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Cloning and expression of a novel cDNA encoding a mannose-binding lectin from *Dendrobium officinale*

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Abstract

Using RNA extracted from *Dendrobium officinale* young leaves and primers designed according to the conservative regions of orchidaceae lectins, the full-length cDNA of *Dendrobium officinale* agglutinin2 (DOA2) was cloned by rapid amplification of cDNA ends (RACE). The full-length cDNA of *doa2* was 777 bp and contained a 513 bp open reading frame (ORF) encoding a lectin precursor of 170 amino acids. Through comparative analysis of *doa2* gene and its deduced amino acid sequence with those of other *orchidaceae* species and *Amaryllidaceae* species, it was found that DOA2 had many common characters of mannose-binding lectin superfamily including three mannose-binding sites. Semi-Quantitative RT-PCR analysis revealed that *doa2* mRNA expression was detected in all tested tissues including root, stem and leaf, however, the expression was higher in stem, and lower in leaf. As the *doa2* mRNA was detected in all the tested plant tissues, the *doa2* was considered to be a constitutively expressed gene. The recombinant protein was expressed in *E. coli* and purified. Anti-fungal assay showed that DOA2 has anti-fungal activity towards *Gibberella zeae*. To our knowledge, this is the first report on cDNA cloning of mannose binding lectin from *Dendrobium officinale*.

Keywords: Mannose-binding lectin; RACE; *Dendrobium officinale*; DOA2

Lectins or agglutinins are a group of non-immunogenic proteins possessing at least one non-catalytic domain that binds reversibly to specific mono- or oligo-saccharide (glycoconjugate) (Peumans and Van Damme, 1995; Carlini and Grossi-de-Sá, 2002). One such group is the so-called monocot mannose-binding lectins. Mannose-binding lectins are widely distributed in higher plants and are believed to play a role in recognition of high-mannose type glycans of foreign microorganisms or plant predators (Barre et al., 2001). Many monocot mannose-binding lectins from families *Amaryllidaceae*, *Alliaceae*, *Araceae*, *Iridaceae*, *Liliaceae* and *Orchidaceae* have been well documented and characterized on protein properties and functions (Van Damme et al., 1991, 1993, 1994, 2000).

In the past few years an increasing interest is drawn to this group of agglutinins for some reasons. One is because of their unique and exclusive specificity towards mannose these lectins have become very interesting tools in glycoconjugate research (Haselbeck et al., 1990). The other is recent discovery that the snowdrop lectin exhibits a striking toxicity to sucking insects (Hilder et al., 1995), which has provided the hope for insect control.

Recently, mannose-binding lectins have been isolated from several *Orchidaceae* species including *Listera ovata*,

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Epipactis helleborine, *Cymbidium hybrid* and Gastrodianin-like mannose-binding proteins (Van Damme et al., 1994; Xu et al., 1998; Wang et al., 2001). Feeding bioassays showed, like *Galanthus nivalis* agglutinin (*Amaryllidaceae*), *L. ovata* agglutinin (LOA) was effective against Maruca pod borer (MPB) larvae when incorporated into artificial diets (Machuka et al., 1999). Gastrodianin-like mannose-binding proteins (GLIPs) such as *Gastrodia elata* anti-fungal protein (GAFP) and *E. helleborine* mannose-binding protein also display in vitro antifungal activity against *Armillaria mellea*. GLIPs are being characterized as a novel class of antifungal proteins (Xu et al., 1998; Wang et al., 2001).

Dendrobium officinale, one of the traditional Chinese medicinal plant species, belongs to *Orchidaceae* family. Its stem has anti-thirst, abirritation, detumescence, antitumor effects on certain illnesses (Yang et al., 2002). Under natural conditions, rare diseases and pest damage are found on the plants of *D. officinale*. Whether its natural resistances to diseases and pests are related or partly related to its mannose-binding lectin content is unknown. The cloning and subsequent functional identification of the mannose-binding lectin gene from *D. officinale* will help to elucidate its anti-disease and anti-pest activities. There is no report on gene cloning from *D. officinale.* Here we report the molecular cloning and characterization of a mannose-binding lectin gene from *D. officinale* by the use of RACE-PCR cloning method.

Plant materials *D. officinale* plants were collected from the Second Military Medical University, China. Total RNA was extracted from young leaves of *D. officinale* using TRIZOL Reagent (GIBCO BRL, USA) according to the manufacturer's instructions. cDNA synthesis was performed with the 3′ RACE System for Rapid Amplification of cDNA ends (RACE PCR Kit, GIBCO BRL, USA). An aliquot of 3 μg RNA was reversely transcribed using primer AP (5′-GGCCACGCGTCGACTAGTAC$(T)_{18}-3'$) provided by the kit. Primer SH001 (5′-ATGCAGGGCGACTG CAACCT-3′) was designed according to the conserved amino acid sequence and synthesized (Shanghai Sangon Biological Engineering Technology and Service Co. Ltd, People's Republic of China). Reverse transcription (RT)-PCR was conducted in terms of kit protocol. The PCR reaction was performed under the following conditions: cDNA was denatured at 94 °C for 3 min followed by 35 cycles of amplification (94 °C for 45 s, 57 °C for 1 min, 72 °C for 1 min) and by 72 °C for 10 min. The PCR positive clone was sequenced using T7/SP6 primers (Sangon).

The 5′ RACE System for Rapid Amplification of cDNA Ends (GIBCO BRL, Life Technologies, USA) was used for 5′ cDNA cloning. According to the sequencing result of 3′ cDNA end, two primers SHR-1 (5′- CTCCTGCTCGTC GTAGATGACAAAGTT -3′) and SHR-2 (5′- GTGAGC CTTGATTAGCAGTGTTCGTTG -3′) were designed and synthesized for 5′ RACE.

By assembling the sequences of 3′ RACE and 5′ RACE, the full-length cDNA sequence of *D. officinale* lectin gene was obtained through RT-PCR reaction using primer SHFL (5′- GCCATGACTTTCTCCATCAGCTC -3′) and AUAP.

Upon sequencing, 3′ cDNA end of 590 bp and 5′ cDNA end of 320 bp were obtained. By assembling them the full length cDNA of 777 bp was obtained and further verified by sequencing. Except for a 53-bp 5′UTR and a 211-bp 3′UTR, the cDNA of *doa2* possessed a 513-bp open reading frame (ORF) from 54 to 566 bp of the sequence (Fig. 1). The 3′UTR possessed typical low G+C content (27.96%) and one polyadenylation signal site (AATAAA) was found within this region.

Doa2 contains a 513 bp open reading frame with one possible initiation codon at positions 1 of the deduced amino acid sequence. Translation starting from the ATG codon yields a 170 amino acid lectin precursor with calculated molecular mass of 18.1 kDa (Fig. 1). The ORF was translated with DNA tools 5.0 (DNA analysis software), which besides the information for the mature peptide also revealed a signal peptide and a C-terminal peptide. The possible cleavage site of a signal peptide was determined by application of the rules of von Heijne (1986). Cleavage of the signal peptide between residues 30 and 31 of the deduced amino acid sequence results in a 15.0 kDa polypeptide. This sequence, which is known to be the processing site for the cleavage of C-terminus in *G. nivalis* is also present in the C-terminal sequence of DOA2 (Van Damme et al., 1991). If we assume that the sequence Gly-Thr will also be the processing site for the cleavage of the C-terminal sequence in *D. officinale*, cleavage at this site will result in a lectin polypeptide of approximately 11.8 kDa. The hydrophobic character of this C-terminal peptide is consistent with the possibility that it is removed. The loss of C-terminal extension has been reported in LOA (*L. ovata* agglutinin) and CHA (*C.* hybrid agglutinin) (Van Damme et al., 1994). However, the possible function of these C-terminal peptides remains unclear. DOA2 cDNA encodes a precursor protein containing one potential glycosylation site within its C-terminal sequence. Generally, C-terminal peptide is removed therefore the mature *D. officinale* lectin is probably not glycosylated.

Doa2 encodes a mature lectin with two cysteins. The cystein content of DOA2 is in good agreement with those of *araceae* lectin AKA (*Amorphophallus konjac* agglutinin) (Fei et al., 2003) and *orchidaceae* lectin GAFP, the position of which coincides with the cysteins known to be involved in an internal disulfide bond in the *Amaryllidaceae G. nivalis* lectin. A third cystein was shown to occur as a free cystein in *G. nivalis* lectin (Van Damme et al., 1991).

Protein–protein BLAST of deduced DOA2 amino acid sequence showed much wider similarity to existing monocot mannose–binding lectins. It showed 27–84% identities and 45–91% positives in local alignments to lectins or agglutinins from *Narcissus* hybrid, *Clivia miniata*, *G. nivalis*, *L. ovata*, *Zephyranthes grandiflora*, *Ananas comosus*, *E. helleborine*, *Zephyranthes candida*, *Allium sativum*.