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Full Length Article

Analysis of Expression Characteristics of Scarecrow-Like Gene *Stsl-1* Elicited by Exogenous Hormone and *Ralstonia solanacearum* Infection in Potato

Xiaojuan Wang†, Yongping Luo†, Li Shi, Pengxiang Pang and Gang Gao*
College of Life Sciences, Shanxi Normal University, Linfen 041000, China
*For correspondence: ggsxnu@126.com; wxjdqy1314@163.com; lyping1234@aliyun.com; 1194813802@qq.com
†These are co-first authors

Abstract

In higher plants, Scarecrow-like protein (SCL) is a transcription factor belonging to the GRAS family. It regulates root growth and cell cycle and also mediates resistance to environmental stresses. To investigate the function of SCL gene in response to plant pathogen infection, the full-length of potato (*Solanum tuberosum*) SCL gene named *Stsl-1* was cloned. Bioinfromatcal analysis of the gene revealed that *Stsl-1* consists of 2,139 bp; including a 1,614 bp open reading frame that encodes a protein with 537 amino acids. *Stsl-1* is highly similar to Scarecrow-like protein 21 (SCL21) in potato and other Solanaceae species. Quantitative real-time polymerase chain reaction assays indicated that *Stsl-1* was induced by *Ralstonia solanacearum* and up-regulated to varying extents by specific concentrations of exogenous phytohormones, including salicylic acid (SA), methyl jasmonate (MeJA) and abscisic acid (ABA). Examination of tissue localization suggested that *Stsl-1* was primarily expressed in specific tissues (the phloem of the stem and leaf vascular systems). Furthermore, the subcellular localization of *Stsl-1* in *Nicotiana benthamiana* leaves proved that the encoded protein was mainly localized in the cell nucleus. The results of the study should lay down the foundation for future research on the functional characterization of the SCL in potato plants.

Keywords: Gene expression; *Solanum tuberosum*; *Stsl-1*; Subcellular localization; Tissue localization; Transcription factor

Introduction

As an important plant-specific transcription factor family, the GRAS gene whose name is an acronym of the first three identified members: GAI (GIBBERELLIC ACID INSENSITIVE), RGA (REPRESSOR OF GAI) and SCR (SCARECROW) have diverse functions in plant growth and development (Cruz-Ramírez *et al*., 2012). As one member of the GRAS proteins, SCR belongs to a plant-specific transcription factor family that have key roles in root meristem and stem cell maintenance have been investigated in some detail in past decades (Salvi *et al*., 2018; Shimotohno *et al*., 2018). The biological function of SCR transcription factor is also involved in the response to external stress in plants. In the root of *Arabidopsis*, SCR was found to be hypersensitive to abscisic acid (ABA) and regulate a set of stress response genes, suggesting that SCR plays an important role in coordinating developmental programs and stress response mechanisms (Cui *et al*., 2012).

As another one member of the GRAS gene family, SCL genes are highly similar to the SCR genes. However, there are some slight differences between SCL and SCR, SCL seems to involve in a more general range of plant physiological functions. Diverse characteristics of the SCL genes have been reported in many aspects, such as gene expression patterns, gene structures, motif compositions, promoter cis-elements identification, alternative splicing of transcripts and phylogenetic relationship (Liu *et al*., 2017) and so on. Studies have shown that SCLs are functionally versatile in regulating plant growth, development, plant information transmission and signal transduction (Heidstra *et al*., 2004) and regulation of plant abiotic stresses, such as drought or salt stress (Zhou *et al*., 2013). For examples, the Arabidopsis GRAS protein SCL14 was indicated to be essential for the activation of stress-inducible endogenous promoters that are inducible by salicylic acid (SA) and other chemicals and be involved in the activation of a general broad-spectrum detoxification network upon challenge of plants with xenobiotics (Fode *et al*., 2008). The GRAS protein SCL13 and SCL21 are positive regulator of phytochrome-dependent red light signaling with a role in signal transduction of phytochrome A (Torres-Galea *et al*., 2013). There are several reports on SCR in response to abiotic stress, SCR genes cloned from different species could enhance cold, drought, and salt tolerance in transgenic plants such as *A. thaliana* (Ma *et al*., 2010), potato (*Solanum lycopersicum*) (Ma *et al*., 2010), *Salvia miltiorrhiza* (Bai *et al*., 2017; Liu *et al*., 2017) and

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rice (*Oryza sativa*) (Xu *et al*., 2015). In addition, studies have shown that some SCL genes are involved in pathogen resistance regulated by microRNAs (miRNAs) responsive to infection with the pathogens such as fungus *Verticillium longisporum* (Shen *et al*., 2014), *Citrus psorosis* virus (Reyes *et al*., 2016) and root-knot nematodes (Huang *et al*., 2006).

To date, SCL genes have been well characterized in several plant species, but they have not been investigated in potato (*Solanum tuberosum*). The molecular function of SCLs is well described especially in *A. thaliana*, but no analysis of biotic stress about this gene in potato has been reported. Potato productivity and quality are influenced by many factors, including biotic and abiotic stresses. Bacterial wilt disease caused by plant pathogen *Ralstonia solanacearum* can lead to severe crop losses (Park *et al*., 2016). Those genes that respond to *R. solanacearum* have always been the focus of interest. Biological functionally determined SCL genes in the species described above would provide us some clues for future characterization of their homologues in potato.

In this study, we built a suppression subtractive hybridization (SSH) cDNA library in which an EST for SCL gene was found and subsequently cloned a full length SCL gene (*Stsl-1*) that was elicited by the pathogen. Bioinformatics analyses were also conducted to examine the sequence structure, evolutionary characteristics and expression patterns of this gene. The expression patterns of the transcription factor scarecrow-like gene *Stsl-1* elicited by salicylic acid (SA), methyl jasmonate (MeJA) and abscisic acid (ABA) were analyzed and discussed for the role in resistance to bacterial wilt in potato.

Materials and Methods

Plant and Bacterial Strains

The potato (diploid genotype ED13) plants and *R. solanacearum* strain PO41 used in this study were provided by the Chinese Academy of Agricultural Sciences. Potato ED13 genotype was resistant to bacterial wilt. Aseptic potato ED13 seedlings were grown on culture medium previously developed in laboratory under the following conditions: 16h light (24°C)/8 h dark (18°C) photoperiod (light intensity approximately 3,000 lx) with 75% relative humidity. When plants reached the 4–5 leaf stage, they underwent a 2-day hardening step. The plants were then transplanted to pots (diameter 10 cm, height, 15 cm) containing about 450 mL peat and vermiculite (3:1, volume ratio). The plants were initially treated with an adequate amount of water; after which they were inoculated with *R. solanacearum* at the 9–10 leaf stage. The highly pathogenic *R. solanacearum* strain PO41, which belonged to physiological race 3 and biochemical variant 2, were grown in complete BG medium or in MP minimal medium as previously described (Lonjon *et al*., 2017).

Inoculation

R. solanacearum strain PO41, which was stored at 4°C, was used to inoculate 50 mL beef extract peptone medium. The inoculated medium was placed in a shaking incubator for 12 h at a temperature of 37°C. Potato seedlings of 9–10 leaves were inoculated with *R. solanacearum* by burying the inoculum in the peat and vermiculite mixture. Specifically, a 30 mL bacterial solution (10^8 colony forming units/mL) prepared from individual colonies was used as the inoculums. Control plants were inoculated with an equal volume of water. The inoculated plants were exposed to a 16 h light (22°C)/8 h dark (18°C) photoperiod. Stem samples were collected at 6, 12, 24, 36, 48, 60, 72, 84 and 96 h after the inoculation, wrapped with aluminum foil, placed in liquid nitrogen for 20 min (Kong *et al*., 2016).

Cloning and Bioinformatics Analysis of Full-length *Stsl-1*

In our previous work, a suppression subtractive hybridization (SSH) cDNA library library was created (Kong *et al*., 2016) to enrich the up-regulated genes induced by *R. solanacearum* in potato. An EST for SCL gene was found to have a complete 5' terminate but a missing 3 'end. Hence, 3′ rapid amplification of cDNA ends (RACE) was performed to obtain full length sequences, using the SMARTer RACE cDNA Amplification Kit (Clontech, Mountain View, C.A., United States) according to the manufacturer's instructions. The PrimeScript™ RT-PCR Kit (TaKaRa Bio Group, Dalian, China) was used to extract total RNA and generate cDNA *via* reverse transcription.

The amino acid sequence determined with the BioEdit program was used as a query for a BLAST search to identify homologous sequences, which were arranged in order from high to low similarity. The source, length and physicochemical properties of the sequences were then checked. The SignalP 4.1 Server (http://www.cbs.dtu.dk/services/SignalP) was used to check for the presence of a signal peptide. The TMHMM Server v.2.0 (http://www.cbs.dtu.dk/services/TMHMM) was used to analyze the transmembrane region, while the NetPhos 2.0 Server (http://www.cbs. dtu.dk/services/NetPhos/) was applied to determine the number and distribution of phosphorylation sites in *Stsl-1*. Additionally, the TargetP 1.1 Server (http://www.cbs.dtu.dk/services/TargetP/) was used to examine the subcellular localization of *Stsl-1*, while the ExPASy (https://web.expasy.org/protparam/) online tool was used to analyze the relative molecular weight and pI of *Stsl-1*. Moreover, the secondary structure domains were examined by SOPMA (https://sopma.expasy.org/), while the CPHmodels (http://www.cbs.dtu.dk/services/CPHmodels/) was applied to investigate the tertiary structure.

A few of the most homologous amino acid sequences detected in a BLAST search were downloaded and used to

construct a phylogenetic tree according to the neighbor-joining method of the MEGA 5.0 program.

Hormone Treatment

Seedlings of 9–10 leaves were treated with MeJA, SA and ABA, which were initially dissolved in 100% ethanol as 50 m*M* stock solutions. Prior to use, the stock solutions were diluted in water to prepare working solutions of 50 μM MeJA, 50 μM SA and 100 μM ABA. Seedling leaves and stems were sprayed to drip with one of the three hormone solutions and then placed it in a black square box for 24 h at a temperature of 25°C. The leaves and stems were treated with the hormone solutions at the same time for 5 days, after which they were placed in 10 mL centrifugation tubes and frozen in liquid nitrogen for 10 min used for RNA extractions. Each treatment was repeated three times.

Quantitative Real-time Polymerase Chain Reaction

TransScript II All-in-One First-Strand cDNA Synthesis SuperMix for qPCR (One-Step gDNA Removal) (Sibomu Biotechnology, Nanjing, China) was used to reverse transcribe the extracted RNA into cDNA. The subsequent quantitative real-time polymerase chain reaction (qRT-PCR) was completed with the following primers: scarecrow-like 21-F: TGTGAAGGGACAGAGAGAGTGG, scarecrow-like 21-R: TCCAGCCAAGATAAAGAACACC; internal reference: I733-Actin-F: TATAA CGAGCTTCGTGTTGCAC, I733-Actin-R: ACTGGCATACA GCGAA AGAA CA.

Fluorescence *in situ* Hybridization

The PO41 bacterial suspension (10^8 colony forming units/mL) was used to infect ED13 seedlings at the 9–10 leaf stage. At 6, 12, 24, 36, 48, 60, 72, 84 and 96 h after the inoculation, a few leaves and two or three stem segments (6–7 cm) were collected from each seedling and placed in 10 mL centrifugation tubes. The plant material was immersed in a fixative comprising 85 mL 50% ethanol, 10 mL 37% formaldehyde, 5 mL glacial acetic acid, and 0.1% DEPC. An RNA probe was generated from the *Stsl-1* gene fragment amplified by PCR, labeled with 5-FAM and added to the paraffin section made from previously prepared samples. The *Stsl-1* probe sequence was 5′-CACTGGCTACCCTGAGCAATCTGGAAAT-3′, and the probe was used at 100 μ*M*.

Subcellular Localization

The ORF of *Stsl-1* sequence excluding the stop codon was inserted into the pCambia1301 vector so it was fused to the 5′-terminal of GFP and under the control of the 35S promoter. The resulting plasmid was inserted into *Agrobacterium tumefaciens* strain GV3101 cells, which were then injected into the leaves of 1-month-old tobacco (*Nicotiana benthamiana*). The leaves of control plants were injected with *A. tumefaciens* cells carrying the empty pCambia1301 vector (*i.e.*, GFP alone). The subcellular localization of *Stsl-1* was analyzed based on the fluorescence from the GFP, which was observed by confocal microscopy (Nikon C2-ER, produced by Nikon Corporation).

Results

***Stsl-1* Cloning and Analysis**

Based on a SMART cDNA library containing a positive SCR-cDNA clone, the full-length *Stsl-1* sequence was cloned and submitted to the NCBI database with an accession number MH684486.1. A BLAST analysis indicated that the sequence was approximately 99% similar to the potato gene encoding scarecrow-like protein 21 (Accession: XM_006346099.2). Thus, the detected *SCR* gene was considered to be a homolog and consisted of 2,139 bp (Fig. 1). Additionally, the 37 potential phosphorylation sites in the encoded protein included 26 serine residues, 5 threonine residues and 6 tyrosine residues. The presence of multiple phosphorylation sites suggests that *Stsl-1* is easily phosphorylated and activated by other substances.

A phylogenetic tree was constructed by screening 20 amino acid sequences with the highest homology to the *stsl-1* gene (Fig. 2) using the MEGA 5.0 program. On the basis of node locations and branch lengths, *Stsl-1* was clustered on the same branch as one potato gene and two tomato genes, which suggested that these genes had the same origin. The next closest evolutionary relationships were with genes from pepper (*Capsicum annuum*) and tobacco (*N. benthamiana*). Relatively distant relationships were observed with genes from lotus (*Nelumbo nucifera*), apple (*Malus domestica*), cassava (*Manihot esvulenta*) and grape (*Vitis vinifera*).

The *Stsl-1* protein was structurally similar to SCL21 proteins from other solanaceae species. Additionally, the predicted pI (about 5.40) of *Stsl-1* was consistent with the pIs of the other SCL21 proteins. Moreover, there are more abundant hydrophilic amino acids in the *Stsl-1* sequence. There are no signal peptides and transmembrane region. This implied that *Stsl-1* is highly hydrophilic and water soluble. Phosphorylation and dephosphorylation events are important for intracellular signal transductions. An analysis of the *Stsl-1* sequence revealed 37 potential phosphorylation sites. Additionally, the predicted tertiary structure indicated (Fig. 3) the nine cysteine residues in the *Stsl-1* sequence may form multiple disulfide bonds that influence the folding and structural stability of the protein. Furthermore, 223 α-helices (including one at the N-terminal), 241 random coils and 73 extended strands were detected in *Stsl-1*.

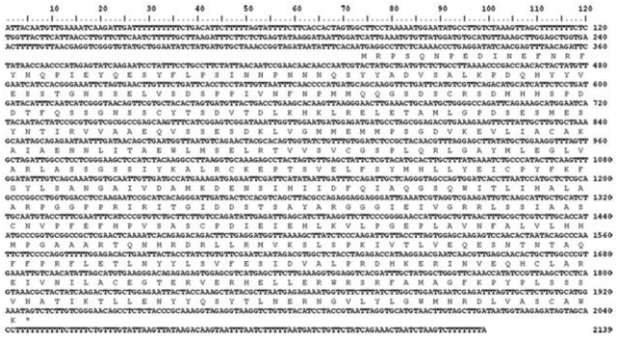

Fig. 1: DNA sequence and the predicted amino acid sequence. The full length of the potato *Stsl-1* sequence was obtained by EST. This figure shows the nucleotide and amino acid sequences of the *Stsl-1* gene, which is 2139 bp and 537 amino acids in total

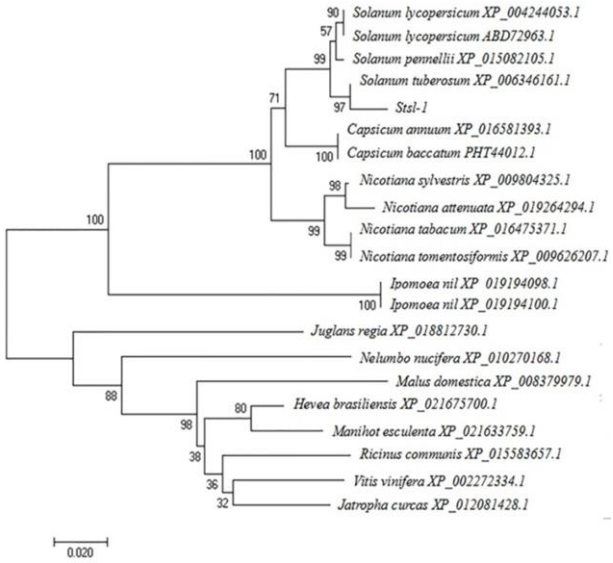

Fig. 2: Phylogenetic relationship of potato *Stsl-1* based on sequence alignment of the encoded proteins. The Neighbour-Joining tree was built using MEGA 5.0 software. The bootstrap value is indicated at the node of the tree and is expressed as a percentage. The scale bar represents the distance calculated from multiple alignments. Data acquisition time expires on July 25, 2018

Stsl-1 Expression Levels Induced by *R. solanacearum*

To explore the early spatial-temporal gene expression patterns induced by the interaction between potato and *R. solanacearum*, a qRT-PCR assay was completed to compare gene expression levels in potato stem tissues collected at different time points after the inoculation (Fig. 4). The *Stsl-1* gene was rapidly and continuously expressed after seedlings were inoculated. The expression level significantly increased between 12 and 36 h after the inoculation, peaking after 36 h (approximately 1.6-fold higher than at the first time point) and then decreasing. The *Stsl-1* gene was involved in the early stage of Potato-*R. solanacearum* interaction.

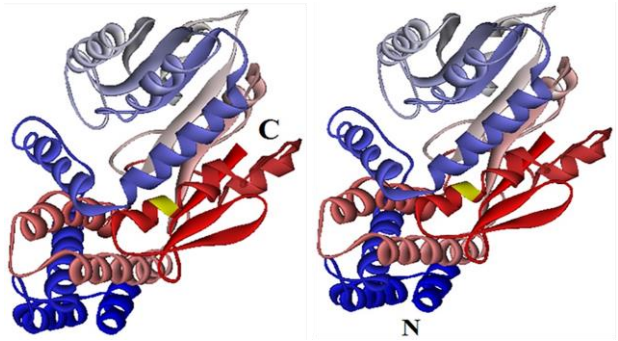

Fig. 3: Three-dimensional structure of the predicted protein of the *Stsl-1*gene
C: Carboxy terminal, N: amino terminal; the three-dimensional model was made by Discovery studio 3.0 software

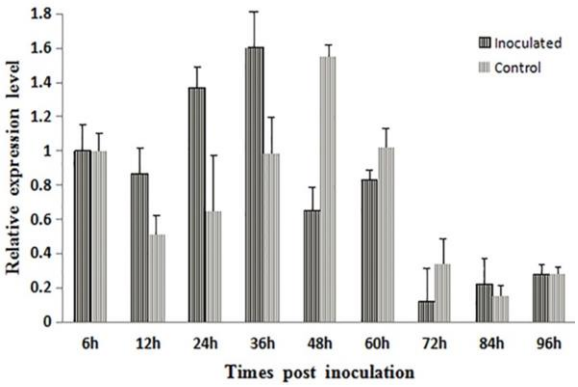

Fig. 4: *Stsl-1* was strongly induced by *R. solanacearum* during the early interaction between potato plants and the pathogen. The abscissa represents the time of inoculation and the ordinate represents the relative expression level of the *Stsl-1* gene. The value of each time point is normalized according to *actin*. (Results are shown by mean ± standard deviation, n = 3, t-test)

Stsl-1 Expression Patterns Elicited by Phytohormones

The expression of *Stsl-1* was up-regulated by exogenously applied hormones SA, MeJA and ABA (Fig. 5). The relative expression of this gene reached the highest peak at almost the same time after treatment with SA, MeJA and ABA, respectively. However, compared with the internal reference gene, the amounts of increase in gene up-regulated expression showed larger difference in the results of the treatment with three plant hormones. ABA could strongly stimulate the up-regulated expression of *Stsl-1* gene, while the other two phytohormones induce the expression of this gene relatively gently. qRT-PCR results showed that the up-regulated expression state could persist for a long time, suggesting that this transcription factor plays an important role in the process of response to *R. solanacearum* pathogens in potato. Thus, our data revealed that ABA can significantly up-regulate *Stsl-1* expression, with transcript levels greater than that induced by SA or MeJA treatment. And these results suggested that the

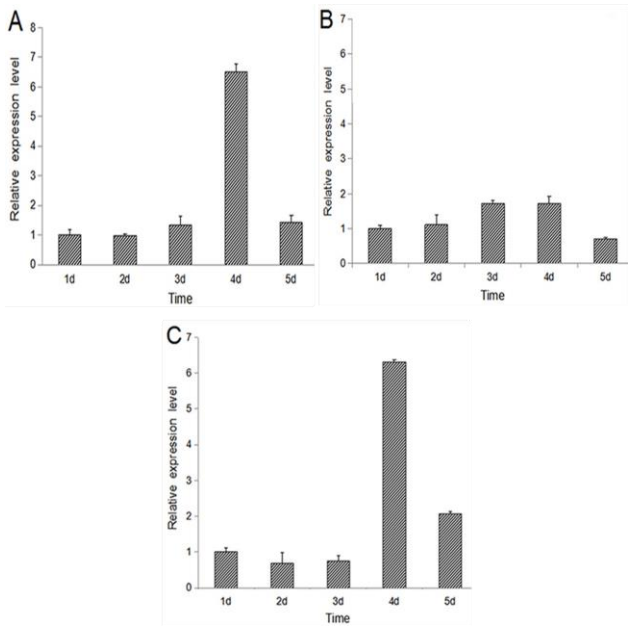

Fig. 5: Differential expression patterns of *Stsl-1* under different plant hormones MeJA, ABA and SA treatment. (**A**) abscisic acid, (**B**) salicylic acid, (**C**) methyl jasmonate treatment, expression analysis of potato *Stsl-1* gene. Statistical values are expressed as mean ± standard deviation (3 replicates, t-test)

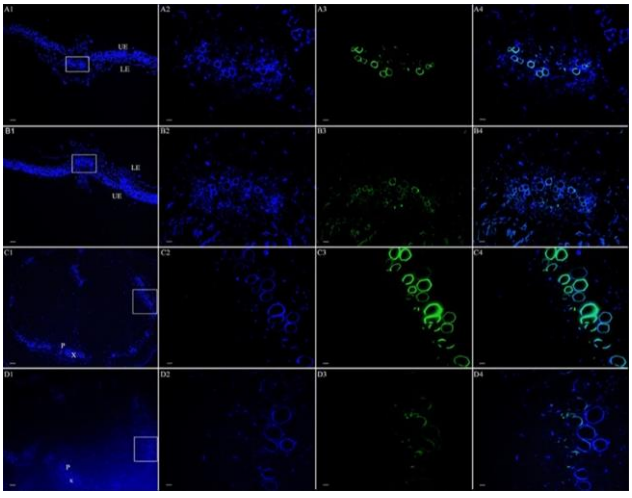

Fig. 6: Fluorescence *in situ* hybridization showed that the localization and expression of *Stsl-1* mRNA in potato stem and leaf tissues

A1: Leaf tissue treated with *R. solanacearum* 100x; A2, A3, A4 400x; B1: Leaf tissue treated with water control 100x; B2, B3, B4 400x; C1: Stem tissue treated with *R. solanacearum* 100x; C2, C3, C4 400x; D1: stem tissue treated with water control 100x; D2, D3, D4 400x; Cross sections were detected using 5-FAM-labeled antisense mRNA and observed under brightfield. The positive hybridization signal was visualized by the green color of the 5-FAM-labeled RNA immunodetection system

P: phloem; X: xylem; UE: upper epidermis; LE: epidermis; 100x scale length: 101 μm; 400 x scale length: 25 μm

regulation of SCL transcription regulator may involve multiple signaling pathways including SA, MeJA and ABA.

Tissue Localization of *Stsl-1* Expression

A fluorescence *in situ* hybridization analysis indicated (Fig. 6) that *Stsl-1* mRNA was mainly distributed in the vascular bundles of leaves (Fig. A3) and in the phloem of stems (Fig. C3). Additionally, a weak hybridization signal was observed in the control plants (Fig. B3 and D3). These results indicated that *Stsl-1* expresses resistance to *R. solanacearum* in this structure while exhibiting tissue-specific expression.

Subcellular Localization of *Stsl-1* Expression

An analysis of the transient expression of *Stsl-1*, which encodes a transcription factor, may rapidly identify the locations in which specific genes are expressed, thereby accelerating investigations of potato gene functions. As shown below (Fig. 7), fluorescence from 35S::GFP was uniformly distributed in the *N. benthamiana* cells transformed by the empty pCambia1301 vector (Fig. 7 B1-B4). In contrast, the fluorescence from the 35S:: *Stsl-1*-GFP fusion protein was detected in the nucleus, with only weak signals in the cytoplasm of some cells (Fig. 7 A1-A4). It is concluded that *Sts1-1*-GFP is localized in the nucleus.

Discussion

In this study, a new SCL21 gene, *Stsl-1*, was cloned from potato inoculated with the pathogen *R. solanacearum* and then characterized using multiple bioinformatics tools to clarify its structural characteristics (Fig. 1 and 3), function, and expression pattern. Phylogenetic tree analysis suggested that *Stsl-1* clustered in the Solanaceae proteins with the same ancestor as the potato gene encoding scarecrow-like protein 21 (Fig. 2). Data presented herein proved that *Stsl-1* is a new SCL gene. SCL proteins are plant specific proteins, for which a large number of reports have shown that they play important roles in plant growth, development and phytohormone signaling pathways including gibberellin (GA), ABA, SA and jasmonate signaling (JA) pathways in stress responses (Heo *et al.*, 2011; Cui *et al*., 2012; Long *et al.*, 2015; Xu *et al.*, 2015; Fode *et al.*, 2008). qRT-PCR data in this study have indicated that *Stsl-1* expression was significantly up-regulated by MeJA and ABA, and while the effect of SA was not significant (Fig. 5B). ABA seems to be more abler to induce strong high-level expression of this gene, the other two phytohormones. From this result, it could be hypothesized that in potato, the signaling pathway involved in ABA may be more likely to stimulate the expression of genes than other signaling pathways, thus showing that the function of the *Stsl-1* gene in the interaction between potato and *R. solanacearum* seems to be more relationship with the ABA pathway. Some literature found that increases in ABA levels at the root tip negatively regulate the local expression of SCR transcription factor genes, which

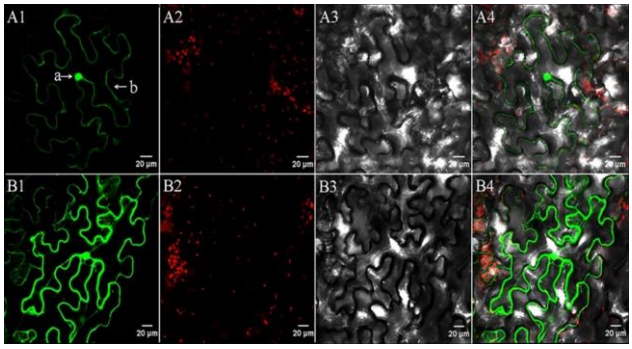

Fig. 7: Subcellular localization showed that *Stsl-1* was mainly localized in the nucleus, less in cytoplasm
a: nucleus; **b**: cytoplasm; A1-A4: pCambia1301-Stsl-1-GFP; A1: GFP fluorescence; A2: Chloroplast; A3: Bright; A4: Merged; B1-B4: pCambia1301-GFP; GFP: excitation wavelength 488 nm, emission wavelength 510 nm
Chloroplast fluorescence signal excitation wavelength: 640nm, emission wavelength: 675 nm

represents a sensitive mechanism for regulating root growth (Harris and Ondzighi-Assoume, 2017). In this study, *Stsl-1* was up-regulated with ABA treatment (Fig. 5A). Recent works had suggested that the SCL proteins integrate generic GA responses into ABA-controlled abiotic stress tolerance and how SCL proteins affect plant development and the multifunctional adaptation of ABA trigger signaling pathways are discussed how GRAS type proteins influence plant development and versatile adaptation as hubs in GA and ABA triggered signaling pathways (Golldack *et al.*, 2013). In a sense, these results are consistent with the experimental results of the present study. This indicated that *Stsl-1* was positively regulated by ABA signal cascade, while the induction of this gene by SA is not obvious, which is worthy of more extensive verification.

However, this needs further experimental verification. Additionally, cis-jasmone can improve the direct and indirect defenses of plants against herbivorous insects, and is a derivative MeJA (Matthes *et al*., 2010). The up-regulated gene expression induced by *cis*-jasmone requires an equal abundance of three transcription factors (TGA2, TGA5 and TGA6) as well as a GRAS regulatory protein (SCL14). This suggests that cis-jasmone and MeJA regulate gene expression *via* diverse pathways (Matthes *et al*., 2010). Thus, the results (Fig. 5C) of this study implied that *Stsl-1* may be associated with MeJA-induced gene expression.

Since there is currently little research on how the SCL gene responds to plant pathogens, which signal pathway in which *Stsl-1* gene is involved remains to be further studied. Recently, on the SCL gene's function of response to abiotic stress, there were several studies reported to be relevant for plant defense signaling pathways. However, an exposure to stress conditions up-regulates *S*CL7 expression, but down-regulates SCL4 expression, suggesting SCL4 and SCL7 are important transcription factors in stress response (Kilian *et al*., 2007). The mir171-SCL module is very important for regulating the gibberellic acid (GA)-DELLA signal and regulating chlorophyll biosynthesis and leaf growth under light conditions. The targeting of SCL genes by miR171 influences chlorophyll biosynthesis under light, but not in darkness (Ma *et al*., 2014). In *Lilium brownii*, SCL affects signal transduction pathways induced by stress (Morohashi *et al*., 2003; Sánchez *et al*., 2007).

Many SCL genes are expressed in the roots, suggesting some of these genes may have important functions related to root biology (Pysh *et al*., 1999). A HISTONE DEACETYLASE 19 (HDA19)-related regulatory factor, SCL15, is mainly localized in vascular bundles. SCL15 is highly expressed in vascular bundle tissue (Gao *et al*., 2015), while is similar to *Stsl-1*. Based on these similar data and results of this study, *Stsl-1* gene was located in the vascular bundle system where *R. solanacearum* colonizes and develops pathogenicity (Fig. 6). These suggested that *Stsl-1* likely provides some possibility against bacterial wilt by defending the pathogen in the vascular system in potato. Most *Stsl-1* proteins were located in the nucleus (Fig. 7) and showed that they are consistent with the subcellular localization of other members of the SCL gene family (Li *et al*., 2018).

There have been very few studies regarding the potato *Stsl-1* gene. In summary, *Stsl-1* was observed to be regulated by *R. solanacearum* (Fig. 4), implying that in response to infections by pathogens, *Stsl-1* expression is regulated by a complex network. It was precisely because the *Stsl-1* expressed mainly in the vascular bundle system where *R. solanacearum* infected and colonized. In that case, it seemed that a corollary could be drawn. That is, its expression may transport a certain signal to defense the pathogen invasion and expansion. And this signal is probably the protein product of the related genes or the SCL gene itself. A recent study showed that ChIP-chip experiments showed that SCR is primarily involved in sugar transport whereas SCL23 functions in mineral transport. SHR is also essential for xylem-related bundle sheath cells cell-fate specification and expressed in the central vascular tissue. However, the SHR protein moves into the bundle sheath cells, where it directly regulates SCR and SCL23 expression (Cui *et al.*, 2012). Although this induction signal molecule has not been determined yet, *Stsl-1* has a continuous high expression at 12–36 h, suggesting that there may be some signal stimulation, which leads to the expression of 24 h and 36 h. Therefore, the molecular mechanism underlying the regulation of *Stsl-1* expression should be explored in future studies. Elucidating this mechanism may be very important for providing a new target for controlling bacterial wilt in potato plants.

Conclusion

The Scarecrow-like protein 21 (SCL21) gene *Stsl-1* cloned from potato during the early stages of Potato-*R.*

solanacearum interaction is structurally similar to SCL21 proteins from other Solanaceae species. Moreover, *Stsl-1* gene was mainly distributed in vascular bundles of leaves and phloem of stems. Subcellular localization results indicated that *Stsl-1* gene is more distributed in nucleus and less in cytoplasm. Likewise, *Stsl-1* gene elicited by SA, MeJA, ABA and *R. solanacearum* infection showed that this gene might be involved in multiple signaling pathways. During the initial stages of the plant-pathogen interaction, *Stsl-1* expression is up-regulated and might be involved in the resistance of potato to bacterial wilt.

Acknowledgments

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