academicJournals

Vol. 14(28), pp. 2234-2243, 15 July, 2015
DOI: 10.5897/AJB2013.13402
Article Number: E3437A354150
ISSN 1684-5315

http://www.academicjournals.org/AJB

African Journal of Biotechnology

Full Length Research Paper

Molecular cloning and expression of a novel gene related to legume lectin from *Salvia miltiorrhiza* Bunge

Wenping Hua[1,2], Limin Han[1,2] and Zhezhi Wang[2]*

[1]Department of Life Sciences, Shaanxi Xueqian Normal University, Xi'an, Shaanxi 710061, P.R. China.
[2]Key Laboratory of the Ministry of Education for Medicinal Resources and Natural Pharmaceutical Chemistry, National Engineering Laboratory for Resource Development of Endangered Crude Drugs in Northwest of China, College of Life Sciences, Shaanxi Normal University, Xi'an 710062, P.R. China.

Received 19 December, 2013; Accepted 13 July, 2015

Lectins have been well studied and proved to play important roles in plant defense but information of legume lectins from non-legume plants has been rarely reported. A new legume lectin gene, designated as *SmL*1, was cloned from *Salvia miltiorrhiza* Bunge, a famous traditional Chinese medicinal plant. The cDNA of *SmL*1 was 919 bp in length and contained an 822 bp open reading frame (ORF) encoding a putative lectin precursor with two legume lectin domains. The deduced SML1 protein of *SmL*1 shared 29 to 43% identities with other legume lectin sequences. Real time PCR analysis revealed that *SmL*1 was predominantly expressed in the leaves and could be induced by pathogens and MeJA. The recombinant protein (rSmL1) of *SmL*1 in *Escherichia coli* M15 was purified and showed agglutination activity towards rabbit and mouse red blood cells, and anti-bacterial activity against *E. coli* (ATCC35218), *Pseudomonas lachrymans* (PSL) and *Xanthomonas campestris* pv. Campestris (Pammel) Dowson (XC-1). Based on these results, SmL1 could play a role in medicinal plant disease control.

Key words: Anti-bacterial activity, gene expression, legume lectin, recombinant protein, *Salvia miltiorrhiza* Bunge.

INTRODUCTION

Plant lectins or agglutinins are a large group of proteins, which possess at least one non-catalytic domain that binds reversibly to a specific mono- or oligosaccharide (Carlini and Grossi-de-Sa, 2002; Peumans and Van Damme, 1995). Lectins exist in most living organisms but were first identified as plant proteins that agglutinate human red blood cells (Van Damme et al., 1998). Now thousands of plant lectins were found and stored in the Lectin database (Lectindb, http://proline.physics.iisc.ernet.in/cgi-bin/lectindb/). Based on their different carbohydrate-binding specificities, plant lectins have been divided into 12 different families, such as (1) *Agaricus bisporus* agglutinin homologs, (2) Amaranthins, (3) Class V chitinase homologs with lectin activity, (4) Cyanovirin family, (5) EEA family, (6) GNA family, (7) proteins with hevein domains, (8) Jacalins, (9) proteins with legume

*Corresponding author. E-mail: zzwang@snnu.edu.cn. Tel: +86-29-85310260. Fax: +86-29-85310546.

Abbreviations: EAPL, Extralong autumn purple bean; **WGA,** wheat germ agglutinin; **ORF,** open reading frame.

lectin domains, (10) LysM domain, (11) Nictaba family (formerly Cucurbitaceae phloem lectins), and (12) Ricin-B family (Van Damme et al., 2008). It has been suggested that plant lectins play important roles due to their abundance in the immune defence, and also that lectins have been coopted to adapt for several functions during evolution (Charungchitrak et al., 2011). More and more attention has been draw onto the antimicrobial activity of plant lectins. Many lectins had been found with anti-microbial activity, such as lectins derived from *Phthirusa pyrifolia* leaves (Costa et al., 2010), *Eugenia uniflora* (EuniSL) (Oliveira et al., 2008) and *Myracrodruon urundeuva* (Sá et al., 2009). In recent years, even some lectins have anti-viral activity, such as lectin from seeds of *Phaseolus vulgaris* L. cv. Extralong Autumn Purple Bean (EAPL) and BanLec isolated from the fruit of bananas, indicated Anti-HIV-1 activities (Fang et al., 2010; Swanson et al., 2010). Some of them, such as a wheat germ agglutinin (WGA) (Ciopraga et al., 1999), Concanavalin A (Safina et al., 2008), *Sebastiania jacobinensis* bark lectin (SejaBL) (Vaz et al., 2010) and *P. pyrifolia* leaf lectin (Costa et al., 2010), have been tried to biologically control plant diseases.

However, different families of plant species, as well as different tissues within the same plant, can contain different lectins with different bioactivities, including different carbohydrate-binding specificities and antimicrobial activity (Charungchitrak et al., 2011). They occur widely in plants but manifest significant differences in bioactivities, which means only a few of them has application prospect. Presently, most of their functions are still unclear in many plants. *Salvia miltiorrhiza* Bunge is a well-known medicinal plant in the Labiatae family. Its dry roots or rhizomes (called 'danshen' or 'tanshen' in China, but better known in the west as Chinese sage or red sage root) has been used in the treatment of cardiovascular, cerebrovascular, hyperlipidemia, and acute ischemic stroke diseases for decades (Kang et al., 2003; Yang et al., 2006; Zhong et al., 2009). One soil-borne disease induced by *Fusarium solani* could lead to rotten roots of *S. miltiorrhiza*, which results in the reduction of yields and the decline of quality. In recent years, the incidence of diseased plants in the field has varied from 10 to 30% in its growth zones. It is difficult to control this disease by farm chemicals, which may pollute this medicinal crop and its environment if applied irrationally. Biocontrol means has attracted people's attention in sustainable environmental development to control this disease compared to the application of chemicals.

To explore the potential lectin protein-encoding genes, we screened our cDNA library of *S. miltiorrhiza* (Hua et al., 2011) and found 30 unigenes encoding putative lectin proteins. Contig1927 (one of lectin unigenes) has also been reported as the highest abundance gene in *S. miltiorrhiza* root EST library (Li et al., 2010). Taking into account that the roots are the part of occurrence of rotten root in *S. miltiorrhiza*, we chose this gene as our object in the present paper. Firstly, we cloned and characterized this novel gene (GenBank Accession Number: EF593952), then we over-expressed it in *Escherichia coli*, eventually its agglutination and antibacterial activity had been identified *in vitro*. The purified recombinant protein showed significant anti-bacterial activity against *E. coli* (ATCC35218), *Pseudomonas lachrymans* and *Xanthomonas campestris pv*. Campestris (Pammel) Dowson. Therefore, these results suggested that the application of this gene in genetically modified plants may be an efficient way to control root rot in *S. miltiorrhiza* and generate more profitable and productive yields without affecting environment.

MATERIALS AND METHODS

Plant materials

The seeds of *S. miltiorrhiza* Bunge were collected from Shangluo County, Shaanxi Province, China. The plants were grown in pots with soil in greenhouse under normal irrigation and fertilization. Two-month-old seedlings were used for DNA extraction and the following two treatments, respectively. For the methyl jasmonate (MeJA) treatment, leaves were sprayed with 5 μM MeJA. Then samples were collected at hour 0, 1, 6, 12, 24 and 48 h after MeJA application. Young leaves nicked with a knife were infected by *P. lachrymans* (PSL, 10^8 cfu·ml^{-1}), and collected at 0, 24, 48, 72 and 96 h post-infection. All collected samples were frozen immediately in liquid nitrogen and stored at -80°C before use.

Isolation of the lectin gene

The genomic DNA was extracted from *S. miltiorrhiza* by CTAB based method (Beji et al., 1987). Total RNAs were extracted respectively from the roots, stems and leaves of control and treated plantlets, using BIOZOL Reagent (BIOER, China) according to the manufacturer's instructions. The first strand cDNA was synthesized using Revert Aid First Strand cDNA Synthesis Kit (MBI, Fermentas). The lectin gene was amplified with the forward primer: 5′-ATGGCCAAGCTTCTCCAAAAC-3′ and the reverse primer: 5′-GTCGATCGCTTAGTCCTTATTGA-3′, both of which were designed according to unigene sequence (Contig1927) from cDNA library of *S. miltiorrhiza*. The PCR reaction was performed under the following conditions: genomic DNA or cDNA was denatured at 94°C for 4 min followed by 30 cycles of amplification (94°C for 30 s, 54°C for 30 s and 72°C for 80 s) and then extention at 72°C for 10 min. The PCR fragments were purified by DNA Gel Extraction Kit (Biospin) and inserted into pGMT-Easy T vector (Promega, USA) and sequenced.

Expression level of *SmL1* gene in different tissues

SYBR Green II dye (Takara, Japan) was used for detecting the expression levels of *SmL*1 under various treatments or in different tissues of *S. miltiorrhiza*. Real-time PCR was conducted with an iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad). The *SmL*1 was amplified with the forward primer: 5′-CATGACATC GTCTCGTGGTATTTC-3′ and the reverse primer: 5′-GATCGCTTAGTCCTTAT TGATTTGC-3′. A housekeeping gene, *SmGAPDH* (glyceraldehydes-3-phosphate dehydrogenase, CV170251), was used as our control and was amplified with forward primer GAPF 5′-CCACCGTCCACTCCATCACT-3′ and

reverse primer GAPR (5′-TG GGAACTCGGAACGACATAC-3′). The amplification of *SmL*1 and *SmGAPDH* gene was performed as: cDNA was denatured at 95°C for 5 min followed by 40 cycles of amplification (95°C for 10 s, 59°C for 15 s and 70°C for 10 s to collected fluorescence). The products were validated by electrophoresis on a 1.5% agarose gel then further sequenced for confirmation. Expression was quantified by the comparative C_T method (Vandesompele et al., 2002). Each data point represented the average of three separate experiments. Statistical analysis was done with SPSS 13.0 software. One-way analysis of variance (ANOVA) and Tukey's pair-wise comparison tests (P<0.05) were successively performed to determine significant differences between means.

Construction of lectin expression vector

The mature protein-encoding region was amplified using the forward primer: 5′-GGGGTACCCAAACGACGTCCTTCACCTA-3′ containing a *Kpn*I restriction site (underlined) and the reverse primer 5′-AACTGCAGGTCGATCGCTTAGTCC TTATTGA-3′ containing a *Pst*I restriction site (underlined). The amplification condition was as described previously. The PCR product digested with *Kpn*I and *Pst*I was ligated with pQE30 vector using T4 DNA ligase (TaKaRa), and then transformed into *E. coli* M15 strain. Positive transformants were screened by plating on Luria-Bertani (LB) agar containing kanamycin and ampicillin antibiotics after growing overnight at 37°C.

SmL1 gene expression and purification

The positive M15 cells were cultured in LB medium containing 100 mg/ml ampicillin and 50 mg/ml kanamycin at 37°C to an absorbance of 0.6 to 0.8 at 600 nm. 1 mM IPTG were applied into culture to induced target gene expression. The cells for SDS-PAGE were harvested at 0, 1, 2, 3, 4, 5 and 6 h after induction, then centrifuged at 4°C, 4,000 g for 8 min. Afterwards, the pellet with 6 h induction was re-suspended in PBS buffer (containing 8 M urea), then the cell walls were fractured by ultrasound (400 w for 7 min). After another centrifuge at 4°C, 10,000 *g* for 20 min, the supernatant was collected for protein purification using equilibrated His-bond Ni Affinity Resin column (Zhuoguan, China) according to the manufacturer's instructions. The elutions were analyzed by SDS-PAGE.

Agglutination activity test of recombinant protein of *SmL1* gene

The erythrocytes from rabbit and mouse (pre-treated with trypsin) were washed with normal saline (0.9%) for three times. The recombinant protein (0.5 mg/ml) solution was serially diluted with two-fold increments. Agglutination assays were carried out in a 96-U-well plate in a final volume of 50 µl containing 25 µl the diluted recombinant protein solutions and 25 µl of a 1% suspension of red blood cells. Elution/washing buffer of the recombinant protein and normal saline were considered negative controls. Agglutination was assessed visually after 2 h at room temperature using microscope. Two separate experiments for every individual were performed and the means were calculated.

Anti-bacterial activity of recombinant protein of *SmL*1 (rSmL1) gene

The anti-bacterial activity of rSmL1 was qualitatively evaluated by optical density. *E. coli* (ATCC35218), PSL, and XC-1 were bought from China General Microbiological Culture Collection Center as test bacteria. The test bacteria were incubated at 28°C for PSL and XC-1, at 37°C for *E. coli* (ATCC35218) overnight at 180 rpm. A secondary propagation of the cells was carried out for another 2 to 3 h, then cultures were divided into equal parts (50 ml for each). Equal amount of purified rSmL1 protein was added into each culture, and boiled purified protein was used as negative control. After inoculation of purified recombinant rSmL1, the density of each medium was measured at OD 595 nm after 0, 1, 2, 3, 4 and 5 h. The experiments were done three times. The *E. coli*, PSL, and XC-1 were cultured in Ordinary Broth Medium at 37°C, KB medium without agar at 28°C, and modified Broth Medium containing sucrose (10 g/L) at 28°C, respectively. Significant differences between treatment and control groups were analyzed using one-way ANOVA with SPSS13.0 software.

RESULTS AND DISCUSSION

Isolation and sequence analysis of *SmL1*

The full-length cDNA of *SmL*1 was amplified by RT-PCR. The cDNA fragment was 919 bp in length and contained an 822 bp open reading frame (ORF) positioned from 33 to 855 bp (Figure 1a). The ORF of *SmL*1 encodes 273 amino acids with the isoelectric point of 5.00 and molecular mass of 29.2 kDa. No intron was found in *SmL*1 sequence by comparing genomic and cDNA sequences. The deduced amino acids sequence (named as SmL1) had two legume lectin domains, a lectin-legB (amino acids 28 to 203) domain, and a lectin-legA (amino acids 211 to 256) domain according to BLASTp search against NCBI (Figure 1b). The amino acid number and molecular mass were identical with other legume lectins as previously reported (Etzler, 1985; Sharon and Lis, 1990; Van Damme et al., 1998). Protein-protein BLAST of deduced *SmL*1 amino acid sequence showed 29 to 43% identities and 49 to 61% positives in local alignments against candidate genes from *Arachis hypogaea* (ABJ15831), *Sophora flavescens* (AF285121), *Sophora alopecuroides* (AAA74572), *Glechoma hederacea* (AAN 050977), *Canavalia ensiformis* (CAA25787), *Cladrastis kentukea* (Q39529), *Sophora japonica* (AAB51442) and *Phaseolus leptostachyus* (CAH60215). All these results suggested that SmL1 belonged to legume lectin family.

Most legume lectins are extensively distributed in legume plants. However, Gleheda (AAN05097) (a legume lectin isolated from *G. hederacea*) and SBoL (a *Salvia bogotensis* seed lectin) had been identified from non-legumes (Vega and Pérez, 2006; Wang et al., 2003) and some of which has been shown to have important physiological activity in plants, such as Gleheda, which indicate insecticidal activity against Colorado potato beetle larvae (Wang et al., 2003). To our knowledge, this is the first time to report legume lectin gene in *S. miltiorrhiza.* And our lectin gene encoding protein sequence was similar to Gleheda, which indicated that it may also played unusual roles in physiology in

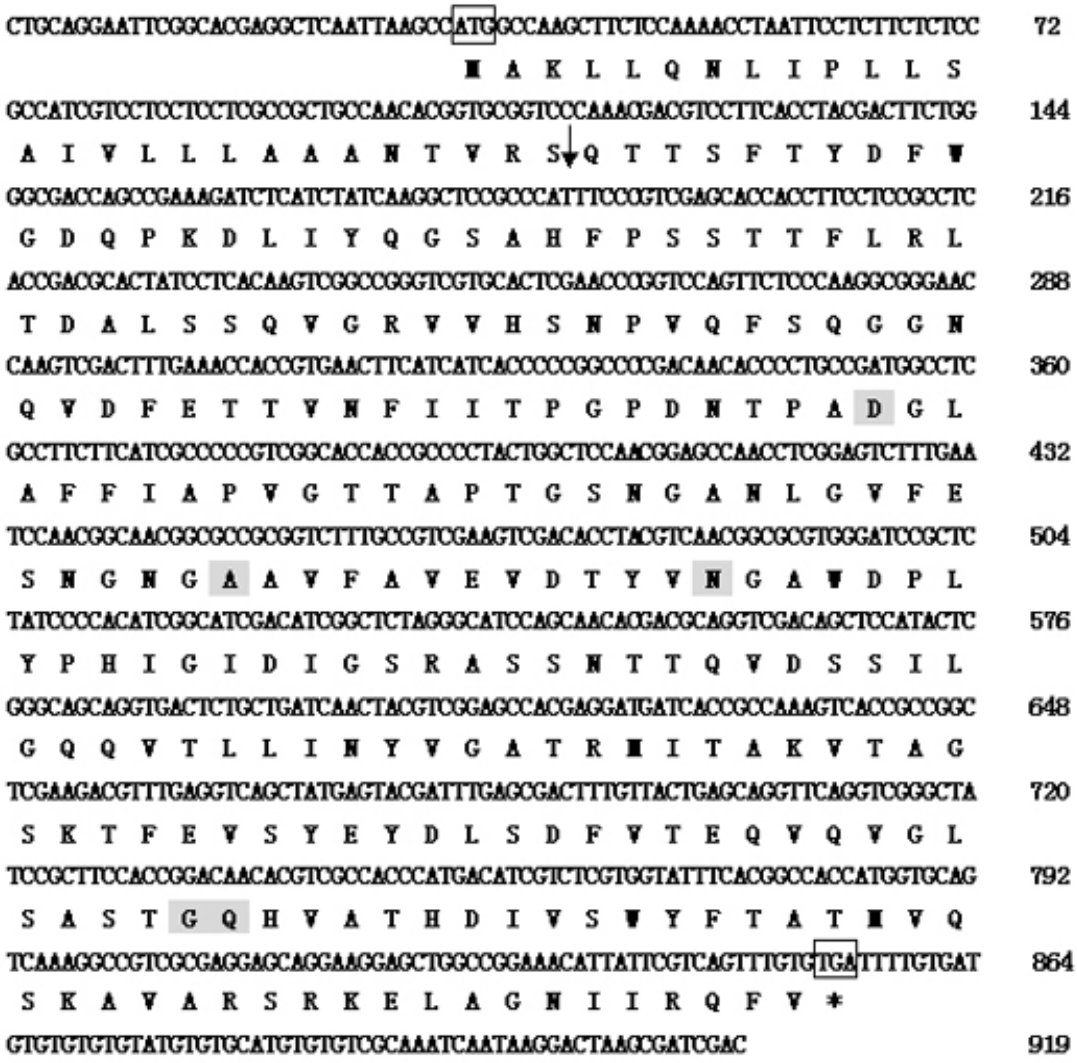

Figure 1a. The full-length cDNA sequence and deduced amino acid sequence of *S. miltiorrhiza* lectin (*SmL*1). The star codon (ATG) and the stop codon are shown by boxes. The putative processing sites for the cleavage of in C-terminal are marked by arrow. The putative N-glycosylation site is shown by black ground.

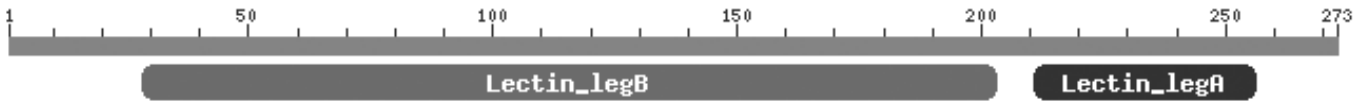

Figure 1b. Putative conserved domain has been detected with the protein-protein BLAST tool of NCBI.

S. miltiorrhiza as Gleheda in *G. hederacea.*

The signal peptide (SignalP, http://www.cbs.dtu.dk/services/SignalP/) prediction on SmL1 revealed a single peptide cleavage site between position 27 and 28th amino acid residues (Ser-Gln) (Figure 1a). The cleavage of the signal peptide in C-terminal sequence of SmL1 resulted in a lectin polypeptide of approximately 26.3 kDa with theoretical pI 4.76.

Sequence analysis of SmL1

Multi-alignment of SmL1 with other legume lectins (Figure 1) was conducted by ClustalW (Figure 2a). SmL1 showed 43, 37, 37 and 37% identity to protein sequences of Gleheda*, S. alopecuroides* lectin, *S. flavescens* lectin, and LecClAII (*C. kentukea* agglutinin), respectively. A further examination on the sequences of SmL1, Gleheda,

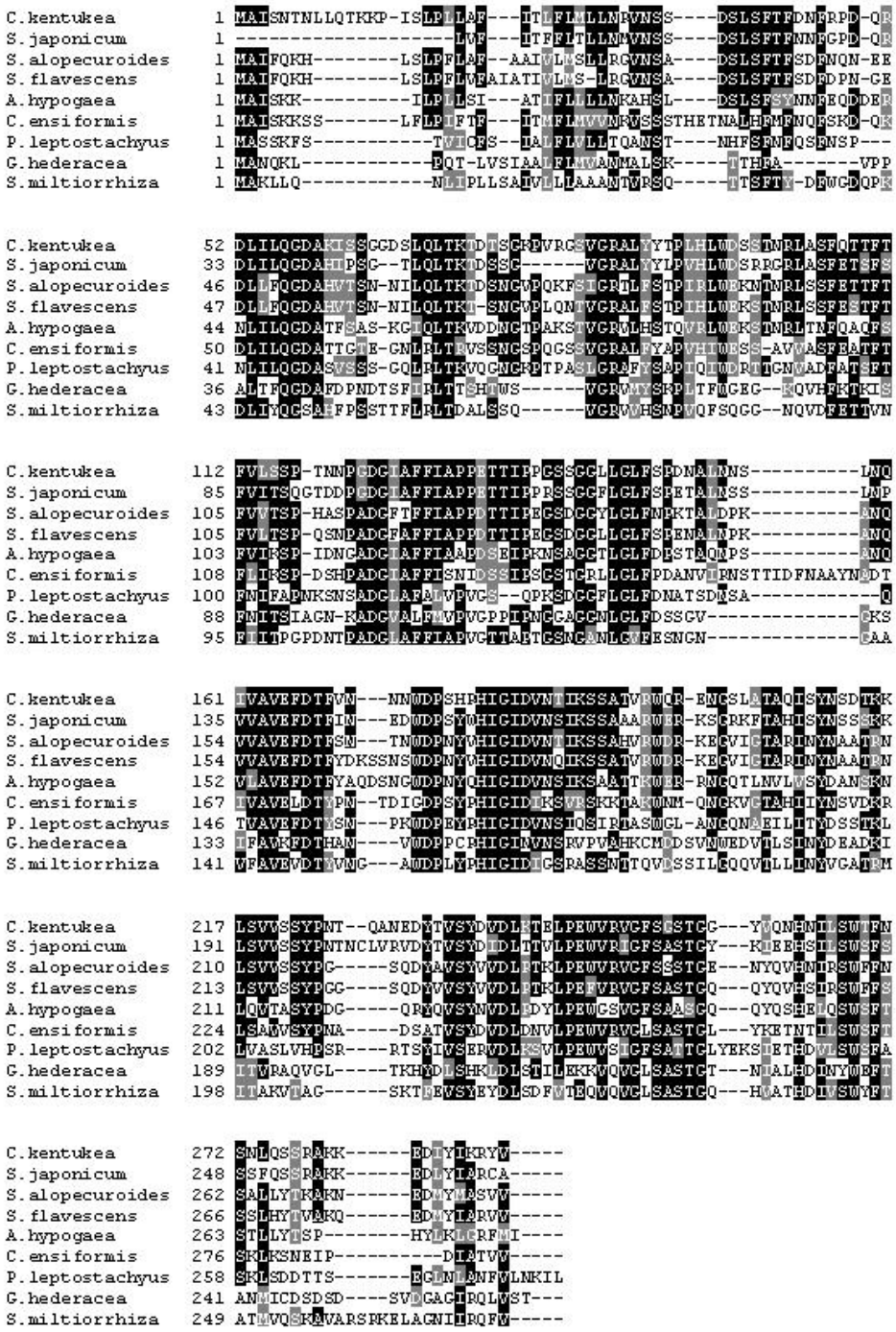

Figure 2a. Multiple sequence alignment of SmL1 with other legume lectin. The multiple sequence alignment was performed by Clustal W (http://www.ebi.ac.uk/clustalw/index.html): *C. kentukea* Agglutinin (Q39529); *S. japonicum* (AAB51442); *S. alopecuroides* lectin (AAA74572); *S. flavescens* (AF285121); *A. hypogaea* (ABJ 15831); *C. ensiformis* (CAA25787); *P. leptostachyus* (CAH60215); *G. hederacea* lectin (AAN050 977); *S. miltiorrhiza* (EF593952).

S. flavescens lectin, and *A. hypogaea* lectin, indicated that the residues forming the mono-saccharide binding sites were highly conserved. The putative carbohydrates binding sites in SmL1 were found as: Asp_{107}, Ala_{139}, Asn_{151}, Gly_{234}, Gln_{235} and His_{236} (Figure 1a). Asp_{107}, Asn_{151}, Gly_{234} were identical to Gleheda, Gln_{235} was

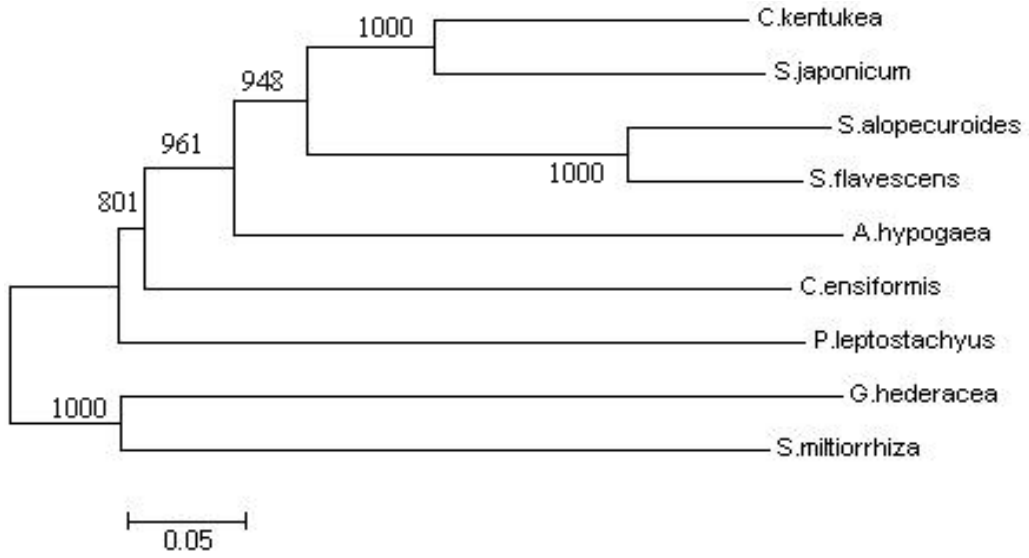

Figure 2b. The phylogenetic relationships of SmL1 with other related proteins. The tree was constructed the alignment resulting from analysis by MEGA4.0.

identical with *S. flavescens* lectin and *A. hypogaea* lectin (Liu et al., 2008). Ala_{139} in SmL1 was replaced by Gly_{93} compared to Gleheda (Wang et al., 2003). Some amino acid residues of monomer ConA (a classical legume lectin from *C. ensiformis*) and legume lectin LoLI from *L. odoratus* L. were highly conserved, which decided the sorts of binding carbohydrate, such as Asp_{81}, Gly_{99}, Asn_{125}, Gly_{208}, Ala_{20s9} and Glu_{210} in LoLI protein. Asp_{81} and Asn_{125} in LoLI were consistent in all known legume lectins, and necessary for carbohydrate activity; while other amino acid residues, Ala_{209} and Glu_{210} (LoLI), were alterable, probably participated in binding carbohydrate (Bourne et al., 1990; Perçin et al., 2012). Some amino acid residues (Asp_{107}, Asn_{151}) of the putative conservative carbohydrates binding sites in SmL1 were invariant, while Ala_{139} and His_{236} were different from other legume lectins, just like ConA and LoLI (Sharon and Lis, 1990).

Furthermore, the phylogenetic relationship of SmL1 with other candidates was determined. The phylogenetic tree was constructed by neighbor-joining method with MEGA 4.0. It shows the SmL1 and Gleheda were clustered into one sub-group; other lectins from legume plants were clustered into another sub-group but SmL1 was closer to legume lectins than Gleheda in molecular evolution (Figure 2b). The amino acid residues on carbohydrate-binding site (in comparison with Gly_{93} in Gleheda, Ala_{139} in SmL1 were more conserved among legume lectins) also supported our assumption. The identification of *SmL*1 gene from *S. miltiorrhiza* showed high similarity with Gleheda, provided another direct evidence that the possibility of finding an ortholog of legume lectins gene outside the family Fabaceae (Wang et al., 2003), which also indicated the evolutionary processes of the same ancestor of modern legume lectins.

Tertiary structure prediction of *SmL*1 amino acid sequence

According to the deduced amino acid sequence of *SmL*1 gene, the three-dimensional model of SmL1 was constructed using SWISS-MODEL (http: //swissmodel.expasy.org/) (Figure 3). The model indicated that the SmL1 monomer consisted of two β-sheets, a curved seven-stranded β-sheet forming the front face, and a flattened six-stranded β-sheet forming the back face of the monomer, which interconnected by turns and loops. Additionally, a four-stranded β-sheet, referred as the S-sheet, was occurred between the two β-sheets at the top of the monomer. The model also showed SmL1 can bind calcium and manganese ions, which could keep the amino acid residues of the sugar-binding site at the required positions (Roopashree et al., 2006). Like other legume lectins (Loris et al., 1998; Sharon and Lis, 1990; Varrot et al., 2011), β-sheets in SmL1 are dominated structure, whereas α-helices are virtually absent. Therefore, it can be concluded that SmL1 adopts the same β-sandwich structure as the classical legume lectins (Wang et al., 2003). Most known legume lectins are homodimers or homotetramers (Li et al., 2012). One monomer cannot form the complex structure with carbohydrates (Eijsden et al., 1992; Li et al., 2012), whether the SmL1 is homodimers or homotetramers still need to be further elucidated.

Expression patterns of *SmL*1 in *S. miltiorrhiza*

According to the report of Li et al. (2010) *SmL*1 have a very high expression level in the *S. miltiorrhiza* root (2010). However, our results of real-time PCR shows that

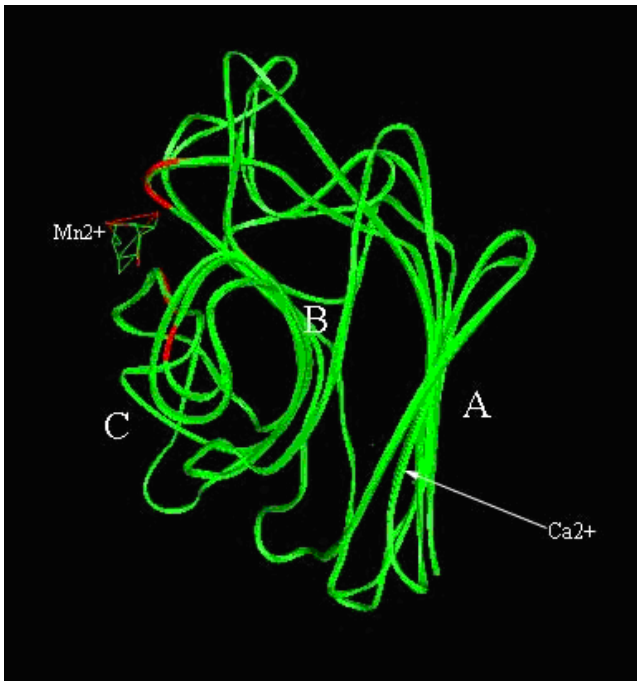

Figure 3. Tertiary structure prediction of *SmL*1 amino acid sequence. **A.** flattened six-stranded β-sheet. **B.** Seven-stranded β-sheet, **C.** The S-sheet. The red fragments on the backbone show the putative carbohydrates binding sites.

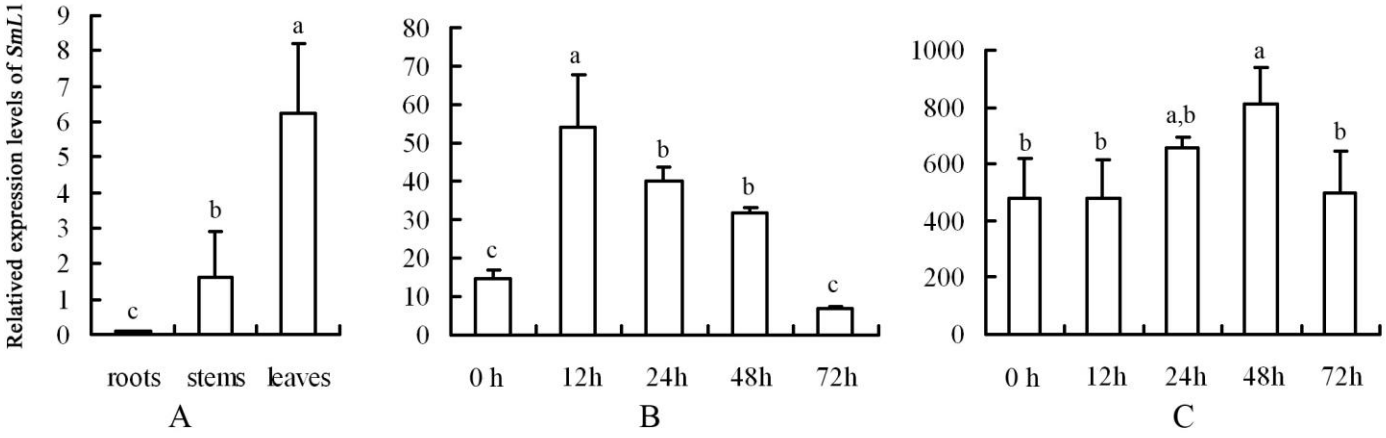

Figure 4. Result of the real-time PCR shows *SmL*1 gene expression in different tissues **(A)** or under different treatments **(B)** PSL, **(C)** MeJA). Bars with the same lowercase letter are not significantly different (P > 0.05).

the expression level of SmL1 was expressed highly in leaves, and but low in stems and roots. The expression level of SmL1 in leaves was approximately 4 times as high as that in stems, and little mRNAs was of *SmL1* were detected in roots (Figure 4A). *Gleheda gene* is also predominantly expressed in the leaves, which encoding the closer legume lectin protein of SmL1 (Wang et al., 2003). This might implied that *SmL1* has high expression levels in various organs, and plant lectins may have important roles according to their abundance. Many legume lectins were served as defense molecules against insect herbivores and pathogens. And the lectin protein-encoding genes can also be induced by insect attack or pathogen infection. To investigate whether *SmL*1 expression can be induced by pathogens, we firstly selected XC-1(a pathogen causing black rot of cabbage)

to infect two-month-old *S. miltiorrhiza* seedlings. After XC-1 infection, *SmL*1 expression was induced to the highest level at 12 h, and gradually returned to normal levels within 72 h (Figure 4B). That result indicated that *SmL*1 was involved in defense against pathogens in *S. miltiorrhiza*.

Jasmonates, as important signal molecules of plant responses to abiotic and biotic stresses, regulate induced defense mechanisms in plants after insect attack and wound response in general (Wasternack et al., 2006). Moreover, the previous studies had showed that the increase of jasmonate levels and the expression of wound-inducible genes after herbivory is a common phenomenon in many plant systems (Qu et al., 2004; Schmidt et al., 2004; Vandenborre et al., 2009). So, we also further determined the change of *SmL1* transcripts level after exposure to methyl jasmonate (MeJA) a derivative of jasmonates) in *S. miltiorrhiza*. Treatment with MeJA only slightly increased transcripts transcript abundance with expression peaking after at 48 h after treatment, and the maximum peak were only about 1.6-fold higher when compared with the control (Figure 4C). So, we concluded that *SmL1* is mainly involved in response against pathogen and may play a small limited role in defense against insect herbivores.

Agglutination and antimicrobial activity test

The result of SDS-PAGE indicated that *SmL*1 gene expressed a protein (named as rSmL1) with the molecular weight of about 26.2 kDa (Figure 5), which was identical with the size of SmL1 mature monomer predicted by bioinformatics method. The agglutination activity assays showed that the recombinant rSmL1 protein could agglutinate mouse and rabbit red blood cells compared to negative control. The minimal concentration required to agglutinate trypsin-treated mouse and rabbit erythrocytes was about 1.99 and 3.91 µg/ml, respectively. Obviously, the agglutination potential of rSmL1 was weaker than ConA (0.98 µg/ml) and Gleheda (0.22 µg/ml) (Jiang et al., 2006; Wang et al., 2003). This may be caused by applying more tyrpsin in our experiment. The bacterial growth experiments showed that the purified protein could inhibit the growth of *E. coli* (ATCC35218), PLS and XC-1 (Figure 6). When the concentration of recombinated SmL1 increased to 4 µg/ml, *E. coli* barely showed any growth (Figure 6A and B). Compared to *E. coli*, the inhibition effects to PLS (Figure 6C) and XC-1 (Figure 6D) could be observed when the medium contained 5 µg/ml purified proteins. This also further elucidated its role in the anti-pathogen responses in *S. miltiorrhiza*.

Conclusion

Plant lectins have been studied over a century. Legume lectin family is the best known lectin family from plants,

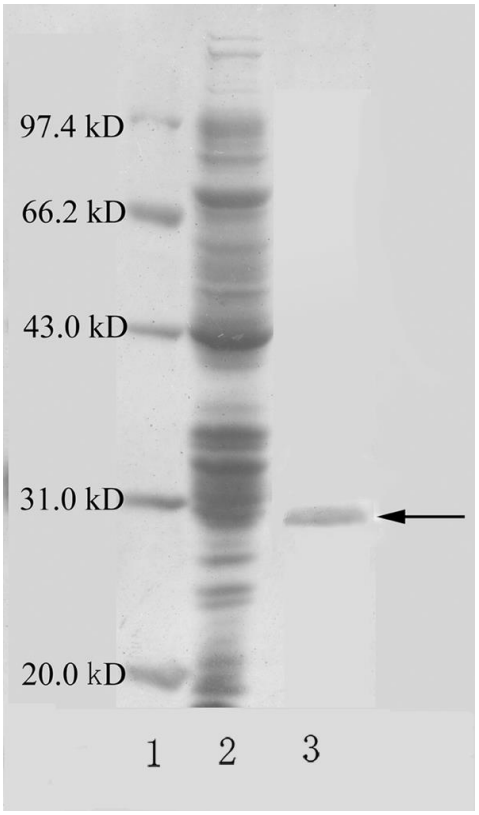

Figure 5. SDS-PAGE analysis of recombinant rSmL1 protein. Line 1, protein marker; line 2, the protein induced after 5 h; line 3: the purified protein (rSmL1).

which are usually, but not exclusively, found in the leguminous plants. In recent years, legume lectins also occur in several non-leguminous species, such as *G. hederacea* and *S. bogotensis*. But limited numbers of legume lectins from non-legume plant were reported. Now, we cloned and characterized a legume lectin gene, *SmL1*, which is expresses abundant in *S. miltiorrhiza* root. And *SmL1* can be induced by pathogen. Its purified recombinant protein from *E. coli* showed significant agglutination and antibacterial activity *in vitro*. These facts indicated that the SmL1 protein might be involved in the defense of plant against pathogen. The application of this gene in future plant genetic modification may be an efficient way to control root rot without damaging the environmental biodiversity.

Conflict of interests

The author(s) did not declare any conflict of interest.

ACKNOWLEDGMENTS

This work benefited from financial support from the postdoctoral fund of Shaanxi Province, the Natural

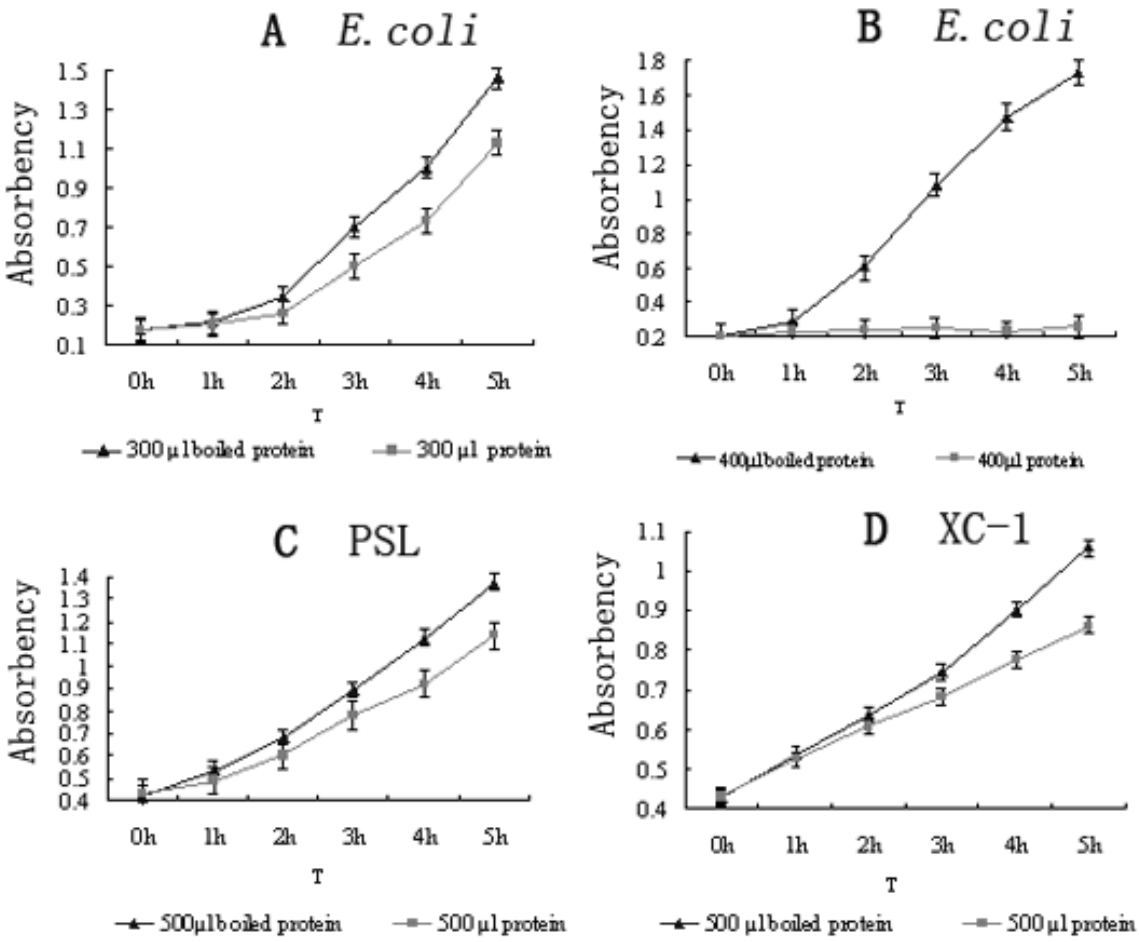

Figure 6. The purified protein inhibits the growth of bacterium. **A)** The growth states of *E. coli* with 2 μg/ml rSmL1 protein. **B)** The growth states of *E. coli* with 4 μg /ml rSmL1 protein. **C)** The growth states of PSL with 4 μg/ml rSmL1 protein, **D)** is the growth states of XC-1 with 4 μg /ml rSmL1 protein.

Science Foundation of Shaanxi Province, China (Grant No. 2014JQ3105) and the Fundamental Research Funds form Shanxi Province Office of Education (14JK1178).

REFERENCES

Beji A, Izard D, Gavini F, Leclerc H, Leseine-Delstanche M, Krembel J, (1987). A rapid chemical procedure for isolation and purification of chromosomal DNA from gram-negative bacilli. Anal. Biochem. 162: 18-23.

Bourne Y, Roussel A, Frey M, Rougé P, Fontecilla-Camps JC, Cambillau C (1990). Three-Dimensionnal structures of complexes of Lathyrus ochrus isolectin I with glucose and mannose: Fine specificity of the monosaccharide-binding site. Proteins 8:365-376.

Carlini CR, Grossi-de-Sa MF (2002). Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. Toxicon 40:1515-1539.

Charungchitrak S, Petsom A, Sangvanich P, Karnchanatat A (2011). Antifungal and antibacterial activities of lectin from the seeds of *Archidendron jiringa* Nielsen. Food Chem. 126:1025-1032.

Ciopraga J, Gozia O, Tudor R, Brezuica L, Doyle RJ (1999). *Fusarium sp*. growth inhibition by wheat germ agglutinin. Biochim. Biophys Acta 1428:424-432..

Costa RMPB, Vaz AFM, Oliva MLV, Coelho LCBB, Correia MTS, Carneiro-da-Cunha MG (2010). A new mistletoe Phthirusa pyrifolia leaf lectin with antimicrobial properties. Process Biochem. 45:526-533.

Eijsden RR, Hoedemaeker FJ, Diaz CL, Lugtenberg BJJ, Sylvia de Pater B, Kijne JW, (1992). Mutational analysis of pea lectin. Substitution of Asn125 for Asp in the monosaccharide-binding site eliminates mannose/glucose-binding activity. Plant mol. Biol. 20: 1049-1058.

Etzler ME (1985). Plant lectins: molecular and biological aspects. Annu. Rev. Plant Physiol. 36:209-234.

Fang EF, Lin P, Wong JH, Tsao SW, Ng TB (2010). A lectin with anti-HIV-1 reverse transcriptase, antitumor, and nitric oxide inducing activities from seeds of *Phaseolus vulgaris cv*. extralong autumn purple bean. J. Agric. Food Chem. 58:2221-2229.

Hua WP, Zhang Y, Song J, Zhao LJ, Wang ZZ (2011). *De novo* transcriptome sequencing in *Salvia miltiorrhiza* to identify genes involved in the biosynthesis of active ingredients. Genomics 98:272-279.

Jiang JF, Han Y, Xing LJ, Xu YY, Xu ZH, Chong K (2006). Cloning and expression of a novel cDNA encoding a mannose-specific jacalin-related lectin from *Oryza sativa*. Toxicon 47:133-139.

Kang DG, Oh H, Chung HT, Lee HS (2003). Inhibition of angiotensin converting enzyme by lithospermic acid B isolated from Radix *Salviae miltiorrhiza* Bunge. Phytother. Res. 17:917-920.

Li T, Yin X, Liu D, Ma X, Lv H, Sun S (2012). Isolation and characterization of a novel lectin with antifungal and antiproliferative

activities from *Sophora alopecuroides* seeds. Acta Biochim. Biophys. Sin. 44(7):606-613.
Li Y, Sun C, Luo HM, Li XW, Niu YY, Chen SL (2010). Transcriptome characterization for *Salvia miltiorrhiza* using 454 GS FLX. Yao Xue Xue Bao (in Chinese) 45:524-529.
Liu Z, Liu B, Zhang ZT, Zhou TT, Bian HJ, Min MW, Liu Y-H, Chen J, Bao JK (2008). A mannose-binding lectin from *Sophora flavescens* induces apoptosis in HeLa cells. Phytomedicine 15:867-875.
Loris R, Hamelryck T, Bouckaert J, Wyns L (1998). Legume lectin structure. Biochim. Biophys. Acta 1383(1): 9-36.
Oliveira MDL, Andrade CAS, Santos-Magalhães NS, Coelho LCBB, Teixeira JA, Carneiro-da-Cunha MG, Correia MTS (2008). Purification of a lectin from Eugenia uniflora L. seeds and its potential antibacterial activity. Lett. Appl. Microbiol. 46:371-376.
Perçin I, Yavuz H, Aksöz E, Denizli A, (2012). Mannose-specific lectin isolation from *Canavalia ensiformis* seeds by PHEMA-based cryogel. Biotechnol. Prog. 28(3):756-761
Peumans WJ, Van Damme E (1995). Lectins as plant defense proteins. Plant Physiol. 109:347.
Qu N, Schittko U, Baldwin IT (2004). Consistency of *Nicotiana attenuata*'s herbivore- and jasmonate-induced transcriptional responses in the allotetraploid species *Nicotiana quadrivalvis* and *Nicotiana clevelandii*. Plant Physiol. 135:539-548.
Roopashree S, Singh SA, Gowda LR, Rao AG (2006). Dual-function protein in plant defence: seed lectin from *Dolichos biflorus* (horse gram) exhibits lipoxygenase activity. Biochem. J. 395:629-639.
Sá R, Gomes F, Napoleão T, Santos N, Melo C, Gusmão N, Coelho L, Paiva P, Bieber L (2009). Antibacterial and antifungal activities of *Myracrodruon urundeuva* heartwood. Wood Sci. Technol. 43:85-95.
Safina G, Van Lier M, Danielsson B (2008). Flow-injection assay of the pathogenic bacteria using lectin-based quartz crystal microbalance biosensor. Talanta 77:468-472.
Schmidt DD, Kessler A, Kessler D, Schmidt S, Lim M, Gase K, Baldwin IT (2004). *Solanum nigrum*: a model ecological expression system and its tools. Mol. Ecol. 13:981-995.
Sharon N, Lis H (1990). Legume lectins--a large family of homologous proteins. FASEB J. 4:3198-3208.
Swanson MD, Winter HC, Goldstein IJ, Markovitz DM (2010). A lectin isolated from bananas is a potent inhibitor of HIV replication. J. Biol. Chem. 285:8646.
Van Damme EJM, Lannoo N, Peumans WJ (2008). Plant lectins. Adv. Bot. Res. 48:107-209.
Van Damme EJM, Peumans WJ, Barre A, Rougé P (1998). Plant lectins: a composite of several distinct families of structurally and evolutionary related proteins with diverse biological roles. Critical Reviews in Plant Sciences 17, 575-692.
Vandenborre G, Miersch O, Hause B, Smagghe G, Wasternack C, Van Damme EJ (2009). *Spodoptera littoralis*-induced lectin expression in tobacco. Plant Cell Physiol. 50:1142-1155.
Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F (2002). Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. Genome Biol. 3(7): RESEARCH0034.
Varrot A, Blanchard B, Imberty A (2011). Lectin binding and its structural basis. Carbohydr. Recogn. 329-347.
Vaz AFM, Costa RMPB, Melo AMMA, Oliva MLV, Santana LA, Silva-Lucca RA, Coelho LCBB, Correia MTS (2010). Biocontrol of *Fusarium* species by a novel lectin with low ecotoxicity isolated from *Sebastiania jacobinensis*. Food Chem. 119:1507-1513.
Vega N, Pérez G (2006). Isolation and characterisation of a *Salvia bogotensis* seed lectin specific for the Tn antigen. Phytochemistry 67: 347-355.
Wang W, Peumans WJ, Rouge P, Rossi C, Proost P, Chen J, Van Damme EJ, (2003). Leaves of the Lamiaceae species Glechoma hederacea (ground ivy) contain a lectin that is structurally and evolutionary related to the legume lectins. Plant J. 33:293-304.
Wasternack C, Stenzel I, Hause B, Hause G, Kutter C, Maucher H, Neumerkel J, Feussner I, Miersch O (2006). The wound response in tomato--role of jasmonic acid. J. Plant Physiol. 163:297-306.
Yang H, Han L, Sheng T, He Q, Liang J, (2006). Effects of replenishing qi, promoting blood circulation and resolving phlegm on vascular endothelial function and blood coagulation system in senile patients with hyperlipemia. J. Tradit. Chin. Med. 26:120-124.
Zhong GX, Li P, Zeng LJ, Guan J, Li DQ, Li SP, (2009). Chemical characteristics of *Salvia miltiorrhiza* (Danshen) collected from different locations in China. J. Agric. Food Chem. 57:6879-6887.