Experiment 1.

Growth and N_2 Fixation Efficiency of Vegetable Soybean

Objective

The objective of this study was to assess quantities and proportions of N composition in vegetable soybean derived from N_2 fixation, N fertilizer and soil.

Materials and Methods

Experimental design.

The experiment was conducted in wet season from July to September 2005 at Tambon Maekhajan, Chiang Rai province on the soil which had the following chemical characteristics: organic matter(OM)1.2%; pH 4.2 ; available P, 35 mg/kg and exchangeable K, 65 mg/kg which was remarkably an acid soil. The treatments consisted of four different nitrogen rates at 0, 16, 32 and 48 kg/rai. Fertilizer application was managed by using urea (46%N) at the rates of 10, 20 and 30 kg/rai as basal fertilizer and top- dressed with urea (46%N) at the rates of 25, 50 and 75 kg/rai. Top-dressing was performed at 12-25 days after planting of soybean (because the soil test indicated excessive P and K). The plots of 2x6 m^2 were arrangred in a randomized complete block design(RCBD) with three replications.

Plant growing.

The vegetable soybeans (No. 75) were seeded at the spacing of 50x20 cm with two plants per hill. Before planting, the seeds were inoculated with the appropriate rhizobium and treated with metalaxyl 25%WP to prevent infection of fungus. A growing plot was divided into two parts. The smaller area of 0.13 m^2 was amended with ^{15}N-labelled (urea 5 atom%) at the rate of 0, 16, 32 and 48 around the plant and the rest of area was amended with normal nitrogen fertilizer

For the standard plant, rice variety RD25 was used. This variety has the harvesting period, root depth and biomass similar to vegetable soybean. The rice plants were grown next to the soybean plot but growing in the same block.

Plant analysis

At V_7 and R_1, soybean plants were sampled to record growth performance in terms of shoot–root fresh and dry weight and number of nodule. Yields of green pods were harvested at 65 days after planting. The soybean plants from the ^{15}N-labelled sub plots were collected to analyse for the concentration of N and ^{15}N isotope ratio. The vegetable soybean and rice RD.25 (reference plant) samples were chopped into 2-3 cm pieces and dried in hot-air oven at 70^0C for 48 hrs, then ground to a fine powder using roller mill machine equipped with 0.6 mesh sieve. A ten mg of ground plant tissues was filled in tin capsules which were prepared for analysis of total nitrogen by elemental analyzer(NC2500) equipped with stable isotope ratio analysis (SIRA) mass spectrometer (Iso Prime) for ^{14}N:^{15}N analysis

^{15}N analysis was performed at Division of Nuclear Technique for Agriculture, Department of Agriculture, Bangkok. The quantity of analysed nitrogen was used for calculation of nitrogen derived from atmosphere (Ndfa) and nitrogen derived from soil (Ndfs) by the following equations (Suwunarit,1988.):

%Ndfa = % Nitrogen derived from air

$$\%Ndfa = 1 - \frac{\left(\text{atom}\%^{15}\text{N excess in plant}\right)}{\text{atom}\%\ ^{15}\text{N excess of reference crop}} \times 100$$

%NdfF = % Nitrogen derived from fertilizer

$$\%NdfF = \frac{\left(\text{atom}\%\ ^{15}\text{N excess in plant}\right)}{\text{atom}\%\ ^{15}\text{N excess of fertilizer}} \times 100$$

%NdfS = % Nitrogen derived from soil

$$\%NdfS = \text{fNdfS} \times 100 = 1 - \frac{\left(\text{atom}\%\ ^{15}\text{N excess in plant}\right)}{\text{atom}\%\ ^{15}\text{N excess of fertilizer}} \times 100$$

$$\text{Nitrogen from air} = \frac{\%NdfA \times \text{total dry weight (kg/rai)}}{100} = \text{kg/rai}$$

$$\text{Nitrogen from soil} = \frac{\%NdfS \times \text{total dry weight (kg/rai)}}{100} = \text{kg/rai}$$

$$\text{Nitrogen from fertilizer} = \frac{\%NdfF \times \text{total dry weight (kg/rai)}}{100} = \text{kg/rai}$$

Results and Discussion

Biomass and Yield of Vegetable Soybean

According to the main purpose of this study which was aimed to utilise the biomass of vegetable soybean for green manuring, only the growth performance of plant, especially biomass of above ground and root, was determined. The plant biomass response to nitrogen fertilizer indicated that there were significant differences among the rates of application. Above-ground biomass, both fresh and dry weight, gradually increased with increassing nitrogen fertilizers. The maximum response was at 32 kgN/rai which gave the biomass of 2,274 and 1,102.51 kg/rai of fresh and dry weight, respectively. The higher rate at 48 kgN/rai did not show significantly higher biomass (Table1). However, additional nitrogen fertilizer application increased biomass of vegetable soybean more than using only rhizobia.

Table 1 Average fresh weight and dry weight of above ground biomass, root and pod yield of soybean (kg/rai).

	Above ground biomass		Root		
Treatments	Fresh wt	Dry wt	Fresh wt	Dry wt	Pod Yield
Control	1,594.67 b	786.99 b	115.73 b	39.35 b	720 b
16 KgN/rai	1,996.67 ab	971.95 ab	142.80 ab	48.60 ab	862 ab
32 KgN/rai	2,274.00 a	1,102.51 a	162.13 a	55.13 a	969 a
48 KgN/rai	2,447.00 a	1,156.91 a	170.13 a	57.85 a	956 a
$LSD_{0.05}$	519.90	209.91	30.72	10.50	160.29
CV(%)	12.52	10.46	10.41	10.46	9.15

Means with the same letter are not significantly different at alpha level =0.05

Similar results were also obtained in increasing root biomass and fresh-pod yield by increasing nitrogen fertilizer. Root biomass was significantly higher than the control (without nitrogen fertiilizer) when growing soybean with additional nitrogen fertilizer. Application of 16 kgN/rai resulted in root fresh weight and dry weight of 142.8 and 48.6 kg/rai, respectively while in the control treatment, it was 115.7 and 39.3 kg/rai of fresh weight and dry weight, respectively. Maximum rate of nitrogen fertilizer at 48 kg/rai gave root biomass of 170.1 and 57.9 kg/rai of fresh and dry weight, respectively. Nitrogen fertilizer less than 48 kg/rai did not significantly affect root biomass as shown in 32 kgN/rai treatment (Table 1). Generally, the vegetable soybean seeds are harvested for consumption at the late growth stage (around R_6 stage). At this stage, the pods are filled with healthy green-colour seeds and the plants are also green so that in other words, vegetable soybean is called green soybean. Total fresh-pod yield is actually harvested at this stage of growth. The marketable yield normally is separated from total fresh-pod yield. However, in this experiment, the yield was determined in terms of total fresh-pod. Total fresh-pod yield was related to the plant biomass which indicated that there were significant differences on yield response to nitrogen fertilizer application rates. The maximum pod yield was found in 32 kgN/rai treatment (969 kg/rai), followed by 48 kgN/rai (956 kg/rai). These yields were significantly higher than the control treatment (720 kg/rai). Nevertheless, there were no significant differences among nitrogen fertilizer application rates on fresh-pod yield (Table 1). This work is in agreement with the studies of Diep (2002) who worked on VL-3 soybean cultivar in Vietnam and Hantolo(1996) who experimented on KPS 292 cultivar in Thailand.

Nodulation and N_2-fixation

Nitrogen fertilizer affected nodulation both in terms of number and dry weight of the legume nodule (Table2). Reduction of nodule biomass (nodule number and dry weight) was significantly found even in 16 kgN/rai treatment.. Nodules were decreased from 174 nodules with 1.89 g dry weight in control treatment(without additional nitrogen fertilizer) to 106 nodules with 0.81g dry weight in the treatment of 16 kgN/rai. More effect was found in higher nitrogen concentration. Decreasing of about half from the control treatment was found in 32 and 48 kgN/rai treatments. Moreover, reduction was not only found in nodule numbers but also in the size of them which was shown in decreasing of nodule dry weight in terms of g/100 nodules (Table2). Much more impact on nodule biomass was significantly found in the treatments of 32 and 48 kgN/rai at 82 and 87 nodules with 0.30 and 0.36 g dry weight, respectively.

Table 2 Number and dry weight of root nodules of vegetable soybean.

Treatments	Number of Nodules	% decrease	Nodules dry weight average (gm.)	Gram/100 nodules
Control	174 a		1.89 a	1.12 a
16 kgN/rai	106 ab	39.0	0.81 b	0.78 ab
32kgN/rai	82 b	52.9	0.30 b	0.43 b
48kgN/rai	87 b	50.0	0.36 b	0.50 ab
$LSD_{0.05}$	67.84		0.72	0.67
CV(%)	30.25		42.91	47.23

Means with the same letter are not significantly different at alpha level =0.05

Choonluchanon (1998) reported that the reduction of nodulation was affected by NO_3^- concentration in soil environment because NO_3^--N plays an important role on reaction with IAA. Malfunctional IAA leads to the difficulty of rhizobium to enter root hair to form nodule. Streeter (1985) also found a significant negative correlation between nitrite concentration in soybean nodules and nodule mass per plant. When nodules were formed by *R.japonicum* lacking nitrate reductase, much lower concentrations of nitrite were found in nodules, the results suggested that nitrite generated by nitrate reductase in the nodule cytosol might be sufficient to interfere with nodule growth and function.

Contribution of Nitrogen in Vegetable Soybean Plant from Different Sources

Nitrogen accumulation in above-ground biomass, as determined by ^{15}N enriched fertilized soybean in microplot, suggested that total N was contributed from three different sources, i.e., atmosphere, fertilizer and soil. Of all nitrogen sources, generally, nitrogen derived from atmosphere (Ndfa) through biological nitrogen fixation (BNF) contributed much more than the other sources, followed by nitrogen derived from soil (Ndfs) and nitrogen derived from fertilizer (Ndff) as shown in Table 3.

Table 3 Contribution of nitrogen in vegetable soybean plant from different sources: atmosphere (Ndfa), fertilizer (Ndff) and soil (Ndfs).

Treatments	Ndfa		Ndff		Ndfs		Total N-uptake
	%	kg/rai	%	kg/rai	%	kg/rai	(kg/rai)
16 kgN/rai	64.5 a	23.35	6.36 b	2.30 b	29.14	10.53 B	36.19 b
32kgN/rai	44.77 b	18.24	19.83 a	8.08 a	35.39	14.43 a	40.75 a
48kgN/rai	40.75 b	16.48	23.51 a	9.19 a	35.74	13.96 a	39.62 a
Average	50.01	19.36	16.57	6.52	33.42	12.98	38.85
$LSD_{0.05}$	11.20	ns	4.28	1.26	ns	2.50	3.18
CV%	9.88	17.65	11.40	8.35	9.16	8.50	3.61

Means with the same letter are not significantly different at alpha level =0.05

From this table, it was also found that higher applied N reduced Ndfa significantly. Ndfa in 16 kgN/rai was about 64.5% while that of 32 and 48 kgN/rai was 44.77 and 40.75%, respectively. Using these percentage of Ndfa, the estimates for weight/area were approximately 23.35, 18.24 and 16.48 kgN/rai. Reduction of nitrogen accumulation was exactly related to reduced nodule biomass. The nodule number was reduced by about 22% when compared between 16 kgN/rai (106 nodules) and 32 kgN/rai (82 nodules) which was of the same decreasing rate of nitrogen accumulation from 23.3 to 18.2 kgN/rai. However, the least reduction was found between 32 kgN/rai and 48 kgN/rai treatments. Such occurence might be because of the highest nitrogen concentration in the soil which impacted nodule formation of the rhizobia. Nevertheless, the infected rhizobia was able to have an activity of nitrogen fixation which was confirmed by the quantity of Ndfa in the high N rate treatments, likewise of 32 and 48 kg/rai (Table3). Danso (1986) reported that this was achieved by comparing %^{15}N atom excess between experimental fertilizers without the application of standard crop. In addition, Oberson *et al.* (2007) found that at maturity of soybean, the %Ndfa ranged from 24 to 54%. Siripin *et al*. (2001) and Yathaphutanon *et al*. (1998) reported that when using soybean line 9614, the %Ndfa was 58.41%,and soybean (CM60), %Ndfa ranged from 67-75%

A part of nitrogen accumulation in above-ground biomass was accounted from chemical fertilizer or Ndff. Greater nitrogen content derived from fertilizer was found in higher nitrogen application. Meanwhile, the Ndff in 16 kgN/rai was 6.36% or 2.30 kgN/rai, the treatments 32 kgN/rai and 48 kgN/rai were increased, respectively, 3 and

4 times at 19.83 and 23.51% or equivalent to 8.08 and 9.19 kgN/rai. Indeed, contribution of nitrogen derived from fertilizer was smallest when compared with that from atmosphere and soil sources . In the case of nitrogen from soil, it was also much more than Ndff in every treatment but it was still lower than Ndfa. For Ndfs, they were 29.14, 35.39 and 35.74% or 10.53 , 14.43 and 13.96 kgN/rai in the treatments of 16 kgN/rai, 32 kgN/rai and 48 kgN/rai, respectively.

The translocation of ^{15}N-enriched N from the isotope labelled fertilizer to soybean biomass was rather different for the rate of N application, there being greater differences in ^{15}N abundant were performed along with N application. Double amount of ^{15}N abundant was found between 16 kgN/rai and 32 kgN/rai treatments at 0.6266 and 1.2853 but the excess of ^{15}N did not come along with this result , they were 0.2946 and 0.9190 atom%. However, the increment to 48 kgN/rai fertilizer led to increase to 1.4563%^{15}N abundant at 1.0896 ^{15}N atom % excess. The amont of ^{15}N enrichement was calculated from total nitrogen content in the soybean above ground biomass which contained at the average of 3.2-3.5% (Table 4).

These evidence reflected that the contribution performance of nitrogen in above-ground soybean biomass was dominantly derived from biological nitrogen fixation (Ndfa), followed by deriving from soil and the lowest was derived from fertilizer.

Table 4 The analysis results of ^{15}N from vegetable soybean biomass using Stable Isotope Ratio Analysis Mass Spectrometer (Isoprime) connected to Elemental analyzer (NC2500).

Treatments	% N 15 abundant	% N 15 excess	Total N (%) (average)
T_1	-	-	3.3167
T_2	0.6267	0.2947	3.54
T_3	1.2853	0.919	3.5267
T_4	1.4563	1.0897	3.2333
Standard crop 1		-	
Standard crop 2	1.197	0.83	
Standard crop 3	2.03	1.664	
Standard crop 4	2.205	1.839	

T_1 = Control ; T_2 = N fertilizer at the rate 16 kgN/rai ; T_3 = N fertilizer at the rate 32 kgN/rai ; T_4 = N fertilizer at the rate 48 kgN/rai