Chapter 3

Interspecific hybridization between cultivated rice (*Oryza sativa* L.) and common wild rice (*O. rufipogon* Griff)

3.1 Introduction

Weedy rice has become a serious weed in rice growing area in Thailand since 2004 (Maneechote *et al*., 2004). Hybridization between cultivated and wild relatives is the one hypothesis for the origin of weedy form. Ellstrand *et al.* (1999) reported that natural hybridization is often observed in crop/ wild ancestor complex in 22 crop species. Generally, cultivated rice (*O. sativa* L.) is a predominantly self-pollination crop, with 0-1% outcrossing rate (Robert *et al.*, 1961). In contrast its progenitor, common wild rice (*O. rufipogon* Griff.), is a cross-pollinated species, with 7 to 55% of outcrossing (Barbier, 1989). In Thailand, evidence of natural gene flow was observed by Oka and Chang since 1961. Waxy gene was found in common wild rice populations that grow in close to glutinous rice field (Oka and Chang, 1961).

Gene flow between cultivated and wild rice has been reported in the range of 1.2-2.19% (Chen *et al.*, 2004) while natural hybridization between cultivated and weedy rice has been reported between 1-52% (Langevin *et al*., 1990). The rate of gene flow is obviously dependent on genotypes and their flowering period. In Thailand, rice farmers grow improved and local varieties in the country's rice growing areas. Farmers choose to grow a particular variety in their field depending on market demand, farmers' own taste preference and suitability of the variety to limitation and

potential of each field. The native wild rice also showed differentiation of populations by geographical distance and ecological factors. The first step for assessment of gene flow is crossability. Therefore, the objectives of this study are as fallows:

1. To evaluate cross compatibility between cultivated rice and wild rice from three major rice growing regions in Thailand.
2. To evaluate fitness of F_1 hybrids between cultivated rice and wild rice.
3. To evaluate the segregation of morphological and physiological characteristics of F_2 progenies between cultivated rice and wild rice.

3.2 Materials and Methods

Genetic materials and seed production

Eight cultivated varieties and three wild rice populations as described in Chapter 2 were used as parents. Crosses were made between all cultivated rice x wild rice i.e. 8 x 3 = 24 combinations. Reciprocal crosses and backcrosses were made for LP wild rice x Suphan Buri 1 and Khao Dawk Mali 105 and reciprocal between F_1(Sew Mae Jan X KC) with SMJ cultivated rice and F_1 (Suphan Buri 1 x KC) with KC wild rice. For all experiments, seeds were pregerminated in petri dishes for 2 days and then seedlings were transferred to pots containing soil. About 5 to 10 plants were sown in pots and maintained as described in Chapter 2.

Experiment 1: Crossability between cultivated rice x wild rice

Twenty plants of cultivated rice were sown in pots, five plants/pot for four planting dates. Wild rice was propagated vegetatively from those used in Chapter 2. At booting, about 5-15 panicles of the each cultivated rice were emasculated and used as female parents. Pollination was made at the ratio of 3 female spikelets: 1 anther of wild rice parent. At maturity, the pollinated panicles were harvested and counted for number of flower pollinated, number of seed fertilized and percent seed set. Seeds from each cross were kept at 4°C and used in Experiment 2.

Experiment 2: Evaluation of F_1 hybrids of cultivated rice x wild rice

F_1 hybrids between cultivated rice x KC or NY wild rice were selected for this study. F_1 hybrids and parents from all combinations were grown in pots, five plants/pot. Pots were arranged in a completely randomized design (CRD) with four

replicates. Seed germination and number of normal seedlings were recorded at four weeks after transplanting. Plants were recorded individually for morphological and physiological traits as described in Chapter 2.

Experiment 3: Evaluation of F_2 generations between cultivated rice and wild rice.

Ten F_2 populations were used in this experiment; including F_2 from crosses;

CNT1 x KC	CNT1 x NY
SPR1 x KC	SPR1 x NY
KDML105 x KC	KDML105 x NY
RD6 x KC	RD6 x NY
KDK x KC	KDK x NY

Two hundred plants of each F_2 population and 20 plants of each parent were grown in pots, 10 plants/pot. Data were recorded in the same manner as in Experiment 2.

Data analysis

Data were analyzed by analysis of variance. Significantly different means was separated at $p < 0.05$ by the least significant difference (LSD) test.

3.3 Results

3.3.1 Crossability between cultivated rice x wild rice

All cultivated rice can cross with wild rice. Seed set of each cross was different depended on cultivated rice x wild rice combinations. About 95-774 spikelets of cultivated rice were pollinated with pollen from wild rice (LP, KC and NY). The seed set ranged from 18 to 59%, 6 to 40% and 6 to 62% when crossed with LP, KC and NY wild rice, respectively (Table 3.1). For the combinations of LP and KC wild rice, the highest seed set were found in CNT1 and SPR1. The rest were between 6.5-46%, with the lowest were those between wild rice and RD6 (18.4% with LP and 6.5% with KC). For combinations with NY wild rice, the highest seed set were again found in SPR1 x NY (62%). The rest were between 6.3-43%. The lowest percent seed set (6.3%) was observed in NSPT x NY combination. For reciprocals and backcrosses, seed set were 14-17% and 42-52%, respectively (Table 3.2).

Table 3.1 Number of spikelets fertilized, number and percent seed set (%) of crosses between eight cultivated rice (as female) and three common wild rice (as male) parents.

Cultivated rice (female)	Wild rice (male)								
	LP			KC			NY		
	Poll.	Set.	%	Poll.	Set.	%	Poll.	Set.	%
CNT1	148	88	59.5	330	116	35.2	109	11	10.1
SPR1	629	354	56.3	248	89	35.9	187	116	62.0
KDML105	103	48	46.6	714	81	11.3	115	50	43.5
RD6	103	19	18.4	400	26	6.5	121	32	26.4
RD10	116	39	33.6	164	66	40.2	-†	-	-
NSPT	190	36	18.9	117	19	16.2	95	6	6.3
SMJ	92	41	44.6	211	48	22.7	-†	-	-
KDK	391	120	30.7	365	89	24.4	103	34	33.0

LP= wild rice from Lamphun (North), KC = wild rice from Kanchanaburi (Central), NY = wild rice from Nakorn Nayok (Central), Poll. = number of pollinated spikelet, Set. = number of seed set, % = seed set percentage, †cross was not produced.

Table 3.2 Number of spikelets fertilized, number of seed set and percent seed set (%) of reciprocal crosses between wild rice x cultivated rice and back crosses between F_1 hybrids to cultivated and wild rice parents.

Cross				
Female	Male	Poll.	Set.	%
Reciprocal cross		448	77	17.2
Wild rice (LP)	SPR1	90	13	14.4
Wild rice (LP)	KDML105			
Back cross				
F_1(SPR1 x KC)	Wild rice (KC)	84	36	42.9
F_1 (SMJ x KC)	Crop rice (SMJ)	184	95	51.6

LP = wild rice from Lamphun (North), Poll. = number of pollinated spikelet, Set. = number of seed set, % = seed set percentage.

3.3.2 Evaluation of F_1 and F_2 generations of cultivated rice x wild rice

Seed germination and seedling survival

All cultivated rice and wild rice seed germinated normally in the range of 85 to 100% (Table 3.3). Seed germination of F_1 hybrids derived from LP, KC and NY wild rice ranged from 20-80%, 20-85% and 33-85%, respectively. For F_1 hybrids between cultivated x LP wild rice, the highest seed germination was found in RD10 x LP (80%). The rest were 20-75%. For F_1 hybrids between cultivated x KC and NY wild rice, the lowest seed germination were observed in NSPT x KC/ NY (20% with KC and 1% with NY). For seedling survival, cultivated rice survived completely after germination while wild rice seedling had a rate of survival about 90-95%. Seedlings of all F_1 hybrids survived in the range of 30 to 100%. Except F_1 hybrids between NSPT with KC and NY wild rice, no seedling survived after germination.

For F_2 populations between cultivated rice x KC and NY wild rice, seed germination were between 92 to 97% and 87 to 97%, respectively (Table 3.4). Seedlings of all F_2 hybrids survived in the range of 71 to 94%.

Table 3.3 Seed germination (%) and seedling survival (%) of F_1 hybrids and their parents.

Cross	Germination (%)				Seedling survival (%)			
	P_1	P_2	F_1	se	P_1	P_2	F_1	se
a) Cultivated rice x LP								
CNT1 x LP	95	95	20		100	95	100	
SPR1 X LP	100		60		100		100	
KDML105 x LP	100		65		100		31	
RD6 x LP	100		40		100		100	
RD10 x LP	100		80		100		69	
NSPT x LP	100		75		100		100	
KDK x LP	100		50	6	100		100	5
b) Cultivated rice x KC								
CNT1 x KC	95	95	25		100	95	86	
SPR1 X KC	100		70		100		86	
KDML105 x KC	100		75		100		100	
RD6 x KC	100		55		100		90	
RD10 x KC	100		65		100		100	
NSPT x KC	100		20		100		0	
SMJ x KC	85		85		100		100	
KDK x KC	100		70	6	100		100	6
c) Cultivated rice x NY								
CNT1 x NY	95	95	60		100	90	100	
SPR1 X NY	100		85		100		82	
KDML105 x NY	100		70		100		86	
RD6 x NY	100		80		100		83	
NSPT x NY	100		33		100		0	
KDK x KC	100		53	12	100		86	7

Table 3.4 Seed germination (%) and seedling survival (%) of F_2 generation and their parents.

	Germination (%)				Seedling survival (%)			
Cross	P_1	P_2	F_2	se	P_1	P_2	F_2	se
a) Cultivated rice x KC								
CNT1 x KC	100	97	98		100	95	91	
SPR1 X KC	97		92		100		79	
KDML105 x KC	98		99		100		79	
RD6 x KC	97		97		100		94	
KDK x KC	100		97	1	100		92	2
b) Cultivated rice x NY								
CNT1 x NY	100	83	92		100	90	79	
SPR1 X NY	97		89		100		80	
KDML105 x NY	98		95		100		71	
RD6 x NY	97		87		100		77	
KDK x KC	100		97	2	100		71	4

Morphological characteristics of F_1s and F_2 s and segregation ratios of F_2 s

Pigmentation in plant parts

Complete dominant gene action for purple, red, dark brown color were found for crosses between wild rice and cultivated rice with single to three gene segregations (Table 3.5 and 3.6). For crosses between wild rice and five cultivated rice (CNT1, SPR1, KDML105, RD6, RD10 and SMJ), single gene control was found in apiculus, stigma, hull and pericarp color, two genes in leaf sheath, apiculus, awn, stigma and hull color and three genes in leaf blade, leaf sheath and stigma color. For crosses between wild rice and purple rice (KDK), single gene were found in leaf blade, leaf sheath, auricle, ligule, stigma, hull and pericarp color, two genes in leaf blade, leaf sheath and pericarp color and three genes in awn color. Chi-square analysis for pigmentation in plant parts of F_2 populations were demonstrated in Appendix 3 – 11.

Table 3.5 Pigment presentation on plant parts of F_1 and segregation of F_2 between six cultivated rice (CR) and wild rice from Kanchanburi (KC) compared with their parents.

	P_1	P_2		F_2	
Plant part	CR	KC	F_1	Character	Ratio
Cultivated rice (var.1-5)† x KC					
Leaf-blade color	green	green	green	green	all green
Leaf-sheath color	green	purple line	purple line	green: purple	15:1, 63:1
Auricle color	colorless	colorless	colorless	colorless	all colorless
Ligule color	colorless	colorless	colorless	colorless	all colorless
Apiculus color	colorless	red	red	colorless: red	3:1, 63:1
Awn color	awnless	white	white	white	all white
Stigma color	white	red	red	white: red	63:1, 3:1, 1:15
Hull color	straw	dark brown	dark brown	straw: dark brown	3: 1, 15: 1, 9: 7
Pericarp color	white	red	red	red: white	3:1
Cultivated rice (var.6) † x KC					
Leaf-blade color	purple	green	green with purple at margin	green: purple	3:1
Leaf-sheath color	purple	green	light purple	green: purple	3:1
Auricle color	purple	colorless	light purple	purple: colorless	3: 1
Ligule color	purple	colorless	light purple	purple: colorless	3: 1
Apiculus color	purple	red	dark red	dark red	all dark red
Awn color	awnless	white	dark red	dark red: white	63:1
Stigma color	purple	red	dark red	red: white	3: 1
Hull color	dark purple	dark brown	dark purple	straw: dark brown	3: 1
Pericarp color	dark purple	red	dark purple	red: white	15: 1

† Cultivated rice variety 1-5=CNT1, SPR1, KDML105, RD6 and RD10, 6=KDK

Table 3.6 Pigment presentation on plant part of F_1 and segregation of F_2 between six cultivated rice (CR) and wild rice from Nakorn Nayok (NY) compared with their parents.

	P_1	P_2		F_2	
Plant part	CR	NY	F_1	Character	Ratio
Cultivated rice (var.1-4) x NY					
Leaf-blade color	green	green with purple at margin	green	green: purple	63:1
Leaf-sheath color	green	light purple	light purple	green: purple	3:1, 15:1
Auricle color	colorless	colorless	colorless	colorless	all colorless
Ligule color	colorless	colorless	colorless	colorless	all colorless
Apiculus color	colorless	red	red	red: colorless	3: 1, 9: 7, 1:3
Awn color	awnless	red	red	red: white	3: 1, 9: 7, 1: 15
Stigma color	white	red	red	red: white	3:1
Hull color	straw	dark brown	dark brown	straw: dark brown	3: 1, 15: 1, 9: 7
Pericarp color	white	light red	light red	white: red	3:1, 15: 1
Cultivated rice (var.5) x NY					
Leaf-blade color	purple	green with purple at margin	green with purple at margin	green: purple	15: 1
Leaf-sheath color	purple	light purple	light purple	green: purple	15: 1
Auricle color	purple	colorless	light purple	purple: colorless	3: 1
Ligule color	purple	colorless	dark red	purple: colorless	9:7
Apiculus color	purple	red	dark red	dark red	all dark red
Awn color	awnless	red	dark red	red: white	63: 1
Stigma color	purple	red	dark red	red	all red
Hull color	dark purple	dark brown	dark brown	straw: dark brown	3: 1
Pericarp color	dark purple	light red	light purple	red: white	3: 1

† Cultivated rice variety 1-4=CNT1, SPR1, KDML105 and RD6, 5=KDK

Physiological characteristics of F_1 and F_2 hybrids and segregation ratios.

Plant type

Intermediate plant type of wild rice was controlled by dominance gene action (Table 3.7). KC wild rice was differed from cultivated rice by two genes (15:1) or three genes (63:1). NY wild rice was differed from cultivated rice by one gene (3:1) or two genes (15:1). Chi-square analysis for plant type of F_2 populations was demonstrated in Appendix 12.

Panicle type

Spread panicle type of wild rice was dominant to compact panicle type of cultivated rice (Table 3.7). KC wild rice was differed from cultivated rice by single gene (1:3 and 3:1). NY wild rice was differed from cultivated by single gene as shown by segregation ratio of 1:3. Chi-square analysis for panicle type of F_2 populations was demonstrated in Appendix 13.

Spikelet awning

Awn on spikelet of wild rice was controlled by dominance gene action (Table 3.7). KC wild rice was difference from cultivated rice by single gene as shown by segregation ratio 1:3 or two genes (1:15). NY wild rice was differed from cultivated rice by single gene (1:3) or threes genes (1:63). Chi-square analysis for spikelet awning of F_2 populations was demonstrated in Appendix 14.

Seed shattering

Seed shattering at maturity of wild rice was dominant to non-shattering of cultivated rice (Table 3.7). KC and NY wild rice were differed from cultivated rice by three genes (1:63). Chi-square analysis for seed shattering of F_2 populations was demonstrated in Appendix 15.

Table 3.7 Morphological characteristics of F_1 hybrids and F_2 populations between cultivated rice and wild rice (KC and NY) compared with their parents.

	P_1	P_2		F_2	
Attribute	Cultivated rice	Wild rice (KC/ NY)	F_1	Character	Ratio
Cultivated x KC					
Plant type	erect	spreading	intermediate	erect: intermediate-spreading	15:1, 63:1
Panicle type	compact	open	intermediate	compact: intermediate-open	3:1, 1:3
Awning	awnless	awned	awned	awnless: awned	3:1, 15:1
Seed shattering	non-shattering	shattering	shattering	shattering: non-shattering:	63:1
Cultivated x NY					
Plant type	erect	spreading	intermediate	erect: intermediate	3:1, 15:1
Panicle type	compact	open	intermediate	compact: intermediate-open	1:3
Awning	awnless	awned	awned	awned: awnless	3:1, 63:1
Seed shattering	non-shattering	shattering	shattering	shattering: non-shattering:	63:1

Days to flowering

KC wild rice was flowering at the same time as KDML105, RD6 and KDK but later than CNT1, SPR1, RD10 and SMJ (Table 3.8). F_1 between CNT1 or SPR1 x KC wild rice were flowering at the same time as wild rice parents whereas days to flowering of F_1 between SMJ x KC were between those of parents. NY wild rice was the latest flowering (105 days). Flowering date of F_1 hybrids between cultivated rice with NY wild rice were closer to cultivated rice for all crosses. Normal, continuous segregation of F_2s were found for all cross. F_2 plants segregated within the range of parents. Segregation of F_2s derived from the photoperiod insensitive SPR1 and CNT1 cultivated rice varieties were larger than the others (Table 3.9 and Figure 3.1).

Culm length (cm)

F_1 hybrids were taller than cultivated rice. Mean of culm length of F_1 hybrids ranged from 123 to 157 cm (Table 3.10). F_2 plants segregated within the range of parents. As found in days to flowering, segregation of culm length of F_2s derived from SPR1 and CNT1 cultivated rice varieties were larger than the others Normal, continuous segregation was found for all cross (Table 3.11 and Figure 3.2).

Table 3.8 Days to flowering of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Days to flowering	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	84 g	99 b	88 f
SPR1	88 f	97 b	89 ef
KDML105	98 b	99 b	97 bc
RD6	97 bc	97 bc	94 cd
RD10	91 e	81 g	†
SMJ	69 i	76 h	†
KDK	97 b	98 b	94 d
Wild rice		99 b	105 a
LSD (0.05)	2.6		

† data not available

Table 3.9 Mean, range and standard deviation of days to flowering of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

Parent	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	19	82	79-87	2	146	90	69-108	7	141	92	71-104	6
SPR1	19	87	83-93	4	134	91	72-111	10	136	91	79-106	5
KDML105	18	98	96-102	2	147	97	90-107	4	128	98	86-107	3
RD6	16	97	93-101	2	155	99	89-112	5	125	90	79-111	5
KDK	19	97	96-99	1	176	95	88-105	4	144	93	76-107	5
Wild rice					18	93	89-103	5	34	104	92-111	4

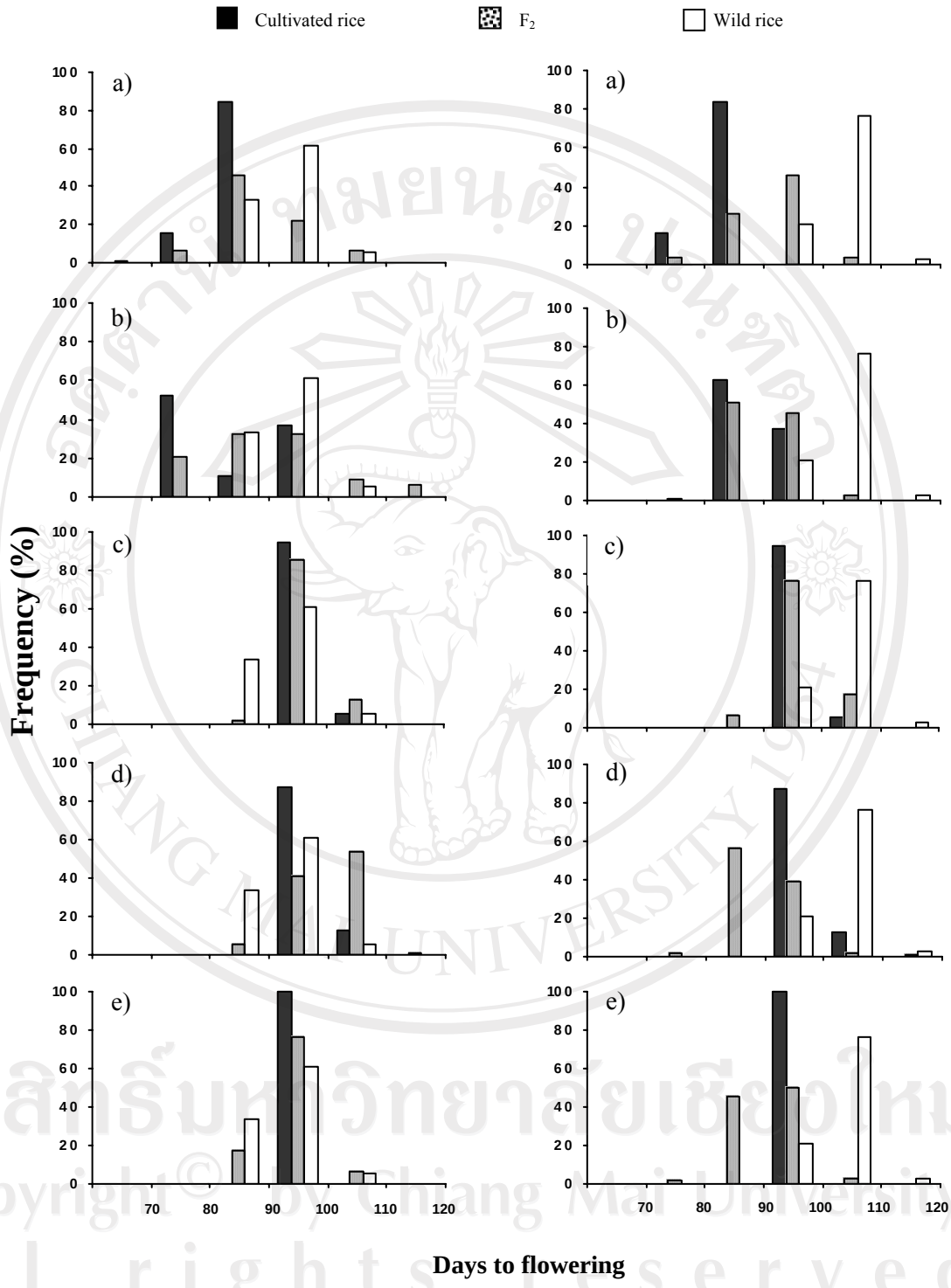

Figure 3.1 Distribution of days to flowering of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Table 3.10 Culm length (cm) of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Culm length (cm)	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	71 l	128 ghi	132 fgh
SPR1	81 k	128 ghi	123 i
KDML105	111 j	136 efg	144 cd
RD6	123 i	143 de	157 ab
RD10	77 kl	125 hi	†
SMJ	106 j	125 hi	†
KDK	134 fg	151 bc	159 a
Wild rice		137 def	139 def
LSD (0.05)	7.8		

† data not available

Table 3.11 Mean, range and standard deviation of culm length (cm) of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
Parent	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	68	60-76	5	178	92	45-130	11	161	103	45-161	24
SPR1	19	81	70-89	6	119	93	49-129	16	150	107	35-153	24
KDML105	18	109	94-122	6	173	92	69-127	11	132	125	95-155	12
RD6	16	114	101-130	8	170	109	60-135	12	130	134	105-170	14
KDK	19	134	118-148	9	183	116	55-150	13	146	146	105-170	14
Wild rice (P_2)					20	129	120-146	7	20	142	126-162	13

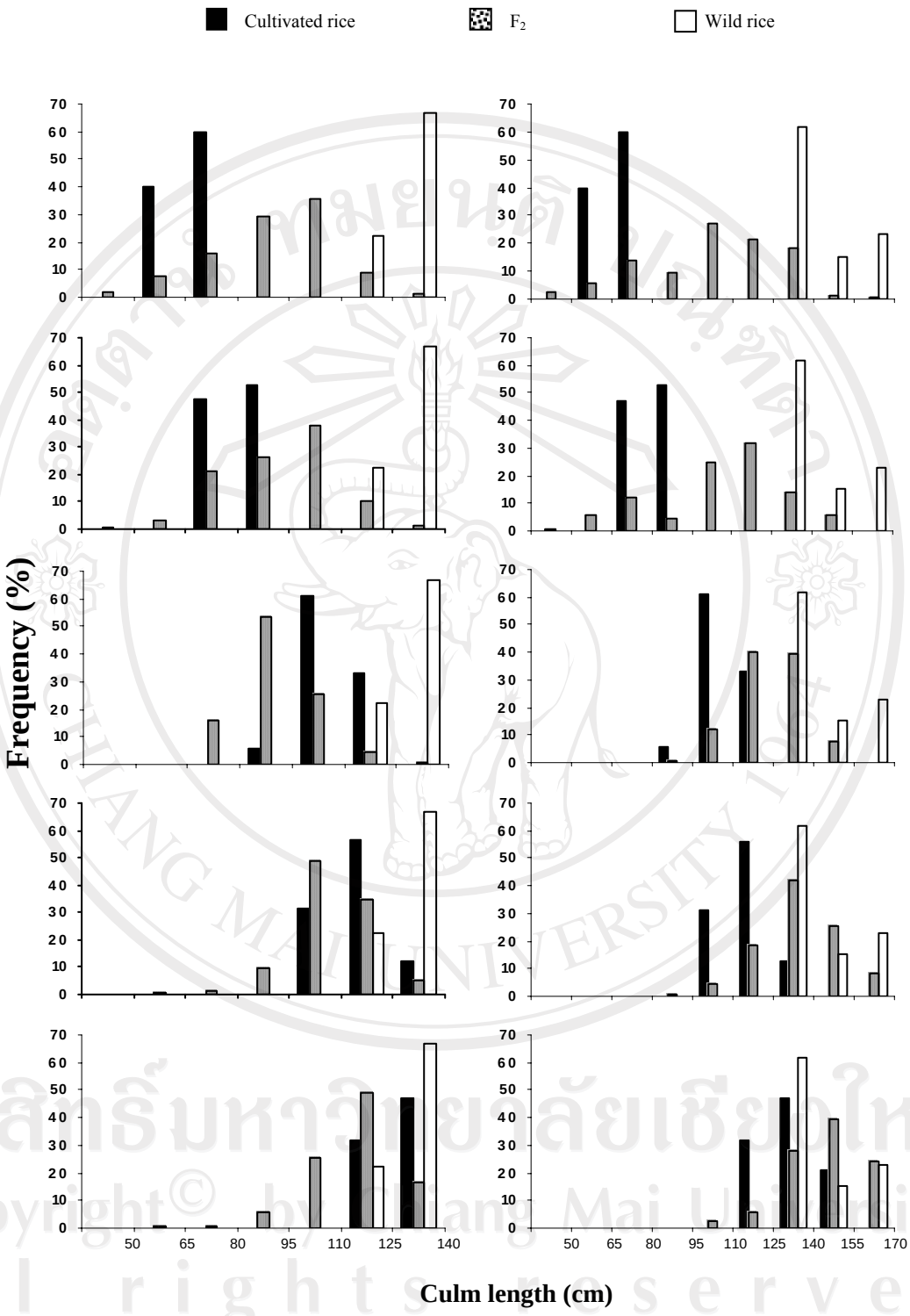

Figure 3.2 Distribution of culm length (cm) of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Panicle length (cm)

Means panicle length of F_1 hybrids between cultivated x KC wild rice were between 22 to 26 cm, which were in the same range of cultivated rice. In contrast, heterosis of F_1 hybrids for panicle length were observed in crosses between cultivated rice x NY wild rice. Panicle length of (CR x NY) F_1 were ranged from 27 to 29 cm. All (CR x NY) F_1 produced larger panicle than their parents (Table 3.12). Normal distribution was found in all F_2 populations for this character. For F_2 populations derived from cultivated rice x NY, transgressive segregations were found for all crosses (Table 3.13 and Figure 3.3).

Number of panicle plant $^{-1}$

Panicles plant^{-1} of cultivated rice were between 6-12 cm. Those of KC and NY wild rice were 7 and 13, respectively (Table 3.14). For cultivated rice x KC wild rice, F_1 from CNT1, SPR1 and SMJ were the same as wild rice parent, the rest were the same as cultivated rice parents. For cultivated x NY combinations, all F_1 except RD6 x NY were intermediate between parents, those from RD6 x NY cross was the same as NY wild rice. F_2 plants produced panicle ranged from less than cultivated rice to the same as of wild rice. Mean of number of panicle of F_2 plants from crosses between CR x NY was higher than F_2 plants from crosses CR x KC (Table 3.15). Trangressive segregation was observed in combination between SPR1 x NY. Fifty to 80% of F_2 plants derived from CR x NY produced panicle in range 7-10, while most of F_2 plants derived from CR x KC produced panicle in range 4-7 (Figure 3.4).

Table 3.12 Panicle length (cm) of F_1 hybrids between eight cultivated rice (CR) varieties and two wild rice (KC and NY) compared with their parents.

Parent	Panicle length (cm)	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	25.0 fgh	22.6 kl	29.4 a
SPR1	24.2 ghij	24.9 fghi	27.4 bcd
KDML105	22.8 jkl	23.9 hijk	28.3 ab
RD6	23.8 hijkl	24.4 ghi	27.5 bcd
RD10	22.3 l	25.1 fgh	†
SMJ	24.2 ghi	26.0 def	†
KDK	23.5 ijkl	25.6 efg	27.9 bc
Wild rice		26.6 cde	24.5 ghi
LSD (0.05)	1.4		

† data not available

Table 3.13 Mean, range and standard deviation of panicle length (cm) of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

Parent	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	23	20-26	2	151	24	17-32	3	168	22	17-34	3
SPR1	15	24	22-26	1	120	23	14-27	2	148	26	17-36	3
KDML105	18	24	20-26	1	174	22	16-28	3	131	27	18-39	3
RD6	16	23	22-26	1	133	24	18-30	2	119	25	15-31	2
KDK	19	23	21-25	1	183	23	12-29	3	137	26	15-32	3
Wild rice (P_2)					23	24	21-30	3	30	24	21-30	2

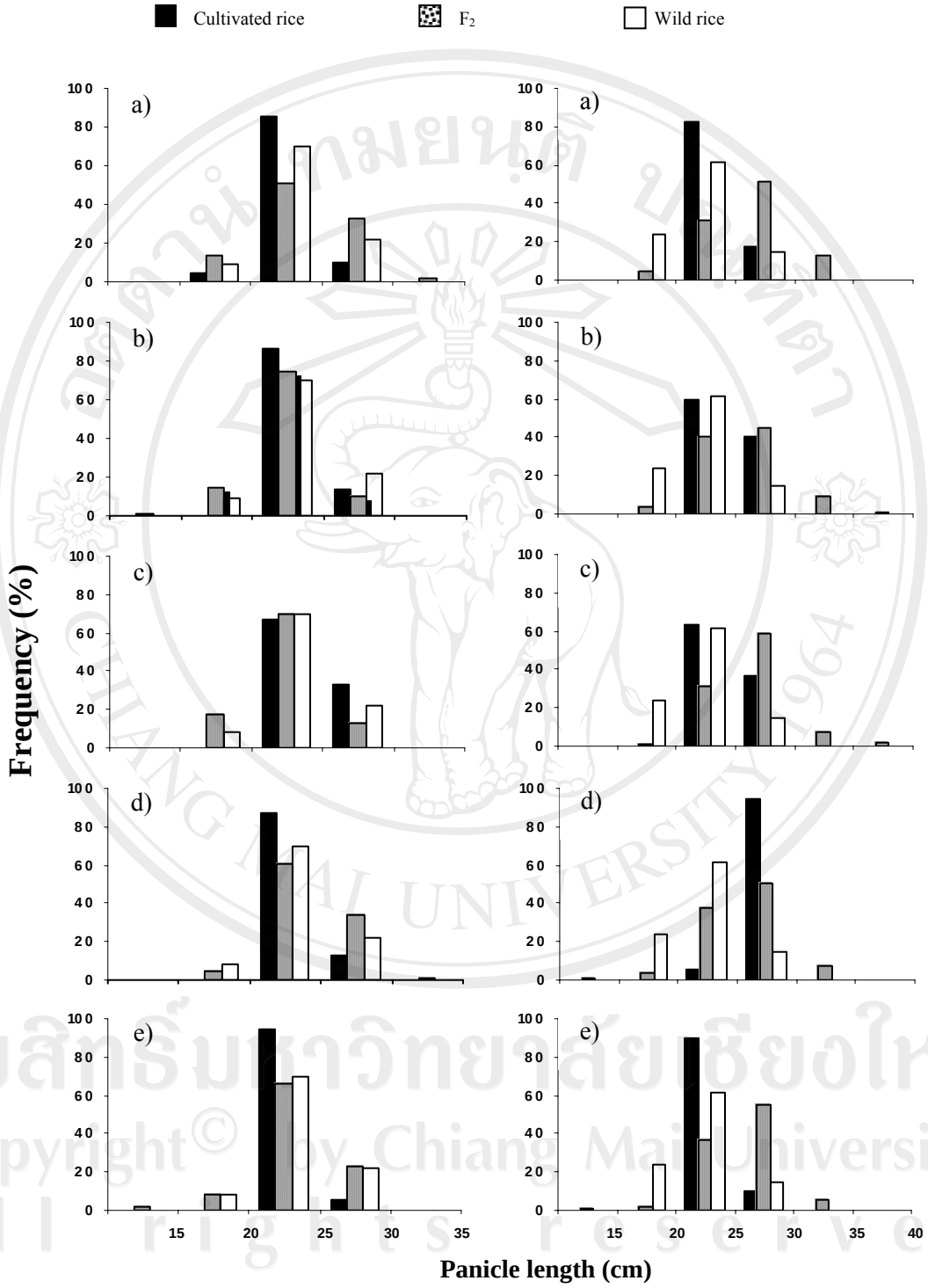

Figure 3.3 Distribution of panicle length (cm) of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Table 3.14 Number of panicles plant^{-1} of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Number of panicle^{-1}	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	10.2 D	7.0 I	11.7 C
SPR1	9.7 DE	7.0 I	11.5 C
KDML105	9.2 EF	9.0 EFG	12.7 B
RD6	9.2 EF	8.7 FGH	13.0 AB
RD10	9.0 EFG	8.5 FGH	†
SMJ	6.0 J	6.7 IJ	†
KDK	8.0 H	8.2 GH	11.7 C
Wild rice		7.0 I	13.7 A
LSD (0.05)	0.84		

† data not available

Table 3.15 Mean, range and standard deviation of number of panicle^{-1} of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

Parent	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	9	7-11	1	151	6	3-9	1	168	9	2-16	2
SPR1	22	8	6-10	1	103	6	2-13	2	148	9	4-21	2
KDML105	20	9	8-12	1	173	6	4-10	1	104	8	3-13	2
RD6	20	9	8-12	1	133	6	4-8	1	148	9	5-13	2
KDK	20	10	8-12	1	184	6	3-10	1	137	8	3-13	2
Wild rice (P_2)					40	7	4-13	2	20	10	6-17	4

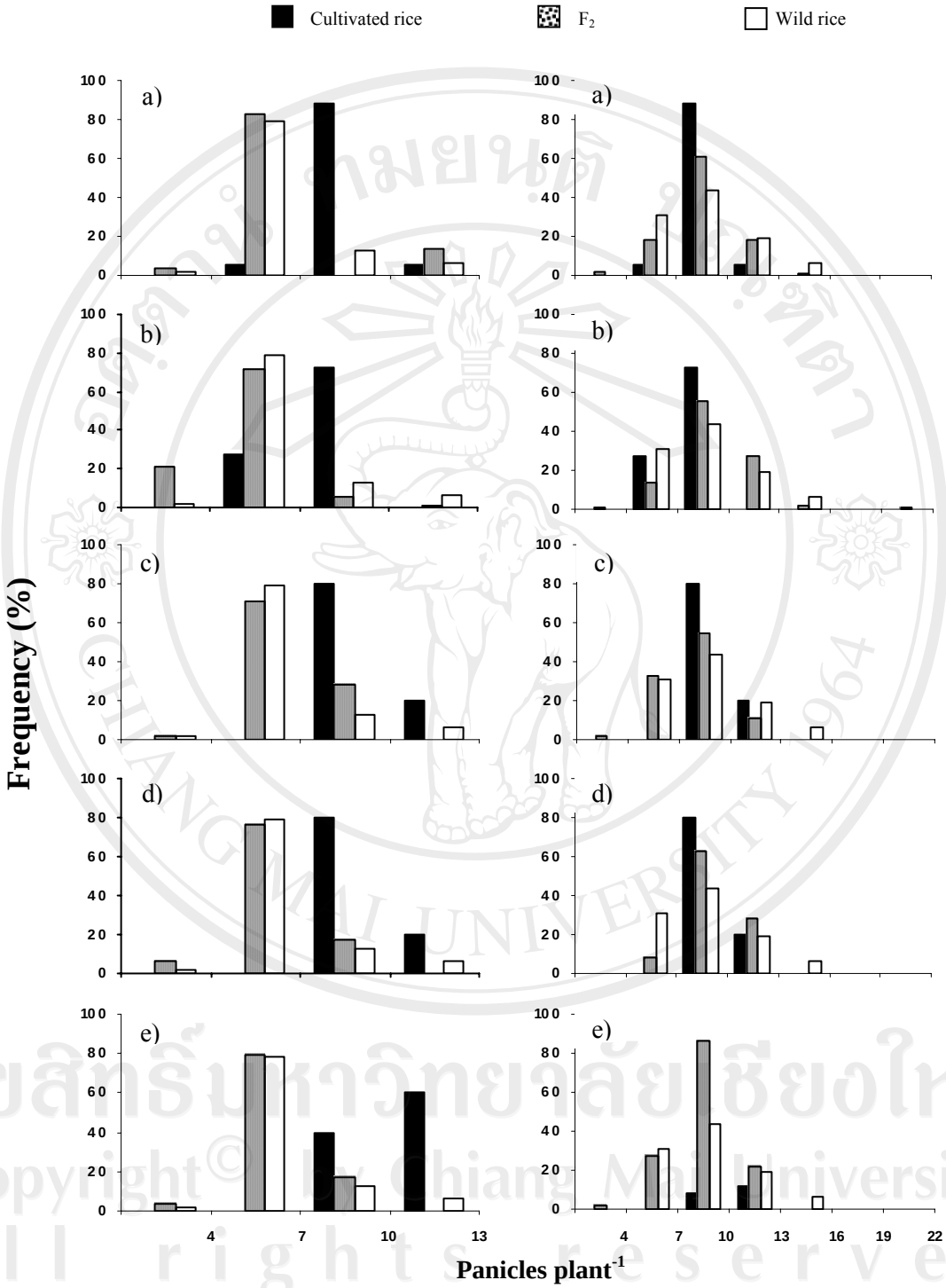

Figure 3.4 Distribution of number of panicles plant^{-1} of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Number of spikelets panicle^{-1}

Number of spikelets panicle^{-1} of cultivated rice were between 108-143. Those of KC and NY wild rice were 125 and 159, respectively (Table 3.16). For cultivated rice x KC wild rice combinations, spikelets panicle^{-1} of F_1 derived from CNT1, RD6 and SMJ were the same as cultivated rice parents. That of F_1 from SPR1 x KC was less than both parents, while that of F_1 derived from KDK were more than parents. For cultivated x NY crosses, spikelets panicle^{-1} of F_1 from all crosses were the same as wild rice, between 116-178 spikelets panicle^{-1}. For F_2 populations derived from cultivated x KC, transgressive segregation was observed with most of the transgressive progenies had less number of spikelets panicle^{-1} than parents (Table 3.17 and Figure 3.5). For F_2 populations derived from cultivated rice x NY, F_2 plants transgressive segregation in both directions were found with the largest range in RD6 x NY and KDK x NY (Table 3.17 and Figure 3.5).

Number of seeds panicle^{-1}

Number of seeds panicle^{-1} of cultivated rice was between 86-124. Those of KC and NY wild rice were 107 and 102 spikelet panicle^{-1} (Table 3.18). Seeds panicle^{-1} of F_1s from all crosses except RD6 x NY were the same as or intermediate between parents. Heterosis was found in RD6 x NY hybrid. For F_2 generations generated from cultivated x KC or NY, more than 50% of F_2 plants which derived from all crosses were as the same and lower than wild rice (Table 3.19). For F_2 populations, transgressive segregations were observed with most of the transgressive progenies had less number of sees panicle^{-1} than parents (Table 3.18 and Figure 3.6).

For F_2s between cultivated x NY, F_2 plants with more seeds panicle^{-1} than both parents were observed in CNT1, SPR1, KDML105 and RD6 (Figure 3.6).

Table 3.16 Number of spikelets panicle^{-1} of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Number of spikelet panicle^{-1}	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	143 cde	142 cde	174 a
SPR1	142 cde	117 fgh	166 ab
KDML105	131 def	120 fgh	178 a
RD6	131 def	149 bcd	178 a
RD10	109 gh	145 cde	†
SMJ	108 gh	104 h	†
KDK	127 efg	170 ab	178 a
Wild rice		125 efg	159 abc
LSD (0.05)	21		

† data not available

Table 3.17 Mean, range and standard deviation of number of spikelets panicle^{-1} of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

Parent	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	139	118-168	14	151	95	42-157	24	166	115	34-251	41
SPR1	20	136	102-163	15	120	88	39-137	22	148	135	46-260	44
KDML105	20	134	100-167	19	174	86	35-163	24	123	137	64-237	35
RD6	20	134	104-166	19	133	111	45-216	30	119	143	57-262	44
KDK	20	138	97-157	18	183	103	30-199	29	137	112	31-202	31
Wild rice (P_2)					45	105	62-192	32	20	123	59-187	49

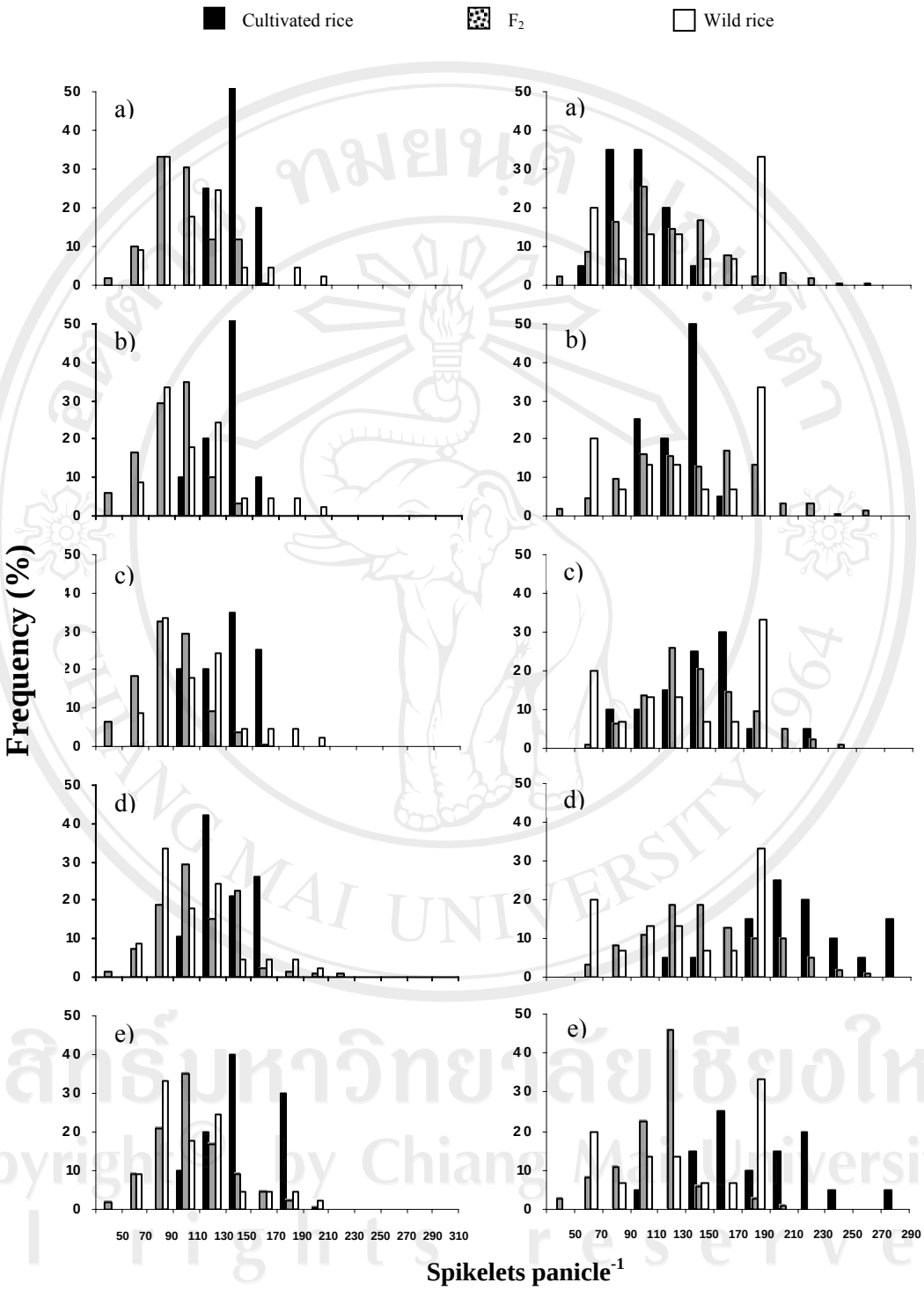

Figure 3.5 Distribution of number of spikelets panicle^{-1} of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Table 3.18 Number of seeds panicle^{-1} of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Number of seed panicle^{-1}	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	131 bc	120 bcde	128 bcd
SPR1	129 bcd	94 fgh	117 bcdef
KDML105	114 cdef	103 efgh	129 bcd
RD6	121 bcde	111 cdefg	160 a
RD10	102 efgh	124 bcde	†
SMJ	89 gh	86 h	†
KDK	114 bcdef	108 cdefgh	138 ab
Wild rice		107 defgh	102 efgh
LSD (0.05)	24		

† data not available

Table 3.19 Mean, range and standard deviation of number of seed panicle^{-1} of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

Parent	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	130	100-153	17	151	67	15-130	24	168	69	0-230	39
SPR1	20	128	105-153	12	101	48	5-127	24	148	71	0-204	38
KDML105	18	125	100-150	16	173	61	10-116	24	123	58	2-184	36
RD6	20	124	98-150	15	133	74	10-177	30	119	90	2-107	42
KDK	20	122	103-150	15	184	61	2-147	20	137	67	4-145	28
Wild rice (P_2)					38	66	37-121	20	20	119	53-169	36

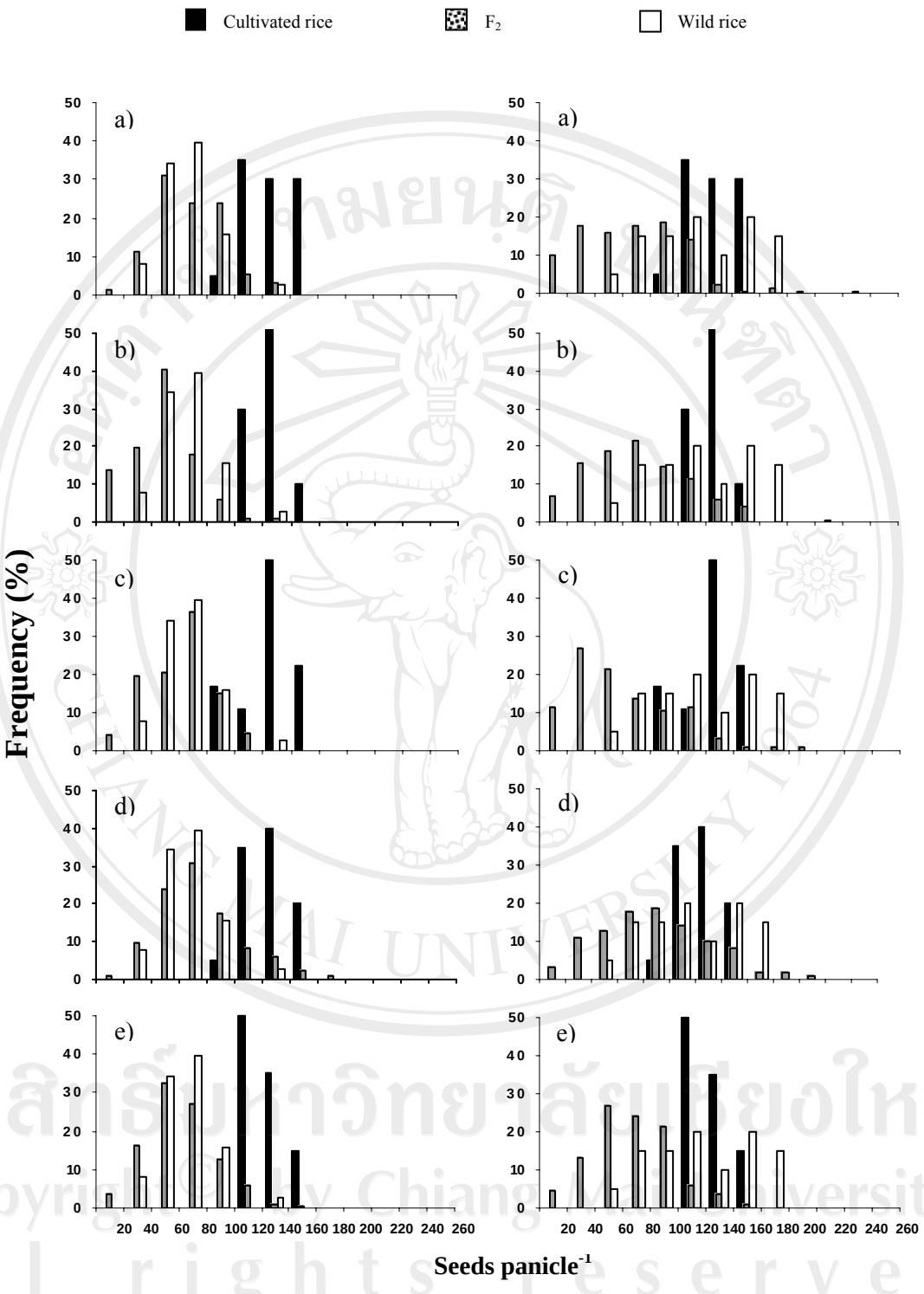

Figure 3.6 Distribution of number of seeds panicle^{-1} of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Seed fertility (%)

Seed fertility of cultivated rice was between 77 to 96%. Those of KC and NY wild rice were lower than cultivated rice, 73 and 67 %, respectively (Table 3.20). For F_1 hybrid between cultivated x KC combinations, F_1 from RD10 was higher than cultivated rice whereas KDML105 was the same as cultivated rice. The rests were intermediate between parents. For cultivated rice x NY combinations, most F_1 crosses were intermediate between parents. For F_2 generations, F_2 plants produced seed ranged from zero to 100. Overall mean of seed fertility of F_2 were ranging from 55 to 73 for F_2 derived from CR x KC and 41 to 60 for F_2 derived from CR x NY (Table 3.21). Transgressive segregations were observed in all F_2 populations (Figure 3.7).

Seed shattering (%)

KC and NY wild rice shattered all seeds (100%). Percent seed shattering of cultivated rice were between 1.4 to 4.6 (Table 3.22). Seed of all F_1 hybrids between cultivated x KC or NY shattered when in the same rate as or close to wild rice parents (Table 3.21). The segregation patterns of seed shattering were difference between cross. Eighty-five to 97% of F_2 plants derived from CR x NY had seed shattering in range 91-100. Three to 47% of F_2 plants derived from CR x KC showed seed shattering fell into 91-100 (Figure 3.8).

Table 3.20 Seed fertility (%) of F_1 hybrids between eight cultivated rice (CR) and two wild rice (KC and NY) compared with their parents.

		F_1	
Parent	Seed fertility (%)	CR x KC	CR x NY
Cultivated rice			
CNT1	96 a	85 def	87 cde
SPR1	94 ab	81 fg	76 dh
KDML105	88 cd	86 def	63 i
RD6	87 cde	75 h	76 gh
RD10	77 gh	86 def	†
SMJ	89 bcd	83 ef	†
KDK	92 abc	63 i	76 gh
Wild rice		73 h	67 i
LSD (0.05)	5		

† data not available

Table 3.21 Mean, range and standard deviation of seed fertility (%) of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
Parent	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	93	86-98	3	151	70	20-97	15	166	56	0-100	23
SPR1	20	92	83-98	3	120	55	0-91	18	148	51	0-92	21
KDML105	20	91	74-98	7	147	73	16-100	17	123	41	2-86	22
RD6	20	86	73-97	6	133	56	19-92	16	119	60	2-92	19
KDK	20	92	75-97	6	182	58	6-94	17	136	58	12-93	18
Wild rice (P_2)					20	55	32-94	17	20	71	43-89	12

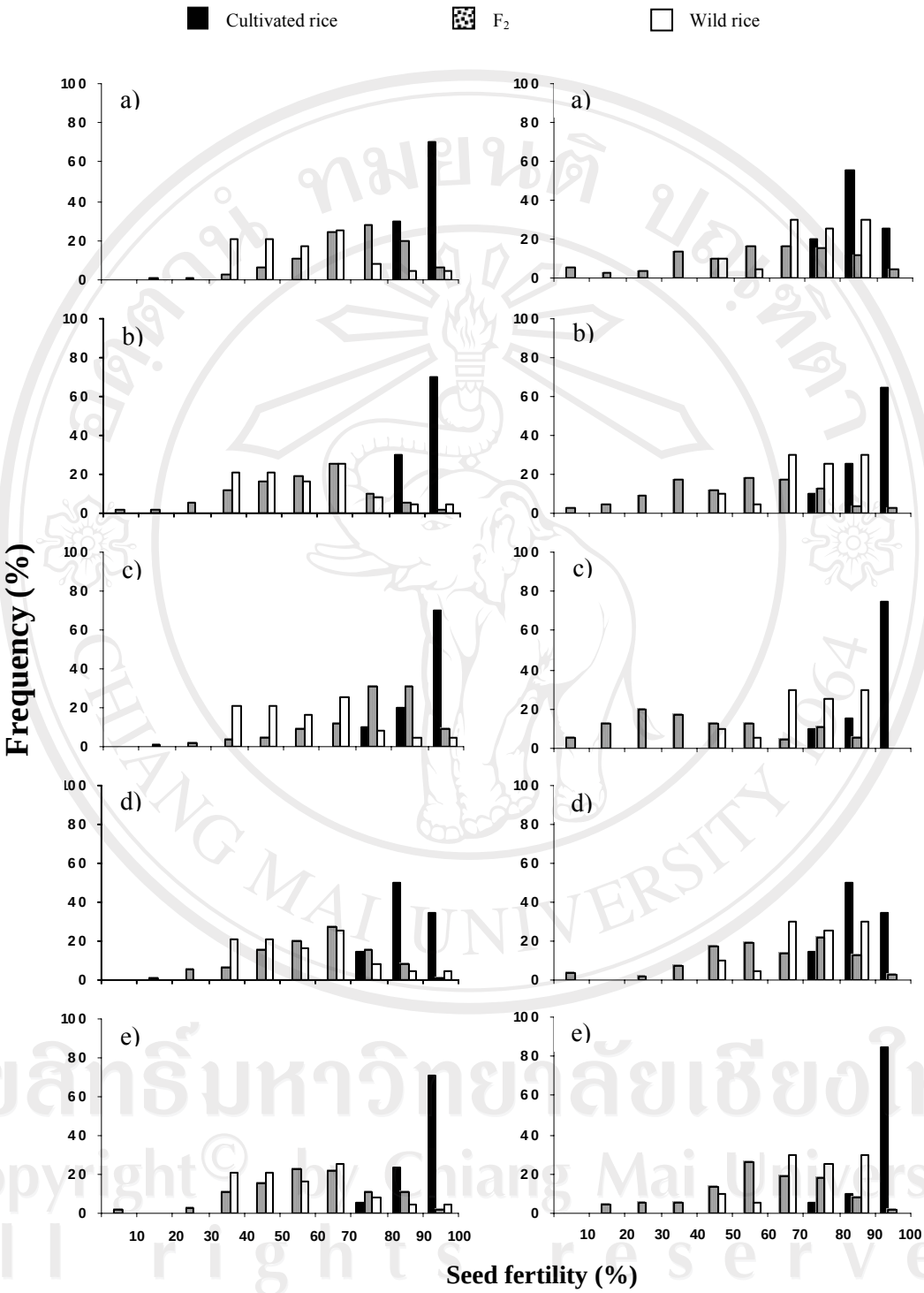

Figure 3.7 Distribution of seed fertility (%) of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Table 3.22 Seed shattering (%) of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Seed shattering (%)	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	1.4 g	99 a	97 a
SPR1	1.3 g	90 de	96 ab
KDML105	3.2 g	93 bcd	86 ef
RD6	2.8 g	86 f	95 abc
RD10	4.6 g	89 def	†
SMJ	3.1 g	98 a	†
KDK	2.2 g	91 cd	96 ab
Wild rice		100 a	100 a
LSD (0.05)	4		

† data not available

Table 3.23 Mean, range and standard deviation of seed shattering (%) of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

Parent	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	2	0-9	2	151	80	24-100	15	165	95	11-100	6
SPR1	20	2	0-9	3	120	67	20-94	14	146	95	11-100	14
KDML105	20	5	1-10	3	174	84	11-125	17	123	99	58-100	5
RD6	20	6	0-10	3	73	78	14-100	22	119	96	17-100	15
KDK	20	2	0-5	1	182	77	0-100	17	137	95	18-100	15
Wild rice (P_2)					20	98	90-100	3	20	99	92-100	2

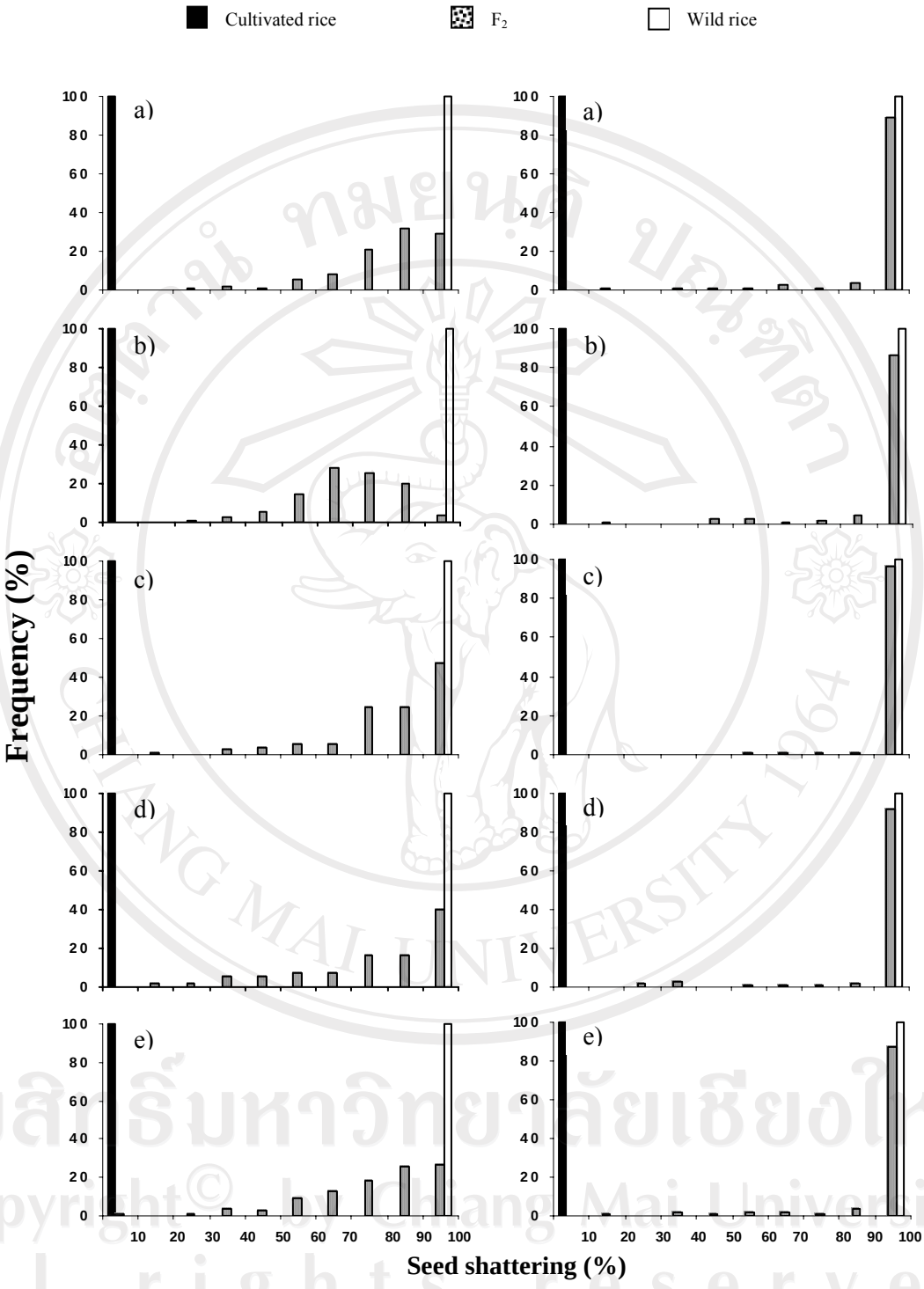

Figure 3.8 Distribution of seed shattering (%) of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

Seed width (mm)

Seed width of cultivated rice parents were between 2.40 to 3.35 mm. Those of KC and NY wild rice were 2.45 and 2.48 mm, respectively (Table 3.24). For CR x KC, F_1s from CNT1, SPR1, RD6 and RD10 were the same as cultivated rice, whereas F_1s from SMJ and KDK were lower than cultivated rice. For CR x NY, F_1s from CNT1, SPR1 and RD6 were the same as cultivated rice. Seed of KDML105 x KC or NY were wider than KDML105 which used as maternal parent. Transgressive segregation of seed width of F_2 seeds were observed in all F_2 populations. Average mean of seed length of F_2 populations were ranging form 2.38 to 2.88 for F_2 populations between CR x KC and 2.34 to 2.91 for F_2 populations between CR x NY (Table 3.25).

Seed length (mm)

Seed length of cultivated rice was between 9.31 to 10.85 mm. Those of KC and NY wild rice were 8,30and 8.12, respectively (Table 3.26). For F_1 hybrids between cultivated x KC or NY, all crosses were shorter than cultivated rice. Seed length of F_1 hybrids between CNT1 x KC was the same as wild rice (8.39 mm). Average mean of seed length of F_2 plants derived from cultivated rice x KC and cultivated rice x NY was ranged from 8.62 to 9.59 and 8.77 to 9.39 mm, respectively. Transgressive segregation was observed in seed length of F_2 populations (Table 3.27).

Seed shape

Cultivated rice and both wild rice (KC and NY) produced slender seed except KDK and SMJ. Those of KDK and SMJ cultivated rice produced large seed (Figure 3.9). For cultivated rice x KC and NY, all F_1 crosses produced slender seed which were in the same class as parents (Figure 3.9). For F_2s derived from cultivated rice and wild rice, all F_2 plants derived from CNT1 and SPR1 x KC or NY and KDML105 x NY produced only slender seed whereas one plant derived from KDML105 x KC and RD6 x NY was large seed type. Seeds of F_2 plants derived from KDK x KC and NY segregated between slender and large seed, only one plant derived from KDK x NY produced round seed (Figure 3.11).

Table 3.24 Seed width (mm) of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Seed width (mm)	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	2.48 ijk	2.49 hij	2.48 ijk
SPR1	2.56 fgh	2.53 ghi	2.56 fgh
KDML105	2.40 k	2.58 efg	2.53 ghi
RD6	2.65 de	2.64 de	2.63 def
RD10	2.69 cd	2.64 de	†
SMJ	2.73 c	2.61 ef	†
KDK	3.35 a	2.91 b	2.88 b
Wild rice		2.45 jk	2.48 ijk
LSD (0.05)	0.07		

Table 3.25 Mean, range and standard deviation of seed length (mm) of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
Parent	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	2.50	2.37-2.63	0.08	89	2.38	1.98-2.69	0.19	89	2.34	1.93-2.72	0.16
SPR1	20	2.44	2.14-2.92	0.17	132	2.48	2.06-2.77	0.14	125	2.52	2.06-3.04	0.18
KDML105	20	2.42	2.26-2.56	0.09	71	2.51	2.18-3.11	0.16	70	2.47	2.18-2.90	0.16
RD6	20	2.62	2.36-2.85	0.13	120	2.71	2.22-3.10	0.14	98	2.63	2.27-3.12	0.17
KDK	20	3.38	3.15-3.53	0.11	108	2.88	2.40-3.42	0.21	92	2.91	2.52-3.21	0.16
Wild rice (P_2)					20	2.52	2.34-2.75	0.11	20	2.51	2.39-2.70	0.08

Table 3.26 Seed length (mm) of F_1 hybrids between cultivated rice (CR) and wild rice (KC and NY) compared with their parents.

Parent	Seed length (mm)	F_1	
		CR x KC	CR x NY
Cultivated rice			
CNT1	10.37 c	8.39 j	9.35 efg
SPR1	9.42 def	8.63 i	8.69 i
KDML105	10.54 bc	9.15 gh	9.25 fg
RD6	9.63 d	9.28 fg	8.97 h
RD10	10.68 ab	9.52 de	†
SMJ	10.85 a	9.46 def	†
KDK	9.31 efg	8.99 h	9.45 def
Wild rice		8.30 jk	8.12 k
LSD (0.05)	0.21		

Table 3.27 Mean, range and standard deviation of seed length (mm) of F_2 populations between cultivated rice (CR) x wild rice (KC and NY).

	P_1				F_2 (CR x KC)				F_2 (CR x NY)			
Parent	n	mean	range	sd	n	mean	range	sd	n	mean	range	sd
Cultivated rice												
CNT1	20	10.36	9.81-10.95	0.34	89	9.29	7.67-10.92	0.62	89	9.38	7.89-11.02	0.66
SPR1	20	9.56	8.23-10.44	0.54	132	8.62	7.82-9.33	0.28	125	8.77	7.82-10.41	0.48
KDML105	20	10.29	9.53-10.18	0.37	71	9.11	8.02-10.80	0.65	70	9.11	7.65-10.31	0.59
RD6	20	9.57	9.04-10.25	0.35	120	9.59	8.40-10.92	0.43	98	9.25	7.79-10.56	0.61
KDK	20	9.42	8.98-9.99	0.30	108	9.38	8.01-10.81	0.49	92	9.39	7.24-10.50	0.54
Wild rice (P_2)					20	9.03	8.40-9.62	0.33	20	8.53	7.68-9.18	0.46

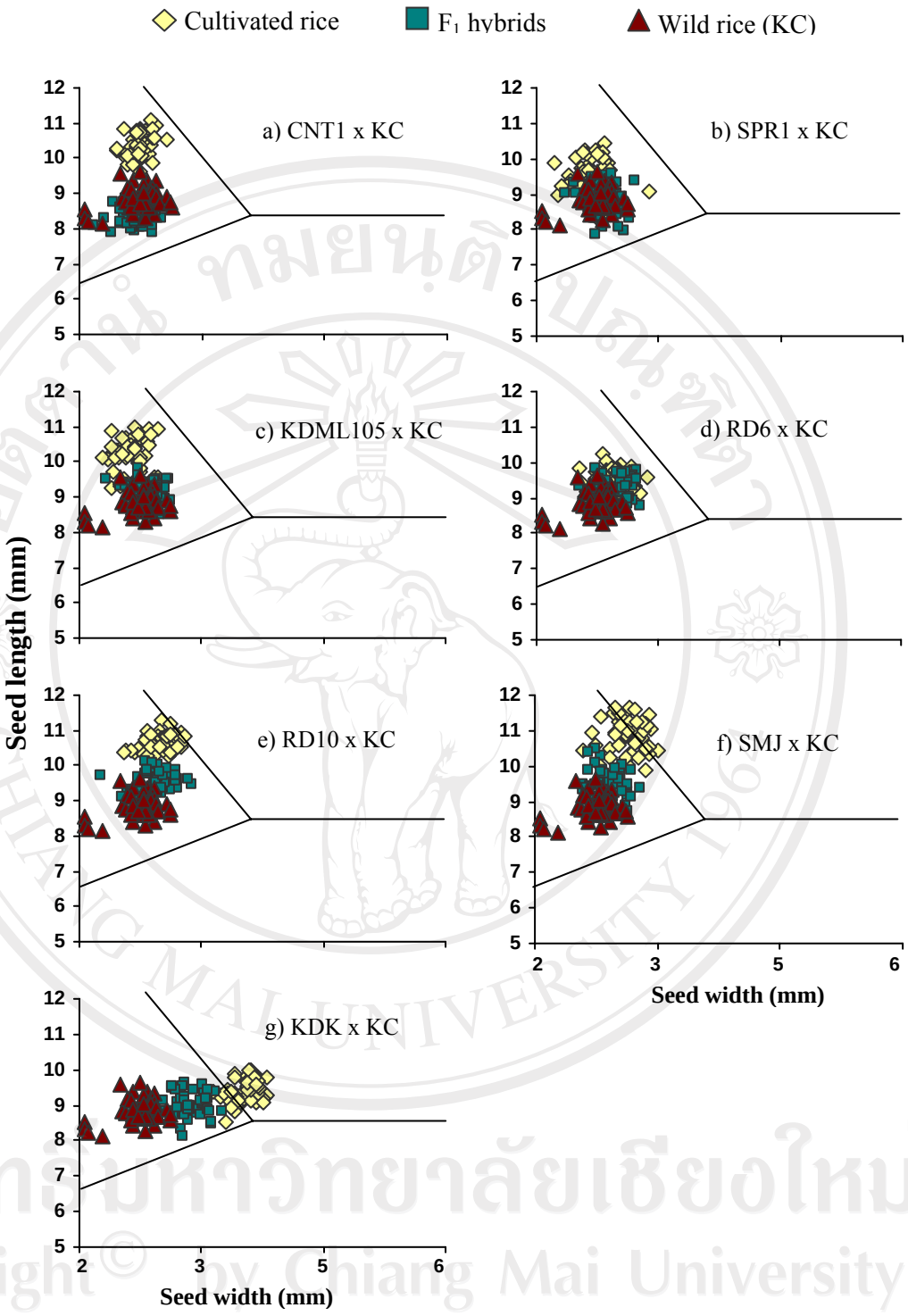

Figure 3.9 Seed shape of F_1 hybrids between cultivated rice x KC wild rice compared with their parents.

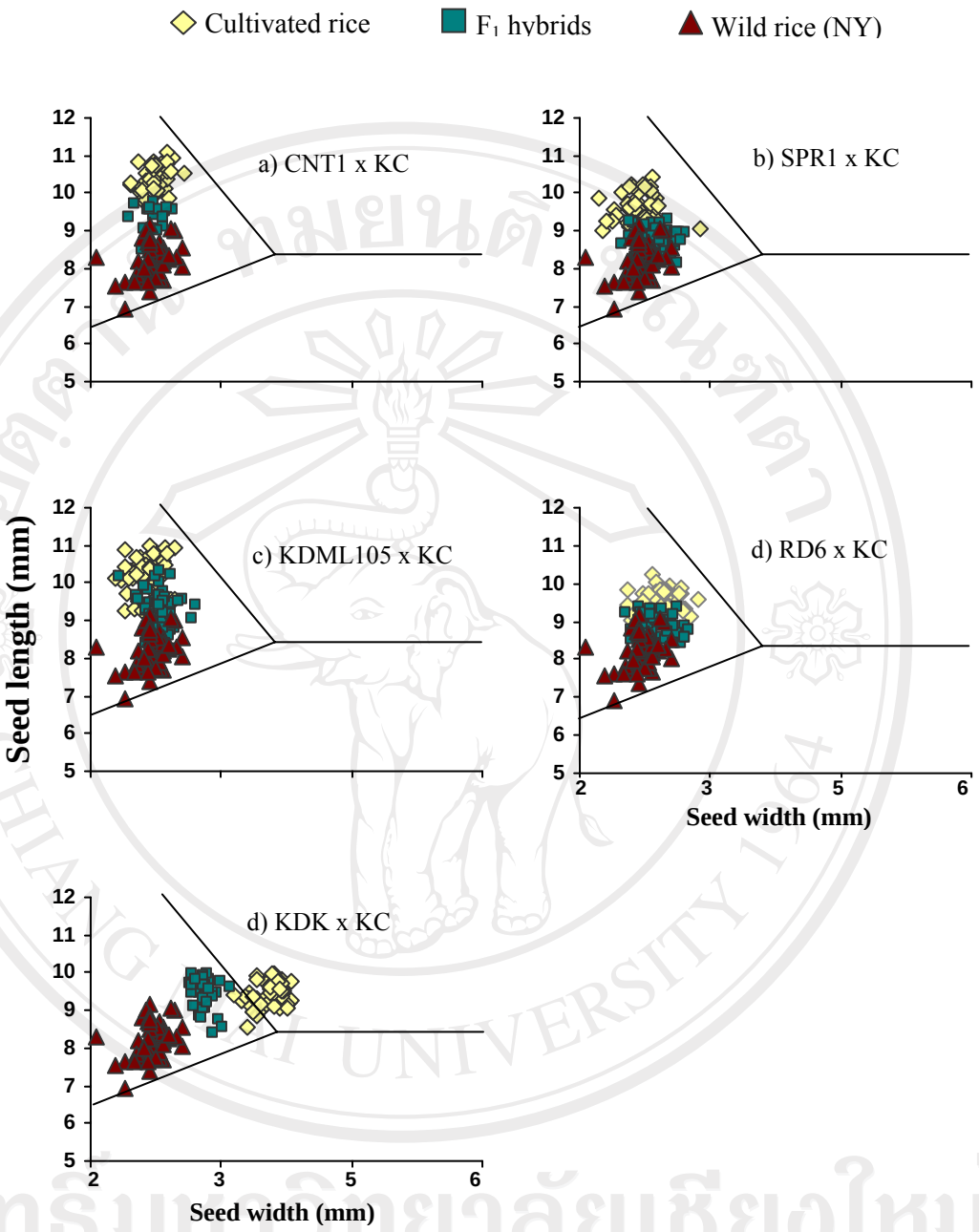

Figure 3.10 Seed shape of F_1 hybrids between cultivated rice x NY wild rice compared with their parents.

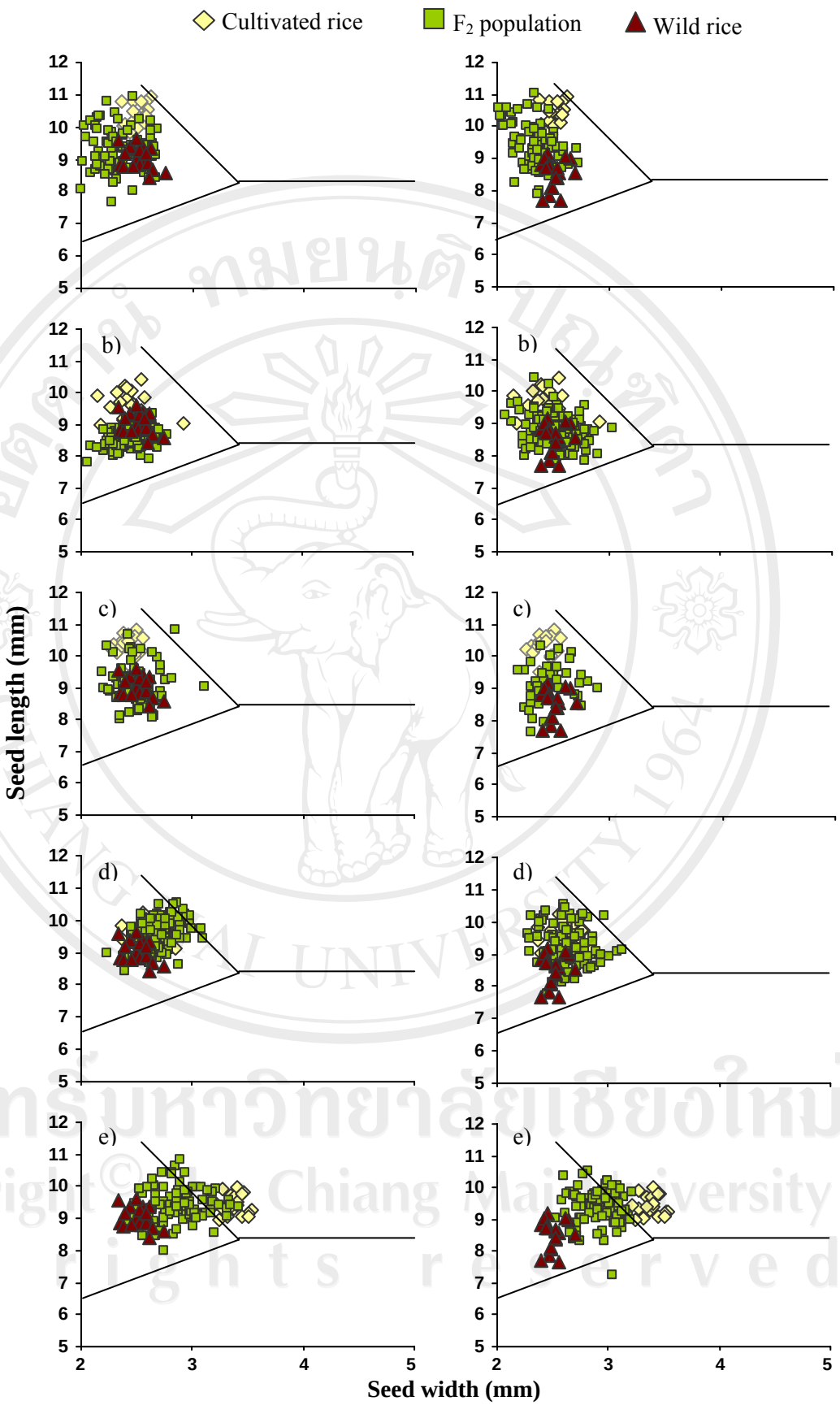

Figure 3.11 Seed shape of F_2 populations between cultivated rice x KC wild rice (left) and cultivated rice x NY wild rice (right). Five cultivated rice (CNT1, SPR1, KDML105, RD6 and KDK) were illustrated in plate a, b, c, d and e, respectively.

3.4 Discussion

Cultivated rice varieties used in this study are classified into high yielding variety (HYVs) and improved/ purified variety. The HYVs, CNT1 and SPR1, usually grown in irrigated, direct-seedinged in central plain with 2-3 crops/year. The other six improved/ purified varieties were grown in both north and northeast (KDML05, RD6 and RD 10) and north (NSPT, SMJ and KDK). Three common wild rice were collected from north (LP) and central (KC and NY) which used as male parents. The results indicate that interspecifeic hybridization between cultivated rice (*O. sativa*) and common wild rice (*O. rufipogon*) can be achieved including reciprocal cross and back cross. It is confirm that high sexually compatibility between the species sharing AA genome (Naredo *et al*., 1997 and 1998). Seed set between cultivated rice x wild rice varied with cross combination with the higest seed set was found when wild rice cross with HYVs varieties, SPR1 or CNT1.

For evaluation of F_1 hybrids and F_2 generations, we found that wild rice traits such as spikelet awning, black hull, red pericarp and seed shattering habit were controlled with few major genes and expressed as dominance gene action over cultivated traits. F_1 hybrids are interfertile, produced normal seedlings for F_2 generation (Sintukhiew, 2004) and expressed the dominant wild characters. When compared with cultivated rice parents, F_1s were taller, had more spikelets panicle^{-1} than parents. All hybrids had dark brown hull, red seeds and shattered all seeds, the key wild traits. In F_2 generation, morphological and physiological characters of most F_2 plants were segregated into wild type and recombined into intermediate between cultivated and wild rice. Plants with cultivated type were quite rare because of the low proportion of recessive phenotypes. Moreover, transgressive segregations were

found in number of panicles plant^{-1}, panicle length, number of spikelets and seeds panicle^{-1} and seed size. Therefore, the interspecific hybridization produced a large source of genetic variation and adaptation in segregating populations. This will be useful in plant breeding program. Similar findings were reported. For example, McCouch *et al.* (2007) studied the source of trait-enhancing alleles in *O .rufipogon* for *O. sativa* by using BC_2F_2 populations derived from *O. sativa*, indica and japonica ssp. with common wild rice (*O. rufipogon*). They found transgressive variation for yield and yield components.

However, when hybridization occurred naturally, negative effects will lead to the build up of weedy rice in the field. From the results demonstrated the area which cultivated rice grown in close proximity and have the chance of flowering synchronization between both species especially, in intensive rice production area, natural hybridization will be occurred and weedy rice will be emerged. It is obvious that the highest seed set was found between the high yielding varieties (HYVs), SPR1 or CNT1 x wild rice and the F_1 hybrids displayed normal fertility. HYVs are photoperiod insensitive, they are generally grown in a system of continuous rice in which 2-3 crops are grown on the same land in one year. This will increase the chance of flowering synchronization and outcrossing between HYVs and wild rice. Hybrids between cultivated rice and wild rice still maintained importance wild rice habits, seed awning and seed shattering. These characters were promoted the dispersal of weedy rice seed and seed bank when weedy rice seed dropped on the soil. Moreover, reciprocal and backcross or hybridization between hybrids and their parents can occur. In Thailand weedy rice described by Maneechote *et al*. (2004). Three types of weedy rice were observed, including, Khao Harng (seeds with long

awns and shattering), Khao Deed or jumping rice (seeds without awns and shattering) and Khao Dang (red pericarp and not shattering). We supposed that if hybrids between cultivated and wild rice has emerged and grown in rice growing system, back cross is the importance process for weedy rice mimicry in cultivated rice. Therefore, areas in which cultivated and wild rice grow in close proximity, gene flow between introduced rice genotypes with new traits and wild rice should be closely monitored to prevent the build up of invasive weedy rice.

From this study, we concluded that cultivated rice can easily cross with wild rice. F_1 hybrids are interfertile and produced normal seedling for F_2 generations. For the next chapter, natural hybridization between the two species will be studied for evaluate the consequence of gene flow to rice gene pool.