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ROT3 and ROT3 homolog, which fine-tune the biosynthesis of brassinosteroids in Arabidopsis, play critical roles in plant morphogenesis

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Objectives

In order to elucidation of the regulation of brassinosteroid biosynthesis, we have identified and characterized two cytochrome P450s, which are possibly involved in brassinosteroid biosynthesis.

Materials and Methods

1. Material
   Plant - *Arabidopsis thaliana* (Col-0), *rot3-1, cpd* mutants
2. Methods: Genetical and biochemical approaches, light experiment

Results and Discussion

Brassinosteroids (BRs) are plant hormones that are essential for a wide range of developmental processes in plants. Many genes responsible for the early and later reactions in thebiosynthesis of BRs have recently been identified. However, the genes for enzymes of several steps in the biosynthesis of BRs remain to be characterized, and none of the genes responsible for the reaction that produces bioactive BR have been identified. Here we found that the ROTUNDIFOLIA3 (ROT3) gene, which is involved in the specific regulation of leaf length in Arabidopsis, encodes the enzyme CYP90C1, which is required for the conversion of typhasterol to bioactive castasterone in BR biosynthesis. We also analyzed the gene most closely related to ROT3, ROT3 homolog/CYP90D1, and found that double mutants for ROT3 and for ROT3 homolog have a synthetic dwarf phenotype, whereas cyp90d1 single knockout mutants do not, suggesting that these two cytochrome P450s act independently at different steps in BR biosynthesis. BR profiling in these mutants revealed that ROT3 homolog is also involved in the early steps of BR biosynthesis. ROT3 and ROT3 homolog were expressed differentially in leaves of Arabidopsis, and the mutants for these two genes differed in defects in elongation of hypocotyls under various light conditions. Dark induced the expression of ROT3 homolog, especially in leaf petiole. These results provide evidence that these two cytochrome P450s, ROT3 and ROT3 homolog, not only play critical roles in BR biosynthesis, but also connect BR biosynthesis to the responses of plants to light.

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