Diversity of Grains of Rice Landraces of Northeastern Thailand

Praweena Maneerattanarungroj, Panadda Jermklang, Boonsong Kongsook, and Pitakpong Maneerattanarungroj

Abstract**—**Diversity of grains of 50 rice landraces of Northeastern Thailand was examined using a model Leo 1450vp scanning electron microscope (SEM). The studied rice varieties were classified into 5 groups including Sticky rice or Khao Neaw group, Khao Chow group, Yellow rice or Khao Luang group, White rice or Khao Khaw group and Khao Dore group. Two commercial varieties of Thailand (KDML 105 and RD 6) were used as standard ones. The data obtained were clustered based on dendrogram analysis (UPGMA) using NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) version 2.10p. Genetic similarity coefficients were confirmed with trichome density and papillae character. The results showed three closed genetic groups of grains of rice landraces with the relatively high genetic similarity index (GSI) of 0.90-1.00 observed for all groups. Cophenetic correlation of cluster was 0.91, indicating good grouping.

Keywords**—**dendrogram, diversity, grains, rice landraces

I. Introduction

RICE is the staple food for nearly half of the world's population. It is the world's third most important food crop (after wheat and maize). Over 90% of rice is produced and consumed in Asia. *Indica* type rice feeds more than two billion people, predominantly in developing countries. The demand for rice has instantly increased due to a sharp increase in the world's population, which is expected to reach nearly 10 billion by 2050 [1], and therefore, producing rice to meet the global demand is of importance.

Rice genetic resources are a key component to breeding programs and farmers have long played an important role, contributing to rice diversity by developing and nurturing thousands of rice varieties [2]. This vast wealth of rice germplasm including landraces and traditional varieties is a good source of important alleles to develop new rice varieties [3].

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Thailand is a major producer of many rice varieties, especially aromatic varieties and some landraces. More than 10,000 rice landraces have been explored in Thailand, which is an important genetic resource for rice improvement in the future. In general, examining genetic diversity and relationship among cultivars is very practical for facilitating resourceful germplasm collection and management [4]. Rice landraces possess several dominant features that allow them to thrive on various natural habitats like tolerance to drought and salinity. In addition, some landraces have some outstanding characters such as disease resistance or insect resistance. Moreover, some landraces have higher level of nutritional or medicinal values than commercial varieties. Inevitably, many rice landraces in Thailand are at risk of extinction and therefore, germplasm collection and management and examination of genetic diversity and relationship are urgently needed. Nowadays several technologies have been developed for rice breeding and improvement such as genetic engineering, cloning and mutations. Unfortunately, some rice varieties occur via natural selection without grain morphological differences easily identified between closed varieties and it is therefore difficult for farmers to identify such rice grains. Thus, examining diversity of grains of rice landraces based on their morphological features is undoubtedly important. [5,6]. This study is the first report on diversity of grains of rice landraces native to Northeastern Thailand using trichome density and papillae height for cluster analysis.

II. Materials and Methods

A. Plant materials

Grains of 50 rice varieties were provided by the Surin Rice Research Center, Thailand. The studied rice varieties were classified into 5 groups based on their local names and characters (Table 1) including Sticky rice or Khao Neaw group (8 varieties), Khao Chow group (9 varieties), Yellow rice or Khao Luang group (10 varieties), White rice or Khao Khaw group (9 varieties) and Khao Dore group (14 varieties). Two commercial varieties of Thailand (KDML 105 and RD 6) were used as standard varieties in this study.

TABLE I
LOCAL NAME AND GENETIC STOCK (G.S.) NUMBER OF ALL RICE VARIETIES

Number	Local name	G.S. number
sticky rice or KhaoNeaw group (8 varieties; KN)		
1	Neaw-Khaw	607
2	Pha-Neaw	611
3	Neaw-Dum	621
4	Neaw-Kheaw-Ngu	5605
5	Neaw-Dum 1	NRM 09001
6	Neaw-Dum 2	NRM 09002
7	Neaw-Dok-Chan	NRM 09003
8	Khao-Neaw-Whan-Yen	none
Khao Chow group (9 varieties; KC)		
9	Khao-Chow- Hangmu-Yai32-27	554
10	Khao-Chow- Hangmu-Yai32-27	555
11	Chow-Luang 27-3-91	564
12	Chow-Phae 31-28-1	566
13	Chow-Dore	7776
14	Chow-Dum	NRM 09007
15	Chow-Dum	NRM 09008
16	Chow-Luang	10166
17	Chow-Khaw	13209
yellow rice or KhaoLuang group (10 varieties; KL)		
18	Luang-Yai	584
19	Luang-Lek30-1-57	586
20	Luang-Noi30-1-39	588
21	Luang-Non6-4-102	589
22	Luang-Tong77-12-82	623
23	E-Luang	3299
24	Luang-Lao	21737
25	Luang11	21738
26	Luang-Nangma	none
27	Luang-Boonma	none
white rice or KhaoKhaw group (9 varieties; KK)		
28	Khaw-Dok-Mali4-1-137	542
29	Khaw-Ta-Hang55-5-55	546
30	Khaw-Yai33-22-61	547
31	Khaw-Pak-More74	548
32	Khaw-Lood-Nhe75-3-2	549
33	Khaw-Med-Yai94-4-87	550
34	Khae-Pung74-3-38	551
35	Khaw-Kod31-24-21	553
36	Khaw-Dok-Mali	2750
Khao Dore group (14 varieties; KD)		
37	Dore-Dok-Mapraw 48-3-123	601
38	Dore-Nang-Nual	3345
39	Dore-Khaw	5641
40	Dore-Pon	5644
41	Dore-Dum	5647
42	Dore-Plasil	5736
43	Dore-Luang	7689
44	Dore-Hua-Chang	7772
45	Dore-Khaw	7780
46	Dore-Non	7783
47	Dore-Dang1 (long seed)	7785
48	Dore-Dang2 (short seed)	7785
49	Dore-Khaw	13236
50	Dore	13309
Standard varieties		
1	KDML 105	none
2	RD 6	none

B. Scanning electron microscope (SEM) and cluster analysis

At least 5 typical and intact grains of each variety were coated with gold-palladium alloy and 4 major parts of rice grains were examined under SEM (Leo 1450vp) including head part, tail part, middle part and papillae character. Trichome density and papillae character were measured in the middle part of grains. The obtained data were clustered based on dendrogram (UPGMA) analysis using NTSYSpc (Numerical Taxonomy and Multivariate Analysis System) version 2.10p. Genetic similarity coefficients were confirmed with trichome density and papillae character.

III. RESULT AND DISCUSSION

A. Sticky rice or Khao Neaw group

SEM analysis showed that the average trichome density observed for Khao Neaw group ranged between 13-22 trichome/mm^2. The highest density of trichomes was seen with Neaw-Dum 2 G.S. number NRM09002 (22±0.77) while the lowest density was observed for Neaw-Dum G.S. number 621 (13±0.67). The average papillae height of this group ranged between 0.018-0.029 mm with a maximum papillae height observed for Neaw-Dum 1 G.S. number 09001 (0.028±0.009 mm) and a minimum papillae height in Khao-Neaw-Whan-Yen (0.029±0.004 mm) (Table 2).

B. Khao Chow group

According to SEM analysis, the average trichome density ranged between 12-19 trichome/mm^2. The greatest density of trichomes was found in Chow-Dum G.S. number NRM09007 (19±1.42) while Chow-Luang 27-3-91 G.S. number 564 and Chow-Khaw G.S. number 13209 showed the lowest trichome density of 12±3.19 and 12±3.13, respectively. The average papillae height of this group ranged between 0.026-0.040 mm with a maximum papillae height observed for Chao-Phae 31-28-1 G.S. number 566 (0.040±0.003 mm) and a minimum papillae height seen with Chow-Dum G.S. number NRM09008 (0.026±0.003 mm) (Table 2).

C. Khao Luang group

All the varieties of Khao Luang group presented the average trichome density of 11-19 trichome/mm^2 (Table 2). Luang-Yai G.S. number 584 showed the lowest trichome density (11±0.72) whereas Luang-Noi 30-1-39 G.S. number 588 showed the highest trichome density (19±0.68). The average papillae height of Khao Luang group was between 0.019-0.032 mm with Luang-Lao G.S. number 21737 exhibiting a minimum papillae height of 0.019±0.001 mm and E-Luang G.S. number 3299 revealing a maximum papillae height of 0.032±0.000mm.

TABLE II
AVERAGE TRICHOME AND PAPILLAE HEIGHT OF ALL RICE VARIETY

varieties	G.S. number	Average trichome density (mm^2) mean ± SD	Average papillae height (mm) mean ± SD
sticky rice or KhaoNeaw group (8 varieties)			
Neaw-Khaw	607	20±0.52	0.027±0.031
Pha-Neaw	611	18±1.55	0.025±0.005
Neaw-Dum	621	13±0.67	0.026±0.002
Neaw-Kheaw-Ngu	5605	17±1.29	0.023±0.002
Neaw-Dum 1	NRM 09001	18±1.50	0.028±0.009
Neaw-Dum 2	NRM 09002	22±0.77	0.024±0.004
Neaw-Dok-Chan	NRM 09003	16±2.92	0.018±0.002
Khao-Neaw-Whan-Yen	none	15±0.45	0.029±0.004
Khao Chow group (9 varieties)			
Khao-Chow- Hangmu-Yai32-27	554	17±1.23	0.032±0.002
Khao-Chow- Hangmu-Yai32-27	555	16±0.76	0.029±0.002
Chow-Luang 27-3-91	564	12±3.19	0.031±0.002
Chow-Phae 31-28-1	566	17±1.10	0.040±0.003
Chow-Dore	7776	15±2.03	0.035±0.005
Chow-Dum	NRM 09007	19±1.42	0.029±0.002
Chow-Dum	NRM 09008	17±3.59	0.026±0.003
Chow-Luang	10166	16±2.39	0.039±0.008
Chow-Khaw	13209	12±3.13	0.029±0.002
KhaoLuang group (10 varieties)			
Luang-Yai	584	11±0.72	0.025±0.002
Luang-Lek30-1-57	586	14±2.13	0.031±0.004
Luang-Noi30-1-39	588	19±0.68	0.022±0.002
Luang-Non6-4-102	589	18±5.66	0.029±0.005
Luang-Tong77-12-82	623	18±2.41	0.029±0.004
E-Luang	3299	18±1.96	0.032±0.000
Luang-Lao	21737	15±2.64	0.019±0.001
Luang11	21738	16±1.23	0.022±0.001
Luang-Nangma	none	15±1.66	0.031±0.001
Luang-Boonma	none	16±3.44	0.026±0.002
white rice or KhaoKhaw group (9 varieties)			
Khaw-Dok-Mali4-1-137	542	16±2.02	0.076±0.084
Khaw-Tahang55-5-55	546	10±0.68	0.028±0.002
Khaw-Yai33-22-61	547	10±2.83	0.023±0.000
Khaw-Pak-More74	548	10±2.97	0.019±0.004
Khaw-Lood-Nhe75-3-2	549	6±2.54	0.022±0.002
Khaw-Med-Yai94-4-87	550	14±4.29	0.027±0.002
Khae-Pung74-3-38	551	13±2.35	0.021±0.002
Khaw-Kod31-24-21	553	8±2.79	0.020±0.005
Khaw-Dok-Mali	2750	12±1.49	0.032±0.005
Khao Dore group (14 varieties)			
Dore-Dok-Mapraw 48-3-123	601	12±0.52	0.030±0.004
Dore-Nang-Nual	3345	17±2.25	0.035±0.002
Dore-Khaw	5641	21±2.73	0.027±0.002
Dore-Pon	5644	22±0.89	0.021±0.004
Dore-Dum	5647	14±2.25	0.024±0.003
Dore-Plasil	5736	16±2.88	0.025±0.003
Dore-Luang	7689	16±1.55	0.021±0.002
Dore-Hua-Chang	7772	18±3.72	0.024±0.002
Dore-Khaw	7780	20±3.22	0.027±0.003
Dore-Non	7783	16±3.58	0.024±0.003
Dore-Dang1 (long seed)	7785	16±1.03	0.023±0.003
Dore-Dang2 (short seed)	7785	16±2.73	0.027±0.002
Dore-Khaw	13236	16±3.61	0.026±0.003
Dore	13309	6±1.55	0.021±0.004
Standard varieties			
KDML105	none	13±2.25	0.038±0.004
RD6	none	14±2.37	0.030±0.004

D. Khao Khaw group

The average trichome density of 9 varieties of Khao Khaw group was between 6-16 trichome/mm^2. The highest density of trichomes was seen with Khaw-Dok-Mali 4-1-137 G.S. number 542 (16±2.02) while Khaw-Lood-Nhe75-3-2 G.S. number 549 showed the lowest trichome density (6±2.54). The average papillae height of this group was between 0.019-0.076 mm. A maximum papillae height of 0.076±0.084 m was observed for Khaw-Dok-Mali 4-1-137 G.S. number 542 whereas a minimum papillae height of 0.019±0.004 mm was seen with Khaw-Pak-More74 G.S. number 548 (Table 2).

E. Khao Dore group

Fourteen varieties of Khao Dore group revealed the average trichome density of 6-22 trichome/mm^2. The lowest trichome density was observed for Dore G.S. number 13309 (6±1.5) whereas Dore-Pon G.S. number 5644 showed the highest trichome density of 22±0.89 trichome/mm^2. Table 2 shows that the average papillae height of this group was between 0.021-0.035 mm. A maximum papillae height of 0.035±0.002 mm was observed for Dore-Nang-Nual G.S. number 3345 while minimum papillae height was seen with three varieties (Dore-Pon G.S. number 5644 (0.021±0.004 mm), Dore-Luang G.S. number 7689 (0.021±0.002 mm) and Dore G.S. number 13309 (0.021±0.004mm).

In this study, trichome density (Fig. 1) on seed coat of rice grains was divided into 3 groups, i.e., 1-10, 11-20 and 21-30 trichome/mm^2, which are designated as number 1, 2 and 3 for UPGMA analysis, respectively. As depicted in Fig. 1, papillae height observed for all varieties was divided into 3 groups, i.e., 0.020-0.010, 0.030-0.021and 0.031 mm, which are designated as number 1, 2 and 3, respectively. Following cluster analysis using trichome density and papillae height, genetic similarity coefficients were confirmed and three clusters were classified; First group contained a majority of rice varieties (41 varieties) including RD 6 and DM 105 that were used as standard varieties (Table 3, Fig.2), Second group contained 6 varieties (Dore G.S. number 13309, Khaw-Ta-Hang 55-5-55 G.S. number 546, Khaw-Lood-Nhe 75-3-2 G.S. number 549, Khaw-Yai 33-22-61 G.S. number 547, Dore-Khaw G.S. number 5641 and Dore-Pon G.S. number 5644), Third group contained 4 varieties (Neaw-Dok-Chan G.S. number NRM 09003, Luang-Lao G.S. number 21737, Khaw-Pak-More 74 G.S. number 548 and Khaw-Kod 31-24-21 G.S. number 553). Genetic similarity index (GSI) of all groups was relatively high (0.90-1.00). Cophenetic correlation of cluster was 0.91, indicating good grouping [7]. The findings in our study were similar to an earlier study [8], which reported variations of trichomes and papillae height in different rice varieties. However, more characters should be done for dendrogram analysis. To our knowledge, this is the first report on trichome density and papillae height of rice landraces native to Northeastern Thailand, which provides a foundation for further morphological characterization of grains of rice landraces in other regions of the country. Our further studies will focus on more comprehensive analyses of morphological characters of rice grains such as seed coat color, shape and size, weight and width of seed coat and endosperm.

TABLE III
LOCAL NAME AND GENETIC STOCK NUMBER OF ALL RICE VARIETYIN DIFFERENT CLUSTERS.

Cluster	Number	Local name	G.S. number
I	1	Neaw-Khaw	607
	2	Pha-Neaw	611
	3	Neaw-Dum	621
	4	Neaw-Kheaw-Ngu	5605
	5	Neaw-Dum 1	NRM 09001
	6	Neaw-Dum 2	NRM 09002
	8	Khao-Neaw-Whan-Yen	none
	9	Khao-Chow- Hangmu-Yai32-27	554
	10	Khao-Chow- Hangmu-Yai32-27	555
	11	Chow-Luang 27-3-91	564
	12	Chow-Phae 31-28-1	566
	13	Chow-Dore	7776
	14	Chow-Dum	NRM 09007
	15	Chow-Dum	NRM 09008
	16	Chow-Luang	10166
	17	Chow-Khaw	13209
	18	Luang-Yai	584
	19	Luang-Lek30-1-57	586
	20	Luang-Noi30-1-39	588
	21	Luang-Non6-4-102	589
	22	Luang-Tong77-12-82	623
	23	E-Luang	3299
	25	Luang11	21738
	26	Luang-Nangma	none
	27	Luang-Boonma	none
	28	Khaw-Dok-Mali4-1-137	542
	31	Khaw-Pak-More74	548
	33	Khaw-Med-Yai94-4-87	550
	34	Khae-Pung74-3-38	551
	36	Khaw-Dok-Mali	2750
	37	Dore-Dok-Mapraw 48-3-123	601
	38	Dore-Nang-Nual	3345
	40	Dore-Pon	5644
	41	Dore-Dum	5647
	42	Dore-Plasil	5736
	43	Dore-Luang	7689
	44	Dore-Hua-Chang	7772
	45	Dore-Khaw	7780
	46	Dore-Non	7783
	47	Dore-Dang1 (long seed)	7785
	48	Dore-Dang2 (short seed)	7785
	49	Dore-Khaw	13236
	-	KDML 105	none
	-	RD 6	none
II	29	Khaw-Ta-Hang55-5-55	546
	30	Khaw-Yai33-22-61	547
	32	Khaw-Lood-Nhe75-3-2	549
	39	Dore-Khaw	5641
	40	Dore-Pon	5644
	50	Dore	13309
III	7	Neaw-Dok-Chan	NRM 09003
	24	Luang-Lao	21737
	31	Khaw-Pak-More74	548
	35	Khaw-Kod31-24-21	553

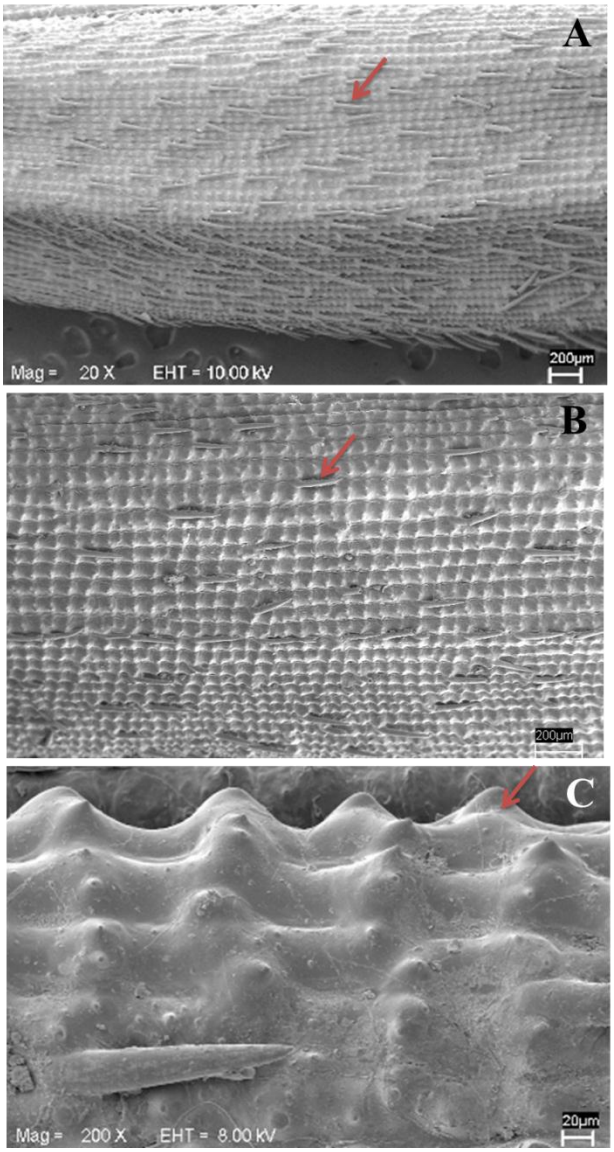

Fig. 1 Trichome and papillae on husk of rice grain;
A-B: areas for observe trichome density (red arrow),
C: papillae characters (red arrow).

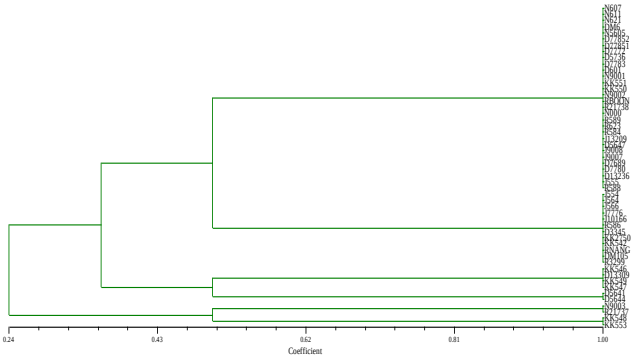

Fig. 2 Dendrogram generated by cluster analysis of morphological characters using Unweighted Pair Group Method with Arithmetic Mean (UPGMA).

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