Plant Science 211 (2013) 17–22

Contents lists available at SciVerse ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Expression of soybean lectin in transgenic tobacco results in enhanced resistance to pathogens and pests

Peipei Guo, Yu Wang, Xiaohui Zhou, Yongli Xie, Huijun Wu, Xuewen Gao*

Department of Plant Pathology, College of Plant Protection, Nanjing Agricultural University, Key Laboratory of Integrated Management of Crop Diseases and Pests, Ministry of Education, Nanjing 210095, PR China

ARTICLE INFO

Article history:
Received 29 January 2013
Received in revised form 10 May 2013
Accepted 4 June 2013
Available online xxx

Keywords:
Soybean lectin
Resistance
Phytophthora nicotianae
Spodoptera exigua
Transgenic tobacco plant

ABSTRACT

Lectins are proteins of non-immune origin that specifically interact with carbohydrates, known to play important roles in the defense system of plants. In this study, in order to study the function of a new soybean lectin (SBL), the corresponding encoding gene *lec-s* was introduced into tobacco plants via *Agrobacterium*-mediated transformation. Southern blot analyses had revealed that the *lec-s* gene was stable integrated into the chromosome of the tobacco. The results of the reverse transcription polymerase chain reaction (RT-PCR) also indicated that the *lec-s* gene in the transgenic tobacco plants could be expressed under the control of the constitutive CaMV35S promoter. Evaluation agronomic of the performance had showed that the transgenic plants could resist to the infection of *Phytophthora nicotianae*. Insect bioassays using detached leaves from transgenic tobacco plants demonstrated that the ectopically expressed SBL significantly (P.0.05) reduced the weight gain of larvae of the beet armyworm (*Spodoptera exigua*). Further on, the lectins retarded the development of the larvae and their metamorphosis. These findings suggest that soybean lectins have potential as a protective agent against pathogens and insect pests through a transgenic approach.

1. Introduction

Carbohydrate-binding proteins, also called lectins or agglutinins, are a large, heterogenous group of proteins that reversibly bind specific mono- or oligosaccha-rides and possess at least one non-catalytic domain [1]. Lectins have been isolated from a variety of plants, animals, bacteria, viruses and fungi [2–5]. Lectins can be classified into seven families of structurally and carbohydrate-binding proteins, including (1) the amaranthins, (2) the cucurbitaceae phloem lectins, (3) the chitin binding lectins composed of hevein domains, (4) the GNA-related lectins, (5) the jacalin-related lectins, (6) the legume lectins and (7) the lectins with ricin-B domains [6]. Recently, researches were reported for the occurrence in plants of homologs of the *Agaricus bisporus* agglutinin [7] and homologs of class V chitinases [8]. The wide distribution of lectins in various tissues of plants and their broad presence in the plant kingdom suggest their important roles. One possible physiological function that has emerged is the defensive role of these carbohydrate-binding proteins against phytopathogenic microorganisms, phytophagous insects and plant-eating animals [9,10].

Legume lectins are the best-studied group of lectins and hundreds of these proteins have been isolated and extensively characterized. Historically, legume lectin refers to proteins that were originally discovered in seeds of legumes like jack bean, common bean, pea, peanut and soybean. Until now, legume lectins were exclusively found in Fabaceae [11]. The family of legume lectins is fairly homogeneous about the molecular structure of the native lectins. Overall, the legume lectins whose structures are known exhibit a similar protein-folding pattern, thus exhibiting three-dimensional structural conservation. They consist of two or four subunits with relative molecular mass of 30 kDa and each subunit has one carbohydrate-binding site. The interaction with sugars requires tightly bound calcium and manganese ions. The structural similarities of these lectins are reported by the primary structural analyses and X-ray crystallographic studies [12,13]. During the last decades, numerous reports have been published on the insecticidal activity of plant lectins against many pest insects belonging to Lepidoptera, Coleoptera, Diptera and Homoptera [14]. Recent discoveries showed that the legume lectin has a striking toxicity to many insects. Many legume lectins have been documented and their protein properties and anti-pest functions are well characterized [15–18]. Besides, some legume also manifested inhibitory activity on fungal growth. Lectins from *Phaseolus vulgaris* exerted a suppressive effect on growth of the fungal species *Fusarium oxysporum*, *Coprinus comatus*, *Rhizoctonia solani*, and *Valsa mali* [19,20].

Soybean lectin (SBL), as the largest number of the Legume lectins family, is a homotetrameric lectin glycoprotein, and can bind to

* Corresponding author. Tel.: +86 25 84395268; fax: +86 25 84395268.
E-mail address: gaoxw@njau.edu.cn (X. Gao).

0168-9452/$ – see front matter © 2013 Elsevier Ireland Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.plantsci.2013.06.001

N-acetyl-D-galactosamine (GalNAc) [21]. It had been reported that SBL could inhibit the sporulation and growth of fungi such as *Trichoderma viride*, *Penicilium notatum*, and *Aspergillus niger* [22]. Shukle and Murdock found that soybean lectin inhibited larval growth of *Manduca sexta* (Lepidoptera: Sphingidae) when added in the diet, which demonstrated that soybean lectin could function as plant defense against insect attack [23]. Singh et al. reported that treating second instar larvae with increasing concentrations of soybean lectin significantly reduced the development period, number of pupae and number of emerging melon fly (*Bactrocera cucurbitae*) [24]. So, soybean lectins are believed to play a role in recognizing the corresponding or similar glycans of plant pathogens or insects, which has provided hope for plant disease or insect control.

Cercospora sojina is a fungal pathogen causing frogeye leaf spot, one of the most important diseases of soybean worldwide. In our previous work, we performed an mRNA differential display technique to screen for soybean genes that were specifically expressed during the incompatible interaction with *Cercospora sojina* compared to the compatible interaction. After analysis, we found out 74 differentially expressed fragments, 46 of them were finally identified according to further re-amplification and Reverse Northern blot analysis. Functional categorization of these genes showed eleven categories including energy production, cell rescue, defense, signal transduction and so on. Then, since fragment DN40M shows highly homologous with soybean lectin, the entire sequence of the corresponding gene was gotten using RACE (rapid amplification of cDNA ends) technique, and was renamed *lec-s* (Accession number: DQ235094) (Unpublished work).

In this study, in order to know whether the *lec-s* gene has functions in plant defence system, the tobacco expression system was used. The resistance to the pathogens and pest insects of the transgenic tobacco was evaluated by using the *Phytophthora nicotianae* and beet armyworm (*Spodoptera exigua*) (Lepidoptera:Noctuidae) as the indicators, respectively. *P. nicotianae* is a soil-borne, hemibiotrophic plant pathogen with a broad host range of over 70 tropical and temperate crops, predominately solanaceous plants [25]. It causes the black shank disease of tobacco, which includes root rot, leaf wilting, stem blackening and eventual death. *S. exigua* originates from south eastern Asia. It is an important pest in numerous crops worldwide and also attacks glasshouse ornamentals and vegetables in northern Europe [26].

2. Materials and methods

2.1. Plant materials

Wild-type tobacco (*Nicotiana tabacum* cv. Samsun NN) and soybean (*Glycine max*) cultivar 'Hefeng 29' plants were grown in vitro on half-strength Murashige and Skoog medium [27]. Young tobacco leaves were used for transformation experiments. Plants were maintained in a growth chamber under 16 h photoperiod at 25 °C.

2.2. Vector construction and Agrobacterium-mediated transformation

A cDNA designated as *lec-s* was isolated from soybean seedlings by RT-PCR. Total RNA was isolated using Trizol reagent (Invitrogen, USA) and Reverse transcriptase-polymerase chain reaction (RT-PCR) was conducted based on the protocol of PrimeScript™ Reverse Transcriptase (TaKaRa, Japan). The 849-bp full-length DNA sequence of *lec-s* was amplified using gene-specific primers *lec-s*-FOR/REV. The PCR product was cloned into pMD18-T Simple vector (TaKaRa, Japan) and subcloned into the binary vector pBI121 at *Sma* I and *Xba* I sites. The recombinant vectors, pBI121-*lec-s* and empty vector, pBI121 were transferred into the *Agrobacterium tumefaciens* strain EHA105 for transformation.

Tobacco leaf discs were infected with *A. tumefaciens* EHA105 harboring the recombinant plasmid as described by Horsch et al. [28]. Transgenic plants were selected on MS medium containing 200 μg mL^{-1} kanamycin and 500 μg mL^{-1} carbenicillin. Kanamycin-resistant and PCR positive, transgenic T0 plants were selected, and transferred to greenhouse and maintained up by self-pollination to T1 generation, which were used for further analysis.

2.3. Molecular analysis of transgenic lines

PCR was employed to screen the transformants carrying *lec-s* gene and the partial pBI121 vector sequence that contains partial 35S promoter, *lec-s* coding region, and partial *gus* coding region. Genomic DNA was extracted from leaves of transgenic plants and wild type plants using the cetyltrimethylammonium bromide (CTAB) method [29]. PCR analysis of *lec-s* was amplified using primers *lec-s*-FOR/REV and PCR amplification of partial pBI121 vector sequence was performed by using the primers pBI-vector -FOR/REV (Table 1).

For RT-PCR, total RNA from transgenic and wild type plants was isolated and cDNA synthesized as mentioned earlier. Eukaryotic elongation factor 1a (*EF-1a*) plays an important role in translation and its sequence is highly conservative, we used it as an internal reference to detect the quality of RNA. PCR amplification of *lec-s* was performed using primers *lec-s*-FOR/REV and amplification of *EF-1a* was amplified by primers *EF-1a*-FOR/REV (Table 1).

For southern blotting, genomic DNA (10 μg) was digested with *EcoR* I, separated on a 1% (w/v) agarose gel, and transferred to Hybond N$^+$ Membrane (Millipore, USA). Labeling of probes, hybridization, and signal detection were performed using DIG High Prime DNA Labeling and Detection Starter Kit I, according to the manufacturer's protocol (Roche, Germany). The probe primers used are described in Table 1.

2.4. Pathogen response assays of transgenic plants

The oomycete pathogen *Phytophthora nicotianae*, was grown in the dark at 26 °C on V8 juice agar medium. The inoculation procedure followed was that described by Edward and Johnp [30].

Table 1
Oligonucleotides used in PCR and RT-PCR.

Gene	Primer sequence	Segment length (bp)
lec-s (DQ235094[a])	FOR:3′-TCTAGAATGGCCACCTCCAACTTCTC-5′ REV:3′-CCCGGGTTAGATGGCCTCATTGAGCAC-5′	849
pBI-vector	FOR:3′-CACTATCCTTCGCAAGACCC-5′ REV:3′-GTGACATCGGCTTCAAATGG-5′	1303 in *lec-s*-trangenic lines 454 in vector-transgenic lines
EF-1a (AJ223969[a])	FOR:3′-AGACCACCAAGTACTACTGCAC-5′ REV:3′-CCACCAATCTTGTACACATCC-5′	495
Probe	FOR:3′-GCACCCAACAAATCAAAC-5′ REV:3′-GAGAAGCAACCAAGAGGC-5′	307

[a] GenBank accession numbers.