

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

Zhong Tang, Guohua Xu*

State Key Laboratory of Crop Genetics and Germplasm Enhancement and Key Laboratory of Plant Nutrition and Fertilization in Low-Middle Reaches of the Yangtze River, Ministry of Agriculture, Nanjing Agricultural University, Nanjing, China

*For correspondence: ghxu@njau.edu.cn

[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. $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (catalog number: 94248-52-9)
6. $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (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. $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (catalog number: 20603-88-7)
11. $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (catalog number: 7758-99-8)
12. $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (catalog number: 7446-20-0)
13. $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (catalog number: 7631-95-0)
14. $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ (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 mm²) 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 NH₄NO₃) 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 CaSO₄) for 1 min, then to a new container with 7 L complete nutrient solution containing 0.5 mM K¹⁵NO₃ (atom% ¹⁵N: 80.25%) for 5 min uptake, and finally to washing solution (0.1 mM CaSO₄) again for 1 min. Make sure the whole root system were soaked in the solution.
3. For analyzing the nitrate accumulation, the N-starved seedlings were transferred to an IRRI nutrient solution containing 0.5 mM K¹⁵NO₃ (atom% ¹⁵N: 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 ¹⁵NO₃⁻ was calculated from the ¹⁵N concentrations of the roots.

Recipes

1. IRRI nutrient solution
 - 1.25 mM NH₄NO₃
 - 0.3 mM KH₂PO₄
 - 0.35 mM K₂SO₄
 - 1 mM CaCl₂·2H₂O
 - 1 mM MgSO₄·7H₂O
 - 0.5 mM Na₂SiO₃
 - 20 μM NaFeEDTA
 - 20 μM H₃BO₃
 - 9 μM MnCl₂·4H₂O
 - 0.32 μM CuSO₄·5H₂O

- 0.77 μM $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$
- 0.39 μM $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$
- pH 5.5
- 2. 0.1 mM CaSO_4 solution (1 L)
 - 0.0172 g $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$
 - Add ddH₂O 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 \$\text{NO}_3^-\$ uptake in soybean plants I. Changes in \$\text{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.