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# Nitrate Absorption by Barley

## II. INFLUENCE OF NITRATE REDUCTASE ACTIVITY[1]

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K. PRASAD RAO AND D. WILLIAM RAINS
*Department of Agronomy and Range Science, University of California, Davis, California 95616*

### ABSTRACT

**The influence of protein synthesis and nitrate reductase activity on nitrate absorption by barley (*Hordeum vulgare* L.) was investigated. Cycloheximide decreased nitrate absorption. Pretreatment studies showed that cycloheximide affects either energy transfer or nitrate reductase activity or both.**

**Illumination increased plant capacity for nitrate absorption, possibly through increased energy supply and/or increased nitrate reductase activity. There was a positive correlation between nitrate reductase activity and light. Inhibiting the development of nitrate reductase activity by tungstate decreased nitrate absorption.**

**At least two nitrate transport systems are thus proposed in barley: one operating in the dark, with little nitrate reductase activity detectable; and one closely correlated with nitrate reductase activity. Total absorption is the sum of dark absorption and absorption facilitated by nitrate reductase.**

Nitrate reductase and nitrite reductase are known to be responsible for the reduction of $NO_3^-$ to $NH_4^+$ form (5), and NR[2] is inducible by $NO_3^-$. Light enhances $NO_3^-$ absorption which in turn is followed by an enhanced NR induction (1). Inhibitors of protein synthesis, such as CHI, effectively suppress NR formation. Cycloheximide also considerably decreases the absorption of $NO_3^-$ (10, 20, 21) and other ions (4).

If NR is linked to $NO_3^-$ absorption, the relative dependence might be determined by using various NR-inducing conditions as well as NR suppressors, such as CHI or $WO_4$. This aspect was studied with special emphasis on the effect of CHI on $NO_3^-$ absorption, and also the relationship between NR activity in shoots and its effect on $NO_3^-$ absorption by roots.

### MATERIALS AND METHODS

Six-day-old barley (*Hordeum vulgare* L. var. Arivat) seedlings were prepared and treated as detailed elsewhere (17).

**Illumination Studies.** For studying illumination effects on $NO_3^-$ absorption, the seedlings were grown in the dark at 25 C for 4 days in 0.2 mM $CaSO_4$ solution. Two days before the absorption studies they were transferred to a growth chamber at about 5000 ft-c. The light experiments were done in the same chamber at 30 C, where as the dark experiments were done in a water bath also at 30 C, which was covered with a black plastic sheet. Other conditions are detailed in the respective results sections. *In vitro* NR activity was assayed as described by Schrader *et al.* (19).

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[2] Abbreviations: NR, nitrate reductase; CHI, cycloheximide; DNP, 2,4-dinitrophenol.

### RESULTS

**Effects of $NO_3^-$ Pretreatment and CHI on $NO_3^-$ Absorption.** Pretreatment with $NO_3^-$ for 2 hr increased $NO_3^-$ absorption about 50% over that of the control (Table I). Various combinations of CHI and $NO_3^-$ were used to determine the relationship of $NO_3^-$ absorption and protein synthesis.

**Pretreatment Studies.** Table I presents data on treatments A through D. The concentration of $CaSO_4$ in all the pretreatment and absorption solutions in this series was 5 mM. A: When CHI was present in the absorption solution, the amount of $NO_3^-$ absorbed by $CaSO_4$-pretreated seedlings was 60% less than that of the seedlings placed in a CHI free solution. B: The seedlings were pretreated for 2 hr in 0.5 mM $KNO_3$ and then transferred to solutions containing either $NO_3^-$ alone or $NO_3^-$ + CHI. There was a significant increase in $NO_3^-$ absorption in the absence of CHI, whereas in the presence of CHI the absorption was decreased by 40%. C: Seedlings were pretreated with 10 mM CHI for 2 hr and then exposed to solutions containing either $NO_3^-$ alone or $NO_3^-$ + CHI. Absorption was nearly normal without added CHI, but decreased by 60% with CHI. D: Seedlings were pretreated with $NO_3^-$ + CHI, and $NO_3^-$ absorption was measured in the presence or absence of CHI in the absorption solutions. In the presence of CHI the absorption was decreased by 80% as compared to 60% in the absence of CHI.

Nitrate absorption was also studied as a function of pretreatment time in either $NO_3^-$ alone or $NO_3^-$ + CHI (Fig. 1). Nitrate alone enhanced absorption, whereas $NO_3^-$ + CHI produced a

Table I. *Effect of $NO_3^-$ Pretreatment and CHI on $NO_3^-$ Absorption*

Pretreatment solutions: A: 5 mM $CaSO_4$; B: 5 mM $CaSO_4$ + 0.5 mM $KNO_3$; C: 5 mM $CaSO_4$ + 10 mM CHI; D: 5 mM $CaSO_4$ + 10 mM CHI + 0.5 mM $KNO_3$. Pretreatment time was 2 hr. Absorption solutions contained 0.5 mM $KNO_3$, 5 mM $CaSO_4$, and CHI (where present) at 10 mM. Absorption time was 3 hr. Temperture of both pretreatment and absorption was 30 C.

| Pretreatment | Absorption Solution | $No_3^-$ Absorbed |
|---|---|---|
| | | *μmoles g⁻¹ fresh wt* |
| A | | |
| Control | $NO_3^-$ | 7.95 |
| Control | $NO_3^-$ + CHI | 3.00 |
| B | | |
| $NO_3^-$ | $NO_3^-$ | 12.50 |
| $NO_3^-$ | $NO_3^-$ + CHI | 7.90 |
| C | | |
| CHI | $NO_3^-$ | 10.15 |
| CHI | $NO_3^-$ + CHI | 4.50 |
| D | | |
| CHI + $NO_3^-$ | $NO_3^-$ | 2.32 |
| CHI + $NO_3^-$ | $NO_3^-$ + CHI | 1.60 |

progressive decrease that was greater still when the absorption solutions also contained CHI.

**Nitrate Absorption as a Function of $NO_3^-$ and Light Pretreatment.** Figure 2 shows the interaction between light and $NO_3^-$. Seedlings pretreated with 0.5 mM $NO_3^-$ in the dark for 2 days absorbed more $NO_3^-$ than the ones pretreated with light alone. Nitrate plus light treatment, however, produced a substantially greater increase than the $NO_3^-$ treatment in the dark.

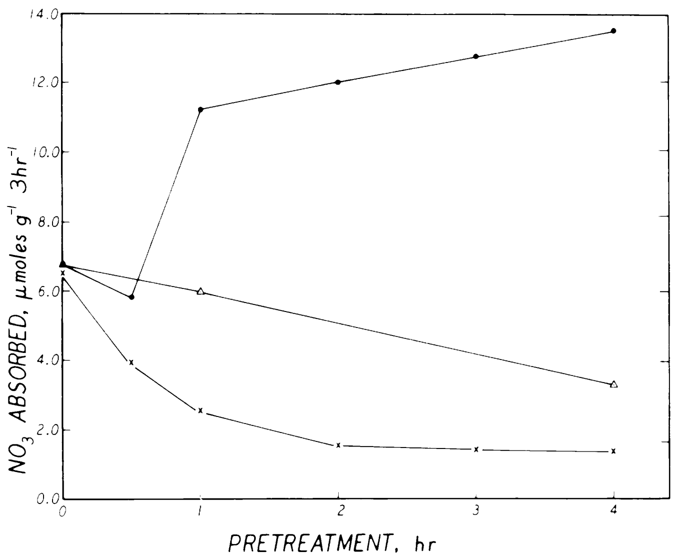


FIG. 1. Effect of $NO_3^-$ and $NO_3^-$ + CHI pretreatment on $NO_3^-$ absorption. $KNO_3$, 0.5 mM; $CaSO_4$, 5 mM; CHI, 10 mM; temperature, 30 C; absorption time, 3 hr. Pretreatment and absorption in $NO_3^-$ (●); pretreatment in $NO_3^-$ + CHI and absorption in $NO_3^-$ (△); pretreament and absorption in $NO_3^-$ + CHI (X).

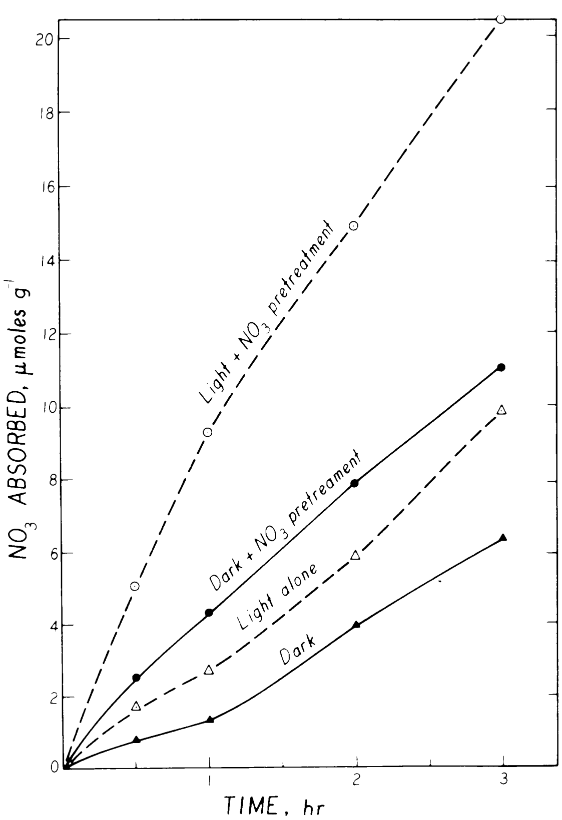


FIG. 2. Nitrate absorption as a function of $NO_3^-$ + light pretreatment. Light intensity was approximately 5000 ft-c. $KNO_3$, 0.5 mM; $CaSO_4$, 5 mM.

In all treatments, roots contained more $NO_3^-$ than shoots (Fig. 3a). In light-treated seedlings, the $NO_3^-$ concentration in the shoots was less than that in the shoots of the dark-treated seedlings. This indicates that more $NO_3^-$ was reduced by the shoots in light than by those in the dark, which is reflected by the higher NR activity in light than in the dark (Fig. 3b).

Absorption of $NO_3^-$ was studied as a function of time in dark- and light-grown seedlings pretreated for 2 days with 0.5 mM $KNO_3$. The four treatments are detailed in the legend to Figure 4. The light-grown seedlings absorbed about 50% more $NO_3^-$ than the dark-grown, irrespective of whether the absorption was in the light or in the dark.

**Effect of $WO_4$ on $NO_3^-$ Absorption.** Nitrate absorption was studied in seedlings exposed to solutions of 0.5 mM $KNO_3$

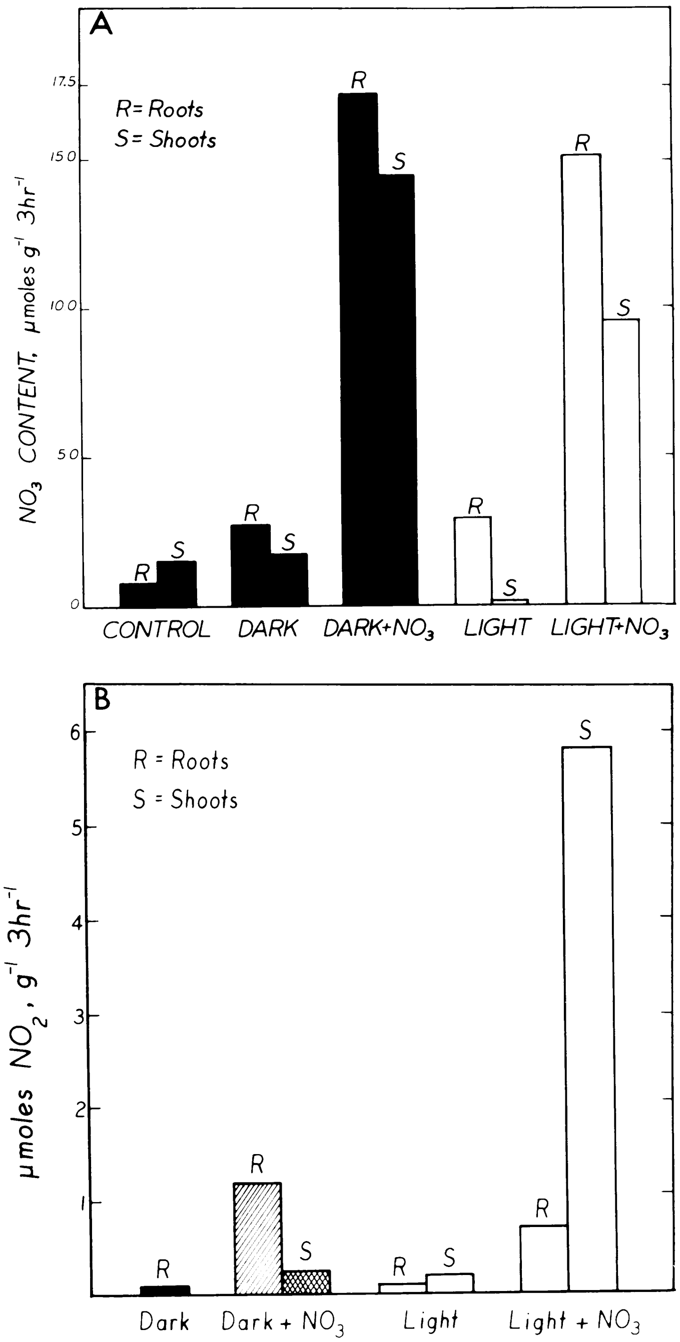


FIG. 3. A: Distribution of $NO_3^-$ among the roots and shoots. Shaded histogram indicates dark treatment. B: Nitrate reductase activity in the roots and shoots. Shaded histogram indicates dark treatment. Experimental conditions were as in Fig. 2.

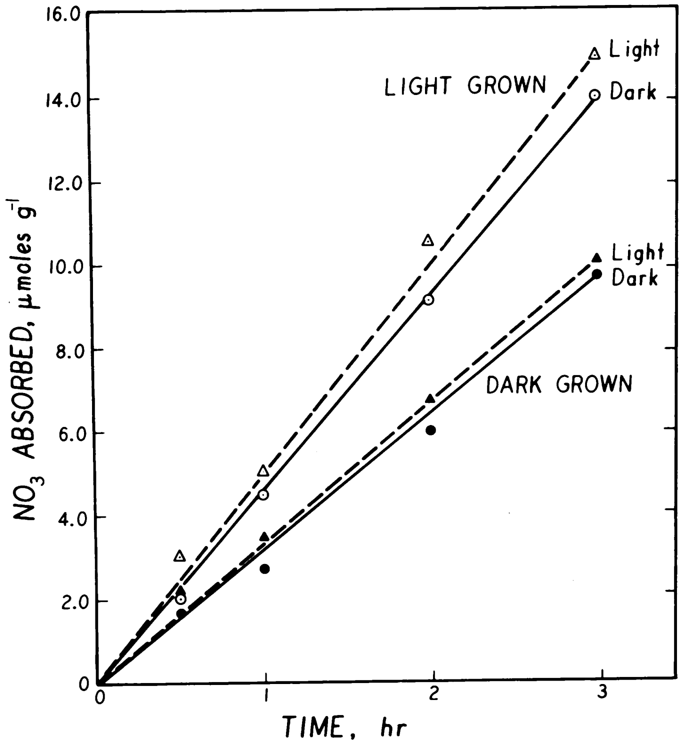


FIG. 4. Nitrate absorption as a function of time by seedlings grown in light or dark. $KNO_3$, 0.5 mM; $CaSO_4$, 5 mM; temperature 30 C. Absorption in light (△, ▲); absorption in dark (○, ●).

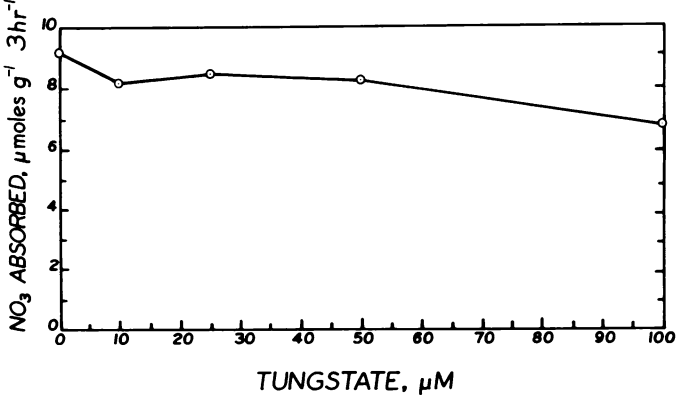


FIG. 5. Effect of $WO_4$ on $NO_3^-$ absorption. $KNO_3$, 0.5 mM; $CaSO_4$, 5 mM; temperature, 30 C.

Table II. *Effect of $WO_4$ on $NO_3^-$ Absorption and NR Activity*

The concentration of $NO_3^-$ and $WO_4$ (where present) in both pretreatment and absorption solutions were 0.5 mM and 100 µM, respectively. Calcium was present at 5 mM throughout.

| Treatment | | $NO_3^-$ Absorbed | NR Activity in Leaves | |
|---|---|---|---|---|
| Pretreatment | Absorption solution | | Before absorption | After absorption |
| | | *µmoles $g^{-1}$ 3 $hr^{-1}$* | *µmoles $NO_2^-$ $g^{-1}$ $hr^{-1}$* | |
| $NO_3^-$ + $WO_4$ | $NO_3^-$ | 6.66 | 0.00 | 0.00 |
| $NO_3^-$ + $WO_4$ | $NO_3^-$ + $WO_4$ | 5.36 | | 0.00 |
| $NO_3^-$ − $WO_4$ | $NO_3^-$ | 12.35 | 5.28 | 5.76 |
| $NO_3^-$ − $WO_4$ | $NO_3^-$ + $WO_4$ | 5.19 | | 4.68 |

containing 0 to 100 µM $WO_4$. Even at 100 µM $WO_4$, there was no appreciable effect on $NO_3^-$ absorption (Fig. 5).

This study was pursued further by first letting the NR enzyme system develop in the presence or absence of 100 µM $WO_4$, followed by absorption of $NO_3^-$ with or without $WO_4$. For this experiment, the seedlings were grown 3 days in dark and 3 days in light in 0.2 mM $CaSO_4$. Before transfer to light (about 3500 ft-c), 0.5 mM $KNO_3$ and ± 100 µM $WO_4$ were added. The leaves were analyzed for NR activity before and after absorption. The absorption solutions contained 0.5 mM $KNO_3$, 5 mM $CaSO_4$, and ± 100 µM $WO_4$. The presence of $WO_4$ during the induction period completely inhibited NR activity (Table II), but $WO_4$ inhibited only 15% of the activity of the already induced NR system, while decreasing $NO_3^-$ absorption by 60%.

## DISCUSSION

**Effect of CHI on $NO_3^-$ Absorption.** The increased $NO_3^-$ absorption from $NO_3^-$ pretreatment in this and other studies (Table I and refs. 6, 12) suggests that the synthesis of components which facilitate $NO_3^-$ absorption depends on $NO_3^-$ entering the root. It has been well established that NR is induced by $NO_3^-$ (1, 7, 22), and CHI inhibits its induction (15, 20). Though CHI is considered as an inhibitor of protein synthesis, under suitable conditions it stimulates $O_2$ uptake in a manner similar to DNP (4). The inhibitory effect of CHI on ion absorption (Table I and refs. 10, 20) is probably mediated via interference with energy transfer and oxidative phosphorylation (11, 14).

Nitrate absorption in barley shows induction-type kinetics, a lag phase followed by an accelerated phase (17). Cycloheximide inhibits the development of the accelerated phase (10), and it also exponentially decreases the already induced $NO_3^-$ transport system (18). Hence, we cannot rule out the possibility that CHI may be preventing the development of the $NO_3^-$ transport system, through or in addition to the interference with energy transfer.

The effect of CHI on $NO_3^-$ absorption is substantial when $NO_3^-$ is present with CHI (Table I and Fig. 1). In this case CHI would inhibit the NR activity (15, 20), in addition to disrupting the energy transfer and the development of the $NO_3^-$ transport system. No matter what the mechanism of CHI inhibition, it interferes with $NO_3^-$ absorption.

**Effect of Light on $NO_3^-$ Absorption and Reduction.** Barley seedlings exposed to light absorbed more $NO_3^-$ than the seedlings kept in the dark (Fig. 2). When no $NO_3^-$ was present during illumination, the seedlings did not develop any significant amount of NR activity to influence absorption. Therefore, the enhanced absorption after illumination might be due to an increased supply of energy for transport of ions through ATP supplied by photophosphorylation (13, 16). When $NO_3^-$ was present during illumination, the seedlings developed substantial NR activity (Fig. 3b), and $NO_3^-$ absorption was twice that of the plants grown in the absence of $NO_3^-$ (Fig. 2 and refs 3, 22). This enhanced absorption may be due to a rapid reduction of the available $NO_3^-$ in the tissue, which subsequently increases the total $NO_3^-$ absorbed. Ben Zioni *et al.* (2) proposed a mechanism to explain how the $NO_3^-$ reduction in the shoots may influence the absorption of $NO_3^-$ by the roots. They suggested that after $KNO_3$ is translocated to the shoots from the roots, a stoichiometric amount of malate is produced for the amount of $NO_3^-$ reduced in the shoots. After the malate is synthesized, part of it moves down to the root system as K-malate, where it is oxidized, yielding $KHCO_3$, which exchanges for $KNO_3$ in the external medium. Thus, $NO_3^-$ reduction in the shoots promotes preferential uptake of $NO_3^-$ by the roots.

**Effect of $WO_4$ on $NO_3^-$ Absorption and Reduction.** Though $WO_4$, an analog of Mo, does not effect the existing NR activity (23), it does inhibit any further development of functional NR (Table II and refs. 8, 9). Also in short term experiments $WO_4$ has no apparent effect on $NO_3^-$ absorption by the seedlings with no detectable NR (Fig. 5 and Table II) and yet it inhibits the enhanced absorption by the seedlings with active NR. IT does so without any appreciable inhibition of either *in vitro* (Table II) or *in vivo* NR activity (data not shown).

How does $WO_4$ affect $NO_3^-$ absorption without affecting the NR system? This question leads to the speculation that there are at least two different $NO_3^-$ absorption systems: one positively correlated with NR, and one independent of the NR system. It is the first system which is in some way affected by $WO_4$. Without understanding the exact mode of action of $WO_4$ on the absorption mechanisms, it is difficult at present to explain the effect of $WO_4$ on $NO_3^-$ absorption.

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