CHAPTER 3

MATERIALS AND METHODS

3.1. Field Survey:

The informal and formal survey were conducted at the Chiang Mai Field Crop Research Center and Ban Ton Ou village of San Pa Tong district, Chiang Mai, respectively. The purposes of the surveys were to understand and analyze the current farmers' practices in vegetable soybean production with respect to nitrogen fertilizer management and variety use.

In order to understand the vegetable soybean production system and to identify the major problems in the farmer' fertilizer practices before the field experiment was conducted, the following procedures were carried out.

3.1.1. Secondary data analysis: Data on system components, production area, fertilizer management and crop yield were collected from the Chiang Mai Field Crop Research Center and the other relevant sources and analyzed.

3.1.2. Exploratory survey: Exploratory survey included on-farm survey and interview with private company

dealing with commercial vegetable soybean production.

3.1.3. **Formal survey:** One major vegetable soybean production area was chosen as the target for the formal survey with the questionnaire, which emphasized on nitrogen fertilizer management practice. A total number of 12 farmers were interviewed.

3.2. Field Experimentation:

This experiment was conducted at the experimental station, MCC, Chiang Mai University during May 19, 1992 to August 4, 1992 on a sandy loam (San Sai series) of pH 6.3, total N in the top 20 cm was 0.061%, O.M. 1.23%, 63.25 ppm exchangeable P and 35.94 ppm exchangeable K. The climate of Chiang Mai is characterized by definite wet and dry seasons. The wet season lasts from May to October; daily mean temperature is 26-29 °C and precipitation averages total 1120 mm.

Based on the field survey, the experiment consisted of twelve treatments, a factorial combinations of 6 nitrogen fertilizer treatments applied to two varieties 301 (V1) and AGS292 (V2). The experiment design was a randomizedcomplete block with four replications.(See Fig. 2)

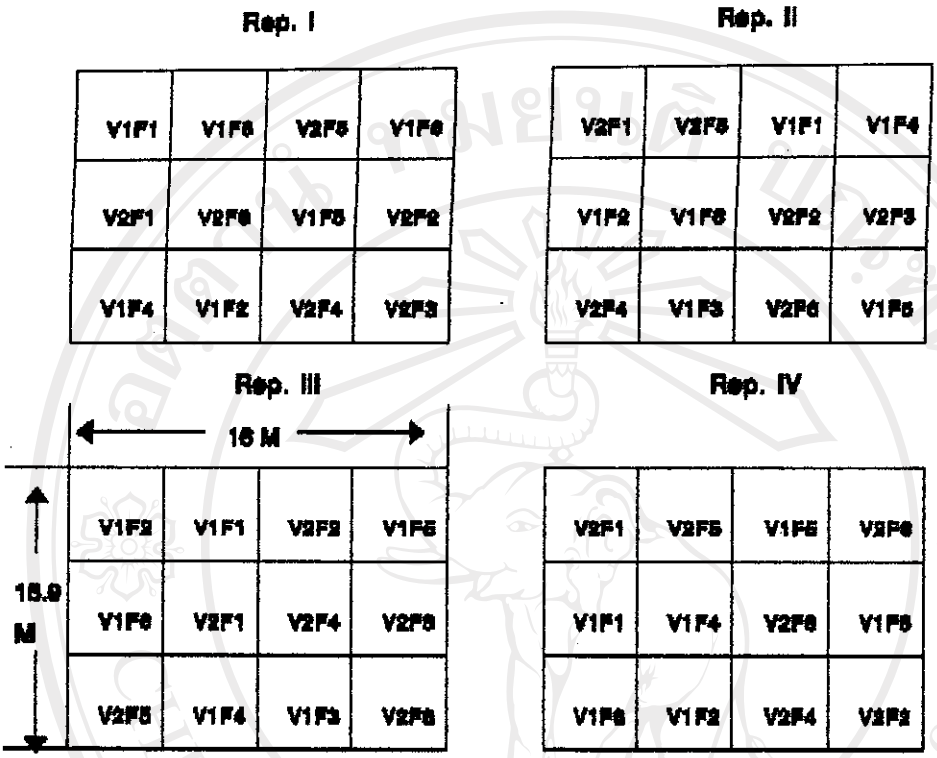

Fig. 2. The layout of a 4m*5.3m factorial experiment involving two varieties (TVB5 and TVB7) and six Nitrogen rates (N1, N2, N3, N4, N5 and N6) in a randomized complete block design with four replications.

Six Nitrogen fertilizer treatments were as follows:

F1: 25 kg N/ha before sowing

F2: 25 kg N/ha before sowing and 50 kg N/ha at R1 stage

F3: 25 kg N/ha before sowing and 50 kg N/ha at 14 days (V1.5) after sowinging.

F4: 25 kg N/ha before sowing, 50 kg N/ha at 14 days (V1.5) after sowing and 25 k/ha at 21 days (V4) after sowing.

F5: 25 kg N/ha before sowing and 50 kg N/ha at 14 days (V1.5) after sowing and 25 kg N/ha at R1 stage.

F6: 25 kg N/ha before sowing and 50 kg N/ha at 14 days (V1.5) after planting and 25 kg N/ha at R4.5 stage.

Plot size was 4 m x 5.3 m with 2 beds in each plot. There were four rows in each bed and the hills were arranged on 40 cm X 20 cm lattice. There were 2 seedlings per hill. For basal P and K applications, at the rate of 40 kg P_2O_5/ha in the form of triple supper phosphate (45% P_2O_5) and at the rate of 60 kg K_2O/ha in the form of KCI (50% K_2O); for the starter N, at the rate of 25 kg N/ha in the form of ammonia sulfate (21% N) were applied before planting. Urea (46% N) was applied as the top dressing during different time according to the treatment requirements. For nitrogen top dressing at both V4 and R1 stage, applying N fertilizer and taking samples were done at the same time.

Before sowing, the seeds were inoculated with a commercial inoculum of *Bradyrhizobium japonicum* with 40% gum arabic solution, fungicide, Dithane M45 were used to protect the seeds from fungi's damage.

3.3. Sampling:

3.3.1. Soil

Four soil samples from each replications at 0-20 cm deep of the experiment area were taken before the start of the experiment and soil samples for each treatments also were taken and placed immediately at -10 °C for later determination the available N content in the soil at each sampling time (V4, R1, R3, R5, R5.5 and R6.5).

3.3.2. Plants:

On 6 occasions during growing season, 1 m^2 quadrant was sampled from each plot for assessments of nodulation, crop growth and nitrogen fixation, the latter based on analysis of xylem sap for nitrogen solutes (Peoples *et al.*, 1989). Sampling commenced in vegetable growth (V4) with final sampling at physiological maturity (R6.5). Plants were sampled by first removing the shoots and leaving the root stumps. Xylem sap and nodulation data were collected from at least 12 plants. Xylem sap was collected as root-bleeding sap from the root stumps (Peoples *et al.*, 1989), and placed immediately at -10 °C for later analysis. The roots were dug from within each sampling area, nodulation was removed from the roots and graded according to the method by People *et al.* (1989), then, dried at 80 °C for 48 hours and weighed. The sample of shoots were dried to constant weight at 80°C, weighed and analyzed for total (Kjeldahl) nitrogen.

At harvesting time as vegetable soybean (R6.5), total fresh pods yield, marketable pod yield (at least two seeds/pod) as well as potential nonmarketable pod yiled (at least two seeds initially, but less than two seeds were developed successfully) measured from the harvest areas of 2 m^2. Yield components (# nodes/plant, # pods/node and 100 pod weight) were determined on a randomly selected subsample of 10 plants.

3.3.3. Chemical analysis, determinations of plant nitrogen derived from nitrogen fixation (Pfix)

Concentrations of ureide (allantoid and allantoic acid) in root-bleeding sap were estimated calorimetrically as the phenylhydrazine of glyoxalase. Nitrate was measured by the salicylic acid technique. The amino-nitrogen content of sap was determined calorimetrically with ninhydrin, using a 1:1, asparagine: glutamine standard (Peoples *et al.*, 1989)

3.3.4. The calculation of relatives abundance of ureide-N in Xylem sap

The relatives abundance of ureide-N in sap was calculates as follows:

Relative ureide-N (RU, %)=(4a/(4a+b+c))*100 (1)

Where a, b and c are respectively the molar concentrations of ureide (ureide contain 4 nitrogen atoms per molecule), nitrate and amino-acid-N (Herridge, 1984). Calculation of the proportion of plant nitrogen derived from nitrogen fixation was based on regressions established from glasshouse calibrations (Peoples *et al.*, 1989) as follows:

P fix (%) = 1.21 *(RU-4.8) (2)

for plants in vegetative and flowering stages (up to R2)

P fix (%) =1.49 *(RU-21.3) (3)

for plants during pod-fill (reproductive stages of development after R2). Where RU is the % relative abundance of ureide-N in root-bleeding sap (Peoples *et al.*, 1989).

The procedure of estimation on nitrogen fixation is summarized as Figure 3.

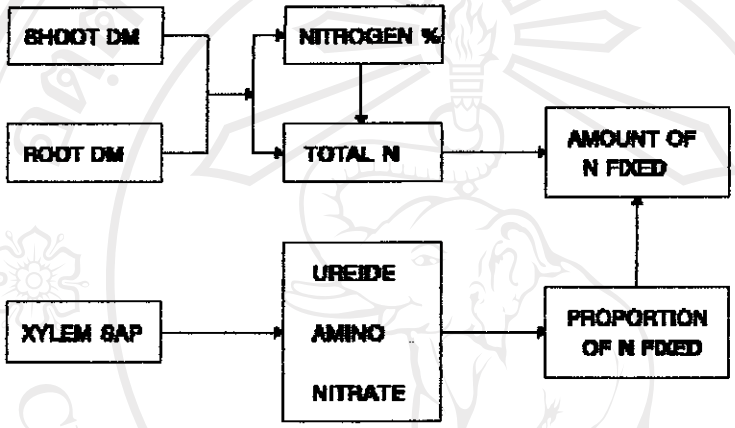

Figure 3. The flow chart of the process of nitrogen fixation estimation.

3.4. Economic analysis:

Economic analysis on the cost of production and return on nitrogen fertilizer managements was conducted.