CHAPTER III

MATERIALS AND METHODS

Methodology

The research consisted of three parts; preliminary farmer survey, field experiment, and analysing farmer production system. The experiment was conducted at Ban Kae Noi, Muang Na sub district, Chiang Dao district, Chiang Mai province (19°14' N Latitude, Longitude 98°49' E). The reason for choosing this area was that 85 percent of the hill tribe farmers in this area grew red kidney bean and this bean could be grown twice, early and late rainy season. It is anticipated that any production improvement in the area would provide significant benefit to the household income.

3.1 Preliminary Farmer Survey

3.1.1 Crop cutting

Preliminary farmer survey, was initiated where by individual farmer's crop yield was estimated by crop cut method from 1 m^2 sample. This estimation was carried out in two seasons in early rainy season and late rainy season. Average farm yield and its variability were then determined. Field observation during crop cutting would also help diagnose overall crop performance.

3.1.2 Farmer survey

Farmer survey was conducted before setting up the experiment. The purposed of this survey was to gather information about production system, to identify farmer production problem in Kae Noi village, and to consider possible

improvements in farmers' farm practices. The information obtained from farmer's management practices helped justifying possible solutions on agronomic practice.

3.2 Field Experiment

The field experiment was conducted at the Royal Project station at Kae Noi during May and September growing seasons, 1994. The factorial experiment consisting of rhizobium inoculation and nitrogen fertilizer application was selected with eight treatment combinations as follows (Table 3.1).

They were two levels of rhizobium, with and without inoculation (RHI, 0), FER_1 represented first application of 25 kg N ha^{-1} at 7 days after planting (DAP) and FER_2 represented second application of the same amount of N at first flowering. Urea was used as N source. The experiment was arranged in a randomized complete block design with four replications. Each experiment unit had a plot size of 16 m^2. Before the May experiment, soil sample was taken at 15 cm depth for chemical analysis. The soil was sandy loam. Chemical analysis revealed the following characteristics, pH 4.9, total nitrogen in soil 0.076 percent, available P 17 ppm and exchangeable K 100 ppm. Liming was carried out by incorporating 6250 kg ha^{-1} of lime during land preparation. Soil samples was taken again after May planting at 15 cm depth. The soil chemical analysis showed that the values of pH, total nitrogen in soil, available P, exchangeable K and soil organic matter were 5.9, 0.098 percent, 17.5 ppm, 97.38 ppm and 1.98 percent respectively. Liming was repeated before the September planting at 1250 kg ha^{-1}.

The local Kae Noi variety of red kidney bean grown at Kae Noi was used for planting at 25×40 cm spacing with three seeds per hill. Ammonium

molybdate 50 g per 20 litter of H_2O was sprayed to provide conditioning for rhizobium activity before sowing in two planting seasons. Super phosphate of 37.5 kg ha^{-1} were applied simultaneously two times to every plot with nitrogen application in May and September plantings. The rhizobium inoculant (*Rhizobium legumeminosarum bv phaseoli*) was used to inoculate the red kidney bean seed at the planting time. The amount of prepared rhizobium was calculated based on the weight of seed required for sowing which was about 30 g of rhizobium inoculated with 266 g of red kidney bean seed for 16 m^2.

Table 3.1 Experimental treatment combinations consisting of two factors of rhizobium and nitrogen management.

Treatment	RHI	FER_1 (kg N / ha)	FER_2 (kg N / ha)
1	0	0	0
2	0	25	0
3	0	0	25
4	0	25	25
5	RHI	0	0
6	RHI	25	0
7	RHI	0	25
8	RHI	25	25

3.2.1 Data collection

The following data were collected:

3.2.1.1 Climatic data:

The data of rainfall, solar radiation and maximum and minimum temperatures were collected during May-September planting.

3.2.1.2 Phenology:

The observation was taken on dates of flowering, seed filling, and harvesting in two planting seasons.

3.2.1.3 Crop management data:

The crop management data were collected from date of sowing and date of fertilizer application in both planting dates.

3.2.1.4 Soil analysis:

The soil samples were taken at 15 cm depth and were analyzed for N, P, and K content, soil organic matter and soil pH.

3.2.1.5 Dry matter:

The plant sample from 1 m^2 was cut close to the first node above ground level. The above ground plant parts were collected at flowering (R_1) and seed filling (R_5) and dried at 75 °C for 48 hours, then weighted for the determination of dry matter.

3.2.1.6 Total plant nitrogen:

Total plant nitrogen was analyzed from plant samples taken from 1 m^2 area at flowering, seed filling and at physiological maturity. The samples of above ground biomass were dried at 75 °C for 48 hour, then weighted and analyzed for total nitrogen by Micro Kjedahl method.

3.2.1.7 Nitrogen fixation:

Relative ureide index was used for evaluation of N_2 fixation. Root bleeding saps were collected from 12 plants per plot at flowering (R_1) and seed

filling (R_4) in September planting. The saps were collected immediately after the plant shoots were detached, by placing a rubber tubing sleeve 4 to 6 cm long with internal diameter slightly smaller than the stem, over each of the exposed root stems. The sap was collected from the sleeve reservoir within 30 minute after shoot detachment (Peoples *et al.*, 1989), and kept in the ice box immediately and then placed in refrigerator at -10 °C for later analysis.

3.2.1.7.1 Plant analysis

(1) Ureide analysis

To carry out ureide assays, xylem sap was obtained by decapitated plants and collected the breeding sap from the cut stump. The contents of ureide, total amino N and nitrate in the sap were estimated by colorimeter assay and the results were expressed as the relative percent ureide content (Herridge, 1984; Peoples *et al.*, 1989).

Sap analysis for ureides, amino acids and nitrate essentially followed the procedure of Peoples *et al.* (1989). The relative abundance of ureides was calculated as four times the molar concentration of ureides divided by the sum of four molar concentration of ureides plus molar concentration of ureides plus molar concentration of nitrate and amino-N time 100 (Herridge, 1984). Thus, the relative abundance of ureide-N in sap was calculated as follow:

$$\text{Relative ureide index } (\%) = \frac{4 \times \text{ureide}}{(4 \times \text{ureide} + \text{amino - N} + \text{nitrate})} \times 100$$

(2) Total plant nitrogen analysis

The procedure of estimation on nitrogen fixation was summarized as follows:

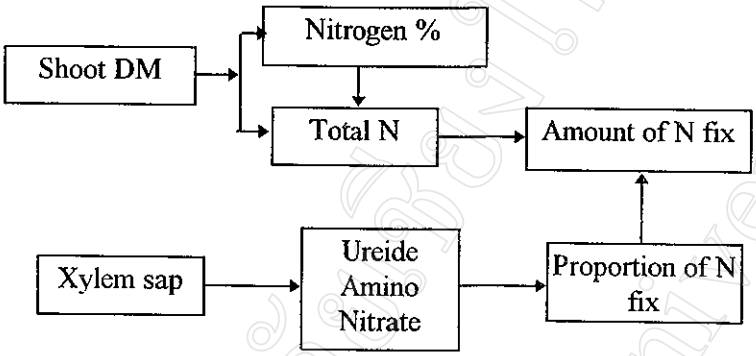

Figure 3.1 Flow chart percent of nitrogen fixation estimation.

Total N measurement was followed by Kjedahl method, which was used with small sub samples of a particular system. The wet Kjedahl digestion was used. In this, organic and mineral N were reduced to NH_3 in hot, concentrated sulfuric acid in the presence of catalyst. The NH_3 was recovered by distillation and estimated by titration (Bergersen, 1980).

3.2.1.8 Yield and yield components:

The final harvest was carried out when plants reached maturity, when 80 percent of the pods had turned brownish yellow and dried (Fehr *et al.*, 1971). Seed yield, plant height, above ground biomass, number of pod per plant, number of seed per pod, 100 seeds weight, number of plant per m^2 and percent of seed damage were determined from 2 m^2 sample in May and September plantings.

3.2.2 Data analysis

The analysis of variance (ANOVA) was used to analyze the treatment effects and their interaction on red kidney bean yield.

3.3 Assessment of Farmer Production Systems

Farmers assessment was done during April to May 1995, the objective was to understand farmers' red kidney bean production system during the 1994 season, particularly with regard to nitrogen management. The formal survey technique was used. The information on farmer production cost was used to help estimate the benefit of nitrogen in the experiment.

3.3.1 Formal survey method

3.3.1.1 Pre-diagnosis

Selection the site in Ban Kae Noi and initial understanding of red kidney bean production system of the area was done through the review of existing information. The information on secondary data such as climate, soil characteristic, topography etc. were collected, examined and incorporated for the study.

3.3.1.2 Formal survey

Preliminary testing of questionnaires was conducted in 10 households and any improvements or changes were then corrected. The number of household to be interviewed was decided on the basis of secondary information. The studied villages within Ban Kae Noi were Pa Bong Kao, Pa Bong Mai, Mae Kan, Huw Tum and Chinese Haw. One hundred households were chosen in order to represent the whole village. The structured questionnaire was used to obtain statistically more precise information.

3.3.2 Data collection

The data collected in the survey were the one year data of 1994-1995 and interviewed during April to May 1995, which included farm size, rate of fertilizer and fertilizer management. Information was collected and focused on agronomic knowledge with respect to fertilizer management.

3.3.3 Data analysis

Descriptive statistics were used to analysis data and the benefit of nitrogen management practice was determine by partial budgeting method.