Tr. J. of Agriculture and Forestry
23 (1999) 389-392

Changes in Nitrogen Status of Soybean Under Influence of Symbiotically Fixed and Bound Nitrogen

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Received: 18.03.1997

Abstract: The contribution of different nitrogen sources (nitrate $^{15}N\text{-}NO_3$ and symbiotic $N\text{-}N_2$) to the nitrogen status of soybean in ontogenesis was studied. Nitrate was assimilated effectively during the vegetative growth, whereas later on the nitrogen-fixation by root nodules became the basic source of nitrogen. The applying of a low dose of nitrate (22.2 mg N/plant) increased the total nitrogen content in the plant and did not depress the nitrogen fixation. Distribution of the symbiotic and nitrate nitrogen among organs of soybean was proportional.

Simbiyotik Fikse Edilmiş Azotun ve Nitrat Dozlarının Soyanın Statüsünün Değişimindeki Etkileri

Özet: Bu çalışmada, farklı azot kaynaklarının (nitrat $^{15}N\text{-}NO_3$ ve simbiyotik $N\text{-}N_2$) ve ontogenesisteki soyanın azot konumunun bağlantısı araştırılmıştır. Büyüme döneminde nitrat belirgin bir şekilde emilmiş, daha sonra, köklerin nodulleri tarafından fikse edilmiş azot, azotun ana kaynağı olmuştur. Azotun düşük dozda (bir bitkide 22.2 mg azot) uygulanmasında, bitkinin toplam azot miktarı yükselmiş ve azot fiksasyonu düşmemiştir. Soya bitkisinin organlarında simbiyotik ve nitrat azotun dağılımı, dengeli karakterli olduğu gösterilmiştir.

Introduction

The leguminous plants acquire the ability to assimilate free nitrogen after the formation of root nodules. However, they can also make use of the bound forms of nitrogen-bearing compounds, e.g. ammonia salts, nitrate, etc. (1). The main role in the establishment of the legume nitrogen status apparently depends on the interaction between symbiotic and asymbiotic nitrogen reduction process. Obviously, involving enzyme systems must include the respective mechanisms to switch over these two processes depending on the level of nitrogen supply, developmental stage, nodulation, and other exogenous factors.

The available data concerning effects of bound nitrogen on the nitrogen-fixing ability of legumes are highly contradictory, and physiological and biochemical mechanisms of inhibitory effects of inorganic nitrogen on the symbiotic system are not well understood. Therefore, the question regarding application of nitrogen fertilizers to legumes remains to be open (2, 3). Elucidation of this problem is important for nitrogen nutrient, understnading the process of utilization of bound and symbiotic nitrogen, and the relative contributions of these processes to the nitrogen supply. In this aspect, a special interest presents contribution of different nitrogen sources to formation of nitrogen status of plants during productive processes in early developmental stages (4, 5).

The objectives of this investigation were to study the effects of different nitrate doses on the accumulation of dry matter and nitrogen in soybean in relation to plant development.

Materials and Methods

Soybean (Glycine max. Merr. Cv. Amber) seeds were grown in green house conditions (25˚C day/20˚C night, 80±5% relative humidity with a natural photoperiod) in containers with quartz and using modified Rinkins media for legumes (6). Nitrogen was added as calcium nitrate enriched with N_{15} izotope at 1/2nd and 1/5th of the complete standard that made up 22.2 and 55.0 mg of N/plant. Nitrogen was not added to control samples. Strain *Bradysizobium japonicum* 2196 was used for inoculation. Samples were taken at the following stages: (a), unfolded cotyledonary leaves; (b-e), 1 to 4th complete leaves; (f), blossoming; (g), initial pod age; (h), ripe seed. Total nitrogen was determined in the dry matter of different soybean organs, and the amount of N^{15}-NO_3 was calculated according to Zamyatina (7). The amount of nitrogen assimilated by soybean from the seed before nodulation was calculated as a difference between total nitrogen and N^{15}-NO_3. These data were taken for constant values and used for N-N_2 determination in samples as a difference between total nitrogen in the plant and nitrogen entered from the seed and nitrate supply. The experiments were carried out three times for each variant and measurements were made on 10 experimental plant levels.

Results

In the present experiments, at all ontogenetic stages, soybean in control samples accumulated less nitrogen and dry matter than in samples with nitrate. The level of the above-the ground dry matter was essentially increased at the beginning of the vegetation under nitrate (22.2 and 55.0 mg of N/plant, Table 1) (O).

Different doses of nitrate affected plant growth and nodulation process. The enhancement of the nitrate amount to 55.0 mg of N/plant delayed the time of nodule appearance for a week and resulted in a decrease in their weight during the first 16-18 days. The differences in the levels of above-the ground parts and nodules were not maintained in the next period of vegetative growth and leveled at the stage of blossoming. Nevertheless, the enhancement of nitrate doses from 22.2 to 55.0 mg of N/plant did not increase crop growth. On the 92nd day after the appearance of sprouts, at the seed ripening stage, dry weights of above-the ground parts were 15.7 and 15.2 g/plant at 22.2 and 55.0 mg of N respectively (Table 2) (●).

No considerable difference was also observed in seed weights. The above-gorund mass increased steadily during seed setting period. Nodule dry weight increased from 0.40 to 0.75 g/plant during the generative period. At seed filling stage, this increase made up additional 30%. The dry weight of nodules reached 0.97±0.02 g when harvested, and there was no considerable difference between treatments (Table 2) (●).

The use of N^{15} showed that in 1-3 leaf period the enhancement of nitrate resulted in greater up-take and accumulation of bound nitrogen in plants. However, enhancement delayed the formation of nodules and reduced their nitrogen-fixing activity. In the 3-4 leaf phase, the up take of nitrate decreased sharply, because of gradual exhaustion of its reserves, and the main role in nitrogen nutrition being switched to symbiotic fixation. In this period, the rate of nitrogen accumulation in greater nitrate doses was lower compared with lesser nitrate dose. This resulted from a weak nitrogen-fixing system

Table 1. (O) Dry biomass accumulation in relation to Soybean plant development and nitrate doses.

Developmental Stage	Total biomass (g)			Above ground mass (g)			Nodule mass (g)		
	0	1/5 N	1/2 N	0	1/5 N	1/2 N	0	1/5 N	1/2 N
cotyledonary leaves	0.146±0.003	0.145±0.001	0.148±0.0035	0.127±0.0015	0.126±0.001	0.128±0.015	--	--	--
1st complete leaf	0.367±0.012	0.400±0.02	0.404±0.028	0.225±0.011	0.263±0.003	0.281±0.016	--	--	--
2nd complete leaf	0.585±0.02	0.773±0.022	0.832±0.034	0.354±0.004	0.552±0.028	0.652±0.030	0.038±0.002	0.024±0.002	--
3rd complete leaf	0.752±0.042	1.245±0.057	1.88±0.041	0.502±0.035	0.830±0.031	1.46±0.065	0.069±0.004	0.103±0.010	0.052±0.007
4th complete leaf	1.309±0.095	2.06±0.084	2.88±0.050	0.897±0.043	1.487±0.06	2.09±0.038	0.124±0.013	0.167±0.010	0.143±0.012
blossoming	4.44±0.094	5.15±0.08	5.16±0.172	3.48±0.087	3.90±0.04	3.77±0.135	0.384±0.020	0.403±0.008	0.417±0.009
initial fruitage	8.21±0.33	9.59±0.15	9.17±0.158	6.41±0.25	7.52±0.14	7.18±0.100	0.765±0.019	0.744±0.022	0.743±0.012
ripe seed	16.18±0.478	17.84±0.30	17.30±0.23	14.15±0.46	15.73±0.28	15.8±0.19	1.003±0.032	0.953±0.031	0.947±0.034

supplying lesser amounts of symbiotically fixed nitrogen (Table 3) (Δ).

During 24-hour preiod average rate of the nitrate nitrogen accumulation (mg N/plant per 24-hours) reached its maximum rate at 4th complete leaf and decreased steadily thereafter (Table 3) (Δ).

The level of symbiotically fixed nitrogen, entered into the plants in the period of nitrate utilization, was negatively correlated with the level of accumulated bound nitrogen (Table 3) (Δ). Small doses of nitrate (22.0 mg) did not depress nitrogen fixation, especially at early stages of the development. These differences between the treatments disappeared gradually after utilization of exogenous nitrate. By the beginning of pod formation, the total nitrogen content in the plants of both treatments became similar and did not differ thereafter.

The contribution of nitrate nitrogen to the final harvest was much lesser as compared with symbiotically fixed nitrogen. Values of total nitrogen showed in the Table 3 included nitrogen of the seed (8.14 mg/plant). Concerning the distribution of bound and symbiotically fixed notrigen (Table 3) (Δ), more than half of nitrogen fixed by nodules over whole vegetation period, entered into soybean during ripe seed stage.

The level of nitrogen assimilation in beans exceeded the one in leaves and stems 2-2.3 times in the ripe seed phase. This proved that needs of beans in nitrogen were provided mainly by symbiotic nitrogen from nodules rather than by reutilization of previously accumulated nitrogen. It was reported that (8) about 70% of nitrogen fixed by soybean nodules during pods included into nitrogen status of forming beans. The greater part of this nitrogen was directed to the seed. The rest of nitrogen directed there by the time redistribution from leaves and stems was completed.

In our experiments, nitrate nitrogen utilized by soybean in the period of seed ripening, was found in seeds. However, its quantity was very small and it dit not actually played any role in the nitrogen status of beans.

Table 2. (●) Dry weight and total nitrogen content of Soybean depending on the nitrate dose.

Phase of development		Nitrate dose, mg N/plant	Dry matter, g/plant			Total N, mg/plant		
			above ground mass	beans + flowers	nodules	above ground mass	beans + flowers	Total biomass
Initial		0	6.4	0.49	0.77	189	18.4	243
fruitage		22.2	7.5	0.49	0.74	214	18.8	271
		55.0	7.2	0.58	0.74	207	23.2	263
	$LSD_{0.95}$		0.6	0.18	0.06	19	5.3	25
Ripe		0	14.2	8.2	1.03	486	341	540
Seed		22.2	15.7	8.9	0.95	542	384	597
		55.0	15.2	8.2	0.95	534	365	586
	$LSD_{0.95}$		1.1	0.54	0.11	39	25	42

Table 3. (Δ) Nitrogen status in Soybean (total biomass) in relation to developmental stage and N doses.

Developmental Stage	Total N, mg/plant			$N\text{-}NO_3^-$, mg/plant			$N\text{-}N_2$, mg/plant		
	0	1/5 N	1/2 N	0	1/5 N	1/2 N	0	1/5 N	1/2 N
cotyledonary leaves	8.27±0.33	11.24±0.49	13.04±0.88	2.35±0.12	3.39±0.14	9.80±0.77			
1st complete leaf	10.90±0.05	19.49±0.85	31.2±1.04	8.36±0.48	9.29±0.79	22.84±0.95	2.63±0.05	2.36±0.25	
2nd complete leaf	22.78±2.21	32.67±1.12	46.3±1.08	9.48±0.69	10.95±0.75	31.9±1.72	14.51±1.39	13.87±0.40	6.08±0.90
3rd complete leaf	39.50±1.72	62.9±2.45	63.1±1.10	11.12±0.51	13.15±0.22	34.5±0.02	31.18±1.72	41.91±2.60	20.25±1.11
4th complete leaf	133.7±3.0	151.6±1.75	149.1±5.34	12.78±0.19	14.12±0.36	33.3±0.88	125.45±3.03	129.6±1.74	107.47±1.14
initial fruitage	243.0±11.4	270.6±3.13	262.8±3.87	10.17±0.75	10.77±0.51	33.3±0.69	234.69±11.45	252.0±2.80	221.42±3.12
ripe seed	539.9±14.5	596.5±14.0	585.9±6.77	9.06±0.64	11.16±0.19	28.9±0.46	531.62±14.45	577.5±13.87	548.65±6.61

Nitrate doses, applied in our experiment, played a significant role in the nitrogen nutrition only in the period of vegetative growth of soybean when fixed noitrogen contributed noticeably to the nitrogen status of the plant.

Since nitrate assimilation by soybean being finished before blossoming, in the preiod of elevated nitrogen requirements, its sole sources were become the symbiotic fixation.

High nitrogen fixation rate was maintained at the beginning of reproduction phase due to the increase of nodule activity. Thereby, both the growth of beans and ripening of seed accured mainly by direct utilization of nodule nitrogen rather than reutilization of previously assimilated nitrogen. As a result, the share of $N-N_2$ in the nitrogen status was proved to be higher in beans than in whole biomass of a plant.

Complete exclusion of bound nitrogen from media slowed down the rate of dry matter accumulation.

The nitrate dose of 22.2 mg/plant proved to be sufficient to alleviate deficiency and to support the normal course of biomass accumulation until activation of root nodules in the process of nitrogen supply. The frequently mentioned lack of positive effects of legumes (4, 9, 10) was caused by temporary relaxation of nitrogen supply by plants till the period of their adaptation to primary utilization of symbiotically fixed nitrogen. Rapid recovery of the nitrogen fixing activity of nodules after removal of exogenous nitrate was meintioned earlier (11). The quantities of nitrogen that depressed the symbiotic relations was extremely small as compared with that of nitrate accumulating in plants during the vegetation (~5%); still it made up considerable amounts in their nitrogen status. One could suggest that the inhibitory effect of nitrate on nitrogen fixation depended on the share which nitrate nitrogen brings to nitrogen status of the plant rather than on the total nitrate quantities. Probably, the role of starting doses of nitrate fertilizers is that they may ensure the plants from possible nitrogen deficiency in the case of delayed formation of nodules or their slowed development under unfavorable conditions.

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