Chapter 2

Literature Review

2.1 Definition of genetic diversity

Genetic diversity refers to the variance among alternative forms of the gene (alleles) at individual gene positions on a chromosome (loci), among several loci, among individual plants in the population among populations (Brown, 2000). Within an individual species, genetic diversity allows populations to adapt to changes in the climate and other environmental conditions.

Genetic diversity refers to the variation of genes within species, and species diversity refers to the variety of species within species within region (World Resources Institute, 1998). The definition of genetic resources of International Plant Genetic Resources Institute (IPGRI) is “genetic material of plants, animals and other organisms that is of value as a source for present and future generations of people”.

2.2 Definition of local varieties

Local varieties (landraces, primitive cultivars, traditional cultivars, folk varieties) are crop populations in balance with their environment and remain relatively stable over long time (Frankel and Bennett, 1970). Populations of landraces are often highly variable in appearance, and are expected to be evolutionary active under changing environmental conditions. A landrace has particular properties or characteristics. Farmers have names for them and different landraces are understood to differ in adaptation to soil type, date of maturity, height and other properties (Harlan, 1992). Landrace is a variety with a high capacity to tolerate biotic and abiotic stress resulting in a high yield stability and an intermediate yield level under a low input agricultural system (Zeven, 1998).

2.3 Structure of genetic diversity in local varieties

The landraces grown in subsistence agriculture are diverse and generally carry a great amount of genetic variability in their populations (Oka, 1988). Populations of landraces are genetically heterogeneous. In contrast, populations of improved varieties are outstandingly homogeneous.

2.3.1 Allelic richness and genotype diversity

Landraces are highly diverse genetically, often having been grown as mixtures of species as well as diverse populations of one species (Hawkes, 1983) Landraces have a certain genetic integrity. They are recognizable morphologically; farmers have names for them and different landraces are understood to differ in adaptation to soil type, time of seeding, date of maturity, height, nutritive value, use and other properties. Most important, they are genetically diverse. Such balanced populations variable, in equilibrium with both environment and pathogens, and genetically dynamic are our heritage from past generations of cultivators (Harlan, 1975).

Bounphanousay (2007) studied 53 accessions of black glutinous rice in Laos. Microsatellite markers were used to assess genetic diversity. She found that a total of 75 alleles were detected by 24 microsatellite markers. The number of alleles per microsatellite marker varied from 2 to 7, on average, for each microsatellite, 3.13.

Supamongkol (2006) studied 83 accessions of Muey Nawng (MN) local cultivars in northern of Thailand. She found that ten morphological characters showed high variation with Shannon-Weaver index (H') were in range of 0.1192 to 4.1569. For molecular analysis, a total of 26 alleles were detected by 5 microsatellite markers. The number of alleles per locus varied from 3 to 10, on average for each locus 5.2. Genetic differentiation among populations (G_{ST}) was 0.227 indicated that three quarters of total genetic differentiation (77.3%) was genetic differentiation within population and only one quarter was distributed among populations. In addition, locally, genetically diverse MN populations had lower level of gall midge infestation and higher yield than purified, improved varieties when grown in areas where rice gall midge is a limiting factor.

2.3.2 Adaptation to the local environment

Much evidence and experience attests that landraces are adapted to their local environment (Frankel *et al*., 1995). Landrace would change when grown in another area. The capacity to adapt to new environment depends on the genetic composition of the mixtures. At each growing cycle directional selection takes place; the selection criteria depend on site and year (Zeven, 1998). Some landraces are able to adapt themselves to wide range of environments, whereas others are able to adapt themselves only to a few environments. For the duration of 1968 to 1972; the International Rice Adaptation Experiment was evaluating 70 improved rice cultivars from different countries at 45 stations in 25 countries. It was concluded from the results that the stability of grain yield under changing environments was genotypically determined (Matsuo, 1975). Phattarakul (2008) studied five local upland rice varieties tolerant to acid soil. She found that Bue Bang (BB) and Bue Mue Ta Bong (BM) tolerant to aluminum (Al) toxicity and adaptation to acid soils in the high mountain areas.

2.3.3 Diversity to meet temporal environmental variation

Diversity itself confers long-term population fitness because it helps population to cope with variable environment. Landraces populations of crops have survived centuries of selection for reliable production in subsistence agriculture, yielding a definite; know but probably limited benefit to the farmers that grow them (Frankel *et al*., 1995).

Appa Rao *et al*. (2006) found that landraces cultivars grown in both rainfed upland and lowland ecosystems differ for a range of characters, in upland contain a mixture of several morphological phenotypes, with variation in flowering, plant height, and panicle and grain characteristics and more tolerant to drought.

2.3.4 Continuing crop evolutionary process

The crop evolutionary processes (mutation, migration, recombination, and selection). It provides scope for ongoing evolution, particularly in response to environmental changes and pathogen and pest pressures fluctuating in numbers and genetic composition (Brush, 2000). As it does conserving not only exist germplasm but also the conditions that allow for the development of new germplasm.

Olsen and Purugganan (2002) studied 105 glutinous and non-glutinous landraces from across Asia, molecular evidence on the origin and evolution of glutinous rice allele genealogy of the *Waxy* locus to trace the evolutionary and geographical origins of glutinous phenotype. They found that many non-glutinous varieties in Northeast Asia also carry the splice donor site mutation, suggesting that partial suppression of this mutation may have played an important role in the development of Northeast Asian non-glutinous rice.

2.4 Threat to local varieties

Local varieties are important genetic resources for breeding program. They provide the source of useful genes for the resistance of pests, diseases, and abiotic stress to modern varieties (Brush, 2000). Genetic erosion in crop is the loss of variability from crop populations (Brush *et al.*, 2003). Widespread adoption of modern high yielding crop varieties has led to a concern about erosion in local crop genetic resources and loss of diversity. Replacement of traditional varieties by high yield improved varieties has accelerated in the past 50 years in what is now commonly known as the Green Revolution (Rerkasem and Rerkasem, 2002). According to IRRI, farmers tend to abandon landraces for modern varieties in Southeast Asia, where the economic situation is rapidly changing. Recent spread of modern varieties over local area and rapid changes of the economic situation in developing country have made it difficult to collect landraces as genetic resources (Sato, 1994). In 1966, the high yield variety of IR8 was released. Since that time, the agro-ecological and human environments relating to rice farming have rapidly changed (Thurston *et al*., 1999).

In recent times "advances" in agriculture have generally been reflected in the establishment of monoculture production systems based on the use of a few profitable varieties, with the concurrent disappearance of local varieties. This is particularly so in countries with a tradition of growing rice. FAO (1993) has estimated the more than 60 % of the world' rice areas are planted to varieties of improved plant type. In Indonesia, Philippines, Srilanka, modern rice varieties are currently estimated to cover 82%, 85%, and 87% of the total rice area, respectively. In the Lao PDR, the spread of improved varieties in farmers' fields in the provinces along the Basin of the Mekong

River has increased from 10% of the provinces' rice area in 1993 to 81% in 2001 (Lao-IRRI, 2003).

2.5 Farmers management of diversity

Farmer's management of diversity as farmers makes decisions in the process of planting, managing, harvesting and processing their crops that affect the genetic diversity of crops populations. Over time they will modify the genetic structure of population by selecting for plants with preferred agromorphological characteristics (Jarvis *et al*., 2000). It is sustainable management of genetic diversity of locally developed traditional crop varieties, with associated wild and weedy species or forms, by farmers within traditional agricultural, horticultural cultivation systems (Maxted *et al.*, 1997).

According to Bellon *et al*. (1997), *in-situ* or on-farm conservation of diversity has been defined as "farmers continued cultivation and management of diverse set of crop populations in the agro-ecosystem where the crop has evolved". On-farm conservation is dynamic because the varieties that farmers manage continue to evolve in response to selection pressures. In on-farm conservation, the role of the farmer is seen in two ways: First, crop variety development is not only the result of natural selection, but also reflects the effects of human selection and management. Secondly, farmers' decisions determine whether populations are maintained. On-farm conservation therefore depends on the active participation of farmers.

Roder *et al*. (1996) undertaken surveys in the northern Lao provinces of Luang Prabang and Oudomxay in early 1991s, indicated that upland farmers continue to use traditional rice varieties only, and generally prefer varieties with large panicles, medium tillering, vigorous early growth, and with glutinous, aromatic grain qualities.

The average number of varieties grown by a household being 2.7, and proportion of areas planted with early, medium and late varieties was 17, 30 and 53%, respectively. Traditional varieties are mainly of the japonica type, have a good yield potential, are well adapted to the local conditions, and represent a wide genetic diversity.

2.6 Factors affecting genetic diversity of local populations

Farmers' management has been identified effecting crop diversity, seed flow, variety selection, variety adaptation and seed selection and storage (Bellon *et al*., 1997). Farmers will influence the survival of the certain genotypes by choosing a particular management practice of planting a crop population in a site with a particular micro-environment. Farmers make decisions on the size of the population of each crop variety to plant each year, the percentage of seed to save from their own stock, and the percentage to buy or exchange from other sources. Each of these decisions, which can affect the genetic diversity of cultivars, is liked to a complex set of environmental and socioeconomic influences on the farmer (Jarvis and Hodgkin, 2000).

Environmental selection as agroecosystems provide the arena in which crop evolution occurs, presenting stresses, but also opportunities, to crop must adapt in order to thrive (Jarvis *et al*., 2000). These factors vary over time, with seasonal, annual and stochastic changes, and in space, from the micro-environmental to the ecoregional scale. As a result, local landraces adapt to the particular conditions of their immediate ecogeographic setting. These adaptations to local environmental stresses are likely to be reflected in the genetic composition of landraces over time.

2.7 Rice diversity base on ecosystem in Laos

Laos is a country with a high level of genetic diversity in rice (*Oryza sativa* L.), both among cultivars used by the Lao farmers and among wild species occurring scattered in forested upland as well as in lowland swamps (Roder, 2001). Lao farmers allocate varieties to particular fields based mainly on crop duration and soil moisture. In general, the range of maturity of upland varieties is considerably less than that for lowland varieties (Appa Rao *et al.*, 2002). Rice cultivation in both rainfed lowland and upland environments is based on systems of minimum inputs or 'natural farming' (Tanaka, 1993), and using family labor as the single most important input. Subsistence and modern farming methods coexist side by side, and transition between these two systems is seen in some locations (Worner, 1997). Upland rice cultivation is primarily by slash-and-burn shifting systems on steep. Farmers' varieties grown under rainfed lowland and upland environments differ considerably for morphological, physiological, and grain quality attributes. The main characteristics used to differentiate between the two types are grain size, aroma, tiller number and stem diameter (Schiller *et al.*, 2006). However a few traditional varieties are grown under both ecosystems.

2.7.1 Rice diversity in the rainfed lowland environment

In traditional system, most lowland farmers grow a group of homogeneous stands of several varieties in small plots in the same field. The varieties grown differ for several characters that include grain quality attributes to various types of food preparations and maturity duration (Appa Rao *et al*., 2006).

Traditional lowland varieties are relatively more uniform in terms of maturity, plant height, and grain and panicle characteristics than upland varieties. Most lowland varieties produce narrow and long leaf blades, many thin culms, panicles that are small and numberous, with varying grain size that is typical to the indica group (Appa Rao *et al*., 2006). Crop duration of the traditional varieties ranges from about 100 to as much 270 days.

2.7.2 Rice diversity in the rainfed upland environment

Upland farmers usually grow upland rice crops, which contain a mixture of several morphological phenotypes, with variation in flowering, plant height, and grain characteristics. Farmers grow between 3 to 6 varieties each year, with the average number of varieties grown by a household being 2.7 (Roder *et al*., 1996). Upland farmers clearly differentiate among early, medium, and later-maturing varieties and most household plant varieties from each group (Appa Rao *et al*., 2006). Farmers also know what varieties are best suited to upland areas, and what varieties can only be planted under upland conditions. Farmers also generally believe that all upland rice varieties will grow well provided that they relate the particular characteristic of varieties to particular soil types. They are generally also aware of which varieties will the highest yields on the best soils. In relation to this, from season-to-season, upland farmers identify plots with varieties or particular groups of varieties (Roder, 2001; Pandey and Sanamongkhoun, 1998).

Based on gross morphology of the plant, most upland varieties belong to the javanica group, as they produce thick culms, dark green, long, and wide leaf blades, few but large panicles, and large grain (Schiller *et al*., 2006). Varieties belonging to this group often have a superior root system when compared with those of the other group, and should be better adapted to areas, such as the upland growing environments, that experience periodic moisture stress.

2.8 Conservation of traditional rice varieties in Laos

Scientists have trended to conserve crop diversity mainly by collecting samples from farmers' fields and storing teem *ex-situ* (off-farm) in genebanks (Holden and Williams, 1984). However, in recent years there has been increasing recognition of the need to complement this with in-situ (on-farm) conservation. The definition of in-situ conservation as 'on-farm conservation' is considered to be sustainable management of genetically of locally developed traditional crop varieties with associated wild and weedy species or forms by farmers with traditional agricultural, horticultural cultivation systems (Maxted *et al*., 1997).

Anticipating changes to this natural biodiversity associated with the development and release of range of improved rice varieties, between 1995 and 2000, the International Rice Research Institute and the Lao Ministry of Agriculture and Forestry, jointly undertook rice germplasm collecting throughout the country. During this period, a total of 13,192 samples of cultivated rice were collected. Most of samples (55.9%) were collected from the upland environment than from lowland sites (44.1%). Most of the samples (85.5%) had glutinous endosperm (Appa Rao *et al*., 2002). The province from which the largest number of samples was collected was Luang Prabang from the northern agricultural region, which 1,243 samples, of which 70.4% were collected from the upland environment.

2.9 Measurement of genetic diversity of local rice varieties

Variety diversity is genetic diversity measured at the level of the variety. It can be measured as the absolute number of rice varieties found in a study area. Diversity can be measured on accession hold in gene-bank, lines or populations utilized in crop breeding programs, or varieties cultivated by farmers. Species diversity has been defined as “the number of species in a community and their relative abundance” (McNaughton and Wolf, 1979). The relative abundance of species is usually referred to as evenness.

For the rice crop, diversity refers to variance among varieties and within populations in a variety. There are several methods of diversity measurement including: morphological characterization, biochemical characteristics (e.g., isozymes), and molecular markers (Brush *et al*., 2003). These methods offer numberous ways to measure the amount of variation that can be identified in a population.

Currently, measurement of genetic is widely used for variance among varieties and within populations in a variety such as:

Margalef’s index is widely used for species richness measures. It is calculated from the following formulae:

$$D_{mg} = (S-1)/ \ln N$$

S = the number of varieties.

N = the number of individuals.

Shannon diversity index is widely using to measure diversity within populations and varieties because it incorporates richness and evenness into a single measure (Brush *et al*., 2003). This index is measured using the following formula:

Shannon diversity index

$$H' = -\Sigma p_i * \ln p_i$$

p_i = the proportional abundance of *i*th characters = (n_i/N)

2.9.1 Measurement of morphological and physiological characteristics

There were several studied of diversity of local rice varieties in morphological characterization such as Dilday *et al*. (1998) evaluated 17,279 accessions from 110 countries. They Found that day of flowering range from 37 to 219 days, kernel length 3.0 to 9.9 mm, kernel length/width ratio (1.0 to 8.0), plant height range from 41 to 208 cm, and 1,000-grains weight from 6.9 to 46.0g, and also Meesin (2004) studied 22 accessions of Bue Chomee local cultivars from five villages in Chiang Mai, northern of Thailand. She found that eighteen morphological characters showed high variation with Shannon-Weaver index (H') were in range of 0.9015 to 5.7452. Mounmeuangxam (2003) evaluated morphological and physiological characters of local rice varieties in Houaphanh province, northern Laos. This study was found that highly diverse both between and within varieties.

2.9.2 Measurement of genetic diversity with DNA level

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 rice. These include Restriction Fragment Length Polymorphism (RFLP) (Bostein *et al*., 1980), Random Amplified Polymorphic DNA (RMPD) (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 widely used to study population and conservation genetic (Olsen & Schaal, 2001). Tajai (2008) studied genetic diversity of local rice varieties 15 populations at Ayomai village. For DNA analysis used four microsatellite loci. She found that high level of genetic variation of local rice varieties h=0.619, and genetic differentiation among populations F_{ST}=0.791 and similar Pusadee *et al*. (2005) studied genetic structure of a local Thai rice (*Oryza sativa* L.) variety. Twenty three populations of the landrace rice variety "Bue Cho Mee" (*Oryza sativa* L.) were collected from farmers in six villages covering four districts in Chiang Mai province, northern Thailand. DNA analysis used six microsatellite loci. They found that high level of genetic variation of local Bue Cho Mee. Gene diversity (h=0.2109) partitioning of total genetic differentiation among population, G_{ST} =0.2426, showed that most microsatellite variation was distributed within population 75.74 % and only 24.26% was between populations. Thomson *et al*. (2007) studied genetic diversity of traditional and improved Indonesian rice germplasm. The 330 rice accessions, consisted of 309 Indonesian varieties (246 Indonesian landraces and 63 Indonesian improved cultivars), using 30 fluorescently-labeled microsatellite markers. They found that genetic diversity analysis characterized the Indonesian landraces as 68% indica and 32% tropical japonica, with an indica gene diversity of 0.53 and tropical japonica gene diversity of 0.56, and a F_{ST} of between the two groups. All of improved varieties sampled were indica, and had an average gene diversity of 0.46.