Farmers Drive Genetic Diversity of Thai Purple Rice (*Oryza sativa* L.) Landraces

Tonapha Pusadee*[,1], Anupong Wongtamee[2], Benjavan Rerkasem[3], Kenneth M. Olsen[4], and Sansanee Jamjod*[,1,5]

[1]Department of Plant and Soil Science, Chiang Mai University, Chiang Mai, Thailand
[2]Department of Agricultural Science, Faculty of Agriculture Natural Resources, Environment Naresuan University, Phitsanulok, Thailand
[3]Plant Genetic Resource and Nutrition Laboratory, Chiang Mai University, Chiang Mai, Thailand
[4]Department of Biology, Washington University in St. Louis, St. Louis, MO, USA
[5]Lanna Rice Research Center, Chiang Mai University, Chiang Mai, Thailand
*Corresponding author; e-mail: sansanee.cm@gmail.com

Purple or black rice (*Oryza sativa* L.) is a culturally important germplasm in Asia with a long history of cultivation in northern Thailand. Purple rice is identified by the color of the rice pericarp, which varies from purple to black with the accumulation of phenolic acids, flavonoids, and anthocyanins. In the present study, we assessed molecular variation within and between wetland purple rice landraces germplasm from northern and northeastern Thailand using 12 microsatellite loci. All purple rice varieties surveyed showed high levels of homozygosity within varieties and strong genetic differentiation among varieties, indicating the fixation of genetic differences among them. This pattern is consistent with purple rice farming practices in northern Thailand, where a small portion of harvested seed is selected and replanted based on farmers' preferences. The reduced genetic diversity and high homozygosity observed for purple rice is also consistent with patterns expected for this inbreeding crop. Genetic differentiation among the varieties showed some degree of structuring based on their geographical origin. Taken together, these data highlight that the genetic diversity and structure of wetland purple rice landraces is shaped by farmer utilization and cultivation through local cultural practices, and that conservation should focus on *ex situ* conservation across its cultivation range, along with on-farm, *in situ* conservation based on farmers' seed-saving practices. *In situ* conservation may prove especially valuable for preserving the genetic identity of local varieties and promote adaptation to local environments.

ข้าวกำหรือข้าวดำเป็นแหล่งพันธุกรรมที่สำคัญทางวัฒนธรรมในเอเชียด้วยมีประวัติศาสตร์อันยาวนานทางการเพาะปลูกในภาคเหนือของประเทศไทย ข้าวก้ำบ่งบอกได้จากสีของเยื่อหุ้มเมล็ดที่มีสีม่วงไปจนถึงสีดำที่มีการสะสมกรดฟีโนลิค ฟลาโวนอยด์ และแอนโทไซยานิน ในการศึกษานี้ได้ประเมินความแปรปรวนทางโมเลกุลดีเอ็นเอภายในและระหว่างแหล่งพันธุกรรมข้าวก้ำนาพื้นเมืองจากภาคเหนือและภาคตะวันออกเฉียงเหนือของประเทศไทยด้วยเครื่องหมายโมเลกุลไมโครแซทเทิลไลท์จำนวน 12 ตำแหน่ง ข้าวก้ำพื้นเมืองทุกพันธุ์ที่ศึกษามีระดับของความเป็นพันธุ์แท้ภายในพันธุ์สูง และมีความแตกต่างทางพันธุกรรมระหว่างพันธุ์ที่ชัดเจน แสดงให้เห็นถึงการคงที่ของความแตกต่างทางพันธุกรรมในข้าวก้ำ ซึ่งแบบแผนดังกล่าวสอดคล้องกับระบบการเพาะปลูกข้าวก้ำในภาคเหนือของประเทศไทย ที่ว่าเมล็ดเพียงส่วนน้อยจะถูกเก็บไว้หลังจากเก็บเกี่ยวและจะถูกนำไปปลูกในฤดูถัดไปตามความชอบของเกษตรกร การลดลงของความหลากหลายทางพันธุกรรมและระดับความเป็นพันธุ์แท้ที่สูงในข้าวก้ำที่พบว่าสอดคล้องกับแบบแผนของระบบการสืบพันธุ์ที่ข้าวเป็นพืชปลูกแบบผสมตัวเอง ความแตกต่างทางพันธุกรรมระหว่างพันธุ์แสดงให้เห็นโครงสร้างที่แบ่งแยกตามภูมิศาสตร์ที่เก็บรวบรวม

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เมื่อนำข้อมูลมาเชื่อมโยงกันแล้วจากการศึกษานี้แสดงให้เห็นว่าความหลากหลายทางพันธุกรรมและโครงสร้างประชากรของข้าวกำนาพื้นเมืองถูกกำหนดโดยการใช้ประโยชน์ และการเพาะปลูกของเกษตรกรผ่านการเพาะปลูกแบบดั้งเดิม งานอนุรักษ์พันธุ์ควรเน้นที่การอนุรักษ์นอกสภาพท้องถิ่นเดิมครอบคลุมพื้นที่ที่มีการเพาะปลูกข้าวกำ พร้อมกับการอนุรักษ์ในไร่นาเกษตรกรโดยอาศัยพื้นฐานวิธีการเก็บรักษาพันธุ์ของเกษตรกร การอนุรักษ์ในสภาพท้องถิ่นเดิมแสดงให้เห็นการเก็บรักษาพันธุกรรมที่เป็นเอกลักษณ์ของข้าวพื้นเมืองอีกทั้งยังเป็นการส่งเสริมการปรับตัวต่อสภาพแวดล้อมในท้องถิ่นอีกด้วย.

Key Words: Genetic structure, Homozygosity, Seed saving, Farmers' managements, Utilization.

Introduction

Purple or black rice is a type of rice (*Oryza sativa* L.) that is typically glutinous and is cultivated mainly in Asia. It is grown in China, Japan, Korea, Sri Lanka, India, Bangladesh, Thailand, Laos, Philippines, and Indonesia (Appa et al. 2006; Sukhonthara et al. 2009). Several varieties have a long history of cultivation in China, India, and Thailand (Kong et al. 2008). In Thailand, purple rice is called "*Kao Kum* – ข้าวกำ" or "*Kao Niaw Dam* – ข้าวเหนียวดำ" where *Kum* means purple, *Dam* means black, *Kao* means rice, and *Neow* means glutinous grain type. Purple rice is mostly used in preparing desserts in Thailand, especially in the northern and northeastern regions of the country. In these regions, each farmer traditionally grows purple rice in a very small portion (< 5%) of their rice cultivation area. After harvest, purple rice is divided into two parts, one for use within the household and the other as the seed source for the next year's crop. Farmers usually cultivate at least one local purple rice variety for consumption within the family for desserts and use in traditional ceremonies. Economic opportunities have recently emerged with increasing interest in the health-food aspects of purple rice, which is sold at premium price in Thailand and Laos, sometimes in mixtures with ordinary white rice.

Purple rice is recognized by its pericarp color, which varies from light to deep purple (termed black) due to pigmentation, and is characterized at the biochemical level by the accumulation of phenolic acids, flavonoids, and anthocyanins that exhibit antioxidant activities (Kaneda et al. 2006). In the past decade, the functional properties of the extracts from pigmented rice have been widely studied, and they have been found to decrease oxidative stress in vivo and in vitro (Lin and Weng 2006), protect endothelial cells (Zhang et al. 2006), prevent heart and cardiovascular diseases, and act as anticancer agents (Leardkamolkarn et al. 2011). These properties have been related to several classes of antioxidant compounds, including tocols, oryzanols, and phenolic compounds (Finocchiaro et al. 2007; Ling et al. 2001). In addition to anthocyanin content in the purple rice grain, some purple rice varieties have also been reported to be rich in minerals including Fe, Zn, Mn, and P (Zhang et al. 2004; Rerkasem et al. 2015).

Much of the research on purple rice has concentrated on its nutritional and pharmacological properties and historic and cultural importance. In contrast, little attention has been paid to characterizing the genetic diversity of this unique germplasm. Effective conservation and utilization of any plant genetic resource requires the ability to characterize the extent of genetic variation within and among varieties or populations (Rao 2004). On-farm purple rice germplasm is a valuable resource that is readily accessible to farmers as well as a potential source of value-adding traits for future breeding programs. The longstanding cultural practice in northern Thailand of saving and replanting local purple rice varieties within families predicts that genetic diversity may be low within varieties, but with potentially high levels of differentiation between varieties due to isolation and drift. The aim of this study was to test this hypothesis by assessing molecular variation within and between local rain-fed lowland purple glutinous rice from northern and northeastern Thailand.

Materials and Methods

Collection of Seed Samples

The purple glutinous rice seed samples collected from farmers' seed stock included eight populations from the northern and 16 from the northeastern Thailand (Table 1 and Fig. 1), where a sample from each farmer was considered a single population. Elite white grain varieties including Chai Nat 1 (CNT1), Khao Dawk Mali 105 (KDML105), San-pah-tawng (SPT1), and RD6 from the Thailand Department of Agriculture were included as

TABLE 1. DESCRIPTION OF THAI WETLAND PURPLE RICE LANDRACES AND ELITE WHITE RICE VARIETIES USED IN THE EXPERIMENT.

Rice samples	Sources	Location (province)	Code	Rice variety name/accession number
Purple rice landraces	North	Chiang Mai	KN1	Kum Muser
		Nan	KN2	Kum Nan
		Phayao	KN3	Kum Phayao
		Chiang Mai	KN4	Kum Omkoi
		Nan	KN5	Niaw Dam Kum Na
		Chiang Mai	KN6	Kum No. 7677
		Chiang Mai	KN7	Kum Doi Sa Ket
		Nan	KN8	Kum Wiang Sa
	Northeast	Genebank	KNE1	Kum No. 5153
		Genebank	KNE2	Kum No. 11875
		Genebank	KNE3	Kum No. 19104
		Genebank	KNE4	Kum No. 19959
		Roi Et	KNE5	Kum Loi
		Genebank	KNE6	Kum No. 87046
		Genebank	KNE7	Kum No. 89057
		Genebank	KNE8	Kum No. 25 KD
		Ubon Ratcha Thani	KNE9	Kum No. 31 KK
		Ubon Ratcha Thani	KNE10	Kum No. 34 K
		Genebank	KNE11	Kum No. 87090
		Genebank	KNE12	Kum No. 89038
		Genebank	KNE13	Kum No. 88069
		Genebank	KNE14	Kum No. 88083
		Genebank	KNE15	Kum No. 87061
		Genebank	KNE16	Kum No. 99151
Elite white rice	Department of Agriculture		CNT 1	Chai Nat 1
	Department of Agriculture		KDML 105	Khao Dawk Mali 105
	Department of Agriculture		SPT1	San-pah-tawng 1
	Department of Agriculture		RD 6	RD 6

outgroups for comparisons to purple rice. Seeds were germinated in petri dishes for 5 days and then transferred to 30-cm-diameter pots, with 10 plants per pot. At the tillering stage, leaves of 10 individuals of each variety were collected. Leaf samples were silica-dried and kept at room temperature until DNA extraction.

DNA EXTRACTION AND MICROSATELLITE ANALYSIS

Total DNA of silica-dried leaf tissue was extracted using a modified CTAB method (Doyle and Doyle 1987). A total of 12 microsatellite markers distributed across the 12 rice chromosomes were chosen at random (Appendix 1). Polymerase chain reactions (PCR) were performed in a total volume of 20-µl reactions consisting of 20–50 ng DNA, 0.25 mM dNTP, 0.2 µM each primers, and 0.5 unit of Taq DNA polymerase (Invitrogen). Reactions were performed by denaturing at 94 °C (4 minutes (min)) followed by 40 cycles of 94 °C, 55 °C or 61 °C, 72 °C, each for 30-second (s) intervals followed by a final 72 °C extension for 5 min, and a 4 °C hold. The PCR products were separated in a 10% polyacrylamide gel electrophoresis in 1× TBE buffer. The gels were then visualized using a BLooK LED transilluminator (Genedirex, Taiwan). Microsatellite alleles were scored based on allele sizes with reference samples included on all gels for consistency in scoring.

GENETIC DIVERSITY ANALYSIS

Genetic diversity indices were calculated at the population level as allelic richness (A_R), expected heterozygosity (h), average gene diversity (H_S), and total gene diversity (H_T) using GENALEX v6.5

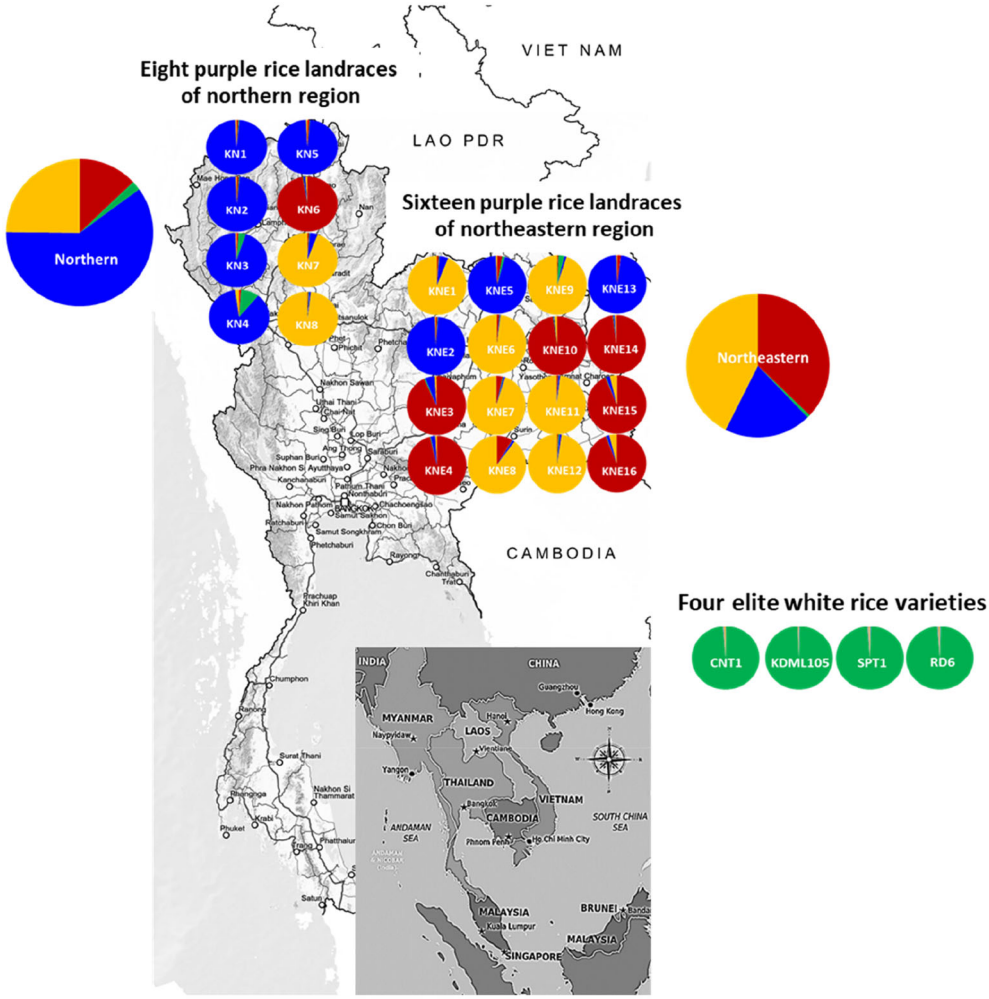

Fig. 1. Population of origins and affiliations of 24 Thai purple rice landraces collected from northern regions (KN), eight populations and northeastern regions (KNE), 16 populations based on the STRUCTURE analysis in 4 clusters ($K = 4$) identified by the colors green, elite white rice varieties; blue, purple rice from northern; yellow, purple rice from northeastern; and red, purple rice from northeastern, located in their regions of origin. Pie chart colors denote different genetic clusters as identified by STRUCTURE and the size of each pie slice shows the average probability of assignment of individuals to the cluster. The large pie chart summarizes the distribution of individual in each region and the smaller pie charts correspond to populations sampled.

(Peakall and Smouse 2012). Genetic differentiation among populations (F_{ST}) was calculated using the FSTAT v2.9.3 (Goudet 2001). Population pairwise F_{ST} values were used to measure the genetic differentiation between populations, with significance assessed through boostrap analysis (10,000 replicates). Values for Wright's inbreeding coefficient per population (F_{IS}), a measure for the deviation from random mating within populations, were computed in FSTAT.

Population Structure Analysis

To investigate population structure and infer the most likely number of genetic clusters (K), we used the model-based clustering algorithm implemented in STRUCTURE v2.3 (Pritchard et al. 2000). In this approach, multilocus genotypic data are used to define a set of clusters with distinct allele frequencies and to assign individuals probabilistically to them. The analyses were run for up to 10 putative clusters

(K = 1–10). Ten independent runs were carried out for each K value with 500,000 burn-ins followed by 500,000 iterations. The probability of best fit into each number of assumed clusters (K) was estimated by an ad hoc statistic ΔK based on the rate of change in the log probability of the data between consecutive K values (Evanno et al. 2005). The parameters of the methods of Evanno et al. (2005) were calculated using the program STRUCTURE HARVESTER v0.6.1 (Earl and vonHoldt 2012), and the clusters were visualized using the program DISTRUCT v1.1 (Rosenberg 2004).

To partition genetic variance among the hierarchical sets of regions of origin and among 24 purple rice populations, we used an analysis of molecular variance (AMOVA) implemented in ARLEQUIN v3.5 (Excoffier and Lischer 2010), which calculates variance components and F statistics for each hierarchical level with 10,000 permutations. The hierarchical analysis considered distributions of genetic variation at four levels: within individuals (heterozygosity), among populations within regions (northern or northeastern Thailand), and between the two sampled regions.

To illustrate the two-dimensional spatial representation of genetic differentiation, we used the principal coordinate analysis (PCoA). The PCoA implemented in GENALEX was used among purple rice populations on all samples, among regions.

Results

Genetic Diversity

A total of 12 SSR markers, randomly distributed across the genome, were used to evaluate the genetic diversity of the purple rice populations. The variability at each microsatellite locus was measured in terms of the number of observed alleles (N_A), expected heterozygosity (H_E), and Shannon's Information Index (I) (Appendix 1). All 12 SSR markers were polymorphic, and 43 alleles were detected across the 24 purple rice populations (240 individuals in total) and the 4 elite (white pericarp) varieties. The average number of alleles per locus (N_A) was 4.1, ranging from 2 (in RM161, RM510, RM11, RM316, RM171, and RM19) to 15 (in RM1). The average expected heterozygosity (H_E) was 0.040, ranging from 0 to 0.180. The average Shannon's Information Index (I) was 0.068, ranging from 0 to 0.345 (Appendix 1).

Table 2 presents genetic diversity measures for individual populations and regional groupings. Although the number of sampled populations in the northern (N = 8) was half that of the northeastern (N = 16), the number of detected alleles was comparable in the two regions (26 in the nothern, and 32 in the northeastern). Within-population genetic diversity was consistently low throughout the sampling range. Allelic richness values were less than 2 for all populations (mean A_R = 1.19), and Nei's gene diversity ranged from 0 for five northeastern populations (KNE3, KNE5, KNE8, KNE13, and KNE14) to a maximum of 0.138 for a single northeastern population (KNE11) (mean h = 0.039). The total gene diversity across all purple rice samples (H_T) was 0.384. At the regional level, the purple rice populations of the northern showed similar level of Nei's genetic diversity (h) to those from the northeastern. However, the purple rice of the northeastern displayed slightly higher level of total gene diversity (H_T = 0.267) than the northern (H_T = 0.218).

Whereas genetic diversity within populations was very low, the level of genetic differentiation among them was substantial. Within-region F_{ST} values were 0.799 and 0.849 for northern and northeastern samples, respectively, and differentiation across all populations was similarly high (F_{ST} = 0.841) (Table 2). Consistent with these values, an analysis of molecular variance (AMOVA) showed that only 4% of the total variation was partitioned between regions, with 80% partitioned among populations within regions; nearly all of the remaining 16% was partitioned among individuals (Table 3).

Population Structure

Bayesian clustering analysis of the 24 local purple rice populations and four elite white rice varieties over a range of K = 1–10 populations revealed optimal values at K = 2 and K = 4 as assessed by the ad hoc measure ΔK (Fig. S1). At K = 2, the two groups corresponded largely to the white rice vs. purple rice samples (Fig. 2a). Since strong support for K = 2 may be an artifact of rejecting very low likelihoods at K = 1 (Vigouroux et al. 2008), we focused on patterns at K = 4 (Fig. 2b). At this K value, each of the 28 sampled rice populations could be assigned primarily to a single genetic subgroup (P1, P2, P3, or P4); all populations had membership assignments of Q > 85%, and all but two populations

TABLE 2. GENETIC DIVERSITY OF 24 PURPLE RICE LANDRACE POPULATIONS COMPARED WITH 4 CHECK ELITE WHITE RICE VARIETIES BASED ON 12 SSR LOCI.

Rice sources	Code	Structure group	*n*	A_R	F_{IS}	*h*	H_S	H_T	F_{ST}
North	KN1	P2	10	1.083	1	0.027			
	KN2	P2	10	1.083	1	0.015			
	KN3	P2	10	1.250	1	0.085			
	KN4	P2	10	1.250	1	0.073			
	KN5	P2	10	1.083	1	0.035			
	KN6	P4	10	1.667	1	0.045			
	KN7	P3	10	1.583	1	0.028			
	KN8	P3	10	1.083	1	0.042			
Total north	8 populations		80	1.229	1	0.044	0.044	0.218	0.799
Northeast	KNE1	P3	10	1.083	1	0.008			
	KNE2	P2	10	1.167	1	0.055			
	KNE3	P4	10	1.000	1	0			
	KNE4	P4	10	1.000	1	0.040			
	KNE5	P2	10	1.000	1	0			
	KNE6	P3	10	1.333	1	0.095			
	KNE7	P3	10	1.167	1	0.062			
	KNE8	P3	10	1.083	1	0			
	KNE9	P3	10	1.083	1	0.053			
	KNE10	P4	10	1.167	1	0.067			
	KNE11	P3	10	1.250	1	0.138			
	KNE12	P3	10	1.167	1	0.062			
	KNE13	P2	10	1.000	1	0			
	KNE14	P4	10	1.000	1	0			
	KNE15	P4	10	1.083	1	0.015			
	KNE16	P4	10	1.083	1	0.050			
Total northeast	16 populations		160	1.172	1	0.040	0.040	0.267	0.849
Total local varieties	24 populations		240	1.191	1	0.039	0.039	0.384	0.841
Elite white rice	4 varieties	P1	40	1	1	0	0	0.213	1
Total	28 populations		280	1	1	0.036	0.044	0.222	0.816

n, number of individuals, A_R allelic richness, F_{IS} inbreeding coefficient, *h* Nei's gene diversity, H_S average gene diversity, H_T total gene diversity, F_{ST} genetic differentiation

had values of $Q > 93\%$ (Appendix 2). Group P1 (green) consisted solely of elite white rice varieties. P2 (blue) populations occurred primarily in the northern, where they comprised five of the eight populations; in the northeastern, they comprised 3 of the 16 populations. In contrast, both P3 (yellow) and P4 (red) occurred primarily in the northeastern. P3 included two populations in the northern and seven populations in the northeastern, while P4 consisted of one population in the northern and six populations in the northeastern.

The PCoA based on allele frequencies at each SSR marker revealed four clusters for the entire sample set which largely correspond to the clusters identified by STRUCTURE at $K = 4$ (Fig. 3). The first and second principal coordinates explained 17.5% and 16.0% of the variance, respectively. The PCoA clustered the purple rice populations

TABLE 3. ANALYSIS OF MOLECULAR VARIANCE (AMOVA) FOR 240 INDIVIDUALS OF 24 THAI PURPLE RICE LANDRACE POPULATIONS FROM TWO REGIONS: NORTH AND NORTHEAST OF THAILAND BASED ON 12 SSR LOCI.

Source	df	Sum of square	Variance component	% of the variance
Among regions	1	41.168	0.066	4
Among populations	22	595.922	1.328	80
Among individuals	216	113.050	0.261	16

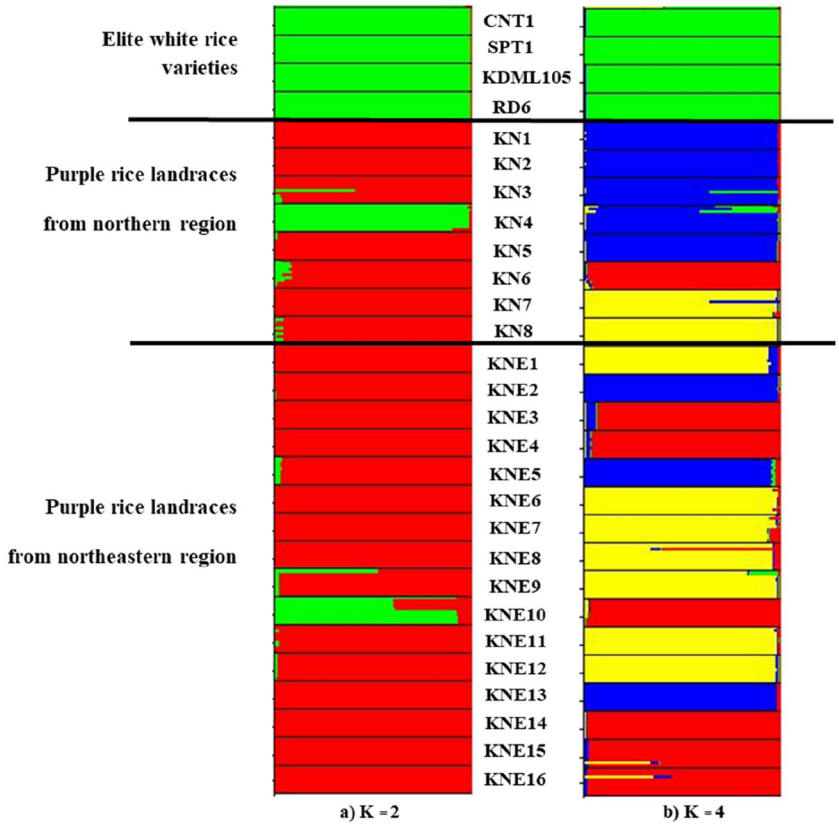

Fig. 2. Population assignment of 24 Thai purple rice landrace populations, northern region (KN), eight populations and northeastern region (KNE), 16 populations, compared with 4 check elite white rice varieties. Each bar represents each population that consisted of 10 individuals. Different colors represent different inferred populations for (**a**) $K = 2$ assigned as green, elite white rice varieties and red, purple rice landraces group and (**b**) $K = 4$ assigned as green, elite white rice varieties (P1); blue, purple rice landraces from northern (P2); yellow, purple rice landraces from northeastern (P3); and red, purple rice landraces from northeastern (P4), and the presence of individuals with genetic admixtures is indicated by more than one color each bar.

from northern Thailand on the upper half of the graph; these correspond to groups P1, P2, and P4 in the STRUCTURE analysis. The purple rice populations from northern Thailand were scattered over the lower half of the graph and correspond broadly to P3 in the STRUCTURE analysis.

Discussion

The molecular characterization of wetland purple rice landraces germplasm of the northern and northeastern regions of Thailand has revealed a diverse array of local genotypes in cultivation. Purple rice is also reportedly diverse in terms of color due to different content, forms, and types of anthocyanin and other morphological characters (Kushwaha 2016), which makes it a valuable germplasm in the production of special quality rice and for rice breeding programs. Efficient use of this germplasm requires knowledge about the patterns of population genetic structure and genetic diversity among the local landraces.

The present study is the first to report on molecular characteristics of a major portion of wetland purple rice landraces diversity available on-farm in northern and northeastern Thailand. Genetic diversity of the surveyed populations, based on 12 microsatellite loci, is low compared to other local rice varieties assessed in previous studies. 'Bue Chomee,' a local non-glutinous variety of the Karen people from northern Thailand, displays a relatively high level of average and total

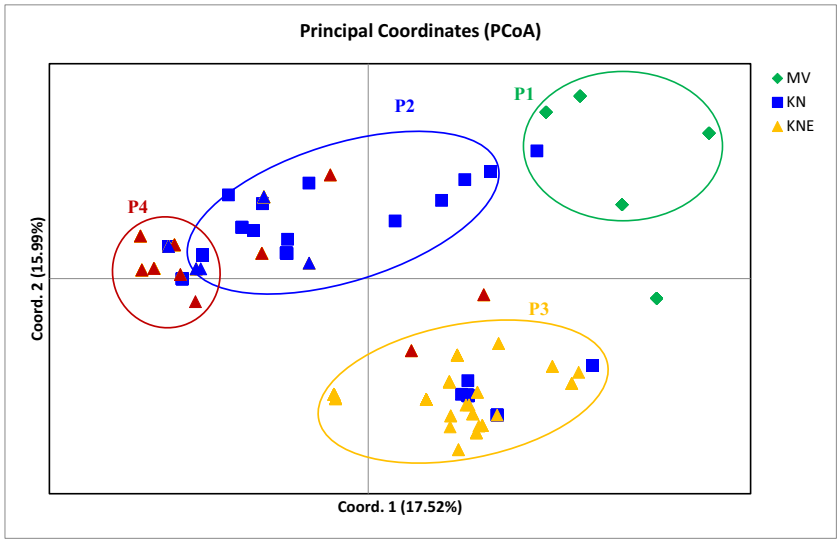

Fig. 3. Principal coordinate analysis (PCoA) of 24 purple rice landraces collected from northern regions (KN), eight populations and northeastern regions (KNE), 16 populations, and 4 check varieties (MV). Different colors represent different groups: green, elite white rice varieties; blue, purple rice landraces populations from northern; yellow, purple rice landraces populations from northeastern; and red, purple rice landraces populations from northeastern Thailand. The shape and color of the symbols correspond to the origin to which the individual belongs, illustrated by the legends of the graphs. P1, P2, P3, and P4 clusters correspond to STRUCTURE result at $K = 4$.

genetic diversity in just one variety name, with diversity values of $H_S = 0.332$ and $H_T = 0.435$, respectively (Pusadee et al. 2009); this diversity is similar to 'Muey Nawng,' a local glutinous variety of local northern Thai people where average genetic diversity was found to be $H_S = 0.202$ and total genetic diversity was $H_T = 0.332$ (Pusadee et al. 2014). The local purple rice in the present study lacks heterozygosity within varieties; this is evident in the observation that $F_{IS} = 1$ for all studied varieties, and by the very low level of average genetic diversity ($H_S = 0.039$) but high level of genetic differentiation ($F_{ST} = 0.841$) (Table 1). These patterns are consistent with genetic fixation among purple rice varieties, a pattern that has likely arisen as an effect of farmers saving and replanting their own family's varieties over multiple generation. The low level of within-variety genetic diversity observed in the present study might be due to limited utilization and cultivation; therefore, genetic variation and pattern of the population structure of local purple rice populations have experienced genetic drift by bottleneck effect due to farmers' selection since only preferable phenotypes were selected and kept by farmers, leading to losing some other traits. In addition to farmer's selection, local adaptation also shapes genetic variation within landrace population.

The patterns of genetic diversity and differentiation observed for wetland purple rice landraces in the present study are notable not only in that it differs from other local landraces, but also because it is quite unlike other landrace rice varieties in the broader Southeast Asian center of rice domestication. Other varieties in this region have typically been found to contain high levels of genetic variation both within and between local or landrace rice varieties. This has been observed in numerous studies, including the following assessments: 517 *indica* landraces rice in China (Huang et al. 2010); 64 umte (large-grained, late maturing) and tening (small-grained, early maturing) hill rice landraces in India (Roy et al. 2016); 175 accessions from upland and lowland ecosystems in Myanmar (Watanabe et al. 2016); 70 accessions of the 'Khao Kai Noi' (KKN) variety in Laos (subdivided based on grain characteristics as 'KKN Deng (red grain),' 'KKN Khao' or 'KKN Khaw (white grain),' 'KKN Leuang (yellow grain),' 'KKN Lai (striped grain),' and 'KKN Dam (black grain)' (Vilayheuang et al. 2016); and two white grain landraces varieties in Thailand, specifically 'Bue Chomee' (a non-glutinous variety) (Pusadee et al. 2009) and 'Muey Nawng' (a glutinous variety) (Pusadee et al. 2014). However, our current results are consistent with

findings by Vilayheuang et al. (2016), who found that black grained 'KKN Dam' contained the lowest level of observed heterozygosity (H_O = 0.001) compared to the others four types of KKN rice, 0.005 in 'KKN Khaw' (white) to 0.038 in 'KKN Hay' (upland).

The results of the STRUCTURE analysis revealed the presence of four subgroups in the sampled purple rice populations (Fig. 2). This observation was in accordance with the clustering observed in the PCoA (Fig. 3). Purple rice from the northeastern showed higher variability and wider distribution on the PCoA graph than the purple rice from the northern. This pattern could suggest that purple rice of the northern region may have originated through introductions from the northeastern; however, further studies need to be performed to test this hypothesis. Important insights could also be gained by performing genetically more detailed analyses using next-generation sequencing approaches to compare Thai purple rice with the much richer purple rice germplasm in Laos, which may have a close relationship with Thai northeastern varieties.

As purple rice is emerging as a high-value crop, further analysis should include detailed phenotypic assessments of special quality characteristics such as anthocyanin content, gamma-oryzonal, and fragrance, and how these traits may add value to the rice crop by genetic manipulation, environmental control, and management. Furthermore, genetic information found here should be taken into account in the conservation of genetic diversity of the purple rice germplasm. Given the importance of landrace germplasm for enhancing crops and the essential role of farmer's practices in maintaining the variation within landraces, the conservation efforts for purple rice should focus on *ex situ* populations across its geographical range, along with on-farm, *in situ* conservation in individual region for the recurrent evolutionary process through local adaptation.

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Compliance with Ethical Standards

Conflict of Interest. The authors declare that they have no conflict of interest.

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