Kasetsart J. (Nat. Sci.) 45 : 385 - 395 (2011)

Effect of Nitrate Levels on Nitrogen Accumulation, Seed Yield and Quality of Soybean Cultivars

Myint Myint Maw[1, 2], Sutkhet Nakasathien[2]* and Ed Sarobol[2]

ABSTRACT

Soybeans (*Glycine max* [L.] Merr.) were grown in nutrient culture containing various concentrations of NO_3^- in the forms of KNO_3 and $Ca(NO_3)_2$. The objective was to evaluate the seed yield and quality responses of soybean cultivars to NO_3^- solution. The experiment was conducted in a hydroponic solution using a split plot in randomized complete blocks with three replications in the greenhouse at the Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand. Three levels of NO_3^- (5, 10, 15 m*M*) formed the main plots and three soybean cultivars (CKB1, SJ5, CM60) were the sub-plots. Nitrate application increased the yield of soybean cultivars and this increase was mainly associated with dry matter accumulation in leaves at R5. In comparison with the control (5 m*M*), the highest NO_3^- level (15 m*M*) resulted in higher leaf N accumulation at the R5 stage by an average of 82, 37 and 32% for SJ5, CKB1 and CM60, respectively. The maximum seed yield (41.43 g $plant^{-1}$) produced by the SJ5 cultivar under the highest NO_3^- level seemed to be supported by the significantly largest value of whole plant biomass and nitrogen content (%) in leaves at the R5 stage.

Keywords: soybean cultivars, nitrate levels, R5-leaf N, seed yields, seed protein content

INTRODUCTION

High yields of soybean are associated with greater nitrogen (N) remobilization from vegetative tissues to the seed. The previous work by Shibles and Sundberg (1998) indicated that since grain yield and N content in leaves during the seed-filling period (SFP) are closely related, the amount of N stored in the vegetative parts at the seed-filling stage is clearly important for development of a large seed yield. Some of the N in the mature seed comes from redistribution of N from vegetative plant parts during seed filling with estimates of this contribution varying from 30 to 100% (Egli *et al*., 1983). Moreover, soybean seeds grow continuously accompanied with the growth of leaves and stems, and a higher percentage of carbon (C) fixed during the SFP is partitioned to the seeds (Hume and Criswell, 1973).

Consequently, during vegetative growth, plants have a high demand for N, which sustains photosynthetic capacity, initiation and expansion of leaves, and growth of stems and roots (Rufty *et al*., 1984). Like other legumes, soybean is capable of utilizing both soil N, mostly in the form of nitrate, and atmospheric N, through symbiotic nitrogen fixation (Harper, 1974). Although N fertilization of soybean is not a common practice, there is speculation that the ability of soybean to fix atmospheric N is not always adequate for

[1] Department of Agricultural Planning, Ministry of Agriculture and Irrigation, Myanmar.
[2] Department of Agronomy, Faculty of Agriculture, Kasetsart University, Bangkok 10900, Thailand.
* Corresponding author, e-mail: agrskn@ku.ac.th

Received date : 26/10/11 Accepted date : 06/01/11

maximum yield (Wesley *et al*., 1998). For this reason, many scientists have investigated the response of soybean to N fertilizer in various forms.

The greenhouse study by Yoshida (1979) utilized five N rates from 15 to 150 mg kg^{-1} NO_3-N in complete hydroponic solutions and a control containing no N. The author concluded that the total seed yield increased from 0.9 g with the control to 81.6 g per pot with 50 mg kg^{-1} N, while the highest N rate, 150 mg kg^{-1}, yielded 76.7 g per pot. Currently, there is interest in increasing the protein concentration of soybean seed to improve its value. Nakasathien *et al*. (2000) used supra-optimal N to regulate seed protein concentration (SPC) in soybean genotypes with different SPCs of normal, intermediate and high lines. The results showed that supra-optimal N supplied from V5 and R5 (Fehr and Caviness, 1977) to maturity increased the SPC of NC 107 (normal) and N87-984-16 (intermediate) by an average of 28%. Clearly, in order to achieve high yield potential or high quality, soybean must accumulate large amounts of N in seeds and requires a large amount of energy provided by N application. The objective of the present study was to investigate the relationships among leaf N accumulation at the beginning of the seed filling period (R5), the final seed yield and the protein content in soybean cultivars under various NO_3^- solution levels.

MATERIALS AND METHODS

Plant culture

Seeds of three soybean cultivars: Chakrabhandhu 1 (CKB1), SJ5 and Chiangmai 60 (CM60), were pre-germinated on germination paper at 28 °C and 95% RH in a room. The moisture of germinating seeds was controlled by capillary action from 0.1 m*M* $CaSO_4$ solution. After 4 d, 81 vigorous uniform-sized seedlings were selected and placed into nine 25 L pipes provided with lids containing nine 2.5 cm holes filled with coarse perlite, hydroponic culture systems. The 25 L pipes were separated into three blocks according to NO_3^- levels. Each block consisted of three 25 L pipes connected to the under table compartment, consisting of a 75 L plastic bucket, provided with a pump to circulate the nutrient solution in a continuous flow. One seedling per hole was suspended in the nutrient solution from lids holding the plants of the three cultivars. Fifteen days after transplanting (DAT), plants were supported against the strings running between the poles to grow upward and to obtain enough light until final harvest. Adjustment of nutrient levels of the under table compartment and measurement of solution pH and electrical conductivity (EC) were monitored daily. To avoid nutrient depletion effects, the nutrient solution was replenished at 18 DAT and 30 DAT. At this time, the solution in each bucket was sampled to determine pH and EC, and then circulation between the two compartments was discontinued. After emptying the solutions in the upper recipients into the under table buckets, the entire solutions in the under table buckets were drained. Fresh nutrient solutions were refilled in the under table buckets, and then the circulation system was continued. This process took less than 10 min to allow for replenishment of fresh nutrient solution into the hydroponic system with minimal disturbance to plant function. Throughout the growing season, plants were exposed to natural light intensity, temperature, humidity and photoperiod in the greenhouse at the Agronomy Department, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand.

Nutritional treatments

Initial concentrations of nutrients in the basic solution (Hoagland's solution) were macronutrients: $Ca(NO_3)_2$ 1.5 m*M*, KNO_3 2 m*M*, $MgSO_4$ 1 m*M*, KH_2PO_4 1 m*M*; micronutrients: H_3BO_3 46.2 μ*M*, $MnCl_2$ 9.2 μ*M*, $CuSO_4$ 0.38 μ*M*, $ZnSO_4$ 2.4 μ*M*, $NaMoO_4$ 1.2 μ*M*; and Fe-EDTA:

$FeSO_4.7H_2O$ 90 m*M*, EDTA 90 m*M*. All solutions were made up using deionized water. The pH of the basic nutrient solution ranged from 5.5 to 7.0 and the EC was about 1.66 mS cm^{-1}. Plants were supplied only basic solution from the day of transplanting until 4 DAT. The concentration of NO_3^- in the basic solution was considered as a control (5 m*M*, N_5). After 4 DAT, the two levels of NO_3^- treatments were started by adding KNO_3 and $Ca(NO_3)_2$ solutions to establish the required concentrations of 10 m*M* (N_{10}) and 15 m*M* (N_{15}) proportionally. The initial pH of all nutrient treatments was maintained with 1N HCl.

Sampling procedures

Three replications of the control and NO_3^--treated plants of the three soybean cultivars were harvested at V5, R5 and R8 (maturity). The roots of plant samples were rinsed thoroughly in tap water, blotted on paper towels and the plants were separated into leaves with petioles, stems and roots at V5, whereas pods were divided into pod walls and seeds at R5. Every plant part was oven-dried at 65 °C for 72 h. The dry mass of the plant parts and the whole plants was measured. At final harvest (R8), the number of branches per main stem, the number of pods per plant, and the dry weight of stems, roots, pod walls and seeds per plant were recorded.

Tissue nitrogen and carbon analysis

For total N and C analysis, subsamples of the leaves, pod walls and seeds at R5 and the pod walls and seeds at R8 were pulverized to a fineness that provided maximum homogeneity and minimum sampling variation. The nitrogen (N), hydrogen (H) and carbon (C) contents were determined with an Elementary CHN Autoanalyzer (TruSpec CHN, LECO. Corp., St. Joseph, Michigan, USA), according to ASTM D5373-93 (2002). The nitrogen percentage in the seeds was converted to a protein percentage by multiplying by the conversion factor (6.25).

Experimental design and statistical analysis

The experiment was conducted in a split plot in a randomized complete block (RCB) design with three replications. The main plots consisted of three levels of nitrate-nitrogen (5 m*M*, 10 m*M*, 15 m*M*). The concentrations of NO_3^- in the three treatments were: 1) N_5 basal solution used as the control (5 m*M* = $Ca(NO_3)_2$ 1.5 m*M* + KNO_3 2 m*M*); 2) N_{10} (10 m*M* = $Ca(NO_3)_2$ 3.5 m*M* + KNO_3 3 m*M*); and 3) N_{15} (15 m*M* = $Ca(NO_3)_2$ 5.5 m*M* + KNO_3 4 m*M*). The three soybean cultivars (CKB1, SJ5, CM60) were arranged in the sub-plots.

Data were subjected to analysis of variance (ANOVA) appropriate for a split plot in an RCB design. Separate analysis of variance was performed for each measurement. Simple correlation coefficients and regression analysis for different parameters were computed. Mean separations were accomplished using a least significant difference test at the 5% level.

RESULTS

Dry matter distribution

There was no interaction between the NO_3^- levels and the cultivars (Table 1). Plants receiving 15 m*M* NO_3^- accumulated progressively higher dry matter (DM) in the whole plant than those receiving 5 and 10 m*M* NO_3^- at the V5 stage. Leaves and stems did not respond to N application for DM accumulation. Therefore, the significant increase in whole plant DM resulted possibly from the highest accumulation of DM in the roots. Dry matter in the leaves (2.382 g) accounted for 68% of the whole plant DM (3.502 g) as shown in Table 1.

At the R5 stage, DM accumulation in the whole plant increased with the increasing NO_3^- level from 5 to 10 m*M*, but no further increase was observed when the NO_3^- level was increased from 10 to 15 m*M*. About half of the total DM accumulated by plants receiving 10 and 15 m*M* NO_3^- was allocated to the leaves. The pod wall

DM was significantly different among NO_3^- levels. However, N application had no significant effect on the DM of seed.

Cultivars showed significant differences in whole plant DM during the vegetative and reproductive stages (Table 1). SJ5 produced the lowest DM (2.612 g plant^{-1}) at V5, but had the highest DW (68.292 g plant^{-1}) at R5 in the three cultivars. This increase in the whole plant DW of the SJ5 cultivar was primarily due to the significant amount of DW in the reproductive part of the plant (the pod wall and seed).

N and C accumulation in leaves at R5

The N content in the leaves of all three soybean cultivars at the R5 stage was significantly different ($P < 0.01$) due to varying NO_3^- levels (Figure 1). The leaf N content positively increased with increasing NO_3^- levels. The maximum N content (4.79%) was achieved by the SJ5 cultivar receiving 15 m*M* NO_3^-. The highest N rate (15 m*M*) resulted in higher leaf N accumulation than the control (5 m*M*). The increment was 82, 37 and 32% for SJ5, CKB1 and CM60, respectively.

Increasing the NO_3^- concentration from 5 to 15 m*M* significantly affected the leaf C content in the three soybean cultivars at the R5 stage as shown in Figure 2. Plants receiving 10 m*M* accumulated higher C in the leaves compared to 5 m*M* (control) by 3 and 1% for the CM60 and SJ5 cultivars, respectively, while the CKB1 cultivar had no significant increase in the leaf C content at the 10 m*M* NO_3^- level. The maximum value (44.2%), however, was observed with the CKB1 cultivar treated with 15 m*M* NO_3^-. CKB1 also produced the highest leaf C content at the lowest

Table 1 Effect of NO_3^- levels on dry weight of plant parts and whole plants in three soybean cultivars at V5 and R5. Values for individual treatments are the means for three replicate plants.

Treatment	Dry weight (g)					
	Leaf	Stem	Root	Pod wall	Seed	Whole plant
			V5 Stage			
NO_3^- levels						
N_5 (5 m*M*)	2.122	0.462	0.453 ^{b}			3.037 ^{b}
N_{10} (10 m*M*)	2.043	0.432	0.461 ^{b}			2.936 ^{b}
N_{15} (15 m*M*)	2.382	0.548	0.572 ^{a}			3.502 ^{a}
Cultivars						
CKB1	2.277 ^{a}	0.613 ^{a}	0.555 ^{a}			3.445 ^{a}
SJ5	1.919 ^{b}	0.276 ^{b}	0.417 ^{b}			2.612 ^{b}
CM60	2.351 ^{a}	0.554 ^{a}	0.515 ^{a}			3.420 ^{a}
			R5 Stage			
NO_3^- levels						
N_5 (5 m*M*)	19.252 ^{b}	9.148 ^{b}	4.209 ^{b}	4.366 ^{b}	0.647	37.622 ^{b}
N_{10} (10 m*M*)	36.802 ^{a}	17.252 ^{a}	8.373 ^{a}	8.722 ^{a}	0.852	72.001 ^{a}
N_{15} (15 m*M*)	39.314 ^{a}	16.260 ^{a}	7.182 ^{a}	10.920 ^{a}	1.123	74.799 ^{a}
Cultivars						
CKB1	30.729	15.786	6.986	5.172 ^{b}	0.483 ^{b}	59.156 ^{b}
SJ5	35.209	13.336	6.791	11.977 ^{a}	0.979 ^{a}	68.292 ^{a}
CM60	29.43	13.539	5.988	6.859 ^{b}	1.158 ^{a}	56.974 ^{b}

Note: Within a column, means followed by the same letter are not significantly different at the 0.05 probability level, based on a least significant difference procedure.

rate of NO_3^-. CM60 showed a slight decrease in the leaf C content when the NO_3^- concentration was increased from 10 to 15 m*M*.

Yield components and seed yield per plant

Nitrogen application had a positive influence on yield components and seed yield per plant (Table 2). The greatest seed yield (48.15 g plant^{-1}) was obtained from plants receiving 15 m*M* NO_3^-, while the lowest (17.72 g plant^{-1}) was obtained from plants receiving 5 m*M* NO_3^- (control). The number of branches per plant

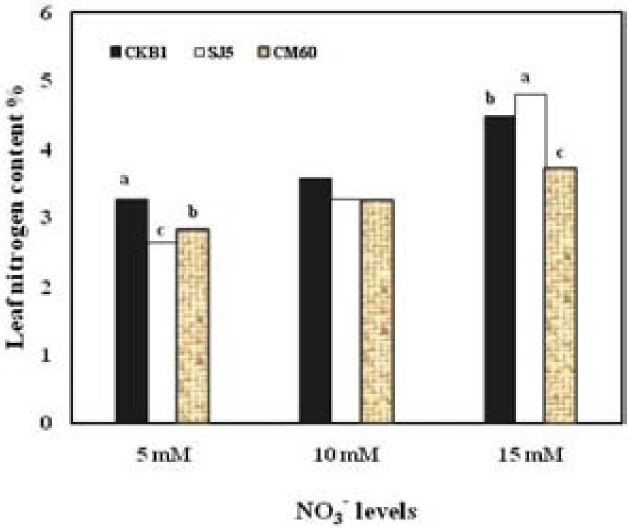

Figure 1 Effect of NO_3^- on leaf N content (%) in three soybean cultivars at R5. Different letters indicate significant differences in cultivars among NO_3^- treatments at the 0.05 probability level.

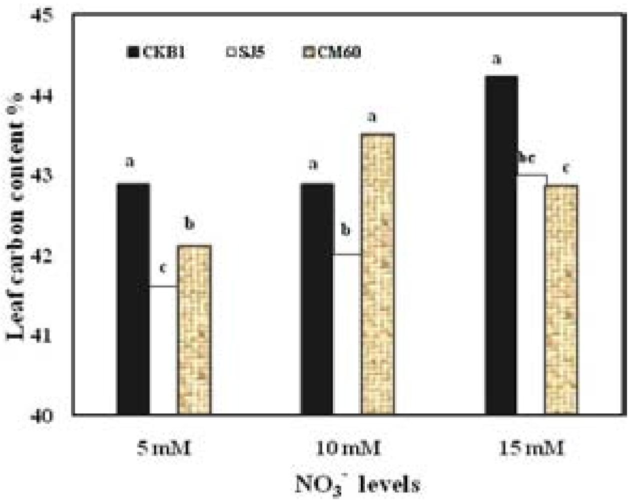

Figure 2 Effect of NO_3^- on leaf C content (%) in three soybean cultivars at R5. Different letters indicate significant differences in cultivars among NO_3^- treatments at the 0.05 probability level.

produced by 15 mM NO_3^- was not significantly different from 10 m*M*, hence the greatest seed yield per plant was mainly associated with the higher number of pods per plant under the application of the highest rate of 15 m*M* NO_3. These results are supported by other research (Schou *et al.*, 1978; Board and Harville, 1993; Board and Tan, 1995) implicating pod number per area as an important factor that influenced yield. The pod wall DM did not increase when the rate of NO_3^- was increased from 10 to 15 m*M*. The effect of NO_3^- on the pod wall DW was consistent at the beginning of the seed filling stage (R5) and at maturity (R8) (Tables 1 and 2).

Cultivars had significant differences in the pod wall DW and seed yield at maturity (Table 2). The seed yield per plant increased with increasing NO_3^- levels in the three cultivars. The maximum average seed yield (41.43 g plant^{-1}) was produced by the SJ5 cultivar. It was clearly noted that the highest seed yield was not obtained by the SJ5 cultivar at the lowest NO_3^- level (control).

Carbon percentage in reproductive parts at R5 and R8

Application of NO_3^- solution had a negative effect on the seed C percentage (Table 3) at the beginning of the seed filling stage (R5). The

Table 2 Effect of NO_3^- levels on yield components and seed yield per plant in three soybean cultivars. Values for individual treatments are the means for three replicate plants.

Treatment	Cultivars	Branches/plant	Pods/plant	Pod wall (g/plant)	Seed yield (g/plant)
5 m*M*		4.56 ^{b}	89.00 ^{c}	15.76 ^{b}	17.72 ^{c}
10 m*M*		6.00 ^{a}	151.56 ^{b}	26.54 ^{a}	37.75 ^{b}
15 m*M*		6.11 ^{a}	184.22 ^{a}	28.79 ^{a}	48.15 ^{a}
	CKB1	5.78	132.22	19.59 ^{c}	29.81 ^{b}
	SJ5	5.56	148.00	28.30 ^{a}	41.43 ^{a}
	CM60	5.33	144.56	23.19 ^{b}	32.35 ^{b}

Note: Within a column, means followed by the same letter are not significantly different at the 0.05 probability level, based on a least significant difference procedure.

Table 3 Effect of supplied with NO_3^- fertilizer levels on the pod wall and seed carbon content (%) in three soybean cultivars at R5 and R8 stages. Values for individual treatments are the means for three replicate plants.

	R5		R8	
	Pod wall C (%)	Seed C (%)	Pod wall C (%)	Seed C (%)
NO_3 levels				
5 m*M*	39.91 ^{c}	45.62 ^{a}	38.87 ^{c}	49.636
10 m*M*	40.42 ^{b}	42.50 ^{b}	39.47 ^{b}	49.792
15 m*M*	40.91 ^{a}	42.66 ^{b}	40.31 ^{a}	50.488
Cultivars				
CKB1	40.38	44.72 ^{a}	39.26 ^{b}	50.796
SJ5	40.31	43.30 ^{b}	40.00 ^{a}	49.645
CM60	40.55	42.78 ^{b}	39.39 ^{b}	49.474

Note: Within a column, means followed by the same letter are not significantly different at the 0.05 probability level, based on a least significant differences procedure.

maximum C content was accumulated in seed receiving 5 m*M* NO_3^-. The seed C percentage decreased with increasing NO_3^- concentration from 5 to 10 m*M* and no further decrease was found when the NO_3^- concentration was increased from 10 to 15 m*M*. At maturity (R8), the C content of seed among NO_3^- treatments showed no statistical differences. The highest percentage of C accumulation was in the pod wall receiving 15 m*M* NO_3^- at both growth stages. In comparison with the beginning of the seed filling stage, the mature seed increased the C percentage after receiving 5, 10 and 15 m*M* NO_3^- by an average of 9, 17 and 18%, respectively.

All cultivars supplied with NO_3^- fertilizer in this experiment showed significant differences in the seed C content at R5. The largest percentage of C (44.72%) was observed in the seed of the CKB1 cultivar. However, there was no significant effect on the seed C content at R8 among cultivars. The SJ5 cultivar had the significantly highest percentage of pod wall C content (40%) at R8.

Protein percentage in reproductive parts at R5 and R8

The seed protein percentage of all three cultivars at the beginning of the seed filling stage was not statistically different, whereas a highly significant increase in the pod wall protein content was observed (Table 4) due to increasing levels of NO_3^- concentration. Conversely, the protein content in the mature seed exhibited a significant response to NO_3^- treatment but there was no response in the pod wall protein content among NO_3^- levels. The maximum protein content (47.06%) in seed was produced by the 15 m*M* NO_3^- treatment which was 15.5% higher than that receiving 5 m*M* (control) but statistically similar to the 10 m*M* NO_3^- treatment. At maturity, the 10 and 15 m*M* NO_3^- treatments increased the seed protein content by an average of 10%. The control treatment slightly reduced the seed protein content when the seed was mature.

Cultivars differed in both the pod wall and seed protein content at the R5 stage (Table 4). The highest seed protein content was produced by the CKB1 cultivar. However, no significant differences among soybean cultivars in terms of the pod wall and seed protein contents at the R8 stage were recorded.

In comparison with the R5 stage, at R8 there was a reduction of 1.42% in the seed protein content for CKB1 and an increase of 3.74 and 4.4% for the SJ5 and CM60 cultivars, respectively.

Table 4 Effect of NO_3^- levels on pod wall and seed protein content (%) in three soybean cultivars at the R5 and R8 stages. Values for individual treatments are the means for three replicate plants.

Treatment	R5		R8	
	Pod wall protein (%)	Seed protein (%)	Pod wall protein (%)	Seed protein (%)
NO_3 levels		%		
5 m*M*	10.92 b	42.35	2.99	40.73 b
10 m*M*	11.85 b	41.49	3.12	45.61 a
15 m*M*	15.06 a	42.85	2.94	47.06 a
Cultivars				
CKB1	12.36 b	45.01 a	2.63	43.59
SJ5	14.62 a	42.64 b	2.48	46.38
CM60	10.85 c	39.03 c	3.95	43.43

Note: Within a column, means followed by the same letter are not significantly different at the 0.05 probability level based on a least significant differences procedure.

Relationship between seed yield and leaf N and C contents at R5

There was a linear relationship between leaf N accumulation at the beginning of seed growth (R5) and the final seed yield (Figure 3). Seed yields in the range 15.50–54.19 g per plant were averaged from the three replications. The coefficient of determination (R^2 = 0.53) showed that 53% of the variance in seed yield among treatments was associated with leaf N content. This result regarding the relationship between potentially mobilized leaf N (that is, leaf N accumulated by the beginning of seed growth) with seed yield was in agreement with the results (R^2 = 0.18 and 0.53 for the two years studied) of Shibles and Sundberg (1998). The accumulation of C in the leaves at R5 was not significantly linked with the final seed yield (R^2 = 0.09; Figure 3).

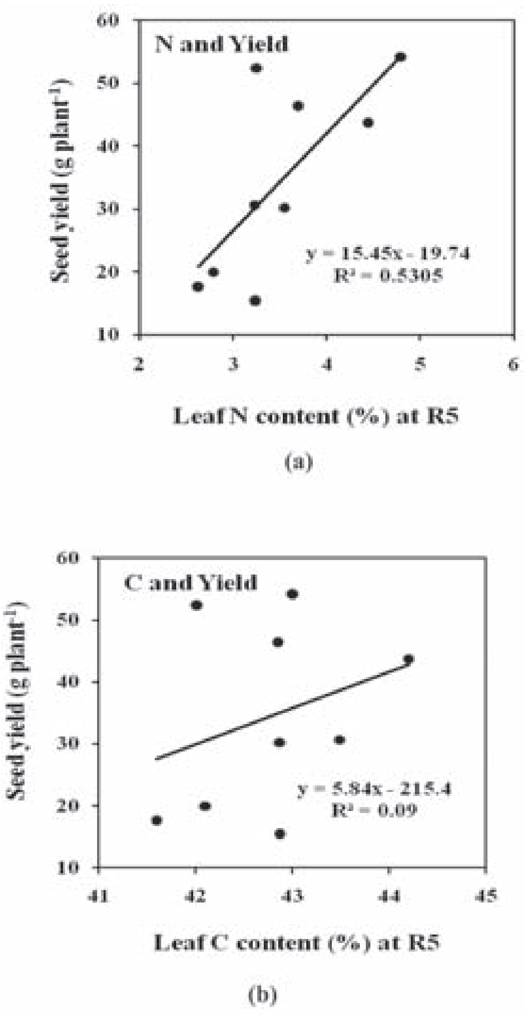

Figure 3 Relationship at R5 between seed yield and (a) leaf N content and (b) leaf C content.

Relationship of seed protein percentage with leaf N and C contents at R5

There were no relationships between the seed protein percentage with the leaf N content and the leaf C content at R5 (Figure 4). The average seed protein percentage in mature seed ranged from 39.34 to 48.96% and showed a slight linear relationship with the leaf N content at R5 (Figure 4; R^2 = 0.41). The leaf C content was not related to the seed protein content (R^2 = 0.04).

DISCUSSION

Biomass and seed yield

The results from this study indicated that increasing the NO_3^- levels in the nutrient solution resulted in significant increases in the dry matter of each plant part and the whole plant at the R5 stage and most of the increase was associated with a greater leaf dry weight. Since R5 marks the end of the period for the accumulation of total vegetative dry matter (TDM; Egli *et al.*, 1987), and greater TDM at R5 resulted in higher seed yields of soybean (Duncun, 1986), the maximum seed yield was obtained from the highest NO_3^- level (15 m*M*) due to the higher number of pods per plant, while significant differences were not found in the whole plant DW at R5 between the treatments involving 10 and 15 m*M* NO_3. This suggests that the possible yield is supported by growth factors (TDM) in combination with yield components (pod number per plant). The leaf N content at R5 was significantly influenced by the application of NO_3^- and the maximum values of all three cultivars were observed at the highest

NO_3^- level. This may have led to the higher seed yield (g plant^{-1}) with increasing NO_3^- levels for all cultivars. The existence of a linear relationship between the final seed yield and the leaf N content at R5 (Figure 3) confirmed the importance on the final seed yield of N accumulation by leaf tissues before the seed filling period (R5). The coefficient of determination ($R^2 = 0.53$) indicated that about half of the yield variance among the observations could be accounted for by differences in the leaf N content at R5.

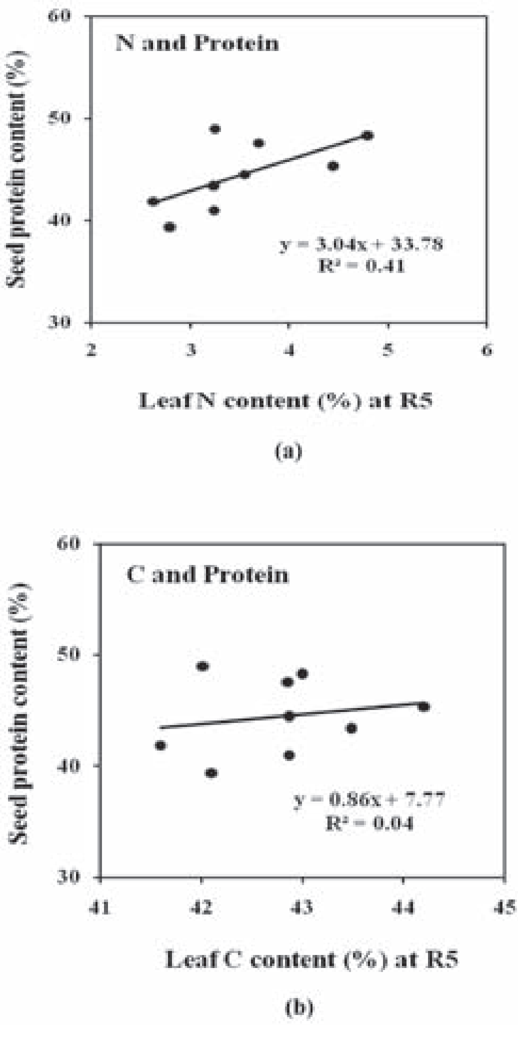

Figure 4 Relationship at R5 between seed protein percentage and (a) leaf N content and (b) leaf C content.

It has been suggested that vegetative N is required for the development of a large yield, but the degree of yield produced by a cultivar depends on differences in the amount of N accumulated in vegetative tissues and in the proportion of vegetative N mobilized for seed growth (Zeiher *et al*., 1982). This was supported in the present study by the SJ5 cultivar having the greatest seed yield per plant among the three cultivars. The positive seed yield that resulted from the response of the SJ5 cultivar to NO_3^- treatment was mainly associated with greater accumulation of whole plant dry matter at R5 (that has been reported by Kakiuchi and Kobata (2004) to be linearly related with an increase in the seed dry matter) and the largest R5 leaf N content (that has been reported by Sinclair and de Wit (1976) as an important determination of seed yield). Although the seed C content at R8 in all cultivars was not statistically different according to the various NO_3^- levels, it increased with seed age until maturation (Table 3). Ho (1988) postulated that in practice, higher yields of economically important crops are the result of both greater dry matter production in the leaves and increased C accumulation in harvestable organs. In the present study, the greater seed yield resulting from the SJ5 cultivar was accompanied with greater dry matter production in the leaves at R5 and higher C accumulation in the pod wall at R8 (Table 3). However, the leaf C content at R5 was not significantly related to the final seed yield for all cultivars supplied with different NO_3^- treatments.

Seed protein percentage

Since the soybean seed protein content is an inherited trait influenced by the environment (Burton, 1989), it can be regulated by the external nutrient supply. Previous reports by various researchers (Ohtake *et al*., 1997; Paek *et al*., 1997; Nakasathien *et al*., 2000) clearly showed that the protein content of soybean increased with increasing levels of N treatment. In the present experiment, an increase in the protein percentage of mature seed was observed at the limited level of NO_3^- because the increment resulting from the 10 and 15 m*M* applications (10%) was statistically the same compared to the control. This indicated that the highest rate of 15 m*M* $NO_3^-NO_3^-$ gave the maximum seed yield per plant, but did not give the greatest seed protein content. Thus, 10 m*M* NO_3^- could be identified as an appropriate concentration for seed protein production. The superior N accumulation in the leaf tissue at R5 would be more closely related to the seed yield than the protein content (Figures 3 and 4). The trend of the variation in the protein content was similar to that of the N content due to conversion to protein by multiplying the N content in seeds by 6.25. Cultivars are more likely to differ in the protein percentage of reproductive parts at the onset of the seed filling period than at maturity with the application of any NO_3^- solution.

CONCLUSION

Collectively, the data from this study indicated that NO_3^- application had a positive impact on the seed yield of all tested soybean cultivars, and the increment could be due to an increase in whole plant biomass and N accumulation in the leaves at the R5 stage. Among the three soybean cultivars tested, SJ5 gave the highest biomass, leaf nitrogen content at R5 and seed yield in response to an increased application of NO_3^-. In addition, the protein contents in the mature seeds of the soybean cultivars increased in response to NO_3^-application. The results of this study suggest that the optimum level of NO_3^- concentration to increase the seed protein content was lower than that for maximizing the seed yield in soybeans.

ACKNOWLEDGEMENTS

The authors are grateful to Professor Dr. Sayan Tudsri for his assistance in the use of the hydroponic apparatus and to Ms. Kanyaporn Noknoy for her help in determining the C, H and N contents in the leaves and seeds. A special thanks goes to the Thailand International Development Cooperation Agency (TICA), Royal Thailand Government for granting a scholarship for this study.

LITERATURE CITED

ASTM D5373-93. 2002. **Standard Test Methods for Instrumental Determination of Carbon, Hydrogen, and Nitrogen in Laboratory Samples of Coal and Coke.** ASTM Committee on Standards, ASTM: Philadelphia, PA, USA.

Board, J.E. and B.G. Harville. 1993. Soybean yield component responses to a light interception gradient during the reproductive period. **Crop Sci.** 33: 772–777.

Board, J.E. and Q. Tan. 1995. Assimilatory capacity effects on soybean yield components and pod number. **Crop Sci.** 35: 846–851.

Burton, J.W. 1989. Breeding soybean cultivars for increased seed protein percentage, pp. 1079-1085. *In* A.J. Pascale, (ed.). **World Soybean Conference IV.** Buenos Aires, 5–9. March 1989. Argentina.

Duncun, W.G. 1986. Planting patterns and soybean yield. **Crop Sci.** 26: 584–588.

Egli, D.B., L. Meckel, R.E. Phillips, D. Radcliffe and J.E. Leggett. 1983. Moisture stress and N redistribution in soybean. **Agron. J.** 75: 1027–1031.

Egli, D.B., R.D. Guffy and J.J. Heitholt. 1987. Factors associated with reduced yields of delayed planting of soybean. **J.Agron. Crop Sci.** 159: 176–185.

Fehr, W.R. and C.E. Caviness. 1977. **Stages of Soybean Development.** Coop. Ext. Serv. Spec. Rep. 80. Iowa State University.

Harper, J.E. 1974. Soil and symbiotic requirements for optimum soybean production. **Crop Sci.** 14: 255–260.

Ho, L.C. 1988. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. **Ann. Rev. Plant Physiol. Plant Mol. Biol.** 39: 355–378.

Hume, D.J. and J.G. Criswell. 1973. Distribution and utilization of ^{14}C-labelled assimilates in soybean. **Crop Sci.** 13: 519–524.

Kakiuchi, J. and T. Kobata. 2004. Shading and thinning effects on seed and shoot dry matter increase in determinate soybean during the seed-filling period. **Agron. J.** 96: 398–405.

Nakasathien, S., D. W. Israel, R. F. Wilson and P. Kwanyuen. 2000. Regulation of seed protein concentration in soybean by supra-optimal nitrogen supply. **Crop Sci.** 40: 1277–1284.

Ohtake, N., S. Yamada, M. Suzuki, N. Takahashi, Y. Takahashi, T. Chinushi and T. Ohyama. 1997. Regulation of accumulation of β-subunit of β-conglycinin in soybean seeds by nitrogen. **Soil Sci. Plant Nutr.** 43: 247–253.

Paek, N.C., J. Imsande, R.C. Shoemaker and R. Shibles. 1997. Nutritional control of soybean seed storage protein. **Crop Sci.** 37: 498–503.

Rufty, T.W., C.D. Raper and S.C. Huber. 1984. Alterations in internal partitioning of carbon in soybean plants in response to nitrogen stress. **Can. J. Bot.** 62: 501–508.

Schou, J.B., D.L. Jeffers and J.G. Streeter. 1978. Effects of reflectors, black boards, or shades applied at different stages of plant development on yield of soybeans. **Crop Sci.** 18: 29–34.

Shibles, R. and D.N. Sundberg. 1998. Relation of leaf nitrogen content and other traits with seed yield of soybean. **Plant Prod. Sci.** 1: 3–7.

Sinclair, T.R. and C.T. de Wit. 1976. Analysis of the carbon and nitrogen limitations to soybean yield. **Agron. J.** 68: 319–324.

Wesley, T.L., R.E. Lamond, V.L. Martin, S.R. Duncan. 1998. Effects of late-season nitrogen fertilizer on irrigated soybean yield and composition. **J. Prod. Agr.** 11: 331–336.

Yoshida, T. 1979. Soil management and nitrogen fertilization for increasing soybean yield. **JARQ.** 13: 163–168.

Zeiher, C., D.B. Egli, J.E. Leggett and D.A. Reicosky. 1982. Cultivar differences in N redistribution in soybeans. **Agron. J.** 74: 375–379.