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Nutritional composition and genetic diversity of Thai Aromatic Rice landraces

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Summary

Fifty Thai aromatic rice landraces and one commercial cultivar (KDML 105) were subjected to proximate nutritional analysis to determine protein, fat, fiber, carbohydrate, ash, moisture, amylose and 2AP contents. Genetic diversity was characterized using random amplified polymorphic DNA (RAPD) and inter simple sequence repeat (ISSR) markers. Fifty-one cultivars were clustered based on RAPD and ISSR markers. Various nutrient compositions of the fifty rice landrace cultivars and one commercial cultivar were checked. Among 100 RAPD primers tested, 15 showed high polymorphism (100%) with an average of 21.2 bands per primer. Fifteen ISSR primers out of 100 produced high polymorphism (99.74%) with an average of 26 bands per primer. The UPGMA dendrogram based on genetic similarity grouped the cultivars into two clusters and several sub-clusters. Clustering of aromatic rice using ISSR markers gave increased clarity and was more effective than using RAPD markers for both nutritional composition and polymorphism level. Findings will provide practical guidelines for nutritionists and plant breeders in selecting suitable cultivars and genetically diverse parents for plant breeding programs.

Keywords: aromatic rice, nutritional composition, genetic diversity, RAPD, ISSR

Introduction

Rice (*Oryza sativa* L.) represents one of the most important cultivated crops since it supplies food for half of the world's population (Mahathanaseth, 2014; Tarang and Gashti, 2016). Thailand is a global center for rice diversity and important rice-producing countries, devoting 66% of its agricultural area for rice production. More than 17,093 rice cultivars have been collected around Thailand since 1937 (Department of Agriculture Thailand, 2000). Rice landraces developed from wild progenitors and still retain high genetic diversity (Ray et al., 2013). They can be identified by their morphological characteristics and many have been named by local farmers. The Thai word "Hom" means aroma.

The genetic structure of rice landraces is heterogeneous; landraces can show variable phenology and are able to grow in both biotic and abiotic stress environments as a valuable trait for crop production improvement (Dwivedi et al., 2016). The diversity of rice landraces serves as a valuable genetic resource for future crop improvement to meet the ever-increasing demand for food production while alleviating poverty and promoting economic growth (Choudhury et al., 2013). Moreover, some landraces or traditional varieties show higher nutritional and medicinal values than general rice distribution in Thailand. Therefore, Thai rice landraces are a necessary and valuable resource for rice breeding programs (Rerkasem and Rerkasem, 2002). Genetic diversity can be evaluated using morphological traits, seed proteins, isozymes and DNA markers (Liu et al., 2016). A number of molecular markers have been used to study genetic diversity in rice (Devi et al., 2015; Emon et al., 2015; Edwards et al., 2016). The random amplified polymorphic DNA (RAPD) technique is simple with low cost and less performing time; prior knowledge on the genotype is not required (Rekha et al., 2011). Inter simple sequence repeat (ISSR) is a microsatellite-based multilocus marker technique, which is simple and useful for estimating genetic diversity in several crop plants. The ISSR technique has advantages over RAPD with a higher level of polymorphism, reproducibility and cost-effectiveness (Kshirsagar et al., 2014). RAPD and ISSR analyses have been used in genetic diversity studies in several crop plants. In rice, RAPD has been used for identification and classification of aromatic rice varieties (Choudhury et al., 2001) and recognition of duplicate accessions within a rice germplasm collection. Meanwhile, ISSR has been utilized to examine genetic diversity and population structure among landraces to improve rice varieties (Kumbhar et al., 2015). Usefulness of RAPD and ISSR markers in assessing the diversity of rice landraces from Northeast Thailand has not been previously examined. Thus, this study was conducted to determine nutritional compositions and elucidate genetic diversity information for 50 aromatic rice landraces from Northeast Thailand and a commercial rice cultivar using these two marker systems. Results will provide a foundation for further research to select appropriate parental genotypes for plant breeding and crop improvement programs.

Materials and methods

Plant materials

Seeds of 50 aromatic rice landraces and one commercial rice cultivar (Hom Mali 105 or KDML 105) were provided by the Surin Rice Research Center, Bureau of Rice Research and Development, Surin Province, Thailand. Codes C1-C50 were used for the cultivars with C51 (KDML 105) as the out group for dendrogram analysis (Tab. 1).

Proximate analysis

Total nitrogen and crude protein were determined by the Kjeldahl Gerhardt method (N x 5.95) (Nitrogen Distillation System): VELP SCIENTIFICA (AOAC, 2000) while amylose content was measured using a UV-1700 Shimadzu spectrophotometer at 620 nm with potato amylose as the standard (AOAC, 1996). Crude lipids were extracted with hexane using a Soxhlet extractor (Buchi E-816, Switzerland), Soxhlet method (AOAC, 2000). Total crude carbohydrate (CHO) was analyzed by the phenol-sulfuric acid method (Dubois et al., 1956). Ash contents (gravimetric) were determined based on methods outlined in AOAC (2000), while moisture content was examined based on Induhara et al. (1971).

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Quantification of 2-Acetyl-1-pyrroline (2AP) using headspace-solid phase microextraction (HS-SPME) followed by gas chromatography coupled with flame ionization detection (GC-FID) in scented rice was investigated based on MATHURE et al. (2011). 2AP concentrations were calculated using the following formula.

$$\text{2APConcentrations (ng/g)} = \frac{\text{2AP area} \times \text{TMP conc. (ng/µl)} \times \text{injection vol. (µl)}}{\text{TMP area} \times \text{sample weight (g)}}$$

Each treatment consisted of three replicates with 100 g of rice per replicate, except for 2AP when 0.50 g of finely ground rice grains was used. Data were expressed as means ± standard deviation and assigned by analysis of mean variance (one-way ANOVA) to investigate nutritional variation. Differences of means between clusters based on RAPD and ISSR markers were separated by LSD Test ($p < 0.05$) using SPSS version 18 software.

Correlation studies

The nutritional composition character associations represented by correlation coefficient between different pairs of characters at the phenotypic levels were calculated based on SEARLE (1961) and SINGH et al. (2018):

$$\text{Phenotypic correlation coefficients (rp)} = \frac{\text{cov.XY (p)}}{\sqrt{\text{var.Xp.var.Yp}}}$$

Where, Cov.XY (p) denotes phenotypic covariance between characters X and Y, respectively. Var.X (p), Var.Y (p) denotes variance for characters X and Y at phenotypic levels, respectively.

Extraction of genomic DNA

Fresh leaves of 4-5-day-old rice seedlings were collected randomly from each rice landrace and used as the source material. Genomic DNA was extracted following LI and MIDMORE (1999). DNA concentrations were determined by measuring the absorbance of diluted DNA solution at 260 nm using a spectrophotometer. DNA samples were checked by 1.5% agarose gel with 0.5X TBE buffer; those with high band intensity and less smear were selected for polymerase chain reaction (PCR).

RAPD/ISSR markers and PCR amplifications

A set of 100 decamer primers was used for RAPD and a set of 100 primers was screened for ISSR. Out of 100 RAPD/ISSR primers screened, 15 were selected for further analysis based on clarity, scorability, and reproducibility of the banding patterns. RAPD and ISSR amplifications were carried out using a touchdown PCR method in 20 µL reaction mixtures containing 10-20 ng of genomic DNA, 2.0 µl of 10X reaction buffer, 1.5 mmol/l $MgCl_2$, 1.13% formamide, 200 µmol/l dNTPs and 1 U *Taq* DNA polymerase with 1.0 µmol/l RAPD or ISSR primer. A touchdown thermal program was set as follows: 94 ºC, 5 min; 35 × (52 ºC to 48 ºC decreased in 1 ºC step, 1 min); and 72 ºC, 5 min. PCR reactions were carried out on a thermal cycler. Amplified products from each sample were separated electrophoretically on 1.5% agarose gel containing SYBR green dye in 0.5X TBE buffer at 100 V for 1½ hours.

Data analysis

RAPD and ISSR bands were scored using PhotoCapt program (Vilber Lourmat, France). Each amplified band was considered as a unit character regardless of its intensity and scored in terms of a binary code based on presence (1) and absence (0) of bands. RAPD and ISSR data matrix was constructed based on presence/absence of bands. A combined data matrix was also produced and used to calculate genetic similarity based on the Nei and Li's similarity coefficient (NEI and LI, 1979) using Biogene version 98 (Bioprofil, Vilber Lourmat, France). The similarity matrix was used to construct a dendrogram using the unweighted pair-group method with arithmetic averages (UPGMA).

Results and discussion

Nutrient composition and 2AP analysis

Proximate protein, fat, fiber, carbohydrate, ash, moisture and amylose contents of 50 cultivars and one commercial rice cultivar were investigated. Average, minimum, maximum, mean and standard deviations are shown in Tab. 1. Nutritional composition showed no significant relationship with low standard deviation except for carbohydrate and amylose content. High variances were shown in carbohydrate and amylose contents with standard deviations also high at 5.23 and 6.38, respectively. Seven cultivars of aromatic rice and KDML 105 showed 2AP content, with highest value in C36 (Hom Mali, GS No. 19380) (Tab. 1). The 2AP contents of C35, C36, C41, C44 and C50 were all higher than KDML 105. This result concurred with HIEN et al. (2006) and PITIJA et al. (2017) with 2AP content of C36 cultivar higher than KDML 105 by a factor of three.

Many rice cultivars showed lower protein content compared to general rice at around 7-8% protein (CHAUDHARI et al., 2018) and other rice cultivars such as Indian aromatic rice (NADIGER and KASTURIBA, 2015), Indian non-aromatic rice (VERMA and SRIVASTAV, 2017), landrace rice cv Njavara (DEEPA et al., 2008), basmati, Siam, and fragrant rice (MOHD FAIRULNIZAL et al., 2015) and Thai jasmine rice, KDML 105 (LAOKULDILOK et al., 2013; MOONGHGARM et al., 2012). This finding presents an opportunity to develop or improve some aromatic rice cultivars as low protein rice, a food with special medical properties to support the renal function of patients with chronic kidney disease. These patients need to reduce the amount of protein ingested from rice (TAKEI et al., 2017).

Correlation studies

The estimates of phenotypic correlation coefficients calculated between seven nutritional composition characters were presented in Tab. 3. Amylose exhibited significant and positive correlation with protein, fat, fiber, carbohydrate and ash. Most of nutritional composition characters showed positive correlation except fat and protein (-0.1205), fat and moisture (-0.0320), fiber and moisture (-0.0319), and carbohydrate and moisture (-0.0274). This significant negative correlation indicated that rice variety high in moisture may probable to be low in fat, fiber and carbohydrate. The correlation between moisture and carbohydrate in this result concurred with VERMA and SRIVASTAV (2017) who reported on aromatic and non-aromatic Indian rice but not similar in correlation between carbohydrate and protein. However, nutritional composition content may not be consistent as milling time (LAOKULDILOK et al., 2013), rice processing (ABBAS et al., 2011), different measurement methods (GRIMM et al., 2011), and diverse varieties, environments and crop management methods all affect nutritional composition content and their correlations. Although many reports have been established about nutritional composition in rice varieties, nutrient correlation between some compositions are poorly perceived, including in Thai rice. However, correlation between some nutritional compositions in this report may useful as selection criteria for Thai aromatic rice breeding program in the future.

Genetic similarities and clustering

Among the 100 RAPD markers used, 15 were selected to characterize and assess the genetic variability among the 50 rice landraces be-

Tab. 1: Nutrient composition and 2AP contents of the rice landraces.

GS No.	Code-local name	Percentage (mean)							
		Protein	Fat	Fiber	CHO	Ash	Moisture	Amylose	2AP (µg/g)
2720	C1-Kee Tom Hom	6.24	0.40	2.67	71.51	0.13	11.90	2.06	0.00
4488	C2-Khao Hom	5.80	0.73	2.67	72.03	0.33	12.29	1.56	0.00
4489	C3-Niaw Hom Mali	4.34	0.60	1.67	65.98	0.47	10.96	1.72	0.00
4869	C4-Khao Hom	6.00	0.73	2.00	74.18	0.53	10.86	2.33	0.00
4870	C5-Khao Hom	4.59	0.73	1.67	80.20	0.33	11.40	1.61	0.00
4878	C6-Khao Hom	7.57	0.40	1.67	71.05	0.47	11.23	2.22	0.00
5595	C7-Niaw Hom	4.97	0.47	3.00	72.00	0.47	10.94	2.61	0.00
5624	C8-Hom Tung	6.54	0.73	2.33	72.25	0.60	11.89	4.33	0.00
5670	C9-Hom Tung	5.42	0.40	1.33	63.80	0.27	12.13	3.61	0.00
6724	C10-Hom Pa Ma	4.34	0.80	2.00	72.11	0.27	13.08	2.61	0.00
7608	C11-Hom Udom	4.55	0.67	2.33	73.91	0.33	11.04	2.72	0.00
12148	C12-Mak Hom	4.14	0.60	1.67	79.69	0.33	12.31	1.94	0.00
12512	C13-Hom Udom	4.73	0.40	1.67	64.11	0.13	11.28	2.67	0.00
13911	C14-Hom	3.90	1.07	1.67	79.12	0.47	11.65	1.56	0.00
13929	C15-Hom	4.05	0.73	2.33	66.21	0.27	11.75	2.39	0.00
13932	C16-Hom Maled Lek	6.76	0.60	2.33	80.99	0.67	11.70	13.89	0.00
13975	C17-Doh Hom	5.82	0.80	2.67	80.00	0.67	10.59	15.50	0.00
14512	C18-Hom Nual	5.02	0.87	3.33	80.73	0.67	12.78	15.67	0.00
14518	C19-Hom Nang Nual	4.64	1.07	3.33	79.40	0.67	11.80	13.00	0.00
14539	C20-Hom Dong	4.83	0.67	3.67	80.20	0.27	10.97	16.17	0.00
16066	C21-Hom Pa Ma	5.84	0.80	2.33	78.02	0.27	11.29	14.89	0.00
16068	C22-Pa Ma Hom	5.72	0.73	2.67	80.53	0.47	14.02	14.33	0.00
18415	C23-Hom Mali	3.85	0.60	3.33	67.62	0.53	12.20	3.28	0.00
18421	C24-Hom Mali	4.73	0.73	2.00	79.03	0.40	11.60	15.78	0.00
18424	C25-Hom Mali	5.12	0.33	2.33	81.21	0.47	10.83	17.78	0.00
19342	C26-Hom Mali	5.83	0.93	2.33	79.98	0.67	12.31	15.06	9.34
19343	C27-Hom Mali	5.82	0.73	2.33	81.64	0.33	13.16	18.22	0.00
19344	C28-Hom Mali	5.82	0.73	2.33	81.04	0.47	11.53	15.22	0.00
19345	C29-Hom Mali	5.74	0.67	2.00	82.48	0.27	13.30	16.61	0.00
19347	C30-Hom Mali	5.39	0.67	3.33	78.20	0.40	13.58	14.28	0.00
19348	C31-Hom Mali	5.16	0.87	2.00	79.64	0.33	11.70	16.61	0.00
19349	C32-Hom Mali	5.55	0.67	2.67	83.54	0.47	12.13	13.61	0.00
19350	C33-Hom Mali	5.12	0.73	2.33	77.10	0.33	12.22	14.61	0.00
19378	C34-Hom Mali	5.83	0.53	3.33	81.55	0.47	12.31	13.39	0.00
19379	C35-Hom Mali	5.22	0.80	2.67	78.29	0.47	11.97	16.78	30.14
19380	C36-Hom Mali	5.68	0.60	3.00	81.36	0.40	10.72	14.67	65.88
19381	C37-Hom Mali	6.16	0.47	3.00	81.31	0.33	12.54	16.61	0.00
19382	C38-Hom Mali	5.24	0.93	2.33	80.07	0.33	13.57	15.56	0.00
19383	C39-Hom Mali	4.21	0.47	1.00	67.41	0.33	13.31	2.72	0.00
19384	C40-Hom Mali	5.19	0.80	3.67	82.88	0.47	9.86	15.44	0.00
19385	C41-Hom Mali	6.67	0.40	1.33	78.25	0.33	12.83	2.17	29.87
19386	C42-Hom Mali	4.27	0.60	1.67	82.50	0.27	10.72	4.83	0.00
19387	C43-Hom Mali	5.94	0.27	2.67	80.27	0.40	11.18	1.56	0.00
21688	C44-Hom Pa Ma	4.80	0.53	1.67	79.44	0.27	10.88	3.50	34.18
21940	C45-Hom Dok Doo	7.11	0.40	3.33	81.07	0.27	12.02	14.89	0.00
22776	C46-Hom Nual	4.72	0.20	2.33	73.19	0.40	13.45	5.94	0.00
22781	C47-Khao Hom	6.74	0.47	2.33	78.30	0.27	14.35	14.50	5.52
21383	C48-Hom Yai	6.55	0.60	1.67	75.99	0.27	12.97	2.39	0.00
23209	C49-Khao Hom	5.51	0.60	3.00	78.04	0.47	12.29	4.89	0.00
23253	C50-Hom Udom	4.77	0.93	1.33	77.99	0.33	10.23	3.89	42.75
none	C51-KDML 105 or Hom Mali 105*	5.03	0.73	2.33	80.81	0.47	10.59	14.89	20.43
	min	3.85	0.20	1.00	63.80	0.13	9.86	1.56	0.00
	max	7.57	1.07	3.67	83.81	0.67	14.35	18.22	65.88
	mean	5.36	0.65	2.36	77.02	0.40	11.93	9.20	4.86
	SD	0.85	0.19	0.65	5.23	0.13	1.01	6.38	13.25

* C51-KDML 105 or Hom Mali 105 is a commercial cultivar used as the out group. (GS No.-Genetic Stock Number; CHO-Carbohydrate)

Tab. 2: Efficiency comparison between RAPD and SSR markers for identifying polymorphism among the 51 rice cultivars.

Fifty-one selected rice cultivars	RAPD	ISSR
Total amplified bands	347	390
Maximum band	5,335	3,580
Minimum band	140	170
Polymorphic bands	347	389
Polymorphic band percentage	100	99.74
Number of primers used	15	15
Mean of polymorphic bands/primers	21.20	26
Nei and Li's similarity coefficient percentage	91	74

Tab. 3: Estimates of phenotypic correlation coefficients between seven nutrient compositions in aromatic rice.

Composition	Protein	Fat	Fiber	CHO	Ash	Moisture
Amylose	0.2001**	0.1722*	0.3638**	0.6606**	0.1843*	0.1263
Protein		-0.1205	0.0948	0.2050*	0.1145	0.1236
Fat			0.0178	0.1437	0.1294	-0.0320
Fiber				0.2463	0.2256	-0.0319
CHO					0.2054	-0.0274
Ash						0.1843

* and **, Correlation is significant at $p < 0.05$ and $p < 0.01$, respectively.

cause of their distinct amplifications and highly reproducible bands. A total of 347 bands were amplified by the selected RAPD primers with an average of 21.2 bands per primer and sizes ranging from 140 bp to 5,335 bp. They were all polymorphic as shown in Fig. 1 and Tab. 2. Out of 100 ISSR markers screened, 15 were selected to assess genetic diversity among the 50 rice landraces. Selected ISSR primers yielded a total of 390 bands with an average of 26 bands per primer, with sizes ranging from 170 bp to 3,580 bp, and 389 as polymorphic (99.74%) as presented Tab. 2.

The RAPD and/or ISSR amplification patterns were used to assess genetic variation among the 50 rice landraces by cluster analysis. A dendrogram was plotted for each of the amplification patterns using the similarity coefficient derived from the RAPD profile data as depicted in Fig. 2-A. The dendrogram constructed for the RAPD amplification pattern was resolved into two clusters at 91% similarity coefficient. Minimum number of rice landraces (6) was represented in Cluster I, whereas Cluster II contained 45 rice landraces. Cluster I was again divided into two sub-clusters and Cluster II into five sub-clusters. Each sub-cluster of Cluster I contained 3 rice landraces, with 12, 14, 13, 5 and 1 rice landrace included in sub-clusters IIA, IIB, IIC, IID and IIE, respectively. The dendrogram constructed for the ISSR amplification pattern was divided into two clusters at 74% similarity coefficient (Fig. 2-B). The minimum number of rice landraces (2) was represented in Cluster I, whereas Cluster II contained 49 rice landraces. Cluster II was again divided into six sub-clusters. Sub-clusters IIA, IIB, IIC, IID, IIE and IIF contained 2, 14, 13, 9, 10 and 1 rice landrace, respectively.

Data quality monitoring is important for various utilized applications, especially in the food industry and nutrition-related medicine. In general, a rice grain consists of 90% flour, 8% protein, 0.4-0.6% fat, 0.3-0.6% fiber and 0.4-0.9% ash (PIYACHOMKWAN et al., 2001). Examples of rice grain applications are as high purity rice flour or low protein flour production which are both useful in the food, pharmaceutical and cosmetics industries.

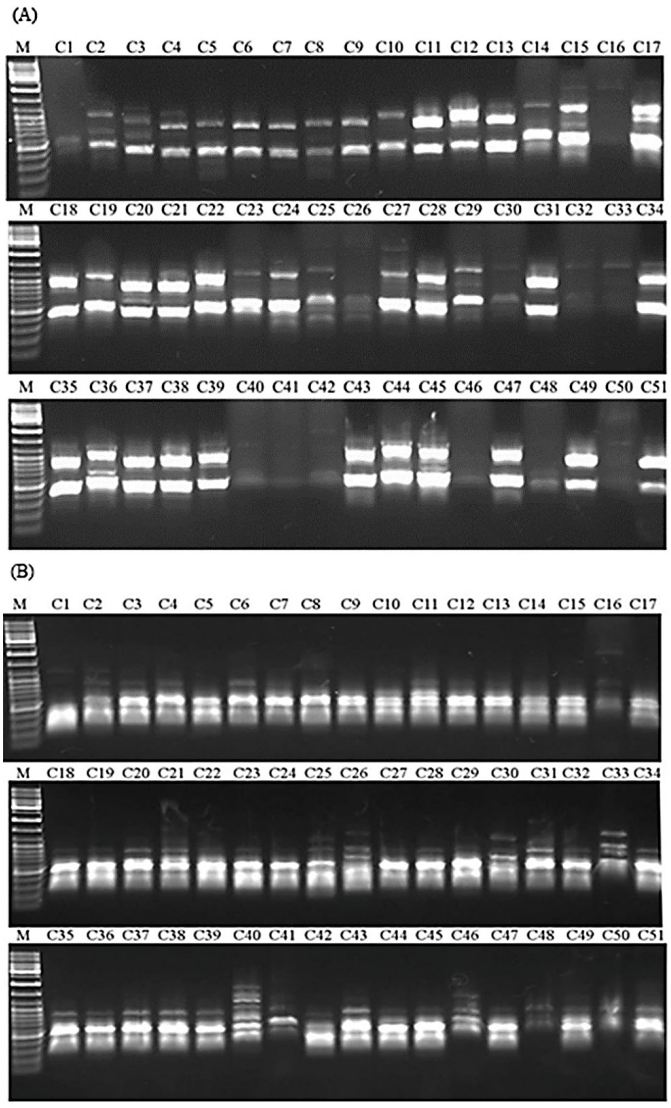

Fig. 1: RAPD and ISSR amplification profiles of 50 rice cultivars and a commercial rice cultivar generated using RAPD primer A01 in panel A and ISSR primer 03 in panel B, where lane M is 100–10,000 bp DNA ladder.

Nutritional composition in this aromatic rice group showed no distinguishing differences except for total carbohydrate and amylose contents. Some rice cultivars recorded low carbohydrate and amylose content although these had the same local names. For example, cultivar codes C23-C43 had the same local name (Home Mali) but different Genetic Stock Number (GS No.), nutritional composition and polymorphism level. Their nutritional compositions showed various measurement values. Hom Mali rice varieties (C23-C43) were found in various clusters using the RAPD and ISSR systems (Fig. 2). This may involve amylose biosynthesis gene expression (GBSS alleles) (FASAHAT et al., 2014) or other genes in the rice genome. Clustering of aromatic rice using the ISSR system showed more clarity and effectiveness than the RAPD system, especially regarding the nutritional composition issue. Average nutritional composition value from ISSR clustering related closely with the measurement data. Our results indicated that the 50 aromatic rice landraces showed both genetic and nutrient diversity; however, genetic diversity remains the cornerstone of crop improvement, providing breeders with options to develop new and improved cultivars with desirable characteristics (GOVINDARAJ et al., 2015). Morphological

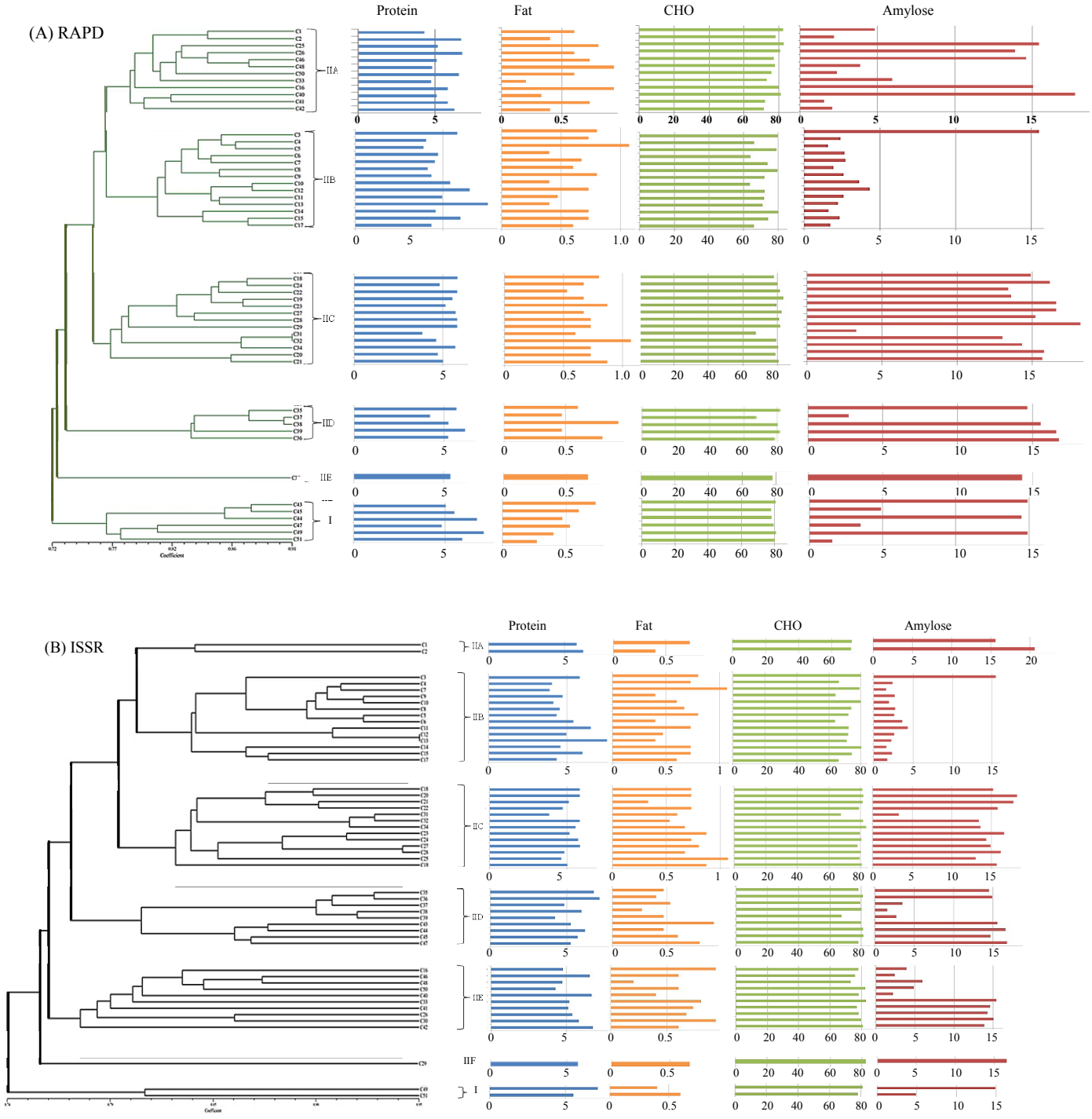

Fig. 2: UPMGA-based dendrograms depicting genetic relationships among 50 rice landraces and a commercial rice cultivar based on RAPD (A) and ISSR (B) profiles in comparison with some nutritional composition (Protein, Fat, CHO-Carbohydrate and Amylose).

trait analyses, as well as chemical characterization, are useful tools to identify differences in plant cultivars affected by phenotypic expression (Roy and Sharma, 2014; Roy et al., 2016). Our results may not reflect real genetic variation due to genotype-environment interaction or unknown genetic control of phylogenic morphological and agronomic traits (Kumbhar et al., 2015). Instead, classification of individual genotypes into different groups based on polymorphisms at the DNA level with molecular markers is considered a powerful tool for estimation of genetic divergence (al-Turki and Basahi, 2015; Roy et al., 2015).

Here, RAPD and ISSR markers were utilized to assess genetic diversity in 50 aromatic rice landraces from Northeast Thailand and a commercial rice cultivar (KDML 105). Selected RAPD and ISSR markers exhibited distinct amplification and highly reproducible bands, thereby suggesting their suitability for genetic diversity study. Grouping of rice landraces based on polymorphic RAPD and ISSR markers indicated low to moderate genetic diversities among the studied genotypes. Moderate genetic diversity among the landraces suggested distinct differences in their genetic architecture.

Among the 100 RAPD primers used, 15 generated several specific

bands in 23 rice landraces. In particular, primer C07 produced only two specific bands in C51, which can be effectively used for further development of sequence characterized amplified region (SCAR) markers for precise identification of this rice landrace in the given set of rice genotypes. Similarly, out of 100 ISSR markers, 15 primers produced several specific bands in 32 rice landraces, with primers ISSR5 and ISSR15 generating 5 specific bands in C41.

In this investigation, 51 rice genotypes were grouped into 2 major clusters through UPGMA-based clustering using Nei and Li's similarity coefficient which indicated low to moderate variation between genotypes. The studied rice landraces belong to different areas of Northeast Thailand and evolved through different biotic and abiotic factors, thereby displaying differences in their genetic compositions. Comparison between RAPD and ISSR markers indicated that clustering using ISSR markers gave a higher level of polymorphism than RAPD markers, similar to Kshirsagar et al. (2014). Our findings showed that genetic diversity assessed using ISSR markers was more reliable than evaluated by RAPD markers due to the larger size of ISSR markers and higher temperature in the annealing step, allowing ISSR markers to generate more specific bands. This is the first report on the nutrient composition of a set of 50 aromatic rice landraces from the northeastern region of Thailand.

Conclusions

Findings will be useful for plant breeders to selected suitable cultivars to achieve high nutrient composition and improved yield through breeding techniques as the first step in rice improvement. Moreover, this study highlighted the utilization of touchdown PCR for RAPD and ISSR amplifications in the identification of 50 aromatic rice landraces and a commercial rice cultivar KDML 105. Both marker systems produced specific bands in the studied genotypes, thereby suggesting their suitability for genetic diversity studies. Findings also showed low to moderate genetic diversities among the studied rice genotypes. Marker-based identification and differentiation of rice genotypes may be applied to maintain the integrity of these rice landraces which will benefit farmers and research workers. This investigation determined five cultivars (C35, C36, C41, C44 and C50) that showed interesting characteristics of low protein and high 2AP content. Moreover, their genetic characteristics were similar when considered on RAPD (cluster I, IIA and IID) and ISSR (cluster IID and IIE) systems. Further improvements in breeding programs and cultivation of these five cultivars are highlighted as a future research area.

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