Zeitschrift: Botanica Helvetica
Herausgeber: Schweizerische Botanische Gesellschaft
Band: 93 (1983)
Heft: 1

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DOI: https://doi.org/10.5169/seals-65234

Botanica Helvetica *93:* 57-65 (1983)

Nitrate uptake by intact, non-nodulated bean plants: Effect of ammonium and of light.*

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Manuscript received January 22, 1983

Abstract

Dicht, M., and U. Feller. 1983. Nitrate uptake by intact, non-nodulated bean plants: Effect of ammonium and of light. Botanica Helvetica *93:* 57-65. Dual-phase kinetics were found for nitrate uptake at pH 5.5 by intact, non-nodulated bean plants. Apparent K_m values for phase I (higher affinity for nitrate) were in the range between 0.2 and 0.4 mM and depended on the nitrogen nutrition of the plants prior to the experiment. Ammonium added to the medium at the same concentration as nitrate had no effect on nitrate uptake rates from nutrient solutions containing 0.1-1.5 mM nitrate. Uptake rates for nitrate were considerably lower in plants preincubated on higher nitrate or ammonium concentrations than used for the experiment, compared to those preincubated on the same medium as used during the experiment. No effect of light on nitrate uptake from solutions containig 0.1-1.5 mM nitrate was observed, although the transpiration rates were about 70% lower in darkness than in light. Nitrate uptake rates from solutions containing more than 2 mM nitrate were slightly lower in darkness than in light.

Introduction

Nitrate and ammonium are major nitrogen sources for plants. Several aquatic plants strongly prefer ammonium, when both ions are present in the medium (Morris and Syrett 1963, Ferguson and Bollard 1969, Feller and Erismann 1971, Tischner and Lorenzen 1981). The nitrate content in *Lemna minor* increases immediately after transfer from ammonium to nitrate medium, but nitrate utilization starts after an initial lag (Kopp et al. 1974). Therefore, it appears likely that in these plants nitrate assimilation rather than its uptake into the cells is affected by ammonium or its assimilation products. In crop plants nitrate and ammonium are often utilized simultaneously (Bloom and Chapin 1981, MacKown et al. 1982). Interferences with the long distance transport should be considered in uptake studies with intact crop plants (Breteler 1977, Huffaker and Rains 1978). Nitrate can serve as a major transport compound in the xylem of bean plants, while ammonium is always present at very low concentrations (Thomas et al. 1979).

*Dedicated to Prof. Dr. K.H. Erismann (University of Bern) in honor of his 60th birthday.

These results indicate that nitrate can either be assimilated in the roots or transported without reduction to the shoot, but ammonium taken up from the medium is assimilated in the roots.

Dual-phase kinetics have been described for nitrate uptake by several plants (Rao and Rains 1976, Doddema and Telkamp 1979). The K_m values for different plants vary widely (Breteler 1977, Doddema 1978). For phase I (higher affinity for nitrate) the apparent K_m was always below 1 mM. Lowest K_m values were determined in short-term experiments with N-starved plants. The nitrogen nutrition of the plants (Jackson et al. 1976, Doddema and Otten 1979, Ullrich et al. 1981) and the carbohydrate status of the roots (Hänisch ten Cate and Breteler 1981) affect uptake and assimilation of nitrogen compounds during the experiment.

The objectives of this work were to investigate nitrate uptake by intact been plants and to examine possible effects of light and of the N-source prior to and during the experiment.

Materials and Methods

Bean seeds (*Phaseolus vulgaris* L., var. «Saxa», Stamm Vatter) were soaked and aerated in tap water for 12 h (day 1) and germinated as described by Thomas et al. (1979). After 6 d in darkness the plants were transferred to a 14 h light/10 h dark cycle as reported earlier (Thomas et al. 1979). Experiments were started 2 h (Fig. 1, 2, 3) or 3 h (Tab. 2) after the beginning of the light phase.

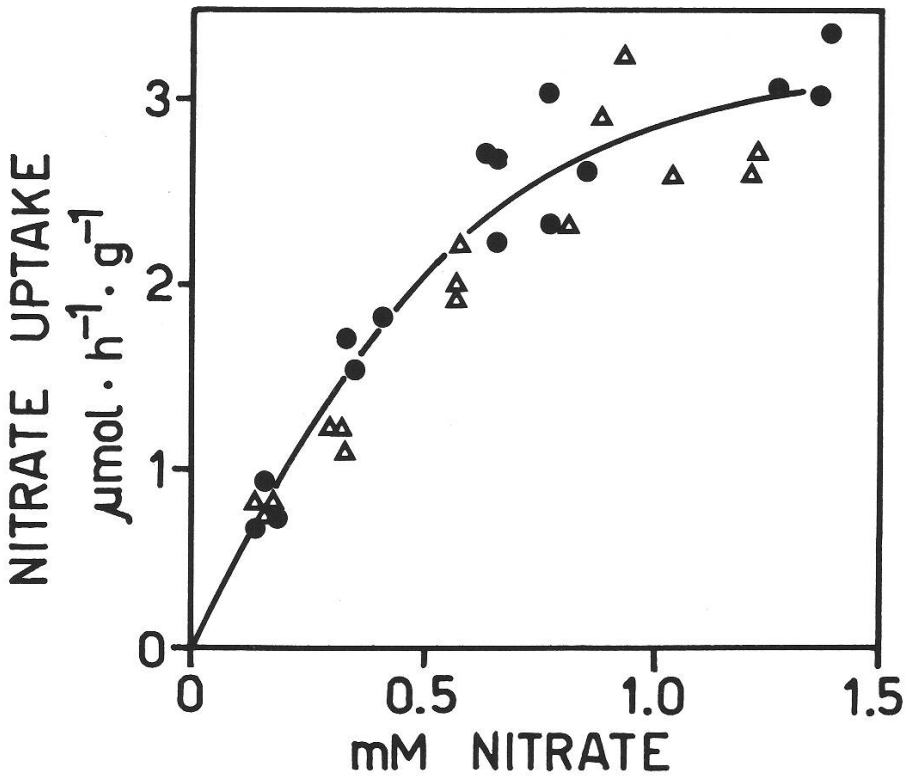
All nutrient solutions contained micronutrients in the following concentrations: 144 μM Fe(III) Na-EDTA, 1 μM $MnCl_2$, 0.17 μM $ZnSO_4$, 0.11 μM $CuSO_4$, 5 μM H_3BO_3 and 0.2 μM MoO_3. Nutrient solution with 3.5 mM NH_4^+ was prepared according to Thomas et al. (1979). The macronutrients in N-free medium and nutrient solutions with different nitrate concentrations are shown in Tab. 1. It was attempted to maintain the cation concentrations as constant as possible and, therefore, sulfate and phosphate concentrations were lowered with increasing nitrate concentration. However, increasing potassium and calcium concentrations were unavoidable for nitrate levels above 15 mM. Ammonium nitrate medium was prepared from N-free (0 mM nitrate) nutrient solution by addition of NH_4NO_3. All experiments were performed at pH 5.5 The plants were grown on 3.5 mM nitrate and then transferred to the preincubation solutions as indicated (60-84 h prior to the experiment). The preincubation solution was replaced daily. The temperature of the solutions was 20-21 °C.

Uptake experiments were performed in brown plasic pots containing 100 ml (up to 2 mM nitrate) or 50 ml (above 2 mM nitrate) nutrient medium. The solutions were permanetly aerated. Transpiration was measured gravimetrically. The values were corrected for evaporation measured in pots without plants. The plants were transferred to the experiment nutrient solution at the beginning of the light phase and the uptake measurements started 2 or 3 h later.Nitrate (Feller et al. 1971) and ammonium (Bohley 1967) were measured colorimetrically. For kinetic studies the nitrate content per pot was determined repeatedly up to 6 or 7 h taking into account the volume reduction by transpiration, evaporation and sampling. Cumulative nitrate uptake was essentially linear. The actual nitrate concentration in the middle of the experiment was used for kinetic plots. K_m and $V_{max.}$ values were determined by linear regressions in the Hanes-Woolf plot (Segel 1975).

Results

Nitrate uptake in the range 0.1-1.5 mM external nitrate was investigated in the presence or absence of ammonium (Fig 1). No major effect of ammonium on nitrate utilization by intact bean plants was observed, when ammonium and nitrate were added

Fig. 1 Effect of ammonium on nitrate uptake by 13 d old bean plants. The plants were preincubated for 60 h on the same nutrient solution as used for the experiment. The symbols represent results obtained with individual plants and uptake rates are expressed per g fresh weight of the whole plant (root + shoot). Nitrate was added to the nutrient solutions as KNO_3 (△; nitrate medium without ammonium)or as NH_4NO_3 (●; medium with nitrate and ammonium in equimolar concentrations).



to the medium in equimolar concentrations. These results indicate that net nitrate uptake was not affected by ammonium under the conditions used.

The type of nitrogen nutrition of the plants prior to the experiment strongly affected nitrate uptake. Net uptake rates in the range 0.1-1.5 mM external nitrate were considerably lower in plants peincubated in 3.5 mM nitrate than in those preincubated on the same nutrient solution as used for the experiment (Fig. 2). K_m and $V_{max.}$ values were affected by the pretreatment. The importance of prior nitrogen nutrition for nitrate uptake experiments becomes evident from these results.

Further combinations of N-sources during preincubation and experiment were investigated and some of these results are presented in Tab. 2 Nitrate uptake rates remained always relatively constant throughout the experiment, but ammonium uptake rates were often initially low and increased during the experiment (data not shown). The data presented in Tab.2 refer to the total amount of nitrogen taken up during the 7 h period. Nitrate uptake was highest when nitrate was present in the preincubation medium in equal or lower concentrations than those used for the experiment. Net nitrate uptake markedly decreased after transition from higher to lower nitrate concentrations. Ammonium present in the preincubation medium at the same concentration as nitrate had little effect on nitrate uptake during the experiment, but extremely low uptake rates for nitrate were caused by the transition from 3.5 mM ammonium to 1.0 mM ammonium

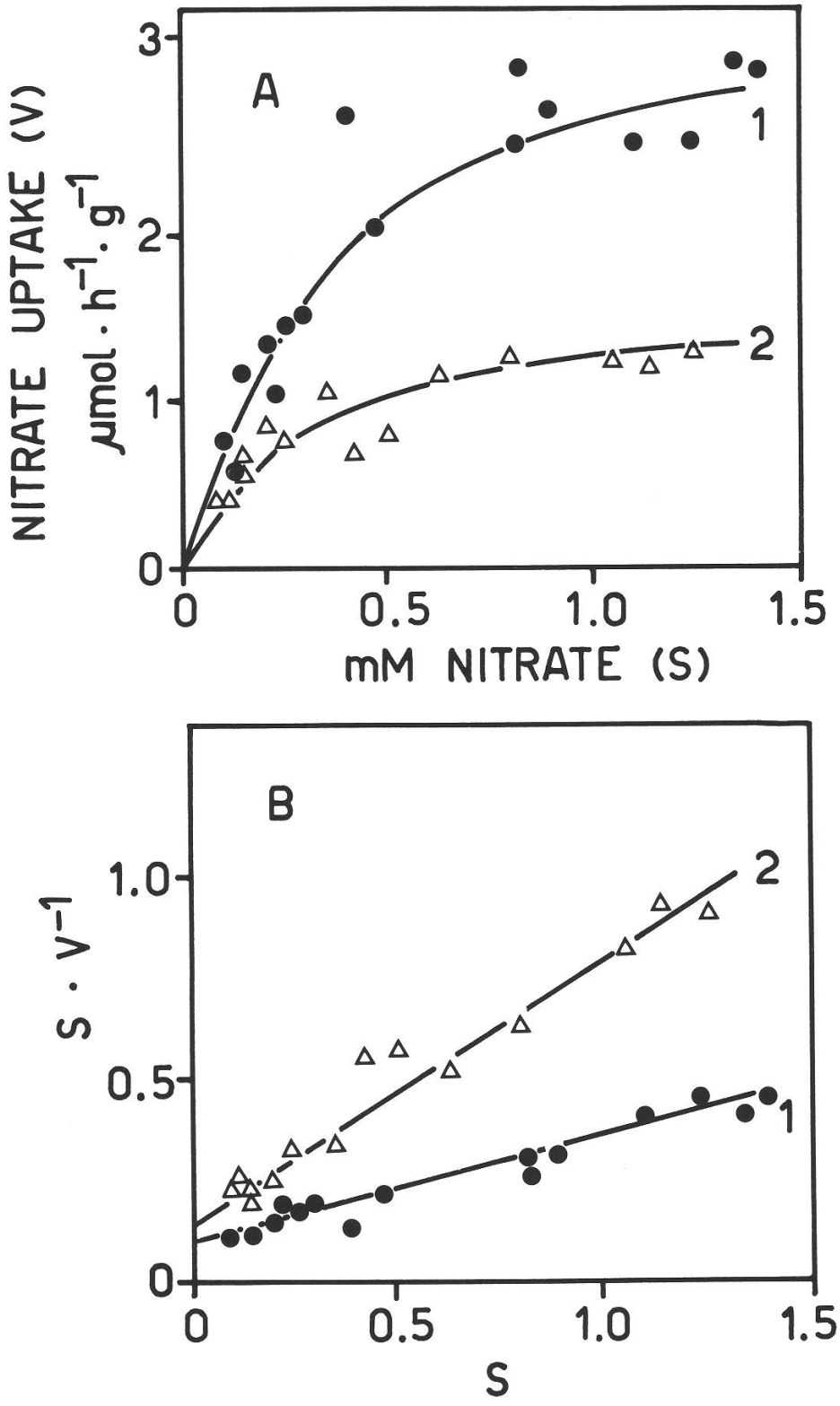


Fig. 2 Effect of the nitrogen nutrition status on nitrate uptake kinetics. The plants were preincubated for 72 h on the same ammonium nitrate medium as used for the experiment (curve 1) or on 3.5 mM nitrate solution (curve 2). The nutrient solutions for the uptake experiments contained ammonium nitrate. The symbols represent results obtained with individual 12 d old plants and uptake rates are expressed per g fresh weight of the whole plant (root + shoot). A: uptake rate versus external nitrate concentration. B: Hanes-Woolf plot; apparent K_m values were 0.39 mM (curve 1) and 0.23 mM (curve 2) and the $V_{max.}$ values in µmol per hour per g fresh weight were 3.9 (curve 1) and 1.54 (curve 2).

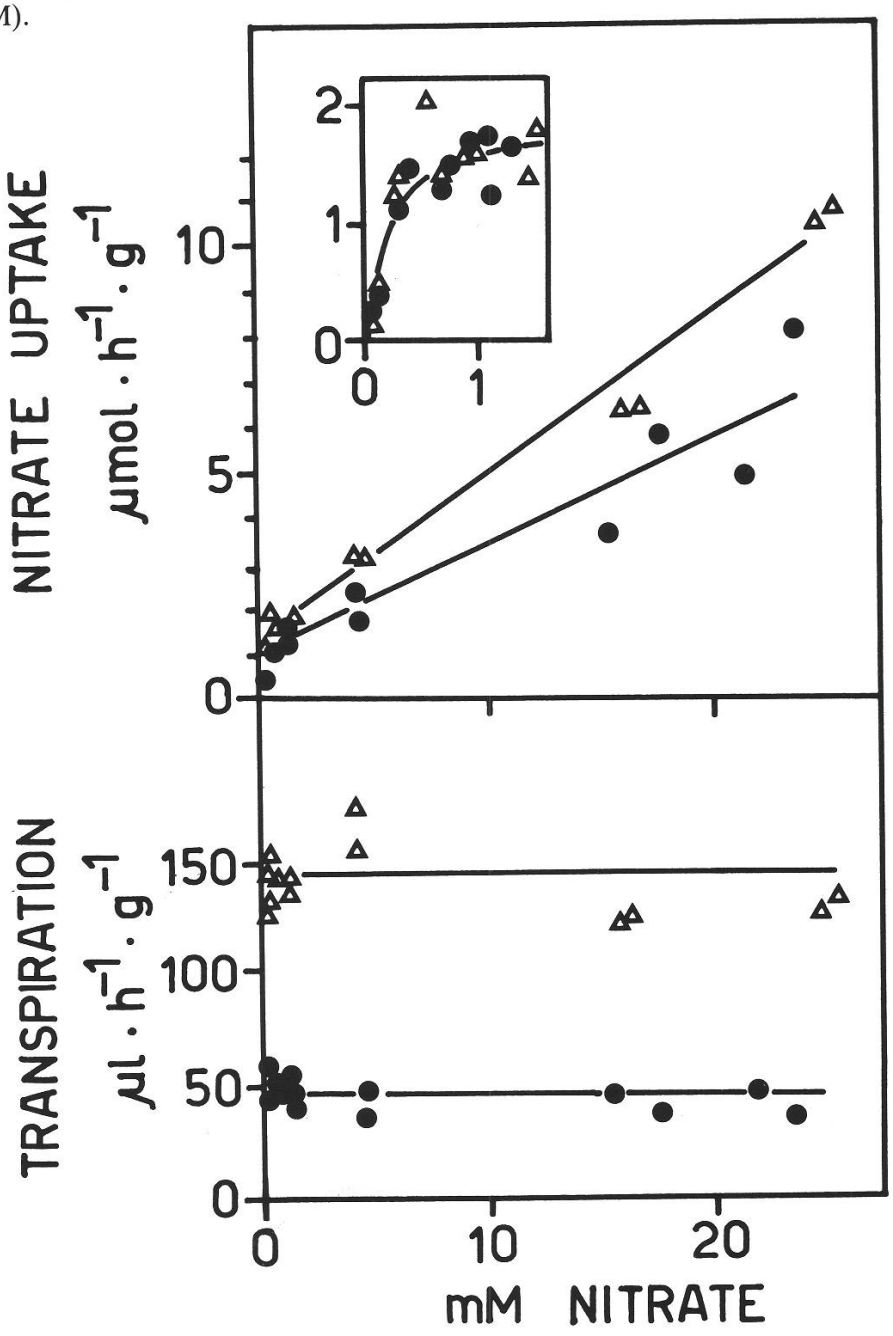
Table 1: Macronutrients in nutrient solution with different nitrate concentrations. The composition of solutions with nitrate concentrations not listed can be found by interpolation.

Nitrate (mM)	Macronutrients (mM)				
	KNO_3	$Ca(NO_3)_2$	KH_2PO_4	$MgSO_4$	$CaSO_4$
0	0	0	6.7	3.3	5.2
0.5	0.5	0	6.2	3.3	5.2
1.0	1.0	0	5.7	3.3	5.2
1.5	1.5	0	5.2	3.3	5.2
3.5	0.9	1.3	5.8	3.3	3.9
5.0	1.2	1.9	5.8	3.3	3.5
10.0	2.4	3.8	4.4	3.3	1.5
15.0	3.6	5.7	2.9	3.3	0
20.0	4.8	7.6	2.9	3.3	0
25.0	6.0	9.5	2.9	3.3	0
30.0	7.2	11.4	2.9	3.3	0

Table 2: Nitrate and ammonium uptake by 12 d old bean plants preincubated for 84 h on different nutrient solutions. Nitrogen uptake during the experiemnt (7 h) was calculated from the initial and final contents per pot. The experiments were performed in triplicate. The data represent the means ± standard deviation and are expressed per g fresh weight of the whole plant (root + shoot).

N-source preincubation	N-source experiment	Nitrate uptake (μmol·7 h^{-1}·g^{-1})	Ammonium uptake (μmol·7 h^{-1}·g^{-1})
3.5 mM NO_3^-	3.5 mM NO_3^-	24.3 ± 1.2	–
3,5 mM NO_3^-	3.5 mM NH_4NO_3	27.2 ± 4.4	7.2 ± 0.4
3.5 mM NH_4^+	3.5 mM NH_4NO_3	26.7 ± 0.8	9.0 ± 1.8
3.5 mM NO_3^-	3.5 mM NH_4^+	–	16.3 ± 2.3
3.5 mM NO_3^-	1.0 mM NO_3^-	6.3 ± 0.7	–
3.5 mM NO_3^-	1.0 mM NH_4NO_3	8.4 ± 0.8	3.1 ± 0.7
3.5 mM NH_4^+	1.0 mM NH_4NO_3	5.0 ± 0.7	4.9 ± 0.4
1.0 mM NO_3^-	1.0 mM NO_3^-	18.5 ± 4.0	–
1.0 mM NH_4NO_3	1.0 mM NH_4NO_3	18.9 ± 1.4	6.5 ± 1.3
N-free	3.5 mM NO_3^-	25.7 ± 2.6	–
N-free	3.5 mM NH_4NO_3	30.8 ± 0.2	6.7 ± 1.1
N-free	3.5 mM NH_4^+	–	15.9 ± 1.6
N-free	1.0 mM NO_3^-	18.9 ± 1.0	–

Fig. 3 Effect of light on rates of nitrate uptake and transpiration over a wide range of external nitrate concentrations. The plants were preincubated for 60 h on nitrate medium containing the same concentration as used for the experiment. The symbols represent data obtained from individual 12 d old plants kept for 2 h prior to and during the whole experiement in light (△) or 12 h prior to and during the whole experiment in darkness (●). Transpiration and nitrate uptake rates are expressed per g fresh weight of the whole plant (root + shoot). Inset: nitrate uptake rates at low external nitrate concentrations (0.1-1.5 mM).



nitrate. Ammonium uptake was highest when no nitrate was present in the medium and was considerably lower when nitrate was added at the same concentration as ammonium. The pretreatment with higher nitrogen concentrations had a smaller effect on ammonium than on nitrate uptake. Uptake rates for ammonium and nitrate depended in a different manner on the pH of the nutrient solution (data not shown). At pH values above 6 ammonium was taken up prefentially, while below pH 5 nitrate was strongly preferred.

Nitrate utilization was not affected in the low concentration range (0.1-1.5 mM, phase I) by light as shown in Fig. 3. From nutrient solutions with more than 2 mM nitrate (phase II) nitrate uptake was slightly lower in darkness than in light. The transpiration rate was relatively constant over a wide range of nitrate concentrations (0.1-28 mM) and was about 70 % lower in darkness than in light. No effect of light on nitrate uptake in phase I was observed, although transpiration and the flow rate in the xylem were considerably lower in darkness. Our data suggest that the illumination of the plants affects nitrate uptake in phase II (higher concentration range) more than in phase I.

Discussion

Dual-phase kinetics were found for nitrate uptake by intact plants. Apparent K_m values for phase I were in the range between 0.2 and 0.4 mM, depending on the nitrogen nutrition of the plants prior to the experiment. It remains unknown, whether the translocation of nitrate through the plasmalemma into the cytoplasm of root cells or other processes (e.g. xylem loading or accumulation of nitrate in root cell vacuoles) were rate limiting under the conditions used. It appears possible that different factors were rate limiting in our investigations and in short-term experiments with N-starved plants, where considerably lower K_m values for nitrate were found. Our data are consistent with K_m values for several crop plants as summarized by Doddema (1978).

Ammonium ions present in the medium at the same concentration as nitrate had little effect on nitrate uptake in phase I at pH 5.5. Since nitrate and ammonium uptake depend on the pH and temperature of the external solution (Rao and Rains 1976, Breteler 1977), nitrate uptake may be influenced differently by ammonium at other pH values and at other temperatures. The strong effect of the nitrogen nutrition prior to the experiment may be due to changes in the C/N-balance within the plants and to a different initial nitrate content of the roots.

The result that nitrate uptake in phase I was not affected by light is consistent with findings from Doddema (1978) and Breteler et al. (1979). At high external nitrate concentrations interferences with transpiration appear likely and should be considered in further experiments. The slower transport of nitrate or of its assimilation products from the shoot may affect nitrate uptake into intact plants. This hypothesis is supported by the observation that nitrate uptake rates into excised bean roots are lower than those into intact plants (Breteler et al. 1979).

Zusammenfassung

Zwei Phasen können in der Konzentrationsabhängigkeit der Nitrataufnahme bei pH 5,5 bei intakten, nicht-nodulierten Bohnenpflanzen unterschieden werden. Die apparenten K_m-Werte für Phase I (höhere Affinität für Nitrat) lagen im Bereich zwischen 0,2 und 0,4 mM und waren abhängig von der Stickstoffversorgung der Pflanzen vor dem Experiment. Ammonium, das in gleicher Konzentration wie das Nitrat dem Nährmedium zugesetzt wurde, hatte keinen Einluß auf die Nitrataufnahmeraten in Medien mit 0,1-1,5 mM Nitrat. Die Aufnahmeraten für Nitrat waren deutlich niedriger bei

Pflanzen, denen in der Vorkultur höhere Nitrat- oder Ammoniumkonzentrationen als im Experiment angeboten wurden, als bei solchen mit gleichem Medium während Vorkultur und Experiment. Obwohl die Transpirationsraten im Dunkeln ungefähr 70% niedriger waren als im Licht, war kein Einfluß des Lichtes auf die Nitrataufnahme aus Lösungen mit 0,1-1,5 mM Nitrat feststellbar. Aus Lösungen mit mehr als 2 mM Nitrat waren die Aufnahmeraten im Dunkeln etwas niedriger als im Licht.

We thank Dr. J. Fuhrer for assistance in preparing the English version of the manuscript. This work was supported by Swiss National Science Foundation grant No. 3.067-0.81.

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