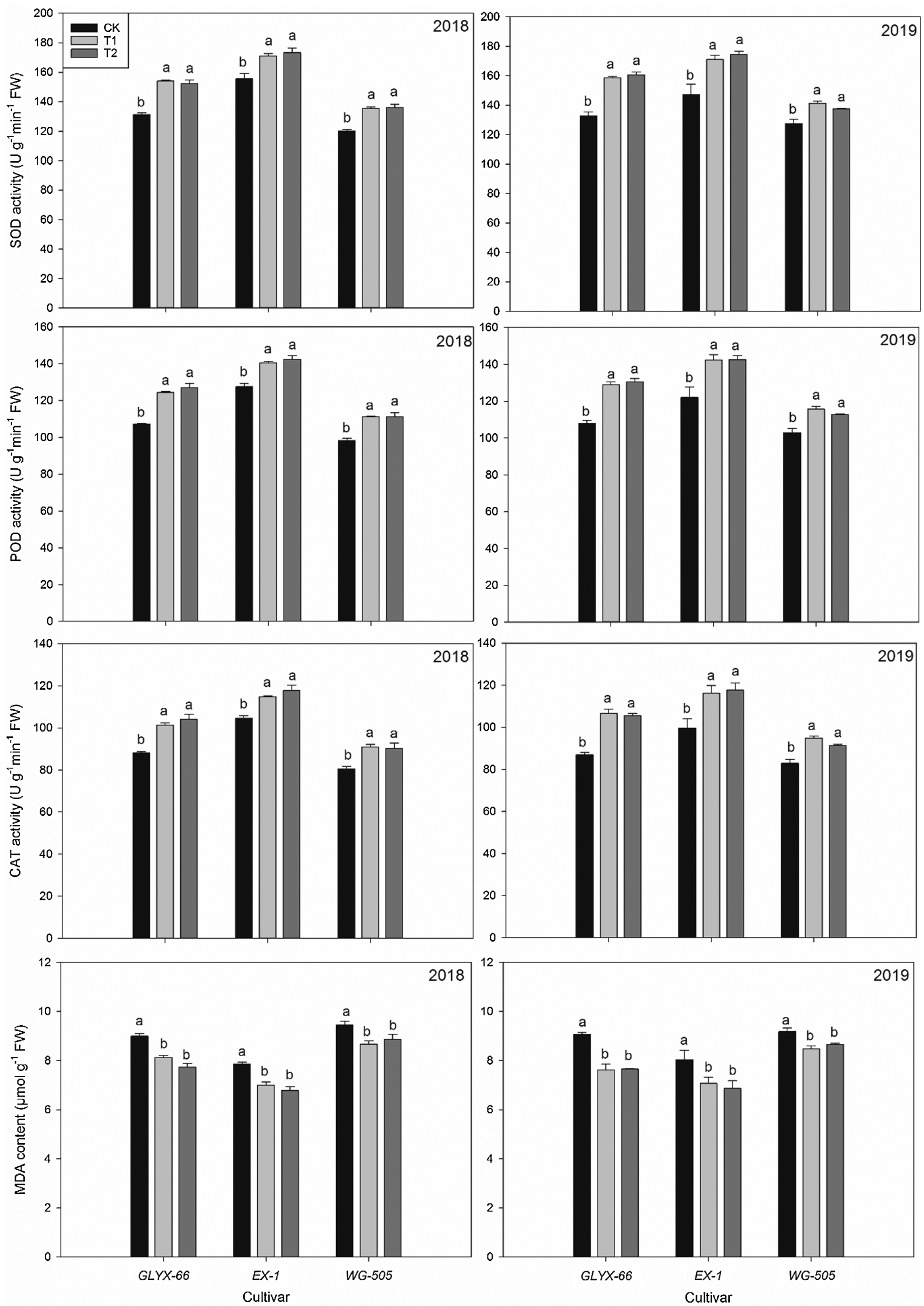

Figure 5: Effects of exogenous α-ketoglutaric acid on MDA content and activities of antioxidant enzymes including SOD, POD and CAT

Table 2: Effects of exogenous α-ketoglutaric acid on yield and related attributes

Year	Cultivar	Treatment	Effective panicle number per m^2	Grain number per panicle	Seed-setting rate (%)	1000-grain weight (g)	Grain yield (t ha^{-1})
2018	*GLYX-66*	CK	229.50a	181.27a	79.09a	29.93a	8.88a
		T1	231.50a	183.03a	78.97a	30.05a	9.00a
		T2	239.00a	179.12a	78.67a	30.14a	8.91a
	EX-1	CK	327.66a	111.88a	66.18a	27.87a	6.62a
		T1	326.00a	112.75a	65.78a	27.84a	6.56a
		T2	325.33a	117.79a	65.81a	28.21a	6.68a
	WG-505	CK	385.33a	79.62a	85.77a	25.40a	6.40a
		T1	392.16a	80.15a	85.66a	25.51a	6.65a
		T2	390.83a	79.32a	85.99a	25.33a	6.23a
2019	*GLYX-66*	CK	230.16a	182.11a	79.12a	30.02a	8.83a
		T1	232.83a	182.96a	78.62a	30.07a	9.07a
		T2	238.16a	178.43a	78.46a	30.24a	8.86a
	EX-1	CK	329.16a	112.87a	66.20a	27.82a	6.52a
		T1	325.66a	113.35a	65.68a	27.73a	6.51a
		T2	325.33a	117.17a	66.24a	28.08a	6.67a
	WG-505	CK	385.00a	77.68a	85.90a	25.30a	6.23a
		T1	392.83a	79.96a	85.44a	25.47a	6.55a
		T2	391.50a	79.42a	86.33a	25.36a	6.17a

Rice is an important protein source for human. Normally, most farmers would choose to apply more nitrogen fertilizer to increase grain protein content of rice [12,13], however, the use of large amounts of nitrogen fertilizer not only increased greenhouse gas emissions, but also caused surface water eutrophication and soil acidification [14]. The results of present study showed that the foliar application of α-ketoglutaric acid in rice production was able to achieve the goal to increase grain protein content. Meanwhile, because α-ketoglutaric acid is an organic matter and the lower dosage applied, less environment pollution would be caused during the application process. On the other hand, present study showed that exogenous α-ketoglutaric acid significantly decreased the grain amylose content which affects the texture of cooked rice, increases hardness and reduces stickiness of rice [15–17]. Lower content of amylose in grain means better texture with less harness and stickiness. Therefore, the application of α-ketoglutaric acid would improve the nutrient and texture of aromatic rice.

In addition, we observed that exogenous α-ketoglutaric acid improved the appearance of aromatic rice for significantly decreasing both chalk rice rate and chalkiness. Chalkiness refers to the white opaque part of rice endosperm formed by the loose tissue. As one of the important characters to measure rice quality, Chalkiness directly affects the appearance quality, commodity circulation and processing quality of rice [18]. The chalky area of rice grain is caused by the accumulation of starch and protein grains in endosperm and easy to be broken during processing [19]. The main reason of the decreased chalkiness might relate to the antioxidative system of aromatic rice during the grain-filling stage. The study of Kong et al. [19] revealed that the conditions of antioxidant system and lipid peroxidation significantly affected the appearance quality of aromatic rice. Normally, because the paddy environment is very complicated, the rice plant would face many and different degrees stress such as unsuitable air or soil temperature, strong wind, pest bite, large temperature difference, unsuitable soil pH and so on during the growth and development while the MDA content is an important indicator of oxidative stress [20–22]. In our study, lower MDA contents was observed in α-ketoglutaric acid treatments than CK. Less MDA means less lipid peroxidation and this might be attributed to the enhanced activities SOD, POD and CAT. Previous studies has revealed that SOD, POD and CAT are the antioxidant enzymes in rice and play significant roles in maintaining cellular structures and functions and protect the rice plant from abiotic stresses [23]. For example, SOD dismutases superoxide radical whereas POD and CAT involved in scavenging H_2O_2. The higher antioxidative enzymatic activities under α-ketoglutaric acid treatments would be beneficial for the grain-filling process and it also could be the reason for the higher head rice rate in present study.

Moreover, there was no remarkable difference between two applied concentrations of α-ketoglutaric acid on aromatic rice performances. Considered the cost, the optimum applied concentration of α-ketoglutaric acid in aromatic rice production might be 0.50 mmol L^{-1} and more studies should be done in the physiological and molecular level to reveal the metabolism of how α-ketoglutaric acid affecting the 2-AP concentration of aromatic rice in the future.

5 Conclusion

Foliar applications of α-ketoglutaric acid not only significantly improved the head rice rate, protein content and 2-AP content, but also significantly decreased the amylose content, chalk rice rate and chalkiness of aromatic rice. But α-ketoglutaric acid applications had no significant influence on grain yield and related attributes of aromatic rice.

Funding Statement: This work is supported by Hubei special fund for agricultural science and technology innovation (2018skjcx01) and the Engineering Research Center of Ecology and Agricultural Use of Wetland, Ministry of Education (KFT201904).

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

References

1. Maraval, I., Mestres, C., Pernin, K., Ribeyre, F., Boulanger, R. et al. (2008). Odor-active compounds in cooked rice cultivars from Camargue (France) analyzed by GC-O and GC-MS. *Journal of Agricultural & Food Chemistry, 56 (13),* 5291–5298. DOI 10.1021/jf7037373.
2. Yang, D. S., Shewfelt, R. L., Lee, K. S., Kays, S. J. (2008). Comparison of odor-active compounds from six distinctly different rice flavor types. *Journal of Agriculture & Food Chemistry, 56(8),* 2780–2787. DOI 10.1021/jf072685t.
3. Poonlaphdecha, J., Gantet, P., Maraval, I., Sauvage, F. O., Menut, C. et al. (2016). Biosynthesis of 2-acetyl-1-pyrroline in rice calli cultures: Demonstration of 1-pyrroline as a limiting substrate. *Food Chemistry, 197,* 965–971. DOI 10.1016/j.foodchem.2015.11.060.
4. Luo, H. W., He, L. X., Du, B., Wang, Z. M., Zheng, A. X. 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. DOI 10.32615/ps.2019.114.
5. Mo, Z. W., Li, Y. H., Nie, J., He, L. X., Tang, X. R. (2019). Nitrogen application and different water regimes at booting stage improved yield and 2-acetyl-1-pyrroline (2AP) formation in fragrant rice. *Rice, 12(1),* 77. DOI 10.1186/s12284-019-0335-5.
6. Li, M. J., Ashraf, U., Tian, H., Mo, Z. W., Pan, S. G. 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 & Biochemistry, 103,* 167–175. DOI 10.1016/j.plaphy.2016.03.009.
7. Chernyavskaya, O. G., Shishkanova, N. V., Il'Chenko, A. P., Finogenova, T. V. (2000). Synthesis of alpha-ketoglutaric acid by *Yarrowia lipolytica* yeast grown on ethanol. *Applied Microbiology & Biotechnology, 53(2),* 152–158. DOI 10.1007/s002530050002.

8. Xie, W. J., Ashraf, U., Zhong, D., Lin, R. B., Xian, P. Q. et al. (2019). Application of γ-aminobutyric acid (GABA) and nitrogen regulates aroma biochemistry in fragrant rice. *Food Science & Nutrition, 7(11),* 3784–3796. DOI 10.1002/fsn3.1240.
9. Kong, L. L., Ashraf, U., Cheng, S. R., Rao, G. S., Tang, X. R. (2017). Short-term water management at early filling stage improves early-season rice performance under high temperature stress in South China. *European Journal of Agronomy, 90,* 117–126. DOI 10.1016/j.eja.2017.07.006.
10. Bao, G. G., Ashraf, U., Wang, C. L., He, L. X., Wei, X. S. et al. (2018). Molecular basis for increased 2-acetyl-1-pyrroline contents under alternate wetting and drying (AWD) conditions in fragrant rice. *Plant Physiology & Biochemistry, 133,* 149–157. DOI 10.1016/j.plaphy.2018.10.032.
11. Chen, S. H., Yang, Y., Shi, W. W., Ji, Q., He, F. et al. (2008). Badh2, encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of 2-acetyl-1-pyrroline, a major component in rice fragrance. *Plant Cell, 20(7),* 1850–1861. DOI 10.1105/tpc.108.058917.
12. Deng, Q. Q., Ashraf, U., Cheng, S. R., Sabir, S. R., Mo, Z. W. 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 & Environmental Research, 16(6),* 7741–7758. DOI 10.15666/aeer/1606_77417758.
13. Xie, W. J., Ashraf, U., Zhong, D. T., Lin, R. B., Xian, P. Q. et al. (2019). Application of γ-aminobutyric acid (GABA) and nitrogen regulates aroma biochemistry in fragrant rice. *Food Science & Nutrition, 7(11),* 3784–3796. DOI 10.1002/fsn3.1240.
14. Huang, M., Chen, J., Cao, F., Zou, Y. B. (2018). Increased hill density can compensate for yield loss from reduced nitrogen input in machine-transplanted double-cropped rice. *Field Crops Research, 221,* 333–338. DOI 10.1016/j.fcr.2017.06.028.
15. Ando, I., Sato, H., Aoki, N., Suzuki, Y., Takeuchi, Y. (2010). Genetic analysis of the low-amylose characteristics of rice cultivars Oborozuki and Hokkai-PL9. *Breeding Science, 60(3),* 187–194. DOI 10.1270/jsbbs.60.187.
16. Yoon, M. R., Koh, H. J., Kang, M. Y. (2009). Pasting and amylose component characteristics of seven rice cultivars. *Journal of the Korean Society for Applied Biological Chemistry, 52(1),* 63–69. DOI 10.3839/jksabc.2009.011.
17. Umemoto, T., Terashima, K. (2002). Research note: Activity of granule-bound starch synthase is an important determinant of amylose content in rice endosperm. *Functional Plant Biology, 29(9),* 1121–1124. DOI 10.1071/PP01145.
18. Dai, L. P., Lan, W., Leng, Y. J., Yang, Y. L., Zeng, D. L. (2016). Quantitative trait loci mapping for appearance quality in short-grain rice. *Crop Science, 56(4),* 1484–1492. DOI 10.2135/cropsci2015.07.0404.
19. Chen, J. L., Tang, L., Shi, P. H., Yang, B. H., Zhu, Y. (2016). Effects of short-term high temperature on grain quality and starch granules of rice (*Oryza sativa* L.) at post-anthesis stage. *Protoplasma, 254(2),* 935–943. DOI 10.1007/s00709-016-1002-y.
20. Liu, K., Deng, J., Lu, J., Wang, X. Y., Lu, B. L. et al. (2019). High nitrogen levels alleviate yield loss of super hybrid rice caused by high temperatures during the flowering stage. *Frontiers in Plant Science, 10,* 122. DOI 10.3389/fpls.2019.00357.
21. Rao, G. S., Ashraf, U., Kong, L. L., Mo, Z. M., Xiao, L. Z. et al. (2019). Low soil temperature and drought stress conditions at flowering stage affect physiology and pollen traits of rice. *Journal of Integrative Agriculture, 18(8),* 1859–1870. DOI 10.1016/S2095-3119(18)62067-2.
22. Liu, K., Yang, R., Lu, J., Wang, X. Y., Lu, B. L. et al. (2019). Radiation use efficiency and source-sink changes of super hybrid rice under shade stress during grain-filling stage. *Agronomy Journal, 111(4),* 1788–1798. DOI 10.2134/agronj2018.10.0662.
23. Luo, H. W., Du, B., Zheng, A. X., Lai, R. F., You, Z. S. et al. (2018). Flooding treatment restrains volunteer rice germination and seedling growth. *Applied Ecology & Environmental Research, 16(5),* 7231–7242. DOI 10.15666/aeer/1605_72317242.