Biochimie 95 (2013) 359–365

Contents lists available at SciVerse ScienceDirect

Biochimie

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

Research paper

Jacalin domain in wheat jasmonate-regulated protein Ta-JA1 confers agglutinating activity and pathogen resistance

Qing-Hu Ma*, Wei-Bo Zhen, Yun-Chao Liu

Key Laboratory of Plant Resources, Institute of Botany, Chinese Academy of Sciences, 20 Nanxincun, Xiangshan, Beijing 100093, China

ARTICLE INFO

Article history:
Received 10 August 2012
Accepted 8 October 2012
Available online 29 October 2012

Keywords:
Chimeric protein
Dirigent domain
Jacalin-related lectin domain (JRL)
Monocot jacalin-related lectins
Triticum aestivum L

ABSTRACT

Ta-JA1 is a jacalin-like lectin from wheat (*Triticum aestivum*) plants. To date, its homologs are only observed in the Gramineae family. Our previous experiments have demonstrated that Ta-JA1 contains a modular structure consisting of an N-terminal dirigent domain and a C-terminal jacalin-related lectin domain (JRL) and this protein exhibits a mannose-specific lectin activity. The over-expression of *Ta-JA1* gene provides transgenic plants a broad-spectrum resistance to diseases. Here, we report the differential activities of the dirigent and JRL domains of Ta-JA1. *In vitro* assay showed that the recombinant JRL domain could effectively agglutinate rabbit erythrocytes and pathogen bacteria *Pseudomonas syringe* pv tabaci. These hemagglutination activities could be inhibited by mannose but not by galactose. In contrast, the recombinant dirigent domain did not show agglutination activity. Corresponding to these differentiations of activities, similar to full-length of *Ta-JA1*, the over-expression of JRL domain in transgenic plants also increased resistance to the infection of *P. syringe*. Unlike JRL, the over-expression of dirigent domain in transgenic plants led to alteration of the seedling sensitivity to salts. In addition, a d_N/d_S ratio analysis of Ta-JA1 and its related proteins showed that this protein family functionally limited to a few crop plants, such as maize, rice and wheat.

1. Introduction

Plants have evolved preventive mechanisms to cope with various environmental stresses, such as low temperature, high salt, drought, and pathogen infections [1]. Certain mechanisms involve small signal molecules such as plant hormone, jasmonates (JAs). JAs are well known to play important roles in plant stress and defense response [2]. To date, JAs have been demonstrated to biochemically derive from a lipoxygenase-dependent oxidation of poly-unsaturated fatty acids. Under stress conditions, bioactive forms of isoleucine-conjugated jasmonoyl-L-isoleucine (JA-Ile) accumulate. These molecules have been demonstrated to bind to the F-box protein CORONATINE INSENSITIVE1 (COI1) in the E3 ubiquitin-ligase Skip-Cullin-F-box complex SCFCOI1 [3]. The binding of JA-Ile to COI1 leads to proteolytic degradation of jasmonate ZIM-domain transcriptional repressor proteins (JAZ) by proteasome [4]. This biochemical process further leads to a cascade regulation activities, e.g. activation of transcriptional factor MYC2 and possibly other transcriptional regulators, the function of which activate the expression of numerous JA-responsive genes [5].

The activation of JA-responsive genes will lead to *de novo* or altering levels of various protein syntheses, these proteins are termed jasmonate-regulated proteins (JRPs). JRPs are associated with numerous biological activities and biochemical processes. These include plant secondary metabolism, formation of cell wall structure, stress adaptation and resistance to pathogens [6]. Besides those well-characterized JRPs such as lipoxygenase, extension and chitinase, a group of proteins with molecular weight 32 kDa namely JRP-32 have received extensive attention recently, as their association with stress and unique existence in monocot plants. To date, JRP-32 proteins identified include beta-glucosidase-aggregating factor (BGAF) from maize and sorghum [7,8], OsJVC1 from rice [9], Crs-1 from creeping bentgrass [10], TaVer2 [11], TaWCI-1 [12], TaHfr-1 [13] and Ta-JA1 [14] from wheat. These proteins are related to jacalin-related lectins and are named as monocot jacalin-related lectins (monocot JRL) [15].

Lectins are a heterogeneous group of carbohydrate-binding proteins that recognize and bind to specific carbohydrate molecules. Based on their molecular structures, biochemical properties and sugar-binding specificities, plant lectins are classified into 12 families [16], one of which is named jacalin-related lectins (JRLs) characterized by one or more JRL domains which contain similar amino acid sequences to the jacalin protein from jackfruit [17]. JRLs

* Corresponding author. Tel.: +86 10 62836236; fax: +86 10 62590839.
E-mail address: mqh@ibcas.ac.cn (Q.-H. Ma).

0300-9084/$ – see front matter © 2012 Elsevier Masson SAS. All rights reserved.
http://dx.doi.org/10.1016/j.biochi.2012.10.014

have been found in both angiosperm and gymnosperms [18]. By contrast, monocot JRLs are only found in the Poaceae family (in wheat, rice, maize and others) and have been demonstrated to be modular proteins that are structurally characterized by an N-terminal dirigent domain (also called disease response domain) followed by a JRL domain, which is distinct from classical JRLs that just have JRL domain [15]. Till now, the accurate functions of the dirigent and JRL domains from wheat have not been characterized. In this report, we biochemically and transgenically characterize the two domains of Ta-JA1 and show their differential functions involved in plant adaptation.

2. Materials and methods

2.1. Heterologous expression and purification of dirigent and JRL domains

To generate the dirigent domain (residues 1–151), *Ta-JA1* cDNA was amplified using a pair of primers, 5′-CGGAATTCATGGCCAATT TCCAGATAAC-3′ (N1) and 5′-CCCAAGCTTTTAGGGACAAAATCCATGG ACAG-3′. The amplified product contained a restriction *Eco*RI site prior to the ATG start codon and a *Hind*III restriction site and stop codon TAA immediately after the residue 151 of Ta-JA1. The amplified PCR product was then cloned into pET-32a expression vector (Novagen, USA) to obtain a recombinant plasmid designated as pET-Dir in this study. To generate the jacalin-related lectin (JRL) domain (residues 152–304), *Ta-JA1* cDNA was amplified using a specific pair of primers consisting of 5′-CCCAAGCTTTTAGAGAGGGAGCACGTA-GAC-3′(C1) and 5′-CGGAATTCATGCTGAAAGGTTCACAGAG-3′. The late one contained an *Eco*RI restriction site immediately prior to the residue 151. Residue 151 is methionine so no additional start codon was added. Then, this cDNA fragment was cloned into pET-32a vector to obtain a recombinant plasmid designated as pET-JRL in this study. *Ta-JA1* was amplified using the pair of primers N1 and C1 and then cloned into pET-32a vector to obtain a recombinant plasmid designated as pET-JA1. After the proof of sequences, three recombinant plasmids were then introduced into competent *E*scherichia *coli* strain BL21 (DE3) cells, respectively. The bacteria culture, protein expression induction using IPTG and protein purification on Ni-NTA His-Bind® Spin Columns (QIAGEN, USA) were conducted as reported previously [15].

2.2. Agglutination and carbohydrate-binding assays

The agglutinating activity of the purified Ta-JA1, dirigent domain and JRL domain was tested using rabbit erythrocytes as previously described [15]. Briefly, freshly washed rabbit red blood cells were tested for the hemagglutination by various proteins. The mixture was kept for 1 h at room temperature and then examined visually for agglutination. The carbohydrate-binding specificity was determined by the inhibition of agglutination of rabbit red blood cells with serially diluted saccharides. The lowest concentration of saccharides that visibly decreased agglutination was defined as the minimum inhibitory concentration (MIC). In addition, the agglutination of bacteria was conducted according to Guan et al. [19]. The carbohydrate-binding specificity was analyzed as the same as rabbit red blood cells.

2.3. Construction of plant expression vectors and generation of transgenic tobacco

The full-length cDNA of *Ta-JA1* and its dirigent and JRL domains were subcloned into the binary plasmid pKYLX71 [20]. To conduct this, artificial *Hind*III and *Xho*I restriction sites were introduced to products at 5′-terminus and 3′-terminus by PCR, respectively. The three primer pairs were 5′ 5′-CCCAAGCTTCTCATAAGCCTACAA-CATCC-3′(N2) and 5′-AACCCTCGAGCTAGGGACAAAATCCAT-3′, 5 5′-CGGAAGCTTGGATTTTGTCCCATGC-3′ and 5′-CCGCTCGAGGCTTGA-TAGGATCTTCTAG-3′ (C2), and N2 and C2, which were designed to amplify the dirigent domain, the JRL domain and *Ta-JA1*, respectively. The resulting PCR products were then inserted at the immediate downstream of the cauliflower mosaic virus (CaMV35S) promoter of the binary vector pKYLX71 to create three recombinant plasmids, designated as pKY-Dir, pKY-JRL and pKY-JA1, correspondingly. The three recombinant binary vectors were introduced into competent *Agrobacterium tumefaciens* strain LBA4404 cells using a routine freeze-thaw transformation method. The resulting positive LBA4404 strains were used to transform tobacco (*Nicotiana tabacum* cv Wisconsin 38) using our established protocols previously described [21]. In addition, transgenic plants were then grown in soil in the greenhouse and allowed self-pollination to obtain T1 progeny.

2.4. Identification of transgenic plants using regular PCR and RT-PCR analyses

Total genomic DNA was isolated from tobacco tissues as described by Edwards et al. [22]. Total RNA was isolated from tobacco tissues using the TRI reagent (Molecular Research Center, Inc, Cincinnati, USA) and following the manufacturer's instructions.

PCR reactions were carried out with 1 μmol/L primers, 0.4 mmol/L of each dNTP and 2.5 U Taq DNA polymerase (Gibco). To identify the transgenes in tobacco, following primers were synthesized according to wheat *Ta-JA1* cDNA sequence: D3: 5′-CTCATAAGCCTACAACATCC-3′, D4: 5′-CTAGGGACAAAATCCAT-3′, J3: 5′-GGATTTTGTCCCATGC-3′ and J4: 5′-GCTTGATAGGATCTTCTAG-3′. The three pairs of D3 plus D4, J3 plus J4, and D3 plus J4 were used to amplify dirigent domain transgene, JRL domain transgene, and *Ta-JA1* transgene, respectively.

For RT-PCR analysis, the first-strand cDNA was synthesized from total RNA using SuperScript™ III Reverse Transcriptase Kit (Invitrogen Corporation, USA). Reverse transcription reactions were carried out at 50 °C for 60 min and terminated by heating at 95 °C for 10 min. One microliter of the reaction mixture was used as template in PCR reaction. Primer pairs for the amplification of cDNAs of *Ta-JA1*, the dirigent and JRL domains were the same as ones for regular PCR described above. A pair of primers consisting of 5′-CTATTCTCCGCTTTGGACTTGGCA-3′ (forward) and 5′-ACCTGC TGGAAGGTGCTGAGGGAA-3′ (reverse) were designed to amplify tobacco actin cDNA as a control to evaluate of quality of RT and loading quantity of temperate cDNA as well as to normalize the expression levels of targeted transgenes.

2.5. Salt tolerance assay

Seeds of T1 transgenic plants were surface-sterilized with 1% NaClO for 8 min, followed by 5 times of washing with autoclaved water. Fifty seeds were then inoculated on to agar-solidified MS medium supplemented with 75 or 125 mM of NaCl. The plates were placed in a culture condition consisting of a photoperiod of 16/8 h (day/night) and a temperature of 25 °C. After 18 days of growth, the root length of seedlings was measured.

2.6. Pathogen resistance assay

The fourth to sixth leaves from top of 2-month-old tobacco plants grown in greenhouse were used to inoculate *Pseudomonas syringae* pv tabaci, which cause tobacco wildfire disease. The growth of *P. syringae* and inoculation on tobacco leaves were according to Guo et al. [23]. The *P. syringae* population inside the

leaf discs was determined based on the numbers of colonies formed on King's B plates after 7 days of the inoculation.

The experiment was repeated three times. The Student's *t*-test for independent samples [24] was applied to determine the difference between the transgenic and the control lines that transferred with pKYLX71 vector alone.

2.7. d_N and d_S analysis

Codeml program at PAL2NAL website (http://www.bork.embl.de/pal2nal/) was used to calculate the non-synonymous substitution rate leading to amino acid replacements (d_N) and synonymous substitution rate leading to silent changes (d_S) [25].

3. Results

3.1. Properties of Ta-JA1, and the dirigent and JRL domains

To study the roles of different domains in Ta-JA1, we introduced each individual domain into plasmid to express recombinant protein in *E. coli*. After induction of expression, oligomeric amino acid products of both dirigent and JRL domains were obtained from engineered *E. coli* (Fig. 1). Compared to the expression of full-length of *Ta-JA1*, SDS-PAGE gel images showed that the protein production of the dirigent and JRL domains were high (Fig. 1). The dirigent and JRL domains plus Ta-JA1 protein from the soluble fraction of the induced *E. coli* extracts was purified to homogeneity (Fig. 1).

The purified dirigent and JRL domains versus Ta-JA1 protein were tested for the lectin activity and carbohydrate specificity. Both JRL domain and Ta-JA1 agglutinated rabbit red blood cells in the tested concentration ranges from 375 to 23.4 μg/mL. The agglutination was also observed when the concentration of these proteins was reduced to 11.7 μg/mL. In contrast, the dirigent domain did not agglutinate red blood cells in the tested concentrations up to 375 μg/mL.

Carbohydrate specificity was determined by inhibition of hemagglutination using three saccharides. Both the full-length Ta-JA1 protein and the JRL domain bound to D-mannose with high preference. In contrast, three types of sugars did not inhibit the hemagglutination activity of the dirigent domain (Table 1).

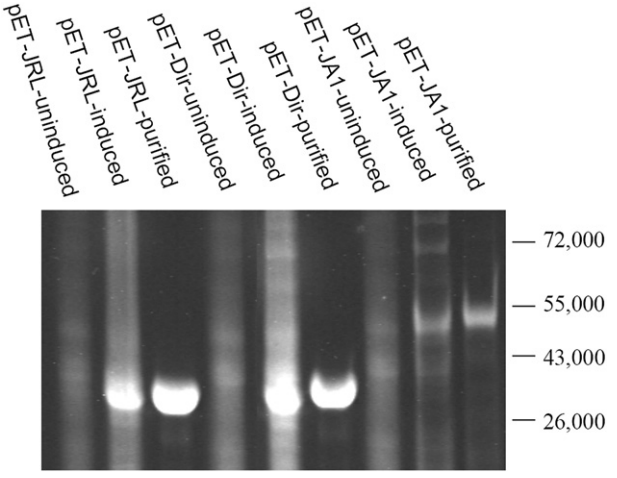

Fig. 1. Gel electrophoresis images showing recombinant *Ta-JA1* and its two domains expressed in *E. coli*. Proteins were separated from the total protein fractions of uninduced and IPTG-induced *E. coli* harboring pET-Dir, pET-JRL and pET-JA1 expression plasmids, and after His-Tag resin purification of the soluble fraction from induced cells. pET-Dir: plasmid with dirigent domain of *Ta-JA1*; pET-JRL: plasmid with JRL domain of *Ta-JA1*; pET-JA1: plasmid with full-length of *Ta-JA1*. Molecular markers are indicated on the right of the figure.

3.2. Agglutination activity to P. syringae cells

To understand whether Ta-JA1 and its two domains agglutinate bacteria cells, *P. syringae* grown to the exponential phase was tested with different diluted proteins. The results showed that in the tested proteins starting with concentration of 11.7 μg/mL, both Ta-JA1 and JRL agglutinated *P. syringe*. In contrast, in the same tested concentration range, the dirigent domain did not show this agglutination activity (Table 2).

Sugar inhibition was performed to characterize the agglutination of the three types of protein to *P. syringae*. The tested sugars included mannose, galactose and glucose. The resulting data showed that mannose strongly inhibited the agglutination of Ta-JA1 and JRL domain to *P. syringe* cells (Table 2).

3.3. Over-expression of Ta-JA1, dirigent or JRL domains in tobacco plants exhibit the normal morphology and development

Approximately 30 putative transgenic tobacco plants were obtained via genetic transformation of *A. tumefaciens* containing pKY-Dir, pKY-JRL or pKY-JA1 expression vectors. PCR analysis amplified DNA fragment of dirigent, JRL and *Ta-JA1* from transgenic plants, respectively, but not from control plants (Supplementary Fig. 1). PCR-positive plants were then chosen for further analysis of RT-PCR.

The putative transgenic plants were further demonstrated by RT-PCR. Three recombinant genes were expressed in PCR-positive plants but not in control plants (Fig. 2). From dirigent domain transgenic plants, four lines Dir-1, Dir-3, Dir-4 and Dir-6 were identified to express the recombinant gene (Fig. 2A). Four transgenic lines, labeled with JRL-1, JRL-5, JRL-6, and JRL-7 were obtained with a high expression level of the JRL recombinant gene (Fig. 2B). Four transgenic lines labeled with JA-1, JA-2, JA-5, and JA-6, were identified with a high expression level of the recombinant *Ta-JA1* (Fig. 2C).

The T1 progeny of transgenic plants of the dirigent domain, JRL domain and *Ta-JA1* transgenes were selected from seeds of self-fertilization of transgenic plants. The seeds were geminated on medium containing kanamycin. The segregation ratio of resistance versus sensitivity to kanamycin was approximately 3:1 in seeds of Dir-3, Dir-4, Dir-6, JRL-1, JRL-5, JRL-6, JA-2, JA-5, and JA-6 progenies, indicating that these transgenic plants contained one copy of T-DNA that was integrated into the genome of these plants (data not shown).

These transgenic lines and pKYLX71 vector control transgenic plants were grown in greenhouse to observe their development. The plant morphology (height and leaf number) was essentially identical between the transgenic tobaccos and controls, with the exception days to first flowering, as the transgenic lines Dir-3, Dir-4 and Dir-6A flowered marginally earlier than the control (Table 3).

Table 1
The carbohydrate-binding specificity of the dirigent domain, JRL domain and Ta-JA1 protein. The carbohydrate-binding specificity was determined using saccharides that inhibit agglutination of rabbit erythrocytes. The purified recombinant proteins and simple sugar solutions were pre-incubated for 1 h, then rabbit erythrocytes was added into the reaction mixture. The agglutination was evaluated after 1 h of reaction at room temperature. The lowest tested concentration of saccharides that visibly inhibit agglutination was defined as MIC.

Sugars	MIC (mM)		
	Ta-JA1	Dirigent	JRL
Mannose	6.25	>50	6.25
Galactose	>50	>50	>50
Glucose	>50	>50	>50

Table 2
Recombinant Ta-JA1 and its two domains agglutinate on *P. syringe* pv tabaci. The bacteria grown to exponential phase ($OD_{600} = 0.4$) was tested with different diluted proteins. The lowest concentration tested was expressed as the minimum agglutinating concentration (MAC). Sugar inhibition on bacteria agglutination was performed with 187 μg/mL of proteins and different diluted saccharides. The lowest concentration of saccharides that visibly inhibit agglutination was defined as MIC.

Proteins	MAC (μg/mL)	MIC(mM)		
		Mannose	Galactose	Glucose
Ta-JA1	11.7	6.25	>50	>50
JRL	11.7	12.5	>50	>50
Dirigent	>375	>50	>50	>50

These results suggest that the over-expression of the JRL domain and Ta-JA1 transgenes do not significantly affect tobacco growth and development under normal conditions. Although the dirigent domain exhibited a minor modification on flower time, the overall morphology of the dirigent transgenes was similar to that of control tobacco.

3.4. Alteration of salt sensitivity of the dirigent domain transgenic plants

To further examine the biological function of Ta-JA1 and its two domains, the transgenic tobacco and control plants were germinated on MS plates supplemented with different concentrations of NaCl. The growth of roots was used to evaluate their sensitivity to NaCl. After 18 days of seed germination, the length of roots was recorded from each individual seedling. Compared to control seedlings, roots of Dir-3, Dir-4 and Dir-6 lines were much shorter on media supplemented with 75 mM and 125 mM NaCl (Fig. 3). In contrast, roots of both Ta-JA1 (JA-2, JA-5 and JA-6) and the JRL domain (JRL-1, JRL-5 and JRL-6) transgenic seedlings grew normally on media containing NaCl. These data indicated that the over-expression of the dirigent domain increase the root sensitivity to salt stress.

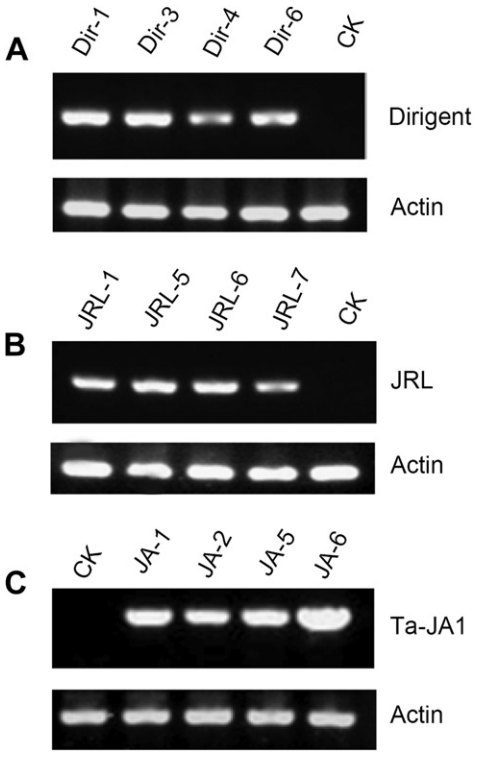

Fig. 2. RT-PCR analyses of dirigent domain, JRL domain and *Ta-JA1* gene expression in transgenic tobacco plants. RT-PCR was performed using the gene-specific primers. Actin RT-PCR was included as an internal control. (A) Dirigent domain expression in the different transgenic tobacco lines Dir-1, Dir-3, Dir-4, and Dir-6, respectively. (B) JRL domain expression in the different transgenic tobacco lines JRL-1, JRL-5, JRL-6, and JRL-7, respectively. (C) The full-length of *Ta-JA1* expression in the different transgenic tobacco lines JA-1, JA-2, JA-5, and JA-6, respectively.

Table 3
Phenotype analysis of transgenic and control tobacco.

Plants	Height (cm)	Leaf number	Days to the first flower
CK	140 ± 13.7	20.3 ± 4.43	115.2 ± 2.88
Dir-3	138 ± 15.4	22.3 ± 4.32	109.7 ± 3.56*
Dir-4	144 ± 15.5	20.8 ± 3.25	107.6 ± 3.25*
Dir-6	136 ± 10.5	18.8 ± 2.25	106.9 ± 3.75*
JRL-1	142 ± 11.5	19.8 ± 3.45	116.7 ± 2.25
JRL-5	145 ± 12.5	18.8 ± 3.75	112.7 ± 4.25
JRL-6	141 ± 13.5	20.1 ± 3.25	110.7 ± 4.50
JA-2	135 ± 9.5	20.8 ± 2.25	112.1 ± 2.95
JA-5	138 ± 13.5	18.1 ± 2.55	111.2 ± 4.95
JA-6	141 ± 12.5	22.1 ± 4.15	113.1 ± 3.65

Values presented here are expressed as mean ± SD, $n = 3$. Probability values were tested using the Student's *t*-test and significant difference at $P_{0.05}$ level between control (CK, with empty pKYLX71) and transgenic plants in the same column is marked by *.

3.5. Resistance performance of dirigent or JRL domains in transgenic tobacco

The over-expression of *Ta-JA1* has been reported to increase disease resistance of transgenic tobacco plants [15]. To understand whether the over-expression of the dirigent and JRL domains altered the resistant manners to pathogen, transgenic plants were inoculated with *P. syringae* pv tabaci. As observed in *Ta-JA1* transgenic plants, the over-expression of JRL domain significantly increased the resistance to this bacterium. After 7 days of inoculation, unlike control plants with obvious yellow lesion, JRL and *Ta-JA1* transgenic plants did not show obvious lesion symptoms (Supplementary Fig. 2). Bacterial growth was further determined 7 days after inoculation. As illustrated in Fig. 4, the growth of *P. syringae* in tobacco cells was inhibited by almost one order of magnitude in the JRL-1, JRL-5, JRL-6, JA-2, JA-5, and JA-6 transgenic lines compared to the control line. JRL and JA plants showed a similar level in inhibition of *P. syringae* growth. By contrast, Dir-3, Dir-4 and Dir-6 transgenic lines exhibited a weak inhibition on *P. syringae* growth (Fig. 4).

3.6. Patterns of Ta-JA1-related protein sequence evolution

The determination of the ratio of the non-synonymous substitution rate d_N to synonymous substitution rate d_S has been used extensively to infer the nature of selection operating on genes of interest [26]. The d_N/d_S ratios for Ta-JA1-related proteins were significantly lower than 1 (Table 4), suggesting potential functional constraints and a strong purifying selection on Ta-JA1-related proteins [27].

4. Discussion

The monocot jacalin-related lectins demonstrate to functionally involve plant defense reactions. Examples include Hfr-1 and Ta-JA1. *Hfr-1* (Hessian fly response gene-1) gene identified from wheat has been shown to inhibit Hessian fly larval feeding and kill larvae [13]. Our previous report showed that the over-expression of *Ta-JA1* increased the resistance of transgenic plants to infection by bacterial and fungal pathogens [15]. The monocot jacalin-related lectins are composed of two distinct domains, which have not

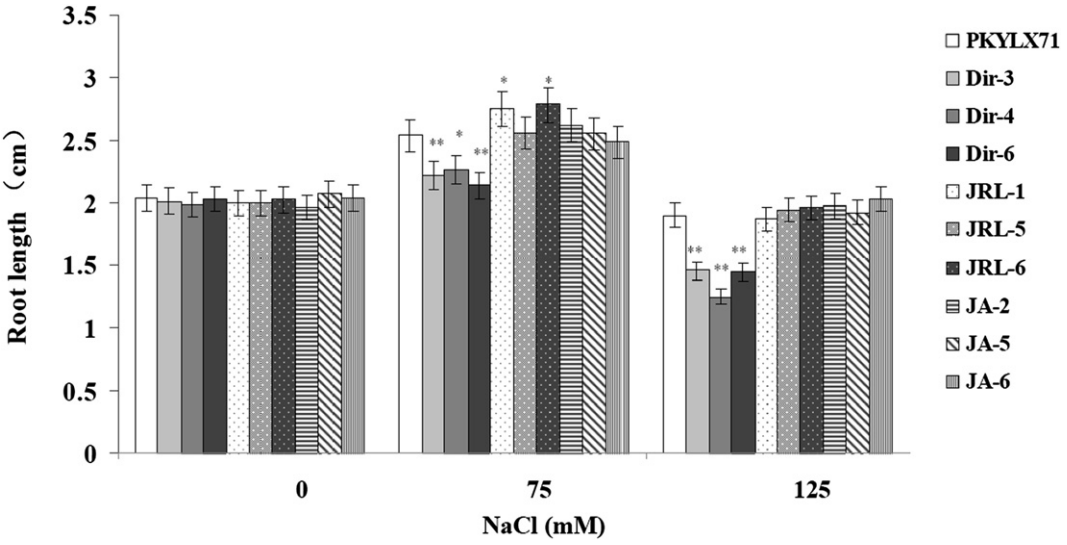

Fig. 3. Root growth of transgenic plants expressing dirigent domain, JRL domain, and *Ta-JA1* under salt stress. Tobacco seeds were germinated on the medium containing 75 or 125 mM NaCl. Root length of seedlings was measured 18 days after seeding. Dir-3, Dir-4, Dir-6: dirigent domain over-expression lines; JRL-1, JRL-5, JRL-6: JRL domain over-expression lines; JA-2, JA-5, JA-6: *Ta-JA1* over-expression lines; the control is pKYLX71: transgenic line with empty pKYLX71 vector. Each column represents an average of three replicates, and bars indicate standard errors. Probability values between the control and transgenic tobacco were estimated by the Student's *t*-test and significant differences at $P_{0.05}$ and $P_{0.01}$ levels are marked by * and **, respectively.

been functionally characterized. In the present study, we express and purify the dirigent domain and JRL domain of Ta-JA1. *In vitro* and transgenic analyses demonstrated the difference of their functions. Our experiments clearly showed that JRL but not the dirigent domain provided the agglutination and pathogen-resistant activities of Ta-JA1. This suggests that JRL domain confers a lectin agglutination activity of Ta-JA1 and this will be directly involved in its biological function in disease resistance. A similar report was obtained from a Ta-JA1 homolog in maize, called BGAF. The JRL domain of BGAF has glucosidase binding and aggregating activities [28]. A mannose-specific jacalin-related lectin (TaJRLL1) was reported in wheat. The over-expression of *TaJRLL1* in *Arabidopsis thaliana* increased resistance to fungal pathogens. Interesting, TaJRLL1 contained two JRL domains without dirigent domain [29]. This is consistent with our data that JRL domain confers the disease resistance property.

Our results showed the dirigent domain not only lacked aggregating activity but also contributed little to disease resistance of Ta-JA1. Therefore, it should be caution to call this domain as disease-related domain as annotated in some protein databases. Instead, the over-expression of dirigent domain in the transgenic plants enhanced their sensitivity to salt stress (Fig. 3), although these variations were not very remarkable. In maize BGAF, although JRL domain could bind to glucosidase directly, but JRL domain alone could not aggregate glucosidase and this aggregation needed the dirigent domain. It suggested that BGAF formed a homodimer by dirigent domain and then one BGAF dimer would bind two β-glucosidase dimers, connecting β-glucosidase dimers in a linear chain which would lead to aggregation [28]. Clearly, the functions of dirigent domain warrant further investigations.

Ta-JA1 represents a new family of protein specific to monocots. Studies on homologs may enhance the understanding of functional

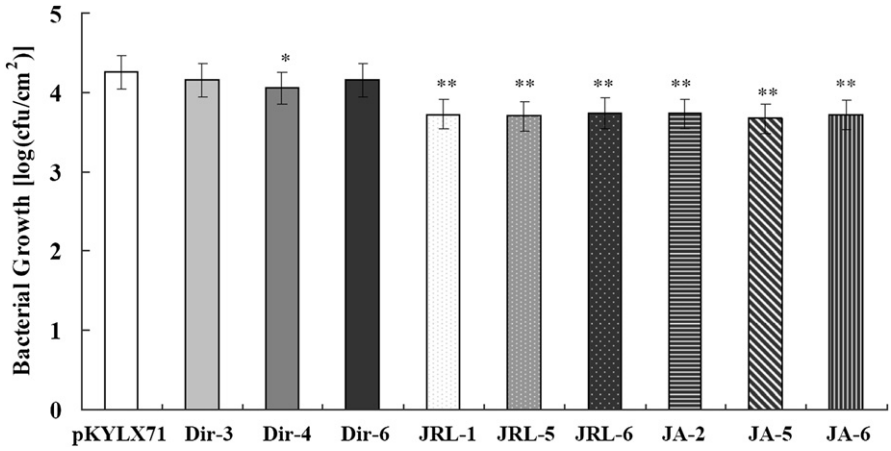

Fig. 4. Analysis of resistance to *P. syringae* tabaci in dirigent domain, JRL domain, and *Ta-JA1*-over-expressing transgenic lines. *P. syringae* (10^7 colony forming units/ml) was inoculated into the fourth to sixth upper leaves of transgenic plants of T_1 progeny. The infected leaves were collected and the bacterial populations were determined 7 days after the inoculation. Dir-3, Dir-4, Dir-6: dirigent domain over-expression lines; JRL-1, JRL-5, JRL-6: JRL domain over-expression lines; JA-2, JA-5, JA-6: *Ta-JA1* over-expression lines; the control is transgenic line with empty pKYLX71 vector. Values are means of three different experiments, and bars are standard errors. Probability values between the control and transgenic tobacco were estimated by the Student's *t*-test and significant differences at $P_{0.05}$ and $P_{0.01}$ levels are marked by * and **, respectively.

Table 4
The d_N/d_S ratios of pairwise comparisons for Ta-JA1-related proteins.

d_N/d_S	Ta-JA1	TaHfr	TaVer2	TaWCI-1	OsJVC1	SbBAGF	ZmBAGF
Ta-JA1							
TaHfr	0.360						
TaVer2	0.340	0.370					
TaWCI-1	0.411	0.338	0.371				
OsJVC2	0.338	0.280	0.338	0.366			
SbBAGF	0.259	0.257	0.325	0.265	0.273		
ZmBAGF	0.147	0.120	0.174	0.136	0.044	0.510	
AsCrs-1	0.237	0.435	0.442	0.489	0.296	0.434	0.099

evolution of this group of proteins. *Crs-1* is a homolog of Ta-JA1 reported in creeping bentgrass [10]. *Crs-1* homologs have isolated from the genomes of rice and maize, which have been considered to have diverged from a common ancestor approximately 50 million years ago. In addition, the tribe Aveneae has been proposed to diverge from rice approximately 46 million years ago, from which the tribe Triticeae diverged approximately 25 million years ago. *Crs-1* homologs have been identified in the genome of wheat but being lost in bentgrass [10]. Why this group of proteins is specific to these monocot plants remains unknown. It has been proposed that this specificity may result from the consequence of long domestication. The d_N/d_S ratio for Ta-JA1-related proteins were significantly lower than neutral selection (Table 4), suggesting strong purifying selection on Ta-JA1 homologs which may contribute the adaptation evolution of this family of protein in crop plants.

Consideration of the d_N/d_S ratio data and the different tissue expression profiles among Ta-JA1-related proteins, a sub-functionalization mechanism for functional evolution may exert on these proteins. Although the high similarity has been found in protein primary sequence and structure, Ta-JA1-related proteins exhibit broad functional diversity. Sorghum BGAF agglutinated rabbit erythrocytes as maize BGAF did but did not interact to β-glucosidases [8]. The dirigent domain in Ta-JA1 (Table 2) and OsJAC1 [30] did not affect the sugar-specificities of their JRL domains, while this deletion abolished binding to galactose and GalNAc in maize and sorghum BGAF [31]. Taking everything together, Ta-JA1-related proteins are formed a special family with restrictive functions to crop plants such as maize, rice and wheat. The JRL domain of Ta-JA1 confers a basic disease resistant property while its dirigent domain may be involved to fine regulation of Ta-JA1. Chimeric genes formed through the combination of existing coding sequences have been demonstrated to have a profound influence on adaptive evolution [32,33]. Ta-JA1 provides a good example for further investigations in this aspect.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (No. 31070235, No. 31170277), the Natural Science Foundation of Beijing (No. 5122024, No. 6082018), and Innovation Project of Chinese Academy of Sciences.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biochi.2012.10.014.

References

[1] I. Ahuja, R.C. de Vos, A.M. Bones, R.D. Hall, Plant molecular stress responses face climate change, Trends Plant Sci. 15 (2010) 664–674.
[2] C. Wasternack, Jasmonates: an update on biosynthesis, signal, transduction and action in plant stress response, growth and development, Ann. Bot. 100 (2007) 681–697.
[3] J. Yan, C. Zhang, M. Gu, Z. Bai, W. Zhang, T. Qi, Z. Cheng, W. Peng, H. Luo, F. Nan, Z. Wang, D. Xie, The *Arabidopsis* CORONATINE INSENSITIVE1 protein is a jasmonate receptor, Plant Cell 21 (2009) 2220–2236.
[4] L.B. Sheard, X. Tan, H.B. Mao, J. Withers, G. Ben-Nissan, T.R. Hinds, Y. Kobayashi, F.F. Hsu, M. Sharon, J. Browse, S.Y. He, J. Rizo, G.A. Howe, N. Zheng, Jasmonate perception by inositol-phosphate- potentiated COI1-JAZ co-receptor, Nature 468 (2010) 400–405.
[5] G.A. Howe, Ubiquitin ligase-coupled receptors extend their reach to jasmonate, Plant Physiol. 154 (2010) 471–474.
[6] L. Pauwels, D. Inze, A. Goossens, Jasmonate-inducible gene: what does it mean? Trends Plant Sci. 14 (2009) 87–91.
[7] A. Esen, D.J. Blanchard, A specific beta-glucosidase-aggregating factor is responsible for the beta-glucosidase null phenotype in maize, Plant Physiol. 122 (2000) 563–572.
[8] F.S. Kittur, H.Y. Yu, D.R. Bevan, A. Esen, Homolog of the maize β-glucosidase aggregating factor from sorghum is a jacalin-related GalNAc-specific lectin but lacks protein aggregating activity, Glycobiology 19 (2009) 277–287.
[9] J.F. Jiang, Y.Y. Xu, K. Chong, Overexpression of OsJAC1, a lectin gene, suppresses the coleoptile and stem elongation in rice, J. Integr. Plant Biol. 49 (2007) 230–237.
[10] H.M. Li, D. Rotter, S.A. Bonos, W.A. Meyer, F.C. Belanger, Identification of a gene in the process of being lost from the genus *Agrostis*, Plant Physiol. 138 (2005) 2386–2395.
[11] W.D. Yong, Y.Y. Xu, W.Z. Xu, X. Wang, N. Li, J.S. Wu, T.B. Liang, K. Chong, Z.H. Xu, K.H. Tan, Z.Q. Zhu, Vernalization-induced flowering in wheat is mediated by a lectin-like gene VER2, Planta 217 (2003) 261–270.
[12] J. Gorlach, S. Volrath, G. Knauf-Beiter, G. Hengy, U. Beckhove, K. Kogel, M. Oostendorp, T. Staub, E. Ward, H. Kessmann, J. Ryals, Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat, Plant Cell 8 (1996) 629–643.
[13] S. Subramanyam, D.F. Smith, J.C. Clemens, M.A. Webb, N. Sardesai, C.E. Williams, Functional characterization of HFR1, a high-mannose N-glycan-specific wheat lectin induced by Hessian fly larvae, Plant Physiol. 147 (2008) 1412–1426.
[14] X.M. Wang, Q.H. Ma, Characterization of a jasmonate-regulated wheat protein related to a beta-glucosidase-aggregating factor, Plant Physiol. Biochem. 43 (2005) 185–192.
[15] Q.H. Ma, B. Tian, Y.L. Li, Overexpression of a wheat jasmonate-regulated lectin increases pathogen resistance, Biochimie 92 (2010) 187–193.
[16] E.J.M. Van Damme, N. Lannoo, W.J. Peumans, Plant lectins, Adv. Bot. Res. 48 (2008) 107–209.
[17] R.A. Moreira, I.L. Ainouz, Lectins from seeds of jack fruit (*Artocarpus integrifolia* L.): isolation and purification of two isolectins from the albumin fraction, Biol. Plant 23 (1981) 186–192.
[18] F. Yagi, T. Iwaya, T. Haraguchi, I.J. Goldstein, The lectin from leaves of Japanese cycad, *Cycas revoluta* Thunb. (gymnosperm) is a member of the jacalin-related family, Eur. J. Biochem. 269 (2002) 4335–4341.
[19] Y.F. Guan, S. Ramalingam, D. Nagegowda, P.W.J. Taylor, M.L. Chye, *Brassica juncea* chitinase BjCHI1 inhibits growth of fungal phytopathogens and agglutinates Gram-negative bacteria, J. Exp. Bot. 59 (2008) 3475–3484.
[20] C.L. Schardl, A.D. Byrd, G. Benzion, M.A. Altschuler, D.F. Hildebrand, A.G. Hunt, Design and construction of a versatile system for the expression of foreign genes in plants, Gene 61 (1987) 1–11.
[21] Q.H. Ma, X.M. Wang, Z.M. Wang, Expression of isopentenyl transferase gene controlled by seed-specific lectin promoter in transgenic tobacco influences seed development, J. Plant Growth Regul. 27 (2008) 68–76.
[22] K. Edwards, C. Johnstone, C. Thompson, A simple rapid method for the preparation of plant genomic DNA for PCR analysis, Nucl. Acids Res. 19 (1991) 1349.
[23] Z.J. Guo, X.J. Chen, X.L. Wu, J.Q. Ling, P. Xu, Overexpression of the AP2/EREBP transcription factor OPBP1 enhances disease resistance and salt tolerance in tobacco, Plant Mol. Biol. 55 (2004) 607–618.
[24] S. Geng, F.G. Hills, Biometrics in Agriculture Science, Kendell Hunt Publisher, Dubuque, Iowa, 1989.
[25] N. Goldman, Z. Yang, A codon-based model of nucleotide substitution for protein-coding DNA sequences, Mol. Biol. Evol. 11 (1994) 725–736.
[26] Z. Yang, Inference of selection from multiple species alignments, Curr. Opin. Genet. Dev. 12 (2002) 688–694.
[27] W.J. Swanson, Z. Yang, M.F. Wolfner, C.F. Aquadro, Positive Darwinian selection drives the evolution of several female reproductive proteins in mammals, Proc. Natl. Acad. Sci. USA 98 (2001) 2509–2514.
[28] F.S. Kittur, M. Lalgondar, H.Y. Yu, D.R. Bevan, A. Esen, Maize β-glucosidase aggregating factor is a polyspecific jacalin-related chimeric lectin, and its lectin domain is responsible for β-glucosidase aggregation, J. Biol. Chem. 282 (2007) 7299–7311.
[29] Y. Xiang, M. Song, Z.Y. Wei, J.H. Tong, L.X. Zhang, L.T. Xiao, Z.Q. Ma, Y. Wang, A jacalin-related lectin-like gene in wheat is a component of the plant defence system, J. Exp. Bot. 62 (2011) 5471–5483.
[30] J.F. Jiang, Y. Han, L.J. Xing, Y.Y. Xu, Z.H. Xu, K. Chong, Cloning and expression of a novel cDNA encoding a mannose-specific jacalin-related lectin from *Oryza sativa*, Toxicon 47 (2006) 133–139.

[31] F.S. Kittur, H.Y. Yu, D.R. Bevan, A. Esen, Deletion of the N-terminal dirigent domain in maize β-glucosidase aggregating factor and its homolog sorghum lectin dramatically alters the sugar-specificities of their lectin domains, Plant Physiol. Biochem. 48 (2010) 731–734.
[32] N. Elrouby, T.E. Bureau, *Bs1*, a new chimeric gene formed by retrotransposon-mediated exon shuffling in maize, Plant Physiol. 153 (2010) 1413–1424.
[33] R.L. Rogers, D.L. Hart, Chimeric genes as a source of rapid evolution in *Drosophila melanogaster*, Mol. Biol. Evol. 29 (2012) 517–529.