

EXOGENOUS APPLICATION OF MIXED MICRO-NUTRIENTS IMPROVES YIELD, QUALITY, AND 2-ACETYL-1-PYRROLINE CONTENTS IN FRAGRANT RICE

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Abstract. Application of micro-nutrient at low concentrations can play significant roles in yield and quality improvements as well as aromatic characters of fragrant rice. Present study investigated the exogenous mixed application of micro-nutrients on yield quality, 2-acetyl-1-pyrroline (2-AP) and mineral content accumulation in the grains of two aromatic rice cultivars i.e., Xiangyaxiangzhan and Guixiangzhan. Two mixtures containing different proportions of micro-nutrients i.e., mixture-I (40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride, 50% proline, and 3% sodium selenite) and mixture-II (containing 1% gibberellic acid, 8% zinc sulfate, potassium 76% di-hydrogen phosphate, 8% manganese sulfate, and 7% copper sulfate) were exogenous applied at full heading stage at 1.5 kg hm⁻² (T1) and 3 kg hm⁻² (T2) diluted in 750 L water while plots with only water application were served as control (CK). Results revealed that exogenous application of mixed micro-nutrients notably increased yield in terms of improved grain numbers per panicle, filled grain (%), 1000-grain weight, grain yield, grain 2-AP, selenium (Se) and zinc (Zn) contents while decreased amylose content, chalky rice percentage and chalkiness degree in both rice cultivars. Furthermore, exogenous application of mixed micro-nutrient enhanced above ground plant dry biomass for both rice cultivars. Significant correlations among 2-AP, Zn and Se ($P \leq 0.05$) suggested that these two micro-nutrients have dynamic roles in rice aroma production, however, to unravel the exact mechanisms of micro-nutrient involvement in 2-AP biosynthesis, further research is needed.

Keywords: *dry weight, rice aroma, nutrient concentration, grain chalkiness, amylose contents*

Introduction

Fragrant rice (*Oryza sativa* L.) is precious for its special aroma, unique flavor and good quality (Baradi et al., 2015; Ashraf et al., 2017a,b). Thai ‘jasmine’ and Pak-Indian ‘basmati’ varieties are highly valued by consumers globally and gaining popularities in international markets (Huang et al., 2012), while the sale-price of aromatic rice is higher than non-aromatic rice (You et al., 2012). Through strummental analyses, it is well recognized that different volatile compounds involved in rice aroma formation (Champagne et al., 2005), of which 2-acetyl-1-pyrroline (2-AP) is a key

aromatic compound (Mo et al., 2015; Li et al., 2016). The organic compounds extracted from different fragrant rice cultivars showed similar aroma profile, nevertheless the levels of different aroma compounds varied significantly (Maraval et al., 2008). Buttery et al. (1983) found 2-AP, (E)-2-nonenal, (E,E)-2,4-decadienal, 4-vinyl-guaiacol, 4-vinylphenol, hexanal, decanal and nonanal as principle compounds in the aroma of cooked rice. Furthermore, 2-AP is synthesized in both aromatic and non-aromatic rice cultivars; however, its level of production is quite lower in non-aromatic rice (Widjaja et al., 1996; Maraval et al., 2010).

Normally, plants absorb inorganic nutrients and metallic ions from soil solution through roots and transport them to different plant organs (Ashraf et al., 2015); however, the nutrient acquisition via roots is interdependent with nutrient assimilation rates in the leaves while efficacy of foliar applied nutrient uptake depends on plant architecture, canopy development and their mobility within the plant system (Saleem et al., 2010; Pandey et al., 2014; Hu et al., 2015). Foliar application of macro and micro-nutrients is more efficient than soil supplementation and led to significant increase in growth and yield of wheat (Zain et al., 2015). Exogenously applied micro-nutrients improved nutrient uptake, phenological events, growth and yield as well as grain micro-nutrient contents in cereals (Bameri et al., 2012). Recently, Habib et al. (2016) resulted that foliar applied nitric oxide improved growth and yield of rice. Micro-nutrient at low concentrations has significant roles in morphological growth, yield formation and quality characters of aromatic rice (Li et al., 2016). For example, Tang and Wu (2006) found that Zn application (foliar or soil applied) could improve the growth and quality of aromatic rice. Tian et al. (2009) found that grain exogenous micro-nutrient application significantly increased seed setting rate and yield, while decreased percentage of chalkiness in rice grains. Except growth and yield, micro-nutrients could also improve rice aroma (Sun and Liu, 1991) by enhancing the biosynthesis of 2-AP in different plant parts of rice.

Most of the previous studies used micro-nutrients to evaluate their effectiveness on plant performance and grain quality (Fan et al., 2010; Wu et al., 2010); nevertheless information about the application of mixed micro-nutrients on the plant growth, yield and 2-AP contents in aromatic rice is very few. Thus, in this study, we used two different mixtures containing a set of micro-nutrients (zinc sulfate, manganese sulfate, ferric chloride, sodium selenite, potassium dihydrogen phosphate, and copper sulfate) along with proline, and gibberellic acid in different proportions to evaluate their effects on growth, yield and quality of fragrant rice. Furthermore, to provide additional insights on grain quality, 2-AP contents in grains and its relationships with applied micro-nutrients have also been studied.

Materials and Methods

Experimental site and growing conditions

This study was conducted in the Experimental Research Farm, South China Agricultural University, Guangzhou, China (23°20' N, 113°30' E and 11 m above the sea

level. The climate is subtropical-monsoon type with mean annual air temperatures of 21.9 °C, mean annual maximum and minimum air temperatures of 31.0 °C (in June) and 15.0 °C (January), respectively (*Fig. 1*). The soil of the experimental site was lateritic red soil type containing 23.34 g kg⁻¹ organic matter, 1.139 g kg⁻¹ total N, 1.136 g kg⁻¹ total P, 24.41 g·kg⁻¹ total K, 114.27 mg kg⁻¹ available N, 61.34 mg kg⁻¹ available P, 127.04 mg kg⁻¹ available K and 6.45 pH.

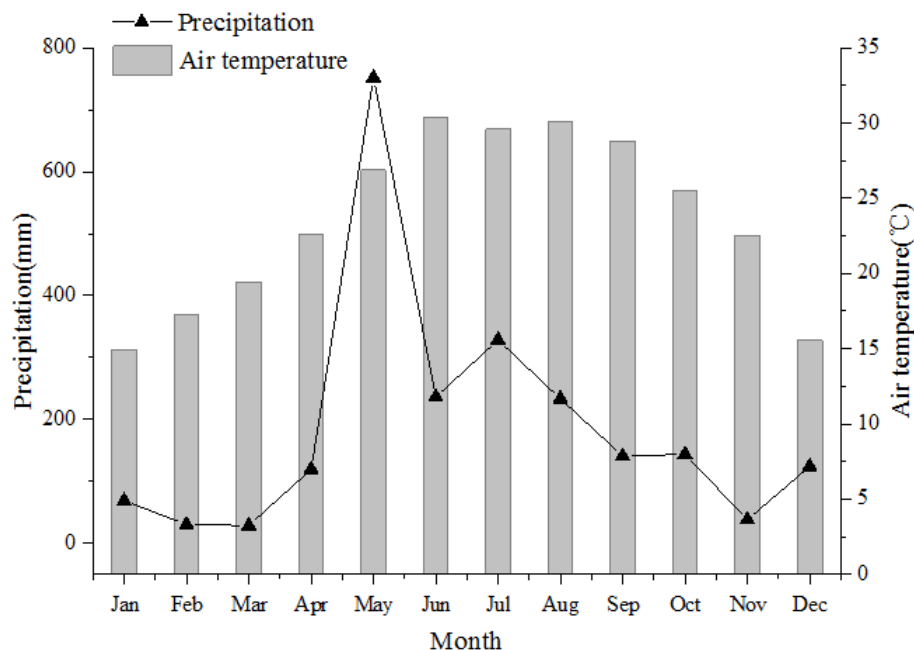


Figure 1. Mean monthly temperature and precipitation in 2015 of the experimental site

Treatment application and crop husbandry

Seeds of two aromatic rice cultivars i.e., Xiangyaxiangzhan and Guixiangzhan (famous due to their aroma and well adopted in South China) were collected from College of Agriculture, South China Agricultural University, Guangzhou China. Both cultivars have almost similar growth period ranging from 108 to 114 days from sowing to physiological maturity. Before sowing, seeds of both these cultivars were soaked in water for 24 h and allowed to germinate in dark chamber at 30°C for next 24 h. Seeds of both rice cultivars were sown in the field (protected by plastic sheet) on March 12th and transplanted on April 5th, 2015 at 20 cm × 20 cm (hill to hill distance) with 4 seedlings per hill. At full heading stage, two micro-nutrient mixtures containing different proportions of micro-nutrients and growth and quality regulators were exogenously applied. Mixture-I containing 40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride, 50% proline, and 3% sodium selenite and mixture-II containing 1% GA₃, 8% zinc sulfate, potassium 76% di-hydrogen phosphate, 8% manganese sulfate, and 7% copper sulfate were obtained from ‘Biotechnology Co., Ltd., Dongguan Foote, China’. Both mixture-I and mixture-II at 1.5 kg hm⁻² (T1) and 3 kg hm⁻² (T2) were diluted in 750

L of water (as recommended by the company) and applied exogenously with the help of a special knapsack electric sprayer (3WBD-Qianfeng Agricultural machinery, Yangjiang, Guangdong, China) with 0.2-0.5 mPa pressure and 16-18L capacity fitted with a special windproof atomizing spray nozzle was used for overhead spray. Both set of mixtures were manually sprayed in the evening (4 p.m.) with uniform movement in the treatment plots. In control (CK) treatments, only water was sprayed exogenously at the same stage. A special fertilizer containing 12.5% N, 6% P₂O₅, 10% K₂O and 15.0% organic matter was applied at 1500 kg ha⁻¹ with 60% as basal dose and 40% at tillering stage. All other standard agronomic practices were followed as recommended by the Guangdong Province, South China. The crop was harvested on July 6th, tied into bundles, threshed and seeds were sun-dried for a week.

Sampling and measurement

Determination of dry weight

For the measurement of aboveground biomass, 3 plants were randomly sampled at 7, 14 and 21 days after spraying (DAS), oven-dried at 80 °C till constant weight. The dry weight of the plants was measured by using an electronic digital balance (BSA224S, Sartorius, Japan).

Determination of grain quality and 2-AP contents

Grain quality was measured after winnowing by NP-4350 winnower (Zhejiang, China). Brown rice rate was measured by rice husker (Jiangsu, China) while milled and head milled rice rates were measured by rice polishing machine (Zhejiang, China). The percentage of chalky grain and chalkiness degree were estimated by using a SDE-A light box (Guangzhou, China). Amylose protein content and alkali of rice grains were estimated by Infratec1241 grain analyzer (FOSS-TECATOR). For 2-AP contents, fresh grains (10 g) from each treatment were obtained and analyzed by synchronization distillation and extraction method (SDE) as devised by Mo et al. (2015).

Determination of yield and yield related traits

At maturity, an area of 5 m² in each plot was reaped, threshed and sun dried to estimate grain yield and expressed as tons/square hectare meter (t hm⁻²). To measure grain numbers per panicle and seed setting rate five hills from each treatment were randomly sampled. From filled seeds lot, five random samples of 1000 grains were taken and weighed to record 1000-grain weight.

Elemental analyses

Fe, Zn, Cr and Mn content in grains were determined as described by Karanam (2011). Briefly, the dried samples of rice grains (1 g) were digested by 25 ml 2mol/L HCL-Vitamin C mixture (0.40 g Vitamin C dissolved in 834 ml distilled water, then added 166 ml concentrated HCl and mixed well). The concentration of Fe, Zn, Cr and

Mn in the solution were then measured by AA-650 Atomic Absorption Spectrometer (Shimadzu Company, Japan). Se content in grain was determined according to Zhang et al. (2014). Dried grain samples (0.30 g) were digested by HNO₃:HClO₄ (5:1 v/v) at 200 °C for 1h and diluted to 20ml with distilled water. The concentration of Se in the solution was measured by 8200 Atomic Fluorescence Spectrometer (JITIAN Company, China).

Experimental design and data analyses

The experiment was carried out in a randomized complete block design with three replications. The net plot size was 6 m × 4 m. Analysis of variance and correlation coefficients were performed using Statistix 8.0 (Analytical software, Tallahassee, Florida, USA) while the differences amongst treatments were separated by using least significant difference (LSD) test at 5% probability level.

Results

Yield and yield related traits

For Xiangyaxiangzhan, T1 caused a significant increment of seed setting rate and yield by 1.11% and 4.81% respectively. T2 resulted in a significant increment of grain numbers per panicle, 1000-grain weight and yield by 10.31%, 2.26% and 11.61% respectively. For Guixiangzhan, T1 significantly increased grain numbers per panicle and yield by 7.78% and 11.59% respectively. T2 significantly increased grain numbers per panicle, 1000-grain weight and yield by 5.72%, 4.20% and 10.26% respectively (Table 1).

Table 1. Effect of exogenous micro-nutrient application on yield and yield related traits of fragrant rice

Rice cultivars	Treatment	Grain numbers per panicle	Filled grain (%)	1000-grain weight (g)	Grain yield (t hm ⁻²)
Xiangyaxiangzhan	CK	122.10b	83.03b	19.87b	6.03c
	T1	124.60b	83.95a	20.06b	6.32b
	T2	134.69a	83.24b	20.32a	6.73a
Guixiangzhan	CK	111.48c	82.44a	22.14b	6.04b
	T1	120.15a	82.98a	22.38b	6.74a
	T2	117.86b	82.32a	23.07a	6.66a

Values sharing a letter in common within a column do not differ significantly according to LSD test at P< 0.05. CK (control); T1 (40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride, 50% proline, and 3% sodium selenite), T2 (1% gibberellic acid, 8% zinc sulfate, potassium 76% di-hydrogen phosphate, 8% manganese sulfate, and 7% copper sulfate).

Grain quality attributes

For Xiangyaxiangzhan, T1 significantly increased protein content by 7.45%. T1 and T2 significantly decreased amylose content, percentage of chalky rice and chalkiness degree. For Guixiangzhan, T1 significantly increased protein content by 8.38%. T1 and T2 resulted in a decrease of amylose content, percentage of chalky rice and chalkiness degree (Table 2).

Table 2. Effect of exogenous micro-nutrient application on grain quality of fragrant rice

Rice cultivars	Treatment	Protein content (%)	Amylose content (%)	Chalky rice (%)	Chalkiness degree
Xiangyaxiangzhan	CK	9.43b	19.27a	5.12a	0.44a
	T1	10.13a	18.33b	2.89c	0.16c
	T2	9.47b	18.03c	3.69b	0.25b
Guixiangzhan	CK	8.83b	19.10a	5.54a	0.38a
	T1	9.57a	17.93b	3.11b	0.14b
	T2	8.87b	17.47c	2.77c	0.07c

Values sharing a letter in common within a column do not differ significantly according to LSD test at $P < 0.05$. CK (control); T1 (40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride, 50% proline, and 3% sodium selenite), T2 (1% gibberellic acid, 8% zinc sulfate, potassium 76% di-hydrogen phosphate, 8% manganese sulfate, and 7% copper sulfate).

Dry matter accumulation

For Xiangyaxiangzhan, T1 caused a significant increment of population dry matter accumulation by 13.21%, 22.77% and 21.87% at 7d, 14d and 21d after treatment respectively. T2 significantly increased population dry matter accumulation by 9.61% at 21d after treatment. For Guixiangzhan, T1 significantly increased population dry matter accumulation by 10.51%, 18.55% and 11.49% at 7d, 14d and 21d after treatment respectively. T2 significantly increased population dry matter accumulation at 7d, 14d and 21d after treatment, increased by 10.51%, 18.55% and 11.49% respectively (Table 3).

Grain 2-AP and nutrient acquisition

Both T1 and T2 significantly improved grain 2-AP contents by 94.32% and 61.69% in Xiangyaxiangzhan and upto 37.44% and 44.01%, in Guixiangzhan respectively compared with control (Fig. 2a). Both cultivars showed a similar response to both set of nutrient mixtures in accumulation of grain Se content. T1 and T2 increased Se contents in the grains for both cultivars considerably. Moreover, T1 showed the highest Se content in grains for both cultivars, with 0.17 mg kg^{-1} and 0.16 mg kg^{-1} , respectively (Fig. 2b). Exogenous micro-nutrient application enhanced grain Fe contents for both rice cultivars. T1 led to 0.79 and 1.56% higher grain Fe contents in Xiangyaxiangzhan and Guixiangzhan, respectively while T2 found statistically similar to CK (only water spray) (Fig. 2c).

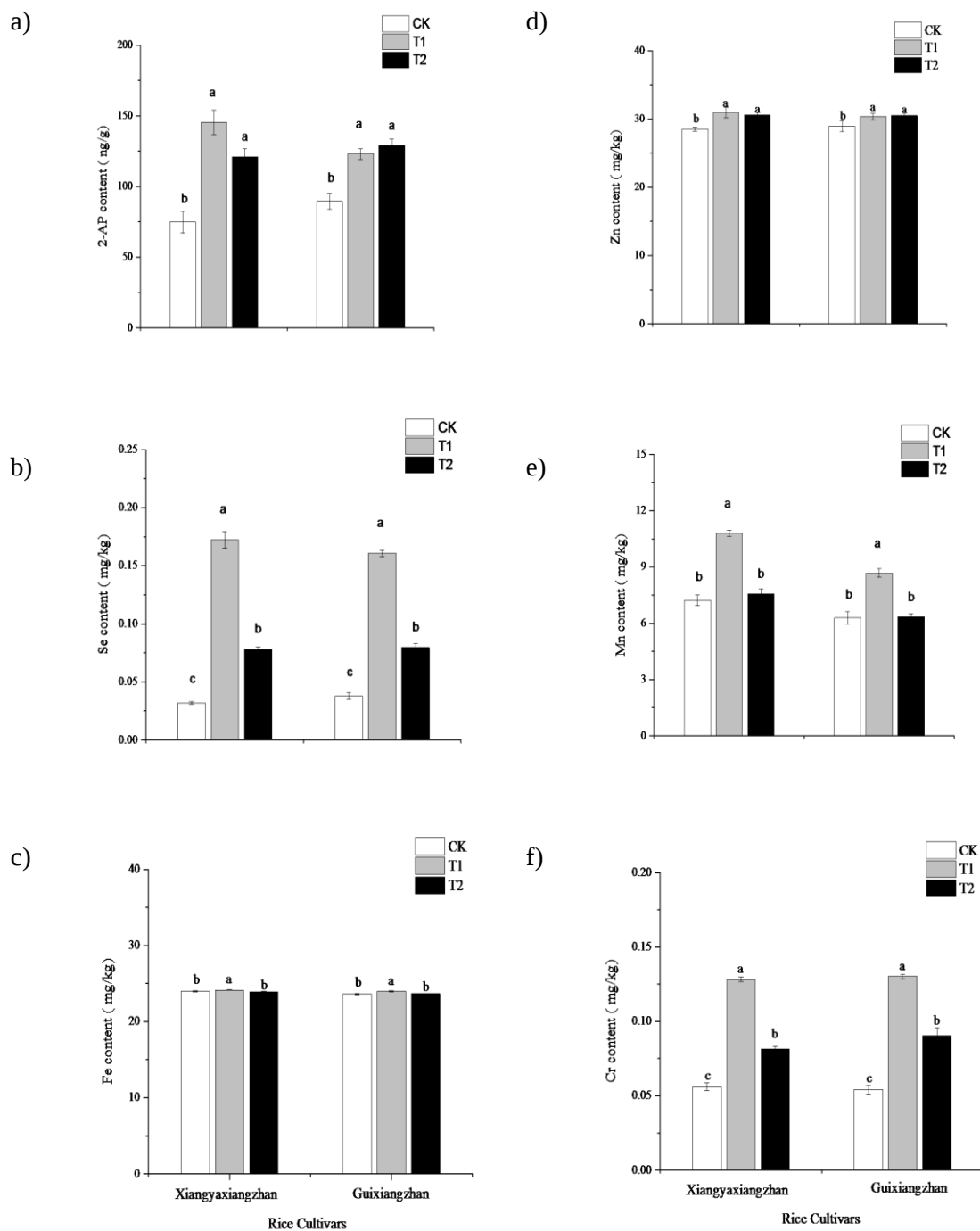


Figure 2. Effects of exogenous micro-nutrient application on grain a) 2-acetyl-1-pyrroline (2-AP), b) Selenium (Se), c) Iron (Fe), d) zinc (Zn), e) Manganese (Mn), and f) chromium (Cr) contents in two aromatic rice cultivars. Means with different lowercase letters differ significantly according to LSD test at $P < 0.05$. Error bars represent SD of three replications. CK (control); T1 (40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride, 50% proline, and 3% sodium selenite), T2 (1% gibberellic acid, 8% zinc sulfate, potassium 76% di-hydrogen phosphate, 8% manganese sulfate, and 7% copper sulfate).

Furthermore, for Xiangyaxiangzhan, T1 and T2 significantly increased Zn content in grains by 8.49% and 7.26%, respectively while for Guixiangzhan, 4.83% and 5.25% higher grain Zn contents were recorded in T1 and T2, respectively (*Fig. 2d*). Grain Mn contents were improved in the grains of micro-nutrient treated rice plants. T1 led to 49.58% and 38.00% increased grain Mn contents in Xiangyaxiangzhan and Guixiangzhan, respectively, however, T2 was remained statistically similar to CK regarding grain Mn contents (*Fig. 2e*). Furthermore, for Xiangyaxiangzhan, T1 and T2 significantly increased grain Cr content by 129.11% and 45.18%, respectively. Similarly, for Guixiangzhan, T1 and T2 significantly increased grain Cr content by 139.96% and 66.67%, respectively. Further, T1 showed the highest grain Cr contents in both cultivars (*Fig. 2f*).

Table 3. *Effect of exogenous micro-nutrient application on dry matter accumulation ($t\text{ hm}^{-2}$) in fragrant rice*

Rice cultivars	Treatment	7 DAS	14 DAS	21 DAS
Xiangyaxiangzhan	CK	7.87b	9.66b	10.61c
	T1	8.91a	11.86a	12.93a
	T2	8.17b	10.08b	11.63b
Guixiangzhan	CK	7.80b	9.65c	11.14b
	T1	8.62a	11.44b	12.42a
	T2	8.79a	11.69a	12.52a

Values sharing a letter in common within a column do not differ significantly according to LSD test at $P < 0.05$. CK (control); T1 (40% zinc sulfate, 6% manganese sulfate, 1% ferric chloride, 50% proline, and 3% sodium selenite), T2 (1% gibberellic acid, 8% zinc sulfate, potassium 76% di-hydrogen phosphate, 8% manganese sulfate, and 7% copper sulfate).

Correlation analyses among 2-AP, micro-nutrients and grain quality attributes

Correlation analyses showed significant positive correlations were among grain 2-AP, Se, Zn and Cr content in response to micro-nutrient application for both rice cultivars. Contrastively, significant negative correlations were noted among 2-AP, amylose, chalky rice percentage and chalkiness degree for both Xiangyaxiangzhan and Guixiangzhan. Furthermore, significant correlations were identified among 2-AP, Mn and protein content in response to micro-nutrient application for Xiangyaxiangzhan but not for Guixiangzhan. Significant correlations were also observed between Zn and Cr in response to exogenous nutrient application for both cultivars (*Table 4*).

Table 4. Correlation relationship among grain 2-AP, micro-nutrient contents and quality traits of fragrant rice

Index	2-AP content	Se content	Fe content	Zn content	Mn content	Cr content	PRO content	AMY content	CHA Per	CHA Deg
Xiangyaxiangzhan										
2-AP content	1									
Se content	0.931**	1								
Fe content	0.564	0.745*	1							
Zn content	0.908**	0.798**	0.307	1						
Mn content	0.799**	0.963**	0.797*	0.644	1					
Cr content	0.937**	0.999**	0.717*	0.817**	0.957**	1				
PRO content	0.779*	0.949**	0.819**	0.645	0.983**	0.939**	1			
AMY content	-0.824**	-0.575	-0.075	-0.885**	-0.344	-0.596	-0.324	1		
CHA Per	-0.997**	-0.934**	-0.560	-0.927**	-0.807**	-0.943**	-0.788*	0.826**	1	
CHA Deg	-0.974**	-0.892**	-0.602	-0.890**	-0.752*	-0.897**	-0.744*	0.829**	0.978**	1
Guixiangzhan										
2-AP content	1									
Se content	0.669*	1								
Fe content	0.470	0.914**	1							
Zn content	0.677*	0.578	0.439	1						
Mn content	0.408	0.936**	0.968**	0.466	1					
Cr content	0.779*	0.982**	0.880**	0.680*	0.884**	1				
PRO content	0.413	0.944**	0.896**	0.461	0.959**	0.882**	1			
AMY content	-0.984**	-0.548	-0.322	-0.599	-0.253	-0.666	-0.276	1		
CHA Per	-0.995**	-0.677*	-0.453	-0.659	-0.402	-0.780*	-0.434	0.983**	1	
CHA Deg	-0.983**	-0.597	-0.361	-0.616	-0.304	-0.704*	-0.343	0.994**	0.988**	1

Significant correlations at *P < 0.05 and **P < 0.01. PRO-Protein; AMY- Amylose; CHA Per- Chalky rice (%); CHA Deg-Chalkiness degree.

Discussion

This study explored the consequential affects foliar applied mixtures of micro-nutrients on the growth, yield, grain quality and accumulated grain nutrient contents in two fragrant rice cultivars i.e., Xiangyaxiangzhan and Guixiangzhan.

We have found that exogenous application of micro-nutrients significantly increased grain 2-AP, and other concentrations of other micro-nutrients i.e., Se, Fe, Zn, Mn, Cr in both Xiangyaxiangzhan and Guixiangzhan, however, mixture-II could not led to significant increase in grain Fe and Mn contents (*Fig. 2 a-f*). Further significant correlations were also identified among 2-AP, Zn and Se in both rice cultivars (*Table 3*). The accumulation of 2-AP, Zn and Se in response to micro-nutrient application suggested that there may be parallel activation of pathways leading to proline (the precursor of 2-AP) accumulation in response to micro-nutrient application in fragrant rice (Thimmaraju et al., 2005; Huang et al., 2012; Mo et al., 2015). In a recent study, Mo et al. (2016) concluded that exogenous Zn supplementation significantly improved 2-AP contents in aromatic rice panicles.

Furthermore, Huang et al. (2012) found that glutamate dehydrogenase, a Zn-containing enzyme present in the mitochondria, promotes the conversion of glutamate to proline and increases grain 2-AP content in fragrant rice. Zn mediates the biosynthesis of auxin plants, which could improve the chlorophyll contents and net photosynthetic rate in flag leaf, thus may may led to enhanced growth (Min et al., 2012). Additionally, application of Zn fertilizer could also promote the conversion of proline to 2-AP and increased the aroma of fragrant rice (Tang and Wu, 2006). Se-supplementation has dynamic roles in the physio-morphological features and yield formation of rice Zhang et al. (2003). Se-related increase in 2-AP contents (significant correlations) might be the confirmation of the results of Zhou et al. (2005) who reported Se-induced up-regulation in proline accumulation which might be converted to 2-AP by the activation of proline dehydrogenase activities. Positive effects of Se on brown rice quality were also reported by Li et al. (2016). Moreover, Arvy (1995) found that Se concentration has synergistic effects on Zn content but not with Fe, and Mn in *Catharanthus roseus*. Li (2016a) found that application of Fe fertilizer significantly increased Zn content in grains of rice. Recently, Li et al. (2016) reported positive effects of Mn application on growth, yield, quality and aromatic aspects of fragrant rice, whereas application of silicon (Si) in soil also led to increase in yield and aroma in fragrant rice (Mo et al., 2017). Additionally, we found significant correlations between grain 2-AP and Cr content in response to foliar applied micro-nutrients for both rice cultivars; however, the exact mechanisms between grain 2-AP and Cr content have not been known yet and needs further study.

We found significant effects of micro-nutrient application on the yield and related attributes in term of grain numbers per panicle, filled grain percentage, 1000-grain weigh and grain yield (*Table 1*). Moreover, exogenous micro-nutrient application also enhanced rice protein contents but reduced grain amylose, grain chalkiness and

chalkiness % (Table 2) as well as enhanced dry biomass accumulation in both rice cultivars (Table 3). Nutrient concentrations have significant roles in regulating the morphological growth and yield formation in rice (Abid et al., 2015). Previous studies have reported dynamic roles of micro-nutrient application on yield and quality of rice (Fang et al., 2008; Tian et al., 2009; Wu et al., 2010). Exogenous application of micro-nutrients increased grain numbers per panicle, seed setting rate and brown rice rate while decreased rice chalkiness (Fan et al., 2010). So, the results from this study revealed that mixed micro-nutrient application not only improved the rice grain mineral contents but also improved growth, yield and quality of rice. In addition, improved grain 2-AP contents in mixed micro-nutrient treated plants suggest that micro-nutrients might involve in 2-AP biosynthesis.

Conclusion

In short, exogenous application of mixed micro-nutrient has significant effects on yield and quality traits in fragrant rice, and led to enhanced grain aroma by improving grain 2-AP and mineral contents. These results further emphasized the importance of relationships among micro-nutrients especially Zn, Se and 2-AP production in aromatic rice. Exploring these relationships and their involvement in rice aroma production has great potential for optimization of both trace elements enrichment aroma production and in fragrant rice cultivars.

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