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# Gene Cloning and Analysis of Invertase Inhibitor Proteins from Plants

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## Objectives

Potato tubers are either treated with dormancy-prolonging chemical or are stored at low temperature to prevent sprouting. During the cold storage, hexose accumulation in potato tuber is thought to be caused by an imbalance between the rate of starch degradation and the rate of glycolysis leading to accumulation of sucrose, which subsequently is divided into glucose and fructose via invertase. In this study, to investigate the relationship between reduction in the activity of invertase and hexose accumulation of tuber, and to inhibit the formation of sucrose-derived hexoses, genes for proteinous invertase inhibitors were cloned and analyzed.

## Materials and Methods

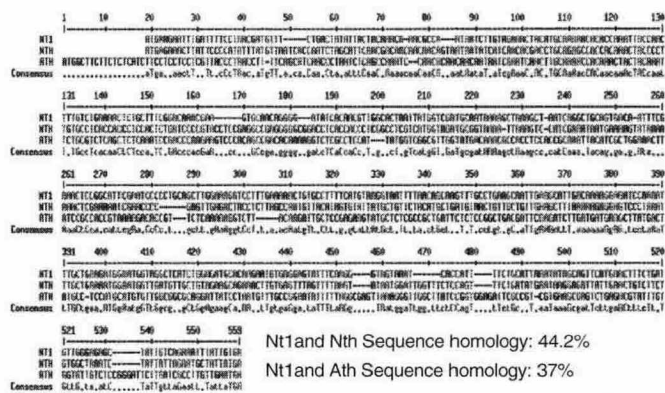
Total RNAs were isolated from *Nicotiana tabacum* L. and *Arabidopsis thaliana* L. by CTAB method and GTC-GHCl method. Gene-specific primers were used for RT-PCR and screening. The amplified DNA fragments were cloned and sequenced. For investigation of characteristics of the invertase inhibitor proteins, overexpression and purification of the recombinant proteins have been performed using prokaryotic overexpression system.

## Results and Discussion

Full length cDNAs of genes for tobacco invertase inhibitor proteins were 501 bp and 519 bp long, respectively, and encoded 166 and 172 amino acids, respectively. ORF of *Arabidopsis* invertase inhibitor proteins was 543 bp long and encoded 180 amino acids. The molecular masses estimated by deduced amino acid sequences and SDS-PAGE were 18.3 kDa, 19 kDa, and 19.8 kDa, respectively. The similarities of nucleotide and amino acid sequences are not so high, however, four cysteine residues are present at positions conserved in three invertase inhibitor proteins. The characterization of the invertase inhibitor proteins have been performed using the recombinant proteins. The gene cloning and investigation of the roles of invertase inhibitor proteins for preventing cold-induced sweetening will be useful for the development of value-added potato and various applications in the field of food industries.

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