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Role of root hairs and aquaporin genes (AtPIPs) in water uptake

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Objectives

The uptake of water from the soil and its delivery to the xylem requires that water move radially across living root tissues like cellular membranes. The root hairs' role as major water and nutrient-uptake sites has also made them an obvious site to study the molecular basis of water transport activities in the root. Here, we are showing an evidences that root hairs are essential for water uptake using several root hairless mutants and seeing the beginnings of a comprehensive molecular expression of transport activities (aquapoeins) in plasma membrane.

Materials and Methods

Seedlings of Arabidopsis (Col-0, trh1-1, and rhd6) were grown under short-day condition 200 μmol m^{-2} s^{-1} light in soil and aerated hydroponic culture. Also seeds were surface sterilized and then planted on plates with modified nutrient medium. After water stress treatments, biomass and transpiration rate were measured and total RNA was isolated and its quality was checked by agarose gel electrophoresis. Two micrograms of DNA free RNA was then reverse transcribed using First-Stand Synthesis System for RT-PCR. cDNA concentrations were then normalized using β-tubulin and ubiquitin primers. Root hair protoplasts were isolated from separately grown seedlings following the method described by Ivashikina et al. (2001) The enzyme solution contained cellulase, pectolyase, bovine serum albumin, polyvinylpyrrolidone, $CaCl_2$, and MES/Tris (pH 5.6). Osmolarity of the enzyme solution and 1 mM $CaCl_2$ buffer (pH 5.6) were adjusted to 280 mosmol kg^{-1} using D-sorbitol. Roots were incubated in enzyme solution at 30°C for 30 min. Protoplasts released from the tip of the root hairs. The protoplast suspension was stored on ice, and aliquots were used for RT-PCR. To confirm that the isolated protoplasts were from root hairs, specific primers were used for a gene that is not expressed in root hairs.

Results and Discussion

We tested two different root hair mutants for water uptake properties under water-sufficient and -limiting conditions. The *trh1-1* (tiny root hairs) mutant had fewer root hairs than the wild type; *rhd6* (root hair defective) almost completely lacked root hairs (Fig. 1). We tested the growth of these lines and found that under water-limiting conditions, the presence of root hairs was important (Fig. 2 and 3). A decrease in whole-plant biomass, root length and transpiration rate in roots and leaves at water-limiting conditions was related to the amount of roots hairs present (Fig. 4). Also, we found that 10 AtPIP genes were expressed in root hairs

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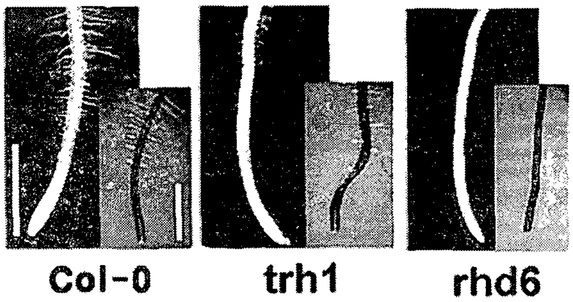

Fig. 1. The difference between root hairs of the Col-0, *trh1-1*, and *rhd6*.

Fig. 2. Recovery analysis of plants after water stress 2 weeks.

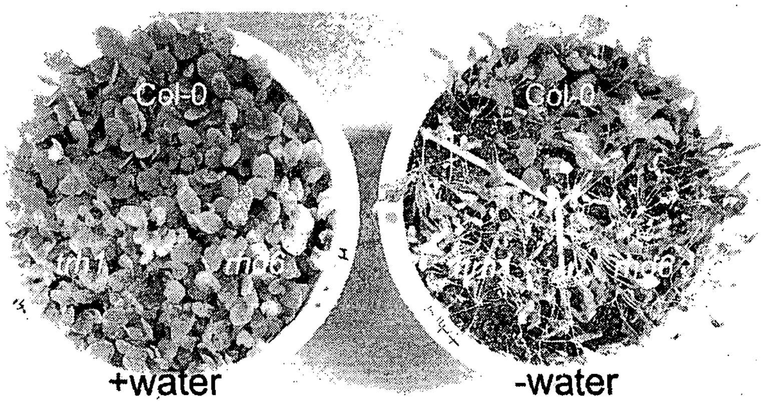

Fig. 3. Comparing with Col-0, *trh1-1* and *rhd6* after water stress for 2 weeks.

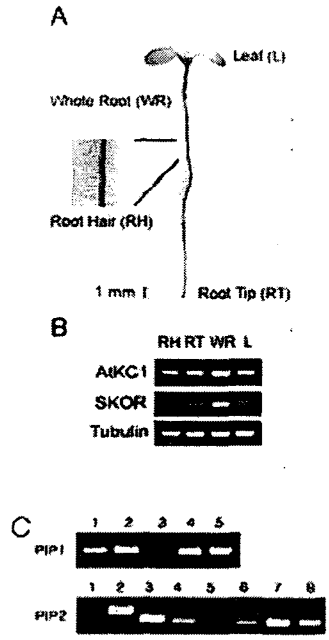

Fig.5 RT-PCR analysis of the expression of AtPIPs in root hairs

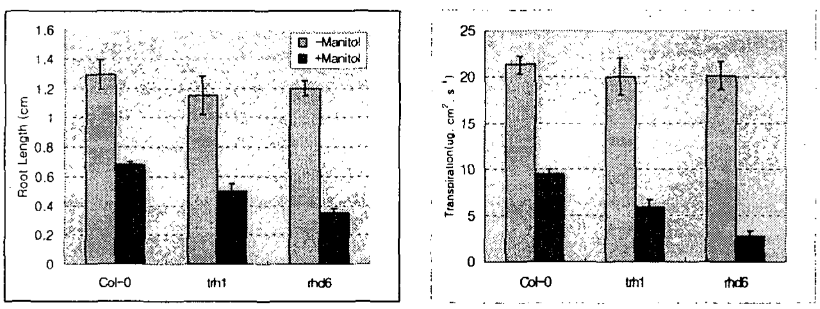

Fig. 4. Growth analysis of root and leaf by water stress (0.25M manitol).