Chapter 3

MATERIALS AND METHODS

3.1 Site, treatments and sowing

The experiment was carried out at the experimental station of the Multiple Cropping Center, Chiang Mai University (19°N, 99°E). The soil used was a sandy loam (San Sai series), pH 5.7-5.9, containing 0.05-0.06% total N, 34-57 ppm P and 91-124 ppm K respectively (Preidsripipat, 1988). The climate at Chiang Mai is characterized by definite wet and dry seasons. Precipitation during the wet season (May to October) totals 1119 mm on average (from 1950 to 1980); temperatures during this time are high (25-28°C, daily means). The dry season includes a cool winter (November to February) and hot summer (March to April); with only 141 mm of rain (Akratanakul, 1987).

The treatments were factorial combinations of irrigation method, soybean variety and starter fertilizer nitrogen, each at two levels. The experimental lay-out was a split-split-plot design. Irrigation methods in mainplots, variety in subplots and combined nitrogen in sub-subplots; each with three replicates.

Seed of the two soybean cultivars, NW1 (early maturing) and SJ5 (mid season) was inoculated with a commercial inoculum of *Bradyrhizobium japonicum* and sown by hand into moist soil on 4 July 1989. Each plot consisted of two beds 8 m long and 2.2 m in

width in which 0.6 m was used as furrow. Seeds were sown at 5-6 per hill and seedling thinned to 2 per hill after emergence; hills were arranged in 10 x 40 cm lattice. Fertilizer nitrogen was applied as urea in bands between the soybean rows at the rate of nil (N0) and 50 kg N/ha (N50) two days after sowing.

Saturated soil culture treatment (SSC) was initiated 12 days after sowing. Water was added daily, at 7-8 h in the morning and 16-18 h in the afternoon so that the water table was maintained within 5-15 cm below the soil surface. Conventional irrigation (CI) supplied sufficient water for growth, but provided adequate drainage so that standing water rarely remained within furrows.

3.2 **Sampling**

On 8 occasions during growth, one m^2 quadrats were sampled from each plot for assessments of nodulation, crop growth and nitrogen uptake and nitrogen fixation. The later measurement was based on analyses of xylem sap for N-solutes (Peoples et al, 1989).

Sampling was commenced in vegetative growth (V6) with final sampling at late pod-fill (R7) (Fehr et al, 1971). An additional sample was taken at V4 to measure shoot dry matter and nodulation.

3.2.1 Dry matter

All plants from one m^2 were cut close to the first node

above ground-level with a pair of secateurs and the root stumps left *in situ*. The plant shoots were collected and dried at 80°C for 48 hours, then weighed for the determination of dry matter.

3.2.2 Sap bleeding.

(1) Immediately after the plant shoots were detached, a rubber tubing sleeve, 4 to 6 cm long with an internal diameter slightly smaller than the stem, was placed over each of the exposed root stems of about 15 plants randomly selected from 1 m^2 sample area.

(2) The sap was collected from the sleeve reservoir with the aid of a Pasteur pipette within 30 minutes and placed in a stoppered vial. Sap samples were chilled on ice during sampling and frozen at -20°C for storage (Peoples et al, 1987 and Norhayati et al, 1988).

3.2.3 Nodulation.

(1) Ten randomly selected roots were dug from within each sampling area with a earth volume of 10 cm width, 30 cm length and 20 cm depth.

(2) The roots were placed in plastic bags and taken to the laboratory where the roots were washed free from soil on a wire screen.

(3) Nodules were carefully removed and placed on tissue paper to absorb excess water. The nodule numbers and fresh

weights were determined.

(4) Nodule dry weights were also recorded after drying at 80°C for 48 hours.

3.2.4 Total nitrogen.

Dry samples were chopped and ground through 1 mm screen, then thoroughly mixed for sub-sampling analysis of nitrogen, using a Kjeldahl method (Peoples et al, 1989).

3.2.5 Yields and yield components.

The final harvest was carried out when plants reached maturity (when 80% of the pods had turned brownish yellow and dried). A sample area of 3 m^2 was taken from each plot for dry matter and seed yield, with yield components determined on a randomly selected subsample of ten plants. Bean yield was expressed in tons per hectare at 14% moisture. The yield components determined were number of pod-bearing nodes per plant, number of pods per node, numbers of seeds per pod and weight of 100 seeds.

Seed nitrogen contents were estimated with the Kjeldahl method.

3.3 Chemical analysis, determinations of plant nitrogen derived from N2 fixation (Pfix)

Concentrations of ureide (allatoin and allantoic acid) in root-bleeding sap were measured using the method of Young and

Conway (1942). Nitrate was measured by the salicylic acid technique (Cataldo et al., 1975). The amino-N content of sap was determined calorimetrically with ninhydrin (Yemm and Cocking, 1955; Herridge et al, 1984), using a 1:1, asparagine:glutamine standard.

The relative abundance of ureide-N in sap was calculated as:

$$\text{Relative ureide-N (\%)} = 4a /(4a+b+c)*100 \qquad [1]$$

where a, b and c are respectively the molar concentrations of ureide (ureide contain 4 nitrogen atoms per molecule), nitrate and α amino-N (Herridge et al, 1984). Calculation of P fix was based on regressions established from glasshouse calibrations (Herridge and Peoples, 1990; Peoples et al, 1989)

$$\text{P fix (\%)} = 1.21\ (RU-4.8) \qquad [2]$$

for plants in vegetative and flowering stages.

$$\text{P fix (\%)} = 1.49\ (RU-21.3) \qquad [3]$$

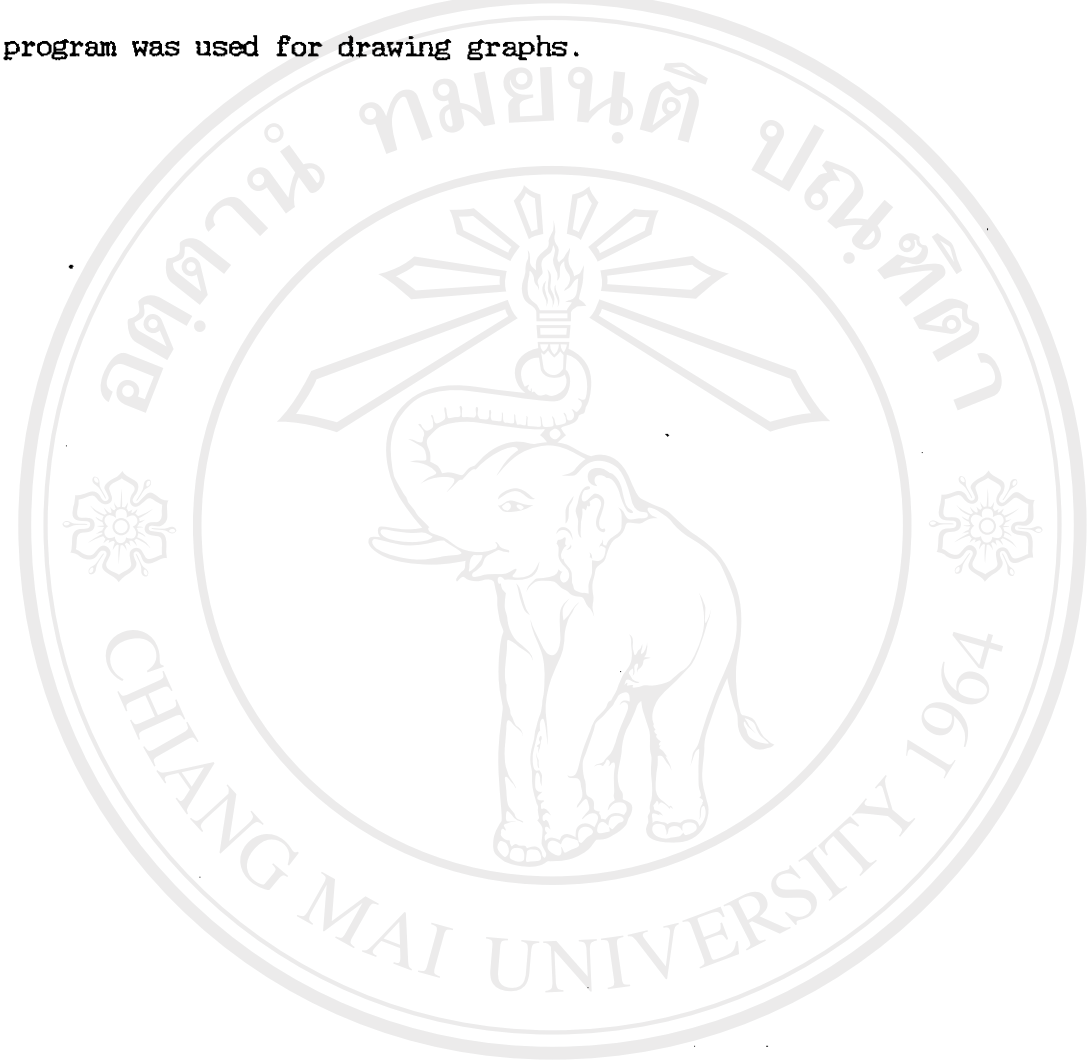
for plants during pod-fill.

Where RU is the % relative abundance of ureide-N in root-bleeding sap (Herridge and Peoples, 1990).

The procedure of estimation on nitrogen fixation is summarized as Figure 1.

3.4 Data analysis

Statistix software was adapted to analyze the variance of observed data and regress shoot dry matter accumulation. Hardard program was used for drawing graphs.



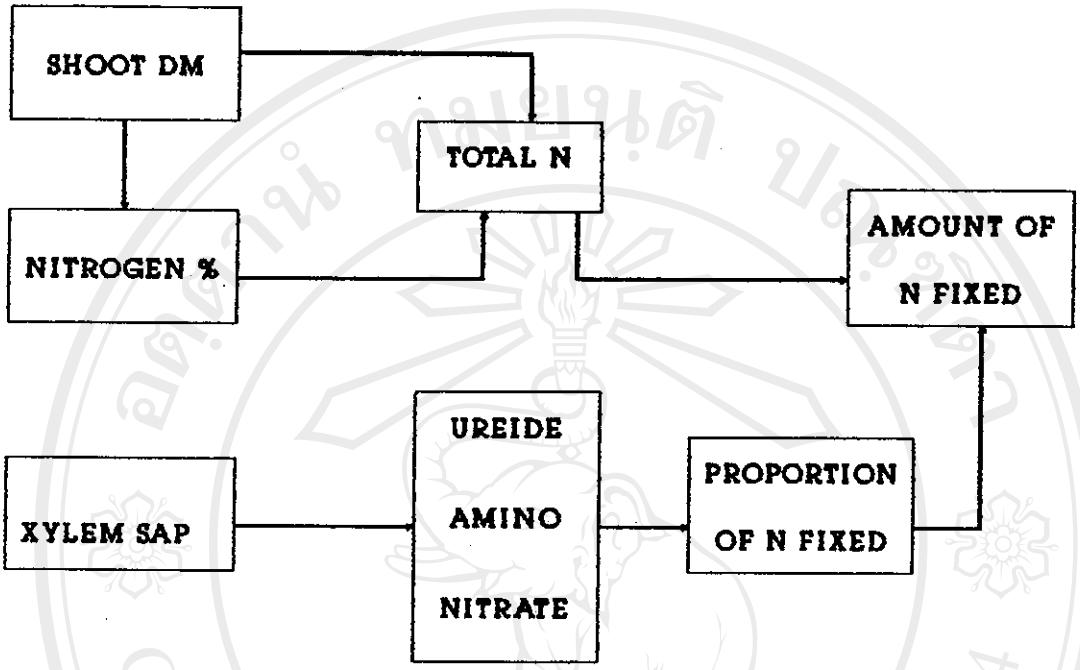


Figure 1. Flow chart of the process of nitrogen fixation estimation.