Bot. Bull. Academia Sinica (1988) **29**: 143-151

Effect of ammonium nitrate on the biosynthesis of leghemoglobin and nitrogen fixation in nodules of soybean plants

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(*Received December 2, 1987; Accepted March 11, 1988*)

Abstract. The combined nitrogen, ammonium nitrate or potassium nitrate, was used to treat soybean plants four weeks after the seed germination. The ammonium nitrate inhibited the nitrogen fixing activity of nitrogenase, but the ammonium nitrate did not have a direct effect on the nitrogen fixation. The nitrogenase activity was closely related with the concentration of leghemoglobin. The reduction of leghemoglobin contents in cytosol of nodules was the limiting factor of the reduction of nitrogen fixation, and the key enzyme for the biosynthesis of heme moiety of leghemoglobin, δ-aminolevulinic acid synthetase, was inhibited by the ammonium nitrate. There were three electrophoretic components in leghemoglobin, and these components were not changed by ammonium nitrate treatment. The inhibitory effect on nitrogen fixation by ammonium nitrate would be released while the ammonium nitrate was exhausted.

Key words: δ-Aminolevulinic acid synthetase; Leghemoglobin; Nitrogen fixation; Nitrogenase; Soybean.

Introduction

It had been known that combined nitrogens inhibit the nitrogen fixation in nodules of pea plants (Oghoghore and Pate, 1971; Chen and phillips, 1977; Bisseling *et al.*, 1978; Houwaard, 1980), soybean plants (Harper and Cooper, 1971), in Lentil (Wong, 1980). It had also reported that combined nitrogens induced the senescence of root nodules (Chen and Phillips, 1977). It was proposed that a low internal carbohydrate to nitrogen ratio in the presence of combined nitrogen caused the reduction of nodulation and nitrogen fixation (Houwaard, 1980; Wong, 1980). Small and Leonard (1969) and Oghoghorie and Pate (1971) proposed the similar conclusion that a diminshed supply of photosynthate to nodules because of the nitrate assimilation reduced the nitrogen fixation.

Leghemoglobin is an oxygen-binding protein with a low Km for oxygen. Therefore, the presence of leghemoglobin increases the rate of oxygen uptake by bacteriods and so increases the nitrogen fixing activity of nitrogenase (Bergersen *et al.*, 1973; Wittenberg *et al.*, 1974; Wittenberg, 1980; Bergersen and Appleby, 1981).

It has been demonstrated that the limiting biosynthetic pathway for protoheme formation

in most cells and tissues is the condensation step of succinyl CoA and glycine by δ-aminolevulinic acid synthetase (ALAS) to form δ-aminolevulinic acid (Nadler and Avissar, 1977; Dilworth and Appleby, 1979).

The combined nitrogen, in addition to an inhibitory effect on nitrogen fixation, also inhibited the biosynthesis of leghemoglobin (Bisseling *et al.*, 1978). In this study, it is attempted to figure-out the inhibitory mechanism of combined nitrogens on the biosynthesis of leghemoglobin, and the inter-relationship between the rate of biosynthesis of leghemoglobin and the activity of nitrogen fixation in nodules of soybean plants treated with ammonium nitrate.

Materials and Methods

Isolation and Culture of Rhizobia

Rhizobium japonicum was isolated from nodules of soybean plants (*Glycine max.* cv. Shih-Shih), and purified by the regular microbiological techniques (Vincent, 1974). The purified rhizobia were cultured on yeast-mannitol agar for four days, then transferred to a liquid medium. This rhizobial suspension was incubated for two days at 30°C, then it was used directly as an inoculum for soybean seeds.

Inoculation and Growth Conditions

Soybean seeds were sterilized with a 0.01% of sodium hypochloride solution for five minutes, then rinsed several times with tap water, and once with distilled water. Those seeds were moistened thoroughtly with the rhizobial suspension, then sown in pots containing a mixture of one part vermiculite and two parts of sand. The growing conditions for the plants in the growth chamber were temperature, day/night, 28/25°C; photoperiod, light/dark, 14/10 hours; light intensity, 6000 lux; and relative humidity, 75%. Seedlings were supplied with a nutrient solution (Dilworth, 1980) containing 2 mM of ammonium nitrate every other day for a week after seed germination. The small amounts of N supplied by the solution provide a source of N which enhanced nodulation (Harper, 1974). Thereafter, the seedlings were watered with a nitrogen-free nutrient solution. An appropriate amount of ammonium nitrate or potassium nitrate solution were added to vermiculite-sand mixture in pots while the leaf water potential was slightly below -6 bars. The leaf water potential was held more or less at -6 bars. The soybean plants had higher photosynthetic activity while their leaf water potentials were held around at -6 bars, and so, their nodules had higher efficiency in nitrogen fixation (Huang *et al.*, 1975). The water potentials were measured by the thermocouple psychrometer (Huang *et al.*, 1975).

Recovery Experiments

Four weeks after the plants had grown in vermiculite-sand mixtures in pots and supplied with nitrogen-free medium, plants were treated with 8 mM ammonium nitrate for five days. This treatment caused the nodules of plants had negligible leghemoglobin content and nitrogen fixing activity. Then, those plants were transferred to pots with vermiculite-sand mixtures containing various concentrations of ammonium nitrate, i. e., 0, 1, 2, 3, 4, and 5 mM, respectively. Five days after the transferring, the leghemoglobin concentration and the nitrogen fixing activity in detached nodules of plants were determined.

Determination of Nitrogen Fixation in Detached Nodules

Plants were harvested four weeks after the seed emergence. The nodules were detached from the root system of intact plants. The nitrogen fixing activity of nitrogenase in nodules was determined as the method used by Huang

et al. (1975). The gas samples (0.1 ml) were withdrawn at 30 minutes intervals and chromatographed immediately. Acetylene and ethylene were separated by the gas chromatograph (Shimazu, Model 3 BF) having a hydrogen ionization detector at an oven temperature of 65°C. A glass column of 0.8 m long and 3 mm i. d. packed with Porapak R was used for the separation. The carrier gas was nitrogen at a flowing rate of 30 ml per minute.

Assay of Nitrate Reductase

One gram of nodules were ground with 1/3 of polyvinylpyrrolidone and 10 ml of 0.02 M K-phosphate buffer (pH 7.5) in a prechilled mortar with pestle. The resulting slurry was squeezed through four layers of cheesecloth and then centrifuged at 10,000 g for 10 minutes. The supernatant was defined as the crude extract. All the operations were run at 4°C. Part of the crude extract was used for the assay for nitrate reductase. The activity of this enzyme was determined as the method described by Scholl *et al.* (1974).

Assay of δ-Aminolevulinic Acid Synthetase (ALAS)

Nodules were also fractionated for the assay of ALAS activity in bacteriods as the method described by Nadler and Avissar (1977), except the bacteriods were broken by the French Press instead of the sonic disruption. All operations were performed at 4°C. The δ-aminolevulinic acid (ALA) concentration was determined by the colorimetric reaction with Ehrlich-Hg reagent (Urata and Granick, 1963). The optical density was read at 550 nm, and the molar absorbancy of the color salt was 68,000 (Urata and Granick, 1963).

Assay of Leghemoglobin

Five gram of nodules were homogenized with 50 ml of precooled Drabkins solution (Wilson and Reisenauer, 1963) in a prechilled mortar with pestle. The homogenate was filtered through four layers of cheesecloth and then centrifuged at 10,000 g for 30 minuts. The absorbance of leghemoglobin in the supernatant was read at 540 nm (Wilson and Reisenauer, 1963). The hemoglobin standard was purchased from Sigma Chemical Company.

Gel Electrophoresis of Leghemoglobin

Leghemoglobin for gel electrophoresis was prepared as the method described by Bergersen *et al.* (1973). The purified leghemoglobin was electrophorezied according to method of Verma *et al.* (1974).

For the comparisons of the effect of N source on plant growth, four weeks after the seed germination, part of soybean plants which had been supplied with a medium free from ammonium nitrtate were treated with 8 mM ammonium nitrate for five days as the N source for plants, and another part of plants were remained untreated with ammonium nitrate, so the nitrogen fixed in nodules was the solely N source for plants. Those plants which were treated or untreated with ammonium nitrate were then harvested for the measurements of nitrogenase activity, concentrations of leghemoglobin, organic matters, chlorophyll, and dry weight of leaf and root systems.

Assay of Organic Compounds

The crude extract from nodules that was likely the crude extract obtained from nodules for assay of nitrate reductase was deproteinized by 16% of trichloroacetic acid and centifuged at 5,000 g for 10 minutes after standing still two hours. The resulting supernatant was used for the determination of soluble carbohydrate, amino acid, and protein contents. The carbohydrate, amino acid, and protein concentrations were determined by anthrone (Koehler, 1952), ninhydrin (Moore and Stein, 1954), and Lowry method (Cooper, 1977), respectively.

Chlorophyll Determination

The extraction and determination of chlorophyll were according to the method of Mayfield and Huff (1986).

Dry Weight Determination

Plants were washed several times with tap water, and dried in an oven with 80°C for twenty-four hours. The leaves and root systems were then separated and their dry weights were determined.

Results

The nitrogen fixing activity (acetylene reduction) of nitrogenase was reduced about 73%, and the activity of nitrate reductase was increased about 3.5 folds in nodules of soybean plants at five days after the plants had treated with ammonium nitrate, but the nitrogenase activity was still increasing significantly, and the nitrate reductase remained steadily low activity in nodules of plants untreated with ammonium nitrate. Obviously, the longer periods of ammonium nitrate treated to plants, the lower activity of nitrogenase, and the higher activity of nitrate reductase were detected (Fig. 1). The inhibitory effect of combined nitrogen on the nitrogen fixing activity was changed with the concentrations of combined nitrogen treated to plants (Fig. 2), The higher concentration of combined nitrogen treated to plants, the lower nitrogen fixing activity was measured. In addition to the concentration, the different forms of combined nitrogen also caused various levels of inhibitory effect on nitrogen fixation. As the data shown in Fig. 2, ammonium nitrate enforced much more severe influence than nitrate on the nitrogen fixation. The nitrogenase in nodules of soybean remained about 71%, 54%, 36% and 8.6% activity while plants were treated with ammonium nitrate at concentrations 2, 4, 6, and 8 mM, respectively.

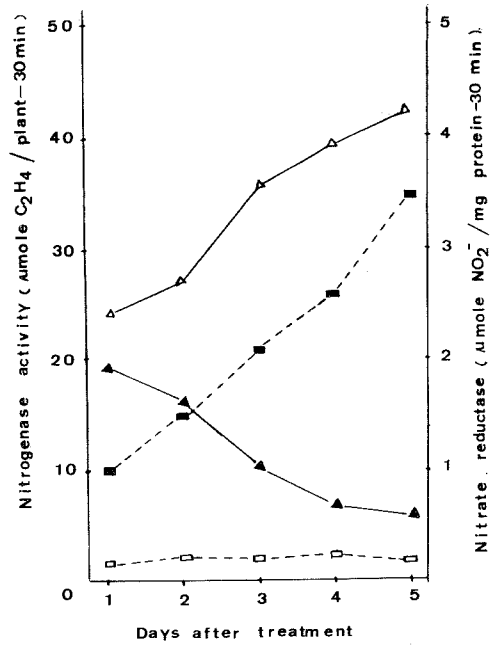

Fig. 1. The nitrogenase and nitrate reductase activity in nodules of soybean plants untreated or treated with ammonium nitrate. Four weeks after seed germination, plants were started to treat with 4 mM of ammonium nitrate. This considered as zero day treatment. Ten plants were harvested for each measurement, and there were triplicate for each measurement. Nitrogenase activity, △, nitrate reductase activity, □, in nodules of plants untreated with ammonium nitrate; Nitrogenase activity, ▲, nitrate reductase activity, ■, in nodules of plants treated with ammonium nitrate.

However, the nodules remained about 92%, 73%, 67%, and 43% activity while plants were treated with potassium nitrate at concentrations of 4, 8, 12, and 16 mM, respectively. The ways of reduction of nitrogenase activity in nodules of soybean plants treated with ammonium nitrate or with K-nitrate were closely related with the reduction of leghemoglobin concentration. The higher nitrogen fixing activity was observed while there was higher concentration of leghemoglobin in nodules, and *vice versa* (Fig. 2).

The inhibition of combined nitrogen on the nitrogen fixation and the reduction of leghem-

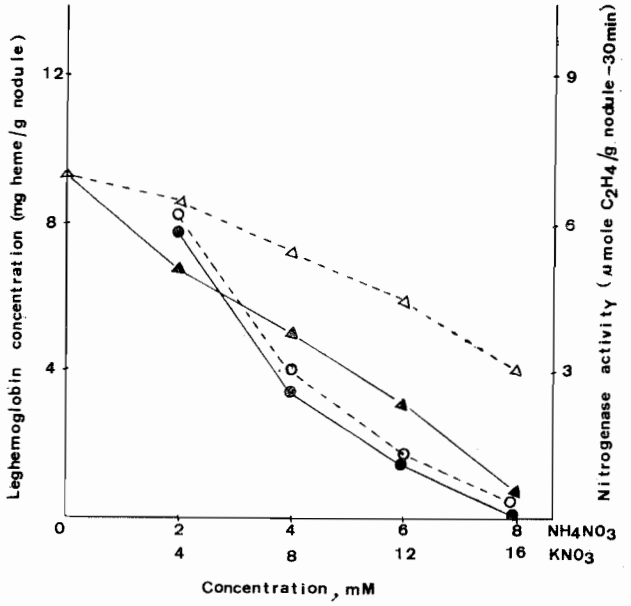

Fig. 2. Leghemoglobin concentration and nitrogenase activity in nodules of soybean plants treated with ammonium nitrate or potassium nitrate. Four weeks after seed germination, plants were treated with various concentrations of ammonium nitrate or potassium nitrate for five days, then root-nodules were harvested for measurements. Leghemoglobin concentration, ●, nitrogenase activity, ▲, in nodules of plants treated with ammonium nitrate at concentrations 2, 4, 6, 8 mM; Leghemoglobin concentration, ○, nitrogenase activity, △, in nodules of plants treated with potassium nitrate at concentrations 4, 8, 12, 16 mM.

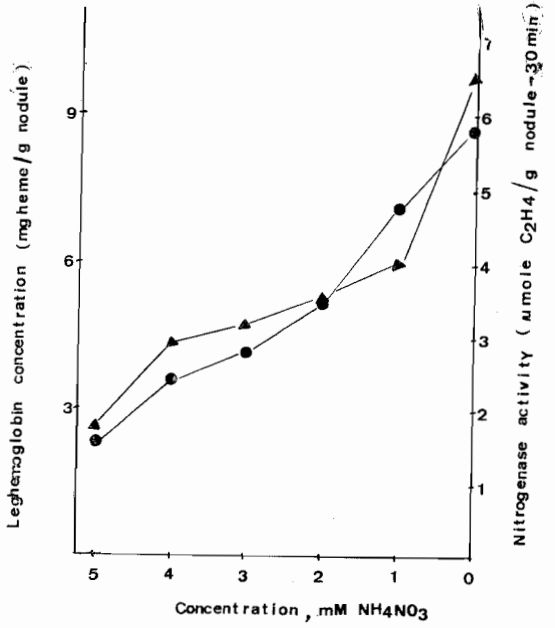

Fig. 3. Recovery experiments. Leghemoglobin concentration. ●, Nitrogenase activity, ▲.

oglobin concentration were reversible effects. As the data shown in Fig. 2, the leghemoglobin concentration and the nitrogenase activity, which had already been severely reduced or inhibited five days after the plants had treated with 8 mM of ammonium nitrate, could be partially or nearly full recovered after those plants were transferred to pots that contained vermiculite-sand mixture with lower concentrations of ammonium nitrate (Fig. 3). The levels of recovery were depended on lower concentrations of ammonium nitrate supplied after the plants had transferred from 8 mM of ammonium nitrate. As the data shown in Fig. 3, there were about 27%, 44% and 94% of leghemoglobin concentration and 26%, 46% and 93% of nitrogenase activity were restored after those plants were released from the treatment of 8 mM ammonium nitrate and transferred to vermiculite-sand mixture supplied with 5 mM, 3 mM, and 0mM of ammonium nitrate, respectively.

The enzyme ALAS is a limiting enzyme for heme biosynthesis in bacteriods of nodules of soybean plant (Dilworth and Appleby, 1979). This enzyme activity was also inhibited by the treatment of ammonium nitrate (Fig. 4). The longer treatment of ammonium nitrate to plants, the lower activity of ALAS was detected. There was negligible activity coulb be measured five days after the plants had treated with ammonium nitrate (Fig. 4). The close interrelatioships among ALAS activity, leghemoglobin concentration, and the nitrogenase activity in nodules of plants untreated or treated with ammonium nitrate are shown in Figs. 4A and 4B, respectively. The leghemoglobin concentration in nodules of treated plants was decreased as the ALAS activity reduced, and

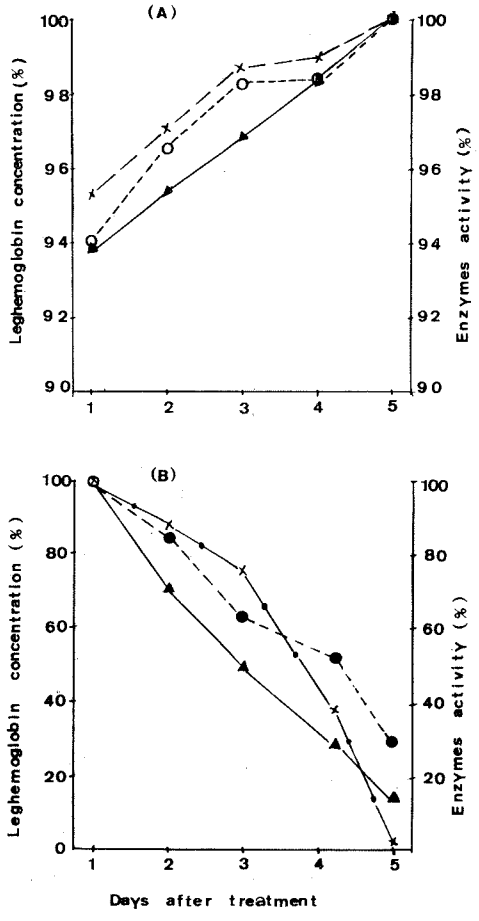

Fig. 4. The relative concentration of leghemoglobin and the relative activity of δ-aminolevulinic acid synthetase (ALAS), and nitrogenase in nodules of soybean plants untreated (A) or treated with ammonium nitrate at a concentration of 8 mM four weeks after seed germination (B). Leghemoglobin concentration, ●, ALAS activity, ×, nitrogenase activity, ▲. Those values measured at 1st day for treated plants (B), and at 5th day for untreated plants (A) were all counted as 100%.

the nitrogenase activity was decreased as the leghemoglobin concentration reduced (Fig. 4B). However, the ALAS activity, leghemoglobin concentration, and the nitrogenase activity in nodules of untreated plants remained slightly increasing four weeks after the seed germinated (Fig. 4A).

There were three components of leghemoglobin could be detected by gel electrophoresis. According to their mobility, there were slow, moderate, and fast electrophoretic components (Fig. 5). These three electrophoretic components of leghemoglobin could not be changed by the ammonium nitrate treatment to plants, and the electrophoretic patterns of leghemoglobin obtained from the nodules of plants untreated and treated with ammonium nitrate were identical (data not shown). Besides, the electrophoretic patterns of a mixed leghemoglobins, i. e., the leghemoglobins extracted from untreated and treated plants were mixed, were looked likely the patterns of leghemoglobin obtained from the untreated plants (Fig. 5).

Although the leghemoglobin concentration and the nitrogenase activity were reduced by the ammonium nitrate, a higher concentration of proteins, free amino acids were measured in nodules of plants treated with ammonium nitrate for five days four weeks after the seed

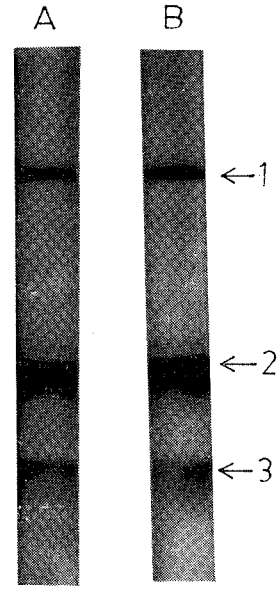

Fig. 5. The electrophoretic patterns of leghemoglobin. (A) The leghemoglobins extracted from nodules of soybean plants treated with ammonium nitrate. (B) A mixture of leghemoglobins extracted from plants treated and untreated with ammonium nitrate. 1. slow electrophoretic component; 2, moderate electrophoretic component; 3. fast electrophoretic component.

Table 1. *Comparison of the nitrogenase activity and concentrations of leghemoglobin and other organic matters in nodules of soybean plants untreated or treated with ammonium nitrate*

Data shown in Table are the average values of triplicate.

Treatment	Nitrogenase activity ($\mu molC_2H_4$/g nodule 30 min)	Leghemoglobin	Protein	Free amino acid	Soluble sugar
		(mg/g nodule)			
Control	6.8	11.5	14.2	5.0	10.4
Ammonium nitrate (8 mM)	0.1	0.2	16.9	6.5	7.4

Table 2. *The chlorophyll concentrations and some morphological parameters of soybean plants treated with ammonium nitrate*

Data shown in Table are the average values of triplicate.

Treatment	Chlorophyll (mg/g dry wt)	Leaf area (cm_2/plant)	Leaf dry weight (g/plant)	Root dry weight (g/plant)
Control	3.26	130.72	0.38	0.40
Ammonium nitrate (8 mM)	3.63	141.84	0.45	0.43

germination (Table 1). Besides, those plants treated with ammonium nitrate grew better than those untreated plants. A higher concentration of chlorophyll in leaves, larger leaf area, and heavier leaf and root dry weights were measured from those treated plants (Table 2). However, a lower concentration of soluble sugars was detected in nodules of plants treated with ammonium nitrate (Table 1).

Discussion

The nitrogen fixing (acetylene reduction) activity of nitrogenase in nodules of soybean plants was significantly inhibited by combined nitrogens (Figs. 1, 2 and 4). The ammonium nitrate had much more harmful effect than the K-nitrate on the nitrogen fixation (Fig. 2). The different levels of inhibition on nitrogen fixation caused by ammonium nitrate or by potassium nitrate were due to the forms of combined nitrogen rather than the concentrations of combined nitrogen treated to plants. However, the nitrate and ammonia equally inhibited the nitrogen fixation had also been reported (Harper and Cooper, 1971). It had been reported or reviewed in papers that nitrogenase biosynthesis could be inhibited by the ammonia (Tubb and Postgate, 1973; Nicholas and Deering, 1976; Eady *et al.*, 1978; Brill 1980; Yates and Eady, 1980; Dixon *et al.*, 1981; Eady, 1981). However, in this study, like the report of Bisseling *et al.* (1978), ammonium nitrate could reduce the concentration of leghemoglobin in nodules (Table 1, Figs. 2 and 4), and the changes of nitrogen fixation was closely matched with changes of leghemoglobin concentration, and the changes of leghemoglobin concentration were intimately related with the change of ALAS activity (Fig. 4). Therefore, the repression of ammonium nitrate on the nitrogen fixing activity of nitrogenase was probably due to the inhibitory effect of ammonium nitrate on the activity of ALAS, and that, consequently reduced the biosynthesis of leghemoglobin in nodules.

The nitrate reductase in nodules was induced and the activity of this enzyme was promoted by ammonium nitrate, but very low activity of this enzyme was detected in nodules of soybean plants untreated with ammonium nitrate (Fig. 1). The activity of nitrate reductase and nitrog-

enase could not have function simultaneously. This indicates that nitrate reduction reaction and nitrogen fixation can not carry on simultaneously in nodules of plants treated with ammonium nitrate under the nitrogen.

The leghemoglobin in nodules of soybean plant composed of three components (Fig. 5). Although the concentration of leghemoglobin was decreased by the ammonium nitrate, the electrophoretic components were not changed by the treatment of ammonium nitrate to plants (Fig. 5). This suggests that certain enzyme(s), such as ALAS, involving in biosynthesis of the heme moiety of leghemoglobin, but not the gene(s) regulating biosynthesis of this enzyme(s), are inactivated or inhibited by ammonium nitrate. Ammonium nitrate decreased the heme concentration in nodules of pea plants has also been reported by Bisseling *et al.* (1978).

The soybean plants treated with 8 mM of ammonium nitrate grew better than those untreated plants (Tables 1 and 2). Besides, the concentrations of organic nitrogenous compounds, such as proteins and amino acids, were about 19% and 30% higher in nodules of the treated plants than in those of untreated ones (Table 1). This might be that quantity of N_2 fixed by nitrogenase was less than that of N supplied to plants in form of ammonium nitrate, or the N in form of ammonium nitrate was preferentially utilized and assimilated by plants. However, the concentration of soluble sugar in nodules of treated plants was 39% lower than in those of untreated ones. This suggests that the total energy cost for the uptake and assimilation of ammonium nitrate is more than those for nitrogen fixation and assimilation of fixed nitrogen.

Literature Cited

Bergersen, F. J., G. L. Turner, and C. A. Appley. 1973. Studies of the physiological role of leghemoglobin in soybean root nodules. Biochim. Biophys. Acta **292**: 271-282.

Bergersen, F. J. and C. A. Appleby. 1981. Leghemoglobin within bacteriod-enclosing membrane envelopes in soybean root nodules. *In* A. H. Gibson and W. E. Newton (eds.), Current Perspectives in Nitrogen Fixation. Canberra: Aust. Acad. Sci., P. 366.

Bisseling, T., R. C. Van Den Bos, and A. Van Kammen. 1978. The effect of ammonium nitrate on the synthesis of nitrogenase and the concentration of leghemoglobin in pea nodules induced by *Rhizobium leguminosarum*. Biochim. Biophys. Acta **539**: 1-11.

Brill, W. J. 1980. Biochemical genetics of nitrogen fixation. Microbiol. Rev. **44**: 449-67.

Chen, Pin-ching and D. A. Phillips. 1977. Induction of root nodules senescence by combined nitrogen in *Pisum sativum* L. Biochim. Biophys. Acta **35**: 140-153.

Cooper, T. C. The Tools of Biochemistry. Wiley-Interscience, pp. 53-55.

Dilworth, M. J. 1980. *In* S.P. Colowick and N.O. Kaplan, (eds.), Methods Enzymol. **69**: 812-823.

Dilworth, M. J. and C. A. Appleby. 1979. Leghemoglobin and rhizobium hemoproteins. *In* R. W. F. Hardy, F. Bottomley and R. C. Burns (eds.), A Treatise on Dinitrogen Fixation, vol. 2. Wiley, New York, pp. 691-764.

Dixon, R., C. Kennedy, M. Merrick. 1980. Genetic control of nitrogen fixation. Symp. Soc. gen. Microbiol. **31**: 161-85.

Eady, R. R., R. Issack, C. Kennepy, J. R. Postgate, and H. D. Ratcliffe. 1978. Nitrogenase synthesis in *Klebsiella pneumoniae;* Comparison of ammonium and oxygen regulation. J. gen. Microbiol. **104**: 277-85.

Eady, R. R. 1981. Regulation of nitrogenase activity. In A. H. Gibson and W. E. Newton, eds., Current perspectives in nitrogen fixation. Canberra: Aust. Acad. Sci., pp. 172-181.

Emerich, D. W., J. E. Lepo, and H. J. Evans. 1983. Nodules metabolism. *In* W. L. Broughton (ed.), Nitrogen Fixation, vol. 3. Oxford University Press, pp. 213-244.

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

Harpder, J. E. and R. L. Cooper, 1971. Nodulation response of soybean (*Glycine max.* L. Merr.) to application rate and placement of combined nitrogen. Crop Sci. **11**: 438-440.

Houwaard, F. 1980. Influence of ammonium and nitrate nitrogen on nitrogenase activity of pea plants as affected by light intensity and sugar addition. Plant and Soil **54**: 271-282.

Huang, C. Y., J. S. Boyer, and L. N. Vancerhoef. 1975. Acetylene reduction and metabolic activities of soybean having various leaf and nodule water potentials. Plant Physiol. **56**: 222-227.

Koehler, L. H. 1952. Differentiation of carbohydrates by anthrone reaction rate and color intensity. Anal. Chem. **24**: 1576-70.

Mayfield, S. P. and A. Huff. 1986. Accumulation of chlorophyll, chloroplastic proteins, and thylakoid membranes during reverse of chromoplasts to chloroplasts in *Citrus sinesis* Epicarp. Plant Physiol. **81**: 30-35.

Moore, S. and W. H. Stein. 1954. A modified ninhydrin reagent for the photometric determination of amino acids and related compounds. J. Biol. Chem. **211**: 907-911.

Nadler, K. D. and Y. J. Avissar. 1977. Heme synthesis in soybean root nodules. Plant Physiol. **60**: 433-36.

Nicholas, D. J. and J. V. Deering. 1976. Repression, derepression, and activation of nitrogenase in *Azotobacter vinelandii*. Aust. J. Biol. Sci. **29**: 147-61.

Oghoghore, C. G. O. and J. S. Pate. 1971. The nitrate stress syndrome of the nodulated field pea. *In* T. A. Lie and E.G. Mulder (eds.), Biological Nitrogen Fixation in Natural and Agricultural Habitats. Plant Soil (special volume), pp. 185-202.

Scholl, R. L., J. E. Harper, and R. H. Hageman. 1974. Improvements of the nitrite color development in assays of nitrate reductase by phenazine methosulfate and zinc acetate. Plant Physiol. **55**: 824-828.

Small, J. G. C. and O. A. Leonard. 1969. Translocation of ^{14}C-labelled photosynthate in nodulated legumes as influenced by nitrate nitrogen. Am. J. Bot. **56**: 187-194.

Tubb, R. S. and J. R. Postgate. 1973. Control of nitrogenase synthesis in *Klebsiella pneumoniae*. J. Gen. Microbiol, **79**: 103-117.

Urata, G. and S. Granick. 1963. Biosynthesis of δ-aminoketones and the metabolism of aminoacetone. J. Biol. Chem. **238**: 811-20.

Verma, D. Pal S., D. T. Nash, and H. M. Schulman. 1974. Isolation kand *in vitro* translation of soybean leghemoglobin mRNA. Nature **251**: 74-77.

Vincent, J. M. A Manual for the Practical Study of Root Nodule Bacteria. IBP handook No. 15. Blackwell Scientific Publications, Oxford Edingburgh.

Wilson, D. O. and H. M. Reisenauer. 1963. Determination of leghemoglobin in legume nodules. Anal. Biochem. **6**: 27-30.

Wittenberg, J. B. 1980. Utilization of leghemoglobin-bound oxygen by rhizobium bacteriods. *In* W. E. Newton and W. H. Orme-Johnson (eds.), Nitrogen Fixation, vol. 2. University Park Press, Baltimore, pp. 53-67.

Wong, P. P. 1980. Nitrate and carbohydrate effects on nodulation and nitrogen fixation activity of Lentil. Plant Physiol. **66**: 78-91.

Yates, M. G. and R. R. Eady. 1980. The physiology and regulation of nitrogen fixation. *In* N.S. Subba Rao (ed.), Recent Advances in Biologicl Nitrogen Fixation. London, pp. 88-121.

硝酸銨處理大豆其根瘤血紅素之合成對固氮作用之影響

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大豆種子發芽後四週開始處理硝酸銨或硝酸鉀，此兩種含氮化學肥料均可抑制根瘤之固氮作用，惟此種抑制作用是可逆的現象。當大豆從高濃度之硝酸銨移到低濃度之硝酸銨生長環境時，其根瘤固氮作用被抑制之現象即可緩和減少。

硝酸銨及硝酸鉀均亦可抑制根瘤血紅素合成酵素如 δ-aminolevulinic acid synthetase（ALAS）之活性，及減少血紅素之合成。由於根瘤血紅素濃度之減低，固氮酶活性因而減少。硝酸銨雖然對 ALAS 活性以及血紅素含量有影響，但是對血紅素電泳圖樣則沒有改變之現象。