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Effect of Nitrogen Application Timing on Growth, Grain Yield and Eating Quality of the KD18 and TH3-3 Rice Varieties

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This study examined the effect of nitrogen application timing on growth characteristics and seed quality in inbred KD18 and hybrid TH3–3 rice varieties. A pot experiment was conducted in 2012 in a greenhouse at Kyushu University, Japan. The treatments were T0 (no nitrogen application), T1 (40:40:20), T2 (50:50:0), T3 (50:30:20), and T4 (50:20:30), with the ratios indicating the percentages of total nitrogen applied at the basal, active tillering, and panicle initiation stages. Tiller number and panicle number were highest in T2. The soil plant analysis development (SPAD) value was higher in T2 until the panicle initiation stage, after which it was higher in T4 for both varieties. KD18 produced more grain weight in T4 and T2, whereas TH3–3 produced the highest grain weight in T3. Late nitrogen application timing (T3 and T4) increased the starch and amylopectin content of the hybrid rice and the protein content of the inbred rice.

Key words: Carbohydrate, eating quality, nitrogen application timing, protein, yield

INTRODUCTION

Rice is the most important food crop and a major food grain for more than one–third of the world's population (Zhao *et al*., 2011). Many factors affect rice production, including climate, physical conditions of the soil, soil fertility, water management, weed control, cultivar, sowing date, seed rate, and fertilization. Nitrogen (N) is the main nutrient associated with yield (Hirzel *et al*., 2011; Angus *et al*., 1994; Wilson *et al*., 1994a; Sahrawat, 2006; Bouman *et al*., 2007; De–Xi *et al*., 2007), as N affects the growth and yield of rice (Yem, 1995). N availability promotes crop growth and tillering and determines the number of panicles and spikelets during the early panicle formation stage. This nutrient also provides a sink during the late panicle formation stage (Mae, 1997; Artacho *et al*., 2009). N fertilizer is a major essential plant nutrient and a key input for increasing crop yield (Dastan *et al*., 2012). As described in Eckert's (2011) Efficient Fertilizer Use Manual, timing has a major effect on the efficiency of N management systems. N should be applied to avoid periods of significant loss and to provide adequate N when the crop needs it most. The rate and timing of applying N are critical for optimum rice grain yield. N increases plant height, panicle number, leaf size, spikelet number, and number of filled spikelets (Dobermann and Fairhurst, 2000).

The nutrient quality of rice is important for human health. Rice is a major source of dietary protein for most people in tropical Asia (Juliano, 1993). Rice quality is determined by the genetic characteristics of a variety, environment conditions, and cultivation practices. The amount and timing of N fertilizer application have obvious effects on caryopsis development and grain quality of rice (Wang, 2003). Research in the 1960s and 1970s demonstrated that grain protein could be significantly increased by ensuring adequate application of N fertilizer up to the panicle initiation stage (Nangju and De Datta, 1970; De Datta *et al*., 1972). Subsequent field research indicated that the rate and timing of applying N fertilizer must be carefully considered to achieve both high yield and high grain quality. In high–yield production systems, improved congruence between N supply and crop demand sometimes requires several split applications, including a final N top–dressing at the flowering stage (Perez *et al*., 1996). Amylose content is the most important factor affecting the cooking and eating quality of milled rice (Juliano, 1979). The composition and cooking quality of rice vary based on genetic, environmental, and agronomical factors. The cooking and eating quality of rice is related to starch content, partially because starch is the main component of milled rice and accounts for up to 95% of the dry matter (Fitzgerald *et al*., 2009). Amylose and amylopectin are two components of starch, and their proportion differs in different cultivars (Singh *et al*., 2006). Prolamin and glutelin are the two major storage proteins in rice grain (Furukawa *et al*., 2003). Baxter *et al*. (2006) reported that prolamin and glutelin have opposite effects on pasting and textural properties of rice flour. Increased prolamin content causes a decrease in pasting temperature, peak and final viscosities, gel hardness, and adhesiveness, but an increase in breakdown viscosity, whereas glutelin shows the opposite effect. Ning *et al*. (2010) reported that albumin and globulin

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are controlled more by genotypes than by N treatment, whereas prolamin and glutelin are largely determined by N.

Different rice cultivars are affected differently by N (Yoshida, 1981). N absorption by hybrid rice at the early growth stage is higher than that in inbred rice because of heterosis of the root system and N absorbing ability (Kobayashi, 1995). The heterosis for absorbing N and N use efficiency of F1 hybrid rice are higher than those of parent and inbred cultivars (Yang and Pham, 2009).

The present study examined the effects of N application timing on the (1) growth characteristics, (2) yield components, (3) and eating quality of the KD18 inbred and TH3–3 hybrid rice varieties.

MATERIALS AND METHODS

Pot preparation

A pot experiment was conducted in a greenhouse at Kyushu University, Japan (33º37'N, 130º25'E, 3 m above the sea level). The soil was Kasuya soil (clay loam) from Kyushu University farm and was collected from the upper 20 cm layer in a rice cultivation field at 30 positions for pot experiment and at six positions by using steel cylinder tubes to determine soil physicochemical properties. The soil was dried under ambient condition, crushed into small pieces, ground to pass through a 2–mm sieve, and used to determine physicochemical properties. The soil physicochemical properties were examined before the experiment was set–up and are shown in Table 1. The salicylic acid–H_2SO_4–H_2O_2 soil digestion method (Ohyama *et al.*, 1991) was used to analyze the total N content, total phosphorous content, and total potassium content. A 400–mg sample of air–dried soil was weighed, put in a test tube, and 2 mL of H_2SO_4 was added to incubate overnight. Then, 100 mg of sodium thiosulfate was added to the tube and vortexed. The tube was heated, and the digested solution was transferred to a 50–mL volumetric flask. This digested solution was used to analyze the total N content by the indophenol method (Cataldo *et al.*, 1974), total phosphorous content by the ascorbic acid method (Murphy and Riley, 1962), and total potassium content by atomic absorption spectrophotometry (Z5300, Hitachi, Tokyo, Japan).

Soil pH_{H_2O} (1:2.5 soil: H_2O) and soil pH_{KCl} (1:2.5 soil: KCl solution) were measured with a pH meter (Beckman Φ360 pH/ Temp/ mV Meter; Fullerton, CA, USA). Cation exchange capacity and exchangeable cations were determined by the ammonium acetate shaking extraction method (Muramoto *et al.*, 1992) followed by the indophenol method (Cataldo *et al.*, 1974) and atomic absorption spectrophotometry, respectively. Mineralizable N was determined using a soil incubation method following Sahrawat (1983) referring to Waring and Bremner (1964) and the indophenol method (Cataldo *et al.*, 1974). Available phosphorous was determined by Truog's (1930) method followed by the ascorbic acid method (Murphy and Riley, 1962). The results of the chemical property evaluation are shown in Table 1.

Table 1. Chemical properties of the soil before the pot study

Physicochemical properties	Value
Soil pH (Soil: H_2O; 1:2.5)	6.37
Soil pH (Soil: KCl; 1:2.5)	5.38
Total N (%)	0.156
Total K_2O (%)	0.594
Total P_2O_5 (%)	0.254
CEC ($cmol_c\ kg^{-1}$)	20.4
Exc. Ca ($cmol_c\ kg^{-1}$)	4.75
Exc. Mg ($cmol_c\ kg^{-1}$)	0.975
Exc. K ($cmol_c\ kg^{-1}$)	0.328
Exc. Na ($cmol_c\ kg^{-1}$)	1.30
Available P (mg P_2O_5/100 g oven dried soil)	21.4
Mineralizable N (mg N/100 g oven dried soil)	3.41

CEC, cation exchangeable capacity; Exc., exchangeable.

In May 2012, the soil was ground to pass through a 2–cm sieve and put in a/2000 Wagner pots. The water content of the soil was measured to determine the weight of wet soil that was equivalent to 12 kg of dried soil. The pots were flooded with water immediately after applying the fertilizers and kept for 3 days prior to transplantation. Rice cultivation was carried out from May to October 2012.

Treatments and experimental design

The rate of N: P_2O_5: K_2O fertilizer application was 100: 60: 90 ($kg\ ha^{-1}$). In the beginning, all phosphorous fertilizer (superphosphate), 50% of the potassium fertilizer (KCl), and urea according to treatment were mixed with half the soil before irrigating. The remaining 50% of the potassium fertilizer (KCl) was applied using the broadcast method at the panicle initiation stage. Nitrogen was broadcast when applied at the later times (active tillering and panicle initiation). The experiment was conducted using five different treatments including T0: no N application; T1 (40:40:20); T2 (50:50:0); T3 (50:30:20); and T4 (50:20:30). The ratios in each treatment indicate the percentages of total N applied at the basal, active tillering (AT), and panicle initiation (PI) stages (Table 2). The pot experiment in the greenhouse followed a randomized complete block design with three replications.

Rice cultivation

The KD18 inbred and TH3–3 hybrid rice varieties were used in this study. KD18 is a Chinese inbred rice imported into Vietnam. This variety is the most popular variety in Vietnam for its stable yield, wide adaptability to severe weather conditions, and high resistance to disease. Its average yield is 5.0–5.5 tons ha^{-1}. The N: P_2O_5: K_2O fertilizer was applied at a rate of 90:60:70 kg ha^{-1}, respectively. The KD18 growth period lasts 95 days during summer/fall season (MARD 2009). The TH3–3 variety is two lines – hybrid rice (T1S–96/R3), created by Tram (2005) at Hanoi University of Agriculture, Hanoi, Vietnam. The TH3–3 growing period lasts 120–125 days

during the spring, and 105–110 days during the summer/fall. Average yield is 6.5–8.0 tons ha^{-1}. The N: P_2O_5: K_2O fertilizer was applied at a rate of 100:70:100 kg ha^{-1}, respectively. Rice seedlings were prepared using a commercial seedbed soil (Kokuryu Baido, Seisin Sangyo Co., Kitakyushu, Japan). Rice seeds were sterilized and incubated at 25°C for 24 h. Three seeds were sown in each shell (1 hill) with seedbed soil and kept in a tray with water. The water level was kept at 2–3 cm. The 21–day–old seedlings were transplanted in two hills per pot on June 20, 2012. Tap water was used for irrigation, and the water level was maintained at 2–3 cm above the soil surface until maturity.

Rice growth characteristics

During the rice cultivation period, the tiller number, plant height, and soil plant analysis development (SPAD) value were recorded weekly during the fast–growing stage (10–75 days after transplant) and at 2–week intervals thereafter. The SPAD value was measured with a chlorophyll meter (SPAD–502, Konica Minolta Sensing Inc., Osaka, Japan). The uppermost fully expanded leaf was used to measure the SPAD value before the panicle initiation stage, and the flag leaf was used subsequently. Dry weight and yield components were recorded at harvesting time. At maturity, the rice plants were cut 2–3 cm above the ground, and dry matter weight and seed weight were determined. Grain weight was estimated at 14% moisture content. The harvest index was calculated by dividing economic yield (seed weight) by biological yield (total dry matter weight) (Yoshida, 1981).

Rice quality

Brown rice was vacuum–freeze dried and ground to a fine powder. The brown rice powder was used to analyze quality parameters, including starch content, amylose and amylopectin contents, total protein, and protein fraction content.

Starch was extracted using perchloric acid and determined using the anthrone method of Yoshida (1976). Starch content was calculated based on the weight of the brown rice powder. A 50–mg of vacuum–freeze dried brown rice powder was placed in a centrifuge tube, 80% ethanol was added, and the tube was kept in an 80–85°C water bath for 30 minutes. Then, the tube was centrifuged at 2,000 rpm for 10 minutes. This extraction procedure was repeated three times. The precipitate was oven dried at 80°C for 2 h, 1 mL of distilled water was added, and the solution was kept in a boiling water bath for 15 minutes (stirred tube occasionally by vortex). When the tube was cool, 1 mL of 9.2 N $HClO_4$ was added while mixing constantly by vortex. The suspension was brought up to 5 mL with distilled water, mixed well by vortexing, centrifuged, and decanted into a 50–mL volumetric flask. Next, 1 mL of 4.6 N $HClO_4$ was added to the residue. The suspension was brought up to 5 mL with distilled water, mixed well by vortex, centrifuged, and decanted to combine with the previous extract in the 50–mL volumetric flask, which was filled up with distilled water. A 0.5–mL aliquot of the starch extract solution was diluted with 4.5 mL of distilled water, kept in an ice bath, and 10 mL of anthrone reagent was added slowly (0.2% anthrone in H_2SO_4 solvent) allowed it ran down the side of tube; the solution was then mixed well. Finally, the tube was kept in a boiling water bath for exactly 7.5 min and immediately cooled down in ice. A glucose standard was used to prepare the standard curve. Starch content and the glucose standard curve were determined by measuring absorbance at a wavelength of 630 nm, and the content was calculated following the method of Holm *et al*. (1986):

$$\text{Starch} = \frac{\mu\text{g of glucose} \times 10^{-3} \times \mathbf{a} \times 0.9}{\text{mg of sample weight}} \times 100 \text{ (where } \mathbf{a} \text{ is the dilution factor)}$$

Amylose and amylopectin contents were determined according to the method of Juliano (1971). For the digestion, 100 mg of brown rice powder was weighed and added to test tubes. Then 1 mL of 95% ethanol and 9 mL of 1 N NaOH were added. The sample glass tubes were heated in a boiling water bath for 10 minutes, cooled, transferred to a 100–mL volumetric flask, filled up, and then transferred to 100 mL plastic boxes. Five milliliters of this starch solution was pipetted into a 100–mL volumetric flask, and 1 mL of 1 N acetic acid and 2 mL of iodine solution (0.2 g of iodine and 2.0 g of potassium iodine in 100 ml of aqueous solution) were added. The solution was brought up to volume with distilled water, shaken, and allowed to stand for 20 min before measuring absorbance at 620 nm using a spectrophotometer. For the standard curve, 100 mg of dried amylose (amylose from potato, Sigma–Aldrich, Co., St. Louis, MO, USA) was mixed with 2.5 mL ethanol and 22.5 mL 1 N NaOH, heated in a boiling water bath for 10 min, cooled, transferred to a 250–mL volumetric flask, and then trans-

Table 2. Amount of N applied per growth stage in the treatments (% of total N amount)

Treatments	Basal	Active tillering stage	Panicle initiation stage	Note
T0 (0:0:0)	0	0	0	No N application
T1 (40:40:20)	40	40	20	Standard in Vietnam
T2 (50:50:0)	50	50	0	
T3 (50:30:20)	50	30	20	
T4 (50:20:30)	50	20	30	

The N percentage calculation was based on 100 kg N ha^{-1}.

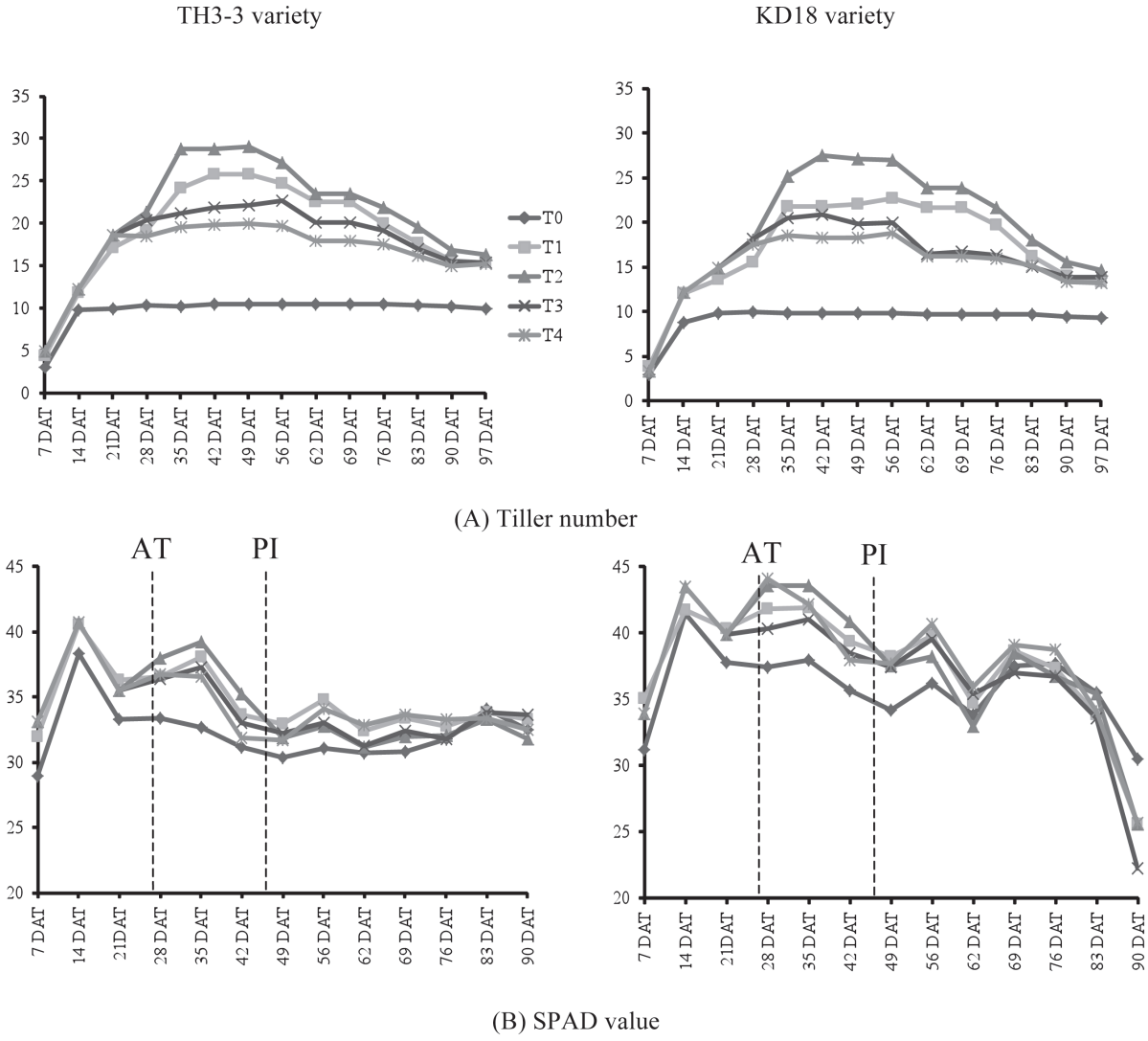

Fig. 1. Changes in tiller number and soil plant analysis development (SPAD) value with differences in nitrogen application timing. T0: none N; T1: 40:40:20; T2: 50:50:0; T3: 50:30:20; T4: 50:20:30; AT, active tillering (2nd application) ; PI, panicle initiation (3rd application); DAT, days after transplant.

ferred to a plastic bottle and maintained at 4°C. A blank without amylose was used for the standard curve. Aliquots of 0, 1, 2, 3, 4, and 5 mL of amylose stock solution were placed in 100–mL volumetric flasks, brought up to 5 mL with blank solution (with a pipet, 5, 4, 3, 2, 1, 0 mL), and then treated in the same manner as the samples above. Absorbance was measured at 620 nm, and amylose content was calculated from the standard curve. Amylopectin content was calculated by subtracting starch content from amylose content.

Total protein content was calculated by multiplying total N by 5.95 (Juliano, 1994). The brown rice powder was digested using H_2SO_4–H_2O_2 digestion (Ohyama *et al*., 1991) and then analyzed for total N content following the indophenol method (Cataldo *et al*., 1974).

The protein fraction content was determined according to Ju (2001) with some modified steps for the extraction. A 200 mg of freeze–dried brown rice powder was added to 12 mL Nalgene centrifuge plastic test tubes. The rice powder was defatted with 2 mL cyclohexane, mixed well by vortex, shaken at 150 rpm for 2 h, and centrifuged at 6,500 rpm for 30 minutes, and then the supernatant was discarded. The defatted rice flour was completely dried under a vacuum. Among four protein fractions, the albumin fraction was first extracted with distilled water. A 2 mL of distilled water was added and mixed well by vortex. Ultrasound was done for 20 sec at 0°C in an ice bath, and then the sample was mixed by vortex. This procedure was repeated nine times (total 3 min of ultrasound). Next, the sample was centrifuged at 6,500 rpm for 30 min. The supernatant was decanted into a 10–mL volumetric flask. This albumin extraction was repeated three times. The volumetric flask was filled up with distilled water, and the residue was used for the next extraction. After extracting the albumin, the globulin, prolamin, and glutelin extractions were performed with 2 mL each of 5% NaCl, 70% ethanol, and 0.4% NaOH, respectively. The extraction method was

the same for all protein fractions. The supernatant of each protein fraction was digested with H_2SO_4–H_2O_2 (Ohyama *et al*., 1991), total N content was analyzed following the indophenol method (Cataldo *et al*., 1974), and protein content was calculated by multiplying by 5.95 (Juliano, 1994).

Statistical analysis

Analysis of variance was used to test for differences, and Tukey's HSD test was used to calculate the least significant difference at the 5% probability level using STATISTIX 8 (Analytical Softwar*et al*.,lahassee, FL, USA).

RESULTS

Plant growth characteristics

Tiller number

The tiller number per hill increased gradually at the early vegetative stage and reached a peak 35 days after transplant (DAT) to 56 DAT. Then, it decreased continuously up to harvest time in both varieties. Total tiller number was affected significantly by N application timing (NAT). The N treatments produced a significantly higher value than the no N application, and TH3–3 produced a higher tiller number than that of KD18 (Fig. 1A). The highest tiller number was observed in T2 (50:50:0) during the whole growth period of both varieties, followed by T1, T3, and T4.

SPAD value

The SPAD value is a very important plant growth characteristic because it provides the relative chlorophyll content of the rice plant leaf. In this study, the KD18 SPAD value was higher than that of TH3–3 during the rice growing period (Fig. 1B). SPAD values for TH3–3 were higher in the N application treatments than in the no N application treatment until 76 DAT, after which the values of all treatments were within the same range. The KD18 SPAD values were higher in the N application treatments until 62 DAT, and then the SPAD value of the no N application treatment was higher than the others at ripening time. Among application treatments, TH3–3 produced the highest SPAD value in T2 after the second N application, followed by T1, T3, and T4. The SPAD value of this variety after the third N application increased in T4 (highest value), followed by T1, T3, and T2. After the N application of KD18 at the AT stage, T2 had the highest value, followed by T1, T3, and T4. After the third N application at the PI stage, the SPAD value increased in T4 (highest value), followed by T1, T2, and T3.

Dry matter accumulation

Dry matter weight was significantly different between the N application treatments and the no N application treatment. TH3–3 produced greater dry matter weight than that of KD18. The NATs did not produce a significant difference in dry matter weight among the N application treatments in either variety. However, T2 produced the highest value (136.93 g pot^{-1}) in KD18, and both T2 (151.33 g pot^{-1}) and T3 (151.43 g pot^{-1}) produced the highest values in TH3–3 (Fig. 2).

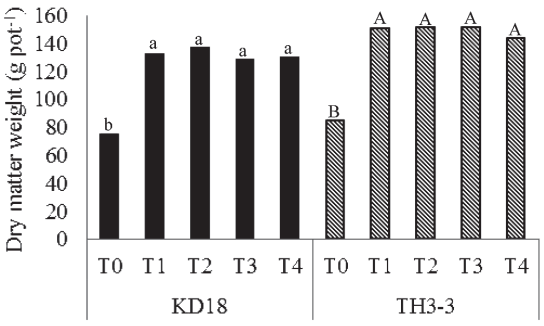

Fig. 2. Dry matter weight with differences in nitrogen application timing. Values with the same cased letter are not significantly different according to Tukey's HSD test ($P < 0.05$).

Yield component and grain weight

Table 3 shows that the NATs produced different responses in the two varieties in terms of panicle number. The panicle number per hill was significantly different among the N application treatments for TH3–3 but was similar for KD18. TH3–3 produced the highest number of panicles in T2 (15.83 hill^{-1}) followed by T3 (14.67 hill^{-1}) and was significantly higher than T1 (14.22 hill^{-1}) and T4 (14.17 hill^{-1}).

The results of the statistical analysis showed that the interaction effects between varieties and NATs on the percentage of filled seed, grain weight, and harvest index were significant. In contrast, no differences were observed in spikelet number per panicle or 1000–grain weight. The percentage of filled seed was higher in KD18 than in TH3–3. The highest filled seed percentage value was recorded in T4 (83.57%) for KD18, and the lowest value was recorded in T4 (64.55%) and T0 (63.53%) for TH3–3. As a result, KD18 produced the highest grain weight in T4 (64.41 g pot^{-1}) and TH3–3 produced the

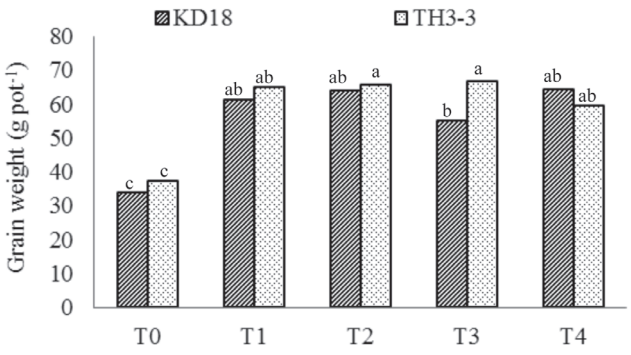

Fig. 3. Changes in grain weight with differences in NAT. Values with the same cased letters are not significantly different according to Tukey's HSD test ($P < 0.05$).

highest yield in T3 (66.71 g pot^{-1}) and T2 (65.63 g pot^{-1}) (Fig. 3). The harvest index ranged from 0.41 (T4, in TH3–3) to 0.49 (T4, in KD18) (Table 3).

Rice quality

Table 4 shows the results for rice quality. The starch and amylopectin contents of TH3–3 significantly responded to the NATs, but those of KD18 did not.

Table 3. Effect of nitrogen application times on yield components and harvest index

Treatments		Panicle number/hill	Spikelet number/panicle	Filled seed (%)	1000–grain weight (g)	Grain weight (g/pot)	Harvest index
KD18	T0 (0:0:0)	9.17 b	147.73	70.34 c	17.97	34.01 c	0.45 ab
	T1 (40:40:20)	13.00 a	164.99	77.96 b	18.35	61.20 ab	0.46 ab
	T2 (50:50:0)	13.50 a	159.92	82.02 ab	18.18	64.09 ab	0.47 ab
	T3 (50:30:20)	13.25 a	157.56	78.65 ab	18.54	55.10 b	0.43 b
	T4 (50:20:30)	12.83 a	165.49	83.57 a	18.21	64.41 ab	0.49 a
Probability		< .0001	ns		ns		
TH3–3	T0 (0:0:0)	9.67 C	130.35	63.53 e	23.27	37.23 c	0.44 ab
	T1 (40:40:20)	14.22 B	142.01	68.99 cd	23.00	64.93 ab	0.44 ab
	T2 (50:50:0)	15.83 A	125.51	72.73 c	22.74	65.63 a	0.43 ab
	T3 (50:30:20)	14.67 AB	143.5	69.17 cd	22.86	66.71 a	0.44 ab
	T4 (50:20:30)	14.17 B	143.35	64.55 de	22.52	59.45 ab	0.41 b
Probability		< .0001	ns		ns		
Source of variance							
N				0.0023		< .0001	0.4896
Variety				< .0001		0.0208	0.0015
N*Variety		ns	ns	0.0427	ns	0.0089	0.0169

Values with the same cased letter in the same column are not significantly different according to Tukey's HSD test ($P < 0.05$).

Table 4. Rice qualities of KD18 and TH3–3 under the different treatments of nitrogen (% of brown rice powder)

Treatments		Carbohydrate content			Protein content				
		Starch	Amylose	Amylopectin	Total	Albumin	Globulin	Prolamin	Glutelin
KD18	T0 (0:0:0)	64.35 c	32.93	31.42 c	5.92 a	0.87	0.57 a	0.16 ab	3.41 b
	T1 (40:40:20)	63.95 c	33.15	30.80 c	5.77 ab	0.75	0.54 ab	0.16 ab	3.53 ab
	T2 (50:50:0)	65.13 c	32.96	32.17 c	5.78 ab	0.80	0.52 bc	0.13 ab	3.41 b
	T3 (50:30:20)	64.11 c	33.11	31.49 c	6.12 a	0.74	0.57 a	0.19 a	3.71 a
	T4 (50:20:30)	64.35 c	32.87	31.47 c	5.93 a	0.79	0.56 ab	0.14 ab	3.44 b
Probability			ns			ns			
TH3–3	T0 (0:0:0)	66.74 bc	29.29	37.45 b	5.36 c	0.79	0.48 cd	0.11 b	3.31 bc
	T1 (40:40:20)	69.27 ab	28.68	40.61 ab	5.26 c	0.77	0.45 d	0.10 b	3.14 cd
	T2 (50:50:0)	66.60 bc	28.98	37.62 b	5.44 bc	0.76	0.47 d	0.12 ab	3.15 cd
	T3 (50:30:20)	70.76 a	29.27	41.49 ab	5.24 c	0.78	0.45 d	0.10 b	3.12 cd
	T4 (50:20:30)	71.32 a	29.53	41.79 a	5.32 c	0.89	0.44 d	0.09 b	2.94 d
Probability			ns			ns			
Source of variance									
N	0.0197		0.0812		0.4302		0.0452	0.3010	0.0066
Variety	<.0001		<.0001		<.0001		<.0001	0.0002	<.0001
N*Variety	0.0028	ns	0.0214		0.0545	ns	0.0066	0.0595	0.0028

Values with the same cased letter in the same column are not significantly different according to Tukey's HSD test ($P<0.05$). Protein content was calculated by multiplying total N by 5.95; N content was determined by salicylic acid–H_2SO_4–H_2O_2 digestion (Ohyama *et al.*, 1991) followed by the indophenol method (Cataldo *et al.*, 1974).

TH3–3 produced greater starch and amylopectin contents than those of KD18, and the highest values were recorded for TH3–3 in T3 (70.76% starch, 41.49% amylopectin) and T4 (71.32% starch, 41.79% amylopectin). In contrast, the total protein of KD18 responded significantly to NATs, whereas that of TH3–3 did not. The highest total protein content was found for KD18 in T3 (6.12%), which was similar to T4 (5.93%) and T0 (5.92%). No differences in amylose content were observed between the two varieties and among all treatments.

The four main rice protein fractions are albumin, globulin, prolamin, and glutelin. In this study, those protein fractions were extracted sequentially to measure their content. The results are shown in Table 4. The interaction effect between varieties and NATs for globulin, prolamin, and glutelin contents was significant, whereas albumin content was not affected by the interaction.

DISCUSSION

Soil

The soil used was clay loam under yearly rice cultivation. The analytical results of the chemical properties in Table 1 show that the soil was slightly acidic (pH 6.37, dry condition) (Horneck, 2011). The optimum soil pH for rice growth is 5.5–6.5 under dry conditions and 5.5–7.2 under flood conditions (Landon, 1991; De Datta, 1981). Total N content was 0.156%, which was in the moderate range (Ilaco, 1985; Landon, 1991). Medium levels of cation exchange capacity (20.4 $cmol_c$ kg^{-1}), exchangeable K (0.328 $cmol_c$ kg^{-1}), and available P (21.4 mg P_2O_5 100 g^{-1} soil) were recorded (Landon 1991). Available N was low (3.4 mg 100 g^{-1} soil) according to the FDDD (1990). Thus, the soil was assumed to be in a low fertility condition.

Plant growth characteristics

In both rice varieties, the NATs had clear effects on tiller production during the growth stage, particularly from the second N application (AT stage) to the third N application (PI stage). Although the amount of total N was the same in all treatments, different NATs caused a difference in the amount of N used during the growth stage. As a result, 100% of the N was applied at the basal and AT stages until the second N application in T2 (50:50:0) in which the highest tiller number was observed. A dose of N at the tillering stage is essential for optimum tiller number in rice because N has a positive influence on the production of tiller number per plant, yield, and yield attributes (BRRI, 1990). In addition, 70% of the N was applied as the lowest N level among the N application treatments until the AT stage in T4 (50:20:30); in this treatment, both rice varieties produced the lowest tiller number. Vaiyapuri *et al.* (1998) and Sathiya and Ramesh (2009) reported that the N requirement is higher during the early transplant to tillering stage in rice. Moreover, the total tiller number per hill was found to increase following the application of N at transplant or by two equal split dressings at transplanting and tillering (Shoo *et al.*, 1989). The N absorbed during the vegetative period mainly promotes early growth of the plant and increases the number of tillers (Makino *et al.*, 1984). After the final application of N fertilizer, total tiller number decreased in both varieties.

Unlike in upland crops, the amount of nitrate in paddy rice leaves is not significant; thus, the N status in the leaf could be measured by the SPAD value (Takebe and Yoneyama, 1989). The SPAD value is very important to evaluate current growth status because it shows the relative chlorophyll content of the rice plant leaf. Because 35 is a critical SPAD value for the IR72 cultivar during a dry season, fertilizer should be applied whenever the SPAD value falls below this number (Peng *et al.*, 1996). In general, the SPAD value was lower in TH3–3 compared with that in KD18. In particular, the SPAD value in TH3–3 was generally < 35 and in the same range as the no N application treatment during the late growth period. This may have occurred because TH3–3 is a hybrid rice, in which growth heterosis started at the beginning and a stronger root system was observed. In addition, good weather conditions in Japan helped to promote rice growth, and no disease or insects were observed during the cultivation period. Kobayashi (1995) reported that N absorption by hybrid rice is higher than that of inbred rice at the early growth stage because of root system heterosis and increased N absorptive ability. In our study, TH3–3 produced a larger plant body during the early growth stage, and the tiller number produced by TH3–3 was significantly higher than that of KD18 during 21–49 DAT (Fig. 1A). Thus, the application of 100 kg N ha^{-1} may not be sufficient for TH3–3 under Japan's cultivation conditions. A higher N level should be applied for further study of TH3–3 in Japan.

The SPAD value of the flag leaf during the later growth stage increased in KD18, whereas it was stable in TH3–3. Many authors have reported that the SPAD value and N content in the flag leaf depend on the growth stage and cultivar and are poorly correlated with plant N demand (Turner and Jund, 1994; Hussain *et al.*, 2000; Yoshida, 1981). Aung *et al.*, (2011) found that the SPAD value increases in the flag leaf of the Manawthuka rice variety, which has growth characteristics similar to those of the KD18 rice variety.

Both rice varieties showed the same pattern of change in the SPAD value according to NATs. The SPAD value was high during the early growth stage and low during the later growth period. A difference in SPAD values was observed among treatments due to the different amounts of applied N. After the second N application (27 DAT), SPAD values were the highest in T2 followed by T3, T1, and T4; in these treatments, the rice received N amounts of 100% (T2), 80% (T3), 80% (T1), and 70% (T4), respectively. After the third N application, T4 produced the highest value because the remaining N amount was highest in this treatment (30%). The SPAD value in the no N application treatment during the late growth period was in the same range as the N application treatments, and this value in KD18 was higher than the N

application treatments at harvesting time. A possible explanation is that in the no N application treatment, the body of the rice plant formed using only N from the soil, producing the minimum tiller number and lowest SPAD value. Because of smallness of the plant body, in the late growth stage, photosynthesis may have been sufficient in providing the plant with organic matter.

Yield components

Panicle number was highest in the T2 treatment in both varieties (Table 3), indicating that more tillers were produced during the early growth stage, and more panicles were obtained during the later growth stage. This result may have occurred because of the number of tillers per hill, and T2 produced the highest value in both varieties (Fig. 1A). Moreover, TH3–3 responded significantly to NATs (highest panicle numbers in T2 and T3), indicating that a heavy application of N during the early growth stage significantly increased the panicle number in the hybrid rice.

The grain yield in the no N application treatment is an indicator of soil N supply capacity; it responds to mineralizable soil N, as pointed out by Wilson *et al*., (1994b). The grain yields were 34.01 g pot^{-1} for KD18 and 37.23 g pot^{-1} for TH3–3 were significantly lower than those in the N application treatments in both varieties (Fig. 3).

Among the yield components, significant differences were observed in the panicle number per hill and the percentage of filled seed (Table 3). Spikelet number per panicle and 1000–grain weight were not different. These results suggest that the significant difference in grain weight mainly came from the difference in panicle number per hill and the percentage of filled seed. The highest number of panicles per hill and the percentage of filled seed in T2 and T4 resulted in the highest grain weight compared with other KD18 treatments. The highest values of those parameters in T2 and T3 resulted in the highest grain weight in TH3–3.

In the T1, T2, and T3 treatments, the TH3–3 hybrid rice produced a significantly higher percentage of filled seed and grain weight than those in T4, indicating that TH3–3 might be sensitive to low N level at the active tillering stage.

Quality parameters

Starch is the main component of milled rice and accounts for up to 95% of the dry matter. Starch is related to the cooking and eating quality of rice (Fitzgerald *et al*., 2009). Starch consists of amylose and amylopectin, and the proportion of these components varies in different cultivars (Singh *et al*., 2006). In our experiment, TH3–3 produced higher starch and amylopectin content in late NATs and was lower in T2 when the fertilizer was applied at the basal and the active tillering stages (Table 4). Chen *et al*. (2006) suggested that adding N during the late reproductive growth stage helps alleviate the negative effects of catabolism; that is, translocation of N from leaves and stems to panicles, allowing plants to maintain higher photosynthetic rates in green tissues. The total protein content in TH3–3 did not respond to NATs. This might be because N was not completely translocated to the sink (spikelets), and it remained in stems and leaves to maintain photosynthetic activity. This could be related to the pattern of change in the SPAD value in this rice cultivar (Fig. 1B). The SPAD value was maintained at about 33 until harvest time. Islam *et al*. (1996) reported that the response of grain protein to an application of N fertilizer is cultivar dependent. In contrast to the TH3–3 hybrid rice, in the KD18 cultivar late NAT resulted in a significant difference in total protein content. The highest total protein content was recorded in the late N applications of T3 and T4. However, no difference in carbohydrate (starch, amylose, and amylopectin) content was observed among treatments.

The main fractions of rice grain protein are albumin, globulin, prolamin, and glutelin based on their solubility. Grain albumin and globulin are mainly stored in the outer layer, whereas glutelin and prolamin are concentrated in the endosperm (Yamagata *et al*., 1982). Rice proteins have good nutritional quality because they are mostly composed of glutelins, which are nutritionally more important than prolamins owing to their greater digestibility by humans and higher lysine concentration (Cagampang *et al*., 1996; Islam *et al*., 1996; Zang *et al*., 2008). Moreover, some albumins and globulins are allergenic proteins in patients with kidney disease; thus a breeding program has been conducted to produce hypoallergenic rice cultivars (Zang *et al*., 2008). Interestingly, we found that the timing of the N application affected the content of the protein fractions in the two rice cultivars (Table 4). However, it is necessary to repeat and continue these experiments with different N application ratios at different critical rice growth stages for additional verification.

CONCLUSION

NATs had significant effects on the growth characteristics of the two rice varieties studied. The highest grain weight was found in T4 (50:20:30) for the inbred rice and in T3 (50:30:20) for the hybrid rice.

The starch and amylopectin content of hybrid rice increased following the late N application. Amylopectin was produced in greater amounts in T3 and T4, and in significantly lower amounts in T2 (50:50:0) without N at the panicle initiation stage. The inbred rice responded to NATs in terms of an increase in protein content in T3. Thus, we suggest that the inbred KD18 rice variety should receive N fertilizer at the late stage to increase protein content. This method would increase starch and amylopectin contents in the TH3–3 hybrid rice cultivar.

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REFERENCES

Angus, J. F., M. Ohnishi, T. Horie and L. Williams 1994 A preliminary study to predict net nitrogen mineralization in a flooded rice soil using anaerobic incubation. *Aust. J. Exp Agr.*, **34**: 995–999

Artacho, P., C. Bonomelli and F. Meza 2009 Nitrogen application in irrigated rice growth in Mediterranean conditions: Effects on grain yield, dry matter production, nitrogen uptake, and nitrogen use efficiency. *J. Plant Nutr.*, **32**: 1574–1593

BRRI (Bangladesh Rice Research Institute) 1990 Nitrogen response of promising variety. *Annual Report Bangladesh Rice Research Institute*, Joydebpur, Gazipur. p. 95

Bouman, B. A. M., E. Humphreys, T. P. Tuong, R. Barker and L. S. Donald 2007 Rice and water. *Adv. Agron.*, **92**: 187–237

Cataldo, D. A., L. E. Schrader and V. L. Youngs 1974 Analysis by digestion and colorimetric assay of total nitrogen in plant tissues high in nitrate. *Crop Sc.*, **14**: 854–856

Dastan, S., M. Siavoshi, D. Zakavi, A. Ghanbaria–malidarreh, R. Yadi, D. E. Ghorbannia and A. R. Nasiri 2012 Application of nitrogen and silicon rates on morphological and chemical lodging related characteristics in rice (*Oryza sativa* L.) north of Iran. *J. Agri. Sci.*, **4**: 12–18

De Datta, S. K. 1981 *Principles and practices of rice production*. New York, USA: John Wiley & Sons publisher

De Datta, S. K., W. N. Obcemea, and R. K. Jana 1972 Protein content of rice grain as affected by nitrogen fertilizer and some triazines and substituted ureas. *Agron. J.*, **64**: 785–788

De–Xi, L., F. Xiao–Hui, H. Fena, Z. Hong–Tao and L. Jia–Fa 2007 Ammonia volatilization and nitrogen utilization efficiency in response to urea application in rice fields of the Taihu Lake Region, China. *Pedosphere*, **17**: 639–645

Dilz, K., D. S. Jenkinson and K. A. Smith 1988 Nitrogen Efficiency in Agricultural Soils. Edited by D. S. Jenkinson and K. A. Smith. Elsevier Applied Science, London and New York, pp. 1–26

Dobermann, A. and T. Fairhurst 2000 Rice: Nutrient disorders & nutrient management. Philippines: International Rice Research Institute. Potash and Photphorous Institute/ Potash and Photphorous Institute of Canada

Eckert, D. 2011 Nitrogen. *In* "Efficient Fertilizer Use Manual". The Mosaic Company. Suite E490, 3033 Campus Drive Plymouth, Minnesota 55441. USA: Atria Corporate Center

FPDD 1990 *Literature review on soil fertility investigation in Nigeria*. Fertilizer Procurement and Distribution Division, Federal Ministry of Agriculture and Natural Resources, Lagos, Nigeria

Fitzgerald, M. A., S. R. McCouch and R. D. Hall 2009 Not just a grain of rice: The quest for quality. *Trends Plant Sci.*, **14**: 133–139

Furukawa, S., T. Mizuma, Y. Kiyokawa, T. Masumura, K. Tanaka and Y. Wakai 2003 Distribution of storage proteins in low–glutelin rice seed determined using a fluorescent antibody. *J. Biosci. Bioeng.*, **96**: 467–473

Hirzel, J., A. Pedreros and K. Corder 2011 Effect of nitrogen rates and split nitrogen fertilization on grain yield and its components in flooded rice. *Chilean J. Agr. Res.*, **71**:437–444

Homl J., I. Bjorck, A. Drews and N. G. Asp 1986 A rapid method for the analysis of starch. *Starch*, **38**: 24–226

Horneck D. A., D. M. Sullivan, J. S. Owen and J. M. Hart 2011 *Soil Test Interpretation Guide*. Extension service, Oregon State University. USA: 1500 SW Jefferson Way, Corvallis, OR 97331

Ilaco, B. V. 1985 *Agricultural Compendium: For rural development in the tropics and subtropics*. Elsevier, Amsterdam/ Oxford/ New York/ Tokyo

Islam, N., S. Inanaga, N. Chishaki and T. Horiguchii 1996 Effect of N top–dressing on protein content in japonica and indica rice grains. *Cereal Chem.*, **73**: 571–573

Jing, Q., B. Bouman, H. van Keulen, H. Hengsdijk, W. Cao and T. Dai 2008 Disentangling the effect of environmental factors on yield and nitrogen uptake of irrigated rice in Asia. *Agr. System*, **98**: 177–188

Juliano, B. O. 1971 A simplified assay for milled–rice amylose. *Cereal Sci. Today*, **16**: 34–38

Juliano, B. O. 1979 The chemical basic of rice grain quality. *In*: Proceedings of the workshop on Chemical Aspects of Rice Grain Quality. Los Banos, Laguna, Philippines: International Rice Research Institute, pp. 69–90

Juliano, B. O. 1993 Rice in Human Nutrition. *Food and Nutrition Series No. 26*. Rome, Italy: Food and Agriculture Organization of the United Nations. Los Banos, Philippines: International Rice Research Institute

Juliano, B. O. 1994 Polysaccharides, proteins, and lipits of rice. *In* "Rice Chemistry and Technology", ed by B. O. Juliano, St. Paul, USA: The American Association of Cereal Chemists, pp. 98–141

Kobayashi, M., F. Kubota, K. Hirao and W. Agata 1995 Characteristic of photosynthesis and matter partitioning in leading hybrid rice, Oryza sativa L., Bred in China. *J. Fac. Agr. Kyushu Univ.*, **39**: 175–182

Landon, J. R. 1991 *Booker Tropical soil manual*: A handbook of soil survey and agriculture land evaluation in the tropical and sub tropical. New York, NY 10017, USA: 8th Floor, 711 3rd Avenue. Routledge publishers

MARD (Ministry of Agriculture and Rural Development). 2009 *575 giong cay trong nong nghiep moi*. Ha Noi, Viet Nam: The Nong Nghiep publisher

Mae, T. 1997 Physiological nitrogen efficiency in rice: Nitrogen utilization, photosynthesis, and yield potential. *Plant Soil*, **196**: 201–210

Makino, A., T. Mae and K. Okira 1984 Relation between nitrogen and ribulose–1,5–bisphosphate carboxylase in rice leaves from emergence through senescence. *Plant Cell Physiol.*, **25**: 429

Motavalli, P. P., K. A. Kelling and J. C. Converse 1989 First–year nutrient availability from injected dairy manure. *J. Environ. Qual.*, **18**: 180–185

Muramoto, J., I. Goto and M. Ninaki 1992 Rapid analysis of exchangeable cation and cation exchange capacity (CEC) of soils by shaking extraction method. *Jpn. J. Soil Sci. Plant Nutr.*, **63**: 210–215 (in Japanease with English summary)

Nangju, D. and S. K. De Datta 1970 Effect of time of harvest and nitrogen level on yield and grain breakage in transplanted rice. *Agron. J.*, **62**: 486–474

Ohyama, T., M. Ito, K. Kobayashi, S. Araki, S. Yasuyoshi, O. Sasaki, T. Yamazaki, K. Soyama, R. Tanemura, Y. Mizuno and T. Ikarashi 1991 Analytical procedures of N, P, K contents in plant and manure materials using H_2SO_4–H_2O_2 Kjeldahl digestion method. *Mem. Fac. Agr. Niigata Univ.*, **43**: 110–120 (in Japanease with English summary)

Peng, S., F. V. Garcia, R. C. Laza, A. L. Sanico, R. M. Visperas and K. G. Cassman 1996 Increased N–use efficiency using a chlorophyll meter on high–yielding irrigated rice. *Field Crop. Res.*, **47**: 243–252

Perez, C. M., B. O. Juliano, S. P. Liboon, J. M. Alcantara and K. G. Cassman 1996 Effects of late nitrogen fertilizer application on head rice yield, protein content, and grain quality of rice. *Cereal Chem.*, **73**: 556–560

Sahrawat, K. L. 1983 Nitrogen availability indexes for submerged rice soil. *Adv. Agron.*, **36**: 415–451

Sahrawat, K. L. 2006 Plants nutrients: sufficiency and requirements. *In* "*Encyclopedia of soil science*", Second edition, ed. by R. Lal, Philadelphia, Pennsylvania, USA: Taylor and Francis, pp. 1306–1310

Shoo, N. C., R. K. Mishra and J. P. Mohanty 1989 Response of aged seedlings of transplanted rice to split application of nitrogen. 1. Effect of single versus split application of nitrogen on growth and physiological growth parameters of rice. *Orissa J. Agr. Res.*, **2**: 194–195

Singh U., J. K. Ladha, E. G. Castillo, G. Punzalan, A. Tirol–Padre and M. Duqueza. 1998 Genotypic variation in nitrogen use

efficiency in medium and long duration rice. *Field Crop. Res*., **58**: 35–53

Singh, N., L. Kaur, K. S. Sandhu, J. Kaur and K. Nishinari 2006 Relationships between physicochemical, morphological, thermal, rheological properties of rice starches. *Food Hydrocolloid*., **20**: 532–542

Singh, N., L. Kaur, N. S. Sodhi and K. S. Sekhon 2005 Physicochemical, cooking and textural properties of milled rice from different Indian rice cultivars. *Food Chem*., **89**: 253–259

Synder, C. 2011 pH. *In* "Efficient Fertilizer Use Manual". The Mosaic Company. Suite E490, 3033 Campus Drive Plymouth, Minnesota 55441. USA: Atria Corporate Center

Takebe, M. and T. Yoneyama 1989 Measurement of leaf color scores and its implication to N nutrition of rice plants. *Japan Agr. Res. Quart*., **23**: 86–93

Truog, E. 1930 The determination of the readily available phosphorus in soil. *Am. Soc. Agron*., **22**: 874–882

Waring, S. A. and J. M. Bremner 1964 Ammonium production in soil under water logged condition as an index of nitrogen availability. *Nature*, **201**: 951–952

Wilson, C. E., R. J. Norman and B. R. Wells 1994a Chemical estimation of nitrogen mineralization in paddy rice soils: I. Comparison to laboratory indices. *Commun. Soil Sci. Plant Anal*., **25**: 573–590

Wilson, C. E., R. J. Norman, B. R. Wells, and M. D. Correll 1994b Chemical estimation of nitrogen mineralization in paddy rice soils. Ⅱ. Comparison to greenhouse variability indices. *Commun. Soil Sci. Plant Anal*., **25**: 591–604

Yem, V. H. 1995 *Phan bon va cach bon phan*. Ha Noi, Viet Nam: The Nong Nghiep publisher, pp. 14–20

Yoshida, S. 1976 *Laboratory manual for physiological studies of rice, third edition*. Los Banos, Philippines: International Rice Research Institute, pp. 46–49

Yoshida, S. 1981 *Fundamentals of rice crop science*. Los Banos, Philippines: International Rice Research Institute, p. 269.

Zhao, L., L. Wu, M. Wu and Y. Li 2011 Nutrient uptake and water use efficiency as effected by modified rice cultivation methods with irrigation. *Paddy Water Environ. J*., **9**: 25–32