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^{15}N-nitrate Uptake Activity and Root-to-shoot Transport Assay in Rice

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[Abstract] ^{15}N is a nonradioactive heavy isotope of nitrogen, widely used for biochemical and physiological research in plants. For instance, ^{15}N-KNO_3 was used as the nitrogen source in plants in order to investigate nitrate uptake activity and transport from roots to shoots (Lin *et al.,* 2008). Here, we describe a detailed pipeline used for labeling living rice (*Oryza sativa*) plants with ^{15}N-KNO_3 and determination of net nitrate uptake and transport activity, and this protocol was proved to be valid in *Arabidopsis* and rice (Lin *et al.,* 2008; Hu *et al.,* 2015).

Materials and Reagents

1. 96-well plate
2. Rice seeds (Zhonghua11, ZH11)
3. NaClO [Sinopharm Chemical Reagent Co.,Ltd (SCRC), catalog number: 7681-52-9]
4. KNO_3 (SCRC, catalog number: 7757-79-1)
5. $K^{15}NO_3$ (Sigma-Aldrich, catalog number: 57654-83-8)
6. $CaCl_2$ (SCRC, catalog number: 10043-52-4)
7. $MgSO_4 \cdot 7H_2O$ (SCRC, catalog number: 10034-99-8)
8. KH_2PO_4 (SCRC, catalog number: 7778-77-0)
9. $FeSO_4 \cdot 7H_2O$ (SCRC, catalog number: 7782-63-0)
10. EDTA-Na_2 (SCRC, catalog number: 6381-92-6)
11. $NaSiO_3 \cdot 9H_2O$ (SCRC, catalog number: 13517-24-3)
12. H_3BO_3 (SCRC, catalog number: 10043-35-3)
13. $CuSO_4 \cdot 5H_2O$ (SCRC, catalog number: 7758-99-8)
14. $ZnSO_4 \cdot 7H_2O$ (SCRC, catalog number: 7446-20-0)
15. $MnCl_2 \cdot 4H_2O$ (SCRC, catalog number: 13446-34-9)
16. $Na_2MoO_4 \cdot 2H_2O$ (SCRC, catalog number: 10102-40-6)
17. $CaSO_4 \cdot 2H_2O$ (SCRC, catalog number: 10101-41-4)
18. Modified Kimura B solution (see Recipes)
19. 5 mM ^{15}N-KNO_3 (see Recipes)
20. 0.1 mM $CaSO_4$ solution (see Recipes)

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Equipment

1. Growth chamber (SANYO, model: MLR-351H)
2. Isotope ratio mass spectrometer(Thermo Fisher Scientific, model: Finnigan Delta Plus XP) with elemental analyzer (Thermo Fisher Scientific, model: Flash EA 1112)

Procedure

1. Seed germination

   Rice seeds are surface-sterilized with 2.5% sodium hypochlorite (NaClO) for 30 min and then soaked in tap water, put in an incubator chamber at 37 °C for 2 days (d), change water every 12 h till the seeds germinate.
2. Seedling growth

   Uniformly germinated seeds are selected and put into 96-well plates, then transferred to clear water until roots length reach 3 cm, after which transplant the seeds to modified Kimura B solution. Rice seedlings are grown in a growth chamber with a 12-h light (30 °C)/12-h dark (28 °C) photoperiod and 70% humidity for about 2 weeks. The solution is changed every day.
3. ^{15}N-nitrate uptake assay

   After 2-week cultivation, rice seedlings are pretreated with modified Kimura B solution for 2 h, after which the rice roots are washed by tap water twice and rice seedlings are transferred to modified Kimura B solution containing 5 mM ^{15}N-KNO_3 for 3 h.
4. Rice seedlings harvest

   After 3 h absorption, rice roots are rinsed with 0.1 mM $CaSO_4$ for 2 min to remove the ^{15}N-NO_3^- on the root surface, then roots and shoots are harvested separately and dried at 70 °C to constant weight in paper bags. Dried samples are ground to fine powder in mortars for subsequent assay.
5. Calculation of nitrogen uptake

   About 0.5 mg dried powder is analyzed by isotope ratio mass spectrometer and the data of ^{15}N content are obtained (Brand, 1996). While detecting the ^{15}N-nitrate uptake activity, a formula [total ^{15}N amount of whole plant (TN)/dry weight (DW) of root (DWR)/3 h] is applied to the calculation, *i.e.,* the amount of ^{15}N take up per unit weight of roots per unit time, total ^{15}N amount of whole plant is derived from the sum of N amount of shoots and roots. The ratio of shoot ^{15}N content (SN) to root ^{15}N content (RN) is used to represent the root-to-shoot transport activity (the higher the value, the higher root-to-shoot transport activity).

   Root-to-shoot transport activity = $\frac{SN}{RN}$

   ^{15}N-nitrate uptake activity = $\frac{SN + RN}{DWR \times 3}$

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Representative data

Figure 1. A 96-well plate that was cut off the bottom well-suited for the growth of rice seedlings. After being put into the 96-well plate, rice roots could grow downward into solution underneath while rice shoots could grow upward tidily along each well.

Figure 2. 10-day-old rice seedlings grown on a 96-well plate. The container under the 96-well plate is full of modified Kimura B solution, usually the container is wrapped with light-tight material (*e.g.,* tinfoil) to protect rice roots from light.

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Table1. ^{15}N content of shoots and roots

Replicates	Sample name	Amount (mg)	^{15}N mM g^{-1}	^{15}N content (μM)
1	shoot1	0.598	0.018658602	0.011157844
	root1	0.434	0.041342353	0.017942581
2	shoot2	0.494	0.020300923	0.010028656
	root2	0.345	0.033636425	0.011604567
3	shoot3	0.519	0.020692085	0.010739192
	root3	0.393	0.038303468	0.015053263

Table2. Calculation of ^{15}N-nitrate uptake activity and root-to-shoot transport activity

Replicates	root-to-shoot transport activity	^{15}N-nitrate uptake activity (mM g^{-1} root DW h^{-1})
1	0.621864	0.022350557
2	0.864199	0.020901664
3	0.713413	0.021876552

Notes

1. Uniformly germinated seeds were selected to make sure the same growing status of different lines.
2. The germinated seeds could be transferred to modified Kimura B solution until seminal root length was about 3 to 5 cm.
3. The aim of pretreating rice seedlings with modified Kimura B solution for 2 h is to make sure the rice seedlings could get back to a relatively normal physiological state before the ^{15}N-nitrate uptake assay.

Recipes

1. Modified Kimura B solution
   5 mM KNO_3
   0.36 mM $CaCl_2$
   0.54 mM $MgSO_4$
   0.18 mM KH_2PO_4
   40 μM $FeSO_4$-EDTA
   18.8 μM H_3BO_3
   13.4 μM $MnCl_2$
   0.32 μM $CuSO_4$
   0.3 μM $ZnSO_4$
   0.03 μM Na_2MoO_4
   1.6 mM Na_2SiO_3

pH 6.0

2. 5 mM ^{15}N-KNO_3
   As mentioned in Procedure 3, 5 mM ^{15}N-KNO_3 was used to replace 5 mM KNO_3 in modified Kimura B solution while other ingredients remain unchanged.
3. 0.1 mM $CaSO_4$ solution
   Dissolve 0.0172 g $CaSO_4 \cdot 2H_2O$ in 1 L deionized water

Acknowledgments

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