

Chapter 4

Evidence of gene flow between cultivated rice (*O. sativa* L.) and wild rice (*O. rufipogon* Griff.)

4.1 Introduction

Gene flow is the natural process of movement of genes between individual organisms and plays an important role in the evolution of crops and their wild relatives. Gene flow from crop to wild can add new genes into wild populations and may have a substantial impact on the evolution of wild populations (Arriola and Ellstrand, 1996). This might be result in more aggressive weed (Langevin *et al.*, 1990; Arriola and Ellstrand, 1996). Cultivated rice and *O. rufipogon* have sympatric distribution and overlap in flowering in many Asian countries and genetically the two species have close relationships and low reproductive isolation (Lu *et al.*, 2003). Natural hybridization between *O. rufipogon* and *O. sativa* has been reported in many locations (Langevin *et al.*, 1990; Shu *et al.*, 1997 and Song *et al.*, 2000), indicating that *O. rufipogon* has high compatible with cultivated rice. Weedy rice has emerged as a growing threat to rice (*O. sativa* L.) farming in Thailand (Maneechote *et al.*, 2004). Outside the natural range of *O. rufipogon* Griff., hybridization between the wild and cultivated rice is of little consequence to the spread of weedy rice (Londo and Schaal 2007). Therefore, the objectives in this study were to identify evidence of gene flow by measuring genetic variation in rice populations and to investigate the origin and spread of weedy rice in Thailand.

4.2 Materials and methods

Genetic materials

Samples of rice and weedy rice seed were taken from 2 farmers' fields growing the semi-dwarf, modern variety Supan Buri 1 (SPR1), and wild rice from natural habitats nearby, in Kanchanaburi in 2001 (Figure 4.1). Weedy rice was recognized as 20-30 cm taller than crop rice plus panicles with awn (Figure 4.2). Twenty panicles of cultivated rice were randomly collected from field 1 (designated SPR1_{FD1}). In field 2, 20 cultivated rice (SPR1_{FD2}) and 50 weedy rice (WR_{FD2}) panicles were collected. Fifty panicles of each two common wild rice population (KC1 and KC2) were also collected, KC1 from an irrigation ditch near field 1 and field 2, KC2 population from near an abandoned rice field 30 km away. Pure, breeder seed of SPR1 and Chainat1 (CNT1) variety, obtained from Thai Rice Research Institute: RRI (designated SPR1_{PURE}, CNT1_{PURE}), was included as check. Seeds of cultivated, weedy and wild rice were germinated in petri-dishes for five days before transplanted into pots.

Experiment procedure

Ninety to 100 of crop from each field, 70 of weedy rice from field 2 and 30 to 60 from each wild rice seedlings was transplanted into pots containing soil, 10 plants per pot. At tillering, 2-3 fresh leaves of each plant were collected separately for DNA analysis. Three microsatellite loci (RM1, RM164 and RM211) were selected to detect alleles specific to crop rice and wild rice. Primers were chosen from previous study in Chapter 2.

Data analysis

For morphological and physiological characteristics, samples were classified by comparing with cultivated rice from RRI and wild rice from natural habitat. By the same for identified by microsatellite markers, DNA pattern of each plant from each sample was classified for compared with DNA pattern of rice from RRI and wild rice from natural habitat. The bands were recorded as A, B, C, ... alleles, homozygous genotypes (AA, BB, CC ...) and heterozygous genotypes (AB, BC, AC ...). Allele frequency and genotype frequency were calculated. Genetic parameters were estimated for as; observed heterozygosity (H_o), expected heterozygosity (H_e) (Nei, 1973). F-statistics (F_{IS} and F_{ST} , Wright, 1965) were computed for polymorphic loci to test for departure from Hardy-Weinberg equilibrium (HWE) and to estimate genetic differentiation among populations. Outcrossing rate (t) for populations were calculated by $t = (1-F_{IS})/(1+F_{IS})$ (Weir, 1996). All parameters except t and F_{IS} were computed using POPGEN version 3.2 (Yeh *et al.*, 1999). F_{IS} was computed using FSTAT version 2.9.3 (Goudet, 2001).

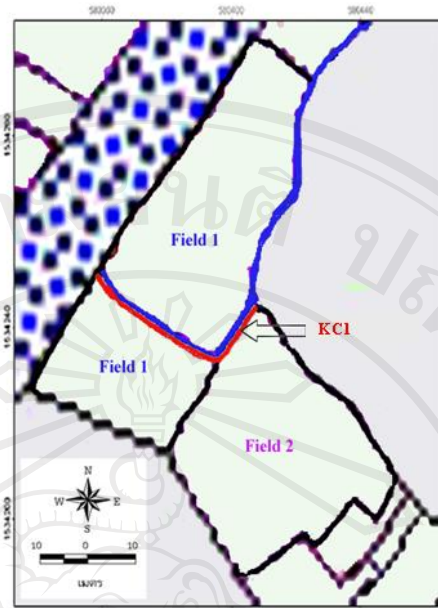


Figure 4.1 Location of rice samples were collected from Kanchanaburi, Thailand.



Figure 4.2 Weedy rice invaded into rice field. Rice plants were taller than cultivated rice plus panicle with awn were recognized as weedy rice.

4.3 Results

Morphological characteristics

Six morphological characteristics that consisted of plant type, panicle type, awned presentation, awn color, hull color and pericarp color were recorded on individual plant in each rice sample. Cultivated rice, SPR1_{PURE}, showed uniformity of erect plant type, compact panicle, awnless, straw color hull and white pericarp. While wild rice KC2 exhibited spreading plant type, open panicle, red and white long awned, dark brown to black hull and predominate red pericarp (Table 4.1). Wild traits were found in farmers' cultivated rice (SPR1_{FD1} and SPR1_{FD2}), 3 to 11% for awning, 3-9% for brown and dark brown to black hull and 3 to 12% for red pericarp. Moreover, intermediate plant type and panicle were observed in both cultivated rice (SPR1_{FD1} and SPR1_{FD2}). Cultivated traits were also observed in weedy (WR_{FD2}) and wild rice (KC1). Eight percent of weedy rice plants had erect plant type, 44% of weedy rice and 32% of wild rice KC 1 had straw color hull and white pericarp (4% for WR_{FD2} and 18% for KC1).

Physiological characteristics

Days to flowering

Mean of days to flowering of SPR1_{PURE} was 86 and flowering date were distributed in 80-100 days (Figure 1). While days to flowering of wild rice KC1 and KC2 were peak at 110-120 days, distributing within the range of >100-130 days. SPR1 from farmers' seed were flowering earlier than SPR1_{PURE} cultivated rice (5% for both SPR1_{FD1} and SPR1_{FD2}). For weedy rice, the distribution of days to flowering

was the same as cultivated rice from farmers' field but with higher proportion (30%) of plants with <80 days flowering.

Culm length (cm)

Culm length of SPR1_{PURE} was distributed in range 60-100 with average culm length at 81 cm (Figure 4.2). Mean culm length of SPR1 from field 1 and 2 were 89 and 88 cm, respectively. Larger distribution was also observed with some plants taller than SPR1_{PURE} (13% for SPR1_{FD1} and 5% for SPR1_{FD2}). The distribution and wild rice KC1 and KC2 were within 100-160 and 80-180 cm, with means of 144 and 126 cm, respectively. For weedy rice WR_{FD2}, culm length was distributed in the range between cultivated rice and wild rice, 80-140 with average culm length at 114 cm. Short plants same as cultivated rice were observed in 3% of weedy rice and 6% of wild rice KC1.

Seed shattering

Mean seed shattering of SPR1_{PURE} was 5% and distributed within the 0-10% class (Figure 4.3). For farmers' SPR1, percent seed shattering was higher than SPR1_{PURE}. About 30% of plants from SPR1_{FD1} and 32% from SPR1_{FD2} shed seeds more than 10%. Wild rice collected from near an abandoned rice field 30 km away (KC2), shattered all seed (100%) at maturity. Mean seed shattering of weedy rice (WR_{FD2}) and the KC1 wild rice were in the same class as KC2 (94-99%), but with wider range of distribution. Decreasing of seed shattering was observed in 3% of WR_{FD2} for 60-90% seed shattering and 20% of KC1 wild rice for 40-90%.

Table 4.1 Frequency of six morphological characteristics in cultivated, weedy and wild rice.

Morphological characters	Crop rice (SPR1)						Weedy rice		Wild rice			
	SPR1 _{PURE} (N=23)		SPR1 _{FD1} (N=100)		SPR1 _{FD2} (N=82)		WR _{FD2} (N=73)		KC1 (N=50)		KC2 (N=35)	
	n	%	n	%	n	%	n	%	n	%	n	%
<i>Plant type</i>												
erect	23	100	95	95	77	94	6	8	0	0	0	0
intermediate	0	0	5	5	5	6	67	92	0	0	0	0
open	0	0	0	0	0	0	0	0	2	4	0	0
spreading	0	0	0	0	0	0	0	0	48	96	35	100
<i>Panicle type</i>												
compact	23	100	94	94	76	93	6	8	0	0	0	0
intermediate	0	0	6	6	6	7	31	43	0	0	0	0
open	0	0	0	0	0	0	36	49	50	100	35	100
<i>Awned presentation</i>												
Awnless	23	100	97	97	73	89	1	1	0	0	0	0
Short awn	0	0	3	3	4	5	23	32	5	10	0	0
Long awn	0	0	0	0	5	6	49	67	45	90	35	100
<i>Awn color</i>												
White	-	-	3	3	9	11	73	100	17	34	19	54
Red	-	-	0	0	0	0	0	0	33	66	16	46
<i>Hull color</i>												
Straw	23	100	97	97	75	91	32	44	16	32	0	0
Brown	0	0	3	3	0	0	16	22	16	32	23	66
Dark brown-black	0	0	0	0	7	9	25	34	18	36	12	34
<i>Pericarp color</i>												
White	23	100	97	97	72	88	2	4	9	18	0	0
Red	0	0	3	3	10	12	71	96	41	82	35	100



Figure 4.3 Intermediate plan type and panicle, hull and pericarp color of weedy rice were recorded in this experiment.

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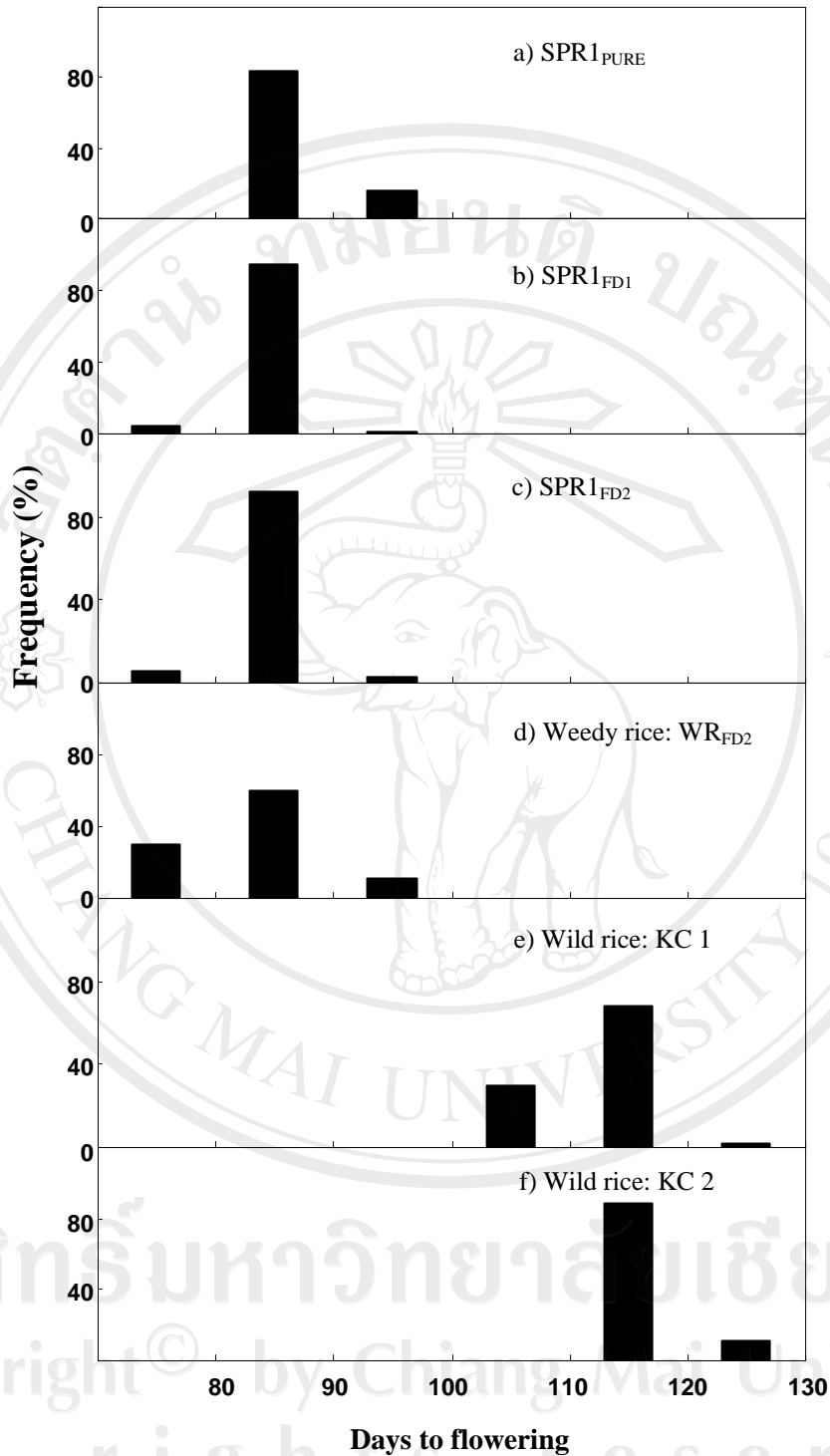


Figure 4.4 Distribution of days to flowering (mean \pm sd) of;

- a) Purified cultivated rice ($SPR1_{PURE}$) from RRI (86 \pm 2)
- b) Cultivated rice ($SPR1_{FD1}$) from farmers' field 1 (85 \pm 3)
- c) Cultivated rice ($SPR1_{FD2}$) from farmers' field 2 (84 \pm 5)
- d) Weedy rice (WR_{FD2}) from farmers' field 2 (84 \pm 5)
- e) Wild rice near the rice field: KC1 (112 \pm 4)
- f) Wild rice 30 km from the rice field: KC2 (115 \pm 3).

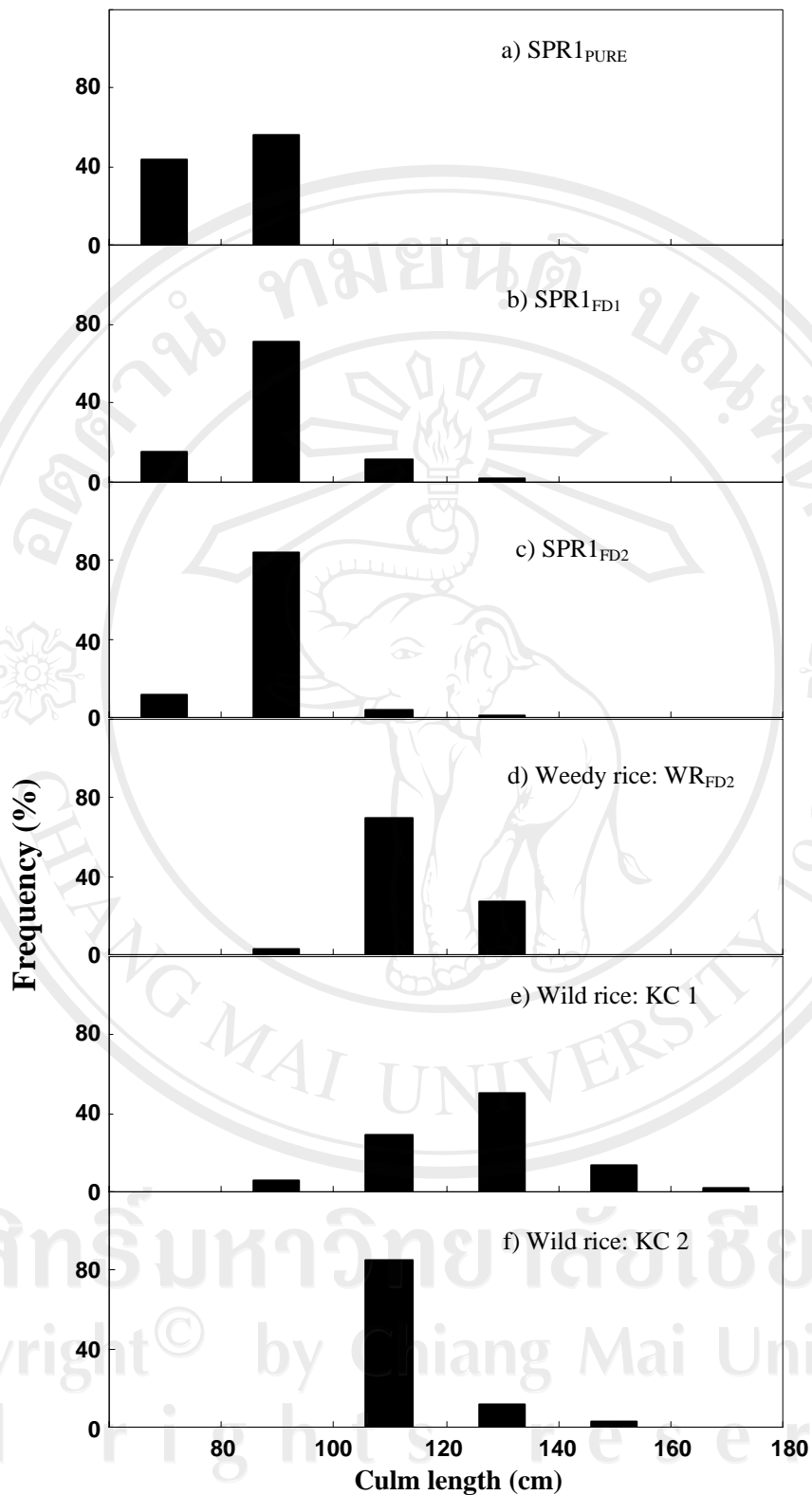


Figure 4.5 Distribution of culm length, cm, (mean±sd) of;
a) Cultivated rice (SPR1_{PURE}) from RRI (81±3)
b) Cultivated rice (SPR1_{FD1}) from farmers' field 1 (89±10)
c) Cultivated rice (SPR1_{FD2}) from farmers' field 2 (88±9)
d) Weedy rice (WR_{FD2}) from farmers' field 2 (114±7)
e) Wild rice near the rice field: KC1 (126±16)
f) Wild rice 30 km from the rice field: KC2 (114±8)

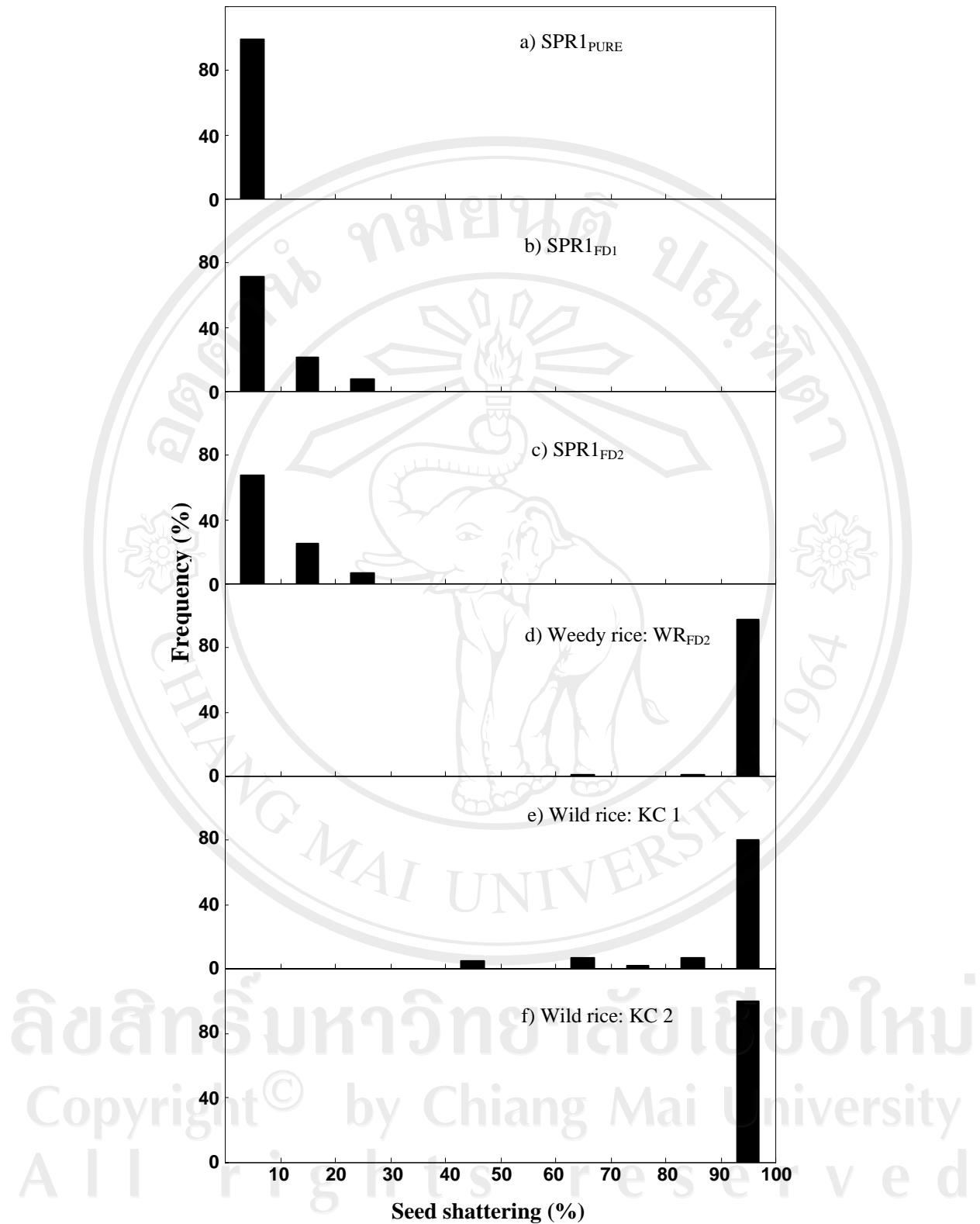


Figure 4.6 Distribution of seed shattering, %, (mean \pm sd) of;

a) Cultivated rice (SPR1_{PURE}) from RRI (5 \pm 3)

b) Cultivated rice (SPR1_{FD1}) from farmers' field 1 (8 \pm 6)

c) Cultivated rice (SPR1_{FD2}) from farmers' field 2 (8 \pm 6)

d) Weedy rice (WR_{FD2}) from farmers' field 2 (99 \pm 5)

e) Wild rice near the rice field: KC1 (94 \pm 14)

f) Wild rice 30 km from the rice field: KC2 (100 \pm 0)

Microsatellite analysis

Allele frequency

Three pairs of primer were used to identify rice sample. SPR1_{PURE} and CNT1_{PURE} varieties that grown from breeder seed showed only one allele each (designated A for SPR1 and B for CNT1) when detected by three pairs of primer. For wild rice, alleles specific to KC1 and/or KC2 were found and assigned to C-J (6) for RM1, C to L (8) for each of RM164 and 211 (Table 4.2). For each marker, alleles of crop rice and wild rice types were summarized in Table 4.3. Wild rice alleles were found in farmers' rice crop of SPR1, 8.6-20.5% in field 1 and 1.3-7.5% in field 2. In wild rice populations, 3.3-5.8% of SPR1 and 1.7-5% of CNT1 alleles were found in KC1. In KC2, 4.8% of CNT1 allele was detected by RM1. In weedy rice, both crop rice (5.3-74.5% of SPR1 and 7.5-81.9% of CNT1) alleles and 12.8-56.4% of wild rice alleles were found (Table 4.3).

Genotype frequency

Both homozygous and heterozygous genotypes were found using three RM markers (Figure 4.7). Frequencies of genotype in each population are listed in Table 4.4 and summarized for all three microsatellite markers in Table 4.5. In farmers' SPR1, 2.5- 3.5% of CNT1, 2.5-3.6% of KC1 and 1-2.5% of KC2 plus 1.0-21.8% of hybrids between SPR1 or CNT1 x wild rice were found. In KC1 wild rice, 6.7 and 3.3% of plants were homozygous for SPR1 and CNT1, respectively. About 7% were hybrids between SPR1 x KC1 and 1% between CNT1 x KC2 (Table 4.5). For KC2, only 3.2% of CNT1 type was found. Weedy rice population contained 40.4% of KC1 types, 10.6 and 14.9% of CNT1 and SPR1 crop rice, respectively. About 27% were hybrids between wild rice near the field, KC1, and SPR1 (23.4%) or CNT1 (4.3).

Table 4.2 Allele frequencies of crop rice, weedy rice and wild rice evaluated with three microsatellite markers, RM1, RM164 and RM211.

Locus/ Allele	Crop rice (SPR1)			Weedy rice	Wild rice		Crop rice (CNT1)
	SPR1 _{PURE} (N=20)	SPR1 _{FD1} (N=110)	SPR1 _{FD2} (N=80)	WR _{FD2} (N=47)	KC1 (N=50)	KC2 (N=35)	CNT1 _{PURE} (N=20)
RM1							
Allele A	1	0.6818	0.8250	0.7447	0.0583	0	0
Allele B	0	0.1136	0.1000	0.0745	0.0167	0.0484	1
Allele C	0	0.0818	0.0750	0	0	0.4194	0
Allele D	0	0.0727	0	0.1170	0.4583	0.4677	0
Allele E	0	0	0	0	0.0917	0	0
Allele F	0	0.0364	0	0.0638	0.0500	0	0
Allele G	0	0	0	0	0.1000	0	0
Allele H	0	0	0	0	0.0667	0	0
Allele I	0	0.0136	0	0	0.1583	0	0
Allele J	0	0	0	0	0	0.0645	0
RM 164							
Allele A	1	0.8500	0.8500	0.1915	0.0333	0	0
Allele B	0	0.0909	0.0750	0.2447	0.0167	0	1
Allele C	0	0	0.0500	0	0.0333	0	0
Allele D	0	0.0364	0.0250	0.2234	0.1417	0.0806	0
Allele E	0	0	0	0.3404	0.0667	0	0
Allele F	0	0	0	0	0.0583	0	0
Allele G	0	0.0182	0	0	0.4083	0	0
Allele H	0	0	0	0	0.1083	0	0
Allele I	0	0	0	0	0.0333	0	0
Allele J	0	0	0	0	0.0333	0	0
Allele K	0	0	0	0	0.0667	0	0
Allele L	0	0.0045	0	0	0	0.9194	0
RM211							
Allele A	1	0.7318	0.8875	0.0532	0.0583	0	0
Allele B	0	0.1818	0.1000	0.8191	0.0500	0	1
Allele C	0	0.0364	0.0125	0.1277	0.3417	0	0
Allele D	0	0.0182	0	0	0.1333	0.9677	0
Allele E	0	0	0	0	0.0500	0	0
Allele F	0	0.0227	0	0	0.1167	0	0
Allele G	0	0	0	0	0.0333	0	0
Allele H	0	0	0	0	0.1167	0	0
Allele I	0	0	0	0	0.0333	0	0
Allele J	0	0.0091	0	0	0.0500	0	0
Allele K	0	0	0	0	0	0.0323	0
Allele L	0	0	0	0	0.0167	0	0

Table 4.3 Frequency (%) of alleles specific to cultivated rice (SPR1 and CNT1) and wild rice (KC1 and KC2) in crop rice, weedy rice and wild rice based on three microsatellite markers.

Primer and allele type	No. of allele	Crop rice (SPR1)			Weedy rice	Wild rice		Crop rice (CNT1)
		SPR1 _{PURE} (n=20)	SPR1 _{FD1} (n=110)	SPR1 _{FD2} (n=80)	WR _{FD2} (n=47)	KC1 (n=50)	KC2 (n=35)	CNT1 _{PURE} (n=20)
RM1								
SPR1	1	100.0	68.2	82.5	74.5	5.8		
CNT1	1		11.4	10.0	7.5	1.7	4.8	100.0
KC1	6		5.0		6.4	46.7	6.5	
KC2	1		8.2	7.5			41.9	
KC1 and 2	1		7.3		11.7	45.8	46.8	
RM164								
SPR1	1	100.0	85.0	85.0	19.2	3.3		
CNT1	1		9.1	7.5	24.5	1.7		100.0
KC1	8		1.8	5.0	34.0	80.8		
KC2	1		0.5				91.9	
KC1 and 2	1		3.6	2.5	22.3	14.2	8.1	
RM211								
SPR1	1	100.0	73.2	88.8	5.3	5.8		
CNT1	1		18.2	10.0	81.9	5.0		100.0
KC1	8		6.8	1.2	12.8	75.8		
KC2	1						3.2	
KC1 and 2	1		1.8			13.3	96.8	
% Wild alleles detected by								
RM1		0	20.5	7.5	18.1	92.5	95.2	0
RM164		0	5.9	7.5	56.4	95.0	100.0	0
RM211		0	8.6	1.2	12.8	89.2	100.0	0
Average		0	11.7	5.4	29.1	92.2	98.4	0
% Crop rice allele detected by								
RM1		100.0	79.6	92.5	82.0	7.5	4.8	100.0
RM164		100.0	94.1	92.5	43.7	5.0	0	100.0
RM211		100.0	91.4	98.8	87.2	10.8	0	100.0
Average		100.0	88.4	94.6	70.9	7.8	1.6	100.0

Table 4.4 Genotype frequencies of crop rice, weedy rice and wild rice evaluated with three microsatellite loci.

Locus	Crop rice (SPR1)			Weedy rice	Wild rice		Crop rice (CNT1)
	SPR1 _{PURE} (N=20)	SPR1 _{FD1} (N=110)	SPR1 _{FD2} (N=80)	WR _{FD2} (N=47)	KC1 (N=50)	KC2 (N=35)	CNT1 _{PURE} (N=20)
RM1							
AA	1.00	0.53	0.78	0.62	0.03	0.00	0.00
BB	0	0.08	0.10	0.06	0.02	0.03	1.00
CC	0	0.02	0.03	0.00	0.00	0.42	0.00
DD	0	0.00	0.00	0.00	0.45	0.45	0.00
EE	0	0.00	0.00	0.00	0.07	0.00	0.00
FF	0	0.00	0.00	0.04	0.05	0.00	0.00
GG	0	0.00	0.00	0.00	0.10	0.00	0.00
HH	0	0.00	0.00	0.00	0.07	0.00	0.00
II	0	0.00	0.00	0.00	0.15	0.00	0.00
JJ	0	0.00	0.00	0.00	0.00	0.06	0.00
AC	0	0.11	0.10	0.00	0.00	0.00	0.00
AD	0	0.10	0.00	0.23	0.00	0.00	0.00
AE	0	0.00	0.00	0.00	0.05	0.00	0.00
AF	0	0.07	0.00	0.02	0.00	0.00	0.00
AI	0	0.03	0.00	0.00	0.00	0.00	0.00
BC	0	0.02	0.00	0.00	0.00	0.00	0.00
BD	0	0.05	0.00	0.00	0.00	0.03	0.00
BF	0	0.00	0.00	0.02	0.00	0.00	0.00
DI	0	0.00	0.00	0.00	0.02	0.00	0.00
RM164							
AA	1.00	0.81	0.85	0.15	0.02	0.00	0.00
BB	0.00	0.07	0.04	0.19	0.00	0.00	1.00
CC	0.00	0.00	0.01	0.00	0.02	0.00	0.00
DD	0.00	0.03	0.03	0.19	0.13	0.06	0.00
EE	0.00	0.00	0.00	0.30	0.07	0.00	0.00
FF	0.00	0.00	0.00	0.00	0.05	0.00	0.00
GG	0.00	0.00	0.00	0.00	0.38	0.00	0.00
HH	0.00	0.00	0.00	0.00	0.08	0.00	0.00
II	0.00	0.00	0.00	0.00	0.03	0.00	0.00
JJ	0.00	0.00	0.00	0.00	0.03	0.00	0.00
KK	0.00	0.00	0.00	0.00	0.07	0.00	0.00
LL	0.00	0.00	0.00	0.00	0.00	0.90	0.00
AB	0.00	0.04	0.00	0.04	0.00	0.00	0.00
AD	0.00	0.01	0.00	0.02	0.02	0.00	0.00

Table 4.4 (Continue).

Locus	Crop rice (SPR1)			Weedy rice	Wild rice		Crop rice (CNT1)
	SPR1 _{PURE} (N=20)	SPR1 _{FD1} (N=110)	SPR1 _{FD2} (N=80)	WR _{FD2} (N=47)	KC1 (N=50)	KC2 (N=35)	CNT1 _{PURE} (N=20)
RM164							
AE	0.00	0.00	0.00	0.02	0.00	0.00	0.00
AG	0.00	0.03	0.00	0.00	0.00	0.00	0.00
AH	0.00	0.00	0.00	0.00	0.02	0.00	0.00
AL	0.00	0.01	0.00	0.00	0.00	0.00	0.00
BC	0.00	0.00	0.08	0.00	0.00	0.00	0.00
BD	0.00	0.00	0.00	0.02	0.00	0.00	0.00
BE	0.00	0.00	0.00	0.04	0.00	0.00	0.00
BH	0.00	0.00	0.00	0.00	0.03	0.00	0.00
CG	0.00	0.00	0.00	0.00	0.03	0.00	0.00
DE	0.00	0.00	0.00	0.02	0.00	0.00	0.00
DG	0.00	0.01	0.00	0.00	0.00	0.00	0.00
DL	0.00	0.00	0.00	0.00	0.00	0.03	0.00
FG	0.00	0.00	0.00	0.00	0.02	0.00	0.00
RM211							
AA	1.00	0.70	0.89	0.04	0.05	0.00	0.00
BB	0.00	0.16	0.10	0.81	0.05	0.00	1.00
CC	0.00	0.02	0.01	0.13	0.32	0.00	0.00
DD	0.00	0.02	0.00	0.00	0.13	0.97	0.00
EE	0.00	0.00	0.00	0.00	0.05	0.00	0.00
FF	0.00	0.02	0.00	0.00	0.12	0.00	0.00
GG	0.00	0.00	0.00	0.00	0.03	0.00	0.00
HH	0.00	0.00	0.00	0.00	0.12	0.00	0.00
II	0.00	0.00	0.00	0.00	0.03	0.00	0.00
JJ	0.00	0.01	0.00	0.00	0.03	0.00	0.00
KK	0.00	0.00	0.00	0.00	0.00	0.03	0.00
LL	0.00	0.00	0.00	0.00	0.02	0.00	0.00
AB	0.00	0.03	0.00	0.02	0.00	0.00	0.00
AC	0.00	0.04	0.00	0.00	0.02	0.00	0.00
BF	0.00	0.01	0.00	0.00	0.00	0.00	0.00
CJ	0.00	0.00	0.00	0.00	0.03	0.00	0.00

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Table 4.5 Genotypic frequency (%) of cultivated rice (SPR1 and CNT1), wild rice (KC1 and KC2) and hybrid types in crop rice, weedy rice and wild rice based on three microsatellite markers.

Genotype	Crop rice (SPR1)			Weedy rice	Wild rice		Crop rice (CNT1)
	SPR1 _{PURE} (n=20)	SPR1 _{FD1} (n=110)	SPR1 _{FD2} (n=80)	WR _{FD2} (n=47)	KC1 (n=50)	KC2 (n=35)	CNT1 _{PURE} (n=20)
Crop rice							
SPR1	100.0	50.9	76.2	14.9	6.7		
CNT1		3.6	2.5	10.6	3.3	3.2	100.0
Wild rice							
KC1		3.6	2.5	40.4	81.6		
KC2		1.0	2.5			90.3	
Hybrids							
SPR1 x KC1		21.8		23.4	6.7		
SPR1 x KC2		10.0	8.8				
CNT1 x KC1		4.5		4.3	1.7		
CNT1 x KC2		1.0	7.5				
SPR1 x CNT1		3.6		6.4			
KC2 (1) x KC2 (2)						6.5	
% heterozygote		40.9	16.3	34.1	8.3	6.5	

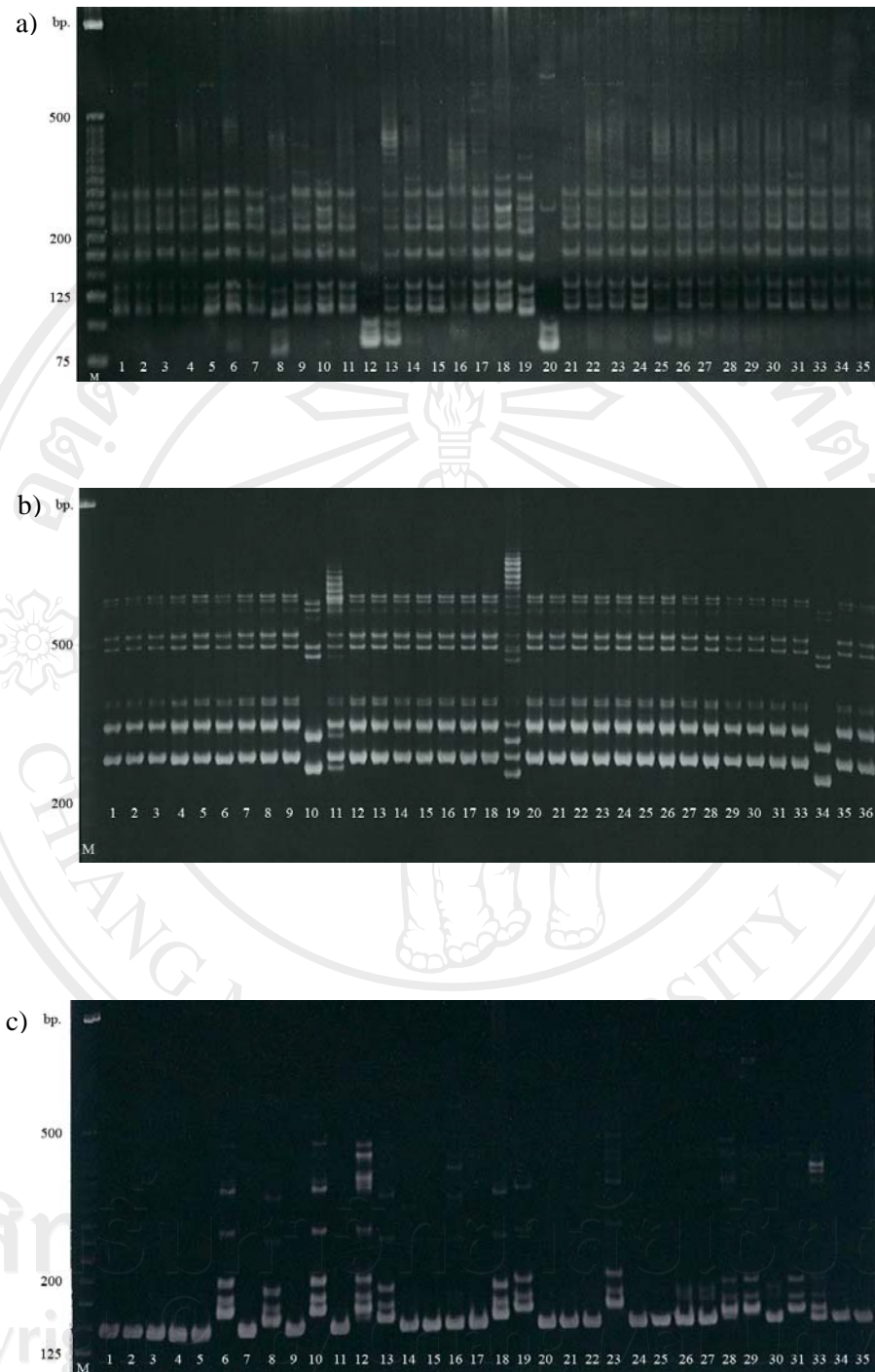


Figure 4.7 Microsatellite amplification products generated from cultivated rice (SPR1) farmers' field genomic DNA with primer RM1 (a), RM164 (b) and RM211 (c). SPR1 from farmers' field contained both homozygous and heterozygous genotypes.

Genetic structure and genetic differentiation of cultivated, weedy and wild rice

Within population genetic structure

Gene diversity index (H_e) was calculated for all population to measure deviation of genetic variation within population which expected from pure line seeds, $SPR1_{PURE}$ and $CNT1_{PURE}$, $H_e = 0$ (Table 4.6). Farmers' crop rice, $SPR1_{FD1}$ and $SPR1_{FD2}$ had mixtures in populations as shown by gene diversity of 0.408 for $SPR1_{FD1}$ and 0.258 for $SPR1_{FD2}$ (Table 4.4). The highest gene diversity was recorded in wild rice near rice field, $KC1$ ($H_e=0.788$) whereas that of wild rice $KC2$ was much lower (0.274). Gene diversity of weedy rice was higher than farmers' crop rice and $KC2$ wild rice but less than $KC1$ (0.489). For the fixation index (F_{IS}), the overall F_{IS} was close to 1, indicating that they deviate from random mating. F_{IS} of farmers' $SPR1$ and weedy rice were lower than those of wild rice $KC1$ and $KC2$ suggest the lower number of heterozygous genotypes in wild rice, compared with farmers' $SPR1$ and weedy rice. The outcrossing rate (t) estimated by F_{IS} for $SPR1_{FD1}$, $SPR1_{FD2}$ and weedy rice, WR_{FD2} were 28, 13 and 18% respectively. Low outcrossing rates were found in wild rice, $KC1$ and $KC2$, 5 and 4%, respectively.

Heterozygote frequency was also estimated and compared among populations. Heterozygote frequency was 0 in $SPR1_{PURE}$ and $CNT1_{PURE}$ (data not shown). The heterozygote frequency was highest in $SPR1_{FD1}$ (40.9%), follow by weedy rice (34%) and $SPR1_{FD2}$ (16.3). Those of $KC1$ and $KC2$ wild rice were 8.5 and 6.5%, respectively.

Between population genetic structure

Genetic differentiation among seven rice populations was represented by F_{ST} values (Table 4.6). With the three RM markers, RM1, RM164 and RM211, pure SPR1 (6) and CNT (1) were completely differentiated to alternate alleles 100% ($F_{ST} = 1.0$). For wild rice, differentiations between KC1 and SPR1_{PURE} and CNT1_{PURE} were 42 and 43%, respectively, while those of KC2 and SPR1_{PURE} and CNT1_{PURE} were both 75%. When compared with crop rice and wild rice, much lower degree of gene differentiation between weedy rice and wild rice in the same area, KC1 (18%) and between weedy rice and farmers' SPR1 (21-29%) than between weedy rice and pure crop rice (42-44%) and wild rice 30 km away, KC2 (44%).

Table 4.6 Gene diversity (H_e), Fixation index (F_{IS}) and outcrossing rate (t) cultivated, weedy and wild rice based on three microsatellite loci (number in parentheses indicate standard deviation).

Population	H_o	H_e	F_{IS}	t^\dagger	Heterozygote frequency (%)
Cultivated rice; field 1 SPR1 _{FD1}	0.178 (0.123)	0.408 (0.168)	0.558	0.283	40.9
Cultivated rice; field 2 SPR1 _{FD2}	0.058 (0.052)	0.258 (0.051)	0.777	0.125	16.3
Weedy rice; field 2 WR _{FD2}	0.156 (0.128)	0.489 (0.224)	0.687	0.185	34.1
Wild rice; KC1	0.077 (0.034)	0.788 (0.045)	0.902	0.05	8.4
Wild rice; KC2	0.021 (0.018)	0.274 (0.292)	0.923	0.04	6.5
Overall	0.101 (0.076)	0.679 (0.016)	0.777	0.138	21.2

$^\dagger t$, outcrossing rate = $(1-F_{IS})/(1+F_{IS})$

Table 4.7 Genetic differentiation among populations (F_{ST}) matrix values determined across three microsatellite loci in crop rice, weedy rice and wild rice.

Population	1	2	3	4	5	6	7
1. Crop rice, SPR1 _{FD1}	0.00						
2. Crop rice, SPR1 _{FD2}	0.02	0.00					
3. Weedy rice; WR _{FD2}	0.21	0.29	0.00				
4. Wild rice; KC1	0.22	0.29	0.18	0.00			
5. Wild rice; KC2	0.49	0.58	0.44	0.26	0.00		
6. Crop rice; SPR1 _{PURE}	0.09	0.06	0.44	0.42	0.75	0.00	
7. Crop rice; CNT1 _{PURE}	0.62	0.75	0.42	0.43	0.75	1.00	0.00

KC1 = wild rice near rice field, KC2 = wild rice 30 km. from rice field

4.4 Discussion

Evidence of successfully hybridization between cultivated and wild rice has been found in farmers' rice crop, in the weedy rice and in the naturalized wild rice populations by morphological, physiological traits and DNA markers. Two-way direction of both crop to wild and wild to crop gene flow were also demonstrated by the presentation of crop rice alleles in wild rice populations and wild rice alleles in crop rice populations. Weedy rice was acting as an intermediate stage, containing all types i.e. homozygous for crop alleles, wild alleles and heterozygous for both wild and crop alleles.

With morphological characteristics, wild traits that were found in the farmers' cultivated rice included some plants with awn, black to brown instead of the straw colour of hull of SPR1, red pericarp and more seed shattering than is expected of SPR1. The weedy rice of farmer's field, identified by the presence of awned spikelets, showed even higher frequency of wild traits mixing with some cultivated rice traits. Even greater extent of cultivated rice and wild rice hybridization can be seen in microsatellite markers that represented alleles other than that of SPR1, SPR1PURE, in the naturalized wild rice, farmers' rice crop of SPR1 and weedy rice in the field. In the KC1 wild rice, alleles of the microsatellite markers that were found those that were specific to SPR1, CNT1, SPR1 x KC1 and CNT1 x KC1 hybrids.

With the genetic structure analysis, close genetic relationships were found between weedy rice, farmers' SPR1 and KC1, demonstrated by shared alleles and low genetic differentiation (F_{ST}) between them. The F_{ST} between farmers' rice crop, weedy and the naturalized wild rice (KC1) were between 0.02-0.75. In contrast, very high genetic differentiation between weedy rice and populations collected outside the

area, KC2 wild rice and pure line crop rice, SPR1_{PURE} and CNT1_{PURE} (F_{ST} 0.42-0.44). Results of this relationship suggested that gene flow between wild rice, weedy rice and farmers' crop rice was an ongoing process. Many reports suggested that the direction of gene flow was predominately from crop to wild because crop rice is self-pollinated plant while wild rice is cross-pollinated plant (Oka and Chang, 1961; Chen *et al.*, 2004). In this study, both crop to wild and wild to crop gene flow were demonstrated with most of hybridization occurred between crop rice and weedy rice. For KC1 wild rice, high F_{IS} together with low outcrossing rate in wild rice suggesting that these populations were quite uniform, cover a very small ditch, and resulting from self fertilization or vegetative propagation. However, with 5% outcrossing together with alleles of SPR1, CNT1 and interspecific hybrids found in plants classified as wild rice at the edge indicated that gene flow did happen at the interface between crop rice and weedy rice with wild rice.

The incidence of hybridization was estimated with the percentage of hybrids found in populations, which were resulting from random hybridization within and between populations. High outcrossing rates were estimated for farmers SPR1 and weedy rice (13-28%) populations, only 4-5% were found in wild rice KC1 and KC2. This is consistent to that report by Langevin *et al.* (1990). Natural hybridization rates between weedy rice and cultivated rice have been estimated to range from 1.08% to 52.18% after examining 12,000 seeds collected on weedy rice plants naturally occurring in fields of six different rice varieties, and determining the incidence of hybrid progeny by isozyme analyses (Langevin *et al.*, 1990). However, very low outcrossing rates were detected in artificial hybridization in experimental fields to evaluate transgene flow between crop rice and wild rice or weedy rice (Chen *et al.*,

2004). Gene flow between weedy rice and transgenic rice in experimental field were 0.04-0.09% depends on wind direction (Messeguer *et al.*, 2004). This may resulted from synchronizing between the sink and source populations, wind direction and the experimental designs. High outcrossing rate of weedy rice population found in this study means that weedy rice will have high chance of introgression. When weed and crop are grown together, more crop traits will be introgressed into weedy rice which will act as a crop trait reservoir. Those with high selective advantage will be retained in the population. This was supported by, where high frequency of brown plant hopper resistance were found in weedy rice in the central plain where crop varieties with BPH resistance are grown (Samanwong *et al.*, 2005). Introduce new variety with new trait into weedy rice area should be monitored closely for the potential harmful effects of gene flow.

High genetic diversity was found in farmers' SPR1 ($He = 0.4081$), weedy rice (0.4899) and wild rice (0.788) compared with that of wild rice KC2. For weedy rice, this value was much higher than weedy rice found in China where He about 0.011-0.038 which found in 30 populations of weedy rice (Cao *et al.*, 2006). High genetic variation of the farmers' crop rice and KC1 wild rice must have resulted from gene exchange between them. High genetic diversity of weedy rice indicates high potential of population to adapt to disturbed situation e.g. habitats, cultivation, farmer practice, herbicide resistance etc. Large range of reproductive successful will increase more serious problem.