Plant Physiol. (1991) 96, 1382–1384
0032-0889/91/96/1382/03/$01.00/0

Received for publication March 19, 1991
Accepted April 3, 1991

Plant Gene Register

Complete Nucleotide Sequence of a *Hordeum vulgare* Gene Encoding (1→3,1→4)-β-Glucanase Isoenzyme II[1]

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Barley (1→3,1→4)-β-glucanases are synthesized in the scutellum and aleurone tissues during germination. The secreted enzymes degrade (1→3,1→4)-β-glucan, a major constituent of the endosperm cell walls. Genes encoding the two barley (1→3,1→4)-β-glucanase isoenzymes have been cloned and sequenced (Table I). The complete nucleotide sequence (5159 bp[2]) of the gene for (1→3,1→4)-β-glucanase isoenzyme II is presented in Figure 1. An open reading frame encoding 334 amino acids is interrupted by a single large intron of 2952 bp. The sequence includes 1035 bp upstream and 171 bp downstream of the coding region. Comparison with the gene encoding barley (1→3,1→4)-β-glucanase isoenzyme I (refs. 1 and 2 and this study [EMBL accession No. X56775]) reveals 92% homology between the two coding regions. Both genes have the large intron disrupting the codon for amino acid No.

[1] This research was supported by the Eureka project (No. Eu 270 ABIN; (Contribution No. 44) and a fellowship in the European Economic Community Training Network on the Application of Genetic Engineering and Molecular Biology for Seed Improvement

[2] Abbreviation: bp, base pair(s).

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Table I. Characteristics of gene Glb2 from *Hordeum vulgare* cv NK 1558

Organism:
Hordeum vulgare cv Northrup King 1558
Gene:
Glb2, encoding (1 → 3, 1 → 4)-β-glucanase isoenzyme II (EC 3.2.1.73).
Chromosomal Location:
Single-copy gene located on chromosome 1 (2) on a 9.5-kilobase *Bam*HI fragment
Techniques:
λ-EMBL3 barley genomic DNA library (Clontech, Palo Alto, CA) was screened with cDNA encoding barley (1 → 3, 1 → 4)-β-glucanase isoenzyme II (5, 6) as hybridization probe. Sequencing of both strands was performed using synthetic primers.
Sequence Identification:
Sequence comparison with cDNA encoding barley (1 → 3, 1 → 4)-β-glucanase isoenzyme II revealed 100% positional identity of the deduced amino acid sequence and 97% homology with the 3′-noncoding region, respectively.
Gene Structure:
The coding region of 1002 bp is interrupted by single large intron of 2952 bp inserted in the codon for amino acid No. 25 of a 28-amino acid signal peptide.
- Promoter Region: putative TATA box at −95 to −89 (+1 is designated as the first base in the coding region); inverted CCAAT motif 34 bp upstream of TATA box; motif homologous to GA_3-responsive element of a barley α-amylase (7) at −264 to −259 and motif homologous to α-amylase GA_3-responsive element in rice (8), inverted, at position −324 to −315; imperfect palindrome at position −470 to −446.
- Intron Region: imperfect palindrome of 327 bp (68% homology of the inverted repeats) from position 165 to 491 with highly complex internal structure: imperfect palindrome of 88 bp (62% homology) from No. 201 to No. 288; inverted repeat of 24 bp (83% homology) from position 175 to 198 and from 307 to 284 (an 18-bp part (179 to 196) of this motif is almost perfectly repeated at position 2190 to 2207 more downstream in the intron); direct repeat of 49 bp (86% homology) is located at position 631 to 679 and at 730 to 778; imperfect palindrome of 29 bp (83% homology) from No. 2055 to No. 2083; direct repeat of 36 bp (100% homology) from 2628 to 2663 and from 2663 to 2698.
- Coding Region: 334 codons with strong bias in codons downstream of the intron for C or G in the wobble position.
- 3′-Noncoding Region: putative polyadenylation signal at position 4097 to 4101.

Protein Structure:
Preprotein of 334 amino acids. The signal peptide consists of 28 residues. The M_r of the mature protein was calculated as 32.121 with a carbohydrate content of 3.6% and a pI of 10.6 (2).
Antibodies:
Monoclonal antibodies were generated that cross-reacted with isoenzyme I and isoenzyme II and isoenzyme I alone.
EMBL accession No.:
M62740

Figure 1. Nucleotide sequence of the gene encoding barley (1→3,1→4)-β-glucanase isoenzyme II.

25 of a 28-amino acid signal peptide. The position of the intron in the signal peptide-coding sequence is conserved in (1→3)- and (1→3,1→4)-β-glucanase genes of other plants: a (1→3)-β-glucanase gene from tobacco has a 669-bp intron at almost the same position (3), and an intron in this region is present in all rice β-glucanase genes studied (4). Alignment of the nucleotide sequences 5′ to the putative TATA boxes of the (1→3,1→4)-β-glucanase genes from barley discloses two highly homologous regions, separated by a 150-bp segment present only in the promoter of the gene encoding isoenzyme I (not shown). Because the two genes are transcribed in a tissue-specific manner (2), the additional segment of 150 bp may be involved in their differential expression. No significant homology was found further upstream.

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