

PHOTOSYNTHATE REQUIREMENTS FOR NITRATE UPTAKE AND REDUCTION IN ROOTS OF *Vicia faba* L. SEEDLINGS

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ABSTRACT

Nitrate uptake was examined in the roots of 15-day old nitrogen depleted broad bean (*Vicia faba* L.) seedlings during a 10 h. exposure to 7 mM KNO₃. The seedlings were subjected to partial defoliation, thermal treatment at stem bases and girdling.

The loss of nitrate from the medium during incubation of the seedlings indicated that about 30% of the nitrates were absorbed during the first 5 h. It was evident that the energy expended for nitrate uptake and reduction came from the tops, since partial defoliation, thermal treatment and girdling restricted nitrate uptake and reduction. The data are interpreted as indicating a close interrelationship between nitrate uptake and reduction and carbohydrate metabolism, particularly in relation to the export of photosynthate from the tops to the roots.

INTRODUCTION

Several investigations have considered the relationship between nitrate assimilation and carbohydrate metabolism in the roots of higher plants (Butt and Beevers, 1961; Weissman, 1972 and Sarkissian and Fowler, 1974). The importance of export of photosynthate products to roots is probably related to nitrate uptake and reduction. Hence, factors associated with photosynthate movement to roots seem to be very crucial for nitrate uptake and reduction. Most higher plant are capable of reducing nitrate in both roots and shoots (Marschner, 1995). But nitrate is reduced more efficiently in leaves than in roots because of the readily available reductants, energy and carbon skeletons produced by photosynthesis (Oaks, 1994 and Chen *et al.*, 2004). Miller and Smith (1996) have shown that nitrate absorbed by plants is either present in the cytosol and referred to as nitrate metabolic pool, and nitrate in the vacuole referred to as nitrate storage pool. This implies that nitrate absorbed by plants is not totally subjected to reduction by nitrate reductase.

This study was initiated to evaluate the effect of factors that limit photosynthate export to the roots of non-modulating broad bean seedlings during initial uptake of nitrate, and the influence of these factors on nitrate uptake and reduction.

MATERIALS AND METHODS

Plant culture:

Seeds of *Vicia faba* L. (cv Ghat) were surface sterilized for 30 min in 3% (w/v) calcium hypochlorite solution, rinsed 5 times with distilled water and germinated in thoroughly prewashed sand placed in black plastic bags. Ten days later, the sand around the roots was removed and the seedlings were placed individually in one litre dimmed glass containers half-filled with nutrient solution permanently bubbled with air. The composition of the nutrient

solution (minus nitrogen) was as in Rigaud and Puppo (1975). The seedlings were left in this solution for 5 days in controlled environment. Natural daylight in green house was supplemented by 200 W lamps with a 16 h photoperiod and temperature was $25^{\circ}\text{C} \pm 2$ and $16^{\circ}\text{C} \pm 4$ during the light and dark periods respectively. For nitrate treatment, (15 days after germination), 7 mM KNO_3 was added to the root medium. In order to limit interaction with the diurnal fluctuations seedlings were treated 4 h after the beginning of the photoperiod. The treatments used to limit photosynthate supply to roots were: i) partial defoliation with elimination of approximately 50% of leaves; ii) thermal treatment with a continuous flow of water at 50°C on a 3 cm zone at stem bases for 30 min; iii) girdling with a razorblade at stem bases.

Assay of nitrate reductase:

Nitrate reductase (EC 1-6.6.1) was extracted from 1 g fresh material/4 ml buffer and assayed in vitro as in Robin (1979) except that centrifugation after grinding was replaced by filtration on nylon mesh 50 nm; NADH was the electron donor. Reaction was stopped with 0.1 ml zinc acetate (1M) and the nitrites produced were measured colorimetrically at 540 nm.

Nitrate assay:

Dried samples were ground and 100 mg of powder were mixed with 10 ml distilled water for 9 h at 45°C . The supernatant after 15 min centrifugation (5000 g) was used for nitrate assay by the salicylic method (Cataldo *et al.*, 1975).

RESULTS AND DISCUSSION

During the 10 h incubation period in potassium nitrate (7 mM), the roots absorbed about 30% of the available nitrate as judged by the loss of nitrate from the medium (Fig. 1). The most rapid uptake occurred in the first 5 h of incubation, after which the uptake rate fell slightly, but remained almost constant to the end of the incubation period. As there was about 70% of the nitrate left in the medium, it is unlikely that the supply of nitrate limited uptake. These results are consistent with those obtained by Sarkissian and Fowler (1974).

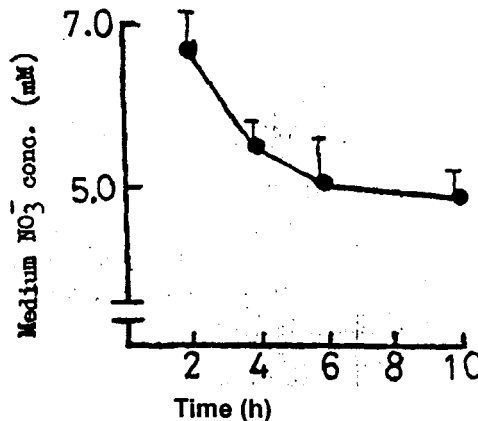


Fig. 1: Loss of nitrate from the medium during incubation of *Vicia faba* roots. Vertical bars indicate + SD, (n = 3).

Partial defoliation (Fig. 2) thermal treatment at stem bases (Fig. 3) and stem girdling (Fig. 4) resulted in significant decline in nitrate content in the roots. It was evident that the addition of glucose (0.2 M) to the nitrate medium in thermally treated seedlings (Fig. 3) alleviated the depressive effect of the thermal treatment.

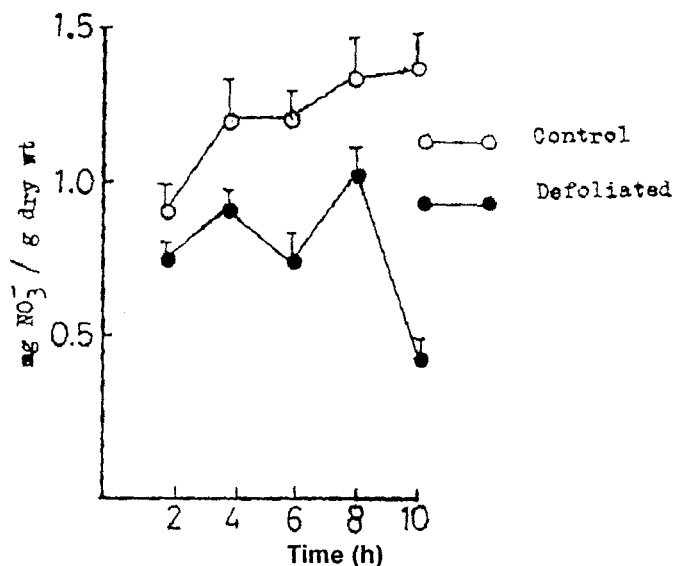


Fig. 2: Effect of partial defoliation on nitrate uptake by roots. Vertical bars indicate \pm SE, (n = 3).

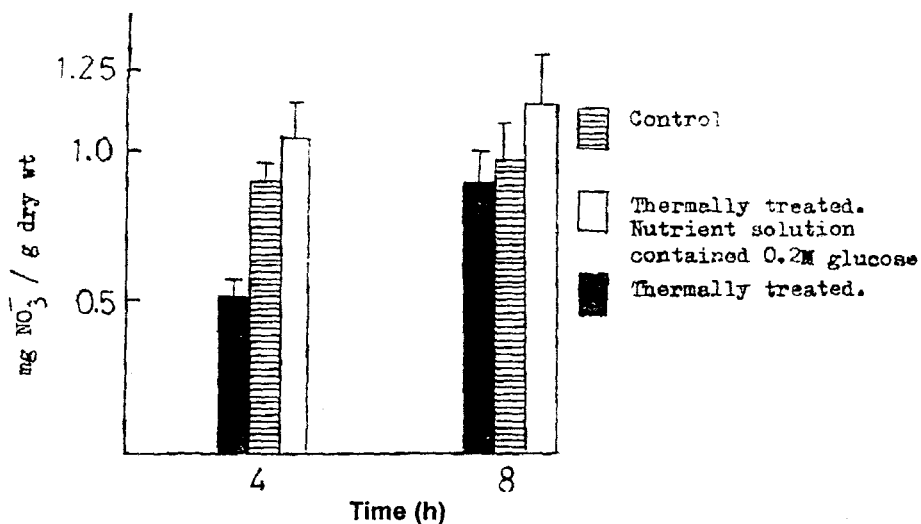


Fig. 3: Effect of thermal treatment at stem bases on nitrate uptake by roots. Vertical bars indicate \pm SE, (n = 3).

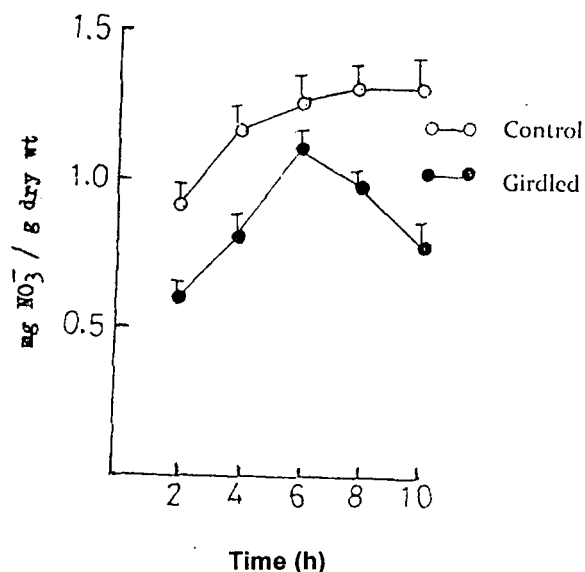


Fig. 4: Effect of girdling at stem bases on nitrate uptake by roots. Vertical bars indicate \pm SE, (n = 3).

In support of the above observation, the addition of glucose (0.2 M) in the nitrate medium of the thermally treated seedlings distinctly enhanced nitrate reductase activity as shown in Fig. 5. Also, Crawford (1995) has shown that the photosynthetic products are the main factors that promote nitrate reductase synthesis.

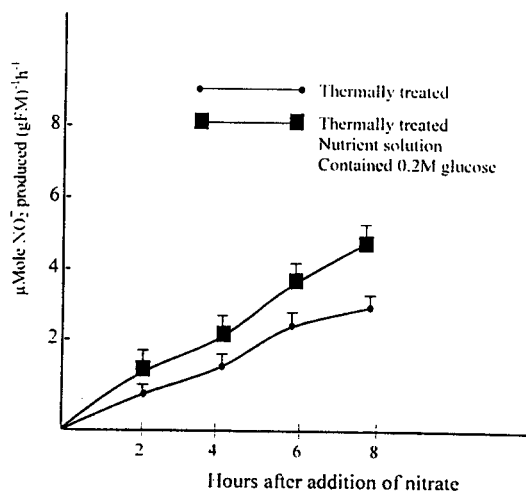


Fig. 5: Effect of glucose (0.2 M) on nitrate reductase activity in roots of thermally treated seedlings. Means from triplicates \pm SE.

The growth conditions followed in this study produced seedlings very low in nitrogen and high in carbohydrate content. Thus, the seedlings would be expected to absorb nitrate at an increased rate during the first few hours after incubation. The nitrate utilization in higher plants is a complex process including uptake, reduction and translocation. These are energy dependent processes as stated by several investigations in higher plant species (Moustafa, 1969; Lawn and Brun, 1974 and Breteler and Hanisch, 1980). The results obtained in this study showed that the energy expended for nitrate uptake and reduction comes from the tops as photosynthate, since partial defoliation, thermal treatment at stem bases and girdling depressed nitrate accumulation and reduction as compared with control seedlings, whereas the addition of glucose alleviated the depressive effect in one of these factors.

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أهمية منتجات البناء الضوئي على امتصاص واختزال النترات في جذور بادرات الفول .

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تمت دراسة عن امتصاص واختزال النترات في بادرات الفول التي كانت تنمو في محلول غذائي منقوص النيتروجين بعد إضافة كلوريد البوتاسيوم بتركيز 0.7 mM. تعرضت البادرات لمعاملات مختلفة من نزع جزئي للأوراق ونزع اللحاء في أسفل الساق وكذلك تسخين أسفل الساق. وضح أن حوالي 30% من النترات قد تم امتصاصها في الخمس ساعات الأولى بعد حضانة البادرات الضابطة في المحلول الغذائي. كذلك فقد تبين أن الطاقة اللازمة لامتصاص واختزال النترات مصدرها منتجات البناء الضوئي المصدرة من الأوراق، فالمعاملات المذكورة أعلاه قد أثرت سلباً على عمليتي امتصاص واختزال النترات. الدراسة أوضحت العلاقة الموجبة بين امتصاص واختزال النترات وأيض الكربوهيدرات المتمثل في تصدير منتجات البناء الضوئي إلى الجذور.