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Cloning of a Gene for Starch Phosphorylase in Potato (*Solanum tuberosum* L. cv. Atlantic)

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Objectives

Potato tubers are either treated with dormancy-prolonging chemical or are stored at low temperature to prevent sprouting and/or cold-induced sweetening during storage. Under the cold conditions, starch is converted into soluble sugars, and hexoses react with free amino acids, thereby negatively affecting the processing quality of potato tubers. In this study, to investigate the relationship between gene expression of starch-degradative enzymes and cold-induced sweetening of tuber, and to prevent the production of reducing sugar by gene alteration, one of the gene for metabolic enzymes in starch degradation, starch phosphorylase, have been cloned and sequenced.

Materials and Methods

Total RNA and polyA⁺ RNA were isolated from *Solanum tuberosum* L. cv. Atlantic by CTAB method and GTC-GHCl

method. cDNA library was constructed using ZAP Express cDNA Synthesis Kit and ZAP Express cDNA GigapackIII Gold Cloning Kit (Stratagene Co. U.S.A.). Gene-specific primers and partial gene fragment of the starch phosphorylase were used for RT-PCR and screening. The isolated clone was sequenced.

Results and Discussion

Full length cDNA of the gene for starch phosphorylase was 2,901 bp long and encoded 966 amino acids. For the gene expression and gene alteration by the antisense inhibition technique, the SacI-digested DNA fragment (5' -half region of the gene, 1.5 kb) of starch phosphorylase was cloned into plant expression vector pBI121 in different orientation (sense and antisense). The transformation and regeneration of potato, and the transgene analysis should be accomplished. This gene for starch phosphorylase will be useful for development of starch variants and metabolic-engineered potato.