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# Genetic classification of upland rice (*Oryza sativa* L.) collected from minority farmers in Thailand using InDel marker

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**Abstract** Upland rice varieties are the most appropriate for rice production in slope areas under rainfed conditions. Upland rice grows in Northern, Central and Northeast Thailand, but genetic information of this type of rice cultivated by minority farmers is still unclear. Thus, the obtained results helped upland rice breeding program. The 15 upland rice accessions were investigated using 23 InDel markers and molecular results to compare traditional morphology method. The morphological characteristics were identified with perplexing outcome and the genetic information obtained by the 25 InDel markers revealed three major groups among the 15 upland rice accessions. The upland rice accessions PLU-SU-0007, PLU-SU-0011, and PLU-SU-0012 collected from Prachuap Khiri Khan were identified as *indica* rice. CM-001, SU-001, and PLU-SU-002 accessions which were collected from Chiang Mai, Petchaburi, and Prachuap Khiri Khan and identified as *japonica* rice. The nine accessions showed closely genetic similarity to *japonica* rice. These data suggested that the InDel marker is an efficient method to classify ecotypes (*indica* or *japonica*) of upland rice, while morphology of seed grains failed to identify the 15 accessions. In addition, the upland rice accessions growing throughout Thailand are genetically diverse. This genetic information will provide strong opportunity for successful study of these accessions *in vitro* and genetic improvement of upland rice.

**Keywords:** InDel markers, Upland rice, *indica, japonica*

## Introduction

Over half of world population consumes rice as main food (Tonini and Cabrera, 2011). Rice is not only a staple food, but it is also related to many people around the world in terms of economic importance providing many benefits, such as income for households and employment opportunities (Okodua, 2018; Anang and Yeboah, 2019). Rice is currently grown all over the world, but almost 90 percent of it is cultivated in Asia (Bandumula, 2017). Southeast Asia is especially important because it constitutes the main planting area of rice germplasm and is where diversity of rice originated (Silva *et al.*,

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2015). Thailand is a country in southeast Asia with a large area of rice cultivation, which has provided income for economic support in the past few decades (Changkid, 2008; Butso and Isvilanonda, 2010). Interestingly, only lowland rice is considered as an economically important rice crop in Thailand (Perret *et al*., 2013). Upland rice is cultivatd for household consumption and serves for culture and wisdom for people in rural and highland areas of the country (Limnirankul *et al*., 2015; Chaichana and Wongtaw, 2018). Upland rice is mainly grown in areas with scarcity of water and poor soil quality (Jamjod *et al*., 2017; Saito *et al*., 2018). In addition, the upland rice varieties that are cultivated in Thailand are very diverse. Cultivation is practiced by farmers who are mainly from minority populations in each planting area, which results in inability to promote the appropriate production technology (Unthong *et al*., 2007; Pusadee *et al*., 2014; Jamjod *et al*., 2017). Therefore, productivity in terms of both quality and quantity of upland rice is very low. These are valid reasons to improve upland rice cultivars.

Proper classification of rice (either *indica* or *japonica*) is necessary in the breeding program for improving quality of upland rice. This is especially important for the varieties that are currently being cultivated in farmers' fields. In addition, many of the highland areas of Thailand border neighbouring countries, which result in high genetic diversity (Keawsomnuk, 2017). The import and distribution of upland rice varieties (in border areas among farmers) and cross-pollination between cultivated and wild upland rice varieties (ancestor species), are the main reasons of genetic variation. Classification of rice can be done using morphological features, such as color, shape, grain weight, awn or awn-less etc. However, morphological classification has low accuracy, especially when tested varieties are closely related. InDel markers have been developed from genome regions with either insertions or deletions. InDel markers have been successfully detected in both *japonica* and *indica* rice cultivars, which truly reflect the genetic differences of the two varieties (Lu *et al*., 2009). Therefore, the objective of this study was to identify upland rice ecotypes (*indica* or *japonica*), both of which are indigenous varieties, using InDel markers. Samples were collected from farmers at Northern, Southern, and Center regions of Thailand. The information from this study will be of benefit for further studies on genetic improvement of upland rice in Thailand.

## Materials and methods

### *Plant materials and morphological study*

Seeds of fifteen upland rice varieties were collected from Northern, Southern, and Center regions of Thailand including Chiang Mai, Chumphon, Phetchaburi, and Prachuap Khiri Khan provinces (Table 1). All seeds were harvested at maturity stage from July to November in 2017. Seeds were heated at 50 ℃ for four days to break dormancy. Subsequently, all seeds were kept in a paper envelope until ready to cultivate. Upland rice seeds were then cultivated by direct seedling method (3-4 seeds/hole) on July-November in 2018 at Silpakorn University, Phetchaburi Campus. The distance of shoots was 25 x 50 cm and 10 meter per row. The experiment was performed using randomized complete block design (RCBD) with four replications. After seeds germinated, only two seedlings per hole were kept. Urea fertilizer (46-0-0) was supplied in a ratio of 25 kg/rai. Seeds were harvested at maturity stage and seed morphology data including seed length, width, thickness, and grain weight for 100 seeds was recorded.

**Table 1.** List of upland rice accessions used in this study

| **Accessions** | **Location** |
|---|---|
| CP-001 | Chumphon |
| CM-001 | Chiang Mai |
| SU-001 | Phetchaburi |
| PLU-SU-001 | Prachuap Khiri Khan |
| PLU-SU-002 | Prachuap Khiri Khan |
| PLU-SU-003 | Prachuap Khiri Khan |
| PLU-SU-004 | Prachuap Khiri Khan |
| PLU-SU-005 | Prachuap Khiri Khan |
| PLU-SU-007 | Prachuap Khiri Khan |
| PLU-SU-009 | Prachuap Khiri Khan |
| PLU-SU-0010 | Prachuap Khiri Khan |
| PLU-SU-0011 | Prachuap Khiri Khan |
| PLU-SU-0012 | Prachuap Khiri Khan |
| PLU-SU-0013 | Prachuap Khiri Khan |
| PLU-SU-0014 | Prachuap Khiri Khan |

### *DNA extraction, PCR and electrophoretic analysis*

Seeds were germinated in moist paper towels in the laboratory. DNA was extracted from one-week seedlings by using PureLink TMPlant DNA purification kit (Thermo Fisher, MA, USA), according to the manufacturer's protocols. Twenty-three InDels primers previously developed by Shen *et al*. (2004) and successfully utilized and validated by Lu *et al*. (2009) and Xingxing *et al*. (2007), were used in PCR amplification (Table 2). The PCR reactions were performed in a 20 µL final volume containing 0.1 µg of genomic DNA, 10 pM of each primer, 1X Taq DNA polymerase buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, and 1 U of Taq DNA polymerase. The following PCR thermal cycle parameters were used: 94 °C for 3 min, 35 cycles of 30 s at 94 °C, 40 s at

55 °C, and 35 s at 72 °C and a final extension at 72 °C for 7 min. The amplified products were examined by electrophoresis in 4.0 % agarose gels in 1XTAE buffer. Electrophoresis banding patterns were recorded using a Gel doc XR+ system (Biorad).

**Table 2.** InDel primers used in this study

| Primer name | Forward primer | Reverse primer | Base pair difference |
|---|---|---|---|
| R1M7 | ATTCCTGGTTCTACATTACTTA | CGCCTCACTAGAATATCGGA | 37 |
| R1M30 | AAGGGGCCCTAATTTATCTAG | TGTTTACTTTGTTCTTGGACTG | 49 |
| R1M37 | ATAGTTCGCCATCGTCAT | ACACGCCATAGCAAGGAA | 65 |
| R1M47 | AATAGAATTACTGATGAAACCTTA | GCCCGTTACCGCTTATGT | 51 |
| R2M10 | CCCAGTCTGCTGCCATCT | GAATGTATTTCAGTTCCAGTAAG | 48 |
| R2M24 | GGGCAACAACGGCTCTG | AGGGAATAAGGCGATACGG | 31 |
| R2M26 | GCAGCAAAGTGCGGAGTA | CAGGTGAATTGCCAATTT | 38 |
| R2M50 | CCTGAAGGAAATGATAGCAATAG | GTTTTGTATGCTCTTCACTTGTC | 42 |
| R3M10 | CCGAGTACCATTGCTTTC | CTGCCATAGTTACTGCTCTGTT | 37 |
| R3M30 | AGGCTAAGTGAAGAAATAATAAG | CTCCGTATTCATTACTGGTTG | 24 |
| R4M13 | TACACGGTAGACATCCAACA | ATGATTTAACCGTAGATTGG | 32 |
| R4M43 | CTTGAACCTGAGTGAGTGG | CGATGAAAATGATGTCTA | 34 |
| R5M30 | CTCAATTTCACCCATCCC | CGCTCCGTCTCCAACCTC | 46 |
| R6M44 | TTAGGAATAAAGGCTGGATA | TTACCGTTAATAGGTGGAA | 34 |
| R7M7 | ACCTTCCCTCCCCTTTTGAT | AACTTGGTCTTCCTGTTTTATTG | 67 |
| R7M37 | CAGCCCTAAATCTAAATACCC | ACGTTGAGACAGGCGAGC | 36 |
| R8M23 | CCTATTCACTCTACCGACAT | GTTTAGTTCCCATTGCTTT | 36 |
| R8M33 | CGAAAGAGGAGAGGGGTAGT | CGAAAACGAGAAACAAATA | 38 |
| R9M42 | CTATAAGACCAAAACGAAAACT | GAAAACCATTGTGTCACTGTA | 48 |
| R10M30 | CCCTAAAAATAGAGCAACCT | ACCCATAATACTACCAATCAAC | 19 |
| R10M40 | GTCCCTAGGCCATCTCTTG | GCGAATAGGGGTGGACAG | 44 |
| R11M23 | AAGGTTGACAAGGACAGAAG | TCGCAGGAATGGATAAAA | 42 |
| R12M27 | ATTTCATTGCCATCAGTT | GTAATCTTCTATCCGTTCA | 33 |

### ***Genotype score and allele frequency calculation***

The seeds of two *indica* varieties including Pathumthani 1 (PTT1) and Chinat1 (CN1) were used as standard reference for the *indica* genotype. Band patterns identical to these two varieties were scored as homozygous *indica* genotype (II). Band patterns identical to those in the two *japonica* varieties (K.Y.P.1 and K.Y.P.2) were scored as homozygous *japonica* genotypes (JJ). Band patterns similar to both *indica* and *japonica* references were scored as heterozygous *indica-japonica* genotypes (IJ). Hierarchical clustering was used to analyze phylogenetic trees with the linkage criteria of complete cluster method and distance set as Euclidean.

## Results

### *Seed morphological traits*

Seed morphology traits of 15 accessions and four reference varieties (PTT1, CN1, K.Y.P.1, and K.Y.P.2) were presented in Table 3-4. Seeds from both *indica* varieties (PTT1 and CN1) had longer seed values (10.65 and 10.44 mm, respectively) than *japonica* seeds (K.Y.P.1 and K.Y.P.2 with 7.20 and 7.85 mm, respectively) (Table 3). However, grain width of *indica* had lower values (2.03-2.09 mm) than *japonica* varieties (3.21-3.39 mm) (Table 3). The two varieties showed differences in their length/width ratio: *indica* (4.995-5.246) had higher values than *japonica* types (2.242-2.315) (Table 3). For 100-grain weight, seeds of *indica* varieties (PTT1 and CN1) were lighter (2.67-2.69 g) when compared with those of *japonica* varieties (3.00-3.01 g) (Table 3). Grain thickness of the two varieties was similar and ranged between 2.00-2.23 cm (Table 3).

**Table 3.** Grain morphology of reference varieties

| Accessions | Grain length (mm) | Grain width (mm) | Length/Width Ratio | Grain thickness (mm) | 100-Grain weight (g) |
|---|---|---|---|---|---|
| PTT1 | 10.65 | 2.03 | 5.246 | 2.01 | 2.69 |
| CN1 | 10.44 | 2.09 | 4.995 | 2.00 | 2.67 |
| K.Y.P.1 | 7.20 | 3.21 | 2.242 | 2.12 | 3.00 |
| K.Y.P.2 | 7.85 | 3.39 | 2.315 | 2.23 | 3.01 |

The grain morphological traits of 15 upland rice accessions were presented in Table 4. Highly significant differences were observed in all five morphological traits for all rice accessions. For instance, the seeds of CP-001, PLU-SU-003, PLU-SU-009, PLU-SU-0010 and PLU-SU-0014, were long and slender ranging from 9.77-10.96 mm in length, and from 2.22-2.47 mm in width (Table 4). On the other hand, the width of seeds of CM-001, SU-001, PLU-SU-0002 and PLU-SU-0013 ranged from 3.00-3.44 mm. Moreover, the rice accessions CM-001, SU-001, PLU-SU-0002 and PLU-SU-0013, had the lowest length/width ratio ranging from 2.90-3.28 mm and were the thicknest, except for SU-001 (1.78-2.26 mm) (Table 4). The grain weight of rice accessions CM-001 and PLU-SU-013 ranged from 2.98-3.70 g.

**Table 4.** Grain morphology of 15 upland rice accessions

| **Accessions** | **Grain length (mm)** | **Grain width (mm)** | **Length/Width Ratio** | **Grain thickness (mm)** | **100-Grain weight (g)** |
|---|---|---|---|---|---|
| CP-001 | 10.13+0.75b | 2.40+0.14 def | 4.22+0.39a | 1.90+0.15d-g | 2.37+0.09de |
| CM-001 | 9.14+0.46d | 3.00+0.11abc | 3.05+0.14cde | 2.10+0.10bc | 2.98+0.07b |
| SU-001 | 8.95+0.79d | 3.44+1.76a | 2.93+0.74de | 1.78+0.27h | 2.31+0.09e |
| PLU-SU-001 | 9.17+0.53d | 2.77+0.33b-e | 3.35+0.36c | 1.91+0.18d-g | 2.65+0.09c |
| PLU-SU-002 | 10.00+0.66b | 3.13+0.56ab | 3.28+0.58cd | 2.14+0.15ab | 2.59+0.22c |
| PLU-SU-003 | 10.96+0.62a | 2.47+0.17def | 4.46+0.43a | 1.97+0.02cde | 2.42+0.16de |
| PLU-SU-004 | 9.48+0.61cd | 2.86+0.15bcd | 3.32+0.25c | 1.97+0.08cde | 2.45+0.07d |
| PLU-SU-005 | 10.17+0.47b | 2.68+0.13b-f | 3.80+0.27b | 2.02+0.04bcd | 2.46+0.06d |
| PLU-SU-007 | 7.98+0.30e | 2.61+0.25c-f | 3.07+0.21cde | 1.82+0.22fgh | 1.80+0.12g |
| PLU-SU-009 | 10.23+0.58b | 2.30+0.18ef | 4.46+0.26a | 1.94+0.08def | 2.14+0.02f |
| PLU-SU-0010 | 9.77+0.58bc | 2.22+0.15f | 4.40+0.23a | 1.84+0.09e-h | 2.11+0.06f |
| PLU-SU-0011 | 8.05+0.36e | 2.41+0.14def | 3.34+0.20c | 1.50+0.20i | 1.10+0.09h |
| PLU-SU-0012 | 7.86+0.48e | 2.69+0.14b-e | 2.93+0.18e | 1.80+0.09fgh | 1.82+0.07g |
| PLU-SU-0013 | 9.97+0.38bc | 3.44+0.15a | 2.90+0.12e | 2.26+0.07a | 3.70+0.12a |
| PLU-SU-0014 | 9.99+0.49bc | 2.28+0.16ef | 4.39+0.33a | 1.78+0.16gh | 2.31+0.11e |
| P-value (F-test) | < 2x10$^{-16}$ ** | 8.36x10$^{-10}$ ** | < 2x10$^{-16}$ ** | < 2x10$^{-16}$ ** | < 2x10$^{-16}$ ** |
| CV(%) | 5.91 | 18.12 | 9.72 | 7.41 | 4.68 |
| Mean | 9.36 | 2.70 | 3.56 | 1.91 | 2.34 |

### *InDel markers*

The 25 InDel markers were tested with known *indica* [Chainat 1 (CN1) and Pathum 1 (PTT1)] and *japonica* varieties (K.Y.P.1, K.Y.P.2). Polymorphisms between *indica* and *japonica* reference varieties were present in 23 InDel markers (Table 2). Thus, these 23 polymorphisms were used for differentiating 15 upland rice accessions collected from Northern, Southern and Center parts of Thailand. The 15 upland rice accessions clustered into three genetically distinct groups. Both *indica* reference varieties (PTT1, and CN1) grouped in the first group along with three upland rice accessions including PLU-SU-007, PLU-SU-0011, and PLU-SU-012 (Figure 1). On the other hand, the upland rice accessions CM-001, SU-001, and PLU-SU-002 clustered into the same group as the two of *japonica* reference varieties (K.Y.P.1 and K.Y.P.2) (Figure 1 and 2). Nine upland rice accessions (CP-001, PLU-SU-001, PLU-SU-003, PLU-SU-004, PLU-SU-005, PLU-SU-009, PLU-SU0010, PLU-SU-0013, and PLU-SU-0014) clustered in a separate, distinct group as a sister clade to *japonica* (Figure 1).

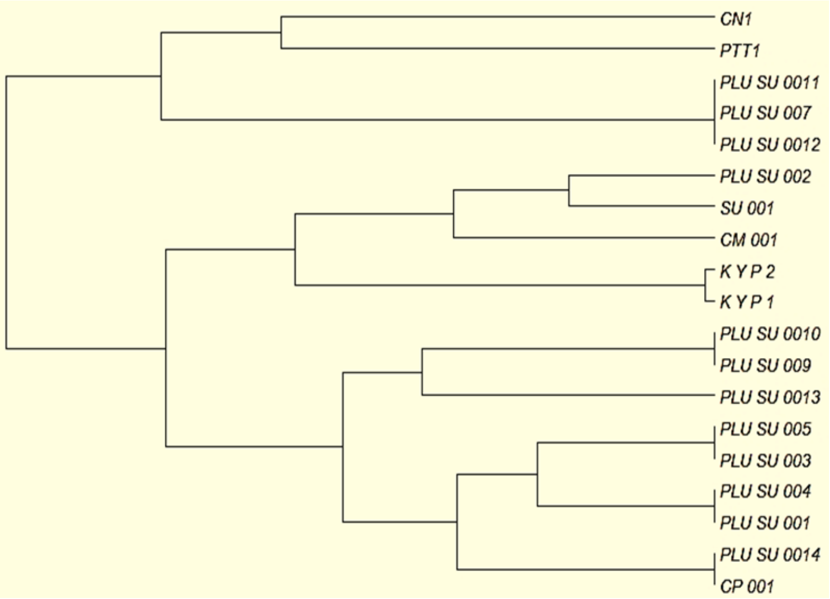

**Figure 1.** Phylogenetic tree of 15 upland rice accessions with *indica* **(**CN1 and PTT1**)** and *japonica* **(**K**.**Y**.**P**.**1 and K**.**Y**.**P**.**2**)** references

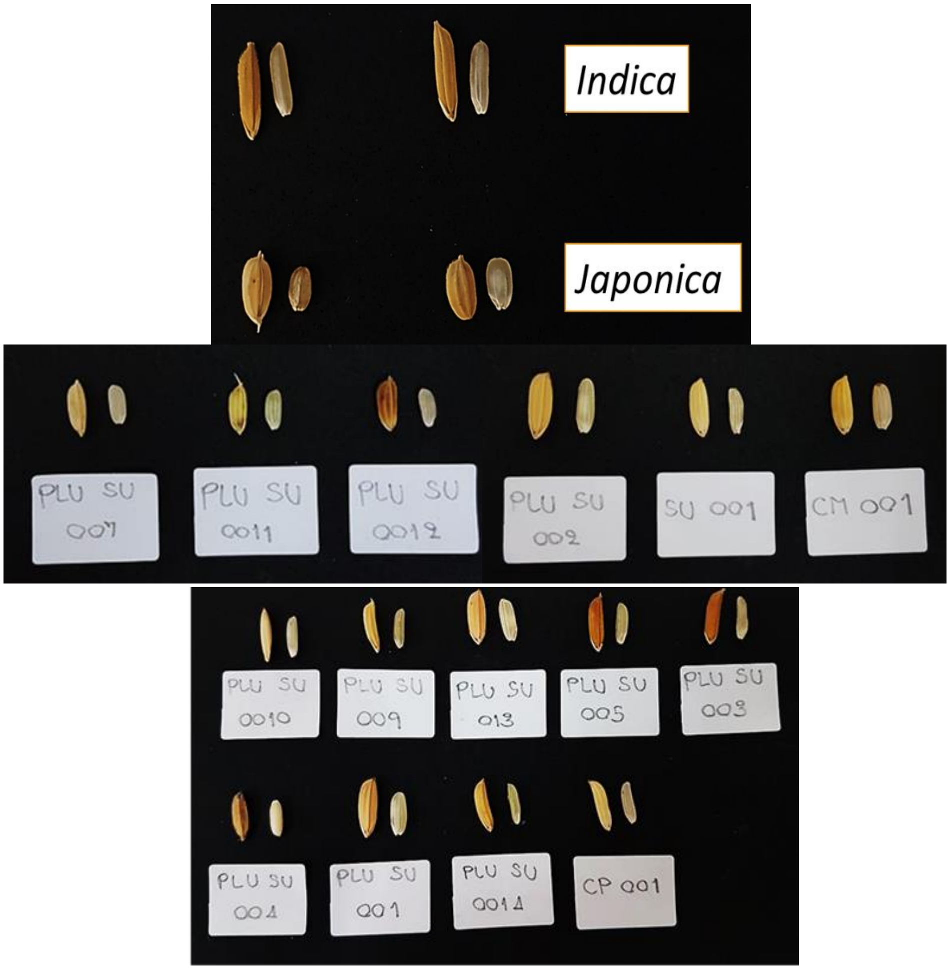

**Figure 2.** Grain morphology of upper row**:** *indica* **(**CN1, PTT1**)** and *japonica* **(**K**.**Y**.**P**.**1 and K**.**Y**.**P**.**2**)**, middle row**:** six accessions that identified as *indic*a or *japonica* rice **(**PLU-SU-007, PLU-SU-0011, and PLU-SU-0012, identified as *indica* rice and PLU-SU-002, SU-001, and CM-001, identified as *japonica* rice**)**, lower row**:** nine accessions that are closely related to *japonica* rice

## Discussion

In the past, morphology of seed grain, and ratio between length and width has been used to classify different types of rice (Cheng *et al*., 1984; Lu *et al*., 2009). Classification based on the ratio of seed length/width has already been reported by Chang (1985). Both *indica* and *japonica* were classified according to this scheme with the ratio of *indica* being between 3.1-3.5 to >3.5, and that of *japonica* <2.0-2.1-2.5. In this study, known *indica* rice varieties (PTT1 and CN1) showed different agronomic traits from those of *japonica* (K.Y.P.1 and K.Y.P.2). Specifically, the length/width grain ratio of *indica* rice varieties (PTT1 and CN1) was approximately double (4.995-5.246) than that of *japonica* accessions (K.Y.P.1 and K.Y.P.2) (2.242-2.315). Grain thickness of both *indica* and *japonica* ricevarieties was similar when compared to other morphological traits examined in this study. Thickness of grain of *indica* rice was 2.00-2.01 mm, which was lower than that of *japonica* rice at 2.12-2.23 mm. The findings resemble those of Lu *et al*. (2013), who reported grain thickness of 2.0 mm and 2.2 mm in *indica* and *japonica* rice varieties, respectively. The results have demonstrated that seed morphology, especially grain length/width ratio and gain thickness can clearly categorize *indica* and *japonica* rice varieties.

Nonetheless, a wide range of morphological variation was found among 15 upland rice accessions in this study. These data suggested that the observed phenotypic characteristics were more due to environmental variables rather genetics (Lu *et al*., 2009). For instance, the seeds of the upland rice accessions CP-001, PLU-SU-003, PLU-SU-009, PLU-SU-0010 and PLU-SU-0014, greater grain length and higher grain length/width ratio (Table 4). Thus, these five accessions had long-slender seeds when compared with the other accessions, and were related to *indica* rice. Although the CM-001, SU-001, PLU-SU-002 and PLU-SU-0013 rice accessions had bold seed, they had higher grain width than other accessions. Their grain length and length/width ratio ranged between 2.90-3.28 was not the lowest among other accession. Thus, it was obscured to classify these 15 accessions as *indica* or *japonica* by considering only grain length, grain width, and grain length/width ratio. As for a hundred grain weight, only two upland rice accessions including CM-001 and PLU-SU-013 were closely related to *japonica* rice. These data suggested that the grain morphology among 15 accessions is widely variable and cannot be used to clearly classify rice varieties as *indica* or *japonica*.

Rice morphology is subject to ecotype or cultivating areas, which include temperate regions with high altitude, low altitude, or mountainous regions with high elevation (Cheng, 1993). Combination of physiological and biochemical characteristics has been used to distinguish between the ecotypes *indica* and *japonica* (Lu *et al*., 2009). “Chen’s index” is well-known for classifying between *indica* and *japonica* rice by using morphology, physical traits, and isozyme analysis. Specific characters used for classification according to “Chen’s index” include lemma hairiness, phenol response in rice grains, inter-node length of panicle axes, color of grain husks, hairiness of leaf-blades, and grain length/width ratio (Morishima and Oka, 1981; Cheng *et al*., 1984;

Glaszman, 1987). However, these characteristics are also variable due to environmental effects, resulting in inaccurate and inconsistent data (Lu *et al*., 2009). For instance, overlap in the morphology of the two ecotypes of *indica* and *japonica* has been observed during some growing seasons (Sato, 1985; Oka, 1988; Cheng, 1993).

Recently, other characters such as biochemical traits, shattering pattern, lodging resistance or metabolic variation have been applied to classify these two ecotypes as well (Garris *et al*., 2005; Hu *et al*., 2014; Yang *et al*., 2014; Zhang *et al*., 2016; Okubo, 2014). Given that some morphological characteristics that are used to identify ecotypes are present during the reproductive stage of rice, this method is considered as time-consuming. Moreover, since the environment greatly influences the morphology of *O. sativa*, morphological identification is considered as inconsistent and less accurate.

Due to the limitations of exclusively using character traits, molecular markers were applied in this study in an attempt to identify 15 upland rice accessions. Using InDel molecular markers showed some notable results. Three accessions (PLU-SU-007, PLU-SU-0011, and PLU-SU-012) had clear genetic proximity with PTT1 and CN1. Thus, these accessions were characterized as *indica* type of rice. Surprisingly, these three accessions didn’t stand out as *indica* when grain morphological traits were considered. Similarly, the CM-001, SU-001, and PLU-SU-002 accessions were closely related to *japonica* varieties (K.Y.P.1 and K.Y.P.2). Therefore, these three accessions were characterized as *japonica* type of rice. Compared with the results obtained from classifying using grain morphological traits, only SU-001 and PLU-SU-002 had bold grain, whereas CM-001 did not.

The rest of the accessions (9 in 15 accessions; CP-001, PLU-SU-001, PLU-SU-003, PLU-SU-004, PLU-SU-005, PLU-SU-009, PLU-SU0010, PLU-SU-0013, and PLU-SU-0014) did not group as either *indica* or *japonica*. Although these nine upland rice accessions were not identified clearly, they were more genetically related to *japonica* rather than *indica* (Figure 1). In support of this, the PLU-SU-003, PLU-SU-009, PLU-SU-0010 and PLU-SU-0014 accessions had grain morphological characteristics matching *indica* rice varieties.

The CM-001, SU-001, PLU-SU-002, and PLU-SU-013 accessions had morphological characteristics of *japonica* rice having thicker seed grain. Using InDel markers, three accessions were identified as *japonica*, while one was closely related to it. However, *grain* thickness was not able to distinguish *indica* from other accessions that were closely related to *japonica*. These data suggested that traits of seed morphology were not sufficient to identify type of rice in this study, while use of InDel markers was an efficient method.

This study found that the accessions were not clearly categorized but were determined by InDel molecular study data. Due to InDel markers having been developed from either insertions or deletions in genome sequences (Lu *et al*., 2009), the observed ambiguous grouping could mean gradual genetic changes of these upland rice accessions. Although rice is a self-pollinating crop, it also shows natural crossing (less or up to 3 percent of outcrossing), depending on genetic and environmental factors (De Silva *et al*., 2005). Moreover, planting behavior and field care in highland areas may support crossbreeding. For example, this woud be the case when cultivating different varieties of field rice in the same area for different uses over a long period of time. Another case when there is residual yield in the field that can contaminate the next cropping, which in turn can promote genetic flow between cultivated and contaminated varieties (Mariam *et al*., 1991). This study has demonstrated high genetic diversity of upland rice in Thailand. More importantly, besides *japonica* and *indica*, there is possibility of other rice varieties as shown in the nine accessions collected from Prachuap Khiri Khan. These accessions are genetically distant from *indica* rice, but at the same time they are not identical to *japonica* either. This could be explained by the geographical location of Prachuap Khiri Khan, which borders Burma. Crossover between upland rice from Burma and Thailand could have occurred resulting in inter-subspecies hybridization between *indica* and other rice varieties. Thus, selection for pure lines/varieties before genetic improvement and promotion of either field or seed management is of great importance.

Compared to the traditional morphological method, using InDel molecular markers was confirmed as efficient (less time consuming, easy and accurate) for classifying between *indica* and *japonica* rice varieties (Lu *et al.*, 2009). Interestingly, Laosutthipong *et al*. (2019) also reported that based on DNA sequence of lignin biosynthesis genes (OsPAL and Os4CL3), the PLU-SU-001, PLU-SU-002, PLU-SU-003, PLU-SU-004, PLU-SU-005, NYP-SU-001 or SU-001 and CM-001 upland rice accessions were more similar to *japonica* than to *indica* rice. These results correspond to this study, in which most accessions (7 accessions) are related to *japonica* rather than *indica* rice. However, the identification by DNA sequence of OsPAL and Os4CL3 is not cost effective when compared to InDel markers. Thus, this study confirms that InDel marker is efficient and cost-effective method for classifying ecotypes of *O. sativa*.

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