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Visualization Of ^{13}N Accumulation In Two Cultivars Of Barley Plants Using A Positron-emitting Tracer Imaging System (petis)

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Abstract: *'The translocation of nitrate in 10-day-old intact plant of barley (Hordeum vulgare L.) Steptoe (wild type) and Az12(mutant), starting from soaking was visualized using the positron-emitting tracer (PETIS), ^{13}N-labelled nitrate (half life of 9.96 min) was supplied to N-deficient and N-sufficient barley seedlings at 9-day-old seedling. Nitrate-deficient barley seedlings showed negligible accumulation of short-lived tracer $^{13}NO^{3}$- in shoots than did N-sufficient barley and in Az12 (mutant) more than in Steptoe (wild type) genotypes revealing that the N-sufficient seedlings caused enhancement of nitrate uptake and translocation to shoots. The mechanism of accumulation will be discussed.*

Keywords: Barley cultivars, ^{13}N, positron-emitting tracer imaging system (PETIS).

INTRODUCTION

Nitrogen is required in the largest amounts in which represents about 2% of plant dry weight as a structural component of proteins, nucleic acids, and many secondary metabolites (Miller and Cramer, 2005). Nitrate is the major source of combined inorganic -N for most plant species, and NO_3- uptake is ATP-dependent substrate, inducible transport and specific channel systems (Flores *et al*., 2000; Forde, 2000 and Meloui *et al*., 2004). Plants exhibit concentration-dependent differences in mechanisms of nitrogen uptake at low concentrations, uptake of both $NH_4^+$and NO_3^- is by a high affinity carrier system (Goyal & Huffaker, 1986; Glass, 1988; Warner & Huffaker, 1989 and Aslam *et al*., 1992). However, at higher concentrations absorption appears to be by either simple diffusion or a low affinity channel-mediated transport system and the rate of absorption is concentration dependent (Glass, 1988).

Wang *et al*. (2001) reported that the increased NO_3^- provision and uptake was associated with stimulated K^+ influx. Also, Debouba *et al*. (2006) stated that the loading of NO_3^- in xylem depends on the xylem ionic composition, particularly K^+ content which functions as a major change balance cation or transporter. Abdel-Latif *et al*. (2004) reported that the rate of NO_3^- uptake is mainly correlated with NO_3^- utilization in roots i.e depend on NR activity.

Imaging technology using high-energy emitting radioisotopes and radionuclide tracers allow researchers to visualize the dynamics of mineral movement in plant. Among this technology, the positron-emitting tracer imaging system (PETIS) was designed for studying plant physiology and agriculture for examining the distribution and translocation of nutrients.(Mori *et a*l., 2000), which was designed for studying plant physiology and agriculture for examining the distribution and translocation of nutrients. The PETIS could detect the two-dimensional and real-time distribution of ^{13}N by measuring the amount of γ-rays emitted from positrons without destruction of the plant (Kiyomiya et al., 2001). Several positron emitting radioisotopes such as ^{11}C and^{13}N can be used in plant biology research. In general, radioisotope tracers are useful tools for analyzing the spatial distribution or temporal change in the amount of a substance in the plant body (Ohtake *et al*., 2001). Two barley cultivars Steptoe (wild type) and Az12 (mutant) are differ in the distribution of nitrate reductase (NR) among plant organs. (Warner & Huffaker, 1989). Nitrate reductase occurs as two isozymes, one specific for NADH and the other NAD(P)H-bispecific. The roots and shoots of Steptoe contain the two characterized isozymes of NR [NADH-specific and NAD(P)H-bispecific], whereas the mutant Az12 genotype lacks (deficient) of these isozymes, yet have low levels of NR activity by some unknown nitrate assimilatory pathway (Kolb & Evans, 2003).

The aim of this study was to employ PETIS to realize direct observations of 13N accumulation in the shoots of two barley genotypes grown hydroponically on N-deficient and N-sufficient nutrient solutions. The two barley (*Hordeum vulgare* L.) genotypes (Steptoe and Az12) were specifically chosen for this investigation because they differ only in the distribution of nitrate reductase activity.

MATERIALS AND METHODS

Plant materials:

Barley (*Hordeum vulgare* L. cv. Steptoe and cv. Az12) grains were soaked and aerated in 1% sodium hypochlorite for 5 min, then rinsed in distilled water and placed on moistened paper in the dark at 25°C in an

environmentally controlled room. After 24h germinated grains were grown hydroponically using 2 liter of 0.5 mM $CaCl_2$ and kept in the dark at 25°C. After 48h the endosperm were removed, then the seedlings were transferred to a N-free basal nutrient solution consisting of 0.5mM $CaCl_2$, 0.2mM $MgSO_4$, 2mM KH_2PO_4, 0.025mM Fe-EDTANa_2 and micronutrients as previously described by (Sueyoshi *et a*l., 1995). The nutrient solution was aerated at all times to provide adequate oxygenation and ensure complete mixing of the nutrient solution. The pH of the medium (5.6) was monitored daily. At 10-day from soaking period, the plastic containers were divided into two sets. The first one was supplemented with 2.3mM KNO_3^- (N-sufficient) and the other set was lacking NO_3^- (N-deficient). The two sets were placed in controlling room for further one day, then ^{13}N (as isotopic KNO_3^-) was supplied to nutrient medium of both sets every 10 min. The ^{13}N spots movements in shoots were traced for 40 min and the data were scored every 0.25 sec, using PETIS imaging apparatus. A typical setup for the imaging experiment with PETIS is shown in Fig. (1). The two opposing detector heads of the PETIS apparatus were set, and the test plant was placed at the mid plane between them. Serial images of the distribution of the tracer in the plant were generated by the following mechanism. A positron emitted from a tracer immediately undergoes annihilation by collision with an electron of an adjacent atom in the plant tissue. A pair of γ-rays is emitted in opposite directions from that point. The detector heads detect the pair of annihilation γ-rays at the same moment. Then the emission point is determined as the middle point of the two incident points. Repeated determination of the emission points reconstructs a static image of the tracer distribution.

The half-life of a substance can be calculated according to manual of international atomic energy agency (L'annunziata, 1998) as follows:

$T = \log_e 2/\lambda$

Where T = half-life

λ = decay constant

$\log_e 2 = 0.6931$

This equation can be used to determine the activity of a particular nuclide at a particular time.

That is:

Decay factor (DF) $= 2^{-T/T1/2}$

Where T = days since T_0 (i.e. T_0 = activity date)

$T_{1/2}$ = half-life in days

Hence, to determine the activity of a particular substance:

Counts at time x = counts at T_0 x D.F.

e.g., Determine the activity of ^{13}N 1.9335 min after its activity date, when the activity was 11.819 MBq/mL.

So, if for example T = 1.9335 min

$T_{1/2}$ = 9.96 min

Then, decay factor (DF) $= 2^{-T/T1/2}$

$= 2^{-1.9335/9.96}$

$= 0.8741$

Therefore, the ^{13}N has an activity = T x MBq /ml x 2

Count /area/ activity = E

Relative counts $= E/2^{-T/T1/2}$

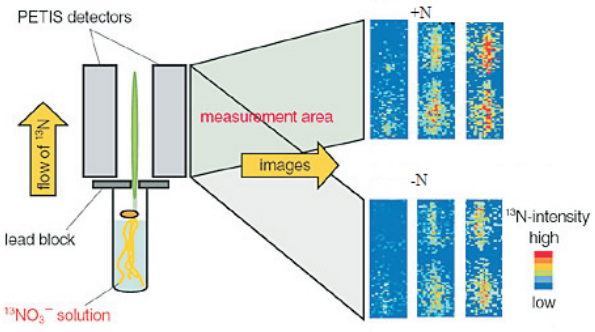
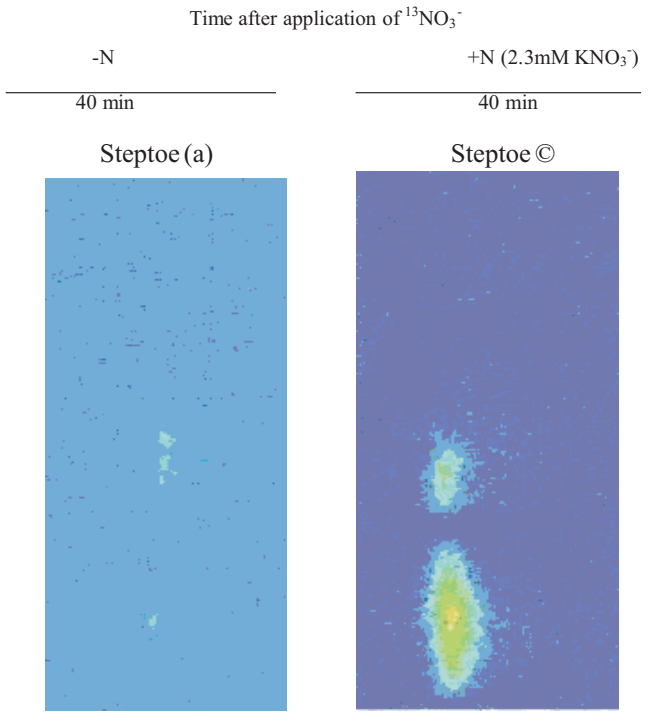


Fig. 1. A scheme of barley plant in front of the PETIS detector to illustrate the imaging of radioactivity of $^{13}NO_3^-$ supply using the positron imaging method.

RESULTS

Autoradiography imaging showed that both N-deficient Steptoe and Az12 containing lower amount than those of N-sufficient plants (Fig. 2). Also, the radioactivity in the shoots of N-sufficient Az12 was markedly higher than that in Steptoe genotype. This meaning that N-sufficient plants take up NO_3^- and translocate it to shoots more than N-deficient one, and in Az12 was greater than in Steptoe plants.



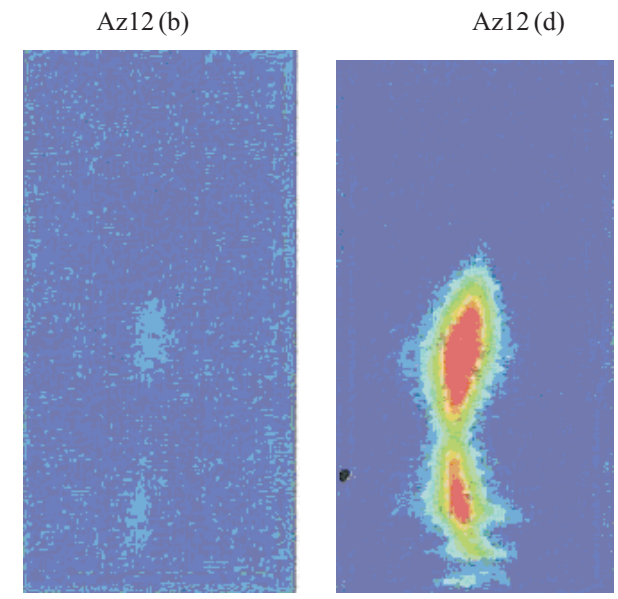


Fig. 2. Autoradiography imaging at 40 min $^{13}NO_3^-$ in the shoots of Steptoe and Az12 barley plants grown on N-deficient (a, b) and N-sufficient (c, d) nutrient solution.

In Fig. (3), it is clear that the radioactivity was detected within 10 min in the N-deficient Az12 plants and then steady decreased till further 30 min. While, in Steptoe genotype the radioactivity appeared during the first 5 min and then disappeared. In contrast, the radioactivity imaging was markedly detected with the first min of 13NO3- incubation for N-sufficient Steptoe and Az12 plants. The accumulation of $^{13}NO_3^-$ was greatly increased during the detection period and in Az12 was greater than in Steptoe plants (Fig. 3b).

The imaging pictures of ^{13}N radioactivity were monitored in N-deficient barley seedlings (Fig. 3a).

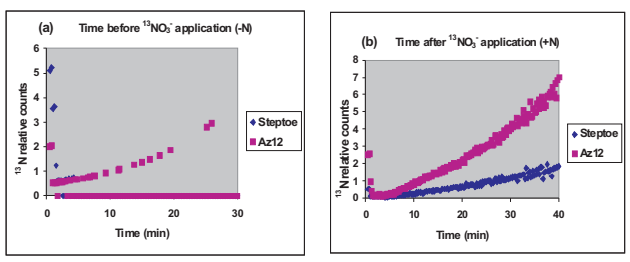


Fig. 3. Autoradioactivity imaging of ^{13}N in N-deficient (a) and N-sufficient (b) Steptoe and Az12 plants.

DISCUSSION

The adsorption and allocation of $^{13}NO_3^-$ observed for Steptoe and Az12 genotypes were similar and showed uniphasic pattern that had been reported by many plants (Aslam *et al*., 1992 and Peuke & Jeschke, 1998). Moreover, the radiocativity in the shoots of both N-sufficient plants was markedly higher than N-deficient and in Az12 was more than in Steptoe plants. These observations might attributed to enhancement of uptake and transport systems in the N-sufficient plants.(Abdel-Latif *et al*., 2004 and Kolb & Evans, 2003) have reported that the rate of NO_3^- absorption and translocation were greatly independent upon the rate of NO_3^- assimilation in roots and shoots. They also stated that Az12 lack or deficient in NAD(P)H-NR, while in Steptoe genotype both NADH-NR and NAD(P)H-NR found in shoots and roots. Thus, the increase of radioactivity of ^{13}N in the shoots of Az12 genotype might be related to: a) lower NR activity in shoots; b) higher NR activity in the roots and that resulted in the allocation of reduced-N to shoots (Abdel-Latif *et al*., 2004) and c) higher NR activity in Steptoe genotype might led to increase of NO_3^- assimilation to reduced-N compounds, and hence lower amount of NO_3^- accumulation in Steptoe shoots.

In conclusion, the result of this study might confirm that NO_3^- uptake and translocation depend upon NR activity in plant organ. (Abdel-Latif *et al*., 2004 and Kolb& Evans (2003). Further, the measurements with short-lived positron-emitting isotopes have led to a better understanding of basic physiological phenomena. The development of quantitative analysis methods for tracer profiles has been crucial to the interpretation of measurements using short-lived radioisotopes. It is clearly shown that, $^{13}NO_3^-$ accumulation in the shoots of Steptoe genotype was markedly disappeared after the short period of ^{13}N accumulation, indicating to induction of NR reducatse activity within few minutes and that resulted in an increase of NR activity leading to reduction of allocated NO_3^-, i.e. disappearing of ^{13}N.

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