

## GENETIC VARIABILITY AND DIVERSITY OF UPLAND RICE LANDRACES

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### ABSTRACT

The objective of this study was to investigate the genetic variability among 43 Thai upland rice genotypes and to estimate the broad-sense heritability for the examined traits. The genetic variability and diversity studies provide important information for plant breeders who manage breeding programs. Forty-three upland rice genotypes in Thailand were assessed for the genetic variability using randomized complete block designs with three replications in two years at the Faculty of Natural Resources, Prince of Songkla University. The seven characterized quantitative traits showed significant variations by genotypes, except rice yield. For the interaction between Genotype and Year was significant for days to maturity, plant height, number of tillers hill<sup>-1</sup>, rice yield, and thousand grain weight. High genetic coefficients of variation were observed for days to flowering, plant height, number of tillers hill<sup>-1</sup>, thousand grain weight, and grain width. High broad-sense heritability was found for days to flowering, days to maturity, plant height, number of tillers hill<sup>-1</sup>, thousand grain weight, grain width, and grain length. The cluster analysis grouped the 43 genotypes into eight groups based on SSR markers at 0.75 similarity level. These results show that the large variability will help rice breeders in selecting the appropriate genotypes for future breeding programs.

**Keywords:** Cluster analysis, genetic coefficient, heritability, molecular marker, *Oryza sativa* L.

### INTRODUCTION

Rice (*Oryza sativa* L.), especially the Indica subspecies, is a staple food in Asian countries including Bangladesh, Indonesia, Philippines, Thailand, and Vietnam. It is the most important cereal grown worldwide (FAO, 2020). Diverse ecosystems are used in rice cultivation, such as irrigated, rain fed lowland, deep water, and upland (Rao et al., 2017). In Thailand, upland rice yields in farmers' fields are normally low; averaging only 2.12 t ha<sup>-1</sup> (DGA, 2022). Plant breeders are interested in improving new high yield cultivars with other desirable agronomic characteristics such as blast disease resistance (Luangmanee et al., 2016). Estimates of the genetic variances of these traits can assist to design a breeding program to develop new cultivars. Broad-sense heritability ( $H^2$ ) is the fraction of genetic variance in the total phenotypic variance (Bernardo, 2002; Ozturk and Yildirim, 2014). Heritability shows the responses to selection. The yield components have been confirmed to be the most effective traits in improving rice yield when these characters have high heritability and positively correlations with each other and rice yield (Surek and Beser, 2003).

The genetic diversity of crops is very essential in crop breeding and germplasm conservation. Crop genetic diversity can be assessed using various methods, such as morphological and molecular markers. Morphological markers are phenotypic characteristics, and these are also impacted by environment, gene expression variability and narrow diversity. Molecular markers are DNA-based, such as random amplified polymorphic DNA (RAPD), microsatellites or simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) (Khan, 2015). DNA markers can improve accuracy and efficiency of genetic diversity analysis.

An SSR marker was effectively indicated to separate genetic diversity and associations in crop germplasms (Powell et al., 1996). The SSR markers are mono-locus, multi-allelic, co-dominant, uncomplicated, rapid, economical and conveniently automated (McCouch et al., 2002; Gonzaga et al., 2015). The SSR markers were advantageous in investigations of diversity and relationships among rice germplasms (Rashmi et al., 2017; Park et al., 2019; Pathaichindachote et al., 2019; Nilthong et al., 2020).

The objective of this study was to investigate the genetic variability among 43 Thai upland rice genotypes, for later use in the future breeding programs.

## MATERIALS and METHODS

### *Plant material and field experimental design*

Forty-three upland rice genotypes selected from one crop of healthy rice by planting in isolated area to prevent the hybridization between each genotype (Table 1) were planted in a randomized complete block design with three replications, at the research field of Faculty of Natural

Resources, Prince of Songkla University, Hat Yai, Songkhla, Thailand (altitude = 22 m, 7°00'31"N, 100°29'46"E). The experiment conducted for two years. Each plot had four rows of 4 m length with 0.30 m spacing between the rows, and 0.25 m between the plants in a row. The NPK (15–15–15) fertilizer was applied at the rate 156 kg ha<sup>-1</sup> before planting and at panicle initiation stages. Urea (46–0–0) fertilizer was applied at the rate 31 kg ha<sup>-1</sup> at panicle initiation stages. Agronomic procedures, such as weed control, were in general done manually, and insect control was through the application of 40 ml cypermethrin 10% v/v EC 20 L water<sup>-1</sup>.

**Table 1.** Genotype name, cultivation type, and location of upland rice

No.	Name (Type)	Region /Province	No.	Name (Type)	Region /Province
NR008	Daeng (G)	N/Nan	NR092	Bow Leb Nahng (NG)	S/Satun
NR025	Jao Yuak (G)	N/Nan	NR093	Gam Niaw (G)	NE/Maharakham
NR030	Nam-ngern (G)	N/Nan	NR094	Maharakham (G)	NE/Maharakham
NR044	Sew Phan (G)	N/Nan	NR100	Niaw Ma-led-lek (G)	N/Chiang Mai
NR048	Gam Pleuak Khao (G)	N/Nan	NR102	Nual Hawm (NG)	S/Songkhla
NR064	Hua Bon (NG)	S/Krabi	NR104	Sew Mae Jan (G)	NE/Sakon Nakhon
NR065	Dawk Kha 50 (NG)	S/Krabi	NR105	Leum Pua (G)	NE/Sakon Nakhon
NR066	Hawm Jet Ban (NG)	S/Krabi	NR108	Hawm Sakon (G)	NE/Sakon Nakhon
NR067	Dawk Pa-yawm (NG)	S/Phatthalung	NR110	Mai Tahk (NG)	S/Songkhla
NR069	Trai (NG)	S/Krabi	NR111	Chaw La-mud (NG)	S/Nakhon Si Thammarat
NR070	Ruang (NG)	S/Krabi	NR112	Ma-led-nai-fai (NG)	S/Nakhon Si Thammarat
NR073	Sahm Deuan (NG)	S/Chumphon	NR115	Niaw Dam-mohr (G)	S/Pattani
NR074	Nahng Kruan (NG)	S/Chumphon	NR116	Niaw Dam Chaw Mai Pai 49 (G)	S/Pattani
NR076	Dawk Kam (NG)	S/Chumphon	NR118	Niaw Peek Peun Meuang (G)	N/Mae Hong Son
NR077	Nahng Kian (NG)	S/Chumphon	NR119	Gum Mong (G)	N/Mae Hong Son
NR078	Phu Khow Thong (NG)	S/Chumphon	NR120	Mong Pleuak Lai (NG)	N/Mae Hong Son
NR079	Nahng Dum (NG)	S/Chumphon	NR122	Mong Pah-sum-ran (NG)	N/Mae Hong Son
NR081	Goo Meuang Luang (NG)	S/Phatthalung	NR123	Jao Khao (NG)	C/Phichit
NR085	Niaw Hawm (G)	S/Songkhla	NR124	Hawm Mali Doi (NG)	N/Chiang Mai
NR086	Niaw Dum (G)	S/Songkhla	NR126	Niaw ngern (G)	C/Phichit
NR088	Hawm Satun (NG)	S/Satun	NR127	Jao Daeng (NG)	C/Phichit
NR089	Niaw Daeng (G)	S/Satun			

NG = Non-glutinous, G = Glutinous, C = Central; N = North, NE = Northeastern, S = South

### *Field data collection*

Eight agro-morphological traits were recorded for ten plants genotype<sup>-1</sup> in each replicate: days to flowering, days to maturity, plant height, number of tillers plant<sup>-1</sup>, yield, thousand grain weight, grain width, and grain length.

### *Sample collection and DNA extraction*

Young leaves of 43 upland rice genotypes were collected and put into plastic bags, placed in ice, and were later frozen and stored at -20°C. DNA from rice leaf tissue was extracted with N-Cetyl-N,N,N-trimethylammonium bromide method (CTAB) of Dellporta et al. (1983) with slight modifications. Approximately 0.3 g of leaf tissue was ground to fine powder in the presence of liquid nitrogen using mortar and pestle. The powder was then transferred into 1.5 ml Oak Ridge tube, mixed with 700 µl extraction CTAB buffer (PVP-40 1%, 1.4 mM NaCl, 20 mM Na2EDTA pH 8.0, and CTAB 2%) as well as 2% β-Mercaptoethanol, and incubated at 65°C for 1 hour. Then 700 µl of Chloroform:Isoamyl alcohol (24:1 v/v) was added with shaking for 20 mins. The mixture was centrifuged at 12000 rpm for 15 mins at 4°C to separate leaf residues. The

supernatant was collected into a new tube and was mixed with 700 µl isopropanol to precipitate the DNA. After discarding the supernatant, the DNA pellet was washed with 70 % ethyl alcohol (500 µl) two times for 5 mins each time, dried, and then 30 µl of TE buffer was added. DNA concentration and purity of these samples were measured using a spectrophotometer at wavelengths 260 and 280 nm.

### *SSR analysis and primer screening*

SSR analysis of genomic DNA was carried out using ten SSR rice primer pairs (Table 2). The PCR reaction blend had 20 ng of genomic DNA, 2 µl of 10X Taq buffer, 0.2 µM of each primer in forward and reverse primer pair, 200 µM of each of the four dNTPs, and 0.7 units of Taq polymerase. PCR amplifications were carried out on a Biometra thermo cycler using the following program: denaturation at 94°C for 3 mins 1 cycle, annealing at 52 or 55°C (depending on the primer used) for 1 min, extension at 72°C for 1 min for 30 cycles, and a final elongation step at 72°C for 5 mins. The products were stored at -20°C. The PCR products were later separated by gel electrophoresis using 3% (W/V) agarose gel, run at a constant 100 V for 60 mins. The gel was stained with ethidium bromide for 15

mins and de-stained with double-distilled water for 15 mins. The bands were detected under UV light.

**Table 2.** The primer pairs used in SSR analysis

Primers	Chr.	Sequence (5' to 3')	TA	Fragments size
RM5	1	(F)TGCAACTTCTAGCTGCTCGA (R)GCATCCGATCTTGATGGG	57	94-138
RM259	1	(F)TGGAGTTTGAGAGGAGGG (R)CTTGTTGCATGGTGCCATGT	55	133-186
RM283	1	(F)GTCTACATGTACCCTTGTGGG (R)CGGCATGAGAGTCTGTGATG	61	130-176
RM307	4	(F)GTACTACCGACCTACCGTTCAC (R)CTGCTATGCATGAACTGCTC	55	116-191
RM413	5	(F)GGCGATTCCTGGATGAAGAG (R)TCCCCACCAATCTTGCTCTC	53	71-114
RM455	7	(F)AACAACCCACCACCTGTCTC (R)AGAAGGAAAAGGGCTCGATC	57	127-144
RM44	8	(F)ACGGGCAATCCGAACAACC (R)TCGGGAAAACCTACCCTACC	53	82-132
RM215	9	(F)CAAAATGGAGCAGCAAGAGC (R)TGAGCACCTCCTTCTCTGTAG	55	126-161
RM316	9	(F)CTAGTTGGGCATACGATGGC (R)ACGCTTATATGTTACGTCAAC	55	194-216
RM19	12	(F)CAAAAACAGAGCAGATGAC (R)CTCAAGATGGACGCCAAGA	55	192-250

Chr. = Chromosome, TA = Temperature of amplification  
From: Anonymous (2021)

#### Statistical and genetic analysis

The agro-morphological traits of genotypes in each year were subjected to analysis of variance (ANOVA). Homogeneity of variance was checked with F-test (Gomez and Gomez, 2001). If it was homogeneous, the quantitative trait means of the genotypes evaluated over two years were used for analysis by year (Table 3). The mean trait values of 43 genotypes were also subjected to the Duncan's Multiple Range test (DMRT). The R program with agricolae package was used for the calculations (Mendiburu and Simon, 2007). Broad-sense heritabilities based on family means ( $H^2$ ) were analyzed with the formulary given by Bernado (2002). The following equations were used to estimate the genetic parameters:

a) Genotypic variance:  $(\sigma_g^2) = \frac{M_1 - M_2}{ry}$

$M_1$ ,  $M_2$  and  $M_3$  = Mean squares

r = Number of replications

y = Number of years

b) Phenotypic variance:  $(\sigma_p^2) = \frac{M_1}{ry}$

c) Coefficient of variance for genotypic:

$(GCV) = \frac{\sigma_g}{\bar{X}} \times 100$

d) Coefficient of variance for phenotypic:

$(PCV) = \frac{\sigma_p}{\bar{X}} \times 100$  where,

$\bar{X}$  = Mean of the trait

e) Heritability:  $H^2 = \frac{\sigma_g^2}{\sigma_p^2} \times 100$

**Table 3.** Combined analysis of variance at several years

Source of variation	d.f.	Mean Squares	Expected Mean Squares
Years (Y)	(y-1)	-	-
Blocks within Years	y (r-1)	-	-
Genotypes (G)	(g-1)	$M_1$	$\sigma_e^2 + r\sigma_{gy}^2 + ry\sigma_g^2$
G×Y	(g-1) (y-1)	$M_2$	$\sigma_e^2 + r\sigma_{gy}^2$
Pooled Error	y (r-1) (g-1)	$M_3$	$\sigma_e^2$

SSR reproducible fragments were scored for presence or absence of an allele in the locus for SSR. The polymorphic information content (PIC) of each SSR primer was analysed as follows:  $PIC = 1 - \sum(P_i)^2$

Here,  $P_i$  is the frequency of the  $i$ th allele computed for each SSR locus (Botstein et al., 1980). The bands were then entered in a computer file as a binary matrix, and analyzed by NTSYS pc-2.1 (Rohlf, 2002). The hierarchical

clustering algorithm UPGMA was used to construct dendrograms separately for the SSR markers, with slightly different results in the similarities of genotypes, showing that these sets of markers provide complementary information on genetic similarity. Therefore, the data from both markers were combined for maximal information (Ahmed et al., 2012; Zhan et al., 2012), and analyzed for the 43 genotypes. The genetic distances were quantified by means of the Jaccard index, to arrive at the similarity matrix used in clustering.

## RESULTS and DISCUSSION

### *Analysis of variance and mean comparison*

The combined analysis of variance for means indicated that the differences among the genotypes were significant

( $p < 0.01$ ) for the seven traits (Tables 4 and 5). The significant differences among the upland rice genotypes showed potential for grouping them into clusters to identify divergent groups. This reasoning was similar to the reports of Ahmad et al. (2015) and Chuchert et al. (2018). The Genotype $\times$ Year interaction was significant for days to maturity, plant height, number of tillers hill<sup>-1</sup>, yield, and thousand grain weight. Banterng and Joralee (2015) reported that rice breeders need to test the genotypes for several years or environments for general adaptability and selecting the optimized traits. Means of days to flowering, days to maturity, plant height, number of tillers hill<sup>-1</sup>, yield, thousand grain weight, grain width and grain length of 43 upland rice genotypes are shown in Table 5.

**Table 4.** Mean square of days to flowering (DF), days to maturity (DM), plant height (PH), number of tillers hill<sup>-1</sup> (NT H<sup>-1</sup>), yield, thousand grain weight (TGW), grain width (GW) and grain length (GL) in upland rice genotypes

Source of variation	d.f.	Mean Squares							
		DF	DM	PH	NT H <sup>-1</sup>	Yield	TGW	GW	GL
Years (Y)	1	4437.60	8003.76	2693.30	10.95	383905507.50	810.48	0.02	0.15
Blocks/Y	4	673.80	281.90	111.80	7.64	1948437.50	8.37	0.07	0.39
Genotypes (G)	42	1292.70**	519.50**	1370.90**	8.13**	4309470.90 <sup>ns</sup>	249.39**	1.42**	4.62**
G $\times$ Y	42	73.20 <sup>ns</sup>	92.90*	232.00**	2.87**	2741325.90**	8.53*	0.07 <sup>ns</sup>	0.59 <sup>ns</sup>
Pooled Error	168	57.20	57.20	13.00	1.43	363700.30	5.56	0.06	0.45
CV (%)		8.60	6.15	3.10	19.44	11.63	8.25	7.90	6.36

\*, \*\* and ns = significant ( $p < 0.05$ ), = significant ( $p < 0.01$ ), and non-significant ( $p > 0.05$ ) by the F test, respectively

### *Genetic variation*

The highest phenotypic coefficient variation was observed for the thousand grain weight (22.52%), followed by the number of tillers hill<sup>-1</sup> (18.93%), and the days to flowering (16.71%). So, the thousand grain weight is one of the most stable features in phenotypic characters. The supreme among genetic coefficients of variation was observed for the thousand grain weight (22.13%), followed by the days to flowering (16.23%), and the number of tillers hill<sup>-1</sup> (15.22%). The phenotypic coefficient variation was greater than the genotypic coefficient of variation, showing large environmental effects on the expression of the traits.

The broad-sense heritability (on a family-mean basis) of days to flowering, days to maturity, plant height, number of tillers hill<sup>-1</sup>, thousand grain weight, grain width, and grain length ranged from 64.70 to 96.58%, indicating very high broad-sense heritability. Yield showed medium broad-sense heritability (36.39%), Robinson (1951) recorded similar broad-sense heritability for grain yield. The heritability estimates are higher when estimated from means of progeny than from individual-plant basis, because individual-plant evaluations of traits are exposed to large non-genetic effects (Bernardo, 2002). Also, Kamara et al. (2017) reported high broad-sense heritability for plant height and thousand grain weight.

### *Diversity analysis using SSR markers*

An SSR marker is generally used to analyze the genetic relationship in rice. A study of the genetic relationship among the 43 genotypes was here based on ten SSR

markers. These markers yielded a total of 43 alleles. The highest (6) and the lowest (3) numbers of alleles were for RM413 as well as RM215 and RM259, respectively, with an average of 4.30 alleles per locus (Table 7). The polymorphism information content (PIC) value is commonly used to study the informativeness by polymorphism of each marker locus. RM5, RM19, RM44, RM259, RM283, RM307, RM316, RM413 and RM455 (PCI 0.56-0.76) were very highly informative and therefore useful in molecular rice breeding, while RM215 (PIC 0.25) was moderately informative according to the criteria of Botstein et al. (1980).

The similarity values from each pairwise comparison of SSR marker data were used to calculate a dendrogram (Figure 1). At 0.75 similarity level, 43 genotypes were clustered into eight groups. The group VI was homogeneous and represented only one geographic region, in southern Thailand. Groups II, III, IV, V, VII and VIII each represented two regions. Groups V, VI and VIII consisted of ten, seven and four genotypes, respectively. Groups II, IV and VII all had three varieties. Cluster group III was the smallest with two genotypes. Group I represented three regions (north, northeast and south) of Thailand and had high heterogeneity. This group was the largest with eleven members. Nilthong et al. (2020) reported that cluster analysis based on SSR data precisely identified total rice cultivars into three groups which related to their collected places. These results indicate that non-glutinous or glutinous upland rice genotypes could be chosen from each group to ensure genetic variability in a future rice breeding program.

**Table 5.** Means of days to flowering (DF), days to maturity (DM), plant height (PH), number of tillers hill<sup>-1</sup> (NT H<sup>-1</sup>), yield, thousand grain weight (TGW), grain width (GW) and grain length (GL) in upland rice genotypes

Genotypes	DF	DM	PH	NT H <sup>-1</sup>	Yield	TGW	GW	GL
	day	day	cm	no.	t ha <sup>-1</sup>	g	mm	mm
NR008	76fghi	112lmnop	99.82ghij	5.18c	5.182	43.88a	3.90bcd	11.39abcdef
NR025	69ghi	114klmno	107.07cdefghi	5.27c	5.201	44.95a	4.18ab	12.02abc
NR030	66ghi	112lmnop	116.69bcdefghi	5.31c	5.782	47.66a	4.25ab	11.61abcde
NR044	67ghi	105op	94.65ij	6.88bc	4.842	25.38hijk	3.01hijkl	10.73cdefghijk
NR048	73ghi	116hijklmn	112.61bcdefghi	6.05bc	5.747	35.39bc	4.44a	10.24defghijklmn
NR064	101abc	130abcdef	125.50bcdefgh	5.95bc	5.787	23.88ijkl	2.79l	10.97bcdef
NR065	99abc	130abcdef	129.25abcdef	6.35bc	6.902	25.93ghij	2.81l	11.18bcdefg
NR066	99abc	125bcdefghijk	119.19bcdefghi	5.63c	6.120	26.65ghij	2.83kl	11.08bcdefg
NR067	100abc	128abcdefg	120.32bcdefghi	5.77c	5.353	23.50jkl	2.83kl	10.79bcdefghij
NR069	99abc	129abcdefg	122.44bcdefghi	5.82bc	5.831	23.72ijkl	2.85kl	10.83bcdefghij
NR070	95abcd	126abcdefghi	135.47abc	5.26c	5.673	26.65ghij	2.85kl	10.93bcdefgh
NR073	69ghi	106nop	108.53cdefghi	6.09bc	5.173	24.03ijkl	2.99ijkl	9.39jklmn
NR074	102abc	129abcdefg	124.34bcdefgh	6.59bc	5.844	24.47ijk	3.17ghijkl	9.43ijklmn
NR076	100abc	129abcdefg	117.01bcdefghi	5.97bc	6.143	23.34jkl	2.81l	10.58defghijkl
NR077	96abcd	128abcdefg	129.39abcdef	6.56bc	6.443	24.82hijk	3.38efghij	10.18efghijklmn
NR078	96abcd	125bcdefghijk	108.83bcdefghi	5.49c	5.242	24.07ijkl	2.74l	10.64cdefghijkl
NR079	95abcd	127abcdefgh	133.87abcd	6.96bc	5.902	25.21hijk	3.22ghijkl	9.99fghijklmn
NR081	107a	134abc	152.76a	5.15c	4.531	31.00cdefg	3.43defghi	11.69abcd
NR085	100abc	132abcdef	129.45abcdef	6.26bc	5.298	25.25ijklh	3.41efghi	10.86bcdefghi
NR086	103abc	138a	117.26bcdefghi	6.11bc	3.700	22.96jkl	3.39efghij	10.08fghijklmn
NR088	100abc	134abcd	132.04abcd	5.51c	5.182	20.05kl	2.87kl	8.98mn
NR089	100abc	131abcdef	116.14bcdefghi	5.45c	3.707	27.30fghij	3.48cdefgh	10.35defghijklm
NR092	98abc	127abcdefgh	126.36bcdefg	6.38bc	7.338	24.29ijk	2.77l	10.48defghijkl
NR093	98abc	132abcdef	121.21bcdefghi	5.65c	3.658	23.71ijkl	3.59cdefg	9.24lmn
NR094	90bcde	124cdefghijk	105.54defghi	7.98bc	3.253	25.90ghij	2.83kl	12.71a
NR100	82defg	128abcdefg	77.05j	8.99b	3.933	28.38efghij	3.23ghijkl	10.51defghijkl
NR102	101abc	127abcdefg	129.61abcdef	5.36	5.267	25.22hijk	2.78l	11.62abcde
NR104	69ghi	108mnop	97.18hij	6.65bc	4.931	25.53hij	3.44defghi	10.78bcdefghij
NR105	63i	104op	110.24bcdefghi	5.54c	5.547	37.38b	3.95bc	11.59abcde
NR108	91bcde	116hijklmn	115.81bcdefghi	6.92bc	4.984	29.04defghi	3.01hijkl	11.57abcde
NR110	101abc	133abcde	115.41bcdefghi	5.83bc	4.712	28.49efghij	3.31fghijk	10.60cdefghijkl
NR111	106ab	132abcdef	130.05abcde	5.19c	5.653	18.94l	2.91jkl	8.84n
NR112	70ghi	122fghijkl	76.56j	11.71a	5.104	23.44jkl	2.99ijkl	10.53defghijkl
NR115	105abc	136ab	137.68ab	5.58c	4.335	24.85hijk	3.40efghij	10.88bcdefghi
NR116	107a	136ab	134.86abc	5.64c	5.156	28.49efghij	3.80bcde	10.27defghijklm
NR118	65hi	112lmnop	102.13efghi	5.37c	4.982	36.58b	4.46a	10.04fghijklmn
NR119	74ghi	119ghijklm	111.10bcdefghi	5.83bc	4.789	32.63bcde	3.96bc	9.74ghijklmn
NR120	71ghi	121fghijkl	111.01bcdefghi	6.73bc	5.483	32.20bcdef	3.93bc	9.32klmn
NR122	80efgh	122fghijkl	114.30bcdefghi	5.16c	4.287	30.09defgh	3.81bcde	9.48hijklmn
NR123	80efgh	115ijklmno	119.36bcdefghi	6.25bc	5.651	33.92bcd	3.55cdefg	10.20efghijklmn
NR124	71ghi	112lmnop	101.51efghij	5.81bc	5.178	32.31bcdef	3.32fghijk	10.28efghijklm
NR126	78efgh	116hijklmn	100.89fghij	5.62c	4.080	36.13bc	3.49cdefgh	12.21ab
NR127	72ghi	114klmno	114.04bcdefghi	5.83bc	5.096	33.44bcde	3.53cdefg	9.79ghijklmn

Within columns, mean values followed by different letters are significantly different by DMRT at  $p < 0.01$

**Table 6.** Genetic parameter estimates for eleven agronomic traits of upland rice genotypes

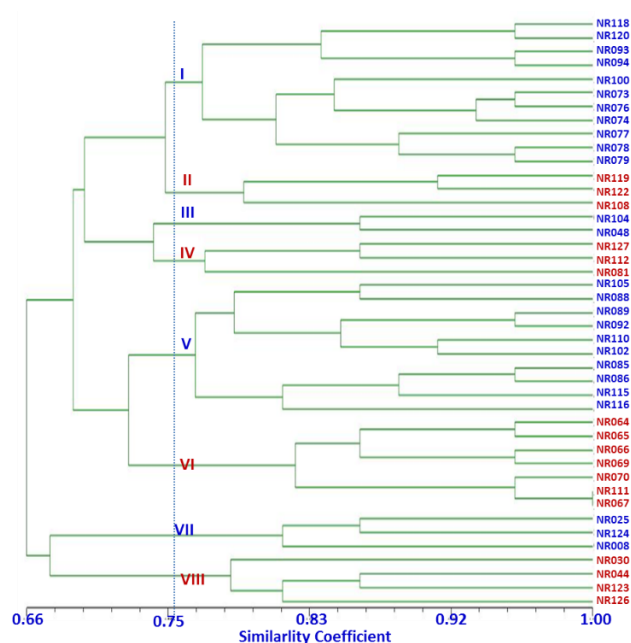
Traits	$\sigma_p^2$	$\sigma_g^2$	PCV %	GCV %	H <sup>2</sup> %
Days to flowering	215.45	203.25	16.71	16.23	94.34
Days to maturity	86.58	71.10	7.57	6.86	82.12
Plant height	228.48	189.82	13.03	11.88	83.08
Number of tillers hill <sup>-1</sup>	1.36	0.88	18.93	15.22	64.70
Yield	718245.15	261357.50	16.38	9.88	36.39
Thousand grain weight	41.57	40.14	22.52	22.13	96.58
Grain width	0.24	0.23	14.56	14.19	95.07
Grain length	0.77	0.67	8.30	7.75	87.23

GCV= Genotypic Coefficient of Variation, PCV= Phenotypic Coefficient of Variation, H<sup>2</sup>= board-sense heritability

**Table 7.** Amplified products and number of polymorphic bands obtained by using SSR markers in 43 genotypes of upland rice

Primers	Polymorphic bands	PIC value
RM5	4	0.65
RM259	3	0.58
RM283	4	0.56
RM307	4	0.72
RM413	6	0.76
RM455	4	0.59
RM44	5	0.73
RM215	3	0.25
RM316	5	0.68
RM19	5	0.72
Total	43	6.25
Average	4.30	0.63

PIC = Polymorphic Information Content



**Figure 1.** Cluster analysis of 43 upland rice genotypes based on Jaccard's similarity

## CONCLUSION

Forty-three Thai rice genotypes were used to study genetic diversity, to design breeding programs. Highly significant differences were found among genotypes for seven major quantitative traits, except yield. No Genotype×Year interaction was found for days to flowering, grain width, and grain length. Broad-sense heritabilities of the agronomic traits ranged from about 36-97%. Genomic based cluster analysis using SSR markers revealed that the Thai upland rice genotypes could be clustered by geographic regions. This method would be useful for rice breeders selecting parents for crossing programs.

According to the results of this investigation, selection maybe based on thousand grain weight, day to maturity, and the number of tillers hill<sup>-1</sup>. Because, these traits have high genetic coefficient variation and broad-sense

heritabilities. The results of SSR marker analysis indicated that non-glutinous or glutinous upland rice genotypes could be chosen from each group to ensure genetic variability in a future rice breeding program.

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## LITERATURE CITED

- Ahmad, F., M.M. Hanafi, M.A. Hakim, M.Y. Rafii, I.W. Arolo and S.N.A. Abdullah. 2015. Genetic divergence and heritability of 42 colored upland rice genotypes (*Oryza sativa*) as revealed by microsatellites marker and agro-morphological traits. PLOS One 10:1-18.
- Anonymous. 2021. Panel of 50 standard SSR markers, [https://archive.gramene.org/markers/microsat/50\\_ssr.html](https://archive.gramene.org/markers/microsat/50_ssr.html), (Accessed Sept 1, 2021)
- Bantern, P. and A. Joralee. 2015. Evaluation of black glutinous rice genotypes for stability of gamma oryzanol and yield in tropical environments. Turk. J. Field Crops 20(2):142-149.
- Bernado, R. 2002. Breeding for Quantitative Traits in Plants. Minnesota: Stemma Press.
- Botstein D.R.L., White, M. Skolnick and R.W. Davis. 1980. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. Am. J. Hum. Genet. 32:314-331.
- Chuchert, S., C. Nualsri, N. Junsawang and W. Soonsuwon. 2018. Genetic diversity, genetic variability, and path analysis for yield and its components in indigenous upland rice (*Oryza sativa* L. var. glutinosa). Songklanakarin J. Sci. Technol. 40:609-616.
- Dellporta, S.L., J. Wood and J.B. Hicks. 1983. A plant DNA minipreparation: Version II. Plant Mol. Biol. Rep. 1:19-21.
- Digital Government Development Agency (DGA). 2022. Crop production data of Thailand in 2019. <https://data.go.th/dataset/production-62>, (Accessed April 29, 2022)
- Food and Agriculture Organization (FAO). 2020. The State of Food Security and Nutrition in the World. Rome: FAO.
- Gomez, K.A. and A.A. Gomez. 1984. Statistical Procedures for Agricultural Research. New York: John Wiley & Sons Inc.
- Gonzaga, Z.J., K. Aslam, E.M. Septiningsih and B.C.Y. Collard. 2015. Evaluation of SSR and SNP markers for molecular breeding in rice. Plant Breed. Biotech. 3:139-152.
- Kamara, N., M.D. Asante, R. Akromah and C.S. Kamara. 2017. Genetic analysis of yield and yield components in *Oryza sativa* x *Oryza sativa* cross. Afr. Crop Sci. J. 25:539-550.
- Khan, F. 2015. Molecular markers: An excellent tool for genetic analysis. J. Mol. Biomark. Diagn. 6:233, doi:10.4172/2155-9929.1000233.
- Luangmanee, J., P. Bantern and A. Wongcharoen. 2016. Blast disease of black glutinous rice germplasms under inoculation

- at seeding and tillering stages. *Turk. J. Field Crops* 21(1):131-138.
- McCouch, S.R., L. Teytelman, Y. Xu, K.B. Lobos, K. Clare, M. Walton, B. Fu, R. Maghirang, Z. Li, Y. Xing, Q. Zhang, I. Kono, M. Yano, R. Fjellstrom, G. DeClerck, D. Schneider, S. Cartinhour, D. Ware and L. Stein. 2002. Development and mapping of 2240 new SSR markers for rice (*Oryza sativa* L.) (supplement). *DNA Res.* 9:257-279.
- Mendiburu, F.D. and R. Simon. 2007. *Agricolae* - a free statistical library for agricultural research. Iowa: Iowa State University.
- Nilthong, S., E. Chukeatirote and R. Nilthong. 2020. Assessment of genetic diversity in Thai upland rice varieties using SSR markers. *Aust. J. Crop Sci.* 14:597-604.
- Ozturk, G. and Z. Yildirim. 2014. Heritability Estimates of Some Quantitative Traits in Potatoes. *Turk. J. Field Crops* 19(2): 262-267.
- Park, J.R., W.T. Yang, Y.S. Kwon, H.N. Kim, K.M. Kim and D.H. Kim. 2019. Assessment of the genetic diversity of rice germplasms characterized by black-purple and red pericarp color using simple sequence repeat markers. *Plants* 8: 47, doi:10.3390/plants8110471.
- Pathaichindachote, W., N. Panyawut, K. Sikaewtung, S. Patarapuwadol and A. Muangprom. 2019. Genetic diversity and allelic frequency of selected Thai and exotic rice germplasm using SSR markers. *Rice Sci.* 26:393-403.
- Powell, W., G.C. Machray and J. Provan. 1996. Polymorphism revealed by simple sequence repeats. *Trend Plant Sci.* 1:215-222.
- Rao, A.N., S.P. Wani, M.S. Ramesha and J.K. Ladha. 2017. Rice Production Systems. In: *Rice production worldwide*, ed. Chauhan, B.S. Jabran, K. and Mahajan, G., 185-205. Aktiengesellschaft: Springer International Publishing.
- Rashmi, D., P. Bisen, S. Saha, B. Loitongbam, S. Singh, Pallavi and P.K. Singh. 2017. Genetic diversity analysis in rice (*Oryza sativa* L.) accessions using SSR markers. *Int. J. Agric. Environ. Biotechnol.* 10:1-11.
- Robinson, H.F., R.E. Comstock and P.H. Harvey. 1951. Genotypic and phenotypic correlations in corn and their implications in selection. *Agron. J.* 43:282-287.
- Rohlf, F.J. 2002. *NTSYS-Pc, Numerical Taxonomy and Multivariate Analysis System, Version 2.1.* New York: Applied Biostatistics.
- Surek, H. and N. Beser. 2003. Selection for grain yield and yield components in early generations for temperate rice. *Philipp. J. Crop. Sci.* 28:3-15.
- Zhan, Q., L. Zhou, N. Bi, H. Wu, J. Li, J. Lu and P. Lin. 2012. A comprehensive analysis of genetic diversity and relationships of 13 sweet sorghum varieties as energy sources. *J. Sustain. Bioeng. Syst.* 2:86-91.