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ASSIMILATION OF NITROGEN BY SOYBEAN GROWN AT DIFFERENT NITRATE-NITROGEN LEVELS

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Abstract: Investigations were carried out to study the effects of varying levels of nitrate-nitrogen on the absorption of nitrogen by different plant parts in soybean [*Glycine max* (L.) Merrill] in greenhouse. The treatments were 0 mM, 2 mM, 5 mM, 10 mM KNO_3. Soybean cultivar RAWAL-1 was used as test plant. Assimilation of nitrogen in shoots and roots increased with concurrent increasing levels of nitrate-nitrogen in the media. Furthermore, nitrogen content in shoot and root increased with the age of crop. Various plant parts maintained the maximum content of nitrogen in plants harvested at middle of pod growth, i.e., 10 weeks after sowing compared to that of harvested at flowering stage, i.e., 5 weeks after sowing.

Keywords: *Glycine max* (L.), growth stages, nitrogen content, nitrate-nitrogen supply, plant parts, soybean.

INTRODUCTION

Soybean [*Glycine max* (L) Merrill] has become the major source of edible vegetable oils and of high protein feed supplements for livestock in the world. Soybean is a legume and when well nodulated is capable of fixing its own nitrogen (N). Harper [1974] found that both symbiotic N_2 fixation and nitrate (NO_3) utilization appear essential for maximum yield. However, it was found that excessive NO_3 appears to be detrimental to maximum yield because symbiotic fixation is completely inhibited. Apparently, most soils can meet NO_3 needs of the plants because soil application of N showed no yield advantage regardless of source of N or time, method, or rate of application [Porter *et al*. 1981]. An exception to this has occurred on soils that are somewhat poorly drained and low in organic matter. These soils have nitrogen rates from 50 to 110 kg ha^{-1} [Bhangoo and Albritton 1976]. Vasilas *et al*. [1980] studied labeled N, applied in combined N with phosphorus, potassium and sulphur to form a relatively low salt NPKS solution. He found that from 44% to 67% of the total N applied was recovered in the plants. The greenhouse research utilizing sand or solution culture has demonstrated the importance of the presence of some soil or fertilizer N for initial growth of soybean, even in the presence of adequate nodulation [Hatfield *et al*. 1974]. Wych and Rains [1979] reported that a small amount of starter N has a synergistic effect on subsequent plant growth and increased nitrogen assimilation by soybean plants. Matthews and Hayes [1982] demonstrated that soybean plants totally deprived of any starter N source undergo a severe N stress period following depletion of cotyledonary N reserves and before functional nodule development occurs. Thus, the optimum plant growth is never attained.

The net rate of nitrogen uptake by intact plants is dependent on NO_3 level in the soil solution, water availability and rate of flow to roots and mineralization rates. Since NO_3 is freely mobile, the moisture status of a soil is likely the predominant factor controlling NO_3 availability to the root [Olson and Kurtz 1982]. The uptake of nitrogen is relatively low during seedling and early vegetative growth stages. Peak uptake rates per plant occur at the time for early to mid-pod fill under conditions of adequate water supply and continuous NO_3 availability [Gibson and Harper 1985]. However, a little information is available about the assimilation of nitrogen by various parts of the plant and at various stages of growth. Therefore, the studies were carried out to quantify the absorption of nitrogen content by various parts of plant under varying levels of nitrate-nitrogen in RAWAL-1 soybean cultivar.

MATERIALS AND METHODS

The experiment was conducted in the greenhouse of the Institute of Pure and Applied Biology, Bahauddin Zakariya University, Multan to investigate the response of soybean cultivar to different levels of nitrate-nitrogen. The treatments consisted of four levels of nitrate-nitrogen (0 mM, 2 mM, 5 mM, 10 mM KNO_3). Soybean cultivar RAWAL-1 was used as test plant. The treatments were arranged in a Completely Randomized Design having six replications. The seeds of the cultivar were collected from National Agricultural Research Center, Islamabad and inoculum from NIBGE, Faisalabad. The seeds of soybean were inoculated with Rhizobium strain (BioPower-BioFertilizer for legumes) and sown in 20cm diameter plastic pots containing washed coarse sand. Ten seeds were sown in each pot on 2nd April, 2003. The media of different concentrations of nitrate-nitrogen were prepared according to the method of Meidner [1984]. A complete nitrogen-free nutrient solution amended with different levels of nitrate-nitrogen was applied once a week. The pots were watered to field capacity, when required.

Table 1: Effect of different levels of nitrate-nitrogen (mM) on nitrogen concentration (%) in different parts of soybean cv. RAWAL-1.

Stage of harvest (weeks after sowing)	Plant part	NO_3-N (mM) level				LSD
		0	2	5	10	($p<0.05$)
Flowering stage (5)	Shoot	1.34	1.60	2.01	2.15	0.08**
	Root	0.90	1.05	1.26	1.40	0.07**
Beginning of pod growth (8)	Shoot	1.82	2.14	2.27	2.42	0.08**
	Root	1.10	1.16	1.32	1.50	0.08**
Middle of pod growth (10)	Shoot	2.17	2.43	2.59	2.91	0.07**
	Root	1.11	1.29	1.73	1.98	0.08**

** = significant at the 0.01 level.

The plants were harvested at three growth stages, i.e., 5 weeks after sowing at flowering stage, 8 weeks after sowing at beginning of pod growth stage, and 10 weeks after sowing at middle of pod growth stage according to the method of Obaton [1983]. The nitrogen concentration in shoots and roots on dry weight basis was determined following the methods of Ryan *et al*. [2001]. Statistical analysis of the data was carried out as outlined by Steel and Torrie [1980]. The least significant differences (LSD) test at the 0.05 and 0.01 probability level was applied to test the significance of the treatment means. Simple correlation co-efficient and regression analysis for different parameters were also computed.

RESULTS AND DISCUSSION

The nitrogen concentration in shoots and roots of the plant differed significantly ($p<0.01$) due to varying nitrate-nitrogen levels (Table 1). The absorption of N by shoots and roots increased with concurrent increasing levels of nitrate-nitrogen in the media. The plants maintained two-third higher concentration of N in their shoot tissues compared to root tissues. Averaged across the growth stages, N concentration increased from 1.78 to 2.49% and 1.04 to 1.63% at 10 mM NO_3-N in shoots and roots, respectively. Crop treated with 10 mM NO_3-N assimilated 39.89% and 56.73% higher N compared to 0 mM NO_3-N in shoots and roots, respectively. The assimilation of N in shoots and roots increased with crop ontogeny. The plants at flowering stage maintained the lowest N concentration in shoots and roots compared to that of plants harvested at middle of pod growth. Maximum N content was found in shoots and roots in plants harvested at middle of pod growth. Averaged across shoots and roots of plant parts and different nitrate levels, the values of N concentration were 1.46, 1.72 and 2.03% at flowering stage, beginning of pod growth and middle of pod growth, respectively. The plants harvested at middle of pod growth maintained 42.1 and 33.0% higher content of N in shoots and roots, respectively compared to that harvested at flowering stage. Averaged across plant parts and nitrogen supply, the plants harvested at middle of pod growth assimilated 39.04% higher N than that of at flowering stage.

The variation in translocation and compartmentalization in maintaining N content in different plant parts resulted due to sustained supply of N in the medium. Crafts-Bradner and Harper [1982] reported that in a view of greater dry matter of stems (including petioles and cotyledons) compared with roots, the absolute quantity of NO_3 storage is greater in the stems. Furthermore, accumulation of N was enhanced with concomitant varying levels of N fertilizer. De Mooy *et al*. [1973] stated that soil and plant water status primarily influence nutrient absorption and accumulation because of their effect on plant growth. In a recent study, Usman and Jamro [2004] also found that sufficient supply of nitrogen in the soil resulted in the enhanced assimilation of nitrogen by various plant parts of soybean plant.

The high degree of correlation coefficient ($p<0.01$) between varying nitrate-nitrogen levels and nitrogen content in shoots and roots signify that maintenance of nitrogen concentration in shoot and roots tissues was closely related to sustained supply of nitrogen in the medium (Table 2). The nitrogen content in various plant parts increased with increasing levels of nitrogen supply.

Table 2: Relationships between nitrate-nitrogen levels (mM) and nitrogen content (%) during growth period in soybean cv. RAWAL-1.

Independent Variable	Dependent Variable	Regression equation	Correlation co-efficient (*r*)	Co-efficient determination (R^2)
		Flowering Stage		
NO_3-N levels	Shoot	Y = 1.340+0.109x	0.94**	0.88
	Root	Y = 0.897+0.093x	0.95**	0.90
		Beginning of Pod Growth		
NO_3-N levels	Shoot	Y = 1.815+0.232x	0.90**	0.81
	Root	Y = 1.096+0.041x	0.96**	0.92
		Middle of Pod Growth		
NO_3-N levels	Shoot	Y = 2.170+0.172x	0.98**	0.96
	Root	Y = 1.110+0.47x	0.97**	0.94

** = significant at 0.01 level.

Table 3: Effect of different levels of nitrate-nitrogen (mM) on nitrogen uptake (g plant^{-1}) by different plant parts during growth period in soybean cv. RAWAL-1.

Stage of harvest (weeks after sowing)	Plant part	NO_3-N (mM) level				LSD (p<0.05)
		0	2	5	10	
Flowering stage (5)	Shoot	0.0040	0.010	0.0140	0.0143	0.00179**
	Root	0.0017	0.0023	0.0029	0.0029	0.00027**
Beginning of pod	Shoot	0.0064	0.0131	0.0209	0.0191	0.00046**
growth (8)	Root	0.0024	0.0028	0.0040	0.0036	0.00027**
Middle of pod growth	Shoot	0.0093	0.0231	0.0360	0.0290	0.00368**
(10)	Root	0.0032	0.0045	0.0063	0.0049	0.00059**

** significant at the 0.01 level.

Data for N uptake by shoot and roots tissues differed significantly due to varying levels of nitrate-nitrogen and stage of plant growth (Table 3). The uptake of N increased gradually at 2 mM and 5 mM NO_3-N and then it declined at 10 mM NO_3-N. Averaged across stages of growth and parts of plants, the plants treated with 5 mM NO_3-N absorbed 0.014 g plant^{-1} N compared to that 0.0123 g plant^{-1} nitrogen by plants receiving 10 mM NO_3-N in the medium. Furthermore, averaged across the stages of growth and various nitrogen levels, the shoots absorbed five times higher quantity of N than that of roots. The uptake of N by plants increased with advancement of crop growth. These results agree with those of Crafts-Bradner and Harper [1982] and Harper [1987].

Data for N distribution percentage in shoot and root tissues differed significantly due to nitrogen supply and various stages of growth (Table 4). The proportion of N absorption by shoot increased with increasing levels of nitrate-nitrogen while it decreased conversely in root tissues at all growth stages. The plants retained two-third of the total N in shoot tissues compared to that of root tissues. The higher percentage of N distribution between shoots and roots was found in plants harvested at middle of pod growth, i.e. 10 weeks after sowing compared to that of at flowering stage i.e. 5 weeks after sowing.

Table 4: Effect of different levels of nitrate-nitrogen (mM) on nitrogen distribution (%) among different plant parts during growth period in soybean cv. RAWAL-1.

Stage of harvest (weeks after sowing)	Plant part	NO_3-N (mM) level				LSD (p<0.05)
		0	2	5	10	
Flowering stage (5)	Shoot	70.2	81.3	82.8	83.1	5.38**
	Root	29.8	18.7	17.2	16.9	3.77**
Beginning of pod	Shoot	72.7	82.4	83.9	84.2	5.13**
growth (8)	Root	27.3	17.6	16.1	15.8	2.86**
Middle of pod growth	Shoot	74.4	83.7	85.1	85.5	4.92**
(10)	Root	25.6	16.3	14.9	14.5	2.71**

** significant at the 0.01 level.

The results obtained in the study corroborate with those of Hanway and Weber [1971]. They reported that amount of N uptake and its relative distribution in various plant parts depended upon the N supply in the soil. They found that maximum accumulation of N occurred near physiological maturity of soybean plant. Various researchers [Sorensen and Penas 1978, Terman 1977] also reported that uptake of N and its distribution in different plant parts was affected by nitrogen nutrition. Similar results have been reported by [Finke *et al*. 1982, Rabie *et al.* 1980 and Harper 1987] that under sustained supply of N, crop produced greater quantity of biomass and shared its greater proportion to stems compared with roots. Moreover, in absolute terms greater quantity of N was accumulated in stems than in the roots.

References

Bhangoo, M.S. and Albritton, D.J. (**1976**) "Nodulating and non-nodulating Lee Soybean isolines response applied nitrogen", *Agron. J*., 68, 642-645.

Crafts-Bradner, S.J. and Harper, J.E. (**1982**) "Nitrate reduction by roots of soybean (Glycine max [L.] Merrill) seedlings", *Plant Physiol*., 69, 1298-1303.

De Mooy, C.J., Pesek, J. and Spaldon, E. (**1973**) "Mineral Nutrition", In: B.E. Caldwell (Ed.), *Soybean Improvement, Production & Uses*, *Agronomy Series* 16, pp. 267-232.

Finke, R.L. Harper, J.E. and Hagesman, R.H. (**1982**) "Efficiency of nitrogen assimilation by N_2 fixing and nitrate grown soybean plants", *Plant Physiol*., 70, 1178-1184.

Gibson, A.H. and Harper, J.E. (**1985**) "Nitrate effect on nodulation of soybean by Brady-*rhizobium japonicum*", *Crop Sci*., 25, 497-501.

Hanway, J.J. and Weber, C.R. (**1971**) "N, P and K percentages in soybean (*Glycine max* (L.) Merrill) plant parts", *Agron. J*., 63, 286-290.

Harper, J.E. (**1974**) "Soil and symbiotic nitrogen requirements for optimum soybean production", *Crop Sci*., 14, 255-260.

Harper, J.E. (**1987**) "Nitrogen metabolism", In: J.R. Wilcox (Ed.), *Soybeans: Improvement, Production, and Uses*, ASA-CSSA-SSSA, Madison, Wisconsin, USA, pp. 497-533.

Hatfield, J.L., Egli, D.B., Leggett, J.E. and Peaslee, D.E. (**1974**) "Effect of applied nitrogen on the nodulation and early growth of soybeans (*Glycine max* [L.] Merrill)", *Agron. J.*, 66, 112-114.

Matthews, D.J. and Hayes, P. (**1982**) "Effect of root zone temperature on early growth, nodulation & nitrogen fixation in soybeans", *J. Agric. Sci.,* 98, 371-376.

Meidner, H. (**1984**) "Class Experimentation in Plant Physiology", George, Allan and Unwin Publisher Ltd., London, pp. 156-157.

Obaton, M. (**1983**) "Technical Handbook on Symbiotic Nitrogen Fixation, Legume/Rhizobium". FAO, Rome, Italy. pp. 13-23.

Olson, R.A. and Kurtz, L.T. (**1982**) "Crop nitrogen requirements, utilization, and fertilization". In: *Nitrogen in Agricultural Soils*, *Agronomy Series.* 22, p. 567-604.

Porter, O.A., Bhangoo, M.S., Albritton, D.J. and Burleigh, J.G. (**1981**) "Influence of nitrogen fertilizer on nodulating and non-nodulating soybeans", *Arkansas Farm Res.*, 30(1), 8.

Rabie, R.K., Arima, Y. and Kumazawa, K. (**1980**) "Effect of application time of labelled combined nitrogen on its absorption and assimilation by nodulated soybeans", *Soil Sci. Plant Nutr.*, 27, 225-235.

Ryan, J., Estefan, G. and Rashid, A. (**2001**) "Soil and Plant Analysis Laboratory Manual", International Center for Agricultural Research in the Dry Areas, Aleppo, Syria, pp. 124-127.

Sorensen, R.C. and Penas, E.J. (**1978**) "Nitrogen fertilization of soybeans", *Agron. J.,* 70, 213-216.

Steel, R.G.D and Torrie, J.H. (**1980**) "Principles and Procedure of Statistics", McGraw Hill Book Co. Inc., New York, pp. 183-236.

Terman, G.L. (**1977**) "Yields and nutrient accumulation by determinant soybeans as affected by applied nutrients", *Agron. J.*, 69, 234-238.

Usman, M. and Jamro, G.H. (**2004**) "Yield components and seed quality of soybean with regards to oil and protein as influenced by different levels of NPK fertilizer with and without Rhizobium inoculation in NWFP", *Proceedings of 10th Congress of Soil Science: Management of Natural Resources for Food Security*, 16-19th March, 2004, Sindh Agriculture University, Tandojam, p. 7.

Vasilas, B.L., Zegg, J.D. and Wolf, D.C. (**1980**) "Foliar fertilization of soybeans: absorption and translocation of ^{15}N labeled urea", *Agron. J.*, 72, 271-275.

Wych, R.D. and Rains, D.W. (**1979**) "Nitrate absorption and acetylene reduction by soybeans during reproductive development", *Physiol. Plant,* 47, 200-204.