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Cloning and expression of a novel cDNA encoding a mannose-specific jacalin-related lectin from *Oryza sativa*

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Abstract

Lectin plays an important role in defense signaling in plants. A few genes of this family have been cloned. Here we report on a mannose-specific jacalin-related lectin in rice. Using sequence information of wheat gene VER2, which we had previously cloned, we were able to amplify a cDNA of *OsJAC1* from *Oryza sativa* by RT-PCR. The cDNA of *OsJAC1* was 1172 bp and contained a 921-bp open reading frame (ORF) encoding dirigent (amino acids 26–139) and jacalin (amino acids 175–305) domains of 306 amino acids. Comparison of the OsJAC1 sequence with those of other lectins (jacalin) from rice, wheat and other species revealed that OsJAC1 had the 12 amino acid positions conserved in all mannose-binding lectins. Semi-quantitative RT-PCR revealed that *OsJAC1* expression was present in stems, leaves and young spikes but not young roots; the expression was high in leaves and low in stems and spikes. And methyl jasmonate could induce the expression of OsJAC1. To test the activity of OsJAC1, the jacalin domain at the C-terminal was expressed in *E. coli*. BL21 (DE3). The purified recombinant protein could agglutinate red blood cells of rabbit, and the agglutination activity was strongly inhibited by mannose compared with other carbohydrates. These results indicate that lectin with dirigent and jacalin domains exists in rice as well as wheat. This is the first report of cDNA cloning of mannose-binding jacalin-related lectin with a dirigent domain in N-terminal region from *O. sativa*.

Keywords: Mannose-binding lectin; *Oryza sativa*; OsJAC1; Jasmonate

1. Introduction

Carbohydrate-binding proteins, also called lectins or agglutinins, are a heterogeneous group of proteins classified together on the basis of their ability to bind in a reversible way to well-defined simple sugars and/or complex carbohydrates (Van Damme et al., 1998). They can be classified into seven families of structurally and evolutionary related proteins (amaranthins, Cucurbitaceae phloem lectins, lectins with hevein domain(s), jacalin-related lectins, legume lectins, monocot mannose-binding lectins and type-2 ribosome-inactivating proteins) (Van Damme et al., 1998; Murdock and Shade, 2002). The wide distribution of lectins in all tissues of plants and their ubiquitous presence in the plant kingdom suggest important roles for these proteins. One possible

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physiological function that has emerged is the defensive role of these carbohydrate-binding proteins against biotic stress (phytopathogenic microorganisms, phytophagous insects and plant-eating animals) (Chrispeels and Raikhel, 1991; Gatehouse et al., 1995) and abiotic stresses such as salinity (Zhang et al., 2000).

In the past few years, increasing interest has focused on mannose-binding lectin as interesting tools in glycoconjugate research (Haselbeck et al., 1990). As well, mannose-binding lectins are believed to play a role in recognizing the high-mannose-type glycans of foreign microorganisms or plant predators (Barre et al., 2001), which has provided hope for insect control. A recent discovery is that the snowdrop lectin has a striking toxicity to sucking insects (Hilder et al., 1995; Nagadhara et al., 2004). The mannose-binding snowdrop lectin binds to the mid-gut of the brown planthopper and the green leafhopper (Foissac et al., 2000), thus preventing nutrient absorption. Many monocot mannose-binding lectins from Amaryllidaceae, Alliaceae, Araceae, Iridaceae, Liliaceae and Orchidaceae have been documented and their protein properties and functions well characterized (Van Damme et al., 1991, 1993, 1994, 2000).

When plants are stimulated by specific biotic or abiotic stimuli, their response is the expression of cytoplasmic and/or nuclear plant lectins (Van Damme et al., 2004). Abiotic stress and biotic stress are major limiting factors in crop growth and grain yield. Wheat and rice are important food grains consumed directly by humans. Recently, some mannose-binding lectins in wheat were cloned and their potential functions in defense discussed. Wheat chemically induced gene 1 (*Wci-1*) showing high expression during systemic acquired resistance (Görlach et al., 1996) may be a plant protection member. Hessian fly-response gene 1 (*Hfr-1*), encoding a jacalin-like mannose-binding lectin, showed increased mRNA levels when the leaves of wheat containing the *H9* resistance gene responded to specific biotypes of the Hessian fly (Williams et al., 2002); the cDNA sequence was similar to that of a maize gene encoding a β-glucosidase aggregating factor (BGAF) (Blanchard and Esen, 2000) and to *Hv-JA1* (a barley gene that responds to jasmonate) (Lee et al., 1996). A jasmonate-regulated protein (JRP-32) related to BGAF, is also a mannose-binding lectin (Wang and Ma, 2005).

Protein structure analysis indicated that Wci-1, together with Hfr-1, maize BGAF, and JRP-32, contain two functional domains: a disease-response domain and a jacalin-related lectin domain. But in rice, only 1 jacalin-related mannose-binding lectin, *sal*T, without a disease-response domain, was isolated from salt-stressed plants, characterized and found to be involved in adaptation to adverse osmotic conditions (Garcia et al., 1998; Zhang et al., 2000). Using sequence information of wheat gene vernalization-related gene 2 (*VER2*) we previously had cloned (Yong et al., 2003), we cloned and expressed a rice cDNA encoding mannose-binding jacalin-related lectin (designated OsJAC1 in this paper) with a disease-response domain in the N-terminal region.

2. Materials and methods

2.1. Isolation of the OsJAC1 gene

Oryza sativa (cv. ZhongHua 10) plants were grown in a greenhouse under normal irrigation and fertilization. Total RNA for semi-quantitative RT-PCR was extracted using TRIZOL Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions from stems, leaves and young spikes of flowering plants and young roots of seedlings (14 days old). To see the effect of Jasmonate in levels of *OsJAC1* expression, rice seedlings (14 days old) were immersed into 50 μM methyl jasmonate solution for some hours and then used to extract total RNA for semi-quantitative RT-PCR. The cDNA synthesis was performed using AMV reverse transcriptase (Promega, USA) according to the manufacturer's protocol. The *OsJAC1* gene was amplified with the forward primer OsJAC1F (5′-gattactcg-cagcgcatcactcacagctac-3′) and the reverse primer OsJAC1R (5′-gatgcatgcaacac actacagacagaaacaactg-3′) according to the sequence of rice cDNA clone (AK066682). The forward primer (TubulinF, 5′-tcagatgccca gtgacagga-3′) and the reverse primer (TubulinR, 5′-ttggtgatctcggcaacaga-3′) of rice tubulin were used to amplify tubulin as an internal standard for RT-PCR. The PCR reaction was performed under the following conditions: cDNA was denatured at 94 °C for 2 min followed by 23–30 cycles of amplification (94 °C for 30 s, 57 °C for 30 s, 72 °C for 1 min) and followed by 72 °C for 10 min. The PCR product was inserted in pGEM®-T Easy vector (Promega, USA) and sequenced.

2.2. Purification of the recombinant protein

To test the activity of OsJAC1, the jacalin domain at the C-terminal of OsJAC1 was expressed in *E. coli* BL21 (DE3) cells. The 3′-terminal sequence (438 bp) of the open reading frame (ORF) added with the start codon (ATG) was inserted into the pGEX4T-1 vector and expressed in *E. coli*. A promoter inducible by IPTG controls the production of the fusion protein in the pGEX expression system. The fragment was amplified by PCR with a pair of primers (5′ga *agatct*atgtgccctgtcaccaagat) containing a *BglII* restriction site (underlined) and (5′ccg *ctcgag* gatcggctgcacgtagacac), an *XhoI* restriction site (underlined). After digestion with *BglII* and *XhoI*, the amplified product was inserted into the pGEX4T-1 vector pre-digested with *BamHI* and *XhoI*. The resulting recombinant plasmid pGEX4T-1 *OsJAC1* was sequenced and transformed into *E. coli*. Transformed BL21 cells were grown in 600 mL Luria-Bertani (LB) liquid