Gibberellin-mediated regulation of major cell-wall proteins in pea roots

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ABSTRACT

Gibberellin (GA), a shoot growth-promoting hormone, is known to regulate root growth in the presence of ancymidol (Anc), an inhibitor of GA biosynthesis. We have analyzed cell wall proteins of pea roots (*Pisum sativum* L.) in view of suggested importance of cell walls during morphological change of root cells by GA and Anc. Major protein bands on SDS-PAGE were compared between Anc-treated and (Anc+GA)-treated roots. The most remarkable was the down-regulation by GA, i.e. up-regulation by Anc-treatment, of 35 kD protein band which had 100 % homologous partial amino acid sequences to peroxidase of white clover (*Trifolium repens*). Other down-regulation by GA were chitinase class III. Up-regulated proteins by GA were pectin methylesterase and another peroxidase. As far as major protein bands from cell walls are compared, Anc-treated (GA-starved) thick roots accumulates peroxidase in the cell walls suggesting the higher level of cell-wall stiffening by peroxidase-mediated cross-linking of cell-wall components. GA may keep more extensible root cell walls by decreasing the peroxidase content and by regulation of pectin methylesterase.
KEY WORDS: apoplast proteins, cell wall, gibberellin, pea, peroxidase, root growth.

INTRODUCTION

Gibberellin (GA), a shoot growth-promoting hormone, is known to regulate root growth (Tanimoto, 2005). GA function in root elongation was discovered by the interaction of GA with ancymidol (Anc), an inhibitor of GA biosynthesis. By the dose-response experiment, GA was found to maintain slender root elongation. Anc treatment, without GA application, induced thickening of roots (Tanimoto, 1994; 2002). Using this experimental system, we have analyzed cell wall proteins in view of suggested importance of cell wall deformation during morphological change of root cells by GA and Anc.

MATERIALS AND METHODS

Pea plants *Pisum sativum L.* cv. Alaska were grown under fluorescent light in hydroponics containing an inhibitor of gibberellin biosynthesis, 10 μM ancymidol (Anc), with or without 1 μM GA_3. The apical 20-mm parts of main and lateral roots were harvested, frozen, homogenized and cell wall fraction was purified. Cell-wall bound proteins were extracted by 2 M NaCl solution and sequentially precipitated by salting out with $(NH_3)_2SO_4$ at 30%, 50%, and 80% saturation. Cell-wall bound proteins were separated by SDS-PAGE and major protein bands were compared between Anc- and (Anc+GA)-treated roots. Amino acid sequences of

major proteins bands affected by GA were analyzed by either N-terminal or internal sequencing and identified by homology analysis on database.

RESULTS AND DISCUSSION

Since GA alone shows little effect on root growth, effect of GA was tested in the presence of Anc (Tanimoto, 1994; 2002). Major cell wall proteins affected by GA were listed in Table 1. Up-regulated proteins by GA were pectin methylesterase, peroxidase, and down regulated were peroxidases and chitinase class III. The most remarkable effect of GA was down-regulation, i.e. up-regulation by Anc-treatment, of 35 kD protein band which had 100 % homologous partial amino acid sequences to peroxidase of white clover (*Trifolium repens*). Since peroxidase mediates cross linking of cell-wall components (Fan *et al*., 2006; Wakabayashi *et al*., 2009), Anc-treatment (GA-starvation) may cause higher level of cell-wall stiffening to inhibit root elongation. These results show GA function to regulate cell wall proteins to keep extensible cell walls. GA-depletion by Anc induces peroxidase to make cell wall less extensible by increasing cross linkages in cell wall phenolics. Since some peroxidases and chitinases function as defense system against pathogens, GA-regulation of these proteins may participate defense activity of roots against pathogens.

Table 1. Gibberellin-regulated major cell wall proteins in pea roots.

Up or down by GA	Name	M.W. (kD)	Identities	Plants
down	Peroxidase	35.5	100 % --12 aa--	*Trifolium repens*
down	Peroxidase	36.0	66% N-15aa--	*Medicago truncatula*
down	Chitinase ClassIII	8.4	83% N-12aa--	*Oriza sativa*
Up	Pectin methylesterase	36.0	60% --15aa--	*Vigna radiata*
Up	Peroxidase	41.2	60% N-15aa--	*Glycine max*

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