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# The effects of light on induction, time courses, and kinetic patterns of net nitrate uptake in barley

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## ABSTRACT

**Barley seedlings (*Hordeum vulgare* L.) were grown hydroponically with (induced) or without (uninduced) nitrate in a light/dark cycle with high photon flux density to determine the effects of light on time courses, induction and kinetics of net nitrate uptake. Nitrate uptake was induced by external nitrate in both light and dark and was prevented by 1 mol $m^{-3}$ p-fluorophenylalanine. In high light, nitrate uptake was about 2-fold higher than in low light. During time course experiments the uptake rates oscillated due to daily light–dark changes. Rates of nitrate uptake also increased at about 2200 h during continuous darkness. This increase coincided approximately with the time at which the dark period started during the previous culture of the plants, indicating that it was due to a mechanism associated with an endogenous diurnal rhythm. When calculating the kinetics of nitrate uptake, a model with two saturable systems, including a high-affinity system (HATS) and a low-affinity system (LATS), gave the best fit to data in all treatments. The apparent affinity of the HATS ranged from 7·7 to 12·2 mmol $m^{-3}$ in induced plants in all light conditions. The effect of light on the HATS was mainly an increase of apparent $V_{max}$ in the step from low to high light. In uninduced plants the HATS operated at a very low activity which was strongly enhanced during induction. Interpretation of the calculated kinetics of the LATS was much more difficult on the basis of net uptake data. The apparent affinity of the LATS increased from 24·3 mol $m^{-3}$ in low light up to 0·17 mol $m^{-3}$ after acceleration in high light. These extreme changes in apparent affinity of the LATS could not be explained satisfactorily, and the nature of this system is also discussed with respect to the method used.**

*Key-words*: *Hordeum vulgare* L.; net nitrate uptake; light (intensity); kinetic parameters; induction time courses; Poaceae.

*Abbreviations:* FPA, p-fluorophenylalanine; $FW_R$, fresh weight of the roots; HATS, high-affinity transport system; $K_d$, rate constant; $K_m$, Michaelis-Menten constant; LATS, low-affinity transport system; $V_c$, constant uptake rate; $V_{max}$, maximal uptake rate.

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## INTRODUCTION

The uptake of nitrate by roots of plants not previously exposed to nitrate is usually at a low, constitutive level, but exposure to external nitrate can increase the rate by 2- to 5-fold above this constitutive level. Nitrate uptake occurs thermodynamically uphill and can be inhibited by processes which depress synthesis of ATP and proteins (for a review, see Clarkson 1986). The energy dependence of nitrate uptake is consistent with a $NO_3^-$: $2H^+$ symport (Glass, Shaff & Kochian 1992). Subsequent steps in nitrate metabolism, nitrate reduction and ammonium assimilation, are also inducible and energy dependent since reducing power, ATP and C-skeletons are needed. The uptake and assimilation of ammonium and nitrate require a significant amount of root respiration (Bloom, Sukrapanna & Warner 1992). Hence in an intact plant, nitrate uptake, N metabolism, C metabolism and ultimately photosynthesis are all linked. These steps in nitrogen metabolism are all life-dependent processes which are influenced by environmental factors like light, temperature or stress conditions as well as variations during ontogeny (Imsande & Touraine 1994). So, if light conditions change, a signal from the shoot, the site of light perception, to the root, the site of nitrate uptake, would be not surprising. Rideout *et al*. (1993) concluded from their experiments with soybean that carbohydrate flows from the shoot to the root may regulate nitrate uptake more than the level of nitrate in the root itself. However, the mechanistic effects of light on nitrate uptake in higher plants have not been studied as extensively as other factors such as concentrations of nitrate, temperature and inhibitors. These results were more descriptive. Rao & Rains (1976b) reported that illumination stimulated nitrate absorption in barley, and Aslam *et al*. (1979) showed that nitrate uptake was 20% faster in the light than in the dark. Clement *et al*. (1978) found that the nitrate uptake in simulated swards of ryegrass was related to diurnal, day-to-day, and seasonal changes in radiation. Delhon *et al*. (1995) recently showed that nitrate influx was down-regulated in the dark. No information is available about the effects of light on the kinetic parameters of nitrate uptake.

A ‘dual uptake system’ for the kinetics of nitrate uptake was first described in maize (Neyra & Hageman 1975), in barley (Rao & Rains 1976a), and in Arabidopsis (Doddema & Telkamp 1979). These and subsequent observations led

to a model with a high-affinity transport system (HATS) and a low-affinity transport system (LATS) (Siddiqi, Glass & Ruth 1991). The HATS is described as a saturable system, whereas the nature of the LATS is still not clear. Doddema & Telkamp (1979) and Goyal & Huffaker (1986b) found a second saturable system, but Siddiqi *et al*. (1990) and Aslam, Travis & Huffaker (1992) observed a linear non-saturable component under higher concentrations of nitrate. Aslam *et al*. (1992) even found two saturable, possibly constitutive systems in the low concentration range and a linear system at more than 500 mmol $m^{-3}$ nitrate. Despite some evidence that the LATS is of a passive nature (Glass *et al*. 1990; Siddiqi *et al*. 1991), it appears more likely that HATS and LATS both proceed thermodynamically uphill (King, Siddiqi & Glass 1992). In the papers mentioned above, two methods were used for exploring the kinetics: the first monitoring the depletion of nitrate from the external medium and the second following the movement of tracers (for a review, see Peuke & Kaiser 1996).

The aim of the present paper is to show the time courses of net nitrate uptake under different light regimes and to explain the observations in terms of how the kinetics of the different nitrate uptake systems are regulated by the light environment. Further, the effect of light on the induction of net nitrate uptake by roots of intact barley plants was examined. Uptake was measured by monitoring the depletion of nitrate in the nutrient solution (see also Aslam *et al*. 1992; Goyal & Huffaker 1986a,b; Behl, Tischner & Raschke 1988; Doddema & Telkamp 1979; Rao & Rains 1976a; Pearson & Steer 1977; Mäck & Tischner 1990) using an automatically computer-controlled HPLC system. For the calculation of kinetic parameters the data were fitted to different equations using a non-linear curve-fitting computer program. Barley plants were grown with or without nitrate (induced or uninduced) in a light–dark cycle from 10 to 20 d prior to analysis in pre-cultivation. Subsequently, during the experiments the plants were illuminated with a low or a high photon flux density or were kept in the dark.

## MATERIALS AND METHODS

### Plant material and growth conditions

Seeds of *Hordeum vulgare* L. cv. 'California Mariout' were germinated in darkness on filter paper moistened with 0·5 mol $m^{-3}$ $CaSO_4$. After 3 to 4 d, 12 seedlings were transferred to 4 $dm^3$ vessels containing aerated nutrient solution. The control medium was based on 'Long Ashton solution' (Hewitt 1966) and contained in mmol $m^{-3}$: $KNO_3$ 333, $Ca(NO_3)_2$ 333, $MgSO_4$ 150, $MnSO_4$ 2, $H_3BO_3$ 10, $Na_2MoO_4$ 0·20, $CoSO_4$ 0·08, $ZnSO_4$ 0·19, $CuSO_4$ 0·13, $FeCl_3$ 10, $Na_2EDTA$ 10 and $NaH_2PO_4$ 150. In N-free medium, nitrate salts were replaced by KCl and $CaCl_2$ to maintain the ionic strength. Nutrient solutions were renewed after 1 week and subsequently every 3 d.

The plants were cultivated for 10 to 20 d in a greenhouse (15–25 °C; 45–75% relative humidity) with a 16 h light and 8 h dark cycle (light on at 0600 h and light off at 2200 h) with supplemental illumination (Osram HQI 400); photon flux density was 300–500 $\mu$mol photon $m^{-2}$ $s^{-1}$. During this time the plants showed no significant changes with respect to internal nitrate concentration or nitrate uptake rates (related to a fresh weight basis). The relative growth rate was about 8% $d^{-1}$. Plants were grown in N-free medium for the last 10 to 14 d before the uptake experiments began. These plants were used for experiments with uninduced nitrate uptake. After exposure to 1 mol $m^{-3}$ nitrate for at least 6 h the plants were termed 'newly induced'.

### HPLC system

Nitrate uptake was measured with a computer-controlled HPLC system including a valve/pump arrangement similar to that described by Goyal & Huffaker (1986a). Three, seven or 15 hydroponic plastic pots (0·5 or 1·0 $dm^3$) were placed in a plexi-glass cuvette (0·6 × 0·6 × 0·8 m, ≈ 0·27 $m^3$) which was used as a growth chamber during the uptake experiments. Each pot was connected with Teflon tubing to a multiposition valve (Valco ECSD16P, VICI Inc., Houston, TX, USA). A peristaltic pump (Micro Perpex Pump 2132, LKB, Broma, Sweden) sampled aliquots (1·14 $cm^3$) from the nutrient solutions for rinsing the tubes and valves and filling the sample loop of an electric switching injector (Valco EC6 W, VICI Inc., Houston, TX, USA). The HPLC system for nitrate analysis corresponded to that of Thayer & Huffaker (1980). Separation was achieved with a strong anion exchanger (Partisil SAX 10, Whatmann, Maidstone, Kent, UK) in a Vertex column (120 × 4 mm, Knauer, Bad Homburg, Germany). The eluent was 30 mol $m^{-3}$ $NaH_2PO_4$ adjusted to pH 2·4 with $H_3PO_4$ and the flow rate was 1·2 $cm^3$ $min^{-1}$ (Waters 501 HPLC pump, Millipore, Eschborn, Germany). Nitrate was detected by a UV detector (Waters 486) at 214 nm. The pump/valve system, data acquisition, and the processing of the chromatograms were controlled by the Waters maxima 820 chromatography workstation. The duration of one analysis, including the sampling time (0·5 min), was about 3·4 min. Using different loop volumes (40–2 $mm^3$) nitrate concentrations between 0·1 mmol $m^{-3}$ and 10·5 mol $m^{-3}$ could be measured.

### Uptake experiments

Two to four hours before starting the experiments (at about 1200 or 2400 h as indicated), plants were transferred to the experimental cuvette and fresh nutrient solution. This was done to adapt the plants to the experimental conditions which were all at 25–30 °C, 35–60% RH and either low light: 50 $\mu$mol photon $m^{-2}$ $s^{-1}$ provided by luminescence lamps (Osram L20 W 3–2 warm white deluxe and L18 W/20 cool white), or high light: 500 $\mu$mol photon $m^{-2}$ $s^{-1}$ provided by an Osram 400 W HQI lamp, or darkness. At the beginning of the measurements, the roots of two to 15 uniform plants were rinsed with uptake solution and the

plants placed into pots with 0·5 or 1·0 $dm^3$ of this solution, the composition of which was identical to that of the nutrient solution (for composition, see above, apart from the nitrate concentration used). For concentrations below 1 mol $m^{-3}$ nitrate, the ionic strength was maintained by adding KCl and $CaCl_2$, while at higher concentrations $KNO_3$ and $Ca(NO_3)_2$ were added. For determining kinetic parameters the nitrate concentration up to 10 mol $m^{-3}$ was divided into three ranges to obtain higher resolution: low range: 5, 10, 30, 50, 100, 200 and 300 mmol $m^{-3}$ initial concentration, middle range: 0·5, 0·7, 1, 1·5, 2, 3 and 4 mol $m^{-3}$, and high range: 5, 6, 7, 8, 9 and 10 mol $m^{-3}$. At high concentrations more plants per pot were used than with lower concentrations. Stirring of the nutrient solution was achieved by bubbling with air.

Nitrate assays were performed every 4·2–7·5 min so that each pot was tested every 34–60 min. For each nitrate concentration evaluated and every time point, a nitrate uptake rate was calculated. In time course experiments (1 to 3 d) the nitrate concentrations were kept constant at the initial concentration by adding $KNO_3$ and $Ca(NO_3)_2$. For kinetic analysis, the decrease from the initial concentration was monitored without further addition of nitrate and data from a day period of about 8 h were taken once the rate of uptake was constant as measured in the time course experiments (see 'Results').

### Statistical treatment and calculations

Every experiment (induction, light–dark cycles, continuous light or dark conditions, and every concentration range for apparent $K_m$ determinations) was repeated at least four times. For the time course experiments, all evaluated uptake data are shown as a function of time and the time course is indicated by calculating a running average. The kinetic parameters were calculated by direct fitting of the raw data (about 2000 data points for each treatment) using the 'nonlinear curve-fitting' option of SigmaPlot 4·1 (Jandel Scientific, Corte Madera, CA, USA) which is based on the Marquardt-Levenberg algorithm. In the table and the text the computed kinetics and errors ($P$ = 0·95) are given. For evaluating the underlying kinetic constants (i.e. apparent $V_{max}$ and apparent $K_m$) five different equations describing two to three uptake systems were provisionally fitted to the data (see also Borstlap 1983; Wang *et al*. 1993):

- a saturable and a linear system

$$V = (V_{max}[NO_3^-])(K_m + [NO_3^-])^{-1} + K_d\,[NO_3^-];$$

- a saturable and a linear system with a constant $V_c$ (< 0)

$$V = (V_{max}[NO_3^-])(K_m + [NO_3^-])^{-1} + K_d[NO_3^-] + V_c;$$

- two saturable systems (HATS and LATS)

$$V = (V_{max1}[NO_3^-])(K_{m1} + [NO_3^-])^{-1} + (V_{max2}[NO_3^-])\,(K_{m2} + [NO_3^-])^{-1};$$

- two saturable (HATS and LATS) and a linear system

$$V = (V_{max1}[NO_3^-])(K_{m1} + [NO_3^-])^{-1} + (V_{max2}[NO_3^-])\,(K_{m2} + [NO_3^-])^{-1} + K_d[NO_3^-];$$

- two saturable (HATS and LATS) and a linear system with a constant $V_c$ (< 0)

$$V = (V_{max1}[NO_3^-])(K_{m1} + [NO_3^-])^{-1} + (V_{max2}[NO_3^-])\,(K_{m2} + [NO_3^-])^{-1} + K_d[NO_3^-] + V_c.$$

The negative constant term ($V_c$) was introduced in the linear system in order to describe a linear component of uptake, which is initiated only at higher concentrations (Siddiqi *et al*. 1990; Aslam *et al*. 1992).

Standard errors and confidence limits (Student's $t$ $P$ = 0·95) are denoted in the text by ± or by horizontal and vertical error bars in figures.

## RESULTS

### Induction of nitrate uptake depending on light conditions

For plants grown for 10 to 14 d in N-free medium, full induction of nitrate uptake (reaching the maximal uptake rate) by 1 mol $m^{-3}$ nitrate required 4–6 h in low light (Fig. 1a) or in high light (Fig. 1b) and led to similar uptake rates (6·6 ± 0·2 or 6·9 ± 0·2 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$, respectively). These rates were 1·5–3-fold higher than in pre-induced control plants (1 mol $m^{-3}$) of the same age and remained at this higher rate for at least 2 d (data not shown). In darkness, induction led to similarly high rates (6·4 ± 0·2 in 5–15 h after starting; Fig. 1c), but then decreased after about 24 h. There was a lag phase in induction under both light conditions and in darkness (Figs 1a–c). In order to calculate the kinetics of the uninduced uptake system, 1 mol $m^{-3}$ fluorophenylalanine (FPA) was added to the medium at the beginning of the experiments. In the presence of 1 mol $m^{-3}$ FPA the nitrate-dependent increase was totally abolished (Fig. 1d). The mean rate of nitrate uptake (1·3 ± 0·1 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$) was several-fold lower than that of newly induced or control plants.

### Effects of light conditions on time courses of net nitrate uptake rates in induced plants

For these experiments, plants were grown in 1 mol $m^{-3}$ nitrate and a 16:8 h light–dark cycle, and were transferred to the uptake conditions 2 to 4 h before starting the uptake measurements (at 1200 h) with 1 mol $m^{-3}$ nitrate.

Under low light conditions, rates of nitrate uptake remained at a low level for 10 h (2·2 ± 0·3 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$) after which a slight increase was observed (Fig. 2a). However, in high light the observed uptake rates at the beginning were more than doubled compared to low light and an acceleration of nitrate uptake occurred at about 2200 h, which corresponded to the time the lights were turned off during the pre-cultivation (Fig. 2b). This effect was even more pronounced at lower nitrate concentrations (data not shown; see 'Discussion'). The uptake rates increased from 4·7 ± 0·4 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$ to a relatively constant level of 5·4 ± 0·3 (same units, 12–26 h after starting).

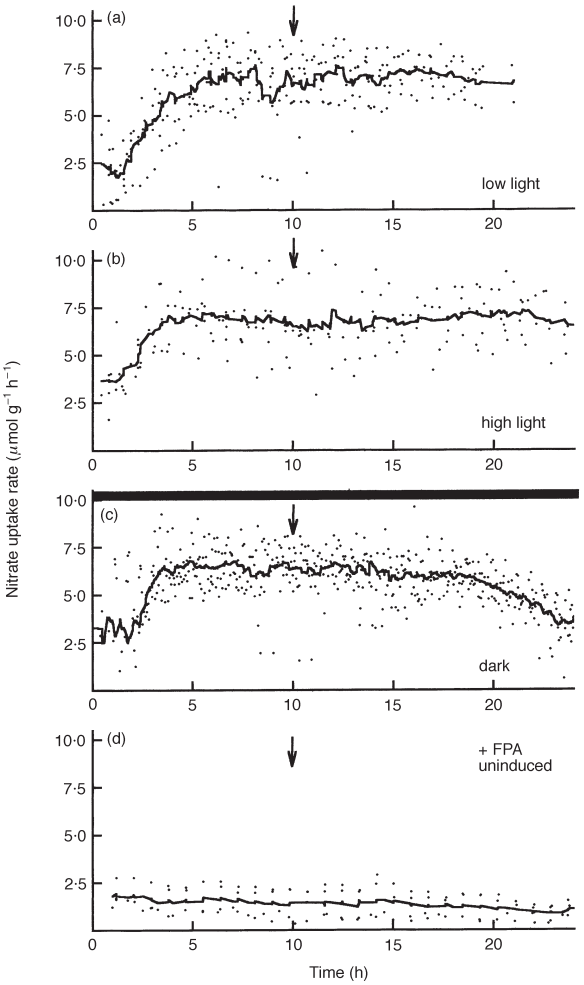


**Figure 1.** Time course of net nitrate uptake rates after transferring uninduced plants (grown for 10 to 14 d in N-free medium) to nitrate-containing medium. Shown is the moving average (period: *n*/25, as solid line) of all calculated uptake rates (symbols) as a function of time. Barley seedlings grown in N-free medium with a light–dark cycle (16 h photoperiod at 300–500 $\mu$mol photon $m^{-2}$ $s^{-1}$) were transferred to 1 mol $m^{-3}$ nitrate-containing medium (a) under continuous low photon flux density (50 $\mu$mol photon $m^{-2}$ $s^{-1}$, $n$ = 263), (b) under continuous high photon flux density (500 $\mu$mol photon $m^{-2}$ $s^{-1}$, $n$ = 329), (c) in the dark ($n$ = 520), and (d) in continuous high photon flux density plus 1 mol $m^{-3}$ FPA in the medium ($n$ = 215). Arrows indicate the time that lights were switched off in pre-cultivation and the black box indicates darkness in the experiments.

A similar acceleration also occurred if high light was switched off at 2200 h, but if plants were kept in continuous darkness, the acceleration was transient and the rate of nitrate uptake decreased markedly to a very low level ($0{\cdot}9 \pm 0{\cdot}1$ $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$) after about 30 h (Fig. 2c).

If a light–dark cycle with high light was maintained, as in pre-cultivation, then nitrate uptake oscillated (Fig. 2d): it increased at about the onset of darkness (2200 h) and decreased after the beginning of the light period (0600 h).

In all long-term experiments (over 3 d), a slight increase in uptake rates was observed (Figs 2b & d) if high light was applied, which was caused by plant growth. For obvious reasons, fresh weights of roots were taken only at the end of the experiment, but these weights were used as a basis for assessing the uptake rates at the beginning of the experiments as well. In experiments conducted over a shorter time (24 h) this played a minor role.

## Effects of photon flux density and induction status on kinetic parameters

The effect of external nitrate concentration (from 5 mmol $m^{-3}$ to 10 mol $m^{-3}$ $NO_3^-$) on net nitrate uptake was

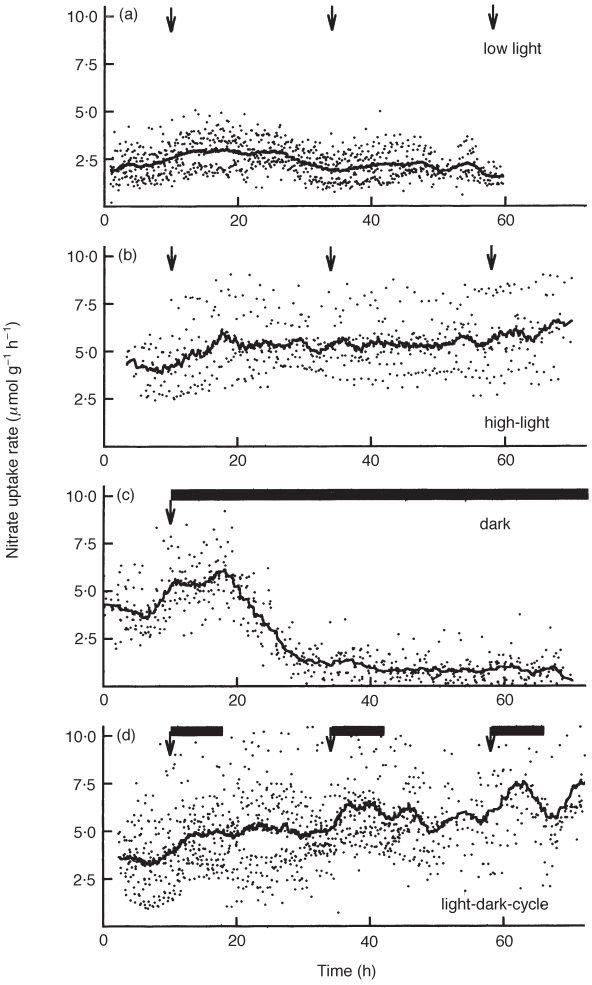


**Figure 2.** Time course of net nitrate uptake rates of induced plants in 1 mol $m^{-3}$ nitrate-containing medium. Shown is the moving average (period: *n*/25, as solid line) of all calculated uptake rates (symbols) as a function of time. Barley seedlings grown in 1 mol $m^{-3}$ nitrate-containing medium with a light–dark cycle (16 h photoperiod at 300–500 $\mu$mol photon $m^{-2}$ $s^{-1}$) were transferred to different light conditions. (a) Continuous low photon flux density (50 $\mu$mol photon $m^{-2}$ $s^{-1}$, $n$ = 912); (b) continuous high photon flux density (500 $\mu$mol photon $m^{-2}$ $s^{-1}$, $n$ = 524); (c) 10 h in high light then in continuous dark conditions ($n$ = 548), and (d) a light–dark cycle with high light conditions during the light period ($n$ = 1113). Arrows indicate the time that lights were switched off in pre-cultivation and black boxes indicate darkness in the experiments.

studied in uninduced and newly induced plants and in induced plants under different light conditions (Fig. 3). Data were taken from an 8 h period during the day in which uptake rates remained constant, as shown in the previous time course experiments (see Figs 1 & 2). Thus for low and high light conditions data were used from 1 to 9 h after the start of the experiment, for accelerated uptake from 12 to 20 h and in newly induced plants from 6 to 15 h. In both high light and low light, uptake was first saturated below 0·5 mol $m^{-3}$ but increased further up to 10 mol $m^{-3}$ (Figs 3a & b), whereas accelerated uptake in high light (compare Fig. 2b after 10 h) seemed to be almost fully saturated at 1 mol $m^{-3}$ $NO_3^-$ (Fig. 3c). Rates were considerably lower in low light than high light conditions over the whole concentration range. Uninduced nitrate uptake (in the presence of FPA for 8 h) showed some saturation at low concentrations but then increased substantially as the external concentration increased. In newly induced plants (at least 6 h after induction) a strong increase in uptake rate in the low concentration range occurred (Fig. 3e) similar to the accelerated uptake (Fig. 3c).

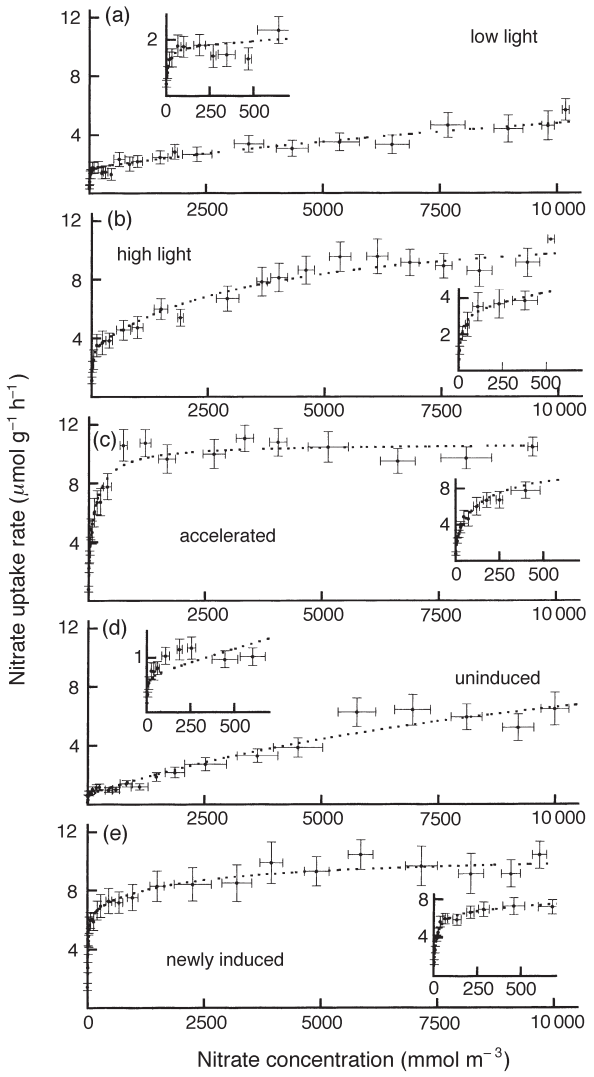


**Figure 3.** Concentration dependence of net nitrate uptake rates of induced and uninduced barley seedlings grown in a medium containing 1 mol $m^{-3}$ nitrate or N-free with a light–dark cycle (16 h photoperiod at 300–500 $\mu$mol photon $m^{-2}$ $s^{-1}$) and then transferred to different light conditions. (a) Continuous low photon flux density (50 $\mu$mol photon $m^{-2}$ $s^{-1}$); (b) continuous high photon flux density (500 $\mu$mol photon $m^{-2}$ $s^{-1}$); (c) accelerated uptake observed 10 h after transfer to high light conditions, (d) high light conditions with uninduced plants plus 1 mol $m^{-3}$ FPA, and (e) high light conditions with newly induced plants after 6 h. The symbols are the means of about 100 data points. Bars indicate confidence limits (Student's *t* $P = 0{\cdot}95$) and the dotted lines are the theoretical curves based on two saturable systems HATS and LATS (compare Table 1 and Fig. 4). The inserts show uptake in the low concentration range.

Fitting of the five equations mentioned in the 'Materials and methods' section to the uptake data led to good agreement with a model of two saturable systems – a high-affinity (HATS) and a low-affinity (LATS) transport system – for all treatments with regard to the calculated standard errors and in accordance with the concentration-dependent uptake rates (Fig. 3). The equations with a saturable and a linear system resulted in the calculation of high apparent $K_m$ values (for example 12 kmol $m^{-3}$ under low light and up to 60 kmol $m^{-3}$ in newly induced plants) which were unphysiological for the saturable system (in particular for high light, accelerated, and newly induced). Furthermore, in many cases addition of a linear component to the models with two saturable systems also led to an unacceptable prediction, in this case calculated $K_d$s which were so low ($< 0{\cdot}1\ 10^{-6}$ $m^3$ $g^{-1}$ $FW_R$ $h^{-1}$) as to result in a negligible uptake activity. Both types of model were omitted from subsequent discussions. Models with the two saturable systems are shown in Fig. 4. Other attempts to fit curves to the experimental data resulted in higher standard errors of parameters. Thus, only the results from the model with two saturable systems will be described (Table 1).

In the case of high light – the 'normal' conditions for growth of barley – the HATS had an apparent $K_{m1}$ of 12·2 mmol $m^{-3}$ and an apparent $V_{max1}$ of 3·4 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$, while parameters for the LATS were higher (apparent $K_{m2}$ 3·7 mol $m^{-3}$ and apparent $V_{max2}$ 8·8 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$). Acceleration of uptake in high light decreased apparent $K_{m1}$ somewhat to 7·7 mmol $m^{-3}$ but apparent $K_{m2}$ (LATS) decreased substantially to 0·17 mol $m^{-3}$ without altering the apparent $V_{max}$ values. Low light conditions, on the other hand, strongly decreased the apparent $V_{max1}$ (HATS) but greatly increased apparent $K_{m2}$ to 24·3 mol $m^{-3}$, resulting in an almost linear concentration dependence for LATS up to 10 mol $m^{-3}$ nitrate. The role of the LATS in total uptake increased with light conditions. The LATS contributed more to total nitrate uptake under control conditions (1 mol $m^{-3}$ nitrate used for example in induction or time course experiments) in high light after acceleration (68%) relative to high light before acceleration (36%) or in low light (19%) (Figs 4a–c).

The effects of newly induced uptake conditions were almost opposite to those of low light, the apparent $V_{max1}$ of HATS being doubled and the apparent $K_{m2}$ and apparent $V_{max2}$ being substantially decreased. Finally, in the case of

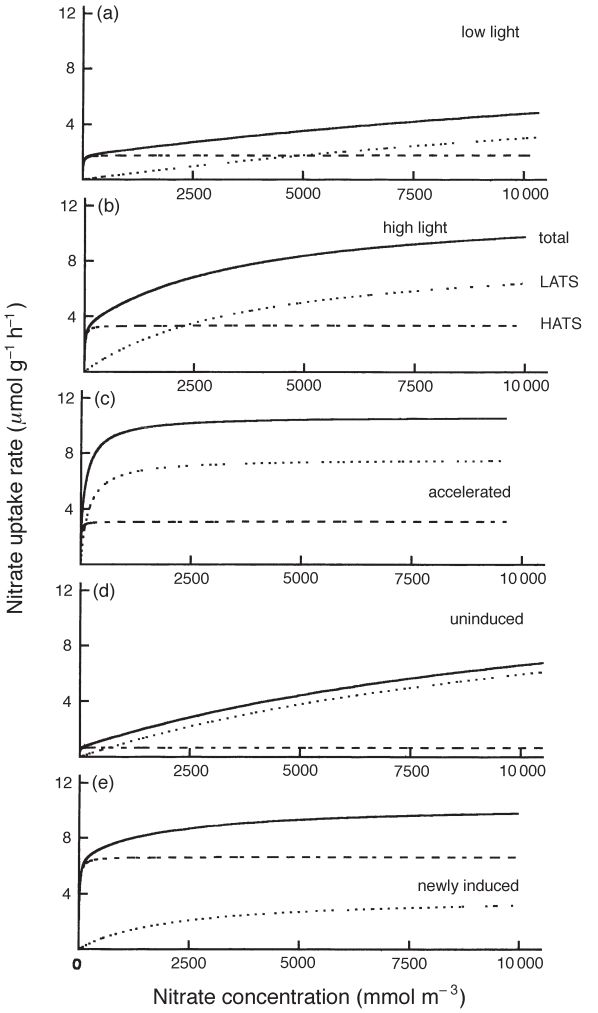


**Figure 4.** Kinetic models of net nitrate uptake in barley in (a) continuous low photon flux density (50 $\mu$mol photon $m^{-2}$ $s^{-1}$), (b) continuous high photon flux density (500 $\mu$mol photon $m^{-2}$ $s^{-1}$), (c) accelerated uptake observed 10 h after transfer to high light conditions, (d) high light conditions with uninduced plants plus 1 mol $m^{-3}$ FPA, and (e) high light conditions with newly induced plants (after 6 h). The models were calculated with an equation assuming two saturable systems (HATS + LATS). Medium dashed lines: HATS; dotted lines: LATS; solid lines: fitting of the total system. The related kinetic parameters are given in Table 1.

nitrate uptake in uninduced plants (in the presence of 1 mol $m^{-3}$ FPA), both the apparent $V_{max1}$ and the apparent $K_{m1}$ of HATS were substantially decreased (the apparent affinity increased), whereas the apparent $K_{m2}$ and the apparent $V_{max2}$ of the LATS were considerably increased, to values similar to those in low light conditions. Due to the induction by external nitrate the contribution of the LATS to total uptake at 1 mol $m^{-3}$ nitrate decreased from 59% in uninduced plants to only 16% in newly induced barley (Figs 4d & e).

In only two cases, in low light and in the uninduced system (in presence of 1 mol $m^{-3}$ FPA), models other than HATS plus LATS can possibly be used for fitting the data. In the case of nitrate uptake under low light, the uptake system in the higher concentration range could also be satisfactorily fitted by a linear component instead of a saturable LATS (HATS apparent $K_m$: 11·1 mmol $m^{-3}$, apparent $V_{max}$: 1·9 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$; LATS $K_d$: 0·31 $10^{-6}$ $m^3$ $g^{-1}$ $FW_R$ $h^{-1}$). In uninduced plants it seems to be possible that only one saturable LATS-like system is working, since in three of the five models a quantitatively relatively significant saturable uptake system with a low apparent affinity was calculated (apparent $K_m$: 5·1–6·5 mol $m^{-3}$, apparent $V_{max}$: 8·3–10·8 $\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$; data not shown in Table 1).

## DISCUSSION

### Effects of photon flux density during induction

At both light intensities as well as in the dark, some 4 to 6 h, possibly including a short lag phase, were required for a full induction of the nitrate uptake system by external nitrate. A similar induction period was found in excised barley roots (4 h, Behl *et al*. 1988) and in sugar beet (5–6 h, Mäck & Tischner 1990). A more rapid induction (1–2 h, Neyra & Hageman 1975) was found in maize and a slower one (10 h, Goyal & Huffaker 1986b) in wheat.

After being induced for more than 6 h, nitrate uptake proceeded at a higher rate than in pre-induced nitrate-fed plants, presumably due to higher demand for N after growth in N-free medium. These high uptake rates were found in all light conditions as well as in the dark. Increased influx/uptake rates in response to nitrogen limitation have been observed previously (Lee & Rudge 1986; Lee & Drew 1986; Siddiqi *et al*. 1990).

As has been found earlier (Behl *et al*. 1988; Mäck & Tischner 1990), the phenylalanine analogue FPA fully depressed nitrate-dependent induction and in its presence uptake occurred at a very low and slowly declining rate (Fig. 1d). This agrees with earlier literature showing that inhibitors of transcription or translation blocked induction of the transport system (Hole *et al*. 1990) and almost every metabolic function. The continued low rate of uptake appears to be mediated by constitutive systems, which may be responsible for the nitrate-dependent induction (Behl *et al*. 1988). Treated plants were therefore used to evaluate the kinetics of the uninduced uptake systems.

### Time courses of net nitrate uptake rates

The light regime was responsible for very significant changes in the rate of net nitrate uptake in induced whole barley seedlings. Whereas in constant low light, uptake occurred at a low rate for the first 10 h (Fig. 2a), it was increased about 2-fold during the first 10 h in continuous high light and then was accelerated further by almost 16% (Fig. 2b). The increase in the rate of uptake was more pronounced at lower nitrate concentrations than under the applied control conditions of 1 mol $m^{-3}$ (data not shown). This acceleration coincided approximately with the time at which the dark period had started previously during plant

| | Low light | High light | Accelerated | Uninduced | Newly induced |
|---|---|---|---|---|---|
| $K_{m1}$ | 9·2 ± 2·4 | 12·2 ± 3·9 | 7·7 ± 5·8 | 3·6 ± 3·0 | 11·9 ± 1·8 |
| $V_{max1}$ | 1·8 ± 0·1 | 3·4 ± 0·2 | 3·1 ± 1·1 | 0·7 ± 0·1 | 6·7 ± 0·3 |
| $K_{m2}$ | 24 300 ± 11 700 | 3740 ± 740 | 170 ± 47 | 13 300 ± 2230 | 2090 ± 890 |
| $V_{max2}$ | 10·4 ± 3·6 | 8·8 ± 0·5 | 7·6 ± 1·0 | 13·9 ± 1·4 | 3·8 ± 0·3 |

**Table 1.** Kinetic parameters of net nitrate uptake in barley (*Hordeum vulgare* L. cv. California Mariout) assuming two saturable systems (HATS and LATS). Kinetic parameters of net nitrate uptake in barley seedlings were measured in a: continuous low photon flux density (50 $\mu$mol photon $m^{-2}$ $s^{-1}$) ($n$ = 2348), b: continuous high photon flux density (500 $\mu$mol photon $m^{-2}$ $s^{-1}$) ($n$ = 2011), c: accelerated uptake observed 10 h after transfer to high light conditions ($n$ = 1915), d: high light conditions with uninduced plants plus 1 mol $m^{-3}$ FPA ($n$ = 2362), and e: in high light conditions with newly induced plants ($n$ = 2127). $K_m$: Michaelis-Menten constant (mmol $m^{-3}$); $V_{max}$: maximal uptake rate ($\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$); standard errors ($P$ = 0·95) calculated by sigmaplot 4·1 are given as ±

growth (2200 h). It occurred not only in continuous light but also if continuous light was followed by continuous dark (Fig. 2c) or by a dark–light cycle (Fig. 2d). These observations suggest that acceleration of uptake was due to an endogenous mechanism associated with a diurnal rhythm, which normally led to a transient increase in the rate of uptake at about the time of the onset of darkness, which persisted in continuous light. The same observation under the same conditions was also found in two other Poaceae, *Leptochloa fusca* and *Triticum durum* (Peuke & Jeschke, unpublished). The acceleration was not, however, found in newly induced plants (Fig. 1) in which uptake already occurred at the 'highest possible' rate. Under low photon flux density and to a greater extent in continuous dark, nitrate uptake apparently was light (energy)-limited and this prevented a strong increase at the time when the dark period would have started.

The marked enhancement of nitrate uptake caused by photon flux density from 0·9 (dark) to 2·2 (low light), 4·7 (high light) and 5·4 $\mu$mol $h^{-1}$ $g^{-1}$ $FW_R$ (high light, accelerated) indicates the energy dependence of nitrate uptake (and assimilation), and the observed increase exceeds by a long way the extent to which light stimulated nitrate uptake in earlier studies (Rao & Rains 1976b: 50%, Aslam *et al*. 1979: 20%, both in barley). In contrast, Clement *et al*. (1978) found the mean nitrate uptake rates in natural light in winter to be only about 5% of those found in summer while photon flux density dropped to 10%. In the summer the diurnal change in uptake due to radiation was only a factor of about 3 at most in these experiments. The reason for the rhythmic stimulation we observed in the dark might be the high energy status of the plants, since a long period of darkening was required before uptake declined substantially (Fig. 2c). The present plants were relatively well supplied with light during the 16 h photoperiod. A similar acceleration of nitrate uptake after the onset of darkness (see Fig. 2d) has been found in *Spinacia oleracea* (Steingröver, Ratering & Siesling 1986) but a contrasting pattern with a decrease in the dark has been observed in *Capsicum annuum* (Pearson & Steer 1977). Delhon *et al*. (1995) showed for the first time that the decrease in net nitrate uptake in soybean in the dark was due to lower influx of nitrate. Additionally, nitrate reduction and xylem transport in soybean were decreased while the efflux remained constant. The authors related this effect to a feedback control. However, Scaife & Schloemer (1994) found no effect of darkness on the time course of nitrate uptake in spinach. An essential point was not only the light conditions during the experiments but also the pre-treatment of the plants with light. Rao & Rains (1976b) showed that nitrate uptake was higher in light- than in dark-grown barley, but the effect during the experiments was less than that of the light treatment during pre-cultivation. The decrease in the rate of nitrate uptake due to darkness was stronger in carbohydrate-depleted plants (grown in the dark) than in control plants (Aslam *et al*. 1979). Rideout & Raper (1994) showed that nitrate uptake declined in association with depletion of carbohydrates in shoots as well as in roots. These authors concluded that nitrate uptake was regulated by the flows of carbohydrates from the shoots to the roots. However, Bloom, Sukrapanna & Warner (1992) calculated that only 5% of root carbon catabolism was coupled to nitrate uptake. In the present investigations the plants were supplied very well with light in a 16 h photoperiod in pre-cultivation compared with other greenhouse plants. This might be one of the main reasons for the observed effects.

## Kinetic parameters

Light acted on the supply of energy, but it was unlikely that the nature of nitrate uptake systems was altered by light conditions. The calculation of the kinetics on the basis of concentration dependence of nitrate uptake in barley revealed the operation of two saturable corresponding uptake systems under all light conditions: a high-affinity (HATS) and a low-affinity (LATS) transport system. This model conformed best with the measured data with regard to the obtained standard errors (data not shown) and the fitting of curves to the means of uptake rates (Fig. 3). Apparent $K_m$ values of HATS in induced plants ranged

between 8 and 12 mmol $m^{-3}$ under all light conditions (Table 1), agreeing well with other values found in depletion experiments in barley (7 mmol $m^{-3}$, Aslam *et al*. 1992), but lower than those found in maize (24 mmol $m^{-3}$, Hole *et al*. 1990), in wheat (27 mmol $m^{-3}$, Goyal & Huffaker 1986b) and in *Arabidopsis* (40 mmol $m^{-3}$, Doddema & Telkamp 1979) or in excised barley roots in earlier reports (110 mmol $m^{-3}$, Rao & Rains 1976a). Similar agreements were found for the $^{13}N$-$NO_3^-$ influx (Lee & Drew 1986: 14 mmol $m^{-3}$; Siddiqi *et al*. 1990: between 30 and 79 mmol $m^{-3}$ depending on the N status of the plants). For further kinetic data and models, see the review by Peuke & Kaiser (1996). The effect of light on the HATS in the present experiments was an increase of the apparent maximal activity only.

It was more difficult to describe the LATS on the basis of net nitrate uptake data. In the higher concentration range (up to 10 mol $m^{-3}$ nitrate) the uptake of nitrate fitted well to a second saturable system (LATS), which had a rather low apparent affinity in low light (apparent $K_m$ = 24·3 mol $m^{-3}$) and a much higher one (apparent $K_m$ = 0·17 mol $m^{-3}$) in high light after the acceleration phase. The apparent affinity of LATS in low light compares well with the apparent $K_m$ (25 mol $m^{-3}$) of the second saturable system found in *Arabidopsis* by Doddema & Telkamp (1979). This large increase in the apparent affinity of the LATS in low light is difficult to explain.

Siddiqi *et al*. (1990) and Aslam *et al*. (1992) proposed that a linear system operated in this concentration range, but only the LATS observed in low light could be alternatively described as having a linear dependence, because the range of measurements (up to 10 mol $m^{-3}$) was far below the apparent $K_m$ (24·3 mol $m^{-3}$). A saturable plus a linear component model resulted in similar kinetic parameters for the HATS (Table 1) and a $K_d$ of $0{\cdot}31 \times 10^{-3}$ $m^3$ $g^{-1}$ $h^{-1}$ for the linear system starting near zero nitrate. This model also fitted relatively well with the nitrate uptake rates depending on external nitrate concentration (data not shown; compare Fig. 3a.). The rate constant was in the range of the rate constant calculated by Siddiqi *et al*. (1990) ($0{\cdot}57\ 10^{-3}$ $m^3$ $g^{-1}$ $h^{-1}$), but here the linear component started at a higher nitrate concentration (> 200 mmol $m^{-3}$). However, since it is rather unlikely that light supply to the foliage would alter the principal systems of nitrate transport in the root, it is suggested that a saturable low-affinity system LATS in addition to HATS operates under all light conditions. An important consideration might be the methods used for investigating the nitrate uptake systems. In the present paper net uptake data (the sum of influx and efflux) were used for kinetic analysis, and from analysis of 'best fit' a system with two saturable system was found. However, different kinetic models have been found with the help of the depletion method as well as for the tracer technique (for a review, see Peuke & Kaiser 1996).

Summarizing the results, high light conditions (a) substantially increased the apparent $V_{max}$ of the HATS without changing its apparent affinity and (b) increased the apparent affinity of the LATS. The increase in the apparent $V_{max}$ of HATS can be compared directly with the action of light versus dark on ion uptake by leaves and is in agreement with an increased energy supply (Jeschke 1976). The increase in apparent affinity of LATS is less easily explained and requires more knowledge of the nature of this low-affinity 'uptake system'. Effects of subsequent energy-dependent steps of nitrate metabolism such as xylem transport, reduction, and amino acid and protein biosynthesis which may regulate uptake cannot be excluded. However, Warner & Huffaker (1989), for example, have excluded the possibility that nitrate reductase had a direct role in nitrate uptake. Another problem arises from the difficulty of measuring the net nitrate uptake by depletion at high nitrate concentrations, which was responsible for the high errors in this range. Additionally, efflux effects became more prominent at high external nitrate.

As a consequence of this dual action of increasing light supply, in the increased apparent $V_{max}$ of the HATS and the increased apparent affinity of the LATS, the light-dependent enhancement of nitrate uptake was much more pronounced at lower than at higher external concentrations of nitrate (see Figs 3a & c), and in particular the acceleration of nitrate uptake when the dark period started (Figs 2b–d) was virtually abolished at 10 mol $m^{-3}$ external nitrate (compare Figs 3b & c).

Whereas different light conditions did not alter the principal pattern of the concentration dependence of nitrate uptake in induced plants displaying HATS and LATS, uninduced plants might possess only one of the two nitrate uptake systems, operating at low rates, as suggested by Behl *et al*. (1988). These authors proposed that uptake in uninduced roots was mediated by a low-capacity system only. The present data, however, suggest the presence of a saturable uptake component at concentrations below 0·5 mol $m^{-3}$ (see Fig. 3d). The kinetic analysis indicates a HATS with low apparent $K_m$ and apparent $V_{max}$ and in addition a low-affinity system with an intermediate apparent affinity and a high apparent $V_{max}$. The low values of kinetic parameters of HATS in uninduced compared to induced plants were in agreement with the findings of Siddiqi *et al*. (1990) Aslam *et al*. (1992). In contrast, in these papers a linear LATS was also proposed in uninduced plants. But in the present experiments the means of uptake rates could never be described satisfactorily by a kinetic model including a linear system. In uninduced plants, the model with one saturable system (Behl *et al*. 1988; Mäck & Tischner 1990) fitted the data in the high concentration range but not in the low concentration range (apparent $K_m$ = 6500 ± 520 mmol $m^{-3}$, $V_{max} = 10{\cdot}7 \pm 0{\cdot}4\,\mu$mol $g^{-1}$ $FW_R$ $h^{-1}$). In essence, our data suggest that, in uninduced barley roots, both basic systems of nitrate uptake are present. It might be that induction by external nitrate increases the number of transporters, particularly those of the HATS. Similar conclusions were drawn from studies of $^{13}NO_3^-$ influx in the low concentration range (Lee & Drew 1986). This is supported by the finding that in uninduced plants the HATS

contributed only 41% to total nitrate uptake at 1 mol $m^{-3}$ (control) and that this percentage was increased to 84% in newly induced plants (Fig. 4).

## Conclusions

The time course at which nitrate uptake *per se* was induced was independent of light conditions. The changes in the time courses of net nitrate uptake with an acceleration in the dark phase was assumed to be due to circadian mechanisms. The observed rates of nitrate uptake in response to light conditions or induction status could be described best with a model of two saturable systems (HATS + LATS). Both systems were present and active under all conditions and contributed, in various proportions, to a single system of at least 16% of the total uptake. The calculated apparent $K_m$s of the HATS were relatively constant under all conditions applied, but the apparent $V_{max}$ varied: it was low in low light and uninduced plants and high in high light and newly induced plants. The calculated kinetic parameters of the HATS were in agreement with literature values. However, description of the LATS was more difficult; the apparent $K_m$ varied by a factor of 140 and the apparent $V_{max}$ also varied, which cannot yet be explained adequately. Several effects may interfere with the calculation of the kinetics of this 'uptake system'.

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