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cDNA CLONING AND EXPRESSION ANALYSES OF THE ISOFLAVONE REDUCTASE-LIKE GENE OF *DENDROBIUM OFFICINALE*

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Abstract

The full length of the isoflavone reductase-like gene (*IRL*) cDNA of *Dendrobium officinale* was cloned by using reverse transcription (RT) PCR combined with cDNA library, the *IRL* function was identified by Bioinformatics and prokaryotic expression analyses, and the *IRL* expression levels in the organs and tissues of *D. officinale* plants with different ages were determined by using real-time quantitative PCR (RT-qPCR). The results indicated that the full length of the cDNA of *D. officinale IRL*, *DoIRL*, was 1238 bp (accession no. KJ661023). Its open reading frame (ORF) was 930 bp which encoded 309 amino acids with a predicted molecular mass of 34 kDa, the 5′ untranslated region (UTR) was 61 bp and the 3′ UTR containing a poly (A) tail was 247 bp. The deduced amino acid sequence of *DoIRL,* DoIRL, was forecast to contain a NAD(P)H-binding motif (GGTGYIG) in the N-terminal region, two conserved N-glycosylation sites, a conserved nitrogen metabolite repression regulator (NmrA) domain and a phenylcoumaran benzylic ether reductase (PCBER) domain, to hold the nearest phylogenetic relationship with the PCBER of *Striga asiatica*, and to share both 73% identity with the isoflavone reductases-like (IRLs) of *Cucumis sativus* and *Striga asiatica*. In *Escherichia coli* 'BL21' cells, the *DoIRL* cDNA expression produced a protein band holding the predicted molecular mass of 34 kDa. *DoIRL* expressed in all organs and tissues of *D. officinale* plants with different ages at comparatively low levels, and the expression level in the leaves of the two-year-old plants was the highest.

Key words: cDNA, Cloning, Reductase-like gene, *Dendrobium officinale*, RT-qPCR.

Introduction

As one of the rare Traditional Chinese Medicines, *Caulis dendrobii officinalis,* i.e., Tiepishihu or Ribbed hedyotis herb, refers to the fresh or dried stems of *Dendrobium officinale* Kimura et Migo which was ever designated *D. candidum* Wall ex Lindl (Wang & Chen, 1996; Bao *et al.*, 2005), and was named because the stem epidermis was iron-like or yellowish green usually mixed with a little golden yellow hue. *Caulis dendrobii officinalis* was honored as the first of Nine Chinese Fairy Herbs in Taoist Sutra which was written within the Kaiyuan Period of Tang Dynasty of China, and as a life-saving fairy herb in Chinese folk. More than 1000 years of medical practice in China have proven that *D. officinale* holds many pharmaceutical activities, such as Yin-nourishing and heat-clearing, saliva secretion-promoting and stomach-tonifying, lung-moistening and cough-relieving, liver-nourishing and eyesight-improving, and so on (Anon., 2010; Ng *et al.*, 2012). Thus, *Caulis dendrobii officinalis* was regarded as the treasure of *Herba Dendrobii* all the time, and, in 2010, it was officially arranged independently in Pharmacopeia of the People's Republic of China (Division 1) (Anon., 2010).

D. officinale is a perennial adnascent herb, and its demands for ecological environment are extremely harsh. It distributes mainly in Anhui, Fujian, Guangdong, Guangxi, Hunan, Sichuan, Yunnan and Zhejiang Province of China, and also in Southeast Asia and Australia (Anon., 1999; Zha *et al.*, 2007). Being fond of warm and humid climate, wild *D. officinale* is not cold-resistant and, therefore, grows only at semi-gloomy and humid cliffs, rocks, trunks or barks (Anon., 1999; Nie & Cai, 2012). *D. officinale* seeds are very small and have no endosperm, and, under natural conditions, they germinate only when the symbiosis between the seeds and some fungi establishes (Jiang *et al.*, 2013). On the other hand, during last decade, wild *D. officinale* was immoderately collected and utilized. So, at present, it is approaching extinction and is called the panda in pharmaceutical kingdom (Nie & Cai, 2012).

Modern pharmaceutical studies have confirmed *Caulis dendrobii officinalis* can strengthen immunity, resist oxidation, fatigue and aging, inhibit tumors and decrease blood sugar and pressure, etc. due to its comprehensive ingredients, including the most important one, polysaccharides, and other minor ones, e.g., alkaloids, amino acids, phenolic compounds, stilbenes, lignans, lactones, flavonones, aldehydes, flavonoids, etc (Li, 2009; Wu *et al.*, 2011; Nie & Cai, 2012; Chen *et al.*, 2013). However, up to now, the roles of the lignans in the pharmacological activities of *Caulis dendrobii officinalis* and in the growth and development of *D. officinale* are completely unclear, forming a blind zone in the research of *D. officinale* all the time. Lots of previous investigations indicated that the lignans of *D. officinale*, e.g., icariol A_2-4-O-β-D-glucopyranoside and (+)-lyoniresinol-3a-O-β-D-glucopyranoside (Li, 2009; Nie & Cai, 2012), are probably related to the anticancer activity of *Caulis dendrobii officinalis* (Griffiths *et al.*, 1996). Biochemically, *D. officinale* lignans are biosynthesized via the crucial catalysis of isoflavone reductases-like (IRLs), even though IRLs are also the key enzymes in isoflavonoid biosynthesis (Paiva *et al.*, 1991; Brandalise *et al.*, 2009). Nevertheless, no isoflavonoid was identified in *D. officinale*

(Li, 2009; Chen *et al*., 2013), which is consistent with the general distribution rule of isoflavonoids (Dixon, 1999). Belonging to short-chain dehydrogenases/reductases (SDRs) family (Kallberg *et al*., 2002), IRLs, mainly including isoflavone reductase (IFR), pinoresinol-lariciresinol reductase (PLR) and phenylcoumaran benzylic ether reductase (PCBER) (Koeduka *et al*., 2006; Kajikawa *et al*., 2009), are phylogenetically related and NAD(P)H-dependent oxidoreductases of aromatic series (Vassão *et al*., 2007). Moreover, IFR, PLR and PCBER form a class of PIP enzyme subfamily of aromatic alcohol reductases with significant homology (Gang *et al*., 1997; Vassão *et al*., 2007). PCBER is typically about 66% similar and 45% identical to PLR, and about 65% similar and 50% identical to IFR, and, furthermore, it is proposed to be the possible progenitor of PLR and IFR (Gang *et al*., 1999; Min *et al*., 2003). In plants, IRLs have been verified to be the key enzymes in the synthesis of isoflavonoids and lignins which hold important pharmacological activities and defense functions (Paiva *et al*., 1991; Brandalise *et al*., 2009). Additionally, IRLs are involved in the biosynthesis of various phytoalexins, e.g., the (-)-medicarpin in alfalfa (*Medicago sativa*) (Paiva *et al*., 1991). Accordingly, it is reasonably presumed that *D. officinale* IRL (designated DoIRL) is associated not only with the pharmacological actions of *Caulis dendrobii officinalis* resulted from the lignans but also with the resistance of *D. officinale* plants to various stresses.

Nevertheless, so far, no paper has published concerning the isoflavone reductase-like gene (*IRL*) of *D. officinale*. For the first time, this paper reported the cDNA cloning of *D. officinale IRL* (designated *DoIRL*), the function-identifying of the *DoIRL* cDNA by Bioinformatics analysis and prokaryotic expression, and the differential expressions of *DoIRL* in the organs and tissues of *D. officinale* plants with different ages, aiming to provide a reference for the research of the pharmacological activity-optimizing, resistance and the plantation of *D. officinale*.

Materials and Methods

Plant materials: The healthy organs and tissues of *D. officinale* 'Hongxin 6#' plants with different ages, including the roots, stems and leaves of one-, two-, three- and four- year-old plants, the apical and lateral meristems of one-year-old plants, and the axillary buds and fruits of three-year-old plants, were randomly collected from the plantation of Yunnan Honghe Qunxin Dendrobium Planting Co., Ltd., China (103.46º E, 22.6º N. Altitude: 1300 m. The space of the plants: 12 cm×15 cm). All organs and tissues were quickly frozen in liquid nitrogen, ground to powder by adding liquid nitrogen, and then stored at -80°C for use.

cDNA cloning of *DoIRL* using reverse transcription (RT) PCR combined with cDNA library: The total RNA of *D. officinale* stems was extracted by using TRIzol® Reagent (Invitrogen, USA) according to the manufacturer's instructions, and treated by DNase □ (BioTeke, China) to remove the genomic DNA (gDNA). RNA concentration and integrity were determined by ultraviolet spectrophotometry and 1% agarose gel electrophoresis. Afterward, by using High Capacity cDNA Archive kit (Applied Biosystems, USA), first-stand cDNA was synthesized by reversely transcribing 1 μg RNA in a final reaction volume of 20 μL. The first pair of the specific primers of *IRL*, i.e., the forward primer 1 (F1): 5′-TCATTATCCTGGGCGATGGCA-3′ and the reverse primer 1 (R1): 5′-ATCGACAGAATAACATTCAGC-3′, which were designed according to the consensus sequences of the *IRLs* of other plants registered in the GenBank were used to clone the partial sequence of *DoIRL*. The reaction system consisted of 2.0 μL cDNA, 0.4 μL *EasyTaq* DNA polymerase (5 U/μL), 3.2 μL dNTPs (2.5 mM), 4 μL *EasyTaq* buffer (10×), 1.2 μL F1 (10 mM), 1.2 μL R1 (10 mM) and 28 μL RNA free water which was added finally. PCR was carried out as follows: 94 , 5 min→35 cycles (94 , 30 s→56 , 30 s→72 , 1 min)→72 , 10 min. The PCR product was sequenced by BGI, and, as such, the second pair of the specific primers of *DoIRL*, i.e., the forward primer 2 (F2): 5′-AACATCTACACGCATAACGA-3′ and the reverse primer 2 (R2): 5′-TGTTGAGGGTTCTTGGGT-3′ were designed. Then, by using the cDNA library of *D. officinale* which was constructed in advance and had a titer of about 1.3×10^6 pfu/mL as template (Attucci *et al*., 1996; Jiang *et al*., 2013), and by using M13F/R2 and F2/M13R as primers, respectively, the second PCR was performed to amplify the 5′- and 3′- end sequences of above partial sequence, other ingredients of the reaction system and the parameters of the second PCR were the same as those of the first one. Finally, the full length of *DoIRL* was accomplished by using Vector NTI9.

Bioinformatics analysis of the sequences of *DoIRL* cDNA and its deduced amino acids: The open reading frame (ORF) of *DoIRL* cDNA was identified by ORF Finder. The homology characteristics of *DoIRL* cDNA and its putative amino acid sequence, i.e., the theoretical *D. officinale* isoflavone reductase (DoIRL), were assessed by NCBI (http://blast.ncbi.nlm.nih.gov/). The transmembrane and other conserved domains of DoIRL were identified by using InterProScan against protein databases (http://smart.embl-heidelberg.de/) and TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/). The protein secondary structure was predicted by SOPMA (http://pbil.ibcp.fr/) (Geourjon & Deléage, 1995). Phylogenetic analysis of DoIRL and other plant IRLs were aligned with Clustal W (http://www.ebi.ac.uk/Tools/msa/clustalw2/) (Thompson *et al*., 1994), and, a neighbor-joining (NJ) tree was constructed by using MEGA 6.0 based on the alignment of full-length protein sequences (Saitou & Nei, 1987). Bootstrap analysis was performed with 1000 replicates (Tamura *et al*., 2001).

Expression of recombinant *DoIRL* cDNA in *Escherichia coli*: The third pair of the specific primers of *DoIRL*, i.e., the forward primer 3 (F3): 5′-GGATCCATGGCTGCAGAGAAGAGTCG-3′ and the reverse primer 3 (R3): 5′-CTCGAGTTACAGAAATCGGTTGAGGT-3′ were designed on the basis of the full length of *DoIRL* cDNA, and the two boxes were the added enzymatic sites of *BamH* and *Xho* , respectively. The reaction system was composed of 2.5 μL cDNA, 0.5 μL *EasyTaq* DNA polymerase (5 U/μL), 4.0 μL dNTPs (2.5 mM), 5 μL *EasyTaq* buffer (10×), 1.5 μL F3 (10 mM), 1.5 μL R1 (10 mM) and 35 μL RNA free water which was added at last. PCR was performed as follows: 94 , 5 min→35 cycles

(94 , 30 s→62 , 30 s→72 , 1 min)→72 , 10 min. The PCR product and the prokaryotic expression vector, pET-42a, were digested with *BamH* (BioTeke, China) and *Xho* (BioTeke, China) at 37 for 6 h, respectively. Then, the product was purified with QIAquick PCR Purification Kit (QIAGEN, Germany), incubated with the linearized pET-42a at 16 overnight. Next, the positive plasmids of pET42a-*DoIRL* cDNA were transformed into the competent cells of *E. coli* 'BL21' and induced by isopropy-β-D-thiogalactoside (IPTG). The expression profiles of the recombinant protein of *DoIRL* cDNA were analyzed by sodium dodecyl sulfate-polyacrylamide gel electropheresis (SDS-PAGE) after 1, 3 and 5 h of isopropy-β-D-thiogalactoside (IPTG) induction.

Determination of the differential expressions of *DoIRL* cDNA in the organs and tissues of *D. officinale* plants with different ages: The differential expressions of *DoIRL* cDNA in the above-mentioned organs and tissues of *D. officinale* plants with different ages were determined by real-time quantitative PCR (RT-qPCR). By employing ABI Prism 7000 Sequence Detection System and software (PE Applied Biosystems, USA), using the SYBR Green technology and regarding glyceraldehyde phosphate dehydrogenase gene (*GAPDH*) as the internal reference one, RT-qPCR was performed on the Corbett Rotor-Gene 3000 (Qiagen, Hilden, Germany). The fourth pair of the specific primers of *DoIRL*, i.e., the forward primer 4 (F4): 5′-TATGTGTCATCCAACTTC-3′ and the reverse primer 4 (R4): 5′-TGTGCCAATATCTTCTTC-3′, and one pair of *GAPDH*-specific primers, i.e., the forward primer (G-F): 5′-CAGCAAGAAGTAGATTAACG-3′, and the reverse primer (G-R): 5′-ACACATCAAACGATACAAAG-3′, were designed. The PCR reaction system consisted of 2.0 μL cDNA, 10 μL SYBR Premix Ex Taq^{TM} II (TaKaRa, Japan) (2×), 0.4 μL forward primer (10 mM), 0.4 μL reverse primer (10 mM), and 12.2 μL RNA free water which was added finally. The RT-q PCR protocol included an initial step of 95 for 5 min, followed by 40 cycles of 95 for 15 s and then annealed at 60 for 45 s, followed by 1 cycle of 95 for 60 s. Products were dissolved by curve analysis (55~95) after 81 cycles. Finally, E^{-Ct} analysis method was employed, the fluorescent threshold was set to 25, and the expression level of *DoIRL* in the apical meristem was set as 1. Three biological replicates for each sample were used for the RT-qPCR and all samples were repeated thrice for each biological replicate.

Results

Length and constitution of *DoIRL* cDNA: The full length of *DoIRL* cDNA was 1238 bp, and contained 711 bp 5′-end and 520 bp 3′-end (Figs. 1 and 2). The open reading frame (ORF) ranging from 62 to 991 bp was 930 bp long, and predicted to encode 309 amino acids (designated DoIRL) (Fig. 2). The 5′ untranslated region (UTR) was 61 bp and the 3′ UTR containing a poly (A) tail was 247 bp.

Physiochemical, structural and evolutionary characteristics of the deduced amino acid sequence of *DoIRL* cDNA: Physiochemically, the molecular mass and isoelectric point (pI) of the putative DoIRL were predicted to be 34 kDa and 5.67, respectively. Based on the alignment of the presumed DoIRL with the IRLs of other four plants, i.e., *Brachypodium distachyon*, *Glycine max*, *Oryza sativa* and *Vitis vinifera,* the structural features of DoIRL were chiefly reflected at its primary and secondary structures. On one hand, three main domains could be forecast in the primary structure. The first was a conservative structure in the N-terminal region, i.e., GGTGYIG, which was a NAD(P)H-binding site and belonged to the conserved GX(X)GXXG sequence of PCBERs (Fig. 3) (Kawamoto *et al.*, 2002; Moummou *et al.*, 2009), indicating DoIRL might be a PCBER of SDRs family. Meanwhile, a PCBER specific hit holding a lysine-contained active site could also be discovered. The second was two conserved N-glycosylation sites, i.e., NAS and NKT (Fig. 2) (Gang *et al.*, 1997). The third was a conserved nitrogen metabolite repression regulator (NmrA) domain (Fig. 4). On the other hand, the secondary structure of DoIRL was inferred to contain 38.19% α-helix, 6.47% β-pleated sheet, 37.54% random coil and 17.80% extended strand. Evolutionarily, the clustering results in the phylogenetic tree of DoIRL and other plant IRLs showed that DoIRL had the highest homology, i.e., 73% similarity, with *Striga asiatica* PCBER (Fig. 5). Thus, it was reasonably suggested that DoIRL has closer relationship with PCBERs than IFRs and might function as a PCBER *In vivo*.

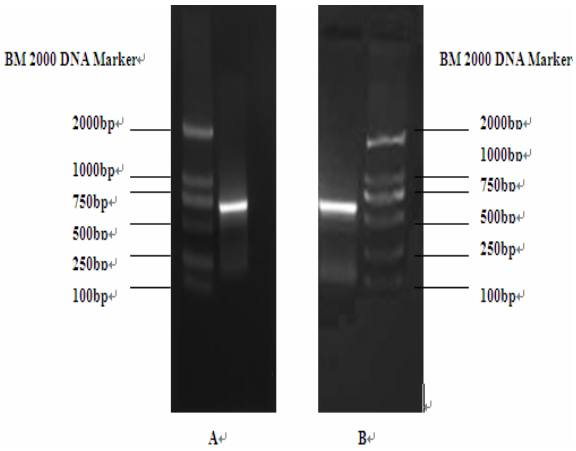

Fig. 1. Agarose gel electropherograms of the 5′- and 3′- ends of *DoIRL* cDNA. A: 5′-end; B: 3′-end.

Prokaryotic expression characteristics of *DoIRL* cDNA: Under the induction of IPTG, the recombinant protein of *DoIRL* cDNA in *E. coli* 'BL21' cells was verified to express steadily, producing a specific protein band with a molecular weight of about 33 kDa in the SDS-PAGE which was consistent with the predicted values (Fig. 6). Furthermore, when the induction time of IPTG ranged from 1 h to 2 h and further to 3 h, the target protein bands in the electropherogram were observed to become more and more obvious (Fig. 6), suggesting that, the longer the induction time of IPTG, the more the expression quantity of the protein.

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CGACCTGCAGCTGGCCATTACGGCCGGGGGAGAGAGGAGAAGGGAAAGAGAAAGAGGAAATATGGCTGCAGAGAAGAGTCGGATTCTGATCATCGGCGGGACGGGCTAC
                                                          M  A  A  E  K  S  R  I  L  I  I  G  G  T  G  Y
ATCGGAAAGTTCATCGTAAAGGCGAGTGCGCAAGAAGGTCACCCCACCTTCGCCCTCGTCAGGGAGAGCACTGCGGCCGATCCAGCCAAGGCCAAGATACTTGACGAA
I  G  K  F  I  V  K  A  S  A  Q  E  G  H  P  T  F  A  L  V  R  E  S  T  A  A  D  P  A  K  A  K  I  L  D  E
TTCAAGGCCCACGGCGTTACTCTCGTTCATGGGGATTTGTATGATCACGATAGCTTGGTGAAGGCGATAAAGGACGTGGATGTGGTTATATCAGCTGTTGGATTCGGG
F  K  A  H  G  V  T  L  V  H  G  D  L  Y  D  H  D  S  L  V  K  A  I  K  D  V  D  V  V  I  S  A  V  G  F  G
CAGCTTGCTGACCAGACCAAGATTATTGCTACCATTAAAGAGGCGGACAACATCAAGAGGTTCCTTCCTTCTGAGTTTGGCAATGATGTTGACCGAACTAACGCTGTT
Q  L  A  D  Q  T  K  I  I  A  T  I  K  E  A  D  N  I  K  R  F  L  P  S  E  F  G  N  D  V  D  R  T  N  A  V
GATCCTGCTAAGTCAGCGTTCGGAATAAAATCACAGATTCGTCGAGCAATTGAGGCAGAGAAAATTCCTTACACTTATGTGTCATCCAACTTCTTTGCCGGGTATTTC
D  P  A  K  S  A  F  G  I  K  S  Q  I  R  R  A  I  E  A  E  K  I  P  Y  T  Y  V  S  S  N  F  F  A  G  Y  F
CTTCCTTCCTTGGCACAAGCGACCACTACGAGCCTTCCAACTGATAAGGTCATTATCCTGGGCGATGGCAACACTAAAGCGATCTTCACTTATGAAGAAGATATTGGC
L  P  S  L  A  Q  A  T  T  T  S  L  P  T  D  K  V  I  I  L  G  D  G  N  T  K  A  I  F  T  Y  E  E  D  I  G
ACATTCACCGTTAAATCTGTGGATGACCCAAGAACCCTCAACAAGGTGTTGTATTTGAGACCTTCTGGAAACATCTACACGCATAACGAACTAGTCTCACTTTGGGAG
T  F  T  V  K  S  V  D  D  P  R  T  L  N  K  V  L  Y  L  R  P  S  G  N  I  Y  T  H  N  E  L  V  S  L  W  E
AAGAAGACTGGAAAGACATTCGAAAGGGTCTATATTTCTGAAGAAGAAATCCTCAAACAGATCCAAGAAGCACCAGCGCCGCTGAATGTTATTCTGTCGATACATCAC
K  K  T  G  K  T  F  E  R  V  Y  I  S  E  E  E  I  L  K  Q  I  Q  E  A  P  A  P  L  N  V  I  L  S  I  H  H
GCGACATTTGTGAAGGGAGATCAAACAAACTTTGAGATACACCAGTCGTTTGGAGTGGAGGCTACAGAGCTTTACCCTGATGTCAAGTATACCTCTGTGGACGAATAC
A  T  F  V  K  G  D  Q  T  N  F  E  I  H  Q  S  F  G  V  E  A  T  E  L  Y  P  D  V  K  Y  T  S  V  D  E  Y
CTCAACCGATTTCTGTAA AGTAACTATTACGGGCGAGGGCATGGACTGGCTTGTTTTCTTCCTGCTTGTAAGAGATATGCCTGTGTATTGTACCTATGGAATTGTCT
L  N  R  F  L  *
TATGTTTTCGTTTGTTGGTCTAGCCAGGTCATGAAGGCTCTTTCGTAGTACCTCAAGAAATTGACTTGTGATTTCCTTCTTTGTCGAGTCAGGTCTTTATGTACTTGTG
TTTTTGTTTAAGTTGATGGGTTCCTCTTGTTAAACAAAAAAAAAAAAAA
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Fig. 2. Full length of *DoIRL* cDNA and its deduced amino acid sequence.
The underlined part indicated the poly (A) tail.

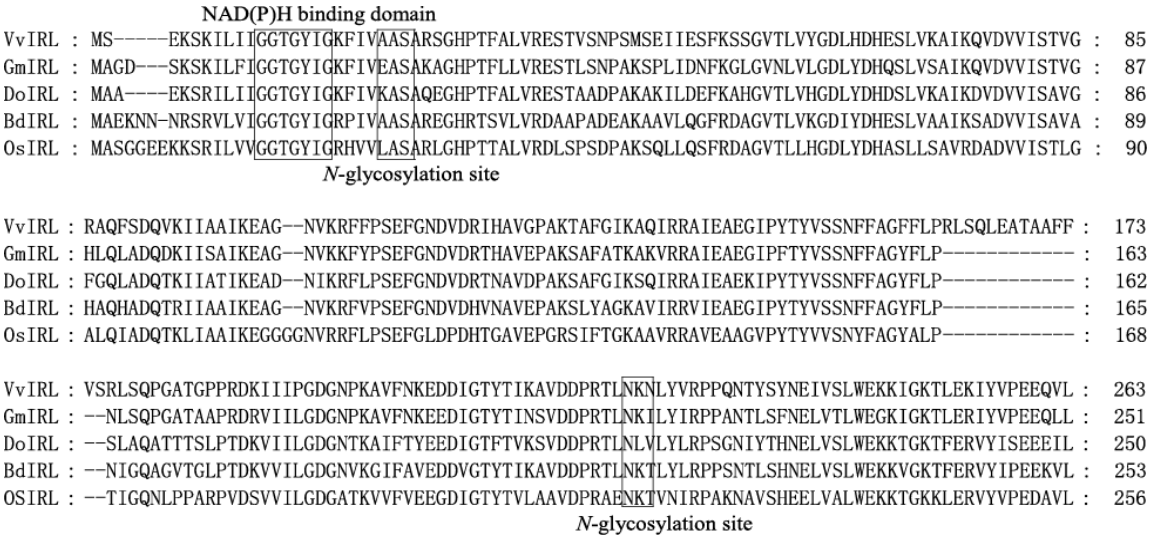

Fig. 3. NAD(P)H-binding and N-glycosylation sites of putative *Dendrobium officinale* isoflavone reductase (DoIRL) revealed by the alignment of DoIRL with the IRLs of other four plants.
VvIRL: *Vitis vinifera* IRL (XM 002283964); GmIRL: *Glycine max* IRL (NM 001250708); DoIRL: *Dendrobium officinale* IRL (KJ661023); BdIRL: *Brachypodium distachyon* IRL (XM 003575628); OsIRL: *Oryza sativa* IRL (AY 071920); The combinations of the letters and digits in the brackets indicated the accession numbers of the IRLs in the GenBank.

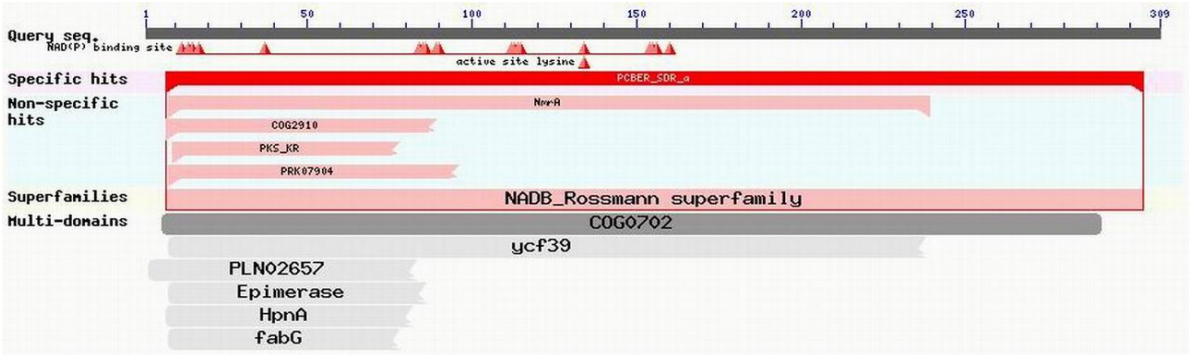

Fig. 4. Main predicted domains of putative *Dendrobium officinale* isoflavone reductase (DoIRL).

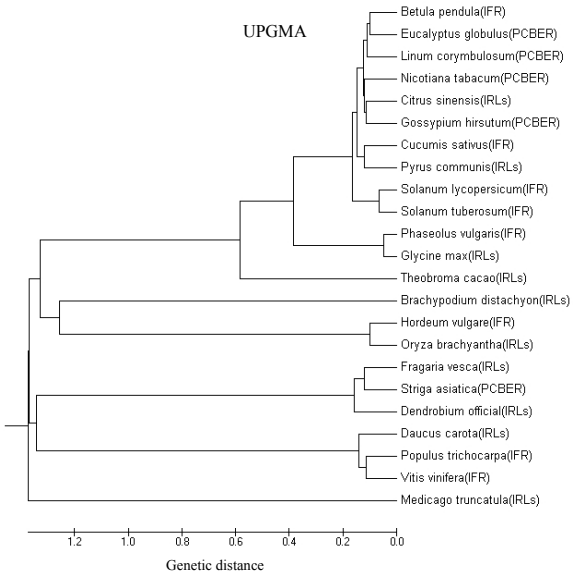

Fig. 5. Phylogenetic tree based on the alignment of putative *Dendrobium officinale* isoflavone reductase (DoIRL) with other plant IRLs.
IFR: isoflavone reductase; IRLs: isoflavone reductase-like proteins; PCBER: phenylcoumaran benzylic ether reductase.

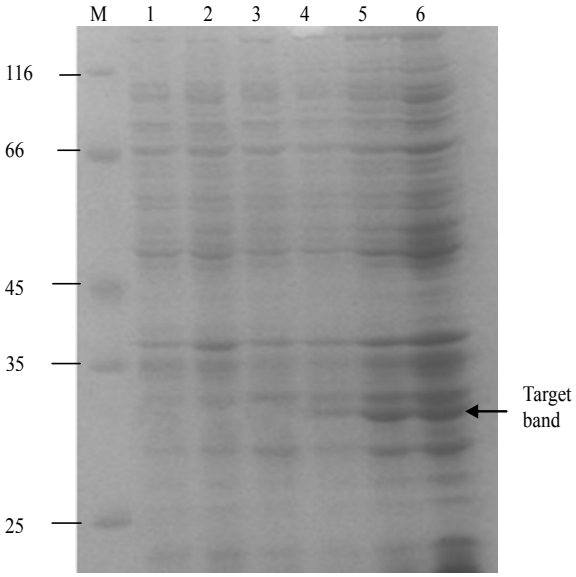

Fig. 6. SDS-PAGE electrophoresis of the recombinant protein of *DoIRL* cDNA in *Escherichia coli* 'BL21' cells.
M: protein marker; Lane 1: non-induced pET-42a; Lane 2: induced pET-42a; Lane 3: non-induced pET-42a-Do*IRL* cDNA; Lane 4-6: pET-42a-*DoIRL* cDNA induced by IPTG for 1, 3 and 5 h, respectively.

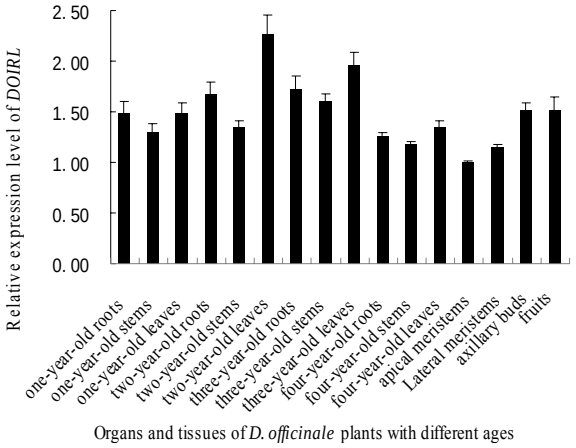

Fig. 7. Differential expressions of the *DoIRL* in the organs and tissues of *D. officinale* plants with different ages.

Differential expressions of *DoIRL* in different organs and tissues of *D. officinale* plants: *DoIRL* expressed differentially in the organs and tissues of *D. officinale* plants with different ages. For the plants of same ages, the expression levels of *DoIRL* in different organs were as follows: leaves>roots>stems (Fig. 7), indicating the leaves were the most crucial organs for *DoIRL* expression. For the plants of different ages, the expression level of two-year-old plants was the highest, next was the three-, one- and four- year-old plants successively (Fig. 7). Additionally, the expression levels of *DoIRL* in the lateral meristems were higher than those in the apical meristems. However, the expression levels in axillary buds and fruits were almost equal (Fig. 7).

Discussion

Many previous studies have verified that *IRL*s and IRLs are related to the responses of plants to various biotic and abiotic stresses. For example, Paiva *et al*. (1991) found IRLs were involved in the biosynthesis of (-)-medicarpin in *M. sativa*. Petrucco *et al*. (1996) discovered a maize (*Zea mays*) *IRL* was activated in response to sulfur starvation. Lers *et al*. (1998) found, under UV irradiation, the transcription level of the *IRL* in gape fruits increased, facilitating the fruit's resistance to pathogen infections and damages. Kim *et al.* (2003) witnessed the expression of rice (*Oryza saliva*) *IRL1* is induced by rice blast fungal elicitor. Marcos *et al*. (2009) confirmed the transcription level of the *IRL* in coffee (*Coffea arabica*) leaves raised significantly due to mechanical damages and fungal infections. It was found in this study that the forecast DoIRL held not signal peptide but a NAD(P)H-binding site in the N-terminal region, two conserved N-glycosylation sites and a conserved NmrA domain. It was a hydrophilic cytoplasm protein and shared high similarities with the IRLs, IFRs, PCBERs of other plants which all belonged to SDR family. At transcription level, *DoIRL* expressed in all organs and tissues of *D. officinale* plants of different ages with comparatively low levels, and the expression level in the leaves of two-year old plants was the highest. Biochemically, PCBERs have been confirmed to be a ubiquitous vascular plant enzyme and catalyze the reductive processes in the biosynthesis of 8-5′ linked lignans, e.g., with dehydrodiconiferyl alcohol (Gang *et al*., 1999; Min *et al*., 2003). However, the functional characteristics of *DoIRL* expression with respect to the resistance of *D. officinale* to various stresses are still waiting to be elucidated, which will favor the optimization of the cultivation measures of *D. officinale*.

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