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### **Cultivar and farming practice affect yield and quality of Thai rice.**

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#### **Abstract**

The objective of this research was to investigate the effects of farming practice and cultivar on yield and quality of 'Khao Dawk Mali 105' rice and its mutant lines derived from fast neutron mutagenesis. It was shown using ISSR markers that the mutations did occur in these lines. The pre-organic farming system resulted in longer husked and brown rice as well as higher 100-grain weight and grain amylose content than the other farming practices. Whereas direct-seeded farming practice with chemical fertilizer produced the higher number of tillers per hill and seeds per panicle. However comparable yields were obtained with pre-organic farming. These studies showed that rice cultivars influenced both grain yield and quality. When compared with the grade standard for Thai jasmine rice, all rice cultivars with different farming practices produced husked rice which were classified as long grain Class 1. 'Khao Dawk Mali 105' was taller and produced higher number of tillers, seeds per panicle and yield than its mutant lines. The grain amylose content of all cultivars ranged between 16.0 and 18.5%.

**Keywords:** organic and conventional farming practice, Thai rice cultivar, yield, quality

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## Introduction

Rice (*Oryza sativa* L.) is an important and traditional cereal in many countries. The most important economic characteristics of agricultural grain crops are their yield, nutritional characteristics and culinary quality. Yield and nutritional value are mostly determined by the synthesis and storage of carbohydrates, proteins and minerals during grain filling, and culinary quality is affected by the interaction of various enzymes to produce the final structure of the starch at the molecular and granule levels (Mazur *et al.*, 1999). 'Khao Dawk Mali 105' (KDML 105) is an important and popular rice cultivar around the world. KDML 105 is widely cultivated in Thailand, but the cultivation in the north-eastern region is claimed to be best. Agricultural practice for rice farming currently performed by conventional farming currently depends on chemical fertilizers and pesticides. The consequence of over using such chemicals has resulted in environmental pollution, lowering of soil organic matter and damage soil physical properties. The Office of Agricultural Economics reported that the farmers in Northeastern of Thailand in Tungkularonghai region are highly reliant on chemical fertilizer and pesticides. This also directly affects farmers' health and indirectly to rice consumers in the food chain. As a result, the farmers indeed require better agricultural practices where organic farming is one such alternative. Organic farming lessens chemical utilization by using organic fertilizers and pathogenic biocontrol. However, organic farming takes more time for soil replenishment and recovering ecological balance. Therefore rice farmers are uncertain to change their agricultural practice. Moreover they are also uncertain to rice grain quality whether it is similar to that from the conventional farming one.

Unlike most other cereals, rice is consumed as a whole grain. Therefore physical properties such as size, shape, uniformity, and general appearance are of utmost importance. In this paper we compared rice grain of KDML 105 and its mutant lines collected from conventional and pre-organic farming, in terms of yield and grain quality such as size, amylose content, aroma, protein.

## Materials and Methods

### Rice sample and preparation

Khao Dawk Mali 105 rice (*Oryza sativa* L.) and its' five mutant lines with different sensory-tested aroma level (Patwibool *et al.*, 2001) were grown in a lowland loamy sand in the rainfed area in Banfang District, Khon Kean Province, located in the Northeastern Thailand, during July to November 2005 and 2006. General chemical soil property was analysed (Table 1).

Conventional farming (transplanting and direct-seeded using the dibbling method) or pre-organic farming (Table 1) were performed in area of 1 x 1.5 m in three replicates for each crop. Chemical (16-16-8) or organic (cow dung) fertilizers were applied at 15, 30 and 60 days.

### Analysis of grain yield component and grain quality

On maturity, plants were harvested and number of tillers per hill, plant height, number of grains per panicle, one hundred grain weight, grain size, amylose content and 2-aceyl-1-pyrroline (2AP) were recorded. Ten samples were collected from each treatment and each sample was treated as a replication. Collected data was analyzed using MSTATC (Michigan State University).

### 2AP analysis

Seed 2AP quantification method was modified from Wongporchai *et al.* (2004) using 10 ppm 2,4,6-trimethylpyridine (TMP) (99% purity; Fluka Chemika) as a standard (Sirisoontaraluk

and Noomhorm, 2006), and comparing the GC-MS data and GC retention times of the rice samples with 2AP from pandan leaves (Yoshihashi, 2002).

### **2D-PAGE analysis**

Rice grains were ground on ice and extracted protein using extraction buffer (20 mM Tris-HCl, [pH 8.0], 4% (w/v) CHAPS, 5 mM EDTA and 2 mM PMSF). The homogenate was vortexed and incubated at -20 °C for 40 minutes, centrifuged at 12,000 x g for 1 hr. Supernatants were collected. Protein was quantified by Bradford assay. After protein extraction, the supernatant was removed the contaminating substance by 2D-Clean up kit protocol (Amersham Biosciences, Sweden). Equivalent samples were loaded into 13 cm isoelectric focusing (IEF) tube gel (pH 3-10) for the first dimension electrophoresis step followed by separation of proteins in the second dimension on 12% polyacrylamide gels. Separated proteins were detected by staining with silver nitrate. Digitized gel images were analyzed using the ImageMaster 6.0 2D Platinum software (Amersham Biosciences AB, Uppsala, Sweden). A minimum of three independent experiments were used.

### **Results and Discussion**

Since rice is produced and marketed according to grain size and shape, the physical dimensions, weight, and uniformity are of prime importance. It was proved by ISSR markers that the mutations occurred on these lines (data not shown). Our result showed that KDML 105, which matured in 124 and 104 days, had 30- and 38-day ripening phase in 2005 and 2006 respectively, whereas mutant lines which matured earlier and had a shorter vegetative phase (Table 2). On average, yields in the pre-organic farming were comparable to that of conventional practices.

The pre-organic organic farming treatment resulted in longer husked and brown rice with grain amylose content than the other farming practices. When compared with the grade standard for Thai jasmine rice, all rice cultivars with different farming practices produced husked rice which was classified as long grain Class 1 (Figure 1A and 1B). The grain amylose content of all cultivars ranged between 16.0 and 18.5%.

The number of grains per panicle, however, can vary genetically from 20-200-4 and 20-200-8 with 144 grains per panicle to KDML 105 with panicles of over 240 grains or more (Figure 1C). Direct-seeded with chemical fertilizer seemed to produce more grains per panicle than the other farming practice when using the same spacing (20 x 20 cm). Management practices can influence the number of grain per panicle. Most commonly, plant density has the greatest influence on panicle size. Rice plants will compensate for low stand densities by producing more grains per panicle as well as by producing more tillers per plant (Hill, 2004).

Pre-organic farming resulted in higher 100-grain weight in most of the mutant lines except No.16. While the cultural practice had no effect on KDML105 100-grain weight which gave the least 100-grain weight (Figure 1D). However this cultivar produced the highest number of grain per panicle and also the number of tillers per hill in all treatments (Figure 1C). In overall, KDML still gave the highest yield. In the field, grain weights are the least variable yield component. They generally cannot be increased by good management practices to compensate for poor tillering or smaller panicles (Hill, 2004).

Our selected area contained coarse-loamy, mixed, subactive, isohyperthermic soil with poor chemical properties was therefore infertile. Our data revealed that different sensory-tested aroma level of some genotypes was not in agreement with Patwibool et al. (2001) for either

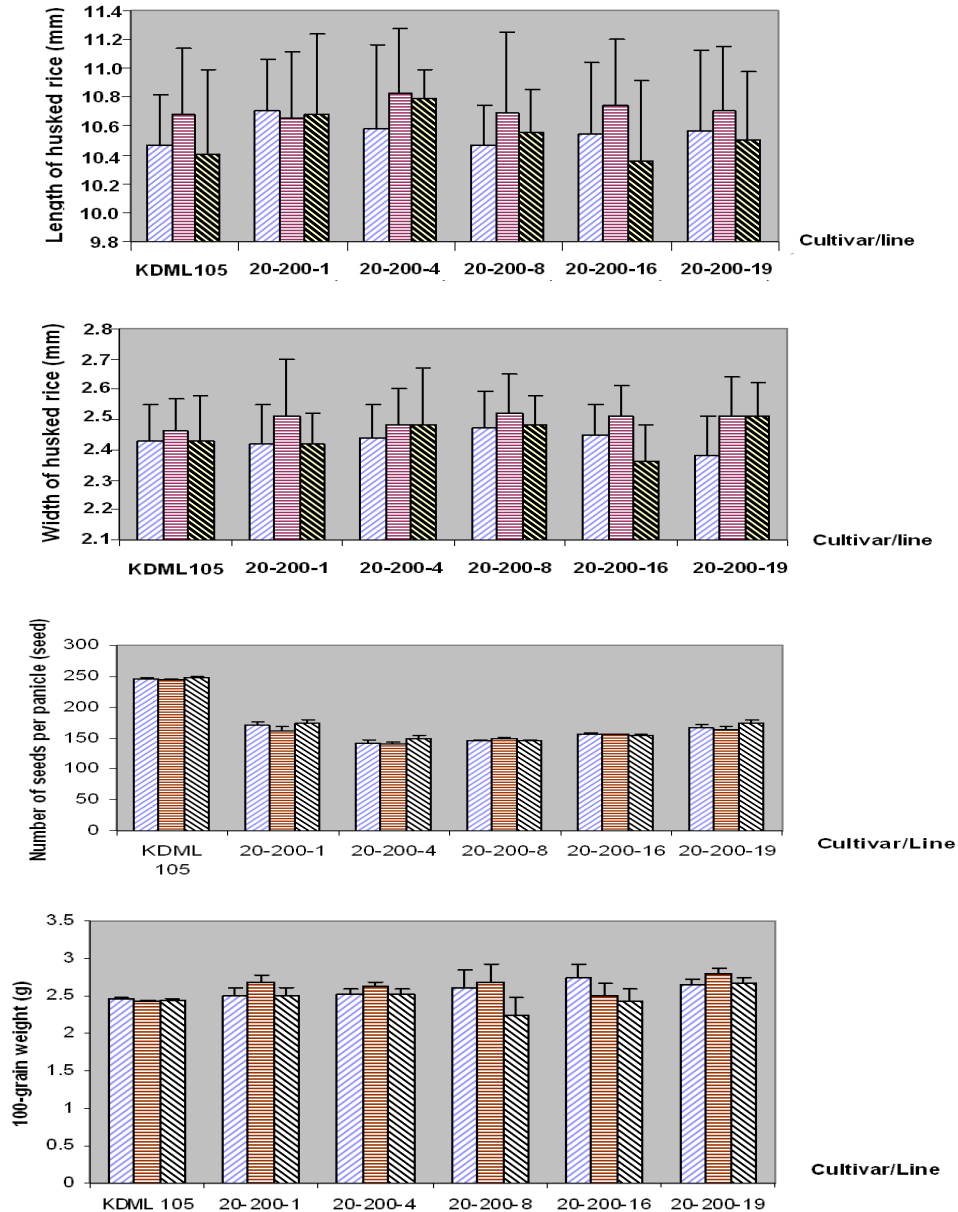
KDML 105 or its mutant lines. Yoshihashi et al. (2004) has reported that 2AP content of KDML105 depends on area of cultivation. Higher 2AP content is detected in KDML105 rice grains produced in rain-fed paddy fields in Tungkula ronghai region, drought and salinity area, more than that of other regions in the Northeastern Thailand.

**Table 1** Chemical properties of the surface soil before sowing.

Farming practice	OM (%)		pH (1:1)		EC (mS cm <sup>-1</sup> )		Total N (%)	
	2005	2006	2005	2006	2005	2006	2005	2006
Transplanting with chemical fertilizer (16-16-8)	0.6483	0.8958	6.4	6.5	0.046	0.055	0.0324	0.0448
Transplanting with cow dung	0.6327	0.7021	6.9	5.9	0.017	0.66	0.0316	0.0351
Direct-seeded with chemical fertilizer (16-16-8)	1.3433	1.345	5.9	5.9	0.012	0.016	0.0627	0.0741

**Table 2** The duration of ripening phase and days to maturity of six different rice genotypes grown in 2005 and 2006.

Cultivar/line	Ripening phase (day)		Day to maturity (day)	
	2005	2006	2005	2006
KDML 105	30	38	124	104
20-200-1	41	42	105	85
20-200-4	41	44	105	85
20-200-8	41	44	105	85
20-200-16	47	54	105	85
20-200-19	47	58	105	85



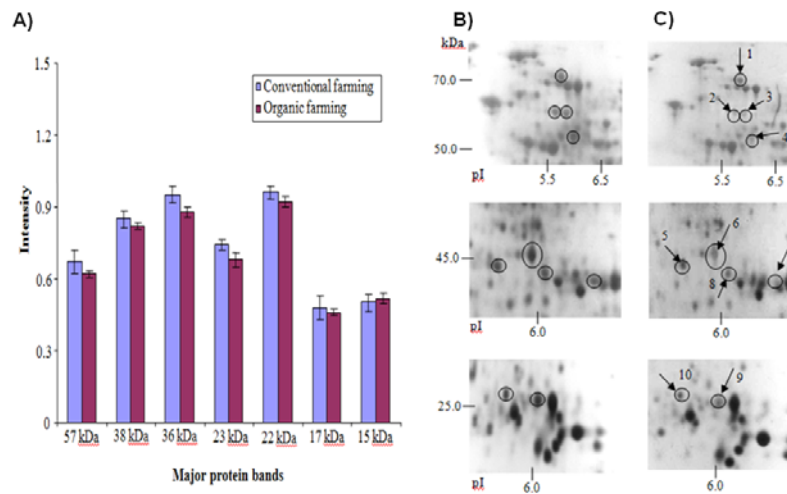
**Figure. 1** The interaction between three different farming practices and genotypes of Thai rice in 2006. ▨ Transplanting with chemical fertilizer

■ Transplanting with cow dung

▨ Direct-seeded with chemical fertilizer

Several proteomic-based studies are available for *Arabidopsis*, tomato, and barley seeds. It was previously shown in barley that not only cultivar differences were observed in seed 2D-gel patterns but also their relation to grain quality (Finnie and Svensson, 2009). We report, for the first time, proteomic analyses of rice (subspecies *indica*) seed proteins harvested from different cultivation practices which is necessary to unravel the precise role of proteins expressed in rice

seed. Approximately, 10 changed (downregulated) proteins were observed (Figure 2B and 2C). These will be selected for further detailed proteomic characterization.



**Figure 2.** Major protein bands (A) and intensity comparison of different protein spots of mature rice seed from (B) conventional farming and (C) pre-organic farming.

## Conclusion

The results presented here from rice grown in Northeast Thailand indicate that under good management practices there is very little difference in yield between the three establishment methods for KDML 105. However cultural practices during growth marked differences in certain grain quality and yield component. However some of these characteristics also depend on genotypes.

The organic transplanted rice provides farmers with the same quality of rice grain (as compared to the normal farmer practice) with better food and is more environmentally friendly. But as this is a pre-organic type of experiment, no conclusion can be made from only two years data. Hence, few more years' research in a large on-farm experiment are needed to see the effects of organic practice on rice grain yield and quality.

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