Autoradiography of Pi Distribution in Barley Seedlings

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[Abstract] Phosphorus-32 and Phosphorus-33 are radioisotopes of phosphorus. These isotopes are used to trace ionic phosphorus and phosphorus compounds. This protocol is used to follow the movement of inorganic phosphate (PO_4^{3-}) from a leaf tip to the rest of the plant.

Materials and Reagents

1. Barley seedlings
2. Radioisotopes ^{32}P or ^{33}P labeled NaH_2PO_4 dissolved in water (MP Biomedicals, PerkinElmer or American Radiolabeled Chemicals)
3. 5 mM $CaSO_4$ solution
4. Hydroponic culture solution (see Recipes)

Equipment

1. Cling film
2. 1.5 ml plastic tubes
3. 15 ml plastic tubes (1.5 ml tube is fitted by opening a hole in the lid) (Figure 1)
4. Cotton
5. Plastic sponge
6. Imaging plate (FCR Imaging Plate for general purpose) (Fujifilm Corporation) and plate cassette (FCR standard cassette) (Fujifilm Corporation)
7. Imaging analyzer (GE Healthcare, model: Typhoon 9400 or other Radioisotope imaging analyzers)

Procedure

1. Barley plants are germinated on moist filter paper for 2-3 days and then seedlings are grown in hydroponic culture for 7-8 days.
2. Cotton is put in a 1.5 ml tube from which the cap has been removed, then a radioisotope medium consisting of 600 µl of 0.2 mM $NaH_2{}^{32}PO_4$ (specific activity 3.7 MBq/nmol) in

5 mM $CaSO_4$ is added. Cotton is put enough to absorb all 600 µl $NaH_2{}^{32}PO_4$. But, do not put too much cotton to keep it moisture.

3. Into the 15 ml tube, an appropriate amount of incubation medium (5 mM $CaSO_4$) is added. A barley plant sandwiched with sponge is put into the medium, and the barley leaf is manipulated into position against the plastic sponge separated from solution, such that when the smaller tube containing the cotton is mounted into a hole in the cap of the 15 ml tube, the tip of the leaf comes into contact with the cotton soaked in radioactive medium (Figure 1).

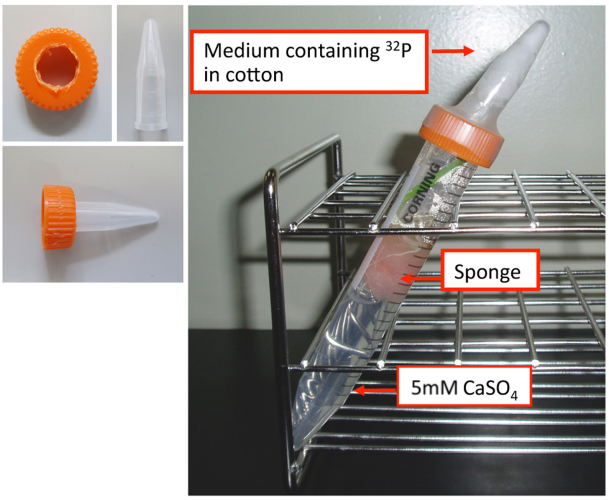

Figure 1. Setup for the radioisotopic labelling of a leaf tip

4. After an appropriate labelling period (about 2 h) at 25 degrees, the sample is washed with water several times and wrapped with cling film. The sample is then set on an imaging plate and exposed (Figure 2).

Figure 2. Setup of the radiolabeled sample on an imaging plate

To avoid contamination of imaging plate with ^{32}P, samples are wrapped by cling film. Wrapped sample is attached to the imaging plate by using binding case to expose.

5. Examine the imaging plate with an imaging analyzer (Figure 3). ^{32}P concentration is indicated by pseudo-color.

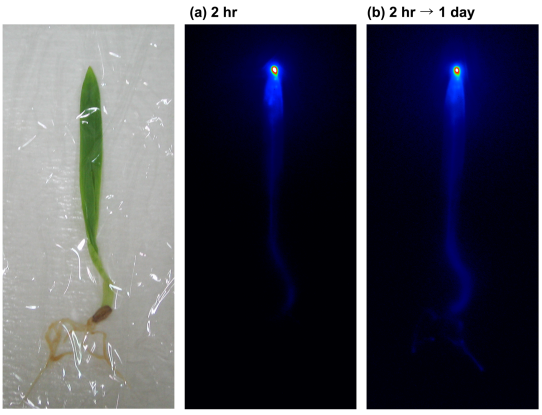

Figure 3. Autoradiogram of ^{32}P distribution in a barley plant which was radiolabeled from the leaf tip

6. Following imaging, plants can continue to be incubated in 5 mM $CaSO_4$ solutions under the same light conditions for 1 or 2 d without cap in order to examine the movement of the radioisotope. In Figure 3 it can be seen that $NaH_2{}^{32}PO_4$ absorbed at the leaf tip was translocated to the root after one day of incubation.

   Figure 4 shows autoradiograms taken every 2 days following the initial incubation of roots in a Pi-deficient barley plant with an appropriate amount of incubation medium mixed $NaH_2{}^{32}PO_4$ (Mimura *et al.*, 1996)

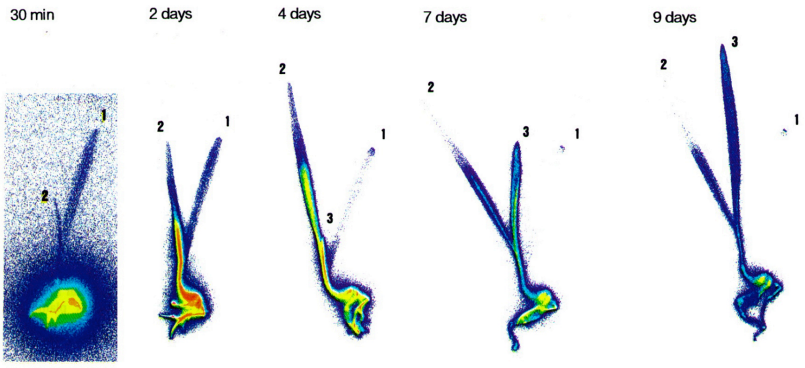

Figure 4. Autoradiogram of ^{32}P distribution in 10 days old barley plant grown in a Pi-deficient medium, which was radiolabeled from the root. The number put at the leaves in order of development.

Notes

1. Resolution and clarity of images are strongly dependent on the radioisotope contents of plants and exposure time on the imaging plate.

Recipes

1. Hydroponic culture solution
   9 mM KNO_3
   6 mM $Ca(NO_3)_2$
   3 mM $MgSO_4$
   1.5 mM KH_2PO_4
   0.125 mM Fe-EDTA
   Micronutrients: 10 µM $MnSO_4$, 1 µM $CuSO_4$, 1 µM $ZnSO_4$, 30 µM H_3BO_3, 30 µM $(NH_4)_6Mo_7O_{24}$, 0.1 µM $CoCl_2$

Acknowledgments

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References

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