Songklanakarin J. Sci. Technol.
46 (4), 370–376, Jul. – Aug. 2024

http://www.sjst.psu.ac.th

Original Article

Examining phenotypic and genetic diversity of Thai upland rice using HAT-RAPD analysis

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Received: 5 September 2023; Revised: 21 April 2024; Accepted: 21 May 2024

Abstract

Quality and productivity improvement of the native upland rice cultivars is necessary for more extensively practiced non-irrigated rice production. This study aimed to genetically and phenotypically characterize the Thai upland rice cultivars that can serve as potential parents in future rice breeding. Ten upland rice cultivars were morphologically characterized based on their agronomic traits. The genetic relationship among the rice cultivars was evaluated by HAT-RAPD analysis. The use of arbitrary primers led to 69.8% of the polymorphic products. Here, we identified some markers that, as a single primer, allowed differentiation of all cultivars. These markers can be utilized for subsequent investigation of rice diversity. Two dendrograms constructed based on the phenotypes and polymorphic bands divided the upland rice cultivars of interest into three clusters. This information will facilitate additional rice breeding programs.

Keywords: HAT-RAPD, upland rice diversity, Thai rice, clustering analysis

1. Introduction

Upland rice is one of the staple crops in the upland production system in Asia. Upland rice is also considered a local crop that farmers and hill tribe people often produce on highlands or farm areas for household consumption. However, hill tribe people in the north and northeast parts of Thailand continue to cultivate upland rice varieties as their main crop. As it requires relatively little water and has low maintenance costs compared to many crops, upland rice is an excellent alternative for food production during drought or in water-stressed, non-irrigated areas.

There has been considerable in understanding the genetic diversity of cultivars of upland rice due to the recognition of multiple desirable traits. Drought resistance in Chinese upland rice has been identified (Guo, Bai, Su, Shi, & Wang, 2013). There are also upland rice cultivars with outstanding taste and unique aromas. In Indonesia, the production of new, aromatic upland rice cultivars through breeding among the selected aromatic varieties and those with high yields have been demonstrated (Haryanto & Yoshida, 2008). Luempua is among the locally popular rice cultivars in Thailand. The taste and aromatic scent granted its name, “Luempua,” which literally translates to *forget the husband*, as *it is so good that the wife forgets to spare some for her husband*. Further, upland rice can be of high nutritional value. Some upland rice varieties in PR Laos have high grain quality in terms of protein, anthocyanin and anti-oxidative capacity

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(Xiongsiyee *et al*., 2018). Moreover, two Thai pigmented-upland rice varieties, Dam-Mong and Ma-led-Fy, have antioxidant and biological activities that can be developed as potential natural substances for dietary supplements or cosmeceutical products (Sripanidkulchai *et al.*, 2022). Despite this potential, upland rice production is hindered by its relatively low yield (Khotasena, Sanitchon, Chankaew, & Monkham, 2022) and the lack of all-round performing cultivars. Thus, breeding and crop improvement are critical for the more widespread production of upland rice. The shortage of genetic information hinders the process of breeding and crop improvement. Further, data on phenotypic traits are pertinent for usage in conjunction with those on genotypic traits. The greater genetic distance often yields heterosis or hybrid vigor (Hochholdinger & Baldauf, 2018), and thus, examining the genetic and phenotypic diversity of the upland rice will facilitate rice breeding and shorten the time for acquiring new, improved, and desirable rice cultivars. In Thailand, there are multiple varieties of upland rice with mixed qualities; some cultivars are better than others depending on the traits evaluated. Thai upland rice has been reported to have improved yield potential and grain quality, such as amylose content (Phapumma *et al.*, 2020). Furthermore, heterosis breeding has been reported to have developed high-yielding Thai upland rice hybrid cultivars (Sari, Nualsri, Junsawang, & Soonsuwon, 2019). To understand the genetic diversity of upland rice varieties for use in future rice breeding programs, DNA technology, such as SSR, and ISSR markers, has recently been successfully applied (Nilthong, Chukeatirote, & Nilthong, 2020; Tanaporn, Sujaree, & Chontira, 2021). However, there has been little documentation on the genetic diversity of Thai upland rice compared to the number of studies conducted on cultivated and irrigated rice, and multiple upland rice cultivars remain unexamined.

Random amplified polymorphic DNA (RAPD) is an easy, inexpensive method for probing plant genetic properties without the necessity for sequencing, and several modifications of the RAPD techniques have been established. In particular, using increased annealing temperature, termed high annealing temperature (HAT-RAPD), has been demonstrated to be effective because it provides higher resolution and stable reproducible gel electrophoretic patterns (Wongsawad, Wongsawad, Chai, & Anuntalabhochai, 2009). HAT-RAPD has also been utilized in various studies of plant genetic diversity including the identification of Ficus species (Phromthep, 2012) and a Thai Jasmine rice mutants (Semsang, Chundet, & Phanchisri, 2013) and determination of the occurrence of a natural hybridization of Roscoea species (Du, Zhang, & Li, 2012).

This study examined the phenotypic and genetic diversity of ten upland rice cultivars grown in the northern region of Thailand, some of which have not previously been studied. In addition to the morphological traits, the total protein content of the rice grain was examined as an indicator of its nutritional value. There are additional opportunities to improve selection efficiency when more upland rice varieties are researched. The results from this study can be utilized for the selection of rice cultivars and further crop improvement; new, improved upland rice cultivars can be obtained to promote upland rice production in the non-irrigated areas.

2. Materials and Methods

2.1 Plant material and growth

Ten Thai upland rice cultivars were collected from the Northern areas of Thailand including Lebnok, Luempua, Kratitia, Jaye, Jeetao, Mooser, Damtuanieow, Sangyod, Khaomalidoi, and Bueka, Healthy mature seeds were germinated in pots containing black soil and cow manure. After 16 days, one seedling of each cultivar was planted into an individual 35-cm pot in the greenhouse at the Department of Crop Production Technology, Maejo University, Phrae Campus, Thailand, under natural daylight with regular watering. This step was performed in three biological replications.

2.2 Phenotypic traits

Eight morphological traits were recorded for all rice cultivars. These included plant height (cm), number of tillers (no.), heading day (DAI, day after imbibition), panicle length (cm), panicle weight (g), number of grains per panicles, spikelet sterility (%), and 1000-grain weight (g). Plant height and number of tillers were observed at booting stage (90 days after planting). The panicle length, the distance from the panicle neck to the ripening tip was measured. The number of grains per panicles was obtained by counting the number of grains in a healthy panicle per plant at the harvesting stage. The percentage of unfilled grains was determined at the harvesting stage. One thousand-grain weight, divide the total weight in grams by the total number of seeds, multiply by 1000. Protein content was determined through Kjeldahl's method (Kjeldahl, 1883) (AOAC (2012), 991.20) using 0.25 grams of dried rice seed powder. The amount of total nitrogen in the rice seed was multiplied with the traditional conversion factor of 6.25.

2.3 DNA extraction and HAT-RAPD analysis

Genomic DNA was extracted from rice leaf tissues with CTAB extraction buffer (Doyle & Doyle, 1987). Initially, 26 arbitrary primers (Operon Technologies, USA) were evaluated for suitability in amplifying rice samples. Selected primers (OPE-03, OPE-06, OPE-12, OPE-14, OPE-17, OPE-18, OPE-19, OPK-07, OPK-08, OPK-11) were used in the HAT-RAPD analysis. The 25-µL PCR reaction contained 12.5 µL of Quick Taq HS DyeMix (Toyobo, Japan), 0.5 µM primer, and 20 ng of genomic DNA. The reaction mix was preheated at 94 °C for 2 min., followed by 39 cycles of 94 °C for 30 sec., 46 °C for 30 sec., and 68 °C for 1 min. with a 10 min. final extension at 68 °C (Bio-RAD, USA). PCR products were separated on 2% (w/v) agarose gel electrophoresis. Images of the ethidium bromide-stained gel were taken using gel documentation (UVP, Taiwan).

2.4 Data analysis

The data were statistically analyzed with analysis of variance (ANOVA) using IBM SPSS statistic software (version 28.0). The treatment differences were performed using Duncan multiple range test (DMRT) at $p<0.05$. Genetic distance among varieties was measured by the Euclidian distance method using STATISTICA 13.3 software. Two dendrograms were constructed using the unweighted pair group method with arithmetic mean (UPGMA) (STATISTICA 13.3).

3. Results and Discussion

3.1 Morphological examination revealed phenotypic diversity among the selected upland rice cultivars

Growth and morphological aspects of 10 Thai upland rice cultivars produced during the months with shorter days were observed. Lebnok and Sangyod rice, which were light-sensitive, neither initiated panicles nor flowered throughout the study (Figure 1). Among the ten cultivars, Luempua rice displayed the highest height growth at an average of 123.9 cm, which was significantly different from Lebnok, Damtuanieow, and Bueka. The upland rice had low numbers of tiller emergence; in general, the average tillering was 3 tillers per the main culm. Luempua had the least tiller numbers (Figure 1). The heading day of the ten rice cultivars, though, was comparable, ranging from 98-113.3 DAI (Table 1). Jeetao and Damtuanieow had the shortest and the longest heading days, respectively.

Luempua had the longest panicle compared to the other. The average panicle length of Luempua rice was 28.1 cm, while the shortest panicles, approximately 19 cm in length, belonged to Mooser and Khaomalidoi rice. Additionally, Luempua rice had the highest panicle weight as well as 1,000-grains weight, indicating that this cultivar had the greatest panicle growth among the upland rice cultivars investigated in this study. Nonetheless, the numbers of grains per panicle were comparable across different cultivars, with Bueka on the higher end and Damtuanieow on the lower end.

In terms of fertility, the spikelet sterility was examined. Damtuanieow and Khaomalidoi possessed the highest percentage of spikelet sterility at approximately 40%. In contrast, Luempua and Jaye had the lowest percentages at about 11% spikelet sterility. Although the long-grain shape was a shared feature among these rice cultivars, the pericarp colors of these ten cultivars were varied. Lebnok, Kratitia, Jaye, Jeetao, and Khaomalidoi possessed non-pigmented pericarps (off-white color); Mooser and Bueka had light brown pericarps; Sangyod had red pericarp, while Luempua and Damtuanieow had purple pericarps (Figure 1).

Rice is generally considered a short-day plant, and its photoperiod sensitivity varies among cultivars (Dogara *et al*., 2018). Remarkably, upland rice in Thailand has photoperiod sensitivity to prevent environmental effects during the growing season (Khotasena *et al*., 2020). Upland rice in this study comprises photoperiod-sensitive and insensitive cultivars, limiting rice production to seasons with shorter days. Among the ten cultivars investigated, Lebnok and Sangyod are the two photoperiod-sensitive ones. Since this study was conducted during a period of longer days, some morphological aspects of Lebnok and Sangyod were omitted from the report.

To determine the rice yield potential, Thai upland rice in this study had low crop yields similar to upland rice in the region of Chiang Rai, Thailand (Karladee, Boonsit, Suriyong, & Jamjod, 2012) and the upland rice germplasm was collected from rice growing areas in Thailand (Khotasena *et al*., 2020). Moreover, Thai upland rice was lower in tiller numbers, with the exception of Lebnok and Sangyod. These two photoperiod-sensitive cultivars were higher in tiller numbers; as the photoperiod inhibited their flowering, they solely grew laterally in the vegetative stage (Dogara *et al*., 2018). Apart from the low number of tillers per hill, some cultivars had notable proportions of underdeveloped kernels, leading to low crop yields.

Similar to the upland rice in Indonesia (Mau *et al*., 2017), Thai upland rice comprises cultivars with varied pericarp colors that stem from the presence of anthocyanins; these antioxidant compounds allow the upland rice to serve as a health-promoting, functional food. In addition, studies of pigments in pericarps of upland rice cultivated in PR Laos reported a significant, positive relationship between anthocyanin and antioxidant concentrations (Xiongsiyee *et al*., 2018). Furthermore, the other study indicated that red and black grains of rice are more nutritious compared to the common white rice (Mau *et al*., 2017), making the cultivars with pigmented pericarps like those in this study attractive for use in crop improvement.

Figure 1. Morphology of ten Thai upland rice cultivars collected from the Northern region of Thailand during the longer-day season

Table 1. Morphological traits of ten Thai upland rice cultivars

Upland rice cultivars	Plant height (cm)	Tiller (no.)	Heading day (DAI)	Panicle length (cm)	Panicle weight (g)	Number of grains per panicle	Spikelet sterility (%)	1,000- grain weight (g)
Lebnok	98.5 ± 7.4^{b}	6.7 ± 0.6^{b}	-	-	-	-	-	-
Luempua	123.9 ± 5.3^{a}	1.7 ± 0.6^{d}	104.7 ± 6.0^{bc}	28.1 ± 0.2^{a}	3.41 ± 0.51^{a}	112.0 ± 17.0^{ab}	11.1 ± 0.6^{b}	29.14 ± 0.23^{a}
Kratitia	104.1 ± 18.9^{ab}	3.3 ± 0.6^{c}	106.3 ± 4.7^{abc}	25.1 ± 0.4^{b}	2.38 ± 0.19^{b}	122.0 ± 4.0^{ab}	30.3 ± 13.4^{ab}	18.57 ± 2.32^{c}
Jaye	104.8 ± 8.5^{ab}	2.7 ± 0.6^{cd}	101.3 ± 4.0^{bc}	23.2 ± 1.2^{d}	2.72 ± 0.30^{ab}	96.7 ± 3.5^{ab}	11.5 ± 3.5^{b}	26.75 ± 1.99^{ab}
Jeetao	107.1 ± 7.6^{ab}	3.3 ± 0.6^{c}	98.0 ± 1.0^{c}	21.9 ± 0.6^{e}	2.78 ± 0.45^{ab}	117.3 ± 27.6^{ab}	14.9 ± 13.3^{b}	22.87 ± 3.06^{bc}
Mooser	108.2 ± 5.8^{ab}	3.0 ± 0.0^{c}	104.3 ± 7.0^{bc}	18.9 ± 0.6^{f}	2.62 ± 0.56^{ab}	118.0 ± 31.0^{ab}	21.6 ± 4.8^{ab}	22.16 ± 0.58^{c}
Damtuanieow	95.9 ± 19.2^{b}	3.0 ± 1.0^{c}	113.3 ± 3.2^{a}	24.8 ± 0.9^{bc}	2.02 ± 0.57^{b}	86.3 ± 10.5^{b}	38.7 ± 15.8^{a}	21.37 ± 4.36^{c}
Sangyod	107.5 ± 2.3^{ab}	8.0 ± 1.0^{a}	-	-	-	-	-	-
Khaomalidoi	103.8 ± 7.6^{ab}	3.3 ± 1.2^{c}	107.7 ± 2.9^{ab}	18.8 ± 0.4^{f}	2.35 ± 0.23^{b}	116.7 ± 8.5^{ab}	39.9 ± 11.9^{a}	18.81 ± 0.25^{c}
Bueka	101.7 ± 7.2^{b}	3.3 ± 0.6^{c}	99.0 ± 2.0^{bc}	23.8 ± 0.4^{cd}	2.88 ± 0.72^{ab}	128.3 ± 18.3^{a}	15.8 ± 4.9^{b}	21.22 ± 2.42^{c}
%CV	9.9070 %	19.2486 %	4.3804 %	2.8201 %	18.4321 %	16.5135 %	44.2567 %	10.5267 %

Standard deviations were calculated from sample sizes of n=3; Means in the columns followed by the same letter(s) are not significantly at $P < 0.05$ different from each other according to Duncan's Multiple Range Test

3.2 Morphological relationship analysis put ten upland rice cultivars into three distinct clusters

The pattern of phenotypic diversity of eight morphological traits was studied in ten Thai upland rice cultivars. The resultant Euclidean distance values ranged from 6.7 to 170.0. Comparatively lower distance was found between Mooser and Khaomalidoi, while the distance between Luempua and Sangyod was highest. Euclidean distances measured from the mean performance data of different quantitative characters established a strong relationship between Mooser and Khaomalidoi, suggesting that these two rice cultivars were highly similar. On the other hand, Luempua and Sangyod were highly different according to the calculated Euclidean distance.

The dendrogram constructed separated ten Thai upland rice into three distinct clusters based on the genotypes' mean values of eight quantitative traits (Figure 2). Cluster 1 consists of four cultivars: Lebnok, Sangyod, Jeetao, and Bueka; Cluster 2 consists of two cultivars: Luempua and Damtuanieow; and Cluster 3 consists of four cultivars: Kratitia, Mooser, Khamalidoi, and Jaye.

Information about genetic distances in crop populations of different characters is crucial for evaluating their phylogenetic relationship and patterns of evolution. The Euclidean distance analysis of ten Thai upland rice cultivars showed results similar to the constructed phenotypic dendrogram (Figure 2): Mooser and Khaomalidoi were with the closest distance and grouped within the same cluster in the phenotypic dendrogram. In contrast, Luempua and Sangyod, which had the longest Euclidean distance, were placed in different phenotypic clusters. In the phenotypic dendrogram, Lebnok and Sangyod were in the same cluster. These two were phenotypically correlated; they both are sensitive to the photoperiod. Further, the cultivars with short heading days (<100 days), Jeetao and Bueka, and the purple-pericarp, sticky rice cultivars, Luempua and Damtuanieow, were placed in the same clusters. The result suggested that the phenotypes grouped under the same clusters shared similarities for multiple traits.

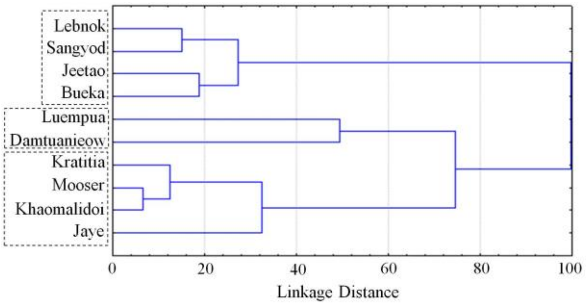

Figure 2. Dendrogram of ten Thai upland rice cultivars based on eight quantitative, morphological traits

3.3 HAT-RAPD and cluster analyses revealed genetic diversity of the Northern-grown Thai upland rice

Ten primers (38.5%) out of 26 primers were potentially capable of differentiating the genetic profiles of the ten upland rice cultivars. The HAT-RAPD genotyping yielded a total of 106 amplified fragments, 73 of which were polymorphic. The percentage of polymorphism produced by the selected primers was 68.9%, while the highest percentage of polymorphism (90%) was recorded by primer OPE-14 (Figure 3). Primer OPE-18 generated the highest number of amplified fragments, and primer OPE-17, as well as OPK-11, generated the lowest number of amplified fragments. Here, primers OPE-12, OPE-14, OPE-18, and OPK-08 were valid for differentiating all cultivars when using them individually. The identified primers in this study can be adopted as DNA markers for upland rice classification.

The HAT-RAPD results were used to conduct the cluster analysis. The resultant Dendrogram using a linkage distance of 5.0 yielded 3 clusters of rice cultivars. The three clusters comprised Lebnok and Sangyod in Cluster 1, Luempua as a sole cultivar in Cluster 2, and the rest, Kratitia, Mooser, Khanalidoi, Bueka, Jaye, Jeetao and Damtuanieow, in Cluster 3 (Figure 4).

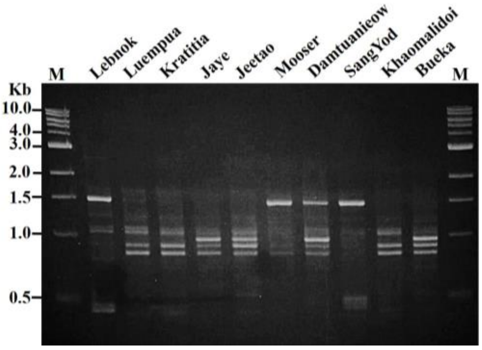

Figure 3. HAT-RAPD profiles of ten Thai upland rice cultivars obtained using OPE-14 primer: M = 1 kb DNA ladder

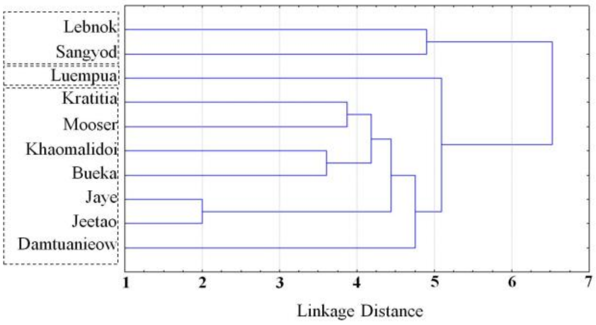

Figure 4. Dendrogram of ten Thai upland rice cultivars based on genotypic traits derived from the HAT-RAPD results

Lebnok and Sangyod were found in the same dendrogram cluster derived from morphological traits and the DNA fingerprint data. Both rice cultivars are photoperiod sensitive and genetically similar based on the HAT-RAPD result. Analogously, Luempua was placed in its own cluster in both dendrograms, while the grouping of Kratitia, Mooser, Kaomalidoi, and Jaye was comparable between two dendrograms. Although, there were some dissimilarities between the two dendrograms as well. In the dendrogram constructed based on rice morphological traits, Luempua and Damtuanieow were placed in the same cluster. While the two rice cultivars shared the same pericarp color, purple, they were placed in different clusters when classified with HAT-RAPD. Jeetao and Bueka were also grouped differently in two dendrograms, suggesting that further experiments, such as morphological classification in different seasons, might be required. The primer library could also be diversified, allowing more product patterns to be established. Dendrograms derived from both morphological traits and genetic marker analysis provide useful data that aid in crop improvement; new rice populations with outbreeding enhancement and heterosis can be achieved.

3.4 Total protein content analysis showed the nutritional value of the selected upland rice grains

Protein is one of the key factors determining the rice nutritional quality. Determining total protein content in the representative upland rice from each cluster, Jaye had the highest amount of total protein, 14.564% (g/100 g of rice grains), while Khaomalidoi possesses the lowest amount of protein (8.318%) among the rice cultivars tested (Table 2).

When selecting rice cultivars for crop improvement, the chemical quality of rice is also a critical factor. Following starch, protein is the second most abundant constituent of milled rice (Bao, 2019). Still, proteins typically comprise 7-10% of the grain's dry weight (Khush, 1997). In this study, the total protein was found to be 8.32-14.56% (w/w) in the grains of selected upland rice. These numbers aligned well with those from previous studies (Aiyswaraya *et al*., 2017). For instance, the total protein content in two cultivated rice in China, *O. sativa* japonica Hexi35 and *O. sativa* indica Dianlong201, was reported to be 9.523% and 11.76% (w/w), respectively.

Table 2. Total protein content in the upland rice grains

Cultivars	Total protein (g/100 g of grains; %)
Lebnok	9.919 ± 0.237^{d}
Luempua	11.363 ± 0.219^{c}
Jaye	14.564 ± 0.195^{a}
Mooser	11.894 ± 0.234^{b}
Sangyod	$10.196 \pm 0.0.25^{d}$
Khaomalidoi	8.318 ± 0.204^{e}

Standard deviations were calculated from sample size of n=3; Means in the columns followed by the same letter(s) are not significantly, at $P < 0.05$, different from each other according to Duncan's Multiple Range Test

Considering the quantitative morphological traits described above, Pearson's correlation coefficient was used to calculate between each trait and the total protein in rice grains. Interestingly, a strong positive correlation was found between the 1,000-grain weight and the grain protein content. Proteins account for about 10% (w/w) of the rice grain (Zhou, Robards, Helliwell, & Blanchard, 2002). Some upland rice cultivars exhibited higher protein content compared to the average cultivated rice. Notably, this increased protein content also substantially influenced the increased 1000-grain weight. It was also reported that high rice grain protein content is associated with high head rice yield, which is desirable for rice breeders (Balindong *et al*., 2018). At the same time, negative correlations were found between the grain protein content and heading day (DAI), the number of grains per panicle, and the spikelet sterility (%) (Table 3). Taken together, Thai upland rice represented by the ten cultivars studied here are quite attractive for breeding and selection for further crop improvement, considering the nutritional value and requirements for growth/cultivation.

4. Conclusions

The morphological and genetic diversity of ten Northern-grown, Thai upland rice cultivars were examined and grouped based on the UPGMA clustering method. The total protein content of the rice grains was assayed from each cluster to gain an insight into the rice's nutritional aspects. Lebnok and Sangyod had a similar amount of total protein. This similarity was found in both dendrograms constructed

Table 3. Pearson’s correlation coefficient of the total protein content (g/100 g grains) and eight quantitative morphological traits (Table 1). Green color indicates strong positive correlation (correlation coefficient = 1) while red color indicates string negative correlation (correlation coefficient = -1)

	1	2	3	4	5	6	7	8	9
1. Plant height (cm)	1.0000								
2. Tiller (no.)	-0.5280	1.0000							
3. Heading day (DAI)	-0.0008	0.3043	1.0000						
4. Panicle length (cm)	0.8405	-0.9720	-0.3537	1.0000					
5. Panicle weight (g)	0.9510	-0.9959	-0.2895	**0.9468****	1.0000				
6. Number of grains per panicle	0.1475	0.2211	**0.8177***	-0.3934	-0.1576	1.0000			
7. Spikelet sterility (%)	-0.5462	0.7621	**0.8381***	-0.7524	-0.7606	0.6012	1.0000		
8. 1000 grain weight (g)	0.7138	-0.9118	-0.6613	**0.9286****	**0.8953***	-0.5642	**-0.9420****	1.0000	
9. Total proteins (g/100 g grains)	0.1805	-0.4004	**-0.9989****	0.3475	0.2987	-0.7893	-0.8457	0.6610	1.0000

Values in bold are different from 0 with a significance level alpha=0.05; * 0.01 < p-value 0.05; ** p-value < 0.01

🟥 Strong negative correlation
🟧 Moderate negative correlation
🟨 No correlation
🟩 Moderate positive correlation
🟩 Strong positive correlation

based on morphological traits and genotypic traits results. The two upland rice cultivars were placed in the same clusters, suggesting that they are genetically close. For breeding and crop improvement purposes, Lebnok and Jaye comprise a recommended pair as they are genetically different, while Jaye possesses high total protein content. This study demonstrated the use of simple methods to examine genotypic and phenotypic traits in the under-studied Thai upland rice cultivars. Thai upland rice's qualities indicate a promising source of nutrition and genetic resources for crop improvement.

Acknowledgements

This research was partially supported by the funding from Maejo University Phrae Campus, Thailand.

References

Aiyswaraya, S., Saraswathi, R., Ramchander, S., Jaivel, N., Uma, D., Sudhakar, D., & Robin, S. (2017). Evaluation of rice (*Oryza sativa* L.) germplasm for the identification of high folate accession using HPLC. *International Journal of Current Microbiology and Applied Sciences*, *6*(11), 5328-5346. doi:10.20546/ijcmas.2017.611.509

Balindong, J. L., Ward, R. M., Rose, T. J., Liu, L., Raymond, C. A., Snell, P. J., . . . Waters, D. L. E. (2018). Rice grain protein composition influences head rice yield. *Cereal Chemistry*, *95*(2), 253-263. doi:10.1002/cche.10031

Bao, J. (2019). *A text book of rice: Chemistry and technology* (4th ed). Saint Paul, MN: AACC International Press.

Dogara, A. M., Tahir, S. M., Shehu, I., Abbah, M., Shitu, U., Ladidi, Z. I., & Jumare, A. I. (2018). Mechanisms of photoperiod in regulation of rice flowering. *International Journal of Technical Research and Applications*, *2*(2), 56-58. Retrieved from https://www.researchgate.net/publication/325153306

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue. *Phytochemical Bulletin*, *19*, 11-15. Retrieved from https://webpages.charlotte.edu/~jweller2/pages/BINF8350f2011/BINF8350_Readings/Doyle_plantDNAextractCTAB_1987.pdf

Du, G. H., Zhang, Z. Q., & Li, Q. J. (2012). Morphological and molecular evidence for natural hybridization in sympatric population of Roscoeahumeana and R. cautleoides (Zingiberaceae). *Journal of Plant Research*, *125*(5), 595-603. doi:10.1007/s10265-012-0478-6

Guo, X. L., Bai, L. R., Su, C. Q., Shi, L. R., & Wang, D. W. (2013). Molecular cloning and expression of drought-induced protein 3 (DIP3) encoding a class III chitinase in upland rice. *Genetics and Molecular Research*, *12*(4), 6860-6870. doi:10.4238/2013.December.19.5.

Haryanto, T. A. D., & Yoshida, T. (2008). Yield stability of aromatic upland rice with high yielding ability in Indonesia. *Plant Production Science*, *11*(1), 96-103. doi:10.1626/pps.11.96

Hochholdinger, F., & Baldauf, J. A. (2018). Heterosis in plants. *Current Biology*. *28*(18), R1089–R1092. doi:10.1016/j.cub.2018.06.041

Karladee, D., Boonsit, P., Suriyong, S., & Jamjod, S. (2012). Population heterogeneity of upland rice in northern Thailand. *Thai Journal of Agricultural Science*, *45*(2), 99-104. Retrieved from https://www.researchgate.net/publication/287927308_Population_heterogeneity_of_upland_rice_in_northern_Thailand

Khotasena, S., Sanitchon, J., Chankaew, S., & Monkham, T. (2022). The basic vegetative phase and photoperiod sensitivity index as the major criteria for indigenous upland rice production in Thailand under unpredictable conditions. *Agronomy*, *12*(4), 957. doi:10.3390/agronomy12040957

Khush, G. S. (1997). Origin, dispersal, cultivation and variation of rice. *Plant Molecular Biology*, *35*, 25-34. doi:10.1023/A:1005810616885

Kjeldahl C. (1883). A new method for the determination of nitrogen in organic matter. *Fresenius' Zeitschrift für Analytische Chemie*, *22*, 366-382. Retrieved from https://doi.org/10.1007/BF01338151

Mau, Y. S., Markus, J. E. R., Shirly, S., Oematan, S., Ndiwa, A. S. S., Handoko, D. D., . . . Makbul, K. (2017). Genetic diversity of red and black upland rice accessions from East Nusa Tenggara, Indonesia as revealed by agro-morphological characters. *Biodiversitas Journal of Biological Diversity, 18*(1), 197-211. doi:10.13057/biodiv/d180127

Nilthong, S., Chukeatirote, E., & Nilthong, R. (2020). Assessment of genetic diversity in Thai upland rice varieties using SSR markers. *Australian Journal of Crop Science*, *14*(4), 597-604. doi:10.21475/ajcs.20.14.04.p2092

Phapumma, A., Monkham, T., Chankaew, S., Kaewpradit, W., Harakotr, P., & Sanitchon, J. (2020). Characterization of indigenous upland rice varieties for high yield potential and grain quality characters under rainfed conditions in Thailand. *Annals of Agricultural Sciences, 65*(2), 179-187. doi:10.1016/j.aoas.2020.09.004

Phromthep, W., (2012). A new genetic analysis of Ficus spp. By HAT-Random amplified Polymorphic DNA Technique. *Procedia Engineering*, *32*, 1073-1079. doi:10.1016/j.proeng.2012.02.057

Sari, W. K., Nualsri, C., Junsawang, N. & Soonsuwon, W. (2019). Heterosis studies for yield and agronomic traits in Thai upland rice. *Indian Journal of Agricultural Research, 53*(3), 255-262. doi:10.18805/A-390

Semsang, N., Chundet, R., & Phanchisri, B. (2013). Development of a SCAR marker for discrimination of a Thai jasmine rice (*Oryza sativa* L. cv. KDML105) mutant, BKOS6, and associated with purple color trait in Thai jasmine rice-related varieties. *American Journal of Plant Sciences*, *4*(9), 1774-1783. doi:10.4236/ajps.2013.49218

Sripanidkulchai, B., Junlatat, J., Tuntiyasawasdikul, S., Fangkrathok, N., Sanitchon, J., & Chankaew, S. (2022). Phytochemical and bioactivity investigation of Thai pigmented-upland rice: Dam-Mong and Ma-led-Fy varieties. *Agriculture and Natural Resources*, *56* (5), 889-898. Retrieved from https://li01.tci-thaijo.org/index.php/anres/article/view/256569.

Tanaporn, K., Sujaree, N., & Chontira, S. (2021). Genetic diversity of thai upland rice germplasm based on inter-simple sequence repeats marker. *Chiang Mai Journal of Science*, *48*(5), 1287-130. Retrieved from http://epg.science.cmu.ac.th/ejournal/ Contributed Paper

Wongsawad, C., Wongsawad, P., Chai, J. Y., & Anuntalabhochai, S. (2009). Haplorchis taichui, Witenberg, 1930: Development of a HAT-RAPD marker for the detection of minute intestinal fluke infection. *Experimental Parasitology*, *123*(2), 158-161. doi: 10.1016/j.exppara.2009.06.016

Xiongsiyee, V., Rerkasem, B., Veeradittakit, J., Saenchai, C., Lordkaew, S., & Prom-u-thai, C. T. (2018). Variation in grain quality of upland rice from Luang prabang province, Lao PDR. *Rice Science, 25*(2), 94-102. doi:10.1016/j.rsci.2018.02.002

Zhou, Z., Robards, K., Helliwell, S. & Blanchard, C. (2002). Composition and functional properties of rice. *International Journal of Food Science*, *37*, 849-868. Retrieved from https://doi.org/10.1046/j.1365-2621.2002.00625.x