Measurement of Net NO_3^- Flux in Rice Plants with the SIET System

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[Abstract] SIET (scanning ion-electrode technique) is a new technique to study the flow rate of the ions and molecules in real time in living biomaterials by using microelectrodes and microsensor. This technique allows non-invasive, simultaneous measurement of fluxes of specific ions at the surface of an intact plant. It has high temporal and spatial resolutions. This protocol uses the SIET system for the measurement of ions flux rate in rice plants.

Materials and Reagents

1. Rice seedlings: Three weeks old seedlings
2. Plastic supporting netting
3. Tributylchlorosilane (Fluka, catalog number: 90796)
4. NH_4NO_3
5. KH_2PO_4
6. K_2SO_4
7. $CaCl_2 \cdot 2H_2O$
8. $MgSO_4 \cdot 7H_2O$
9. Na_2SiO_3
10. NaFeEDTA
11. H_3BO_3
12. $MnCl_2 \cdot 4H_2O$
13. $CuSO_4 \cdot 5H_2O$
14. $ZnSO_4 \cdot 7H_2O$
15. $Na_2MoO_4 \cdot 2H_2O$
16. International Rice Research Institute (IRRI) nutrient solution (see Recipes)
17. Calibrate solution (see Recipes)
18. Measuring solution (see Recipes)

Equipment

1. Small plastic dish (6 cm diameter)
2. Measuring chamber
3. Ion-selective electrodes (Clark Electromedical, model: GC150-10)
4. BIO-IM (NMT-YG-100, Younger USA LLC, Amherst, model: MA01002) with ASET 2.0

Software

1. ASET 2.0 (Sciencewares, Falmouth, catalog number: MA 02540)
2. iFluxes 1.0 (YoungerUSA, LLC, Amherst, catalog number: MA 01002) software

Procedure

1. Rice seeds were surface sterilized with 10% (v/v) hydrogen peroxide for 30 min and then rinsed thoroughly with deionized water.
2. The sterilized seeds were germinated on plastic supporting netting (mesh of 1 mm^2) mounted in plastic containers for 1 week 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%. Uniform seedlings were selected and then transferred to IRRI nutrient solution.
3. Rice seedlings were grown in IRRI nutrient solution for 2 weeks and then deprived of N (IRRI nutrient solution without NH_4NO_3) for 3 d. 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%.
4. The roots of seedlings were equilibrated in measuring solution 1 (without NO_3^-) for 20 to 30 min before measuring at room temperature (24 °C-26 °C).
5. The equilibrated seedlings were then transferred to the measuring chamber, and a small plastic dish (6 cm diameter) was filled with 10 ml of fresh measuring solution 2 containing 0.25 mM NO_3^-.
6. Ion-selective Electrodes were made from 1.5 mm (external diameter) borosilicate blanks. The blanks were pulled to < 1 µm diameter tips using a vertical pipette puller and then silanized with tributylchlorosilane. The tips of electrode blanks were broken to a diameter of 2-3 µm, and then back-filled with appropriate solutions. The back-filling solutions for NO_3^- were 0.5 M KNO_3 and 0.1 M KCl. The electrodes were calibrated with calibrate solution 1 (0.05 mM NO_3^-) and calibrate solution 2 (0.5 mM NO_3^-) prior to flux measurements.

7. When the root became immobilized at the bottom of the dish, the microelectrode was vibrated in the measuring solution between two positions, 5 and 35 μm from the primary root surface, along an axis perpendicular to the root meristem zone. The background was recorded by vibrating the electrode in measuring solution not containing roots. The measuring lasted for 15 min.
8. The data obtained were analyzed and converted into NO_3^- influx (negative) (pmol/cm^2/s) using the MageFlux program (http://www.xuyue.net/mageflux). The ion flux assay around each type of transformed cells was replicated independently five times.

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 $ZnSO_4·7H_2O$
   0.39 μM $Na_2MoO_4·2H_2O$
   pH 5.5
2. Calibrate solution
   a. Calibrate solution 1
      H^+ (pH 6.5)
      0.05 mM NO_3^-
      0.025 mM $Ca(NO_3)_2$
      0.1 mM $CaCl_2$
      0.1 mM NaCl
      0.1 mM $MgSO_4$
      0.3 mM MES
      pH 6.5
   b. Calibrate solution 2
      H^+ (pH 5.5)

0.5 mM NO_3^-
0.25 mM $Ca(NO_3)_2$
0.1 mM $CaCl_2$
0.1 mM NaCl
0.1 mM $MgSO_4$
0.3 mM MES
pH 5.5

3. Measuring solution
   a. Measuring solution 1
      0.2 mM $CaCl_2$
      0.1 mM NaCl
      0.1 mM $MgSO_4$
      0.3 mM MES
      pH 6.0
   b. Measuring solution 2
      0.125 mM $Ca(NO_3)_2$
      0.1 mM $CaCl_2$
      0.1 mM NaCl
      0.1 mM $MgSO_4$
      0.3 mM MES
      pH 6.0

Acknowledgments

This protocol is adapted from Xu *et al*. (2006); Sun *et al*. (2009) and Tang *et al*. (2012).

References

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