

CHAPTER III

SELECTING THE PARENTS

3.1 Introduction

Rice, the staple food crop for people in Lao PDR, is mostly cultivated in the rainfed lowlands of Lao PDR (Inthapanya et al., 2006) and it is almost all waxy (otherwise called glutinous). Waxy rice contains no amylose due to a mutation in the *Waxy* gene (Wanchana et al., 2003). The Green Revolution, which changed rice-growing in so many countries, had little or no impact in Lao PDR because the germplasm of the Green Revolution was non-waxy, so it was not adopted by Lao rice farmers or consumers because they prefer glutinous rice (Roder et al., 1996). For many years, Lao farmers have preserved and cultivated a diverse suite of traditional waxy varieties. Recently, 13192 traditional varieties were systematically collected from Lao farmers in order to conserve them and preserve their diversity; 85.5% of these are waxy (Appa Rao et al., 2002a). These figures illustrate (i) the importance of waxy rice to people of Lao PDR, and (ii) that enormous diversity potentially exists in the quality of waxy rice.

Traditional varieties are generally low-yielding, so for many farming families, there is often insufficient rice to last from one crop to the next. Before 1990, traditional varieties were cultivated on more than 95% of the rainfed lowland areas of Lao PDR (Inthapanya et al., 2006). In 1991, the Swiss Government commissioned the 15-year Lao-IRRI project to improve yield and food security in Lao PDR, particularly in the rainfed lowlands. The rainfed lowland still produces 72% of the Lao rice crop, but the varieties grown have changed significantly as a result of that project. During the Lao-IRRI project, 18 varieties were released for rainfed lowland production (Inthapanya et al., 2006), and now two of them, Thadokkham 1 (TDK 1) and Thasano 1 (TSN 1), account for most of Lao's rainfed lowland production, and most of the rainfed area that is sown to improved varieties (Inthapanya et al., 2006). These improved varieties have had an enormous impact on food security in Lao PDR, and the traditional varieties that they replaced have entered gene banks for conservation

(Appa Rao et al., 2002a). However, two of the traditional varieties, Hom Nang Nouane (HNN) and Kai Noy Leuang (KNL) have continued to be cultivated. HNN is popular in central and southern areas while KNL is popular in central and northern regions of the country (Bounphanousay et al., 2009). These two varieties are cultivated for their quality, principally their fragrance and the softness of their grains after cooking (Appa Rao et al., 2002b; Bounphanousay, 2007). By contrast, TDK 1 and TSN 1 are not fragrant.

The political situation in Lao PDR has changed over the years, at the same time, communication technologies and transportation networks (such as roads and vehicles) have undergone rapid development. As a consequence of the former, Lao PDR has opened its doors to tourism, and aided by the latter, the tourist industry has burgeoned (Phakdisoth and Kim, 2007). Thus, Lao PDR has been able to introduce visitors from all over the world to the high quality and uniqueness of the traditional waxy, aromatic rice of the country. This has prompted Lao PDR to explore export opportunities for these rice, and led to a growing awareness among Lao agriculturists that these traditional varieties must be investigated for ways to improve their yield, understand their special quality traits, and include these traits into improved varieties that are high-yielding and resistant to pests and diseases, such as the popular TDK 1 or TSN 1 (Bestari et al., 2006).

One of the first recommendations to farmers to increase yield is to increase the nitrogen (N) application to the crop, which can alter the quality of non-waxy rice (Champagne et al., 2009). Although quality evaluation programs for rice have mostly focussed on developing methods for non-waxy varieties, a number of analyses commonly used for non-waxy rice could indicate the effect of N fertilizer on the eating and cooking quality of the waxy rice. For example, the texture of cooked grains is important to Lao rice consumers (Bounphanousay, 2007), and N affects the flavour (Champagne et al., 2009) and texture (Okadome, 2005) of the cooked rice. Viscosity traits can predict some sensory properties of rice (Champagne et al., 1999) and viscosity profiles are affected by N (Martin and Fitzgerald, 2002). Also, gelatinisation temperature relates to cooking time of the rice (Cuevas et al., 2010b) but has been shown to be unaffected by N (Bahmaniar and Ranjbar, 2007; Borrell et al., 1999). The objective of this study is to investigate the effect of N on the yield and quality traits of

the two traditional varieties, HNN and KNL, and the two popular improved varieties, TDK 1 and TSN 1, in order to determine strategies for improving the yield as well as the quality of Lao varieties.

3.2 Materials and methods

3.2.1 Plant growth and grain processing

Seeds of TDK 1, TSN 1, HNN and KNL were sown in the rainy season of 2006 in Lao PDR. One month later, seedlings were transplanted in a split plot design of sub-plots within main plots, in three replications at the Agriculture Research Centre, Vientiane, Lao PDR. Different amounts of N fertilizer were applied to each of the main plots and each main plot contained all the varieties, in three replications, in sub-plots. Each sub-plot was 2 m × 5 m, and 10 rows of 25 plants were transplanted at a spacing of 20 cm between plants and between rows. N was applied at four levels to each main plot, and the same amount of phosphorous and the same amount of potassium were applied for all plots as described in Table 3.1

The date of flowering was recorded and the panicle number per hill counted. Just before harvest, plant height was measured and the proportion of filled grains per panicle counted. At maturity, 21 plants in the middle six rows of each sub-plot were harvested and yield and yield components were recorded.

The harvested grain was then sent to the International Rice Research Institute in the Philippines for analysis of quality traits. Paddy from each sample was dehulled (Satake Rice Machine, Tokyo, Japan), milled (Grainman 60-230-60-2AT, Grain Machinery Mfg. Corp., Miami, FL), and a sub-sample ground to flour (Udy Cyclone Sample Mill 3010-030, Fort Collins, CO) to pass through a 0.5 mm sieve. Reagent-grade chemicals were used. Reverse osmosis water, filtered through a 0.22 µm Millipore (Billerica, MA) filter, was used throughout the study.

3.2.2 Protein

Protein content of flour from each treatment and variety was measured by Near-Infrared Transmission (NIT). Flour was placed in 4.5/3 mm (uncompressed / compressed path length) sample cups and scanned in transmission mode on a near-infrared scanning monochromator (Infratec 1241 Grain Analyzer, Foss, Sweden).

Table 3.1 Fertilizer and timing regime for the four N treatments

Main plot	Amount: N – P - K (kg ha ⁻¹)	Amount and time of application
N1	0-30-30	All amount applied as basal fertilizer (the same time transplanting)
N2	30-30-30	0-30-30 kg ha ⁻¹ applied as basal fertilizer and 2 top dressings each of 15 kg ha ⁻¹ N (25 days and 45 days after transplanting)
N3	60-30-30	30-30-30 kg ha ⁻¹ apply as basal fertilizer and 2 top dressings: each of 15 kg ha ⁻¹ N (25 days and 45 days after transplanting)
N4	90-30-30	Amount of 30-30-30 kg ha ⁻¹ apply as basal fertilizer and 2 top dressing: each 30 kg ha ⁻¹ N (25 days and 45 days after transplanting)

Scans were obtained from 570 to 1100 nm. The protein contents of the samples were determined based on the manufacturer's application model for polished rice using the flour module. Each sample was scanned in triplicate.

3.2.3 Gelatinisation temperature

Gelatinisation temperature was measured by differential scanning calorimetry (DSC) (Q100 TA Instruments, New Castle, DE). Flour (4 mg) was mixed with water (8 μ L) in an aluminium hermetic pan which was then hermetically sealed. The temperature was raised from 25 °C to 100 °C at 10 °C min⁻¹. Thermal transitions were recorded and analysed using Universal Analysis 2000 software. The gelatinisation temperature is reported as the peak of the gelatinisation endotherm.

3.2.4 Aroma

The aromatic compound, 2-acetyl-1-pyrroline (2AP) was measured by gas chromatography (Agilent 6890N, Santa Clara, CA, USA), equipped with a mass spectrometer, exactly as previously described (Kovach et al., 2009). Chemically

synthesised 2AP was a kind gift from Dr. T. Yoshihashi (Japan International Research Centre for Agricultural Sciences, Ibaraki, Japan) and was used to quantify 2AP in the samples. 2AP was only measured and quantified using KNL and HNN grains because the two improved varieties are not aromatic.

3.2.5 Texture

Polished grains of each variety (25 grains) were cooked in the same way as is done in the Lao PDR. Polished grains were soaked for 2h in water, then the soaked grains were steamed for 30 mins. For each N treatment, three grains were analysed for hardness and stickiness using a Ta.XT-Plus Texture analyser equipped with a cylindrical probe (diameter = 35mm, Stable Micro Systems Ltd., Surrey, UK) immediately after cooking. Strain was set at 90% and the test speed was 0.5 mm s⁻¹. Measurements were conducted in triplicate. Not all samples of TDK 1 could be analysed for texture because not enough whole grains remained after polishing.

3.2.6 Viscosity

Flour of each sample (3 g) was mixed with water (25 g) in a Rapid Visco-Analyser (RVA) canister. Viscosity was measured by RVA (Newport Scientific Model 4D) using the Approved Method 61-02 (American Association of Cereal Chemists, 2000).

3.2.7 Statistical analysis

Balanced analysis of variance (ANOVA), which is conducted when there are equal numbers of observations, was performed using *R* statistic software (version 2.11.0) for yield, yield components, and protein content. For gelatinisation temperature, hardness, and stickiness, unbalanced ANOVA was conducted using SAS (version 9.1). Pair-wise comparison of means was done using least significant difference (LSD) at 5% level of significance. For each variety and N treatment, all three replicates were included for each analysis. The correlation matrix among RVA parameters, hardness, and stickiness was generated also using *R* statistic software, pooling values for all the replicates and all the varieties.

3.3 Results

3.3.1 Yield

Figure 3.1 shows the increase in yield associated with N fertilizer rate of the traditional and the improved varieties. Yields were not significantly different between 0 and 30 kg N ha⁻¹, but became significantly different between 30 and 60 kg N ha⁻¹. The yield at 90 kg N ha⁻¹ was similar to that at 60 kg N ha⁻¹. The interaction between nitrogen treatment and variety was not significant (data not shown). The two traditional varieties were less responsive to N than the two improved varieties (Figure 3.1): the yields of TDK 1 and TSN 1 increased by 40%; by contrast, HNN and KNL showed a yield increase of only 18% between the lowest and highest N treatment. Moreover, TSN 1 always showed a higher yield than TDK 1 (Figure 3.1).

Yield components, particularly 1000-grain weight and number of panicles per plant, were not significantly responsive to N treatment (Tables 3.2C and 3.2D). On the other hand, the proportion of filled grains per panicle significantly increased between N rates of 0 and 30 kg ha⁻¹ (Table 3.2A). Interactions between N treatment and variety were not significant for these three yield components. The only yield component with significant interaction between N rate and variety was the number of grains per panicle (Table 3.2B). Number of grains per panicle in HNN and TDK 1 did not increase with N treatment; in KNL, an increase in this yield component was observed between 0 and 90 kg N ha⁻¹; in TSN 1, the number of grains per panicle increased between 30 and 60 kg N ha⁻¹. N fertilizer increased plant height significantly for all varieties, but did not alter the days to flowering (data not shown).

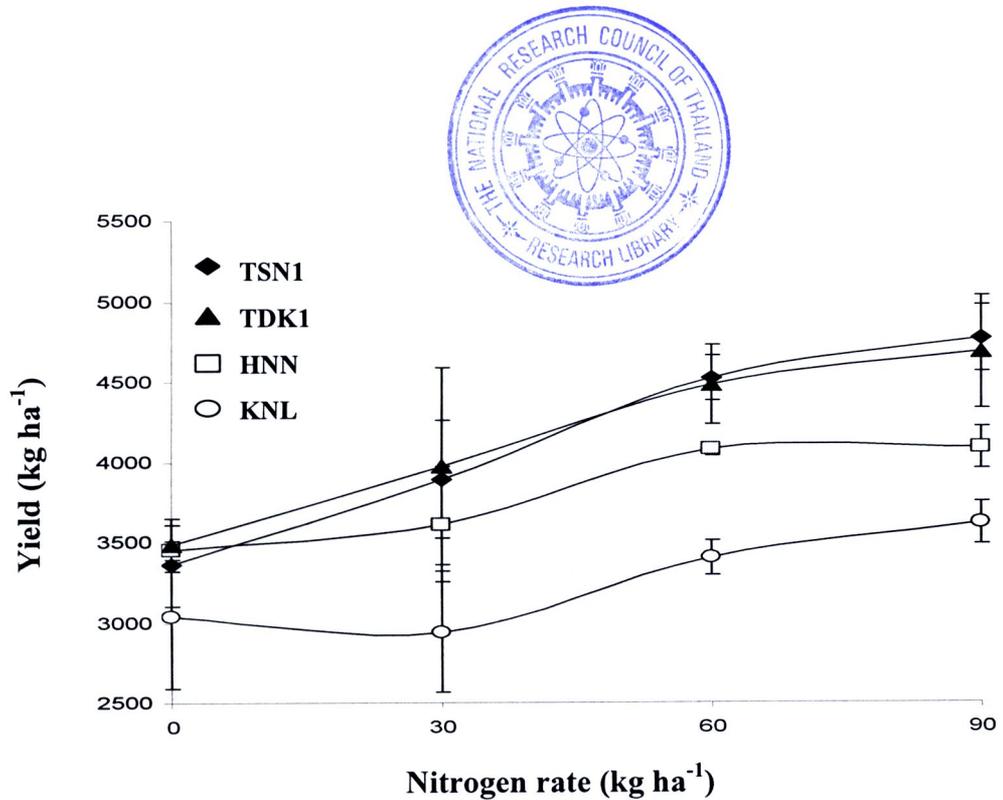


Figure 3.1 Yield of improved varieties responded much more to N fertilizer than traditional varieties, and improved varieties were significantly higher yielding at N treatments greater than 30 kg N ha⁻¹ (LSD_{0.05} = 366 kg ha⁻¹)

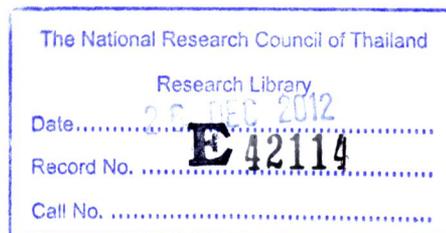


Table 3.2 Yield and yield components of the four varieties across four N treatments**A. % Filled grains per panicle**

Variety (V)	Nitrogen (N) (kg ha ⁻¹)				Means
	0	30	60	90	
HNN	91.7	90.3	90.9	90.7	90.9 ^a
KNL	82.7	86.9	81.9	84.9	84.1 ^b
TDK 1	77.9	84.2	83.0	80.7	81.4 ^b
TSN 1	66.4	81.0	77.1	80.7	76.3 ^c
Means	79.7 ^b	85.6 ^a	83.2 ^{ab}	84.2 ^a	
Comparison	LSD _{0.05}				
2 V means	4.3				
2 N means	3.6				

B. Number of grains per panicle

Variety (V)	Nitrogen (N) (kg ha ⁻¹)				Mean
	0	30	60	90	
HNN	106.0 ^b	126.0 ^a	124.3 ^{bc}	118.3 ^b	118.7
KNL	117.3 ^b	124.7 ^a	137.7 ^{ab}	150.7 ^a	132.6
TDK 1	116.0 ^b	111.0 ^a	115.7 ^c	116.0 ^b	114.7
TSN 1	168.0 ^a	112.3 ^a	151.7 ^a	141.0 ^a	143.3
Comparison	LSD _{0.05}				
2 V means at the same N level	20.6				
Nitrogen (N)		Variety (V)			
(kg ha ⁻¹)	HNN	KNL	TDK 1	TSN 1	Mean
0	106.0 ^a	117.3 ^b	116.0 ^a	168.0 ^a	126.8
30	126.0 ^a	124.7 ^{ab}	111.0 ^a	112.3 ^c	118.5
60	124.3 ^a	137.7 ^{ab}	115.7 ^a	151.7 ^{ab}	132.3
90	118.3 ^a	150.7 ^a	116.0 ^a	141.0 ^b	131.5
Comparison	LSD _{0.05}				
2 N means at the same V level	26.3				

Table 3.2 Yield and yield components of the four varieties across four N treatments
(Cont.)

C. 1000-grain weight (g)

Variety (V)	Nitrogen (N) (kg ha ⁻¹)				Mean
	0	30	60	90	
HNN	31.4	31.9	31.8	31.8	31.7 ^a
KNL	26.6	26.0	25.6	26.3	26.1 ^c
TDK 1	30.6	30.0	31.3	32.5	31.1 ^a
TSN 1	27.1	28.3	28.4	29.6	28.3 ^b
Mean	28.9	29.0	29.3	30.0	
Comparison	LSD _{0.05}				
2 V means	0.9				
2 N means	NS				

D. Number of panicles per hill

Variety (V)	Nitrogen (N) (kg ha ⁻¹)				Mean
	0	30	60	90	
HNN	6.0	5.4	6.3	6.1	6.0 ^b
KNL	5.6	5.3	6.3	6.0	5.8 ^b
TDK 1	7.4	6.7	7.4	9.4	7.7 ^a
TSN 1	5.5	5.5	6.3	6.9	6.0 ^b
Mean	6.1	5.7	6.6	7.1	
Comparison	LSD _{0.05}				
2 V means	0.7				
2 N means	NS				

† In a column, means followed by the same letter (a, b, c, d) are not significantly different at 5% level of LSD.

‡ In yield components where LSD_{0.05} values for comparing two N means are indicated as non significant (NS), main effects of nitrogen are not significant (p>0.05).

3.3.2 Quality

The amount of protein in each variety differed significantly (Table 3.3B). The N treatments did not affect the amount of protein in the grains until the N rate was 90 kg ha⁻¹ (Table 3.3). Gelatinisation temperature of the four varieties was not significantly different, and the rate of N fertilizer did not alter it (Table 3.3A). The rate of N fertilizer did not affect hardness or stickiness of cooked rice (Tables 3.3C and 3.3D). The only other significant differences in traits of texture were that cooked grains of HNN were harder than those of KNL and TSN 1 and KNL was less sticky than HNN and TSN 1. Possibly the texture of the cooked grains of TDK 1 is similar to that of HNN, but we were unable to obtain sufficient whole grains of TDK 1 after milling to carry out enough replication at each N level to determine statistical significance.

Table 3.3 Effect of variety and nitrogen on gelatinisation temperature (GT), protein content, hardness and stickiness of freshly cooked rice grains

A. Gelatinisation temperature (°C)

Variety (V)	Nitrogen (N) (kg ha ⁻¹)				Mean
	0	30	60	90	
HNN	69.0 ^c	70.0 ^b	69.0 ^c	70.2 ^b	69.6
KNL	69.9 ^b	70.9 ^a	71.5 ^a	71.4 ^a	70.9
TDK 1	70.9 ^a	70.7 ^{ab}	70.6 ^{ab}	70.1 ^b	70.6
TSN 1	69.8 ^b	70.5 ^{ab}	69.8 ^b	70.1 ^b	70.1
Comparison	LSD _{0.05}				
2 V means at the same N level	0.8				
Nitrogen (N) (kg ha ⁻¹)	Variety (V)				Mean
	HNN	KNL	TDK 1	TSN 1	
0	69.0 ^b	69.9 ^b	70.9 ^a	69.8 ^a	69.9
30	70.0 ^a	70.9 ^a	70.7 ^a	70.5 ^a	70.5
60	69.0 ^b	71.5 ^a	70.6 ^a	69.8 ^a	70.2
90	70.2 ^a	71.4 ^a	70.1 ^a	70.1 ^a	70.4
Comparison	LSD _{0.05}				
2 N means at the same V level	0.9				

Table 3.3 Effect of variety and nitrogen on gelatinisation temperature (GT), protein content, hardness and stickiness of freshly cooked rice grains (Cont.)

B. Protein content (%)

Variety (V)	Nitrogen (N) (kg ha ⁻¹)				Mean
	0	30	60	90	
HNN	7.6	7.9	7.8	7.9	7.8 ^c
KNL	7.3	7.5	8.6	8.8	8.1 ^{bc}
TDK 1	8.5	8.1	8.3	8.5	8.3 ^b
TSN 1	8.6	8.5	8.6	9.3	8.8 ^a
Mean	8.0 ^b	8.0 ^b	8.3 ^{ab}	8.6 ^a	
Comparison	LSD _{0.05}				
2 V means	0.3				
2 N means	0.4				

C. Hardness (g)

Variety (V)	Nitrogen (N) (kg ha ⁻¹)				Mean
	0	30	60	90	
HNN	1910.1	1841.3	1874.6	1944.3	1892.6 ^a
KNL	1738.1	1677.9	1746.2	1713.0	1718.8 ^b
TDK 1	NA	1855.5	1990.1	1962.4	NA
TSN 1	1546.5	1720.2	1856.6	1916.9	1760.1 ^b
Mean	NA	1773.7	1866.9	1884.1	
Comparison	LSD _{0.05}				
2 V means	92.8				
2 N means	NS				

Note: NA - non analysis

Table 3.3 Effect of variety and nitrogen on gelatinisation temperature (GT), protein content, hardness and stickiness of freshly cooked rice grains (Cont.)

D. Stickiness (g)

	Nitrogen (N) (kg ha ⁻¹)				Mean
	0	30	60	90	
HNN	-1047.9	-1014.7	-968.0	-983.6	-1003.6 ^a
KNL	-898.6	-900.0	-935.6	-854.0	-897.1 ^b
TDK 1	NA	-1157.8	-1193.7	-1464.7	NA
TSN 1	-995.8	-1088.8	-1124.3	-1046.5	-1063.9 ^a
Mean	NA	1040.3	1055.4	1087.2	
Comparison	LSD _{0.05}				
2 V means	72.2				
2 N means	NS				

† In a column, means followed by the same letter (a, b, c, d) are not significantly different at 5% level of LSD.

‡ In quality indicators where LSD_{0.05} values for comparing two N means are indicated as NS, main effects of nitrogen are not significant ($p > 0.05$).

Figure 3.2 shows the 2AP content of KNL and HNN grains at each N treatment. The amount of 2AP in each variety was not significantly different at each N treatment (0 to 90 kg ha⁻¹), but the amount of 2AP from KNL grains was significantly greater than that from HNN grains at the three N treatments above zero (Figure 3.2).

The RVA traces in Figure 3.3 show significant differences (LSD_{0.05}) between varieties for some parameters. The viscosity parameters (and values derived from these parameters) of KNL were significantly lower than those of the three other varieties. Also, peak, trough, and final viscosities and the derived value retrogradation (the difference between final and trough viscosities) were all significantly higher in TDK 1 than in TSN 1. The trough viscosity and the retrogradation of HNN were significantly different from those of TDK 1 and TSN 1. However, there were no significant differences among TDK 1, TSN 1, and HNN for setback and breakdown.

In contrast, the RVA traces in Figure 3.3 show no significant difference due to N treatment for any of the viscosity parameters in TDK 1, TSN 1, and most parameters for HNN. For KNL, on the other hand, the peak, trough, and final viscosities of grains grown at 0 and at 30 kg N ha⁻¹ were lower than of those grown the higher rates of N (Figure 3.3).

Correlations among viscosity parameters, hardness, and stickiness were obtained for the four waxy varieties (Figure 3.4). The direct values of peak, trough, and final viscosity values correlated very well with each other, as well as with the derived values of setback, retrogradation, and breakdown. Hardness correlates weakly with the RVA parameters of peak, trough, and final viscosities, and with retrogradation, but stickiness correlates slightly with hardness (Figure 3.4).

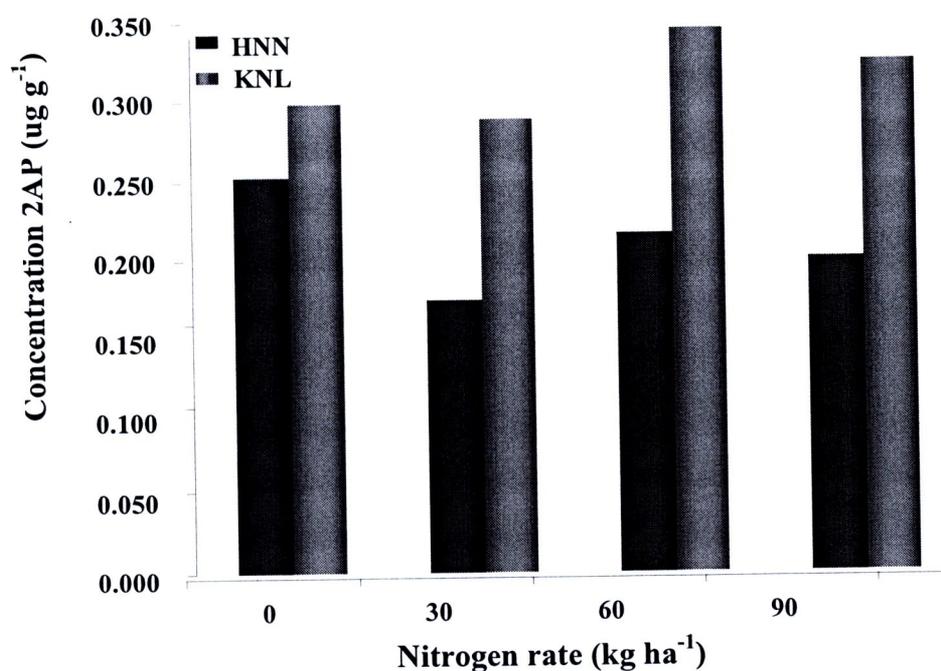


Figure 3.2 Concentration of 2AP in the grains of the two aromatic traditional varieties at each N treatment. Differences between varieties and between N treatments are not significant ($LSD_{0.05} = 0.144$)

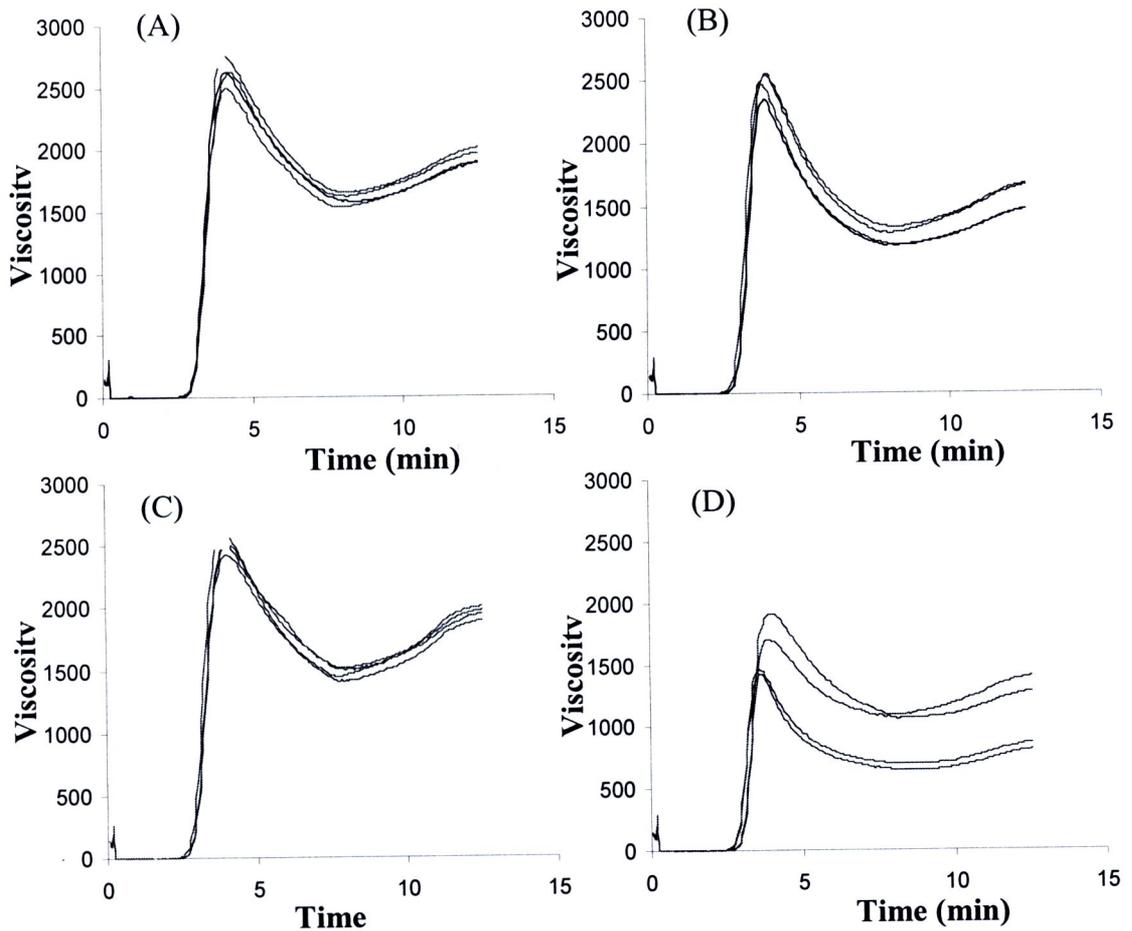


Figure 3.3 RVA traces of each variety, TDK 1 (A), TSN 1 (B), HNN (C), KNL (D) at each of the four N treatments. Each curve is the average of 3 biological replicates. For KNL, the two lower curves are the lowest N treatments and the two higher curves are the higher N treatments

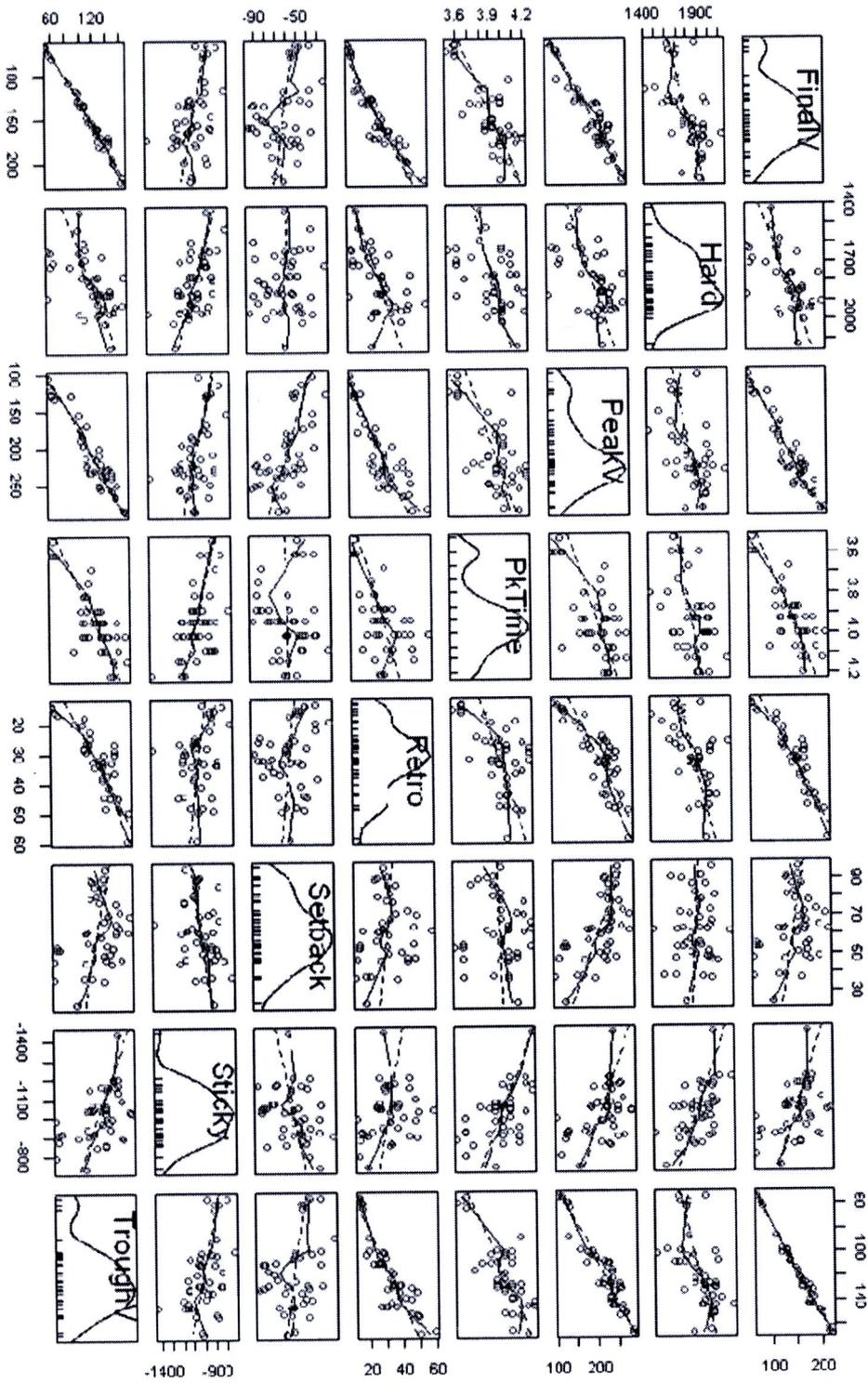


Figure 3.4 Correlation matrix of RVA parameters, hardness, and stickiness for the four waxy varieties

3.4 Discussion

3.4.1 Yield

The two improved varieties were more responsive to N treatment than the two traditional varieties in terms of yield. Number of panicles per plant was not significantly different among the N treatments (Table 3.2D). However, it has previously been reported to be one of the most important N responsive yield components (Bond et al., 2008). Consistent with this, the yield increase of TDK 1 and TSN 1 with N might be attributed to an increased number of panicles per plant, as well as a minor effect of an increase in thousand-grain weight (Table 3.2C), suggesting greater translocation of substrates to the grain during grain-filling. Both of these varieties offer potential as parents in quality improvement programs; however, the enhanced responsiveness of TSN 1 to N treatment in terms of yield makes it a more promising candidate than TDK 1.

Yield of the two traditional varieties increased by 18% over the N treatments. This difference was significant (Figure 3.1), but none of the measured yield components were significant (Table 3.2). Nevertheless, the main difference in yield components of KNL between the highest and lowest N treatment was the number of grains per panicle (Table 3.2B), which explains the yield increase for this variety. The data suggest that KNL cannot increase the number of panicles initiated in response to N, but is able to increase the size of the panicles in response to N, and maintain the proportion of filled grain in the larger panicles.

KNL showed the lowest yield of the four varieties (Figure 3.1), and relative to HNN and the improved varieties, the data in Table 2 indicates that this is due to the lower grain weight of KNL. The lower yield of HNN relative to the improved varieties is most likely to be due to the lower number of grains per panicle (Table 3.2B).

3.4.2 Quality

The main quality trait of the traditional varieties used in the present study that is prized by Lao consumers is fragrance. Although over 100 volatile compounds have been detected from rice, the compound that contributes the most to fragrance is 2AP (Buttery et al., 1983a). It has previously been reported that the amount of 2AP in KNL is higher than in HNN (Fitzgerald et al., 2008). This was also found in the present

study at N levels of 30 kg ha⁻¹ and greater (Figure 3.2). The major gene for aroma is *betaine aldehyde dehydrogenase (BADH2)* (Bradbury et al., 2005). At least 10 alleles of the *BADH2* gene are known, that lead to different amounts of 2AP (Kovach et al., 2009). HNN has the most common allele for *BADH2*, but the genetic basis of aroma in KNL is yet to be identified (Fitzgerald et al., 2008; Kovach et al., 2009). Perhaps the gene for aroma in KNL is more active than in HNN, leading to higher amounts of 2AP.

The amount of 2AP that accumulates in grains is affected by environmental and management conditions (Yoshihashi et al., 2004). Figure 3.2 shows that the concentration of 2AP was not affected by N treatment for either variety, so either these two varieties are not affected by N or the accumulation of aroma is not affected by 2AP. The *BADH2* gene is expressed in the grain (Chen et al., 2008), so differences in panicle number or grains per panicle are not expected to dilute the amount of 2AP produced in grains of HNN. Though the genetic basis of aroma in KNL is unknown, the increased panicle size of KNL in the different N treatments did not have any significant affect on the high level of 2AP in the grains of KNL (Figure 3.2) suggesting that the gene for aroma in KNL is also expressed in the endosperm.

Instrumental measurements of hardness and stickiness indicate that the samples used in this study did not respond to N treatment in terms of hardness and stickiness. Varietal differences, however, were observed. The hardness of TSN 1 was similar to that of KNL (Table 3.3C) and its stickiness was similar to that of HNN (Table 3.3D). Comparisons with TDK 1 could not be conducted in this study because the amount of milled whole grains was too low in the 0 kg N ha⁻¹. Nevertheless, hardness and stickiness of grains from N treatments with sufficient samples suggest that the texture of TDK 1 is similar to that of HNN.

Rapid viscosity analysis (RVA) is generally used to predict a number of traits of eating quality of non-waxy rice (Champagne et al., 1999). The development of the viscosity profile has been described previously (Fitzgerald et al., 2003). In brief, viscosity begins as starch granules absorb water and proteins swell. As temperature increases, the starch granules swell rapidly and amylose leaches from granules, resulting in the initial increase in viscosity. Peak viscosity is reached when the rate at which the starch granules swell is about the same as the rate at which they burst. After

which, disrupted granules start to align, under constant shear and under constant temperature, leading to a decrease in viscosity. The lowest point of this decrease is the trough viscosity. As the temperature decreases, viscosity begins to increase as the starch granules retrograde. At this stage, the leached amylose and other molecules form networks. The last point in the viscosity curve is the final viscosity.

It has previously been shown using a number of non-waxy varieties that N fertilizer decreases the peak and final viscosity of the curve disproportionately (Martin and Fitzgerald, 2002) suggesting that there may be grain components in non-waxy varieties that could be responsive to N. However, waxy varieties show quite different curves from non-waxy varieties (Merca and Juliano, 1981). In particular, the viscosity profiles of KNL grown in the lower N treatments were lower than those of grains grown in the higher N treatments (Figure 3.3D). Moreover, curves from KNL had the most positive setback. These observations show that the relationship between N fertilizer and viscosity in non-waxy varieties was not observed in most of the waxy varieties used in the present study, which suggests that the usual correlations between RVA and quality traits might not apply to waxy rice.

The difference in viscosity profiles between KNL grown in high and low N treatments is perhaps due to the significantly lower protein content of the grains from the two lower rates of N compared with the grains from the two higher rates (Table 3.3B). Proteins absorb a large amount of water as they swell during heating (Baxter et al., 2004; Bushuk, 1998), and the more protein in the grain, the more water required for cooking (Sun et al., 2008). When water content is not increased to account for protein, as is usually the case when using the RVA, higher levels of protein cause the paste to become thicker, leading to higher viscosity curves (Martin and Fitzgerald, 2002). The three other varieties did not show significant differences in protein content due to N rate, and perhaps consequently, did not show significant differences in the thickness of the paste formed during the RVA.

Small differences are found in some of the derived viscosity parameters between the varieties (Figure 3.3), in particular, breakdown, setback and retrogradation. The values for KNL are lower than for the other varieties, and perhaps this is because the whole viscosity curve is lower (Figure 3.3), suggesting a more dilute paste requiring less force to stir. The cooked rice grains of KNL are slightly

softer than those of the other varieties (Table 3.3C), but softness is more usually correlated with setback than with any other parameter of the curve (Bao and Xia, 1999; Bao et al., 2000; Champagne et al., 1999; Rani and Bhattacharya, 1989; Sandhya Rani and Bhattacharya, 1995; Varavinit et al., 2003). N rate did not significantly affect the derived parameters tested within a variety, suggesting that the lower curves of the low N KNL grains are actually the same shape as the curves of the higher N grains, but made from a weaker paste.

Figure 3.3C shows the viscosity traces of HNN and shows that the final viscosity rises from the trough significantly more than it does for the other varieties, leading to a higher final viscosity. This rise from the trough to the final is usually associated with firmness of the cooked rice (Champagne et al., 1999), which is usually associated with amylose content (Allahgholipour et al., 2006; Chen et al., 2003; Fitzgerald et al., 2003; Varavinit et al., 2003). Waxy rice does not contain amylose due to a mutation in the *Waxy* gene (Wanchana et al., 2003), but Figure 4 shows that there is an association between hardness of cooked grains and the rise from the trough to the final viscosity; this must be due to structures in the grain other than amylose. HNN grains have the lowest average protein than the other three varieties (Table 3.3B). Examination of the data reported by Martin and Fitzgerald (Martin and Fitzgerald, 2002) indicates that the lower the protein content of the grain, the higher is the difference between final and trough. However, removal of proteins from waxy varieties of rice can have very different effects, depending on variety (Fitzgerald et al., 2003; Xie et al., 2008).

By only using waxy varieties to construct Figure 4, interesting information about viscosity curves emerges. Strong correlations were found between all the direct parameters of peak, trough and final viscosities and the derived parameters of breakdown, retrogradation and setback (Figure 3.4). These correlations indicate that the initial swelling of starch granules to reach a balance between shear and swelling, the peak, determines the degree to which the molecules undergo shear-thinning to the trough, and then this determines how the molecules of protein and amylopectin interact to rise to the final viscosity. Therefore, using RVA curves to predict the quality of waxy rice requires an understanding of what, in a waxy matrix, causes the differences in the ability of the granules from different varieties to swell and to resist

shear. Hardness of the cooked grains correlates well with peak and trough viscosity and quite well with other parameters. Thus, understanding how the peak forms will then provide a screening tool to differentiate between the hardness of cooked waxy rice.

3.5 Conclusion

In the present study, it was demonstrated that N treatment affected the yield of the four varieties used, but an effect on quality was less apparent. The improved varieties used in the present paper, TDK 1 and TSN 1, were more responsive to N treatment than the traditional varieties HNN and KNL. The increase in yield among the varieties arose through different responses in yield components, such as grain weight and number of grains per panicle. TSN 1's yield increase in response to N treatment makes it a more viable candidate in improving grain quality in Lao PDR. Quality parameters measured in this study were mostly affected by varietal effects rather than by N treatment. Aroma, textural attributes (hardness and stickiness), gelatinisation temperature, and viscosity parameters were mostly non-responsive to N treatment. However, the viscosity response to N in KNL is attributed to changes in protein content associated with differences in N treatment. Taken together, the data indicate that further improvements to food export opportunities in Lao PDR could be achieved by combining the quality of KNL or HNN with the yield of TSN 1. Moreover, a breeding program attempting to capture the quality traits of a waxy variety into a high-yielding variety requires that tools to evaluate quality be developed and current tools customized, to screen for the desired quality traits in waxy rice, to enable breeders to use quality as a selection tool.