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Identification of differently expressed genes in a natural fruit-abscission apple, Akane, compared to Fuji

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Objectives

Differently from Fuji cultivar, Akane cultivar shows a characteristic of severe early-season lateral fruit drop. Through identification of genes involved in early-season lateral fruit drop, we expect to obtain the information in molecular level on the early-season fruit drop in Akane cultivar.

Materials and Methods

1. Material

Plant: Malus domestica cv. Akane, Malus domestica cv. Fuji
- cultivated under field condition at the Taegu Apple
Research Institute

PCR subtraction: PCR-Select cDNA Subtraction Kit (Clontech)

2. Methods:

Subtractive hybridization, Plasmid library construction, Electro-transformation, Dot blotting, Genomic library construction, Plaque hybridization, Comparative RT-PCR

Results and Discussion

Akane, an apple cultivar, has a characteristic of natural abscission in lateral fruit, not in central fruit. Abscission is a fundamental and a highly regulated process of plant development. Though abscission in leaf, flower, and fruit has been studied in tomato and Arabidopsis, little information about abscission of woody plant has been known. To get information about molecular mechanism on lateral fruit drop in Akane, firstly, we have carried out PCR subtraction using PCR-Select cDNA Subtraction Kit, and Akane and Fuji central and lateral fruit/peduncle mRNA. The PCR products were subcloned into pGEM-T easy vector and transformed into E. coli SURE strain. Through dot blot analysis using plasmid pool, and Akane and Fuji cDNA probe, we isolated several upregulated cDNAs in Akane including cellulase, beta-1,3glucanase, phosphate transporter, chitinase, nuclease, glyoxal oxidase, protein serine/threonine kinase, and RING finger protein, and several down-regulated cDNAs including glycerol kinase, hydrolase, and sorbitol dehydrogenase. Most of upregulated cDNAs are stress-responsive genes. Especially it is known that cellulase and beta-1,3-glucanase are involved in abscission as cell wall-degrading enzymes. Three cDNAs and one genomic clone encoding protein serine/threonine kinase and phosphate transporter showing the strongest signal in dot blot analysis were isolated from Fuji cDNA and genomic libraries. We constructed binary vector and transformed it into micro-tomato. Glycerol kinase, a down-regulated gene in Akane, plays key role in lipid metabolism and the cDNA and genomic DNA are being isolated. The research on how the genes are involved in early-season lateral fruit drop is on progress.

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