

Original Research Paper

Effects of Nitrogen Fertilizer on Grain Yield and Fragrant Contents in two Thai Rice Varieties

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Abstract: Nitrogen (N) is commonly applied in rice crop production for improving grain quality and productivity. This experiment aimed to evaluate how applying N fertilizer affects the concentration of 2-Acetyl-1-Pyrroline (2AP), the major fragrant compound in rice grains, between two selected rice varieties with high yield potential and fragrant scent, Pathum Thani 1 (PTT1) and RD22. Rice plants were grown in pot conditions and N fertilizer was applied in three rates at 2.44, 3.82 and 5.66 g N/pot designated as N1, N2 and N3, respectively. Rice samples were harvested at maturity and evaluated for grain yield, grain N and 2AP concentration. The results revealed that grain yield and concentration of N and 2AP were affected by N rates differently between the varieties; RD22 had higher grain yield and the concentrations of N and 2AP than PTT1 with all N rates. Grain N concentration in both varieties increased when increasing N rates. There was highly positive correlation between grain N and grain 2AP concentration among the N rates and varieties. We conclude that applying N in rice cultivation not only improves productivity but also enhances the quality of rice grains by improving the concentrations of N and 2AP, the magnitude of the effect depending on the rice variety.

Keywords: Fragrant Rice, Aroma Rice, Nitrogen Fertilizer, 2-Acetyl-1-Pyrroline (2AP)

Introduction

Fragrant rice is a major type of rice that has a high value in the global rice markets (Roy *et al.*, 2020). The price of fragrant milled rice, e.g., Hom mali (jasmine rice), a popular Thai fragrant rice, is about double that of the non-fragrant rice (TREA, 2021). Thai fragrant rice is famous worldwide not only for its premium quality and soft texture of cooked grains but also for the unique scent (Giraud, 2013). The 2-Acetyl-1-Pyrroline (2AP) in rice grains is a key compound determining the scent and is strongly correlated with the fragrance (Nadaf *et al.*, 2014). The 2AP content in grains is under tight genetic control but can be affected by cultivation management (Roy *et al.*, 2020). There are many aromatic rice varieties in the world that have been well recognized by consumers, e.g., Basmati from India, Jasmine rice from Thailand, Bahra from Afghanistan and Della, Texamati and Kasmati from the United States (Singh *et al.*, 2000). In Thailand, the prevalence of fragrant varieties can be found in both photosensitive (e.g., Khao Dawk Mali 105 and RD6) and non-photosensitive (e.g., Pathum Thani 1 and RD

22) rice, where the fragrant type is more dominant in the photosensitive varieties compared with the non-photosensitive varieties. However, due to the greater fragrance but limited potential for productivity in the photosensitive varieties, new varieties of fragrant rice with non-photosensitive characteristics have been bred according to the demands of farmers and consumers (Vanavichit *et al.*, 2018). Although the price and popularity of the non-photosensitive fragrant rice varieties are lower than those of the photosensitive varieties, the cultivation and consumption are continuing to increase due to the better agronomic characteristics and yield potential (Boontakham *et al.*, 2019). The Thailand Rice Department suggests that for N fertilizer application in non-photosensitive fragrant rice varieties such as Pathum Thani 1 and RD 22, the response to fertilizer application should be double that associated with the same application rate among the photosensitive varieties such as Khao Dawk Mali 105 and RD15, but the exact rates should be based on soil property testing or “tailor-made fertilizer” techniques (RD, 2016).

Grain 2AP content depends on both the rice variety and environmental factors during cultivation that impact 2AP concentration. Kongpun *et al.* (2020) reported that growing rice variety Khao Dawk Mali 105 in a slightly saline soil enhanced 2AP accumulation in rice grains. Additionally, growing rice crops under drought stress conditions during the milky ripening stage was reported to enhance grain 2AP content (Yoshihashi *et al.*, 2002). Nitrogen is a common fertilizer applied in rice crop cultivation together with other nutrients, mainly phosphorus and potassium, for boosting productivity and grain quality. Applying N fertilizer at booting stage has been found to improve grain aroma content, but overapplication of N could decrease the content (Mo *et al.*, 2018). The effect of N fertilizer on the aroma has been reported in several studies, but there is limited information concerning how applying N affects the aroma. We hypothesized that applying N may affect grain N accumulation and that this may be associated with the grain aroma. This study set out to evaluate the effects of N application rates on grain yield and the contents of N and 2AP in non-photosensitive fragrant rice varieties. The results from this study should be useful for improving the fragrant rice varieties with high 2AP content by adjusting N fertilizer management during cultivation.

Materials and Methods

Experimental Design and Plant Culture

A pot experiment was conducted at the Department of Plant Production Technology, Kalasin University, Kalasin Province, Thailand during the dry season from December 2018 to April 2019. The experiment was arranged in a factorial design with two factors and three independent replications. The first factor was the rice varieties of Pathum Thani 1 (PTT1) and RD22, high-yield varieties with photoperiod insensitivity. The second factor was three N fertilizer rates of 2.44, 3.82 and 5.66 g N/pot designated as N1, N2 and N3, respectively, where N2 represents the common rate for N requirement among the varieties (Ali *et al.*, 2020). Rice seeds were directly sown in 10 L plastic pots containing 13 kg soil (4.4% moisture content). Soil samples were taken for analysis profiling before being used in the experiment. Eight seeds were sown in each pot and the seedlings were thinned to two plants per pot at 11 days after sowing. The water level in each pot was maintained at 2 - 5 cm above the soil surface until harvesting. Nitrogen fertilizer was applied equally by splitting into four applications at 16, 48, 77 and 104 day after sowing; these corresponded to the seedling, floral initiation, booting and grain filling stages, respectively. The phosphorus (1.39 g P/pot) and potassium (2.63 g K/pot) were applied four times as for the N application. At maturity, grain yield and yield components were evaluated and seeds samples were kept in a cold room at 18°C before being subjected to chemical analysis.

Table 1: The fertility profile of soil used in this experiment

Soil properties	
Organic matter (OM) (%)	0.180
Total N (%)	0.010
Extractable P (Bray II) (mg/kg)	1.330
Exchangeable K (mg/kg)	12.000
Electrical conductivity (1:5, soil: water) (mS/cm)	0.095
pH	5.200

Soil Properties

The soil in this experiment was a sandy loam (68.5% Sand, 16.5% Silt and 15.0% Clay); the properties are given in Table 1. The soil pH was slightly acidic with low organic matter and N, P, K concentrations, with no salinity stress on rice growth based on the EC (Wolf, 1999).

Chemical Analysis

About 10 g of each paddy rice sample was de-husked to yield brown rice grain. A key aroma compound, 2-acetyl-1-pyrroline (2AP), was evaluated based on the fresh extract of uncooked brown rice via capillary gas chromatography-mass spectrometry using the method of Mahatheeranon (2001). Grain N concentration in brown rice was evaluated by the Kjeldahl method.

Statistical Analysis

Data were analyzed using two-way analysis of variance. The differences among treatment means were compared using least significant difference at $P < 0.05$. Associations between datasets were determined using correlation analysis.

Results and Discussion

Yield and Yield Components

Grain yield was affected by N application rates differently between the two fragrant varieties (Fig. 1). The variety RD22 had higher grain yield than PTT1 in all applied N rates. In PTT1, grain yield increased from 35.4 to 55.8 g/pot when increasing N rates from N1 to N2, but yield was decreased to 34.9 g/pot when applied with N3. In contrast, grain yield of RD22 was increased from 62.5 to 93.2 g/pot when increasing the N rate from N1 to N2, while increasing N rate from N2 to N3 had no effect on grain yield. Nitrogen fertilizer is commonly used to increase crop yield (Liu *et al.*, 2018; Duncan *et al.*, 2018) due to it being an integral constituent of proteins, nucleic acids, chlorophyll, co-enzymes, phytohormones and secondary metabolites (Marschner *et al.*, 2011). The efficacy of N fertilizer on yield potential varies according to rice variety and many other factors; over application of

the fertilizer can be toxic and reduce yield in rice crops (Jaksomsak *et al.*, 2017; Duncan *et al.*, 2018).

Yield components are major parameters that reflect on the final grain yield in rice crops and these vary among rice varieties. It is well established that the yield components of rice varieties with high yield potential are much more responsive to N fertilizer than varieties with low yield potential (Ladha *et al.*, 1998). This study found that the yield components were affected by N rates differently between the two varieties (Table 2). The number of panicles per plant of PTT1 (24.7) was higher than in RD22 (17.7). Increasing N rates from N1 to N2 and N3 increased panicle number from 15.1 to 21.9 and 26.5 per plant, respectively. Number of grains per panicle of PTT1 was not affected by increasing N rates from N1 to N2 (46.0 and 49.2 grain/panicle, respectively) but was depressed to 23.4 grains/panicle by applying the higher N rate (N3). In RD22, increasing N rates from N1 to N2 increased the number of grains per panicle from 91.7 to 101.9, but increasing N rates to N3 decreased it to 97.7 grains per panicle. The thousand grain weight of PTT1 was not affected by N rate, but in RD22 increasing the N rate from N1 to N2 and N3 decreased the thousand grain weight from 25.0 to 22.3 and 22.2 g, respectively.

Grain N and 2AP Concentration

Applying N affected grain N concentration differently between the varieties (Fig. 2A). Grain N concentration

of PTT1 was the lowest when applied at the rate N1 (1.42%) and was increased to 1.53 and 1.55% when applied with N2 and N3, respectively. In RD22, application with N1 resulted in grain N at 1.58%. Increasing the N rate to N2 had no effect on grain N (1.65%), while grain N was increased to 1.81% when applied with N3 (Fig. 2A). The results were in accordance with a previous study reporting that applying N at 120 kg ha⁻¹ to a rice crop resulted in higher grain N concentrations, but the increase percentage depended on N rate and rice variety (Jaksomsak *et al.*, 2017).

Grain 2AP concentration affected by N rates differently between the varieties (Fig. 2B). In PTT1, increasing N rate from N1 to N2 increased grain 2AP concentration from 0.88 to 1.35 mg/kg, but the concentration was not affected from N2 to N3 (1.32 mg/kg). In RD22, increasing N rate from N1 to N2 decreased grain 2AP from 3.23 to 2.89 mg/kg, but applying N2 increased grain 2AP to 3.55 mg/kg.

Relationship between Grain Yield, Grain N and Grain 2AP Concentration

There were significant positive linear relationships between grain yield and grain nitrogen concentration ($r = 0.83$, $P < 0.01$) (Fig. 3A), grain yield and grain 2AP concentration ($r = 0.81$, $P < 0.01$) (Fig. 3B) and grain N and grain 2AP concentration ($r = 0.82$, $P < 0.01$) (Fig. 3C).

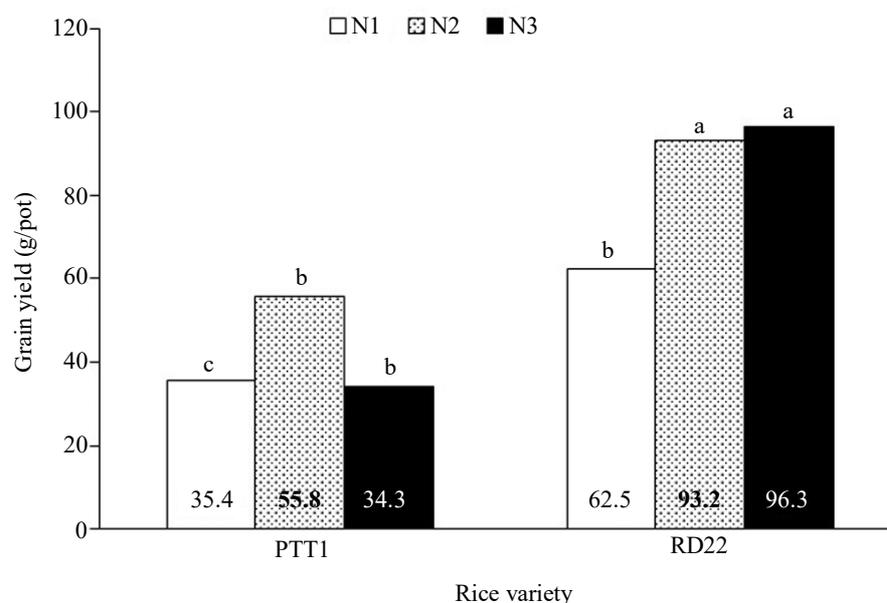


Fig. 1: Effects of N rates on grain yield in Pathum Thani 1 (PTT1) and RD22 rice varieties. The values are means of three replications. The different lowercase letters indicate significant differences by LSD_{0.05}

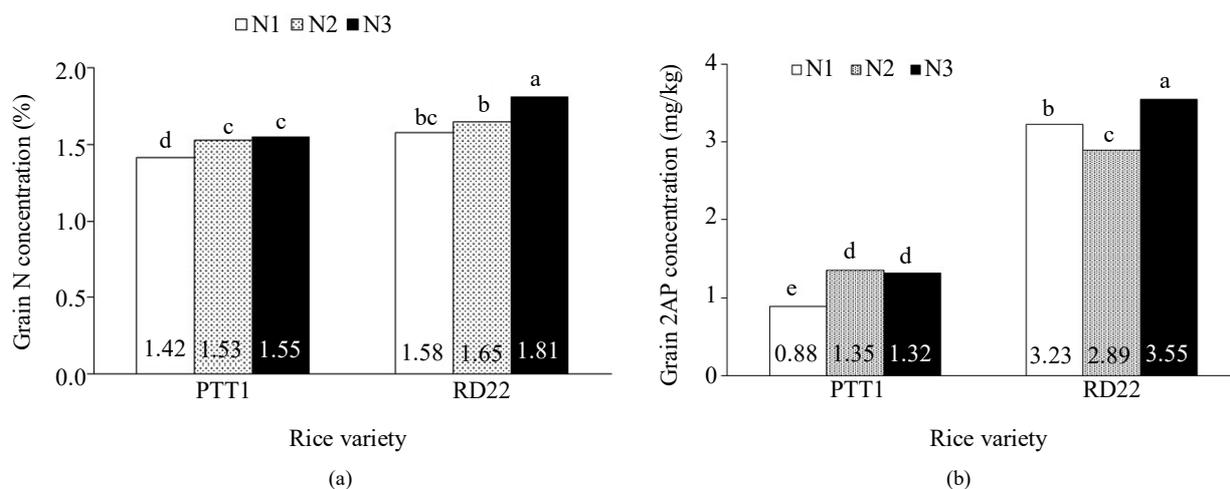
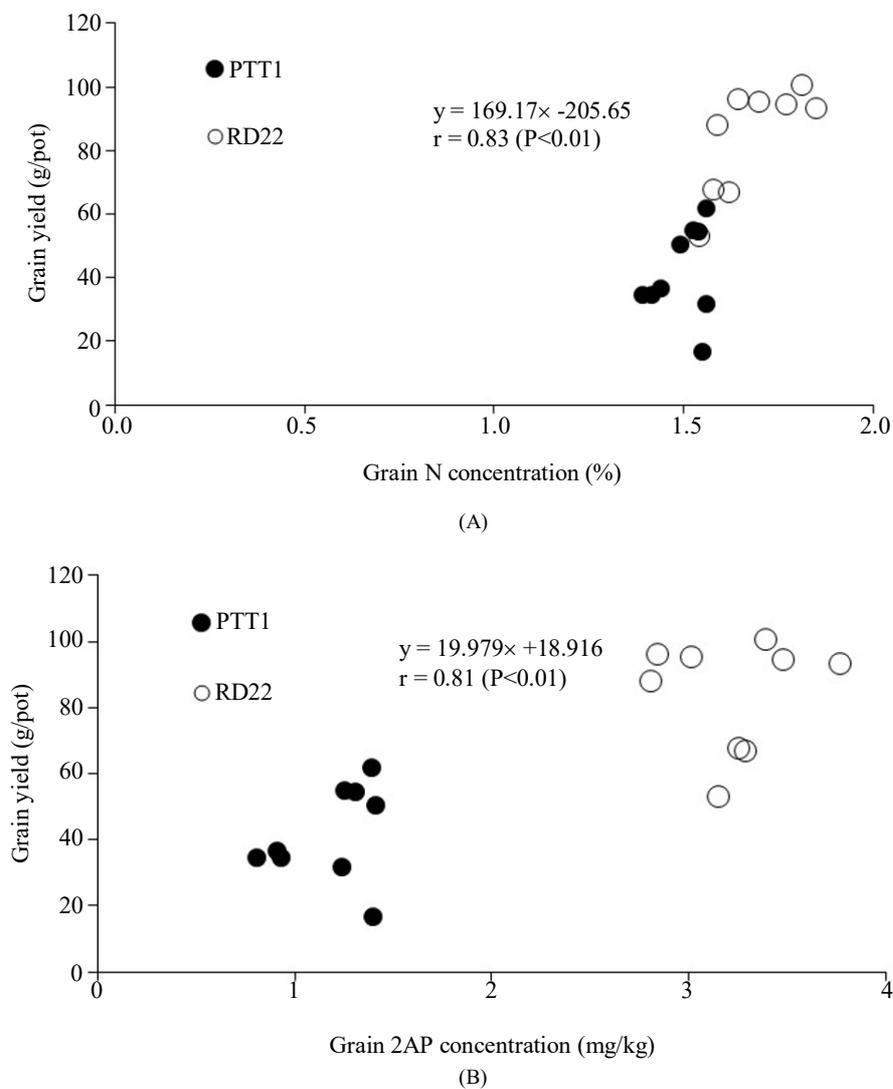


Fig. 2: Effects of N rates on grain N concentration (A) and grain 2AP concentration (B) of Pathum Thani 1 (PTT1) and RD22 rice varieties. The values are means of three replications. The different lowercase letters indicate significant differences by LSD_{0.05}



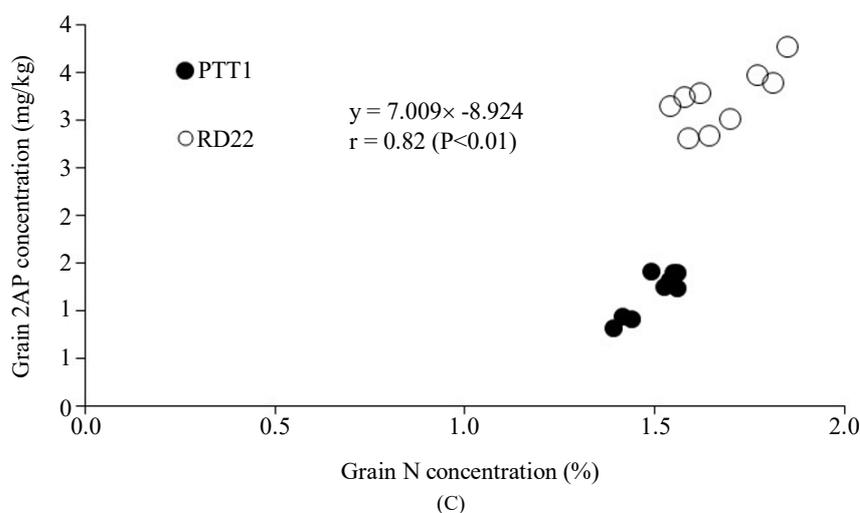


Fig. 3: Relationship between grain yield and grain N concentration (A), grain yield and grain 2AP concentration (B) and grain N and grain 2AP concentration (C), r = correlation coefficient (highly significant difference at $P < 0.01$)

Table 2: Number of panicles per plant and number of filled grains per panicle of two rice varieties grown under different nitrogen fertilizer rates

Variety	Nitrogen rate			mean
	N1	N2	N3	
Number of panicles per plant				
PTT 1	17.5	25.7	30.9	24.7a
RD22	12.7	18.2	22.3	17.7b
Mean	15.1 C	21.9B	26.6 A	
F-test	V**	N**	V x N ^{NS}	
Number of filled grains per panicle				
PTT 1	46.0 c	49.2c	23.4 d	39.5
RD22	97.1 b	101.9a	97.7 b	98.9
Mean	71.8	75.5	60.5	
F-test	V**	N**	V x N*	
1,000 grain weight (g)				
PTT 1	19.4 d	20.8 cd	19.3 d	19.8
RD22	25.0 a	23.3 b	22.2 bc	35.2
Mean	22.2	22.0	20.7	
F-test	V*	N**	V x N*	

N = nitrogen rate, V = rice variety, N x V = interaction effects between nitrogen rate and rice variety, * = significant ($P < 0.05$), ** = highly significant ($P < 0.01$), ns = non-significant ($P > 0.05$). Means in the same column followed by different letters indicate significant differences at $P < 0.05$

This experiment has established that applying N fertilizer enhanced grain 2AP accumulation in the high yield and non-photosensitive fragrant rice varieties. This may be the result of increasing grain N concentration due to the strong positive correlation between grain N and 2AP concentration found in this study. It has been documented that proline compounds and amino acids having N as the main component are major precursors in the pathway of 2AP synthesis (Nadaf *et al.*, 2014). Several studies have confirmed that applying N fertilizer to rice crops during cultivation increased the proline compounds and 2AP concentrations in rice grains, but most such conclusions were based on the relationship between N

application rates and grain 2AP concentration (Mo *et al.*, 2018; Yang *et al.*, 2012). Additionally, the effect of N application on grain 2AP content depends on growing conditions of the rice crop, as it has been reported that applying nitrogen to lead (Pb) contaminated soil increased grain yield and 2AP content differently in three fragrant rice varieties (Huoyi *et al.*, 2020). A recent study has reported that applying N fertilizer with less irrigation water at tillering stage not only increased head rice yield but also enhanced 2AP content in rice grains (Li *et al.*, 2021). Thus, applying N fertilizer under varied growing conditions may have different effects on the available N concentration in the soil for the plant's absorption and

uptake, which may consequently impact grain 2AP concentration. In fact, N application rates and/or soil N concentration are not always associated with the total N uptake in rice plants and grain accumulation, but rather are indicated by N concentrations in specific plant tissues (Reuter and Robinson, 1997). Additionally, this study has shown that the variety PTT1 accumulated less grain 2AP than in RD22, although both rice varieties were applied with the same rates of N, indicating that the effect of N rates on grain 2AP concentration is highly dependent on the rice variety. Therefore, for high grain fragrance, the appropriate rate of N fertilizer application should be carefully considered when dealing with different rice varieties and cultivation strategies. A highly positive correlation between grain N and grain 2AP concentration was found among N rates and rice varieties, indicating that grain N concentration is a promising indicator for determining grain 2AP concentration. A recent study found that applying Molybdenum (Mo) fertilizer enhanced the utilization and assimilation of N in rice plants and consequently improved yield and 2AP concentration in rice grains in two fragrant varieties grown under Cadmium (Cd) stress (Imran *et al.*, 2021). This confirmed that the factors enhancing N absorption, transport and accumulation in plant tissues may be the key parameters for increasing grain 2AP concentration among fragrant rice varieties. However, similar experiments with more rates of N application and fragrant rice varieties are required for confirmation of the relationship between grain N and 2AP concentration in rice crops. The results of this study will be useful for determining the proper application of N fertilizer for improving grain yield and quality in fragrant rice.

Conclusion

Grain N concentration is a promising indicator for determining grain 2AP accumulation in fragrant rice varieties and N concentration can be improved by applying the appropriate rate of N fertilizer. The magnitude of the increase in grain 2AP concentration by N application in fragrant rice depends on the rice variety and growing conditions. We conclude that increasing N application rate in rice cultivation would help to improve grain yield and grain N concentration, thereby increasing the 2AP concentration in rice grains. This study has further indicated that the fragrant rice variety RD22 showed higher efficiency of N localization and fragrance quality in the grains than PTT 1.

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Author's Contributions

Ayut Kongpun: Designed the experiment, conducted the research and contributed to results analysis and drafting the manuscript.

Chanakan Thebault Prom-u-Thai: Analyzed grain chemical properties, edited the manuscript and submitted the manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that the coauthor has read and approved the manuscript and that there are no ethical issues involved.

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