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### Chapter

# Nitrogen Transport in Barley

Salwa Abdel-latif, Hanan Abou-Zeid and Kuni Sueyoshi

# Abstract

The translocation of nitrate in intact plant of barley (*Hordeum vulgare* L. cv.) two genotypes, wild type Steptoe and a mutant Az12, was visualized by a positron-emitting tracer imaging system (PETIS) after supplying positron-emitting <sup>13</sup>N-labelled nitrate (<sup>13</sup>NO<sub>3</sub><sup>-</sup>) to the seedlings. <sup>13</sup>N movement was monitored to visualize the distribution of radioactivity in the two genotypes. N sufficient seed-lings causes enhanced N uptake and translocation to shoots in time course from (0, 10, 20, 30, 40 min). The N-depleted seedlings were exposed to a nutrient solution containing nitrate and nitrite, and were labeled with <sup>15</sup>N for 38 h under (14L/10D) cycles. The two genotypes utilized <sup>15</sup>NO<sub>3</sub><sup>-</sup> and accumulated it as reduced <sup>15</sup>N, predominately in the shoots. In the Az12, nitrate accumulation in shoots was 78% higher than that in the Steptoe. Accumulation of reduced <sup>15</sup>N in the Az12 roots was nearly similar to that of the Steptoe roots, but 8% lower in the Az12 shoots than in the Steptoe shoots at the end of the experiment.

**Keywords:** barley, mutant, nitrate reduction, light/dark, <sup>15</sup>N incorporation model, PETIS—a positron-emitting tracer imaging system

## 1. Introduction

The two barley genotypes were specifically chosen since they differ only in the distribution of nitrate reductase (NR). Imaging technologies using high-energy emitting radio isotopes and radionuclide tracers allow researchers to visualize the dynamics (absorption, translocation and distribution) of mineral movement in plant, understand the dynamics of water, nutrient, pollutants in plants and to analyze the plant physiology of a test plant. The use of  $\gamma$ -rays emitted from positrons in <sup>11</sup>CO<sub>2</sub> [1–3] <sup>13</sup>NH or <sup>13</sup>NO [4, 5] and H<sub>2</sub><sup>18</sup>F<sup>-</sup> [6] has been adopted in plant nutrition research. Among these technologies, the positron-emitting tracer imaging system (PETIS) [7] which was designed for studying plant physiology and agriculture, has often been used to examine the distribution and translocation of nutrients using positron-emitting tracers. Several positron emitting radioisotopes such as <sup>11</sup>C and <sup>13</sup>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.

The PETIS was recently used to visualize the accumulation of photo assimilates in grains of a wheat ear [8] with 2.3 mm resolution. In recent years, the PETIS has been employed to study various physiological functions in intact, living plants [9, 10] one of the most advanced radiotracer-based imaging methods available today. This system enables not only monitoring of the real-time movement of the tracer in living plants as a video camera might, but also quantitative analyses of the movement of the substance of interest by freely selecting a region of interest on the image data obtained. Short-lived radioisotope techniques provide data that are crucial for developing models that quantitatively link the underlying biochemical reactions to physiological responses.

# 2. Visualization of <sup>13</sup>N accumulation in barley using (PETIS)

## 2.1 <sup>13</sup>N analysis in Steptoe

Short incubation times are used in order to determine the fate of nitrate transported in the shoot of two barley genotypes. Therefore, <sup>13</sup>NO<sub>3</sub><sup>-</sup> was applied to barley seedling and short-term <sup>13</sup>N distribution was determined using PETIS. The imaging pictures of <sup>13</sup>N radioactivity were monitored after <sup>13</sup>NO<sub>3</sub><sup>-</sup> was supplied to the medium containing 2.0 mM KNO<sub>3</sub> PETIS images show high <sup>13</sup>N accumulation in Az12 shoot than in Steptoe shoot (**Figure 1**).



Figure 1.

Imaging of radioactivity in barley shoot supplied with <sup>13</sup>NO<sub>3</sub><sup>-</sup> for 40 min using PETIS.



Figure 2.

Changes in the relative counts radioactivity for 40 min in barley shoot of wild type Steptoe and mutant Az12 measured by PETIS.

The <sup>13</sup>NO<sub>3</sub><sup>-</sup> was supplied to barley seedlings for the two genotypes and the translocation of <sup>13</sup>NO<sub>3</sub><sup>-</sup> were monitored using PETIS. The seedlings were incubated with 2.3 mM KNO<sub>3</sub><sup>-</sup>, one seedling was selected and transferred to a feeding container that contained 8 mL of pretreatment solution. Subsequently, 100  $\mu$ L of 2.3 mM KNO<sub>3</sub><sup>-</sup> and 2 mL of <sup>13</sup>NO<sub>3</sub><sup>-</sup> solution (113 MBq) were immediately added (final concentration of KNO<sub>3</sub><sup>-</sup> is 2.0 mM).

A plot of relative radioactivity was shown in **Figure 2**. The radioactivity was shown at 40 min after the addition of  ${}^{13}NO_3^-$  containing 2.0 mM KNO<sub>3</sub><sup>-</sup> in the Steptoe and Az12 plants. The radioactivity linearly increased over time after a lag of several minutes for both genotypes. The count of radioactivity of Az12 shoots at 40 min after  ${}^{13}NO_3^-$  supply was about four times higher than that of Steptoe. These results suggest that the excess nitrate accumulation in Az12 plants shoots is probably due to the lower capacity of the mutant to reduce nitrate [11, 12].

## 3. Nitrate reduction and allocation of reduced nitrogen in roots and shoots of the wild type Steptoe and the mutant Az12 using <sup>15</sup>N-tracing method to determine accumulation, uptake, translocation and reduction of nitrate, together with transport of reduced <sup>15</sup>N in intact N-starved seedlings under light/dark cycles

Barley besides its importance as a crop is an established model plant for both genetic and physiological studies [13]. In the two barley genotypes (Hordeum vulgare L.) wild type Steptoe and the mutant Az12 using <sup>15</sup>N-tracing method to determine accumulation, uptake, translocation and reduction of nitrate, together with transport of reduced <sup>15</sup>N in intact N-starved seedlings under light/dark cycles. Also for both genotypes root contribution increased during L/D cycles and decreased during the subsequent light cycle. Shoot nitrate accumulation in Az12 was higher than in Steptoe. Nitrate-deficient barley seedlings showed negligible accumulation of short-lived tracer <sup>13</sup>NO<sub>3</sub>- in shoots than did N-sufficient barley and in Az12 more than in Steptoe genotypes revealing that the N sufficient seedlings caused enhancement of nitrate uptake and translocation to shoots [14]. Barley is a highly adaptable cereal grain and ranks 5<sup>th</sup> among all crops for dry matter production in the world since it is an important food source of protein in many parts of the world. Nitrogen is considered as one of the three macronutrients required for high crop yields and has a critical role in plant growth and development [15, 16]. Three quarters of our atmosphere consists of nitrogen gas  $(N_2)$  and elemental nitrogen must be transformed to usable forms before it is available for plant uptake. Addition of  $NO_3^-$  to the incubation medium of dark-grown NO<sub>3</sub><sup>-</sup>-starved Steptoe seedlings resulted in a greater accumulation of NO<sub>3</sub><sup>-</sup> in leaves and roots more than those of illuminatedgrown seedlings [17]. Plants require more nitrogen than any other nutrient [18]. Nitrogen is an important component of various compounds such as amino acids, amides and proteins, quaternary ammonium compounds and polyamines [19-21]. Nitrate  $(NO_3^{-})$  is the major N-source for cultivated plants and is an important signaling ion that influences plant growth and differentiation [22]. NO<sub>3</sub><sup>-</sup> uptake, transport, and responses have been a major focus of research. In addition to its role as a nutrient, NO<sub>3</sub><sup>-</sup> can act as a signaling molecule that modulates gene expression and a wide range of processes including plant growth, root system architecture [23, 24]. Roots are crucial for perception and uptake of nitrate in plants [25–27].

The first enzymatic step of nitrate after its active transport into the cell, is the reduction to nitrite which achieved by nitrate reductase (NR). In higher plants NR exists in two forms: NADH:NR (E.C 1.6.6.1) and NAD(P)H:NR (E.C.1.6.6.2). The bispecific NAD(P)H:NR is found in the non-green tissues of monocotyledons and in both green and non-green tissues of legume. NR is a key enzyme in a plant's

nitrogen assimilation pathway, this step of the reduction often considered to be rate limiting step and nitrite transfers to plastids where it quickly reduced to NH<sub>4</sub><sup>+</sup> by NiR enzyme [28]. In a second step, nitrite reductase which is localized in the chloroplast, catalyses the reduction of nitrite to ammonia before incorporation into amino compounds so, for protein synthesis [29–31]. This reduction can take place in either roots or leaves, depending on plant species, age and nitrate supply rate. It has been reported that the factors which regulate nitrate reductase enzyme are inorganic salts and ions, antibiotics and metabolic inhibitor, fungicides and herbicides, seedling age and diurnal rhythms, temperature, water stress and gaseous environment, atmospheric pollutants and external pH [32]. For higher plants, nitrate is the major source of inorganic nitrogen, which is translocated to the leaf, and assimilated and metabolized into various organic compounds utilizing reductant provided by photosynthesis. The reduced nitrogen compounds are incorporated into various biomacromolecules, such as proteins and nucleic acids. Nitrate uptake and reduction are considered the initial processes by which NO<sub>3</sub><sup>-</sup> is metabolized by higher plants, are modulated by light and dark [33] and also of interest in understanding plant nitrogen nutrition and the plant nitrate assimilation pathway. Nitrogen assimilation is a fundamental biological process that has a marked effect on plant productivity, biomass and crop yield. It is well established that plants supplied with excess nitrate of current demand have the ability to accumulate nitrate. The manner in which nitrate stored or reduced and assimilated in both roots and shoots depends on plant species [34]. Depending on the nitrogen demand, nitrate is directed into several routes after uptake into the root cells: it can be translocated to the shoot, stored in the vacuole, or added to the cytosolic pool. Some aspects of plant nitrogen metabolism have been studied in detail in barley [5, 35, 36]. Several studies with mutants or trasformants with altered NR expression clearly showed that there is no direct correlation between plant growth and the nitrate reduction capacity of the plant also showed a reverse relation between nitrate content and NR activity, i.e., plants with decreased NR activity contain more nitrate and are phenotypically not different. It is only when NR activity is decreased below 10% of the wild type level is plant growth and protein affected [37–39]. Both roots and shoots of wild-type Steptoe contain the two characterized isozymes of NR (NADH-specific and NAD(P)H-bispecific) typically found in barely, where the NAD(P)H-specific NR is not expressed in leaves, but is induced by nitrate in roots. In Az12, NAD(P) H-specific NR is present in roots and shoots, since Az12 is a mutant that is affected by partial or complete loss of its capacity to either reduce nitrate or produce nitrite under NR assay conditions, yet has low levels of NR activity by some unknown nitrate assimilatory pathway [11].

Nitrate assimilation is the primary pathway by which plants obtained reduced nitrogen. In many species of higher plants most organic N is derived from the assimilation of nitrate in the shoots [12, 40]. Short-time labelings are generally used by several authors [41–44] to prevent the minor allocation of reduced <sup>15</sup>N onto and out of the roots. Most studies showed that when plants are exposed to nitrate a continuous increase of nitrate uptake was achieved by the roots. However long time exposure to nitrate may inhibit nitrate uptake [45]. Use of Az12 mutant provide a simpler system to study the characteristics of nitrate reduction since the two genotypes are phenotypically the same but differ only in the distribution of NR [46]. For that the whole plant contribution to nitrate reduction occurred upon the early stages of N utilization when the induction is not fully achieved.

Since many studies on the physiological characterizations of over expression and under expression NR genotypes include no measurements of the in vivo nitrate reduction rate. The fact that using whole plant in experiments to investigate the contribution between shoots and roots to reduced <sup>15</sup>N is complicated although it has an advantage for measuring the assimilation and translocation of reduced N in intact seedlings and consequently to the measurement of the whole plant nitrate reduction. Since some limitations and disadvantages may be involved as a result of using



#### Figure 3.

Balance sheets for uptake, accumulation, reduction, and translocation of nitrate, and accumulation and translocation of reduced <sup>15</sup>N in barley seedlings.

excised tissues [47]. The present study was conducted to investigate the differences between the wild-type (Steptoe) and the mutant (Az12) in nitrate reduction, uptake and the transport of reduced <sup>15</sup>N between roots and shoots using <sup>15</sup>N labeling in a split root experiment. Also, to study the upward and downward translocation of reduced <sup>15</sup>N in intact barley seedlings which were assimilating nitrate from a mixed N-medium, by using the <sup>15</sup>N incorporation model [48, 49]. The mutant Az12 plants deficient in NADH-specific NR was used with wild-type Steptoe as a control.

Nitrate reduction during the first 14 h light period accounted for 54 and 46% of total nitrate absorbed by the plants, respectively, in Steptoe and Az12. In both genotypes, nitrate accumulation in root was occurred mainly during the first 14 h light period and accounted for about 19% of the total amount of absorbed nitrate. In the other hand, reduced <sup>15</sup>N accumulation in roots was low and quit constant during whole period as amounted to about 8% of total absorbed nitrate. Shoot nitrate reduction during the first 14 h light period accounted for 33 and 27% of total nitrate uptake, respectively, in Steptoe and Az12, and for 25 and 30% during the dark period, and for 86 and 75% during the second light period. In both genotypes about 60% of absorbed nitrate was transported to the shoot via xylem during the first 14 h light period and the proportion decreased during dark period, then increased again at the subsequent light period and this may be due to light/dark transition. From the same reason also fluctuation was observed in both shoot reduced <sup>15</sup>N accumulation and shoot nitrate reduction in Steptoe where at first 14 h light period, shoot nitrate reduction accounted for 33, 25 and 86% of total nitrate uptake, respectively, in light/ dark/light periods. On the other hand, shoot nitrate reduction in Az12 accounted for 27, 30 and 75% of total nitrate, respectively, in light/dark/light periods. Nitrate translocation to shoots at 14 h light for both genotypes was 59% in Steptoe and 62% in Az12 of total nitrate uptake then decreased to about 34% for both genotypes at dark period and increased to 91% in Steptoe and 75% in Az12 at subsequent second light period (**Figure 3**).

Az12 was used as a tool in order to assess the importance of root and shoot nitrate reduction and allocation of reduced N for the N-nutrition of barley plants. Although some differences between Az12 and Steptoe in this study, it could be concluded that the overall fate of the absorbed nitrate was basically similar between the two genotypes under light/dark cycle.

### 4. Nitrate accumulation

The accumulation of nitrate in barley (*Hordeum vulgare* L. cv. Giza 123) changed in roots and leaves at light/dark 14:10 h cycle (**Table 1**). The root nitrate content was 0.65–1.68 µmol plant<sup>-1</sup> respectively during the 14 h light and 10 h dark periods after the transfer to nutrient solution containing nitrate. In the dark nitrate content of both roots and leaves was elevated. However, the nitrate content decreased in roots

Plant part	14 h (light)	24 h (light-dark)	38 h (light-dark-light)
Roots Shoots	0.65 ± 0.06 0.79 ± 0.11	$1.68 \pm 0.13$ $1.23 \pm 0.11$	1.12 ± 0.15 0.53 ± 0.17
Total plant	1.44 ± 0.14	2.91 ± 0.23	1.65 ± 0.27

N-depleted seedlings (9-d-old) were treated with a nutrient solution containing 2.5 mM KNO<sub>3</sub><sup>-</sup> under a light-dark cycle of 14:10 h at 25°C. Leaves and roots were harvested after 14, 24, and 38 h in the nutrient solution. Results are the means of three replicates ( $3 \times 5$  plants) ± SE.

#### Table 1.

Nitrate accumulation in roots and leaves of barley seedlings after treatment with a N-medium.

and leaves during the 38 h light period and significantly low nitrate contents were measured in roots and leaves. On a whole plant basis, roots accounted for 45 (14 h), 58 (24 h) and 68% (38 h) of the nitrate accumulation of the whole plant during light/dark/light period [17].

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# **Conflict of interest**

No potential conflict of interest was reported by the authors.

## Abbreviations

Anl	accumulation of reduced <sup>15</sup> N from <sup>15</sup> NO <sub>3</sub> <sup>-</sup> in non-labeled roots of split roots
Ar	accumulation in roots of reduced <sup>15</sup> N from <sup>15</sup> NO <sub>3</sub> <sup>-</sup>
As	accumulation in shoots of reduced <sup>15</sup> N from <sup>15</sup> NO <sub>3</sub> <sup>-</sup>
Rr	<sup>15</sup> NO <sub>3</sub> <sup>-</sup> reduction in roots
Rs	<sup>15</sup> NO <sub>3</sub> <sup>-</sup> reduction in shoots
Тр	translocation to root of shoot reduced <sup>15</sup> N from <sup>15</sup> NO <sub>3</sub> <sup>-</sup> in phloem
Tx	translocation to shoot of root-reduced <sup>15</sup> N from <sup>15</sup> NO <sub>3</sub> <sup>-</sup> in xylem
PETIS	a positron-emitting tracer imaging system

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