

## Introduction

Rice (*Oryza sativa* L.) is unusual among major cultivated crops in that it may be found growing side by side with its closest wild relative (*O. rufipogon* Griff.), as in Thailand (Chitrakon, 1995; Jamjod *et al.*, 2002). The common wild rice has long been grown in close proximity to cultivated rice field. Although, cultivated rice is predominate self-pollinated plant, 0-1% outcrossing rate (Robert *et al.*, 1961). Natural hybridization between *O. rufipogon* and *O. sativa* has been reported in many locations (Oka and Chang, 1961; Langevin *et al.*, 1990; Shu *et al.*, 1997; Song *et al.*, 2000), indicating that *O. rufipogon* is highly compatible with cultivated rice. This is expected from the relatively high sexual compatibility between the species sharing the AA genome, with complete chromosome pairing in meiosis of F<sub>1</sub> interspecific hybrids (Naredo *et al.*, 1997, 1998). In Thailand, gene flow between cultivated rice and wild rice has been documented (Oka and Chang, 1961; Akimoto *et al.*, 1999). Hybrid swarms were frequently observed in farmers' rice crop in Thailand (Morishima *et al.*, 1980; Rerkasem and Rerkasem, 1984).

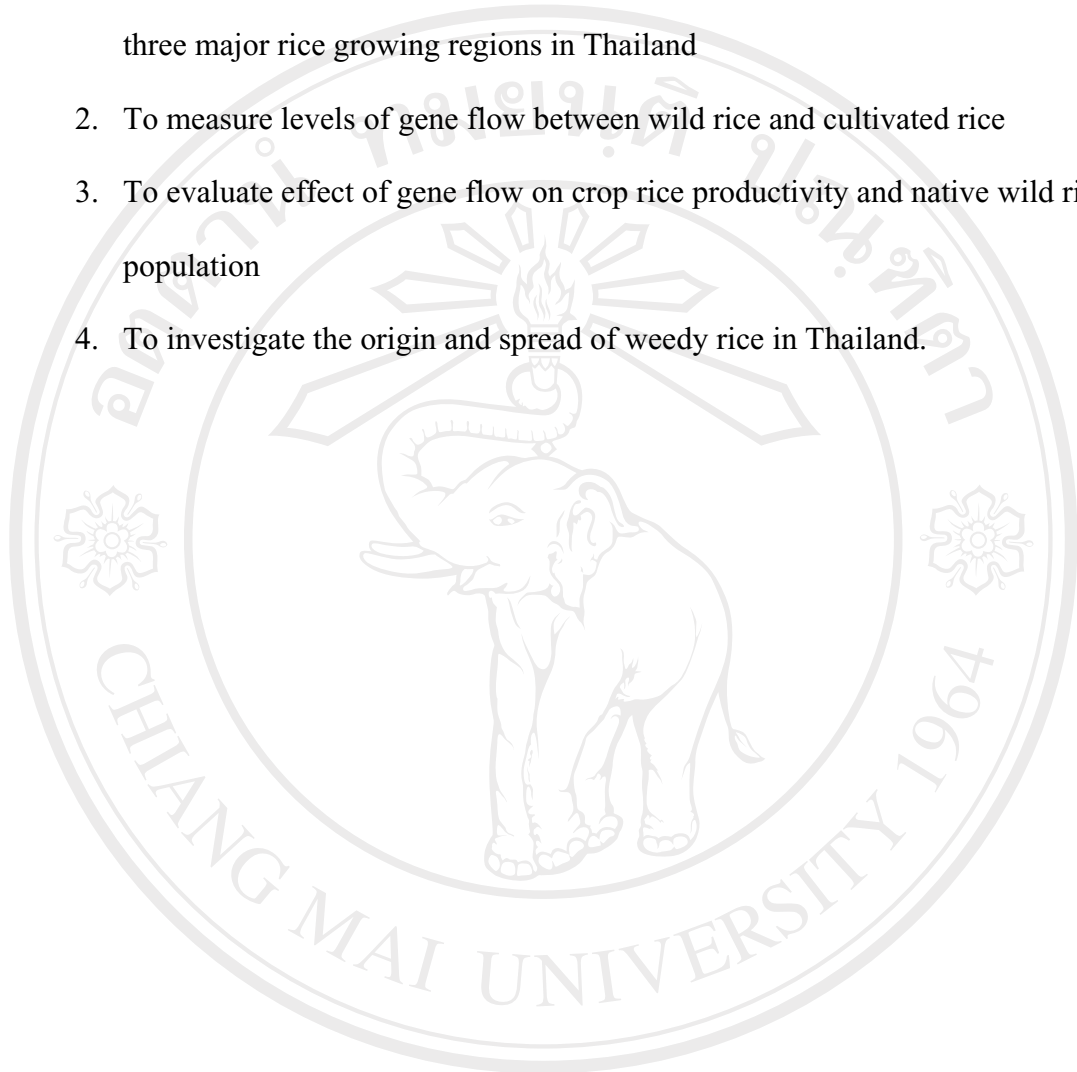
Rice production systems are distinguished according to geographical regions and irrigation. Cultivated rice (*O. sativa* L.) varieties are adapted to rice growing areas. Improved and local rice varieties still grow in farmers' field. Farmers decide to grow rice in their field depending on rice market demand, farmers' preference including the suitability of each improved rice variety for each location. Wild rice (*O.*

*rufipogon* Griff.) also showed differentiation of population by geographical distance and ecological factors.

Gene flow between the cultivated and wild rice can have important impacts on both the common gene pool of the two species as well on rice production. However, it has been pointed out that gene flow may cause a loss of genetic diversity (Akimoto *et al.*, 1999) or extinction of wild rice populations, or producing an aggressive weed in cultivated systems (Ellstrand *et al.*, 1999). A negative outcome of the gene flow between cultivated and wild rice is suggested by the emergence of weedy rice as a serious weed of rice fields in the central Thailand since 2001 (Maneechote *et al.*, 2004), causing severe economic losses (Phaokreung, 2004). In this area, improved rice or local rice varieties were grown in direct-seeded fields and consistent uses of herbicide for weed control. Weedy rice continues to spread from field to field and has become a serious problem in Thailand. Moreover, controlling weedy rice by herbicide is difficult because of the close genetic background between cultivated rice and weedy rice. For this reason, the proposed study will evaluate compatibility between popular rice cultivars and wild rice populations from each region in Thailand, and will investigate the origin and spread of weedy rice in Thailand by examining gene flow between cultivated rice (*O. sativa*) and wild rice (*O. rufipogon*). The results will be useful in the development of strategies for protection against weedy rice infestation. Furthermore, this study will improve our understanding of the genetic structure in rice populations that have experienced gene flow between wild and cultivated rice.

The objectives of this investigation are as follows:

1. To evaluate cross compatibility between cultivated rice and wild rice from three major rice growing regions in Thailand
2. To measure levels of gene flow between wild rice and cultivated rice
3. To evaluate effect of gene flow on crop rice productivity and native wild rice population
4. To investigate the origin and spread of weedy rice in Thailand.



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# Chapter 1

## Literature review

Rice is the important food grain in the world. The rice complex with the AA genome in the *Oryza* gene pool includes cultigens and their wild and weedy relatives. Cultivated rice comprise two species, *O. sativa* and *O. glaberrima*. Common wild rice, *O. rufipogon* Griff. and *O. barthii* Chev. are the putative ancestors of Asian cultivated rice (*O. sativa*) and Africa rice (*O. glaberrima*), respectively.

### 1.1 Cultivated rice

Cultivated rice is an autogamous plant with a low outcrossing rate of 0-1% (Robert *et al.*, 1961), which nevertheless has significant potential impact on rice farming and natural environment. Crop rice includes two taxonomically distinct species: *O. sativa* L., found throughout the world and *O. glaberrima* Sted., whose economic importance is limited to West and Central African, from Sénégal to Sudan.

#### 1.1.1 Asian cultivated rice (*O. sativa* L.)

The species *O. sativa* is grouped into three varietal types: indica, which predominate in the tropics and subtropics; japonicas, grown only in the temperate and subtropical zones; and javanica, found mostly in Indonesia (De Datta, 1981). The history of Asian cultivated rice's domestication was explained by Londo *et al.* (2006) Their results showed that *O. sativa indica* was domesticated within a region south of

the Himalayan mountain range, whereas *O. sativa japonica* was domesticated from wild rice in Southern China.

### **1.1.2 African rice (*O. glaberrima* Stedu.)**

The African rice (*O. glaberrima*) is thought to have been domesticated from the wild ancestor *O. barthii* (formerly known as *O. brevilugata*) by people living in the floodplains at the bend of the Niger River some 2,000-3,000 years ago (Portères, 1976). African *O. glaberrima* varieties have certain negative features when compared with the Asian *O. sativa*: the seed scatters easily, the grain is brittle and difficult to mill and most importantly, and yields are lower. But the *O. glaberrima* types also offer distinct advantages: the plants have luxurious wide leaves that shade out weeds and the species is more resistant than its Asian cousin to diseases and pests (Linares, 2002).

## **1.2 Wild rice**

### **1.2.1 Common wild rice (*O. rufipogon* Griff.)**

The common wild rice, *O. rufipogon* Griff., is the putative ancestor of the Asian cultivated rice (*O. sativa*) and the most important genetic resources for rice improvement in terms of its accessibility for gene transfer through sexual means (Oka, 1988). Habitats of the wild rice overlaps that of cultivated rice. The wild rice is found in and around rice fields (either under cultivation or abandoned), as well as in ditches, canals, marshes and riverbanks (Vaughan, 1994).

*O. rufipogon* is a close relative of cultivated rice, *O. sativa*, and shares many of the characteristics of the crop. This species is found in shallow water, irrigated fields, pools, ditches and sites with stagnant or slow, running water. *O. rufipogon* is

an erect, perennial tufted grass, 150-400 cm tall, with spongy lower culms below, the lower parts floating and rooting at the nodes, the upper parts sub-erect (NAPPO, 2003, <http://www.nappo.org/PRA-sheets/Oryzarufipogon.pdf>). It occurs at altitude from 0 to 1000 m and is suited to sites that support populations of cultivated rice and shows a range of variation from perennial to annual types. Generally, perennial populations contain more gene diversity than annual ones (Oka, 1988). The perennial type is found in swampy habitats, which remain inundated throughout the year. The annual form is found in temporal swamps, open ditches which are parched in the dry season (Oka, 1988). The perennial type has higher outcrossing rate (30 – 50%) than annual type (5 – 25%), but seed productivity is lower in the perennial type. Barbier (1989) found that wild rice in Thailand to outcross in varying degrees, from 7.2% to 55.9%.

### 1.2.2 *O. barthii* A. Chev. (formerly known as *O. brevilugata*)

*O. barthii* is a close relative of the African cultivated rice, *O. glaberrima* (Linares, 2002). *O. barthii* was found in open habitat and grown in deep water, seasonally flooded land, stagnant water and slowly flowing water or pool. This species is also closely related to *O. longistaminata*, which sometimes grows in the same habitat and leads to speculations on the possibility of hybridization having occurred. Usually show long red awn, large spikelet and seed shattering (Vaughan, 1994).

### 1.3 Cultivated rice and wild rice in Thailand

Thailand has about 51.8 million ha of land area and rice cultivation area is about 8.4 million ha. About 1.9 million ha of the rice land is irrigated and the rest (77%) is nonirrigated or rainfed (Somrith and Awakul, 1979). The country is divided into four regions: central, north, northeast, and south. Rice production systems are distinguished according to geographical regions and irrigation, and consists of upland rice, deepwater rice, irrigated and rainfed lowland rice. Two planting methods, broadcast and transplanting are generally used in rice cultivation. Most of the north, northeast and south rice-growing areas grow transplanted rice during the monsoon season. In the central plain, rice cultivation is changed from transplanting method to direct seeding with double cropping system in larger fields. All irrigated rice lands are planted to modern semi-dwarf high yielding varieties. Most of the rainfed lowland and deepwater areas are planted to improved traditional or local photoperiod-sensitive varieties.

Common wild rice, *O. rufipogon*, is widely distributed in north, northeast, central and south part of Thailand (Chitrakorn, 1995; Jamjod *et al.*, 2002). This wild species consists of two types, perennial and annual. They are always found near and around the rice field, in ditches, canals and roadside (Vaughan, 1994; Jamjod *et al.*, 2002). Perennial wild rice flowers around mid-November to the end of December while annual wild rice is started flowering on mid-October and dying out soon after seed set (Jamjod *et al.*, 2002). Punyalue (2006) studied about diversity of common wild rice populations from main growing regions of Thailand. The results showed that common wild rice was separated into three groups by using morphological

characters consist of perennial type, annual-intermediate and spontanea form. Those wild rice start flowers on the end of September to the begining of December.

#### 1.4. Weedy rice

Weedy rice is generally believed to originate from crop-to-wild rice hybridization or degenerated individuals of cultivated rice. It is mostly found in the rice field alongside cultivated rice, but also occurs in the vicinity of rice fields, in ditches, or in sympatric regions of cultivated and wild rice (Vaughan, 1994). Weedy rice plants share most of the features of the two cultivated species *O. sativa* and *O. glaberrima* (Khush, 1997). The weedy rice are more or less mimics of the cultivars with which they associate and reproduced themselves by their dispersed seed or by being sown together with the cultivar seed by man. They often occur in direct-seeded fields but are rare in transplanted paddies. They have a high seed-shedding rate and the seeds buried in soil germinate in the next season (Oka, 1988). Most plants are awned and have red pericarp (red rice).

The weedy rice is distributed over a wider area than the common wild rice in Asia. They can be classified into two categories, one occurring together with common wild rice and the other distributed in the region where no wild rice occur. The former category is commonly found in India and Thailand and is referred to as the weedy type of common wild rice. They seem to have arisen from the hybrids between wild and cultivated rice (Oka and Chang, 1961). Studies of natural weedy Asian rice (*O. sativa*) populations showed introgression into local populations of wild *O. rufipogon* in Thailand (Shu *et.al.*, 1997). Langevin *et al.* (1990) confirmed that red rice or weedy rice– rice hybrid can introgress substantially into rice populations



within two growing seasons. In Thailand weedy rice was observed in farmers' rice field in Kanchaburi, northwest of Bangkok since 2001. Weedy rice showed many different forms and farmers classified them by apparent characters such as Khao Hang (rice with awn), Khao Deed (jumping rice) and Khao Daeng (red rice) (Maneechote *et al.*, 2004).

### **1.5. Gene flow**

Gene flow is the movement of gametes, zygotes (seeds), individuals or groups of individuals from one place to another and their subsequent incorporation in the gene pool of new locality (Slatkin, 1987). Gene flow is a natural biological process and in plants it primarily occurs via pollen and seed dispersal. In flowering plants, pollen dispersal is the main mode of gene flow (Levin and Kerster, 1974). In cultivated species, however, seed exchange among farmers also contributes significantly to gene flow (Dennis, 1987; Louette 1998; Brush, 1999; Sirabanchongkran *et al.*, 2004).

#### **1.5.1 Crop-to-wild gene flow**

The direction of gene flow is predominantly from crop to wild because of the differentiation of mating system among them. Gene flow between two species can occur when both of them are in sympatric region or are in contact. Outcrossing has been shown to occur between crop and wild and weedy relatives, especially in rice (Langevin *et al.*, 1990; Lu *et al.*, 2003; Gealy *et al.*, 2003; Song *et al.*, 2003; Chen *et al.*, 2004; Messeguer *et al.*, 2004). Many researches reported that natural hybridization between *O. rufipogon* and *O. sativa* has been frequently found in many locations (Langevin *et al.*, 1990; Majumder *et al.*, 1997 and Song *et al.*, 2002). Rate

of gene flow between crop and wild rice was shown to vary from 1.21% to 2.54%, depending on the genotypes and their flowering period (Song *et al.*, 2003; Chen *et al.*, 2004).

### 1.5.2 Crop-to-weed gene flow

Langevin *et al.* (1990) found that natural hybridization between cultivated rice and weedy rice was between 1-52% depending on varieties and time to flowering and hybrids were generally taller and had longer, wilder flag leaves than either cultivated rice and weedy rice. Lower rates (<1%) of gene flow between crop rice and weedy rice were observed by Zhang *et al.* (2003). Even lower rates of gene flow from crop rice to weedy rice, of 0.011% to 0.046%, were measured by Chen *et al.* (2004) in experimental fields.

### 1.5.3 Crop-to-crop gene flow

Although crop rice is predominantly a self-pollinating crop, outcrossing can occur between crop rice plants. Natural outcrossing among rice plants is generally lower than between wild and cultivated rice. Most of the pollen dispersal studies published to date indicated that rice x rice outcrossing rates were less than 1.0% (Beachell *et al.*; 1938; Robert *et al.*; 1961; Messeguer *et al.*; 2001; Zhang *et al.*;

2003). Gene flow in cultivated rice (*O. sativa*) was observed by Gealy *et al.* (2003).

Gealy's report although suggested that outcrossing rates between rice and red rice can be highly variable but usually are similar to or lower than this level. However, even 0.1% outcrossing can have a significant impact on genetic changes in the population.

This 0.1% represents 2 cross fertilized seeds from 1 m length of two adjacent rows of different genotypes that happen to flower in synchrony.

## 1.6 Gene flow assessment

Jenczewski *et al.* (2003) suggested that effective crop-wild gene flow depends on the completion of a number of successive steps required for gene dispersal: (i) the presence of crops and close relatives growing nearby, (ii) their biology and phenology in relation to pollen and seed dispersal, (iii) the production of viable and fertile F<sub>1</sub> hybrids, (iv) the production of fertile successive generations, (v) the opportunity for gene transmission, chromosome recombination and crop genes introgression into the wild genetic background and ultimately (vi) the persistence of introgressed crop genes in natural communities.

Gene flow can be estimated using direct and indirect methods. Pollen and seed for flowering plants are the two major vectors of gene flow. In direct method, observations of pollen or seed dispersal are used to estimate range of gene flow. Indirect methods using genetic markers are the primary measure of gene flow levels among interbreeding populations (Nilsson *et al.*, 1992). Gene flow can be measured by setting up experiments for detecting gene migration. Another way is to focus on genetic evidence of past gene flow by measuring variation in populations.

Genetic analysis of the wild intermediates has been conducted using isozyme, RAPDs and progeny segregation studies of morphological and physiological traits. Hybrid formed between wild rice and cultivated rice or between red rice and rice varieties in the field were identified by morphology and isozyme (Langevin *et al.*, 1990). A range of classical morphological and molecular techniques have been used to document natural hybridization and introgression between cultivated rice and their wild relatives (Oka and Chang, 1961; Chu and Oka, 1970; Oka and Morishima, 1971; Langevin *et al.*, 1990).

Application of molecular markers to detect gene flow among different varieties of the same species or different species has been extensively reported (Song *et al.*, 2002; Messeguer *et al.*, 2004). Microsatellites or simple sequence repeats (SSRs) are tandem repeats of short nucleotide repeats with 1-5 bp. Simple sequence repeats are characterized as codominant, highly polymorphic, abundant and randomly distributed markers in genome. To date, more than 2000 SSR loci of cultivated rice are available (McCouch *et al.*, 2002), providing a powerful tool for studying gene flow among rice varieties. The SSR markers can be amplified easily by polymerase chain reaction (PCR) and probably selectively neutral (Akagi *et al.*, 1997), which is preferred for quantitative detection of gene flow.

### 1.7 Interspecific hybridization

In natural environment, hybridization between cultivated rice and wild rice occurs because the species are closely related (Harlan, 1992). The study of interspecific hybridization between cultivated, wild and weedy rice has important implications for germplasm conservation as well as for the persistence of novel transgene in wild populations. Normally, intraspecific hybridization show that most of the wild rice species with AA genome have relatively high compatibility with cultivated rice (Naredo *et al.*, 1997, 1998). However, reproductive barriers were observed in cross within AA genome (Naredo *et al.*, 1997), crosses between Australian *O. meridionalis* with cultivated rice (*O. sativa*) has crossability of less than 5% with the cultivated rice and F<sub>1</sub> hybrids did not germinate. Many reproductive barriers have been identified.

### 1.7.1 Reproductive barriers

The reproductive barriers are comprised of premating and postmating ones. Generally, distantly related organisms do not mate, or even if they mate, fertilization does not take place due to disorder in protein interaction. There are different mechanisms preventing such mating; if the two mate and produce a hybrid, it may suffer from various postmating disorders. The postmating barriers are classified into  $F_1$  inviability (crossing barriers),  $F_1$  weakness,  $F_1$  sterility and hybrid breakdown (Oka, 1988). All these have been found in cultivate rice and their wild relatives, although they have the AA genome in common and their  $F_1$  plants show no significant disturbances in meiotic chromosome pairing (Chu *et al.*, 1970 and Majumder *et al.*, 1997).

### 1.7.2 $F_1$ fitness

Studies of hybrid fitness can be used to evaluate the potential for introgression to occur following episode of interspecific hybridization. Introgression breeding or directed introgression can be a powerful model to examine this process in an artificial setting and to predict how it might function when transgenes are moved from crops to wild relatives.

A direct method to assess the potential consequence of crop-wild/weed gene flow, estimation of fitness components of crop-wild hybrids and their parents, particularly the wild parents, is an important step (Snow *et al.*, 1998). Among the large number of studies that have examined fitness-related traits of crop-wild-weed hybrids (Langevin *et al.*, 1990; Snow *et al.*, 1998), most have found crop-wild/weed hybrids to be nearly as fit as their wild parents. These results generally suggest that crop genes might persist in wild populations.  $F_1$  fitnesses from crosses between wild

and cultivated rice are generally high, but hybrids from certain crosses show reduced fertility. Spontaneous intermediates between cultivated rice species and their wild relatives occur frequently in and near rice fields when wild taxa are present. Song *et al.* (2004) found that the hybrids performed poorly at the stage of sexual reproduction, although they had slightly higher hybrids vigor at the vegetative growth stage and better tillering ability than their wild parents and there were no significant difference in composite fitness across the whole life-history between the hybrids and their wild parental species.

Thus, the relative fitness, as estimated by the performance of hybrids in comparison with their parents, is essential for predicting the fate of hybrids and understanding the evolutionary significance of interspecific hybridization. Data on fitness of interspecific hybrids are extremely valuable for predicting the destiny of the hybrids in natural habitats, particularly in the context of environmental biosafety where alien transgenes might be included in the hybrid and cause safety concern (Snow *et al.*, 1998).

The emergence of weedy rice is a serious threat to rice farming in this country.

Local wild rice, on the other hand, remains one of Thailand's most valuable natural resource. Understanding the gene flow between popular cultivars and local wild rice populations should be the basis on which strategies for controlling weedy and protection of the wild rice as a genetic resource that is important to rice eaters and growers worldwide as well as in Thailand.