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Seven members of the (1→3)-β-glucanase gene family in barley (*Hordeum vulgare*) are clustered on the long arm of chromosome 3 (3HL)

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Abstract Members of the (1→3)-β-glucan glucanohydrolase (EC 3.2.1.39) gene family have been mapped on the barley genome using three doubled haploid populations and seven wheat-barley addition lines. Specific probes or polymerase chain reaction (PCR) primers were generated for the seven barley (1→3)-β-glucanase genes for which cDNA or genomic clones are currently available. The seven genes are all located on the long arm of chromosome 3 (3HL), and genes encoding isoenzymes GI, GII, GIII, GIV, GV and GVII (ABG2) are clustered in a region less than 20 cM in length. The region is flanked by the RFLP marker MWG2099 on the proximal side and the Barley Yellow Mosaic Virus (BYMV) resistance gene *ym4* at the distal end. The gene encoding isoenzyme GVI lies approximately 50 cM outside this cluster, towards the centromere. With the exception of the gene encoding isoenzyme GIV, all of the (1→3)-β-glucanase genes are represented by single copies on the barley genome. The probe for the isoenzyme GIV gene hybridized with four DNA bands during Southern blot analysis, only one of which could be incorporated into the consensus linkage map.

Key words Barley DNA · (1→3)-β-Glucanase · Linkage map · Pathogenesis-related proteins · Gene family

Introduction

(1→3)-β-Glucanases are widely distributed in higher plants, where they function in normal developmental processes such as microsporogenesis, pollen-tube growth, senescence and in the removal of dormancy or wound callose (Bucciaglia and Smith 1994; Hird et al. 1993; Stone and Clarke 1993). However, most attention has been focussed on their role in plant-pathogen interactions. The (1→3)-β-glucanases are classified with the pathogenesis-related (PR) group of proteins that are expressed when higher plants are challenged by a broad range of fungi, bacteria, viruses or viroids (Boller 1987; Stintzi et al. 1993). There is no evidence to suggest that (1→3)-β-glucanase genes represent plant resistance genes of the type envisaged by the gene-for-gene model of plant-pathogen interactions (Staskawicz et al. 1995; Godiard et al. 1994; Jones 1994). It is more likely that the enzymes participate in a general, non-specific response to pathogen attack through their ability to hydrolyse the substituted or branched (1→3)-β-glucans that are common constituents of fungal cell walls (Wessels 1993).

In barley, (1→3)-β-glucanase isoenzymes are encoded by at least seven genes (Høj et al. 1989; Xu et al. 1992; Wang et al. 1992; Malehorn et al. 1993; Xu et al. 1994). The products of these genes have been designated isoenzymes GI-GVI (Xu et al. 1992) and ABG2 (Malehorn et al. 1993). Here, the ABG2 isoform is referred to as isoenzyme GVII. In the work presented here we have used DNA probes that are specific for genes encoding isoenzymes GI-GVI and polymerase chain reaction (PCR) primers designed to specifically amplify a fragment of the isoenzyme GVII gene to map the barley (1→3)-β-glucanase gene family. All of the genes map to the long arm of chromosome 3 (3HL).

Materials and methods

Plant material

Wheat-barley addition lines described by Islam et al. (1981) were provided by Drs K. W. Shepherd and A. K. R. M. Islam, and the barley chromosome 5 (1H*) addition line, which is a double monosomic addition containing one copy each of chromosomes 5 (1H) and 6 (6H) (Islam and Shepherd 1990), was made available by Dr. A. K. R. M. Islam. The wheat (*Triticum aestivum* cv 'Chinese Spring')

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Table 1 Probes used to map (1→3)-β-glucanase genes

Isoenzyme	Type of clone	Position of probe[a]	Length of probe
GI	cDNA	3′-Untranslated region	121 bp
GII	cDNA	3′-Untranslated region	215 bp
GIII	Genomic	8 to 1236 bp	1229 bp
GIV	Genomic	1684–2218 bp	535 bp
GV	cDNA	3′-Untranslated region	216 bp
GVI	Genomic	Entire clone	1948 bp

[a] The base pair numbering system follows Xu et al. (1992)

and barley (*Hordeum vulgare* cv. 'Betzes') parents of the addition lines were provided by Dr. K.W. Shepherd. Throughout this paper the numbers of the equivalent wheat homoeologous group of chromosomes are placed in parentheses after the traditional barley chromosome number.

Three doubled haploid (DH) populations were used for mapping the barley (1→3)-β-glucanase genes. One population consisted of 60 DH lines generated using the *Hordeum bulbosum* method (Hayes and Chen 1989) from the cross 'Clipper'×'Sahara' and was generously provided by Dr. A. K. R. M. Islam. Two other populations, consisting of 114 DH lines from a 'Haruna Nijo'×'Galleon' cross and 120 DH lines from a 'Chebec'×'Harrington' cross, were very kindly provided by Dr. S. Logue.

DNA isolation and Southern blot analysis

Large-scale DNA isolations were made from approximately 5 g fresh barley leaves using the procedure of Guidet et al. (1991). Gel electrophoresis, blotting and hybridization were performed as described by Reed and Mann (1985). Gene-specific probes for isoenzymes GI-GVI were as described in Table 1. The shorter DNA fragments for the genes encoding isoenzymes GI, GII and GV were labelled with α-[^{32}P]dCTP using specific oligonucleotide primers, while the longer probes for genes encoding isoenzymes GIII, GIV and GVI were labelled using random primers (Table 1). To identify polymorphism amongst the six parental varieties, DNA preparations from those varieties were digested with restriction endonucleases *Eco*RI, *Eco*RV, *Dra*I, *Bam*HI or *Hin*dIII. If no polymorphisms were detected, the DNA samples were subsequently digested with *Sal*I, *Sac*I, *Xba*I, *Hae*III, *Dde*I or *Alu*I. Linkage analyses were conducted with Mapmaker (Lander et al. 1987) and JoinMap software (Stam 1993). Crossover units were converted into map distances (centiMorgan, cM) using the Kosambi function (Kosambi 1944).

PCR analysis

A DNA clone encoding barley (1→3)-β-glucanase isoenzyme GVII was not available, although the sequence of the gene has been published (Malehorn et al. 1993). As an alternative to restriction fragment length polymorphism (RFLP) mapping, PCR was used to map the isoenzyme GVII gene. Primers corresponding to the flanking regions of the single intron of the gene were used to amplify the intron (Fig. 1), which was likely to represent that portion of the gene most divergent from other (1→3)-β-glucanase gene sequences (Weining and Langridge 1991). The PCR reaction was carried out in a total reaction volume of 25 µl, which contained 0.2 µg genomic DNA template, 0.2 µ*M* each primer, 200 µ*M* each of dATP, dCTP, dGTP and dTTP, 1.5 m*M* $MgCl_2$ and 0.5 units *Taq* polymerase. After 4 min at 94°C, 35 cycles of 1 min at 94°C, 2 min at 50°C and 2 min at 72°C were performed and the reaction terminated at 72°C for 5 min followed by 5 min at 25°C. The PCR products were separated on 1.5% agarose gels and DNA bands were examined under UV light after ethidium bromide staining.

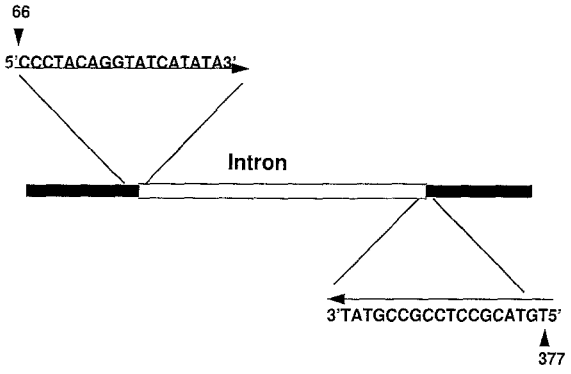

Fig. 1 Schematic representation of the locations of oligonucleotide primers for PCR amplification of the β-glucanase isoenzyme GVII gene. The numbering system is based on that of Malehorn et al. (1993)

Results

Specificity of probes

Overall positional identities of amino acid sequences in mature (1→3)-β-glucanases from barley range from 44% to 78%, and the enzymes are characterized by regions of up to ten identical amino acids that are interspersed with variable regions (Xu et al. 1992). To ensure that the DNA probes (Table 1) used to map individual (1→3)-β-glucanase genes were specific, we tested each probe against cDNA or genomic DNA dot blots under the same stringency conditions used in the Southern blot hybridizations. Probes for genes encoding isoenzymes GI, GII, GIV, GV and GVI exhibited no cross-hybridization with any other genes (Fig. 2). In the case of the probe used to detect the gene for isoenzyme GIII, some hybridization with other barley (1→3)-β-glucanase genes or cDNAs was evident (Fig. 2). Nevertheless, the hybridization signal with this probe was very much stronger against the isoenzyme GIII gene than against the other cDNAs or genes (Fig. 2), and the probe could therefore be used in Southern blot analyses to locate the isoenzyme GIII gene.

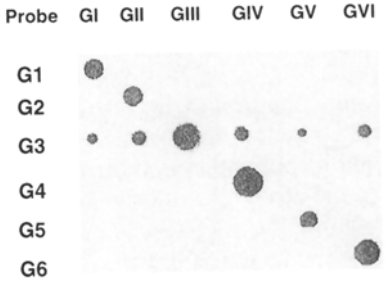

Fig. 2 Specificity of probes for mapping the barley (1→3)-β-glucanase genes encoding isoenzymes GI to GVI. DNA from cDNAs or genes (Xu et al. 1992) was spotted on nitrocellulose paper strips, and individual strips were probed with "gene-specific" oligonucleotides or DNA fragments shown in Table 1

RFLP mapping of genes encoding isoenzymes GI to GVI

The genes encoding isoenzymes GI and GVI were found to be highly polymorphic, with at least two of the six parental varieties from the mapping populations showing polymorphism with each restriction enzyme used (data not shown). The genes for isoenzymes GII and GIII showed polymorphisms when DNA from vars 'Clipper' and 'Sahara' were digested with *Xba*I or *Sal*I, the gene for isoenzyme GIV showed polymorphism between 'Chebec' and 'Harrington' digested with *Dra*I and the isoenzyme GV gene showed polymorphism between 'Galleon' and 'Haruna Nijo' digested with *Xba*I (data not shown).

All genes except the gene encoding isoenzyme GIV were detected as single DNA bands after hybridization with the specific DNA probes. This indicated that the genes for isoenzymes GI, GII, GIII, GV and GVI are represented by single copies on the barley genome. In contrast, Southern blots probed with the isoenzyme GIV DNA revealed a relatively complex banding pattern; the probe hybridized with four genomic DNA fragments, only one of which was polymorphic and could be mapped. When the alternative procedure of PCR mapping was used to amplify the 5′-untranslated region of the isoenzyme GIV gene, several PCR products were again detected (data not shown).

Linkage analysis of the RFLP banding patterns showed that the genes for barley (1→3)-β-glucanase isoenzymes GI to GVI are all located on the long arm of chromosome 3 (3HL). The positions of the genes in a consensus linkage map are shown in Fig. 3. The five genes encoding isoenzymes GI to GV lie clustered in a region of less than 20 cM, between the RFLP marker MWG2099 and the *ym4* gene for Barley Yellow Mosaic Virus (BYMV) resistance (Fig. 3). The distance between the genes for isoenzymes GII and GIII is only 0.5 cM. Of the genes within this cluster the isoenzyme GI gene is closest to the centromere (76 cM), and the most distal gene is isoenzyme GV. The isoenzyme GV gene is closely linked with both the *ym4* gene (6.6 cM) and the alcohol dehydrogenase *Adh10* gene (3.0 cM). The RFLP markers most closely linked with the genes encoding barley (1→3)-β-glucanase isoenzymes GI to GVI are cMWG693 (0.1 cM), BCD131 (0.2 cM), BCD131 (0.7 cM), MWG41 (0.5 cM), MWG838 (0.8 cM) and CDO178 (1.8 cM), respectively. The gene encoding isoenzyme GVI is located outside the cluster, in a position between the centromere and the isoenzyme GI gene (Fig. 3). The isoenzyme GVI gene is 26 cM from the centromere region and 50 cM from the isoenzyme GI gene.

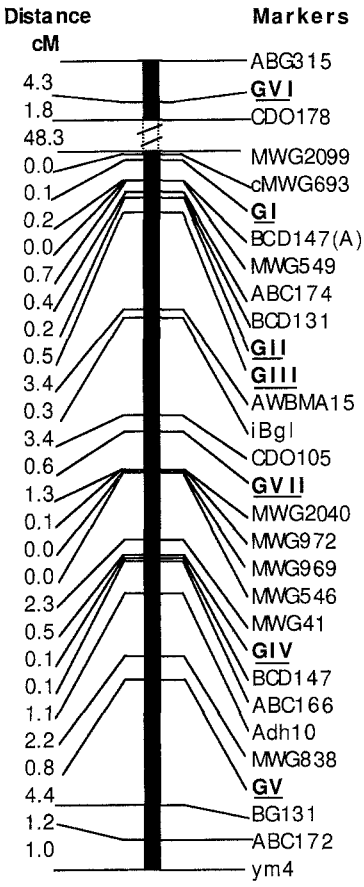

Fig. 3 Consensus linkage map of the seven barley (1→3)-β-glucanase genes, encoding isoenzymes GI to GVII, on the long arm of chromosome 3 (3H), in which GI, GII, GIII, GVI and GVII were mapped in the 'Clipper'×'Sahara' DH population, and GIV and GV were mapped in the 'Chebec'×'Harrington' and 'Haruna Nijo'× 'Galleon' DH populations, respectively

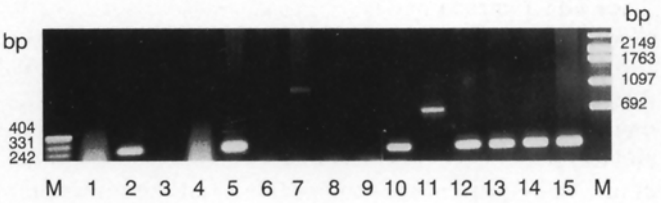

Fig. 4 PCR products of wheat, barley and wheat-barley addition lines using isoenzyme GVII primers (Fig. 1). *Lane 1* Wheat (cv 'Chinese Spring'), *lane 2* barley (cv 'Betzes'), *lanes 3–9* wheat-barley addition lines 1H–7H. *lanes 10–15* barley cvs 'Clipper', 'Sahara', 'Galleon', 'Haruna Nijo', 'Chebec' and 'Harrington', respectively. DNA size markers are shown in lanes *M*

PCR mapping of the gene for isoenzyme GVII

The primers shown in Fig. 1 were used in PCRs to amplify DNA fragments from the intron of the gene for isoenzyme GVII, with the templates being genomic DNA preparations from wheat, barley and the wheat-barley addition lines. The isoenzyme GVII gene corresponds to the *ABG2* gene isolated and characterized by Malehorn et al.. (1993), and a fragment of 310 bp would be predicted from their sequence data. A PCR product of approximately 310 bp was amplified from barley DNA and from DNA of the wheat-barley addition line 3H; this product was not detected in DNA from wheat or the other wheat-barley addition lines. These results indicated that the isoenzyme GVII gene is located on barley chromosome 3 (3H). However, the PCR-amplified product from the 'Sahara' parent of the

'Clipper'×'Sahara' DH lines was approximately 600 bp in length (Fig. 4), and this polymorphism allowed the isoenzyme GVII gene to be mapped. Linkage analysis confirmed that the isoenzyme GVII gene is located on chromosome 3 (3H). It is found in the region of the (1→3)-β-glucanase gene cluster, 7.7 cM from the isoenzyme GIII gene and 4.2 cM from the isoenzyme GIV gene (Fig. 3). The RFLP marker nearest the isoenzyme GVII gene is CDO105 (0.6 cM).

Discussion

Using RFLP and PCR mapping procedures we have been able to incorporate the genes encoding seven individual (1→3)-β-glucanase genes into a high-density consensus linkage map for barley (Fig. 3). The genes are all located on the long arm of chromosome 3 (3HL), and six of the genes are grouped within 18.2 cM of each other. The gene encoding isoenzyme GVI is found between the major group of (1→3)-β-glucanase genes and the centromere (Fig. 3). Each of the genes is represented on the barley genome by a single copy, with the possible exception of the isoenzyme GIV gene, for which multiple DNA fragments were detected during both RFLP and PCR mapping experiments. It is not clear whether this result indicates the presence of additional (1→3)-β-glucanase genes which share a high degree of sequence identity with the isoenzyme GIV probe or whether the isoenzyme GIV gene is a representative of a subfamily. Polymorphisms were detected on only one of the DNA fragments that hybridized with the isoenzyme GIV probe. This precluded the mapping of the other three genes, if they indeed exist, and until these effects are investigated in more detail, we are unable to make firm conclusions about the number of (1→3)-β-glucanase isoenzyme GIV genes in barley.

In an earlier study, Kleinhofs et al. (1993) used a near full-length cDNA encoding isoenzyme GII (Høj et al. 1989) to map barley (1→3)-β-glucanase genes. Two loci were mapped to the long arm of barley chromosome 3 (3H), and the genes were designated *Glb3* and *Glb4* (Kleinhofs et al. 1993), but the lack of specificity of the probe prevented the assignment of the two loci to individual (1→3)-β-glucanase genes. However, on the basis of the relative map positions (Fig. 3 cf. Kleinhofs et al. 1993) and the high degree of sequence identity between isoenzymes GI and GII (Xu et al. 1992), it is likely that *Glb3* and *Glb4* of Kleinhofs et al. (1993) correspond to the genes encoding (1→3)-β-glucanase isoenzymes GII and GI, respectively.

The use of the designations *Glb3* and *Glb4* by Kleinhofs et al. (1993) raises a general problem with the nomenclature for β-glucanase genes. Cereal β-glucan endohydrolases can be divided into three major classes: the (1→3)-β-glucanases (EC 3.2.1.39), the (1→3,1→4)-β-glucanases (EC 3.2.1.73) and the (1→4)-β-glucanases (EC 3.2.1.4) or endo-cellulases (Høj and Fincher 1995). Given that two (1→3,1→4)-β-glucanase isoenzymes, designated isoenzymes EI and EII (Woodward and Fincher 1982; Slakeski et al. 1990), and seven (1→3)-β-glucanase isoenzymes, designated isoenzymes GI to GVII (or in the case of isoenzyme GVII, ABG2) (Xu et al. 1992; Malehorn et al. 1993), have so far been characterized and that there is every possibility that barley cellulases will be described in the future, any systematic nomenclature for the genes encoding these related polysaccharide hydrolases must not only take account of their different specificities but must also allow for the identification of multiple isoforms within each enzyme class. We therefore propose that the (1→3)-β-glucanase genes be designated *Glb31* to *Glb3x*, where 3 indicates that the (1→3)-linkage is cleaved and *x* is the number of the individual isoenzyme. In this system the gene for (1→3)-β-glucanase isoenzyme GV would therefore be *Glb35*; for isoenzyme GVII, *Glb37*, etc. Similarly, the genes for (1→4)-β-glucanases, or cellulases, could be designated *Glb41* to *Glb4x*, where the *4* indicates that the (1→4)-linkage is hydrolysed and *x* represents the individual isoform. In the case of the (1→3, 1→4)-β-glucanases, of which there are only two isoforms in wheat and barley (Slakeski et al. 1990), the simplest designations for isoenzymes EI and EII would be *Glb1* and *Glb2*, respectively. This nomenclature has been adopted in earlier work (Søgaard and von Wettstein-Knowles 1987; von Wettstein-Knowles 1992).

Comparative studies on barley (1→3)-β-glucanases and (1→3,1→4)-β-glucanases suggest that these enzymes are members of a single "super-gene" family and that, in all likelihood, the (1→3,1→4)-β-glucanases diverged from the (1→3)-β-glucanases during the appearance of the graminaceous monocotyledons (Høj and Fincher 1995). The evidence is based on similarities in their three-dimensional structures (Varghese et al. 1994), in the topology of their substrate binding domains (Hrmova et al. 1995), in their catalytic mechanisms (Chen et al. 1993 1995) and in the structure of their corresponding genes (Høj et al. 1989; Slakeski et al. 1990; Xu et al. 1992; Wang et al. 1992). In considering possible evolutionary pathways of the barley β-glucanases, Høj and Fincher (1995) used the algorithms of Hein (1990) to reconstruct a phylogenetic tree, of which a modified version is shown in Fig. 5. The question arises as to whether the position of individual (1→3)-β-glucanase genes on the phylogenetic tree can be reconciled with their positions on the linkage map shown in Fig. 3. Comparisons of the tree generated by sequence similarity and the linkage distances revealed many similarities (Figs. 5 and 3), in particular the close relatedness of isoenzymes GI and GII, isoenzymes GIII and GVII, and isoenzymes GIV and GV. Furthermore, isoenzyme GVI is placed furthest from the other isoenzymes in both cases (Figs. 3 and 5). The major difference is the relative proximity of the isoenzyme GII/GIII pair in the linkage map analysis (Fig. 3 cf. Fig. 5).

The two barley (1→3,1→4)-β-glucanases have been classified with the (1→3)-β-glucanases in a single gene family (Høj and Fincher 1995), but the genes encoding the (1→3,1→4)-β-glucanase isoenzymes EI and EII are located on the long arms of chromosome 5 (1H) and chromosome 1 (7H), respectively (Loi et al. 1988; MacLeod

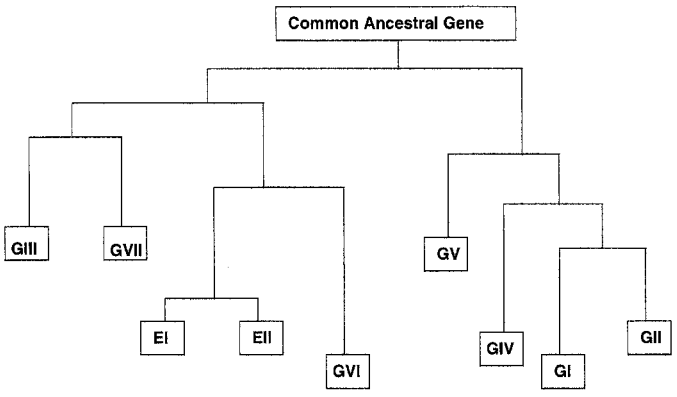

Fig. 5 Possible phylogeny of the barley (1→3)- and (1→3,1→4)-β-glucanase genes, based on alignments of amino acid sequences and the algorithm of Hein (1990). (1→3)-β-Glucanase genes are designated GI to GVII and (1→3,1→4)-β-glucanase genes are designated EI and EII, in accordance with the corresponding isoenzymes they encode (Xu et al. 1992; Høj and Fincher 1995; Malehorn et al. 1993)

et al. 1991). If the genes encoding (1→3,1→4)-β-glucanases are indeed derived from (1→3)-β-glucanase genes (Høj and Fincher 1995), all of which are located on chromosome 3L (3HL) (Fig. 3), it is clear that multiple chromosomal translocations accompanied (1→3,1→4)-β-glucanase evolution.

The distribution of (1→3)-β-glucanase genes along a single barley chromosome arm implies that duplication of the ancestral gene occurred via a type of illegitimate recombination rather than by an excision, duplication and reinsertion mechanism. It is possible that gene duplication occurred initially by tandem duplication of the ancestral gene. Subsequent dispersal along the chromosome could have been affected by chromosome rearrangements. It is noteworthy that the organization of the barley (1→3)-β-glucanase genes on chromosome 3 (3H) is similar to that of the barley hordein genes on chromosome 5 (1H) (Shewry and Miflin 1982; Bunce et al. 1986; Shewry et al. 1990) and the clustering of α-amylase genes (*Amy1*) on chromosome 6 (6H) (Khursheed and Rogers 1988; Takano and Takeda 1987). The α-amylase genes may represent an early stage in the evolutionary process, where the duplicated genes are still closely linked.

The final point for consideration in the evolution of the barley (1→3)-β-glucanase gene family is the apparent need for multiple isoforms of the enzyme. There are at least seven (1→3)-β-glucanase genes in barley (Xu et al. 1992; Malehorn et al. 1993). Apart from the isoenzymes that are involved in normal plant (1→3)-β-glucan metabolism during wounding, senescence, microsporogenesis and pollen-tube growth (Stone and Clarke 1993), "pathogenesis-related" (1→3)-β-glucanases might be expressed either constitutively or only after pathogen attack, and there may be requirements for different responses in different tissues. Furthermore, the enzymes might be transported either to the extracellular space or to different internal compartments of the cell. In barley, (1→3)-β-glucanase isoenzymes GII and GIII are extracellular (Høj et al. 1989; Wang et al. 1992), isoenzyme GIV is vacuolar (Xu et al. 1992) and isoenzymes GI and GV may be cytosolic (Xu et al. 1994). The multiplicity of (1→3)-β-glucanase functions, together with the apparent advantages afforded to the plant by having several lines of defence against invading microorganisms at the cellular level (Mauch and Staehelin 1989; Van den Bulcke et al. 1989), presumably explains the need for multiple genes with independent controlling mechanisms.

In the context of (1→3)-β-glucanase expression during plant-microbe interactions, there is increasing evidence from analyses of infection of barley with the leaf scald fungus *Rhynchosporium secalis* (Roulin, Brown and Fincher, unpublished) and the powdery mildew fungus *Erysiphe graminis* (Xu et al. 1992; Malehorn et al. 1993), and from the induction of (1→3)-β-glucanases with *N*-acetylchitooligosaccharides of fungal cell-wall origin (Kaku, Shibuya, Aryan and Fincher, unpublished), that the gene encoding isoenzyme GII is particularly important in the plant's response to pathogen attack. It is noteworthy that the scald resistance genes *Rrs1* and *Rrs2* are also located on the long arm of chromosome 3 (3HL) together with resistance genes for leaf rust and net blotch (Søgaard and von-Wettstein-Knowles 1987). However, at this stage we are unable to conclude whether or not any relationship exists between the disease resistant genes and the (1→3)-β-glucanase genes that have been mapped in the present work.

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References

Boller T (1987) Hydrolytic enzymes in disease resistance. In: Kosuge T, Nester EW (eds) Plant-microbe interactions: molecular and genetic perspectives, vol. 2. MacMillan, New York, pp 385–413

Bucciaglia PA, Smith AG (1994) Cloning and characterization of *Tag-1*, a tobacco anther β-1,3-glucanase expressed during tetrad dissolution. Plant Mol Biol 24:903–914

Bunce NAC, Forde BG, Kreis M, Shewry PR (1986) DNA restriction fragement length polymorphism at hordein loci: application to identifying and fingerprinting barley cultivars. Seed Sci Technol 14:419–429

Chen L, Fincher GB, Høj PB (1993) Evolution of polysaccharide hydrolase substrate specificity. J Biol Chem 268:13318–13326

Chen L, Garrett TPJ, Varghese JN, Fincher GB, Høj PB (1995) A tetrad of ionisable amino acids is important for catalysis in barley β-glucanases. J Biol Chem 270:8093–8101

Godiard L, Grant MR, Dietrich RA, Kiedrowski S, Dangl JL (1994) Perception and response in plant disease resistance. Curr Opinion Genet Dev 4:662–671

Guidet F, Rogowsky P, Taylor C, Song W, Langridge P (1991) Cloning and characterisation of a new rye-specific repeated sequence. Genome 34:81–87

Hayes PM, Chen FQ (1989) Genotypic variation for *Hordeum bulbosum L.*-mediated haploid production in winter and facultative barley. Crop Sci 29:1184–1188

Hein J (1990) Unified approach to alignment and phylogenies. Methods Enzymol 183:626–645

Hird DL, Worral D, Hodge R, Smart S, Paul W Scott R (1993) The anther-specific protein encoded by *Brassica napus* and *Arabidopsis thaliana* A6 gene displays similarity to (1→3)-β-glucanase. Plant J 4:1023–1033

Høj PB, Fincher GB (1995) Molecular evolution of plant β-glucan endohydrolases. Plant J 7:367–379

Høj PB, Hartman DJ, Morrice NA, Doan DNP, Fincher GB (1989) Purification of (1→3)-β-glucan endohydrolase isoenzyme II from germinated barley and determination of its primary structure from a cDNA clone. Plant Mol Biol 13:31–42

Hrmova M, Garrett TPJ, Fincher GB (1995) Subsite affinities and disposition of catalytic amino acids in the substrate-binding region of barley 1,3-β-glucanases: implications in plant-pathogen interactions. J Biol Chem 270:14556–14563

Islam AKMR, Shepherd KW (1990) Incorporation of barley chromosomes into wheat. In: Bajaj YPS (ed) Biotechnology in agriculture and forestry, Vol 13: Wheat. Springer, Berlin Heidelberg New York, pp 128–151

Islam AKMR, Shepherd KW, Sparrow DHB (1981) Isolation and characterization of euplasmic wheat-barley chromosome addition lines. Heredity 46:161–174

Jones JDG (1994) Resistance crumbles? A plant disease resistance gene, of the kind involved in 'gene for gene' interactions with pathogens, has been cloned and found to encode a putative serine/threonine kinase. Curr Biol 4:67–69

Khursheed B, Rogers JC (1988) Barley α-amylase genes. J Biol Chem 263:18953–18960

Kleinhofs A, Kilian A, Saghai Maroof MA, Biyashev RM, Hays P, Chen FQ, Lapitan N, Fenwick A, Blake TK, Kanazin V, Ananiev E, Dahleen L, Kudrna D, Bollinger J, Knapp SJ, Liu B, Sorrells M, Heun M, Franckowiak JD, Hoffmann D, Skadsen R, and Steffenson BJ (1993) A molecular, isozyme and morphological map of barley (*Hordeum vulgare*) genome. Theor Appl Genet 86: 705–711

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

Lander ES, Green P, Abrahamson J, Barlow A, Daly MJ, Lincoln SE, Newberg L (1987) MAPMAKER: an interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics 1:174-181

Loi L, Ahluwalia B, Fincher GB (1988) Chromosomal location of the genes encoding barley (1→3,1→4)-β-glucan 4-glucanohydrolases. Plant Physiol 87:300–302

MacLeod LC, Lance RCM, Brown AHD (1991) Chromosomal mapping of the *Glb1* locus encoding (1→3,1→4)-β-D-glucan 4-glucanohydrolase EI in barley. J Cereal Sci 13:291–298

Malehorn DE, Scott KJ, Shah M (1993) Structure and expression of a barley acidic β-glucanase gene. Plant Mol Biol 22:347–360

Mauch F, Staehelin LA (1989) Functional implications of the subcellular localization of ethylene-induced chitinase and β-1,3-glucanase in bean leaves. Plant Cell 1:447–457

Reed KC, Mann DA (1985) Rapid transfer of DNA from agarose gels to nylon membranes. Nucleic Acids Res 20: 7207–7221

Shewry PR, Miflin BJ (1982) Genes for the storage proteins of barley. Qual Plant Plant Foods Hum Nutr 31:251–267

Shewry PR, Parmar S, Franklin J, Burgess SR (1990) Analysis of a rare recombination event within the multigenic *Hor2* locus of barley (*Hordeum vulgare*). Geneti Res 55:171–176

Slakeski N, Baulcombe DC, Devos KM, Ahluwalia B, Doan DNP, Fincher GB (1990) Structure and tissue-specific regulation of genes encoding barley (1→3,1→4)-β-glucan endohydrolases. Mol Gen Genet 224:437–449

Søgaard B, Von Wettstein-Knowles P (1987) Barley: genes and chromosomes. Carlsberg Res Commun 52:126–196

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Staskawicz BJ, Ausubel FM, Baker BJ, Ellis JG, Jones JDG (1995) Molecular genetics of plant disease resistance. Science 268: 661–667

Stintzi A, Heitz T, Prasad V, Wiedemann-Merdinoglu S, Kauffmann S, Geoffroy P, Legrand M, Fritig B (1993) Plant "pathogenesis-related" proteins and their role in defense against pathogens. Biochimie 75:687–706

Stone BA, Clarke AE (1993) Chemistry and biology of (1→3)-β-glucans. La Trobe University Press, Bundoora, Victoria, Australia

Takano T, Takeda G (1987) Genetic variation of α-amylase in germinating barley seeds. Barley Genet 5:787–792

Van den Bulcke M, Bauw G, Castresana C, Van Montagu M, Vandekerckhove J (1989) Characterization of vacuolar and extracellular β-1,3-glucanase of tobacco: evidence for a strictly compartmentalised plant defence system. Proc Natl Acad Sci USA 86: 2673–2677

Varghese JN, Garrett TPJ, Colman PM, Chen L, Høj PB Fincher GB (1994) Three-dimensional structures of two plant β-glucan endohydrolases with distinct substrate specifities. Proc Natl Acad Sci USA 91:2785–2789

Von Wettstein-Knowles P (1992) Cloned and mapped genes: current status. In: Shewry P (ed) Barley: genetics, biochemistry, molecular biology and biotechnology. CAB Int, Wallingford, Oxon, pp 73–98

Wang J, Xu P, Fincher GB (1992) Purification, characterisation and gene structure of (1→3)-β-glucanase isoenzyme GIII from barley (*Hordeum vulgare*). Eur J Biochem 209:103–109

Weining S, Langridge P (1990) Identification and mapping of polymorphisms in cereals based on the polymerase chain reaction. Theor Appl Genet 82:209–216

Wessels JGH (1993) Wall growth, protein excretion and morphogenesis in fungi. New Phytol 123:379–413

Woodward JR, Fincher GB (1982) Purification and chemical properties of two 1,3-1,4-β-glucan endohydrolases from germinating barley. Eur J Biochem 121: 663–669

Xu P, Wang J, Fincher GB (1992) Evolution and differential expression of the (1→3)-β-glucan endohydrolase-encoding gene family in barley, *Hordeum vulgare*. Gene 120:157–165

Xu P, Harvey AJ, Fincher GB (1994) Heterologous expression of cDNAs encoding barley (*Hordeum vulgare*) (1→3)-β-glucanase isoenzyme GV. FEBS Lett 348:206–210