BIOLOGIA PLANTARUM 37 (1): 57-64, 1995

Effect of form and level of applied nitrogen on nitrogenase and nitrate reductase activities in faba beans

A.M. ABDEL WAHAB and M.H. ABD-ALLA

Department of Botany, Faculty of Science, Assiut University, 71516, Assiut, Egypt

Abstract

The effects of nitrogen applied at increasing levels of 0, 4, 8, 16 and 32 mM N (KNO_3 or NH_4Cl) were studied in faba bean (*Vicia faba*) nodulated by *Rhizobium leguminosarum* bv. *viceae* RCR lool. Nitrogenase activity was higher at 4 and 8 mM N than the zero N treatment (control), but 16 and 32 mM N significantly reduced the efficiency of nodule functions. Nitrate reductase activities (NRA) of leaves, stems, roots, nodules and nodule fractions (bacteroid and cytosol) were increased with rising the NO_3^- or NH_4^+ levels. NRA decreased in the order of nodules > leaves > stems > roots. Cytosolic NR was markedly higher than that recorded in the bacteroid fractions. Nitrate levels were linearly correlated to NRA of nodules. Accumulation of NO_2^- within nodules suggests that NO_2^- inhibits nodule's activity after feeding plants with NO_3^- or NH_4^+.

Key words: ammonium, nitrate, nodulation, *Rhizobium leguminosarum*, *Vicia faba*

Introduction

Fertilization by different forms of nitrogen is inhibitory to nearly all stages of the *Rhizobium*- and *Bradyrhizobium*-legume symbiosis (Streeter 1988). Both NH_4^+ and NO_3^- exhibit adverse effects on the legume symbioses, but NO_3^- is generally more inhibitory than NH_4^+ to both nodulation and nodule functions (Rys and Phung 1984, Imsand 1986). Despite the abundance of studies on the inhibitory effects of NO_3^- on N_2 fixation (Wasfi and Prioul 1986, Silsbury *et al.* 1986, Drevon *et al.* 1988), the mechanism(s) implicated in the inhibition of nodule functions are not well understood. Moreover, it is often difficult to differentiate the respective roles of the legume host and the rhizobial isolate in the inhibition process.

The addition of NO_3^- to nodulated legumes induces the activity of nitrate reductase (NRA), the first enzymatic step in the assimilatory reduction of nitrate. Nitrite, the first product of NO_3^- reduction, has been investigated as a potential inhibitor of N_2 fixation since it inhibits nitrogenase activity when added to the

Received 20 July 1994, *accepted* 19 October 1994.

Abbreviations: ARA - acetylene-reducing activity; DAP - days after planting; N_2ase - nitrogenase, NA - nitrogenase activity; NR - nitrate reductase; NRA - nitrate reductase activity.

purified enzyme, bacteroids *in vitro* or when supplied to intact or detached nodules (Kamberger 1977, Chalifour and Nelson 1987). The expression of NRA in nodule bacteroids depends on the specific host-isolate combination and leads to NO_2^- accumulation in nodules of legumes supplied with NO_3^- (Streeter 1985, Becana *et al.* 1985). An inverse relationship between nitrate accumulation in nodules and nitrogenase activity has been reported in soybean (Wasfi and Prioul 1986). While nitrite is considered to have direct effects on nitrogenase activity, the reduction of NO_3^- in various sites of the symbiotic system may indirectly inhibit N_2 fixation by competing with nitrogenase for reductants and carbon derived from photosynthesis (Houwaard 1980).

Both legumes and rhizobia show variation in their tolerance to nitrogen compounds (Gibson and Harper 1985). Chalifour and Nelson (1988) showed that faba bean was consistently more tolerant to the nitrogen fertilizer than pea when both plants were inoculated with different *R. leguminosarum* bv. *viceae* strains.

Our experiments were designed to study the effect of NO_3^- and NH_4^+ application on nitrogenase activity, NRA, nitrite accumulation in nodules and their fractions in faba bean with established symbiosis.

Materials and methods

Plant cultivation: Surface sterilized faba bean (*Vicia faba* L. cv. Giza 3) seeds were inoculated with *Rhizobium leguminosarum* bv. *viceae* RCR lool (from Rothamsted Experimental Station, Harpenden, UK) and planted into plastic pots containing autoclaved sand soil. Plants were grown in a greenhouse (24 ± 4 °C, natural day light). Five levels of nitrogen fertilization (NH_4Cl or KNO_3) of 0, 4, 8, 16 and 32 mM N were applied at 19 d after planting (DAP). These represent the actual N concentrations in soil extract after N additions.

Nitrogenase activity: Nitrogenase activity (NA) was determined on a detached root system, using the acetylene reduction assay (Hardy *et al.* 1968). Assays were conducted in closed system (Wu and Harper 1990, Abd-Alla 1992). Roots were cut-off at cotyledonary nodes, gently shaken to remove soil particles, immediately placed in 556 cm^3 bottles and sealed with a rubber septum. 55 cm^3 of acetylene were injected into the bottles which were then incubated at 28 °C for 1 h. The reaction was terminated using HCl (6 M). A 0.5 cm^3 gas samples for each bottle was injected into a *Pye Unicam 104 FID Gas Chromatograph* with 122 cm glass column packed with activated alumina at 150 °C. Afterwards nodules of each individual root were weighed and NA was expressed in [nmol C_2H_4 g^{-1}(nodule f.m.) s^{-1}].

Preparation of bacteroids and cytosol for enzyme assay: For the extraction of bacteroids, 1 g of nodules was rinsed thoroughly with deionized water and ground in an ice-chilled mortar with 8 cm^3 of an extraction buffer consisting of 100 mM phosphate buffer, pH 7.5, 200 mM Na ascorbate, and 2 g polyvinylpyrrolidone-10. The homogenate was filtered through four layers of cheese cloth and the filtrate was centrifuged at 12 000 *g* for 15 min to sediment the bacteroids. The supernatant is

referred to as nodule cytosol. Bacteroids were disrupted in 5 cm^3 of the same buffer with an *M.S.E. Ultrasonicator* at 0 - 4 °C for 8 min. The soluble fraction was obtained from the supernatant following centrifugation at 20 000 *g* for 20 min.

NRA of bacteroids was assayed by measuring the amount of nitrite formed with methyl viologen as electron donor (Kennedy *et al.* 1975). In a total volume of 2 cm^3, the reaction medium contained 50 mM potassium phosphate buffer, 20 mM KNO_3, 0.2 cm^3 extract and 0.2 cm^3 sodium dithionite (5.75 mg per cm^3 of distilled water). After incubation for 60 min at 30 °C, the reaction was terminated by vigorous shaking until complete oxidation of the electron donor system.

NRA of cytosol was determined as reported by Streeter (1982). The reaction mixture, in a total volume of 2 cm^3 comprised 0.2 mM K-phosphate buffer, pH 7.5, 10 mM KNO_3, 0.75 μM NADH, and 0.3 cm^3 cytosol. Boiled cytosol and bacteroids served as control. After incubation of mixtures for 1 h at 30 °C, nitrite formation was determined spectrophotometrically at 540 nm after addition of 2 cm^3 Griess-Ilosvay reagent. NRA of bacteroids and cytosol was expressed in [nmol NO_2^- g^{-1}(nodule f.m.) s^{-1}].

Measurements of *in vivo* NRA: Whole nodules, or small pieces of leaves, stems and roots were put in glass tubes with 5 cm^3 of the mixture assay (100 mM phosphate buffer, pH 7.5, 100 mM KNO_3). Assays were run in darkness by wrapping the tube in an aluminum foil and incubated aerobically in water bath at 27 °C for 1 h. After the incubation period, samples were put in a bath of boiling water for 1 min to terminate the reaction. 1 cm^3 of each sample was add to 2 cm^3 of Griess-Ilosvay reagent and nitrite concentrations were determined as described above.

Nitrite contents of the bacteroid soluble fractions and cytosol were analysed after diazotation by adding 2 cm^3 of Griess-Ilosvay reagent to 1 cm^3 of samples. Data were expressed in [mg NO_2^- g^{-1} (nodule f.m.)].

Results

Acetylene-reducing activity (ARA) of pre-existing nodules throughout seven harvests of faba bean plants (Figs. 1 A,C) was markedly stimulated by 4 and 8 mM N. Maximum NA was achieved at the second and fourth week (14 - 28 d) after treatment (33 - 47 DAP). The application of 16 and 32 mM N, however, significantly suppressed specific NA.

Statistical analysis (Tables 1 and 2) showed that both N sources had similar effect, nevertheless KNO_3 had a more depressive effect on ARA.

Since N_2ase and NR are both involved in the assimilation of nitrogen and both enzymes compete for reducing power (photosynthates), it was thus necessary to study the effect of KNO_3 and NH_4Cl on NRA in different organs as well as in nodules and their fractions. NRA was, therefore, detected in whole nodules (Figs. 1 C,D) and also in nodule fractions (Tables 1 and 2).

NRA in nodules increased in nearly to a linear fashion with increase of mineral N levels (Figs. 1. B,D) to reach a maximum at 16 mM N.

The highest levels of NRA were detected in nodules. NRA was then recorded in the decreasing order of leaves > stems > roots. NRA of all organs differed significantly with the different levels of the same nitrogen form. The activity of this enzyme also showed significant variations between KNO_3 and NH_4Cl. Higher NRA was recorded at the early sampling date (Table 1 and Figs. 1 C,D); it declined gradually as plants approached maturity but it reached a maximum in plants at the fullbloom to mid-pod stage (Fig. 1 C,D). NRA of plant tissue (each organ) showed a transient increase 14 d after addition of N fertilizer.

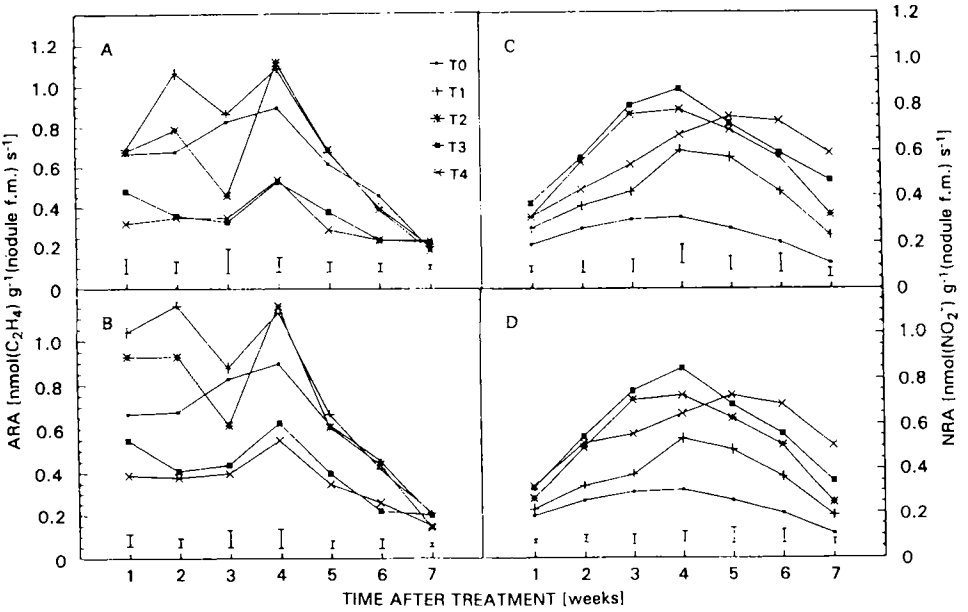

Fig. 1. Effect of different concentrations (0, 4, 8, 16, 32 mM) of KNO_3 [A,C) or NH_4Cl [B,D] on specific nitrogenase (A,B) and nitrate reductase [C,D] activites of nodules of *Vicia faba* cv. Giza 3. Bars indicate LSD ($P = 0.05$) for comparsion between all levels of nitrogen at each recording date.

NRA of nodule cytosol were higher than those estimated in the bacteroid fractions. Further NRA in cytosol increased linearly and substantially with the applied N levels, whereas NRA in the bacteroid fractions increased only to a certain extent (10 % of cytosol).

Nodule content of NO_2^- consistently increased in response to KNO_3 and NH_4Cl supply in both fractions of nodules. However, the levels of nitrite accumulating in the cytosol were much higher than those found in the bacteroids (Tables 1 and 2).

Discussion

Results presented in this report confirm many of the previous observations that the judicious application of N fertilizer enhanced NA of established nodules. Thus the application of low or moderate levels of either KNO_3 or NH_4Cl (Fig. 1 A,B) have

promoted NA. NA of the cultivar used was considerably inhibited at higher doses of N at all stages of plant growth (Tables 1 and 2). This result is supported by the

Table 1. Effect of nitrogen form and level on nitrogenase activity [nmol C_2H_2 g^{-1}(nodule f.m.) s^{-1}], nitrate reductase activity [nmol NO_2^- g^{-1}(f.m.) s^{-1}] and nitrite contents [μg g^{-1}(f.m.)] of nodules or plant organs of *Vicia faba* cv. Giza 3 harvested 28 d after treatments. Each value represents the mean of three replicates.

Nitrogen level [mM]	Nitrogenase activity	Nitrate reductase activity *in vivo*				*in vitro*		Nitrite content	
		leaves	stems	roots	nodules	cytosol	bacteroids	cytosol	bacteroids
Control	0.90b	0.19e	0.10c	0.19c	0.31d	0.04e	0.01c	0.65e	0.15d
KNO_3									
4	1.09a	0.50c	0.30b	0.22bc	0.54c	0.54d	0.05b	1.23d	0.60d
8	1.14a	0.63b	0.32a	0.25b	0.78a	0.78a	0.06ab	2.84d	1.43c
16	0.53c	0.77a	0.34a	0.32a	0.87a	0.82d	0.08a	5.19b	2.72b
32	0.54c	0.55b	0.31a	0.32a	0.67b	0.89a	0.08a	7.08a	3.19a
NH_4Cl									
4	1.13a	0.42d	0.29b	0.20bc	0.54c	0.41c	0.04b	0.86e	0.47d
8	1.16a	0.56b	0.30b	0.23b	0.73b	0.78b	0.05b	1.99d	1.21c
16	0.63b	0.73a	0.32a	0.29a	0.85a	0.66b	0.07a	4.33c	1.45c
32	0.55c	0.52c	0.32a	0.32a	0.65b	0.69b	0.08a	4.96c	1.85c

Table 2. Effect of nitrogen form and level on nitrogenase activity [nmol C_2H_2 g^{-1}(nodule f.m.) s^{-1}], nitrate reductase activity [nmol NO_2^- g^{-1}(f.m.) s^{-1}] and nitrite contents [μg g^{-1}(f.m.)] of nodules or plant organs of *Vicia faba* cv. Giza 3 harvested 42 d after treatments. Each value represents the mean of three replicates.

Nitrogen level [mM]	Nitrogenase activity	Nitrate reductase activity *in vivo*				*in vitro*		Nitrite content	
		leaves	stems	roots	nodules	cytosol	bacteroids	cytosol	bacteroids
Control	0.46a	0.06c	0.05d	0.10d	0.20d	0.02c	0.01c	0.40d	0.08e
KNO_3									
4	0.40a	0.29b	0.18c	0.22bc	0.42c	0.22b	0.02bc	0.51d	0.19d
8	0.33a	0.35ab	0.22b	0.14c	0.57b	0.50a	0.03b	1.25c	0.56c
16	0.24b	0.33ab	0.26b	0.16c	0.59b	0.53a	0.03b	5.41a	1.85a
32	0.24b	0.51a	0.32a	0.32a	0.73a	0.53a	0.05a	6.70a	1.98a
NH_4Cl									
4	0.42a	0.21b	0.14c	0.11d	0.37c	0.03c	0.01c	0.65d	0.12d
8	0.44a	0.26b	0.20c	0.13c	0.51b	0.33ab	0.02bc	1.20c	0.42c
16	0.22b	0.32b	0.23b	0.19b	0.56b	0.33ab	0.03b	4.44b	0.68b
32	0.26b	0.44a	0.31a	0.31a	0.69a	0.34ab	0.03b	5.51a	0.82b

Values within the same column followed by the same letter are not significantly different at the 5 % level by Duncan's multiple range test.

findings of Jessop *et al.* (1984) and Eskew *et al.* (1989). These authors reported that the application of increasing levels of NO_3^- substantially repressed nodulation and

symbiotic N_2 fixation by *Cicer arietinum* and nitrate tolerant mutants of *Glycine max*. Several other publications (Nelson 1987, Chalifour and Nelson 1988, Herdina and Silsbury 1989) reported similar results.

The mechanisms responsible for the inhibition of symbiotic N_2 fixation are not well understood but a number of hypotheses have been proposed involving direct and indirect effects of NO_3^- metabolism (Silsbury *et al.* 1986, Becana and Sprent 1987, Drevon *et al.* 1988, Streeter 1988, Heckmann *et al.* 1989). One of these known as carbohydrate deprivation theory states that N_2ase function is impaired through the diversion of photosynthetically derived energy and reductants from nodules to the sites of NO_3^- assimilation in the plant (Wasfi and Prioul 1986, Nelson and Edie 1991, Jensen 1986).

Because N_2ase and NR are both involved in the assimilation of N, the competition of these two enzymes for reducing power is well established by data in Fig. 1. The overall pattern of decline in N_2ase with the concomitant high potential of NR is in favour of the carbohydrate deprivation theory. The inverse relationship (low ARA-high NRA) observed here is supported by several recent publications (Williams *et al.* 1988, Neyra and Stephens 1985 and Vessey *et al.* 1988).

In this investigation, emphasis was given to nitrate metabolism in shoots, roots and nodules, with a major consideration of the nodule cytosolic NR or bacteroids. Data obtained here (Tables 1 and 2) indicate that the highest NRA was found in nodules followed by leaves, stems and roots.

Eskew *et al.* (1989) reported that NRA is much greater in roots than shoots of four soybean genotypes. They suggested that the greater tolerance of soybean to N addition is related to higher NRA in roots although there was insufficient evidence to conclude this relationship. In contrast to our results, Hervas *et al.* (1991) found that NRA in different organs of peas was in the order of stems > roots > leaves. These discrepancies in NRA of different organs might be linked to differences in the assays used by different authors to measure NRA, *i.e.* to the use of *in vivo* as opposed to *in vitro* assays (Chalifour and Nelson 1988) although NRA determined *in vivo* and *in vitro* is generally correlated (Timpo and Neyra 1983). Differences in NRA has also been attributed to plant age, cultivar differences and to variations in growth conditions (Andrews *et al.* 1984). Hervas *et al.* (1991) ascribed the expression of NRA in different organs of pea principally to NO_3^- treatments (concentration in the growth medium), to the nodulation status of the legume plant and to the *Rhizobium* strain. However, the physiological mechanism remains to be established.

The NRA measured in nodule extract supernatant, cytosol, was markedly higher (10-fold) compared to the bacterial NRA (Tables 1 and 2). This results is in a good agreement with Heckmann and Drevon (1987) and Chalifour and Nelson (1988).

The accumulation of NO_2^- in both bactereoids and cytosol as observed in the present study (Tables 1 and 2) depends on the relative rates of NO_3^- reduction. The accumulation of NO_2^- would be mostly related to the cytosolic nitrate reduction. These findings would explain the increase in nodule level of NO_2^- with increasing NO_3^- application.

Such an accumulation of nitrites could directly cause supression or inhibition of NA since NO_2^- is a potential inhibitor not only to NA but also to the synthesis of this

enzyme. Main arguments in support of the hypothesis that NO_2^- is inhibitory metabolite of NA are: (*a*) NO_2^- strongly depresses the activity of purified N_2ase (Trinchant and Rigaud 1980) as well as the N_2-fixing activity of crude bacteroids or isolated bacteroids (Trinchant and Rigaud 1981) and intact nodules (Kamberger 1977). This was explained by the capacity of NO_2^- to bind the Fe-Mo component of N_2-ase reversibly, thus by inhibiting N_2ase competitively (Trinchant and Rigaud 1989). (*b*) The second possibility is that NO_2^- accumulated in the nodules combines reversibly with the oxidized form of leghaemoglobin. Thus indirectly inhibiting N_2ase (Trinchant and Rigaud 1980), such suggestions will be examined in our forthcoming publication.

In conclusion, our findings support the idea that one of the ways to avoid inhibitory effects of N fertilization on N_2 fixation is by limitation of nitrate reduction. This could be achieved by the selection of plant cultivars tolerant to NO_3^- (Liuch *et al.* 1988, Gremaud and Harper 1989) or by inoculating rhizobial symbionts lacking NR (Liuch *et al.* 1989).

References

Abd-Alla, M.H.: Nodulation and nitrogen fixation in faba bean (*Vicia faba* L.) plants under salt stress. - Symbiosis **12**: 311-319, 1992.

Andrews, M.A., Sutherland, J.M., Thomas, R.J., Sprent, J.I.: Distribution of nitrate reductase activity in six legumes: The importance of the stem. - New Phytol. **98**: 301-310, 1984.

Becana, M., Sprent, J.I.: Nitrogen fixation and nitrate reduction in the root nodules of the legumes. - Physiol. Plant. **70**: 757-765, 1987.

Becana, H., Aparicio-Tejo, P.M., Sanchez-Diazo, M.: Nitrate and nitrite reduction in the plant fraction of alfalfa root nodules. - Physiol. Plant. **65**:185-188, 1985.

Chalifour, F.P., Nelson, L.M.: Effects of continuous combined nitrogen supply on symbiotic dinitrogen fixation of faba bean and pea inoculated with different rhizobial isolates. - Can. J. Bot. **65**: 2542-2548, 1987.

Chalifour, F.P., Nelson, L.M.: Effect of time of nitrate application on nitrate reductase activity, nitrite uptake and symbiotic dinitrogen fixation in faba bean and pea. - Can. J. Bot. **66**:1646-1652, 1988.

Drevon, J.J., Heckmann, M.O., Soussana, J.F., Salsac, L.: Inhibition of nitrogen fixation by nitrate assimilation in legume-*Rhizobium* symbiosis. - Plant Physiol. Biochem. **154**: 1403-1413, 1988.

Eskew, D.L., Kapuya, J., Danso, S.K.A.: Nitrate inhibition of nodulation and nitrogen fixation by supernodulating nitrate-tolerant symbiosis mutants of soybean. - Crop Sci. **29**:1491-1496, 1989.

Gibson, A.H., Harper, J.E.: Nitrate effect on nodulation of soybean by *Bradyrhizobium japonicum*. - Crop Sci. **25**: 497-501, 1985.

Gremaud, M.F., Harper, J.E.: Selection and initial characterization of partially nitrate tolerant nodulating mutation of soybean. - Plant Physiol. **89**: 169-173, 1989.

Heckmann, M.O., Drevon, J.J.: Nitrate metabolism in soybean root nodules. - Physiol. Plant. **69**: 721-725, 1987.

Heckmann, M.O., Drevon, J.J., Saglia, P., Salsac, L.: Effect of oxygen and malate on NO_3^- inhibition of nitrogenase in soybean nodules. - Plant Physiol. **90**: 224-229, 1989.

Herdina, Silsbury, J.H.: Nodulation and early growth of faba bean (*Vicia faba* L.) and pea (*Pisum sativum* L.) as affected by strain of *Rhizobium*, NO_3^- supply and growth temperature. - Aust. J. agr. Res. **40**: 991-1000, 1989.

Hervas, A., Ligera, F., Liuch, C.: Nitrate reduction in pea plants: Effects of nitrate application and *Rhizobium* strains. - Soil Biol. Biochem. **23**: 695-699, 1991.

Houwaard, F.: Influence of ammonia and nitrate nitrogen on nitrogenase activity of pea plants as

affected by light intensity and sugar addition. - Plant Soil **54**: 271-273, 1980.

Imsande, J.: Inhibition of nodule development in soybean by nitrate or reduced nitrogen. - J. exp. Bot. **37**: 348-355, 1988.

Jensen, E.S.: The influence of rate and time of nitrate supply on nitrogen fixation and yield in pea (*Pisum sativum* L.). - Fert. Res. **10**: 193-202, 1986.

Jessop, R.S., Hetheringtan, S.J., Hault, R.H.: The effect of soil nitrate on growth, nodulation and nitrogen fixation of chickpeas (*Circer arietinum*). - Plant Soil **82**: 205-214, 1984.

Kamberger, W.: Regulation of symbiotic nitrogen fixation in root nodules of alfalfa (*Medicago sativa*) infected with *Rhizobium meliloti*. - Arch. Microbiol. **115**: 103-107, 1977.

Liuch, C., Caba, J.M., Hervas, A., Ligero, F.: Effect of nitrate and cultivar genotype on nitrogen metabolism in nodules of *Vicia faba minor* L. - In: Bothe, H., de Bruijn., F.J., Newton, W.E. (ed.): Nitrogen Fixation: Hundred Years After. P. 572. Gustav Fischer, Stuttgart - New York 1988.

Liuch, C., Caba, J.M., Perez, M.C., Ligero, F.: *Rhizobium* strain, cultivar and short-term nitrate applications effects on the enzymes involved in nitrogen assimilation in root nodules of *P. sativum*. - In: Advanced Course on Inorganic Nitrogen Metabolism. P. 151. Napoli 1989.

Nelson, L.M.: Variation in *Rhizobium leguminosarum* response to short term application of NH_4NO_3 to nodulated *Pisum sativum* L. - Plant Soil **98**: 275-284, 1987.

Nelson, L.M., Edie, S.A.: Nodule carbohydrate composition and nitrogen fixation in pea (*Pisum sativum* L.); Effect of *Rhizobium* strain and NH_4NO_3. - Soil Biol. Biochem. **23**: 681-688, 1991.

Neyra, C.A., Stephens, B.D.: Interactions between nitrate reduction and nitrogen in grain legumes. - In: Harper, J.E., Schrader, L.E., Howell, R.W. (ed.): Exploitation of Physiological and Genetic Variability to Enhance Crop Productivity. Pp. 12-22. American Society of Plant Physiologists, Rockwille 1985.

Rys, G.J., Phung, T.: Effect of nitrogen form and counterion on establishment of *Rhizobium trifolii-Trifolium repens* symbiosis. - J. exp. Bot. **35**: 1811-1819, 1989.

Silsbury, J.H., Catchpoole, D.W., Wallace, W.: Effect of nitrate and ammonium on nitrogenase (C_2H_2 reduction) activity of subterranean clover, *Trifolium subterraneum* L. - Aust. J. Plant Physiol. **13**: 257-173, 1986.

Streeter, J.G.: Synthesis and accumulation of nitrite in soybean nodules supplied with nitrate. - Plant Physiol. **64**: 1429-1434, 1982.

Streeter, J.G.: Nitrate inhibition of legume growth and activity. II. Short term with high nitrate supply. - Plant Physiol. 77: 325-328, 1985.

Streeter, J.G.: Inhibition of legume nodule formation and N_2 fixation by nitrate. - CRC crit. Rev. Plant Sci. 7: 1-32, 1988.

Timpo, E.E., Neyra, C.A.: Expression of nitrate and nitrite reductase activities under various forms of nitrogen nutrition in *Phaseolus vulgaris* L. - Plant Physiol. 72: 71-75, 1983.

Trinchant, J.C., Rigaud, J.: Nitrate inhibition of nitrogenase from soybean bacteroids. - Arch. Microbiol. **124**: 49-54, 1980.

Trinchant, J.C., Rigaud, J.: Acetylene reduction and respiration of bacteroid isolated from French beans receiving nitrate. - Physiol. Plant. **53**: 511-517, 1981.

Trinchant, J.C., Rigaud, J. : Nitrogen fixation in presence of nitrate: Effect on bacteroid respiration and comparison with nitrate. - J. Plant Physiol. **116**: 209-214, 1984.

Vessey, J.K., Walsh, K.B., Layzell, D.B.: Can a limitation in phloem supply to nodules account for the inhibitory effect of nitrate on nitrogenase activity in soybean. - Physiol. Plant. **74**: 137-146, 1988.

Wasfi, M., Prioul, J.-L.: A comparison of inhibition of French-bean and soybean nitrogen fixation by nitrate, 1 % oxygen or direct assimilate deprivation. - Physiol. Plant. **66**: 481-490, 1986.

Williams, W.M., Hoh, C.H., Lenz, F., Broughton, W.J.: Rhizobia in tropical legumes: environmental factors and the reduction of nitrogen. - Soil Biol. Biochem. **20**: 667-675, 1988.

Wu, S., Harper, J.E.: Nitrogen fixation of nodulation mutants of soybean as affected by nitrate. - Plant Physiol. **92**: 1142-1147, 1990.