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# Analysis of Plastid Subtype ID Sequences in Traditional Upland and Lowland Rice Cultivars from Thailand

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**Abstract:** Geographically, different rice ecosystems are found throughout the various regions of Thailand and since ancient times, traditional upland and lowland rice cultivars from these regions have played a key role in both production as a staple food and rice germplasm for variety improvement. In this study, a polymorphism of chloroplast DNA and the physiological marker of 74 upland and lowland rice accessions were collected and examined from all regions of the country. Based on the results of the phenol reaction, the hulls of all upland rice cultivars from the northern region of Thailand, but not those from the northeastern region, showed unchanged color of the hulls when soaked in a 1.5% phenol solution and were scored as positive ($Ph^-$). Lowland rice cultivars from other regions in Thailand turned to black of hull color ($Ph^+$). Upland rice from the Northern and northeastern regions carried Non-Deletion (ND) type ORF100, but only a few lowland rice samples from the central region carried the ND type. Most of the lowland rice cultivars showed deletion type (D type) of the region. Six plastid subtype ID sequences (6C7A, 7C7A, 7C6A, 8C8A, 9C7A and 9C8A) were found in the collected samples. Upland rice from both the northern and northeastern regions carried three (6C7A, 7C7A and 7C6A) PS-ID sequences. The subtype 8C8A was not found in the northern region samples, but predominated in the northeastern samples. The PS-ID sequences of upland (6C7A and 7C6A) and lowland (8C8A) black rice cultivars were different, suggesting that this molecular marker is useful in terms of DNA marker-assisted selection for black rice variety improvement.

**Key words:** Upland rice, lowland rice, chloroplast DNA, *Oryza sativa* L.

## INTRODUCTION

Thailand is situated at the center of one of the world's most diverse and productive rice growing regions. Because of its strategic location, it is widely recognized as the originator and primary distributor of Asian rice (*Oryza sativa* L.). Throughout recorded history, this region, known as Suwannabhum or the golden peninsula of Southeast Asia, has cultivated rice. The region produced a multitude of different rice varieties during the eras of Dhavaravadi, Srivichai, Lopburi, Chiangsaen, Sukothai and Ayudhaya and to this day, the production that began in the Ratanakosin era continues (Chitrakon and Somrith, 2003). There are a large number of rice varieties in Thailand. The Rice Research Institute in Bangkok alone has collected more than 19,000 samples of Thai cultivated rice and wild rice. Among these samples are approximately 3,500 individual names, each with their own different characteristics. These characteristics are in part attributable to the dissimilar ecological environments and/or agroecological conditions found in the growing regions. Each region is typically cultivated by a number of local tribes. In the country's northern region for example, there are a number of minority tribes living mainly in mountainous areas such as *Khamu*, *Mien*, *Lisu*, *Hmong* and *Karen*. These groups have traditionally cultivated local upland rice cultivars. Their rice fields have been maintained by the slash and burn farming system, in which forests are first slashed and then burned to create the upland fields (Ishikawa *et al*., 2006). A previous report revealed that these minority peoples grew both glutinous and non-glutinous rice (Prathepha and Baimai, 2004). According to local farmers, the minority people ate the non-glutinous rice while the glutinous rice was used mainly in festivals. As opposed to the upland cultivars that are grown in the mountainous north, rain fed and irrigated-lowland cultivars are grown in the lowlands and irrigated lands of the many other regions in the country. Observations on rice consumption habits show that in contrast to the minority groups of the mountainous region, the local people of the Northern and Northeastern regions consume glutinous rice as a staple food, while the Thai people living in the central and southern regions eat non-glutinous rice in a variety of forms. People living in the southern region prefer rice cultivars with intermediate (20-25%) and high (>25%) amylose content, while Thai

people in the central region favor non-glutinous rice with low (<20%) amylose content. These regional preferences coupled with cultural differences in rice consumption vary considerably among Thai people. As a result, numerous rice cultivars have been created by local farmers through a process of primitive selection and domestication. The fluctuating economy and changes in the agroecological environment, i.e., pest control, have also prompted the development of modern rice varieties with high yields and resistance to pests. The new varieties, particularly cv. Pathum Thani 1 (PTT1) and Chai-nat 1, have proven to be resistant to the brown plant hopper, a menacing pest found in all regions, but particularly troublesome in the county's central region. This pest has caused a reduction in the number of local rice varieties, particularly in the northeastern region of the country. To counter this reduction, the extension of an improved glutinous variety RD 6 and non-glutinous KDML 105, has been used in recent years to replace the old local glutinous rice varieties.

Since basic information about the genetics of Thai rice cultivars is very limited, especially information about the chloroplast subtype, which is of interest in the study of the origin and phylogeny of rice cultivars (Nakamura *et al*., 1998) and as a DNA marker to determine the plastid origin of rice cultivars (Ishikawa *et al*., 2006), as well as providing genetic evidence from DNA analysis data, it is a good candidate to reflect the diversity of Thai rice germplasm. As such, the present study attempts to investigate the diversity of chloroplast DNA contained in the Thai rice germplasm of both traditional rice cultivars and modern varieties. This information is valuable for rice genetic breeders of the country because these makers can be used as the DNA markers in breeding programs and also for shedding light on questions of rice diversity in Thailand.

## MATERIALS AND METHODS

**Plant materials:** The seeds of both traditional and modern rice cultivars were gathered during 2004-2005. The collection included 74 accessions from the hilly areas of the northern region and upland and lowland cultivars from the Northeastern, Central and Southern regions of the country. Names, localities and endosperm types of these cultivars are shown in Table 1. All accessions were examined for the phenol reaction following the procedure of Ishikawa *et al.* (2005). Hulls of each accession were soaked in 1.5% phenol solution for 3 h and then dried and observed. The hulls of the samples that turned black were scored as positive ($Ph^+$), while the hulls that were unchanged in color were scored as negative ($Ph^-$) for the phenol reaction. This characteristic is regulated by the Ph gene on chromosome 4 of the rice genome. The seeds of each accession were placed on one sheet of Whatman no. 3 filter paper and moistened with 10 mL distilled water for 7 days. The seedlings were then transplanted to plastic pots for fresh young leaf production.

**DNA extraction, DNA markers analysis:** Total genomic DNA was extracted from the fresh young leaves of each rice accession based on the CTAB method (Doyle and Doyle, 1987). To detect the ORF100 region of the rice chloroplast DNA, a new primer set was designed to amplify this region in the rice plasmid DNA: ORF100-f (5'-atgaaattgtataagtgg-3')) and ORF100-r (5'-cagccgaggtcgtggtaaatcc-3')). The INDEL marker can discriminate *Indica-japonica* differentiation (Ishikawa *et al*., 2006). The PCR was carried out using 0.5 units of *Taq* DNA polymerase, 150 ng of template DNA, 10 pmol of each primer, 1.5 mM $MgCl_2$, 0.1 mM dNTPs in a final volume of 20 μL The PCR reactions were performed using the following profile: 94°C, 1 min; 50°C, 1 min and 72°C, 1.5 min for 35 cycles and a final extension of 5 min at 72°C. After PCR, the amplified products were electrophoresed for 45 min at 75 V and stained with ethidium bromide. The plasmid marker was classified into Deletion (D) and Non-Deletion (ND) types. The rice strain with D type showed a 69 bp deletion in the ORF100, while the rice with ND type possessed this 69 bp site (Kanno *et al*., 1993). To detect the polymorphism of the linking sequences between the two rice plastid genes, PCR amplification of the plastid DNA fragment containing rpl16 and rpl14 was performed using a primer pair (forward primer: 5'-aaagatctagatttcgtaaacaacatagaggaagaa-3'; reverse primer: 5'-atctgcagcatttaaaagggttctgaggttgaatcat-3') (Nakamura *et al*., 1998). The reaction mixture contained 300 ng of template DNA, 10 pm of each primer, 0.2 mM of each dNTPs, 2 mM $MgCl_2$, 0.5 units of *Taq* polymerase in a final volume of 20 μL. The amplification conditions were 45 cycles of 98°C for 10 sec, 55°C for 30 sec and 72°C for 1 min. A final extension step at 72°C for 5 min was performed after the 45 cycles. PCR products were resolved using 2.0% agarose gel electrophoresis. The amplified DNA fragments corresponding to the expected size (ca. 450 bp) were cut from the gel and purified with a purification kit (Pharmacia) according to the manufacturer's instructions. The purified PCR chloroplast products *rpl16* and *rpl14* were sequenced directly on an ABI PRISM® 310 Genetic Analyzer (Applied Biosystems, Foster City, CA).

## RESULTS

**Variation in the physiological character in Thai rice germplasm:** Based on the examined rice accessions, a negative reaction for phenol ($Ph^-$) was found in most of upland rice cultivars; all rice cultivars (100%) from lowland areas showed a positive reaction (Fig. 1, Table 1). The

Table 1: Characteristics of the 74 rice strains from Thailand used in this study

| Cultivars' name/type of rice[a] | Species/ Subspecies[b] | Cultural type/ Ethnic group | Locality/ Province | Endosperm type[c]/%AC | Chloroplast sub-type | Phenol reaction |
|---|---|---|---|---|---|---|
| Ang Jerng Jahn/T | *O. sativa*/J | Rainfed lowland/Thai | Central/Phetburi | NW (15.6%) | 7C7A | $Ph^+$ |
| Bael Jao Juor/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (12.9%) | 6C7A | $Ph^-$ |
| Bael Der/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (15%) | 7C7A | $Ph^-$ |
| Bael Jai/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (14.3%) | 6C7A | $Ph^-$ |
| Bael La Mi/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (15.1%) | 6C7A | $Ph^-$ |
| Bael Leu/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | G (3.5%) | 6C7A | $Ph^-$ |
| Bael Jai/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (14.3%) | 6C7A | $Ph^-$ |
| Bael Jao Jau/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (12.9%) | 6C7A | $Ph^-$ |
| Bael Ma Kael/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (14.7%) | 6C7A | $Ph^-$ |
| Biaw Bud/T | *O. sativa*/J | Upland/Mien | Northern/Chiang Rai | G (3.6%) | 6C7A | $Ph^-$ |
| Biaw Pae/T | *O. sativa*/J | Upland/Mien | Northern/Chiang Rai | NG (12.9%) | 6C7A | $Ph^-$ |
| Be Dao/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (14.3%) | 6C7A | $Ph^-$ |
| Be Dao Derk/T | *O. sativa*/J | Upland/Hmong | Northern/Tak | G (4.0%) | 6C7A | $Ph^-$ |
| Be Jah/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | G (2.4%) | 7C6A | $Ph^-$ |
| Be Lao Da/T | *O. sativa*/J | Upland/Hmong | Northern/Tak | G (2.5%) | 6C7A | $Ph^-$ |
| Be Lia Tia Tao/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | NG (15.6%) | 6C7A | $Ph^-$ |
| Biaw Mai Yan Rai/T | *O. sativa*/J | Upland/Mien | Northern/Chiang Rai | NG (16.5%) | 6C7A | $Ph^-$ |
| Bao Bud/T | *O. sativa*/J | Upland/Mien | Northern/Chiang Rai | G (3.6%) | 6C7A | $Ph^-$ |
| Bao Bae/T | *O. sativa*/J | Upland/Mien | Northern/Chiang Rai | NG (12.9%) | 6C7A | $Ph^+$ |
| Chawlung/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Pattani | NG (23.4%) | 9C7A | $Ph^+$ |
| Chiang Phatthalung/T | *O. sativa*/J | Rainfed lowland/Thai | Southern/Phatthalung | NG (28.9%) | 7C7A | $Ph^+$ |
| E-Khao-Yai/T | *O. sativa*/I | Rainfed lowland | Northeastern/Kalasin | G (6.5%) | 9C7A | $Ph^+$ |
| E-Long-Mah/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Kalasin | G (6.5%) | 9C7A | $Ph^+$ |
| E-Tia/T | *O. sativa*/I | Rainfed lowland | Northeastern/Kalasin | G (7.1%) | 8C8A | $Ph^+$ |
| Ga Saen/T | *O. sativa*/J | Upland/Thai | Northeastern/Mukdahan | G (7.5%) | 6C7A | $Ph^+$ |
| Gaen Jahn/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (29.3%) | 8C8A | $Ph^+$ |
| Hao' Kaen Du/T | *O. sativa*/J | Upland/Thai | Northeastern/Mukdahan | G (8.7%) | 6C7A | $Ph^+$ |
| Hawm Jan/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (26%) | 8C8A | $Ph^+$ |
| Hawm Mali/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Yasothon | NG (15%) | 8C8A | $Ph^+$ |
| Hua Nah/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phattalung | NG (24.9%) | 8C8A | $Ph^+$ |
| Ja Lai/T | *O. sativa*/J | Upland/Khamu | Northern/Chiang Rai | G (4.3%) | 6C7A | $Ph^-$ |
| Ja Ngai/T | *O. sativa*/J | Upland/Khamu | Northern/Chiang Rai | G (4.1%) | 6C7A | $Ph^-$ |
| Jaw Haw/T | *O. sativa*/J | Upland/Lisu | Northern/Chiang Rai | NG (17.5%) | 6C7A | $Ph^-$ |
| Jek Chuey/T | *O. sativa*/I | Rainfed lowland/Thai | Central/Phetchaburi | NG(27.5%) | 7C7A | $Ph^+$ |
| Kala/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | G (3.6%) | 6C7A | $Ph^-$ |
| KDML105/M | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Nakhon Phanom | NG (14.7%) | 8C8A | $Ph^+$ |
| KDML105/M | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Roi Et | NG (13.5%) | 8C8A | $Ph^+$ |
| KDML105/M | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Mahasarakham | NG (14%) | 8C8A | $Ph^+$ |
| Kha Jao/T | *O. sativa*/J | Upland/Khamu | Northern/Chiang Rai | NG (11%) | 6C7A | $Ph^-$ |
| Khai Mod Rin | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (23.3%) | 7C7A | $Ph^+$ |
| Khao Kam (black rice)/T | *O. sativa*/J | Upland/Hmong | Luang Prabang/Laos | G (4.5%) | 6C7A | $Ph^-$ |
| Khao Kam (black rice)/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | G (5.2%) | 6C7A | $Ph^-$ |
| Khao Kam (KN3) (black rice)/T | *O. sativa*/J | Upland/Hmong | Northern/Chiang Rai | G (5.8%) | 7C6A | $Ph^-$ |
| Khao Kam (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Kalasin | G (6.8%) | 8C8A | $Ph^+$ |
| Kam Poon acc.1 (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Kalasin | G (6.2%) | 8C8A | $Ph^+$ |
| Khao Kam acc. 2 (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Kalasin | G (6.8%) | 8C8A | $Ph^+$ |
| Khao Kam (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Mukdahan | G (5.6%) | 8C8A | $Ph^+$ |
| Khao Kam acc. 8 (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Ubol Ratchathani | G (7.2%) | 8C8A | $Ph^+$ |
| Khao Kam acc. 31 (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Ubol Ratchathani | G (5.8%) | 8C8A | $Ph^+$ |
| Kam Nai (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Ubol Ratchathani | G (4.8%) | 8C8A | $Ph^+$ |
| Kam Nork (black rice)/T | *O. sativa*/I | Rainfed lowland/Laos | Naxaythong/Vientiane | G (4.5%) | 8C8A | $Ph^+$ |
| Kam Ngan (black rice)/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Mukdahan | G (5.5%) | 8C8A | $Ph^+$ |
| Khao' Chao/T | *O. sativa*/J | Rainfed upland/Tai Dam | Western/Vietnam | NG (20%) | 6C7A | $Ph^-$ |
| Khao' Niaw/T | *O. sativa*/J | Rainfed upland/Tai Dam | Western/Vietnam | G (5.9%) | 6C7A | $Ph^-$ |
| Khao Pong Krai/T | *O. sativa*/J | Upland/Thai | Northern/Chiang Mai | G (4.5%) | 7C7A | $Ph^-$ |
| Khao' Rai Kaset/M | *O. sativa*/I | Upland/Thai | Northeastern/Mukdahan | G (9.8%) | 8C8A | $Ph^+$ |
| Khem Tawng/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (26.1%) | 8C8A | $Ph^+$ |
| Khitom Yai/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Mukdahan | G (8.5%) | 8C8A | $Ph^+$ |
| Lai Noi/T | *O. sativa*/J | Upland/Khamu | Northern/Chiang Rai | G (3.9%) | 6C7A | $Ph^-$ |
| Lao Taek/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Kalasin | G (6.8%) | 8C8A | $Ph^+$ |
| Mali Daeng/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Surin | NG (25.7%) | 8C8A | $Ph^+$ |
| Mali Hawm/T | *O. sativa*/I | Rainfed lowland/Thai | Central/Suphanburi | NG (15.6%) | 8C8A | $Ph^+$ |
| Nam Pa/T | *O. sativa*/I | Rainfed/Lowland/Laos | Kasi/Vientiane | G (7.8%) | 8C8A | $Ph^+$ |
| Nahng Roi Yai/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Buriram | NG (26.5%) | 9C7A | $Ph^+$ |
| Niaw Daeng/T | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Kalasin | G (7.3%) | 6C7A | $Ph^+$ |
| Paung Tawng/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (38.3%) | 9C7A | $Ph^+$ |

Table 1: Continued

| Cultivars' name/type of rice[a] | Species/ Subspecies[b] | Cultural type/ Ethnic group | Locality/ Province | Endosperm type[c]/%AC | Chloroplast sub-type | Phenol reaction |
|---|---|---|---|---|---|---|
| Pathumthani 1/M | *O. sativa*/I | Rainfed lowland/Thai | Central/Pathumthani | NG (17.5%) | 9C8A | $Ph^+$ |
| Phatthalung 60/M | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (27.5%) | 8C8A | $Ph^+$ |
| Pheuak Nam/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (30.5%) | 8C8A | $Ph^+$ |
| Pirnneonklog/T | *O. sativa*/J | Upland/Khamu | Northern/Chiang Rai | G (2.8%) | 6C7A | $Ph^-$ |
| RD 15/M | *O. sativa*/I | Rainfed lowland/Thai | Northeastern/Yasothon | NG (16.5%) | 8C8A | $Ph^+$ |
| Sungyod/T | *O. sativa*/I | Rainfed lowland/Thai | Southern/Phatthalung | NG (14.5%) | 8C8A | $Ph^+$ |
| Yuan Daw/T | *O. sativa*/I | Rainfed lowland | Northeastern/Kalasin | G (5.6%) | 9C7A | $Ph^+$ |

[a]T: Traditional rice cultivar, M= modern rice cultivar. [b]I: *Indica*; J: *japonica*, assessed by deletion or addition of ORF100 in cp-DNA. [c]G: Glutinous rice, NG: Non-Glutinous rice

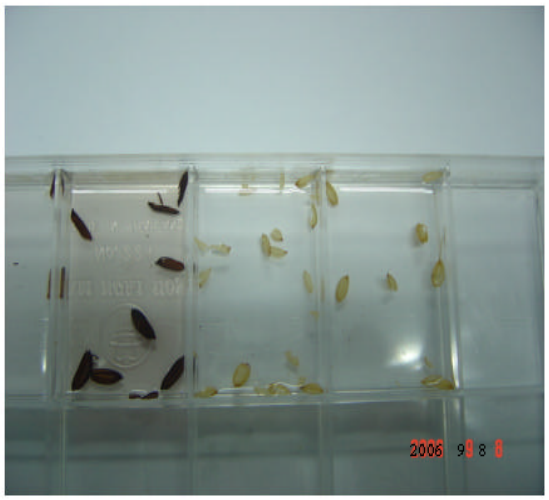

Fig. 1: The hulls of rice cultivar *KDML105* (indica type) that turned black (left) and unchanged color hulls of cultivar *Tamasakae* (*japonica* type) (right) for phenol reaction when soaked in 1.5% phenol solution

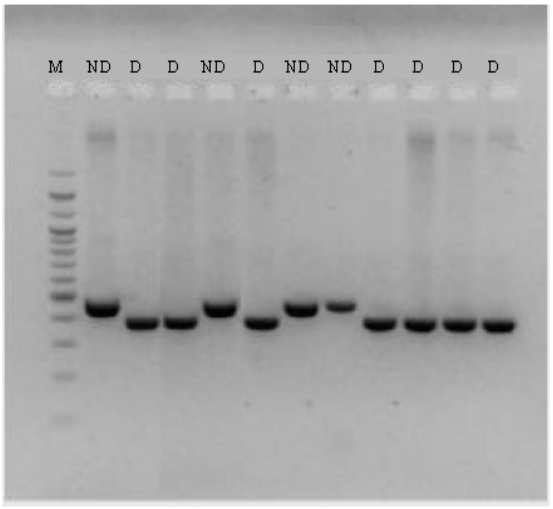


Fig. 2: Amplicons generated by PCR and separated by electrophoresis of plastid type of ORF100 classified into deletion (D) and non-deletion (ND) of rice cultivars used as materials. M = the molecular marker, a 100 bp ladder

variation in the phenol reaction between upland and lowland rice cultivars from Thailand was significant

**Cytoplasmic genotypes of Thai upland and lowland rice cultivars:** Maternal genotypes of the rice cultivars were investigated by the length of the polymorphism of the amplicon of the ORF100 region (D or ND types), as shown in Fig. 2 and the plastid subtype ID (PS-ID) sequences, CA repeats between genes *rpl16* and *rpl14* (Fig. 3). The combined data for 74 rice accessions were summarized in Table 1. All upland rice cultivars and a few lowland cultivars showed the ND type, whereas the D type predominated in the lowland rice cultivars.

Generally, the D type and ND types are used to distinguish indica and japonica plastid types, respectively. Based on the present study, the variation in plastid types showed a significant difference between upland and lowland rice cultivars. The *indica* cultivars predominated in lowland areas of Thailand and the *japonica* cultivars predominated in the highlands. Further analysis of the PS-ID sequence indicated that the 74 rice accessions examined carried six plastid subtypes, 6C7A, 7C7A, 7C6A, 8C8A, 9C8A and 9C7A. These CA repeats for rice (*O. sativa* L.) have been reported previously (Nakamura *et al*., 1998; Ishikawa *et al*., 2002). The two subtypes 6C7A and 7C6A are specific genotypes which predominated to upland cultivars originating from both the Northern and Northeastern regions of Thailand. The subtype 7C6A was not found in the Japanese lowland rice as reported by Ishikawa *et al*. (2002). The subtypes 6C7A and 7C6A were consistent with *japonica* cultivars (ND type), which were grown in the highland areas of Thailand. Three types, 7C7A, 8C8A and 9C7A, are known as *indica*-specific subtypes and are consistent with the D type in the ORF100 region (Nakamura *et al*., 1998). In contrast, the subtype 7C7A was found in traditional rice cultivars which carried either the D type (*indica* cultivar) or the ND type (*japonica* cultivar) used in this study,

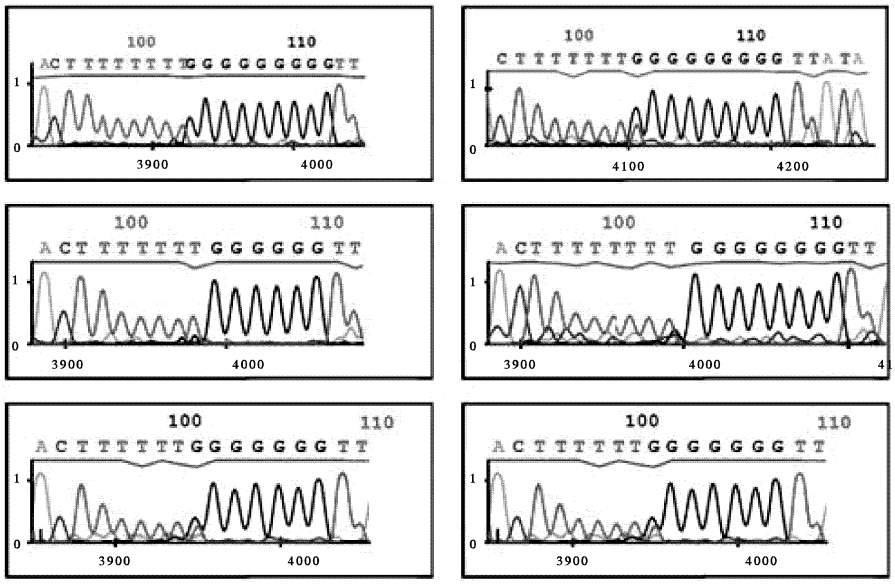


Fig. 3: Six plastid subtype ID sequences (9C8A, 6C7A, 7C6A, 9C7A, 8C8A and 7C6A) found in Thai rice cultivars

whereas the two subtypes 8C8A and 9C7A are consistent to the D type. According to endosperm types, both upland and lowland rice cultivars from all regions of the country share common plastid subtypes. Eleven traditional rice cultivars with intermediate (20-25%) and high (>25%) amylose content preferred by people of the Southern region were examined. These cultivars did not carry the 6C7A subtype, which predominated in the Northern region of Thailand. This absence of the 6C7A subtype indicates that the cultivars have not been transferred between the two regions. Traditionally, people who lived in the Northern region preferred glutinous and non-glutinous rice with low amylose content (<20%), whereas non-glutinous rice with intermediate and high amylose was preferred by the people of Southern Thailand. Among the 12 accessions of black rice studied, including rainfed upland and rainfed lowland cultivars, three upland rice accessions carried the subtype 6C7A or 7C6A and the remaining nine lowland accessions carried subtype 8C8A. This suggests that the maternal origins of upland black rice and lowland black rice are different. This finding for the origins of black rice is consistent with variations in the ORF100 region between upland and lowland black rice strains. The subtypes 6C7A and 8C8A were associated with non-deletion (*japonica* type) and deletion (*indica* type) in the ORF100 region, respectively.

## DISCUSSION

**The importance of traditional cultivars in terms of a breeding program in Thailand:** In Thailand, the traditional upland and lowland rice cultivars, which have been cultivated for many years, are very useful in rice breeding programs. A number of today's improved rice cultivars originated from rice landraces that have been growing in different regions of the country since ancient times. For example, the glutinous rice cv. Khao Pong Krai and non-glutinous rice cv. Jaw Haw were developed from the collection of local varieties in northern Thailand. In another example, the lowland rice cv. Nahng Pa-yah 132 and Gaen Jan were developed from local rice varieties which have been growing in the southern region of the country since 1962 and 1983, respectively (Chitrakon and Somrith, 2003).

With respect to the importance of traditional rice varieties that have been recognized as genetic resources, basic information obtained from the present study about their genetic background will be useful for rice variety improvement through biotechnology. Rice is the world's most important cereal. As such, knowledge of biotechnology is a key factor in developing genetic improvements in rice, particularly as it applies to the development of rice strains using DNA molecular markers. As mentioned above, Thailand is recognized worldwide as an important source of genetically diversified rice. Natural

wild rice, such as Khao Jaw Loi and cultivated rice have been collected by Thai farmers since ancient times as can be seen from the variety in local rice in all regions of the country. This genetic diversity can be used to improve rice varieties. It is critical to maintain this natural biodiversity because modern varieties are regularly being introduced into upland and lowland fields. Ishikawa *et al.* (2006) stated that traditional rice cultivars as well as traditional cultivation styles may soon be lost. Genetic erosion of Thailand's traditional rice cultivars is the major reason for giving a high priority to the conservation, characterization and evaluation of the genetic resources as outlined in this report.

**The importance of molecular data for further application in the characterization of rice cultivars:** In previous studies, molecular markers have been successfully applied to characterize rice cultivars and to describe genetic diversity and its distribution. As an example, molecular markers, including isozymes and PCR mediated molecular markers, were used as genetic markers for evaluating rice genetic resources in Nan province, northern Thailand (Ishikawa *et al.*, 2006). In that report, the Thai rice strains showed an association between cytoplasmic genotype and nuclear genotype. Rice strains with non-deletion (ND type) of the 69 bp of the ORF100 region of chloroplast DNA were consistent to the subtype 6C7A of linker sequences between rice plastid genes *rpl16* and *rpl14*, whereas rice strains with deletion (D type) of the region showed the sequence 8C8A of the linker sequences.

Intraspecific classification of rice has been of importance to rice genetics and breeders (Garris *et al.*, 2005). In Thailand, for example, the two cultivars, cv. PSL60-1 and PSL60-2, which were developed by crossing and selecting for yield, originated from different maternal origins. cv. PSL60-1 was classified as *indica* and cv. PSL60-2 as *japonica*. The classifications were based on the INDEL marker of the ORF100 region of the chloroplast DNA. From pedigree analysis, their chloroplast type reflected their maternal origin (Prathepha and Baimai, 2004). The INDEL molecular marker could be useful for classifying rice germplasm in a breeding program. D and ND differentiations have also been detected with chloroplast DNA variation using PCR analysis, however, INDEL molecular markers were preferred for recognition of the *indica* and *japonica* types because the markers accurately reflected their chloroplast compositions. The INDEL method was previously applied to evaluate the genetic resources of upland rice in northern Laos (Yamanaka *et al.*, 2001), to examine the *indica-japonica* differentiation of Chinese rice landraces (Chen *et al.*, 1994), to determine the origin of cytoplasm substitute in Japanese rice cultivars and to determine the maternal origins of Japanese lowland and upland rice (Ishikawa *et al.*, 2002). In Thailand, developing improved rice varieties by selecting the pedigree of traditional varieties has been practiced by farmers over centuries. There are a number of improved varieties that have been released to farmers by the Rice Department in the Ministry of Agriculture and Cooperatives. Because there is a lack of basic genetic information about Thai rice germplasm, the present study has applied a variety of molecular techniques to provide much greater detail in the diversity of rice varieties.

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## REFERENCES

Chen, W.B., I. Nakamura, Y.I. Sato and H. Naki, 1994. *Indica-japonica* differentiation in Chinese rice land races. Euphytica, 74: 195-201.

Chitrakon, S. and B. Somrith, 2003. The Development of Thai Rice Varieties. In: Science and Technology with Thai Rice, Lorlowhahakarn, S. (Ed.). The Public Information Department, Thailand's National Science and Technology Development Agency, Bangkok, pp: 29-76.

Doyle, J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19: 11-15.

Garris, A.J., T.H. Tai, J. Coburn, S. Kresovich and S. McCouch, 2005. Genetic structure and diversity in *Oryza sativa* L. Genetics, 169: 1631-1638.

Ishikawa, R., Y.I. Sato, T. Tang and I. Nakamura, 2002. Different maternal origins of Japanese lowland and upland rice populations. Theor. Applied Genet., 104: 976-980.

Ishikawa, R., N. Toki, K. Imai, Y.I. Sato, H. Yamagishi, Y. Shimamoto, K. Ueno, H. Morishima and T. Sato, 2005. Origin of weedy rice grown in Bhutan and the force of genetic diversity. Genet. Resour. Crop Evol., 52: 395-403.

Ishikawa, R., S. Yamanaka, Y. Fukuta, S. Chitrakon, C. Bounphanousay, K. Kanyavong, L.H. Tang, I. Nakamura, T. Sato and Y.I. Sato, 2006. Genetic erosion from modern varieties into traditional upland rice cultivars (*Oryza sativa* L.) in Northern Thailand. Genet. Resour. Crop Evol., 53: 245-252.

Kanno, A., N. Watanabe, I. Nakamura and A. Hirai, 1993. Variations in chloroplast DNA from rice (*Oryza sativa*): Differences between deletions mediated by short direct-repeat sequences within a single species. Theor. Applied Genet., 86: 579-584.

Nakamura, I., H. Urairong, N. Kameya, Y. Fukuta, S. Chitrakon and Y.I. Sato, 1998. Six different plastid subtypes were found in *O. sativa-O. rufipogon* complex. Rice Genet. Newslett., 15: 80-83.

Prathepha, P. and V. Baimai, 2004. Variation of *Wx* microsatellite allele, waxy allele distribution and differentiation of chloroplast DNA in a collection of Thai rice (*Oryza sativa* L.). Euphytica, 140: 231-237.

Yamanaka, S., R. Ishikawa, Y. Fukuta, I. Nakamura, T. Sato and Y.I. Sato, 2001. Evaluation of upland rice genetic resources in Northern Laos. Rice Genet. Newslett., 18: 9-10.



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