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Contributed Paper

Genetic Diversity of Thai Upland Rice Germplasm Based on Inter-Simple Sequence Repeats Marker

Kajonphol Tanaporn [a], Nontakod Sujaree [a] and Sangsiri Chontira*[b]

[a] Faculty of Natural Resources and Agro-Industry, Kasetsart University, Chalermphrakiat Sakon Nakhon Province Campus, Sakon Nakhon 47000, Thailand

[b] Agricultural Sciences Program, Mahidol University, Kanchanaburi Campus, Kanchanaburi 71150, Thailand

*Author for correspondence; e-mail: chontira_sangsiri@yahoo.com

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ABSTRACT

Upland rice is an important plant genetic resource for rice breeding programs due to the high level of genetic diversity with inter-simple sequence repeats (ISSR), drought tolerance and resistance to diseases and pests in Thailand. The aim of this study was to investigate the genetic diversity of local rice at the molecular level using ISSR markers. The ISSR technique is simple based on high polymorphism in the genetic background. Fifty-four local rice accessions were genetically analyzed based on ISSR markers. All accessions were collected from upland areas in 14 provinces of Thailand. Thirty-seven primers were selected with 10 being high polymorphic primers. Ninety-six alleles were found in all germplasms with 53 polymorphic bands (55.35%). The ISSR841 primer had the highest number of bands (13), while ISSR834, ISSR835, ISSR841 and ISSR864 had the lowest number of bands (8). The similarity index was in the range 0.84-0.99. Clustering analysis of local rice using the unweighted pair group method with arithmetic mean produced two main groups. The matrix correlation was 0.86. This research should be useful for evaluating rice germplasms from various areas and can be used as a database for rice breeding programs in the future.

Keywords: genetic diversity, inter-simple sequence repeats (ISSR) marker, local rice

1. INTRODUCTION

Rice is an important cereal crop of the world and it is a major crop in Thailand. The genetic diversity of rice is due to its high polymorphism and its adaptability to grow in all types of terrain including arid areas, flood plains or on mountains. Local rice is a good source of some characters such as disease, pests and environmental resistance which can be used in rice breeding programs to obtain a high yield, high quality or other requirements.

Rice diversity can be classified by the chemical components in the grain or it can rely on morphological characteristics such as height, seed size, seed shape, seed color, days to flowering and harvesting date. The morphological characters are variable traits based on the environment and can involve complex types for analyzing between species [1]. Accurate measurement at the molecular genetic level can be used to classify rice varieties.

The molecular genetic level of local rice varieties provides a good opportunity to identify the genetic relationship of rice varieties based on different kinds of genetic markers because

it is accurate and precise. The current research was conducted using inter-simple sequence repeats (ISSR) markers which involves using microsatellites to increase the amount of DNA in the polymerase chain reaction (PCR). The ISSR technique can replicate in a highly efficient manner the polymorphic markers and has even greater potential for the identification on plant species or genetic diversity [2].

There have been several reviews on applications of ISSR markers in rice. [3] studied the genetic diversity of three wild rice populations and three cultivated rice varieties of Ethiopia. All samples were studied using inter simple sequence repeats (ISSRs) markers. The clustering contained six groups based on populations of origin. All the clustering analysis showed the Ethiopian wild rice was different from cultivated rice. [4] studied the genetic diversity of 27 landraces of Hassawi rice in Saudi Arabia, with the analysis of all accessions based on the ISSR molecular markers. The results revealed 11 of 14 primers were polymorphic markers. The average polymorphism based on 11 primers was more than 75%. The clustering could be divided into two distinct groups. These results indicated that ISSR analysis is efficient for studying genetic diversity.

The genetic information will be useful in many aspects including in rice fields to develop rice varieties. The purpose of this study was to study the genetic diversity of newly collected Thai local rice at the molecular level using ISSR markers. The results that will identify genetic diversity in local rice varieties should be useful for rice database breeding programs in the future.

2. MATERIALS AND METHODS

2.1 Plant Materials

Fifty-four accessions were collected from 14 provinces within three regions of Thailand (Figure 1, Table 2 and Table 3). All seeds were planted over 3 years and collected as pure lines of each accession. Thirty seeds of pure lines were planted in plastic boxes. DNA extraction was tested at 2 weeks after sowing at a seedling height between 10 cm and 15 cm.

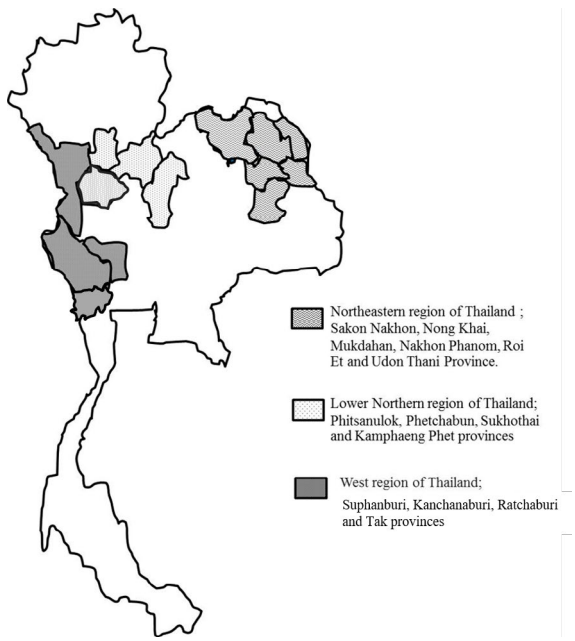

Figure 1. Collection sites of upland rice fields in 14 provinces of Thailand.

2.2 Extraction of Genomic DNA

Fresh leaves of 54 selected local rice accessions were extracted for genomic DNA using the modified CTAB method [5]. One gram of each leaf sample was ground with liquid nitrogen in a mortar. Then 10 mg of leaf powder were separated in a 1.5 ml micro centrifuge tube and 600 µl of extraction buffer containing 2% CTAB, 100mM Tris-HCl (pH8.0), 200mM EDTA (pH8.0), 1.4M NaCl, and 1% PVP were added and vortexed. The tubes were incubated in a water bath at 65 °C for 1 hr after which 600 µl of chloroform:isoamyl alcohol (24:1) were added and the tubes were gently inverted to mix the aliquot. The tubes were centrifuged at 4 °C and 13,000×*g* for 30 min. Then, 600 µl of the aqueous layer was transferred into a new tube and 500 µl of chilled absolute ethanol were added. The DNA was pelleted using centrifugation at 4 °C and 12,000×*g* for 10 min. The DNA pellet was washed twice using 300 µl of 70% ethanol. The supernatant was discarded and the DNA pellet was dried at room temperature and then dissolved in 50 µl of TE plus RNAse buffer (99 µl TE + 1 µl RNAse (10mg/ml)). The DNA qualification was checked using 0.8% agarose gel electrophotoresis and comparing with the intensity of λ DNA and diluted DNA concentration of 10ng/µl for ISSR analysis.

2.3 ISSR Analysis

Thirty-three ISSR primers were initially screened and 10 primers were selected among these that had clear reproducibility and polymorphism for the germplasm study (Table 1). The PCR amplifications were carried out with 20 µl of reaction mixture containing 20 ng genomic DNA, 1× PCR buffer, 15mM $MgCl_2$, 10mM dNTPs, 0.2µM primer and 0.5U of Taq polymerase enzymes. The PCR conditions were initial denaturation at 95 ºC for 5 minutes, followed by 35 cycles of 94 ºC for 60 s, 48-52 ºC for 60 s, 72 ºC for 2 min and final extension at 72 ºC for 15 min and a temperature step down to 4 ºC. Agarose gel electrophoresis was carried out at 75 V for 90 min with 1× TBE buffer on 1.5 % agarose gel. The gel was stained with a solution of ethidium bromide for 15 minutes. After that, photographs were taken using ultraviolet light and the DNA size were confirmed using a 1kb DNA ladder.

2.4 Data Analysis

The DNA banding pattern was scored as 1 for the presence and 0 for the absence of a band. The ISSR binary data matrix was used with the Jaccard similarity coefficient [6]. Cluster analysis was grouped based on similarity using UPGMA (unweighted pair group method using arithmetic average). The dendrogram was generated using the SAHN program (sequential agglomerative hierarchic non-overlapping clustering). Cophenetic correlation and bootstrapping (10,000 replications) were performed to check the fit of the dendrograms obtained using the NTSYS-pc version 2.1e. [7]. The polymorphic information content (PIC) value of each marker was calculated based on the formula as PIC = 1-Sp_i^2, where p_i is the frequency of the *i*th allele of the locus in the set of the 54 rice genotypes was calculated according to the formula of [8]. The resolving power (Rp) was calculated according to the formula, Rp =SI_b, where I_b is informative band and $I_b = 1-[2 \times |0.5-p|]$, where *p* is the proportion of genotypes containing the band [9].

3. RESULTS AND DISCUSSION

The genetic diversity of Thai local rice accessions by using the 33 ISSR primers, selected 10 primers gave the good productivity and which produced total 96 bands with 53 polymorphic bands.

The DNA product from the PCR was separated using agarose gel electrophoresis. DNA fragments were obtained from 250 to 1,750 base pairs. The dendrogram obtained based on ISSR data produced similar results, separating the rice germplasms studied. The UPGMA tree with the highest cophenetic correlation (r = 0.86) was identified and classified the genetic diversity of

Table 1. Primer name, primer sequence, number of bands, percentage of polymorphic bands, polymorphic information content (PIC) and resolving power of 10 polymorphic primers.

Primer Name	Sequence (5' -> 3')*	Number of bands	Number of polymorphic bands	% of polymorphism	PIC	Resolving power (Rp)
ISSR009	TCT CTC TCT CTC TCT CTC G	10	7	70	0.492	11.29
ISSR010	CCT CCT CCT CCT CCT CCT A	9	6	66.67	0.497	9.67
ISSR812	GAG AGA GAG AGA GAG AA	12	6	50	0.282	12.00
ISSR822	TCT CTC TCT CTC TCT CA	9	9	100	0.490	10.22
ISSR834	AGA GAG AGA GAG AGA GYT	8	5	62.5	0.455	11.85
ISSR835	AGA GAG AGA GAG AGA GYC	8	2	25	0.398	6.14
ISSR840	GAG AGA GAG AGA GAG AYT	10	7	70	0.333	15.78
ISSR841	GAG AGA GAG AGA GAG AYC	13	5	38.46	0.195	6.67
ISSR859	TGT GTG TGT GTG TGT GRC	9	3	33.33	0.210	7.89
ISSR864	ATG ATG ATG ATG ATG ATG	8	3	37.5	0.163	7.07
Average				**55.35**	**0.3515**	

*Y = (C, T); R = (A, G)

the Thai rice germplasms into main two groups. The Jaccard similarity coefficient ranged from 0.84 to 0.99 among these germplasms (Figure 4). The ISSR primers used in this study were composed of di- and tri-nucleotide repeats sequences. The results revealed varying primers showing different levels of polymorphism. The ISSR841 primer had the highest allele number with 13 bands. The ISSR834, ISSR835 and ISSR864 primers had the lowest of band number with 8 bands. The polymorphism for each ISSR primer is presented in Table 1. Five ISSR primers produced high levels of polymorphism: ISSR822 (100%), ISSR009 (70%), ISSR840 (70%), ISSR010 (66.67%) and ISSR834 (62.5%). This was in agreement with [4], [9] and [10] who reported the percentage of polymorphic bands ranged between 60 and 100 in high level polymorphism primers. In addition, [11] reported that dinucleotides repeats had the highest average polymorphism rate at 84.4%. Others similar reports, [10] and [12] revealed the highest average polymorphism rate at 85.71% and 82.96%. The high levels of polymorphism bands in each primer were due to the tandem motif repeats. The current results were in agreement with [4], [9] and [12] that the primers based on dinucleotides $(GA)_n$, $(AG)_n$ and $(TC)_n$ produced a high level of polymorphism, especially in rice [12] and wheat [9]. The current results supported [4] who used 11 selected primers to estimate the genetic diversity among landraces of rice. Moreover, [10] reported the analysis of the genetic relationship among forty-six Indo-China rice varieties using 17 selected ISSR primers. In addition, [11] reported that dinucleotide primers were more suitable for estimating genetic rice diversity. ISSR markers based on $(AG)_n$ and $(GA)_n$ have been reported to be very informative and cost-effective for determining genetic relationships among diverse accessions of rice germplasms [13] and repeats of the $(GA)_n$ dinucleotide is the most abundant in plant species [14]. For the Thai population, our results agreed with [15] who analyzed 90 rice genotypes including 65 accessions of *O. rufipogon*, 9 accessions of *O. nivara*, 2 accessions of *O. longistaminata* and 14 accessions of cultivated rice which included

Table 2. Accession number, local name, collection location and region of Thailand of local rice clustered in group I.

Group	Cluster	Accession	Local name	Collection location	Region of Thailand
I	I-a	Rice015	Hom rai	Bong Ti Village, Bong Ti Sub District, Saiyok District, Kanchanaburi Province, Thailand	Western Thailand
I	I-b	Rice100	Khao Boa	Mae Kra Bung Sub District, Si Sawat District, Kanchanaburi Province, Thailand	Western Thailand
I	I-b	Rice234	Bue Serna (jamook dam)	Mai Wang Yaw Village, Wang Yaw Sub District, Dan Chang District, Suphan Buri Province, Thailand	Western Thailand
I	I-b	Rice314	Khao Nieow khaw	hek Noi Sub District, Khao Kho District, Phetchabun Province, Thailand	Lower Northern Thailand
I	I-b	Rice325	Khao Nieow khaw	Huai Hia Sub District, Nakhonthai District, Phitsanulok Province, Thailand	Lower Northern Thailand
I	I-b	Rice336	Khao Bai tok (Khao rai)	Tha Kradan Sub District, Si Sawat District, Kanchanaburi Province, Thailand	Western Thailand
I	I-b	Rice145	Khao Chao Pleak Deang	Hui Sai Village, Noen Phoem Sub District, Nakhon Thai District, Phitsanulok Province, Thailand	Lower Northern Thailand
I	I-b	Rice394	Khao Nieow Dam (Phu Kho)	Mae Sam Sub District, Si Satchanalai District, Sukhothai Province, Thailand	Lower Northern Thailand
I	I-b	Rice246	Khao Lay	Mai Wang Yaw Village, Wang Yaw Sub District, Dan Chang District, Suphan Buri Province, Thailand	Western Thailand
I	I-b	Rice172	Prae sawan	Muang Sub District, Sangkhom District, Nong Khai Province, Thailand	Upper Northeastern Thailand
I	I-c	Rice235	Leaung Tong	Mai Wang Yaw Village, Wang Yaw Sub District, Dan Chang District, Suphan Buri Province, Thailand	Western Thailand
I	I-c	Rice322	Khao Hom Doi	Noen Phoem Sub District, Nakhonthai District, Phitsanulok Province, Thailand	Lower Northern Thailand

Table 2. (Continued).

Group	Cluster	Accession	Local name	Collection location	Region of Thailand
I	I-c	Rice323	Khao Stern Nam Stern Bok	Noen Phoem Sub District, Nakhonthai District, Phitsanulok Province, Thailand	Lower Northern Thailand
I	I-c	Rice392	Khao Doi	Chiang Thong Sub District, Wang Chao District, Tak Province, Thailand	Western Thailand
I	I-c	Rice354	Hom Mali Doi	Khao Kho Sub District, Khao Kho District, Phetchabun Province, Thailand	Lower Northern Thailand
I	I-c	Rice250	Khao Nieow Dam	Mai Wang Yaw Village, Wang Yaw Sub District, Dan Chang District, Suphan Buri Province, Thailand	Western Thailand
I	I-c	Rice335	Khao Nieow dam (khao rai)	Tha Kradan Sub District, Si Sawat District, Kanchanaburi Province, Thailand	Western Thailand
I	I-c	Rice345	Khao Nieow Dam	Tha Kradan Sub District, Si Sawat District, Kanchanaburi Province, Thailand	Western Thailand
I	I-c	Rice251	Khao Nieow Dam	Hui Din Dam Village, Wang Yaw Sub District, Dan Chang District, Suphan Buri Province, Thailand	Western Thailand
I	I-c	Rice313	Khao dam	Khao kho Sub District, Khao Kho District, Phetchabun Province, Thailand	Lower Northern Thailand
I	I-c	Rice352	Khao Nieow Dam	Mae Sam Sub District, Si Satchanalai District, Sukhothai Province, Thailand	Lower Northern Thailand
I	I-c	Rice327	Khao Nieow Dam	Khao Kho Sub District, Khao Kho District, Phetchabun Province, Thailand	Lower Northern Thailand
I	I-c	Rice320	Khao Dam	Khek Noi Sub District, Khao Kho District, Phetchabun Province, Thailand	Lower Northern Thailand
I	I-c	Rice351	Khao Pleuak Dam	Chong Khaep Sub District, Phop Phra District, Tak Province, Thailand	Western Thailand

Table 2. (Continued).

Group	Cluster	Accession	Local name	Collection location	Region of Thailand
I	I-c	Rice347	Khao Khaw	Chiang Thong Sub District, Wang Chao District, Tak Province, Thailand	Western Thailand
I	I-c	Rice376	Khao Nieow dam	Pong Nam Ron Sub District, Khlong Lan District, Kamphaeng Phet Province, Thailand	Lower Northern Thailand
I	I-c	Rice355	Khao Nieow Dam	Khirirat Sub District, Phop Pra District, Tak Province, Thailand	Western Thailand
I	I-c	Rice379	Khao Doi (Khao chao)	Tambon Mae Sam, Si Satchanalai District, Sukhothai Province, Thailand	Lower Northern Thailand
I	I-c	Rice380	Khao Nieow dam	Chiang Thong, Wang Chao District, Tak Province, Thailand	Western Thailand
I	I-c	Rice381	Khao Khaw (Khao Chao)	Chiang Thong, Wang Chao District, Tak Province, Thailand	Western Thailand
I	I-c	Rice382	Khao Hom Dong (Khao Chao)	Pong Nam Ron Sub District, Khlong Lan District, Kamphaeng Phet Province, Thailand	Lower Northern Thailand
I	I-d	Rice096	Beung-E-Wong Bong	Kong Mong Ta Village, Nong Loo Sub District, Sangkhla Buri District, Kanchanaburi Province, Thailand	Western Thailand
I	I-d	Rice066	Beung Wong Song	Kong Mong Ta Village, Tambon Nong Loo Sub District, Sangkhla Buri District, Kanchanaburi Province, Thailand	Western Thailand

Table 3. Accession number, local name, collection location and region of Thailand of local rice clustered in group II

Group	Cluster	Accession	Local name	Collection location	Region of Thailand
II	I-a	Rice083	EI-Leaw	Kong Mong Ta Village, Nong Loo Sub District, Sangkhla Buri District, Kanchanaburi Province, Thailand	Western Thailand
II	I-a	Rice243	Khao Pae	Hui Din Dam Village, Tambon Wangyaw, Dan Chang District, Suphan Buri Province, Thailand	Western Thailand
II	I-a	Rice217	Khao Pleuak Dam	Nongwang Village, Chai Wan Sub District, Chai Wan District, Udon Thani Province, Thailand	Upper Northeastern Thailand
II	I-a	Rice148	HangYee	Tha Kon Village, Tha Kon Sub District, Akat Amnui District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-a	Rice173	Khao maekung	Muang Sub District, Sangkhom District, Nong Khai Province, Thailand	Upper Northeastern Thailand
II	I-a	Rice 177	Krasean	Tao Ngoi Sub District, Tao Ngoi District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-a	Rice183	Khao Kam	Bead Village, Dong Luang Sub District, Dong Luang District, Mukdahan Province, Thailand	Upper Northeastern Thailand
II	I-a	Rice212	E-deang	Na Luk Village, Pung Deang Sub District, Dong Luang District, Mukdahan Province, Thailand	Upper Northeastern Thailand
II	I-a	Rice370	E-pee (Khao Nieow)	Mae Tuen Sub District, Mae Ramat District, Tak Province, Thailand	Western Thailand
II	I-a	Rice208	E-deang	Huai Yang Sub District, Muang District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice146	Khao Lao Teak	Akat Amnui village, Akat Amnui Sub District, Akat Amnui District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice202	Hang yee	Huai Yang Sub District, Muang District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand

Table 3. (Continued).

Group	Cluster	Accession	Local name	Collection location	Region of Thailand
II	I-b	rice197	Ton Mey Khoa	Ban Na PhiangKao, Kusuman District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice158	E-khaonoi	Nong Din Dam Village, Na Hua Bo Sub District, Phannanikhom District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice187	Ban wern	Na Neua Village, Phra Song Sub District, Nakae District, Nakhon Phanom Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice188	Kang Loahug	Seangsoa Village, Ban Ueang Sub District, Si Songkhram District, Nakhon Phanom Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice147	E-noi	Nayo Village, Akat Amnui Sub District, Akat Amnui District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice194	Maledsin	Ban Na Seang, Selaphum District, Roi Et Province, Thailand	Upper Northeastern Thailand
II	I-b	Rice168	Khao nisoun	Phon Pheng Sub District, Akat Amnuai District, Sakon Nakhon Province, Thailand	Upper Northeastern Thailand
II	I-c	Rice192	Leo	Ban Kho Sub District, Phon Sawan District, Nakhon Phanom Province, Thailand	Upper Northeastern Thailand
II	I-c	Rice372	Khao Yang Jer (Khao Chao)	Pong Nam Ron Sub District, Khlong Lan District, Kamphaeng Phet Province, Thailand	Lower Northern Thailand

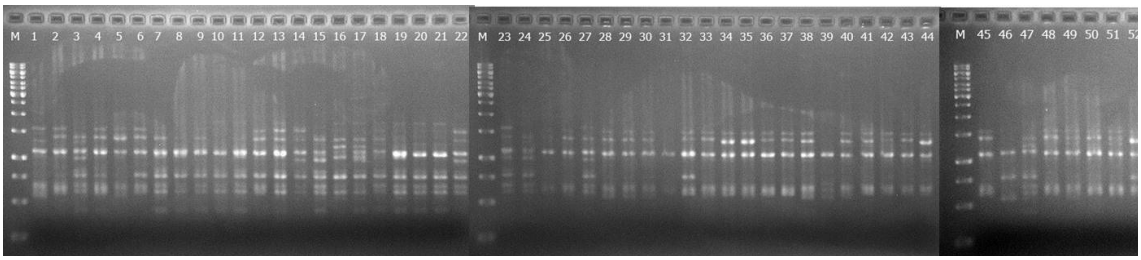

Figure 2. DNA band pattern of ISSR009 primer under UV light after staining with ethidium bromide compared with 1kb DNA Ladder (M lane = λDNA marker) (1-54 lanes = rice accession numbers).

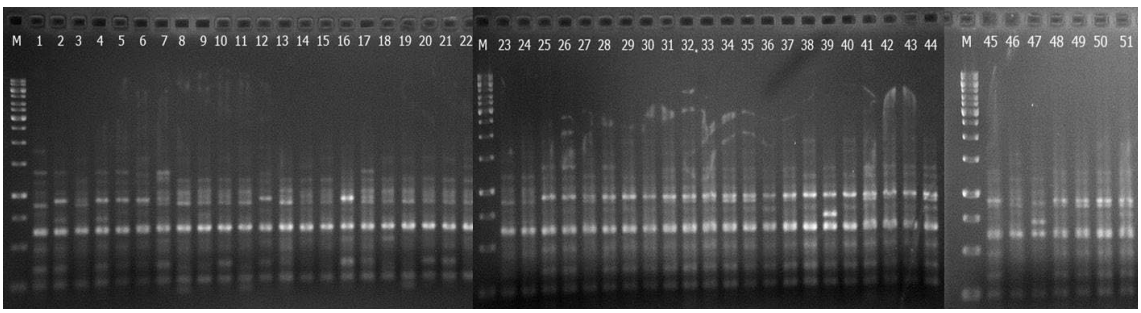

Figure 3. DNA band pattern of ISSR840 primer when illuminated with UV light after staining with ethidium bromide compared with 1kb DNA Ladder (M lane = λDNA marker) (1-54 lanes = rice accession numbers).

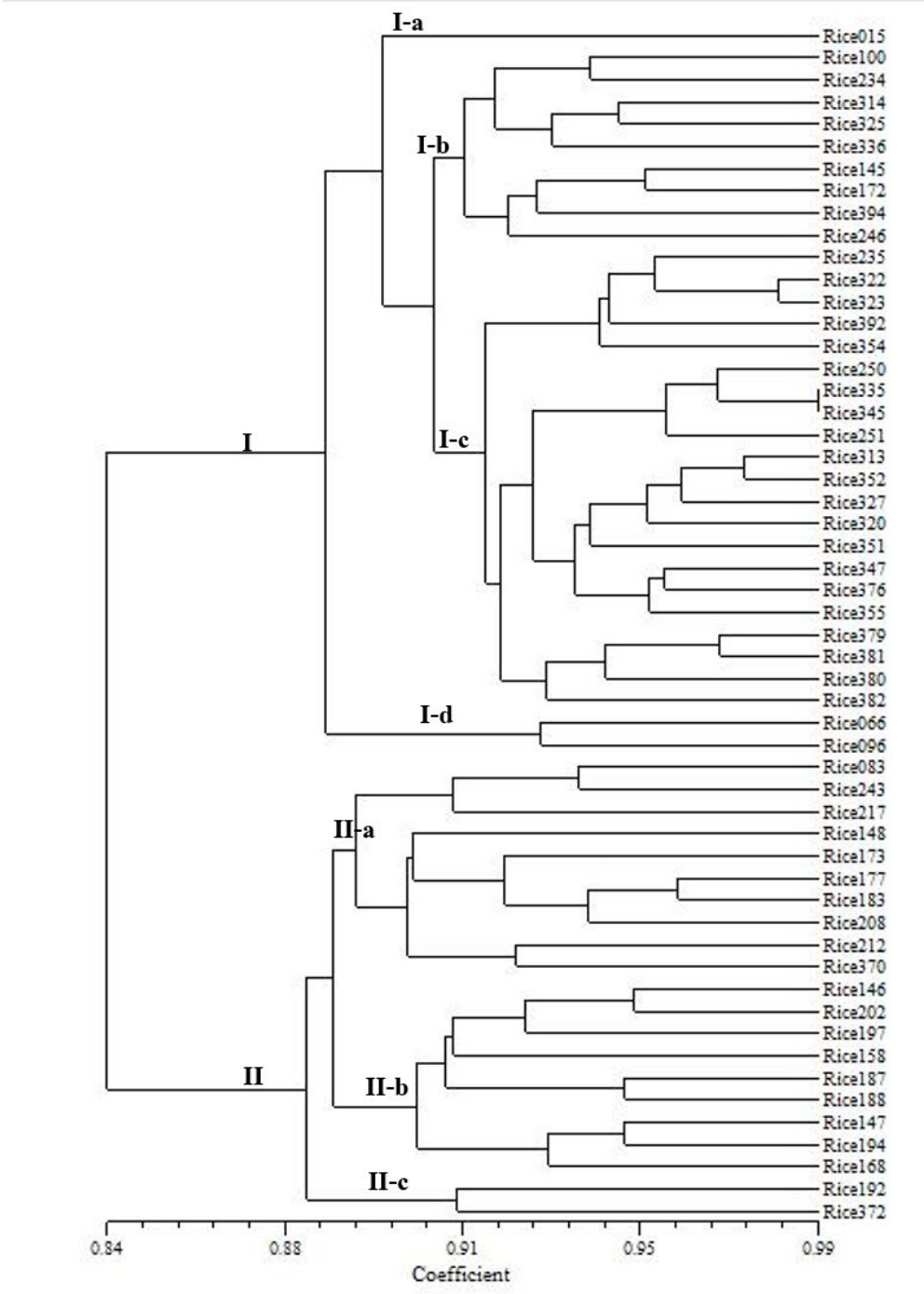

Figure 4. Genetic diversity of 54 upland rice accessions analyzed using UPMGA based on 10 ISSR primers.

5 subspecies consisting of indica (IR64, Swarna, Rasi and 9314), temperate japonica, (Nipponbare and Taipei 309), tropical japonica, (BSI115 and Moroberekan), aus (N22, FR13A and Dular), and aromatic (Basmati 370, Type 3 and CSR30). They reported a dendrogram based on the 17 ISSR primers separated all genotypes into 4 major clusters with a genetic similarity of 53%–100%. Six primers (UBC-809, UBC-812, UBC-842, UBC-840, UBC-885 and UBC-834) based on AG and GA repeats with high Rp values were highly informative. In addition, [10] who used ISSR marker to separate forty-six Indo-China cultivars into three major groups at a similarity index value of 0.659. The genetic similarity was lower than this report, which may be due to the collection of the germplasm from larger geographical area throughout the Indo-China.

The polymorphic information content (PIC) index has been used for primers in genetic diversity. The PIC value of markers indicates the usefulness of DNA markers for plant breeding and germplasm evaluation [11]. In the current study, the PIC value of 10 selected primers ranged from 0.163 to 0.497 with an average of 0.3315 (Table 1). Among all the primers, ISSR010 produced the highest polymorphic information content (PIC value of 0.497), while the lowest PIC value was for ISSR864 (0.163), as shown in Table 1. This result was in agreement with [9-10] and [12-13] who determined genetic diversity and phylogenetic relationships using ISSR primers with PIC values ranging from 0.13 to 0.42, 0.18 to 0.70, 0.0-0.498 and 0.07-0.38 respectively.

The estimates of resolving power (Rp) of each primer ranged from 6.14 to 15.78. The highest Rp was for the primer ISSR840 (15.78) followed by ISSR834 (11.85) and the lowest Rp value was for ISSR835 (6.14), as shown in Table 1. Similar results were reported by [13] with the Rp values of the ISSR primers analyzed in rice having a range of 3.8 to 14.3. The current results were also in agreement with [9] who analyzed genetic diversity in wheat cultivars and breeding lines using ISSR primers, with Rp values ranging from 7.2 to 16.5 and ISSR834 and ISSR840 recording high values (15.7 and 14.9, respectively).

The information obtained in the current work is highly informative for genetic studies as the genotypes used in the current study were more diverse due to differences in origin, ecotype and speciation [16]. It was observed that markers with a lower number of alleles showed lower gene diversity than those with a higher number of alleles that showed higher gene diversity. Thus, this approach can be regarded as a powerful tool to understand genetic variation in rice cultivars [10], [12] and [17].

3.1 Analysis of Genetic Relationships

The genetic relationships of 54 local rice accessions were calculated using UPGMA. The Jaccard similarity index was 0.84 to 0.99. We found the two major groups with different genetic diversity (Figure 4).

3.2 Intra-genetic Diversity of Novel Thai Upland rice

Analysis of the genetic relationships between Thai local rice accessions based on UPGMA identified two groups: group I had 33 local rice accessions and group II had 21 local rice accessions. The genetic relationship of the local accessions of each group revealed they were related to topographical slope-complex and upland areas based on in-situ conservation of rice genetic resources.

The intra-genetic structure of the local rice accessions revealed that most of the accessions in group I were located in the western and lower northern regions of Thailand. The 33 accessions in group I were in-situ cultivated on highland areas and in villages (Figure 4 and Table 2). The similarity coefficients within populations were in the range from 0.88 to 0.99. In group I, cluster I-c had 11 purple and black rice seed germplasms with a similarity coefficient value of 0.92. The highest genetic relationship within the cluster had the local name of Khao Nieow Dam (sticky rice with black

seed) closely located in Western Thailand (Rice IDs 250, 335, 345, 251, 351, 347 355 and 380) and closely located in Lower Northern Thailand (Rice IDs 313, 352 and 327), as shown in Table 2. However, group I, Rice 015 in cluster I-a and Rice IDs 096 and 066 in cluster I-d were located in a distant valley of Karen rice in Bong Ti village of Saiyok district and Kong Mong Ta village of Sangkhla Buri district, Kanchanaburi province in Western Thailand, which suggested that these rice germplasms had adapted to climate and could be characterized by strong adaptability to drought, soli salinity and genetic variation outspread in the germplasms. This was consistent with [18] who reported on the population genetic structure of a single variety of landrace rice, Bue Chomee, cultivated by the Karen people of Thailand based on microsatellite markers that revealed a high level of genetic variation despite predominant inbreeding in the crop.

Group II consisted of 21 accessions commonly located in the upland areas in Northeastern Thailand (Figure 4). The Jaccard similarity coefficient within this population was in the range 0.88 to 0.99 (Figure 4 and Table 3). In this group, the germplasms were closely located as in group I. This was named the Khao Bao group (short group duration rice), as shown in Table 3. This result is supported by the report from [12] who revealed that even though this group of rice lines had the same name but they were not genetically identical and they were collected and maintained lines at Thailand Rice germplasm bank from different parts of Thailand. Our result was perhaps the result of the population genetic structure reported in [18] where landrace rice was cultivated in a dynamic genetic system that responded to evolutionary forces, both natural and those imposed by humans.

The current results overall showed the usefulness of ISSR primers to analyze collected Thai rice germplasm. In the current study, the markers revealed that $(AG)_n$, $(GA)_n$ and $(TC)_n$ had high polymorphism. The results suggested that the diversity of the collected Thai rice germplasm samples was associated with their isolation so that they could be separated into two groups. The accessions in group I were determined as an upland rice landrace, growing in valleys and on slope-complexes, while the accessions in group II had adapted to local rice germplasms "Khao Bao (the local name)" meaning a short growth duration lines. Thai rice germplasms were characterized by strong adaptability to drought and soil properties perhaps due to being forced to adapt using a dynamic genetic system to suitable cultivation areas imposed by human management. This research should be useful for evaluating local rice varieties from various areas and can be used as a database for rice breeding programs in the future [19].

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