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Isolation and characterization of a proteinase inhibitor II gene that is not wound-inducible (Accession No. [U45450](#)).

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From a Russet Burbank Potato DNA library, we have isolated several proteinase inhibitor II genes (pin2). One of these, pin2T, was subcloned, and a 2068 nucleotide insert was sequenced. This fragment contained the complete Inhibitor II gene including 1013 bp of the flanking DNA upstream from the gene and 454 bp of flanking DNA downstream from the gene. The open reading frame encodes a protein similar to other reported Proteinase Inhibitor II proteins (Graham *et al.*, 1985). The DNA sequence of the 5' flanking region of pin2T from -714 to +1 is highly homologous (Table 1) with that of the previously isolated wound-inducible pin2K (Thornburg *et al.*, 1987). There are however, four small deletions [22 bp (-221..-200), 20 bp (-263..-254), 65 bp (-523..-426), and 52 bp (-759..-708)] in the pin2T promoter.

Chimeric constructs containing the pin2T promoter (-1012 to +24) linked to chloramphenicol acetyl transferase (CAT; pRT198) and b-glucuronidase (GUS; pRT193). Promoter deletions were also prepared at various restriction enzyme sites in the promoter, and GUS constructs were made pRT192, -777 to +24; pRT191, -657 to +24; pRT196, -417 to +24; and pRT194, -198 to +24. *Nicotiana tabacum* cv Xanthi was transformed to kanamycin resistance by *Agrobacterium tumefaciens* containing each of the transgenes. The presence of the transgenes was verified by PCR. CAT (Gorman *et al.*, 1982) or GUS (Jefferson 1989) analyses of these plants indicated that pin2T is not a wound-inducible gene, but is expressed at low levels. The promoter deletions did not affect the mode of expression. All plants showed low constitutive expression and were not wound-inducible. These transgenic plants were not capable of being induced by sucrose, or ABA, nor were the genes derepressed by low auxin concentrations.

The nucleotide sequence of the pin2T promoter is highly homologous (91% identity) with that of the wound-inducible pin2K from -767 to +29. Yet the pin2T promoter was neither induced by mechanical wounding, nor by various biochemicals known to induce the expression of the wound-inducible pin2K. This weak promoter activity was not due to the presence of silencer sequences in the upstream region of the pin2T promoter because removal of upstream portions of pin2T promoter did not activate the remaining core promoter. Rather, the loss of sequences in the four small promoter deletions are likely responsible for the loss of enzyme activity.

Three independent studies have examined the promoter of the potato Inhibitor II genes (Keil *et al.*, 1990; Palm *et al.*, 1990; Lorberth *et al.*, 1992). Some discrepancy exists in these studies, but the net results show

that sequences controlling the wound-inducible phenotype are localized between -557 and -506. This region of overlap is deleted (-523..-426) in the promoter of the noninducible pin2T gene.

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Table 1. Characteristics of the Potato Proteinase Inhibitor II T gene

Organism:

Solanum tuberosum

Gene Product:

Proteinase Inhibitor II, inhibits the enzymatic action of trypsin and chymotrypsin

Characteristics of the gene:

The sequenced DNA fragment of the pin2T gene was 2068 nucleotides covering the entire pin2T structural gene, 1013 nucleotides of the 5' upstream region, and 454 nucleotides of the 3' downstream region. By matrix analysis, the sequence of the pin2T promoter shows 91% identity with the pin2K promoter (Thornburg, *et al.* , 1987).

Upstream Region -- 1013 Nucleotides (1..1013)

Putative promoter elements:

Direct Repeats

No large repeated regions were identified in the pin2T promoter by matrix analysis. Several small (15 bp or less) direct repeats were identified if mismatches were allowed.

Hairpin Loops

2 hairpin loops with stems equal to 10 bp were detected.

1) 1 mismatch - (555..564) - (573..582)

2) 1 mismatch - (898..907) - (908..917)

TATA box

(978..990) (10/13) to canonical plant TATA box (Joshi, 1987a)

bZIP Binding Sites (ACGT)

104, 144, 544

5' end of pin2T cDNA -- nucleotide 1014 (based upon homology with tomato Proteinase Inhibitor II cDNA (Graham, *et al.* , 1986).

5' untranslated region -- 44 nucleotides (1014..1057)

ATG start codon -- 1058..1060

Intron 1 -- 113 Nucleotides (1110..1222)

TAA stop codon -- 1612..1614

3' untranslated region -- 454 Nucleotides (1615..2068)

putative polyadenylation signals: (Joshi, 1987b)

AATAAG -- (1912..1917)

CAYTG -- 1 mismatch (1905..1909)

Literature Cited

Gorman, CM, Moffat, LF and Howard, BH (1982) Recombinant genomes which express chloramphenicol acetyltransferase in mammalian cells. *Mol Cell Biol* **2**: 1044-1051

Graham, J, Pearce, G, Merryweather, J, Titani, K, Ericsson, L and Ryan, C (1985) Wound-induced proteinase inhibitors from tomato-leaves. II. The cDNA-deduced primary structure of pre-inhibitor II. *J Biol Chem* **260**: 6561-6564

Jefferson, RA (1989) The GUS reporter gene system., *Nature* **342**: 837-838

Joshi, PC (1987) Putative polyadenylation signals in nuclear genes of higher plants: A compilation and analysis. *Nucl Acids Res* **15**: 9627-9640

Joshi, CP (1987) An inspection of the domain between putative TATA box and translation start site in 79 plant genes. *Nucl Acids Res* **15**: 6643-6653

Keil, M, Sanchez-Serrano, J, Schell, J and Willmitzer, L (1990) Localization of elements important for the wound-inducible expression of a chimeric potato proteinase inhibitor II-CAT gene in transgenic tobacco plants. *Plant Cell* **2**: 61-70

Lorberth, R, Cammann, C, Ebneith, M, Amati, S and Sanchez-Serrano, JJ (1992) Promoter elements involved in environmental and developmental control of potato proteinase inhibitor II expression. *Plant Journal* **2**: 477-486

Palm, CJ, Costa, MA, An, G and Ryan, CA (1990) Wound-inducible nuclear protein binds DNA fragments that regulate a proteinase inhibitor II gene from potato. *Proc Natl Acad Sci USA* **87**: 603-307

Thornburg, RW, An, G, Cleveland, TE, Johnson, R and Ryan, CA (1987) Wound-inducible expression of a potato inhibitor II-chloramphenicol acetyltransferase gene fusion in transgenic tobacco plants. *Proc Natl Acad Sci USA* **84**: 744-748



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