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Nitrate accumulation by wheat (*Triticum aestivum*) in relation to growth and tissue N concentrations

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Abstract

The accumulation of nitrate in relation to total N concentrations ($[N]_i$) in tissues of wheat (*Triticum aestivum* L., cv Sicco) grown in solution culture was investigated. Root, shoot and leaf tissues showed qualitatively similar relationships between internal nitrate concentrations and $[N]_i$, both expressed on a tissue water basis. At low $[N]_i$, no nitrate was detectable but once a particular $[N]_i$ was exceeded, nitrate accumulated as a linear function of $[N]_i$. The threshold $[N]_i$ values for nitrate accumulation were 110, 450, and 550 m*M* for roots, total shoot and leaf 4, respectively. The slope of the relationship between nitrate and $[N]_i$ indicated that in all tissues nitrate accounted for 50–55% of the extra N accumulated above the threshold $[N]_i$. All growth requirements for N were satisfied before nitrate accumulated.

Introduction

Nitrogen supply is often the most important factor limiting plant growth and crop yield. Enhanced N supply increases both growth and the concentration of N in dry matter (Leigh and Johnston, 1985). However, N will continue to accumulate beyond the level needed to achieve maximum growth and the extra N is accumulated in storage forms, particularly as nitrate. This nitrate is located in the vacuole (Granstedt and Huffaker, 1982; Martinoia *et al.*, 1981) and may be made available for metabolism if exogenous N becomes limiting.

Understanding the physiological basis for nitrate accumulation in agricultural crops is important for a number of reasons. Firstly, tissue nitrate might be useful as an indicator of sufficiency of N supply. Thus, understanding its accumulation in relation to supply and growth might provide a diagnostic tool that could be used to assist in the design of fertilizer regimes that maximise growth and yield but minimise environmental impact. Secondly, maximising nitrate storage in plant tissues offers a route for removing nitrate from the soil and so decreasing the opportunity for leaching.

In this paper, we describe the results of some experiments designed to investigate the relationships between nitrate and total N ($[N]_i$) concentrations in wheat. A particular aim was to determine whether nitrate only began to accumulate when particular $[N]_i$ concentrations were achieved. Concentrations of nitrate and total N were both expressed on the basis of tissue water, rather than as a % in dry matter, because the former has been shown to provide a more physiologically-relevant basis for expressing crop N concentrations (Leigh and Johnston, 1985).

Material and methods

Seeds of wheat (*Triticum aestivum* L. cv. Sicco) were germinated for 5 d on moist tissue paper at 25°C in continuous light and then were transplanted to 1-litre pots containing nutrient solution. In the first type of experiment, eleven levels of nutrient solution containing 0.05 to 20 m*M* nitrate were used and 9 seedlings were transplanted to each pot. In the second type of experiment, four treatments were imposed, HH: 10 m*M* nitrate provided throughout the experiment; HO: 10 m*M* nitrate provided for the first 2 weeks, with no N thereafter; LL: 2 m*M* nitrate provided throughout the experiment; LO: 2 m*M* nitrate provided for the first 2 weeks, with no N thereafter. Solutions were changed every 2 days. All experiments were conducted in a controlled environment at 20°C with a 16 h photoperiod, 70% and 90% day/night relative humidity, and a photon flux density of 450–480 $\mu E\,m^{-2}\,s^{-1}$ at plant level.

All sampling was begun 8 h after the start of the light period. In the first experiment, plants were harvested when leaf 4 was fully expanded. Harvested plants were divided into roots and shoots, and leaf 4 was removed for separate analysis. Fresh and dry weights were measured on all samples and the length and area of leaf 4 were determined. In the second experiment, plants were harvested after 10 and 14 days, and then the experimental solutions were changed to impose new treatments (see above) and further samples were taken on days 15, 17, 19 and 21. Harvested plants were divided into roots and shoots, and fresh and dry weights were measured.

Total-N content of dried plant material was determined using a Carlo Erba automatic nitrogen analyzer. Nitrate was extracted from dried plant material by boiling in water for 1 min, and was analyzed colorimetrically (Lichfield, 1967).

Results

Growth increased with external nitrate concentrations between 0.05 and 4 m*M* and then no further increase was observed (data not shown). This was accompanied by an increase in $[N]_i$. For whole shoots, $[N]_i$ expressed on a dry weight basis increased from 1.5 to 6.0%; on a tissue water basis (Leigh and Johnston, 1985) the increase was from 200 to 600 m*M*. For leaf and root tissue, the corresponding increases were from 300 to 800 m*M* and from 50 to 250 m*M*, respectively.

Figure 1 shows the relationship between internal nitrate concentrations and $[N]_i$ for shoots from the first experiment. At low $[N]_i$, no nitrate was detectable but above a particular, tissue-specific value of $[N]_i$, nitrate accumulated proportionately with changes in $[N]_i$. Similar relationships were observed for roots and leaf 4 but the threshold $[N]_i$ for nitrate accumulation were different. For roots, shoots and leaf 4, the threshold values of $[N]_i$ above which nitrate began to accumulate were 110, 450, and 550 m*M*, respectively. In all tissues, the slope of the relationship above the threshold indicated that nitrate represented 50–55% of the extra N accumulated after the threshold was reached. The threshold values of $[N]_i$ at which nitrate began to accumulate and the percentage of the extra N that was stored as nitrate were very similar in a number of experiments (Table 1).

Figure 2a shows the relationship between fresh weight of shoots and $[N]_i$. The vertical dashed line marks the threshold value at which nitrate begins to accumulate in shoots. The largest growth response to N occured before $[N]_i$ reached the threshold value; there was relatively little change in fresh weight at values of $[N]_i$ above the threshold. This indicates that nitrate

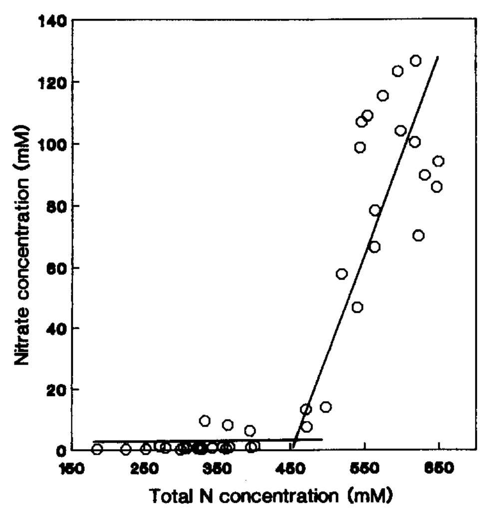

Fig. 1. Relationships between $[N]_i$ and internal nitrate concentrations in shoots of wheat plants grown continuously in nutrient solution containing different nitrate concentrations.

Table 1. Mean (±s.e.) values for the threshold $[N]_i$ at which nitrate begins to accumulate in different tissues of wheat, and the percentage of extra N accumulated above the threshold that was stored as nitrate

Tissue	Threshold $[N]_i$ (m*M*)	Percentage of extra N stored as nitrate	Number of experiments
Root	118 ± 5	55 ± 4	3
Shoots	443 ± 15	52 ± 5	3
Leaf 4	538	53	2

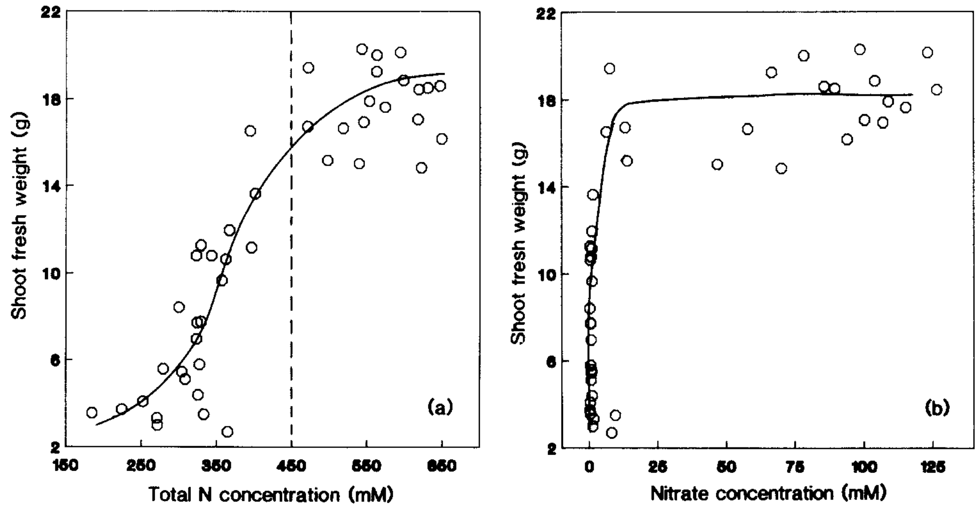

Fig. 2. The relationships between shoot fresh weight and (a) $[N]_i$; and (b) nitrate concentrations in shoots of wheat. The vertical dashed line in (a) indicates the threshold value of $[N]_i$ for nitrate accumulation in shoots.

only begins to accumulate once the N requirements of growth are satisfied. This is confirmed by the data in Figure 2b which show that there is little difference in shoot fresh weight between plants containing 10 or 120 m*M* nitrate.

The second type of experiment was designed to determine whether the relationship between $[N]_i$ and nitrate concentrations changed as plants mobilised nitrate. Plants were grown at either 2 or 10 m*M* external nitrate for 14 d and then some were transferred to solutions containing no nitrate. Transfer to nitrate-free solution caused a large decrease in internal nitrate concentrations over the next 3 days (Fig. 3). However, the relationships between $[N]_i$ and nitrate were not changed significantly compared to those in the first type of experiment in which different concentrations of nitrate were provided continuously (compare Figs. 1 and 4). The threshold values of $[N]_i$ measured in this depletion experiment were 425 and 120 m*M* for shoots and roots, respective-

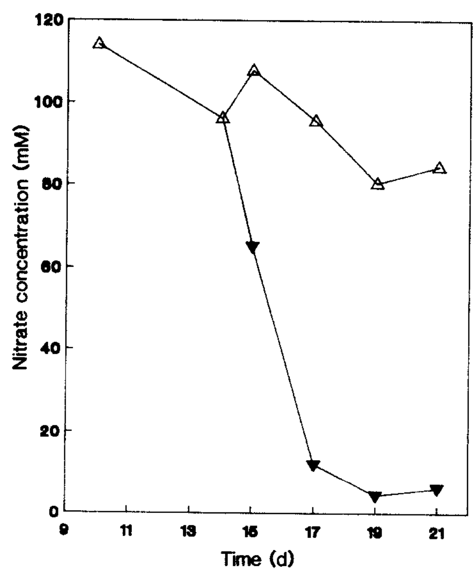

Fig. 3. Changes in the concentrations of nitrate in shoots of wheat grown continuously on nutrient solution containing 10 m*M* nitrate (△) or transferred after 14 days to solutions containing no nitrate (▼).

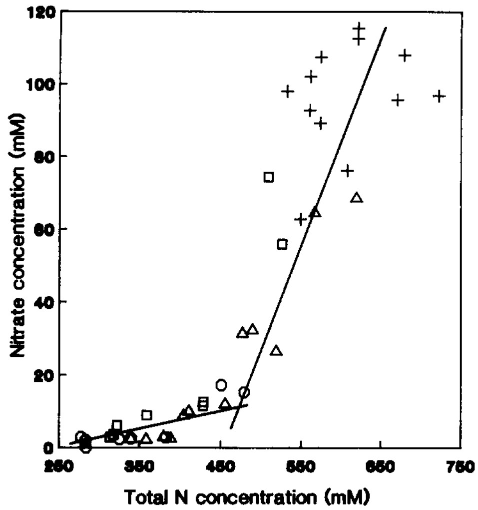

Fig. 4. The relationship between $[N]_i$ and internal nitrate concentrations in shoots of wheat grown continuously on 2 (△) or 10 m*M* (+) nitrate or transferred to solutions with no nitrate after 14 days growth in 2 (○) or 10 m*M* (□) nitrate.

ly, in good agreement with those observed in other experiments (Table 1).

Discussion

Roots, shoots and leaves all show qualitatively similar relationships between tissue nitrate concentrations and $[N]_i$. Two parameters may be determined from these relationships; a threshold value of $[N]_i$ above which nitrate accumulates, and the percentage of extra N that is stored as nitrate. The threshold value of $[N]_i$ was tissue-specific, being lowest in roots and highest in leaves (Table 1). This presumably reflects differences in the amount of enzymes in different tissues. Leaves have much higher $[N]_i$ than roots because they must invest a large amount of N in photosynthetic enzymes, particularly ribulose 1,5-bisphosphate carboxylase (Schmitt and Edwards, 1981). The threshold values for different tissues were reasonably constant between experiments which suggests that they have physiological significance. Increases in $[N]_i$ upto the threshold value are accompanied by increases in growth (Fig. 2). The lack of growth response once the threshold for nitrate accumulation is exceeded indicates that storage of N only occurs once growth requirements are satisfied, as expected if stored N is excess to metabolic requirements. Surprisingly, the proportion of the extra N that is stored as nitrate is similar in all tissues, despite the large differences in $[N]_i$ between tissues. The form of the N that is not stored as nitrate was not determined in these experiments but it was presumably accumulated as free amino acids or proteins.

The ability to define a threshold at which growth requirements for N are satisfied may have practical benefits. At this threshold, N supply is sufficient to maintain maximal vegetative growth and the N that is accumulated above the threshold concentration is excess to requirements. As nitrate only accumulates above the threshold, it can be used as an intrinsic marker for N-sufficiency. Maintaining a low but detectable level of nitrate in crops in the field could provide a means of ensuring that N fertilizer supply is maximised for growth and minimised for environmental protection.

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