Determination of Nitrate Uptake and Accumulation Using ^{15}N in Rice Seedlings

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[Abstract] Nitrogen-15 is a rare stable isotope of nitrogen. This isotope is often used in agricultural research. For example, Nitrogen-15 is used to trace mineral nitrogen compounds and translocate the nitrogen molecule in plants. This protocol is used to determine nitrate uptake and accumulation in rice seedlings by using Nitrogen-15.

Materials and Reagents

1. Rice seedlings: Four weeks old seedlings
2. NH_4NO_3 (catalog number: 6484-52-2)
3. KH_2PO_4 (catalog number: 7778-77-0)
4. K_2SO_4 (catalog number: 7778-80-5)
5. $CaCl_2 \cdot 2H_2O$ (catalog number: 94248-52-9)
6. $MgSO_4 \cdot 7H_2O$ (catalog number: 10034-99-8)
7. Na_2SiO_3 (catalog number: 1344-09-8)
8. NaFeEDTA (catalog number: 7720-78-7; 139-33-3)
9. H_3BO_3 (catalog number: 10043-35-3)
10. $MnCl_2 \cdot 4H_2O$ (catalog number: 20603-88-7)
11. $CuSO_4 \cdot 5H_2O$ (catalog number: 7758-99-8)
12. $ZnSO_4 \cdot 7H_2O$ (catalog number: 7446-20-0)
13. $Na_2MoO_4 \cdot 2H_2O$ (catalog number: 7631-95-0)
14. $CaSO_4 \cdot 2H_2O$ (catalog number: 10101-41-4)
15. IRRI nutrient solution (see Recipes)
16. 0.1 mM $CaSO_4$ solution (see Recipes)
17. $K^{15}NO_3$ (catalog number: 57654-83-8) (see Recipes)

Equipment

1. Isotope Ratio Mass Spectrometer (Thermo Fisher Scientific, model: MAT253)

2. Elemental Analyzer (Thermo Fisher Scientific, model: Flash EA1112 HT)

Procedure

1. The rice seeds were surface sterilized with 10% (v/v) hydrogen peroxide for 30 min and then rinsed thoroughly with deionized water. The sterilized seeds were germinated on plastic supporting netting (mesh of 1 mm^2) mounted in plastic containers for 1 week. Uniform seedlings were selected and then transferred to a tank containing 7 L of International Rice Research Institute (IRRI) nutrient solution for 4 weeks and then deprived of N (IRRI nutrient solution without NH_4NO_3) for 1 week. All the plants were grown in a growth room with a 16-h-light (30 °C)/8-h-dark (22 °C) photoperiod, and the relative humidity was controlled at approximately 70%.
2. The plants were transferred first to a container with 7 L washing solution (0.1 mM $CaSO_4$) for 1 min, then to a new container with 7 L complete nutrient solution containing 0.5 mM $K^{15}NO_3$ (atom% ^{15}N: 80.25%) for 5 min uptake, and finally to washing solution (0.1 mM $CaSO_4$) again for 1 min. Make sure the whole root system were socked in the solution.
3. For analyzing the nitrate accumulation, the N-starved seedlings were transferred to an IRRI nutrient solution containing 0.5 mM $K^{15}NO_3$ (atom% ^{15}N: 80.25%) for 24 h before the harvest.
4. Harvest the roots and shoots respectively and grinding in liquid N, the powder was dried to a constant weight at 70 °C. About 10 mg of powder of each sample was analyzed using the Isotope Ratio Mass Spectrometer system.
5. Influx or accumulation of $^{15}NO_3^-$ was calculated from the ^{15}N concentrations of the roots.

Recipes

1. IRRI nutrient solution
   1.25 mM NH_4NO_3
   0.3 mM KH_2PO_4
   0.35 mM K_2SO_4
   1 mM $CaCl_2·2H_2O$
   1 mM $MgSO_4·7H_2O$
   0.5 mM Na_2SiO_3
   20 μM NaFeEDTA
   20 μM H_3BO_3
   9 μM $MnCl_2·4H_2O$
   0.32 μM $CuSO_4·5H_2O$

0.77 µM $ZnSO_4 \cdot 7H_2O$

0.39 µM $Na_2MoO_4 \cdot 2H_2O$

pH 5.5

2. 0.1 mM $CaSO_4$ solution (1 L)

   0.0172 g $CaSO_4 \cdot 2H_2O$

   Add ddH_2O to final volume

3. $K^{15}NO_3$

   0.5 mM $K^{15}NO_3$

Acknowledgments

This protocol is adapted from Delhon *et al*. (1995) and Tang *et al*. (2012).

References

1. Delhon, P., Gojon, A., Tillard, P. and Passama, L. (1995). Diurnal regulation of NO_3^- uptake in soybean plants I. Changes in NO_3^- influx, efflux, and N utilization in the plant during the day/night cycle. *J Exp Bot* 46(10): 1585-1594.
2. Tang, Z., Fan, X., Li, Q., Feng, H., Miller, A. J., Shen, Q. and Xu, G. (2012). Knockdown of a rice stelar nitrate transporter alters long-distance translocation but not root influx. *Plant Physiol* 160(4): 2052-2063.

