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Cloning and Characterization of a Promoter Region of Gene for Tuber-specifically Expressed Lipoxygenase in potato

Soonhwa Kwak, Jeongmin Lee, Tae Young Chung, Moosik Kwon*

Department of Genetic Engineering, Sungkyunkwan University, Suwon 440-746, Korea

Objectives

Potato (*Solanum tuberosum* L.) is one of the most important crops worldwide. Using *Agrobacterium*-mediated gene transformation, development of value-added potato and various applications in the field of food industries have been performed. In development of transgenic potatoes, tuber-specific promoter is very important for effective gene expression, protein targeting, and plant growth. In this study, tuber-specific gene expression of lipoxygenase 1 (*lox*1) was investigated, and 5'-flanking region of the gene was cloned and analyzed.

Materials and Methods

Total genomic DNA was isolated from potato (*Solanum tuberosum* L. cv. Atlantic) by CTAB method. Gene-specific primers and partial gene fragment of *lox*1 were used for amplification of DNA fragment, and 5'-flanking region of *lox*1 gene was cloned using Genome Walker kit (Clonetech Co., USA) and was analyzed.

Results and Discussion

According to the results of RNA gel blot analysis by Kolomiets *et al.* (The Plant Cell 13:613-26, 2001), l*ox*1 transcripts were detected only in undergroud organs, not in leaves, stems or flowers, and the highest levels of mRNA occurred in actively growing tubers. A portion of 5'-flanking region (1.5 kb) of Lox1 was cloned and sequenced. Within this region several putative *cis*-acting elements were identified. 1,285-1,289 and 1,409-1,414 regions of cloned DNA fragment are predicted as CAAT-boxes, and 1,498-1,504 region is predicted as a TATA-box by homology search in sequence database (PlantCARE: http://intra.psb.ugent.be:8080/PlantCARE/). Putative promoter sequence and putative transcription start site are in 1,530-1,551 region of the clone. To confirm the promoter activity and essential sequence motif, the proximal 1.5kb and progressively 5'-deleted fragments were fused with a β-glucuronidase (GUS) reporter gene. The constructs of putative *lox*1 promoter has been tested by transient expression assays in tobacco leaves. The tissue-specific expression patterns will be investigated using transgenic tobacco and potato. Development of tuber-specific promoter will be useful to control the foreign gene expression in potato.

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Corresponding author : Moosik Kwon, TEL: 031-290-7861, E-mail: mskwon@skku.edu