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Comparison of grain yield and 2-acetyl-1-pyrroline (2AP) content in leaves and grain of two Thai fragrant rice cultivars cultivated at greenhouse and open-air conditions

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Abstract

To verify how different growing environments affect the aroma quality and production yield of fragrant rice, two commercial Thai fragrant rice cultivars, Khao Dawk Mali 105 (KDML 105) and Pathum Thani 1 (PTT 1) were grown in a greenhouse and also open air. Between the two growing environments, the temperature difference averaged 6 ºC. Each rice cultivar was grown in clay loam and sandy loam soils. In addition, a water stress treatment was applied at the beginning of the grain filling stage and onward. Determination of 2-acetyl-1pyrroline (2AP) in both rice leaves and grain was accomplished using automated headspace-gas chromatography with selective nitrogen/phosphorus detection. The results showed that throughout all growth stages the rice leaf 2AP content was different for both rice cultivars depending on the soil type. The KDML 105 cultivar had higher 2AP content in clay loam soil compared to PTT 1. A reverse trend was observed in the sandy loam soil. The grain 2AP content and grain yield of both rice cultivars were lower for those plants grown in the greenhouse condition. The water stress treatment led to higher 2AP content in both the leaves and grain of the two rice cultivars, which averaged 19% higher for rice leaves and 22% higher for rice grain. However, the water stress treatment dramatically decreased grain yield for all growing conditions. The interaction effects of these environmental factors were analysed using a full-factorial design. The interaction between rice cultivar × temperature had the strongest effect on the 2AP content, whereas rice cultivar × soil type greatly affected rice grain yield.

Keywords: Fragrant rice, 2-acetyl-1-pyrroline, grain yield, static headspace-gas chromatography, greenhouse, full factorial analysis.
Abbreviations: 2AP_2-acetyl-1-pyrroline, SHS-GC_static headspace-gas chromatography, NPD_nitrogen phosphorus detector, KDML 105_Khao Dawk Mali 105, PTT 1_Pathum Thani 1, 2,6-DMP_2,6-dimethylpyridine

Introduction

Rice is considered the world's most important staple food source. More than 90% of the world's rice is grown and consumed in Asia, where 60% of the Earth's population lives (Khush, 1997). Fragrant rice is a type of rice that produces a pleasant floral odour during and after cooking. Most fragrant rice varieties also possess a characteristic eating quality, which is highly desired by consumers and can attract premium prices in the world market. Among all fragrant rice varieties, Basmati rice of India and Pakistan and Jasmine rice of Thailand account for the greatest volume in the global fragrant rice trade.

Jasmine rice is grown primarily in Thailand and is now called Thai Hom Mali rice. In the Thai language, Hom means "sweet smell" and Mali is the Thai name for the Jasmine flower, which is white like rice. Thus, the distinctive characteristic of Jasmine rice is a result of its white seed, good eating quality, and its sweet pleasant odour. Thai Jasmine rice includes two rice cultivars, Khao Dawk Mali 105 (KDML 105) and Rice Department 15 (RD 15). Only these two cultivars are exported to the world market. They have similar characteristics and are similar in quality. Unfortunately, they are photoperiod-sensitive cultivars and can only be grown once a year. An alternative fragrant rice variety, Pathum Thani 1 (PTT 1), is the second most popular rice after Thai Jasmine rice. It has grain almost identical in appearance to KDML 105 but with an inferior fragrance. PTT 1 has an advantage over Thai Jasmine rice because it is insensitive to photoperiod; therefore, it can be grown at any time of year, including dry season.

The aroma quality of fragrant rice is related to the content of an impact aroma compound, 2-acetyle-1-pyrroline, 2AP (Buttery et al., 1983; Widjaja et al., 1996; Maraval et al., 2008). This compound was firstly identified as a product of Maillard degradation in cooked rice and was found later in many processed and cooked foods (Wakte K et al., 2017). In contrast, our research group believes that 2AP is a naturally occurring compound and it is present in uncooked Jasmine rice (Mahatheeranont et al., 2001), soybeans (Juwattanasomran et al., 2011), sorghum (Yundaeng et al., 2013), coconuts (Saensuk et al., 2016) and winter melon (Ruangnam et al., 2017). The compound was also detected as an impact aroma compound found in some aromatic plants, such as pandan leaves and bread flowers (Wongpornchai et al., 2003).

Fragrant foods have gained increasing popularity throughout Asian food markets. Thai rice breeders have attempted to improve the effectiveness of their aromatic rice breeding programmes, which has led to higher productivity, yield and aroma quality. A clearer understanding of the biochemical processes in the 2AP biosynthesis pathways and the identification of genes associated with the 2AP biosynthesis pathway have led to more effective breeding programmes. Briefly, the inactivation of betaine aldehyde dehydrogenase (*BADH2*) is responsible for the accumulation of 2AP in fragrant rice varieties (Bradbury et al., 2008). Proline is one of the amino acid precursors to 2AP in rice (Yoshihashi et al., 2002a) and γ-aminobutyraldehyde (*GABald*) is likely the direct precursor to 2AP production via *BADH2*.

The influence of environmental factors on the aroma quality and yield of fragrant rice has been investigated over the past few decades. An early report in 1985 stated that Indian Basmati rice was not aromatic when grown in some specific geographical regions (Efferson, 1985). In terms of the effect of temperature, aroma is best developed when fragrant rice is grown in areas, where the weather is cooler during the ripening stage (Dela Cruz et al., 1989). A difference in the 2AP content of Jasmine rice, KDML 105, was found when the rice was grown in different areas in the north and northeast parts of Thailand (Yoshihashi et al., 2004). Stresses during cultivation, such as drought (Yoshihashi et al., 2002b) and salinity stress (Gay et al., 2010; Poonlaphdecha et al., 2012), has led to higher 2AP content in the grain at harvest. A more recent study reported that shading during grain filling decreased grain yield but increased the aroma quality of the rice (Mo et al., 2015). Rice aroma is a result of both genetic and environmental factors. Hence, better control of the aroma quality of fragrant rice can be achieved by good management practices, which are governed by important environmental factors during rice growth. Further, careful consideration should be paid to the factors that co-exist. For example, two fragrant rice varieties may respond differently to changes in temperature when they are grown in different soil types.

The purpose of this study was to compare the response of two Thai fragrant rice cultivars, in terms of 2AP production and grain yield, grown at the same location and time but in two different conditions, greenhouse and open-air. The experiment was conducted so that the interaction effect of rice cultivar and some environmental factors, including temperature, soil type and water stress, on 2AP content and grain yield could be characterized by applying statistical analyses based on a full factorial design. The 2AP concentration in rice leaves was determined across all growth stages of rice by utilizing a rapid and reliable method that employs automatic static headspace-gas chromatography (SHS-GC) with a selective nitrogen/ phosphorus detector (NPD) (Sriseadka et al., 2006). A convenient headspace solid-phase microextraction (SPME) method has been utilized for food volatile extraction during the past decade. Despite its widespread application, SPME has rarely been a successful analytical tool for the quantitation of 2AP in the grain of fragrant rice. SPME is limited by its poor extraction reproducibility and recovery (Grimm et al., 2001). The advantages of the SHS-GC-NPD method are ease of sample preparation, a short total analysis time, fully automation and the fact that it is a solvent-free method. In this study, a SHS-GC-NPD was developed and shown to be a suitable tool, in terms of accuracy and precision, for the evaluation of 2AP concentrations in groups of various rice leaf samples.

Results and Discussion

SHS-GC-NPD method validation for the quantitative analysis of 2AP in rice leaves and grain

In this study, the method of employing static headspace sampling prior to GC analysis was developed for a rapid determination of the 2AP content of raw rice leaves. Optimization of the sample headspace conditions was performed to improve recovery of 2AP. The use of NPD as a selective GC detector for *N*-containing compounds can eliminate problems with peak overlap, which are usually caused by the complex nature of rice leaf headspace volatiles. Thus, the GC chromatogram of the headspace volatiles of KDML 105 leaves obtained by SHS-GC-NPD (Fig 1a) showed less complex peak signals than those obtained by SPME-GC-MS (Fig 1b). In the SHS-GC-NPD chromatogram, 2,6-DMP, used as internal standard, and 2AP were eluted at 6.55 and 7.27 min, respectively. The use of NPD as a detector for GC not only provides a higher detection sensitivity for 2AP but also allows for a shorter analysis time and higher chromatographic resolution. Validation of the SHS-GC-NPD method for the determination of 2AP in fragrant rice leaves revealed that detector responses, in terms of peak area ratios between 2AP and 2,6-DMP and the amount of standard 2AP, were linear and reproducible with a correlation coefficient (r^2) of 0.9998. The effective linear concentration ranged from 0.50 to 50 µg of 2AP/g of rice leaf sample. Sensitivity was reflected by the limit of detection (LOD) and limit of quantitation (LOQ). The LOD and LOQ were defined as the concentration at which S/N g 3 in terms of the least amount of 2AP and the concentration at which S/N g 10 in terms of the least amount of rice leaf sample, respectively. The LOD and LOQ were 10 ng with a relative standard deviation (RSD) of 2.55 % and 0.01 g with RSD of 3.28 %, respectively. Reproducibility was calculated as intraday and interday coefficients of variation, being 1.93% RSD (n = 10) and 2.40% RSD (n = 25), respectively. The validation data confirmed that GC-NPD was more sensitive compared to SPME-GC-MS even when it was operated in a selected ion monitoring (SIM) mode (LOD = 100 µg and LOQ = 0.5 g of rice sample). This newly developed method was

Table 1. Physicochemical characteristics of the experimental soils

Soil characteristics	Clay loam	Sandy loam
Soil texture		
- Clay content (%)	32.78	13.18
- Silt content (%)	36.00	30.00
- Sand content (%)	31.22	56.82
Organic matter (%)	1.04	0.62
Organic carbon (%)	0.60	0.36
Total nitrogen (%)	0.06	0.04
C/N ratio	10.00	9.00
Cation exchange capacity (me/100g)	11.46	4.94
Electric conductivity (µS/cm)	74.63	130.40

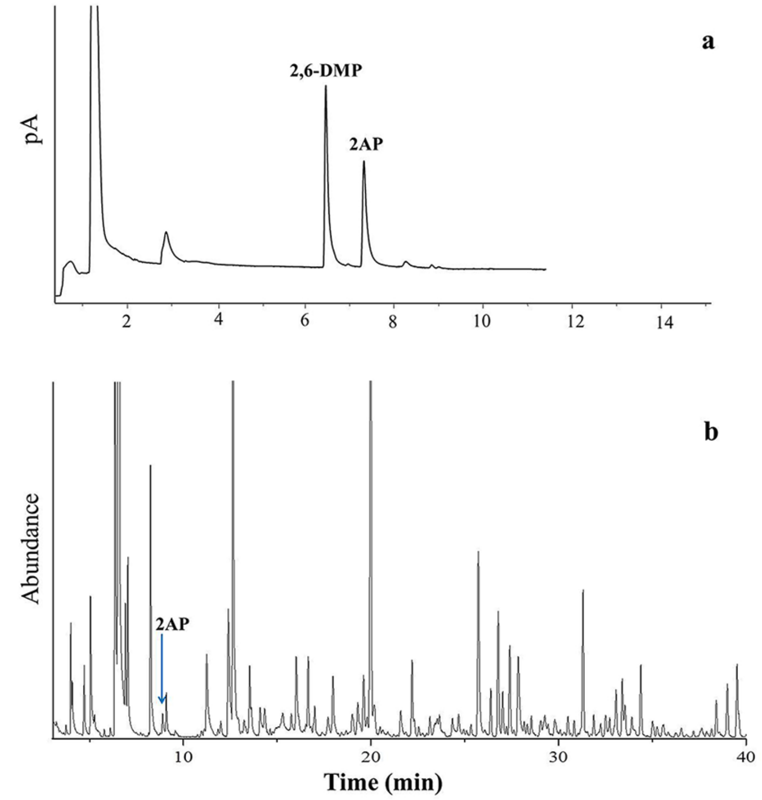

Fig 1. GC Chromatograms of headspace of KDML 105 rice leaf volatiles obtained by static headspace sampling with a specific nitrogen-phosphorus detector (a) and dynamic headspace sampling (SPME) with mass spectrometric detection (b)

further applied to evaluate the aroma quality, based on 2AP concentrations, of both fragrant rice leaf and grain samples.

2AP content in rice leaves and grain at each growth stage

The 2AP content in the leaves and grain of PTT 1 and KDML 105 at different growth stages are shown in Fig 2a, b, respectively. For all growth stages of rice, the average 2AP content of PTT 1 was approximately one-third of that in KDML 105. For both PTT 1 and KDML 105, the 2AP content continuously increased from the seedling to the booting stage, at which the highest amount of 2AP was occurred. A higher level of 2AP has been reported to accumulate during the booting stage (Hinge et al., 2016). In addition, the 2AP content decreased during heading stage before rising again throughout the grain filling stage. This decrease was probably due to the translocation of 2AP from the leaves to the grain or to a lower rate of secondary metabolite production during later stages of the rice plant growth (Poonlaphdecha et al., 2012; Hinge et al., 2016; Mo et al., 2016). Finally, the 2AP content decreased to its lowest level at the end of grain filling stage.

2AP content under different growing conditions e.g. different temperatures in greenhouse and open air

The effect of the temperature difference between the greenhouse and open-air conditions on the 2AP content in leaves and grain of two fragrant rice cultivars, PTT 1 and KDML 105, are shown in Fig 2a, b. The leaf samples of rice plants grown at the higher temperatures in the greenhouse had 2AP contents ranging from 2.24-9.20 µg/g and 3.08-16.39 µg/g for PTT 1 and KDML 105, respectively. At lower temperature in open air, the 2AP content ranged from 2.89-12.36 µg/g for PTT 1 and 4.76-21.52 µg/g for KDML 105.

Table 2. Effect of growing condition on yield components of two Thai fragrant rice cultivars.

Growing conditions	Plant height (cm)	Grain number/panicle		Panicle weight (g)		100 Grain weight (g)		Grain yield (g/m^2)	
		Control	Water stress	Control	Water stress	Control	Water stress	Control	Water stress
PTT 1									
I	66.9±3.88 ^{a}	78±3.00 ^{abd}	68±16.09 ^{ad}	1.16±0.03 ^{ad}	0.99±0.11 ^{de}	1.48±0.11 ^{a}	1.24±0.05 ^{d}	105.13±20.60 ^{a}	70.96±12.80 ^{a}
II	75.4±2.93 ^{b}	77±11.06 ^{abd}	60±5.13 ^{d}	0.62±0.02 ^{b}	0.52±0.02 ^{b}	1.00±0.08 ^{be}	0.87±0.06 ^{e}	81.55±13.71 ^{a}	47.37±7.49 ^{a}
III	77.0±1.47 ^{b}	104±6.66 ^{bc}	89±11.55 ^{abe}	1.90±0.05 ^{c}	1.83±0.09 ^{c}	1.83±0.11 ^{c}	1.74±0.04 ^{c}	449.11±53.67 ^{b}	286.58±15.87 ^{c}
IV	98.6±1.94 ^{c}	118±8.19 ^{c}	111±11.50 ^{ce}	1.19±0.10 ^{a}	0.81±0.01 ^{e}	1.16±0.06 ^{bd}	0.89±0.07 ^{e}	417.00±28.73 ^{b}	258.34±17.68 ^{c}
KDML 105									
Growing conditions	Plant height (cm)	Grain number/panicle		Panicle weight (g)		100 Grain weight (g)		Grain yield (g/m^2)	
		Control	Water stress	Control	Water stress	Control	Water stress	Control	Water stress
I	126.2±1.47 ^{a}	165±22.65 ^{a}	152±19.14 ^{a}	4.43±0.10 ^{a}	3.59±0.12 ^{e}	2.71±0.07 ^{a}	2.31±0.08 ^{c}	449.08±21.78 ^{a}	244.56±52.13 ^{c}
II	140.1± 1.94 ^{b}	150±18.19 ^{a}	138±4.51 ^{a}	2.92±0.28 ^{bc}	2.27±0.19 ^{f}	1.99±0.05 ^{be}	1.72±0.07 ^{f}	367.01±60.00 ^{a}	214.67±29.58 ^{bc}
III	107.1±2.64 ^{c}	137±30.05 ^{a}	123±9.85 ^{a}	3.02±0.08 ^{ce}	2.42±0.30 ^{bf}	2.17±0.03 ^{ce}	1.90±0.07 ^{bf}	170.69±11.41 ^{bce}	99.62±23.10 ^{ef}
IV	127.4±1.94 ^{a}	131±27.00 ^{a}	112±14.50 ^{a}	1.69±0.21 ^{d}	1.28±0.24 ^{d}	1.22±0.10 ^{d}	1.07±0.06 ^{d}	120.26±37.46 ^{bde}	69.58±3.34 ^{df}

The data are presented as mean value ± standard deviation. Values followed by different letters in the same column are significantly different (p ≤ 0.05). Four sets of growing conditions were clay loam in open air (I), clay loam in a greenhouse (II), sandy loam in open air (III) and sandy loam in a greenhouse (IV).

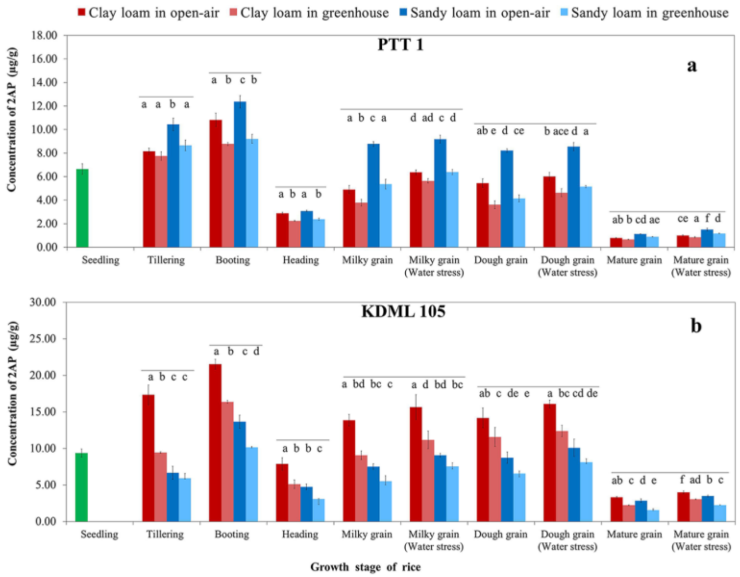

Fig 2. 2AP content in leaves from seedling to dough grain stage and in grains at mature grain stage of PTT 1 (a) and KDML 105 (b) grown under greenhouse and open-air conditions. The four sets of growing conditions were clay loam in open air (I), clay loam in a greenhouse (II), sandy loam in open air (III) and sandy loam in a greenhouse (IV). Data are presented as mean value ± standard deviation. Values with different letters in the same growth stage are significantly different (p ≤ 0.05).

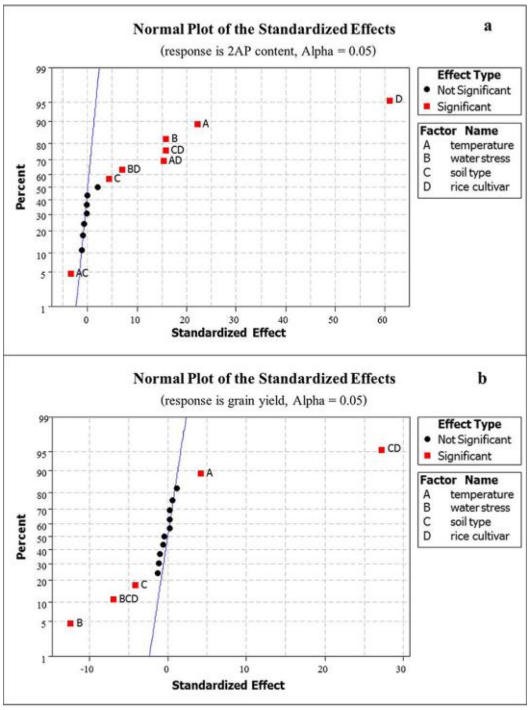

Fig 3. Normal probability plots of standardized effects for 2AP content (a) and grain yield (b) for all factors. The fitted line indicates the expected position of points, in which the effects is zero. Significant effects have a label and fall either left or right of this line. A greater distance from the fitted line indicates a greater significant effect.

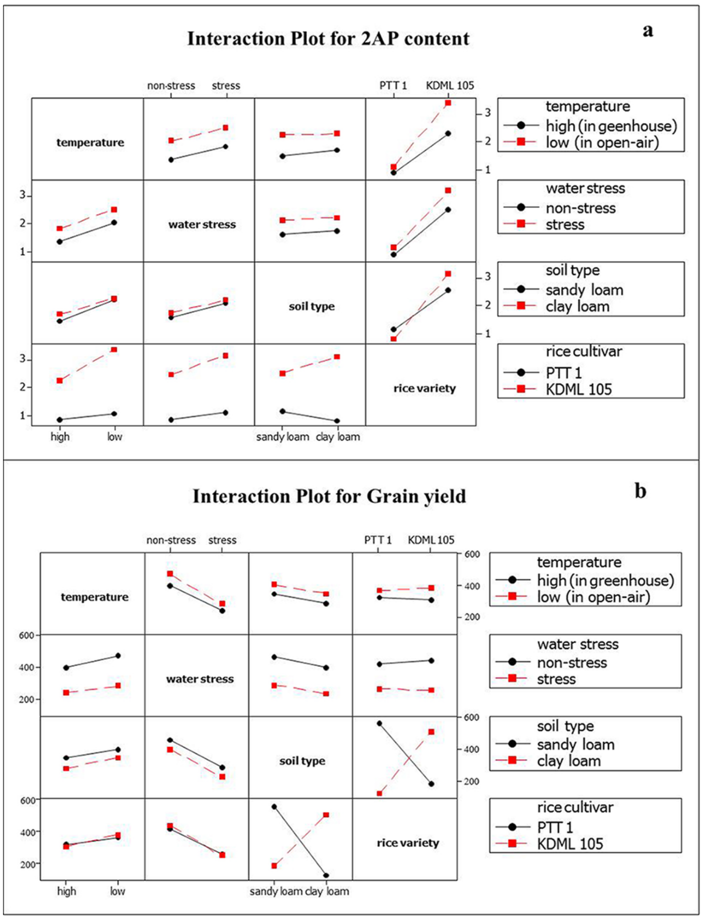

Fig 4. Interaction plots for 2AP content (a) and grain yield (b) of all factors. When the lines are parallel, the interaction effect is zero. An increase in deviation from being parallel indicates that interactions among control factors increase.

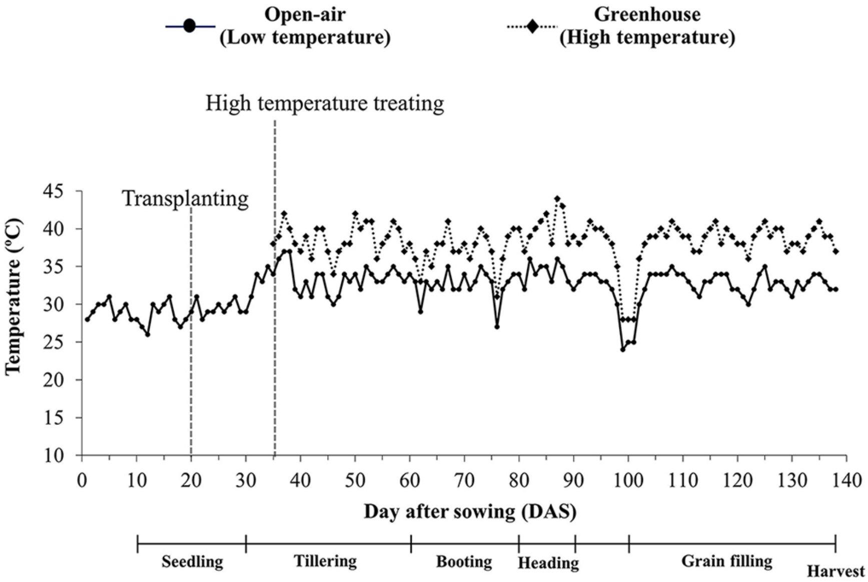

Fig 5. Daily daytime temperature in greenhouse and open-air conditions.

The high temperature reduced the 2AP content in rice leaves at all growth stages. In rice grain, as expected, the content of 2AP was lower for the greenhouse group. The 2AP content in the grain of the two rice cultivars grown in greenhouse and open-air conditions ranged from 0.65-1.17 and 0.79-1.50 µg/g for PTT 1 and 1.58-3.06 and 2.87-4.01 µg/g for KDML 105, respectively.

Comparing the two groups of KDML 105 grown under different conditions, the average content of 2AP was much lower for the group under a high temperature in the greenhouse, which was approximately two-thirds the level of the open-air group. For PTT 1, the 2AP content of the high temperature group was only one-fifth less than that of the low temperature group before the heading stage, but the 2AP content of the high temperature group was decreased to nearly half of the content of the low temperature group during grain filling stage. Thus, the strong influence of temperature generated different responses for different cultivars of fragrant rice. Previous studies reported a similar result that temperature dramatically reduced the grain aroma quality of fragrant rice although none of previous studies was conducted at the same growing location and time as in our study. For example, Pusa Basmati rice grown during the summer had no detectable 2AP but contained 0.03 ppm of 2AP when grown at a lower temperature during the rainy season (Nadaf et al., 2006). Additionally, Rambir Basmati and Rato Basmati grown in the Indian sub-continent (day night average temperature 22-23 ºC) showed higher aroma scores compared to those cultivars grown in Malaysian tropical environment (day night average 28-30 ºC) (Golam et al., 2010). Being a highly volatile compound, 2AP might be easily liberated from leaf tissues. Regardless of location and seasonal differences, our study suggests that high temperature might accelerate the rate of 2AP volatilization. However, there are many factors that could affect 2AP biosynthesis, including a fragrance gene (Bradbury et al., 2005) and additional stress effects such as salt and drought stress (Yoshihashi et al., 2002b).

Under different soil types

In the same experimental design, the two fragrant rice cultivars were grown in two different soil types, clay loam and sandy loam. As shown in Fig 2a, there was no difference in the 2AP content in the leaves of PTT 1 grown in clay loam and sandy loam; the 2AP content ranged from 2.24-10.82 µg/g and 2.38-12.36 µg/g in clay loam and sandy loam, respectively. In contrast, KDML 105 (Fig 2b) had a higher 2AP content in clay loam, which ranged from 5.13-21.52 µg/g, than in the sandy loam (3.08-13.66 µg/g). The average 2AP content in the grain of PTT 1 was only half of that in KDML 105. Considering the low temperature in open air, 2AP content in PTT 1 grain grown in clay loam and sandy loam were 0.79 and 1.12 µg/g, respectively. Conversely, KDML 105 grain had a higher 2AP content when grown in clay loam (3.32 µg/g) than in sandy loam (2.87 µg/g). Thus, among the greenhouse and open-air samples, the greatest differences in the 2AP content occurred between KDML 105 in clay loam and PTT 1 in sandy loam.

Table 1 shows additional information on soil texture, organic matter content, organic carbon content, total nitrogen content and electric conductivity of the clay loam and sandy loam soils. The differences in soil property may have contributed to the difference in the 2AP level of the Thai fragrant rice cultivars. Soil properties affect aroma quality of many food plants, although a clear explanation of their roles in aroma production have not been properly defined yet. For more than a decade, lighter soils and upland conditions with soils low in nitrogen were generally perceived to favour aroma formation in rice (Singh et al., 2000; Singh et al., 2003). Recently, Jedrum et al. (2014) reported that the concentration of aromatic substances in Jasmine rice grown in sodic soil was higher than that of Jasmine rice grown in saline soil. Although, soil properties affect the 2AP content differently, our study shows that this effect is greatly dependant on the cultivar of rice.

Drought stress

To assess all basic stresses that naturally occur during rice cultivation, the effect of water stress on both the 2AP content and the grain yield was also investigated. A set of experiments was performed on two fragrant rice cultivars, PTT 1 and KDML 105, to determine the effect of water stress. One group was the control group where a constant water level or non-stress was maintained, while the other group was the experimental group, where water stress was simulated by reducing the regular water supply at the start of the grain filling stage. For the water stress treatment, the 2AP content in rice leaves was higher than that in the control group. The average 2AP content in the leaves grown under water stress were 20% and 18% higher than the control for PTT 1 and KDML 105, respectively. Water stress also increased the 2AP content in grain samples for both rice cultivars, which on average was 22% higher compared to the control. The results indicate that water stress during the period of rice grain filling can lead to high 2AP production in both leaves and grain (Fig 2). However, water stress during the grain filling stage decreased the grain yield of both rice cultivars.

Although some previous studies have reported that the 2AP content was significantly increased under water stress (Yoshihashi et al., 2002b), our results showed that water stress was only a minor factor affecting the 2AP content in rice leaves of both fragrant rice cultivars. However, an explanation for the higher 2AP content during osmotic stress, such as drought and salt stress, is that a high level of proline is biosynthesized via intermediate Δ^1-pyrroline-5-carboxylic acid by both glutamate and ornithine pathways during osmotic stress (Kavi Kishor et al., 2005). The accumulation of proline could lead to enhanced 2AP production in fragrant rice because proline is an amino acid precursor of 2AP.

Effect of different growing conditions on rice growth and yield

The growth and yield component data were obtained from the same sets of fragrant rice samples as those used for evaluating the rice aroma quality. Generally, PTT 1 rice grew well in sandy loam soil, whereas KDML 105 preferred clay loam soil. The height of KDML 105 plants was higher than that of PTT 1.

Grain yield from plants grown in clay loam soil ranged from 47.37-105.13 g/m^2 for PTT 1 and 214.67-449.08 g/m^2 for KDML 105, as shown in Table 2. In contrast, PTT 1 rice grown in sandy loam had higher grain yields, 258.34-449.11 g/m^2, than those grown in clay loam soil. These results confirmed that PTT 1 prefers sandy loam soil, while KDML 105 prefers clay loam for the best growth and maximal yield. In clay loam soil, PTT 1 had lower plant height, grain number per panicle, panicle weight, 100-grain weight and grain yield than KDML 105.

The data in Table 2 shows that both cultivars grew taller in the greenhouse than in open air because of the high temperature in the greenhouse. Also growing these two fragrant rice cultivars in a greenhouse caused significant reductions in panicle weight, 44%, 100-grain weight, 36%, and grain yield, 23%, compared to growing these rice cultivars in open-air condition. Furthermore, the yield components of rice were decreased because of water stress treatment during the grain filling stage. A reduction in grain number per panicle, panicle weight and 100-grain weight of both rice cultivars was occurred under the water stress treatment. Compared to the control, the water stress treatment decreased the grain yield of PTT 1 by 37% and decreased the grain yield of KDML 105 by 42%.

Usually, clay soil contains more organic matter than sandy soil because of its greater physical protection, including its lower porosity and higher bulk density (Six et al., 2000). Recently, Dou et al. (2016) reported that the grain yield of Rondo cultivar, a long-grain indica cultivar, was 22% higher when planted in clay soil than sandy loam soil. However, growing long-grain Cocodrie cultivar in clay soil with an aerobic water regime caused significant yield losses. Thus, soil texture has been reported to influence rice grain yield. Rice should be grown in soils suitable for high yield and good quality. However, further information is needed to determine the appropriate soil type for fragrant rice that results in both high yield and good aroma quality.

High temperature conditions caused a significant increase in plant height for both KDML 105 and PTT 1. This result agrees with the report of Oh-e et al. (2007) that plants grew taller at higher temperatures compared to ambient condition. Nevertheless, at a low temperature, rice had a greater grain number per panicle, panicle weight, 100-grain weight and grain yield than rice grown at high temperature. High temperatures have also been reported to induce sterility and lead to a low harvest index and grain yield (Peng et al., 2004; Prasad et al., 2006; Jagadish et al., 2007; Oh-e et al., 2007). Moreover, higher night temperatures have been reported to decrease spikelet fertility and 100-grain weight, which lead to a significant reduction in rice yields (Cheng et al., 2009; Nagarajana et al., 2010; Mohammed and Tarpley, 2011). In addition, Zhang et al. (2013) reported that a high temperature during night negatively affects grain yield, resulting in a lower grain weight. Such warm conditions also reduced rice growth, resulting in low grain yields.

Our results also indicated that water stress reduced the grain number per panicle, panicle weight, 100-grain weight and grain yields for both PTT 1 and KDML 105 varieties (Table 2). This reduction in grain number agrees with the results obtained by Sarvestani et al. (2008), which showed that water stress during the grain filling stage reduces the grain number and 100-grain weight. Mannan et al. (2012) reported that more grain per panicle was found in crops that were grown continuously in standing water. Since it is well established that drought stress reduces rice growth and grain yield, it also severely affects the seedling biomass, photosynthesis, stomatal conductance, plant water relations and starch metabolism (Pandey et al., 2014). Depending on the duration and severity of the water deficit, the grain yield of some rice genotypes may be decreased (Pantuwan et al., 2002).

Interaction effect of environmental factors on 2AP content and grain yield

The cultivation factors such as rice cultivar and other environmental factors have influenced the 2AP content. The grain yield was evaluated using a normal plot of standardized effects, as well as interaction effect plots with a 95% confidence level using Minitab software (version 16).

A normal probability plot of standardized effects is shown in Fig 3, which displays negative and positive effects on the left or right of the normal probability straight line. The effects that lie along the normal probability straight line are negligible, whereas significant effects are the square points that fall to the left and right of the normal probability straight line. The larger significant effects are more distant from the straight line. Fig 3a shows a normal probability plot of the effects of different factors and their combinations on the 2AP content. Considering each single environmental factor, temperature (A), water stress (B), soil type (C) and rice cultivar (D), have shown significant effects on 2AP content. The order of significance is D > A > B > C, which means that the rice cultivar (D) had the greatest effect on 2AP content and is followed by temperature (A), water stress (B) and soil type (C). The interaction terms of the model had three positive effects, CD, AD and BD, and one negative effect, AC. These results indicated a synergistic effect between paired factors on rice 2AP content. Fig 3b shows a normal probability plot of standardized effects for the different environmental factors on the grain yield. Only three single factors, A, B and C, had a significant effect on grain yield, with B > A > C. It was remarkable that the rice cultivar (D) did not significantly affect grain yield. However, the rice cultivar (D) had a significant effect on the interactions of factors CD and BCD.

To evaluate the interaction effects of rice cultivar, temperature, water stress and soil type, an interaction effect graph was plotted as shown in Fig 4. All possible combinations and mean responses of two factors are shown in the graph. If the lines in the interaction plots are parallel to each other, there is no interaction between the factors (Srinivasan and Viraraghavan, 2010). Increasing deviation from parallel indicates increased interactions among the control factors. These results indicated that two-way factor interactions affected the 2AP content and grain yield. Fig 4a indicates that rice cultivar interacted with all factors. Thus, rice cultivar had the greatest influence on 2AP content. The non-parallel lines of the interaction between rice cultivar × temperature, followed by rice cultivar × water stress and rice cultivar × soil type, represent strong two-way interactions. Minor interactions were occurred between temperature × water stress, temperature × soil type and water stress × soil type. The interaction effect plots for all factors on grain yield are shown in Fig 4b. Rice cultivar also had the most interactions with other factors for grain yield. The maximum interaction effect that occurred was between rice cultivar × soil type. The interactions between rice cultivar × water stress and rice cultivar × temperature had minor impacts on grain yield.

Considering the effect of each single factor from normal probability plot of standardized effects in Fig 3, the rice cultivar (D) had the greatest effect on the 2AP content, but it did not significantly affect grain yield under both greenhouse and open-air conditions. Water stress (B) also had the greatest effect on grain yield. Since the experiment was conducted under multiple simultaneous environmental factors, an interaction was occurred when individual factors affected results differently. Rice cultivar × temperature had the greatest impact on the 2AP content, while rice cultivar × soil type affected grain yield (Fig 4). The influences of the two-factor interactions are different, when single main factors are considered. The two strongest interaction effects involved at least one factor with a strong main effect (rice cultivar) and are; therefore, plausible. However, there are many other cultivation factors that affect rice production and seed quality, which should be studied in the future researches. The results from this study suggest that rice cultivation based on a statistical design approach is beneficial, particularly when many environmental factors are involved. Applying such an approach allows for the production of fragrant rice with high aroma quality and grain yield.

Materials and methods

Chemicals

2-Acetylpyrole (98% purity), 5% rhodium on an activated alumina catalyst (99% purity) and celite were purchased from Fluka (Switzerland). Silver carbonate was purchased from Aldrich (Milwaukee, WI). 2AP standard compound was synthesized as outlined by Sriseadka et al. (2006). 2,6-Dimethylpyridine (2,6-DMP) used as internal standard was purchased from BHD (UK). All solvents used were analytical reagent grade and purchased from Merck (Germany).

Plant materials and experiment site

The experiment was conducted under greenhouse and open-air conditions with natural sunlight in the experimental field of the Department of Agronomy, Faculty of Agriculture, Chiang Mai University, Thailand from July to November 2015. Two commercial Thai fragrant rice cultivars, KDML 105 and PTT 1 were used in this study. The soils types were clay loam and sandy loam in Chiang Mai Province, Thailand, which were provided by the Department of Plant and Soil Sciences, Faculty of Agriculture, Chiang Mai University. The soil physicochemical characteristics are shown in Table 1.

Greenhouse and treatment

The experiment used two growing conditions: outside (open air) and inside a greenhouse. Thus, the two conditions were low temperature (average day temperatures of 32 ºC) and high temperature (average day temperatures of 38 ºC), respectively, with a temperature difference averaging 6 ºC (Fig 5). The relative humidity during the day ranged from 45-62% and 40-53% in greenhouse and open air, respectively. The dimensions of the acrylic greenhouse were 4×2×2 m in length, width and height, respectively. Each rice cultivar was grown in two different soil types, clay loam and sandy loam. Altogether, four basic sets of growing conditions were obtained: clay loam in open air (I), clay loam in a greenhouse (II), sandy loam in open air (III) and sandy loam in a greenhouse (IV). Pre-germinated seeds were sown in seeding trays to produce uniform seedlings. Twenty-day-old seedlings were transplanted; two seedlings were grown in each plastic pot, which was 25 cm in height and 17 cm in diameter, at a 20 cm soil depth. A fertilizer (46% N_2, 18% P_2O_5 and 50% K_2O) was applied to the pots at planting at a rate of 0.3 mg/kg of soil. Thirty-five-day old tillers were then divided into greenhouse and open-air groups. From the grain filling stage onwards, the experiment was designed with two additional treatments, a control and water stress treatment, which were applied to both growing conditions until the maturity stage. For control (non-water stress condition), a constant water level was maintained at 3 cm above the soil surface throughout the rice growth stages. For the water stress treatment, the water level of the plot was kept at the same level as the control treatment until the early stages of grain filling when water supply was stopped.

Sampling and measurement

The seven growth stages selected for 2AP analysis were seedling (20 day after sowing, DAS), tillering (45 DAS), booting (68 and 70 DAS, greenhouse and open air, respectively), heading (82 and 85 DAS), milky grain (100 and 105 DAS), dough grain (119 and 123 DAS) and mature grain (134 and 138 DAS). Leaf blade samples were collected from the seedling to dough grain stage and grains were collected at the mature grain stage. Three replicates of leaf samples were collected at 10.00 AM. Mature grain was collected, sun-dried to approximately 14% moisture and then de-hulled using a rice husker machine (TR-200; Kett, Tokyo, Japan) prior to quantitative analysis of 2AP by SHS-GC-NPD. Rice yield and yield components, including plant height at the booting stage (cm), grain number per panicle, panicle weight (g), 100-grain weight (g) and grain yield (g/m^2), were also determined at physiological maturity.

Analysis of 2AP in rice leaves and grains

SHS-GC-NPD

Each leaf sample (0.20 g) was cut and ground in a blender (Y46; Moulinex, Paris, France). Unpolished grains (1.00 g) were ground and screened through an Endecotts test sieve (Endecotts Ltd., London) to obtain uniform particle size. The samples were then placed in a 20 mL headspace vial containing 1.0 µl of 500 ppm 2,6-DMP as an internal standard. The headspace vials were immediately sealed with PTFE/silicone septa and aluminum caps prior to analysis by static headspace-gas chromatography coupled with a nitrogen/phosphorus detector (SHS-GC-NPD).

The 2AP content in rice leaves was quantified following the method of Sriseadka et al. (2006). A static headspace (Model G1888, Agilent Technologies, CA, USA) coupled to an Agilent 6890 Series GC system equipped with a NPD detector was used. The optimum headspace operating conditions were: oven temperature 120 ºC; loop temperature 130 ºC; transfer line temperature 140 ºC; vial equilibration time 5 min with high speed shaking; pressurizing time 0.05 min; loop filling time 0.01 min; loop equilibration time 0.40 min; and inject time 0.40 min.

The headspace volatiles were separated using an HP-5 (30 m × 0.53 mm i.d. × 1.5 µm film thickness) column (J&W Scientific, Folsom, CA, USA). The optimum GC conditions were achieved using an HP-5 column with a splitless injection at 250 ºC. The column temperature programme began at 50 ºC and increased to 125 ºC at 8 ºC/min. Purified helium was used as the GC carrier gas at a flow rate of 5 mL/min. The NPD temperature was set at 300 ºC.

A calibration curve of the 2AP standard in rice leaves was made by diluting a stock solution with toluene to yield 0.5-50.0 µg/mL concentration series, which was added to headspace vials containing 0.20 g of non-aromatic rice leaves (cv. Supanburi 2), used as the solid phase material. The internal standard, 1 µL of 0.50 mg/mL 2,6-DMP in toluene, was added to each vial and the vials were immediately sealed with PTFE/silicone septa and aluminum caps prior to analysis by the SHS-GC-NPD. Five replicates of each of the 2AP standard concentrations were analyzed.

SPME-GC-MS

Each rice leaf sample was cut and ground in the blender. A portion of five grams of the ground leaves was placed in a 50 mL Duran bottle. These samples were then heated at 75 ºC for 20 min. Solid phase microextraction (SPME) holder with a divinylbenzene/carboxen/ polydimethylsiloxane (DVB/CAR/ PDMS) fibre was (Bellefonte, PA, USA) then exposed to the headspace above the rice leaf samples for 30 min prior to thermal desorption of the volatile analysts from the fibre to the GC system.

GC-MS analysis was performed with an HP model 6890 gas chromatograph (Agilent Technologies, Palo Alto, CA, USA) coupled with mass-selective detector, HP model 5973. An HP-5MS capillary column (J&W Scientific, Folsom, CA, USA), 30 m × 0.25 mm i.d. × 0.25 µm film thickness was used. The oven temperature was initially held at 45 ºC then increased at a rate of 2 ºC/min to a final temperature of 230 ºC, which was maintained for 13 min. The injector temperature was 230 ºC. Purified helium was used as the carrier gas at a flow rate of 1 ml/min. EI mass spectra were collected at 70 eV ionization voltages over the range of m/z 29-550 with 0.1 s/scan. The electron multiplier voltage was 1350 V. The ion source and quadrupole temperatures were set at 230 ºC and 150 ºC, respectively.

Statistical analysis

Each experiment was carried out three times and results were expressed as the mean ± SD. Statistical differences among treatments were calculated by one-way analysis of variance (ANOVA) and significant differences were assessed by post hoc tests (least significant difference, LSD, defined at $p < 0.05$) using SPSS software, version 17.0 (SPSS Inc., Chicago, IL). The interaction effects of the environmental factors on the 2AP content and grain yield were analyzed by applying a full 4×2 factorial design with MINITAB version 16.0 (Minitab Inc., USA).

Conclusion

The environment during rice cultivation and production plays an important role in producing fragrant rice with a high aroma quality and a high yield. Two Thai fragrant rice cultivars responded similarly to the influence of temperature difference between greenhouse and open-air conditions and to water stress. However, the two rice cultivars responded differently to soil type, in terms of the 2AP content and grain yield. Using an interaction model with a full factorial design, the cultivar of rice was the single main factor that had the greatest influence on the aroma quality of fragrant rice, while water stress had the greatest effect on grain yield. When considering the two-factor interaction effects, the most significant interaction concerning the 2AP content was rice cultivar × temperature, whereas rice grain yield was strongly influenced by the interaction between cultivar × soil type. Thus, to find an optimal cultivation condition for fragrant rice cultivars, identification of interactions between environmental factors that can improve rice aroma quality and productivity is a prerequisite.

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References

Bradbury LM, Fitzgerald TL, Henry RJ, Jin Q, Waters DL (2005) The gene for fragrance in rice. Plant Biotechnol J. 3:363-370.

Bradbury LM, Gillies SA, Brushett DJ, Waters DL, Henry RJ (2008) Inactivation of an aminoaldehyde dehydrogenase is responsible for fragrance in rice. Plant Mol Biol. 68(4-5):439-449.

Buttery RG, Ling LC, Juliano BO, Turnbaugh JG (1983) Cooked rice aroma and 2-acetyl-1-pyrroline. J Agr Food Chem. 31:823-828.

Cheng W, Sakai H, Yagi K, Hasegawa T (2009) Interactions of elevated CO_2 and night temperature on rice growth and yield. Agr Forest Meteorol. 149:51-58.

Dela Cruz N, Kumar I, Kaushik RP, Khush GS (1989) Effect of temperature during grain development on stability of cooking quality components in rice. Jpn J Breed. 39:299-306.

Dou F, Soriano J, Tabien RE, Chen K (2016) Soil texture and cultivar effects on rice (*Oryza sativa*, L.) grain yield, yield components and water productivity in three water regimes. Plos One. 11(3):e0150549.

Efferson JN (1985) Rice quality in world markets. Papers presented at the international rice research conference, International rice research institute, Manila, 1-5 June 1985.

Gay F, Maraval I, Roques S, Gunata Z, Boulanger R, Audebert A, Mestres C (2010) Effect of salinity on yield and 2-acetyl-1-pyrroline content in the grains of three fragrant rice cultivars (*Oryza sativa* L.) in Camargue (France). Field Crop Res. 117(1):154-160.

Golam F, Norzulaani K, Jennifer AH, Subha B, Zulqarnain M, Osman M, Nazia AM, Zulqarnian M, Mohammad O (2010) Evaluation of kernel elongation ratio and aroma association in global popular aromatic rice cultivars in tropical environment. Afr J Agr Res. 5(12):1515-1522.

Grimm CC, Bergman C, Delgado JT, Bryant R (2001) Screening for 2-acetyl-l-pyrroline in the headspace of rice using SPME/GC/MS. J Agr Food Chem. 49:245-249.

Hinge VR, Patil HB, Nadaf AB (2016) Aroma volatile analyses and 2AP characterization at various developmental stages in Basmati and non-Basmati scented rice (*Oryza sativa* L.) cultivars. Rice. 9(1):38.

Jagadish SVK, Craufurd PQ, Wheeler TR (2007) High temperature stress and spikelet fertility in rice (*Oryza sativa* L.). J Exp Bot. 58:1627-1635.

Jedrum S, Thanachit S, Anusontpornperm S, Wiriyakitnateekul W (2014) Soil amendments effect on yield and quality of Jasmine rice grown on typic natraqualfs, Northeast Thailand. Int J Soil Sci. 9(2):37-54.

Juwattanasomran R, Somta P, Chankaew S, Shimizu T, Wongpornchai S, Kaga A, Srinives P (2011) A SNP in $GmBADH_2$ gene associates with fragrance in vegetable soybean variety ''Kaori'' and SNAP marker development for the fragrance. Theor Appl Genet. 122(3):533-541.

Kavi Kishor PB, Sangarn S, Amrutha RN, Sri Laxrni P, Naidu KR, Roa KRSS, Rao S, Reddy KJ, Theriappan P, Sreenivasulu N (2005) Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: its implications in plant growth and abiotic stress tolerance. Curr Sci India. 88(3):424-438.

Khush GS (1997) Origin, dispersal, cultivation and variation of rice. Plant Mol Biol. 35:25-34.

Mahatheeranont S, Keawsa-ard S, Dumri, K (2001) Quantification of the rice aroma compound, 2-acetyl-1-pyrroline, in uncooked Khao Dawk Mali 105 brown rice. J Agr Food Chem. 49:773-779.

Mannan MA, Bhuiya MSU, Akhand MIM, Zaman MM (2012) Growth and yield of Basmati and traditional aromatic rice as influenced by water stress and nitrogen level. J Sci Found. 10(2):52-62.

Maraval I, Mestres C, Pernin K, Ribeyre F, Boulanger R, Guichard E, Gunata Z (2008) Odor-active compounds in cooked rice cultivars from Camargue (France) analyzed by GC-O and GC-MS. J Agr Food Chem. 56:5291-5298.

Mo Z, Huang J, Xiao D, Ashraf U, Duan M, Pan S, Tian H, Xiao L, Zhong K, Tang X (2016) Supplementation of 2-AP, Zn and La improves 2-acetyl-1-pyrroline concentrations in detached aromatic rice panicles in vitro. Plos One. 11(2):e0149523.

Mo Z, Li W, Pan S, Fitzgerald TL, Xiao F, Tang Y, Wang Y, Duan M, Tian H, Tang X (2015) Shading during the grain filling period increases 2-acetyl-1-pyrroline content in fragrant rice. Rice. 8:9.

Mohammed AR, Tarpley L (2011) High night temperature and plant growth regulator effects on spikelet sterility, grain characteristics and yield of rice (*Oryza sativa* L.) plants. Can J Plant Sci. 91:283-291.

Nadaf AB, Krishnan S, Wakte KV (2006) Histochemical and biochemical analysis of major aroma compound (2-acetyl-1-pyrroline) in Basmati and other scented rice (*Oryza sativa* L.). Curr Sci India. 91:1533-1536.

Nagarajan S, Jagadish SVK, Hari Prasad AS, Thomar AK, Anjali A, Madan P, Agarwal PK (2010) Local climate affects growth, yield and grain quality of aromatic and non-aromatic rice in northwestern India. Agr Ecosyst Environ.138: 274-281.

Oh-e I, Saitoh K, Kuroda T (2007) Effects of high temperature on growth, yield and day-matter production of rice grown in the paddy field. Plant Prod Sci. 10: 412-422.

Pandey A, Kumar A, Pandey DS, Thongbam PD (2014) Rice quality under water stress. Indian J Adv Plant Res. 1(2):23-26.

Pantuwan G, Fukai S, Cooper M, Rajatasereekul S, O'Toole JC (2002) Yield response of rice (*Oryza sativa* L.) genotypes to different types of drought under rainfed lowlands: Part 1. Grain yield and yield components. Field Crop Res. 73(2-3):153-168.

Peng S, Huang J, Sheehy JE, Laza RC, Visperas RM, Zhong X, Centeno GS, Khush GS, Cassman KG (2004) Rice yields decline with higher night temperature from global warming. P Natl Acad Sci USA. 101(27):9971-9975.

Poonlaphdecha J, Maraval I, Roques S, Audebert A, Boulanger R, Bry X, Gunata Z (2012) Effect of timing and duration of salt treatment during growth of a fragrant rice variety on yield and 2-acetyl-1-pyrroline, proline, and GABA levels. J Agr Food Chem. 60(15):3824-3830.

Prasad PVV, Boote KJ, Allen LH, Sheehy JE, Thomas JMG (2006) Species, ecotype and cultivar differences in spikelet fertility and harvest index of rice in response to high temperature stress. Field Crop Res. 95:398-411.

Ruangnam S, Wanchana S, Phoka N, Saeansuk C, Mahatheeranont S, de Hoop SJ, Toojinda T, Vanavichit A, Arikit S (2017) A deletion of the gene encoding amino aldehyde dehydrogenase enhances the "pandan-like" aroma of winter melon (*Benincasa hispida*) and is a functional marker for the development of the aroma. Theor Appl Genet. Available at: https://link.springer.com/article/10.1007%2Fs00122-017-2976-3.

Saensuk C, Wanchana S, Choowongkomon K, Wongpornchai S, Kraithong T, Imsabai W, Chaichoompu E, Ruanjaichon V, Toojinda T, Vanavichit A, Arikit S (2016) De novo transcriptome assembly and identification of the gene conferring a "pandan-like" aroma in coconut (*Cocos nucifera* L.). Plant Sci. 252:324-334.

Sarvestani ZT, Pirdashti H, Sanavy SA, Balouchi H (2008) Study of water stress effects in different growth stages on yield and yield components of different rice (*Oryza sativa* L.) cultivars. Pakistan J Biol Sci. 11(10):1303-1309.

Singh RK, Singh US, Khush GS, Rohilla R, Singh JP, Singh G, Shekhar KS (2000) Small and medium grained aromatic rices of India. In: Singh RK, Singh US, Khush GS (eds) Aromatic rices, Oxford & IBH, New Delhi, p 155-177.

Singh US, Rohilla R, Srivastava PC, Singh N, Singh RK (2003) Environmental factors affecting aroma and other quality traits. In: Singh RK, Singh US (eds) A treatise on the scented rices of India, Kalyani, Ludhiana, p 143-164.

Six J, Paustian K, Elloitt ET, Combrink C (2000) Soil structure and soil organic matter, I. distribution of aggregate size classes and aggregate associated carbon. Soil Sci Soc Am J. 64:681-689.

Srinivasan A, Viraraghavan T (2010) Oil removal from water by fungal biomass: a factorial design analysis. J Hazard Mater. 175:695-702.

Sriseadka T, Wongpornchai S, Kitsawatpaiboon P (2006) Rapid method for quantitative analysis of the aroma impact compound, 2-acetyl-1-pyrroline, in fragrant rice using automated headspace gas chromatography. J Agr Food Chem. 54:8183-8189.

Wakte K, Zanan R, Hinge V, Khandagale K, Nadaf A, Henry R (2017) Thirty-three years of 2-acetyl-1-pyrroline, a principal basmati aroma compound in scented rice (*Oryza sativa* L.): a status review. J Sci Food Agr. 97:384-395.

Widjaja R, Craske JD, Wootton M (1996) Changes in volatile components of paddy, brown and white fragrant rice during storage. J Sci Food Agr. 71:218-224.

Wongpornchai S, Sriseadka T, Choonvisase S (2003) Identification and quantitation of the rice aroma compound, 2-acetyl-1-pyrroline, in bread flowers (*Vallaris glabra* Ktze). J Agr Food Chem. 51:457-462.

Yoshihashi T, Huong NT, Inatomi H (2002a) Precursors of 2-Acetyl-1-pyrroline, a potent flavor compound of an aromatic rice variety. J Agr Food Chem. 50: 2001-2004.

Yoshihashi T, Kabaki N, Nguyen TTH, Inatomi H (2002b) Formation of flavor compound in aromatic rice and its fluctuations with drought stress. In: Research highlights JIRCAS 2002-2003, JIRCAS, Tsukuba, p 32-33.

Yoshihashi T, Nguyen TTH, Kabaki N (2004) Area dependency of 2-acetyl-1-pyrroline content in an aromatic rice variety, Khao Dawk Mali 105. Jpn Agr Res Q. 38(2):105-109.

Yundaeng C, Somta P, Tangphatsornruang S, Wongpornchai S, Srinives P (2013) Gene discovery and functional marker development for fragrance in sorghum (*Sorghum bicolor* (L.) Moench). Theor Appl Genet. 126:2897-2906.

Zhang Y, Tang Q, Peng S, Zou Y, Chen S, Shi W, Qin J, Laza MRC (2013) Effects of high night temperature on yield and agronomic traits of irrigated rice under field chamber system condition. Aust J Crop Sci. 7:7-13.