J. Plant Biol. (2015) 58:52-60
DOI 10.1007/s12374-014-0407-4

ORIGINAL ARTICLE

# Over-expression of *SlWRKY39* Leads to Enhanced Resistance to Multiple Stress Factors in Tomato

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Received: September 1, 2014 / Accepted: October 15, 2014


**Abstract** The WRKY transcription factors are one of the well-characterized classes of plant transcription factors, which participated in various biotic and abiotc stress responses. Previous study showed that there are 81 WRKY genes in tomato, wherein a number of *SlWRKY* genes including *SlWRKY39* were significantly up-regulated under salt, drought stress and *PstDC3000* infection. However little is known about their physiological role in tomato. In this study, by using a forward genetic approach, we demonstrated transgenic plants over-expressing *SlWRKY39* showing enhanced resistance to multiple stress factors including *PstDC3000*, salt and drought. Transgenic plants accumulated higher level of proline and lower level of malonic dialdehyde. Compared with wild type, the expression of pathogenesis-related genes *SlPR1*, *SlPR1a1* and environmental stress related genes *SlRD22*, *SlDREB2A* were up-regulated in the transgenic plants. These results indicated that *SlWRKY39* is a positive regulatory component of tomato against biotic and abiotic stress probably via activating the expression of both pathogenesis-related genes and stress related genes.

**Key words:** Pathogenesis-related genes, *PstDC3000*, Salt/drought stress, Stress related genes, *SlWRKY39*, Tomato

## Introduction

During the whole life cycle, plants encounter various abiotic stress including drought, high salinity, cold, and biotic stress such as pathogen attacks, insect herbivory (Vinocur and Altman 2005; Mittler and Blumwald 2010). Evolutionarily, plants have developed a series of complex responsive mechanisms, and have possessed the ability to perceive and respond to external signals via multiple signaling pathways (Zhu 2001; Hasegawa et al. 2000). Various transcription factors have been implicated in regulation of plant adaptability to environmental stresses (Narsai et al. 2013). The WRKY transcription factor form one of the largest families and play a broad-spectrum regulatory role in the responses to abiotic and biotic stresses in plants (Pandey and Somssich 2009; Pan and Jiang 2014; Eulgem and Somssich 2007).

The function and molecular mechanisms of WRKY transcription factors in biotic stress have been well studied to date (Xing et al. 2008; Vinocur and Altman 2005). Most WRKY transcription factors bind to a consensus *cis*-element termed the W-box (TTGACT/C), which is found in the promoters of many defense-associated genes, and regulates their transcription (Agarwal et al. 2011). Over-expression of the Arabidopsis *WRKY18* gene leads to constitutive pathogenesis-related (PR) gene expression and enhanced disease resistance in a developmentally regulated manner (Chen and Chen, 2002). *AtWRKY25* functions as a negative regulator of defense responses to *Pseudomonas syringae* with reduced expression of *PR1* gene after the pathogen infection (Zheng et al. 2007). Most PR genes exhibit antifungal, antibacterial, insecticidal, and antiviral activities (Cutt et al. 1989; Durner et al. 1997). PR genes are highly conserved in plant and are widely used as markers in studies of signal transduction processes involved in plant pathogenesis (Durrant and Dong 2004).

In addition, WRKY transcription factors were shown to be involved in regulating plant responses to abiotic stress, such as drought and high salinity, the major stress factors limiting plant growth and productivity (Chen et al. 2010; Zhou et al. 2008; Wang et al. 2013; Niu et al. 2012). Expression of a number of genes has been demonstrated to be induced by abiotic stresses (Shinozaki and Yamaguchi-Shinozaki 1996). These genes can be classified into two groups: those that directly protect against environmental stresses like *RD22*, *RD29*, *Cor6.6*, and those that regulate gene expression and

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signal transduction in the stress response such as DREBs, MYB (Shinozaki and Yamaguchi-Shinozaki 1997). Transgenic Arabidopsis plants over-expressing *TaWRKY19* conferred tolerance to salt, drought and freezing stresses in transgenic plants by activating expressions of *DREB2A*, *RD29A*, *RD29B* and *Cor6.6*, and bounding to *DREB2A* and *Cor6.6* promoters (Niu et al. 2012).

Previous study showed that there are 81 WRKY genes in tomato, wherein a number of *SlWRKY* genes including *SlWRKY39* were significantly up-regulated under salt, drought stress and *Pseudomonas syringae pv.tomato DC3000* (*Pst DC3000*) infection (Huang et al. 2012). However little is known about their physiological role in tomato. In the current study, by using a forward genetic approach, we demonstrated transgenic plants over-expressing *SlWRKY39* showing enhanced resistance to *PstDC3000*, salt and drought stress compared with wild type plants. We found that *SlWRKY39* is a positive regulatory component of tomato conferring resistant ability to biotic and abiotic stress probably via activating expression of pathogenesis-related genes *SlPR1*, *SlPR1a1* and environmental stress related genes *SlRD22*, *SlDREB2A.*

## Results

### Expression Pattern of *SlWRKY39*

The temporal and spatial expression pattern of a gene is associated with its function. In order to elucidate *SlWRKY39* function, we first investigated the expression pattern of *SlWRKY39* in root, stem, leaf, flower and fruit at different developmental stages of wild type plant using real-time PCR analysis. As shown in Fig. 1A, *SlWRKY39* was mainly expressed in leaf, flower, immature and breaker fruits.

To investigate the expression of *SlWRKY39* under various conditions, 6-week-old seedlings were subjected to salt, drought, abscisic acid (ABA), salicylic acid (SA), jasmonic acid (JA), *Pst DC3000* treatment, total RNAs were extracted for real-time PCR analysis. As shown in Fig. 1B, *SlWRKY39* was induced by salt, drought, ABA, SA, JA and *Pst DC3000* infection. The response of *SlWRKY39* to a broad range of stress and hormones implied that *SlWRKY39* might be involved in different stress signaling pathways.

### Molecular Analysis of *SlWRKY39* Over-expression Transgenic Tomato Lines

The sequence and bioinformatics analysis revealed that *SlWRKY39* was classied as the WRKY group II member and had a single WRKY domain containing $C_2H_2$ zinc finger. In order to determine the function of *SlWRKY39*, we generated transgenic tomato over-expressing *SlWRKY39* under the control of the constitutive CaMV *35S* promoter. A 1053 bp fragment containing a complete open reading frame was obtained and designated as *SlWRKY39* by reverse transcription PCR. The fragment was cloned into plant expressing vector pBI121 under the direction of CaMV35S promoter and verified by restriction enzyme digestion. The transgenic lines were obtained using *Agrobacterium tumefaciens*-mediated transformation and verified by PCR amplification with primers designed to NPTII. Three independent *35S::SlWRKY39* (WRKY39-7, WRKY39-11 and WRKY39-16) transgenic lines were verified by real-time PCR analysis and selected for further study. As shown in Fig. 2, the expression levels of *SlWRKY39* were significantly increased compared to wild type plants.

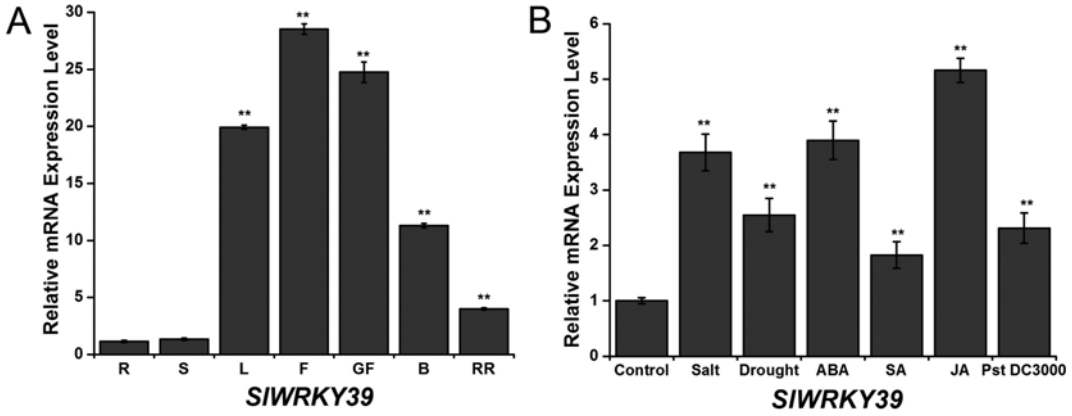


**Fig. 1.** The expression pattern of *SlWRKY39*. The mRNA levels of *SlWRKY39* were analyzed by real-time PCR. (**A**) Total RNAs were extracted from roots (R), stems (S), leaf (L), flower (F), immature fruit pericarps (GF), breaker (B) and red ripe fruit pericarps (RR) of wild type plants. (**B**) Total RNAs were extracted from wild type plants which were treated with NaCl, drought, ABA, SA, JA, *PstDC3000* respectively. Standard error bars represent three independent experiments.

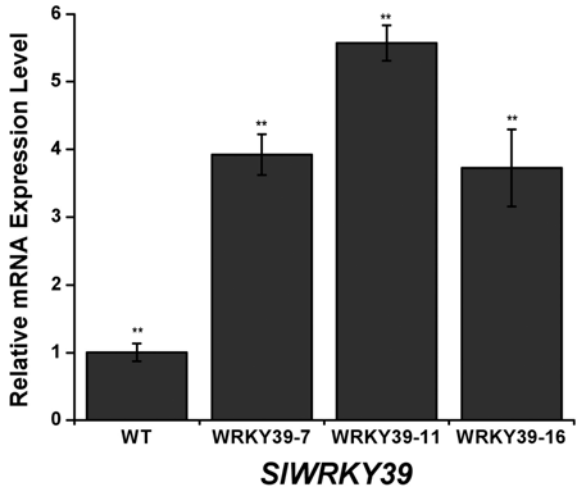


**Fig. 2.** Real-time PCR analysis of *SlWRKY39* expression levels in wild type and transgenic plants. mRNA levels were detected in mature leaves derived from wild type (WT) and three *35S::SlWRKY39* transgenic lines (WRKY39-7, WRKY39-11 and WRKY39-16)

## Over-expression of *SlWRKY39* Enhances the Resistance to *PstDC3000* Infection of Transgenic Plants

To address the role of *SlWRKY39* in resistance to *PstDC3000*, symptoms of the disease and the number of *PstDC3000* in the leaves of wild type and transgenic plants were monitored for 3 d post inoculation. As shown in Fig. 3A and 3B, over-expression of *SlWRKY39* resulted in reductions in symptoms of disease and also in the numbers of bacteria. In addition, the expression levels of pathogenesis-related genes *SlPR1* and *SlPR1a1* were analyzed of wild type and transgenic lines. The transgenic lines had up-regulated expression of *SlPR1* and *SlPR1a1* relative to wild type plants at all the examined time-points (Fig. 3C, 3D). These results indicate that over-expression of *SlWRKY39* enhanced resistance to *PstDC3000* infection.

## Over-expression of *SlWRKY39* Improves Salt and Drought Tolerance of Transgenic Plants

In order to study the function of *SlWRKY39*, the performances of wild type and transgenic plants under NaCl and mannitol stress were evaluated. Wild type and transgenic seeds were surface-sterilized, germinated and grown for 2-4 d on 1/2MS medium. Then seedlings with similar primary root lengths were transferred to 1/2MS medium supplemented with different concentrations of NaCl or mannitol. The root and seedling length were measured after an additional 5 d of

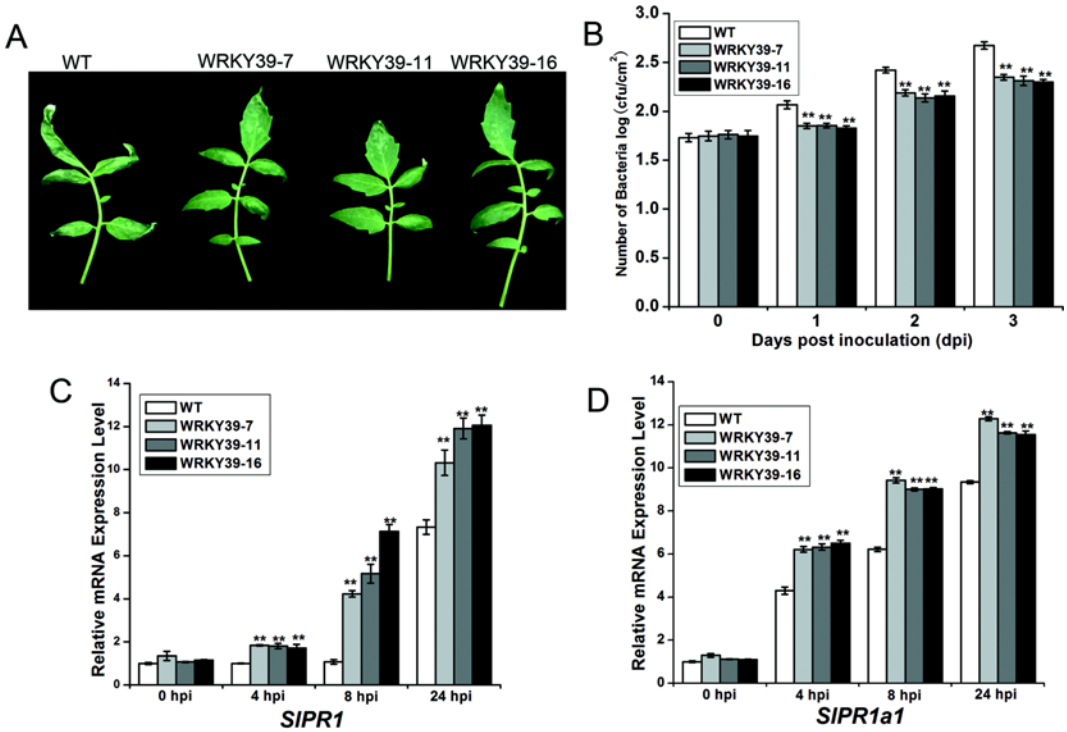


**Fig. 3.** Response of wild type and *SlWRKY39* transgenic plants to *Pst DC3000.* (**A**) Disease symptoms of WT and transgenic plants infected by *PstDC3000*. Photographs were taken 2 d post infection. (**B**) Bacterial growth of WT and transgenic plants infected by *PstDC3000* which was determined at 0-3 d post inoculation. (**C**) The expression of pathogenesis-related gene *SlPR1* in WT and transgenic plants. (**D**) The expression of pathogenesis-related gene *SlPR1a1* in WT and transgenic plants. The statistical significance of the difference was confirmed by Student's t-test (**P < 0.01).

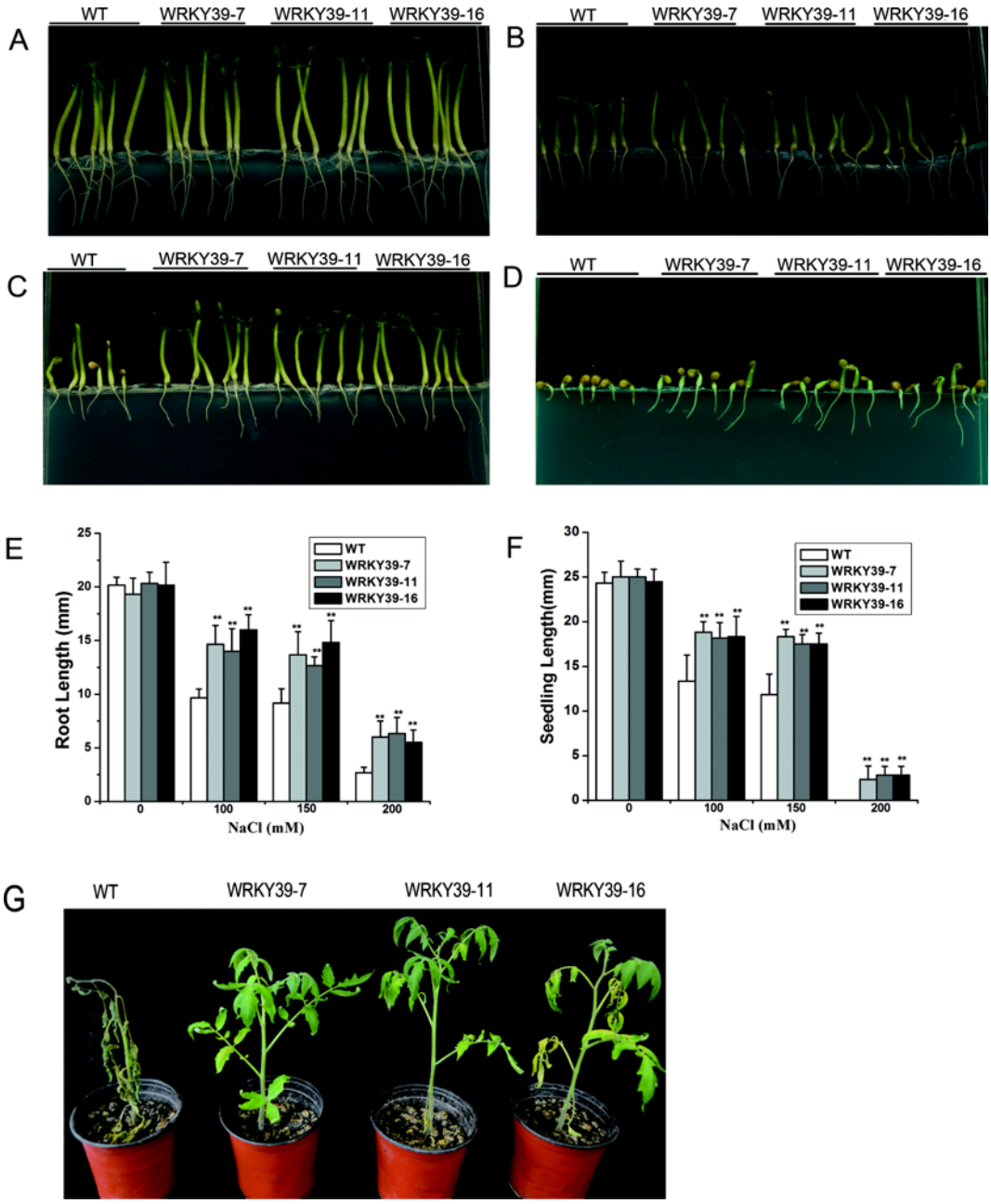


**Fig. 4.** Salt tolerance assay of wild type and transgenic tomato over-expressing *SlWRKY39*. (**A**) Phenotypes of WT and transgenic seedlings grown on 1/2MS medium without NaCl. (**B**) Phenotypes of WT and transgenic seedlings grown on 1/2MS medium adding 100 mM NaCl. (**C**) Phenotypes of WT and transgenic seedlings grown on 1/2MS medium adding 150 mM NaCl. (**D**) Phenotypes of WT and transgenic seedlings grown on 1/2MS medium adding 200 mM NaCl. (**E**) Root length of 5-d-old WT and transgenic seedlings grown on 1/2MS medium with 0, 100, 150, 200 mM NaCl. (**F**) Seedling length of 5-d-old WT and transgenic seedlings grown on 1/2MS medium with 0, 100, 150, 200 mM NaCl. (**G**) Phenotypes of WT and transgenic plants grown in soil with salt stress for 2 weeks. The experiment was repeated three times with similar results. Standard error bars represent three independent experiments. The statistical significance of the difference was confirmed by Student's t-test (**P < 0.01).

growth. As shown in Fig. 4A and 5A, root and seedling length did not show any significant difference on 1/2MS medium without adding stress factors. As the concentration of NaCl increased, the seedling growth of wild type was inhibited more severely than transgenic plants measured by root and seedling length (Fig. 4B-4F). Under 300 mM mannitol treatment, the root length of transgenic plants was significantly longer than wild type, showing increased tolerance to mannitol stress (Fig. 5B, 5C).

We also investigated salt and drought tolerance of wild

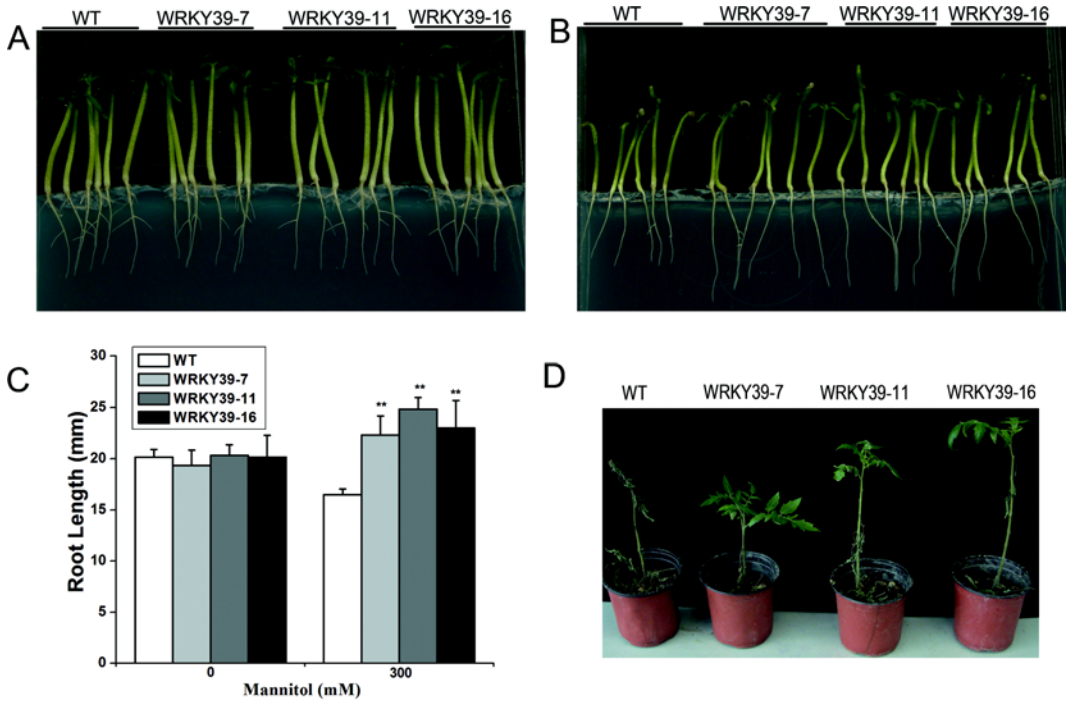


**Fig. 5.** Drought tolerance assay of wild type and transgenic tomato over-expressing *SlWRKY39*. (**A**) Phenotypes of WT and transgenic seedlings grown on 1/2MS medium without mannitol. (**B**) Phenotypes of WT and transgenic seedlings grown on 1/2MS medium adding 300 mM mannitol. (**C**) Root length of 5-d-old WT and transgenic seedlings grown on 1/2MS medium with 0 and 300 mM mannitol. (**D**) Phenotypes of WT and transgenic plants after drought stress, the plants were rewatered after drought stress for 3 weeks. The experiment was repeated three times with similar results. Standard error bars represent three independent experiments.The statistical significance of the difference was confirmed by Student's t-test (*P< 0.05, **P < 0.01).

type and transgenic plants grown in soil. Under salt stress, the transgenic plants showed better growth than wild type plants. After 2 weeks of salt stress, wild type plants were dead and transgenic plants turned yellow (Fig. 4G). For drought stress, wild type and transgenic plants were exposed to drought stress withholding water for 3 weeks, wild type plants appeared heavily wilted, and in contrast, transgenic plants appeared less affected. After rewatering, the wild type plants died and transgenic plants recovered (Fig. 5D). These results indicate that over-expression of the *SlWRKY39* gene improves salt and drought tolerance of the transgenic plants.

### The Contents of Proline, MDA and Expressions of Stress Related Genes

Physiological parameters changes and expression of stress related genes have been demonstrated to be induced by abiotic stresses. To this end, the contents of proline, malonic dialdehyde (MDA) and stress related genes *SlRD22*, *SlDREB2A* were analyzed for wild type and transgenic plants under normal and stress conditions. As shown in Fig. 6A, 6B and Fig. 7A, 7B, no significant difference in the content of proline and MDA was observed between wild type and transgenic plants under normal growth condition. After salt or drought treatment, the accumulation of proline increased while MDA decreased in transgenic lines compared with wild type plants. Under normal growth condition, the expression of *SlRD22*, *SlDREB2A* was up-regulated in transgenic plants in comparison with wild type plants (Fig. 6C, 6D, 7C, 7D). Under salt stress, the expression of *SlRD22* was up-regulated in transgenic plants compared with wild type (Fig. 6C). The expression of *SlRD22* and *SlDREB2A* was up-regulated in transgenic plants compared with wild type after drought stress (Fig. 7C, 7D). These results suggested that increased expression of *SlWRKY39* could enhance drought and salt stress tolerances in transgenic plants. The functions are probably achieved by accumulating proline and reducing MDA contents, through enhancing the expression of stress related genes *SlRD22* and *SlDREB2A*, thus protecting plants from damage.

## Discussion

Phytohormones such as salicylic acid (SA), ethylene (ET), and jasmonic acid (JA) play important roles in the regulation of plant responses to pathogen infection (Durrant and Dong 2004). Despite the existing cross-talk between signaling

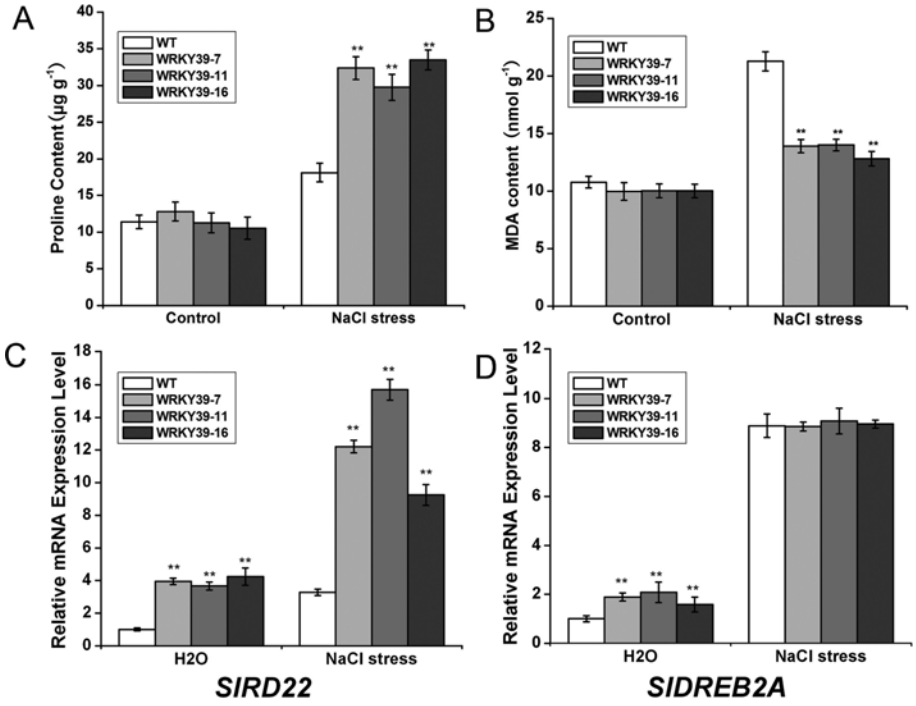


**Fig. 6.** The contents of proline, MDA and expressions of stress related genes of wild type and transgenic plants under salt stress. (**A**) The proline content of WT and transgenic plants under normal and salt stress. (**B**) The MDA content of WT and transgenic plants under normal and salt stress. (**C**) Expression profiles of *SlRD22* in WT and transgenic plants under normal and salt stress. (**D**) Expression profiles of *SlDREB2A* in WT and transgenic plants under normal and salt stress. Standard error bars represent three independent experiments. The statistical significance of the difference was confirmed by Student's t-test (**P < 0.01).

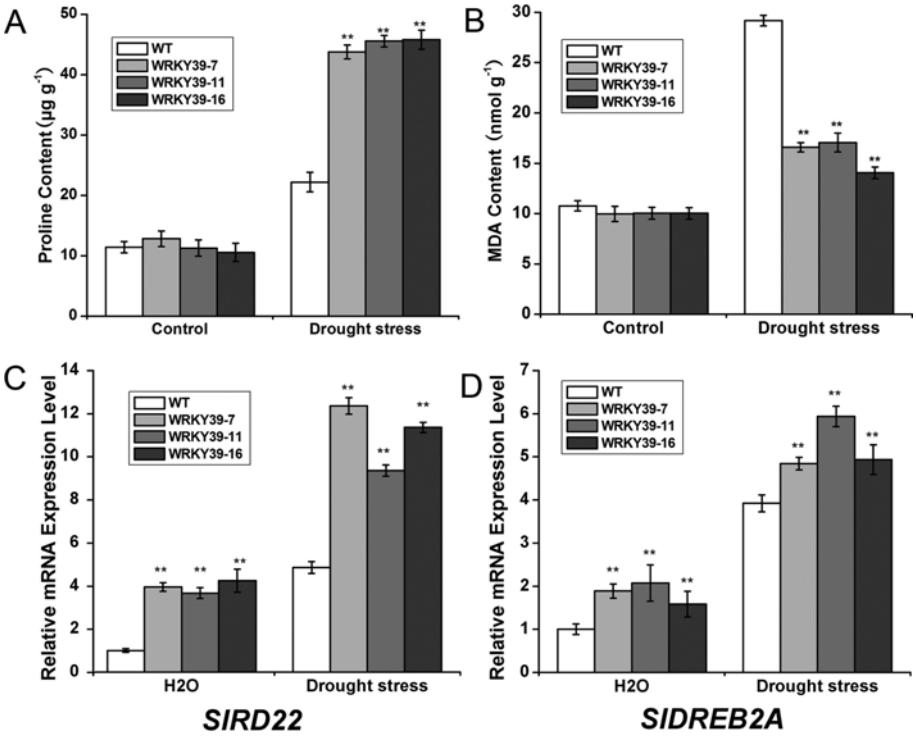


**Fig. 7.** The contents of proline, MDA and expressions of stress related genes of wild type and transgenic plants under drought stress. (**A**) The proline content of WT and transgenic plants under normal and drought stress. (**B**) The MDA content of WT and transgenic plants under normal and drought stress. (**C**) Expression profiles of *SlRD22* in WT and transgenic plants under normal and drought stress. (**D**) Expression profiles of *SlDREB2A* in WT and transgenic plants under normal and drought stress. Standard error bars represent three independent experiments. The statistical significance of the difference was confirmed by Student's t-test (**P < 0.01).

pathways mediated by these phytohormones, it is generally thought that SA-regulated defense signaling is associated with defense responses against biotrophic pathogens, which parasitize on plant living tissue (Cao et al. 1997; Wildermuth et al. 2001; Durrant and Dong 2004). ET- and JA-mediated signaling pathways, on the other hand, often mediate plant defense against necrotrophic pathogens that promote host cell death at early stages of infection (Glazebrook 2005). Over-expression of *SlWRKY39* enhanced resistance to *PstDC3000* infection which is a biotrophic pathogen (Fig. 3), and upon SA treatment, the expression of *SlWRKY39* was up-regulated (Fig. 1B), indicating that *SlWRKY39* probably participated in SA-mediated signaling pathway. Plant defense responses are associated with the transcriptional activation of a large number of plant host genes after pathogen infection. Induction of certain pathogenesis-related (PR) proteins is dependent upon SA-mediated defense signaling pathways and is associated with resistance to biotrophic and hemibiotrophic pathogens (Durrant and Dong 2004). PR genes contain W-box elements in their promoter regions which are specifically recognized by WRKY proteins and are necessary for the inducible expression of these genes (Eulgem and Somssich 2007). The transgenic lines had up-regulated expression of *SlPR1* and *SlPR1a1* relative to wild type plants at all the examined time-points (Fig. 3C, 3D), suggesting that over-expression of *SlWRKY39* enhanced resistance to *PstDC3000* infection probably via up-regulation of pathogenesis-related genes *SlPR1* and *SlPR1a1*.

The WRKY transcription factors are not only involved in biotic stress response but also abiotic stress responses. Over-expression of *SlWRKY39* improves salt and drought tolerance of transgenic plants (Fig. 4, 5). It has been shown that salinity and drought stresses cause detrimental changes in cellular components, consequently accumulation of a wide range of metabolites including amino acids like proline can alleviate these detrimental changes (Shao et al. 2006; Wang et al. 2013). It has been proved that free proline in plants plays multiple roles in osmotic adjustment, protection of cellular macromolecules and scavenging of hydroxyl radicals (Shao et al. 2006). In the current study, our transgenic plants accumulated higher level of proline and displayed enhanced tolerance under salt or drought stress than wild type plants (Fig. 6A, 7A). In addition, it has been reported that abiotic stress causes lipid peroxidation, leading to MDA accumulation (Kong et al. 2011; Wu et al. 2008). MDA content could be used as a marker of the damage caused by abiotic stresses (Sathiyaraj et al. 2011). In our experiment, the MDA content of transgenic plants was significantly lower than in wild type plants after the drought and salinity stress treatment (Fig. 6B, 7B), indicating that over-expression of the *SlWRKY39* gene protected the cell membrane from damage imposed by drought/salinity stress.

The expression of the *RD22* (responsive to dehydration), encoding a BURP domain-containing protein, was found to be induced by water deficit, salinity stress and the application of ABA, but not by cold or heat stress (Yamaguchi-Shinozaki and Shinozaki 1993). The transcription factors DREBs (DRE binding protein) can bind to DRE (dehydration-responsive element) *cis*-elements in the promoters and activate transcription of the stress related genes (Cui et al. 2011; Sakuma et al. 2006). In the present study, we observed that increased expression of *SlWRKY39* in the transgenic plants enhanced the expression of both *SlDREB2A* and *SlRD22* under normal growth condition (Fig. 6C, 6D, 7C, 7D). And the expression of *SlDREB2A* and *SlRD22* were up-regulated under mimic drought stress condition (Fig.7C, 7D), in contrast, only *SlRD22* was up-regulated under salt stress compared with wild type plants (Fig. 6C, 6D). This is consistent with previous observations that inducible expression of *ZmDREB2A* could improve tolerance to drought stress in *Arabidopsis* (Qin et al. 2007), and that *GmRD22* can alleviate salinity and osmotic stress (Wang et al. 2012).

Plant response to biotic and abiotic stress is a very complex trait involving different signaling pathways and gene regulation. In this study, we demonstrated that *SlWRKY39* is a positive regulatory component of tomato against multiple stresses including *PstDC3000* infection, drought and salt stress. The underlying mechanism for the complicated regulation of both biotic and abiotic stress responses by *SlWRKY39* requires further study.

## Materials and Methods

### Plant material and Growth Conditions

Tomato cv. Ailsa Craig was used for transformation material. Primary transformed plants (T0) were first planted in green house with a standard conditions (26°C d, 18°C night; 16 h light, 8 h dark) and transplanted to the experimental field 35 d later. T2 transgenic lines were used for *Pseudomonas* inoculation, drought and salt tolerance assay.

To investigate the expression pattern of *SlWRKY39* in different tissues, including root, stem, leaf, flower, immature fruit, breaker and red ripe fruit, these tissues were collected from wild type plants and immediately frozen in liquid nitrogen then stored at -80ºC until further analyses.

For the induction experiment, 6-week-old WT seedlings were exposed to high-salinity (plants were irrigated with 400 mM NaCl every other day for 8 d), drought stress (withhold water for 8 d). For hormone treatments, seedlings were sprayed with 0.5 mM ABA, SA, JA respectively, leaves were collected 30 min later. For *PstDC3000* infection, WT plants were inoculated with *PstDC3000* at $OD_{600}$= 0.02, leaves were collected 4 h later. The leaves were collected and immediately frozen in liquid nitrogen then stored at -80°C until further analyses.

### Plasmid Construction and Tomato Transformation

DNA manipulations were carried out by using standard procedures

(Sambrook and Russell 2001). To generate the over-expression constructs, the full-length cDNA of *SlWRKY39* was amplified by using PCR primers WRKY39-F0 (5'-CTTCCAAACTTCAATTCCCCC -3'), WRKY39-R0 (5'-GTCCTAGTGGCTGCCTGTCTT-3'), WRKY39-F1 (5'-TCTAGAATGGAGTTCACAAGTTTAGTTG -3'), WRKY39-R1 (5'-GAGCTCCATTTCAATGTGTCAACATAAT-3'). The cDNA was cloned into the GUS-deleted pBI121 vector (driven by the CaMV 35S promoter) at the XbaI and SacI restriction enzyme sites. The recombinant vector was verified by restriction enzyme digestion and sequencing. Transgenic plants were generated by *Agrobacterium tumefaciens*-mediated transformation according to the method described by Fillatti et al. (1987). The transgenic lines were screened for kanamycin (70 mg $L^{-1}$) resistance and confirmed by PCR using NPTII (GenBank accession no. AF485783) specific primers (5'-ATTGAACAAGATGGATTGCACG-3' and 5'-CTCGTCAAGAAG-GCGATAGAAG-3'). PCR was performed by using Taq DNA Polymerase (TIANGEN BIOTECH, China) in MJ Mini™ PCR (BioRad, USA), following the instruction given by the manufacturer.

### Real-time PCR Analysis

For real-time PCR, total RNA was extracted with TRIzol reagent (Invitrogen) and treated with DNase. The first-strand cDNA was synthesized according the protocol provided by the manufacturer (TransGen Biotech, China). Primers for real-time PCR were designed for *SlWRKY39* (SGN-U566776; SlWRKY39-F, 5'-GCGGTAATGCC-AAGACAAAC-3'; SlWRKY39-R, 5'-TCAGTTCCTGGTGATTTA-CGC-3'), *SlRD22* (SGN-U580823; SlRD22-F, 5'-CAAGTTTTCA-GTGGATGAGGATAG-3'; SlRD22-R, 5'-TGTTTCCTAACTTTGAT-GTGGTGA-3'), *SlDREB2A* (SGN-U565259; SlDREB2A-F, 5'- GA-AGGAAGCCAGTGCGTAAAGT-3'; SlDREB2A-R, 5'- TCAGCAA-CCCATTTACCCCA-3'), *SlPR1a1* (SGN U577839; SlPR1a1-F, 5'-TGCTGGTGCTGTGAAGATGTG-3'; SlPR1a1-R, 5'-CAGACTT-TACCTGGAGCACACG-3'), *SlPR1* (SGN U579345; SlPR1-F, 5'-GCTGTGAAGATGTGGGTTGATG-3'; SlPR1-R, 5'-CCTAGCAC-AACCAAGACGTACC-3'), *SlUBI3* (GenBank accession no. X58253; SlUBI3-F, 5'-AGGTTGATGACACTGGAAAGGTT-3'; SlUBI3-R, 5'-AATCGCCTCCAGCCTTGTTGTA-3'). The real-time PCR was performed using an SsoFast EvaGreen Supermix (Biorad catalog #172-5203). Each sample was amplified in triplicate and all PCR reactions were performed on the Applied Biosystems StepOne Real-Time PCR System (Applied biosystems, USA). Dissociation curve analysis was performed at the end of each run to ensure that unique products were amplified. The tomato *SlUBI3* gene was used as an internal reference. The RT-PCR conditions were as follows: 95ºC for 30 s, followed by 40 cycles of 95ºC for 5 s, 60ºC for 20 s. The expression level was normalized to the *SlUBI3* control, and relative expression values were determined against the buffer-treated sample or the WT sample using the $2^{-\Delta\Delta Ct}$ method. To confirm the specificity of the PCR reaction, PCR products were verified on a 1% agarose gel for the accurate amplification product size.

### *Pseudomonas* Inoculation

The *Pseudomonas syringae pv. tomato DC3000* (*PstDC3000*) was incubated overnight at 28ºC on King's B medium plates containing rifampicin (50 μg $mL^{-1}$).The bacteria was collected and resuspended in 10 mM $MgCl_2$ buffer containing 0.006% Silwet L-77 to $OD_{600}$= 0.00001. The prepared bacteria solution was used for vacuum infiltration on 6-week-old seedlings. Samples were collected at 0, 1, 2 and 3 d post inoculation and homogenized in 10 mM $MgCl_2$. Diluted leaf extracts were plated on King's B medium containing rifampicin (50 μg $mL^{-1}$). The number of bacteria (cfu/$cm^2$) was counted 2 d after the plates had been kept at 28°C. In the case of expression assessments of *SlPR1* and *SlPR1a1*, the inoculation of *PstDC3000* at $OD_{600}$= 0.02 was used.

### Drought and Salt Tolerance Assay

For NaCl or mannitol treatement, wild type and transgenic seeds were surface-sterilized, germinated and grown for 2-4 d on 1/2MS medium. Germinated seeds of radicle protruding were then transferred to square plates supplemented with different concentrations of NaCl (0, 100, 150, 200 mM) and mannitol (0, 300 mM). The root length and seedling length were measured on the fifth day.

For salt stress treatment of soil-grown plants, 6-week-old wild type and transgenic plants were irrigated with 400 mM NaCl every other day for 2 weeks.

For drought stress treatment of soil-grown plants, 6-week-old wild type and transgenic plants were imposed to drought stress by withholding water for 3 weeks and rewatered.

### Proline and MDA Measurements

6-week-old wild type and transgenic plants with salt or drought stress for 8 d were used for proline and MDA determination. Proline content analysis was carried out by the ninhydrin reaction method. Fresh leaf material was extracted with sulfosalicylic acid, and the acetic acid and acid ninhydrin reagent were added into the solution and heated at 100ºC for 30 min. After cooling to room temperature, the optical density of organic phase was determined at 520 nm. The MDA content analysis was carried out by the thiobarbituric acid method. Fresh leaves were extracted with 10% trichloroacetic acid. After centrifugation at 4,000 *g*, supernatant was boiled with 0.5% thiobarbituric acid. Optical density readings of organic phase were taken at 532 nm, 600 nm and 450 nm and calculated as described by Draper et al.

## Acknowledgements

This work was supported by National Science Fund for Distinguished Young Scholars (Grant No. 30825030), the National Natural Science Foundation of China (Grant Nos. 31171179, & 90717110), the 973 Program of China (Grant No. 2011CB100401) and Advanced Program of Doctoral Fund of Ministry of Education of China (20110181130009).

## Author's Contributions

XS, YG and YL designed the experiments. XS, YG and HL performed the experiments. XS analyzed the data and drafted the initial manuscript. YL revised the paper. All the authors agreed on the contents of the paper and post no conflicting interest.

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