CHAPTER 3

Genetic Structure and Dynamics of Sympatric Wild-Weed-Crop Rice Populations in Thailand

3.1 Introduction

Some domesticated plants occur as part of a crop-weed-wild complex. A wild-weed-domesticated complex in sympatric habitat results from genetic compatibility between wild and cultivated populations by introgression of genes from wild populations to domesticated plants (Zizumbo-Villarreal *et al*., 2005). Both weeds and crops often begin with a common wild progenitor as in those complexes where each crop has a companion weed. The evolution of weeds often parallels the evolution of crops and the same principles apply to both (Harlan, 1992).

Natural introgression among the wild, weedy and domesticated of *Oryza sativa* is an ongoing process in sympatric habitats could result in gene flow among the several biological components of them. As weedy types may be the result of introgression between wild types and the types of cultivated varieties in sympatric habitat. Hybrids, therefore, especially aggressive weedy types may in turn both derive and contribute genetic diversity in relation to wild relatives and companion cultivated types (Ellstrand *et al*., 1999). Over time, the hybrids mimic the companion cultivated rice in morphological characters making it resemble closely the companion cultivars varieties. Most of the weedy rice types are essentially adapt and mimic to companion cultivated rice that are highly successful as weeds in terms survival and distribution. They show high adaptation to the agronomic practices and ecological conditions favored for the cultivars that it infests because a life cycle that is closely synchronized with the cultivars (Cao *et al*., 2006). An interesting adaptation syndrome of weedy rice is an important one in which the weedy rice mimics to the companion cultivated rice sufficiently well that the seeds are harvested along with the cultivar rice and sown with it at next planting season. The weedy rice is mimics to the companion cultivated rice in morphological characters which it so similar to the cultivars varieties from seedling until the reproductive stage (Vaughan *et al*., 2005). They are difficult to recognize during the periodic weeding of the cultivars. These adaptations and crop mimicries of weedy rice could arise from the cultivar genes added to the gene pool of the weedy rice populations by introgressive hybridization in sympatric habitats (Cao *et al*., 2006). In addition, this additional genetic material have a substantial impact on the evolution of weedy rice populations in cultivated habitats by allowing the weeds to become more adapted to man-made habitats and increased their weediness (Ellstrand *et al*., 1999).

Recently, common wild rice habitats were disturbed by farmers for expansion of rice cultivation areas in Thailand. The wild-weed-crop rice populations were frequently found in sympatric habitat. Therefore, the following two questions of population genetic structure and dynamics of the sympatric wild-weed-crop rice populations in Thailand in rice field scale were asked:

1. How are the patterns of population genetic structure and dynamics in spatial and temporal terms of weedy rice when coexisted with

their companion crop rice and native common wild rice in sympatric rice fields in Thailand?

2. How demonstrate the affect of gene flow and other ecological conditions influence the population genetic structure and dynamics of weedy rice when coexisted with their companion crop rice and native common wild rice in sympatric rice fields in Thailand at the rice field scale?

In present study, weedy rice, companion crop rice and native common wild rice with occur in sympatric rice fields from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005, 2008 and 2009 were used to evaluated population genetic structure dynamics and the evolutionary factors by partitioned into the following components: (a) for spatial term analysis; within and between regions in each season of within and among rice groups including, weedy rice, companion crop rice and native common wild rice and (b) for temporal analysis; within and between regions among seasons of within and among rice groups.

3.2 Materials and Methods

Surveying and collection of weedy rice, companion crop rice and native common wild rice samples from three regions of Thailand

Weedy rice, companion crop rice and native common wild rice populations with occur in sympatric rice field were surveyed and studied from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet of 2005, 2008 and 2009.

Population sampling

In each field, Leaves of individual plant of crop, weedy and wild rice were collected and preserved as described in Chapter 2. Seed of each crop and weedy rice population were collected and bulked for the pot experiment. Plant samples with node and root of wild rice, 10 plants per population were also collected.

Table 3.1.1 Location and description of weedy rice, companion crop rice and native common wild rice populations with occur in sympatric rice field

Type	Sample Accession No.	Year collection	Region	Province	UTM
Companion crop rice	CrLN501	2005	Lower North	Phitsanulok	N17.07133 E100.81639
	CrLN502	2005		Phichit	N16.36638 E100.33548
Weedy rice	WeLN501	2005		Phitsanulok	N17.07133 E100.81639
	WeLN502	2005		Phichit	N16.36638 E100.33548
Native common wild rice	WiLN501	2005		Phitsanulok	N17.07133 E100.81639
	WiLN502	2005		Phichit	N16.36638 E100.33548
Companion crop rice	CrNE501	2005	Northeast	Si Saket	N14.95359 E104.20601
	CrNE502	2005		Buri Rum	N14.62856 E103.24280
Weedy rice	WeNE501	2005		Si Saket	N14.95359 E104.20601
	WeNE502	2005		Buri Rum	N14.62856 E103.24280
Native common wild rice	WiNE501	2005		Si Saket	N14.95359 E104.20601
	WiNE502	2005		Buri Rum	N14.62856 E103.24280
Companion crop rice	CrCP501	2005	Central Plain	Suphan Buri	N14.01070 E101.77691
	CrCP502	2005		Sing Buri	N14.96109 E100.36876
Weedy rice	WeCP501	2005		Suphan Buri	N14.01070 E101.77691
	WeCP502	2005		Sing Buri	N14.96109 E100.36876
Native common wild rice	WiCP501	2005		Suphan Buri	N14.01070 E101.77691
	WiCP502	2005		Sing Buri	N14.96109 E100.36876
Companion crop rice	CrLN801	2008	Lower North	Phitsanulok	N17.07133 E100.81639
	CrLN802	2008		Phichit	N16.36638 E100.33548
Weedy rice	WeLN801	2008		Phitsanulok	N17.07133 E100.81639
	WeLN802	2008		Phichit	N16.36638 E100.33548
Native common wild rice	WiLN801	2005		Phitsanulok	N17.07133 E100.81639
	WiLN802	2005		Phichit	N16.36638 E100.33548
Companion crop rice	CrNE801	2008	Northeast	Si Saket	N14.95359 E104.20601
	CrNE802	2008		Buri Rum	N14.62856 E103.24280
Weedy rice	WeNE801	2008		Si Saket	N14.95359 E104.20601
	WeNE802	2008		Buri Rum	N14.62856 E103.24280
Native common wild rice	WiNE801	2005		Si Saket	N14.95359 E104.20601
	WiNE802	2005		Buri Rum	N14.62856 E103.24280
Companion crop rice	CrCP801	2008	Central Plain	Suphan Buri	N14.01070 E101.77691
	CrCP802	2008		Sing Buri	N14.96109 E100.36876
Weedy rice	WeCP801	2008		Suphan Buri	N14.01070 E101.77691
	WeCP802	2008		Sing Buri	N14.96109 E100.36876
Native common wild rice	WiCP801	2005		Suphan Buri	N14.01070 E101.77691
	WiCP802	2005		Sing Buri	N14.96109 E100.36876

Table 3.1.1 (*continued*)

Type	Sample Accession No.	Year collection	Region	Province	UTM
Companion crop rice	CrLN901	2009	Lower North	Phitsanulok	N17.07133 E100.81639
	CrLN902	2009		Phichit	N16.36638 E100.33548
Weedy rice	WeLN901	2009		Phitsanulok	N17.07133 E100.81639
	WeLN902	2009		Phichit	N16.36638 E100.33548
Native common wild rice	WiLN901	2005		Phitsanulok	N17.07133 E100.81639
	WiLN902	2005		Phichit	N16.36638 E100.33548
Companion crop rice	CrNE901	2009	Northeast	Si Saket	N14.95359 E104.20601
	CrNE902	2009		Buri Rum	N14.62856 E103.24280
Weedy rice	WeNE901	2009		Si Saket	N14.95359 E104.20601
	WeNE902	2009		Buri Rum	N14.62856 E103.24280
Native common wild rice	WiNE901	2005		Si Saket	N14.95359 E104.20601
	WiNE902	2005		Buri Rum	N14.62856 E103.24280
Companion crop rice	CrCP901	2009	Central Plain	Suphan Buri	N14.01070 E101.77691
	CrCP902	2009		Sing Buri	N14.96109 E100.36876
Weedy rice	WeCP901	2009		Suphan Buri	N14.01070 E101.77691
	WeCP902	2009		Sing Buri	N14.96109 E100.36876
Native common wild rice	WiCP901	2005		Suphan Buri	N14.01070 E101.77691
	WiCP902	2005		Sing Buri	N14.96109 E100.36876

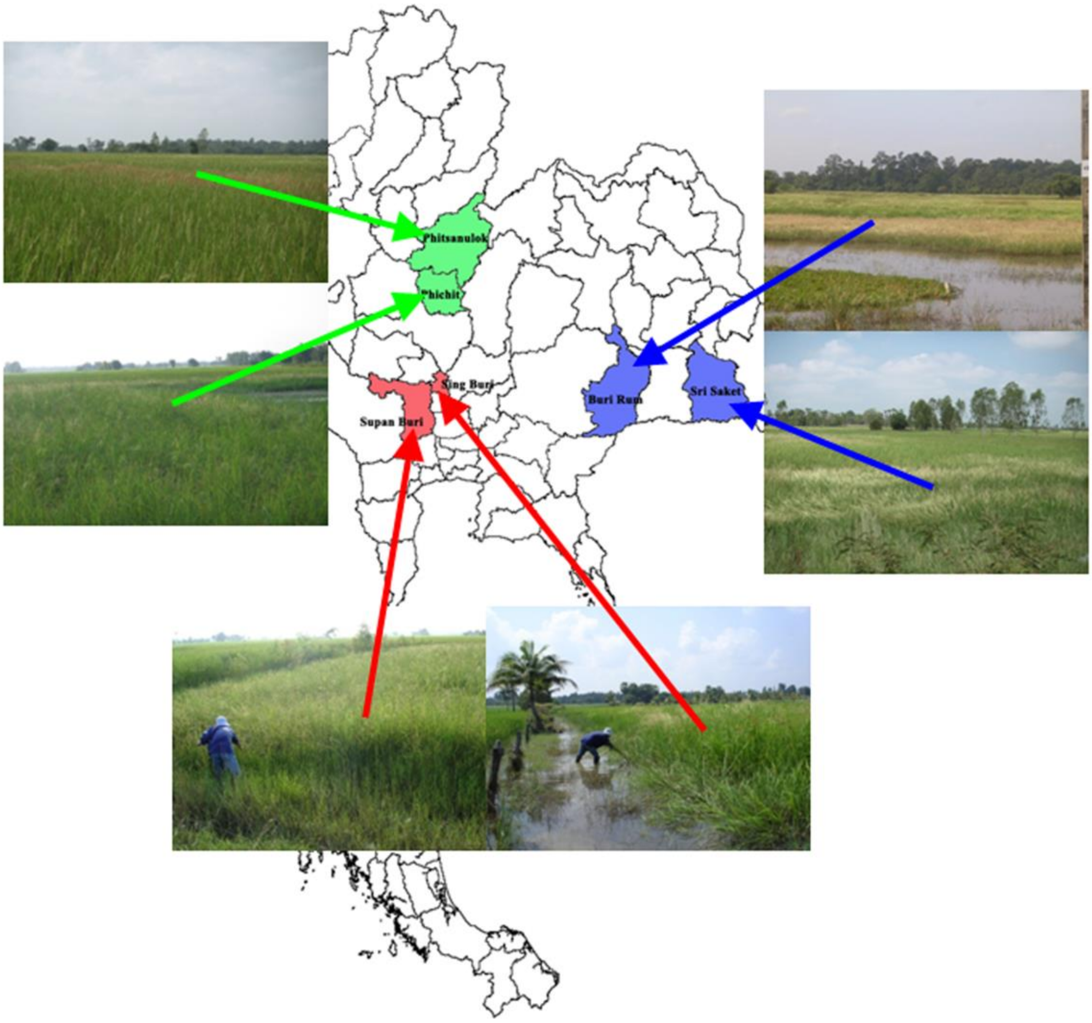


Figure 3.1.1 Location of weedy rice, companion crop rice and native common wild rice sampling with occur in sympatric rice field in Thailand in wet 2005, 2008 and 2009 of the present study

Characterization of populations

The experiment was set up in pots. Seeds if each companion crop rice and weedy rice population were sown in pot as described in Chapter 2 while native common wild rice were grown by plant samples, 10 plants per population of each type. Twenty five morphological and physiological characteristics were recorded individually of weedy rice, companion crop rice and native common wild rice were carried out as described in Chapter 2.

For DNA analysis, genomic DNA individually (10 plants per population) was extracted from dry leaves sample of weedy rice, companion crop rice and native common wild rice populations using modify method from Doyle and Doyle (1987) and the PCR reactions were performed following the description of Panaud *et al*. (1996). Twelve microsatellite loci distributed on 12 chromosomes (as described in Table 2.1.4) were chosen (Pusadee, 2009). Amplification of DNA was carried out as described in Chapter 2.

Data analysis

Morphological and physiological analysis

For morphological characters, diversity in morphological characters of weedy rice, companion crop rice and native common wild rice was calculated by using Shannon-Weaver Index (Shannon and Weaver, 1949 cited by Coffey, 2002). Neighbor-joining method of phylogenetic tree was used to calculate illustrating taxonomy relationships among rice groups by using UPGMA method on the basis of C.S. Chord (1967) genetic distance based on 6 morphological traits.

Physiological characters were calculated for mean and standard deviation (sd). Means were compared the differences between weedy rice populations and check populations were determined by using F-test at a significance level of 95% and 99%.

Population genetic structure and dynamics in spatial and temporal terms of weedy rice, companion crop rice and native common wild rice in Thailand

Weedy rice, companion crop rice and native common wild rice populations occur in sympatric rice field collected from the surveys in three rice cultivation seasons including, wet 2005, 2008 and 2009, were used to examine gene flow, population genetic structure and dynamics in spatial and temporal terms of weedy rice, companion crop rice and native common wild rice populations (Table 3.1.1). Genetic variation, genetic structure and population structure were used to analyze by partitioned into the following components: (a) for spatial term analysis; within and between regions in each season of within and among rice groups including, weedy rice, companion crop rice and native common wild rice and (b) for temporal analysis; within and between regions among seasons of within and among rice groups.

Genetic diversity analysis

Genetic parameters were calculated base on microsatellite data by using FSTAT version 2.9.3 (Goudet, 2001) and POPGENE version 1.32 (Yeh *et al*., 1999).

Genetic structure analysis

Genetic structure was analyzed by hierarchical analysis of molecular variance (AMOVA) implemented in the software of GeneAlEx version 6.1 (Peakall and

Smouse 2006 cited by Pusadee, 2009). In addition, Total genetic diversity variance of F_{ST} estimator was partitioned into the following components: between and within companion crop rice, weedy rice and native common wild rice populations in each season.

Population Structure

Population structure was assessed based on three different analysis methods. Bayesian clustering analysis was undertaken using the program STRUCTURE version 2.2. The principal coordinate analysis (PCA) was conducted on the basis of EIGEN method in GeneAlEx version 6.1. In addition, Cavalli-Sforza Chord genetic distance was obtained by POWERMARKER version 3.0 and subsequently visualized the Neighbor-joining (NJ) tree using MEGA version 4. (The detail of each analysis is described in Chapter 2 Data analysis).

Table 3.1.2 Popular crop rice varieties, companion crop rice, weedy rice and native common wild rice populations in each rice growing region and year

Region	Cultivated rice		Weedy rice	Native common wild rice	Location	Year
	Pure line rice variety	Companion crop rice				
Lower North	CNT1	CrLN501	WeLN501	WiLN501	Phitsanulok	2005
	SPR1	CrLN502	WeLN502	WiLN502	Phichit	2005
	PTT1	CrLN801	WeLN801	WiLN801	Phitsanulok	2008
	PSL2	CrLN802	WeLN802	WiLN802	Phichit	2008
		CrLN901	WeLN901	WiLN901	Phitsanulok	2009
		CrLN902	WeLN902	WiLN902	Phichit	2009
Northeast	CNT1	CrNE501	WeNE501	WiNE501	Si Saket	2005
	SPR1	CrNE502	WeNE502	WiNE502	Buri Rum	2005
	PTT1	CrNE801	WeNE801	WiNE801	Si Saket	2008
	KDML105	CrNE802	WeNE802	WiNE802	Buri Rum	2008
	RD6	CrNE901	WeNE901	WiNE901	Si Saket	2009
	RD15	CrNE902	WeNE902	WiNE902	Buri Rum	2009
Central Plain	CNT1	CrCP501	WeCP501	WiCP501	Suphan Buri	2005
	SPR1	CrCP502	WeCP502	WiCP502	Sing Buri	2005
	PTT1	CrCP801	WeCP801	WiCP801	Suphan Buri	2008
	PSL2	CrCP802	WeCP802	WiCP802	Sing Buri	2008
		CrCP901	WeCP901	WiCP901	Suphan Buri	2009
		CrCP902	WeCP902	WiCP902	Sing Buri	2009

3.3 Results

Morphological and physiological characterization

Morphological characterization

Characteristics of stem, panicle, spikelets and seeds of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand were recorded and compared among three growing seasons in wet 2005, 2008 and 2009 (Table 3.2.1 and Figure 3.2.1). Companion crop rice showed uniformity of erect plant type, compact panicle, awnless seeds, straw hull and white pericarp while spreading plant type, open panicle, red and white long awned, dark brown to black hull and predominate red pericarp were found in native common wild rice. For weedy rice, large variation were found in all six characters including, erect to spreading plant type, compact to open panicle, awnless to red and white long awn, straw to black hull and white to red pericarp (Table 3.2.1). Cultivar traits in weedy rice populations tended to increase over years.

All morphological characteristics were subjected to cluster analysis using UPGMA method (Table 3.2.1). In 2005 and 2008, companion crop rice populations were classified as a distinct group from native common wild rice and weedy rice at the distance parameter around 0.36. However, weedy rice and companion crop rice were increased the morphological relationship between them with year of infestation, with the distance around 0.36 in 2005 and 2008 while in 2009 was decreased to 0.25, respectively. Within the weedy rice populations, there was no relationship between locations of collection and morphological distance in all seasons (Figure 3.2.1)

Table 3.2.1 Frequency (%) of six morphological characteristics of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009.

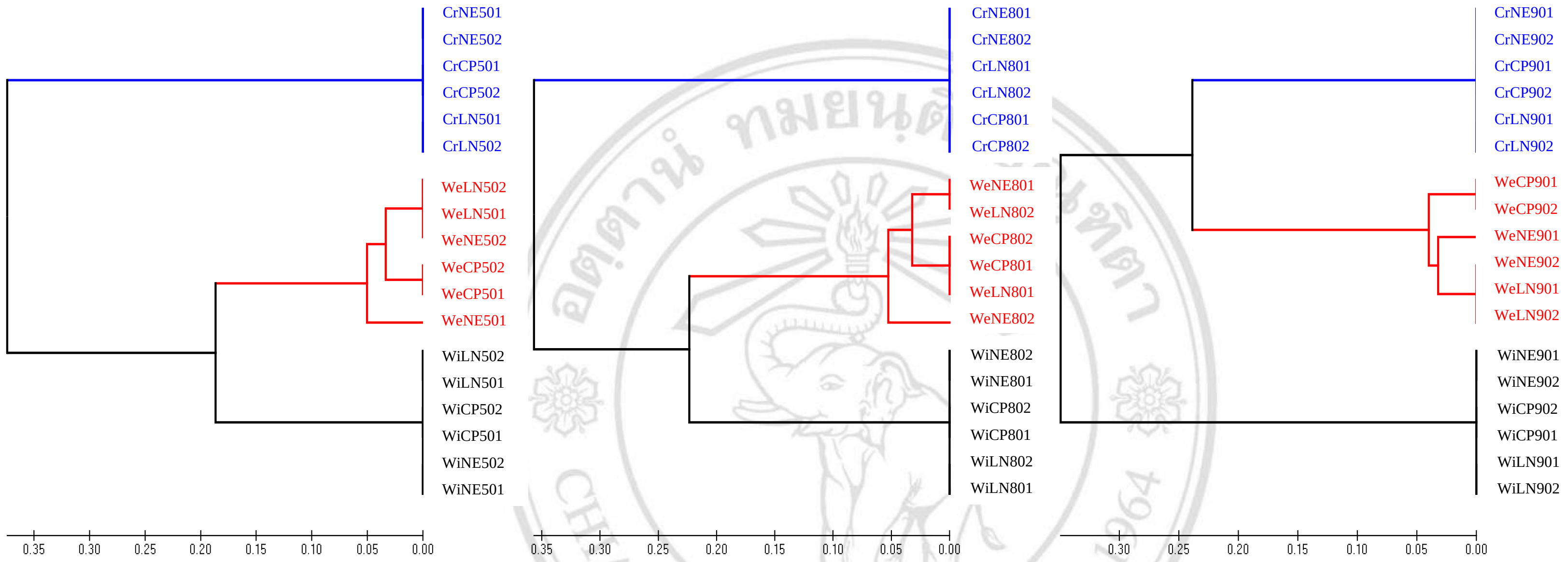
Region	**Year**	**Type***	**N**	**n**	**Plant type**		**Panicle type**		**Awned presentation**		
	collection				**erect**	**spreading**	**compact**	**open**	**awnless**	**<5 cm**	**>5 cm**
Lower North	2005	Cr	2	20	100	0	100	0	100	0	0
	2008	Cr	2	20	100	0	100	0	100	0	0
	2009	Cr	2	20	100	0	100	0	100	0	0
	2005	We	2	20	40	60	0	100	0	65	35
	2008	We	2	20	70	30	10	90	15	60	25
	2009	We	2	20	75	25	10	90	35	65	0
	2005	Wi	2	20	0	100	0	100	0	0	100
	2008	Wi	2	20	0	100	0	100	0	0	100
	2009	Wi	2	20	0	100	0	100	0	0	100
Northeast	2005	Cr	2	20	100	0	100	0	100	0	0
	2008	Cr	2	20	100	0	100	0	100	0	0
	2009	Cr	2	20	100	0	100	0	100	0	0
	2005	We	2	20	35	65	0	100	0	50	50
	2008	We	2	20	50	50	5	95	5	75	20
	2009	We	2	20	60	40	10	90	30	60	10
	2005	Wi	2	20	0	100	0	100	0	0	100
	2008	Wi	2	20	0	100	0	100	0	0	100
	2009	Wi	2	20	0	100	0	100	0	5	95
Central Plain	2005	Cr	2	20	100	0	100	0	100	0	0
	2008	Cr	2	20	100	0	100	0	100	0	0
	2009	Cr	2	20	100	0	100	0	100	0	0
	2005	We	2	20	50	50	0	100	5	65	30
	2008	We	2	20	80	20	10	90	25	55	20
	2009	We	2	20	85	15	15	85	50	45	5
	2005	Wi	2	20	0	100	0	100	0	0	100
	2008	Wi	2	20	0	100	0	100	0	0	100
	2009	Wi	2	20	0	100	0	100	0	0	100

*Cr = Companion crop rice, We = Weedy rice, Wi = Native common wild rice

Table 3.2.1 *(continued)*

Region	Year collection	Type*	N	n	Awn color		Hull color		Pericarp color	
					white	red	straw	dark brown-black	white	red
Lower North	2005	Cr	2	20	-	-	100	0	100	0
	2008	Cr	2	20	-	-	100	0	100	0
	2009	Cr	2	20	-	-	100	0	100	0
	2005	We	2	20	65	35	65	35	0	100
	2008	We	2	20	85	15	80	20	0	100
	2009	We	2	20	90	10	90	10	5	95
	2005	Wi	2	20	10	80	0	100	0	100
	2008	Wi	2	20	10	80	0	100	0	100
	2009	Wi	2	20	15	85	0	100	0	100
Northeast	2005	Cr	2	20	-	-	100	0	100	0
	2008	Cr	2	20	-	-	100	0	100	0
	2009	Cr	2	20	-	-	100	0	100	0
	2005	We	2	20	60	40	50	50	0	100
	2008	We	2	20	80	20	70	30	0	100
	2009	We	2	20	80	20	80	20	0	100
	2005	Wi	2	20	25	75	0	100	0	100
	2008	Wi	2	20	15	85	0	100	0	100
	2009	Wi	2	20	15	85	0	100	0	100
Central Plain	2005	Cr	2	20	-	-	100	0	100	0
	2008	Cr	2	20	-	-	100	0	100	0
	2009	Cr	2	20	-	-	100	0	100	0
	2005	We	2	20	65	35	65	35	0	100
	2008	We	2	20	100	0	90	10	0	100
	2009	We	2	20	100	0	100	0	10	90
	2005	Wi	2	20	25	75	0	100	0	100
	2008	Wi	2	20	15	85	0	100	0	100
	2009	Wi	2	20	25	75	0	100	0	100

*Cr = Companion crop rice, We = Weedy rice, Wi = Native common wild rice



***(a)* 2005** ***(b)* 2008** ***(c)* 2009**

Figure 3.2.1 Cluster analysis using UPGMA method on the basis of C.S. Chord (1967) genetic distance among sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005 *(a)*, 2008 *(b)* and 2009 *(c)* constructed based on six morphological traits.

Physiological characterization

Days to flowering

Days to flowering of sympatric wild-weed-crop rice populations from three rice production areas; Lower North (LN), Northeast (NE) and Central Plain (CP) among three growing seasons in wet 2005, 2008 and 2009 was recorded (Table 3.2.2 and Figure 3.2.2). The earliest of day to flowering was found in companion crop rice populations ranged from 80 (in both the LN and the CP) to 90 (in the NE) days after sowing while native common wild rice populations were latest were peak about 110 days, distributing within the range of >100-120 days. For weedy rice populations, the distribution of days to flowering was earlier than both companion crop rice and native common wild rice but there different among locations with ranged from 68 (in both the LN and the CP) to 80 (in the NE) days. However, weedy rice populations were no different among growing seasons (Table 3.2.2 and Figure 3.2.2). However, day to transplant in pot of wet 2005 populations was July 2008, wet 2008 populations was August 2009, wet 2009 and dry 2009/10 were August 2010.

Number of tillers plant^{-1}

The largest of number of tillers plant^{-1} was found in native common wild rice populations (ranged 16-18 tillers) while companion crop rice populations were smallest of number of tillers plant^{-1} (ranged 5-6 tillers). For weedy rice populations, number of tillers plant^{-1} was distributed in the range between companion crop rice and native common wild rice, 7-8 tillers but there were no variation between both locations and growing seasons (Table 3.2.2 and Figure 3.2.3).

Culm length (cm)

Culm length of companion crop rice populations was distributed in range from about 85 (in both the LN and the CP) to 108 (in the NE) cm while native common wild rice populations were ranged from 135 to 142 cm. For weedy rice populations, culm length was distributed in the range between companion crop rice and native common wild rice but there different among locations with 83-111 cm in both the LN and the CP while 101-120 cm in the NE. Interesting, weedy rice populations were decreased with year of infestation in each location (Table 3.2.2 and Figure 3.2.4).

Seed shattering (%)

Very low rate of seed shattering was recorded in companion crop rice populations (2-3%) while all seeds of native common wild rice populations were shattered at maturity (100%). Weedy rice populations (91-97%) were in the same class as native common wild rice but with wider range of distribution. However, weedy rice populations were no different between both locations and growing seasons (Table 3.2.2 and Figure 3.2.5).

Table 3.2.2 Physiological characteristics of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009 (mean±sd).

Region	**Year collection**	**Type***	**N**	**n**	**Days to Flowering$^{\$}$**	**Tillers plant^{-1}**	**Culm length (cm)**	**Seed shattering (%)**
Lower North	2005	Cr	2	20	80±2	6±1	88±5	3±2
	2008	Cr	2	20	82±2	6±1	89±5	3±3
	2009	Cr	2	20	83±4	6±2	85±5	3±2
	2005	We	2	20	68±6	8±3	111±7	94±9
	2008	We	2	20	69±7	7±4	95±7	92±6
	2009	We	2	20	71±6	7±3	83±5	92±4
	2005	Wi	2	20	108±7	18±8	137±7	100
	2008	Wi	2	20	107±8	17±9	135±13	100
	2009	Wi	2	20	107±7	17±8	136±11	100
Northeast	2005	Cr	2	20	89±2	6±1	105±6	2±2
	2008	Cr	2	20	89±2	6±1	107±4	2±2
	2009	Cr	2	20	90±2	5±2	108±5	3±2
	2005	We	2	20	80±5	8±4	120±8	94±8
	2008	We	2	20	78±6	8±3	111±7	92±8
	2009	We	2	20	80±4	7±4	101±5	95±9
	2005	Wi	2	20	108±6	17±6	141±13	100
	2008	Wi	2	20	109±7	16±7	140±14	100
	2009	Wi	2	20	106±6	17±4	142±13	100
Central Plain	2005	Cr	2	20	81±3	6±2	80±6	2±2
	2008	Cr	2	20	84±4	6±2	83±5	3±2
	2009	Cr	2	20	82±5	6±2	86±3	2±2
	2005	We	2	20	68±3	7±3	106±9	91±7
	2008	We	2	20	70±5	8±3	95±8	97±5
	2009	We	2	20	70±7	8±4	87±8	96±6
	2005	Wi	2	20	107±7	18±8	140±12	100
	2008	Wi	2	20	109±8	17±9	142±9	100
	2009	Wi	2	20	106±6	18±8	137±11	100

*Cr = Companion crop rice, We = Weedy rice, Wi = Native common wild rice

$^{\$}$ Day to transplant in pots; Wet 2005 – July 2008, Wet 2008 – August 2009, Wet 2009 - August 2010

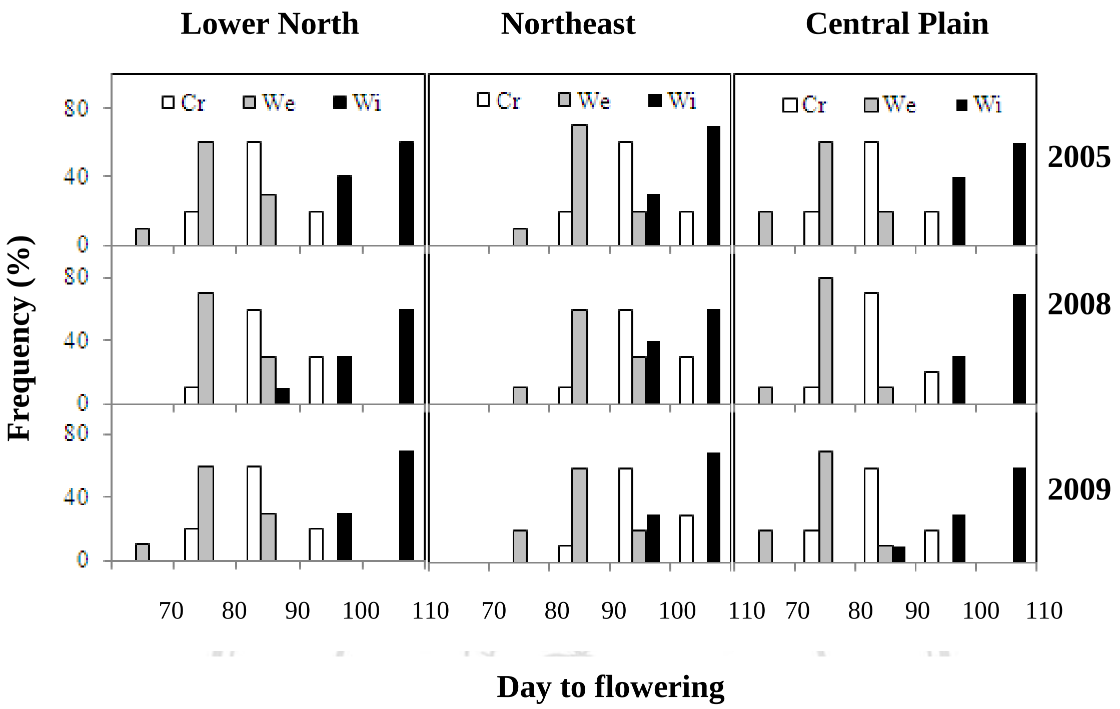


Figure 3.2.2 Distribution of days to flowering of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009.

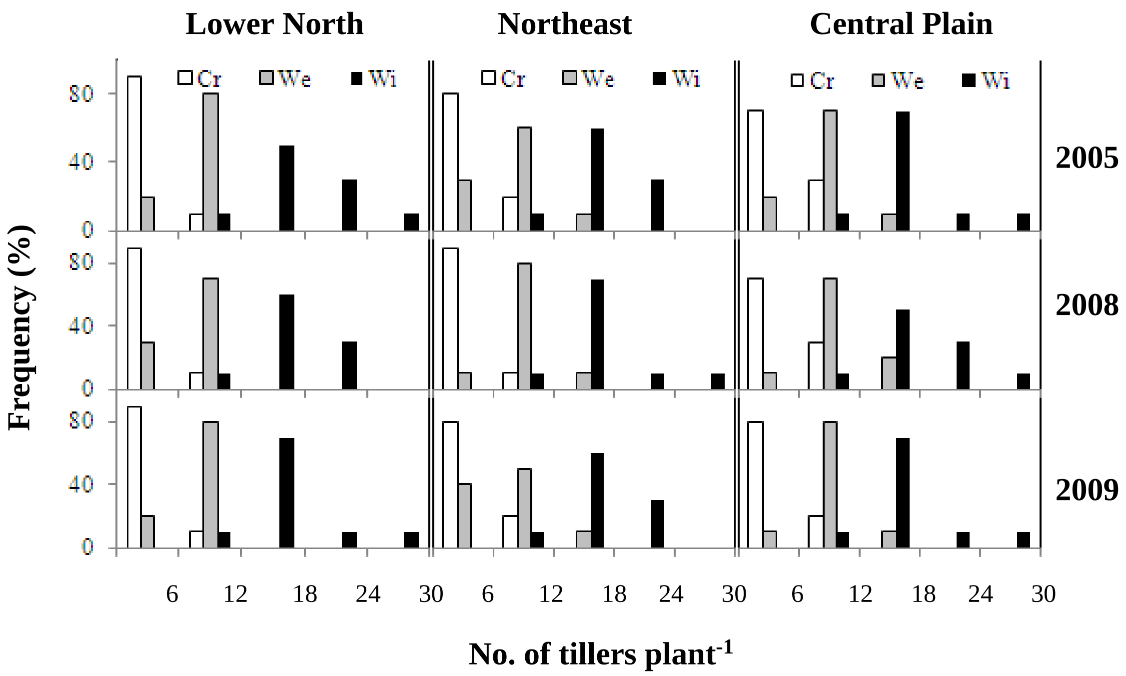


Figure 3.2.3 Distribution of tillers plant^{-1} of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009.

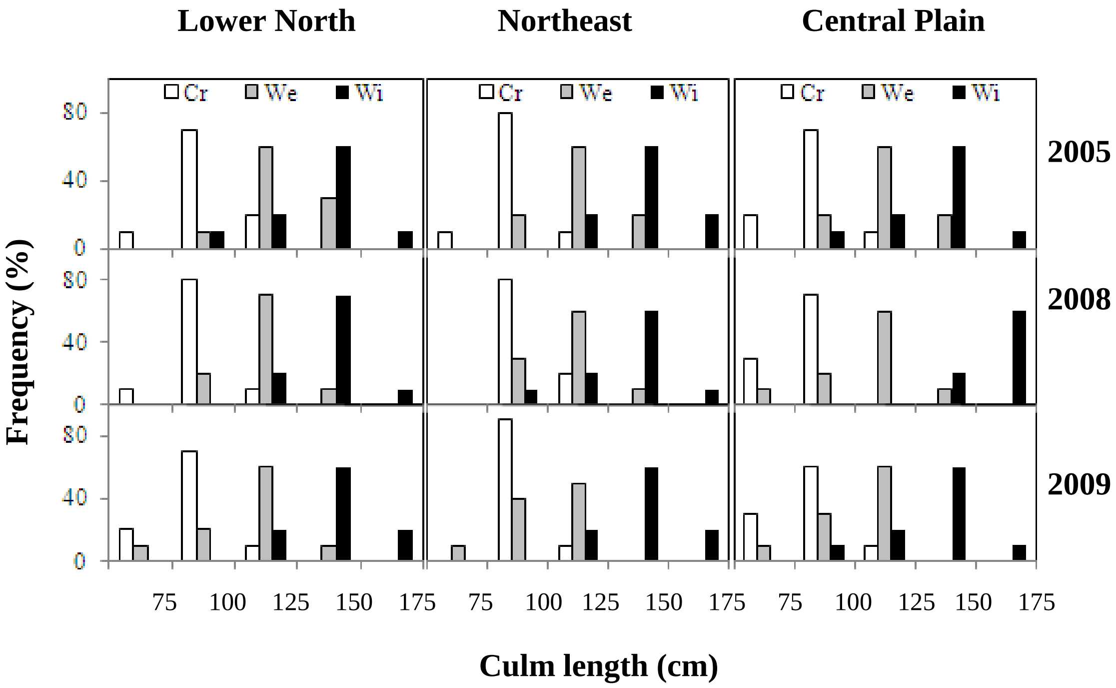


Figure 3.2.4 Distribution of culm length (cm) of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009.

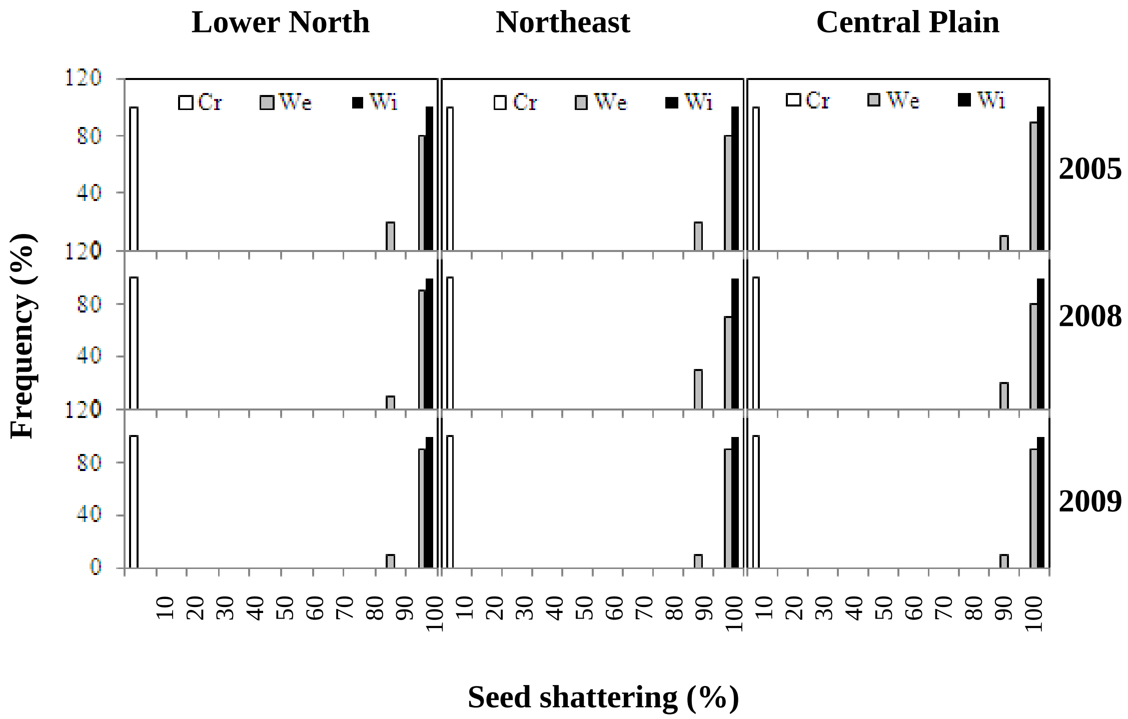


Figure 3.2.5 Distribution of seed shattering (%) of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009.

Population genetic structure and dynamic in spatial term of sympatric wild-weed-crop rice populations in Thailand

<u>*Allele specific to crop and wild types*</u>

Twelve pairs of primer were used to identify alleles for specific to crop and wild types in sympatric wild-weed-crop rice populations from three rice production areas; Lower North (LN), Northeast (NE) and Central Plain (CP) of Thailand among three growing seasons in wet 2005, 2008 and 2009 (Table 3.3.1). Companion crop rice from all populations showed allele for specific to crop varieties with commonly cultivated in paddy fields of each location. Both Crop1 (SPR1 or CNT1) and Crop2 (PTT1 or PSL2) alleles were found in companion crop rice in the LN while the NE found allele specific to Crop3 (KDML105 or Rd15 or RD6). The CP populations found allele specific to Crop1 (SPR1 or CNT1). Furthermore, some alleles specific to wild type were found in companion crop rice populations. The allele in native common wild rice populations was specific to wild type but few (<3%) alleles of companion crop rice were found together (Table 3.3.1). For weedy rice, allele specific to crop and wild types were found but crop alleles were increased with year of infestation. Crop alleles in weedy rice from each location were different with the varieties of companion crop rice each region. Interesting, in 2008 and 2009, some alleles of alien crop rice were introduced into weedy rice populations such as in the NE found allele specific to Crop1 (7% in 2008 and 10% in 2009) and Crop2 (1% in 2008 and 3% in 2009) (Table 3.3.1).

Genotype frequency

Both homozygous and heterozygous genotypes were found using twelve RM markers (Table 3.3.2). All genotypes with found in companion crop rice populations were homozygous genotype with the same as genotype of popular varieties in each location. Both Crop1 (SPR1 or CNT1) and Crop2 (PTT1 or PSL2) genotypes were found in companion crop rice in the Lower North (LN) while the Northeast (NE) found genotype specific to Crop3 (KDML105 or Rd15 or RD6). The Central Plain (CP) populations found allele specific to Crop1 (SPR1 or CNT1). Furthermore, a few (<1%) heterozygous genotype were found in companion crop rice populations. In contrast, both homozygous genotypes specific to wild and heterozygous genotype were found in native common wild rice populations but few (<5%) homozygous genotypes specific to companion crop rice were found together (Table 3.3.2). For weedy rice, all three types of genotype, including homozygous genotypes specific to wild and companion crop rice and heterozygous genotype, were found. Homozygous genotypes specific to companion crop rice were increased with year of infestation while homozygous genotypes specific to wild and heterozygous genotype were decreased with year of infestation. Crop genotypes in weedy rice from each location were different with the varieties of companion crop rice each region. Interesting, in 2008 and 2009, some genotypes of alien crop rice were introduced into weedy rice populations such as in the NE found genotypes specific to Crop1 (4% in 2008 and 9% in 2009) and Crop2 (1% in 2009) (Table 3.3.2).

Table 3.3.1 Frequency of alleles specific to crop types (SPR1, CNT1, PTT1, PSL2, KDML105, RD15 and RD6) and wild types (WiLN, WiNE and WiCP) in sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009 based on 12 microsatellite markers.

Year	**Allele type**[*]	**Companion crop rice**[**]			**Common wild rice**[+]			**Weedy rice**[$]		
		CrLN	**CrNE**	**CrCP**	**WiLN**	**WiNE**	**WiCP**	**WeLN**	**WeNE**	**WeCP**
2005	Wild	0.016	0.006	0.014	0.975	0.983	0.971	0.492	0.630	0.545
	Crop1	0.492	0	0.986	0.025	0	0.021	0.426	0.004	0.450
	Crop2	0.492	0	0	0	0	0	0.082	0.005	0.005
	Crop3	0	0.994	0	0	0.017	0.008	0	0.361	0
2008	Wild	0.004	0.008	0	0.996	0.990	0.994	0.321	0.444	0.315
	Crop1	0.498	0	1.000	0.004	0	0.006	0.315	0.066	0.617
	Crop2	0.498	0	0	0	0	0	0.347	0.009	0.046
	Crop3	0	0.992	0	0	0.010	0	0.017	0.481	0.023
2009	Wild	0.006	0.006	0.010	0.996	0.990	0.992	0.308	0.355	0.267
	Crop1	0.497	0	0.990	0.004	0	0.008	0.155	0.103	0.515
	Crop2	0.497	0	0	0	0	0	0.517	0.027	0.138
	Crop3	0	0.994	0	0	0.010	0	0.020	0.515	0.080

[*] Different crop group represent 3 groups referred to the assignment obtained from STRUCTURE; wild = all allele types of wild, Crop1 = SPR1/CNT1, Crop2 = PTT1/PSL2, Crop3 = KDML105/RD15/RD6

[**] CrLN = Companion crop rice from the Lower North, CrNE = Companion crop rice from the Northeast, CrCP = Companion crop rice from the Central Plain

[+] WiLN = native common wild rice from the Lower North, WiNE = native common wild rice from the Northeast, WiCP = native common wild rice from the Central Plain

[$] WeLN = weedy rice from the Lower North, WeNE = weedy rice from the Northeast, WeCP = weedy rice from the Central Plain

Table 3.3.2 Genotypic frequency of crop types (SPR1, CNT1, PTT1, PSL2, KDML105, RD15 and RD6) wild types (WiLN, WiNE and WiCP) and hybrid types in sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand among three growing seasons in wet 2005, 2008 and 2009 based on 12 microsatellite markers.

Year	**Genotype**[*]	**Companion crop rice**[**]			**Common wild rice**[+]			**Weedy rice**[$]		
		CrLN	**CrNE**	**CrCP**	**WiLN**	**WiNE**	**WiCP**	**WeLN**	**WeNE**	**WeCP**
2005	Wild	0	0	0	0.387	0.366	0.451	0.379	0.346	0.238
	Crop1	0.496	0	0.993	0.013	0	0.011	0.203	0	0.350
	Crop2	0.496	0	0	0	0	0	0.051	0	0
	Crop3	0	0.997	0	0	0.009	0.004	0	0.217	0
	Hybrids	0.008	0.003	0.007	0.601	0.634	0.535	0.367	0.438	0.413
2008	Wild	0	0	0	0.408	0.355	0.484	0.298	0.231	0.244
	Crop1	0.499	0	1.000	0.002	0	0.003	0.227	0.040	0.517
	Crop2	0.499	0	0	0	0	0	0.237	0	0.028
	Crop3	0	0.996	0	0	0.005	0	0.015	0.464	0.006
	Hybrids	0.002	0.004	0	0.590	0.640	0.514	0.198	0.265	0.206
2009	Wild	0	0	0	0.422	0.299	0.683	0.242	0.203	0.170
	Crop1	0.499	0	0.995	0.002	0	0.004	0.084	0.087	0.506
	Crop2	0.499	0	0	0	0	0	0.467	0.012	0.101
	Crop3	0	0.997	0	0	0.005	0	0.011	0.482	0.025
	Hybrids	0.003	0.003	0.005	0.576	0.696	0.314	0.195	0.216	0.197

[*] Different crop group represent 3 groups referred to the assignment obtained from STRUCTURE; wild = all genotypes of wild, Crop1 = SPR1/CNT1, Crop2 = PTT1/PSL2, Crop3 = KDML105/RD15/RD6

[**] CrLN = Companion crop rice from the Lower North, CrNE = Companion crop rice from the Northeast, CrCP = Companion crop rice from the Central Plain

[+] WiLN = native common wild rice from the Lower North, WiNE = native common wild rice from the Northeast, WiCP = native common wild rice from the Central Plain

[$] WeLN = weedy rice from the Lower North, WeNE = weedy rice from the Northeast, WeCP = weedy rice from the Central Plain

Populations from wet 2005

Genetic diversity

Genetic diversity with comparison among sympatric wild-weed-crop rice populations from three rice production areas; Lower North (LN), Northeast (NE) and Central Plain (CP) of Thailand in wet 2005 was analyzed by 12 microsatellite markers (Table 3.3.3). Average in three regions, native common wild rice populations showed the highest level of average and total genetic diversity (H_S = 0.605 and H_T = 0.784, respectively) while no variation was detected within companion crop rice populations but high level of total genetic diversity (0.639) was found. Weedy rice populations showed intermediate value of genetic diversity (H_S = 0.555) between native common wild rice and companion crop rice populations but have the same value of total genetic diversity (0.764) as native common wild rice populations. Considering mating system, inbreeding coefficient (F_{IS} = 0.208) was the lowest in native common wild rice, on the other hand, outcrossing rate (t = 66%) was the highest. In contrast, companion crop rice was autogamy illustrated by F_{IS} = 1.0, and t = 0%. Whereas weedy rice showed intermediate level of both F_{IS} (0.509) and t (33%) between native common wild rice and companion crop rice (Table 3.3.3).

For among regions of weedy rice populations, the NE showed highest level of average and total genetic diversity (H_S = 0.617 and H_T = 0.796, respectively) following with the CP (H_S = 0.541 and H_T = 0.771, respectively) while the lowest was found in the LN (H_S = 0.507 and H_T = 0.724, respectively). The genetic differentiation (F_{ST}) was highest in the NE (0.507) followed by the CP (0.427) and the LN (0.331) was the lowest with 0.155 in among three regions illustrated that about 16% of genetic variation of those found in weedy rice was the differences between three locations while the rest 84% was distributed between six populations within each location. In mating system, the highest level of F_{IS} was found in the LN (0.578) following with the CP (0.532) and the lowest was the NE (0.433). Conversely, the NE weedy rice exhibited the highest level of t (40%) following with the CP (31%) and the lowest in the LN (27%) (Table 3.3.3).

Genetic structure

(a) Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) was conducted to investigate the overall distribution of genetic diversity among sympatric wild-weed-crop rice populations (Table 3.3.4). About 7% of total variance was partitioned among rice groups. For within each rice group, about 31%, 100% and 32% were the distribution of genetic variation among populations within weedy rice, companion crop rice and native common wild rice, respectively. For within weedy rice, about 33%, 51% and 43% were the distribution of genetic variation among weedy rice populations within the Lower North, the Northeast and the Central Plain, respectively (Table 3.3.4).

(b) Genetic differentiation (F_{ST})

In term of genetic differentiation (F_{ST}), weedy rice exhibited higher level of F_{ST} within region than between regions. Degrees of genetic differentiation (F_{ST}) from the highest to the lowest were weedy rice in the Northeast (0.507), the Central Plain (0.427) and the Lower North (0.331), respectively. Pairwised F_{ST} was highest between weedy rice in the Northeast vs the Central Plain (0.210) while the lowest was found between weedy rice in the Lower North vs the Central Plain (0.100) (Table 3.3.5).

Table 3.3.3 Genetic parameters of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005 based on 12 microsatellite markers.

Type	N	n	*h*	H_S	H_T	F_{ST}	H_O	F_{IS}	*t*
Companion cultivated rice	**6**	**60**	**0**	**0**	**0.639**	**1**	**0**	**1**	**0**
Lower North	2	20	0	0	0.325	1	0	1	0
Northeast	2	20	0	0	0.376	1	0	1	0
Central Plain	2	20	0	0	0.315	1	0	1	0
Weedy rice	**6**	**60**	**0.560**	**0.555**	**0.764**	**0.155**	**0.275**	**0.509**	**0.325**
Lower North	2	20	0.512	0.507	0.724	0.331	0.216	0.578	0.267
Northeast	2	20	0.623	0.617	0.796	0.507	0.353	0.433	0.396
Central Plain	2	20	0.546	0.541	0.771	0.427	0.255	0.532	0.305
Native common wild rice	**6**	**60**	**0.611**	**0.605**	**0.784**	**0.036**	**0.484**	**0.208**	**0.656**
Lower North	2	20	0.632	0.626	0.769	0.355	0.508	0.196	0.673
Northeast	2	20	0.679	0.672	0.830	0.399	0.529	0.221	0.638
Central Plain	2	20	0.623	0.618	0.753	0.316	0.515	0.205	0.659

Number of populations (N), Number of individuals (n), Observed heterozygosity (H_O), Nei's (1973) gene diversity (*h*), Average gene diversity (H_S), Total gene diversity (H_T), Inbreeding coefficient (F_{IS}) and Outcrossing rate (*t*)

Genetic structure

Table 3.3.4 Analysis of molecular variance (AMOVA) among sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005 based on 12 microsatellite markers.

Source	df	SS	Variance component	% of the total variance
Among rice groups	2	798.333	1.577	7%
Populations/Group	15	10275.130	10.632	66%
Populations/Weedy rice	5	419.300	4.585	31%
Lower North	*1*	*135.582*	*6.692*	*33%*
Northeast	*1*	*163.200*	*8.055*	*51%*
Central Plain	*1*	*120.517*	*5.948*	*43%*
Populations/Cultivated rice	5	484.676	6.429	100%
Lower North	*1*	*164.790*	*6.301*	*100%*
Northeast	*1*	*179.330*	*6.365*	*100%*
Central Plain	*1*	*140.556*	*6.108*	*100%*
Populations/Common wild rice	5	2055.026	5.992	32%
Lower North	*1*	*703.035*	*6.465*	*36%*
Northeast	*1*	*757.115*	*6.962*	*40%*
Central Plain	*1*	*594.876*	*5.470*	*32%*
Individuals/Population	162	4203.462	5.929	27%

Table 3.3.5 Pairwise of genetic differentiation (F_{ST}) within and between sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005 based on 12 microsatellite markers.

Pairwise F_{ST}		**Weedy rice**		**Companion crop rice**		**Native common wild rice**	
		Within	**Between**	**Within**	**Between**	**Within**	**Between**
Lower North		0.331		1.000		0.355	
	Northeast		0.187		1.000		0.108
	Central Plain		0.100		1.000		0.079
Northeast		0.507		1.000		0.399	
	Central Plain		0.210		1.000		0.093
Central Plain		0.427		1.000		0.316	

Population structure

Seven pure line rice varieties were structured into 3 inferred populations (K = 3) (Figure 3.3.1) on the basis of 12 microsatellite loci using STRUCTURE program, the first inferred population was SPR1 or CNT1, the second inferred population was PTT1 or PSL2 and the third inferred population was KDML105 or RD15 or RD6, each interred population represent by the presence of red, green and blue colors, respectively. Six companion crop rice (Cr) from three regions were structured as the same popular rice varieties each region such as companion crop rice from the Lower North (LN) was SPR1 or CNT1 (CrLN1) and PTT1 or PSL2 (CrLN1), the Northeast (NE) was KDML105 or RD15 or RD6 (CrNE1 and 2) and the Central Plain (CP) was SPR1 or CNT1 (CrCP1 and 2). Six native common wild rice (Wi) from three regions were structured into 1 inferred populations (K = 1), represent by the presence of gray color (Figure 3.3.1).

Six weedy rice populations from three regions in 2005 resulted from hybridization between native common wild rice and companion crop rice in each region. Evidence of their genetic admixtures in STRUCTURE model was composed genetic proportion between native common wild rice and companion crop rice (Figure 3.3.1 and Figure 3.3.2). Consequently, the population structure of weedy rice populations can be structured into 2 clusters according to its companion crop rice varieties of each region (*K* = 2) (Figure 3.3.1). The admixtures of the first inferred populations consisted of weedy rice populations from the Lower North and the Central Plain. These admixtures contained companion crop genotypes, CNT1 or SPR1 or PTT1 or PSL2, and wild genotype. These modern cultivated rice varieties are commonly found cultivation in paddy fields of these regions (Figure 3.3.1 and

Figure 3.3.2). The rest of second inferred population consisted of weedy rice populations from the Northeast. The admixtures contained companion crop genotypes, KDML105 or RD6 or RD15, and wild genotype. These improved traditional rice varieties are commonly found cultivation in paddy fields of this region (Figure 3.3.1 and Figure 3.3.2). Furthermore, the cultivar alleles were also detected in native common wild rice populations.

The result of genetic admixtures of sympatric wild-weed-crop rice populations by STRUCTURE is consistent with the distribution of those populations by principal coordinate analysis (PCA) analysis (Figure 3.3.3). The relationship among wild-weed-crop rice populations was displayed that companion crop rice and native common wild rice were widely distributed across the graph while weedy rice groups were distributed between the groups of companion crop rice and native common wild rice in each region but most weedy rice were closely distributed with the group of wild. Neighbor-join tree also revealed relationship among sympatric wild-weed-crop rice groups illustrated that weedy rice populations were also divided among the groups of companion crop rice varieties and native common wild rice populations but most weedy rice were closely relationship with the group of wild (Figure 3.3.4).

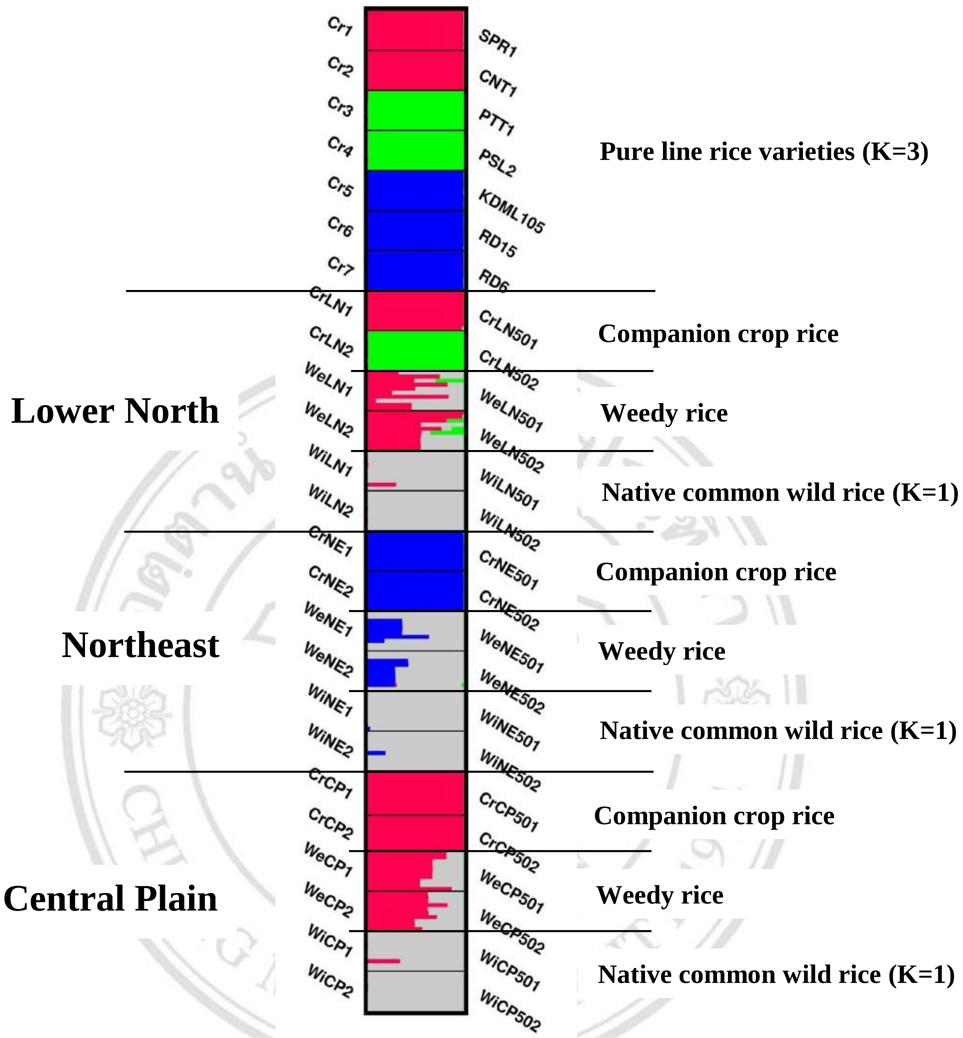


Figure 3.3.1 Population assignment of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005. Each bar represent each population consisted 10 individuals. Different colors represent different inferred populations, referred to different K.

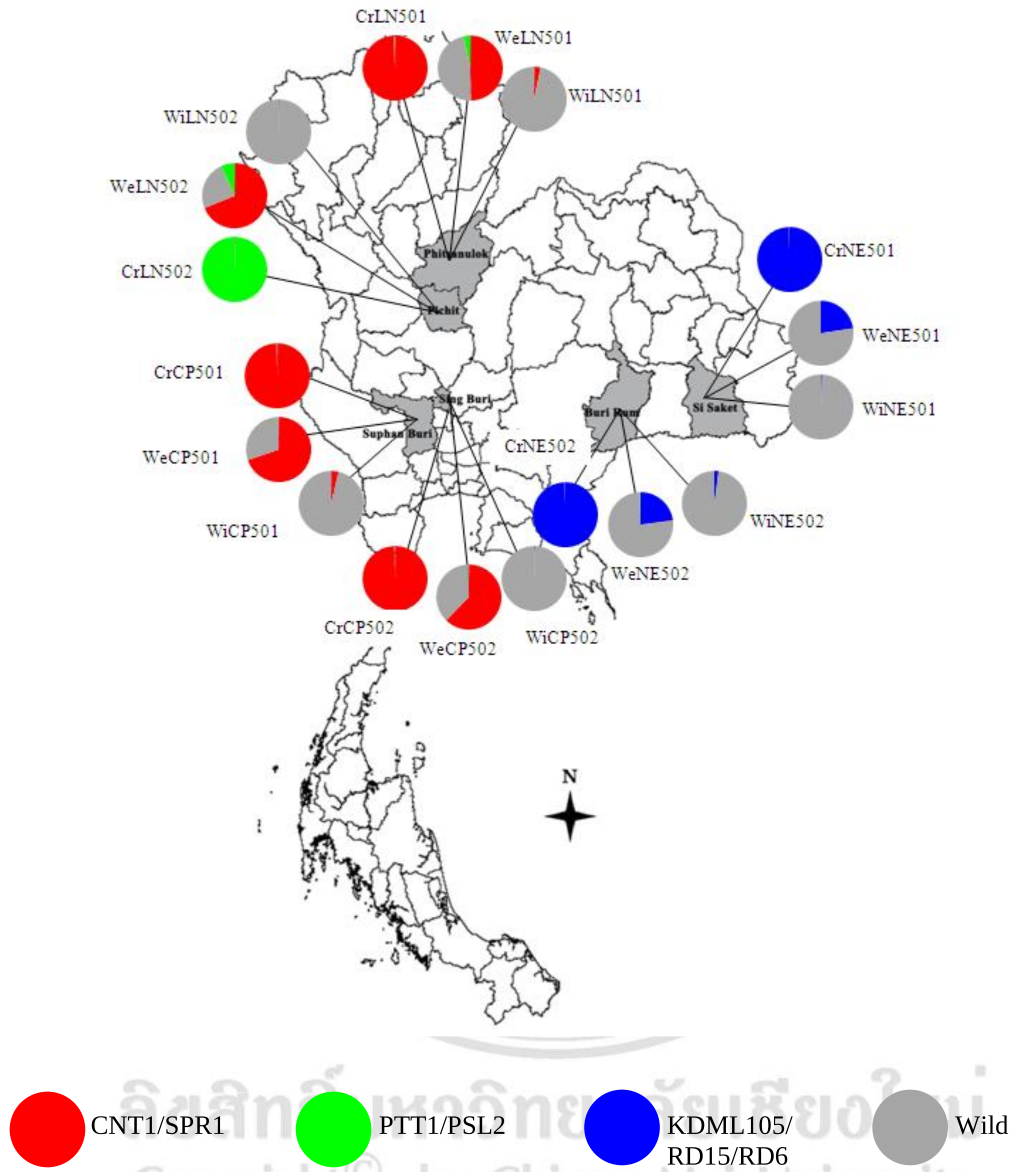
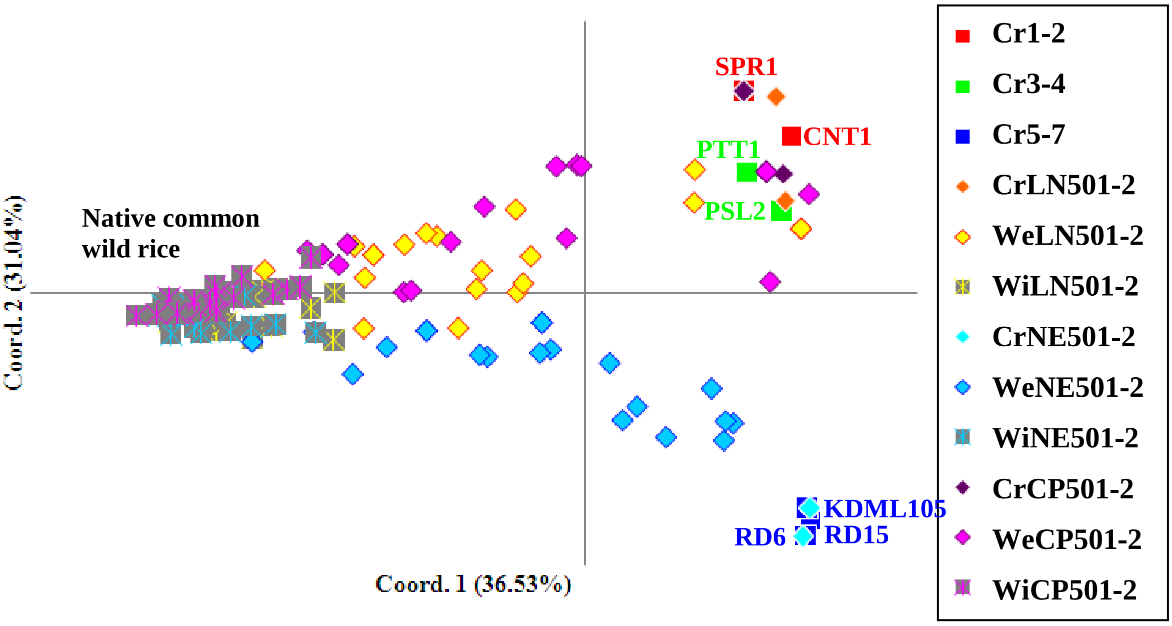


Figure 3.3.2 Genetic proportion of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005.



Cr1-2= SPR1, CNT1 **Cr3-4 = PTT1, PSL2** **Cr5-7 = KDML105, RD15, RD6**

Figure 3.3.3 Distribution of 180 individuals of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005 formed by the principal coordinate analysis (PCA) on the basis of 12 microsatellite markers.

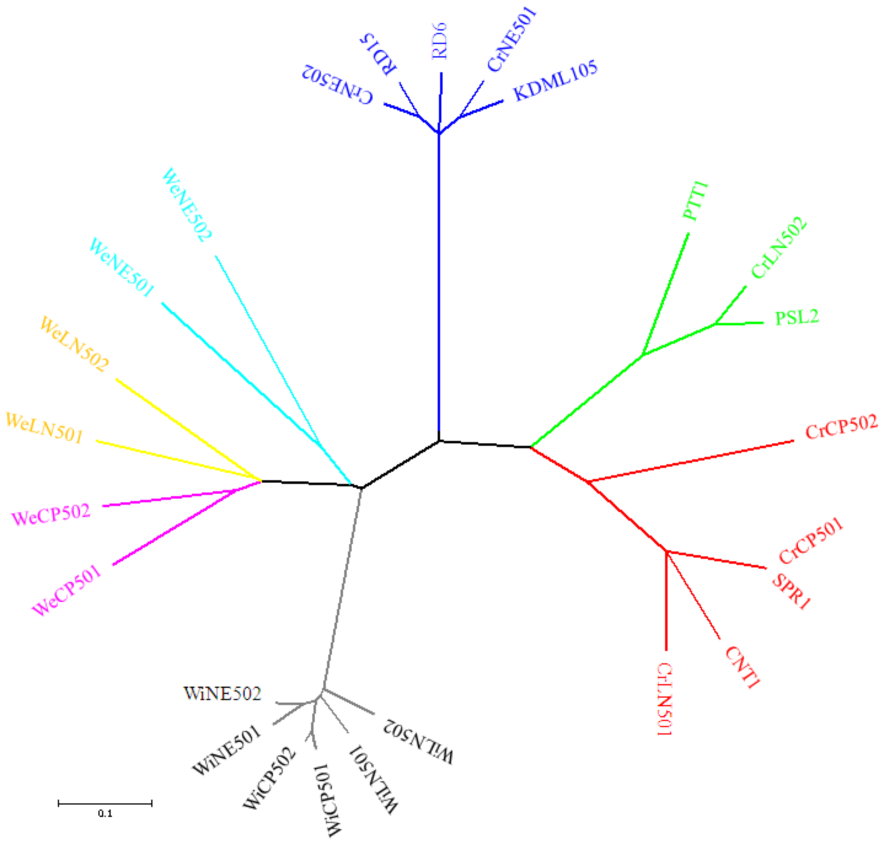


Figure 3.3.4 Cluster analysis using NJ method based on C.S. Chord (1967) genetic distance among sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2005

Populations from wet 2008

Genetic diversity

Genetic diversity with comparison among sympatric wild-weed-crop rice populations from three rice production areas; Lower North (LN), Northeast (NE) and Central Plain (CP) of Thailand in wet 2008 was analyzed by 12 microsatellite markers (Table 3.3.6). Average in three regions, native common wild rice populations showed the highest level of average and total genetic diversity (H_S = 0.603 and H_T = 0.768, respectively) while no variation was detected within companion crop rice populations but high level of total genetic diversity (0.632) was found. Weedy rice populations showed intermediate value of genetic diversity (H_S = 0.447 and H_T = 0.650, respectively) between native common wild rice and companion crop rice populations. Considering mating system, inbreeding coefficient (F_{IS} = 0.217) was the lowest in native common wild rice, on the other hand, outcrossing rate (t = 64%) was the highest. In contrast, companion crop rice was autogamy illustrated by F_{IS} = 1.0, and t = 0%. Whereas weedy rice showed intermediate level of both F_{IS} (0.571) and t (27%) between native common wild rice and companion crop rice (Table 3.3.6).

For among regions of weedy rice populations, the NE showed highest level of average and total genetic diversity (H_S = 0.488 and H_T = 0.691, respectively) following with the CP (H_S = 0.443 and H_T = 0.656, respectively) while the lowest was found in the LN (H_S = 0.421 and H_T = 0.602, respectively). The genetic differentiation (F_{ST}) was highest in the NE (0.613) followed by the CP (0.587) and the LN (0.466) was the lowest with 0.273 in among three regions illustrated that about 27% of genetic variation of those found in weedy rice was the differences between three locations while the rest 73% was distributed between six populations within each location. In mating system, the highest level of F_{IS} was found in the LN (0.603) following with the CP (0.598) and the lowest was the NE (0.521). Conversely, the NE weedy rice exhibited the highest level of t (32%) while the CP (25%) and the LN (27%) were the same as those level (Table 3.3.6).

Genetic structure

(a) Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) was conducted to investigate the overall distribution of genetic diversity among sympatric wild-weed-crop rice populations (Table 3.3.7). About 8% of total variance was partitioned among rice groups. For within each rice group, about 34%, 100% and 32% were the distribution of genetic variation among populations within weedy rice, companion crop rice and native common wild rice, respectively. For within weedy rice, about 47%, 61% and 59% were the distribution of genetic variation among weedy rice populations within the Lower North, the Northeast and the Central Plain, respectively (Table 3.3.7).

(b) Genetic differentiation (F_{ST})

In term of genetic differentiation (F_{ST}), weedy rice exhibited higher level of F_{ST} within region than between regions. Degrees of genetic differentiation (F_{ST}) from the highest to the lowest were weedy rice in the Northeast (0.613), the Central Plain (0.587) and the Lower North (0.466), respectively. Pairwised F_{ST} was highest between weedy rice in the Northeast vs the Central Plain (0.255) while the lowest was found between weedy rice in the Lower North vs the Central Plain (0.124) (Table 3.3.8).

Table 3.3.6 Genetic parameters of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2008 based on 12 microsatellite markers.

Type	N	n	*h*	H_S	H_T	F_{ST}	H_O	F_{IS}	*t*
Companion cultivated rice	**6**	**60**	**0**	**0**	**0.632**	**1**	**0**	**1**	**0**
Lower North	2	20	0	0	0.353	1	0	1	0
Northeast	2	20	0	0	0.343	1	0	1	0
Central Plain	2	20	0	0	0.312	1	0	1	0
Weedy rice	**6**	**60**	**0.452**	**0.447**	**0.650**	**0.273**	**0.194**	**0.571**	**0.273**
Lower North	2	20	0.416	0.412	0.602	0.466	0.165	0.603	0.248
Northeast	2	20	0.493	0.488	0.691	0.613	0.236	0.521	0.315
Central Plain	2	20	0.447	0.443	0.656	0.587	0.180	0.598	0.252
Native common wild rice	**6**	**60**	**0.609**	**0.603**	**0.768**	**0.039**	**0.477**	**0.217**	**0.644**
Lower North	2	20	0.665	0.659	0.727	0.318	0.485	0.271	0.574
Northeast	2	20	0.657	0.650	0.803	0.395	0.542	0.175	0.702
Central Plain	2	20	0.605	0.600	0.775	0.359	0.404	0.199	0.668

Number of populations (N), Number of individuals (n), Observed heterozygosity (H_O), Nei's (1973) gene diversity (*h*), Average gene diversity (H_S), Total gene diversity (H_T), Inbreeding coefficient (F_{IS}) and Outcrossing rate (*t*)

Genetic structure

Table 3.3.7 Analysis of molecular variance (AMOVA) among sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2008 based on 12 microsatellite markers.

Source	df	SS	Variance component	% of the total variance
Among rice groups	2	718.333	1.502	8%
Populations/Group	15	11020.761	17.273	67%
Populations/Weedy rice	5	195.925	1.527	34%
Lower North	*1*	*67.215*	*4.783*	*47%*
Northeast	*1*	*74.366*	*5.292*	*61%*
Central Plain	*1*	*54.344*	*3.867*	*59%*
Populations/Cultivated rice	5	519.847	6.429	100%
Lower North	*1*	*176.748*	*6.301*	*100%*
Northeast	*1*	*192.343*	*6.365*	*100%*
Central Plain	*1*	*150.756*	*6.108*	*100%*
Populations/Common wild rice	5	1886.120	6.065	32%
Lower North	*1*	*645.252*	*8.388*	*32%*
Northeast	*1*	*694.886*	*9.034*	*40%*
Central Plain	*1*	*545.982*	*7.098*	*36%*
Individuals/Population	162	3993.029	6.258	25%

Table 3.3.8 Pairwise of genetic differentiation (F_{ST}) within and between sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2008 based on 12 microsatellite markers.

Pairwise F_{ST}		**Weedy rice**		**Companion crop rice**		**Native common wild rice**	
		Within	**Between**	**Within**	**Between**	**Within**	**Between**
Lower North		0.466		1.000		0.318	
	Northeast		0.206		1.000		0.112
	Central Plain		0.124		1.000		0.081
Northeast		0.613		1.000		0.395	
	Central Plain		0.225		1.000		0.083
Central Plain		0.587		1.000		0.359	

Population structure

Seven pure line rice varieties were structured into 3 inferred populations (K = 3) (Figure 3.3.5) on the basis of 12 microsatellite loci using STRUCTURE program, the first inferred population was SPR1 or CNT1, the second inferred population was PTT1 or PSL2 and the third inferred population was KDML105 or RD15 or RD6, each interred population represent by the presence of red, green and blue colors, respectively. Six companion crop rice (Cr) from three regions were structured as the same popular rice varieties each region such as companion crop rice from the Lower North (LN) was SPR1 or CNT1 (CrLN1) and PTT1 or PSL2 (CrLN1), the Northeast (NE) was KDML105 or RD15 or RD6 (CrNE1 and 2) and the Central Plain (CP) was SPR1 or CNT1 (CrCP1 and 2). Six native common wild rice (Wi) from three regions were structured into 1 inferred populations (K = 1), represent by the presence of gray color (Figure 3.3.5).

Six weedy rice populations from three regions in 2008 resulted from hybridization between native common wild rice and companion crop rice in each region. Evidence of their genetic admixtures in STRUCTURE model was composed genetic proportion between native common wild rice and companion crop rice (Figure 3.3.5 and Figure 3.3.6). Consequently, the population structure of weedy rice populations can be structured into 2 clusters according to its companion crop rice varieties of each region (*K* = 2) (Figure 3.3.5). The admixtures of the first inferred populations consisted of weedy rice populations from the Lower North and the Central Plain. These admixtures contained companion crop genotypes, CNT1 or SPR1 or PTT1 or PSL2, and wild genotype but represented a few genotypes of KDML105 or RD6 or RD15 in some populations of these two regions (Figure 3.3.5

and Figure 3.3.6). The rest of the second inferred population consisted of weedy rice populations from the Northeast. The admixtures contained companion crop genotypes, KDML105 or RD6 or RD15, and wild genotype but represented a few genotypes of CNT1 or SPR1 and PTT1 or PSL2 in populations of this region (Figure 3.3.5 and Figure 3.3.6).

The result of genetic admixtures of sympatric wild-weed-crop rice populations by STRUCTURE is consistent with the distribution of those populations by principal coordinate analysis (PCA) analysis (Figure 3.3.7). The relationship among wild-weed-crop rice populations was displayed that companion crop rice and native common wild rice were widely distributed across the graph while weedy rice groups were distributed between the groups of companion crop rice and native common wild rice in each region but most weedy rice were closely distributed with the group of companion crop rice. Neighbor-join tree also revealed relationship among sympatric wild-weed-crop rice groups illustrated that weedy rice populations were also divided among the groups of companion crop rice varieties and native common wild rice populations but most weedy rice were closely relationship with the group of companion crop rice (Figure 3.3.8).

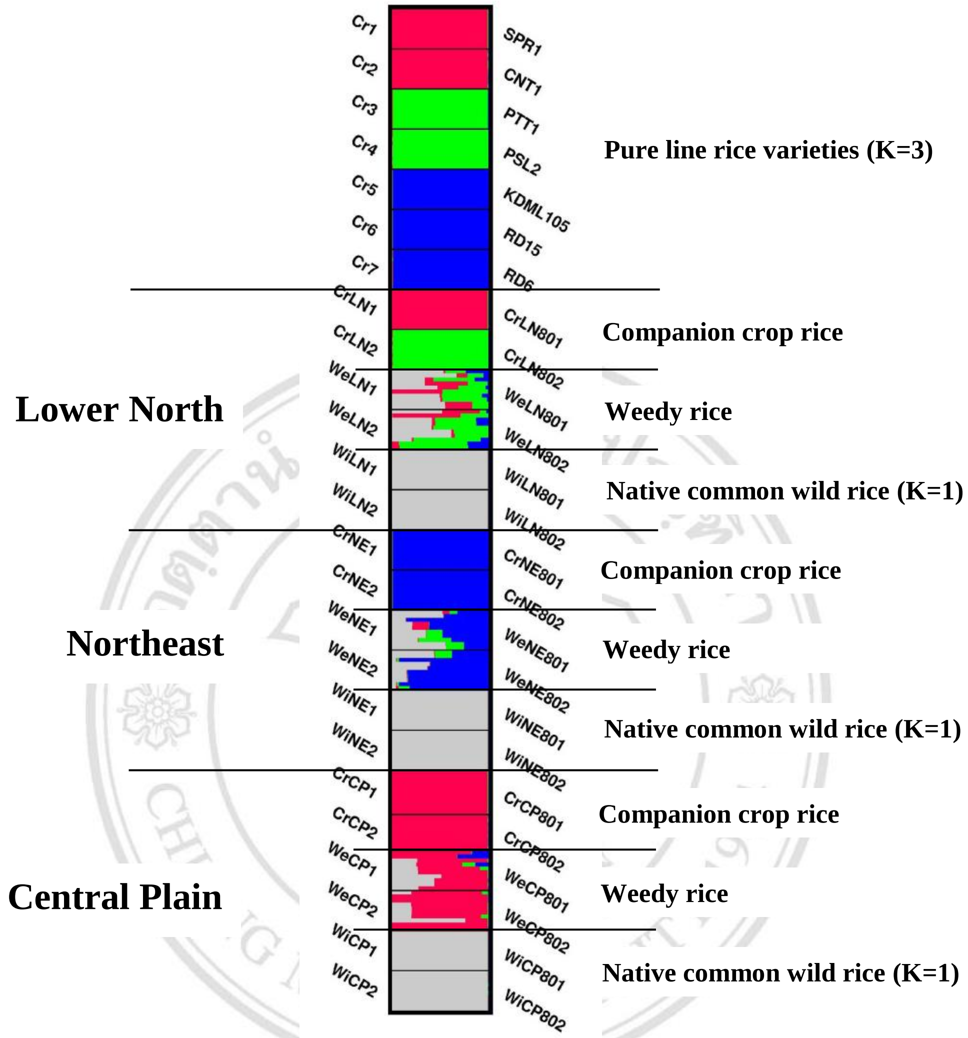


Figure 3.3.5 Population assignment of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2008. Each bar represent each population consisted 10 individuals. Different colors represent different inferred populations, referred to different K.

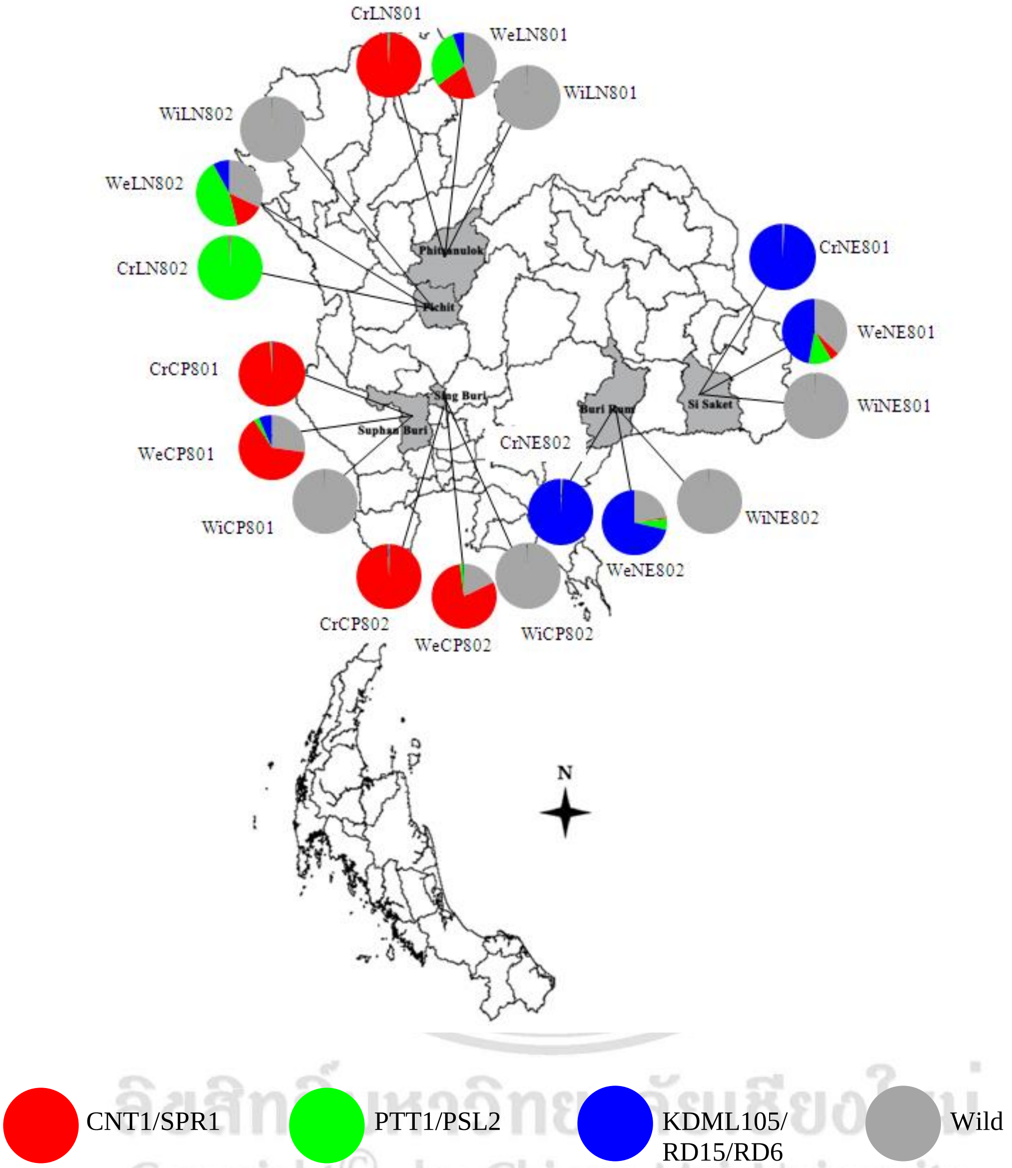
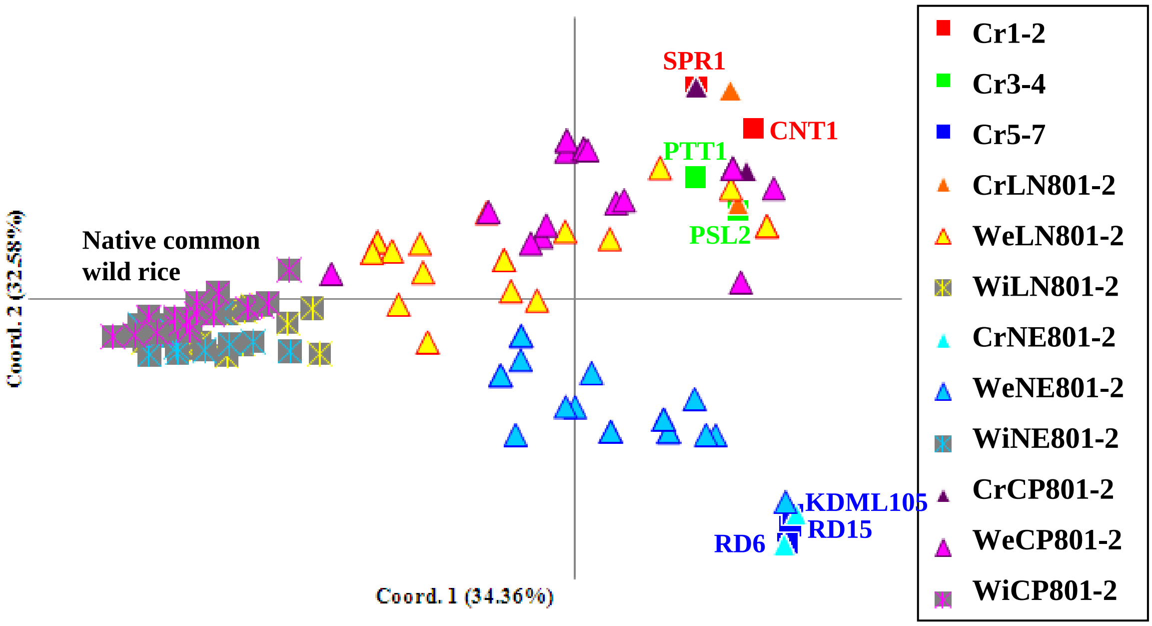


Figure 3.3.6 Genetic proportion of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2008.



Cr1-2= SPR1, CNT1 **Cr3-4 = PTT1, PSL2** **Cr5-7 = KDML105, RD15, RD6**

Figure 3.3.7 Distribution of 180 individuals of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2008 formed by the principal coordinate analysis (PCA) on the basis of 12 microsatellite markers.

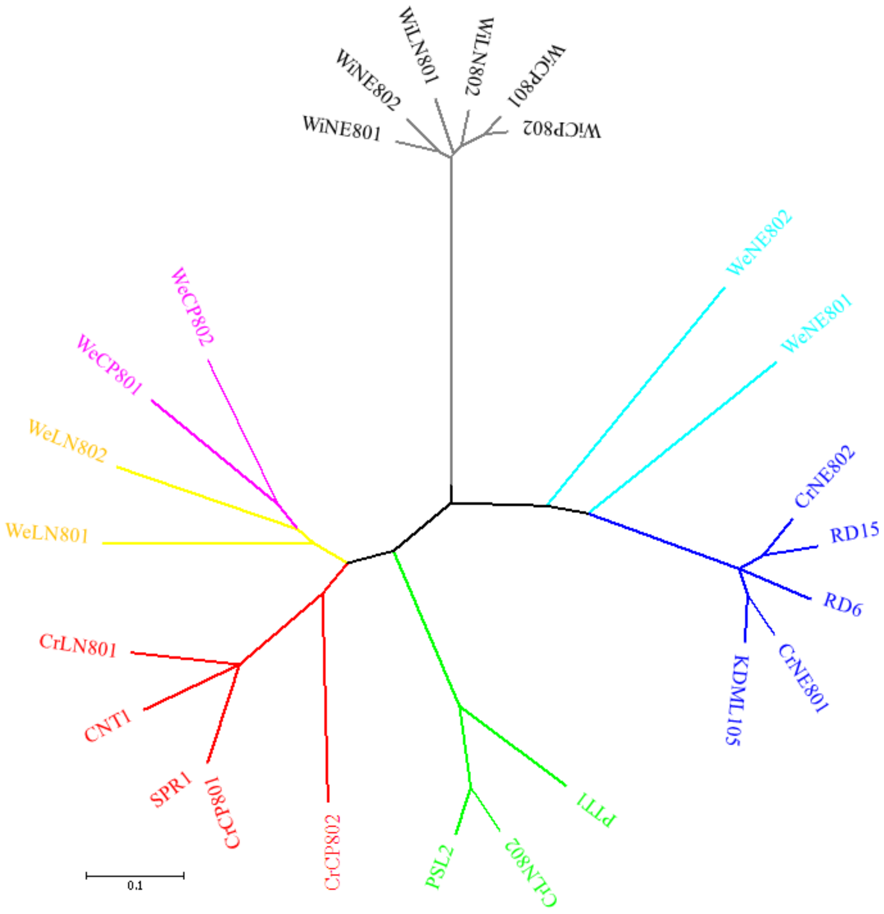


Figure 3.3.8 Cluster analysis using NJ method based on C.S. Chord (1967) genetic distance among sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2008.

<u>*Populations from wet 2009*</u>

Genetic diversity

Genetic diversity with comparison among sympatric wild-weed-crop rice populations from three rice production areas; Lower North (LN), Northeast (NE) and Central Plain (CP) of Thailand in wet 2009 was analyzed by 12 microsatellite markers (Table 3.3.9). Average in three regions, native common wild rice populations showed the highest level of average and total genetic diversity ($H_S = 0.592$ and $H_T = 0.788$, respectively) while no variation was detected within companion crop rice populations but high level of total genetic diversity (0.637) was found. Weedy rice populations showed intermediate value of genetic diversity ($H_S = 0.418$ and $H_T = 0.624$, respectively) between native common wild rice and companion crop rice populations. Considering mating system, inbreeding coefficient ($F_{IS} = 0.214$) was the lowest in native common wild rice, on the other hand, outcrossing rate (t = 65%) was the highest. In contrast, companion crop rice was autogamy illustrated by $F_{IS} = 1.0$, and t = 0%. Whereas weedy rice showed intermediate level of both F_{IS} (0.594) and t (25%) between native common wild rice and companion crop rice (Table 3.3.9).

For among regions of weedy rice populations, the NE showed highest level of average and total genetic diversity ($H_S = 0.472$ and $H_T = 0.659$, respectively) following with the CP ($H_S = 0.392$ and $H_T = 0.615$, respectively) while the lowest was found in the LN ($H_S = 0.390$ and $H_T = 0.598$, respectively). The genetic differentiation (F_{ST}) was highest in NE (0.626) followed by the CP (0.598) and the LN (0.477) was the lowest with 0.318 in among three regions illustrated that about 32% of genetic variation of those found in weedy rice was the differences between three locations while the rest 68% was distributed between six populations within each location. In mating system, the highest level of F_{IS} was found in the LN (0.587) following with the CP (0.568) and the lowest was the NE (0.564). Conversely, the NE and the CP weedy rice exhibited the same as of highest level of t (28%) while the LN (26%) was the lowest of those level (Table 3.3.9).

Genetic structure

(a) Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) was conducted to investigate the overall distribution of genetic diversity among sympatric wild-weed-crop rice populations (Table 3.3.10). About 8% of total variance was partitioned among rice groups. For within each rice group, about 34%, 100% and 33% were the distribution of genetic variation among populations within weedy rice, companion crop rice and native common wild rice, respectively. For within weedy rice, about 48%, 63% and 60% were the distribution of genetic variation among weedy rice populations within the Lower North, the Northeast and the Central Plain, respectively (Table 3.3.10).

(b) Genetic differentiation (F_{ST})

In term of genetic differentiation (F_{ST}), weedy rice exhibited higher level of F_{ST} within region than between regions. Degrees of genetic differentiation (F_{ST}) from the highest to the lowest were weedy rice in the Northeast (0.626), the Central Plain (0.598) and the Lower North (0.477), respectively. Pairwised F_{ST} was highest between weedy rice in the Northeast vs the Central Plain (0.253) while the lowest was found between weedy rice in the Lower North vs the Central Plain (0.142) (Table 3.3.11).

Table 3.3.9 Genetic parameters of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2009 based on 12 microsatellite markers.

Type	N	n	*h*	H_S	H_T	F_{ST}	H_O	F_{IS}	*t*
Companion cultivated rice	**6**	**60**	**0**	**0**	**0.637**	**1**	**0**	**1**	**0**
Lower North	2	20	0	0	0.353	1	0	1	0
Northeast	2	20	0	0	0.376	1	0	1	0
Central Plain	2	20	0	0	0.315	1	0	1	0
Weedy rice	**6**	**60**	**0.422**	**0.418**	**0.624**	**0.318**	**0.171**	**0.594**	**0.254**
Lower North	2	20	0.394	0.390	0.598	0.477	0.163	0.587	0.260
Northeast	2	20	0.477	0.472	0.659	0.626	0.206	0.564	0.279
Central Plain	2	20	0.396	0.392	0.615	0.598	0.173	0.568	0.276
Native common wild rice	**6**	**60**	**0.598**	**0.592**	**0.788**	**0.038**	**0.470**	**0.214**	**0.647**
Lower North	2	20	0.644	0.638	0.737	0.355	0.476	0.261	0.586
Northeast	2	20	0.644	0.637	0.863	0.389	0.553	0.141	0.753
Central Plain	2	20	0.607	0.602	0.765	0.352	0.432	0.247	0.604

Number of populations (N), Number of individuals (n), Observed heterozygosity (H_O), Nei's (1973) gene diversity (*h*), Average gene diversity (H_S), Total gene diversity (H_T), Inbreeding coefficient (F_{IS}) and Outcrossing rate (*t*)

Genetic structure

Table 3.3.10 Analysis of molecular variance (AMOVA) among sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2009 based on 12 microsatellite markers.

Source	df	SS	Variance component	% of the total variance
Among rice groups	2	698.333	1.489	8%
Populations/Group	15	11873.461	12.657	68%
Populations/Weedy rice	5	165.973	1.564	34%
Lower North	*1*	*54.067*	*6.114*	*48%*
Northeast	*1*	*64.126*	*7.252*	*63%*
Central Plain	*1*	*47.780*	*5.403*	*60%*
Populations/Cultivated rice	5	560.069	6.429	100%
Lower North	*1*	*190.423*	*6.301*	*100%*
Northeast	*1*	*207.226*	*6.365*	*100%*
Central Plain	*1*	*162.420*	*6.108*	*100%*
Populations/Common wild rice	5	1722.354	6.555	33%
Lower North	*1*	*589.226*	*9.065*	*35%*
Northeast	*1*	*634.552*	*9.763*	*39%*
Central Plain	*1*	*498.576*	*7.671*	*35%*
Individuals/Population	162	4301.979	6.204	24%

Table 3.3.11 Pairwise of genetic differentiation (F_{ST}) within and between sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2009 based on 12 microsatellite markers.

Pairwise F_{ST}		**Weedy rice**		**Companion crop rice**		**Native common wild rice**	
		Within	**Between**	**Within**	**Between**	**Within**	**Between**
Lower North		0.477		1.000		0.355	
	Northeast		0.240		1.000		0.108
	Central Plain		0.142		1.000		0.084
Northeast		0.626		1.000		0.389	
	Central Plain		0.253		1.000		0.087
Central Plain		0.598		1.000		0.352	

Population structure

Seven pure line rice varieties were structured into 3 inferred populations (K = 3) (Figure 3.3.9) on the basis of 12 microsatellite loci using STRUCTURE program, the first inferred population was SPR1 or CNT1, the second inferred population was PTT1 or PSL2 and the third inferred population was KDML105 or RD15 or RD6, each interred population represent by the presence of red, green and blue colors, respectively. Six companion crop rice (Cr) from three regions were structured as the same popular rice varieties each region such as companion crop rice from the Lower North (LN) was PTT1 or PSL2 (CrLN1 and 2), the Northeast (NE) was KDML105 or RD15 or RD6 (CrNE1 and 2) and the Central Plain (CP) was SPR1 or CNT1 (CrCP1 and 2). Six native common wild rice (Wi) from three regions were structured into 1 inferred populations (K = 1), represent by the presence of gray color (Figure 3.3.9).

Six weedy rice populations from three regions in 2009 resulted from hybridization between native common wild rice and companion crop rice in each region. Evidence of their genetic admixtures in STRUCTURE model was composed genetic proportion between native common wild rice and companion crop rice (Figure 3.3.9 and Figure 3.3.10). Consequently, the population structure of weedy rice populations can be structured into 2 clusters according to its companion crop rice varieties of each region (K = 2) (Figure 3.3.9). The admixtures of the first inferred populations consisted of weedy rice populations from the Lower North and the Central Plain. These admixtures contained companion crop genotypes, CNT1 or SPR1 or PTT1 or PSL2, and wild genotype but represented a few genotypes of KDML105 or RD6 or RD15 in populations of these two regions (Figure 3.3.9 and Figure 3.3.10). The rest of the second inferred population consisted of weedy rice populations from the Northeast. The admixtures contained companion crop genotypes, KDML105 or RD6 or RD15, and wild genotype but represented a few genotypes of CNT1 or SPR1 and PTT1 or PSL2 in populations of this region (Figure 3.3.9 and Figure 3.3.10).

The result of genetic admixtures of sympatric wild-weed-crop rice populations by STRUCTURE is consistent with the distribution of those populations by principal coordinate analysis (PCA) analysis (Figure 3.3.11). The relationship among wild-weed-crop rice populations was displayed that companion crop rice and native common wild rice were widely distributed across the graph while weedy rice groups were distributed between the groups of companion crop rice and native common wild rice in each region but most weedy rice were closely distributed with the group of companion crop rice each region. Neighbor-join tree also revealed relationship among sympatric wild-weed-crop rice groups illustrated that weedy rice populations were also divided among the groups of companion crop rice varieties and native common wild rice populations but most weedy rice were closely relationship with the group of companion crop rice each region (Figure 3.3.12).

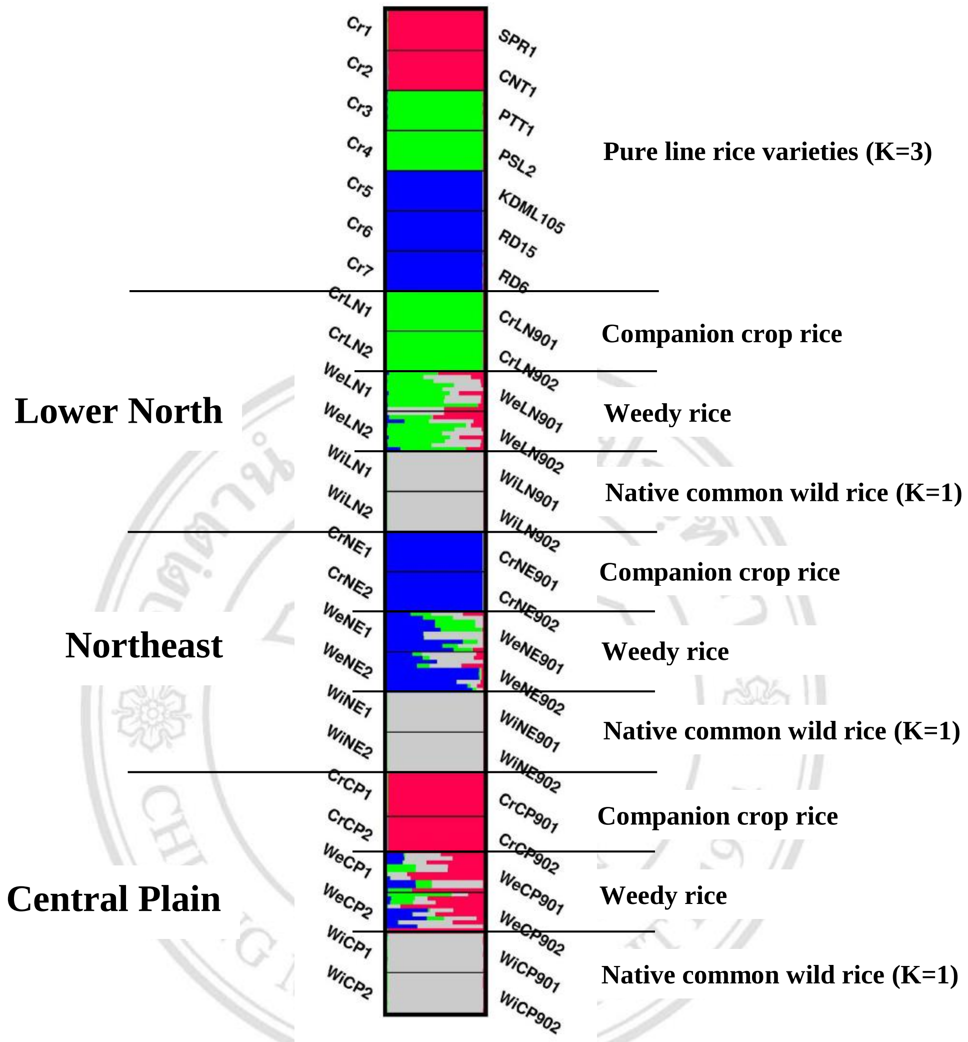


Figure 3.3.9 Population assignment of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2009. Each bar represent each population consisted 10 individuals. Different colors represent different inferred populations, referred to different K.

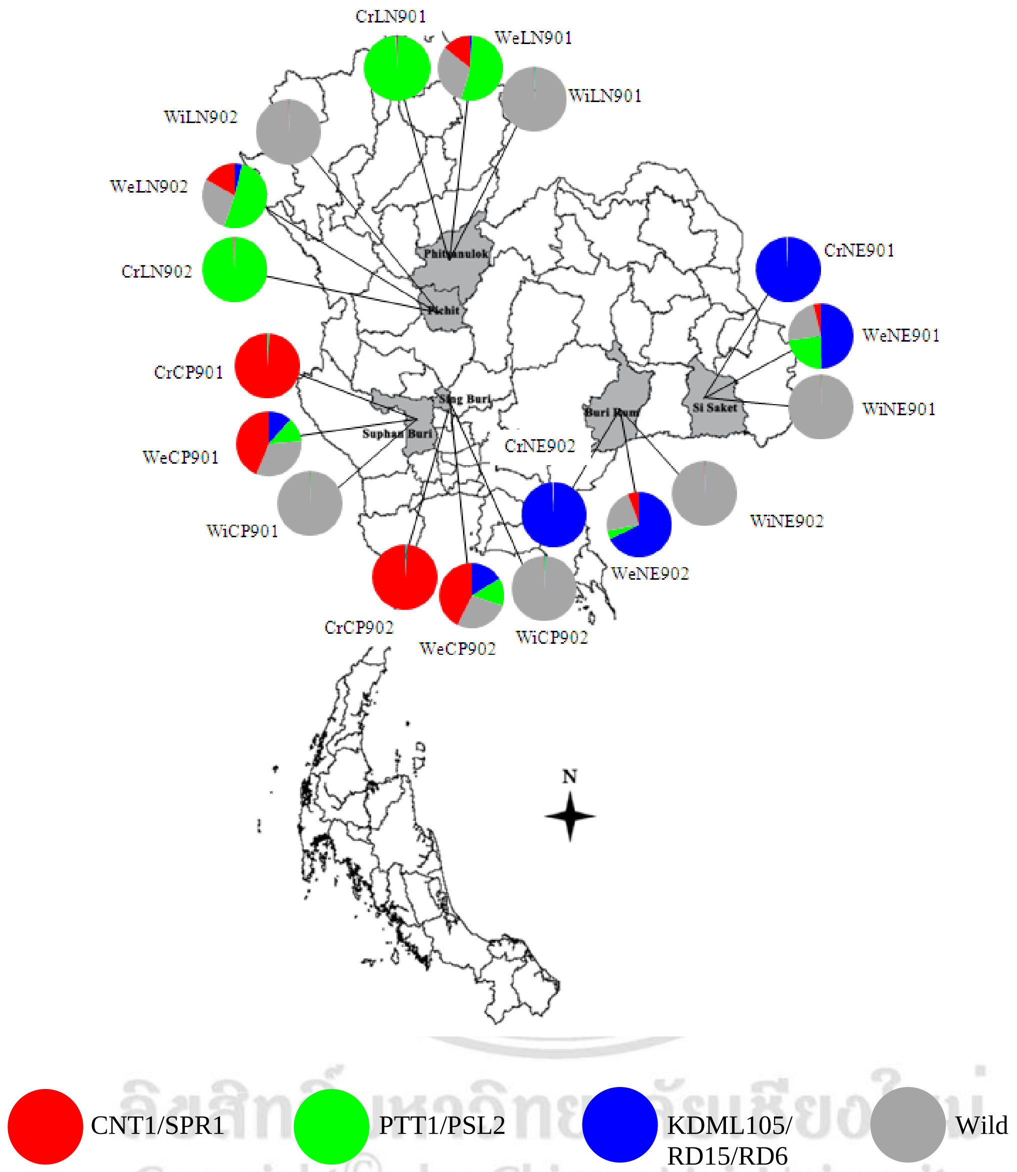
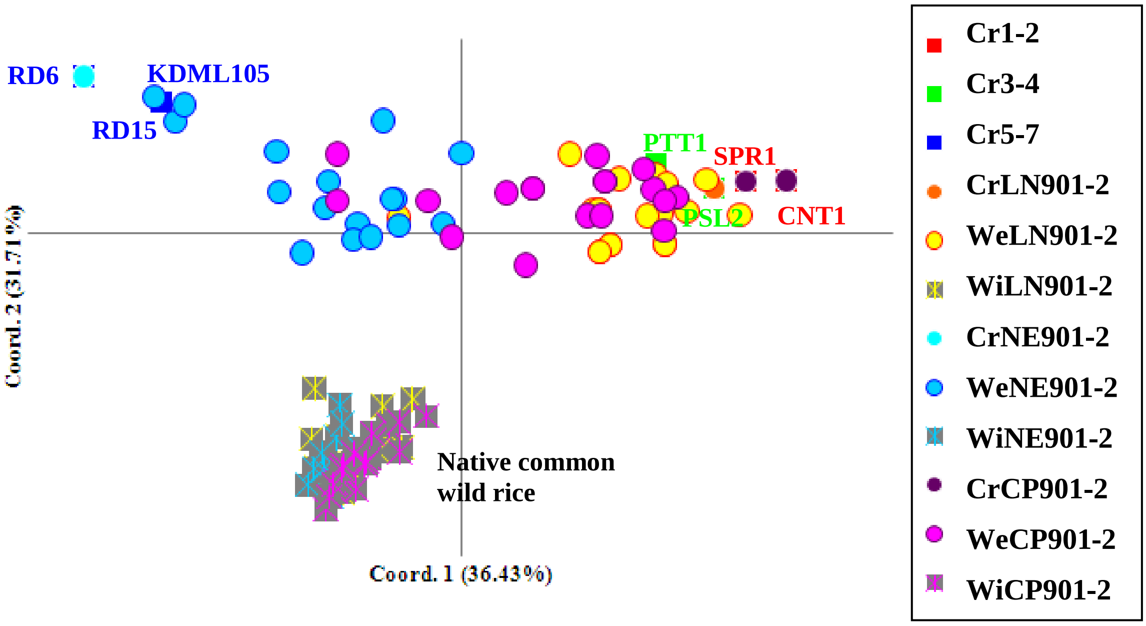


Figure 3.3.10 Genetic proportion of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2009.



Cr1-2= SPR1, CNT1 **Cr3-4 = PTT1, PSL2** **Cr5-7 = KDML105, RD15, RD6**

Figure 3.3.11 Distribution of 180 individuals of sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2009 formed by the principal coordinate analysis (PCA) on the basis of 12 microsatellite markers.

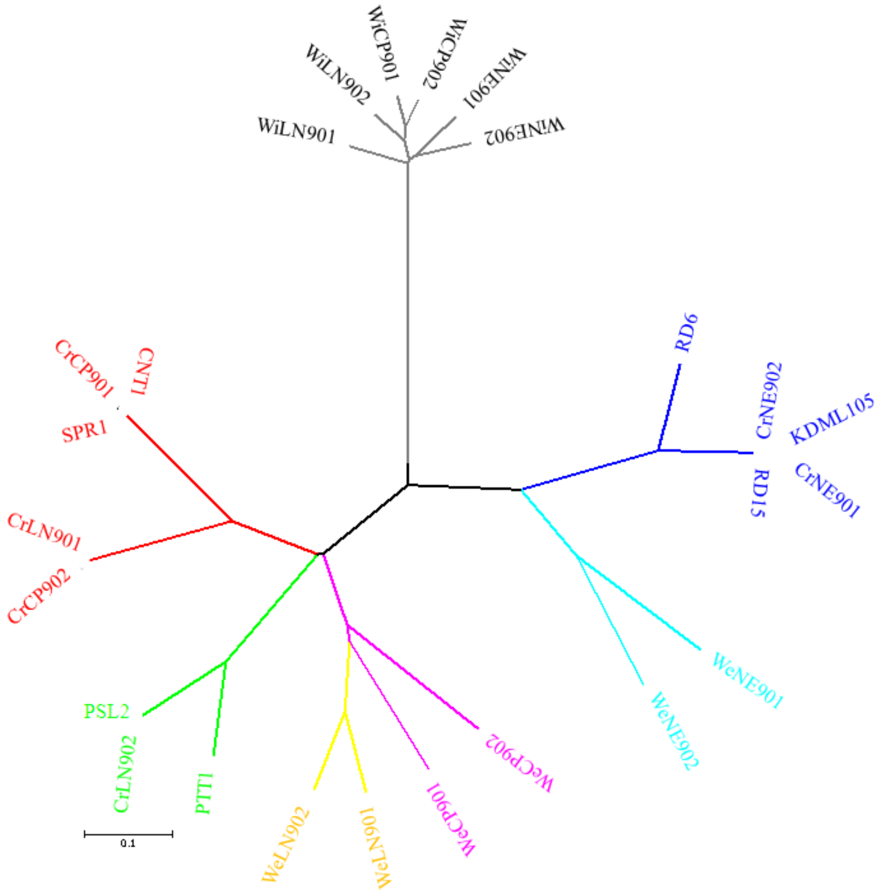


Figure 3.3.12 Cluster analysis using NJ method based on C.S. Chord (1967) genetic distance among sympatric wild-weed-crop rice populations from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet 2009.

Population genetic structure and dynamics in temporal term of sympatric wild-weed-crop rice populations in Thailand

Genetic diversity

Genetic diversity from all sympatric wild-weed-crop rice populations in three growing seasons were analyzed together (Figure 3.3.13 and Table 3.3.12). No variation was detected within eighteen populations of companion crop rice but high level of total genetic diversity was found (H_S = 0 and H_T = 0.652, respectively). In contrast, eighteen native common wild rice populations showed the highest level of average and total genetic diversity (H_S = 0.605 and H_T = 0.825, respectively). Eighteen weedy rice populations showed intermediate value of genetic diversity (H_S = 0.473 and H_T = 0.784, respectively) between each eighteen native common wild rice and companion crop rice populations (Table 3.3.12).

For compared among three seasons, genetic diversity of eighteen weedy rice populations was decreased with year of infestation (Figure 3.3.13 and Table 3.3.12). Weedy rice in 2005 exhibited the highest genetic variation including observed heterozygosity (H_O = 0.275), Nei's gene diversity (h = 0.560), average gene diversity (H_S = 0.553) and total gene diversity (H_T = 0.764), following in populations of 2008 (H_O = 0.194, h = 0.452, H_S = 0.447 and H_T = 0.650) while the populations of 2009 was the lowest (H_O = 0.171, h = 0.422, H_S = 0.418 and H_T = 0.624). On the other hand, the highest level of genetic differentiation (F_{ST}) was found in 2009 (0.567) following with in 2008 (0.555) and the lowest in 2005 (0.422). Considering mating system, inbreeding coefficient (F_{IS}) was the highest in weedy rice in 2009 (0.594) following in 2008 (0.571) and the lowest in 2005 (0.509), respectively. Conversely, outcrossing rate (*t*) was the highest in weedy rice of 2005 (33%) while the lowest in 2009 was 25% (Figure 3.3.13 and Table 3.3.12).

Genetic structure

(a) Analysis of Molecular Variance (AMOVA)

Analysis of molecular variance (AMOVA) was conducted to investigate the overall distribution of genetic diversity among sympatric wild-weed-crop rice populations (Table 3.3.13). About 7% of total variance was partitioned among rice groups. For within each rice group, about 33%, 100% and 36% were the distribution of genetic variation among populations within weedy rice, companion crop rice and native common wild rice, respectively. For within weedy rice, about 42%, 56% and 57% were the distribution of genetic variation among weedy rice populations within 2005, 2008 and 2009, respectively (Table 3.3.13).

(c) Genetic differentiation (F_{ST})

In term of genetic differentiation (F_{ST}), weedy rice exhibited higher level of F_{ST} within season than between seasons. Degrees of genetic differentiation (F_{ST}) from the highest to the lowest were weedy rice in 2009, 2008 and 2005, respectively. Pairwised F_{ST} was highest between weedy rice in 2008 vs 2009 (0.081) while the lowest was found between weedy rice in 2005 vs 2009 (0.145) (Table 3.3.14).

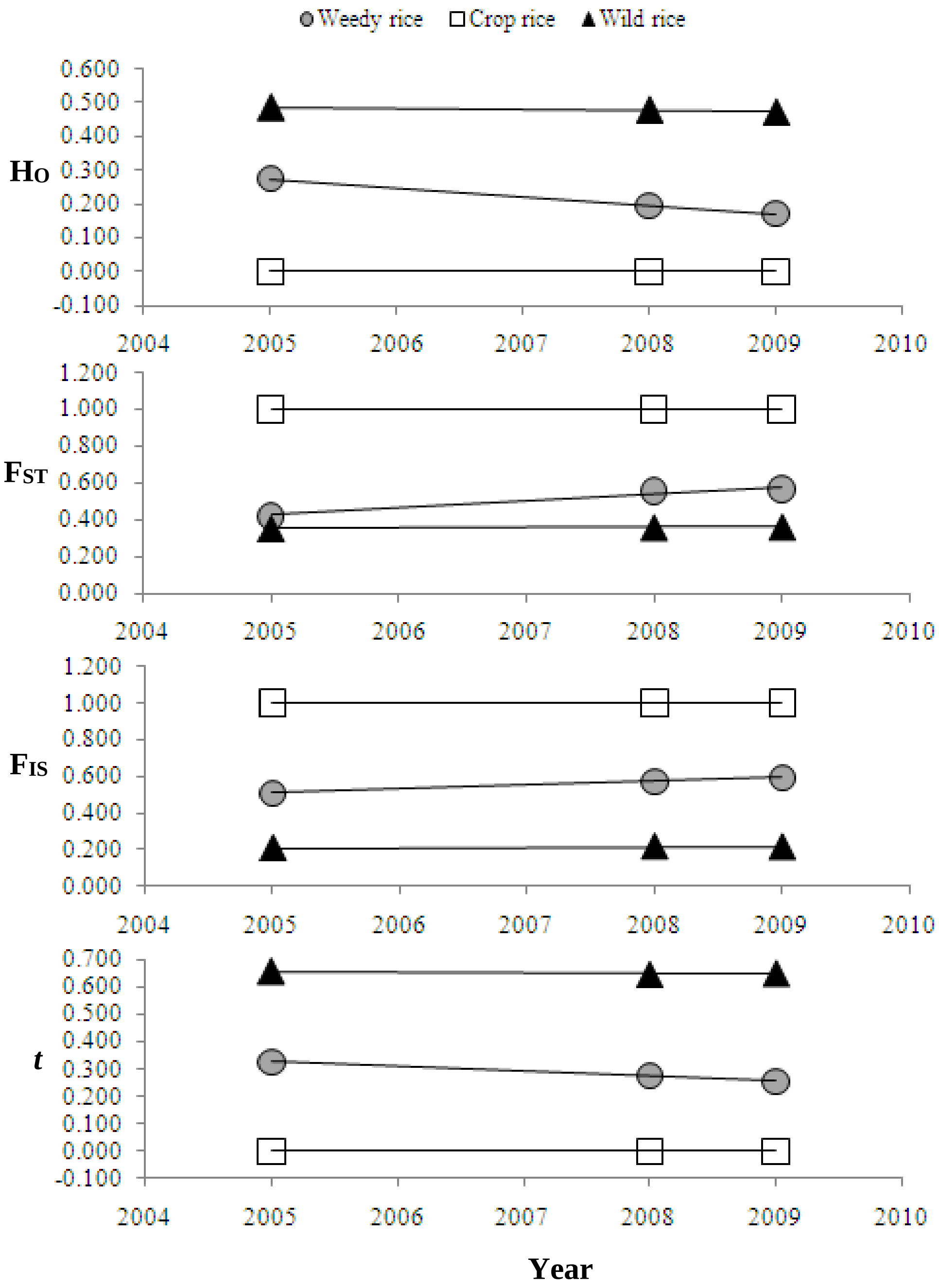


Figure 3.3.13 Observed heterozygosity (H_O), Genetic differentiation (F_{ST}), Inbreeding coefficient (F_{IS}) and Outcrossing rate (*t*) among companion crop rice, weedy rice and native common wild rice populations collected from three growing seasons in 2005, 2008 and 2009, using 12 SSR markers.

Table 3.3.12 Genetic parameters of 18 companion crop rice, 18 weedy rice and 18 native common wild rice populations collected from three growing seasons in 2005, 2008 and 2009, using 12 SSR markers.

Population	**N**	**n**	***h***	**H_S**	**H_T**	**F_{ST}**	**H_O**	**F_{IS}**	***t***
Weedy rice	**18**	**180**	**0.478**	**0.473**	**0.784**	**0.515**	**0.213**	**0.554**	**0.287**
wet 2005	6	60	0.560	0.553	0.764	0.422	0.275	0.509	0.325
wet 2008	6	60	0.452	0.447	0.650	0.555	0.194	0.571	0.273
wet 2009	6	60	0.422	0.418	0.624	0.567	0.171	0.594	0.254
Cultivated rice	**18**	**180**	**0**	**0**	**0.652**	**1.000**	**0**	**1**	**0**
wet 2005	6	60	0	0	0.639	1.000	0	1	0
wet 2008	6	60	0	0	0.632	1.000	0	1	0
wet 2009	6	60	0	0	0.637	1.000	0	1	0
Common wild rice	**18**	**180**	**0.611**	**0.605**	**0.825**	**0.360**	**0.484**	**0.208**	**0.656**
wet 2005	6	60	0.611	0.605	0.784	0.357	0.484	0.208	0.656
wet 2008	6	60	0.609	0.603	0.768	0.363	0.477	0.217	0.644
wet 2009	6	60	0.598	0.592	0.788	0.365	0.470	0.214	0.647

Number of populations (N), Number of individuals (n), Observed heterozygosity (H_O), Nei’s (1973) gene diversity (*h*), Average gene diversity (H_S), Total gene diversity (H_T), Inbreeding coefficient (F_{IS}) and Outcrossing rate (*t*)

Genetic structure

Table 3.3.13 Analysis of molecular variance (AMOVA) among populations of each for 540 individuals of 18 companion crop rice, 18 weedy rice and 18 native common wild rice populations collected from three growing seasons in 2005, 2008 and 2009, using 12 SSR markers.

Source	df	SS	Variance component	% of the total variance
Among rice groups	2	1232.897	2.170	7%
Populations/Group	15	11800.590	7.234	67%
Populations/Weedy rice	5	2671.832	6.944	33%
2005	*1*	*768.609*	*6.076*	*42%*
2008	*1*	*1024.812*	*8.102*	*56%*
2009	*1*	*1043.112*	*8.247*	*57%*
Populations/Cultivated rice	5	556.632	6.294	100%
2005	*1*	*189.255*	*6.168*	*100%*
2008	*1*	*205.954*	*6.231*	*100%*
2009	*1*	*161.423*	*5.979*	*100%*
Populations/Common wild rice	5	3562.442	9.259	36%
2005	*1*	*1166.782*	*6.597*	*36%*
2008	*1*	*1186.391*	*6.635*	*36%*
2009	*1*	*1209.269*	*6.754*	*37%*
Individuals/Population	162	4579.333	5.064	26%

Table 3.3.14 Pairwise of genetic differentiation (F_{ST}) within and between 18 companion crop rice, 18 weedy rice and 18 native common wild rice populations collected from three growing seasons in 2005, 2008 and 2009, using 12 SSR markers.

Pairwise F_{ST}		**Weedy rice**		**Companion crop rice**		**Native common wild rice**	
		Within	**Between**	**Within**	**Between**	**Within**	**Between**
2005		0.422		1.000		0.357	
	2008		0.136		1.000		0.190
	2009		0.145		1.000		0.260
2008		0.555		1.000		0.363	
	2009		0.081		1.000		0.071
2009		0.567		1.000		0.365	

Population structure

Population structure from all sympatric wild-weed-crop rice populations in three growing seasons were analyzed together (Figure 3.3.14 to Figure 3.3.15). Seven pure line rice varieties were structured into 3 inferred populations (K = 3) (Figure 3.3.14) on the basis of 12 microsatellite loci using STRUCTURE program, the first inferred population was SPR1 or CNT1, the second inferred population was PTT1 or PSL2 and the third inferred population was KDML105 or RD15 or RD6, each interred population represent by the presence of red, green and blue colors, respectively. Six companion crop rice (Cr) from three regions were structured as the same popular rice varieties each region such as companion crop rice from the Lower North (LN) was SPR1 or CNT1 (CrLN1, in 2005 and 2008 but in 2009 was PTT1 or PSL2) and PTT1 or PSL2 (CrLN2), the Northeast (NE) was KDML105 or RD15 or RD6 (CrNE1 and 2) and the Central Plain (CP) was SPR1 or CNT1 (CrCP1 and 2). Six native common wild rice (Wi) from three regions were structured into 1 inferred populations (K = 1), represent by the presence of gray color (Figure 3.3.14).

Eighteen weedy rice populations from three growing seasons in 2005, 2008 and 2009 resulted from hybridization between native common wild rice and companion crop rice in each region. Evidence of their genetic admixtures in STRUCTURE model was composed genetic proportion between native common wild rice and companion crop rice but the higher proportions of companion crop genotypes were found than wild genotype and tended to increase with years of infestation (Figure 3.3.14 to Figure 3.3.15). Consequently, the population structure of weedy rice populations can be structured into 2 clusters according to its companion crop rice varieties of each region (*K* = 2) (Figure 3.3.14). The admixtures of the first inferred populations consisted of weedy rice populations from the Lower North and the Central Plain. These admixtures contained companion crop genotypes, CNT1 or SPR1 and PTT1 or PSL2, and wild genotype but genotype of PTT1 or PSL2 was increased with year of infestation. In 2008 and 2009, weedy rice populations from the Lower North and the Central Plain represented a few genotypes of KDML105 or RD6 or RD15 in populations of these two regions (Figure 3.3.14 to Figure 3.3.15). The rest of the second inferred population consisted of weedy rice populations from the Northeast. The admixtures contained companion crop genotypes, KDML105 or RD6 or RD15, and wild genotype but represented a few genotypes of CNT1 or SPR1 and PTT1 or PSL2 in populations of this region and tended to increase with year of infestation (Figure 3.3.14 to Figure 3.3.15). When considering among seasons, the proportions of cultivar genotypes in weedy rice from all regions were found more than wild genotype and trend to increase with years of infestation. This increasing of the proportion of cultivar genotypes may caused by the subsequently backcross of weedy rice to companion crop rice in rice fields resulting the rapidly loss of wild traits in segregating populations.

The result of genetic admixtures of sympatric wild-weed-crop rice populations by STRUCTURE is consistent with the distribution of those populations by principal coordinate analysis (PCA) analysis (Figure 3.3.16). The relationship among wild-weed-crop rice populations was displayed that companion crop rice and native common wild rice were widely distributed across the graph while weedy rice groups were distributed between the groups of companion crop rice and native common wild rice in each region but tended to closer toward the group of companion crop rice with years of infestation in all regions. Neighbor-join tree also revealed relationship among sympatric wild-weed-crop rice groups illustrated that weedy rice populations were also divided among the groups of companion crop rice varieties and native common wild rice populations but weedy rice were tended to closely relationship with the group of companion crop rice each region (Figure 3.3.17).

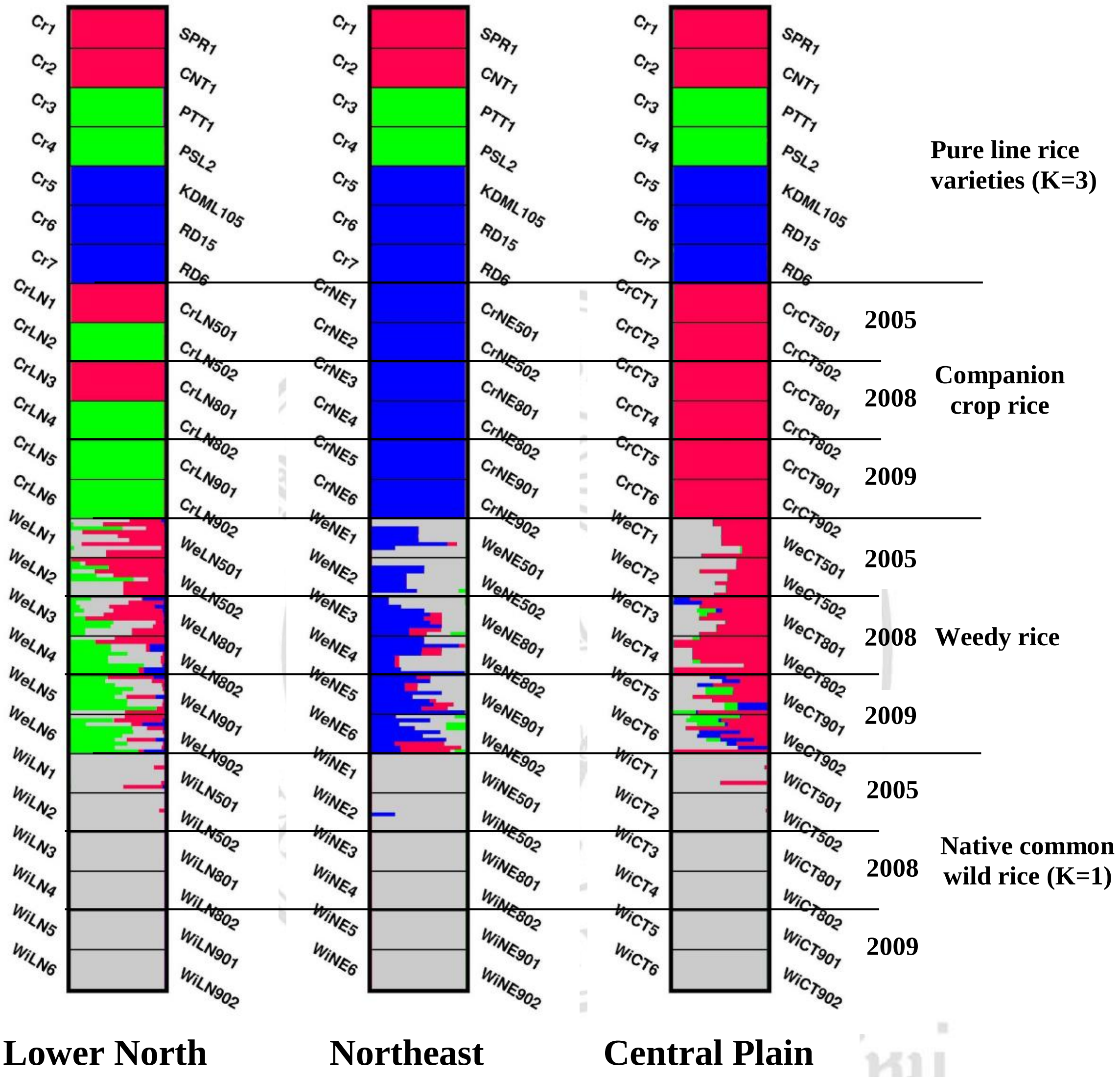
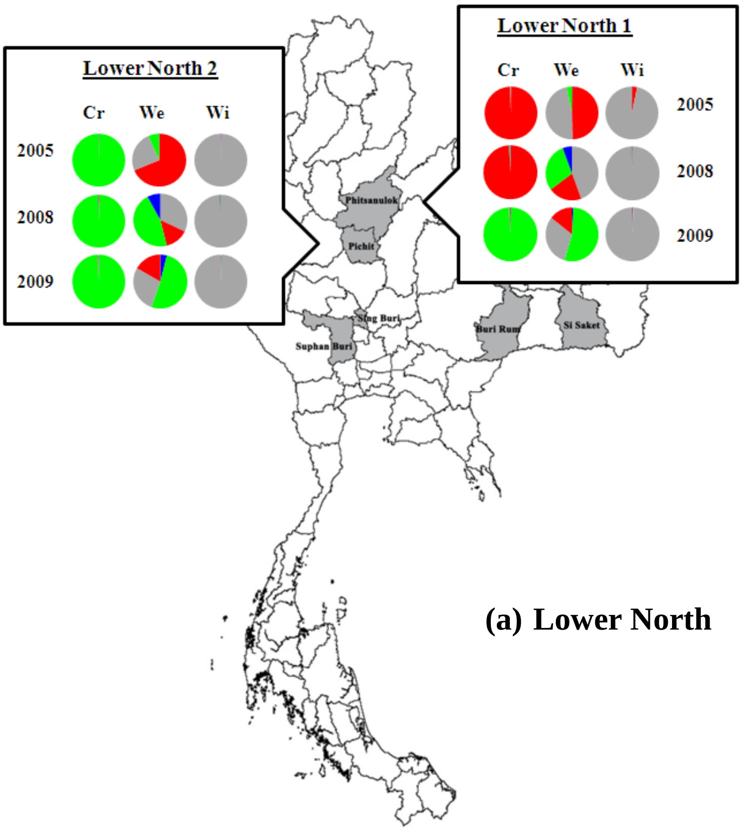
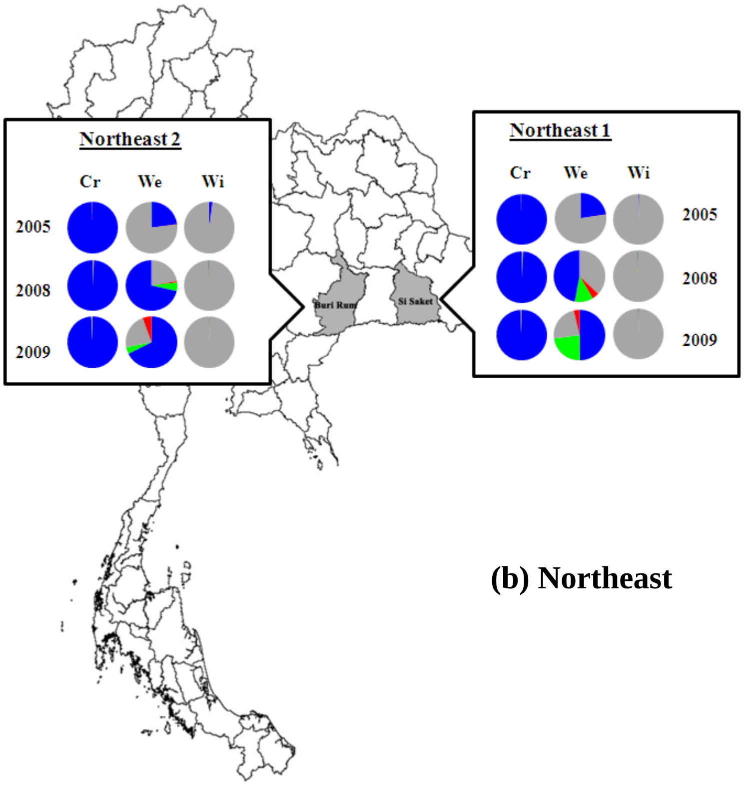


Figure 3.3.14 Population assignment using STRUCTURE analysis of 18 companion crop rice, 18 weedy rice and 18 native common wild rice populations collected from three rice production areas; Lower North (LN), Northeast (NE) and Central Plain (CT), in three growing seasons in 2005, 2008 and 2009, compared with 7 pure line cultivated rice varieties. Each bar represented each population consist 10 individuals. Different colors represent different inferred populations (*K* = 4).

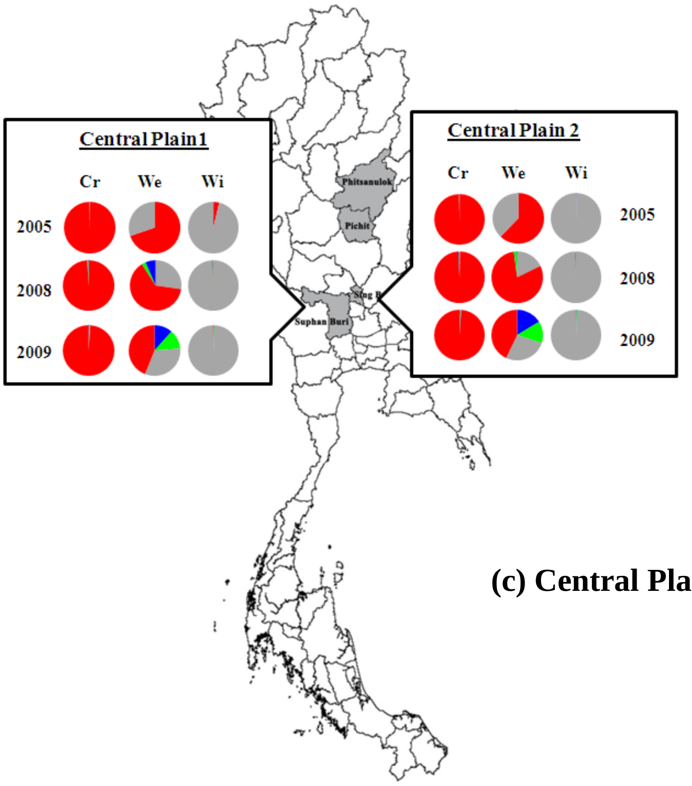


(a) Lower North

Figure 3.3.15 Genetic proportion of 18 companion crop rice, 18 weedy rice and 18 native common wild rice populations collected from three rice production areas; Lower North (a), Northeast (b) and Central Plain (c), in three growing seasons in 2005, 2008 and 2009.

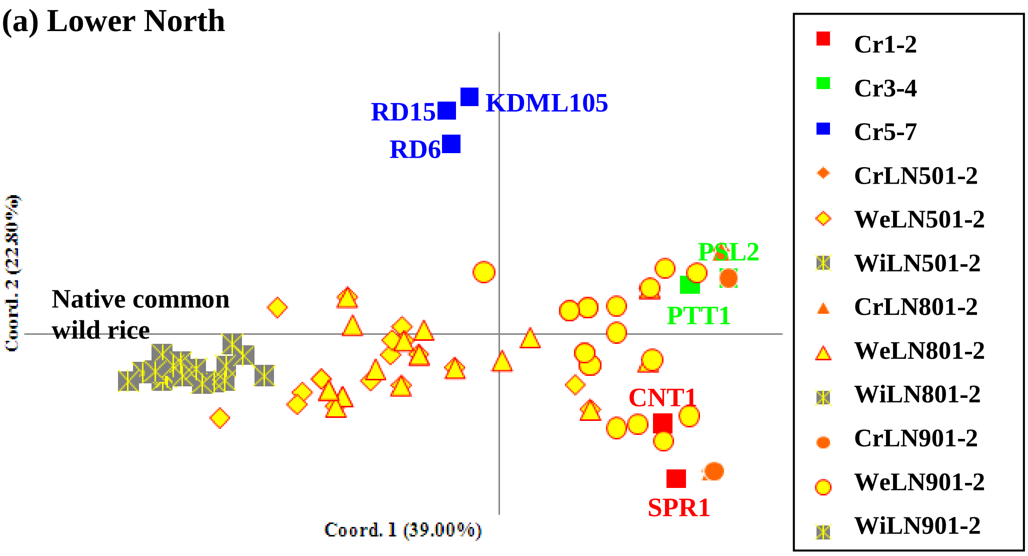


(b) Northeast

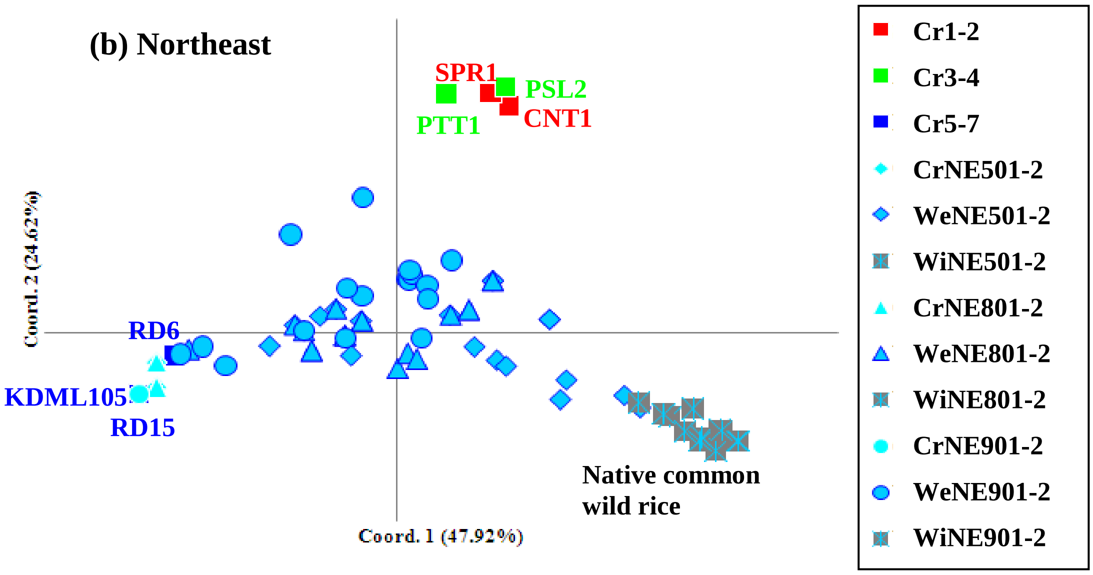


(c) Central Plain

Figure 3.3.15 *(continued)*



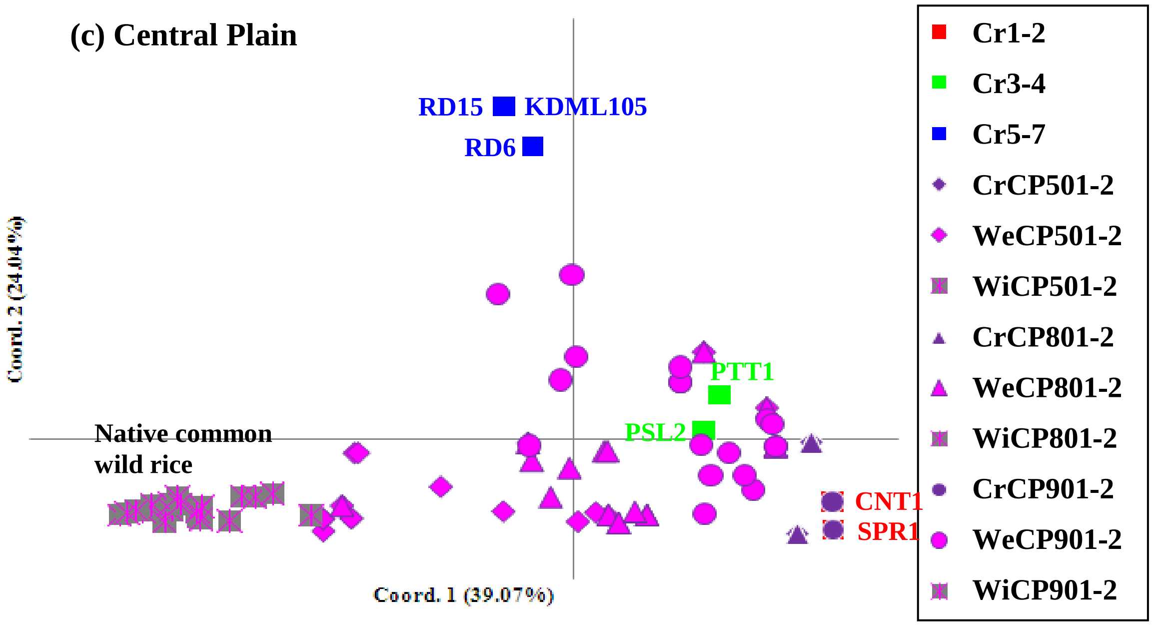
(a) Lower North



(b) Northeast

Cr1-2= SPR1, CNT1 **Cr3-4 = PTT1, PSL2** **Cr5-7 = KDML105, RD15, RD6**

Figure 3.3.16 Principle component analysis (PCA) of 18 companion crop rice, 18 weedy rice and 18 native common wild rice populations collected from three rice production areas; Lower North (a), Northeast (b) and Central Plain (c) in different three growing seasons in wet 2005, 2008 and 2009 compared with 7 pure line cultivated rice varieties. Different colors of weedy rice samples represent different regions.



Cr1-2= SPR1, CNT1

Cr3-4 = PTT1, PSL2

Cr5-7 = KDML105, RD15, RD6

Figure 3.3.16 *(continued)*

(a) Lower North

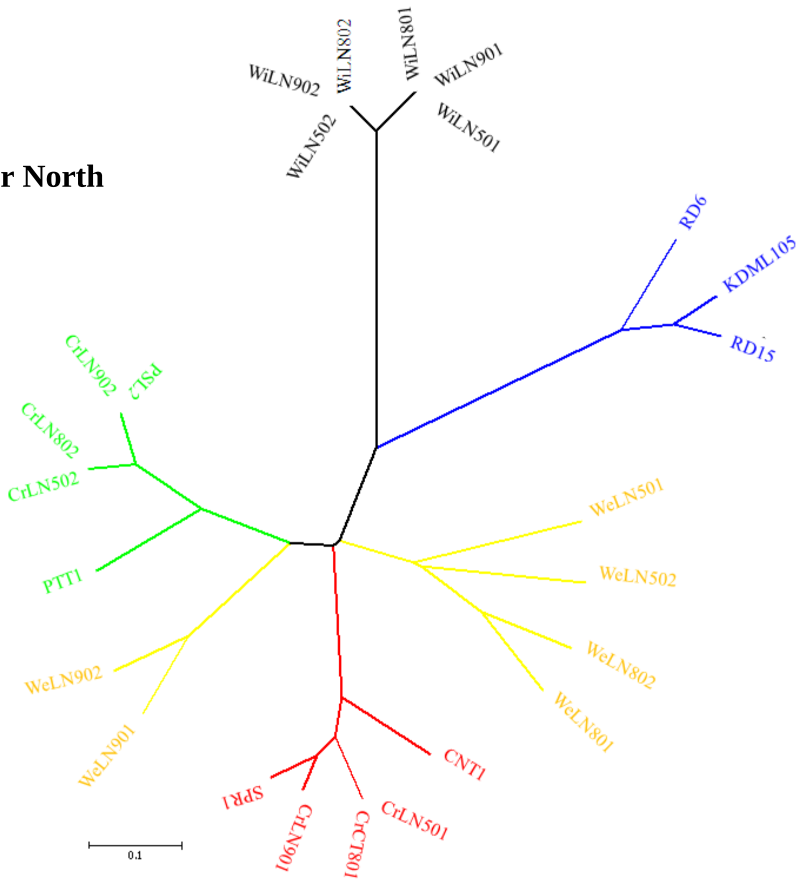
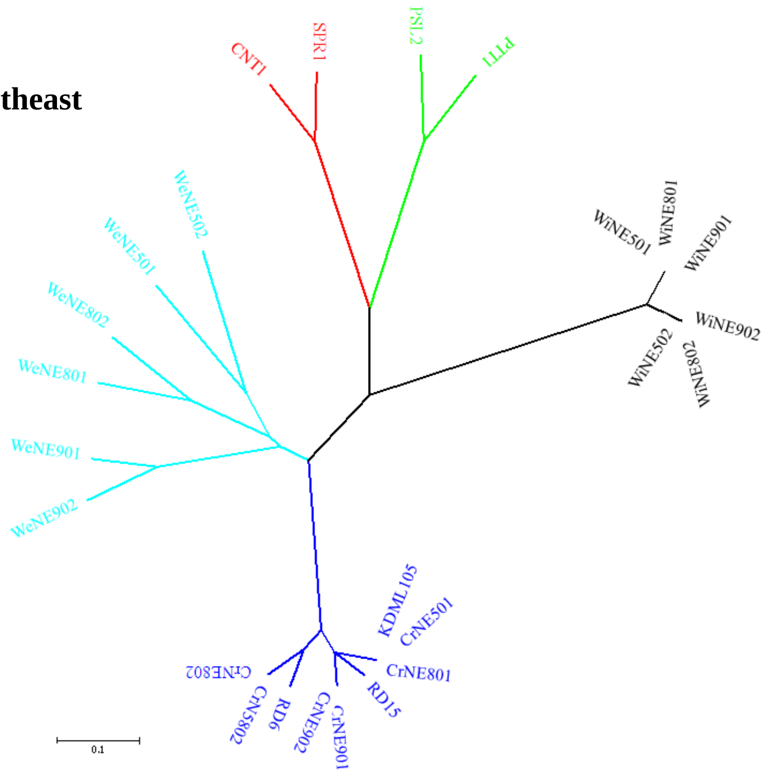


Figure 3.3.17 Cluster analysis using NJ method based on C.S. Chord (1967) genetic distance among 18 companion crop rice, 18 weedy rice and 18 native common wild rice populations collected from three rice production areas; Lower North (a), Northeast (b) and Central Plain (c) in different three growing seasons in wet 2005, 2008 and 2009 compared with 7 pure line cultivated rice varieties. Different colors of weedy rice samples represent different regions; WeLN: yellow, WeNE: light blue, WeCP: pink, Cr1-2: red, Cr3-4: green, Cr5-7: blue and Wi: gray.

(b) Northeast



(c) Central Plain

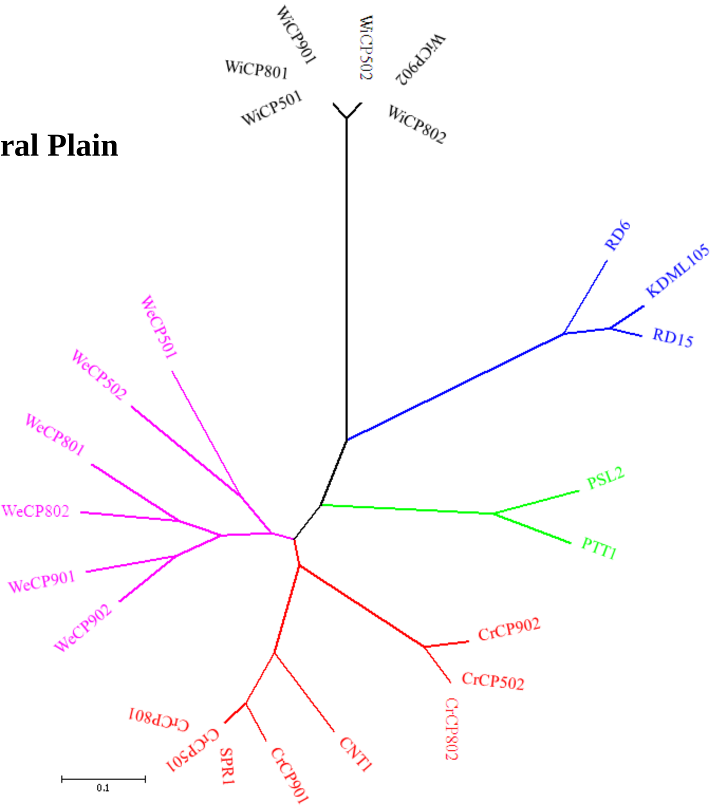


Figure 3.3.17 *(continued)*

3.4 Discussion

Weedy rice populations in Thailand showed high adaptations and mimics to crop rice which was clearly reflected this results from morphological relationship, genetic diversity and population structure when considering at the regional scale from previous chapter. Population genetics structure and dynamics is result from distribution and change of allele frequency in population under the influence of gene flow and other ecological conditions (Postlethwalt, 2009). Weedy rice when coexisted with their companion crop rice and native common wild rice in sympatric rice fields is often interacting between them because they have genetic compatibility (Zizumbo-Villarreal *et al*., 2005). Thus, the patterns of population genetic structure and dynamics in spatial and temporal terms of weedy rice under gene flow process and the other ecological conditions influence their population genetic structure and dynamics when coexisted with their companion crop rice and native common wild rice in sympatric rice fields in Thailand at the rice field scale were evaluated at the field scale in this chapter.

Generally, the sympatric of crop-weed-wild rice populations have mutually influencing each other by means of natural hybridization and introgression lead to gene flow between them (Anderson and Stebbins, 1952 cited by Ellstrand and Schierenbeck, 2000). In all studied fields, the flowering periods of weedy rice and their companion crop rice largely overlapped, providing the opportunity for hybridization. The other studies of flowering overlap and variable opportunities for crop rice to weedy rice gene flow have been reported in many areas such as China (Chen *et al.,* 2004) and USA (Burgos *et al.,* 2008) and in other plants such as sorghum (Barnaud *et al.*, 2009) and sunflower (Roumet *et al.,* 2012). In addition, native common wild rice populations were mainly late flowering than weedy rice and crop rice. Thus, weedy rice, mainly outcross habit, coexisted with their companion crop rice in sympatric rice fields led to the possibility of intercross between them.

Gene flow promotes genetic diversity, genetic variation (Anderson and Stebbins, 1952 cited by Ellstrand and Schierenbeck, 2000) and affects to structure of population (Ellstrand *et al.,* 1999). Weedy rice in the present study showed high level of genetic diversity both within and between populations over their companion crop rice but slightly lower than their native common wild rice. These were also demonstrated by various levels of genetic variation within weedy rice population were detected with slightly lower total genetic diversity than their native common wild rice. Similar relatively high levels of genetic diversity for SSR markers were also detected in other studies of weedy rice in Thailand by Niruntrayakul (2007) and Pusadee (2009).

When considering among different geographical regions within each season indicated that weedy rice populations from different regions showed different levels of genetic diversity. These were demonstrated by the Northeast showed highest levels of total genetic diversity (H_T) and observed heterozygosity (H_O), allowed by the Central Plain while the Lower North shown the lowest levels. These different levels of total genetic diversity and observed heterozygosity are resulted from different level of outcrossing rate from each region, some levels of outcrossing as common wild rice progenitor was highest in the Northeast while the lowest level was found in the Lower North. This result suggested that these differing levels of genetic diversity, genetic variation and mating system of weedy rice collected from different regions in each growing seasons resulted from farmer’s management in each region such as pressure to intensify rice production and changing planting systems in each region. The Central Plain and the Lower North have adopted a high intensify rice production, five crops in two years, as well as used direct seeding method in fields while farmers in the Northeast have still grown just one crop a year only in the wet season with transplanting method. Recently, due to the irrigation development, a double-crop planting in some areas and changing from transplanting to direct seeding method have adopted in the Northeast (Konchan and Kano, 1996). Thus, the generations of weedy rice in the Lower North and the Central Plain with direct seeding method and high intensify rice production would be more infested and possibility hybridized with popular companion crop rice varieties lead to the convergence of homogeneity of weedy rice population toward the cultivar than weedy rice in the Northeast.

Our results from population structure analysis indicated weedy rice populations in the present study were highly structured by its companion crop rice varieties popular in each region. This is clearly reflected by the results from the genetic assignments from the STRUCTURE model. The distribution of weedy rice plants on PCA graph and the grouped weedy rice from the cluster analysis also confirmed that weedy rice in present study showed a relatively close genetic relationship of weedy rice populations with their companion crop rice. This result suggested that weedy rice in the present study resulted from the subsequently backcross of weedy rice to their companion crop rice in rice fields with through a farmer's selection. These results were consistent with Pusadee (2009) suggestion that weedy rice in Thailand strongly structured by its companion cultivated rice varieties in each region.

Due to the crop-weed-wild complex is under ongoing of gene flow (Jarvis *et al.,* 1999). Thus, weedy rice from different growing seasons needed to be considered with their sympatric components. Weedy rice collected in 2005 displayed high genetic diversity, genetic variation and intermediate form of population structure between their native common wild rice and companion crop rice that was the consequence of early hybrid stage of intercross between native common wild rice and companion crop rice in the sympatric habitats (Langevin *et al.*, 1990). Long term, the evolution of weeds often parallels the evolution of companion crops by gene flow between them (Harlan, 1992; Ellstrand et *al.,* 1999). After few years of invasion in the rice fields, weedy rice populations collected in 2008 and 2009, respectively, showed increasing the proportions of cultivar genotypes in STRUCTURE model and self-pollinated habit as cultivated rice but decreasing the genetic diversity, genetic variation, wild and hybrids genotypes frequency. Consequently, the dynamics of these genetic measurements indicated that weedy rice in recently years showed high adaptation to mimicry to crop rice. These caused by accumulating continuous backcrossing of weedy rice to companion crop rice in the sympatric rice fields and share the same period of flowering time resulting the rapidly loss of wild genotypes in segregating populations (Ellstrand *et al.,* 1999)

Interestingly, the genetic admixtures of the third group (specific to KDML105 or RD6 or RD15 varieties) in STRUCTURE model were mixed with first group (specific to CNT1 or SPR1 or PTT1 or PSL2 varieties) in some weedy rice populations from the Northeast in 2008 and 2009. From the Northeast cultivation, the improved traditional varieties, KDML105, RD6 and RD15, are commonly planted in wet season. Recently, the modern rice variety, CNT1 or SPR1 or PTT1 or PSL2, are used to plant in some areas of the Northeast where throughout irrigated system in dry season. Thus, this result can be supported an ongoing gene flow of weedy rice with their companion crop rice. However, from the results of STRUCTURE and allele specific to crop/wild indicated that native common wild rice populations from three regions in three growing seasons were no threatened by introgressed genes of crop. This may caused by lately flowering periods of native common wild rice populations than crop and weedy rice though they are co-existed in sympatric habitats. Thus, native common wild rice populations would be saved from gene flow, if the flowering periods of wild rice is not always overlapped with their crop and weed types.

In addition, the introgressed genes that through a farmer selective advantage may enhanced fitness and adaptability of weedy rice populations (Campbell *et al.*, 2006) and stimulating the potential evolution of invasiveness in weedy plants (Ellstrand and Schierenbeck, 2000). The present study demonstrated some morphological and physiological characters of weedy rice showed adaptive evolutionary dynamics with trend toward as their companion crop rice. However, some physiological characters were strongly maintained in some weedy rice plants such as slightly earlier flowering and high seed shattering for adapt to the agronomic practices and enhance distribution in rice fields.

In conclusion, although all weedy rice populations and their companion crop rice and native common wild rice in present study coexisted in the sympatric rice fields but the evolution of weedy rice often parallel with their companion crop rice because their flowering periods largely overlapped. Thus, weedy rice populations from different regions are structured base on their companion crop rice varieties in each region. Long term, some morphological, physiological characters, population genetic structure and dynamics of weedy rice has tended toward with their companion crop rice. This may caused by accumulate the backcrossing to their companion crop rice with has a farmer’s selection lead to high adaptation by mimic to crop rice. Therefore, gene flow is the major process in the adaptive evolutionary dynamics of weedy rice populations when they are co-existed with their companion crop rice in the sympatric rice fields through intensive other ecological conditions such as agronomic practices and selection. Gene flow and ecological conditions in weedy rice plants contributed to increase adaptation to farmers’ management for highly their weediness. However, weedy rice plants were strongly high seed shattering for increasing their distribution in rice fields.