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Characterization of a jasmonate-regulated wheat protein related to a beta-glucosidase-aggregating factor

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Abstract

Jasmonates are distributed throughout higher plants, where they play an important role in the activation of signal transduction pathways in response to wounding and pathogen attack. Jasmonates are known to induce the transcriptional activation of plant defense genes, produce jasmonate-regulated proteins (JRP). One class of 32 kDa JRP (designated as JRP-32 in this paper) is present in the Gramineae family, although the function of these proteins is still unclear. A cDNA was cloned from wheat (*Triticum aestivum* L.) and designated as *Ta-JA1*. Sequence comparison indicated that *Ta-JA1* encodes a JRP-32 protein. *Ta-JA1* exists as a single-copy gene, but other genes with low sequence similarity to *Ta-JA1* may be present in the wheat genome. The expression of *Ta-JA1* was confined to stem tissues and barely detected in leaf and root tissues. Western blot analyses demonstrated that the recombinant Ta-JA1 protein cross-reacts with maize beta-glucosidase-aggregating factor (BGAF) antibody. Molecular modeling showed that Ta-JA1 and BGAF have a very similar three-dimensional structure. Protein structure analysis indicated that Ta-JA1 together with some related proteins (maize BGAF, wheat Ver2, WCI-1 and Hfr-1) contain two functional domains: a disease response domain and jacalin-related lectin (JRL) domain. A mannose-binding site was also well conserved in these proteins. The data support the hypothesis that JRP-32 and related proteins from Gramineae form a small protein family related to JRLs. This small protein family may have evolved from mannose-specific jacalin-related lectins (mJRLs) by developing a disease response domain in their N-terminus, which may have broadened the functional role of these proteins to include the plant defense response.

Keywords: Beta-glucosidase-aggregating factor; Jacalin-related lectins; Jasmonate-regulated proteins; *Triticum aestivum*

1. Introduction

Jasmonates are cyclopentanone derivatives, which originate biosynthetically from linolenic acid via an inducible octadecanoid pathway [20]. The physiologically active substance, jasmonic acid (JA) and its methyl ester (methyl jasmonate, JM) can be detected throughout the plant, with the highest concentrations found in growing tissues such as the shoot apex, root tips, immature fruits and young leaves [4]. The importance of jasmonates as signaling molecules was first noted by their ability to promote leaf senescence. Higher plants adapt to changes in abiotic or biotic factors in the environment by altering morphological, physiological and developmental processes. It is generally believed that jasmonates are involved in signaling processes that link environmental changes to an intracellular response, particularly in the wound response and plant defense [20].

Many jasmonate responses are manifested by changes in gene expression. The first observation of jasmonate-induced gene expression was made with jasmonate-treated barley leaf segments [15,25]. Jasmonate treatment resulted in symptoms of senescence and gel electrophoresis revealed *de novo* synthesis of several unique proteins [15]. In tomato and potato, it has been shown that jasmonates are required for wound-induced transcription of proteinase-inhibitor II and other

Abbreviations: BGAF, beta-glucosidase-aggregating factor; BTH, benzol (1,2,3) thiadiazole-7-carbothioic acid *S*-methyl ester; IPTG, isopropyl-1-thio-D-galactopyranoside; gJRLs, galactose-specific jacalin-related lectins; JA, jasmonic acid; JM, methyl jasmonate; JRD, jacalin-related domain; JRLs, jacalin-related lectins; JRP, jasmonate-regulated proteins; mJRLs, mannose-specific jacalin-related lectins; ORF, open reading frame; PBS, phosphate-buffered saline; SAR, systemic acquired resistance.

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responsive genes [17]. A similar induction pattern was reported for vegetative storage proteins in soybean [21].

Known jasmonate-regulated proteins (JRP) have diverse physiological functions and include a proteinase-inhibitor, pathogenesis-related proteins, phytoalexin-synthesizing enzymes, cell wall proteins, osmotin, lipoxygenase and many others [24]. However, the function of some JRPs, including the 32 kDa protein JRP-32, is still unclear. JRP-32 was first detected in jasmonate-treated leaves of barley (GenBank accession numbers **AF021256**, **AF021257**, **AF021258**). Expression of JRP-32 was only induced by jamonates, but not by ABA. We are interested in determining the identity of JRP-32 to provide a more thorough understanding of the response to jamonates.

Wheat is the number one food grain consumed directly by humans and its production leads all crops, including maize, rice, and potato. Taxonomically, wheat is closely related to barley. When wheat leaf segments were floated on jasmonate solution or sprayed with jasmonate, the leaves exhibited a similar senescence phenotype as previously observed in barley (our unpublished data). Considering wheat's importance to biology and agriculture, we chose to identify JRP-32 from wheat. Here we report the cloning and characterization of JRP-32 from wheat.

2. Results

2.1. Isolation and sequence analysis of wheat JRP-32 cDNA clone

After RT-PCR with primers J1 plus C2, and nested-PCR with primers J2 and J3, a single fragment of about 600 bp in size was detected (data not shown). This fragment was ligated to the pGEM-Easy T vector. Sequencing analysis indicated that this fragment showed high similarity (more than 80%) with the corresponding region of JRP-32 cDNAs from barley. Using this cDNA fragment as a probe, three positively hybridizing plaques were detected after high-stringency hybridization of a wheat cDNA library. Restriction analysis showed that all of the isolates had the same restriction fragment pattern. Complete DNA sequence analysis of these three clones showed that they belonged to the same cDNA, designated as *Ta-JA1* (accession no. **AY372111**).

The *Ta-JA1* cDNA is 1158 nucleotides in length with a single open reading frame (ORF) of 912 nucleotides flanked by 5′ and 3′ untranslated regions of 57 and 189 nucleotides, respectively. The ORF encodes a predicted protein of 304 amino acids with a molecular mass of 32.7 kDa and a calculated isoelectric point of 8.7. Based on the Kozak consensus sequence for eukaryotes, the ATG at position 58 is the real initiation codon with an A at position –3, and a C at position +5, as frequently observed in plants [11]. The 3′ end terminates in a 17 bp poly(A) tail, preceded by a 172 bp untranslated A + T rich (67%) region. One potential polyadenylation signal (AATAAAA) is located 21 bp upstream of the polyadenylation site. This 3′ end structure of *Ta-JA1* is in accordance with what is generally described in other plant genes [10].

When compared with sequences in the GenBank database, *Ta-JA1* showed very high homology with sequences that were isolated from barley (GenBank accession numbers **AF021256**, **AF021257**, **AF021258**). These sequences were putatively identified as JRP with a molecular mass of approximately 32 kDa (JRP-32). However, the identities of these proteins are still quite unclear since no research work concerning these sequences has been reported. For the convenience of comparison in this paper, we designate sequences from barley as *B-JRP1* (**AF021256**), *B-JRP2* (**AF021257**) and *B-JRP3* (**AF021258**), respectively. The amino acid identity of Ta-JA1 with B-JRP 1–3 is over 81%, and similarity is over 92% (Fig. 1).

After searching the TIGR wheat EST database (http://www.tigr.org/tdb/tgi/tagi), *Ta-JA1* was found to be almost identical to the EST **TC129967**. The amino acid identity of Ta-JA1 with **TC129967** is 99%, with only three amino

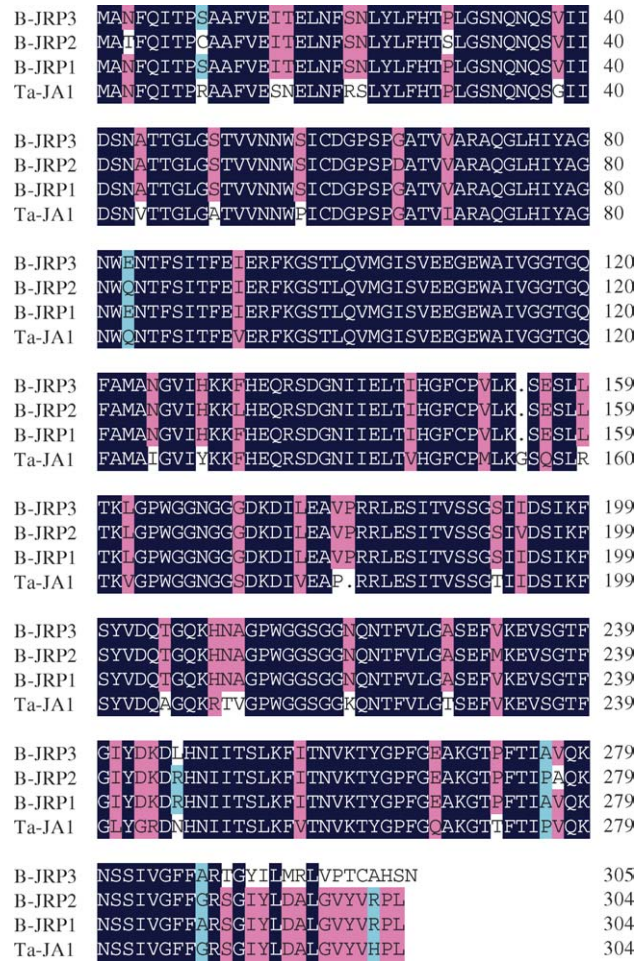

Fig. 1. Alignment of the predicted amino acid sequence of Ta-JA1 with JRP-32 proteins from barley. Gaps, indicated by dots, were inserted to maximize the alignment. B-JRP1 (GenBank accession no. **AF021256**), B-JRP2 (**AF021257**) and B-JRP3 (**AF021258**) are from barley. Ta-JA1 (**AY372111**) is from wheat.