

Plant Gene Register

Complete Nucleotide Sequence of a *Hordeum vulgare* Gene Encoding (1→3,1→4)-β-Glucanase Isoenzyme II¹

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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

bp²) 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.

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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 GA₃-responsive element of a barley α-amylase (7) at -264 to -259 and motif homologous to α-amylase GA₃-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

TTACTTGTAACTTATTATAAATTATCTTGGTATCAAACACTCTTACAACACTTGCATGAAACCTTACTAAAACGCAATTCCTTCGGCTCCTTGGCCGGG -935
TTCGACACTCTTAATTGTCAAAGAGCTGTGATTGATCCCTTACTCGTGGGTATCAACAAGCCGTCTGCTCCTTGTAGAATCCTCACCTTTCATGC -835
GGCATCATGATTTTTCAAATCGATCTATCAAAGCTTGCCTTGGCCCTTTGTAAGTGCCTCCACCGAGAGTGGGGTGTATACGTCTCAAACGCTTGTAT -735
AATTTTAGTACAATTCCTGCTATGTTGCTATCAATTATAAACATTTGGGCATACTTTTATGAGTTTATGATAAACTAAATGTTGATTTCTGCAAA -635
ATGGTCACAAAAGAATTATATGTAGAAATAAGAGTAAACATAGATCCTAGCATGAGGCAGTAAAAGTGTAGAGTACGAAAGGCATCTAGTGTACACCGA -535
AATTTTTAAATGACACGGATATTATAGTAACTACGTGAGTCATGTCGGATACCGTAGAAGACGGTCTTCTATGAAAAACCGTACCAAATGTC -435
GTTGAGTAAGACGTGAACACATCCGGACATCCGGTGTACGCAAAACCACTTGTGCTAAAACTTGTCTCTCATGGTACATTCGTGGAGTCCAAACTC -335
AAACTATTTTCTCAAAGGTTGTTGGTGTACTATGAATGAATTAGCCACCACTCTCTACTTGAATAACAACCTGGATAACCTTGCACCACCG -235
ACACTACGAGAAGGGGTGACGCATGCATGCAACGGCTAGTGCCTATCAACAAGCTAAATACGAAATAAATGTCCTGGAGACGTTGGGCTTGC -135
AAGTGGATTGGACCAAATTAACCTCTGCTCCTAGGAGCCTATATAAGGGGCATGGCCTCACCATAGAAGAAAGACGAGAGTCTTACTTGCCTCCAA -35
CGCAGGTAGAGAGAAAGAGAGTTCGAGACCAATGGCAGCQAAGGCGTTCCTCCTACTCTCGCATTGCTTCTCGGAGCCTTCGCGTCTAT 65
M A S Q G V A S M F T L A L L L G A T S I
CCCAAGtgattccccttctcccttctctctcgcccaaatgattgcttacagagtttttttccatgcaagtcatgcatatagcttttttgagagt 165
P P
accctccttttctaataaaggtttttagagatttcattaggagactacatacgaagcaaatgatgaattataatttaaagtgtgtctatatacat 265
ccgtatgtactcttttagtttaaaacttataattagtaataaagaagtagaagataaataaattcttgttgcgaataaagaccttctatagatt 365
ccactaaaaactatatacggatcgacatatatagacactatagagtagcgtgctttatattttatataatagttcataataaaatctcaaaaaa 465
tcttaatttagaagcggaggagtaacgtacactaatcttagattttatagagaagcattatagctagataggtacgttaactctaggcacaat 565
taactcaggggtacactctttagccggccacacctagatgtagtgcacgtgtaacgacctcaggtgtaaaagataacggcgcaagaagtagttccgc 665
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attcttttaagttaaagaaaatgatgcaccagctagtaactgactctttgagcttctgtgtatcataaactcaaaagaccacacacacacacac 1465
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aacagctcaagcctcggcctcgccttgttgtgttcatgaaatcggcctcattatatttggcttagggcatctcgctgaccccaagaattctgagttt 2065
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tgaacgcaaac 2865
tttttctcgtcacatgcatcacggggaatttctcattggcaatggaaggaacagtagctactaccctggtgaaatttttttaaaaaattgcatgt 2965
ggatgatacaaggttctaaccaagctacatgcatggaatcaccgcatgagggctggagtcgagtcgggggtgctacgggcatgagcggcaaa 3065
S V E S I G V C Y G M S A N
CAACTGCCGGCGGAGCACCGTCTGAGCATGTTCAAGTCCAACGGGATCAAATCGATGCGGCTGTACGCTCCCAACAGGGCGCTGACGGCCGTC 3165
N L P A A S T V V S M F K S N G I K S M R L Y A P N Q A A L Q A V
GGCGACGGGCATCAACGTCGTCTGCGGGGCTCCTAACGACGTCCTTCCAACCTCGCCGACGCCGGCAGCGGGCGCCTCGTGGGTCAAGAGCAACA 3265
G G T G V D V G A P N D V L S N L A S P A A A S W V K S N
TCCAGGGTACCCCAAGGTTTCTTCCGGTACGTCGCTCGGAACAGAGGTCGCGGGCGGCCACCCGGAACCTCGTCCGGCAATGAAGAACGTGCA 3365
I Q A Y P K V S F R Y V C V G N E V A G G A T R N L V P A M K N V H
TGGCGGCTCGTCCGCGTGGGCTGGGCCATCAAGGTGACCAGTGGTGTGCGAGGCCATCCTCGGCGTTCAGCCCGCCTCCGCGGGTCTTCC 3465
G A L V A A G L G H I K V T T S V S Q A I L G V F S P P S A G S F
ACCGGGAGGGCGCGGCTTATGGGCCCCGTGGTGCAGTCTCTTCCCGCCACCAACGCGCCGCTCATGGCCAATTTACCCGTACCTGGCTGGGCTT 3565
T G E A A A A F M G P V F Q F L A R T N A P L M A N I Y P Y L A W A
ACAACCCGAGCGCATGGACATGGGCTACGCTCTCTTCAACGCGTCCGGCACCGTGGTACGGGACGGCGCTACGGGTACCAGAACCTGTCGACACCAC 3665
Y N P S A M D M G Y A L F N A S G T V V R D G A Y G Y Q N L F D T T
CGTGGACGCTTCTACAGGCCATGGGCAAGCAGCGGGCTCCAGCGTGAAGCTGGTGGTGTGCGGAGAGCGGGTGGCGCTGGGGCGGCGCACGGCGGG 3765
V D A F A T A M G K H G G S A S V K L V S E S G W P S G G G T A A
ACTCCGGCAACGCTAGGTTCTACAACAGCACCTCATCAACACGTCGGGCGGGCACCCCAACCGCCACCCGGGCGCCATCGAGACCTACATCTTCGCA 3865
T P A N A R F Y N Q H L I N H V G R G T P R H P G A I E T Y I F A
TGTCAACGAGAACCAGAAGGACAGCGGCTGGAGCAGAATGGGGACTCTTCTACCCCAACATGCAGCAGCTACCCCATCAACTTCTGACGGAGCTC 3965
M F N E N Q K D S G V E Q N W G L F Y P N M Q H V Y P I N F
GTGCTCGTTAAGTCCCTACTTGTCTTGTAAACGAGTAAAAGTCATGTTACGCAACTGACGAGCTACTCGTTGGAGAGCCTCTTAATACCTCCTC 4065
TTCCACATGAGGGATGAGAACGTATGAGTTAATAACAGACCCCACTACTGTGAATTC

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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