Chapter 3

# MATERIALS AND METHODS

This investigation consisted of three sets of experiment: one screenhouse experiment and two field experiments.

## 3.1 Experiment I

### Screenhouse experiment

A screenhouse experiment was conducted in November, 1990 to January, 1991 to quantify the relationship between the relative abundance of ureides (RU) in xylem exudates of rice bean and the proportions of plant N derived from N2 fixation (Pfix).

Plants were grown in naturally-lighted screenhouse with maximum and minimum temperatures of 30$^{0}$C and 17 $^{0}$C, respectively.

Ten rice bean seeds, inoculated with Rhizobium spp., were sown in each of 18 pots each containing 17 kg of sterilized sand. The plants were thinned to five per pot, two weeks after emergence. N-free nutrient solution was supplied to the plants. Calculations of plant nutrient solution was based from Broughton and Dillworth (1970) in

Somasegaran and Hoben (1985).

Fourteen days after emergence, 6 pots were supplied with nutrient containing 8 mM nitrate solution and another 6 pots for 12 mM nitrate solution, 1 liter/day at one day interval; while the other pots continued to receive N-free nutrient solution.

There were two sampling periods, each sampling consisted of three replications. Plants were sampled at the commencement of flowering (R1), 24 days after sowing and during seed set (R6), 28 days later.

Xylem exudate was collected at each sampling as root bleeding from intact root stumps under root pressure from freshly detached stems (Peoples et al., 1987). The symbiotic dependence of plants grown at each nitrate level was estimated by calculating the proportion of N fixed by the plant.

## 3.2 Experiment II

## Intercropping upland rice and rice bean

### 3.2.1 Field experiment

The experiment was carried out on a sandy loam (San sai series) under rainfed conditions at the Experiment Station of the Multiple Cropping Center, Chiang Mai University ($19^0$N, $99^0$E) in May 1990 to January 1991 (wet and cool seasons). The soil was characterized by a pH of 6.2, .04 % total N, .93 % OM, 74 ppm of available P, and 67 ppm of exchangeable K. Precipitation during the wet season (May to October) was 904 mm and mean temperature of $27^0$C, while the cool season (November to January) had a total precipitation of 141 mm and mean temperature of $23^0$C (Table 1).

Table 1. Mean monthly temperature and rainfall during the experimental period*.

| Year 1990 | May | June | July | Aug | Sept | Oct | Nov | Dec |
|---|---|---|---|---|---|---|---|---|
| Temperature ($^0$C) | 27.2 | 27.5 | 26.9 | 27.5 | 26.8 | 26.3 | 24.4 | 21.2 |
| Rainfall (mm) | 268.1 | 63.0 | 93.7 | 213.5 | 133.0 | 132.8 | 135.1 | 0 |
| **Year 1991** | **Jan** | **Feb** | **March** | **April** | **May** | **June** | **July** | **Aug** |
| Temperature ($^0$C) | 21.1 | 22.9 | 26.9 | 29.0 | 29.4 | 25.2 | 27.3 | 26.8 |
| Rainfall (mm) | 6.1 | 0 | 3.0 | 78.0 | 89.7 | 112.9 | 123.8 | 336.1 |

* Recorded at the Experiment Station of the Multiple Cropping Center in 1990 and 1991.

The additive series design (Singh and Gilliver, 1988; Vandermeer, 1989) was used to quantify the effects of rice bean competition on upland rice. There were seven treatments (Table 2) arranged in four completely randomized blocks.

Table 2. The treatment combinations used in the experiment.

| Cropping System | Time of rice bean introduction* |
|---|---|
| Sole rice | |
| Sole rice bean | Simultaneous[1] |
| | Intermediate[2] |
| | Late[3] |
| Intercrop | Simultaneous |
| | Intermediate |
| | Late |

* time of sowing rice bean in relation to rice sowing
1. Rice and bean sown at the same time
2. Bean sown two weeks after rice
3. Bean sown four weeks after rice

In both monoculture and intercrop plots, upland rice was sown at the same time, while rice bean was sown in three planting schedules: simultaneous sowing with rice, and 2 (intermediate sowing) and 4 weeks (late sowing) after rice sowing. Each plot measured 10 x 1.5 m.

Seeds of rice cultivar RD 258, a 110-cm-tall variety which matures in 120 days, was used in the experiment. The seeds were sown at 8 seeds/hill and the seedlings were thinned to 5 plants/ hill one week after emergence; the hills were spaced at 25 x 25cm (160,000 hills/ha).

The local red-seeded variety of rice bean (unnamed cultivar) was used in the experiment. In the monocrop plots, the same spacing was used as the upland rice, while in the intercrop plots, rice bean was sown in between rows of rice (320,000 hills/ha). There were also 5 plants/hill.

The crops were planted in 16 May 1990. Replanting of missing hills both for rice and rice bean was done two weeks after sowing.

Rice bean roots were nodulated by the indigenous soil rhizobia. No fertilizer was applied in compliance with local farm practice. There was only one time full irrigation during panicle initiation because of extra dryness in the soil and weeding was done once, one month after rice emergence.

### 3.2.2 Sampling procedures and data collection

**Dry matter**

Sampling for both upland rice and rice bean was done during the vegetative stages of rice bean, V6, V12 and V20, and during the reproductive stages, R1 (flowering) and R3 (pod development) (adapted from Fehr et al., 1971). Rice bean crops should have been harvested up to stage R7 (physiological maturity), but the crops were severely damaged by insects that sampling could not be done.

Plant samples were harvested from randomly pre-determined $1m^2$ areas for dry matter determination. The shoots were ovendried at 80°C for 48 hours.

**Sap bleeding**

Immediately after rice bean shoots were cut, a rubber tubing (4 to 6 cm long) was fitted to exposed stems of about ten plants from each plot randomly selected from $1m^2$ sampled areas. The sap was collected from the sleeve reservoir with Pasture pipette within 30 minutes and placed in a stoppered vial. Sap samples were chilled immediately on ice and frozen at -15°C for storage and later analysis (Peoples et al., 1989).

**Nodulation**

The roots of plants used in sap bleeding collection were dug from within each sampling area. The roots were placed in plastic bags and were taken to the laboratory where the roots were washed free of soil using a wire screen. Nodules were carefully removed and placed on tissue paper to absorb excess water. Nodule number per plant was determined. Nodule dry weight was also recorded after drying at 80°C to constant weight.

### Total nitrogen

Dry samples of upland rice and rice bean were chopped and ground using the Willey Mill No. 3 passing through 1 mm screen, then thoroughly mixed for sub-sampling analysis of nitrogen, using the Kjeldahl method (Peoples et al., 1989).

Seed nitrogen contents of both crops were also estimated with the Kjeldahl method.

### Grain yield and yield components

A sample area of $2m^2$ was taken from each plot for seed yield and another $1m^2$ for yield components. The yield components determined for rice were panicles/hill, seeds/panicle, seed weight/panicle, and 1,000 seed weight and number of seeds/pod, seed weight/pod, and 100 seed weight were determined for rice bean.

Rice in all treatments was harvested 120 days after sowing while for rice bean, the final harvest was done when the plants reached maturity (when 80% of the bean pods turned brownish to black and were dry). Rice bean pods were harvested in 1-2 pickings. The second picking was done 20 days later. Bean harvesting was done in the morning because it was cooler, in the afternoon the temperature was high, and the bean pods easily cracked.

Harvested grains were sundried. Moisture content of the seeds was determined with a moisture meter. Grain yields were adjusted to 14% for rice and 12% for rice bean.

Both yields were expressed in kg/ha. The yields were calculated per hectare in terms of grain yield and relative to respective monoculture yield (RY, relative yield). Relative yields were expressed as decimal ratios of the yield of the species to the yield of the monocrop of that species.

Considering the additive design used in the experiment, the Land Equivalent Ratio (LER) is used to evaluate intercrop advantage. The calculation is LER = (Yri/Yrm) + (Yrbi/Yrbm), where: Yri/Yrbi = yields of rice and rice bean in intercrops Yrm/Yrbm = yields of rice and rice bean in monocrops.

### 3.2.3. Chemical analysis, determinations of plant nitrogen derived from N2 fixation (Pfix)

Concentrations of the sap nitrogen compound were determined: ureides (allantoin and allantoic acid) colorimetrically as the phenylhydrazone derivative of glyoxalate; amino-N colorimet- rically with ninhydrin using 1:1, asparagine:glutamine standard; and, nitrates by salicylic acid method (Peoples et al., 1989). The relative abundance of ureide-N sap (RU %) was calculated as:

$$\text{Relative ureide-N (\%)} = 4a / (4a+b+c) * 100 \qquad [2]$$

where, a, b, and c are respectively the molar concentrations of ureide (one ureide molecule contains 4 nitrogen atoms), amino-N and nitrate-N (Peoples et al., 1989). Calculation of P fix was based from screenhouse calibration where

$$\text{P fix (\%)} = 1.33\ (\text{RU} - 10) \qquad [3]$$

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

The same Pfix computation was used for all the stages considering the indeterminate characteristic of rice bean. It kept on producing new leaves and branches even after pod development until harvest. The process of estimating nitrogen fixation is shown in Figure 1.

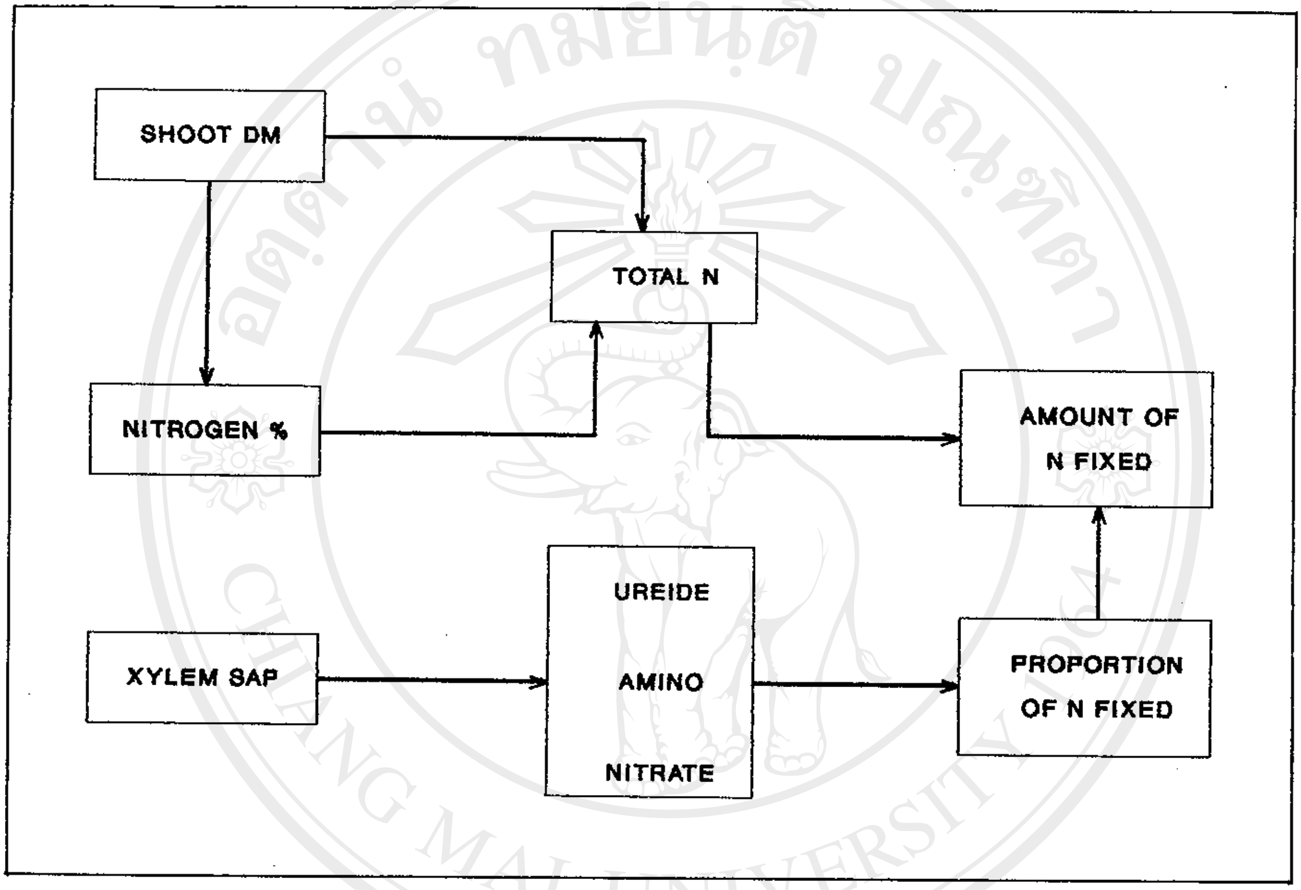


Figure 1. Flow chart on the process of estimating nitrogen fixation (Wang, 1990)

## 3.3 Experiment III

**Residual effects of the bean treatments on the subsequent corn crop**

A field experiment was conducted at the same site of the first experiment using the same treatment plots in May to August 1991. Mean monthly temperatures ranged from 23$^{0}$ C to 33$^{0}$C and a total rainfall of 662.5 mm.

Green manure provided by rice bean was estimated by measuring the shoot dry matter after harvest. Samples were taken from 1 $m^2$ areas; dried to constant weight and were weighed.

Two weeks after rice bean grain harvest, the remaining shoots were plowed into the soil and the area was flooded for three days . It was allowed to decompose for 112 days.

Rotary plowing was done a week before seed sowing. The area was sprayed with pre-emergence herbicide (Alachlor, a.i. 2-chloro-2′,6′-diethyl-N (methoxymethyl) acetanilide, 48% w/v E.C.) to control the weeds. There was no irrigation applied; weeding was done one month after sowing.

Corn variety, Suwan 1, was used in the experiment. The seeds were sown at three/hill on May 8, 1991, and were thinned to 1 plant/hill two weeks after emergence. Replanting of missing hills was done one week after germination.

The plants were sown in rows spaced at .75m apart and .25m between plants (53,333 plants/ha) on raised beds to allow free drainage of water. There were three beds in each treatment. Drainage furrows were made to separate the treatment beds and to allow free flow of water .

Corn was harvested for dry matter production and total N in three occasions, one month after sowing (30 DAS), at silking (55 DAS) and at physiological maturity (88 DAS) from a representative area of .75m x 1.5m (6 plants). Fresh samples were chopped first then dried to constant weight and weighed. Dried samples were ground through 1 mm screen and thoroughly mixed for sub-sampling analysis of total N using the Kjeldahl method.

The ears were harvested on 23 August 1991. Grain yield was obtained from 1.5m x 6m area (24 plants) and yield components (ears/plant, seeds/ear, and 100 seed weight) were taken from .75 x 1.5m area (6 plants). Yield was expressed in t/ha.

### 3.4 Statistical analyses

Analyses of variance procedures with appropriate single degree of freedom (using STATISTIX Package Program) were used to analyze data in the study. If treatment effects were significant, LSD's were used to separate means for particular comparisons. All differences reported are significant at P = 0.05 unless otherwise indicated.

Simple linear regressions from standard readings of ureides, amino-N, and nitrates were computed using Lotus Package Program.

Harvard Graphics Package Program was used in drawing graphs.