P56 Cloning and Sequence Analysis of Tuber-specific Lipoxygenase Promoter from Potato (*Solanum tuberosum* L. cv. Atlantic)

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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 (Lox1) was investigated, and 5´-flanking region of the gene was cloned and analyzed.

Materials and Methods

Total RNA and polyA+RNA were isolated from *Solanum tuberosum* L. cv. Atlantic by CTAB method and GTC-GHCl method. Gene-specific primers and partial gene fragment of Lox were used for RT-PCR and cloning. 5´-flanking region of Lox1 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), Lox1 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. 1,285-1,289 and 1,409-1,414 regions are predicted as CAAT-boxes, and 1,498-1,504 region is predicted as a TATA-box by homology search in sequence database. 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, construction of reporter gene system and plant transformation of Lox1 promoter region has been performed. Development of tuber-specific promoter is very useful to control the foreign gene expression in potato.

Acknowledgement

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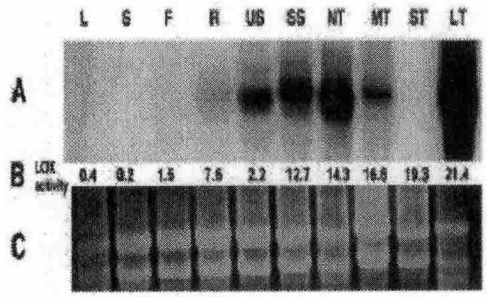

Figure 1. RNA gel bolt analysis of Lox1 in potato (Kolomiets *et al.* 2001)

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ATTGTAATACGACTCACTATAGGGCACGCGTGGTCGACGGCCCGGGCTGGTATCTGGGATATGAGTTAGTTTGGTAGTTT   160
GGTTGTGGAGCTTGATAGTTCGAGGGGGTATTTTGGTAAAGTGGAGCAAGGGTTTGGTACGTTGGGGAAGGCGTTTTGTA   240
ATTTGTCTGAATATTGGCACAGTTGGGGGCGGCTTTTCGGTAGTGATGAGATGAAGTCTGGTAAACTGGAGGGGGAGTTT   320
GGTAGATAGTAGGGGGAGTTTGGTAAGTAGGAGAAGGAGTATTTTGATAACTAGGAGTAGGGGTATTTTGGTAACTGGGT   400
GGAGGGGTATTCTGGTGATCAGCATGTGCATAACAAGTTGGGTATAAACTCTGTGAAGGTCGAGAACGACCTTGGTATGA   480
CAAGGATTTCCTTGGGGTCTTCTTCCCCTCGTAAGAGATAGATGACACATCCTCTCTTTTCTTCTTTAACAAGCCCGAAG   560
ATCCGGGCGATGCAGCAACACGGGCAATCTTCCCGGTTCTCAATCCATCTTCGATAGTCTCACCTACCTTGACTATCTCG   640
GCAAATTTTGCTCCAACGAGCAACATGATTCTGTCATAATACTCGGGCTCTTGCACCCGCACAAACACCTCGACAATTTC   720
CTTTTCTGACATAGGAGGTTTAACTCTAGCTGCCTCCTTTCGCCATCTATATGCGAATTTTCTGTAACTTTCCGTCGACT   800
TCTGCTTCATCTTCTCCAATAAGTAACGATCAGGAACAATCTCCACATTGTAGGCGAATCTTTCGATGAAGTCCTTAAGC   880
CAGAGCATTCCATCTAGACCACTGTCTCGTTTCACGTGATGTAAACAATTCTAGGGCCTCTCCACTCAGACTTCGGCTAA   960
AAAACCGCATTAACAAAGTTTCGTCTCTCCCAACTCCCTCGAGTTGGTCACAGTAAGCCCTCAGGTGTGCTAAGGGTTCC  1040
CCATTCCTCCGGAGATGTCACATTTTGGCACCTTGAACCCTTCCGGGAGGGTACAAATTCGGATGAATGCACAGGTCTTC  1120
GTAACTCAACCTGGAGACCTCAGGAATGCAGTTCAACTCCTTCATGGCTTTTCTGATTTCCTCTTTCATATCTATCTTGA  1200
CGGTCTCTTCCTTTTACCTCCACTCTCTCTCCCTCTTCTCATAGTGGTCGATCTCCAAACCATGGGCGTCATGCTCGTTG  1280
GGGACAAATATTTGGAAAGTGGCCTTTTGTGGTAGTGGTGGAGAAATAGAGGGACTTTGGGAGTTTTAAGTGGCTTGGTA  1360
   CAAT-box
ATTTTGAGGGAAAGTCTGGGGGTTGGTATGTTGATTTTGGTGATGGAGCAAAATGTGTGGGTTATTGGCATCTTGGCTGT  1440
                                                    CAAT-box
GAGGGGGTAAGGTAGTTTGGTGGTTTGCGTCGGGGGGAGGGAGTGGTGCTTGGATGTTGGTAATTGAGGAGGGGGTGGT  1520
                                                    TATA-box
GTTTGATAAGAGGCTGAGGCGGAGTGGGGGTTCTGAGTGGTAAGGTCAATGATGGAGGGATTACGAGCGGACTATTTGGT  1600
  Promoter Sequence/Transcription start site  Start codon
GGCCATAATCACTAGTGAATTCGCGGCCGCCTGCAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCAAGCTTG  1680
AG                                                                                  1682
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Figure 2. Sequence analysis of Lox1 promoter region

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