Chapter 3

Materials and Methods

This section describes research methods, which were separately carried out in two parts, the household survey and the characterization of local rice varieties.

3.1 Household survey

3.1.1 Site selection

Four villages, Ban Ladthahea (LTH) and Ban Houayleung (HL) in Pak Ou district (PO), Ban Houayman (HM) and Ban Thapho (TP) in Phonxay district (PX) of Luang Prabang province, northern Laos were selected for the survey. The PO and PX districts are located about 30 km north and 64 km east of Luang Prabang City, respectively (Figure 3.1). The distant between villages in each district is about 5 km. The altitudes of LTH, HL, HM, and TP were 307, 315, 363 and 370m, respectively.

3.1.2 Methodology

General information on the villages and current cultivation practices and use of rice varieties, was obtained in discussions with leader and elder of the four villages, and in more general meetings with villagers. The survey was carried out in March 2007 during the period of field fallow. More detailed information was obtained by means of interviews with household heads of a total 112 households in the four villages. The survey questionnaires focused on germplasm management (Appendix 1), 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 landholdings 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. Seeds of each variety within each village were collected and classified of each variety based on endosperm type and ecosystem (Table 3.1).

3.1.3 Data analysis

The data from the survey was checked for normality, descriptive analysis was used to qualify and quantify the significant relationships between selected variables.

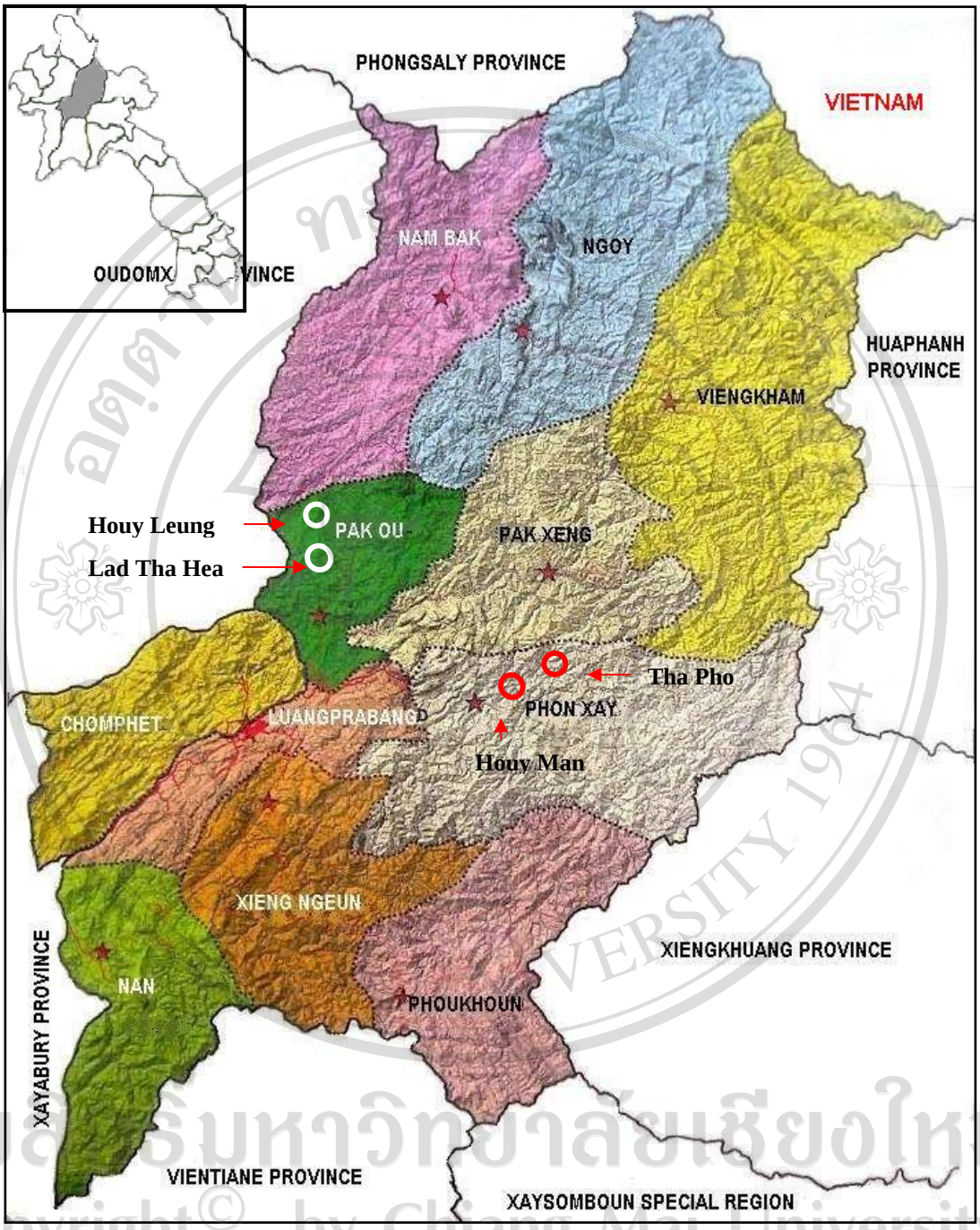

Figure 3.1 Location of Luang prabang province in Lao PDR and the villages in this study.

Table 3.1 Description of local rice in the study areas

	Varieties name	Abbreviation	Ecosystem type	Endosperm Type
Ban Houy man (**HM**), **Phon Xay district (PX)**				
1	Mak Khuea Yai	MKY	Upland	Glutinous
2	Kao Chuk	KCH	Upland	Glutinous
3	Do Deng	DD	Upland	Glutinous
4	Mak Khuea Noi	MKN	Upland	Glutinous
5	Nam Man	NM	Upland	Glutinous
6	Mai Hok	MH	Upland	Glutinous
7	Kao Bung	KB	Upland	Glutinous
8	Deng Phuey	DP	Upland	Glutinous
9	Kao Kum	KKu	Upland	Glutinous
10	Kao Tum	KT	Upland	Glutinous
11	Chao Do	CHD	Upland	Non-glutinous
12	Kao Phae	KP	Upland	Glutinous
13	Mak Hin Soung	MHS	Upland	Glutinous
14	Kao Mee	KM	Upland	Glutinous
15	Kao Dum	KDu	Upland	Glutinous
16	Kao Deng	KD	Upland	Glutinous
17	Do Khao	DKH	Lowland	Glutinous
Ban Tha pho (TP), **Phon Xay district (PX)**				
18	Kao Chuk	KCH	Upland	Glutinous
19	Mak Khuea Yai	MKY	Upland	Glutinous
20	Luem Phouw	LP	Upland	Glutinous
21	Mak Khuea Noi	MHN	Upland	Glutinous
22	Kao Deng	KD	Upland	Glutinous
23	Deng Phuey	DP	Upland	Glutinous
24	Kao bung	KB	Upland	Glutinous
25	Nam Man	NM	Upland	Glutinous
26	Kao Louang	KLO	Upland	Glutinous
27	Kao Leung	KLE	Upland	Glutinous
28	Pak Lueng	PL	Upland	Glutinous
29	Chao Tum	CHT	Lowland	Non-glutinous
30	Thadokham	TDK	Lowland	Glutinous
Ban Lad tha hae (LTH), **Pak Ou district (PO)**				
31	Phae Pee	PP	Upland	Glutinous
32	Phae Do	PD	Upland	Glutinous
33	Kao Nok	KN	Upland	Glutinous
34	Phae Kang	PK	Upland	Glutinous
35	Man Pou	MP	Upland	Glutinous
36	Nam Mak	NMA	Upland	Glutinous
37	Leung Ban	LB	Upland	Glutinous
38	Kao Hea	KH	Upland	Glutinous
39	Kao Kan	KK	Upland	Glutinous
40	Kao Deng	KD	Upland	Glutinous
41	Mon Do	MD	Upland	Glutinous
42	Chao Do	CHD	Upland	Non-glutinous
43	RD16	RD16	Lowland	Glutinous
44	Nam Paa	NP	Lowland	Glutinous
45	Do Dai	DDa	Lowland	Glutinous
46	Na Phon	NPH	Lowland	Glutinous
Ban Houy Leung (HL), Pak Ou district (PO)				
47	Phae Pee	PP	Upland	Glutinous
48	Phae Do	PD	Upland	Glutinous
49	Phae Kang	PK	Upland	Glutinous
50	Kao Nok	KN	Upland	Glutinous
51	Lai Yai	LY	Upland	Glutinous
52	Nok Khor	NKH	Upland	Glutinous
53	Kao Do Det	KDD	Upland	Glutinous
54	Chao Lao Soung	CHLS	Upland	Non-glutinous
55	Kao Kum	Kku	Upland	Glutinous

Table 3.1 (continued).

	Varieties name	Abbreviation	Ecosystem type	Endosperm Type
56	Kao Kan	KK	Upland	Glutinous
57	La Boun	LB	Upland	Glutinous
58	Chao Peek	CHP	Upland	Non-glutinous
59	Kao Deng	KD	Upland	Glutinous
60	Taa Loy	TL	Upland	Glutinous
61	Nam Paa	NP	Lowland	Glutinous
62	Kao Khao	KKH	Lowland	Glutinous
63	Do Khao	DKH	Lowland	Glutinous

3.2 Characterization of local rice varieties

3.2.1 Farmers' seed characterization

Sixty-three samples of local rice varieties from 3.1 were evaluated. One hundred grains in each sample were characterized based on husk color, pericarp color, grain weight, endosperm type, grain width and grain length.

3.2.2 Progeny tested

Seed from sixty-three samples of local rice varieties from 3.2.1 were sown in pots. Planted at the Agronomy Department, Faculty of Agriculture, Chiang Mai University, Thailand on 29 June 2007. Seeds from each sample were pre-germinated in petri-dishes before transplanted into pots, 10 plants/pot. Twenty plants of each sample were grown, e.g. two pots/sample.

Morphological and physiological characteristics were recorded individually using method of IRRI-IBPGR (1980) at three stages;

Tillering: color at different plant parts including, leaf blade, basal leaf sheath, blade pubescence, collar, auricle and ligule, and number of tillers per plant.

Flowering: days to flowering, presentation of awn, color of apiculus and stigma.

Maturity: culm length (cm), number of panicles/plant.

Harvesting: each plant was harvested individually. Two panicles from each plant was randomly collected and measured for panicle length (cm), number of seeds/panicle, filled seed (%). About ten seeds from each plant were determined for seed width, seed length, husk color, husk pubescence, and pericarp color.

3.2.3 DNA analysis

Five varieties with the same name, two to four samples for each variety were selected for DNA analysis. Leaf samples were collected from 10 plants/sample. Genomic DNA were extracted using modified method from Doyle and Doyle (1987) and the PCR (Polymerase Chain Reaction) was performed following the description of Panaud *et al*. (1996). Microsatellite markers with 6 SSR (Simple Sequence Repeat) primer pairs, RM1, RM5, RM22, RM164, RM259 and RM 316 were used (Table 3.2). Amplification of DNA was performed in 20 μl reaction consisted of 20-50 ng DNA, 0.25 mM of each dNTP, 2% formamide, 0.2 μM of each primers and 0.5 unit of Taq DNA polymerase in reaction buffer [10 mM of Tris-HCl pH 8.5, 50 mM KCl, 1.5 mM $MgCl_2$, 0.1mM EDTA, 50%(v/v) glycerol]. The amplified polymorphism alleles were distinguishable with the electrophoresis in 10% Polyacrylamide Gel Electrophoresis (PAGE).

Table 3.2 Primer sequences, repeat motif, expected PCR product size, annealing temperature and chromosomal locations of six microsatellite primers.

Name	Primer sequences (5′→3′)	Repeat	Expected PCR product size (bp)	Annealing Temperature (◦c)	Chromosomal location	References
RM1	GCGAAAACACAATGCAAAAA GCGTTGGTTGGACCTGAC	(AG)26	113	55	1	Panaud *et al*. (1996)
RM5	TGCAACTTCTAGCTGCTCGA GCATCCGATCTTGATGGG	(GA)14	113	55	1	Panaud *et al*. (1996)
RM22	GGTTTGGGAGCCCATAATCT CTGGGCTTCTTTCACTCGTC	(GA)22	194	55	3	Panaud *et al*. (1996)
RM164	TCTTGCCCGTCACTGCAGATATCC GCAGCCCTAATGCTACAATTCTTC	(GT)16TT(GT)4	246	58	5	Wu and Temnykh (1993)
RM259	TGGAGTTTGAGAGGAGGG CTTGTTGCATGGTGCCATGT	(CT)17	162	55	1	Chen *et al*. (1997)
RM316	CTAGTTGGGCATACGATGGC ACGCTTATATGTTACGTCAAC	(GT)8-(TG)9(TTTG)4(TG)4	192	55	9	Temnykh *et al*. (2000)

3.2.4 Data analysis

Physiological characters were calculated for mean, standard deviation (sd), range and coefficient of variance (CV, %). For qualitative characters, Shannon-Weaver Index (Shannon and Weaver, 1949 cited by Power and McSorley, 2000) was used to calculate diversity.

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

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

N = sample size

For DNA analysis, an estimate of the genetic diversity was calculated for each rice population including the effective number of alleles (n_e), Nei's (1973) gene diversity or heterozygosity (h) and percentage of polymorphic loci (P),using POPGENE version 1.32 programs (Yeh *et al*., 1999). Total number of alleles (A), allelic richness (A_R), inbreeding coefficients (F_{IS}), average gene diversity within population (H_S), total gene diversity (H_T) and degree of genetic differentiation among populations (F_{ST}), were calculated using FSTAT version 2.9.3 program (Goudet, 2001).

Population relationships were inferred using the UPGMA clustering methods on the basis of Nei's (1978) unbiased genetic distance with POPGENE program. The tree subsequently visualized with MEGA 2 (Kumar, *et al.*, 2001).