

## The effect of light on nitrate uptake by wheat roots

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### Abstract

Illuminating shoots stimulates nitrate uptake by wheat (*Triticum aestivum* L. cv. 'EM18') roots. A method with a high time resolution (minutes), non-invasive technique, has enabled to measure the nitrate uptake time coarsely. The nitrate uptake by wheat roots increases in the light and decreases in the dark. The mechanism is thought to be via a signal carried in phloem, probably a sugar.

**Keywords:** *Triticum aestivum* L., nitrate uptake, non-invasive, light, time course

### Introduction

The growth of plants depends on taking up carbon from the air and nitrogen from the soil in a ratio of approximately 30C: 1N (plus other essential elements at much lower rates). Thus the assimilatory mechanisms of plants are dominated by photosynthesis and nitrate uptake. If photosynthesis is perturbed (by changes in light intensity or CO<sub>2</sub> concentration, for instance) then nitrate uptake must change to maintain the N supply at the necessary rate, and if nitrate uptake is perturbed it must recover its former rate in order to continue matching the rate of photosynthesis (or photosynthesis must respond). Such adjustments have been observed [1,2].

C/N interactions are a matter of control, as distinct from mechanisms of processes per se, about which much is already known. Understanding control depends on knowing not only pathways of signaling, but also the time courses of adjustments of cellular processes involved in the pathway, and the latter is our main concern here.

We are interested in the way changes in light intensity on the shoot directly affect nitrate uptake by the root, rather than in inbuilt daily rhythms which can also affect N uptake [3].

Previous work has shown that light received by shoots can stimulate nitrate uptake by roots, (measured as net nitrate influx or as net 15N influx) in all species examined, but the magnitude of the response varies with species, environmental conditions and light intensity. For instance, nitrate uptake increases 20% to 40% within a few hours and then becomes steady when plants

are transferred from dark to light in barley [4] and decreases by a similar amount on transfer from light to dark in tobacco and soybean [5,6]. In prolonged darkness, the nitrate uptake rate may subsequently decline even further [7,8]. In tomato the initial adjustment of nitrate uptake takes longer, increasing in the light and decreasing in darkness continuously over a period of 6 h or more [9].

No detailed time courses of these responses have been reported although they are important characteristics of C/N interactions, one of which is the response of plant nutrient uptake to the demands from growth.

In examining time courses of responses to experimental perturbations, the time scale is critical. One needs to measure changes in nitrate uptake rates with sufficiently high time resolution to identify and characterize lags and initial changes in uptake rates. Some steps in signaling pathways, for instance gene expression and protein phosphorylation, may be initiated virtually instantaneously after an experimental perturbation and may be completed in a matter of minutes, though the length of time varies from one process to another (e.g., [10,11]). Measurements of nitrate uptake with a time resolution of minutes are therefore needed. However, in most published work on nitrate responses to light, time courses have generally been measured with relatively few points, none less than 1 h apart, and often after 4 or 6 h. Peuke and Jeschke have provided systematic time courses with more frequent measurement, but a lag of the order of minutes would not be visible in their system because of the variability of the measured uptake rates [8].

In order to obtain the time courses with a high enough resolution to answer the questions posed above, we have used a new technique, which enables very detailed measurement of changes in net nitrate uptake by the root system of intact, growing wheat plants. We present the first systematic set of detailed time courses of the response of net nitrate uptake to the fundamental experimental perturbations – varying illumination of the shoot.

In the periods of growth and pretreatment, one twentieth Long Ashton solution containing 0.3 mM nitrate will have fully induced the nitrate transporters (e.g., [12]). Moreover, given that

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the  $K_m$  of both induced and constitutive high affinity nitrate transport systems is 20–50  $\mu\text{M}$  (e.g., [13]), the concentration used in these experiments will saturate them. On the other hand, the low affinity transport systems, with  $K_m$  above 1 mM, will transport little at 0.3 mM nitrate. For example, with a  $K_m$  of 3.7 mM as in barley [8] the low affinity transport system would be operating at only 4% of its maximum capacity and add only a few percent to the total measured nitrate uptake in these experiments. The results of the paper therefore concern the characteristics of the fully induced, high affinity nitrate transport systems.

## Material and methods

### Plant culture

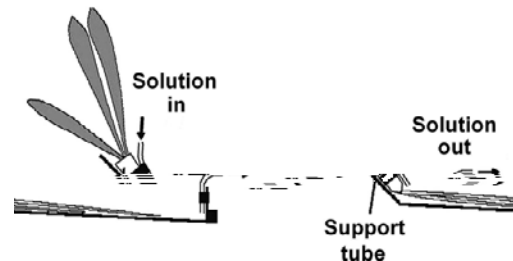
Wheat (*Triticum aestivum* L. cv. 'EM18') plants were grown in one fourth Long Ashton nutrient solution [14] at 20°C ( $\pm 2^\circ\text{C}$ ) with a 16 h photoperiod (Tab. 1), light flux density of 400  $\text{mol m}^{-2} \text{s}^{-1}$  (TPS-1 portable photosynthesis meter, PP Systems International Ltd., England). The bottom and sides of the container were enclosed in black paper, to minimize algal growth. The top of the container was covered with black sponge, into which small plastic tubes, open at both ends, could be inserted. Wheat seedlings (germinated for 3–5 days) were placed using tweezers with the seed in these small plastic tubes, to provide support and allow transfer from culture solution to experimental set-up with minimal handling. Air was pumped through the culture solution using an aquarium pump with the inflow tube plugged through sponge. The water level was replenished every morning and the solution replaced regularly.

**Tab. 1** The full Long-Ashton nutrient solution used in this study as taken from Hewitt [14].

Salt	Concentration (mg/l)
$\text{KNO}_3$	202
$\text{K}_2\text{SO}_4$	86
$\text{Ca}(\text{NO}_3)_2$	328
$\text{CaCl}_2$	222
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	184
$\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$	208
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	2.24
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	0.25
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.29
$\text{H}_3\text{BO}_3$	3.1
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	0.121
$\text{NaCl}$	5.86
$\text{Fe-Citrate}(3\text{H}_2\text{O})$	5.98

To measure nitrate uptake, 20–30 days old plants were removed from the culture vessel and laid in a large tray with culture solution. Individual roots were gently teased apart with a toothpick. The plant was then transferred to a Perspex tray 20 mm wide, 450 mm long, with raised sides 5 mm high, and held a slope of 5° from the horizontal. The roots were aligned along it and covered with transparent polythene film to eliminate “dead” volume, maximize flow over the whole root system and

minimize evaporation of the nutrient solution. The trays were enclosed in black paper kept the roots in the dark during the experiments. The base of the shoot was held in the same small tube as during initial growth. This holder containing the plant was attached to the upper end of the tray, which was bent up at a slightly greater angle as illustrated in Fig. 1. The leaves were nearly upright. Enclosing in black paper also reduced light falling on the root system to near zero, and thus minimized algal growth on the roots. No algal growth was visible on cleaned roots once set up in the experimental trays.



**Fig. 1** Set-up for non-invasive measurement of net nitrate influx.

Gentle physical disturbance of plants can inhibit nitrate uptake for more than 6 h [15]. To minimize the transplant shock, we set up the intact wheat plant in the experimental trays at least 14 h before starting experiments. Solutions were changed by shifting the inflow tube from one solution to another. Plants were regularly used for several successive days. Roots and leaves visibly extended day by day. Growth rates in the experimental trays were estimated from the slope of the plot of  $\ln$  (final plant wt) vs. time.

### Non-invasive measurement of net nitrate influx

To avoid any ambiguity, net nitrate influx = influx across the plasma membrane – efflux across the plasma membrane, detected by loss of nitrate from the bathing solution.

A technique for measuring net nitrate uptake rates by root systems of intact plants growing at a normal rate was used (Cram and Minchin, unpublished data). This method has high time resolution as is necessary for the experiments reported here. Fig. 1 shows the set-up. Various dilutions of the nutrient solution were pumped in at the top of the root system and flowed over the roots from top to bottom of the tray, then pumped out to an automatic sample collector BS-100A (Shanghai Hu Xi Analysis Instrument Factory Co. Ltd., China). The outflow pump rate was three times greater than the inflow rate to ensure there was no overflow.

The nitrate concentration in the outflow solution ( $C_{out}$ /mM) collected after flowing over the root system was measured using an Ion/pH-Analyser ELIT 9801 (EA Instruments Ltd., UK), calibrated regularly against the inflow solution ( $C_{in}$ /mM) to allow for drift. The volume flow rate ( $V \text{ ml min}^{-1}$ ) regulated by a peristaltic pump BT03-DG-8 (Tianjin City Xieda Weiye Ltd. Co., China) was checked by obtaining the difference between the weight of each test tube before and after collecting solution, then dividing by the time collecting each sample. The root system was cut off and weighed ( $W_R$ , g fwt) at the end of a set of measurements, each of which lasted 3–5 days. Net nitrate uptake rate was calculated as:

$$\Phi_{1-\#} = \frac{(C_{in} - C_{out})V}{W_R} \mu\text{mol g fwt}^{-1} \text{ h}^{-1}$$

The speed of turnover of solution flowing over the roots was checked by injecting a pulse of Indian ink at the inflow end and following its clearance. Clearance of the solution round the root system was 80% completed in 1 min and virtually no ink was visible in the tray after 2 min. At any instant the volume of solution surrounding the root system was 1–2 ml, depending on the size of the roots.

To test for artificial changes in solution concentration, for instance by evaporation from the unenclosed collecting end of the trough, roots were replaced by inert plastic strips. No change was then observed in the concentration of solution when it flowed over the system. Changes observed in experiments must therefore have been due to uptake of nitrate by the root system itself. The plant will be transpiring at a normal rate during the experiments. This would concentrate the solution as it flows over the root. For every 1 g dry wt, with a transpiration ratio of 200 g H<sub>2</sub>O [16] and a nitrogen content of 2 mmol, the ratio of water to nitrate uptake is 100 l/mol N. A root of 1.5 g fwt taking up nitrate at 7 mol g fwt<sup>-1</sup> h<sup>-1</sup> would then take up water at 1.5 × 7 × 100 l ml h<sup>-1</sup>. With a flow rate of 180 ml h<sup>-1</sup> transpiration would concentrate the nutrient solution by about 0.5%, which is insignificant. It should be noted, however, that with slower flow rates than used in these experiments or if the solution is recycled, then significant errors could arise due to transpirational uptake of water that would have to be measured and corrected for.

#### Light-dark, dark-light treatments

For each experiment, two plants were used, each set up in an individual tray as described above. In principle, one plant was used to measure the time course of the effect of a treatment on net nitrate influx while the other plant acted as a control, though the detail varied from experiment to experiment as described below.

In the laboratory, lamps were switched on at 6.00 h and off at 22.00 h. Measurements of net nitrate influx were started at 8.00 h, and treatments were applied after 5 h or more when the net nitrate influx was steady (cf. Fig. 2).

To measure effects of transfer from light to dark, a black box was placed over the whole plant. In these experiments one plant was transferred to darkness at 13.00 h or 15.00 h, the other remaining in the light as control. Alternatively, plants were transferred to dark at 22.00 h, at the start of the normal night.

#### Replication and statistical analysis of data

Net nitrate influx rates were expressed relative to the net influx in the control plant or relative to a linear extrapolation of the rates before a change in light intensity.

All experiments were repeated at least four times. Values in tables and elsewhere are presented as mean ± SE of the mean (number of replicates). Significance of differences between means was tested using the *t*-test.

## Results

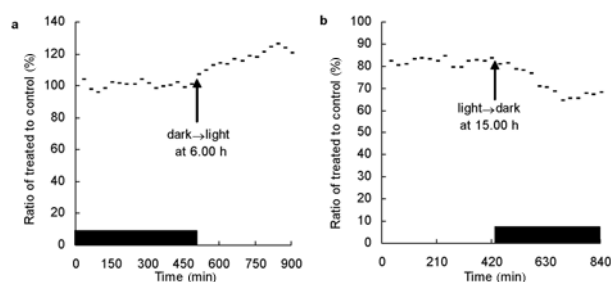
#### The time course of the effect of illuminating the shoot on net nitrate influx

After the plant had been in the dark overnight it was transferred to the light at 6.00 h. Net nitrate influx started to increase immediately (Fig. 2a) and reached a new steady state, 15% to 20% higher, after about 4 h (Tab. 2). After transfer from light to dark, net nitrate influx began to decrease after a lag of about

2 h (Fig. 2b, Tab. 2) and took about 3 h to reach a new steady state (Tab. 2). The overall time to reach a new steady state after light to dark transfer (about 5 h) is significantly longer than that after dark to light transfer (about 4 h; *P* = 0.01). This, with the different time courses, shows that the two responses do not involve a simple reversal of a single process.

The relatively small increase in net nitrate influx after transfer to the light is not significantly different from the decrease after transfer back to the dark, as expected. This is comparable to but slightly lower than the changes of 20–50% reported in some other species [4–6,17].

To make sure this is a genuine light/dark effect rather than a diel rhythm, we have also compared the light-to-dark transition during the daytime with that at night. The time courses of net nitrate influx and particularly the maximum change in rate after transfer to the dark are shown in Tab. 2. There is no significant difference between the response in the middle of the day and that at 22.00 h, showing that there is no appreciable diel effect.



## Discussion

#### What determines the speed of response of nitrate uptake by the root to illumination of the shoot?

Nitrate uptake by the root responds to light on the shoot in a relatively short time, during which a signal must be transported over several tens of cm. This long distance transport cannot be

by diffusion, which is too slow over long distances, and upwards xylem transport is irrelevant. A pressure-propagated signal is most probably an alarm signal [18] and could not carry much information and so cannot be involved. By a process of elimination we conclude that the signal must move in the phloem.

The mechanism is thought to be via a signal carried in phloem, most probably sugar. Sucrose is the primary product of photosynthesis and the main transported substance in the phloem, and consequently it is in the best position to serve as a transported signal (as well as a general substrate). This hypothesis has been verified in recently [19].

An increase in sucrose supply in the phloem (as would be expected to occur in the root after illuminating the shoot) would alter the sucrose flow into the cytoplasm of root cortical cells, with the same consequences as supplying sucrose in the solution around the root. Phloem transport might cause a lag before the root responds to a change in illumination of the shoot. However, no significant lag was found (Fig. 2), so it appears that phloem transport in wheat is relatively fast.

The final question is why there was a lag in response to darkening the shoot (2.3 h) before net nitrate influx began to decrease. The change of net nitrate influx began more or less immediately after illumination. Therefore, the observed lag after dark is unlikely to be due to any feature of the nitrate uptake, but most probably depends on a delayed change in the concentration of signal molecular.

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## Authors' contributions

The following declarations about authors' contributions to the research have been made: conducting experiments and writing the manuscript: JL; research designing: JG; data analysis: MJ.

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