Mol Biol Rep (2009) 36:2365–2374
DOI 10.1007/s11033-009-9459-9

A novel leucine-rich repeat receptor-like kinase gene in potato, *StLRPK1*, is involved in response to diverse stresses

Tian Wu · Zhendong Tian · Jun Liu · Conghua Xie

Received: 20 October 2008 / Accepted: 2 February 2009 / Published online: 13 February 2009

Abstract A potato gene, *StLRPK1* (*Solanum tuberosum* L. leucine-rich-repeat receptor-like protein kinases 1), encoding a protein belonging to leucine-rich repeat receptor-like kinases (LRR-RLKs) was identified. It encodes 796 amino acids with 88% of identity to SRF3 of *Arabidopsis thaliana* and contains a signal peptide, five LRR motifs, a transmembrane domain, two proline-rich regions and a serine/threonine protein kinase domain. The transcripts were present at high levels in flowers and young leaves, while low in other tested organs. The mRNA of *StLRPK1* was inducible in potato leaves by *Phytophthora infestans*, a pathogen causing late blight disease, and showed different profiles after treatment with salicylic acid, methyl jasmonate, ethylene, abscissic acid, wounding, 40°C, 4°C and a salinity stress. The results suggest that *StLRPK1* may participate in the responses against environmental stresses and disease resistance in potato.

Keywords *StLRPK1* · Leucine-rich-repeat receptor-like kinases · RACE · Biotic and abiotic stresses · *Solanum tuberosum* L.

Introduction

Plants respond to continuously changing environmental factors to ensure survival. For instance, protein kinases, which possess an extracellular receptor domain, transfer external signals into cells by protein phosphorylation [1]. Receptor-like protein kinases (RLKs) are involved in the perception and transmission of an external stimulus through signaling cascades to elicit appropriate cellular responses [2]. One of the largest and well-studied classes of RLKs is characterized by the leucine-rich-repeat (LRR) known to be involved in protein–protein interaction [3], and a large number of LRR-RLKs genes have been isolated from plant species [4]. Most of LRR-RLKs have a receptor configuration with an extracellular domain, which is comprised of tandem repeats of a leucine-rich motif, a transmembrane domain, and an intracellular kinase domain with serine/threonine kinase activity [5]. Most of the RLKs identified belong to the LRR class until 2008, for example, 216 among the 417 RLKs identified in *Arabidopsis thaliana* have LRR receptor domains [6].

The LRR-RLKs play important roles in protein–protein interactions. Studies regarding the function of LRR-RLKs have revealed their roles in a wide range of signal responses, such as Xa21 in disease resistance [7], SRK in pollen–pistil interaction [8], and BRI1 in hormone perception [9]. In the process of plant responses to pathogen attack, the quick recognition of the pathogen starts inducible defensive measures. Several resistance genes such as tomato genes *Cf-2*, *Cf-4*, and *Cf-9* share the LRR and protein kinase domain [10], which strongly suggest that the proteins encoded by resistance genes are involved in pathogen and plant interactions, and specific recognition and signal transduction leads to rapid inductions of defense resistance.

Recently, we have isolated a LRR-RLKs-encoding cDNA fragment from potato (*Solanum tuberosum* L.) leaves with quantitative resistance to *Phytophthora infestans* based on the profiles of gene microarray [11]. A preliminary study revealed that LRR-RLKs seem to be involved in disease resistance because the expression of its RNA was highly induced. In this study, we described the isolation and

T. Wu · Z. Tian · J. Liu · C. Xie (✉)
Key Laboratory of Horticulture Plant Biology, College of Horticulture and Forestry, Huazhong Agricultural University, Wuhan 430070, People's Republic of China
e-mail: xiech@mail.hzau.edu.cn

identification of a new full-length cDNA, *StLRPK1* (*Solanum tuberosum* L. leucine-rich-repeat receptor-like protein kinases 1), using the technique of Rapid Amplification of cDNA Ends (RACE), which identified a RLK with five LRR units in its receptor domain. We showed that the transcription of this RLK gene was increased during the inoculation of *P. infestans*. To get an insight into its behavior and regulation by environmental stimuli, we have characterized the mRNA expression profiles of this gene treated with plant growth regulators (salicylic acid, methyl jasmonate, ethylene and abscissic acid), wounding, cold (4°C), heat-shock (40°C) and salinity stress.

Materials and methods

Plant materials and treatments

A potato clone, 386209.10 was employed to isolate the resistance-associated genes against *P. infestans*. It was derived from the Population B without R1–R11 genes of Solanum demissum from the International Potato Center (CIP) [12] and previously used for the construction of the suppression subtractive hybridization (SSH) library [13]. The tubers were grown in 20 cm × 20 cm plastic pots in a naturally illuminated glasshouse. Six-week-old healthy potato plants were transferred to a growth chamber at 18°C under a 16 h photoperiod. In the stage of tuber development, samples of roots, stems, stolons, tubers, young leaves, mid leaves, aged leaves and flowers were collected for assessment of gene expression.

The third fully expanded compound leaf of 6-week-old healthy potato plants was used for the treatments of distilled water (control), 10 mM salicylic acid (SA), 50 mM methyl jasmonate (MJ), 200 μl/l ethylene (ETH) and 100 mM abscissic acid (ABA) (all purchased from Sigma) and the inoculation of *P. infestans* zoospores at 2×10^5/ml of mixture of the races 1, 3, 4, and 1.3. The leaves were sprayed from the abaxial side. For mechanical wounding, a single leaf of the compound leaf was cut a snip and sprayed with distilled water. All of the treated samples were covered with a transparent polyethylene plastic bag to maintain humidity. Each treatment included three potato plants.

For salinity stress, six compound leaves were sampled as above from the potato plants and placed directly into a container of 150 mM NaCl; for cold and heat treatments, the leaves were placed directly into a container of water and respectively, kept at 4, 40 or 18°C for the control.

The samples infected with *P. infestans* were harvested at 0, 1, 8, 12, 24, 36, 48 and 72 h after the inoculations. Those of SA, MJ, ETH, ABA and wounding were harvested at 0, 2, 8, 12, 24 and 36 h, and those for salinity stresses, cold and heat at 0, 5, 15, 30, 60, 360 and 720 min after treatments. All the samples were snap-frozen in liquid nitrogen and stored at −70°C until RNA extraction.

RNA isolation

Total RNA was extracted from potato samples with Trizol reagent (Invitrogen, USA). Briefly, the protocol entailed grinding the sample in liquid nitrogen in a 2 ml tube. About 1 ml of Trizol reagent was added, followed by 0.2 ml of chloroform, vigorous shaking and centrifugation. About 0.6 ml of the aqueous phase was transferred to a fresh tube and mix with 0.4 ml isopropanol. RNA is precipitated after centrifugation. Dissolve RNA with DEPC-treated water. The RNA was then treated with DnaseI (Promega, USA) to remove the residual DNA. At last, purify the RNA sample.

Cloning of potato *StLRPK1* CK277361, which includes the full-length DR751888

The purified total RNA prepared from the pathogen inoculated leaves harvested at 36 h were used to synthesize single-stranded cDNA using the MMLV reverse transcriptase (Clontech, USA) for the template of RACE. A cDNA fragment of 303 bp with GenBank accession number DR751888 was identified from the cDNA microarray analysis [11]. This fragment shows 99% identity with another *Solanum tuberosum* cDNA clone POADZ30 5′ end from potato abiotic stress cDNA library with GenBank accession number

Table 1 The primers used in this study

Abbreviation	Sequence (5′–3′)	Description
LRPKF1	ACAATCGCATCTCTTCAGCAATACACC	Forward; for 3 RACE
LRPKR1	TCTGACCAAGAATTGCTCTCCTCG	Reverse
LRPKR2	GCACAATAGCCCATCAACTCGACAACG	Reverse; for 5 RACE
LRPKF	ATGGGTTGGAAGAGATCT	Gene specific primer, Forward
LRPKR	TCATTCTTCATCGGATCT	Gene specific primer, Reverse
TUB1	TTGGACAGTCTGGTGCTGGGAATA	Gene specific primer as internal control, Forward
TUB3	TGGCCAGGGAATCTCAAACAGCAAG	Gene specific primer as internal control, Reverse

CK277361, which includes the full-length DR751888. So we used the 789 bp CK277361 to design gene specific primers LRPKF1 and LRPKR1 (Table 1). The PCR amplification was performed in a 20 μl tube containing 100 ng cDNA, 0.2 μM LRPKF1 and LRPKR1, 0.2 mM dNTPs, 1× PCR buffer (plus $MgCl_2$) and 2U LA *Taq*™ DNA polymerase (TaKaRa, Japan). The PCR cycle included denaturation at 95°C for 3 min and amplification of 32 cycles (94°C for 45 s, 58°C for 45 s, and 72°C for 60 s), followed by a final extension step of 72°C for 10 min and then maintained at 4°C until needed.

5′ and 3′-RACE were performed with the primer LRPKF1 and a new reverse gene specific primer LRPKR2, which was designed by the result amplified above, respectively, using the SMART™ RACE cDNA Amplification Kit (Clontech, USA). After obtaining the full-length cDNA of *StLRPK1* through the splicing of 5′ and 3′-fragment based on overlap, we designed a pair of specific primers, LRPKF and LRPKR (Table 1), in the region of the open reading frame (ORF), which included the start and stop codons. Conditions for PCR were as follows: 95°C for 3 min; 35 cycles of 94°C for 45 s, 58°C for 45 s and 72°C for 90 s; extension at 72°C for 10 min. PCR reaction components were as above. The resulting PCR products were gel purified and cloned into pMD18-T (TaKaRa, Japan) plasmid vector for sequencing.

Sequencing and analysis

The amplified products were sequenced at Shanghai GeneCore Biotechnologies Co., Ltd (Shanghai, China) and the data was analyzed using the programs provide by NCBI

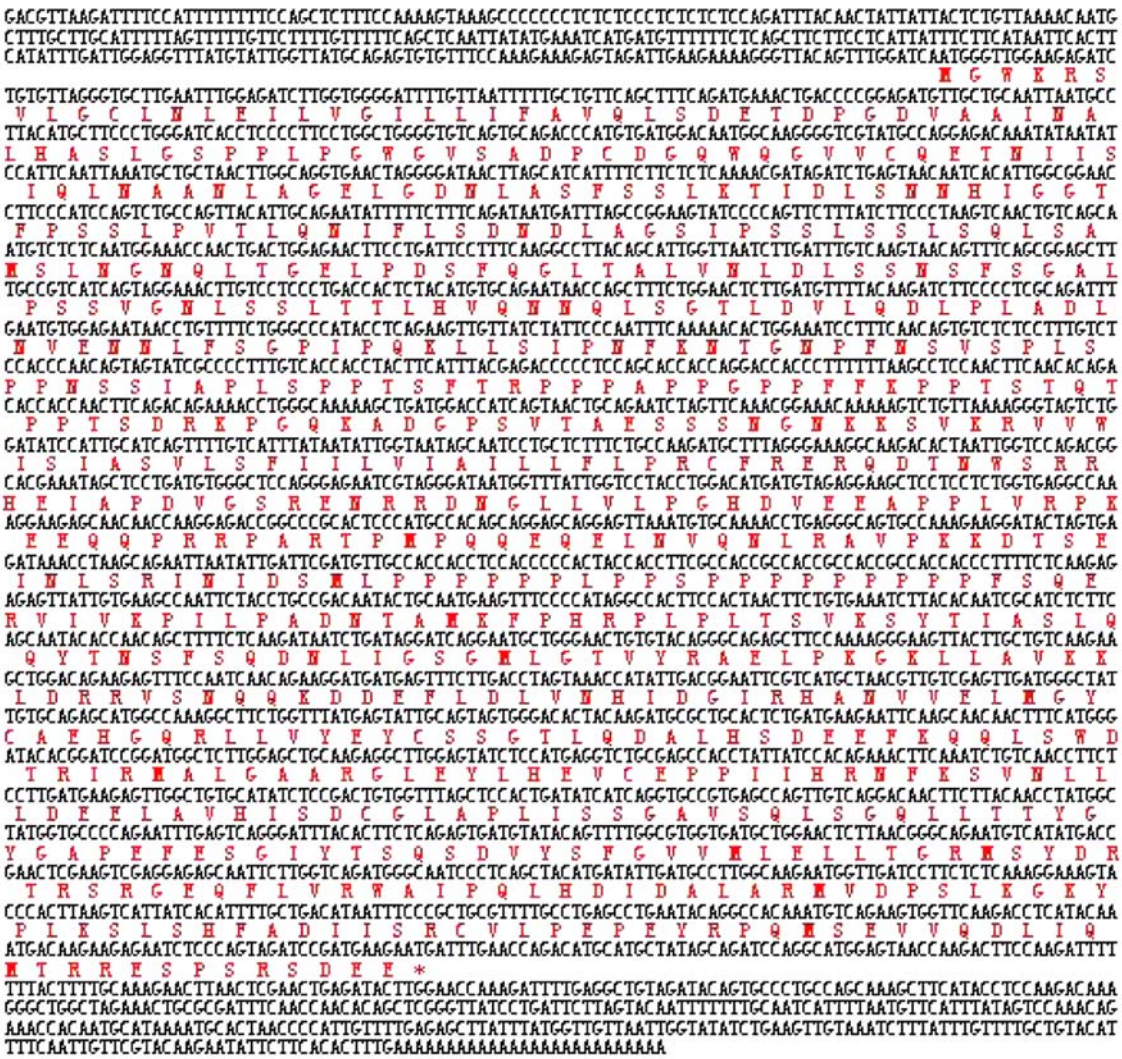

Fig. 1 Nucleotide and deduced amino acid sequences of *StLRPK1*. Full-length *StLRPK1* (GenBank Accession No. EU049848) nucleotide sequence and its deduced amino acid sequence are presented

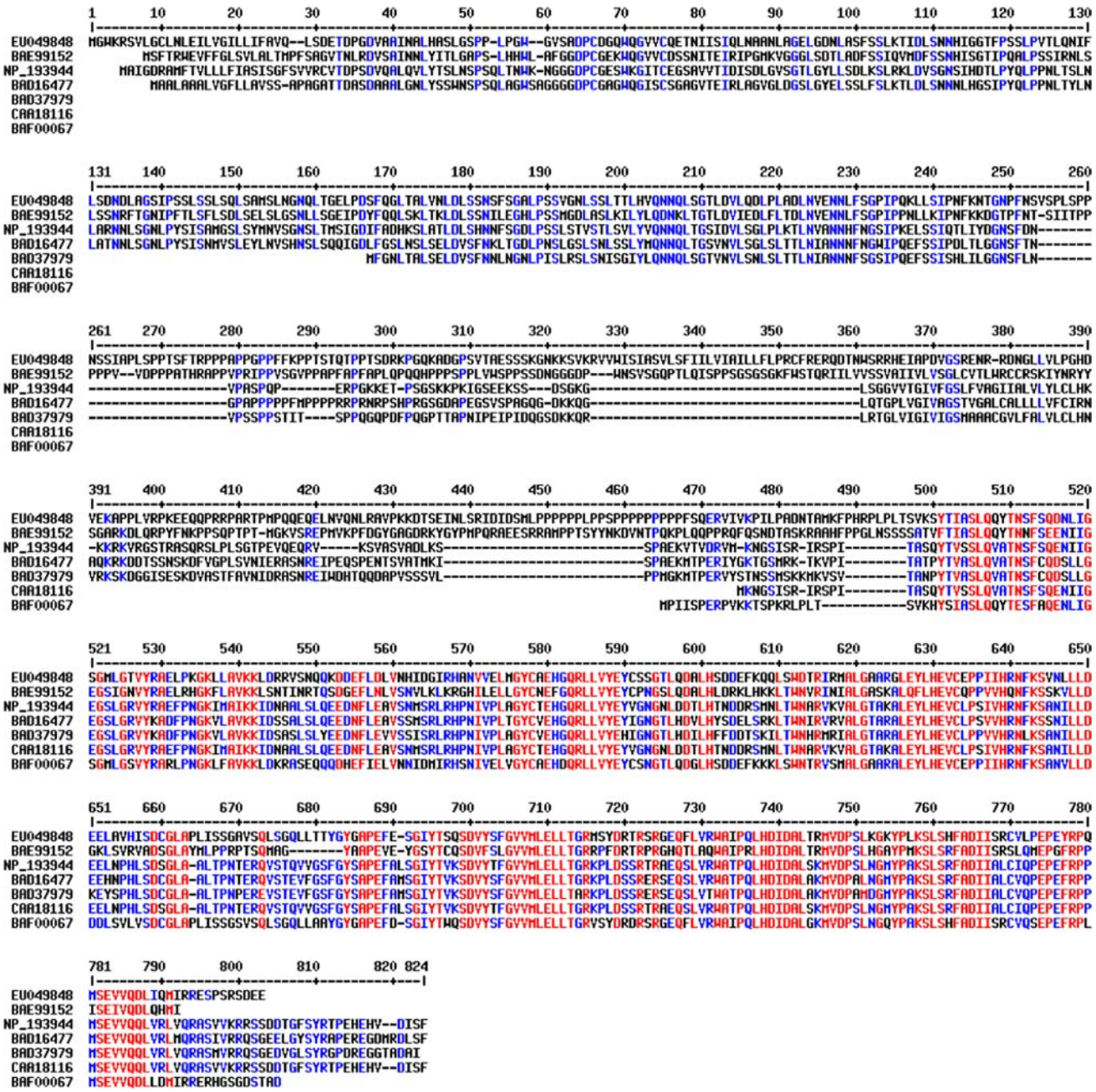

Fig. 2 Alignment of the StLRPK1 protein with other LRR-RLKs from plant species. The plant LRR-RLKs used for alignment are: SRF3 (GenBank Accession No. BAF00067), strubbelig receptor family 8 (GenBank Accession No. NP_193944), serine/threonine protein kinase-like protein (GenBank Accession No. CAA18116), LRR receptor-like protein kinase strubbelig (GenBank Accession No. BAD16477), LRR receptor-like protein kinase strubbelig (GenBank Accession No. BAE99152) from *Arabidopsis thaliana* and LRR transmembrane protein kinase 1 (GenBank Accession No. BAD37979) from *Oryza sativa*

BLAST http://www.ncbi.nlm.nih.gov [14]. The protein conserved domain was analyzed using SMART http://smart.embl-heidelberg.de/smart/change_mode.pl [15] and PlantP http://plantsp.genomics.purdue.edu/html/ [16]. The protein structure, theoretical molecular weight and isoelectric point were predicted using MotifScan and Compute pI/Mw http://au.expasy.org/tools/ [17]. The deduced amino acid sequence was aligned and the phylogenetic tree was generated by the ClustalW method using MEGA4.0 software http://www.megasoftware.net/ [18]. The signal peptide of the putative protein was forecasted using SignalP 3.0 Server http://www.cbs.dtu.dk/services/SignalP/ [19] and prediction of protein localization sites was performed using PSORT http://psort.ims.u-tokyo.ac.jp/form.html [20].

RT-PCR analysis

Reverse transcription reactions for RT-PCR analysis were carried out using the MMLV reverse transcriptase (Toyobo, Japan) as recommended by the manufacturer. The products of reverse transcription were used as templates for the RT-PCR analysis. The specific primers were LRPKF1 and LRPKR1. Primers of the *ß-tubulin* gene [21] TUB1 and TUB3 (Table 1) were used as an internal control. RT-PCR for *StLRPK1* and *ß-tubulin* was carried out with the following cycle: 95°C for 3 min; 27 cycles of 94°C for 45 s, 58°C for 45 s and 72°C for 1 min; 72°C for 5 min and 4°C until needed. Relative expression of *StLRPK1* mRNA was *StLRPK1* to *ß-tubulin* ratio and analyzed by densitometric measurement using Quantity One 1-D Analysis Software (Bio-Rad, USA). The mean density for each band was calculated and expressed as the ratio of *StLRPK1* to *ß-tubulin*. Error bars indicate standard deviation values of RT-PCR analyses.

The software package SPSS 10.0 was applied for statistical analyses. One way analysis of variance with LSD (least significant difference) test was performed to evaluate the differences of treatment with the control. Differences were considered statistically significant for a P value less than 0.05 or extremely significant for a P value less than 0.01.

Results

Cloning and characterization of *StLRPK1* cDNA

About 700 bp PCR product was amplified with LRPKF1 and LRPKR1, and the sequence was predicted to be 687 bp. LRPKF1 and LRPKR2 were used to respectively to amplify the 5′ and 3′ flanking regions by RACE. About 1,000 bp 3′ fragment and about 2,000 bp 5′ fragment were obtained and the sequenced results showed that they were respectively 1,351 bp and 2,019 bp with an overlapping sequence of 233 bp. The full-length cDNA of *StLRPK1* was obtained through splicing, and is 3,137 bp containing a 301 bp 5′-untranslated region and a 448 bp 3′-untranslated region with a 26 bp polyA tail (Fig. 1). To confirm that *StLRPK1* corresponded to a real transcript and was not an artifact of cloning, two primers from the spliced sequence of *StLRPK1*, LRPKF and LRPKR, were used to amplify the ORF which included the start and stop codons of *StLRPK1*. A fragment of about 2,300 bp was obtained and sequencing revealed that it was 99.5% identity to the splicing result.

The gene has been submitted to the GenBank database with accession number EU049848. The open reading frame of the *StLRPK1* gene encodes 796 amino acids with an estimated molecular mass of 86.9 kDa and an isoelectric point of 5.62. The deduced protein, StLRPK1 has 12.6% leucine, which is due to the leucine-rich repeats. PSORT showed a high probability of insertion into the membrane.

Searching NCBI, the amino acid sequence of StLRPK1 compared with other leucine-rich repeat receptor kinase proteins from different higher plants showed 88% identity to SRF3 (GenBank accession number BAF00067) of *Arabidopsis thaliana*, and 76% to strubbelig receptor family 8 (GenBank accession number NP_193944) and serine/threonine protein kinase-like protein (GenBank accession number CAA18116) of *Arabidopsis thaliana* (Fig. 2).

Zhang examined 42 plant LRR-RLKs from databases and made phylogenetic tree, which indicated that plant LRR-RLKs form four subgroups A, B, C, and D [2]. In order to investigate the evolutionary relationships of StLRPK1 to the 42 LRR-RLKs, a phylogenetic analysis was performed. The result indicated that StLRPK1 was assigned to the subgroup C and showed a close relationship with Xa21 of *Oryza sativa* (GenBank accession number U37133) and F23E13.70 of *Arabidopsis thaliana* (GenBank accession number AL022141) (Fig. 3).

SignalP 3.0 Server program analysis indicated the existence of a signal peptide at the N terminus of *StLRPK1*, with the most likely cleavage site located between the 28th and 29th aa. In addition, a strong transmembrane helix from 322 to 344 aa was predicted by SMART and PlantP program. The programs revealed the presence of five LRR motifs from 98 to 213 aa in the extracelullar region and a protein kinase domain from 507 to 781 aa in the cytoplasmic region, and two proline-rich regions from 228 to 291 aa and from 443 to 488 aa (Fig. 4).

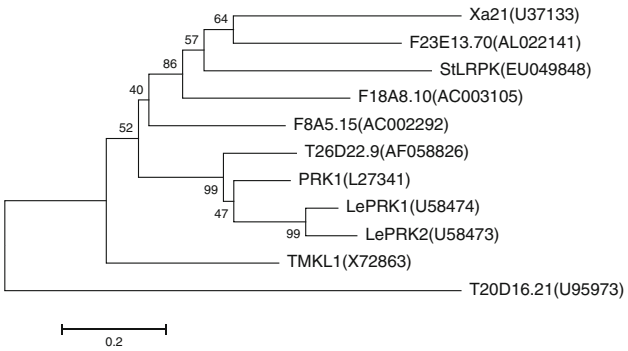

Fig. 3 The phylogenetic relationship of the StLRPK1 protein with other LRR-RLKs from plant species. A phylogenetic tree based on the genetic distance of the protein sequences was constructed by the ClustalW method using MEGA4.0 software. The proteins came from the subgroup C of 42 plant LRR-RLKs [2]. The *symbol* in parenthesis indicates the corresponding GenBank accession number. The numbers above the branches are the percentage of bootstrap trials that support the clade, and branches without number means less than 60%. Xa21 from *Oryza sativa*, PRK1 from *Petunia inflata*, LePRK1 and LePRK2 from *Lycopersicon estulentum*, and others from *Arabidopsis thaliana*

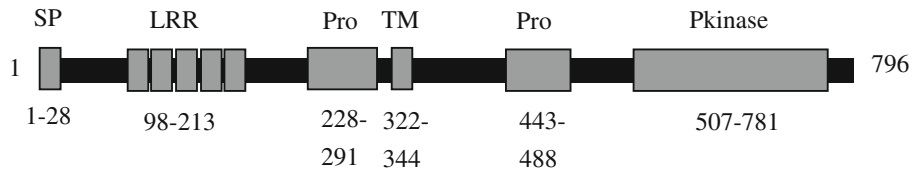

Fig. 4 The structural illustration of *StLRPK1*. The predicted structural and functional motifs including the signal peptide (SP), LRRs, Proline-rich region (Pro), transmembrane domain (TM) and kinase domain (Pkinase) are illustrated

Expression pattern of *StLRPK1* in different potato organs

The spatial expression pattern of *StLRPK1* was investigated in different potato organs by RT-PCR. The results showed that *StLRPK1* transcripts were present at a high level in flowers and young leaves, while low expression was detected in roots, stems, stolons, tubers, aged leaves and mid leaves (Fig. 5).

Expression pattern of *StLRPK1* under biotic- and abiotic-stresses

To investigate the expression pattern of the *StLRPK1* mRNA in response to *P. infestans* infection, RT-PCR was performed with total RNA extracted at different times after inoculation of *P. infestans*. The results indicated that *P. infestans* could induce a rapid and high expression of *StLRPK1* RNA in potato leaves (Fig. 6). After a rapid response in the first hour, the *StLRPK1* mRNA levels were 4.9-fold higher in the inoculated leaves compared with the controls, and the mRNA level of inoculated leaves was increased progressively and significantly over controls from 8 to 72 h.

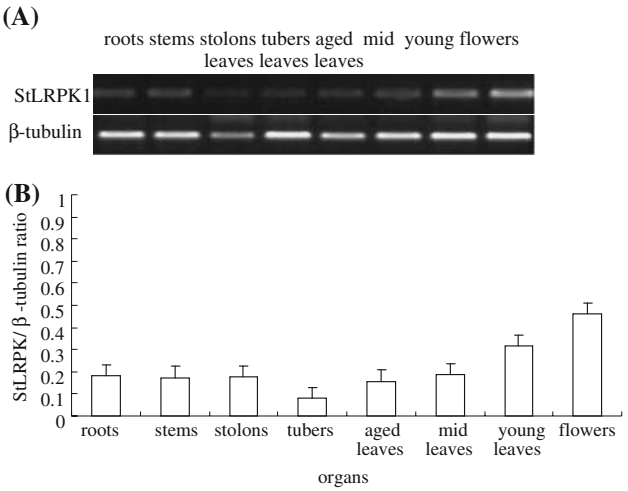

Fig. 5 Expression of StLRPK1 transcript in different organs of healthy potato plants. **a** RT-PCR analysis, *β-tubulin* as the internal control. **b** Relative expression of StLRPK1 mRNA by densitometric analysis using Quantity One 1-D Analysis Software (Bio-Rad, USA). The mean density for each band was calculated and expressed as the ratio of *StLRPK1* to *β-tubulin*. *Error bars* indicate standard deviation values of three independent RT-PCR replicates from a pooled sample. Total RNA was isolated from roots, stems, stolons, tubers, aged leaves, mid leaves, young leaves and flowers

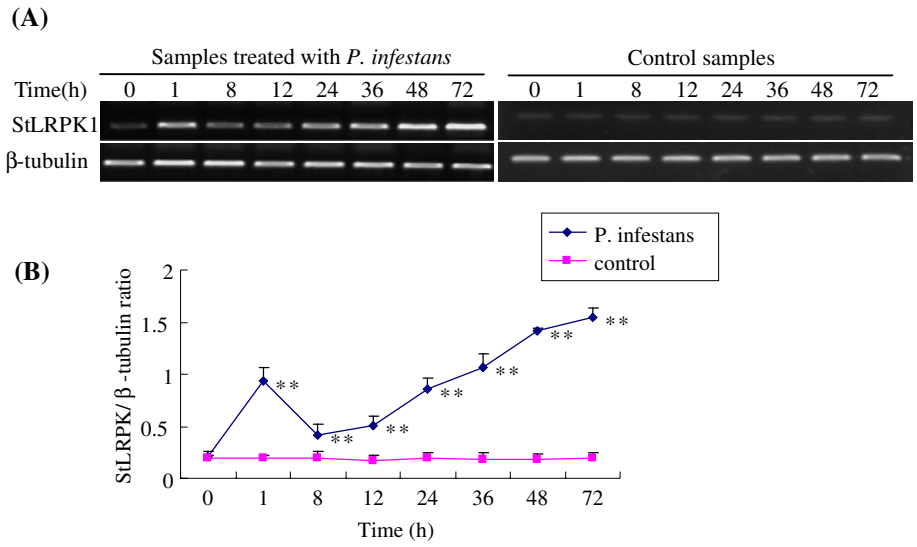

Fig. 6 Expression pattern of *StLRPK1* in potato leaves during *P. infestans* infection and water as control. **a** RT-PCR analysis, *β-tubulin* as the internal control. **b** Relative expression of StLRPK1 mRNA by densitometric analysis using Quantity One 1-D Analysis Software (Bio-Rad, USA). The mean density for each band was calculated and expressed as the ratio of *StLRPK1* to *β-tubulin*. *Error bars* indicate standard deviation values of three independent RT-PCR replicates from a pooled sample. **Means extremely significant at $P < 0.01$

The expression pattern of *StLRPK1* in response to SA, JA and ETH was investigated. In the leaves treated with 10 mM SA, *StLRPK1* mRNA levels were significantly higher ($P < 0.01$) than the controls from 2 to 12 h and decreased to a similar level to the controls thereafter (Fig. 7a). The results also showed that the potato leaves treated with 50 μM MJ induced a high *StLRPK1* expression at 12 h reaching 3.2-fold higher levels than the controls (Fig. 7b). Meanwhile, a

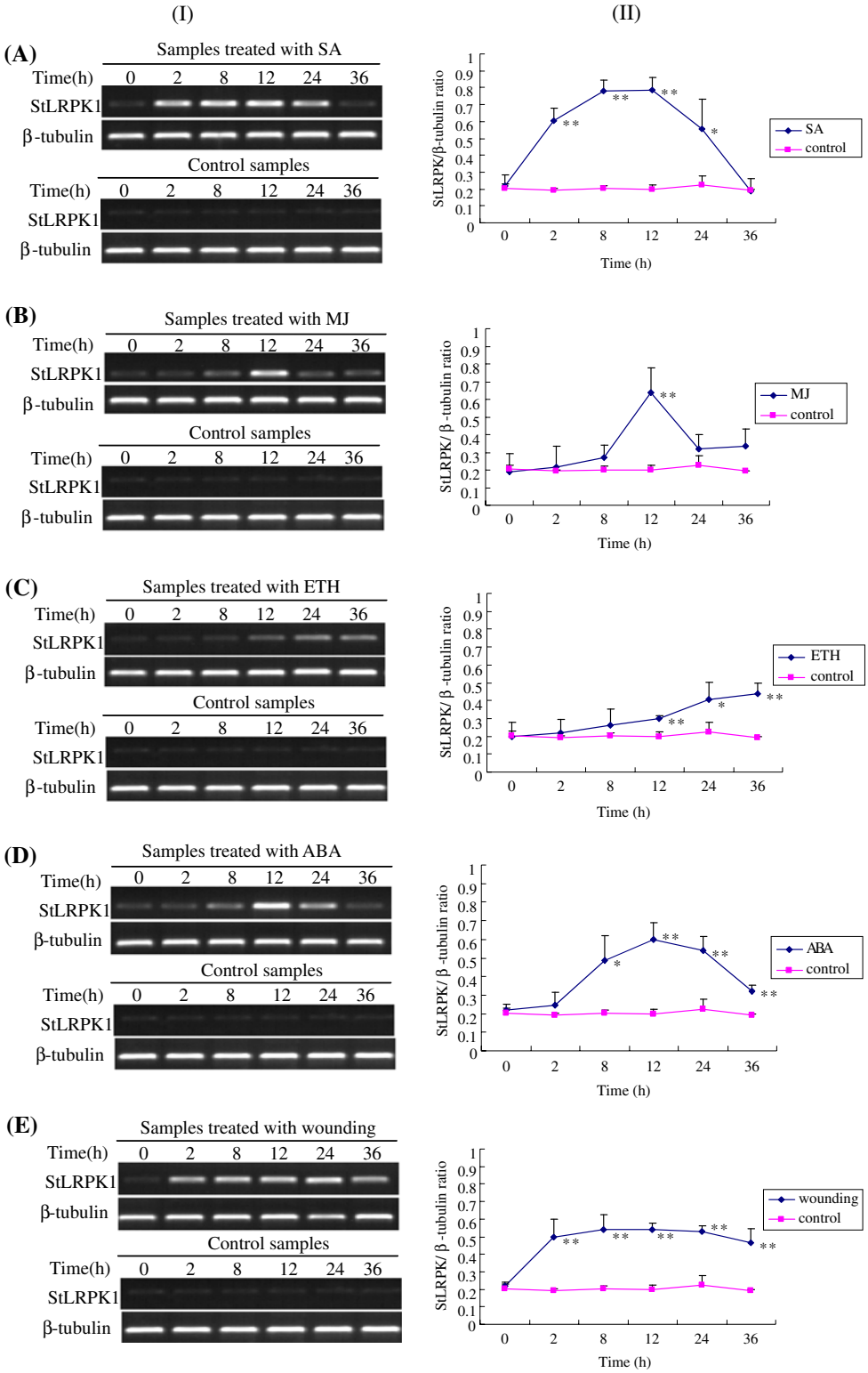

Fig. 7 **a–e** Expression pattern of *StLRPK1* in potato leaves treated with SA, MJ, ETH, ABA and wounding, and water as control. (1) RT-PCR analysis, *β-tubulin* as the internal control. (2) Relative expression of *StLRPK1* mRNA by densitometric analysis using Quantity One 1-D Analysis Software (Bio-Rad, USA). The mean density for each band was calculated and expressed as the ratio of *StLRPK1* to *β-tubulin*. *Error bars* indicate standard deviation values of three independent RT-PCR replicates from a pooled sample. *Means significant at $P < 0.05$, **means extremely significant at $P < 0.01$

steady increase in *StLRPK1* mRNA level was observed in the leaves treated with 200 μl/l ETH with significance after 12 h (Fig. 7c).

Treating potato leaves with 100 μM ABA and mechanical wounding yielded a similar pattern of *StLRPK1* expression in that a rapid increase in mRNA was maintained until 24 h, although a relatively slow increase occurred in ABA treatment (Fig. 7d) in comparison to wounding (Fig. 7e).

To investigate the *StLRPK1* transcript levels in response to other abiotic stress, potato leaves were subjected to heat-shock, cold-shock and salinity stress with 150 mM NaCl. The *StLRPK1* mRNA levels of corresponding controls were slowly rising at all six sampled points (5, 15, 30, 60, 360 and 720 min) but treated samples showed different patterns. Cold-shock varied *StLRPK1* expression but only significantly different ($P < 0.05$) to control at 30 and 360 min (Fig. 8a). A similar pattern was found in the heat-shock treatment but the difference between treated sample and control was more significant in late time points (Fig. 8b). The results also demonstrated that the salinity stress induce *StLRPK1* expression with significantly higher ($P < 0.01$) abundance of mRNA in the treated leaves than in controls that were detected from 60 to 720 min (Fig. 8c).

Discussion

Very few LRR-RLKs genes have been isolated from potato (*Solanum tuberosum*), and so far as we know, *StSERK1* [22] was the first isolation. In our study, we identified a novel LRR-RLKs gene, *StLRPK1*, from potato (*Solanum tuberosum*) leaves inoculated with *P. infestans* by 5′ and 3′-RACE PCR. The extracellular domains of StLRPK1 protein was characterized by common features of plant LRR-RLKs proteins, including a signal peptide, tandem repeats of a leucine-rich motif, a transmembrane domain, and a serine/threonine kinase domain. Besides, the deduced protein contains two proline-rich regions, which are conserved in all somatic embryogenesis receptor-like kinases (SERKs) identified so far, and are seen as a unique feature

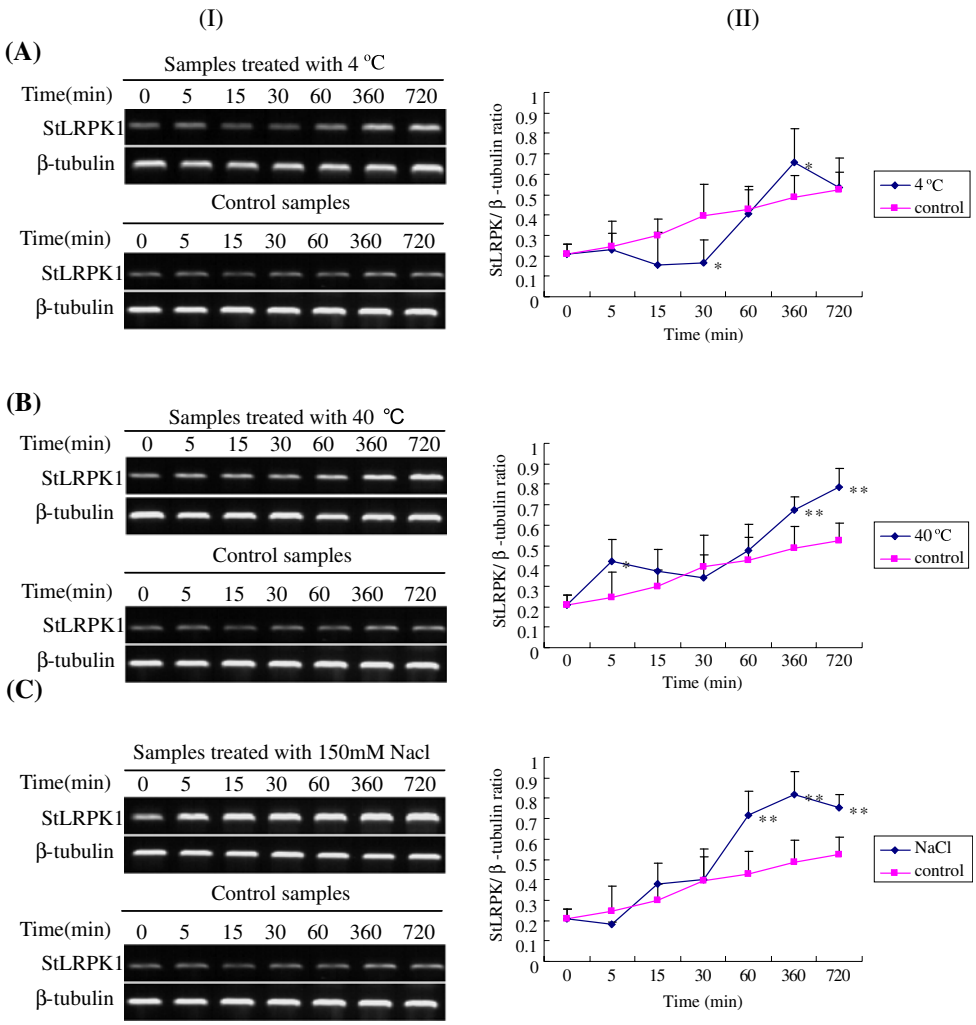

Fig. 8 **a–c** Expression pattern of *StLRPK1* in potato leaves treated with 4°C, 40°C and 150 mM NaCl or water as control. (1) RT-PCR analysis, *β-tubulin* as the internal control. (2) Relative expression of *StLRPK1* mRNA by densitometric analysis using Quantity One 1-D Analysis Software (Bio-Rad, USA). The mean density for each band was calculated and expressed as the ratio of *StLRPK1* to *β-tubulin*. *Error bars* indicate standard deviation values of three independent RT-PCR replicates from a pooled sample. *Means significant at $P < 0.05$, **means extremely significant at $P < 0.01$

distinguishing SERKs from other RLKs [23]. Accordingly, *StLRPK1* can be classified into the subgroup SERKs. Several plant proteins containing both proline-rich domains and LRR domains have been characterized, for example, the maize genes *ZmSERK1* and *ZmSERK2* [24], and a rice gene *OsBISERK1* [25]. Proline-rich regions are generally involved in various aspects of plant development, plant–microbe interactions and the response to pathogen attack [24, 26]. However, the biochemical function of *StLRPK1* extracellular domains remains to be investigated further.

LRR-RLKs are found to play important roles in recognition between plant and pathogen, resulting in the activation of defense-related responses [4]. For instance, the *ERECTA* gene of *Arabidopsis thaliana*, which encodes a leucine-rich repeat receptor-like kinase, provides resistance to bacterial wilt, one of the most devastating bacterial diseases of plants worldwide, caused by *Ralstonia solanacearum* [27]. The rice *Xa21* gene, which carries a leucine-rich repeat motif and a serine/threonine kinase-like domain, is involved in recognition of rice leaf blight bacteria and confers resistance to *Xanthomonas oryzae pv. oryzae* race 6 [7]. The result of the molecular phylogenetic tree revealed that StLRPK1 was closely related to Xa21 in deduced amino acid sequence (Fig. 3), and the RT-PCR showed this gene responded to *P. infestans* infection quickly and strongly at the transcriptional level (Fig. 6), implying a possible function of *StLRPK1* with late blight resistance in potato.

Furthermore, the spatial expression analysis showed that *StLRPK1* was expressed in flowers and young leaves, and was also detectable in other organs suggesting that *StLRPK1* has a feature of constitutive expression and might be involved in plant development, similar to the *ERECTA* gene which is involved in both resistance and developmental pathways [27].

The plant hormones salicylic acid [28], jasmonic acid [29], ethylene [30] and to a lesser extent, abscisic acid [31], have been reported to be effective in inducing defense responses in plants. However, the mechanisms of this resistance and the signal transduction pathways are not well known, especially the involvement of LRR-RLKs. In the previous study, it was reported that *OsSERK1*, a leucine-rich repeat receptor-like kinase from rice, was induced by a number of defense signaling molecules, including benzothiadiazole, salicylic acid, jasmonic acid and abscisic acid [23]. In the present study, we found that expression of *StLRPK1* was induced significantly by SA, MJ, ETH and ABA, which is in accordance with the results gained in our lab with the microarray [11], implying that a crosstalk may exist among these pathways to activate *StLRPK1* in potato leaves in response to *P. infestans* attack.

Acknowledgments We thank the International Potato Center (CIP) for providing the potato clone 386209.10 with moderate late blight resistance. This work was funded by a grant from the National 863 Project of China (No. 2006AA10Z149). We also thank the anonymous reviewers for comments on the manuscript.

References

1. Kruijt M, De Kock MJD, De Wit PJGM (2005) Receptor-like proteins involved in plant disease resistance. Mol Plant Pathol 6:85–97. doi:10.1111/j.1364-3703.2004.00264.x
2. Zhang XR (1998) Leucine-rich repeat receptor-like kinases in plants. Plant Mol Biol Rep 16:301–311. doi:10.1023/A:1007540610933
3. Walker JC (1994) Structure and function of the receptor-like protein kinases of higher plants. Plant Mol Biol 26:1599–1609. doi:10.1007/BF00016492
4. Morris ER, Walker JC (2003) Receptor-like protein kinases: the keys to response. Curr Opin Plant Biol 6:339–342. doi:10.1016/S1369-5266(03)00055-4
5. Yin Y, Wu DY, Chory J (2002) Plant receptor kinases: system in receptor identified. Proc Natl Acad Sci USA 99:9090–9092. doi:10.1073/pnas.152330799
6. Diévart A, Clark SE (2003) Using mutant alleles to determine the structure and function of leucine-rich repeat receptor-like kinases. Curr Opin Plant Biol 6:507–516. doi:10.1016/S1369-5266(03)00089-X
7. Song WY, Wang GL, Chen LL, Kim HS, Pi LY, Holsten T, Gardner J, Wang B, Zhai WX, Zhu LH, Fauquet C, Ronald P (1995) A receptor kinase-like protein encoded by the rice disease resistance gene *Xa21*. Science 270:661–667. doi:10.1126/science.270.5243.1804
8. Kachroo A, Nasrallah ME, Nasrallah JB (2002) Self-incompatibility in the Brassicaceae: receptor-ligand signaling and cell-to-cell communication. Plant Cell 14(Supplement):227–238
9. Scheer JM, Ryan CA (2002) The systemin receptor SR160 from *Lycopersicon peruvianum* is a member of the LRR receptor kinase family. Proc Natl Acad Sci USA 99:9585–9590. doi:10.1073/pnas.132266499
10. Zhang LP, Khan A, Nino-Liu D, Foolad MR (2002) A molecular linkage map of tomato displaying chromosomal locations of resistance gene analogs based on a *Lycopersicon esculentum* × *Lycopersicon hirsutum* cross. Genome 45:133–146. doi:10.1139/g01-124
11. Wang BL, Liu J, Tian ZD, Song BT, Xie CH (2005) Monitoring the expression patterns of potato genes associated with quantitative resistance to late blight during *Phytophthora infestans* infection using cDNA microarrays. Plant Sci 169:1155–1167. doi:10.1016/j.plantsci.2005.07.020
12. Cruickshank K, Stewant HE, Wastie RL (1982) A illustrated assessment key for foliage blight of potatoes. Potato Res 25:213–214. doi:10.1007/BF02359807
13. Tian ZD, Liu J, Xie CH (2003) Isolation of resistance-related genes to *P. infestans* with suppression subtractive hybridization in the R-gene-free potato. Acta Genetica Sin 30:597–605 in Chinese with an English abstract
14. Altschul SF, Madden TL, Schaffer AA, Zhang JH, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402. doi:10.1093/nar/25.17.3389
15. Schultz J, Milpetz F, Bork P, Ponting CP (1998) SMART, a simple modular architecture research tool: identification of signaling domains. Proc Natl Acad Sci USA 95:5857–5864. doi:10.1073/pnas.95.11.5857

16. Gribskov M, Fana F, Harper J, Hope DA, Harmon AC, Smith DW, Tax FE, Zhang GF (2001) PlantsP: a functional genomics database for plant phosphorylation. Nucleic Acids Res 29:111–113. doi:10.1093/nar/29.1.111
17. Gasteiger E, Gattiker A, Hoogland C, Ivanyi I, Appel RD, Bairoch A (2003) ExPASy: the proteomics server for in-depth protein knowledge and analysis. Nucleic Acids Res 31:3784–3788. doi:10.1093/nar/gkg563
18. Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis. Mol Biol Evol 24:1596–1599. doi:10.1093/molbev/msm092
19. Bendtsen JD, Nielsen H, Heijne GV, Brunak S (2004) Improved prediction of signal peptides: signalP 3.0. J Mol Biol 340:783–795. doi:10.1016/j.jmb.2004.05.028
20. Nakai K, Horton P (1999) PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. Trends Biochem Sci 24:34–36. doi:10.1016/S0968-0004(98)01336-X
21. Taylor MA, Wright F, Davies HV (1994) Characterization of the cDNA clones of two beta-tubulin genes and their expression in the potato plant (*Solanum tuberosum L.*). Plant Mol Biol 26:1013–1018. doi:10.1007/BF00028869
22. Sharma SK, Millam S, Hein I, Bryan GJ (2008) Cloning and molecular characterization of a potato *SERK* gene transcriptionally induced during initiation of somatic embryogenesis. Planta 228:319–330. doi:10.1007/s00425-008-0739-8
23. Hu H, Xiong L, Yang Y (2005) Rice *SERK1* gene positively regulates somatic embryogenesis of cultured cell and host defense response against fungal infection. Planta 222:107–117. doi:10.1007/s00425-005-1534-4
24. Baudino S, Hansen S, Brettschneider R, Hecht VFG, Dresselhaus T, Lörz H, Dumas C, Rogowsky PM (2001) Molecular characterisation of two novel maize LRR receptor-like kinase, which belong to the *SERK* gene family. Planta 213:1–10. doi:10.1007/s004250000471
25. Song DH, Li GJ, Song FM, Zheng Z (2008) Molecular characterization and expression analysis of *OsBISERK1*, a gene encoding a leucine-rich repeat receptor-like kinase, during disease resistance responses in rice. Mol Biol Rep 35:275–283. doi:10.1007/s11033-007-9080-8
26. Battaglia M, Solórzano RM, Hernández M, Cuéllar-Ortiz S, García-Gómez B, Márquez J, Covarrubias AA (2007) Proline-rich cell wall proteins accumulate in growing regions and phloem tissue in response to water deficit in common bean seedlings. Planta 225:1121–1133. doi:10.1007/s00425-006-0423-9
27. Godiard L, Sauviac L, Torii KU, Grenon O, Mangin B, Grimsley NH, Marco Y (2003) ERECTA, an LRR receptor-like kinase protein controlling development pleiotropically affects resistance to bacterial wilt. Plant J 36:353–365. doi:10.1046/j.1365-313X.2003.01877.x
28. Dempsey DA, Shah J, Klessig DF (1999) Salicylic acid and disease resistance in plants. Crit Rev Plant Sci 18:547–575. doi:10.1080/07352689991309397
29. Turner JG, Ellis C, Devoto A (2002) The jasmonate signal pathway. Plant Cell 14(Supplement):153–164
30. Lu YJ, Wang X, Lou YG, Cheng JA (2006) Role of ethylene signaling in the production of rice volatiles induced by the rice brown planthopper *Nilaparvata lugens*. Chin Sci Bull 51:2457–2465. doi:10.1007/s11434-006-2148-3
31. Birkenmeier GF, Ryan CA (1998) Wound signaling in tomato plants-evidence that ABA is not a primary signal for defense gene activation. Plant Physiol 117:687–693. doi:10.1104/pp.117.2.687