Italian Journal of Food Science, 2023; 35 (4): 111–118

Investigation of differential 2-acetyl-1-pyrroline concentration in inferior and superior spikelets of fragrant rice

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Received: 5 September 2023; Accepted: 10 November 2023; Published 1 December 2023

PAPER

Abstract

Fragrant rice is popular globally due to its desirable grain quality and strong fragrance. 2-Acetyl-1-pyrroline (2AP) is believed to be one of the key aromatic compounds found in fragrant rice. The present study investigated the differential 2AP concentration in inferior and superior spikelets of fragrant rice. Results showed that inferior spikelets produced higher aroma than superior spikelets, but opposite trend was observed in grain yield. A significant positive correlation was observed between grain yield and contribution ratio to yield, number of grains/m^2, filled number of grains/m^2, percentage of filled grains, and total nitrogen content in grains. Besides, 2AP content at different panicle positions showed a significant negative relationship with contribution ratio to yield but a significant positive correlation with moisture content. The findings demonstrated that it was feasible to improve fragrance and maintain grain yield by reducing the difference between superior and inferior spikelets during grain filling period. The results of the study revealed fragrance in grains in relation to superior and inferior spikelets in fragrant rice panicles.

Keywords: 2-acetyl-1-pyrroline; aromatic rice; grain position; nitrogen

Introduction

Rice is one of the main staple foods consumed globally, and a study has indicated that its demand is increasing (Krishnan *et al.*, 2007). Fragrant rice is a popular rice type because of its pleasant smell, with its rapidly increasing global demand (Bryant and McClung, 2010; Sakthivel *et al.*, 2009). Among various discovered volatile compounds, 2-acetyl-l-pyrroline (2AP) has been recognized as the key fragrant compound (Buttery *et al.*, 1988). 2AP content is discovered in grain, leaf, straw, and root parts in fragrant rice (Maraval *et al.*, 2008, 2010).

Presence of nitrogen (N) and microelements, irrigation, temperature, and light affect the biosynthesis and accumulation of 2AP (Li *et al.*, 2016; Mo *et al.*, 2015, 2016, 2017 and 2018; Ren *et al.*, 2017; Itani *et al*, 2004; Yang *et al.*, 2012; Yoshihashi, 2002). Nitrogen is one of the most important elements that positively affect the grain yield and fragrance of rice. For example, Zhong and Tang (2014) indicated that application of nitrogen at different growth stages affected 2AP formation in fragrant rice. Li *et al.* (2014) reported that water–nitrogen interaction at tillering stage significantly influenced 2AP content in brown rice. Besides, Ren *et al.* (2017) reported that the application of nitrogen (60 kg·per hectare [hm^{-2}]) with less water irrigation at tillering stage could remarkably increase 2AP content in grain yield. Moreover, Mo *et al.* (2018) revealed that different nitrogen application levels at booting stage affected 2AP content in different plant parts of fragrant rice. However, how to balance grain yield and fragrance in fragrant rice by nitrogen input has not been discovered.

Grain filling rate and development of spikelets vary with their positions on panicles in rice. Generally, superior spikelets with high grain filling rate and grain weight are located on upper main branch, while inferior spikelets invariably with low grain filling rate and grain weight are positioned on lower secondary branches (Sekhar *et al.*, 2015; Yang *et al.*, 2000). Reduction in grain filling rate is related to poor grain filling of inferior spikelets, which finally causes decline in grain yield or grain quality (Dong *et al.*, 2013; Fu *et al.*, 2011; Mohapatra *et al.*, 1993; Yoshinaga *et al.*, 2013). Previous studies have discovered the effects of geographical origin, irrigation, temperature, and plant growth regulators on the grain filling of superior and inferior spikelets (Chen *et al.*, 2016a, 2016b, Fu *et al.*, 2016; Hussain *et al.*, 2018). Moreover, physiological events in relation to the poor grain filling of inferior spikelet were also evaluated (Finkelstein, 2010; Kato *et al.*, 1993; Nakamura *et al.*, 1989, 1992a, 1992b; Peng *et al.*, 2011; Yang *et al.*, 2006; Zhu *et al.*, 2011). Iwasaki *et al.* (1992) discussed nitrogen accumulation between superior and inferior spikelets. However, differential 2-acetyl-1-pyrroline (2AP) content in inferior and superior spikelets of fragrant rice has not been studied till now.

Hence, this study aimed to investigate fragrance differences in inferior and superior spikelets of fragrant rice. It further explored the relationship between fragrance and grain yield and other investigated parameters in inferior and superior spikelets of fragrant rice.

Materials and Methods

Experimental description

A small-plot (plot size: 15 m^2) experiment was conducted at the experimental farm of South China Agricultural University. The seeds of Nongxiang18 were collected from the College of Agriculture, South China Agricultural University, Guangzhou, China. This region has a monsoonal climate with a mean temperature of 23.7°C, an average RH of 75.3%, total precipitation of 711.1 mm, and total sunshine period of 980.6 h (Mo *et al.*, 2018). The soil of the experimental plot was sandy loam texture containing soil organic matter, 18.65 $g \cdot kg^{-1}$, total nitrogen, 1.17 $g \cdot kg^{-1}$, available phosphorus, 32.69 $mg \cdot kg^{-1}$, and available potassium, 185.28 $mg \cdot kg^{-1}$, with a pH of 6.44.

Experimental design

The 30-day-old wet raising seedlings were transplanted to experimental plot. Nitrogen (90 $kg \cdot N \cdot hm^{-2}$), phosphorus (90 $kg \cdot P_2O_5 \cdot hm^{-2}$), and potassium (195 $kg \cdot K_2O \cdot hm^{-2}$) were applied as basic fertilizers in the form of urea, superphosphate, and potassium chloride, respectively. Five days after transplanting, nitrogen fertilizer of 30 $kg \cdot hm^{-2}$ was added. At booting stage, three nitrogen levels were applied: 0 $kg \cdot hm^{-2}$ (N0), 30 $kg \cdot hm^{-2}$ (N1), and 60 $kg \cdot hm^{-2}$ (N2). The experiment was arranged in a randomized complete block design (RCBD) with three replicates. Crop management practices, such as irrigation, and pest, disease, and weed control, applied to experimental plots were uniform to avoid yield and fragrance differences (Mo *et al.*, 2018).

Sampling and measurements

Yield and its components

At maturity, 25 hills of plants in each plot were harvested to measure yield and yield-related traits. All primary branches were evenly divided into three parts—upper, middle, and base parts—according to the method described by Dong *et al.* (2008). Grain yield, yield ratio, grain number per square meter (m^2), filled grain number per m^2, filled grain percentage, and grain weight were measured.

2AP concentration, nitrogen content, and moisture content in grains

In all, 12 hills of plants were harvested for measuring 2AP concentration, and nitrogen and moisture contents in grains. The primary branches were divided evenly into three parts according to the method described by Dong *et al.* (2008). Then the grains of each part were separated into two sections—the first for determining 2AP concentration and the second for moisture content measurement. After measuring the moisture content, the grain sample was dried by stoving apparatus and crunched to powder for measuring total nitrogen content.

The 2AP concentration and nitrogen content were measured according to the methods described by

Mo *et al.* (2018). The 2AP concentration was determined by using synchronization distillation and extraction (SDE) method combined with gas chromatography–mass spectrometry (GC-MS) analysis (QP 2010 plus; Shimadzu Corporation, Kyoto, Japan). Digested with concentrated sulfuric acid, the total nitrogen content in the digestion solution was investigated by Kjeldahl method using a 2300 Kjeltec analyzer unit (Foss Tecator AB, Hilleroed, Denmark). The moisture content in grains was measured by using oven-dried method (Chen, 2003).

Statistical analysis

The Statixtics 8.0 program (Analytical Software, Tallahassee, FL, USA) was used for data analysis. The least significant difference (LSD) test was used to compare treatment mean values and determine significant differences ($p < 0.05$).

Results

2AP content and contribution in grains at different positions on fragrant rice panicles

The lowest content of 2AP was recorded in grains at the upper position of fragrant rice panicles. Compared to the upper position, the middle and base positions showed 20.32% and 69.87% higher 2AP content, respectively. The trend of 2AP content in grains at different positions on fragrant rice panicles was recorded as: base > middle > upper positions. The contribution rate of 2AP in grains at different positions on fragrant rice panicles to the mean 2AP content for base, middle, and upper positions was 30.60%, -23.12%, and -7.49%, respectively (Figure 1).

Compared to the upper position, the total 2AP accumulation in grains at 1 m^2 was significantly higher in grains at middle and base positions on fragrant rice panicles. The contribution ratio of 2AP to the total 2AP accumulation in grains at different positions on fragrant rice panicles were recorded as 36.88%, 35.57%, and 27.55% for base, middle and upper positions, respectively (Figure 2).

Moisture and total nitrogen contents in grains at different positions on fragrant rice panicles

Nitrogen affected the moisture and total nitrogen contents in grains. Position (P) on panicles significantly affected moisture content in grains. The moisture content in grains differed significantly with different positions, and moisture content was 16.63%, 18.58%, and 20.36% for upper, middle, and base positions, respectively. There was no significant difference in total nitrogen content between different positions on fragrant rice panicles (Table 1).

Grain yield and yield ratio of different positions on fragrant rice panicles

Both nitrogen and position significantly affected grain yield. Grain yield at upper and middle positions on fragrant rice panicles was significantly higher than that at the base position. Likewise, position significantly impacted yield ratio. Both upper and middle positions contributed a higher yield ratio than the base position, with the yield ratio at upper, middle, and base positions being 35.28%, 37.50%, and 27.51%, respectively. Moreover, both nitrogen and position significantly affected grain number per m^2, filled grain number per m^2, and filled grain percentage. A similar trend for grain number per m^2 and filled grain number per m^2 was observed, which was recorded as: middle > upper > base positions. The middle and upper positions showed significantly higher filled grain proportions than the base position. Furthermore, the

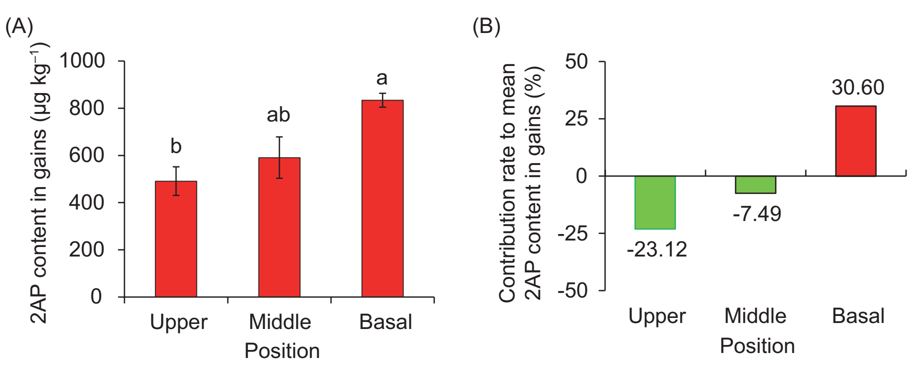

Figure 1. (A) 2AP content in grains at different positions on fragrant rice panicles, and (B) the contribution ratio of 2AP in grains at different positions on fragrant rice panicles to the mean 2AP content. Vertical bars with different lowercase letters above are significantly different at *p* = 0.05 by using the least significant difference (LSD) test. Capped bars represent SD.

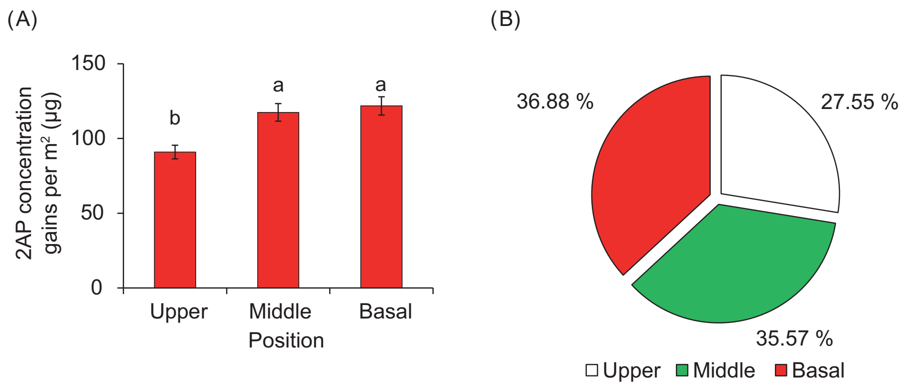

Figure 2. (A) The total 2AP concentration in grains per m^2, and (A) the contribution ratio of 2AP in grains at different positions on fragrant rice panicles to the total 2AP accumulation. Vertical bars with different lowercase letters above are significantly different at *p* = 0.05 by the least significant difference (LSD) test. Capped bars represent SD.

Table 1. Moisture content and total nitrogen content of grains at different positions on fragrant rice panicles.

Position	Nitrogen	Moisture content (%)	Total nitrogen content (mg·kg^{-1})
Upper	N2	17.38	1.3130
	N1	16.38	0.7760
	N0	16.13	0.6300
	Mean	**16.63**^{c}	**0.9063**^{a}
Middle	N2	19.19	0.9210
	N1	18.02	0.8950
	N0	18.54	0.7840
	Mean	**18.58**^{b}	**0.8667**^{a}
Basal	N2	20.49	0.8620
	N1	19.89	0.7830
	N0	20.70	0.7360
	Mean	**20.36**^{a}	**0.7937**^{a}
Variance	N	**	**
analysis	P	**	ns
	N×P	**	ns

Within a column, mean values followed by the same letter were not significantly different at a probability level of 0.05 according to least significant different (LSD) test. *Significant at $p < 0.05$, and **significant at $p < 0.01$; ns: nonsignificant at $p < 0.05$.

position and the interactive effect of both nitrogen and position (N×P) significantly affected grain weight. Grain weight at upper position was significantly higher than that at middle and base positions (Table 2).

Correlation analysis

A significant positive correlation was observed between 2AP and moisture content, but a significant negative correlation was discovered between 2AP and yield ratio. The grain yield was significantly associated in a positive manner with yield ratio, grain number per m^2, filled grain number per m^2, filled grain percentage, and total nitrogen content. Filled grain number per m^2 and filled grain percentage were significantly related in a positive manner to yield ratio; however, moisture content showed a significant negative correlation with yield ratio. Furthermore, total nitrogen content showed a significant positive correlation with both grain number per m^2 and filled grain number per m^2 (Table 3).

Evaluated effects of changing proportions of the ratio of grain yield to grain yield and 2AP accumulation in grains

For evaluating the effects of changing proportions of the ratio of grain yield to grain yield and 2AP accumulation in grains, we first had a hypothesis that the contribution ratio presented in this study was the ideal ratio; then, the evaluation was based on increasing contribution ratio to yield at different positions by 10%, 30%, and 50%. With increasing 10%, 30%, and 50% of contribution ratio to yield at any position on fragrant rice panicles, the grain yield did not change significantly, but with a small change range of -0.790%–-0.003%. However, with an increasing 10% contribution ratio to yield at upper, middle, and base positions, the 2AP accumulation change was -3.659%, -1.073%, and 4.482%, respectively. Furthermore, with an increasing 30% contribution ratio to yield at upper, middle, and base positions, the 2AP accumulation change was -10.330%, -2.625%, and 13.948%, respectively. In addition, with an increasing 50% contribution ratio to yield at upper, middle, and base positions, the 2AP accumulation change was -17.001%, -4.177%, and 23.415%, respectively (Table 4).

Table 2. Grain yield and contribution ratio to grain yield at different positions and yield-related traits.

Position	Nitrogen	Grain yield ($g{\cdot}m^{-2}$)	Yield ratio (%)	Grain number per m^2	Filled grain number per m^2	Filled grain percentage (%)	Grain weight (mg)
Upper	N2	216.42	35.92	9,670	8,340	86.43	25.94
	N1	182.27	34.44	8,131	7,084	86.81	25.83
	N0	157.12	35.47	6,944	5,969	85.83	26.33
	Mean	**185.27[a]**	**35.28[a]**	**8,248[b]**	**7,131[b]**	**86.36[a]**	**26.03[a]**
Middle	N2	226.34	37.46	10,030	8,748	87.25	25.86
	N1	205.02	38.26	9,921	8,440	84.72	24.41
	N0	165.02	36.77	7,547	6,422	84.62	25.8
	Mean	**198.79[a]**	**37.50[a]**	**9,166[a]**	**7,870[a]**	**85.53a**	**25.36[b]**
Basal	N2	161.97	26.59	7,513	6,301	83.91	25.66
	N1	149.14	27.58	7,582	6,184	81.58	24.21
	N0	126.98	28.37	6,338	4,985	78.32	25.46
	Mean	**146.03[b]**	**27.51[b]**	**7,144[c]**	**5,823[c]**	**81.27[b]**	**25.11[b]**
Variance analysis	N	*	ns	**	*	**	ns
	P	**	**	**	**	**	**
	N×P	ns	ns	ns	ns	ns	*

Within a column, mean values followed by the same letter are not significantly different at a probability level of 0.05 according to least significant different test (LSD). *Significant at $p < 0.05$ and **significant at $p < 0.01$; ns, nonsignificant at $p < 0.05$.

Table 3. Evaluated effects of changing proportions of ratio of grain yield to grain yield and 2AP accumulation in grains. Evaluation was based on increase in contribution ratio to yield at different positions by 10%, 30%, and 50%.

Positions	Yield			2AP		
	10%	30%	50%	10%	30%	50%
Upper	–0.393	–0.592	–0.790	–3.659	–10.330	–17.001
Middle	–0.247	–0.152	–0.058	–1.073	–2.625	–4.177
Lower	–0.233	–0.115	0.003	**4.482**	**13.948**	**23.415**

Table 4. Correlation analysis of investigated parameters at different positions (2AP, grain yield, yield ratio, grain weight, grain number per m^2, filled grain number per m^2, filled grain percentage, moisture content, and total nitrogen content).

Index	2AP	GY	YR	GW	GN	FGN	FGP	MC	TN
2AP	1.0000	–0.3459ns	–0.8097**	–0.4914 ns	–0.2096 ns	–0.2880 ns	–0.6546 ns	0.9265**	–0.0130 ns
GY	–0.3459 ns	1.0000	0.7168*	0.1144 ns	0.9662**	0.9869**	0.7903*	–0.3724 ns	0.6870*
YR	–0.8097**	0.7168*	1.0000	0.2641 ns	0.6457 ns	0.6938*	0.7115*	–0.6789*	0.2538 ns
GW	–0.4914 ns	0.1144 ns	0.2641 ns	1.0000	–0.1373 ns	–0.0444 ns	0.4538 ns	–0.4077 ns	0.0427 ns
GN	–0.2096 ns	0.9662**	0.6457 ns	–0.1373 ns	1.0000	0.9930**	0.6440 ns	–0.2426 ns	0.6960*
FGN	–0.2880 ns	0.9869**	0.6938*	–0.0444 ns	0.9930**	1.0000	0.7281*	–0.3253 ns	0.6727*
FGP	–0.6546 ns	0.7903*	0.7115*	0.4538 ns	0.6440 ns	0.7281*	1.0000	–0.7059*	0.3461 ns
MC	0.9265**	–0.3724 ns	–0.6789*	–0.4077 ns	–0.2426 ns	–0.3253 ns	–0.7059*	1.0000	–0.0538 ns
TN	–0.0130 ns	0.6870*	0.2538 ns	0.0427 ns	0.6960*	0.6727*	0.3461 ns	–0.0538 ns	1.0000

GY: grain yield, YR: yield ratio, GW: grain weight, GN: grain number per m^2, FGN: filled grain number per m^2, FGP: filled grain percentage, MC: moisture content, TN: total nitrogen content. *Significant at $p < 0.05$ and **significant at $p < 0.01$; ns, nonsignificant at $p < 0.05$ level.

Discussion

The effects of nitrogen application on crop yield are well established (Hou *et al.*, 2018; Sun *et al.*, 2018; Zhang *et al.*, 2018). As a major factor limiting the yield potential of rice, nitrogen application enhanced grain yield by improving the number of panicles and spikelets (Kropff *et al.*,1993; Wang *et al.*, 2016). In this study, nitrogen application affected grain yield, grain number per m^2, filled grain number per m^2, and filled grain percentage. No significant effect of nitrogen was observed on grain weight and yield ratio (Table 2). Besides, grain yield and yield ratio were significantly affected by the positions and both showed the following trend: upper > middle > basal positions. Moreover, grain number per m^2, filled grain number per m^2, and filled grain percentage showed the following trend: middle > upper > basal positions. Grain weight at upper position was significantly higher than that at middle and basal positions (Table 2). Furthermore, grain yield showed a significant positive correlation with yield ratio, grain number per m^2, filled grain number per m^2, filled grain percentage, and total nitrogen content (Table 3). It suggested that grain yield was strongly associated with superior and inferior spikelets on rice panicles; the result was highly consistent with previous reports (Chen *et al.*, 2016a, 2016b; Fu *et al.*, 2016; Hussain *et al.*, 2018).

On the other hand, nitrogen application not only regulated grain yield but also modulated the 2AP content in the grains of fragrant rice (Itani *et al.*, 2004; Li *et al.*, 2014; Mo *et al.*, 2018; Ren *et al.*, 2017; Zhong and Tang, 2014). In this study, the 2AP content of grains under different nitrogen treatments showed the following trend: N2 > N1 > N0 (data not shown). Further analysis of 2AP at different positions on fragrant rice panicles indicated the following trend: base > middle > upper positions. Contribution proportion to mean 2AP content in grains at base, middle, and upper positions was discovered as 30.60%, -23.12%, and -7.49%, respectively. Besides, significant higher 2AP accumulation in grains per m^2 was observed in grains at middle and base positions than upper position on fragrant rice panicles. In addition, the contribution ratio of 2AP to the total 2AP accumulation in grains at different positions on fragrant rice panicles was recorded as 36.88%, 35.57%, and 27.55% for base, middle, and upper positions, respectively (Figures 1 and 2). Therefore, a certain relationship existed between yield formation and 2AP formation as affected by superior and inferior spikelets of rice panicles. This could support the results of previous studies reporting that fragrance increase in grains was associated with yield decrease in fragrant rice under salt treatment (Fitzgerald *et al.*, 2010; Gay *et al.*, 2010). Moreover, previous studies reported increase in 2AP under (slight) stress conditions, such as shading and salinity, which could lead to yield loss (Gay *et al.*, 2010; Mo *et al.*, 2015).

In order to have the highest economic return, it is important to balance grain yield and fragrance in fragrant rice. Analysis revealed a significant positive correlation between 2AP and moisture content but a significant negative correlation between 2AP and yield ratio (Table 3). Furthermore, in this study, effects of the changing proportions of the ratio of grain yield to grain yield and 2AP accumulation in grains was evaluated. This suggested that a balance between superior and inferior spikelets on rice panicles was essential to produce a strong fragrance without significantly reducing grain yield (Table 4). Thus, it showed that during grain filling period, approaches to reduce difference between superior and inferior spikelets were available for a specific fragrant rice variety to improve grain fragrance while maintaining grain yield. However, a study is required on grain quality difference in superior and inferior spikelets to achieve the grain yield–fragrance–quality balance.

Conclusion

Both basal and middle positions produced significantly higher 2AP accumulation than the upper position. The contribution to total 2AP accumulation at basal, middle, and upper positions was 36.88%, 35.57%, and 27.55%, respectively. Besides, the 2AP content in grains on fragrant rice panicles showed the following trend: basal (834.06 μg·kg^{-1}) > middle (590.82 μg·kg^{-1}) > upper (491.00 μg·kg^{-1}) positions, and the basal (30.60%), middle (-7.49%), and upper (-23.12%) positions contributed to the mean 2AP content in grains differently. Both grain yield and 2AP balance were evaluated by changing the proportions of grain yield ratio. Therefore, approaches to reduce differences between superior and inferior spikelets during grain filling period are available for a specific fragrant rice variety in order to improve grain fragrance while maintaining grain yield.

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