INTRODUCTION

Weedy forms of rice are known as weedy rice (*Oryza sativa* f. *spontanea*). In the rice fields, it is one of the most serious weed, adversely affecting rice areas and yield worldwide, particularly in South and Southeast Asia, South and North America, and southern Europe (Noldin, 2000; Vaughan *et al.,* 2001; Gealy *et al.,* 2002). In Thailand, weedy rice is one of the most noxious weeds and spread rapidly in many direct-seeded rice production areas. Although, weedy rice is taxonomically classified into the same species as cultivated rice (*Oryza sativa* L.), they are still strongly characterized by their seed shattering and dormancy (Cao *et al.*, 2006). It is commonly causes severe yield reduction and affects the quality of rice grains by its high competitive ability and persistence in rice fields due to its high fecundity, high seed shattering and high seed dormancy (Maneechote *et al*., 2004).

Weedy rice in Thailand is originates from interspecific hybridization between common wild rice (*Oryza rufipogon* Griff.) and cultivated rice (Niruntrayakul, 2007) due to Thailand lies partly within the center of genetic diversity of *Oryza*. Weedy rice is frequently found in the sympatric habitats where wild and cultivated rice are co-exist, and occur in the area where absence of wild populations (Oka, 1988; Song *et al.,* 2006). Such hybridization between cultivated rice and common wild rice is a common occurrence when their flowering period overlaps, through the time of flowering peak of wild rice is either earlier or later than the cultivated rice (Chitrakon, 1995).

Some domesticated plants occur as part of a crop-weed-wild complex. A wild-weed-domesticated complex in sympatric habitat is 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. Naturally 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).

Commonly, weeds and crops often share the same evolution between them (Harlan, 1992). Long term, hybrids are mimics to the companion cultivated rice in morphological characters which it so similar to 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 of them 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).

Various evolutionary forces influenced the population genetic structure of weedy rice. Knowledge of the changing of genetic structure of populations is essential for understanding the adaptive evolutions of weedy rice. Population genetic structure and dynamics of weedy rice in the center of diversity is the ongoing process where hybridization and introgression among wild relative, companion crop and weedy rice that are growing in the sympatric rice fields (Ellstrand *et al*., 1999). Gene flow among them is one of the expected evolutionary events that play the major role in the complex variation through the other forces such as selection, domestication, and farmers' managements.

In the past decade, weedy rice was firstly observed in rice production areas in Thailand (Maneechote, 2004) and became a serious weed problem where first found in Central plain. Recently, weedy rices have been increasingly reported widespread in many directed-seed paddy fields in Lower North and Northeast. In present study, therefore, weedy rice from three rice production areas; Lower North, Northeast and Central Plain of Thailand in wet seasons of 2005, 2008, 2009 and dry season of 2009/10 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 weedy rice and compared with crop rice and common wild rice and (b) for temporal analysis; within and between regions among seasons of weedy rice and compared with crop rice and common wild rice.

Population structure and dynamics of weedy rice populations in Thailand were revealed based on microsatellite analysis presented in Chapter 2 and Chapter 3. Chapter 2 illustrated morphological and physiological characters, population genetic structure and dynamics in spatial and temporal terms of weedy rice populations in Thailand compared with common wild rice populations and cultivated rice varieties and demonstrated the adaptive evolutions those were under this population genetic structure and dynamics of weedy rice at the region scale. Finally, Chapter 3 displayed the patterns of population genetic structure and dynamics in spatial and temporal terms of the sympatric wild-weed-crop rice populations in Thailand and demonstrated the affect of gene flow and 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 at the rice field scale.

The objectives of this study are as follows:

1. To evaluate genetic variation of morphological and physiological characters and microsatellite loci of weedy rice populations in Thailand 2. To demonstrate how the adaptive evolutions were under those population genetic structure and dynamics of weedy rice at the region scale.

2. To determine population genetic structure and dynamics of weedy rice populations in spatial and temporal terms at the regional scale.
3. To evaluate 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.
4. To 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.

CHAPTER 1

Literature Reviews

1.1 *Oryza* genus

Oryza is including twenty-three species within the family *Poaceae* (Vaughan *et al*., 2003). This genus includes both annual and perennial species. They are distributed in the humid tropics of Asia, Africa, America, or Oceania, some of them being confined to Madagascar or New Guinea. The genus is divided into four species complexes; including the *O. sativa*, *O. officinalis*, *O. ridelyi* and *O. granulate* species complexes. The basic number of chromosome of the genus is 12 and interspecific crossing is possible within each complex (Vaughan *et al*., 2003).

Rice belongs to the genus *Oryza*. Therefore, one important complex as the *O. sativa* complex composes two cultivated species; *O. sativa* L. and *O. glaberrima* Steud., and five species; *O. rufipogon* Griff. (*O. nivara* for the annual form of *O. rufipogon* Griff.), *O. barthii*, *O. longistaminata*, *O. meridionalis* and *O. glumaepatula*, all of them are diploids with same the AA genome (Oka, 1988). The cultivated rice, *O. sativa* L., is distributed in worldwide especially in Asia as a major food crop, while *O. glaberrima* is distributed only in West Africa. *O. rufipogon* (with annual form is *O. nivara*) can be found throughout Asia and some region of America and Oceania. *O. barthii* and *O. longistaminata* are wild species of African, *O. barthii* endemic in West Africa and *O. longistaminata* is found throughout Africa. *O.*

meridionalis is native to tropical Australia and *O. glumaepatula* is endemic in Central and South America. From these distributions, they are easy to find origin of the modern rice from the location of ancestral pools. The common rice, *O. sativa* was domesticated from *O. rufipogon* (Oka, 1988) and the African cultivars were domesticated from *O. barthii* (formally called *O. breviligulata*) (Semon *et al*., 2005).

1.2 *Oryza sativa* primary gene pool

The rice gene pool in the genus *Oryza* compose different genome types including, diploids (2n = 2x = 24) and tetraploids (2n = 4x = 48) (Vaughan, 1994; Ge *et al*., 1999). These different genome types of *Oryza* species have significant to limit reproductive isolation by cannot hybridize with each other.

Following the gene pool concept for cultivated plants (Harlan and de Wet, 1971), the rice gene pool can be divided into three categories; primary gene pool (PG-1, crossing within this gene pool is easy and hybrids are generally fertile), secondary gene pool (PG-2, races crossable with cultivars but gene flow is restricted and hybrids trend to be sterile), and tertiary gene pool (PG-3, crosses with cultivars are difficult, and gene transfer is not possible without special procedures) according to the genetic relationship between cultivated and their wild and weed relatives. According to the classification of gene pool, therefore, the primary gene pool of Asian cultivated rice composes cultivated rice (*Oryza sativa*), ancestral wild species (*O. rufipogon* and *O. nivara*) and its weedy types (referred to as *O. sativa* f. *spontanea*) (Harlan, 1992).

1.2.1 Common wild rice

Common wild rice (*O. rufipogon* Griff.), the wild progenitor of Asian cultivated rice (*O. sativa* L.), is widely distributed in the humid tropical Asia, America and Oceania (Oka, 1988). *O. rufipogon* is classified into two ecotypes, perennial and annual types, because of a differentiation between them is related to variation in life-history traits, mating system and habitat condition (Oka, 1988). They are an important genetic resource for rice improvement because they share the same AA genome and are highly compatible sexually with cultivated rice.

Genetic diversity of common wild rice

High level of genetic diversity of common wild rice, compared to cultivated rice has been studied using morphology, allozymes and DNA markers (Oka, 1988; Morishima and Barbier, 1990; Sun *et al.*, 2001). Generally, perennial populations of common wild rice contain more gene diversity than annual populations (Morishima, 1986). Kuroda (2004) confirmed that perennial populations showed higher gene diversity within population and lower gene diversity among populations than annual populations. This was caused by the different in outcrossing rate between perennial and annual types, 7% in annual to 56% in perennial types (Oka, 1988).

Genetic diversity of common wild rice populations have been reported using and allozyme SSR markers. In China, genetic diversity within populations of common wild rice were between 0.493 – 0.708 (Gao, 2004; Song *et al.*, 2003). In Thailand, Morishima and Oka (1970) analyzed quantitative traits of common wild rice population Barbier (1989a; 1989b) and Morishima and Barbier (1990) studied life-history traits and genetic diversity using allozymes markers. Recently, Punyalue (2006) assessed genetic diversity of 12 common wild rice populations using seven microsatellite loci showed that total gene diversity (H_T) over all twelve populations was 0.225. Wongtamee (2008) found that 37 common wild rice populations in Thailand were also based on five microsatellite markers and showed moderate to high level of gene diversity.

In Thailand, five wild rice species are detected in Thailand including; *O. rufipogon, O. nivara, O. officinalis, O. ridleyi* and *O. granulate.* Common wild rice, *O. rufipogon*, is distributed in canals, swamp areas and moist lands throughout Thailand (Chitrakon, 1995; Jamjod *et al*., 2003). Both perennial and annual types (*O. nivara*) were found (Chitrakon, 1995). Annual type is flowering earlier than perennial type at around mid-October until drying as soon as after seed set while perennial type is started flowering on mid-November to the end of December (Jamjod *et al.*, 2003).

1.2.2 Cultivated rice

Cultivated rice is an autogamous plant with low out-crossing rate (Robert *et al*., 1961). Cultivated rice includes two species in *Oryza* genus; *O. sativa* can be found throughout the world and *O. glaberrima* is important food crop which is limited to West and Central African. The common rice or Asian cultivated rice (*O. sativa*) are divisible into two major subspecies; the sticky or short grained as *japonica* variety and the non-sticky or long grained as *indica* variety. *Japonica* is commonly cultivated in dry field, in temperate East Asia, upland areas of South and Southeast Asia, while *indica* is mainly lowland rice, grown mostly submerged in throughout tropical Asia (Oka, 1988). The third subspecies, which is broad grained and thrives under tropical condition, was identified based on morphology and initially called *javanica* variety (De Datta, 1981).

In Thailand, rice cultivation area has about about 10.8 million ha. About 33 percent of the rice land is irrigated and the rest is nonirrigated or rainfed (Agricultural Futures Trading Commission, 2007). Cultivated rice in Thailand is differentiated into two ecotypes with different characteristics. Rice production systems are separated according to geographical regions and irrigation including; upland rice, deepwater rice, irrigated and rainfed lowland rice. All irrigated rice lands, especially in central and recently in lower north and some part of northeast, are planted to modern semi-dwarf high yielding varieties (HYVs). Most of the rainfed lowland and deepwater areas are planted to improved traditional or local photoperiod sensitive varieties. Two planting methods; direct seeding and transplanting, are generally used in cultivation in Thailand. Transplanting method is used in most of the north, northeast and south rice-growing areas during the rainy season. While direct seeding with double cropping system in larger fields is changed from transplanting method in central and recently in lower north and some part of northeast rice-growing areas (Maneechote *et al*., 2004).

1.2.3 Weedy rice

In a region where a crop shows a high genetic diversity, the crop is often related with its companion weed. Various weedy forms are found in rice (Ellstrand *et al*., 1999). Weedy forms of rice related with *O. sativa*. All of them may be considered annual and they reproduce themselves by their dispersed seeds or by being sown together with the cultivar seed by man. They often occur in direct seeded fields, but are rare in transplanted paddies. Weedy rice are so morphologically similar to cultivated rice varieties that they are difficult to remove and because of weedy forms and the cultivars are so closely related, herbicides that would kill weedy forms would also kill the cultivars. The cause damages to both quantity and quality of the grain harvested (Oka, 1988).

Among the weedy spontanea form of rice, the invasive weedy rice (*Oryza sativa* f. *spontanea*) is one of the most serious agricultural weed. It is adversely affecting rice areas and yield worldwide, particularly in South and Southeast Asia, South and North America, and southern Europe (Vaughan *et al*., 2001). Although, weedy rice is taxonomically classified as the same species as cultivated rice (*Oryza sativa*), they are still strongly characterized by their seed shattering and dormancy (Cao *et al*., 2006). Morphologically, weedy rice is highly variable and appears to be an intermediate between wild and cultivated rice. Moreover, weedy rice is phenotypically and genotypically diverse and changeable, very vigorous and competitive with cultivated rice, able to spread rapidly and reduce both grain yield of rice and quality (Maneechote *et al*., 2004)

The origin of weeds has been proposed by de Wet and Harlan (1975). Three principle ways of evolution of weeds are: 1) from wild colonizing plant through selection toward adaptation to continuous habitats disturbance, along with the evolution of domesticates; 2) as derivatives of hybrids between wild and cultivated races and 3) from abandoned domesticates through selection toward a less intimate association with man.

1.2.3.1 Specific characters of weedy rice

Adaptation and crop mimicry

Most of the weedy rice types are essentially adapt and mimic to 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 of them 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 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 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 are 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).

Shattering and dormancy

Of all the adaptations that separate wild and weed from cultivated cereals, the shattering trait of wild and weed races is the most conspicuous. Although, weedy rice is taxonomically classified into the species of cultivated rice (*O. sativa*) and mimics to cultivated rice in many morphological characteristics, they are still strongly characterized by their seed shattering and dormancy (Cao *et al*., 2006). A race of weedy rice is brittle that it has most seeds shatter and fall to the ground infesting the soil for escape the harvest. The remainder seeds are non-shattering and are harvested along with the cultivated rice. Generally, an annual species, including weedy rice, is dependent entirely on seed in terms heavy seed production with early and heavy shattering and intense and prolonged seed dormancy (de Wet and Harlan, 1975). The shattering of seeds as they mature in the inflorescence and the ability of the grain to remain viable in the soil during long adverse periods are an important mechanism for their dispersal and distribution. Seed shattering and dormancy increase not only the area distribution but also the survivability of weedy rice (Constantin, 1960). In their review of Asian rice and weedy rice, Vaughan *et al*. (2005) confirmed that seed shattering before the cultivated rice mature is a single characteristic of all weedy rice in the rice field and most of them are maintained the germinability in the soil through during unfavorable seasons until conditions become favorable again in the next crop and incorporated in the soil seed bank for multiple years for start its annual life cycle.

Competition

The negative impact of weedy rice is competitive with the cultivated rice in planting rice on their infested land. Competitiveness is ability to decrease rapidly consumable resources, such as water and nutrients, or grow better than associated species when these resources limiting (Radosevich *et al.*, 1997). Many of the morphological traits that contribute to the competitiveness of weedy rice in rice fields are generally taller, produce more tillers and have a larger leaf area than cultivated rice (Noldin *et al.*, 1999).

Tallness is competitive trait in plant communities. Weeds taller than the crop are usually most competitive during the latter half of the season and have a greater effect on the yield components than growth of the crop as a result of shading effect (Smith, 1968). Weedy rice produces many tillers, especially compared with the modern varieties. The high tillering capacity of weedy rice is a competitive advantage important for their success as weeds (Noldin, 1995). Weedy rice germinated and emerges more slowly than cultivated rice varieties but seedling development was greater for weedy rice than cultivated rice. However, they were superior to the varieties in terms of root length and speed germination (Wague, 1992).

Dispersion

Weed invasion begins with dispersal. Many weed species have seeds with well adapted to assist them moving to great distances. However, weed distribution processes are strongly associated with farmers' managements and weed reproduction can move great distances as contaminants of seeds or transported by machinery or irrigation water (Cousens and Mortimer, 1995).

- Contaminated seed

The spread of weedy rice is increasing given the similarity between weedy rice and rice cultivar variety. Contaminated crop seed is a primary factor in the dissemination of weedy rice. For instance, red rice (one important type of weedy rice) has been introduced into the USA by a contamination in seed lots (Dodson, 1990). In the early 1900, red rice was found in California is the evidence of seed contamination from the southern states of the USA (Bellue, 1932). Similarly, the origin of red rice in Latin America and Caribbean imported from the USA (Dominguez, 1999). In Spain, Netherland, France and Portugal, red rice spread to other areas though the exchange of seeds. In Asia, the origins of weedy rice had different from in the Americas and Europe. However, the spread of weedy rice in several is the same way as in the Americas. For example, weedy rice contaminated seed in Viet Nam is considered to be the main mean of infestation in rice cultivation areas (Chin *et al*., 1999).

1.2.3.2 Gene flow or migration by hybridization in plants

Gene flow can be a potent evolution force. Gene flow, also known as gene migration, is the move of alleles of genes from one population to another (Ellstrand *et al*., 1999). Gene flow is a combination process of the two different gene pools lead to reducing the genetic variation between the two populations. The introgressed genes from different populations through gene flow increases variability within the population and make possible new combinations of traits (Messeguer *et al*., 2004).

Crop-to-wild gene flow

Since cultivated rice and the wild progenitor are diploid (2n=24) and share the same AA genome (Vaughan and Morishima, 2003). They are highly compatible sexually, leading to natural hybridization between them when they have overlapping flowering periods in sympatric habitats (Oka, 1988). Such hybridization may lead to gene flow. If crop alleles from cultivated rice persist in common wild rice population, gene flow from crop to wild may have an influence on the evolution of wild rice populations (Ellstrand *et al*., 1999). This might cause ecological problems by producing aggressive weedy rice, which now threatens rice production in many regions of Thailand, resulting in reduction of quality and quantity of rice yield (Maneechote *et al*., 2004). In some cases, hybrid swarms with better ecological fitness, may lead to their aggressive spread in wild rice habitats and replacement and extinction of the original wild rice populations (Ellstrand *et al*., 1999).

Crop-to-weed gene flow

Crop-to-weed gene flow has the role in the evolution of weedy types. Pollen from domesticated type can fertilize closely related wild or weedy types, potentially enhancing the ability of hybrid weed to compete with the crop and other weeds (de

Wet and Harlan, 1975; Barrett, 1983). The potential problem in crop fields is the transfer of crop alleles to a weedy species to create a more aggressive weed. Crop-to-weed gene flow has been implicated in the evolution of enhanced weediness in wild relatives of 7 of the world's 13 most important crops, especially in rice (Ellstrand *et al*., 1999). The evolutions of enhanced weediness arise when a crop is grown in proximity to a cross-compatible relative. Certain novel traits in weed populations, such as herbicide tolerance, disease resistance and insect resistance, will enhance particular fitness components of a weed in particular environments (Bergelson and Purrington, 1996). The fitness impact of the novel crop alleles will enhance weediness to a point that would have important practical consequences. Therefore, bringing a novel cultivar or a new crop species into contact with a cross-compatible weed may lead to evolution of enhanced weediness if hybridization occurs under field conditions, the hybrids persist to reproduce, and one or more crop alleles give a fitness boost to the weed populations (Ellstrand *et al*., 1999).

Crop-to-crop gene flow

Although most rice cultivars worldwide are highly self-pollinating, outcrossing between them plants can occur. Generally, natural outcrossing among cultivated rice plants is lower than between wild or weedy and cultivated rice (Messeguer *et al*., 2001; Zhang *et al*., 2003). Hybridization among cultivar plants lead to crop-to-crop gene flow in the proximity habitats. Such proximity of two varieties depends not only on the distance between cultivated fields but also on the inadvertent planting of a few seeds of one variety within a field of a different variety (Papa and Gepts, 2004). Sometime, when farmers exchange seeds or save part of their crop for replanting in next crop, transgenes could disperse further by means of both pollen and seeds (Sirabanchongkran *et al.*, 2004). In positive advantage, the movement of herbicide tolerance gene to a neighboring crop could have an impact on weed management in certain crop rotation systems whether the herbicide tolerance exists by biotech or conventional methods (Rong *et al.*, 2006). On other hand, cross-pollination could potentially have a negative impact on the long-term productivity of native farmers by it decreased the genetic diversity of local cultivars and landraces.

1.2.3.3 Selection: Agricultural practices as selection forces

In the context of evolution, many traits or alleles of genes segregating within a population may be influence from selection. Under selection, individuals with advantageous or adaptive traits tend to be more successful than others. It means that they give more offspring to the succeeding generation than others do by produce more seeds or increase the dispersion ability into new habitats (Harlan, 1982). Since offspring will inherit those traits from their parents, so selection can increase the prevalence of those traits when these traits have a genetic basis (Guglielmini *et al.*, 2007). Progressive changes in systems of crop cultivation continually modify the selective forces acting on weed populations. Changes in the composition of weed communities associated with farmer were probably gradual by artificial selection through cultivation practices, hand-weeding, threshing and winnowing (Spencer and Barrett, 1983). Some crop-weed associations are so closely that their interaction with human has led to the evolution of mimetic forms of weeds. These often resemble the crop in morphology and behavior and thereby avoid they were removed. However, human selection (or artificial selection) might impact on weed traits by changes in the genetic structure of weed populations for their survival (Palumbi, 2001).