Characterization of LhSorTGA2, a novel TGA2-like protein that interacts with LhSorNPR1 in oriental hybrid lily Sorbonne

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Abstract

Background: Non-expressor of pathogenesis-related genes 1 (NPR1) regulates expression of pathogenesis-related (PR) genes by interacting with TGA family proteins during systemic acquired resistance (SAR). However, no TGA-like proteins or their interacting partners have been characterized in lily.

Results: In the present study, LhSorTGA2, a novel TGA-like protein, was identified as an interacting partner of LhSorNPR1 (an NPR-like protein) by bimolecular fluorescence complementation (BIFC) and yeast two-hybrid assay (Y2H). Subcellular localization of GFP-tagged proteins targeted LhSorTGA2 to the nucleus, whereas GFP-labeled LhSorNPR1 was observed both in the nucleus and at the cytomembrane. Sequence alignment revealed that LhSorTGA2 was featured with a basic leucine zipper (bZIP) domain and two glutamine rich acid domains (QI and QII). Further phylogenetic analysis showed that TGA family proteins can be grouped into three subclades, within which LhSorTGA2 was clustered into subclade I, together with AtTGA2/5/6. Expression of LhSorTGA2 was investigated in different tissues by qPCR, and the highest expression level was observed in stem. Besides, when treated with phytohormones (SA, MeJA, ETH and ABA) or fungal pathogen Botrytis elliptica, LhSorTGA2 expression was also induced at different time points post treatments.

Conclusions: Collectively, these results suggested that LhSorTGA2 was an interacting partner of LhSorNPR1, which might function in regulating expression of PR genes in lily during SAR.

Keywords: Non-expressor of pathogenesis-related genes 1, TGA-like proteins, Protein interaction, Gene expression, Subcellular localization, Lilium spp.

Background

Infections at local sites often induce resistance against further invasions of pathogenic organisms in the distal uninfected parts of plants. This primed resistance, which protects plants from further invasion of a broad spectrum of pathogens at the whole-plant level, is referred to as systemic acquired resistance (SAR) (Fu and Dong 2013). The establishment of SAR is accompanied by accumulation of salicylic acid (SA) (Gao et al. 2015), and induction of pathogenesis-related (PR) genes. PRs encode for small proteins, of which some are endowed with direct antimicrobial activities. Since SA level, PR accumulation, and SAR are tightly linked, SAR-conferred resistance could be severely compromised either by interfering the level of SA synthesis or by disrupting signaling pathways controlling PR gene expression (Gaffney et al. 1993). Mutation of the non-expressor of PR genes 1 (NPR1) gene, a central node in SA-mediated defense signaling, blocks the priming of SAR (Cao et al. 1997). NPR1 lacks a DNA binding domain itself, but features with an ankyrin repeat domain and a BTB/POZ domain, both of which mediate protein–protein interaction (Pieterse and Van Loon 2004). The ankyrin repeat at C-terminus interacts with TGA2...
to activate PRI transcription, which is critical to plant defense against biotrophic pathogens in the state of SAR (Fan and Dong 2002; Zhang et al. 1999).

TGA proteins are members of the group D basic region/leucine zipper (bZIP) transcription factors (TFs) (Jakoby et al. 2002), which recognize the TGACG motif (also known as activation sequence 1; as-1) in the promoter region (Gatz 2013). With the identification of as-1 elements in promoters of a number of genes, the crucial roles TGA TFs played in gene expression regulation were unveiled. Mutagenesis of LS7 element, which contained a TGA-binding site, resulted in complete abolishment of PRI expression. On the contrary, mutation of LS5, another TGACG motif containing element, augmented PRI expression (Kesarwani et al. 2007). The opposite effects exerted by these two TGACG motifs to PRI expression implied that transcription of genes containing as-1 elements in the adjacent promoter, might subject to complex regulation by different TGAs in the genome.

Lilies are herbaceous perennials that renowned for their elegant flowers. However, bulb and cut flower production in genus Lilium, are both under severe threat of pathogens in different types. For instance, lily bulbs can be infected by some soil-borne fungal pathogens (Fusarium oxysporum, Penicillium alboareum, and Penicillium tulipae), causing rot of scales, bulb bases, and buds (Lecomte et al. 2016; Wang et al. 2017a). Furthermore, infection of air-borne fungi, such as Botrytis cinerea and Botrytis elliptica, could lead to fire blight disease, which is destructive to cut flower production (Huang et al. 2012; Wang et al. 2017d). In addition, multiplication of viruses (lily symptomless virus, cucumber mosaic virus, and lily mottle virus) also affects plant growth, resulting in leaf mottle and contortion, as well as stunted growth of lily plants (Zhang et al. 2016). To counteract pathogenic organisms, a comprehensive understanding of defense related genes that govern plant immunity in lily would be of great necessity.

In the present study, LhSorTGA2, a TGA2-like protein that interacted with LhSorNPR1 (Wang et al. 2017b) was characterized. Subcellular localization revealed that presence of GFP-tagged LhSorNPR1 was detected both in the nucleus and at the cytomembrane, but the GFP-tagged LhSorTGA2 was predominantly detected in the nucleus. LhSorTGA2 expression in response to various phytohormone treatments and fungal pathogen B. elliptica infection was also explored.

**Methods**

**Plant material and treatments**

Bulbs (10–12 cm in diameter) of oriental hybrid cultivar ‘Sorbonne’ were planted in plastic pots containing peat moss as a growing substrate. All pots were then transferred to the growth chamber of Gaolan Station of Agricultural and Ecological Experiment, to keep plants under controlled conditions (16 h light/8 h dark; 22 °C). Nicotiana benthamiana plants were raised under the same conditions for observation of protein localization in planta.

**Full-length cloning of LhSorTGA2**

Total RNA was extracted from the leaves of 1-month-old seedlings using the RNAprep Pure Kit for plants (TIANGEN Corporation, Beijing, China). Partial coding sequence of LhSorTGA2, which was annotated in our previous RNA-seq data, was used to design gene specific primers (5RACE-1, 5RACE-2, 3RACE-1, 3RACE-2; Table 1) for rapid amplification of cDNA ends (RACE). 5’ RACE and 3’ RACE were conducted according to the instructions of the SMARTer® RACE 5/3’ Kit (Takara, China). The cDNA full-length sequence of LhSorTGA2 was obtained by assembling the 5’-RACE sequence, the partial coding sequence, and the 3’-RACE sequence.

**In silico analysis of LhSorTGA2**

The open reading frame (ORF) of the assembled LhSorTGA2 full-length cDNA was predicted by ORF finder. The theoretical isoelectric point and molecular weight were calculated by using the Compute pl/Mw tool of ExPASy. Sequences of TGA proteins were aligned with the Clustal W program. Construction of phylogenetic tree was carried out by Mega 5 software using the neighbor-joining method (Tamura et al. 2011).

**Subcellular localization and protein–protein interaction validated by BIFC**

The ORF of LhSorTGA2 without stop codon was amplified using primers LhSorTGA2-sub-F and LhSorTGA2-sub-R, and inserted into SpeI digested pCAMBIA1302 vector to express LhSorTGA2 in frame with GFP at the N-terminus. For the vector construction of LhSorNPR1, the ORF of LhSorNPR1 was amplified with primers LhSorNPR1-sub-F and LhSorNPR1-sub-R, and cloned into Ncol and SpeI digested pCAMBIA1302 vector after sequencing. The recombinant plasmids pCAMBIA1302-LhSorTGA2 and pCAMBIA1302-LhSorNPR1 were each introduced into Agrobacterium tumefaciens (strain GV3101) competent cells via the freeze-thaw method. GV3101 cells harbouring pCAMBIA1302-LhSorTGA2 or pCAMBIA1302-LhSorNPR1 construct were infiltrated into leaf epidermal cells of 4-week-old N. benthamiana plants using a 1-ml needleless syringe. The pCAMBIA1302 empty vector was used as the control for protein localization.

To validate the protein–protein interaction between LhSorTGA2 and LhSorNPR1, the bimolecular
fluorescence complementation (BiFC) assay was performed using vectors described previously. *LhSorNPR1* was constructed into the pSPYNE( R)173 vector to express *LhSorNPR1* in fusion with the N-terminus of YFP (LhSorNPR1-YFPN), and *LhSorTGA2* was inserted into the pSPYCE(M) vector to express *LhSorTGA2* in fusion with the C-terminus of YFP (LhSorTGA2-YFPC). The resulting recombinant plasmids, pSPYNE-LhSorNPR1 and pSPYCE-LhSorTGA2, were transformed into GV3101 and co-infiltrated into *N. benthamiana* leaf epidermal cells using a protocol described previously (Kerppola 2013). Fluorescence of YFP or GFP was observed 72 h post infiltration using a confocal laser scanning microscope (Leica TCS SP8, Leica Microsystems, Germany).

**Yeast two-hybrid assay**

To further confirm the protein interaction between *LhSorTGA2* and *LhSorNPR1*, the ORF of *LhSorNPR1* was amplified (LhSorNPR1-GBKT7-F, LhSorNPR1-GBKT7-R; Table 1) and inserted into pGBK vector to construct the pGBK-LhSorNPR1 bait plasmid. The prey plasmid, pGADT7-LhSorTGA2, was generated by cloning the *LhSorTGA2* ORF into the pGADT7 vector after amplification with primers (LhSorTGA2-GADT7-F, LhSorTGA2-GADT7-R; Table 1). Constructed vectors were co-transformed into AH109 competent yeast cells in pairs, and transformants were plated on DDO and QDO mediums to test protein interactions. Transformants were also streaked on QDO medium supplemented with 40 μg/ml 5-bromo-4-chloro-3-indoxyl-α-D-galactopyranoside (X-α-Gal) to further confirm interaction of different co-transformants.

**Gene expression analysis by qPCR**

For tissue-specific expression of *LhSorTGA2*, root, stem, leaf, petal, and scale were sampled from 2-month-old fully bloomed plants and subjected to total RNA isolation as described above. For *LhSorTGA2* expression in respond to hormone or pathogen treatment, lily seedlings of 1-month-old were subjected to 20 mM methyl jasmonate (MeJA), 10 mM sodium salicylate (SA), 5 mM ethephon (ETH), 2 μM abscisic acid (ABA) or *B. elliptica* (8 × 10⁵ conidia/ml) inoculation treatments. Preparation of phytohormone solutions, the *B. elliptica* inoculum, and the corresponding mock treatments were performed according our previous report (Wang et al. 2017a). Leaves were sampled at 0, 2, 4, 8, 12, and 24 h post treatments, and froze immediately in liquid nitrogen and kept in a refrigerator at −80 °C until use.

After RNA extraction, 500 ng of total RNA was reversely transcribed to cDNA with the HiScript II Q RT SuperMix for qPCR kit (Vazyme Biotech, Nanjing, China). *LhSorTGA2* specific primers (LhSorTGA2 qPCR-F, LhSorTGA2 qPCR-R; Table 1) were used for qPCR amplification, and the housekeeping gene *polyubiquitin 4* was used as an internal reference (Wang et al. 2017c). All PCR reactions were conducted in a MX3000P qPCR thermocycler system (Stratagene Corp., USA). The following protocol

### Table 1 List of primers used in the experiment

| Experiment        | Primer name       | Primer sequence (5′–3′)                      |
|-------------------|-------------------|---------------------------------------------|
| LhSorTGA2 5′ RACE | LhSorTGA2-5RACE-1 | CAGGTGACTGACGATCCTCTCAATTTCGG               |
|                   | LhSorTGA2-5RACE-2 | ATAGCCCCGAAGTCAGAAACCCCGG                  |
| LhSorTGA2 3′ RACE | LhSorTGA2-3RACE-1 | GAACCTCAGAGCTCCTCTCCAGACAG                 |
|                   | LhSorTGA2-3RACE-2 | GGGTCAGATGCTAGCGCGATGCG                   |
| BIFC              | LhSorTGA2-BIFC-F  | CGCTCATGATGACTCCTCTCCCGGTTTTCT            |
|                   | LhSorTGA2-BIFC-R  | CCGTAGCTCTTCCGCGGCTAGCGG                  |
|                   | LhSorNPR1-BIFC-F  | CCGGATCTCATTCCCGCTACATCTACCCAGACA         |
|                   | LhSorNPR1-BIFC-R  | CCGGATCTCATTCTCCGCTACATCTACCCAGACA         |
| Y2H               | LhSorTGA2-GADT7-F | CCGAATCTAGCAGCTCTCCCGGTTTTCT              |
|                   | LhSorTGA2-GADT7-R | CCGTAGCTCTTCCGCGGCTAGCGG                  |
|                   | LhSorNPR1-GBKT7-F | CCGCATGGAGATCGCCAGCGCCCGGCGGA             |
|                   | LhSorNPR1-GBKT7-R | CCGGATCTCATTCTCCCGCTACATCTACCCAGACA        |
| Subcellular localization | LhSorNPR1-sub-F | ATATCCCATGTAATGGCAGCGGCGGCGGA             |
|                   | LhSorNPR1-sub-R  | CGACTAGTTTCTCCGCTACATCTACCGAGCGACAGA       |
|                   | LhSorTGA2-sub-F  | CCGATGTTTTCTCCGCTACATCTACCGAGCGACAGA       |
|                   | LhSorTGA2-sub-R  | CCGCATGTATGAGCTCCTCTCCCGGTTTTCTGA         |
| LhSorTGA2 qPCR    | LhSorTGA2 qPCR-F  | TCAGATGGCAGCTGCGGATGG                   |
|                   | LhSorTGA2 qPCR-R  | CGGATGGCAGCTGCGGATGG                   |

Restriction sites for vector construction are underlined.
was used for amplification: pre-denaturation for 10 min at 95 °C, followed by 40 cycles of 94 °C for 15 s, 55 °C for 15 s, and 72 °C for 30 s. The relative expression software tool (REST) was used for gene expression data analysis, and we applied P(H1) testing for statistical analysis. The Origin Pro 8.1 software was used for data graphing.

Results

Molecular cloning and sequence analysis of *LhSorTGA2*

The partial coding sequence of *LhSorTGA2* was retrieved from sequences annotated in our previous RNA-Seq data. Gene specific primers were designed for 5′ RACE and 3′ RACE PCR to obtain the *LhSorTGA2* full-length sequence. DNA fragments amplified during RACE cloning were shown in Additional file 1: Figure S1. The *LhSorTGA2* cloned was 1010 bp in length, comprising a 5′ untranslated region (UTR) of 337 bp, a predicted open reading frame (ORF) of 1278 bp, and a 3′ UTR of 395 bp. The ORF encoded a protein of 425 amino acids with a theoretical molecular mass of 47.34 kDa and a predicted isoelectric point (pI) of 6.74. The *LhSorTGA2* full-length cDNA sequence has been submitted to GenBank and deposited under the Accession Number MF685037.

Alignment of *LhSorTGA2* protein sequence to TGAs in *Arabidopsis thaliana* revealed that *LhSorTGA2* was highly similar to members of the TGA family protein (Fig. 1). *LhSorTGA2* contained the feature structures of bZIP transcription factors: a DNA binding domain (DBD) and a leucine zipper that enables protein dimerization. Within the DBD, glutamine, alanine, and serine residues, which were highly conserved for all TGA homologues were identified. Phosphorylation of the serine residue within the DBD led to DNA binding impediment, suggesting that phosphorylation might involve in the fine tune of transcription control of *LhSorTGA2*-regulated downstream genes (Kirchler et al. 2010). Leucine zippers of TGA proteins were formed by four heptads, within which three conserved leucine residues and one glycine residue were identified (Fig. 1). Two glutamine rich acid domains (QI and QII), which functioned in transcription activation, were also found in *LhSorTGA2*. The signature sequence for group D bZIP transcription factors, Yx₂RL[RQ]ALSS[LS]W, was identified at the C-terminus of all TGA homologues. A phylogenetic tree was built to analyze the evolutionary relationship of *LhSorTGA2* with other TGA family proteins in plants. As shown in Fig. 2, TGA family proteins were subdivided into three clades, and *LhSorTGA2* was most closely related to *GhTGA2* in subclade I.

Subcellular localization of *LhSorTGA2* and *LhSorNPR1*, and protein–protein interaction validated by BIFC

*LhSorTGA2* and *LhSorNPR1* (GenBank Accession: KY073343) were fused to the N-terminus of GFP respectively, and the fusion proteins were transiently expressed in *N. benthamiana* leaf epidermal cells under the control of CaMV 35S promoter to examine their subcellular localization. As shown in Fig. 3a, fluorescence of GFP-tagged *LhSorTGA2* was predominantly detected in the nucleus. Different from *LhSorTGA2*, fluorescence of the *LhSorNPR1*-GFP infusion protein was observed both in the nucleus and at the cytomembrane. Strong green fluorescence was detected throughout the entire cell for the pCAMBIA1302-GFP control as expected. Our observation of subcellular localization of *LhSorTGA2* and *LhSorNPR1* was in agreement with their corresponding orthologues in *Gladiolus hybridus* from previous report (Zhong et al. 2015).

Protein–protein interaction between *NPR1* and TGA2 was first identified in *Arabidopsis*. To test whether the interaction between *LhSorNPR1* and *LhSorTGA2* exists or not, we performed BIFC experiment by co-expressing *LhSorNPR1*-YFP₁ and *LhSorTGA2*-YFP₂ transiently in *N. benthamiana* leaf epidermal cells. As shown in Fig. 3b, strong fluorescence was detected in the *LhSorNPR1*-YFP₁ and *LhSorTGA2*-YFP₂ co-infiltration treatment, indicating reconstitution of YFP fluorophore by the interaction between *LhSorNPR1* and *LhSorTGA2*. In contrast, co-infiltration of *LhSorNPR1*-YFP₁ and YFP₂ or YFP₁ and *LhSorTGA2*-YFP₂, did not produce any detectable fluorescent signal. These findings indicate that *LhSorNPR1* interacts with *LhSorTGA2*.

Confirmation of *LhSorNPR1–LhSorTGA2* interaction by Y2H

To further confirm the interaction between *LhSorTGA2* and *LhSorNPR1*, the prey plasmid pGADT7-*LhSorTGA2* and the bait plasmid pGBKKT7-*LhSorNPR1* were co-transformed into AH109 competent yeast cells. Transformants were selected on DDO and QDO mediums, and protein–protein interaction between *LhSorTGA2* and *LhSorNPR1* was identified (Fig. 4a). In contrast, no positive interaction was detected in a series of co-transformants used as negative controls. All co-transformants were streaked on QDO medium supplemented with X-α-Gal chromogenic substrate, and blue colonies were only observed for the pGADT7- *LhSorTGA2* and pGBKKT7-*LhSorNPR1* co-transformant and the positive control (pGADT7-T and pGBKKT7-53 co-transformant) (Fig. 4b, c).

Expression analysis of *LhSorTGA2* in different tissues and in response to pathogen infection and various hormone treatments

Expression of *LhSorTGA2* was detected in all the five tissues explored, but the transcript abundance varied from tissue to tissue (Fig. 5). The transcript levels of *LhSorTGA2* observed in leaf, petal, root, and scale, were
Fig. 1 Sequence alignment of LhSorTGA2 with proteins of the TGA family in Arabidopsis. Sequences were aligned using Clustal W. Identical residues are shaded in black, highly similar residues are shaded in dark gray, and similar residues are shaded in gray. The DNA binding domain (DBD) is indicated by a dashed line box. The conserved glutamine residues (black arrow), alanine residues (blue arrow) and serine residues (red arrow), are indicated by arrows. Three leucine residues and one glycine residue for the four heptad repeats in leucine zipper are highlighted by triangles. Two glutamine rich acid domains (QI and QII) that mediate transcription activation are highlighted by solid line boxes. The signature sequence for group D bZIP transcription factors, Yx2R[RGALSSLSW], is underlined. The accession numbers of all the TGA proteins used for sequence alignment are listed in Additional file 2: Table S1.
all lower than that in stem, making stem the most abundant tissue expressing \textit{LhSorTGA2}.

To investigate \textit{LhSorTGA2} expression in respond to pathogen infection or hormone treatments, transcript levels of \textit{LhSorTGA2} were quantified after subjected to different treatments (MeJA, SA, ETH, ABA, and \textit{B. elliptica} infection). As shown in Fig. 6a, when subjected to MeJA treatment, \textit{LhSorTGA2} transcription was significantly induced, reaching its peak level 4 h after treatment. Foliar spray of SA also stimulated the accumulation of \textit{LhSorTGA2}, resulting in a 4.15 times increase of \textit{LhSorTGA2} transcript level 24 h after treatment (Fig. 6b). Following the ETH treatment, \textit{LhSorTGA2} was up-regulated 4.02 times the control level (Fig. 6c). For the ABA treatment, \textit{LhSorTGA2} expression was rapidly induced 2 h post treatment, reaching a level 2.17 times that of in the control. When exposed to pathogenic fungus \textit{B. elliptica}, the expression of \textit{LhSorTGA2} was also up-regulated, reaching its highest level 12 h after treatment (by a factor of 2.77 compared to the control). Up-regulation of \textit{LhSorTGA2} in respond to fungal infection and hormone treatments suggested that \textit{LhSorTGA2} might involve in diverse defense pathways regulated by multiple phytohormones.

\section*{Discussion}

Transcriptional reprogramming during SAR is predominantly mediated by NPR1. The interactions between NPR1 and TGA family proteins modulate expression of \textit{PR} genes during SAR. bZIP TFs in \textit{Arabidopsis} are subdivided into 10 groups (A–I and S), among which group...
Fig. 4  LhSorNPR1 and LhSorTGA2 protein interaction assessed by the yeast two hybrid assay. a Yeast AH109 cells co-transformed with bait and prey vectors were grown on double dropout (DDO, SD/-Leu/-Trp) and quadruple dropout (QDO, SD/-Ade/-His/-Leu/-Trp) mediums. b The diagram indicates the corresponding co-transformants for the assay in c. c Transformants were streaked on QDO medium supplemented with 40 µg/ml X-a-Gal. pGBK7-LhSorNPR1 and pGADT7-LhSorTGA2 co-transformant was used as the positive control. Yeast cells co-transformed with pGBK7 and pGADT7, pGBK7-LhSorNPR1 and pGADT7, pGBK7 and pGADT7-LhSorTGA2, or pGBK7-Lam and pGADT7-T were used as negative controls.
D proteins are crucial participants in response to pathogen infection (Jakoby et al. 2002). Previous survey of the Arabidopsis genome identified 10 members of the group D bZIP TFs (TGA family proteins), of which seven have been confirmed to exist interaction with NPR1 (Kesarwani et al. 2007).

Lilies are one of the most important floriculture crops in cut flower market and are valued for their elegant flowers emitting fragrance. However, a lack of genome information hinders the genetic improvement targeting genes of interest, for instance, genes related to ornamental traits or disease resistance. Species in the genus Lilium have huge genomes (around 36 GB), which makes sequencing and assembly of their genomes a technical challenge (Du et al. 2017). In light of the constraint in availability of the genomic data, RNA-seq seems to be the most practical approach to profile the transcriptional change of genes of interest in lily. In the present study, the partial coding sequence of LhSorTGA2 was retrieved from our previous transcriptome sequencing data, and the LhSorTGA2 full-length cDNA was obtained by RACE. Subsequent in silicon analysis confirmed the homology of LhSorTGA2 to TGA-like proteins in other plants, such as AtTGA2 (72.23% similarity) in Arabidopsis and GhTGA2 (85.55% similarity) in Gladiolus hybrid cultivar. Sequence alignment of LhSorTGA2 with TGA proteins in Arabidopsis revealed the structural basis for TGA2-mediated transcription control. The leucine zipper domain determines the dimerization specificity of bZIP TFs (Vinson et al. 2006), forming homo- or heterodimeric coiled-coil structures (zipper). The contiguous basic region, which contains a predicted nuclear localization signal (amino acids 143–159 in LhSorTGA2), contacts the DNA. Whereas the glutamine rich acid domains (QI and QII) have been proven to transactivate PR1 expression in a NPR1-dependent way during SAR (Fan and Dong 2002).

A phylogenetic tree was built to characterize the evolutionary relationship of TGA-like proteins originated from different species. LhSorTGA2 was grouped in subclade I, together with AtTGA2, AtTGA5 and AtTGA6. These three closely related TGAs have been proposed as transcriptional repressors of PRI, which are required for the basal repression of PR1. However, in the state of SAR, their biological functions are both redundant and essential, since only the tga2 tga5 tga6 triple mutant is blocked from induction of PR1 during SAR (Zhang et al. 2003).

To further investigate the function of LhSorTGA2 and LhSorNPR1, subcellular localization of GFP-tagged proteins was examined using confocal microscopy. As mentioned above, a NLS was predicted in the middle of LhSorTGA2, which probably making it a nucleus-localized protein. Consistent with the prediction, GFP-tagged LhSorTGA2 was predomnately detected in the nucleus. A NLS was also predicted in LhSorNPR1 at the C terminus, but presence of GFP-tagged LhSorNPR1 was detected both in the nucleus and at the cytomembrane. Consecutive expression of GFP-tagged AtNPR1 or TcNPR1 in Arabidopsis has proven that NPR1 localized in the cytoplasma if the seedlings were not treated with SA (Mou et al. 2003; Shi et al. 2010). However, under SA treatment (in the state of SAR), both AtNPR1-GFP and TcNPR1-GFP fusion proteins were translocated into the nucleus. Agroinfiltration causes immune response. Hence, SAR might be triggered during subcellular localization of GFP-labeled proteins using tabacco transient expression system. Our observation of LhSorNPR1-GFP in the nucleus was consistent with previous reports (GhNPR1 and VvNPR1), which might be caused by the translocation of LhSorNPR1-GFP into the nucleus in the state of SAR during agroinfiltration (Le Henanff et al. 2009; Zhong et al. 2015).

In Arabidopsis, interactions between NPR1 and TGA2 clade proteins modulated the expression of PRI. To validate the possible interaction between LhSorTGA2 and LhSorNPR1, vectors were constructed to test interaction in two different systems. As shown in Figs. 3 and 4, both BIFC and Y2H tests confirmed the interaction between LhSorTGA2 and LhSorNPR1. However, whether transcription of PR genes, especially PR1, is regulated by LhSorTGA2–LhSorNPR1 interaction in lily still needs to be confirmed.

Expression of LhSorTGA2 was detected in all the five tissues (leaf, stem, root, petal and scale) explored, but
Fig. 6  \( LhSorTGA2 \) expression in response to  

- **a** methyl jasmonate (MeJA),  
- **b** sodium salicylate (SS),  
- **c** ethephon (ETH),  
- **d** abscisic acid (ABA) and  
- **e** *Botrytis elliptica* inoculation treatments. Foliar spray of MeJA, SS, ETH, ABA, or Spores of *B. elliptica* (\( 8 \times 10^5 \) conidia/ml), was applied to 1-month-old lily seedlings. Leaves were sampled 0, 2, 4, 8, 12, 24 h post treatments to explore \( LhSorTGA2 \) expression. Expression at 0 h was used as the calibrator (designated as 1.0) to determine the relative expression of the target gene at different time points. Values are shown as mean ± standard deviations (SD) for three replicates. Bars labeled with an asterisk indicate significant differences from the control (0 h) at \( P < 0.05 \) (the REST statistical randomization test).
the transcription levels varied substantially (Fig. 5). TGA2 participated in SA-regulated SAR induction, and the expression of TGA2 itself has been shown to be responsive to SA in Arabidopsis (Fan and Dong 2002). In our case, LhSorTGA2 expression was responsive to SA, MeJA, ETH, ABA, and B. elliptica inoculation treatments, implying diverse roles it might play in defense pathways regulated by different hormones. Phytohormones are tightly linked to physiological processes in plant (Weyers and Paterson 2001). Induction of LhSorTGA2 in response to multiple hormones suggested that LhSorTGA2 might relate to physiological processes contributing to plant defense. Although there is still no direct evidence linking expression of TGA2-like genes to physiological changes under pathologic conditions, AtTGA10 and AtTGA9, two other members of the TGA family in Arabidopsis, has been shown to be involved in reactive oxygen species (ROS)-mediated responses to bacterial PAMP flg22 (Noshi et al. 2016). Accumulation of ROS is associated with hypersensitive response (HR) that restricts the spread of pathogens (Zurbriggen et al. 2010). However, whether TGA family proteins in lily modulate disease resistance through ROS accumulation is unknown. Among these TGA proteins, which one is the key regulator involved in ROS-mediated HR response to pathogen? Answers to these questions are mysteries awaited to be uncovered in future research.

Conclusions
LhSorTGA2, a novel TGA-like protein, was identified as an interacting partner of LhSorNPR1 in lily. Sequence alignment revealed that LhSorTGA2 was a member of the group D bZIP transcription factors, which featured with a conserved bZIP domain and two glutamine rich acid domains. LhSorTGA2 was differentially expressed in various tissues, and its expression was responsive to SA, MeJA, ETH, ABA, and B. elliptica inoculation treatments. However, whether transcription of PR genes is modulated by the interaction between LhSorTGA2 and LhSorNPR1 still needs to be tested.

Additional files

**Additional file 1: Figure S1.** Cloning of the LhSorTGA2 gene by RACE. a partial coding sequence of LhSorTGA2, b 5′ LhSorTGA2 RACE PCR products, c 3′ LhSorTGA2 RACE PCR products; d The LhSorTGA2 open reading frame amplified. M: DNA marker.

**Additional file 2: Table S1.** TGA-like protein sequences retrieved from GenBank used for phylogram construction and sequence alignment.

**Abbreviations**
SAR: systemic acquired resistance; NPR1: non-expressor of pathogenesis-related genes 1; bZIP: basic leucine-zipper; RACE: rapid amplification of cDNA ends; BIFC: bimolecular fluorescence complementation; Y2H: yeast two-hybrid; QDO: SD/-Ade/-His/-Leu/-Trp, DDO: SD/-Leu/-Trp, ABA: abscissic acid; SA: salicylic acid; SS: sodium salicylate; MeJA: methyl jasmonate; ETH: ethephon.

**Authors’ contributions**
ZX and YW conceived and designed the experiments; LW and ZG performed the experiments; YZ, LW and WL analyzed the data; LW, GY and RW wrote the manuscript. All authors read and approved the final manuscript.

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**Competing interests**
The authors declare that they have no competing interests.

**Availability of data and materials**
The data and material obtained in this paper are available.

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Not applicable.

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Not applicable.

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