IRRIGATION AND NITROGEN MANAGEMENT PRACTICES AFFECT GRAIN YIELD AND 2-ACETYL-1-PYRROLINE CONTENT IN AROMATIC RICE

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Abstract. Aromatic rice has a high market value due to its special fragrance and the most important compound in the grains of aromatic rice is 2-acetyl-1-pyrroline (2-AP). In an effort to improve the 2-AP content and the yield of aromatic rice, two known rice cultivars, *Yungao* and *Yundi,* were cultivated across two seasons, and two irrigation and nitrogen management practices were investigated. The results showed that the treatment management practice (TNW) treatment improved panicle number per hill, seed setting rate, and 1000 grain weight and grain yield for both cultivars in early and late season. Significant improvement in grain yield was only observed in *Yundi* in both seasons. Moreover, the 2-AP content in grains was increased for both cultivars in both seasons during grain filling period. Significant increase in 2-AP in grains was observed for *Yungao* at maturity in both seasons, at 7 d AH in early season and at 14 d AH in late season whilst for *Yundi* at 7 d AH, 14 d AH and 21 d AH in early season, and at 14 d AH in late season. Furthermore, 2-AP content in leaves and grains was decrease during grain filling period while proline content in grains was enhanced during grain filling period. Significant relationships were also observed between 2-AP and proline contents in the leaves/grains of both rice cultivars.

Keywords: *aromatic rice; 2-acetyl-1-pyrroline; proline; nitrogen; irrigation*

Introduction

Rice is one of the most important cereal crops and feeds billions of people around the world. Aromatic rice, such as Basmati and Jasmine, has a characteristic fragrance and good grain quality (Ashraf et al., 2017; Ashraf and Tang, 2017). The aromatic rice grains valued at a higher market price than non-aromatic rice varieties (Zhang et al., 2008). Since the 1980s, numerous studies have investigated hundreds of fragrant compounds in scented rice grains and identified the 2-acetyl-1-pyrroline (2-AP) as the key fragrant

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compound of aromatic rice (Yajima et al., 1979; Buttery et al., 1982; Widjaja et al., 1996; Champagne, 2008).

The 2-AP accumulation in aromatic rice is affected by many environmental factors during aromatic rice growth. For example, high night temperature reduced grain quality remarkably (Mohammed and Tarpley, 2010; Nagarajan et al., 2010). During grain filling period, shading treatments significantly improved the 2-AP content and γ-aminobutyric acid (GABA) content in aromatic rice grains (Mo et al., 2015). With salt stress treatment, the popular aromatic rice (*Khao Dawk Mali 105*) cells accumulated Na^+ and proline (Summart et al., 2010) whilst the reduction of fragrant rice yield was related to the fragrance in rice (Fitzgerald et al., 2010). Moreover, some studies reported that salt stress can increase 2-AP content in grains (Gay et al., 2010; Poonlaphdecha et al., 2012). Some minor elements were also found to be associated with 2-AP accumulation in aromatic rice (Lei et al., 2017). Manganese (Mn) application had a positive effect on rice yield, quality and 2-AP content (Li et al. 2016). 2-AP content and proline dehydrogenase (ProDH) activity were increased by moderate concentration of zinc (Zn) and lanthanum (La) treatment (Mo et al., 2016). Furthermore, silicon fertilization also modulates 2-acetyl-1-pyrroline content n fragrant rice (Mo et al., 2017).

Many previous studies have reported that nitrogen and water as important aspects for aromatic rice growth and development. The nitrogen utilization efficiency is associated to the rice genotypes and locations (Djaman et al., 2016). Sikdar et al. (2008) found that N level at 80 kg/hm^2 improved grain quality and soil fertility. However, high nitrogen can lead to lodging in rice (Mahajan et al., 2010). In Asia, rice production is limited due to water shortage (Arora et al., 2006), thus, irrigation patterns directly influence rice production. Alternating wetitnng and drying could regulate rice yield (Zhang et al., 2012), whilst moderate irrigation control can also have a positive impact on 2-AP content and grain yield of aromatic rice (Yoshihashi et al., 2002; Tian et al., 2014). Hakoomat et al. (2014) found the N and Zn interactions had the most significant improvement on yield and yield related traits when the N level was $12kg/hm^2$ and Zn level was $14kg/hm^2$. Moreover, moderate irrigation at tillering, booting, and grain filling stage could increase the 2-AP content in grains (Tian et al., 2010; Wang et al., 2013a, b). Yang et al. (2012) reported high aroma content in grains was associated to high total nitrogen in soil. Zhong and Tang (2014) found that 2-AP content in grains was increased with increasing nitrogen application. Li et al. (2014) found the 2-AP content in brown rice was highest when the N supply was $60kg/hm^2$ and the irrigation potential was -20 kPa at tillering stage.

In our previous studies, we have investigated the interaction effect of water and nitrogen at tillering stage (Li et al., 2014), booting stage and grain filling stage on grain yield and 2-AP accumulation in grains (data unpublished), then, we selected the best water and nitrogen treatment of the three stages and combined the treatments together for investigating the additive effect of the nitrogen and water management treatment on aromatic rice yield and 2-AP accumulation. In this study, we aimed to identify the affects of the combinations of water and nitrogen applications implemented at tillering stage, booting stage, and grain filling stage on 2-AP accumulation and grain yield.

Materials and Methods

Plant materials and growing condition

Two popular aromatic rice cultivars, *Yungao* and *Yundi*, having 115 -120 days of growth period were planted in early season and late season in 2015 at the College of Agriculture, South China Agricultural University, Guangzhou, China (23°20’ N, 113°30’ E and 11 m above the sea level. The experimental site has a subtropical-monsoon type climate with mean annual air temperatures of 22.4 ºC and mean annual precipitation of 2638.3 mm (Fig. 1). The seeds were soaked in water for 24 h, germinated in manual climatic box for the next 24h, and raised at the Research Farm of the College Agriculture. 30-day-old seedlings were transplanted to the field at the recommended planting distance (30 cm × 12 cm). The experimetal soil was sandy loam with of 25.65% organic matter content, 1.362% total N, 0.958% total P, and 17.520% total K.

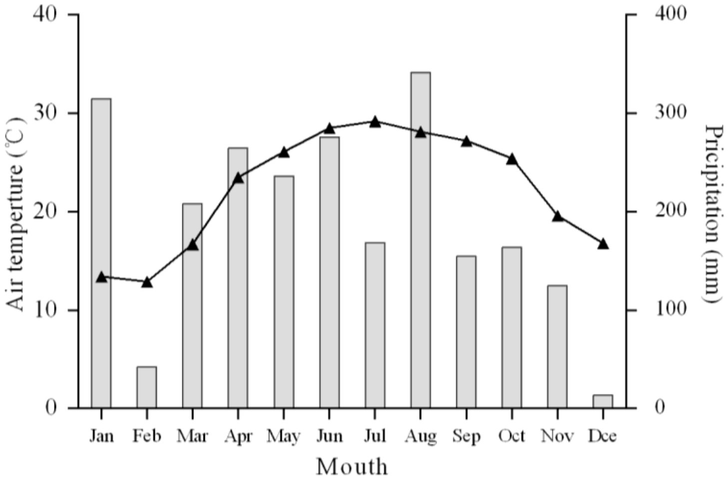

Figure 1. *Mean monthly temperature and precipitation in 2016 of the experimental site*

Treatments and plant sampling

The experiment was conducted in early season and repeated in late season. Experimental treatments are as follows: (i) The conventional management practice (CK) involves applying nitrogen (30 kg/hm^2) at tillering stage, booting stage, and grain filling stage; the water flow at these three stages was 0 kPa in both early and late season rice. (ii) The treatment management practice involved applying nitrogen (60 kg/hm^2) at tillering stage with heavy drought conditions (water potential of - (25±5) kPa), applying 60 kg/hm^2 nitrogen at the booting stage with feebly arid conditions (water potential of - (15±5) kPa), and applying no nitrogen at the grain filling stage with shallow-watered irrigation (water flow of 0 kPa) (TNW). P_2O_5 (90 kg/hm^2), K_2O (195 kg/hm^2) were applied as basic fertilizer. The fresh leaves and grains were sampled from the rice at the end of tillering stage; after 7, 14, and 21 d heading stage; and at maturity. Samples were immediately stored at -20°C for 2-AP analysis.

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Yield and yield related traits

At maturity, the grains were harvested from one unit sampling area (8.1 m^2) in each plot and threshed by machine. The harvested grains were sundried, and the dry weight was used to estimate the grain yield. Thirty hills of rice plants from different locations in each plot were sampled for calculating the average effective panicles number per hill. Then, three hills representative plants were taken for determination of the yield related traits.

Proline estimation

The proline content in leaves and grains were measured according to the method established by Bates et al. (1973). Leaves or grains in which the weight was almost 0.3 g, were homogenized in a 4 ml solution of 3% sulfosalicylic acid and cooled after bringing to a boil for 10 min. Samples were filtered and 2 ml of the filtrate was mixed with 3 mL ninhydrin reagent (2.5 g ninhydrin in 60 mL glacial acetic acid and 40 mL 6 M phosphoric acid) and 2 mL glacial acetic acid. For the extraction of proline, the mixture was boiled for 30 min and 4 mL toluene was added to the cooled liquid. The extract was centrifuged at 4000rpm for 5 min, and proline absorbance was detected at 520 nm. The proline concentration, expressed as $\mu g \cdot g^{-1}$, was assayed by using standard curve to calibrate.

2-AP estimation

The 2-AP content was estimated using the method described by Huang et al. (2012), Prior to analysis, leaves were cut into sections and grains were ground by mortar and pestle. Approximately 5 g leaves or 10 g grains were mixed homogeneously with 150 mL purified water into a 500 mL round-bottom flask attached to a continuous steam distillation extraction head. The mixture was boiled at 150°C in an oil pot. A 30 mL aliquot of dichloromethane was used as the extraction solvent and was added to a 500 mL round-bottom flask attached the other head of the continuous steam distillation apparatus, and this flask was boiled in a water pot at 53°C. The continuous steam distillation extraction was linked with a cold water circulation machine in order to keep temperature at 10°C. After approximately 35 min, the extraction was complete. Anhydrous sodium sulfite was added to the extract to absorb the water. The dried extract was filtered by organic needle filter and analyzed for 2-AP content by GCMS-QP 2010 Plus. High purity helium gas was used as the carrier gas at flow rate of 2 mL/min. The temperature gradient of the GC oven was as follows: 40°C (1 min), increased at $2°C \cdot min^{-1}$ to 65°C and held at 65°C for 1 min, and then increased to 220°C at $10°C \cdot min^{-1}$, and held at 220°C for 10 min. The retention time of 2-AP was confirmed at 7.5 min. Each sample had three replicates, and 2-AP was expressed as $\mu g \cdot kg^{-1}$.

Treatment design and statistical analysis

This study was arranged as a randomized complete block design (RCBD) with three replicates (n=3). Data were analyzed by SPSS 16.0 software (SPSS Inc., Chicago, USA), and the probability level was set at 5% ($P < 0.05$).

Results

Grain yield and yield related traits

As shown in *Table 1*, the panicle number per hill, seed-setting rate, and 1000-grain weight and yield were higher in TNW treatment than in CK for *Yungao* and *Yundi* in both seasons. The seed setting rate was significantly improved by 11.75% in TNW treatment for *Yundi* in late season. The grain yield was dramatically increased by 12.13% and 21.00% for *Yundi* in early and late season, respectively. CK treatment had higher grain number per panicle than TNW treatment in *Yungao* and *Yundi* in both seasons.

Table 1. *Effects of irrigation and nitrogen management practices on yield and yield related traits in aromatic rice*

Season	Cultivar	Treatment	Panicle number per hill	Grains number per panicle	Seed-setting rate (%)	1000-grain weight (g)	Yield (t hm^{-2})
Early season	Yungao	CK	14.03±0.62a	82.52±2.07a	87.24±0.81a	25.13±0.08a	5.16±0.45a
		TNW	15.48±0.71a	74.92±1.50a	88.58±2.37a	26.20±0.51a	5.74±0.38a
	Yundi	CK	13.05±0.48a	108.80±8.96a	88.60±1.70a	24.68±0.12a	5.36±0.20b
		TNW	13.30±0.55a	93.68±4.00a	90.25±0.96a	24.85±0.30a	6.01±0.07a
Late season	Yungao	CK	12.76±0.49a	111.00±5.41a	81.98±4.72a	25.88±0.22a	4.59±0.27a
		TNW	13.00±0.57a	108.49±4.12a	86.10±0.78a	26.50±0.44a	4.73±0.14a
	Yundi	CK	12.69±0.47a	114.82±3.40a	79.97±2.33b	24.03±0.13a	4.81±0.08b
		TNW	12.77±0.47a	109.65±10.79a	89.37±2.34a	24.60±0.44a	5.82±0.07a

Means in the same column followed by different lower case letters for the same variety differ significantly at $P < 0.05$ by T-test. CK (control); TNW (60 kg/hm^2 nitrogen and water potential of - (25±5) kPa) were applied at the tilling stage, 60 kg/hm^2 nitrogen and water potential of - (15±5) kPa were applied at the booting stage, no nitrogen and water flow of 0 kPa were applied at the grain filling stage)

2-AP content in grains

We observed higher 2-AP content in grains in TNW treatment than CK for both cultivars at all the sampling stages in the early and late season. For *Yungao*, the 2-AP content in grains were significantly increased by 61.88% and 31.10% at 7 d AH and maturity in early season, respectively; significant increase in 2-AP content in grains by

79.02% and 13.88% were observed at 14 d AH and maturity in late season, respectively. For *Yundi*, the 2-AP content in grains in TNW treatment were remarkably higher than CK at 7 d AH, 14 d AH and 21 d AH in early season; significant improved in 2-AP content in grains by 12.07% was found at 14 d AH in late season.

Table 2. *Effects of irrigation and nitrogen management practices on 2-AP content of grains at different stages (μg·kg^{-1})*

Season	Cultivar	Treatment	7 d AH	14 d AH	21 d AH	Maturity
Early season	Yungao	CK	102.01±0.46b	115.56±5.75a	145.84±8.09a	387.30±24.94b
		TNW	165.13±6.80a	129.26±5.21a	146.11±3.41a	507.76±27.14a
	Yundi	CK	119.68±2.35b	67.55±1.01b	94.46±5.75b	104.42±11.50a
		TNW	176.14±1.82a	124.14±3.96a	119.07±2.21a	120.91±2.88a
Late season	Yungao	CK	139.24±0.22a	126.77±1.12b	140.77±3.17a	255.99±3.01b
		TNW	140.65±1.22a	226.94±5.48a	146.54±0.07a	291.51±8.48a
	Yundi	CK	146.43±0.85a	233.22±4.00b	164.51±7.47a	327.59±1.44a
		TNW	164.93±5.17a	261.36±5.93a	176.73±6.73a	336.91±3.11a

Means in the same column followed by different lower case letters for the same variety differ significantly at P <0.05 by T-test. AH=after heading; CK (control); TNW (60 kg/hm^2 nitrogen and water potential of - (25±5) kPa) were applied at the tilling stage, 60 kg/hm^2 nitrogen and water potential of - (15±5) kPa were applied at the booting stage, no nitrogen and water flow of 0 kPa were applied at the grain filling stage)

2-AP content in leaves

Table 3 shows that TNW treatment decrease 2-AP content in leaves at all the sampling stages. In early season, 2-AP content in leaves were significantly decreased except for 21 d AH and 14 d AH for *Yungao* and *Yundi*, respectively. The 2-AP content in leaves in TNW treatment decreased for both rice cultivars at 14 d AH, and for *Yungao* at the maturity.

Table 3. *Effects of irrigation and nitrogen management practices on 2-AP content of leaves at different stages (μg·kg^{-1})*

Season	Cultivar	Treatment	7 d AH	14 d AH	21 d AH	Maturity
Early season	Yungao	CK	401.96±4.12a	245.84±16.62a	109.84±2.61a	418.81±7.14a
		TNW	342.24±11.31b	121.43±4.21b	109.51±0.16a	284.18±0.58b
	Yundi	CK	356.64±6.33a	212.29±7.24a	221.28±6.60a	217.18±5.18a
		TNW	112.16±6.57b	211.61±3.73a	117.84±6.60b	112.13±3.30b
Late season	Yungao	CK	347.59±5.19a	200.41±6.73a	238.54±4.61a	238.57±10.19a
		TNW	239.16±11.31b	190.39±2.90a	217.04±6.33a	199.82±2.70b
	Yundi	CK	343.59±2.56a	170.41±4.97a	209.46±12.09a	198.72±15.5a
		TNW	283.23±11.49b	167.32±5.78a	199.58±0.85a	197.52±5.77a

Means in the same column followed by different lower case letters for the same variety differ significantly at $P < 0.05$ by T-test. AH=after heading; CK (control); TNW (60 kg/hm^2 nitrogen and water potential of - (25±5) kPa) were applied at the tilling stage, 60 kg/hm^2 nitrogen and water potential of - (15±5) kPa were applied at the booting stage, no nitrogen and water flow of 0 kPa were applied at the grain filling stage)

Proline content in grains

The proline content in grains increased at all the sampling stages during grain filling in the early and late season. For *Yungao*, the proline content significantly enhanced at 21 d AH and maturity in early season and remarkably increased at 7 d AH, 14 d AH and 21 d AH in the late season. For *Yundi*, TNW treatment significantly increased proline content in grains at 7 d AH in early season, significantly improved proline content in grains at 7 d AH and 14 d AH in late season was observed (*Table 4*).

Table 4. *Effects of irrigation and nitrogen management practices on proline content on grains at different stages (μg·g−1)*

Season	Cultivar	Treatment	7 d AH	14 d AH	21 d AH	Maturity
Early season	Yungao	CK	41.17±3.72a	46.82±2.44a	28.11±0.17b	25.31±0.04b
		TNW	48.89±0.39a	47.45±3.13a	36.77±0.27a	26.53±0.05a
	Yundi	CK	20.60±0.46b	14.69±0.98a	22.58±0.07a	22.52±0.03a
		TNW	45.66±1.73a	20.91±4.30a	22.71±1.21a	23.64±0.77a
Late season	Yungao	CK	43.23±1.80b	61.02±3.10b	43.84±0.85b	7.36±0.80a
		TNW	52.97±1.97a	79.79±1.53a	60.42±1.37a	9.50±0.79a
	Yundi	CK	44.70±6.39b	42.22±3.03b	30.41±4.00a	5.26±1.56a
		TNW	48.84±2.57a	56.09±2.30a	35.51±0.98a	8.13±1.76a

Means in the same column followed by different lower case letters for the same variety differ significantly at $P < 0.05$ by T-test. AH=after heading; CK (control); TNW (60 kg/hm^2 nitrogen and water potential of - (25±5) kPa) were applied at the tilling stage, 60 kg/hm^2 nitrogen and water potential of - (15±5) kPa were applied at the booting stage, no nitrogen and water flow of 0 kPa were applied at the grain filling stage).

Proline content in leaves

We observed a decrease in proline content in leaves at all the sampling stages in the early and late season. For *Yungao*, significant decreased in proline in leaves was found at the 7 d AH and maturity in the early season; a significant decrease in proline in leaves was detected at 7 d AH, 14 d AH and 21 d AH in late season. For *Yundi*, TNW treatment showed significantly lower in proline content in leaves than CK at 7 d AH, 21 d AH and Maturity in early season, and at 7 d AH and 14 d AH in late season (*Table 5*).

Table 5. *Effects of irrigation and nitrogen management practices on proline content in leaves at different stages (μg·g^{-1})*

Season	Cultivar	Treatment	7 d AH	14 d AH	21 d AH	Maturity
Early season	Yungao	CK	99.37±4.04a	52.14±3.59a	15.31±1.32a	22.08±0.82a
		TNW	86.78±0.40b	45.99±2.77a	14.33±0.09a	17.62±0.81b
	Yundi	CK	94.55±1.00a	67.94±0.32a	20.72±0.03a	20.75±0.01a
		TNW	59.64±1.76b	54.27±5.20a	12.54±0.80b	11.82±0.36b
Late season	Yungao	CK	20.53±0.72a	27.79±0.35a	17.66±0.97a	12.30±0.55a
		TNW	17.77±0.30b	9.29±0.46b	13.63±0.15b	8.69±1.40a
	Yundi	CK	23.11±1.08a	15.86±0.03a	16.05±0.73a	16.75±0.06a
		TNW	17.01±1.18b	14.67±0.14b	15.09±0.34a	15.05±1.47a

Means in the same column followed by different lower case letters for the same variety differ significantly at P <0.05 by T-test. AH=after heading; CK (control); TNW (60 kg/hm^2 nitrogen and water potential of - (25±5) kPa) were applied at the tilling stage; 60 kg/hm^2 nitrogen and water potential of - (15±5) kPa were applied at the booting stage; no nitrogen and water flow of 0 kPa were applied at the grain filling stage).

Correlation analysis

There existed a significant positive correlation between yield and seed-setting rate (r=0.8402, P<0.01) and a significant negative correlation between effective panicles and grain number per panicle (r=-0.9335, P<0.01) was observed.

Table 6. *Relationship between yield and yield related traits of two rice cultivars*

Paramaters	Panicle number per hill	Grains number per panicle	Seed-setting rate	1000-grain weight
Grains number per panicle	-0.9335**			
Seed-setting rate	0.3906	-0.5014		
1000-grain weight	0.4348	-0.3419	0.0870	
Yield	0.3979	-0.4836	0.8402**	-0.2389

Significant correlations at *P < 0.05 and **P < 0.01. AH= after heading; MS= maturity stage.

The 2-AP content in grains at maturity was significantly positive correlated to the 2-AP content in grains at 21 d AH (P <0.01), the 2-AP content in grains at 14 d AH (P <0.01 for early season, P <0.05 for late season), and the proline content in grains at 7 d AH in early season (P <0.05). However, the 2-AP content in grain at maturity showed significant negative correlation with the proline content in leaves at 14 d AH (P <0.05), the proline content in leaves at 21 d AH (P <0.05 in early season, P <0.01 in late season)(*Fig. 2 a-e*).

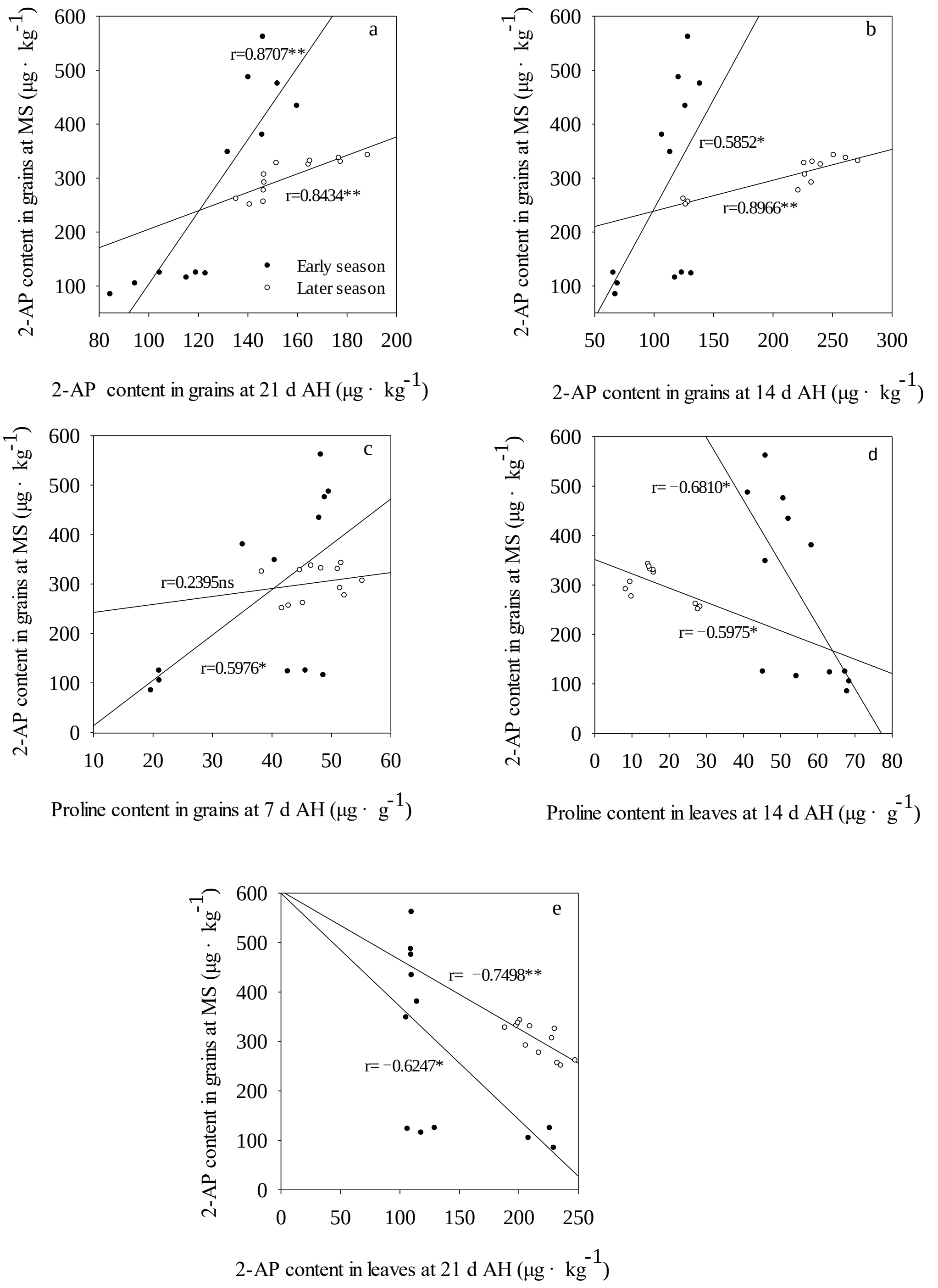

Figure 2. *Correlation analyses between the 2-AP content in grains at maturity and (a) the 2-AP content in grains at 21 d AH, (b) the 2-AP content in grains at 14 d AH, (c) the proline content in grains at 7 d AH, (d) the proline content in leaves at 14d AH, (e) the 2-AP content in leaves at 21 d AH. Significant correlations at *P < 0.05 and **P < 0.01. AH= after heading; MS= maturity stage*

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The 2-AP content in leaves at 21 d AH showed significant positive correlation with the proline content in leaves at 14 d AH and 21 d AH ($P < 0.01$ in early season, $P < 0.05$ in later season) (*Figure 3 a-b*).

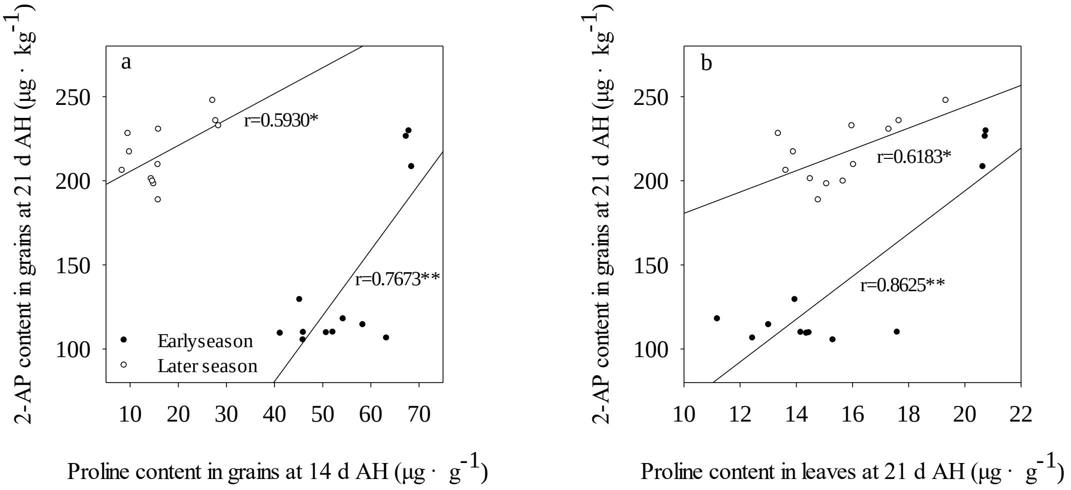

Figure 3. *Correlation analyses between the 2-AP content in leaves and proline content in leaves at 14 d AH and 21 d AH. Significant correlations at *$P < 0.05$ and **$P < 0.01$. AH= after heading; MS= maturity stage*

Discussion

The biosynthesis and accumulation of 2-AP in aromatic rice is an important phenomenon that is affected by several factors (Mo et al., 2016). Precious studies have reported some precursors in the pathways of 2-AP biosynthesis, for example, Seitz et al. (1993) and Huang et al. (2012) found proline was the most important precursor and directly involved in 2-AP formation, then, ornithine and glutamic acid were found to have relevant relationship with the fragrance constitution (Yoshihashi, 2002). The fragrance of aromatic rice is affected by environmental factors, in particular, the irrigation model and nitrogen application. For water irrigation, Wang et al. (2013b) found that moderate soil water potential (- (25±5) kPa) at the tillering stage significantly enhanced in 2-AP accumulation in grain by 22.70%-29.76%. Similar findings were also observed when the soil water potential was - (25±5) kPa at the booting stage, the 2-AP content in grain was significantly increased by 35.88%-47.02% (Wang et al., 2013a). Moreover, when the shallow-water irrigation method was applied at grain filling stage, the 2-AP content in grains was markedly increased by 54.80%-322.90% (Tian et al., 2010). The enhancement in 2-AP content in grains was associated to the improvement in proline content in grains (Tian et al., 2010; Wang et al., 2013a,b). Moreover, nitrogen application also has positive influence on the accumulation of proline and 2-AP. Yang et al. (2012) reported higher proline was found in aromatic rice grains which was planted in paddy filed contains higher total nitrogen, and higher proline content contributed to the accumulation of higher aroma.

Li et al. (2014) found that 2-AP content in grains increased significantly by 10.33%-23.50% when 60 kg/hm^2 nitrogen fertilizer was applied at the tillering stage. Zhong and Tang (2014) also found that the grains 2-AP content was increased, because of the proline concentrations in leaves and grains were improved with the increasing nitrogen application. Meanwhile, the effects of water deficit and nitrogen interaction on proline content have been reported in some previous studies. Lalelou and Fateh (2014) reported that under severe water conditions (60% field capacity loss) and 80 kg/hm^2 nitrogen, the proline content in flag leaf of wheat was increased (15.76-20.39 fold). All the previous indicated the single water or nitrogen treatment could improve the gain 2-AP content. According our previous studies (data unpublished), we have found the water-nitrogen treatment improved the grain 2-AP content at tillering stage, booting stage, and grain filling stage. In this study, we have further found that 2-AP content in grains was increased by TNW (The treatment management practice involved applying nitrogen (60 kg/hm^2) at tillering stage with heavy drought conditions (water potential of - (25±5) kPa), applying 60 kg/hm^2 nitrogen at the booting stage with feebly arid conditions (water potential of - (15±5) kPa), and applying no nitrogen at the grain filling stage with shallow-watered irrigation (water flow of 0 kPa)) (*Table 2*). Similarly, the proline concentration in grains at the four sampling stages was increased in early and later seasons (*Table 3*). Moreover, the 2-AP content in grains at maturity was significantly positive correlated to the 2-AP content in grains at 21 d AH, the 2-AP content in grains at 14 d AH, and positive correlation between 2-AP in grain at maturity and proline content in grain at 7 d AH was also observed (*Fig. 2 a-c, e*). This indicated that the TNW treatment can increase 2-AP content in grains at maturity due to the improvement of 2-AP content in grains at 14 d AH and 21 d AH and the proline content in grains at 7 d AH.

Interestingly, we observed that the proline content and 2-AP content in leaves at the four sampling stages was decreased (*Table 4, 5*). Correlation analysis indicated that the 2-AP content in grains at maturity showed significant negative correlation with the proline content in leaves at 14 d AH and the proline content in leaves at 21 d AH (*Fig. 2d*). Further, the 2-AP content in leaves at 21 d AH showed significant positive correlation with the proline content in leaves leaf at 14 d AH and 21 d AH (*Fig. 3 a-b*). This suggested that proline or 2-AP may possibly transport from leaves to grains (Buttery et al., 1983; Maraval et al., 2010; Mo et al., 2016).

The water and nitrogen effects on crop yield have been investigated by many researchers (Wang et al., 2003; Cai et al., 2006; Yang et al., 2016). Wang et al. (2016) found that 200 kg/hm^2 nitrogen fertilizer application and alternate wetting and moderate drying regime increased grain yield due to more panicle and more spikelet number of per panicle; 300 kg/hm^2 nitrogen fertilizer application and alternate wetting and serve drying regime treatment increased the number of panicle, spikelet number per panicle and the percentage of filled grains and produced high yield. Li et al. (2014) found that the yield aromatic rice varieties were increased under the interaction of 60 kg/hm^2 nitrogen fertilizer application and water flow of - 10 kPa because of higher seed-setting rate, 1000-grains weight or number of panicles. Our results also confirmed that water and nitrogen applications have a positive effect on the yield of two fragrant rice cultivars. The improvement in grain yield is associated to the higher panicle number per hill, seed-setting rate, and 1000 grain weight. Moreover, we found significant positive correlation between grain yield and seed-setting rate (r=0.8402, P <0.01) (*Table 1, 6*).

Conclusion

In conclusion, the TNW treatment increased grain yield, and some yield parameters, 2-AP and proline in grains, and led to improve the grain aroma. For revealing the mechanism of 2-AP transportation by irrigation and nitrogen management practice treatment, much work should be done at molecular and physiological level.

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