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RESEARCH ARTICLE

Overexpression of *LhSorNPR1*, a *NPR1*-like gene from the oriental hybrid lily 'Sorbonne', conferred enhanced resistance to *Pseudomonas syringae* pv. tomato DC3000 in *Arabidopsis*

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Abstract The *non-expressor of the pathogenesis-related genes 1* (*NPR1*) is a master regulator in defense signaling of plants and plays a key role in basal and systemic acquired resistance. In this study, we isolated a *NPR1*-like gene from the oriental hybrid lily 'Sorbonne' (designated as *LhSorNPR1*) using rapid amplification of cDNA ends (RACE). The open reading frame of *LhSorNPR1* consisted of 1854 bp, encoding a protein of 617 amino acids. Multiple sequence alignment revealed that LhSorNPR1 shares high similarity to NPR1-like proteins and characteristics of the BTB/POZ domain and ankyrin repeats. A comparison between the intron/exon organization of *LhSorNPR1* and orthologs from other plant species demonstrated that *NPR1* genomic fragments (including *LhSorNPR1*) are all composed of 4 exons and 3 introns. We also identified sequence motifs involved in hormone response and binding sites for RAV1 proteins and WRKY transcription factors through the prediction of *cis*-regulatory elements in the *LhSorNPR1* promoter. Our gene expression analysis showed that *LhSorNPR1* transcript levels significantly differed in various tissues, and that *LhSorNPR1* expressions were induced by sodium salicylate, ethephon, and methyl jasmonate. Furthermore, we transformed *LhSorNPR1* into Col-0 wild-type *Arabidopsis* to conduct function analysis, and we observed enhanced resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* DC3000 in the *Arabidopsis* expressing *LhSorNPR1* gene. The enhanced disease resistance of *LhSorNPR1* expressing plants could correlate to elevated expression levels in *pathogenesis-related* genes (*PR1*, *PR2*, and *PR5*) in vivo.

Keywords *LhSorNPR1* · Lily · Systemic acquired resistance · Gene expression analysis · Transgenic *Arabidopsis*

Introduction

Signals from pathogen attacks at local infection sites are often transmitted to uninfected distal sites to protect plants from further invasion. This long-lasting, broad-spectrum defense response is referred to as systemic acquired resistance (SAR) (Fu and Dong 2013). The onset of SAR is accompanied by elevated levels of in vivo salicylic acid (SA) and induced expressions of *pathogenesis-related* (*PR*) genes (Gaffney et al. 1993; Rochon et al. 2006). Moreover, SA accumulation, at both infected and uninfected sites, has led to speculation that SA is the SAR signal molecule (Metraux et al. 1990; Rasmussen et al. 1991). Although much effort has been made in understanding the relationship between SA and SAR, there is to date no direct evidence of this. However, reducing SA levels by disrupting SA synthesis genes (Wildermuth et al. 2001) or by expressing bacterial salicylate hydroxylase genes (Vernooij et al. 1994) has compromised SAR phenotypes.

The *non-expressor of PR genes 1* (*NPR1*) was first identified in *Arabidopsis* by screening mutants that were not sensitive to SA or its analogues (Cao et al. 1994, 1997).

Le Wang and Zhihong Guo have contributed equally to this work.

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Analysis showed that the mutation of the *NPR1* gene reduced SA-mediated *PR* genes expression and enhanced the susceptibility of plant species to pathogens (Delaney et al. 1995; Ryals et al. 1997). Furthermore, protein sequence analysis showed that NPR1 was characteristic of two domains, the BTB/POZ domain and the ankyrin repeat, both of which have been shown to mediate protein to protein interactions (Albagli et al. 1995; Aravind and Koonin 1999; Cao et al. 1997). However, DNA-binding domains (DBD), which regulate target gene expressions through promoter bindings, have not been detected in NPR1. The screening of interacting partners identified TGA transcription factors as being candidates that interplay with NPR1 (Kesarwani et al. 2007). The interaction between TGA family proteins and NPR1 revealed their roles in controlling expression of *PR* genes in SAR. NPR1 serves as a cofactor in enhancing the binding activity of TGA transcription factors in the promoter region to regulate *PR* genes expression (Despres et al. 2000).

As a key regulator of the plant defense signaling network (Pieterse and Van Loon 2004), *NPR1* overexpression has been employed to enhance pathogen resistance in a number of plant species. For example, the ectopic expression of the *StoNPR1* gene in potatoes provides enhanced resistance to the fungal pathogen *Verticillium dahliae* (Jue et al. 2014). In *Vitis vinifera*, *VvNPR1.1* was identified as the functional ortholog of *AtNPR1*, and the overexpression of *VvNPR1.1-GFP*, a *GFP* labeled *VvNPR1.1*, in *V. vinifera* exhibited enhanced resistance to powdery mildew (Le Henanff et al. 2011). Moreover, transgenic carrots expressing *AtNPR1* provided enhanced resistance to different types of pathogens (Wally et al. 2009), including necrotic fungi (*Botrytis cinerea*, *Alternaria radicina*, and *Sclerotinia sclerotiorum*) and biotrophic fungal pathogens (*Erysiphe heraclei* and *Xanthomonas hortorum*). Most previous studies have confirmed the critical role that *NPR1* played in biotic stress, but only limited attention has been paid to the function of *NPR1* on abiotic stress. When heterologously expressed in tobacco, *AtNPR1* enhanced the oxidative stress tolerance of tobacco transgenic lines (Srinivasan et al. 2009). Similarly, increased tolerance to salt and osmotic stress was observed in tobacco plants overexpressing *MhNPR1* (Zhang et al. 2014). These studies have uncovered the important roles that *NPR1* play in both biotic and abiotic stresses, which will subsequently be used in future genetic engineering research.

Lilies are perennial bulbous plants that produce prized flowers. However, pathogens severely affect lily production in both bulbs and cut flowers. Lily bulbs can be infected by soil-borne pathogens, such as *Fusarium oxyporum* (Lecomte et al. 2016), *Penicillium albocoremium*, and *Penicillium tulipae* (Kim et al. 2006), causing scale, bulb base, and bud rot. *Botrytis cinerea* and *Botrytis elliptica* (Huang et al. 2012), causal agents of leaf and flower blight, severely affect cut flower quality and production. At the same time, viruses, such as the lily mottle virus (LMoV) and the lily symptomless virus (LSV), cause leaf mottling and stunted growth in lilies (Zhang et al. 2015a, b). Regardless of their differences in type, all these pathogens are major threats to lily production. Hence, a comprehensive understanding of the immunity-related mechanisms of lilies and their parasitic pathogens could facilitate lily bulb and flower production.

In this study, we characterized a *NPR1*-like gene from the oriental hybrid lily 'Sorbonne' (designated as *LhSorNPR1*). Sequence alignments showed that LhSorNPR1 shared domain characteristics of NPR1 orthologs, including the BTB/POZ domain and the ankyrin repeats. Analysis of the promoter sequence identified potential *cis*-regulatory elements (CREs) involved in the expression control of *LhSorNPR1*. Subsequent expression analysis showed that *LhSorNRP1* was differentially expressed in various tissues, and its expression was responsive to sodium salicylate (SA), methyl jasmonate (MeJA), and ethephon (ETH). When consecutively expressed in *Arabidopsis*, *LhSorNPR1* provided enhanced resistance to the bacterial pathogen *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000). Furthermore, the enhanced disease resistance of *Arabidopsis* transgenic lines could correlate to elevated expression levels for in vivo *PR* genes.

Materials and methods

Plant material and growth conditions

We used the oriental hybrid lily cultivar 'Sorbonne' in this study. We planted one bulb from 10 to 12 cm in diameter per pot (pot = 25 cm in diameter) containing peat moss as a growing substrate. We stored all bulbs used in this study at 4 °C for over 2 months to end the lily dormant state before the experiment started. We then transferred all pots to a growth chamber that was maintained at optimum growth conditions (16 h light/8 h dark; 22 °C).

Genomic DNA extraction, total RNA isolation, and first-strand cDNA synthesis

We conducted genomic DNA extraction as previously described (Clarke 2009) with slight modifications. In brief, we milled 500 mg of leaf tissue to a powder using a mortar and pestle. The powdered tissue was then homogenized in an extraction buffer (2% CTAB, 1% PVP, 1.4 M NaCl, 10 mM EDTA, 100 mM Tris–HCl, 1% β-mercaptoethanol) and incubated for 2 h at 65 °C. After incubation, we added 1 volume of chloroform to the mixture and

centrifuged it to remove proteins. Residual RNA that could interfere with downstream PCR was degraded using RNase A. The quality of the precipitated DNA was verified using 1% (w/v) agarose gel electrophoresis and a Nanodrop 2000c UV–Vis spectrophotometer (Thermo Fisher Scientific, USA).

We conducted total RNA isolation using the RNAprep Pure Kit (for plants) (TIANGEN Corporation, Beijing, China) following the manufacturer's instructions. We removed residual DNA in the isolated RNA using the DNase I treatment. We determined concentrations of extracted RNA using Nanodrop 2000c, and we assessed RNA quality applying 260/280 and 260/230 absorbance ratios. Furthermore, we reversely transcribed 5 μg of total RNA into cDNA using the PrimeScript II First Strand cDNA Synthesis Kit (Takara Biotechnology, Dalian, China). For cDNA synthesis of rapid amplification of cDNA ends (RACE) cloning, we used 1 μg of total RNA for first strand cDNA synthesis. RACE cloning was conducted with the SMARTer® RACE 5′/3′ Kit (Clontech Laboratories, USA) according to the manufacturer's instructions.

Full-length *LhSorNPR1* cloning

To obtain the partial coding sequence of *LhSorNPR1*, we designed a pair of degenerate primers (NPR1-deg-F and NPR1-deg-R; Table 1) based on the conserved regions of NPR1 orthologs. We designed gene-specific primers (GSP) for RACE (Table 1) based on the partial *LhSorNPR1* coding sequence obtained. We conducted primary RACE PCR in a 25 μL reaction mixture containing 20 ng of 3′ RACE template cDNA (or 5′ RACE template cDNA), 1.0 μmol L^{-1} of 3′ RACE-GSP-1 (or 5′ RACE-GSP-1), 200 μmol L^{-1} of dNTPs, 0.04 μmol L^{-1} Long Universal Primer A (Long UP), 0.2 μmol L^{-1} Short Universal Primer A (Short UP), and 1.5 U Taq DNA polymerase. We diluted PCR products of primary PCR by a ratio of 50:1 and used this as a template for the next step (Nested PCR). We conducted Nested PCR for 5′ RACE (or 3′ RACE) in a 25 μL reaction mixture containing 2 μL of diluted primary 5′ RACE (or 3′ RACE) PCR products, 1.0 μmol L^{-1} of 5′ RACE-GSP-2 (or 3′ RACE-GSP-2), 1.0 μmol L^{-1} of Nested Universal Primer A, 200 μmol L^{-1} of dNTPs, and 1.25 U Taq DNA polymerase. Amplification conditions for primary and nested RACE PCR followed the same protocol: 94 °C for 30 s, 68 °C for 30 s, and 72 °C for 3 min for 25 cycles. PCR products were gel purified and ligated into pMD18T vectors (Takara Biotechnology, Dalian, China) for sequencing (Sangon Biotech, Shanghai, China). We obtained the full-length sequence of *LhSorNPR1* by assembling the 5′ RACE sequence, the partial coding sequence, and the 3′ RACE sequence. We used the primer set (LhSorNPR1-full-length-F and LhSorNPR1-full-length-R) for full-length cDNA and full-length genomic DNA *LhSorNPR1* cloning.

LhSorNPR1 promoter isolation and *cis*-regulatory elements prediction

We conducted promoter cloning using hiTAIL-PCR according to the protocol previously proposed (Liu and Chen 2007). Initially, we conducted preamplifications in 25 μL reaction mixtures, each containing 200 μmol L^{-1} of dNTPs, 0.3 μM LhSorNPR1-promoter-TAIL-1, 1.0 μM of any one of the LAD primers (Table 1), 1.25 U Taq DNA polymerase, and 50 ng of the lily genomic DNA. We diluted PCR preamplification products by a ratio of 40:1 and used it as a template for the next step (primary amplification). We conducted PCR reactions for primary amplification in a 25 μL reaction mixture containing 200 μmol L^{-1} of dNTPs, 0.3 μM LhSorNPR1-promoter-TAIL-2 and AC1, 1 μL of diluted preamplification product, and 1.25 U Taq DNA polymerase. We conducted secondary amplifications of TAIL PCR in a 25 μL reaction volume, each containing 200 μmol L^{-1} of dNTPs, 0.3 μM LhSorNPR1-promoter-TAIL-3 and AC1, 1 μL of primary product (diluted by a ratio of 10:1), and 1.25 U Taq DNA polymerase. Thermal conditions for all hiTAIL-PCR reactions followed the protocol proposed by Liu and Chen. We submitted the sequence of the isolated *LhSorNPR1* promoter to PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and PLACE (http://www.dna.affrc.go.jp/PLACE/) databases for CREs prediction.

In silico analysis of *LhSorNPR1*

We predicted the open reading frame (ORF) of *LhSorNPR1* using the ORF finder (https://www.ncbi.nlm.nih.gov/orffinder/). Homology of the LhSorNPR1 putative protein sequence to other proteins was confirmed by querying the National Center for Biotechnology Information (NCBI) database using the Basic Local Alignment Search Tool (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi). We calculated the theoretical isoelectric point (pI) and molecular weight (MW) of LhSorNPR1 using the Compute pI/Mw tool (http://web.expasy.org/compute_pi/). The exon/intron organization of NPR1 genes was generated using the Gene Structure Display Server 2.0 (http://gsds.cbi.pku.edu.cn/). We conducted LhSorNPR1 sequence alignment with other NPR1 orthologs using the ClustalW program (http://www.ebi.ac.uk/clustalw). We constructed the phylogram of LhSorNPR1 and other NPR1 homologues from different species applying the neighbor-joining method using Mega 4.1 software (Tamura et al. 2007).

Table 1 List of primers used in the experiment

Experiment	Primer name	Primer sequence (5′–3′)
NPR1 degenerate PCR	NPR1-deg-F	CAYCGNGCNCTNGAYTCNGAYGA
	NPR1-deg-R	CGNCGNCCNAGYTCNACNGT
LhSorNPR1 5′ RACE	LhSorNPR1-5RACE-GSP-1	CAGTCGGGAGCAGCTCTACAAGCGC
	LhSorNPR1-5RACE-GSP-2	CGCTGAAACAGCGAGACCAGCTCGG
LhSorNPR1 3′ RACE	LhSorNPR1-3RACE-GSP-1	GGGTGACGAGAATCCACCGTGCTCTGG
	LhSorNPR1-3RACE-GSP-2	GGAGCGGGAGATGATGAGGAACCCC
LhSorNPR1 full-length cloning	LhSorNPR1-full-length-F	ATGGCCGACGCCGCCGAG
	LhSorNPR1-full-length-R	TCATTCTTCCATATCTACCAGACAGAC
5′ RACE and 3′ RACE	Universal Primer A (Long)	CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT
	Universal Primer A (Short)	CTAATACGACTCACTATAGGGC
5′ RACE and 3′ RACE	Nested Universal Primer A	AAGCAGTGGTATCAACGCAGAGT
LhSorNPR1 promoter cloning	AC1	ACGATGGACTCCAGAG
	LAD1-1	ACGATGGACTCCAGAGCGGCCGCVNVNNNGGAA
	LAD1-2	ACGATGGACTCCAGAGCGGCCGCBNBNNNGGTT
	LAD1-3	ACGATGGACTCCAGAGCGGCCGCVVNVNNNCCAA
	LAD1-4	ACGATGGACTCCAGAGCGGCCGCBDNBNNNCGGT
	LhSorNPR1-promoter-TAIL-1	GGCGCGTGAGGCTGACGACCTCGAGG
	LhSorNPR1-promoter-TAIL-2	ACGATGGACTCCAGTCCGGCCTGGTGCCGTTGGAGACGTAGGATGAGGT
	LhSorNPR1-promoter-TAIL-3	GGACCACTCGGCGGCGTCGGCCAT
LhSorNPR1 qPCR	LhSorNPR1 qPCR-F	CTTGATAAGTTCTTGGAGGACGAT
	LhSorNPR1 qPCR-R	GATGTAGACGATGATGACGATGAT
Construction of the LhSorNPR1 expression vector	LhSorNPR1-OE-F	TAGGATCCATGGCCGACGCCGCCGA
	LhSorNPR1-OE-R	CGGGTACCTCATTCTTCCATATCTACCAGACAG
AtPR1 qPCR	AtPR1-qPCR-F	GCTCTTGTTCTTCCCTCGAAAG
	AtPR1- qPCR-R	GCCTCTTAGTTGTTCTGCGTAGCT
AtPR2 qPCR	AtPR2- qPCR-F	ATCTCCCTTGCTCGTGAATCTCT
	AtPR2- qPCR-R	TCGAGATTTGCGTCGAATAGG
AtPR5 qPCR	AtPR5- qPCR-F	GAGGATCGGGAGATTGCAAA
	AtPR5- qPCR-R	CTCCACGGCAGCAATATTGA
AtACTIN2 qPCR	AtACTIN2-F	ACGGTAACATTGTGCTCAGTGGTG
	AtACTIN2-R	CTTGGAGATCCACATCTGCTGGA

Restriction sites for vector construction are underlined

Expression analysis of *LhSorNPR1* in different tissues and under various phytohormone treatments

For the tissue-specific expression of *LhSorNPR1*, we sampled roots, stems, leaves, petals, and scales from 2-month-old lilies that had fully bloomed. For gene expression analysis of *LhSorNPR1* under different phytohormone treatments, we subjected 30-day-old seedlings of lily plants to foliar application of 5 mM ETH, 10 mM SA, or 20 mM MeJA. For the latter treatment, MeJA was first dissolved in ethanol and then

diluted to the desired concentration in ddH2O. Pure water containing the same concentration of ethanol (1%) was used as the mock for MeJA treatment. For ETH and SA treatments, we used a foliar spray of pure water as the mock treatment. We sampled leaves 0, 2, 4, 8, 12, and 24 h after the phytohormone (or mock) treatments and subjected them to total RNA extraction. Furthermore, we reversely transcribed 500 ng of total RNA into cDNA using the HiScript II Q RT SuperMix for qPCR kit (Vazyme Biotech Co., Nanjing, China), following the manufacturer's instructions. We diluted the synthesized cDNA with RNase-free water and used it as a template for the subsequent qPCR test. We conducted real-time PCRs for gene expression level quantification in a reaction of 20 μL using the AceQ qPCR SYBR Green Master Mix kit (Vazyme Biotech Co., Nanjing, China). We designed the *LhSorNPR1* primer set for qPCR (LhSorNPR1 qPCR-F and LhSorNPR1 qPCR-R) using OligoArchitect (http://www.sigmaaldrich.com/china-mainland/zh/technical-documents/articles/biology/oligoarchitect-online). We used the *polyubquitin4* gene (GenBank accession no. DW718023) of the oriental hybrid lily 'Sorbonne' as the internal standard. We conducted qPCR reactions using the MX3000P qPCR thermo cycler (Stratagene, USA). We used the following thermal conditions for amplification: pre-denaturation for 10 min at 95 °C, followed by 40 cycles at 94 °C for 15 s and 60 °C for 30 s. After amplification, we charted melting curves (from 60 to 95 °C) to detect the existence of non-specific amplicons. We determined application efficiency from the slope of the plotted *LhSorNPR1* standard curve. We conducted calculations of gene expression levels using the relative expression software tool (REST) 2009, and we used the P(H1) test for statistical analysis of gene expressions (Pfaffl et al. 2002).

Overexpression of *LhSorNPR1* in *Arabidopsis*

To construct the LhSorNPR1-pBI121 overexpression vector, ORF primers (Table 1) with *Bam*HI and *Kpn*I sites were designed, and the amplified PCR product was inserted into a modified pBI121 vector preserved in our lab. After verification through sequencing, we introduced the LhSorNPR1-pBI121 recombinant plasmid into the *Agrobacterium tumefaciens* strain GV3101 using the heat-shock method. We used the resulting *A. tumefaciens* strain containing the LhSorNPR1-pBI121 vector to transform *Arabidopsis* Col-0 accession plants as previously described (Clough and Bent 1998). We screened *Arabidopsis* transformants on an Murashige and Skoog (MS) medium containing 50 μg/ml kanamycin. We used T3 homozygous lines for the subsequent experiments.

Expression analysis of *pathogenesis-related* genes in transgenic *Arabidopsis* plants

We extracted total RNA of 3-week-old *Arabidopsis* seedlings and reversely transcribed them into cDNA as described above. We quantified expression levels of *PR* genes (*PR1*, *PR2*, and *PR5*) by qPCR using the *Arabidopsis actin* gene (*AtACTIN2*) as the internal standard. We conducted calculations of gene expression levels and statistical analysis using REST 2009 software as described above.

Challenges of transgenic *Arabidopsis* to *Pseudomonas syringae* pv. tomato DC3000

The *Pseudomonas syringae* pv. tomato DC3000 (*Pst* DC3000) bacteria was streaked from a glycerol stock on agar plates (low salt LB) supplemented with 50 μg mL^{-1} rifampicin. After 2 d incubation at 28 °C, a single colony was inoculated in the liquid LB supplemented by 50 μg mL^{-1} rifampicin and incubated on a shaker, set at 28 °C for 8 to 12 h until OD600 reached 0.6 (Katagiri et al. 2002). The culture was centrifuged at 5000 g for 10 min to remove the supernatant, and the bacteria pellet was then resuspended in sterile water. We adjusted the bacteria concentration in a water suspension to $\sim 10^6$ cfu mL^{-1} (OD600 = 0.001), and this was used for leaf infiltration of 3-week-old plants (applying a needleless syringe). In order to further compare bacterial infection quantitatively, discs were punched from the leaves of wild-type and transgenic plants 3 days after infiltration. The leaf punches were surface sterilized and then milled in 1 mL distilled water using a mortar and pestle. The resultant suspensions were serially diluted and plated on LB agar plates supplemented in 50 μg mL^{-1} rifampicin. After being incubated at 28 °C for 2 days, the number of colonies was counted to calculate the colony forming units (CFU) per square centimeter of leaf tissue. Five days after inoculation, the leaves of Col-0 wild-type and *LhSorNPR1* transgenic plants were photographed to compare phenotypic changes.

Results

Isolation of the full-length *LhSorNPR1* cDNA

To obtain the partial coding sequence of the *NPR1* gene in lilies, we designed a pair of degenerate primers (Table 1) based on the conserved regions of the NPR1 orthologs from different plants. As predicted, we amplified a DNA fragment ~700 bp in the degenerate PCR (Fig. 1; L1). Using

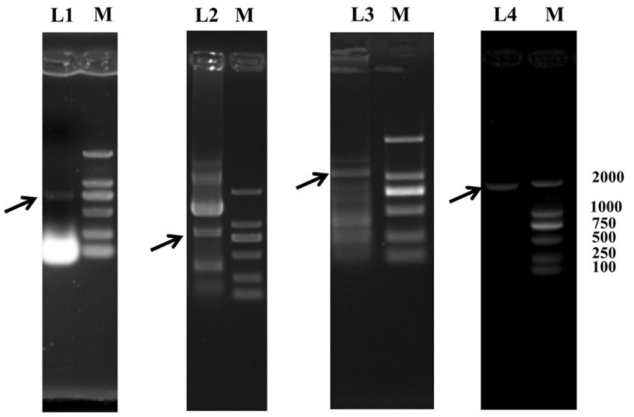


Fig. 1 Cloning of the *LhSorNPR1* gene by RACE. M: DL2000 marker; *L1*: product of degenerate PCR. The amplified partial coding sequence of *LhSorNPR1* is indicated by an *arrow*. *L2*: 5′ *LhSorNPR1* RACE products. The 5′ end fragment amplified by Nested PCR is indicated by an *arrow*. *L3*: 3′ *LhSorNPR1* RACE products. The 3′ end fragment amplified by Nested PCR is indicated by an *arrow*. *L4*: the 1854 bp LhSorNPR1 full-length cDNA amplified. The fragment amplified is indicated by an *arrow*

the partial coding sequence obtained, we designed 5′ RACE and 3′ RACE GSP primers. As indicated by the arrows in Fig. 1, we obtained amplicons of 864 bp and 1143 bp in size for the 5′ RACE (Fig. 1; L2) and the 3′ RACE (Fig. 1; L3) PCRs, respectively. We verified the assembled full-length *LhSorNPR1* sequence using PCR, and we amplified a 1854 bp DNA fragment corresponding to the *LhSorNPR1* ORF (Fig. 1; L4). The ORF encoded a protein of 617 amino acids with a predicted pI of 5.95 and a theoretical molecular mass of 68.87 kDa. The 5′ and 3′ untranslated regions (UTR) flanking the ORF were 140 and 250 bp, respectively. The verified full-length LhSorNPR1 sequence was submitted to GenBank and deposited under the accession number KY073343.

Genomic structure of *LhSorNPR1*

Using the *LhSorNPR1* full-length cloning primers (Table 1), we successfully amplified the genomic DNA fragment of *LhSorNPR1*, which was 5751 bp in length from the start codon to the stop codon. Further sequence alignments of *LhSorNPR1* cDNA and genomic DNA showed that there were four exons in the *LhSorNPR1* genomic DNA fragment, which essentially means that these exons were flanked by three introns. The exon/intron organization of *NPR1* genes was similar in different plants species, since *NPR1* orthologs in both *Arabidopsis* and rice share the same genomic structure with *LhSorNPR1* (Fig. 2). The conservation in genomic DNA structure suggested that *LhSorNPR1* could provide the same function as their orthologs.

Protein sequence analysis of LhSorNPR1

Multiple alignments of the deduced LhSorNPR1 protein sequence with NPR1 orthologs in *Arabidopsis* and rice showed that the NPR1 protein family shared several domains, such as the BTB/POZ domain (Fig. 3; dashed-line box) and the ankyrin repeats (Fig. 3; solid-line box). The BTB/POZ domain functions in the homodimerization of AtNPR1 in *Arabidopsis*, and the ankyrin repeats mediate interactions with the TGA2 transcription factor to control *PR* genes expression. In addition to these two domains that are well conserved, five cys residues (C82, C150, C155, C160, and C216) have been shown to play important roles in the oligomerization of AtNPR1 (Mou et al. 2003; Tada et al. 2008). Among these five residues, C82 and C216 were critical in the oligomerization of AtNPR1 (Fig. 3; solid triangles). Moreover, C82, C150, C155, and C160 were conserved in LhSorNPR1 (as shown in Fig. 3), but gln was substituted for C216. Post-translational modifications (PTM), such as phosphorylation, ubiquitination and sumoylation, also have profound impact on plant immunity regulated by NPR1. The S11/S15 (in AtNPR1) IκB-like phosphodegron (Spoel et al. 2009), whose phosphorylation was required for AtNPR1 ubiquitination and proteasome-mediated degradation was conserved in LhSorNPR1 (S15/S19; Fig. 3). While the second IκB-like motif (S55/S59 in AtNPR1), whose phosphorylation was critical for the inhibition of AtNPR1 sumoylation and degradation (Saleh et al. 2015), was not conserved in LhSorNPR1(Fig. 3). However, the small ubiquitin-like modifier (SUMO) interaction motif (SIM), which mediated conjugation of

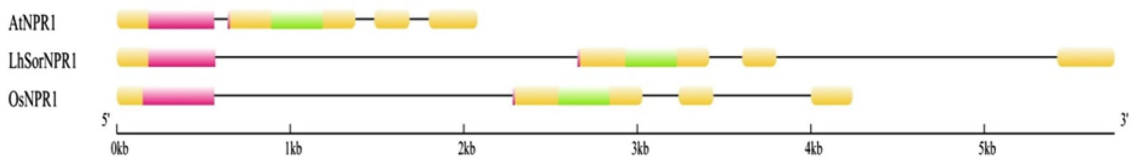


Fig. 2 Comparison of the genomic structure of *LhSorNPR1* between *AtNPR1* (U87794) and *OsNPR1* (DQ450948). Exons (*boxes*) are connected by introns (*black lines*) between them. The BTB/POZ domain and ankyrin repeats domain are represented by *boxes shaded* in *purple and green* respectively (color figure online)

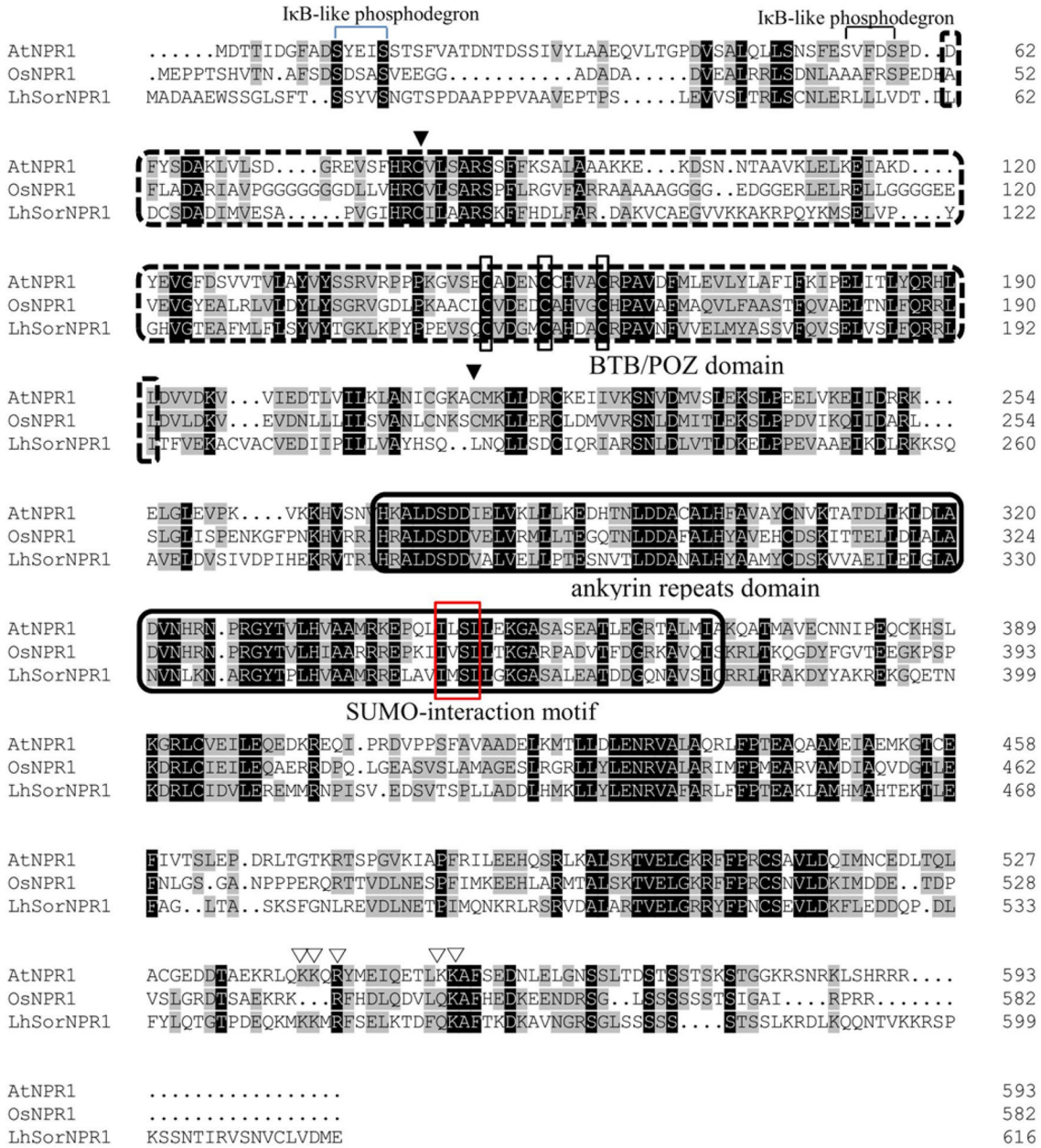


Fig. 3 LhSorNPR1 sequence alignment with NPR1s in *Arabidopsis* and rice. LhSorNPR1 was aligned with AtNPR1 (*Arabidopsis thaliana* NPR1; NP176610) and OsNPR1 (*Oryza sativa* NPR1; ABE11614) using ClustalW. Identical residues are shaded in *black*, and highly similar residues are shaded in *gray*. *Dashed-line boxes* represent the BTB/POZ domain. *Solid-lines* represent the ankyrin repeats. *Solid triangles* represent C82 and C216, which are essential to AtNPR1 oligomerization in cytoplasm. *Rectangular boxes* represent C150, C155, and C160, which also function in oligomer-monomer exchanges. *Hollow triangles* represent amino acid residues required for the nuclear translocation of AtNPR1. The small ubiquitin-like modifier (SUMO) interaction motif (SIM), which mediate conjugation of SUMO3 to the lysine residue(s) in AtNPR1, is highlighted by a *red box*. The S11/S15 IκB-like phosphodegron, whose phosphorylation is required for AtNPR1 ubiquitination and degradation, is highlighted with a *blue bracket*. The second IκB-like motif, S55/S59, whose phosphorylation is critical for the inhibition of AtNPR1 sumoylation and degradation, is highlighted with a *black bracket* (color figure online)

SUMO3 to the lysine residue(s) in AtNPR1, was conserved in LhSorNPR1. The translocation of NPR1 required the participation of the nuclear localization signal (NLS) at the C-terminus. Five basic residues (Fig. 3; hollow triangles), which were found to be critical for the nuclear localization of AtNPR1, were conserved in LhSorNPR1. The conservation of these functional domains in the NPR1 protein family implies that LhSorNPR1 could render similar functions for NPR1 orthologs in lilies.

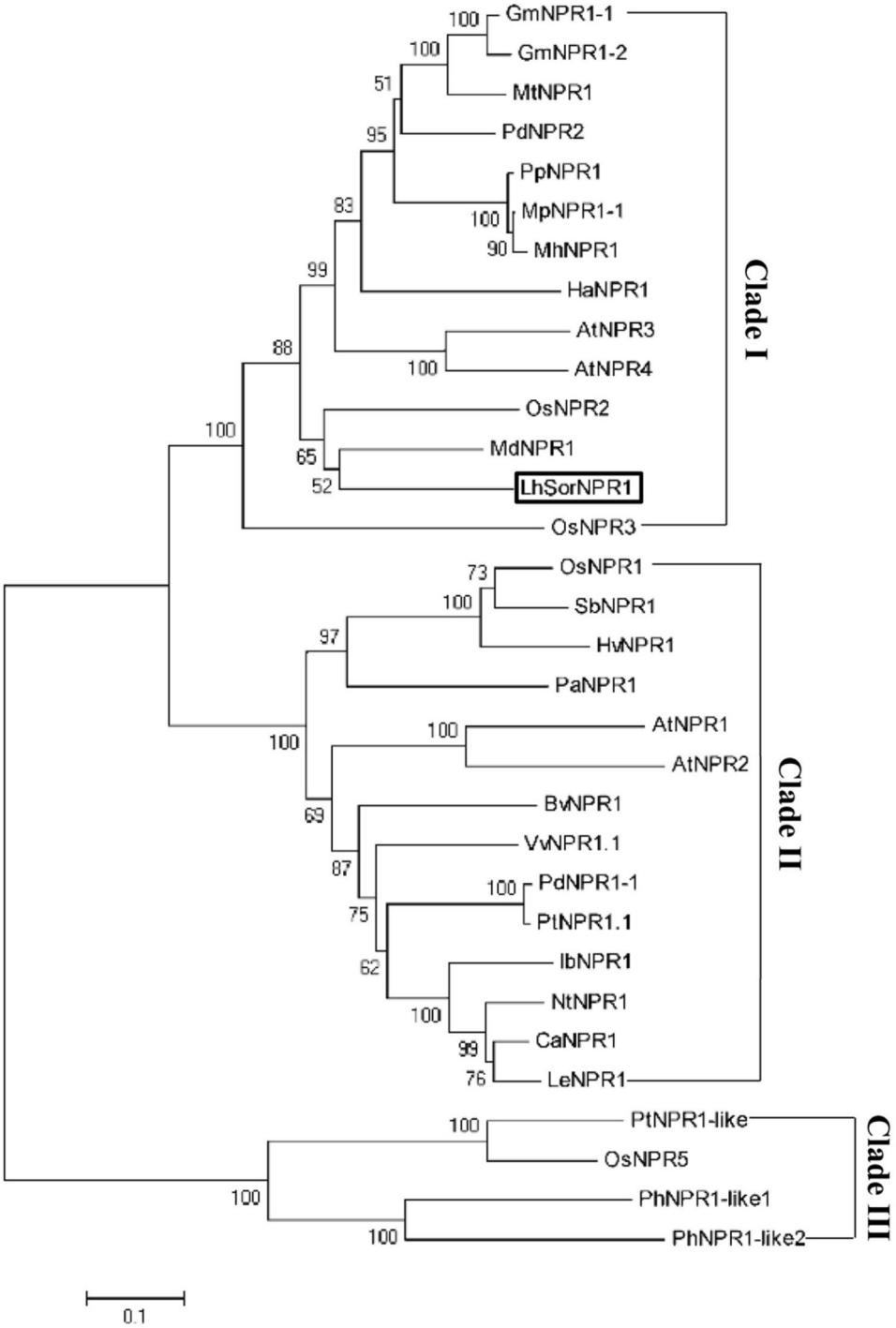


Fig. 4 Phylogram of LhSorNPR1 and other NPR1 homologues from different species. The Phylogenetic tree was constructed with MEGA4 using neighbor-joining (NJ) method. Branch length is proportional to time of divergence. The *scale bar* represents a 5% change. The accession numbers corresponding to all protein sequences used in the *phylogram* were summaried in Table 2

Phylogenetic analysis

Protein sequences of 32 NPR1 homologues in different plants were retrieved from Genbank to construct the phylogram. As shown in Fig. 4, NPR1-like genes from these species mainly grouped into three clades. LhSoNPR1 was most closely related to MdNPR1, a NPR1 ortholog originated from *Musa spp. ABB*.

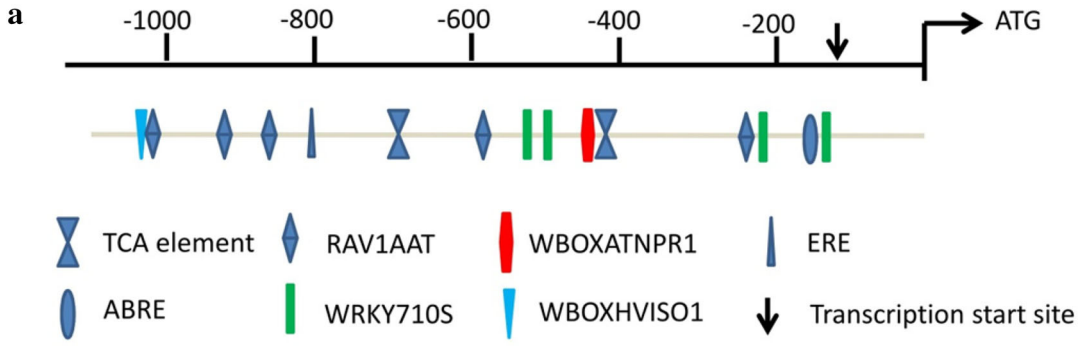
Analysis of potential *cis*-regulatory elements involved in the regulation of gene expression for the *LhSorNPR1* promoter

The *LhSorNPR1* promoter sequence, which corresponds to a DNA fragment of 1196 bp upstream of the start codon, was successfully isolated by employing the hiTAIL-PCR method. Subsequent sequence alignments of the promoter

Table 2 NPR1-like protein sequences retrieved from GenBank used for phylogram construction

Indentifier	Accession number	Species
AtNPR1	NP_176610	*Arabidopsis thaliana*
AtNPR2	NP_194342	*A. thaliana*
AtNPR3	NP_199324	*A. thaliana*
AtNPR4	NP_193701	*A. thaliana*
OsNPR1	AAX18700	*Oryza sativa*
OsNPR2	ABE11616	*O. sativa*
OsNPR3	ABE11618	*O. sativa*
OsNPR5	ABE11622	*O. sativa*
GmNPR1-1	ACJ45013	*Glycine max*
GmNPR1-2	ACJ45015	*G. max*
MtNPR1	XP_003594464	*Medicago truncatula*
PdNPR1-1	AEY99652	*Populus deltoides*
PdNPR2	AEE81755	*P. deltoides*
PpNPR1-1	ABK62792	*Pyrus pyrifolia*
PtNPR1.1	XP_002308281	*Populus trichocarpa*
PtNPR1-like	XP_002323261	*P. trichocarpa*
MhNPR1	ACU78081	*Malus hupehensis*
HaNPR1	AAT57642]	*Helianthus annuus*
MdNPR1	ACJ04030	*Musa spp. ABB*
SbNPR1	XP_002455011	*Sorghum bicolor*
HvNPR1	CAJ19095	*Hordeum vulgare subsp. vulgare*
PaNPR1	AEP68016	*Phalaenopsis aphrodite subsp. formosana*
BvNPR1	AAT57640	*Beta vulgaris*
VvNPR1.1	XP_002281475	*Vitis vinifera*
IbNPR1	ABM64782	*Ipomoea batatas*
NtNPR1	AAM62410	*Nicotiana tabacum*
CaNPR1	ABG38308	*Capsicum annum*
LeNPR1	AAT57637	*Solanum lycopersicum*
PhNPR-like1	XP_001757508	*Physcomitrella patens*
PhNPR-like2	XP_001759240	*P. patens*
MpNPR1-1	ACC77697	*Malus x domestica*

and the *LhSorNPR1* 5′ UTR region showed that the transcription start site (TSS) of *LhSorNPR1* was located at −140 bp upstream of the start codon (Fig. 5b). We found a TATA box (Fig. 5b), which is the binding site of general transcription factors or histones, located at −29 bp upstream of the TSS (an adenine). Several CAAT boxes, as shown in Fig. 5b, were also found in the *LhSorNPR1* promoter. In addition to these two CREs involved in general transcription, the presence of hormone responsive elements indicated that the *LhSorNPR1* expression was subjected to regulation by phytohormones in different ways (Fig. 5a). These CREs include the TCA element involved in SA response, the ABRE element involved in ABA response, and the ERE element involved in ethylene response. We also found several CREs types involved in defense signaling in the *LhSorNPR1* promoter. For example, we detected five potential RAV1AAT element sites (Fig. 5a) that could enable the binding of RAV1 proteins. RAV1 transcription factors regulated expressions of multiple defense-related genes through the binding of RAV1AAT CREs in the promoter region (Sohn et al. 2006). The presence of W boxes in the *LhSorNPR1* promoter showed that WRKY family proteins were involved in *NPR1* expression regulation in lilies. Furthermore, we detected a WBOXATNPR1 motif, the W box for WRKY protein binding in *AtNPR1*, in the *LhSorNPR1* promoter. This seemed to imply that similar mechanisms should exist in *LhSorNPR1* in relation to the WRKY transcription factor contributed expression control. In addition to the WBOXATNPR1 motif, we also detected W boxes in two other different types (WRKY71OS and WBOXHVISO1) in the *LhSorNPR1* promoter. The sequence of *LhSorNPR1* promoter and all potential cis-elements predicted are shown in Fig. 5b.



b

TCCAAGACGAT**CAAAT**CGAATTCCTCTACAACTTCTATATTTTGTAGATCTTCGAGTTCTTACGTTTAGGTCCT

CAAAACATAGTAA**CAAT**AATAGCAACTTGTAAACGCATTAACCTGGACTTATTCGCATAACTATGGATTACTGC

ACACCGAATATGGATT**ATTG**CACCCG**AGTCA**C**CAACA**TGTCGCACG**ATTG**AGTCTCTTGAATCGGACCACT

CGAGAGCCCGAGTATGCCTTCGCATAAGGAAGGACATACC**CAACA**TGAAGTACATGCTTTTGGTCTCATTTA

TGAGACTATAGGAGCTGGGAATAGTCCCCCTCCATTT**CAACA**GCTTTTAGGGATTTAAACATGGT**ATTG**TAT

GGTTTTGTGATGAAGTTCATCA**TTTGAATT**ATAGTATTACATTCTCAGGTATCGAACTAATATATCATTTTAGTT

GAGATACCCCAAAATTAGATATATTTA**TATACTTCTC**AAAAGCGTCGTACGACAAGTCCCCATTCCTCACCTTC

TCATCCACAAAGCATCGCGACTTCTTGAATCATATAGAATACAACTGCTTTCTCGTCTTATCTTTCCATCCTTCT

TTGTATAGC**CAACA**CAAACATCTTCCAAGGTCTCTCTCATCCTT**GTCA**CTATTTCCTAATG**TGA**C**AAT**ACATGA

TATTCACATCTAATTACTGTGAATCATCTAACTAATAGGAGATAATTATG**ATTG**AATAATCTTATTTACATGATTTA

TCAA**GTCAA**TAATTATGG**AAAAATATGG**CTTACCCATTAGTGATAAAGACCATTAAGCTACTTATAACACCTA

AATGTGAAGCTACACTTCTACCACCATGCT**CAAAT**CATTTTCTCACTACTTGAAATCATATATTAC**CAAAT**CAA

AATATGATAAATAAGGCAAACCAGAGACTCACTGCTCGGAAAGGAAACTA**TGTTGTCA**TATATCCATTCTATT

AGGGATCATATTCTCTATAGTACTATTTCTCAGGGTTTATGCCCAAAAAAACTCACCTCCTCTGCCCTCTATTAC

GTGTCATGGACTTTC**CAAT**CCATAGTCAAGGTCTTTGTACCCTTCCCCAGTCTATCAGTTTCTCCCC**CAAAT**T

CATCCCCCTGCT**CAAT**CAATCGAATTTACTCTCAAATTTTCTTGAATCCACTACCTC**CAAT**GGCGATCCGAATC

TAGTCACTCCGTCGCC**ATG**GCCGACGCCGCCGATGGTCC

◀**Fig. 5** Prediction of Cis-regulatory elements in *LhSorNPR1* promoter. **a** Schematic diagram of *cis*-regulatory elements involved in the regulation of *LhSorNPR1* expression in the promoter region. Distribution of core elements involved in *LhSorNPR1* regulation are shown as indicated. The scale on *top* of the bar represents the distance from the *LhSorNPR1* start codon. **b** Sequence analysis of *LhSorNPR1* promoter. 1: WBOXATNPR1, 2: RAV1AAT, 3: ERE, 4: TCA-element, 5: ABRE, 6: Transcription start site, 7: WBOXHVISO1, 8: TATA BOX, 9: CAAT BOX, 10: WRKY71OS. We obtained a 1196 bp DNA fragment upstream of the start codon using hiTAIL-PCR. *Cis*-regulatory elements in the obtained sequence were predicted by querying PlantCARE and PLACE databases

Expression analysis of *LhSorNPR1* in different tissues and under various phytohormone treatments

For *LhSorNPR1* expression analysis (using qPCR), we first plotted standard curves and dissociation curves to calculate the amplification efficiency and to test the amplification specificity. We calculated *LhSorNPR1* gene expression levels in different tissues relative to the calibrator (the stem expression level was set at 1.0). As shown in Fig. 6a, we found that *LhSorNPR1* expression levels in petals and roots were significantly higher than in stems (by a magnitude of 2.76 and 2.33, respectively). However, *LhSorNPR1* transcript levels in leaves and scales were not significantly different from stems.

Phytohormones, such as JA, SA, and ethylene, are major contributors to biotic stress signaling in plants. Hence, we investigated temporal expression patterns of *LhSorNPR1* in response to these three phytohormones. Exogenous application of MeJA, a functional analogue of JA, stimulated *LhSorNPR1* transcript accumulation in leaves. As shown in Fig. 6b, the *LhSorNPR1* expression significantly increased

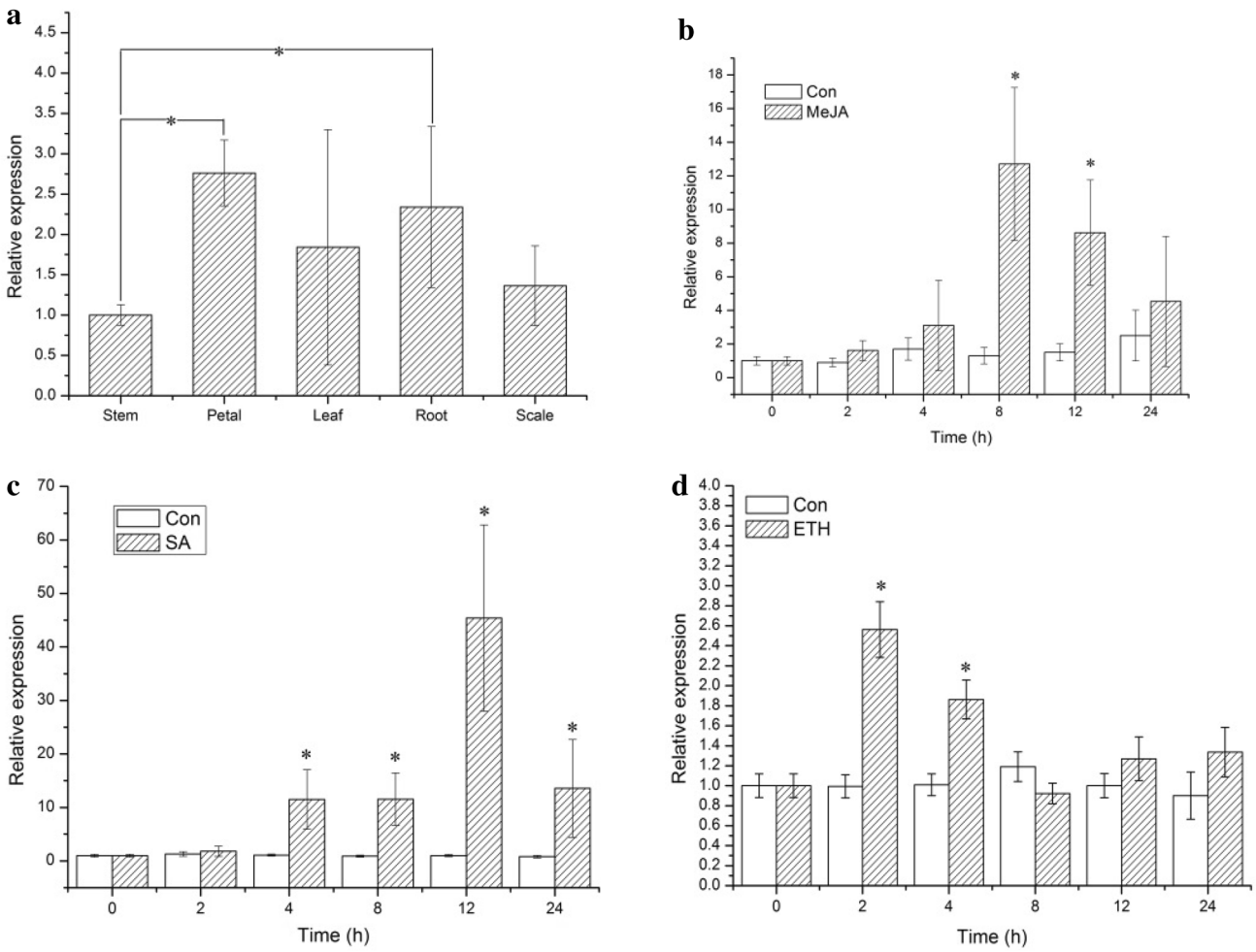
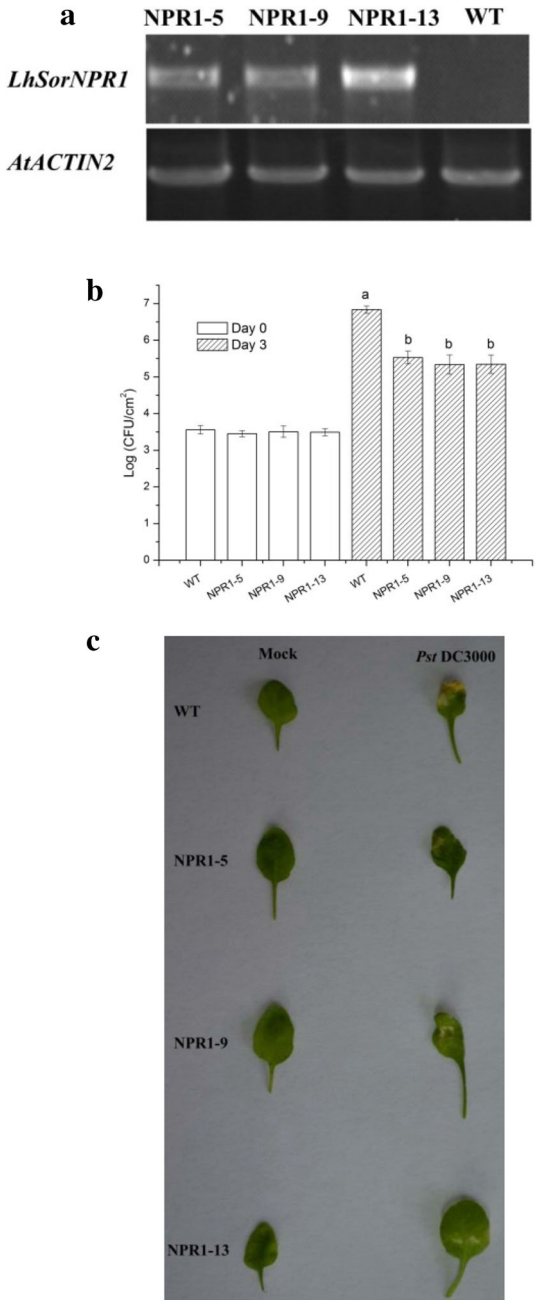


Fig. 6 Expression analysis of *LhSorNPR1* in different tissues and under various phytohormone treatments. **a** The tissue-specific expression of *LhSorNPR1*; **b** the temporal expression of *LhSorNPR1* in response to the methyl jasmonate (MeJA) treatment; **c** the temporal expression of *LhSorNPR1* in response to the sodium salicylate (SA) treatment; **d** the temporal expression of *LhSorNPR1* in response to the ethephon (ETH) treatment. We used the *LhSorNPR1* expression at 0 h as a calibrator (designated as 1.0) to determine the relative expression of the target gene at different time points. Values are presented as mean ± standard deviation (SD) for the three replicates. *Bars* labeled with an *asterisk* (*) indicate significant differences from the control at $P < 0.05$ (the REST statistical randomization test)



◄**Fig. 7** Resistance of Arabidopsis *LhSorNPR1* transgenic lines to *Pseudomonas syringae* pv. tomato DC3000. **a** Detection of the *LhSorNPR1* expression in transgenic *Arabidopsis*; total RNA was isolated from three T3 homozygous lines and the Col-0 wild-type plant; after reverse transcription, the full-length *LhSorNPR1* sequence was specifically amplified from *LhSorNPR1* transgenic plant species, but we did not detect *LhSorNPR1* expressions from the Col-0 wild-type. **b** Multiplication of *Pst* DC3000 in leaves of *LhSorNPR1* transgenic lines and the Col-0 wild-type plant; we collected leaf punches at infection sites 0 and 3 days after inoculation to quantify bacterial growth. Values represent mean ± standard deviation (SD) for the three replicates. *Bars* labeled with different letters differed significantly ($P < 0.05$; *t* test). **c** Disease symptoms of three independent transgenic lines and the Col-0 wild-type plant; leaves of transgenic lines and the wild-type plant species were inoculated with *Pseudomonas syringae* pv. tomato DC3000 ($OD_{600} = 0.001$) or sterile water (mock); we observed disease symptoms 5 days after inoculation

with a lapse in MeJA treatment time, and reached its peak level 8 h after treatment began (at a magnitude of 12.7 compared to the control). For the SA treatment (Fig. 6c), the *LhSorNPR1* expression was significantly up-regulated 4 h after treatment began, reaching a magnitude of 11.49 compared to the control, and then attained its highest expression level at 12 h (at a magnitude of 45.4 compared to the control). The exogenous application of ETH stimulated the *LhSorNPR1* expression, producing with a relatively lower intensity compared to MeJA and SA. After 2 h ETH treatment, the *LhSorNPR1* expression significantly increased, reaching a magnitude of 2.56 compared to the control. Although the *LhSorNPR1* expression was also significantly different from the control after 4 h, we observed no significant time point changes in gene expression levels after this point in time. These findings suggested that multiple phytohormones could induce the *LhSorNPR1* expression, and this inducibility could be correlated to hormone-responsive CREs in the promoter.

Enhanced resistance to *Pseudomonas syringae* pv. tomato DC3000 was observed in *Arabidopsis* expressing *LhSorNPR1*

Transgenic *Arabidopsis* lines constitutively expressing *LhSorNPR1* were produced to validate the gene function. We transformed *LhSorNPR1* into the Col-0 wild-type *Arabidopsis* under the control of CaMV 35S promoter. As shown in Fig. 7a, we observed high *LhSorNPR1* expressions in three transgenic lines of *LhSorNPR1*, but the *LhSorNPR1* expression was not detectable in the untransformed Col-0 wild-type plant. We then challenged these three transgenic lines (NPR1-5, NPR1-9, and NPR1-13) with virulent *Pst* DC3000. Three days after inoculation, the number of *Pst* DC3000 per cm^2 in leaf discs was quantified to compare pathogen multiplication statistically. As shown in Fig. 7b, the Col-0 wild-type and *LhSorNPR1* transgenic lines did not differ in the number of *Pst* DC3000 that were quantified immediately after infiltration (0 day). However, *Pst* DC3000 in the Col-0 wild-type multiplied to a significantly higher level than observed for *LhSorNPR1* transgenic lines 3 days after inoculation (3 days). Five days after inoculation, Col-0 wild-type *Arabidopsis* leaves showed severe signs of necrosis (Fig. 7c). The three *LhSorNPR1* transgenic lines, however, displayed less severe signs of necrotic phenotypes upon *Pst* DC3000 infection. The enhanced disease resistance provided by the

LhSorNPR1 overexpression in *Arabidopsis* suggested that *LhSorNPR1* had a *NPR1* ortholog encoded within it that promoted lily disease resistance.

LhSorNPR1 overexpression elevated the transcript levels of *pathogenesis-related* genes in *Arabidopsis*

To determine whether the *LhSorNPR1* overexpression elevated expression levels of *PR* genes, we quantified transcript levels of three marker genes (*PR1*, *PR2*, and *PR5*) for SA-mediated defense pathways (using qPCR). As shown in Fig. 8a, we found that *LhSorNPR1* expression significantly enhanced the *PR1* expression in all three transgenic lines. The transcript level of *PR2* was significantly elevated in NPR1-13 transgenic line, reaching a magnitude of 2.7 compared to the wild-type control (Fig. 8b). For the *PR5* expression, the NPR1-5 and NPR1-13 transgenic lines showed a significant increase in *PR5* transcript level compared to the WT control (Fig. 8c). Elevated transcript levels of *PR1*, *PR2*, and *PR5* in conjunction with the enhanced resistance to *Pst* DC3000 in *LhSorNPR1* transgenic lines indicated that *LhSorNPR1* disease resistance could be correlated to the enhanced expression of *PR* genes.

Discussion

The aim of this study was to characterize LhSorNPR1, a novel member of the NPR1 protein family from the oriental hybrid lily 'Sorbonne'. Multiple sequence alignment showed that LhSorNPR1 exhibited a high level of identity and similarity to other NPR1 orthologs in plant species. For example, LhSorNPR1 shared 64.96% identity and 73.09% similarity to GhNPR1, a NPR1 ortholog originating from *Gladiolus hybridus*. A phylogenetic investigation of 32 NPR1s showed that LhSorNPR1 was phylogenetically closely related GdNPR1, a NPR1 orthologs originating from *Musa spp. ABB* (Fig. 4). Analysis of the intron/exon organization showed that LhSorNPR1 consisted of four exons and three introns. The intron/exon structure of *NPR1* was conserved in *Arabidopsis* and rice as well as in other species, such as *Theobroma cacao* (Shi et al. 2010), *Musa* spp. ABB (Zhao et al. 2009), *Malus hupehensis* (Zhang et al. 2012), and soybean (Sandhu et al. 2009). Conservation of *NPR1* in the intron/exon organization across different species (including lilies) indicated that the genomic structure of the NPR1 protein family was conserved. NPR1 orthologs share several domains, including the BTB/POZ domain, the ankyrin repeat, and the NLS, which were all found in LhSorNPR1. Disulfide bridges played a critical role in protein folding and structure stability maintenance (Patil et al. 2015). Previous studies demonstrated that five

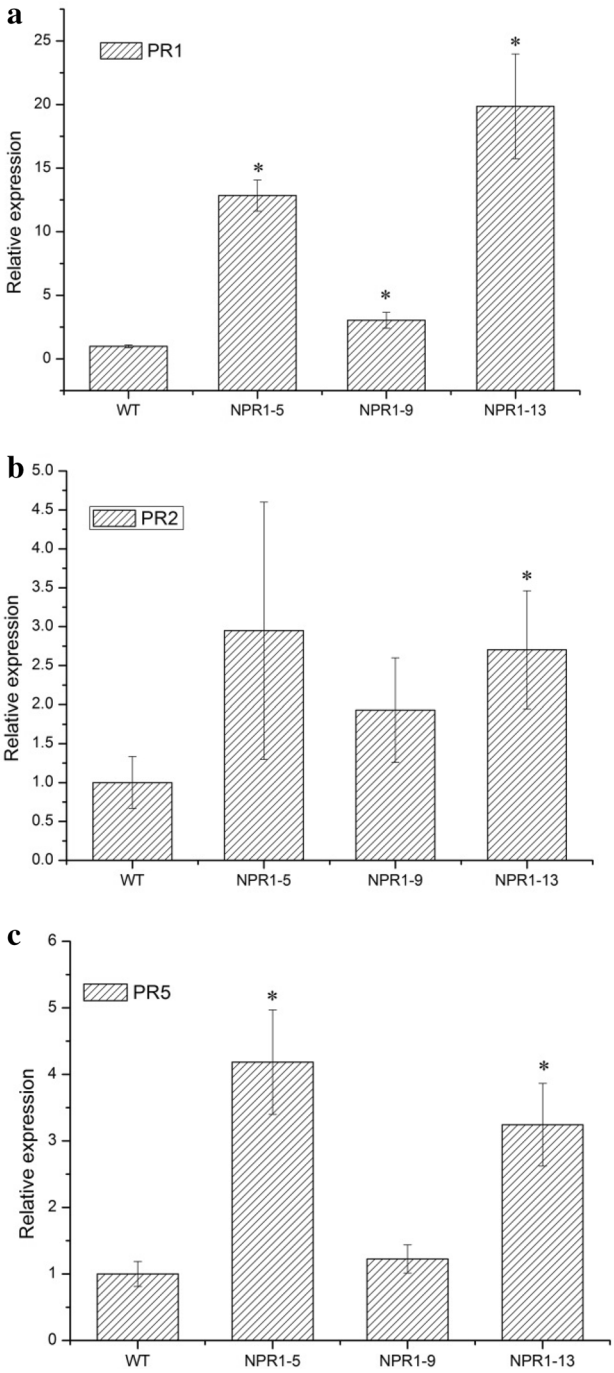


Fig. 8 Expression analysis of *PR* genes in *Arabidopsis* transgenic lines. **a** *PR1* transcript levels in *LhSorNPR1*-expressing transgenic plants and untransformed Col-0 wild-type *Arabidopsis*. **b** *PR2* transcript levels in *LhSorNPR1*-expressing transgenic plants and untransformed Col-0 wild-type Arabidopsis. **c** *PR5* transcript levels in *LhSorNPR1*-expressing transgenic plants and untransformed Col-0 wild-type *Arabidopsis*. We used the expression level in the Col-0 wild-type Arabidopsis as a calibrator (set at 1.0) to calculate the relative expression of *PR1*, *PR2*, and *PR5* in transgenic lines. Values are shown as mean ± standard deviation (SD) for the three replicates. *Bars* labeled with an *asterisk* (*) indicate significant differences from the Col-0 wild-type control at $P < 0.05$ (REST statistical randomization test)

cysteine residues (cys82, cys150, cys155, cys160, and cys216) were important for the oligomer-monomer exchange of AtNPR1 (Mou et al. 2003; Tada et al. 2008). Among these five cysteine residues, cys82 and cys216 were found to be critical for the oligomer formation of NPR1 in *Arabidopsis*. Site-directed mutagenesis in either of these two cysteine residues caused monomerization and nuclear translocation of AtNPR1 to occur (Mou et al. 2003). In LhSorNPR1, four of these cys residues were conserved, cys216 being the exception. In point of fact, LhSorNPR1 was not the only NPR1 ortholog reported to have a non-conserved cys216. MhNPR1 (Zhang et al. 2012), GmNPR1-1 (Sandhu et al. 2009), GmNPR1-2, and MpNPR1 (Zhao et al. 2009) were all found to be inconsistent for cys216 residue after sequence alignment. Taking into consideration the importance of cys216, the inconsistence of this residue in different NPR1 orthologs indicated that additional cys residue(s) might be involved in facilitating oligomerization of NPR1 in cytoplasm. The SIM domain, which functioned in mediating conjugation of SUMO3 to the lysine residue(s) in AtNPR1 was conserved in LhSorNPR1 and OsNPR1. However, a IκB-like phosphodegron (S55/S59 in AtNPR1), whose phosphorylation is critical for the inhibition of AtNPR1 sumoylation was not conserved in LhSorNPR1. In light of the conservativeness of the SIM domain in NPR1 orthologues, it is likely that some other serine residues might play critical roles in regulation of sumoylation in LhSorNPR1.

The expression of a specific gene was mainly driven by the adjacent promoter. Analysis of the *LhSorNPR1* promoter sequence uncovered CREs contributing to *LhSorNPR1* expression regulation. The presence of the TCA element in the *LhSorNPR1* promoter indicated that the *LhSorNPR1* expression was responsive to SA, which was confirmed by qPCR analysis. At the same time, the *LhSorNPR1* promoter also contains potential elements responsible for ethylene and abscisic acid. Although we did not find the motif for JA response, the *LhSorNPR1* expression was responsive to the exogenous MeJA treatment (Fig. 6b). The presence of multiple phytohormone responsive elements in conjunction with the inducibility of *LhSorNPR1* by MeJA, SA, and ETH revealed that the *LhSorNPR1* expression was regulated by multiple hormones. Moreover, transcription factors, such as WRKY family proteins (Hwang and Hwang 2010), RAV1 proteins (Zhong et al. 2015), and ASF-1 proteins, have been reported to play important roles in *NPR1* induction in different species. W boxes, especially the WBOXATNPR1 motif (Yu et al. 2001), within the *LhSorNPR1* promoter, indicated that similar regulation mechanisms could exist for WRKY-involved expression regulation in *Arabidopsis* and lilies.

As a key node of SA-mediated signal transduction, *NPR1* is a suitable candidate for genetic engineering in enhancing disease resistance in plants. Previous studies of carrots (Wally et al. 2009), citrus (Dutt et al. 2015), rice (Bai et al. 2011), and apples (Malnoy et al. 2007) have shown the feasibility of enhanced disease resistance by overexpressing *NPR1*. The *NPR1* conferred resistance to *Pseudomonas syringae* and *Peronospora parasitica* was reported to exhibit a dosage-dependent manner (Cao et al. 1998) in transgenic *Arabidopsis*. Hence, it is possible that the overexpression of *LhSorNPR1* could be used as a strategy to enhance disease resistance in lilies. Recently, the pivotal roles *NPR1* played in salt and oxidative stress tolerance were also uncovered (Jayakannan et al. 2015). The findings of this study provide evidence of the importance of *NPR1* in SA-mediated defense signaling. Moreover, our research and data on *LhSorNPR1* could facilitate the genetic engineering of lilies in the future.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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