Chapter III

Materials and Methods

3.1 Field Survey

The objectives of this section were to examine rice diversity, seed management practices, and agricultural production systems in the study area. At the same time, the common varieties were selected for more detailed study under controlled experimental conditions at the University of Chiang Mai.

3.1.1 Site Selection

Three villages Ban Ong, and Ban Kan, and Ban Lak Sipsong in Samneua district of Houaphan province Laos were selected for the study. Ban Ong and Ban Kan are inhabited by Lao Lum (lowland Lao), Ban Lak Sipsong is inhabited by Lao Sung (highland Lao). These villages are all located in the range 900 to 1200 m asl. These villages are targeted areas for development by the province and are known to suffer a chronic rice deficit; they are also known to be representative of rice growing areas in Samneua district of Houaphanh province. Ban Ong and Ban Kan are situated about 5 km and 10 km to the north of Samneua town, respectively. Both villages cultivate both upland and lowland rice. The third village, Ban Lak Sipsong, is situated 12 km south of the Samneua town. The inhabitants of this village grow a combination of glutinous and non-glutinous varieties, while in the two villages Ban Ong and Ban Kan; most households grow only glutinous varieties.

3.1.2 Methodology

General information on the villages and current cultivation practices and use of rice varieties, was obtained in discussions with leaders and elders of the three villages, and in more general meetings with villagers. More detailed information was obtained by means of interviews with household heads of a total of 36 households in the three villages. Discussions with village committees resulted in the selection of

three primary criterions in defining their socio- economic status: (i) household self-sufficiency of rice for the year; (ii) area of land per household used for rice cultivation; and (iii) housing conditions. Based on these criteria, village households were stratified into rich, medium and poor. In each village, four households were selected from each category for detailed study, a total of 12 households in each village. The questionnaire used contained a number of open-ended questions, which focused on the reasons for villagers planting several varieties, sources of seed of the varieties used, details of any seed exchange, seed selection and seed storage processes. Basic information on the number of varieties used, endosperm type and details of land holdings, education, and seed lot management were also collected.

Community meetings were used to gather information on the total number of rice varieties present in the community, as well the number of households growing each variety, classification of each variety based on endosperm type and ecosystem. Positive and negative of the varieties were also discussed during these meetings. The varieties were also ranked in order of importance within individual households. Ten grains of all varieties found in each village were measured in grain length and width, grain length and width ratio, and 1000-grain weight. Nine seed lots of 6 common different cultivars were also collected for detailed studies of grain morphology, and for evaluation under experimental conditions at Chiang Mai University.

3.2 Field Experimentation

The objective of the field experiment undertaken at Chiang Mai University was to measure the genetic diversity within samples of the most popular varieties collected during the field survey, and to compare the genetic diversity between samples of seed bearing the same varietal name but obtained from different villages, and from different farmers from within the same village.

3.2.1 Experimental Location

The field experiment was undertaken at The Multiple Cropping Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai, Thailand.

3.2.2 Methodology

A total of 9 samples representing 6 different cultivar names were compared in the study. Six samples came from the rainfed upland environment and three from the rainfed lowland environment. Seven of the nine samples were glutinous types; the two non-glutinous types came from the rainfed upland environment (Table 1). 50 grains in each 9 seed lots was characterized based on: width and length, Lemma and palea pubescence, lemma and palea color, awning and awn color, sterile lemma color, apiculus color, and seed coat color. Each character was calculated for diversity index by using the Shannon diversity index (H').

Table 1 Variety samples studied in the field experiment

Growing environment	Glutinous 'samples'	Non-glutinous 'samples
Rainfed upland environment		
1. *Kainoyhai* (KNH)	1	-
2. *Kaopu* (KP),	1	-
3. *Kaodon* (KD)	1	-
4. *Kaolai* (KL)	-	1
5. *Kaochaohang* (KCH).	-	2
Ranfed lowland environment		
1. *Kainoyleuang* (KNL)	3	0
Total	6	3

The upland rice samples were planted in dry-land conditions, on 5 July 2002, while the lowland rice samples were planted in wet- land conditions on 30 June 2002. Each sample was planted in plots 3×6 m^2, using a 25×25 cm plant spacing; a total 288 hills were grown in each plot, each hill having one plant. In the upland field conditions, the plots were kept weed free by hand weeding at 30, 50 and 70 days after planting. No weeding in the lowland field conditions.

3.2.3 Data Collection

Detailed of the data was collected from 40 plantss in each plot using the Standard Evaluation System for Rice. Tillering was counted at 45 days after planting. Plant height was measured in the milk stage by using actual measurement (cm) from soil surface to the tip of tallest panicle. Maturity was recorded in mature grain stage by using the number of days from sowing to grain ripening (85% of grains on panicle are matured. Detailed morphological information collected related to leaf length and width by enter actual measurements, in centimeters of the leaf just below the flag leaf during heading stage, leaf blade color was observed in the stem elongation, awning, awn color were observed in the heading and mature grain stages, apiculus color was observed in milk stage, lemma and palea color in the mature grain stage, sterile lemma color in the mature grain stage, seed coat color mature grain stage, 1000-grain weight in the mature grain by enter measurement in grams of 100 well-developed whole grains dried to 14 % moisture content, grain yield was weighted after 3 days in sunshine and 15 day in the shade, and harvest index is fraction between grain yield and total dry weight by using grain yield at 14% moisture content and straw weight at 3 day dried in sunshine and 15 days in the shade, grain length, grain width, and ratio of grain length and width were measured in millimeters. The detail of these characteristics is contained in Appendix 5.

3.3 Crop Survey

The objective of the crop survey was to measure the diversity of most popular rice variety (KNL) among farmers' field, and to estimate the yield components for this variety under on-farm conditions. It was anticipated that the results of the survey would provide information on improvement strategies for future research under on-farming conditions.

3.3.1 Farm Selection

The Ban Kane community in Samneua district of Houaphanh province was selected for this detailed study. The selection of this village was based on the fact that all households grow the variety KNL, and has grown it for a longer period (25 years) than the other two villages also covered by the initial survey (Ban Ong and Ban Lak

Sipsong). Farmers had also indicated that they had themselves observed some differences in characteristics of the variety, between farmers. The variety is also regarded as being well suited to the cool conditions that prevail in much of northern Laos. The diversity that is now being observed possibly reflects a combination of natural selection as well as selection by individual farmers.

3.3.2 Methodology

A total 23 farmers or fields growing KNL were selected for the study. Three x $1m^2$ plot samples were harvested in each field for detailed study. In each plot, grain and straw yield were estimated, for grain yield based on 14% moisture content and straw was sun dried for 3 days before weighting. Yield components were determined, for number of hills/m^2, tillers/m^2, panicles/m^2, spikelets/panicle, percentage of filled grain. Four panicles were selected from each sample, a total 12 panicles per farmers' field, for estimates of spikelets/panicle and percent of filled grain.

Records were also kept for each sample on – the numbers of tillers with leaf pubescence, the presence of awns, and the numbers of off-type plants. Using this data, the diversity within the population of KNL was then estimated under farmer's field conditions using the Shannon Diversity Index (H).

Information on farmer management practices was obtained by interviewing the 23 farmers prior to the crop sampling. Using both a semi-structured interview form and general discussion, information was obtained on seeding practices, soil preparation, fertilizer use, weeds management, water management, pests and disease control, and seed selection.

At the time of harvesting samples of the farmer's crops, soil samples were also taken for characterization. The samples were taken from soil categories identified by the village farmers. In Ban Kan, the farmers have divided their paddy fields into two levels of fertility – low and medium. Samples of soils from fields in both these categories were taken for detailed study. Analyses were undertaken under laboratory conditions for P, K, organic matter and pH (Table 10).

Environmental data approximating that for the village was obtain from the nearest meteorological station, the provincial town of Samneua, approximately 6 km from Ban Kan. The information collected included - rainfall, temperature, sunshine duration and altitude (Appendix 7).

3.4 Data Analysis

3.4.1 Field Survey

The design of the field survey was such that the data from the entire survey could be aggregated. The data was aggregated at the village level to provide information on generalizations and comparisons. Significant social and economic trends for each village covered by the survey are presented, together with their respective seed management strategies.

The Margalef diversity index was used for varietal diversity at the village level. This model measures a richness of varieties in a given area. The formula for calculating the index is as follows:

$$\text{Margalef' index} = M_{mg} = (S-1)/\ln N$$

Where S= the number of varieties and N= the number of seed lots found in respondents

3.4.2 Crop Survey

For the crop survey, the Shannon diversity index was used as the measure of diversity, based on selected morphological characteristics, which allowed the identification of differences between individual plants; variance analysis was used to analyze the yield components according to the following formulae:

Yield = dry grain weight in grams per square meter

To get an estimate of yield the following components were used: Npl = Number of plants per square meter; Npa = Number of panicles per a plant; Nsp = Number of spikelets per a panicle; % FG = Percentage of filled grains; WG = Weight of one grain (gram)

3.4.3 Field Experiment.

Variance analysis (ANOVA) and Shannon diversity index (H') were used to analyze and interpret the data from the field experiment. This model incorporates *richness* and *evenness* into a single measure. The Shannon index is maximized when the total number of individuals in a sample is evenly distributed among the species represented in a sample. This maximum is given by the natural logarithm of the number of species. In this study, a number of different morphological characteristics present in a rice sample represent the species. In calculating the diversity index between and within a variety, the following formula for the Shannon diversity index was applied.

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

Where p_i = the relative abundance of the *i*th characters= (ni/N).

ln = natural logarithm.

N= sample size

The formulae was used to measure diversity between rice varieties and within populations of the same species, but collected from different farmers and villages. The results of calculation showed the degree of diversity among varieties as well as among populations within a variety.