W. J. Horst et al.(Eds.), Plant nutrition – Food security and sustainability of agro-ecosystems. 654~655, 2001

N_2 fixation estimated by ^{15}N natural abundance can be erroneous because of changes in ^{15}N discrimination during N uptake

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Key words: ^{15}N discrimination, ^{15}N natural abundance, N_2 fixation, N form

Abstract

Determining ^{15}N discrimination (ε) during N_2 fixation is a must for the use of ^{15}N natural abundance (NA) to estimate N_2 fixation, while ε during uptake of mineral N is neglected because of low supply of N the in the field. In the greenhouse a nutrient-solution experiment was run to measure ε during sole NO_3^- nutrition (8 mM) of white lupin (lup_{NO3}), oat and flax. Additionally ε was determined for white lupin solely supplied with N_2-N (lup_{N2}). Effects of plant age and N distribution within the plant were investigated by sequential harvests and separating the plants into their parts. Besides of strong growth of all plants only lupin reached the reproductive stage. More than double the N acquired by lup_{NO3} and lup_{N2} was taken up by oat. Mean $\varepsilon_{P/S}$ [ε ^{15}N ‰] increased from flax (–3.9) via lup_{NO3} (–3.1) and oat (–2.4) to lup_{N2} (–1.5). In oat and flax ε oscillated around its mean, no change in lup_{N2} and showed a steady increase in lup_{NO3}. A close relationship between ε and the shoot share to total N gain was found for all treatments. Therefore, N demand from the shoot determined ε. The impact of these findings for field use of NA is discussed.

Introduction

Using NA to estimate N_2 fixation of legumes in the field the differences in ^{15}N enrichment are small. Therefore data are needed about the discrimination between ^{14}N and ^{15}N during N uptake of either the fixing or the non-fixing plants (Danso *et al.*, 1993; Shearer and Kohl, 1993; Högberg, 1997). For the B-value, i.e. ε of a fixing plant only supplied with N_2-N, Shearer and Kohl (1993) emphasise that it should be measured for the plant part sampled in field. ^{15}N discrimination during uptake of mineral N is regularly observed at high N supplies and explained by NO_3^- efflux (Högberg, 1997). In the field N supply is usually low and therefore ε during uptake of mineral N negligible (Evans *et al.*, 1996). Differences in ^{15}N enrichment between plant parts are often explained by the place where N was assimilated (Evans *et al.*, 1996; Högberg, 1997). Depending on plant species and N supply, the distribution of nitrate reductase-activity can vary distinctly between root and shoot and may influence the internal ^{15}N distribution (Högberg, 1997). This will influence NA if not the whole plant is sampled.

The paper presents data from an experiment which was run to measure ε during N_2 fixation of white lupin along with white lupin, oat and flax growing solely on NO_3^- N. Effects of plant age and plant part on ε were investigated.

Materials and methods

Plants (*Lupinus albus* L. 'Amiga', *Avena sativa* L. 'Jumbo' and *Linum usitatissimum* L. 'Barbara') grew in a greenhouse (21 °C/16 °C day/night with supplementary light) from mid of February to beginning of April 1995. After 24 days of germination and preculture in half strength solution, day 0 of the experiment (0 DoE) was determined by a harvest. Three treatments with NO_3^- nutrition: lupin (lup_{NO3}), oat and flax were established and one with N_2 fixation (lup_{N2}). Six days after germination lup_{N2}-plants were inoculated with a mixture of rhizobia (NPPL, Agricultural Genetics Company Limited, Cambridge, UK).

Table 1. Number of plants harvested (plh) depending on treatment and day of experiment (DoE). Details see text.

Flax and oat		lup_{NO3}		lup_{N2}	
DoE	plh	DoE	plh	DoE	plh
14	2·3	12	1*4	16	1·24
21	6·1	24	4*1	29	8·1
28	6·1	44	3*1	48	8·1
35	6·1	50	3*1	54	8·1

Experiments were run with five containers ($3 \cdot NO_3^-$, $2 \cdot N_2$) of 150 l circulating nutrient solution. Solution with nitrate contained (mM): 1 KNO_3, 1.5 $Ca(NO_3)_2$, 1 KCl, 0.5 $CaCl_2$, 0.75 K_2SO_4, 0.5 KH_2PO_4, 1 $MgSO_4$, 0.05 Fe (Fetrilon®), 0.03 H_3BO_3, $5 \cdot 10^{-3}$ $MnSO_4$, $1 \cdot 10^{-3}$ $ZnSO_4$, $1 \cdot 10^{-3}$ $CuSO_4$, $0.2 \cdot 10^{-3}$ Na_2MoO_4, and for lup_{N2} no nitrate and KCl was added, but 2 $CaCl_2$ and 1.75 K_2SO_4. Nitrate concentration was doubled from day 14 of the experiment. Solutions were changed weekly and regularly checked for N content. At harvest the number of randomly selected plants varied depending on plant species, DoE and N form (Tab. 1) and were combined for the first harvest. Roots were placed in distilled water (3 s) to remove adhering nutrient solution. Plants were at least separated into shoots and roots. Fresh and dry weight (60 °C > 48 h) was determined.

Treble analysis to determine ^{15}N enrichment and total N of powdered plant samples and nitrate stock standard solutions was done on an IRMS combined with a preparation unit (ANCA SL 20-20, Europe Scientific,

Crewe UK). ^{15}N enrichment was expressed in ε ^{15}N and calculated for the whole plant according to Shearer and Kohl (1993). Discrimination ($\varepsilon_{P/S}$) was determined as described by Mariotti *et al.* (1981). Comparison of means was performed with the Scheffé-test.

Results

Lupin bloomed from 20 DoE almost to the end of experiment and developed pods, while oat and flax did not reach the reproductive stage. Within one treatment the relation of the increase in biomass to the increase in N content was stable during the whole experiment (data not shown). Lup_{N2} showed a smaller increase than lup_{NO3} at a much higher variation (Fig. 1). Relative to the other plants oat took up more than double the N. Therefore the average nitrogen concentration of the biomass accumulated during the experiment decreased from oat (6.3 %) followed by flax (5%) and lup_{NO3} (2.9 %) to lup_{N2} (2.5 %).

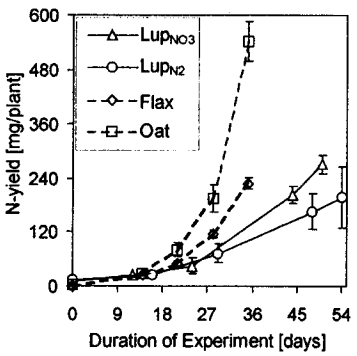

Figure 1. Development of N yield of all treatments in nutrient solution (8 ± s; n = 3 to 8, see Tab. 1).

Table 2. ^{15}N discrimination ($\varepsilon_{P/S}$ [$\varepsilon^{15}N$ ‰]) of all treatments depending on the growing period (Per). Values were determined by comparing the harvested plants to the calculated average of the harvest before (8 ; n =3 to 8, see Tab. 1). ε for the duration of the experiment was compared by the Scheffé test ($P < 0.05$) within the NO_3^- treatments.

Per	Linseed	Oat	Per	Lup_{NO3}	Per	Lup_{N2}
0–14	–3.1	–3.1	0–12	–0.4	0–16	–0.5
14–21	–4.2	–2.1	12–24	–1.7	16–29	–2.9
21–28	–3.3	–3.3	24–44	–3.2	29–48	–1.6
28–35	–4.4	–2.1	44–50	–4.2	29–54[#]	–1.7
0–35	–3.9[b]	–2.4[a]	0–50	–3.1[ab]	0–54	–1.5

[#] N yield at 54 DoE was not significant different from 48 DoE (see Fig. 1)

^{15}N discrimination of oat and flax oscillated around their overall mean (Tab. 2). Nevertheless, their mean for the duration of the experiment was significantly different. During the experiment ε steadily increased in Lup_{NO3} but did not change in lup_{N2}. The share of the shoot to the total N gain during a growing period was highly significantly correlated to the ε determined for the whole plant (Fig. 2).

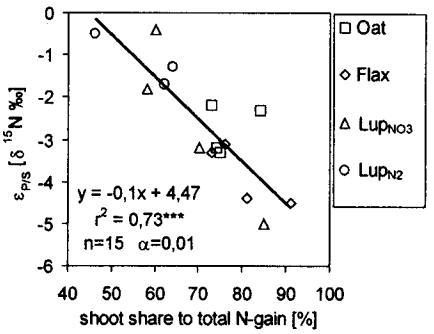

Figure 2. Relationship between the shoot share to the total N gain of a growing period and its ^{15}N discrimination (further details see Tab. 2).

Discussion

The level and stability of ε of lup_{N2} is in agreement with other findings (Shearer and Kohl, 1993; Högberg, 1997). Despite the similarities in growth and yield between lup_{NO3} and flax there was a striking difference in ε which might be in relation to the differences in phenology. According to Evans *et al.* (1996) an increase in NO_3^- efflux may result from a decreasing nitrate reductase activity (NRA) in the root. However, at the high NO_3^- supply used in this experiment, this is an unlikely explanation for the steadily increasing ε in lup_{NO3}. Even less it will explain the oscillating patterns in oat and flax. Instead, the strong relationship between the share of the shoot to the N gain and ε showed that the N demand of the shoot determined NRA in the root and thereby NO_3^- efflux. The argument that N supply controls ε during NO_3^- uptake, which is often put forward for neglecting it in the use of NA (Högberg, 1997), was not supported by the data found. Still, this will not be relevant in the field as long as N-limiting conditions determine a strong N demand from the shoot. This is valid for the non-fixing plants but has to be questioned in the case of the well supplied legume. If there is a flush of mineral-N the legume may discriminate ^{15}N strongly because N demand from the shoot is not that high.

Acknowledgement

Research was funded by the Federal Ministry of Education and Research, Berlin, Germany (BMBF 0339370I).

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