CHAPTER II

LITERATURE REVIEW

The wild relatives are called “wild rice”, which consists of twenty species belonging to genus Oryza, distributed in the tropics and the sub-tropics of Asia as well as Africa, Oceania and America (Vaughan 1994).

2.1. Classification of *Oryza* species

The genus *Oryza* approximately originated about 130 million years ago in Gondwanaland and is classified under the tribe Oryzeae, subfamily Oryzoideae. Twenty are wild species and two are cultivated (*Oryza glaberrima* and *O. sativa*) species. *O. sativa*, the Asian cultivated rice is grown all over the world and *O. glaberrima*, the African cultivated rice is grown on a small scale in West Africa (Khush, 1997). The cultivated species originated from a common ancestor with AA genome. Perennial and annual ancestors of *O. sativa* are *O. rufipogon* and those of *O. glaberrima* are *O. longistaminata*, and *O. barthii* (Chang, 1976).

Oryza genus is also divided into four species complexes; *O. sativa*, *O. officialis*, *O. ridelyi* and *O. granulata*. The basic chromosome number is 12. *O. sativa*, *O. glaberrima* and 14 wild species are diploids with 24 chromosomes, and 8 wild species are tetraploids with 48 chromosomes and while interspecific crossing is possible within each complex (Oka, 1988 and Vaughan *et al.*, 2003) (Table 2.1).

Table 2.1. *Oryza* species with their chromosome number, genome group and geographical distribution.

Section, Species	Other Name Commonly Found in the Literature	Chromosome Number	Genome Group	Geographical distribution
Oryza				
Oryza sativa complex				
Oryza sativa L.		24	AA	Worldwide, cultivated
O. rufipogon sensu lacto	*O. nivara* for the annual form, *O. rufipogon* sensu stricto for the perennial form	24	AA	Tropical Asia, America
O. glaberrima Steud.		24	AA	West Africa, cultivated
O. barthii A. Chev.	*O. breviligulata*	24	AA	Africa
O. longistaminata Chev. Et Roeher	*O. barthii*	24	AA	Africa
O. meridionalis Ng		24	AA	Tropical Australia
O. glumaepatula Steud.	*O. rufipogon*	24	AA	South America
O. officinalis complex				
O. officinalis Wall ex Watt	*O. minuta*	24	CC	Tropical Asia to Papua New Guinea
O. minuta J. S. Presl ex C. B. Presl.	*O. officinalis*	24	BBCC	Philippines, Papua New Guinea
O. rhizomatis D. A. Vaughan		24	CC	Sri Lanka
O. eichingeri Peter	*O. collina* for the Sri Lankan form	24	CC	East and West Africa
O. punctata Kotschy ex. Steud.	*O. schweinfurthiana*	24, 48	BB, BBCC	Africa
O. latifolia Desv.		48	CCDD	Central and South America
O. alta Swallen		48	CCDD	Central and South America
O. grandiglumis (Doell.) Proehr.		48	CCDD	South America
O. australiensis Domin		24	EE	Australia
Ridleyanae Tateoka				
O. brachyantha Chev. et Roehr.		24	FF	Africa
O. schlechteri Pilger		48	Unknown	Papua New Guinea
O. ridleyi complex				
O. ridleyi Hook.		48	HHJJ	Southeast Asia
O. longiglumis Jansen		48	HHJJ	Papua New Guinea
Granulata Roschev.				
O. granulata complex				
O. granulata Nees et Arn ex Watt		24	GG	South and Southeast Asia
O. meyeriana (Zoll. et Mor. ex Steud.) Baill.		24	GG	Southeast Asia

Source: (Oka, 1988 and Vaughan et al., 2003)

2.2. Identification of wild species

Wild species are taxonomically identified by examination of their key characters. For each of the major crops the wild progenitor has been identified through a combination of morphological, biochemical, and genetic studies (Harlan, 1992). On the basis of morphological and ecological data, multivariate analysis has been applied to classify wild plants into appropriate wild species groups (Morishima and Oka, 1960). Wild species are distinguished from *O. sativa* by traits such as habitat, plant type, spikelet, anther length and shape of ligule and auricle, panicle type, awnedness and shattering seeds. Isozyme patterns are also useful to distinguish wild species from *O. sativa* (Oka, 1988). Basis on RFLP analysis of nuclear DNA, Sun *et al.* (1997) suggested that common wild rice from China could be classified into three types: primitive types, indica-like types and japonica-like types.

2.3 Asian common wild rice (*O. rufipogon*)

2.3.1. Habitat of common wild rice

O. rufipogon is a close relative of cultivated rice, *O. sativa*, and it is found in and around rice fields or abandoned, as well as in ditches, canals, marshes and riverbanks (Vaughan, 1994). It occurs at altitude from 0 to 1000 m and tolerant of flooding and acid soils (Mandal and Gupta, 1997). 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 because of perennial type was mainly propagated by clone and annual type was propagated only by seeds (Sano et al., 1980).

2.3.2. Genetic diversity of common wild rice

Genetic diversity refers to the variation of genes within species, that is, the heritable variation within and between populations of organisms. Diversity by dentition is measurable by the statistical term of “variance”. In applied genetics, it refers to the variance of “a gene” within a population. Thus the variance may be measured among alternative forms (polymorphism) of a gene (alleles) at individual gene positions on a chromosome (loci), among several loci, among individual plants in a population or among population (IPGRI, 2003). With the advent of molecular genetics, we can measure the variance of actual DNA sequences of a gene or a specific length of DNA (a DNA marker) and the morphological diversity analyzing based on Shannon-Weaver Index (H'). In addition, gene frequency and genotype frequency in locus or several loci help us to elucidate population dynamics such as mode of reproduction, gene flow from other populations and environmental heterogeneity, and thus the fate of the population (Jain 1975, cited in Kuroda, 2004). Several studies based on morphology, cytogenetics, ecology and biochemical and molecular markers have significantly contributed in complementary ways to the current knowledge of genus *Oryza* (Vaughan, 1994). Molecular markers based genetic diversity analysis also has potential for assessing changes in genetic diversity over time and space. Several types of molecular markers are available for evaluating the extent of genetic variation in within species. These include Restriction Fragment Length Polymorphism (RFLP) (Bostein *et al.*, 1980), Random

Amplified Polymorphic DNA (RAPD) (Williams et al., 1990), Amplified Fragment Length Polymorphism (AFLP) (Vos *et al.*, 1995), and Microsatellite or Simple Sequence Repeat (SSR) (Tautz, 1989). Microsatellite Markers are PCR-based markers that are technically efficient and cost-effective to use and have been wildly used to study population and conservation genetic (Olsen & Schaal, 2001).

Studying on genetic diversity within and among common wild rice populations were carried out by many researchers. In Thailand, Punyalue (2005) showed that 12 common wild rice populations were separated into three groups; perennial type, annual type and spontanea form based on morphological and physiological characters. Molecular analysis using 7 microsatellite loci found that total genetic diversity (H_T) were 0.225. In addition, Wongtamee (2008) evaluated structure of genetic diversity of 36 common wild rice populations (14 annual, 11perennial, and 11 annual-perennial intermediate) from northeastern Thailand using 5 microsatellite markers and indicated that perennial and intermediate types showed higher genetic diversity (0.3) than the annual type (0.18). Similar study in China, Song *et al.* (2003) evaluated the genetic diversity 6 populations of *O. rufipogon* collected from north of China analyzed by using 23 primers. The result, revealed that genetic diversity among 6 common wild rice populations were high with expected heterozygosity (He) over all populations was 0.480, and the average and genetic different among these populations ranged from 0.066-0.276. In addition, Kuroda *et al*. (2006) analyzed population structure of 10 perennial, and 10 annual populations, collected from Vientiane Plain, Laos. Higher genetic differentiation was detected among annual populations (G_{ST} = 0.77) than perennial populations (G_{ST} =

0.29), whereas genetic diversity all populations of these two wild species shown similar values (H_T = 0.77 and 0.64 in perennial and annual, respectively). By the way, knowledge of between and within population genetic variation in natural is strategies to develop the cultivated crop such, provide resistance to pests and diseases, increase yield, improve quality and *in situ* and *ex situ* conservation of agricultural biodiversity (Brown, 2000).

2.3.3. Gene flow

Generally, cultivated rice (*O. sativa*) is a predominantly self-pollination crop, with 0-1 % out-crossing rate (Robert *et al.,* 1961), while the its progenitor, common wild rice (*O. rufipogon*) is a cross-pollinated species, with 7-55% of out-crossing (Barbier, 1989). Gene flow or cross pollination between wild rice and cultivated rice in natural is often found in areas where both species are coexisted (Ellstrand et al., 1999). It may alter genetic structure, gametes, zygotes (seeds), and their subsequent integration in the gene pool of new locality as compatibly by pollen flow and seed dispersal (Slatkin, 1987). For hybridization between *O. rufipogon* and *O. sativa*, gene flow between them has been frequently found in many locations (Majumder *et al.,* 1997; Song *et al.*, 2003, Niruntrayakul, 2008; Wongtamee, 2008). 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), between cultivated rice and weedy rice was between 1-52% depending on varieties and time to flowering (Langevin *et al.* 1990).

2.3.4. The importance of wild rice growth stage

Seed dormancy

Seed of wild rice show strong dormancy at maturity, because of the hard seed coat (Oka, 1988). After being dispersed and buried in the soil, they do not germinate for certain periods until their dormancy is overcome. In wild plant species, variable dormancy can be a useful adaptation, preventing competition between seedlings resulting from simultaneous germination (Oka 1988). For rice, seed dormancy is also important in tropical species and cultivars as a means of preventing preharvest sprouting (germination of the seed in the panicle) if conditions are warm and wet before harvest (Gu et al. 2004). For wild relative, shattered seeds with dormancy may remain dormant and viable for up to 3 years or longevity for several seasons under field conditions (Chen, 2001).

Photoperiod response

Photoperiod response, some species of common wild rice are photoperiod-sensitive. Short or shortening days promote flowering in those species. Thus, the best time to grow them is when day-lengths are shortening. Allow sufficient time for slow growing wild species to become well established before panicle initiation. At Los Banos, Philippine, seed are germinated in June and July with maximum flowering of the photoperiod-sensitive species from October to December when day-length is shortening to a minimum of 11 h 20 min. (Vaughan 1994). In Thailand (Chitrakon 1995) reported, the sensitive to photoperiod of *O. rufipogon* perennial were found (72%), annual 68% and spontanea form (60%).

Seed shattering

Seed shattering of wild rice is one of the most greatly changed traits compared with cultivated rice. Seeds disperse immediately by shattering at maturity, and buried in

the soil for subsequent germination (Oka, 1988). Wild rice typically display long awns and severe shattering for seed dispersal, whereas the domesticated type have short awns and reduced shattering to maximize the number of seeds that can be harvested (Cai and Morishima, 2000). According to the process of domestication, cultivated rice has lost many of the traits associated with other wild grass species; in addition to a shift from perennial to annual populations, cultivated rice differs from *O. rufipogon* in its non-shattering seeds, lack of awns, erect habit, and high grain yield. In contrast, the characteristics of (*O. rufipogon* Griff.) are high of seed shattering at mature, and high of cross-pollinated with cultivated rice (Chitrakon 1995). Seeds fall near the parent plants but may disperse across greater distances as rice seed contaminants and with human activities, water and soil movement, and possibly by birds (Oka 1988).

Mode of common wild rice (*O. rufipogon*) propagation

In general, wild rice has two ways of propagation, vegetative propagation and seed propagation. *O. rufipogon* was found in permanently deep water tend to be perennial and mainly propagated by vegetative (tillers ratooning from old plants), annual propagated by seed and intermediate propagated by seed and vegetative propagation (Sano *et al.*, 1980). However, the mode of this propagation is influenced by water depth. Perennial the water depth was more than 50cm, whereas for annual less than 50cm (Oka 1988). In this case, the different genetic structures between two species are caused by the different ways of propagation, since the water depth in a wild rice habitat is more than 50 cm for perennial and less than 10 cm for annual type (Kuroda 2004). Otherwise, annuals has a medium to high reproductive effort (0.25 - 0.70) associated with a low regenerative

ability (most individuals below 0.3), while most perennials were characterized by a low reproductive effort associated with a medium to high regenerative ability (Barbier 1989II).

2.4 Germplasm and conservation of common wild rice

The wild species are in danger of extinction as their habitats are being destroyed by many factors especially, by human activities. Responding to this concern, conservation, either in situ or ex situ, ensures that varieties are saved for the use of future generation.

Ex-situ conservation

The ex-situ conservation is strategies for conservation of components of biological diversity outside their natural habitat's (UNEP 2003). With the passport data and a set of all the samples collected is to be conserved at the genebanks, in vitro and cold storage for the medium and long term preservation of germplasm at national or international centers belonging to the Consultative Group for International Agriculture Research (CGIAR), which now constitutes a total of 16 research institutes. Of these, the International Rice Research Institute (IRRI) has played the most importance role in conserving rice genetic resources (Jackson 1997). However, the conservation of seed in genebanks will not be appropriate for some wild rice species, which as the vegetative reproduction and not safeguard genetic diversity in natural habitat (Kuroda *et al.*, 2003b).

In-situ conservation

To compensate for the disadvantages of the ex-situ strategies, attention has been recently paid to in-situ conservation (Vaughan and Chang 1992). According to the United

Nations Convention on Biological Diversity (UNCBD), in-situ conservation of germplasm involves “the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties” (Reid *et al.,* 1993). For natural population of wild species, the genetic diversity of in situ conserved populations should be maintained dynamically in changeable environments, long-term habitat protection is most important to prevent further loss of genetic variation and a decrease in population size. In China, the in-situ conservation of *O. rufipogon* at the population level was considering the extensive genetic erosion that has occurred in *O. rufipogon* populations during recent decades (Song *et al.* 2003). Up to date, *O. rufipogon* in-situ conservation has been implemented at several locations such as in Thailand, Laos, Nepal, and especially widely accepted in China (Kuroda 2004.).

2.5. Genetic diversity used for microsatellite analysis

The microsatellite technique is characterized as co-dominant, highly polymorphic, abundant and randomly distributed markers in genomes. Microsatellite markers can be easily amplified by PCR and are probably selectively neutral. Microsatellites have been used for studies of genetic mapping and breeding (McCouch *et al.* 1997), gene flow (Wongtamee 2007), genetic diversity and population differentiation (Kuroda 2004, Song *et al.* 2003). Relating to the evaluation of genetic diversity of *O. rufipogon* used by microsatellite to compare with the isozyme Gao *et al.* (2002) were found that, microsatellite had genetic diversity and population differentiation higher than isozyme.

Kuroda (2004) were found that the polymorphisms of microsatellite showed higher values than isozyme (Barbier 1989b) and allozyme (Gao and Hong 2000). Similar to Yu *et al.* (2005) reported the polymorphism of microsatellite was 47.62% higher values than RAPD was 3.70%. So in case of my study genetic diversity of common wild rice in Cambodia is appropriate to microsatellite marker use.

2.6. Usefulness of wild rice in plant breeding program

The wild relatives of cultivated rice are an important constituent of the rice gene pool and have contributed significantly to breeding programs. Evaluation of the populations continues to reveal new sources of resistance to diseases and pests and increased as techniques to transfer genes from wild species to cultivated rice improve as increase yield and improve quality (Khush, 1995). Moreover, the wild genus *Oryza* has provided many essential to improve yield in cultivated rice (*O. sativa*) based on a number of physiological and morphological traits such as tolerant to abiotic stresses, cytoplasmic genetic male sterility, potassium chlorate resistance, phenol reaction, resistance to insect and disease (Table 2.2). For example, four lines including MTL98, MTL103, MTL105 and MTL110 derived from crossing between *O. sativa* and *O. officinalis* are resistance to brown planthoppers, and resistance to dwarf disease derived from crossing between cultivated (IR36) and *O. nivara* (Vaughan, 1994). In China, *O. rufipogon* is the large scale adoption of hybrid rice technology, which successful transfer of the male sterility gene to produce cytoplasmic genetic male-sterile (CMS) lines. This technology increased rice production by about 10-20% over the past 20 years in China (Yuan 1993).