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Effects of lateral mannitol treatment on the development of tissues in rice seminal roots

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ABSTRACT

Plants modulate their body plan through modulation of cellular developmental processes in response to environmental changes. However, it is not easy to determine whether time-course of cell differentiation process was changed under a certain environment in an organ *in situ*. We previously proposed a unique method to monitor changes in the rate of cell differentiation by monitoring cell production rate. However, measuring cell production rate needs a laborious anatomical procedure. To avoid this difficulty, we have developed another convenient method in this study. Rice seeds were sandwiched between two agar plates, only one of which contained 270 mM mannitol solution, and roots were allowed to grow for four days attaching to both agar plates. Cross sections were cut basipetally from the root apex and they were observed under a fluorescence microscope to examine whether there are any differences in the cellular developmental state between the two sides, the mannitol side and the control side. As a result, indicators of cell differentiation, such as, widening of the Casparian strip in the radial wall of the endodermal cells, suberization of the inner tangential wall of these cells, and formation of aerenchyma were promoted in the mannitol side while appearance of the Casparian strip as fluorescent dots did not differ between the two sides.
KEYWORDS: rice, endodermis, Casparian strip, aerenchyma, osmotic stress

1. INTRODUCTIONS

One of central questions in the study of plant physiology is how plants modulate their body plan through modulation of cellular developmental processes in response to environmental changes. It is not generally easy to determine whether time-course of cell differentiation process can be modulated responding to environmental stimuli in a developing organ *in situ*. However, an individual root provides a good model system for such analysis because of its simple cell tissue organization and its one-dimensional growth. We have previously proposed a method to monitor changes in the rate of differentiation of a particular cell type in roots (Karahara et al., 2008). However, even for the purpose of analyzing only cell differentiation, we have to measure cell production rate as far as using the previous method. And measuring cell production rate needs a laborious procedure. To avoid this difficulty, we have developed another convenient method. Although this method is applicable to detecting change only of cell differentiation, it is possible to compare the change in response to environmental stimuli precisely at the same cell age.

2. METHODS

2.1. Plant Materials

Rice (*Oryza sativa* ssp. *japonica* cv. Nipponbare) caryopses were sterilized in 2.5% (w/v) sodium hypochlorite solution for 5 min, washed with water, imbibed for first 4 days at 4ºC and then for 1

day at 30ºC for germination. Imbibed rice caryopses were sandwiched between two 2% (w/v) agar plates containing 1/10 strength Hoagland medium, only one of which contained 270 mM mannitol solution. Roots were allowed to grow at 25ºC in darkness for three to five days attaching to both agar plates.

2.2. Observation of root tissues

Cross sections in 100 μm thickness were cut basipetally from the root apex at 2.5 mm intervals using a linear slicer (PRO7; Dosaka EM, Kyoto, Japan). The sections were stained with 0.2% (w/v) berberine hemisulfate and then with 4% (w/v) aniline blue to visualize suberin localization. The stained sections were observed under a fluorescence microscope equipped with a filter assembly (WBV) for excitation by UV light. Formation of aerenchyma and root hairs were also observed using bright field optics.

3. RESULTS AND DISCUSSIONS

3.1. Effects of mannitol treatment on the tissue development

Seminal root growth was monitored and confirmed to continue during three to five days after sowing. Mean length of four-day-old seminal roots treated one side for mannitol is ca. 50 mm. Therefore, the seminal roots of 42-58 mm in the length were selected for the observation. Cell wall development of endodermal cells in rice seminal roots were classified into three stages; 1st, only the Casparian strip formed in the radial walls; 2nd, suberization occurred additionally in whole radial walls (or, the Casparian strip widened entirely); 3rd, suberization occurred additionally in inner tangential wall. Development of cell walls of endodermal cells and aerenchyma showed differences between the control side and the mannitol-treated side on cross sections, indicating that the rice root tissues responded locally to the mannitol treatment.

3.2. Quantification of tissue development on a cross section

A cross section was divided into two sides (control side and mannitol-treated side) according to the mark. Each side was then divided into eight unit areas. Percentages of the stages of endodermal cell wall development and of aerenchyma formation were plotted against the distance from root tip. The distances were compared when the percentage reached 40% in the cases of aerenchyma and suberized radial wall, 80% in the case of Casparian strip and root hairs, 30% in the case of suberized inner radial wall.

As a result, appearance of the Casparian strip as fluorescent dots did not differ between the both sides, suggesting that the Casparian strip formation might be determined as an ordinary root tissue development. In contrast, mannitol treatment promoted the suberization of whole radial walls and suberization of the inner tangential wall of the endodermal cells, suggesting an enhancement of apoplastic barriers by mannitol treatment. Mannitol treatment also promoted the formation of aerenchyma, which might contribute to reduce apoplastic pathway itself by reducing apoplast volume.

REFERENCES

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