Jul 13, 2020

Root nitrate influx

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1 Works for me dx.doi.org/10.17504/protocols.io.biibkcan

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ABSTRACT

Nitrogen (N) in the form of nitrate (NO_3^-) can move radially from roots to the vascular tissue from where it is transported to the above-ground organs. Roots are the first to sense the scarcity of nitrogen in the soil. The following protocol describes the step-wise procedure for performing unidirectional root NO_3^- influx with the use of ^{15}N labelled NO_3^- at a concentration that stimulates the inducible high-affinity nitrate transporters. Stable isotopes such as ^{15}N can be used to trace the movement of NO_3^- through the plant. This protocol uses a split-root design where only embryonic roots are treated with the ^{15}N label. The remaining, non-embryonic roots are not labelled but similarly treated. this split-root design can be modified to select and label specific root types based on your interest.

DOI

dx.doi.org/10.17504/protocols.io.biibkcan

PROTOCOL CITATION

Prajakta Bendre, Vanessa Melino 2020. Root nitrate influx . **protocols.io** dx.doi.org/10.17504/protocols.io.biibkcan

KEYWORDS

Split-root design, root nitrate influx., 15N label

CREATED

Jul 13, 2020

LAST MODIFIED

Jul 13, 2020

PROTOCOL INTEGER ID

39203

GUIDELINES

0.1 mM N in the form of $K^{15}NO_3$ was applied to the plants in this protocol. If using potassium nitrate, $K^{15}NO_3$ (Sigma-aldrich), as the label, balance the K^+ across all solutions using K_2SO_4. Each of the pre-label, label and wash steps are performed in 50 ml centrifuge tubes. A single, thin aquarium tube hose is placed in each falcon tube and connected to a pump to provide aeration.

MATERIALS TEXT

1. Pre-wash solution:
Use nutrient growth solution (ie. modified strength Johnson nutrient solution, Johnson et al. 1957) maintaining the same treatments supplied during the growth of the plants.

2. Label solution:
Pre-wash solution except that the nitrogen source is replaced with 0.1 mM N in the form of $K^{15}NO_3$. The concentration of all other nutrient elements in the original growth solution are maintained.

3. Post-label solution:
As per pre-wash solution.

4. Wash solution:
0.01 M calcium sulphate.

1 Grow barley plants hydroponically according to the desired growth conditions.

2 Prepare the set up by pre-filling 50 ml centrifuge tubes with solutions (1-4 above) and aerate.

DAY 18

3 18- day old barley plants were removed from their collars and two plants were bundled together at the stem. These two barley plants provide sufficient material for downstream nitrogen analysis

4 Identify and separate the plant roots based on their type. The split root design here was used to separate the embryonic roots (ER) from the remaining roots (RR). Roots were bundled using nylon wire clips (Figure 1).

5 Return the plants to their hydroponic growth tanks.

DAY 21

6 On sampling day, between 11:00 and 13:00 hours, remove plants from their tanks and immerse them into the pre-wash solution for 5 min (Figure 1).

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7 Transfer the bundled ER to a ^{15}N labeled solution for 10 min (Figure 1).

8 Transfer the bundled ER and the RR to separate tubes of post-label solution for 2 min to avoid contamination of the unlabeled roots with the ^{15}N label (Figure 1).

9 Transfer the bundled ER and the RR to their own 50 ml tubes for a final 5-sec dip in wash solution (Figure 1).

10 Remove the plants and divide into shoots, embryonic roots and non-embryonic roots. Dry them at 70^{o}C for 72 hours. After drying, homogenize the samples into a powder.

11 Maintain a dry powder by storing samples in a desiccator until the preparation of samples for elemental analysis of nitrogen.

12 For elemental analysis of ^{15}N and ^{14}N isotopes, weigh 1-5 mg of sample in a tin capsule and fold.

13 Perform elemental analysis of total nitrogen content using an elemental analyser paired to a mass spectrometer.

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