Chapter 2

Characterization of cultivated and common wild rice populations

2.1 Introduction

Thailand is recognized as the one of center of rice diversity (Chang, 1976). 23, 903 samples of cultivated rice were collected and conserved in the National Gene bank of Thailand (DOA, 2000). The system of rice growing classified depends on the different environment, geographical and source of water supply such as upland, lowland and deepwater or rainfed and irrigated (Datta, 1981). In Thailand, rice cultivation is difference in each region, central, north, northeast, and south. Two planting method, broadcast and transplanting are general used in rice cultivation. In northern region, contains almost one-third of the land area of Thailand. Upland rice is grown in the lower altitudes of high hills and in upland areas. Lowland rice is grown mainly in lower valleys and on some terraced fields where water is available. Rice varieties are improved and released for suitable for each rice growing system by Rice Research Institute (RRI). High yielding rice varieties are usually grown in irrigated areas especially central region. Improved, premium high quality KDML105, its derivatives RD6 and RD10 are grown in rainfed system in north and northeast (OAE, 1998). Cultivated rice is predominated as self pollinated crop with 0-1% of outcrossing rate (Roberto *et al*., 1961). Genetic structure of commercial cultivated rice varieties supposed to be homozygous homogenous population.

Common wild rice (*Oryza rufipogon* Griff.) is usually known as the ancestor of Asian cultivated rice that was distributed in all part of the country and found in vary habitats such as pound, ditches, roadside, abandoned rice field, canal and longan orchard (Chitrakon, 1995; Jamjod *et al*., 2002). Wild rice was adapted to their environment (Oka, 1988) and the conditions of habitat might impose a strong pressure of selection on the populations of wild rice. Reproductive system of wild rice is cross pollinated with high outcrossing rate ranged from 7.2-55.9% for wild rice in Thailand (Barbier *et al.*, 1989).

Therefore, the aims of this study are to characterize cultivated rice and wild rice populations, selected/adapted for different regions of Thailand using morphological and physiological characters, and DNA fingerprint.

2.2 Materials and Methods

Genetic materials

Locally popular crop rice varieties in three regions of Thailand (north, northeast and central) were selected for this experiment. Cultivated rice varieties were improved or bred for different locations consisted of Khao Dawk Mali 105 (KDML 105), RD6, RD10, Chai Nat 1 (CNT1) and Suphan Buri 1 (SPR1), Niaw San-pah-tawng (NSPT), Sew Mae Jan (SMJ) and Kum Doi Sa Ket (KDK) (Table 2.1). Cultivated rice samples were provided from Agronomy Department, Faculty of Agriculture, Chiang Mai University and Thai Rice Research Institute. Wild rice populations were collected from different regions, including, Pa Kam village, Lumphun (LP), Rom Luang village, Sansai, Chiang Mai (CM) from north of Thailand, Kanchanaburi (KC) and Nakorn Na Yok (NY) from central part of Thailand. Description of rice sample was provided in Table 2.1.

Experimental procedure

The experiment was set up in pots. Each population was germinated in petri-dish before being transplanted into pot, 10 plants/pot. Twenty to 30 plants of each cultivated rice variety and wild rice populations were grown. Leaves sample of each plant was collected at 2 weeks after transplanting for DNA analysis. Morphological and physiological characteristics were recorded individually using the method of IRRI-IBPGR (1980). At flowering, plants were recorded for color at different plant parts including, leaf blade, leaf sheath, auricle, ligule, internode, apiculus, awn and stigma, the present of awn on spikelet and days to heading. At maturity, culm length (cm) of each plant was measured. Each plant was harvested separately. Two panicles from each plant were randomly collected and measured for seed fertility (%), seed

shattering (%), 100 seed weight (g) and scored for hull color and pericarp color. At harvesting, rice culm was cut off remaining for 10-15 cm above ground surface for determining regeneration ability on individually plant.

For DNA analysis, genomic DNA individually was extracted from dry leaf sample using modified method from Doyle and Doyle (1987) and the PCR reactions were perform following the description of Panaud *et al*. (1996). Microsatellite markers with seven SSR primer pairs, RM1, RM164, RM167, RM 211, RM225, RM341 and RM588 were used (Table 2.2). Amplification of DNA was performed in 20 μl reaction consisted of 20-50 ng DNA, 0.25 mM of each dNTP, 2% formamide, 0.2 μM of each primers and 0.5 unit of Taq DNA polymerase in reaction buffer [10 mM of Tris-HCl pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1mM EDTA, 50%(v/v) glycerol]. The amplified polymorphism alleles were distinguishable with the electrophoresis in 10% Polyacrylamide Gel Electrophoresis (PAGE).

Data analysis

For morphological characters, Shannon-Weaver Index (Shannon and Weaver, 1949 cited by Power and McSorley, 2000) was used to calculate diversity. Physiological characters were calculated for mean and standard deviation (sd). For DNA analysis, an estimate of the genetic diversity index (H_e) was calculated for each rice population according to Nei (1973) as;

$$H_e = 1 - \sum P_i^2$$

Where Pi is the ith allele frequency. The distribution of variability between and within populations was calculated according to Nei *et al.* (1983) for each microsatellite locus. The total diversity estimate (H_T was partitioned into within population diversity (H_S) and between population diversity (D_{ST}) components, where

$H_T = H_S + D_{ST}$. Gene diversity between populations was expressed as relative to total population diversity or genetic differentiation index (F_{ST}), where $F_{ST} = D_{ST}/H_T$, according to Nei *et al.* (2000). Analyses of genetic diversity indices were perform using POPGENE version 3.2.program (Yeh *et al*., 1999).

Table 2.1 Description of cultivated rice varieties and common wild rice populations used in the experiment.

Cultivated/ Wild rice	Abbreviation	Year released	Description	Photoperiod sensitivity	Production system	Growing area/ Source (Habitat)
Cultivated rice (*O. sativa*)						
High yielding variety						
Chai Nat 1	CNT1	1993	derived from three way cross between F_1 (IR13146-158-1 X IR5314-43-2-3-3) with BKN6995-16-1-1-2	No	Irrigated and rainfed lowland	Central
Suphan Buri 1	SPR 1	1994	derived from cross between three way cross (IR25393-57-2-3/RD23//IR27316-96-3-2-2) whit single cross between SPRLP77205-3-2-1/SPRLP79134-51-2-2	No	Irrigated and rainfed lowland	Central
Improved/ Purified variety						
RD 6	RD6	1977	improved by mutation breeding from irradiated KDML105	Yes	Rainfed lowland	North and Northeast
RD 10	RD10	1981	improved by mutation breeding from irradiated RD1	No	Irrigated lowland	North and Northeast
Khao Dawk Mali 105	KDML105	1959	purified from indigenous KDML	Yes	Rainfed lowland	All regions
Niaw San-pah-tawng	NSPT	1962	selected and purified from non-glutinous Luang Yi 10-137-1	Yes	Rainfed lowland	North
Sew Mae Jan	SMJ	1979	selected and purified from farmer's rice	Yes	Upland	North
Kum Doi Sa Ket	KDK	2005	purified from indigenous KDK	Yes	Rainfed lowland	North
Wild rice (*O. rufipogon*)						
Natural population:						
Lamphun	LP	-	canal in longan orchard	Yes	-	North
Chiang Mai	CM	-	along the main Mae faek irrigation canal	Yes	-	North
Kanchanaburi	KC	-	canal near rice field	Yes	-	Central
Nakorn Na Yok	NY	-	abandoned rice field	Yes	-	Central

Table 2.2 Primer sequences, repeat motif, expected PCR product size, annealing temperature and chromosomal locations of seven microsatellite primers.

Name	Primer sequences (5′→3′)	Repeat	Expected PCR product size (bp)	Annealing Temperature (◦c)	Chromosomal location
RM1	GCGAAAACACAATGCAAAAA GCGTTGGTTGGACCTGAC	(AG)26	128	55	1
RM164	TCTTGCCCGTCACTGCAGATATCC GCAGCCCTAATGCTACAATTCTTC	(GT)16TT(GT)4	246	58	5
RM167	GATCCAGCGTGAGGAACACGT AGTCCGACCACAAGGTGCGTTGTC	(GA)16	113	55	11
RM211	CCGATCTCATCAACCAACTG CTTCACGAGGATCTCAAAGG	(TC)3A(TC)18	161	55	2
RM225	TGCCCATATGGTCTGGATG GAAAGTGGATCAGGAAGGC	(GA)16	128	52	6
RM341	CAAGAAACCTCAATCCGAGC CTCCTCCCGATCCCAATC	(CTT)20	172	52	2
RM588	GTTGCTCTGCCTCACTCTTG AACGAGCCAACGAAGCAG	(TGC)9	126	55	6

2.3 Results

Morphological characters

All cultivated rice (*O. sativa*) showed erect plant type, green leaf-blade and leaf-sheath, colorless auricle and ligule, green internode except in Kum Doi Saket (KDK) and Sew Mae Jan (SMJ) varieties (Table 2.3, Figure 2.1). KDK showed purple color in leaf-blade, leaf-sheath, auricle, ligule and internode (Figure 2.2) while SMJ showed light purple auricle. For panicle, spikelet and seed characters, cultivated rice had compact panicle with awnless spikelet, colorless apiculus and stigma. Straw color hull and white pericarp were unique in most cultivated rice. Except for KDK and SMJ, they showed purple apiculus and stigma. Hull color of KDK was purple with dark purple pericarp while SMJ showed straw hull with purple apiculus but still had white pericarp. No variation within varieties for all morphological traits was observed (H′=0).

In contrast, wild rice (*O. rufipogon*) exhibited spreading and prostrate plant type, had open panicle with long awn on spikelet and red pericarp (Table 2.3 and Figure 2.3). Variation within population were observed in leaf blade color and leaf sheath color, auricle color, ligule color, apiculus color, stigma color and hull color. For LP wild rice, variation was observed in leaf blade color (H′=0.5004), leaf sheath color (H′=0.5004), stigma color (H′=0.5004) and hull color (H′=0.4227). For KC wild rice, variation was observed in leaf blade color (H′=0.4227), leaf sheath color (H′=0.3250), auricle color (H′=0.6881), ligule color (H′=0.1985), apiculus color (H′=0.4227), and stigma color (H′=0.5004). For NY wild rice, variation were observed in only hull color (H′=0.8864).

Table 2.3 Morphological characteristics and Shannon-Weaver Index (H') of cultivated rice varieties and common wild rice populations.

	Stem and Leaf					
Genetic	Plant type	Leaf-blade color	Leaf-sheath color	Auricle color	Ligule color	Internode color
Cultivated rice (*O. sativa*)*						
CNT1	erect	green	green	colorless	colorless	green
SPR1	erect	green	green	colorless	colorless	green
KDML105	erect	green	green	colorless	colorless	green
RD6	erect	green	green	colorless	colorless	green
RD10	erect	green	green	colorless	colorless	green
NSPT	erect	green	green	colorless	colorless	green
SMJ	erect	green	green	light purple	colorless	green
KDK	erect	purple	purple	purple	purple	purple
Wild rice (*O. rufipogon*)						
LP	prostrate	green/green with purple at margin	green/green with purple at margin	colorless	colorless	green
	(0)	(0.5004)	(0.5004)	(0)	(0)	(0)
CM	prostrate	green	green	colorless	colorless	green
	(0)	(0)	(0)	(0)	(0)	(0)
KC	spreading	green/green with purple at margin	green/green with purple at margin	colorless/ light purple	colorless/ light purple	green
	(0)	(0.4227)	(0.3250)	(0.6881)	(0.1985)	(0)
NY	spreading	green with purple at margin	green	light purple	colorless	green with purple strip
	(0)	(0)	(0)	(0)	(0)	(0)

Table 2.3 (continued).

	Panicle and Spikelet				Seed	
Genetic	Panicle type	Apiculus color	Spikelet awning	Stigma color	Hull color	Pericarp color
Cultivated rice (*O. sativa*)*						
CNT1	compact	colorless	awnless	colorless	straw	white
SPR1	compact	colorless	awnless	colorless	straw	white
KDML105	compact	colorless	awnless	colorless	straw	white
RD6	compact	colorless	awnless	colorless	straw	white
RD10	compact	colorless	awnless	colorless	straw	white
NSPT	compact	colorless	awnless	colorless	straw	white
SMJ	compact	purple	awnless	purple	straw with purple apiculus	white
KDK	compact	purple	awnless	purple	purple	dark purple
Wild rice (*O. rufipogon*)						
LP	open	red	long awn	colorless/purple	gray/dark gray	red
	(0)	(0)	(0)	(0.5004)	(0.4227)	(0)
CM	open	red	long awn	purple	gray/dark gray	red
	(0)	(0)	(0)	(0)	(0.6128)	(0)
KC	open	colorless/red	long awn	colorless/purple	dark gray	red
	(0)	(0.4227)	(0)	(0.5004)	(0)	(0)
NY	open	red	long awn	purple	brown/gray/dark gray	red
	(0)	(0)	(0)	(0)	(0.8864)	(0)

* H' for all morphological characters of cultivated rice was zero (0).

Figure 2.1 Morphological characteristics of cultivated rice. Cultivated rice exhibited erect plant type, awnless spikelet, straw color hull and white pericarp.

Figure 2.2 Pigment presentation on different plant parts of purple rice (Kum Doi Sa Ket): purple leaf-blade, leaf sheath, apiculus and dark purple hull and pericarp.

Figure 2.3 Morphological characteristics of wild rice. Wild rice exhibited prostrate to spreading plant type, open panicle with long awn, black hull, red pericarp and all seed shattered when ripe.

Physiological characters

Days to flowering

Overall mean of days to flowering for cultivated rice variety ranged from 69 to 99 days after sowing (1st August 2003). Those of the two photoperiod insensitive varieties, CNT1 and SPR1, were 82 and 87 days, respectively. The earliest variety in this study was SMJ (69 days) and the latest was NSPT (99 days). Days to flowering of wild rice were between 100 to 108 days (Table 2.4).

Number of tillers plant^{-1}

Number of tillers plant^{-1} of cultivated rice was ranging from 3 to 10. The highest number of tillers plant^{-1} was recorded in KDML105 and the lowest was found in KDK. Number of tillers plant^{-1} of wild rice KC and NY were 8 and 5, respectively. Wild rice from LP and CM had the highest number of tiller (37-42) (Table 2.4).

Number of panicle plant^{-1}

The highest number of panicles plant^{-1} (9) was found in CNT1, SPR1, and KDML105. Number of panicle plant^{-1} for the other six varieties ranged from 3 to 5. The lowest number of panicle was observed in KDK. Panicle plant^{-1} of wild rice KC and NY was 8 and 5, respectively (Table 2.4).

Culm length (cm)

Average culm length at harvesting of improved and purified varieties ranged from 91 to 133 cm (Table 2.4). High yielding variety (CNT1 and SPR1) were shorter than improved and purified variety (68 and 81 cm). Average culm length of LP, KC and NY wild rice were 137, 132, 104 cm, respectively.

Panicle length (cm)

Average panicle length of high yielding varieties was 24 cm (Table 2.4). Improved and purified variety had large panicle and mean of panicle ranged from 23 to 25 cm, except RD10 variety had short panicle (21 cm). Average panicle length of wild rice ranged from 24 to 30 cm with wild rice, LP had largest.

Number of primary branches panicle^{-1}

Number of primary branches panicle^{-1} of all cultivated rice was 9, except for SMJ which only 6 branches panicle^{-1} were found (Table 2.4) LP and NY wild rice had the highest primary branch number (14), while the rest were ranging from 7 to 8.

Number of spikelets panicle^{-1}

Mean number of spikelets panicle^{-1} of cultivated rice were between 107 to 140 (Table 2.4). The highest number of spikelet was found in CNT1 and the lowest found in SMJ. Number spikelets panicle^{-1} of wild rice ranged from 81 in CM to 174 in LP.

Number of seeds panicle^{-1}

Mean number of seeds panicle^{-1} of cultivated rice were between 97 to 127 (Table 2.4). Seeds panicle^{-1} for both high yielding varieties, CNT1 and SPR1, were 125, while those of the improved/ purified variety were ranging between 97 to 128. Mean number of seeds panicle^{-1} of wild rice were between 40 to 118. The lowest seeds panicle^{-1} was found in CM wild rice and the highest found in NY.

Seed fertility (%)

High seed fertility rate, 82-95%, were shown in cultivated rice (Table 2.4). Wild rice from LP and CM showed low percent seed fertility (49-55%). Seed fertility of wild rice KC and NY were 81 and 71, respectively.

Seed shattering (%)

Low seed shattering (%) were recorded in all cultivated rice (2-5%). For wild rice, all seeds were shattered at maturity (100%).

100 seed weight (g)

Mean 100 seed weight of cultivated rice varieties ranged from 2.4 to 2.8 g. All wild rice had smaller seed (1.5-2.1 g/100 seed) than cultivated rice (Table 2.4).

Regeneration ability (%)

High regenerating ability was observed in wild rice (75-100%) (Table 2.4). Variation in regenerating ability was observed in cultivated rice varieties. Very high regeneration ability (95-100%) were recorded in RD6 and KDK cultivated rice while the lowest (15%) was found in NSPT. Regeneration ability of the rest cultivated varieties were between 65-75%.

Table 2.4 Physiological characteristics of eight cultivated rice varieties and four common wild rice populations.

Genetic	Days to flowering	Tillers plant^{-1}	Panicle plant^{-1}	Culm length (cm)	Panicle length (cm)	Number of primary branches panicle^{-1}
Cultivated rice (*O. sativa*)						
CNT1	82 ± 2	9 ± 1	9 ± 3	68 ± 5	24 ± 1	9 ± 1
SPR1	87 ± 4	6 ± 2	9 ± 2	81 ± 5	24 ± 3	9 ± 1
KDML105	98 ± 1	10 ± 3	9 ± 3	109 ± 6	23 ± 3	9 ± 1
RD6	97 ± 2	8 ± 3	5 ± 1	117 ± 11	23 ± 3	9 ± 1
RD10	91 ± 2	5 ± 2	5 ± 2	91 ± 2	21 ± 2	9 ± 1
NSPT	99 ± 1	5 ± 1	5 ± 1	126 ± 7	25 ± 2	9 ± 2
SMJ	69 ± 2	4 ± 1	4 ± 1	106 ± 10	24 ± 2	6 ± 1
KDK	97 ± 1	3 ± 1	3 ± 1	133 ± 11	23 ± 2	9 ± 2
Wild rice (*O. rufipogon*)						
LP	108 ± 12	37 ± 9	NA	139 ± 18	30 ± 3	14 ± 2
CM	106 ± 13	42 ± 13	NA	127 ± 12	25 ± 4	7 ± 2
KC	100 ± 1	8 ± 4	8 ± 2	132 ± 7	25 ± 2	8 ± 1
NY	105 ± 2	5 ± 2	5 ± 2	105 ± 2	24 ± 2	14 ± 2

Table 2.4 (continued).

Genetic	Number of spikelets panicle^{-1}	Number of seeds panicle^{-1}	Seed fertility (%)	Seed shattering (%)	100 seeds weight (g)	Regeneration ability (%)
Cultivated rice (*O. sativa*)						
CNT1	140 ± 11	125 ± 12	95 ± 2	2 ± 2	2.6 ± 0.04	59 ± 14
SPR1	134 ± 15	125 ± 12	92 ± 4	2 ± 2	2.7 ± 0.03	75 ± 25
KDML105	137 ± 22	128 ± 30	91 ± 7	4 ± 2	2.5 ± 0.03	72 ± 22
RD6	135 ± 17	119 ± 14	86 ± 7	5 ± 2	2.4 ± 0.19	100 ± 0
RD10	136 ± 17	109 ± 14	82 ± 7	3 ± 2	2.8 ± 0.04	65 ± 19
NSPT	130 ± 18	115 ± 18	92 ± 2	4 ± 2	2.6 ± 0.05	15 ± 10
SMJ	107 ± 12	97 ± 16	91 ± 8	4 ± 3	2.4 ± 0.09	70 ± 25
KDK	128 ± 19	113 ± 17	94 ± 6	2 ± 2	2.7 ± 0.07	95 ± 10
Wild rice (*O. rufipogon*)						
LP	174 ± 32	96 ± 38	55± 18	100 ± 0	1.5 ± 0.3	100 ± 0
CM	81 ± 28	40 ± 24	49 ± 23	100 ± 0	1.5 ± 0.1	100 ± 0
KC	143 ± 23	116 ± 21	81 ± 7	100 ± 0	2.1 ± 0.1	75 ± 25
NY	155 ± 33	118 ± 35	72 ± 12	100 ± 0	1.7 ± 0.2	100 ± 0

Data are mean ± sd.
NA = data not available.

DNA analysis of cultivated and wild rice.

Distribution of alleles at polymorphic microsatellite loci

Genetic polymorphisms were found from seven microsatellite markers used in this study (Figure 2.1-2.7 and Table 2.5). For cultivated rice, alleles were fixed to one allele in all loci while in wild rice populations, allele sizes were varied within populations (Table 2.5). A total of 65 amplified fragments were detected over all loci. The largest number of alleles (15) was detected for RM1 and the lowest (4) for RM558. The other RMs detected about 6-13 alleles/locus (Table 2.6 and Appendix 1). For genotypic frequency, a total 76 genotypes were detected (Appendix 2). The highest number of genotypes were detected for RM1 and RM341 (15) and the lowest for the locus RM167 and RM588 (7). Heterozygous genotypes were observed in wild rice populations when detected by RM1, RM164, RM167, RM225 and RM341. Detailed information of allele and genotype frequencies are listed in the Appendix 1 and 2.

Microsatellite variation

For cultivated rice, no variation within population was found (Table 2.5). However, between populations variation in fragment size were found at 95-118 bp in RM1, 242-300 bp in RM164, 122 and 145 bp in RM167, 142 and 155 bp in RM211, 147-170 bp in RM341 and, 117 and 130 bp in RM588. For wild rice, both within and between populations variation were found. The largest difference in fragment size was observed in KC wild rice for locus RM1, with fragments ranging from 77 to 118 bp. The second largest difference was observed in NY wild rice for locus RM164 ranging from 259 to 295 bp (Table 2.5).

In eight cultivars and three wild rice populations, four to 15 alleles were detected per locus with an average of 9.3 (Table 2.6). In wild rice revealed a large number of unique alleles (63.1%) with doubled those detected in eight cultivated rice (30.8%). The alleles were divided in cultivar specific, wild rice specific and shared alleles with an average of 2.8, 5.9 and 0.6, respectively. Three primers consisted of RM1, RM225 and RM341 detected shared alleles in range of 1-2 (Table 2.6).

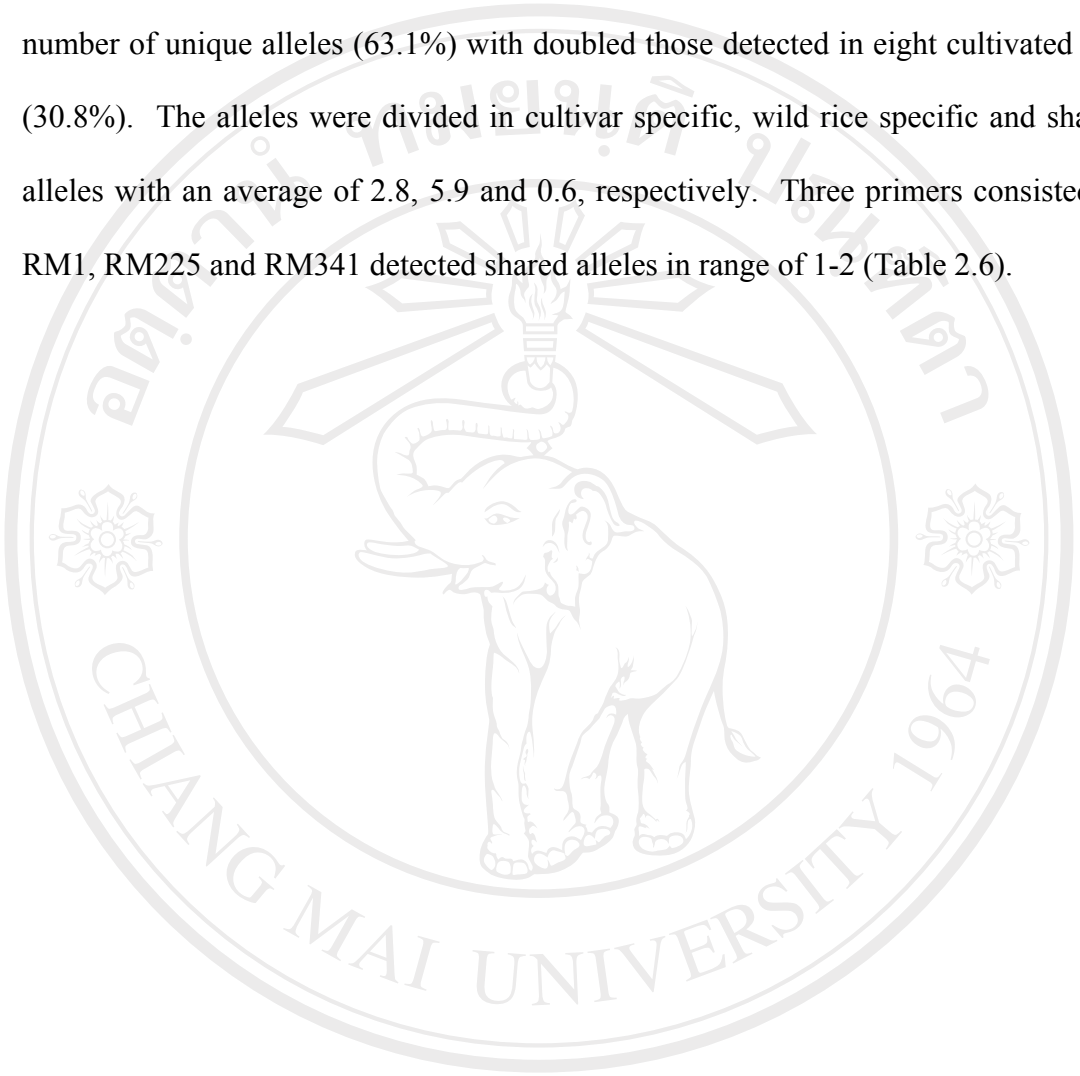

Table 2.5 Allele size ranges in base pairs of seven microsatellite loci in eight cultivated varieties and three wild rice populations.

Genetic	RM1	RM164	RM167	RM211	RM225	RM341	RM588
Cultivated rice							
CNT1	100	242	122	155	140	147	117
SPR1	105	263	122	142	125	165	117
KDML105	110	255	122	142	140	170	130
RD6	118	255	122	142	145	170	130
RD10	95	250	122	142	140	150	130
NSPT	118	242	122	142	145	147	130
SMJ	103	250	122	142	140	165	130
KDK	112	300	145	155	145	150	130
Wild rice							
LP	80-97	282-285	134-150	145-150	125-140	115-138	114-121
KC	77-118	264-272	125	152-160	127-137	162-175	114
NY	93-118	259-295	125-150	145-147	140-145	142-170	114-130

Table 2.6 Number of specific and shared alleles (% in parentheses) in seven microsatellite loci based on eight cultivated rice varieties and three wild rice populations.

Locus	Cultivar-specific alleles	Shared alleles	Wild rice specific alleles	Total
RM1	6	1	8	15
RM164	5	0	7	12
RM167	2	0	4	6
RM211	2	0	6	8
RM225	1	2	4	7
RM341	3	1	9	13
RM588	1	0	3	4
Total	20 (30.8)	4 (6.1)	41 (63.1)	65 (100)
Average	2.8	0.6	5.9	9.3

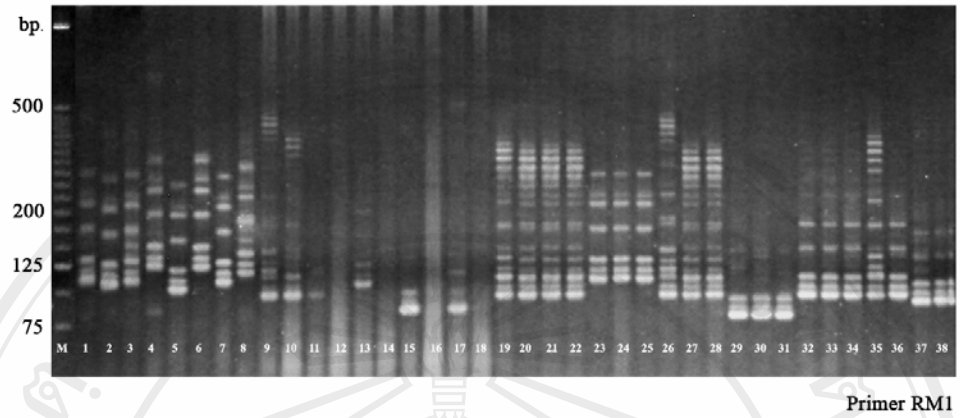

Figure 2.4 Microsatellite amplification products from eight cultivated and three wild rice genomic DNA with primer RM1. *M* 25bp ladder, *Lane 1* CNT1, *Lane 2* SPR1, *Lane* 3 KDML105, *Lane 4* RD6, *Lane 5* RD10, *Lane 6* NSPT, *Lane 7* SMJ, *Lane 8* KDK, *Lane 9 to 18* wild rice LP, *Lane 19 to 28* wild rice KC, *Lane 29 to 38* wild rice NY.

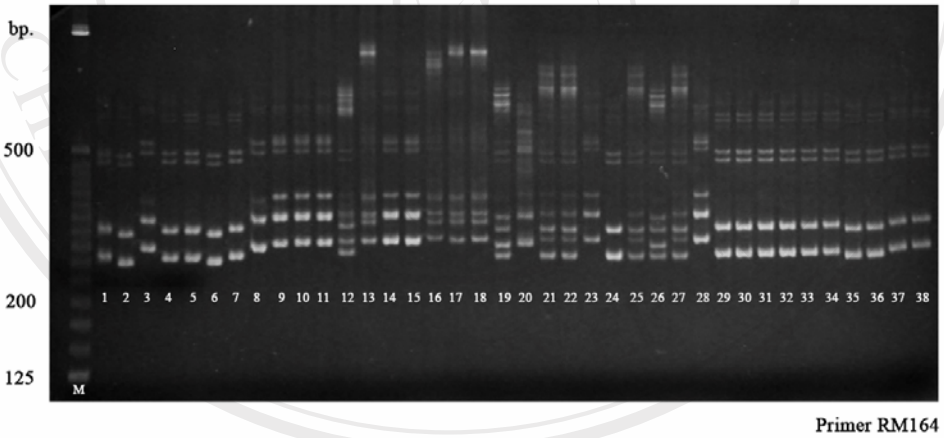

Figure 2.5 Microsatellite amplification products from eight cultivated and three wild rice genomic DNA with primer RM164. *M* 25bp ladder, *Lane 1* CNT1, *Lane 2* SPR1, *Lane 3* KDML105, *Lane 4* RD6, *Lane 5* RD10, *Lane 6* NSPT, *Lane 7* SMJ, *Lane 8* KDK, *Lane 9 to 18* wild rice LP, *Lane 19 to 28* wild rice KC, *Lane 29 to 38* wild rice NY.

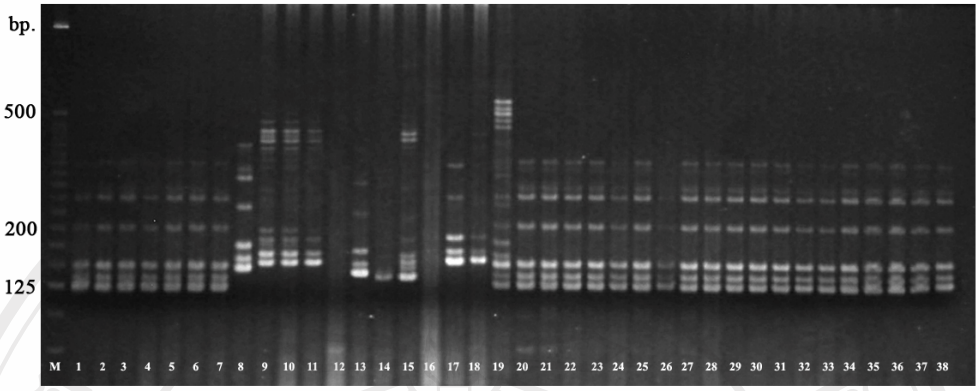

Figure 2.6 Microsatellite amplification products from eight cultivated and three wild rice genomic DNA with primer RM167. *M* 25bp ladder, *Lane 1* CNT1, *Lane 2* SPR1, *Lane 3* KDML105, *Lane 4* RD6, *Lane 5* RD10, *Lane 6* NSPT, *Lane 7* SMJ, *Lane 8* KDK, *Lane 9 to 18* wild rice LP, *Lane 19 to 28* wild rice KC, *Lane 29 to 38* wild rice NY.

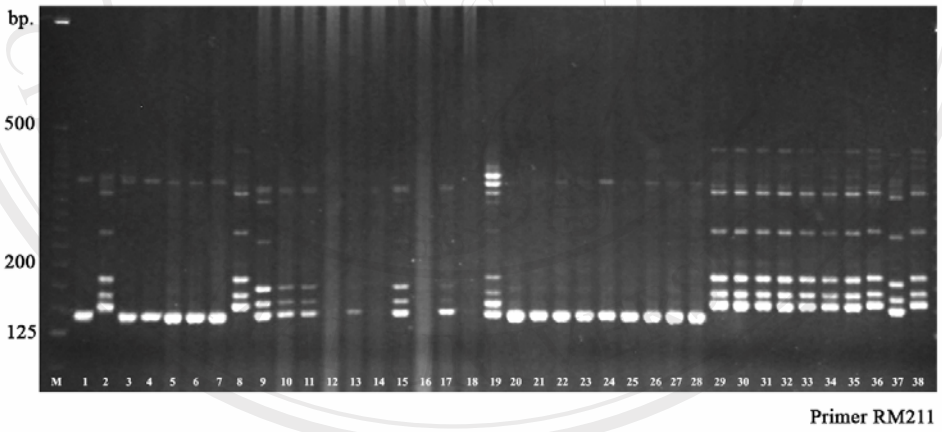

Figure 2.7 Microsatellite amplification products from eight cultivated and three wild rice genomic DNA with primer RM211. *M* 25bp ladder, *Lane 1* CNT1, *Lane 2* SPR1, *Lane 3* KDML105, *Lane 4* RD6, *Lane 5* RD10, *Lane 6* NSPT, *Lane 7* SMJ, *Lane 8* KDK, *Lane 9 to 18* wild rice LP, *Lane 19 to 28* wild rice KC, *Lane 29 to 38* wild rice NY.

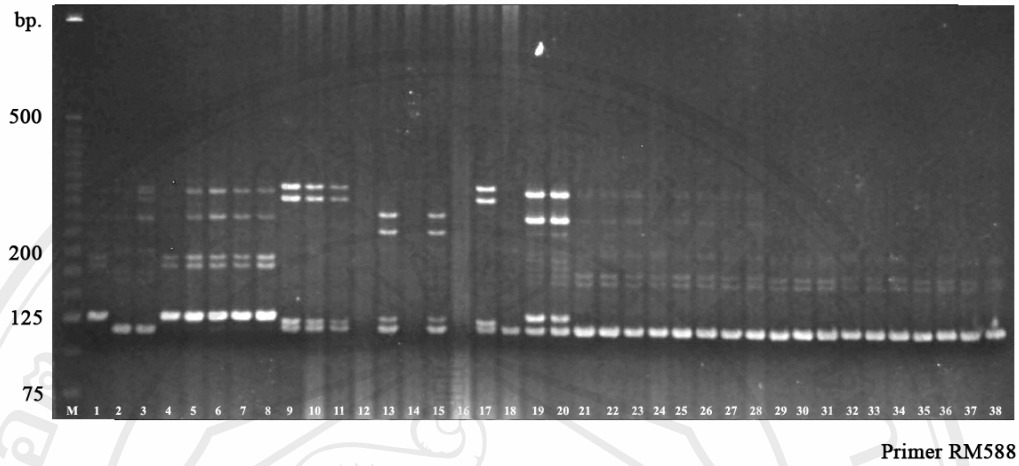

Figure 2.8 Microsatellite amplification products from eight cultivated and three wild rice genomic DNA with primer RM588. *M* 25bp ladder, *Lane 1* CNT1, *Lane 2* SPR1, *Lane 3* KDML105, *Lane 4* RD6, *Lane 5* RD10, *Lane 6* NSPT, *Lane 7* SMJ, *Lane 8* KDK, *Lane 9 to 18* wild rice LP, *Lane 19 to 28* wild rice KC, *Lane 29 to 38* wild rice NY.

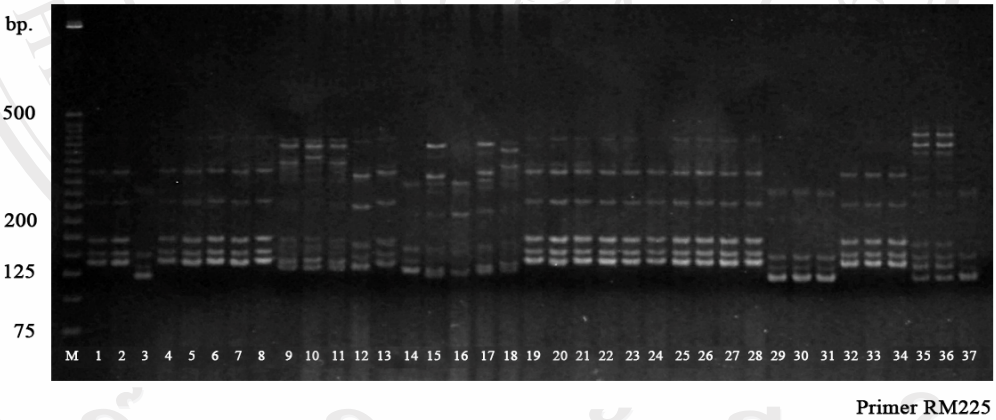

Figure 2.9 Microsatellite amplification products from eight cultivated and three wild rice genomic DNA with primer RM225. *M* 25bp ladder, *Lane 1* CNT1, *Lane 2* SPR1, *Lane 3* KDML105, *Lane 4* RD6, *Lane 5* RD10, *Lane 6* NSPT, *Lane 7* SMJ, *Lane 8* KDK, *Lane 9 to 18* wild rice LP, *Lane 19 to 28* wild rice KC, *Lane 29 to 37* wild rice NY.

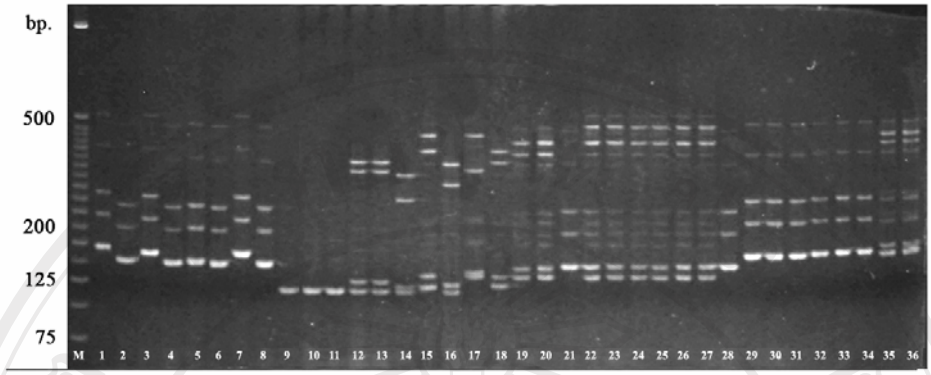

Figure 2.10 Microsatellite amplification products from eight cultivated and three wild rice genomic DNA with primer RM341. *M* 25bp ladder, *Lane 1* CNT1, *Lane 2* SPR1, *Lane 3* KDML105, *Lane 4* RD6, *Lane 5* RD10, *Lane 6* NSPT, *Lane 7* SMJ, *Lane 8* KDK, *Lane 9 to 18* wild rice LP, *Lane 19 to 28* wild rice KC, *Lane 29 to 36* wild rice NY.

Microsatellite gene diversity

Gene diversity index was determined for all populations of each locus (Table 2.7). Gene diversity index of cultivated rice for all variety was fixed (0) for all loci. In wild rice, the LP populations expressed the highest gene diversity across all loci (0.65) while those from KC and NY were 0.32 and 0.34, respectively.

Genetic structure of cultivated and wild rice

In cultivated rice, mean total genetic diversity (H_T) between populations across all loci was 0.58 with fixed allele within population (H_S = 0). Therefore, the differentiation between variety was resulting from between population variation (F_{ST} = 1) (Table 2.8). In contrast, both within population diversity (mean H_S = 0.42) and between population diversity (mean D_{ST} = 0.27) were found with made mean total genetic diversity (H_T = 0.74). The genetic differentiation among wild rice populations was 0.43. It suggested that approximately 43% of variation were resulted from the differentiation between population and 57% remaining was differentiated within population (Table 2.8).

For each locus, total genetic diversity in cultivated rice ranged from 0.22 to 0.84. The highest total genetic diversity was observed when detected by RM1 (H_T = 0.84), fallowed by RM164 (H_T = 0.78). The lowest total genetic diversity was observed when detected by RM167 (H_T = 0.22). For wild rice, genetic diversity within population (H_S) was observed in range of 0.30 to 0.55 and total genetic diversity (H_T) ranged from 0.53 to 0.85. The highest and lowest H_S were observed when detected by RM1 and RM558, respectively. For H_T, highest value was 0.85 when detected by RM341 and lowest value was 0.53 when detected by RM167. Genetic diversity between wild rice populations was determined by D_{ST} value. It was

found that high D_{ST} was observed when detected by RM211 (D_{ST}= 0.40) and lowest D_{ST} was 0.01 when detected by RM167. The differentiation among population value (F_{ST}) ranged from 0.30 to 0.55.

Table 2.7 Gene diversity (H_e) of eight cultivated and three wild rice populations based on seven microsatellite loci.

Population	RM1	RM164	RM167	RM211	RM225	RM341	RM588	Mean (sd)
Cultivated rice[1]	0	0	0	0	0	0	0	0
Wild rice								
LP	0.72	0.58	0.62	0.48	0.72	0.82	0.58	0.65 (0.11)
KC	0.50	0.32	0	0.46	0.48	0.48	0	0.32 (0.23)
NY	0.44	0.70	0.09	0.18	0.32	0.32	0.32	0.34 (0.20)

[1]Gene diversity of each cultivated rice variety was 0.
H_e = gene diversity index according to Nei (1973).

Table 2.8 Partition of genetic diversity of cultivated and wild rice within and between eight cultivated rice and three wild rice populations based on seven microsatellite loci.

Locus	H_T		H_S		D_{ST}		F_{ST}	
	Crop	Wild	Crop	Wild	Crop	Wild	Crop	Wild
RM1	0.84	0.81	0	0.55	0.84	0.26	1	0.30
RM164	0.78	0.84	0	0.53	0.78	0.30	1	0.36
RM167	0.22	0.53	0	0.52	0.22	0.01	1	0.55
RM211	0.38	0.78	0	0.37	0.38	0.40	1	0.52
RM225	0.75	0.82	0	0.46	0.75	0.36	1	0.44
RM341	0.75	0.85	0	0.54	0.75	0.31	1	0.36
RM588	0.38	0.55	0	0.30	0.38	0.25	1	0.45
Mean	0.58	0.74	0	0.42	0.54	0.27	1	0.43

H_S = average gene diversity within population, H_T = average gene diversity for all populations, F_{ST} = degree of genetic differentiation among subpopulation.

2.4 Discussion

Morphological and physiological characterization

The differentiation between cultivated and common wild rice populations were demonstrated in morphological, physiological characteristics and DNA. Specific characteristics of wild rice which were observed visually including, prostrate and spreading plant types, open panicle with long awn on spikelet, gray-dark gray hull with red pericarp. At maturity, all wild rice plants shattered their seed to the ground. These characters were not observed in cultivated rice in this study. Wild rice also showed the presentation of anthocyanin on many parts of the plant such as leaf-blade, leaf-sheath, node, internode, apiculus, stigma and pericarp. In two cultivated rice varieties, SMJ and KDK, the presentation of anthocyanin was also observed in leaf-blade, leaf-sheath, ligule, auricle, apiculus and stigma. Therefore, these characters can be used to study further in progeny from crosses between wild rice x cultivated rice involving populations described in this chapter. The easy, qualitative, traits can be used as morphological markers for identifing wild rice/cultivated rice traits in mixture such as natural or segregating populations.

In general, it was found that wild rice produced more tillers, had larger panicle, higher regeneration ability, lower seed fertility and has smaller seed than cultivated rice (Oka, 1998). In this study, wild rice populations tended to behave the same way as those reported in the literatures but were varied between populations. For example, very high number of tillers plants was found in wild rice from LP and CM (37-42) while those from KC and NY were the same as cultivated rice (5-8) (Table 2.4). Mean for most physiological characteristics of wild rice were overlapped with cultivated rice except seed shattering and seed size. Higher variation within

populations were found in wild rice for days to flowering, tillers plant^{-1}, spikelets panicle^{-1}, seeds panicle^{-1} and seed fertility when compared with cultivated rice. Segregation patterns for physiological characters in crosses between cultivated and wild rice need to be studied to examine the extent of variation in progenies. Understanding scope and extent of variation of adaptive characteristic will help to determine fitness, survival ability in natural population and competition in cultivated fields.

DNA polymorphism

For DNA analysis, genetic polymorphism was found from seven microsatellite markers used in this study. The most polymorphic locus was RM1 having 15 alleles. RM341 and RM164 also provided high (12-13) number of alleles, while RM588 gave the lowest number of alleles. The variation in distribution of allele suggested that the loci differ in their capacity to detect variation in the populations. Markers which detect high number of alleles should be selected for genetic diversity study. For populations derived from wild rice x cultivated rice, markers that can detect specific alleles specific to wild rice populations/plants and cultivated rice variety should be used.

Genetic structure of cultivated rice and wild rice

Differentiation of genetic structure between cultivated and wild rice was shown based on eight cultivated rice and three wild rice populations. Partitioning of genetic variation by using gene diversity statistics reveal that total genetic diversity average over all loci of wild rice and crop rice were 0.74 and 0.58, respectively. For cultivated rice, the genetic diversity within population was zero (H_S=0). Therefore, genetic diversity resulting from the variation between population (F_{ST} = 1). For wild

rice, mean genetic differentiation index (F_{ST}) over all loci was 0.43 indicated that 57% of microsatellite diversity was distributed within the three wild rice populations and 43% between populations. This is consistent with the findings from other studies that most of the genetic diversity of wild rice is partitioned within population rather than between populations (Oka, 1988; Gao and Hong, 2000; Sun *et al.*, 2001).

In conclusion, specific traits and markers that can be used to distinguish wild rice and cultivated rice were demonstrated. Continuous variation for some physiological was also found. Crosses between the three wild rice and cultivated rice were produced, F_1 and F_2 progenies were evaluated for segregation in various traits in the next chapter.