Nucleotide Sequence of a Pea (*Pisum sativum* L.)
**β-1,3-Glucanase Gene**

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Table 1. Characteristics of a β-1,3-glucanase gene from pea

| Organism: | Pisum sativum L. cv Alcan. |
| Location: | Nuclear-encoded, single-copy gene, based on Southern blot hybridization. The gene was found on a 5100-bp EcoRI fragment. |
| Techniques: | The gene was isolated by screening an unamplified partial Sau3A genomic library in λDASHII using an insert from the partial pea cDNA clone, pPIG312, as probe (Chang et al., 1992). The gene was subcloned into Bluescript KS+, and the plasmid DNA was sequenced by the dideoxy nucleotide method. The transcription start sites were determined by primer extension. |

Method of Identification:

The exons were identified by comparing the amino acid sequence with β-1,3-glucanases from beans (Edington et al., 1991) and tobacco (Shinshi et al., 1988).

Expression:

A transcript of approximately 1400 bases induced by chitosan or fungal challenges (Chang et al., 1992).

Features of Amino Acid Sequence:

Calculated mol wt 41,805; 370 amino acids; a putative 32-amino acid signal peptide, based on the mature protein size and homology with the tobacco basic β-1,3-glucanase (Shinshi et al., 1988). The amino acid sequence has high homology with other plant β-1,3-glucanases (De Loose et al., 1988; Shinshi et al., 1988; Takeuchi et al., 1990; Edington et al., 1991). A potential N-glycosylation site identified in tobacco β-1,3-glucanase (Shinshi et al., 1988) is also present in the pea β-glucanase sequence at position 3649 (Asn). Antibodies: Not available in our laboratory.

Subcellular Location:

Not tested, but the presence of a putative signal peptide sequence suggests that transmembrane transport may occur.

β-1,3-Glucanase (EC 3.2.1.39) may be involved in the defense of plants against pathogens through its ability to degrade the cell walls of fungal pathogens (Mauch and Staehelin, 1989). In addition, some of the carbohydrates released from the fungal cell walls can elicit other plant defense responses (Ayers et al., 1976; Yoshikawa et al., 1990). Results of our previous study (Chang et al., 1992) indicated that the deduced amino acid sequence for a pea *Pisum sativum* glucanase clone, pPIG4-3, is highly homologous to those found in other plant species. However, pPIG4-3 was a partial genomic clone containing only 21 bp of the intron, the second exon coding for the mature protein, and the 3′ flanking region. In this paper we report the complete sequencing of a 4132-bp pea β-1,3-glucanase clone, pPIG5-4, isolated from the same genomic library described previously (Chang et al., 1992).

A primer extension reaction with mRNA isolated from fungus-induced pea pods yielded two DNA fragments that were not observed with mRNA from uninduced pods. This confirms the inductive expression pattern of this gene and indicates that two transcription initiation sites occur at 27 bp (major band, +1) and 32 bp (minor band, −5) upstream from the first ATG of the coding sequence.

The pPIG5-4 clone (Table I) contains 1469 bp of the 5′ flanking promoter region (−1442 to +27 relative to the major transcription initiation site), two exons (+28 to +118 and +1218 to +2412) interrupted by a 1099-bp intron (+119 to +2656), and 451 bp of the 3′ flanking region (+2240 to +2412) interrupted by a 1099-bp intron (+119 to +2656). There is a putative TATA box (−TATATATAT-) at position −35 and a putative polyadenylation signal (−AA-TATA-) at position +2372. A comparison of the nucleotide sequence of the 3′ flanking region of pPIG5-4 with the 3′ untranslated region of a pea β-1,3-glucanase cDNA clone, pPIG3-2 (Chang et al., 1992), indicates that a polyadenylation site occurs at position +2412 in pPIG5-4. In the region +1197 to +2656, the pPIG5-4 clone has 100% identity with pPIG4-3 at the nucleotide level and contains the complete sequence of pPIG4-3.

The pPIG5-4 pea glucanase gene encodes 370 amino acids, including a putative 32-amino acid leader peptide and a cleavage site comparable to the leader peptide of a tobacco glucanase (Shinshi et al., 1988). According to Henrissat (1991) and Ori et al. (1990), all known β-1,3-glucanases belong to the glycosyl hydrolase family 17, members of which share the consensus sequence (LIVM)-X-(LIVMFYW)-(ST)-G-W-P-(ST)-X-G. This sequence is found in the pea glucanase gene beginning at position +1922 (amino acid 266, VNYVVSSEGWPSPDG). The deduced amino acid sequence of pPIG5-4 contains two conserved Trp residues at positions +1439 and +1949 (amino acids 105 and 275) that have been hypothesized to be involved in hydrogen bond formation.
with the glucan substrate (Ori et al., 1990). Several conserved Glu and Asp residues, suggested to be involved in the \( \beta \)-glucanase-catalyzed hydrolysis of glycosidic bonds (Ori et al., 1990), are also present in the pPIG5-4 protein.

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**LITERATURE CITED**

Edelstone BV, Lamb CJ, Dixon RA (1991) cDNA cloning and characterization of a putative 1,3-\( \beta \)-glucanase transcript induced by fungal elicitor in bean cell suspension cultures. Plant Mol Biol 16: 81–94  
Ori N, Sessa G, Lotan T, Himmelhoch S, Fluhr R (1990) A major stylar matrix polypeptide (sp41) is a member of the pathogenesis-related proteins superclass. EMBO J 9: 3429–3436  