Multifaceted regulatory function of tomato SlTAF1 in the response to salinity stress

Vikas Devkar, Venkatesh P. Thirumalaikumar, Gang-Ping Xue, José G. Vallarino, Veronika Turečková, Miroslav Strnad, Alisdair R. Fernie, Rainer Hoefgen, Bernd Mueller-Roeber and Salma Balazadeh

1Max Planck Institute of Molecular Plant Physiology, Am Mühlenberg 1, 14476, Potsdam-Golm, Germany; 2Institute of Biochemistry and Biology, University of Potsdam, Karl-Liebknecht-Straße 24-25, Haus 20, 14476, Potsdam-Golm, Germany; 3CSIRO Agriculture and Food, St Lucia, Qld 4067, Australia; 4Laboratory of Growth Regulators, The Czech Academy of Sciences, Institute of Experimental Botany, Palacký University, Šlechtitělu 27, CZ-78371, Olomouc, Czech Republic; 5Institute of Biology, Leiden University, Sylviusweg 72, 2333 BE, Leiden, the Netherlands

Author for correspondence: Salma Balazadeh
Tel: +49 331 5678632
Email: balazadeh@mpimp-golm.mpg.de

Received: 28 July 2019
Accepted: 29 September 2019

New Phytologist (2020) 225: 1681–1698
doi: 10.1111/nph.16247

Key words: abscisic acid (ABA), ion homeostasis, NAC, proline, salt stress, SlTAF1, transcription factors.

Introduction

Salt stress adversely affects plant growth, development and crop productivity and is a major challenge to agriculture production (Munns & Tester, 2008; Shabala, 2013). Stress engenders both osmotic and ionic stress in plants. Excess soil salinity hinders water uptake by the plant roots and decreases turgor pressure due to water efflux from the vacuole, thereby resulting in an insufficient osmotic adjustment. Furthermore, high salinity stress enforces the accumulation of Na+ ions, leading to tissue toxicity. Na+ ion concentration increases gradually in aerial parts of the plants via transportation from root to shoot through the transpiration stream. Salinity-induced stress results in an immediate reduction in growth mainly via reduction of cell expansion in root tips and younger leaves, and stomatal closure in leaves, whereas salinity-induced ion toxicity promotes premature senescence or programmed cell death (Munns and Tester, 2008; Shabala, 2009).

To endure salinity stress, diverse adaptive mechanisms have evolved in plants including, for example, an efficient exclusion of Na+ ions from cells, their compartmentalisation in the vacuole by specific transporters, adjustment of the osmotic balance of the cells by accumulating osmoprotectants, a change in photosynthetic activity, enhanced antioxidant and reactive oxygen species (ROS) scavenging capacity, and changes in hormonal response. Salinity-induced changes in tricarboxylic acid cycle intermediates and amino acids are more pronounced in SlTAF1 overexpressors than knockdown plants. The osmoprotectant proline accumulates more in SlTAF1 overexpressors than knockdown plants.

In summary, SlTAF1 controls the tomato’s response to salinity stress by combating both osmotic stress and ion toxicity, highlighting this gene as a promising candidate for the future breeding of stress-tolerant crops.

To endure salinity stress, diverse adaptive mechanisms have evolved in plants including, for example, an efficient exclusion of Na+ ions from cells, their compartmentalisation in the vacuole by specific transporters, adjustment of the osmotic balance of the cells by accumulating osmoprotectants, a change in photosynthetic activity, enhanced antioxidant and reactive oxygen species (ROS) scavenging capacity, and changes in hormonal response. Salinity-induced changes in tricarboxylic acid cycle intermediates and amino acids are more pronounced in SlTAF1 overexpressors than knockdown plants. The osmoprotectant proline accumulates more in SlTAF1 overexpressors than knockdown plants.

In summary, SlTAF1 controls the tomato’s response to salinity stress by combating both osmotic stress and ion toxicity, highlighting this gene as a promising candidate for the future breeding of stress-tolerant crops.

Summary

- Salinity stress limits plant growth and has a major impact on agricultural productivity. Here, we identify NAC transcription factor SlTAF1 as a regulator of salt tolerance in cultivated tomato (Solanum lycopersicum).
- While overexpression of SlTAF1 improves salinity tolerance compared with wild-type, lowering SlTAF1 expression causes stronger salinity-induced damage. Under salt stress, shoots of SlTAF1 knockdown plants accumulate more toxic Na+ ions, while SlTAF1 overexpressors accumulate less ions, in accordance with an altered expression of the Na+ transporter genes SIHKT1;1 and SIHKT1;2. Furthermore, stomatal conductance and pore area are increased in SlTAF1 knockdown plants during salinity stress, but decreased in SlTAF1 overexpressors.
- We identified stress-related transcription factor, abscisic acid metabolism and defence-related genes as potential direct targets of SlTAF1, correlating it with reactive oxygen species scavenging capacity and changes in hormonal response. Salinity-induced changes in tricarboxylic acid cycle intermediates and amino acids are more pronounced in SlTAF1 overexpressors than knockdown plants, but less so in SlTAF1 overexpressors. The osmoprotectant proline accumulates more in SlTAF1 overexpressors than knockdown plants.

In summary, SlTAF1 controls the tomato’s response to salinity stress by combating both osmotic stress and ion toxicity, highlighting this gene as a promising candidate for the future breeding of stress-tolerant crops.
factor), CUC2 (cup-shaped cotyledon) family has attracted particular attention due to its roles in responses to diverse environmental stresses (Olsen et al; 2005; Jensen et al., 2010; Pérez-Rodríguez et al., 2010; Puranik et al., 2012). The NAC family typically encompasses more than a 100 members in higher plants (Jin et al., 2017). NAC TFs have a highly conserved N-terminal NAM domain that includes a dimerisation motif and confers DNA-binding activity, while their C-terminal region has a transcription activation function and shows high sequence variability (Ooka et al., 2003; Ernst et al., 2004; Jensen et al., 2010). In different plant species, NAC TFs control responses to biotic and abiotic stresses, including salinity. For example, in Arabidopsis thaliana, JUNGBRUNNEN1 (JUB1), Arabidopsis NAC transcription factor 19 (ANAC019), ANAC055 and ANAC072 (also called RD26, RESPONSIVE TO DESICCATION 26) positively regulate the tolerance to salt stress (A. Wu et al., 2012; Li et al., 2014), while ANAC092 (also called ORESARA1, ORE1) and ANAC016 are negative regulators of the response to salinity (Balazadeh et al., 2007; Kim et al., 2013). JUB1 directly controls the expression of DREB2A (DEHYDRATION-RESPONSIVE ELEMENT-BINDING PROTEIN 2A), an Apetala 2/ethylene-responsive element-binding protein (AP2/EREBP) TF with an important function in the regulation of drought, salinity and osmotic stress tolerance (Dubouzet et al., 2003; Sakuma et al., 2006; Chen et al., 2008; Lata and Prasad, 2011; Zhang et al., 2016). ANAC019, ANAC055 and RD26 bind to the promoter of ERD1 (EARLY RESPONSE TO DEHYDRATION1, a drought responsive gene) and enhance drought stress tolerance when over-expressed in Arabidopsis (Tran et al., 2004). Loss-of-function mutants of ANAC019, ANAC055 and RD26 exhibit increased sensitivity to salinity stress (Li et al., 2014).

In rice, STRESS RESPONSIVE NAC1 (SNAC1), SNAC2 (OsNAC6), OsNAC045, OsNAC5, OsNAC106 and ONAC022 function as positive regulators of salt tolerance (Hu et al., 2006; Nakashima et al., 2007; Zheng et al., 2009; Takasaki et al., 2010; Sakuraba et al., 2015; Hong et al., 2016). Enhanced salt tolerance of ONAC022 overexpression plants was accompanied by reduced levels of Na⁺ ions in roots and shoots, and enhanced expression of abscisic acid (ABA) biosynthetic and signalling genes and several stress-responsive TFs, including OsDREB2A (Hong et al., 2016). By contrast, OsNAC2 functions as a negative regulator of the response to severe salinity. OsNAC2 directly activates transcription of OsAP37 (Oryza sativa ASPARTIC PROTEASE 37, encoding a caspase-like protease), but triggers repression of OsCOX1 (Oryza sativa CYTOCHROME OXIDASE 11, involved in ROS scavenging), leading to enhanced caspase activity and accumulation of ROS and subsequently programmed cell death during severe salinity stress (Mao et al., 2018).

Tomato (Solanum lycopersicum) is an important vegetable crop that is rich in antioxidant molecules such as carotenoids, vitamin E, vitamin C, ascorbic acid and phenolic compounds, mainly flavonoids (Fruscianti et al., 2007). Seed germination, growth, biomass allocation and fruit yield of tomato plants are negatively affected by salinity stress (Sholi, 2012; Zhang et al., 2016; Masse et al., 2018). Attempts have been made to enhance salinity tolerance in tomatoes by genetic engineering of genes that encode the plasma membrane Na⁺/H⁺ antiporter SISOS1 (S. lycopersicum SALT OVERLY SENSITIVE 1; Olias et al., 2009), the endosomal Na⁺/H⁺ antiporter LeNHX2 (Huertas et al., 2013), and also regulatory proteins including serine/threonine protein kinase SiSOS2 (S. lycopersicum SALT OVERLY SENSITIVE 2; Belver et al., 2012; Huertas et al., 2012) and TFs of diverse families (e.g. SIAREB1, S. lycopersicum ABA-responsive element-binding protein 1; SiARS1, S. lycopersicum altered response to salt stress 1; SIDREB2, S. lycopersicum dehydration-responsive element-binding protein 2; and SlbZIP1, S. lycopersicum basic leucine zipper 1; Orellana et al., 2010; Campos et al., 2016; Hichri et al., 2016; Zhu et al., 2018). Additionally, some NAC TFs, including SINAC4, SINAC35 and SINAC11, have been shown to affect salt tolerance in tomato (Zhu et al., 2014; Wang et al., 2016; Wang et al., 2017). Silencing of SINAC4 has led to an increased sensitivity of plants to drought and salt stress, and a decreased expression of stress-responsive genes including genes encoding antioxidants (CATALASE 1, CAT1 and CAT2) and proline biosynthesis enzymes (PYRROLINE-5-CARBOXYLATE SYNTHASE, P5CS; Zhu et al., 2014). Similarly, silencing of SINAC11 reduces salt stress tolerance in tomato (Wang et al., 2017), while ectopic expression of SINAC35 elevates salt tolerance in tobacco (Wang et al., 2016). However, molecular knowledge of the signalling pathways and downstream targets of those TFs is scarce.

Here, we demonstrate an important role of tomato NAC transcription factor SITA1 (Solanum lycopersicum Transcription Activation Factor 1, Solyc06g060230) for establishing tolerance to salinity stress. We show that enhanced salt tolerance conferred by SITA1 is associated with increased levels of the osmolyte proline, reduced stomatal conductance and stomatal pore area, reduced accumulation of Na⁺ ions in shoots, and upregulation of salt stress-responsive and ABA biosynthesis genes, including various TFs. Collectively, our results demonstrated that SITA1 is a key regulatory hub that controls diverse circuities of defence-related events in the salinity stress response in tomato, highlighting this gene as a promising candidate for breeding stress-tolerant crops.

Materials and Methods

Plant material and growth conditions

Solanum lycopersicum cv Moneymaker wild-type was used as the control in this study. Seeds of the wild tomato species S. pimpinellifolium and S. cheesmaniae were obtained from the Tomato Genetics Resource Center (https://tgrc.ucdavis.edu).

For seed production, phenotyping and detached leaf experiments, Murashige and Skoog (MS) medium-grown wild-type and SITA1 transgenic tomato seedlings were transferred to soil, as previously reported (Schwarz et al., 2014; Thirumalai Kumar et al., 2018) and grown in a greenhouse under a 16 h : 8 h day : night regime, 450 μmol photons m⁻² s⁻¹ light, 24°C, and 65% relative humidity.
Hydroponic culture system and liquid nutrient medium

For aerated hydroponics, standard nutrient medium was used for *S. lycopersicum* cv Moneymaker. Briefly, macronutrients: 1.25 mM Ca(NO₃)₂·4H₂O; 0.83 mM K₂HPO₄; 1.5 mM KNO₃; 0.75 mM MgSO₄, and micronutrients: 50 µM Na₂FeEDTA; 11.6 µM H₂BO₃; 2.4 µM MnSO₄·H₂O; 200 mM ZnSO₄; 100 nM CuSO₄·5H₂O; 100 nM Na₂MoO₄·2H₂O. After preparation of the nutrient medium, the pH was adjusted to 5.8 using H₂SO₄. Generally, roots of tomato plants are sensitive to hypoxia; they need adequate air around the root zone for proper growth (Klaring & Zude 2009). To this end, an air pump was utilised to achieve an aeration for healthy root growth in the hydroponic culture system via the formation of air bubbles and waves. Aerated hydroponic trays comprising nutrient medium with tomato seedlings were grown in a controlled growth chamber (photoperiod 16 h : 8 h, day : night; light 350 µmol photons m⁻² s⁻¹; temperature 22°C: 18°C, day : night; and 70% relative humidity). After 7 d of seedling transplantation, medium was replenished every third day to avoid depletion of nutrients.

Salt treatment in a hydroponic culture system and by salt-water irrigation

For salinity treatment in hydroponics, 1-wk-old MS medium-grown tomato seedlings (wild-type and *SlTAF1* transgenic plants) were transplanted to an aerated hydroponic culture system containing nutrient medium, and grown in a growth chamber. Salt treatment was induced by supplementing nutrient medium with NaCl; plants grown in nutrient medium without NaCl (0 mM) were used as controls. For salt-water irrigation, wild-type and *SlTAF1* transgenic tomato seedlings were grown in a growth chamber and supplemented with NaCl (200 mM) or without NaCl.

Treatments

For gene expression analysis in different tomato species (*S. lycopersicum, S. pimpinellifolium* and *S. cheesmaniae*) after salt treatment, 2-wk-old seedlings were transferred to MS liquid medium containing 120 mM NaCl (NaCl was omitted in control treatments) and incubated for 4 h. Dehydration treatment was performed as previously described (Thirumalaikumar et al., 2018). For gene expression analysis upon different treatments, 2-wk-old seedlings of wild-type *S. lycopersicum* cv Moneymaker were initially grown on MS medium and thereafter transferred to liquid MS medium flasks and treated with salt (NaCl 120 mM; for 2, 4, 6 or 10 h), H₂O₂ (10 mM; for 4 h), and ABA (100 µM; for 0, 2, 4, 6, 12, 24 or 36 h). For expression analysis of *SlTAF1* early responsive genes, 3-wk-old seedlings of *SITAF1-Ioe* were treated with 15 µM estradiol (EST) for 6 h in liquid MS medium (mock treatment: 0.15% (v/v) ethanol, used to dissolve EST). To test salt-dependent expression of potential target genes of *SlTAF1*, 3-wk-old *SITAF1-Ioe* seedlings were transferred to liquid MS medium containing 200 mM NaCl and 15 µM EST and incubated for 6 h on a shaker (without EST in mock treatment). After the treatments, samples were harvested and immediately plunged into liquid nitrogen.

Determination of sodium (Na⁺) and potassium (K⁺) ions

Na⁺ and K⁺ ion concentrations were measured in tomato shoot and root using ion chromatography (Dionex ICS-3000). Briefly, oven dried (shoot and root) plant material was ground into fine powder using a Retsch mill (Retsch, Haan, Germany). Next, 20 mg of ground material was weighed using an analytical weighing balance and homogenised in 1 ml of ULC/MS grade deionised water by vortexing for 2 min. Subsequently, ultrasonication was performed for 10 min. Afterwards, samples were centrifuged for 30 min and supernatant was filtered through Nanosep Centrifugal Devices ( Pall Corp.; VMR International, Darmstadt, Germany). Filtered samples were diluted 1 : 100 in ULC/MS water. Ion chromatography was calibrated by injecting different concentration solutions of NaCl and KClO₃ (3.125, 6.25, 12.5, 25, 50 and 100 µM). Data were collected and processed using CHROMLEON v.6.8 software (Dionex). Standard curves for Na⁺ and K⁺ ions were calculated from standard solutions. An equation derived from the standard curve was used to calculate the ion concentration in the samples.

Metabolite profile analysis by GC-MS

To perform metabolite profiling, plants were grown in a controlled growth chamber. Leaf samples were harvested and immediately frozen in liquid nitrogen. After grinding samples, the extraction and relative levels indicated in metabolite profile results were obtained by gas chromatography time-of-flight mass spectrometry (GC-TOF/MS) (Osorio et al., 2012). Both, chromatograms and mass spectra were evaluated using CHROMATOOF v.4.51.6 (LECO Corp., St Joseph, MI, USA) and TAGFINDER v.4.0. (Luedemann et al., 2008). Each compound was annotated based on its unique mass spectrum (Kopka et al., 2005).

Data availability statement

Gene IDs are listed in Supporting Information Table S1.

Detailed descriptions of DNA constructs, plant transformation, identification of the *SITAF1* binding motif, RNA extraction, gene expression analysis by qRT-PCR, ABA determination and others are available in supporting Methods S1.

Results

*SITAF1* expression is induced by abiotic stresses

*SITAF1* is a tomato NAC transcription factor. It is a close homologue of Arabidopsis *ATAF1* (*Arabidopsis thaliana ACTIVATING FACTOR 1*, also called *Arabidopsis NAC002*, *ANAC002*; 68% identity and 72% similarity at the amino acid level) whose expression is induced during leaf senescence and by various abiotic stresses, such as H₂O₂ treatment, drought, salinity, prolonged darkness and that mediates multiple functions in...
the adaptation to abiotic and biotic stresses (Jensen et al., 2007; Lu et al., 2007; Jensen et al., 2008; Wang et al., 2009; Garapati et al., 2015; Li et al., 2016) (PLAZA 3.0; http://bioinformatics.psb.ugent.be/plaza).

SlTAF1 is expressed in all organs throughout tomato development, however its expression is considerably higher in roots, open flowers and during fruit ripening than in other organs (Tomato eFP Browser; Rohrmann et al., 2011; Shinozaki et al., 2018). In leaves, expression of SlTAF1 is induced during leaf senescence (Fig. S1a). To assess the response of SlTAF1 to abiotic stresses, we examined the effect of H₂O₂, drought and salinity on its expression in tomato (cv Moneymaker) by qRT-PCR. As shown in Fig. 1a, SlTAF1 expression was significantly enhanced after exposure of 2-wk-old tomato seedlings to a 4 h H₂O₂ treatment. Also, dehydration (2 h) resulted in a significant increase in SlTAF1 transcript level (Fig. 1b). With respect to salinity stress, we analysed 2-wk-old tomato seedlings subjected to 120 mM NaCl for different times (2, 4, 6 or 10 h). SlTAF1 transcript abundance highly (c. 13-fold) increased already after 2 h of salt stress, and it steadily increased peaking at the end of the treatment (c. 57-fold increase at 10 h). (Fig. 1c) We also examined SlTAF1 expression in 2-wk-old seedlings of the wild tomato species S. pimpinellifolium and S. cheesmaniae. After 4 h of NaCl treatment (200 mM), SlTAF1 expression was strongly enhanced in both species, as well as in S. lycopersicum, compared with the control (Fig. 1d).

Salinity and drought led to an accumulation of ABA (Takahashi et al., 2018). Accordingly, treatment with ABA (100 μM) stimulated SlTAF1 expression (Fig. 1e), suggesting that SlTAF1 acts downstream of ABA. Taken together, SlTAF1 is an early dehydration-responsive and salinity stress-responsive gene suggesting that it plays a role in the response to these stresses in tomato.

SlTAF1 promotes tolerance to salt stress

The rapid and strong transcriptional response of SlTAF1 to salinity stress in both, cultivated and wild tomato prompted us to investigate its potential function in combating this environmental stress. To characterise the function of SlTAF1 for the response to salt stress, transgenic lines with altered expression of SlTAF1 were generated. First, we obtained several transgenic tomato lines that overexpressed SlTAF1, compared with wild-type, under the control of the largely constitutive cauliflower mosaic virus (CaMV) 35S promoter (Fig. S1b). All 35S overexpression lines exhibited a severe growth retardation and dwarf phenotype (Fig. S1c,d). To uncouple the pleiotropic effects of SlTAF1 on plant growth and its role in stress tolerance, we next generated plants expressing an SlTAF1 in-frame fusion to green fluorescent protein (GFP), under the control of the native SlTAF1 promoter (from this point forward, pTAF1:TAF1-GFP). Two lines (L1 and L2), exhibiting increased expression of SlTAF1 and GFP after 4 h of NaCl treatment, were selected for further investigation (Fig. S2a, b). Confocal microscope visualisation illustrated a SlTAF1-GFP signal in the nucleus of leaf epidermal cell after 4 h of NaCl treatment in agreement with the function of SlTAF1 as a transcription factor (Fig. S2b).

To generate SlTAF1 transgenic lines with reduced SlTAF1 expression level, the artificial micro-RNA (amiRNA) silencing technology was used. A 21-bp amiRNA sequence was chosen to target the third exon of SlTAF1 (Fig. S2c). Two independent transgenic lines (hereafter named kd-L1 and kd-L2) with reduced expression of SlTAF1 were selected for analysis of salt tolerance. Expression of the closely homologous genes SINAC1 and SINAC4 (c. 62% and 72% identity with SlTAF1 at the nucleic acid level; PLAZA 3.0) were not altered in the selected SlTAF1 knockdown lines (Fig. S2c), indicating that the amiRNA which we designed specifically targets SlTAF1.

To evaluate the function of SlTAF1 in salinity tolerance, SlTAF1 transgenic lines (pTAF1:TAF1-GFP-L1 and L2, kd-L1 and kd-L2) and wild-type plants were subjected to salinity stress. For this purpose, 10-d-old seedlings grown on agar were transferred to an aerated hydroponic nutrient solution and, after 10 d, a subset of plants was subjected to salinity stress (120 mM NaCl) for 5 d. As shown in Fig. 2, all genotypes showed symptoms of salt stress, such as yellowing, reduction of chlorophyll content and biomass. However, the effects were remarkably stronger in the kd plants: wild-type and pTAF1:TAF1-GFP-L1 plants showed a c. 45% decrease in total wet biomass, while a 70% reduction of biomass was observed for the kd lines (Fig. 2d). The chlorophyll content was significantly higher in the pTAF1:TAF1-GFP-L1 line, but in direct contrast lower in the kd-L1 and kd-L2 lines compared with wild-type upon 5 d of salt stress (Fig. 2d). Accordingly, the expression of the senescence-associated genes SAG13 and SAG15 was reduced in pTAF1:TAF1-GFP-L1 but enhanced in SlTAF1-kd lines compared with the wild-type plants following 5 d of salt stress (Fig. 2e). Importantly, hypersensitivity of SlTAF1-kd lines to salinity became even more evident when the exposure to salt stress was extended to 8 d (Fig. S3), strongly supporting the role of SlTAF1 for protecting against the otherwise deleterious effects of salinity stress.

Abiotic stresses including salinity cause an accumulation of ROS which eventually leads to programmed cell death (Petrov et al., 2015). Detection of H₂O₂ by diaminobenzidine (DAB) staining revealed reduced ROS (H₂O₂) levels in pTAF1:TAF1-GFP-L1 plants but increased levels in kd-L1 lines compared with wild-type after 5 d of salt stress (Fig. 2f,g).

We also tested the effect of salt stress on the transgenic lines grown in soil. To this end, 25-d-old soil-grown tomato plants were subjected to salt stress (200 mM NaCl) at three intervals (each time 72 h) for a period of 10 d. As illustrated in Fig. S4a, b, kd-L1 and kd-L2 were severely affected by salt stress while pTAF1:TAF1-GFP-L1 and -L2 plants showed a less sensitive phenotype compared with wild-type. The chlorophyll content remained significantly higher in pTAF1:TAF1-GFP-L1 and -L2 than wild-type after salt treatment, while it dropped in kd-L1 and kd-L2 plants (Fig. S4c). Ion leakage (as an indicator of membrane integrity) was significantly higher in kd-L1 and kd-L2 plants than in wild-type, while it was lower in pTAF1:TAF1-GFP-L1 and -L2 plants (Fig. S4d). These data provide further support for the
**Fig. 1** *SlTAF1* expression under different stress treatments in tomato. (a) Transcript level of *SlTAF1* (*Solanum lycopersicum* TRANSCRIPTION ACTIVATION FACTOR 1) in 2-wk-old wild-type seedlings after H$_2$O$_2$ treatment for 4 h. (b) Transcript level of *SlTAF1* in detached leaflets (terminal leaflet of leaf no. 5) of 42-d-old wild-type plants after 2 h dehydration. (c) Expression level of *SlTAF1* in 2-wk-old wild-type seedlings (*S. lycopersicum* cv Moneymaker) at different time points after NaCl (120 mM) treatment. (d) Expression of *SlTAF1* in 2-wk-old wild-type seedlings of *S. lycopersicum*, *S. pimpinellifolium* and *S. cheesmaniae* after 4 h of 200 mM NaCl treatment. (e) *SlTAF1* expression upon ABA treatment. Two-week-old wild-type seedlings were treated with 100 µM ABA for 2, 4 or 6 h; 0 h indicates the time point before treatment (control). (a–e) Data represent the means of three biological replicates ± SE. Asterisks indicate significant difference (Student’s t-test; **, *P* < 0.01) from controls. Expression analysis was carried out using qRT-PCR. Expression of *SlTAF1* was determined relative to the *SlGAPDH* (*S. lycopersicum* GLYCERALDEHYDE 3-PHOSPHATE DEHYDROGENASE; Solyc04g009030) reference gene. The Y-axis indicates expression level (40-dCt). Values are expressed as the difference between an arbitrary value of 40 and dCt, so that high 40-dCt values indicate high gene expression levels.
**Fig. 2** SlTAF1 promotes salt stress tolerance in tomato. (a) Representative images of 22-d-old wild-type and SlTAF1 transgenic plants grown in aerated hydroponics nutrient solutions for 15 d. Tomato wild-type (Solanum lycopersicum cv Moneymaker) and transgenic seeds (T3, homozygous) were germinated on MS medium and grown for 7 d before transfer of the seedlings to the hydroponics system. (b) Wild-type and SlTAF1 transgenic plants were grown as in (a) for 10 d in aerated hydroponics and later supplemented with 120 mM NaCl for 5 d. The black-boxed areas are enlarged in the lower panels. Note the generally less healthy phenotype of SlTAF1-kd lines (kd-L1 and kd-L2). (c) Total plant biomass (FW) of control plants as shown in (a) and salt-treated plants as shown in (b). Values are means of eight biological replicates ± SE. (d) Chlorophyll content of the third leaf (counted from the bottom of the stem) of plants grown in (a) and (b). Chlorophyll content was measured by SPAD meter. Values are means of eight biological replicates ± SE. (e) Expression of SENESCENCE-ASSOCIATED GENES (SAGs) in SlTAF1-kd-L1 and pTAF1:TAF1-GFP-L1 plants compared with wild-type after 5 d of salt treatment (120 mM NaCl). Expression analysis was carried out using qRT-PCR. Values were normalised to those determined in the control plants. Y-axis denotes expression values on a log₂ fold change (FC) scale. Data represent means of three biological replicates ± SE. (f) DAB (3,3′-diaminobenzidine) staining after 5 d of salt stress in wild-type and SlTAF1 transgenic plants. (g) Percentage of dark-brown spot coloration relative to the total leaf area after DAB staining. Note, stronger DAB staining indicates higher level of H₂O₂. Values are means of three biological replicates ± SE in control and of five biological replicates ± SE in NaCl treatment experiments. Asterisks (c–e,g) denote a significant difference between transgenic lines and wild-type (Student’s t-test; *, *P < 0.05).
model that SITAF1 functions as a positive regulator of salt tolerance in tomato plants also when grown in soil.

To further evaluate the role of SITAF1 for the response to salinity stress, we generated transgenic lines with a deletion at the SITAF1 locus, using CRISPR/Cas9 editing (Belhaj et al., 2013; Brooks et al., 2014), and evaluated their response to salt stress (Fig. S5a–d). Like SITAF1-kd plants, CR-taf1-L18 plants were significantly more sensitive to salt than wild-type when grown in a hydroponics system (120 mM NaCl for 5 d) or in soil (200 mM NaCl for 6 d) (Fig. S5e–j).

Taken together, the results presented provide compelling evidence that SITAF1 is a key regulatory component of salinity stress tolerance in tomato.

**SITAF1 regulates ion homeostasis under salinity stress**

During salinity stress, the excessive accumulation of sodium (Na⁺) ions in leaves leads to ion toxicity which negatively affects plant growth (Maathuis, 2014). Here, we quantified Na⁺ and K⁺ levels in shoots (youngest leaves number 4, 5 and 6, counted from the bottom of the stem) and roots of 22-d-old wild-type and SITAF1 transgenic plants exposed to salinity stress, 120 mM NaCl, for 5 d (and no NaCl as control). As expected, salt stress led to higher accumulation of Na⁺ and decreased K⁺ levels in shoots and roots of the wild-type plants (Fig. 3 and Fig. S6). In shoots, Na⁺ accumulation was drastically higher in SITAF1-kd plants than in wild-type. By contrast, significantly lower Na⁺ level was detected in pTAF1:TAF1-GFP-L1 compared with wild-type (Fig. 3a). No considerable difference in the level of K⁺ was observed between the transgenic lines (Fig. 3b). As a result, the deduced Na⁺/K⁺ ratio was higher in SITAF1-kd and lower in pTAF1:TAF1-GFP-L1 than in wild-type (Fig. 3c). The higher accumulation of Na⁺ in the leaves of SITAF1-kd plants could explain their salinity-hypersensitive phenotype.

In roots, no significant differences were observed for Na⁺ and K⁺ contents between wild-type and the SITAF1 transgenic plants. Similarly, the Na⁺/K⁺ ratios were not altered (Fig. S6).

To investigate the mechanisms underlying the altered accumulation of Na⁺ in leaves of SITAF1 transgenic lines, we determined the expression of the xylem parenchyma localised Na⁺ transporters SIHKT1;1 (S. lycopersicum HIGH-AFFINITY K⁺ TRANSPORTER 1;1) and SIHKT1;2 (Asins et al., 2013), plasma membrane-localised Na⁺/H⁺ antiporter SISO1 (Olias et al., 2009), and vacuolar antiporter LeNHX4 (Gálvez et al., 2012) in shoots (sixth leaf) and roots of SITAF1 transgenic lines subjected to 120 mM NaCl for 2 d in hydroponic culture. Expression of SIHKT1;1 and SIHKT1;2 was significantly lower in SITAF1-kd than wild-type, but higher in pTAF1:TAF1-GFP-L1 in both, shoots and roots. Expression of SISO1 was higher in SITAF1-kd than wild-type shoots, while no difference was observed in roots. LeNHX4 expression was slightly upregulated in SITAF1-kd compared with wild-type shoots, while no change was detected in roots (Fig. 3d,e).

In tomato, it has been reported that Na⁺/K⁺ homeostasis in the aerial part is mainly regulated by the Na⁺ transporter SIHKT1;2. Silencing of SIHKT1;2 increased the leaf Na⁺/K⁺ ratio and resulted in hypersensitivity to salinity (Asins et al., 2013). Differential accumulation of Na⁺ in the leaves of SITAF1-kd and pTAF1:TAF1-GFP-L1 could be a consequence of altered HKT1 expression in those lines.

**SITAF1 controls stomatal aperture in response to salinity stress**

Na⁺ moves from roots to shoots via the transpiration stream. Enhanced leaf transpiration and, therefore, water loss leads to massive transport of Na⁺ to leaves (Campos et al., 2016). Therefore, the ability to prevent water loss is one of the mechanisms to enhance salinity tolerance (H. J. Wu et al., 2012; Koenig et al., 2013; Shabala, 2013).

To test whether the elevated Na⁺ level in shoots of SITAF1-kd plants may be due to alteration in water loss via transpiration, we determined the stomatal pore area in SITAF1 transgenic and wild-type plants. To this end, the abaxial leaf epidermis of wild-type, SITAF1-kd and pTAF1:TAF1-GFP-L1 was imprinted with dental resins after 48 h of 120 mM NaCl treatment (and without NaCl as control) and analysed by microscopy. As shown in Fig. 4a,b, SITAF1-kd displayed significantly larger stomatal pore area than wild-type, whereas pore area in pTAF1:TAF1-GFP-L1 was significantly lower. We did not observe a difference in stomatal pore area between wild-type and SITAF1 transgenic lines at the control condition (Fig. 4a,b). We also assessed stomatal conductance of SITAF1 transgenic lines during salinity stress. A significantly higher stomatal conductance was observed in SITAF1-kd after salt stress (48 h and 15 d) than wild-type, while pTAF1:TAF1-GFP-L1 exhibited lower stomatal conductance (Fig. 4c).

As ABA is an important phytohormone involved in stomatal closure, we checked whether treatment of ABA affects stomatal response in SITAF1 transgenic plants. Peeled abaxial epidermal leaf strips of wild-type, SITAF1-kd and pTAF1:TAF1-GFP-L1 plants were treated with ABA (100 µM) and examined for stomatal closure. Application of ABA led to reduction of stomatal pore area in all genotypes, however the reduction was significantly lower in SITAF1-kd-L1 than wild-type, but substantially higher in pTAF1:TAF1-GFP-L1 (Fig. S7). These results indicated that SITAF1 is involved in ABA-mediated stomatal closure during salinity stress.

**SITAF1 alters primary metabolism upon salt treatment in tomato**

To elucidate potential additional mechanisms involved in the regulation of salinity tolerance by SITAF1, we investigated the primary metabolite profile of SITAF1 transgenic and wild-type plants following salt stress treatment by gas chromatography coupled to mass spectrometry (GC-MS). To this end, 54 primary metabolites were characterised in the leaves (sixth leaf counted from the bottom of the stem) of 22-d-old wild-type and transgenic lines after 5 d of salt (120 mM NaCl) stress. In wild-type plants, 39 metabolites were significantly altered upon salt treatment in those lines.

To elucidate potential additional mechanisms involved in the regulation of salinity tolerance by SITAF1, we investigated the primary metabolite profile of SITAF1 transgenic and wild-type plants following salt stress treatment by gas chromatography coupled to mass spectrometry (GC-MS). To this end, 54 primary metabolites were characterised in the leaves (sixth leaf counted from the bottom of the stem) of 22-d-old wild-type and transgenic lines after 5 d of salt (120 mM NaCl) stress. In wild-type plants, 39 metabolites were significantly altered upon salt treatment in those lines.
Fig. 3 Effect of salt stress on Na⁺ and K⁺ ions in leaves of SlTAF1 transgenic tomato plants. (a) Na⁺ and (b) K⁺ concentrations (nmol/mg DW) in leaves no. 4, 5 and 6 (counted from the bottom of the stem; top three leaves) of wild-type and SlTAF1 transgenic plants (as grown in Fig. 2a,b) after 5 d of salt (120 mM NaCl) treatment and under control condition (no salt added). (c) Ratio of Na⁺ (a) and K⁺ (b) concentrations in leaves of wild-type and SlTAF1 transgenic lines. Data represent the mean of four biological replicates ± SE. (d,e) Expression of ion transporter genes SlHKT1;1 (Solanum lycopersicum HIGH-AFFINITY K⁺ TRANSPORTER 1;1), SlHKT1;2, SISOS1 (S. lycopersicum SALT OVERLY SENSITIVE 1) and LeNHX4 (Lycopersicon esculentum Na⁺/H⁺ ANTIPORTER 4) in (d) sixth leaf (counted from the bottom of the stem) and (e) roots of SlTAF1-kd-L1 and pTAF1:TAF1-GFP-L1 plants compared with wild-type after treatment for 2 d with NaCl (120 mM) in a hydroponic culture system. Expression analysis was carried out using qRT-PCR. Expression values were normalised to those in the corresponding control plants. Y-axis denotes log₂ fold change. Expression values represent means of three biological replicates ± SE. Asterisks indicate a significant difference between wild-type and kd-L1, and pTAF1:TAF1-GFP-L1 plants (Student’s t-test; *, P < 0.05).
significantly different abundances between wild-type and SlTAF1 transgenic plants (kd-L1, kd-L2 and pTAF1:TAF1-GFP-L1) upon salt stress were plotted in histograms (Fig. 5 and Table S2). Among all examined metabolites, the compatible osmolytes proline and 4-hydroxyproline showed dramatic induction upon salt stress in wild-type plants. Accumulation of proline by salt stress has been reported in several plant species including tomato (Verbruggen and Hermans, 2008; Gharsallah et al., 2016). However, upregulation of proline and 4-hydroxyproline by salt treatment was significantly diminished in SlTAF1-kd. By contrast, induction of proline by salt stress was considerably higher in pTAF1:TAF1-GFP-L1 plants compared with wild-type.

The level of the majority of other amino acids decreased in wild-type plants after salt stress, with the exception of tyrosine and serine. This reduction was less significant in pTAF1:TAF1-GFP-L1 but more prominent in SlTAF1-kd plants (in comparison with wild-type).

Among the sugars, xylose and rhamnose declined in SlTAF1-kd, but remained unchanged in pTAF1:TAF1-GFP-L1 compared with wild-type, while maltose increased in SlTAF1-kd, but decreased in pTAF1:TAF1-GFP-L1. Tricarboxylic acid (TCA) cycle intermediates malate and fumarate were significantly lower in the SlTAF1-kd plants and higher in pTAF1:TAF1-GFP-L1 compared with wild-type upon salt treatment.

When taken together these data suggested that SlTAF1 is an important component of the control of cellular metabolism under salt stress since modification of its expression levels either dampens (in the case of deficiency of SlTAF1 expression) or exacerbates (in the case of SlTAF1 overexpression) the wild-type metabolic response to salt stress.

Fig. 4 Stomatal aperture and conductance in wild-type and SlTAF1 transgenic tomato plants during salinity stress. Wild-type, SlTAF1-kd-L1 and pTAF1:TAF1-GFP-L1 plants were grown hydroponically as shown in Fig. 2(a, b). (a) Representative images of stomatal aperture of terminal leaflets of the third leaf after 48 h of 120 mM NaCl treatment (without NaCl as control). (b) Stomatal pore area of wild-type and SlTAF1 transgenic plants. Measurements were taken at 3 h after the beginning of the photoperiod. Data are means of three biological replicates ± SE in control; while four biological replicates ± SE in salt treatment. Each biological replicate included the measurement of c. 120 stomata. (c, d) Stomatal conductance, determined with a porometer, of the third leaf of wild-type, SlTAF1-kd-L1 and pTAF1:TAF1-GFP-L1 plants after 48 h and 15 d of 120 mM NaCl treatment, respectively (without NaCl treatment as control). (c, d) Data represent means of six biological replicates ± SE. Asterisks indicate a significant difference between wild-type and kd-L1, and pTAF1:TAF1-GFP-L1 plants (Student’s t-test; *, P < 0.05).
SITAF1 regulates salt-responsive genes in tomato

To acquire further insight into salt-tolerance mechanisms regulated by SITAF1 and to identify the early responses at the gene expression level, we generated estradiol-inducible SITAF1 overexpression lines (hereafter, SITAF1-IOE; Fig. S2e) and checked the expression of 23 stress-relevant genes in SITAF1-IOE after 6 h of estradiol (EST) or EST in combination with salt (200 mM NaCl) treatments. The examined genes include genes that encoded TFs whose expression is strongly induced by salt (Table S3) and genes involved in ABA metabolism. Moreover, genes encoding alternative oxidases (AOX) were included in our study as manipulation...
of alternative oxidases has been reported to influence salt and drought tolerance in different species (Smith et al., 2009; Hu et al., 2018; Zhu et al., 2018).

Based on the expression profile, genes were categorised into two groups. The first group corresponds to salt-independent SITAF1 early induced/responsive genes (nine genes); and the second group compiles salt-dependent early induced/responsive genes (seven genes) whose significant rapid induction by SITAF1 required salt treatment (Fig. 6a). Overall, transcript levels of several genes encoding TFs such as SiJUB1 (S. lycopersicum JUNGBRUNNEN 1), SiJUB2, SiH87 (S. lycopersicum HOMEBOX 7), SiJ2A (S. lycopersicum JASMONIC ACID 2), SiDREB2A1 and SiDREB2A2 as well as ABA-signalling TFs such as SlABF1 (S. lycopersicum ABA-RESPONSIVE ELEMENT-BINDING FACTOR 1), SiAREB1/SiABF2 and SiABF3 were rapidly and significantly induced by SITAF1 either in a salt-dependent or salt-independent manner. Among ABA biosynthesis genes SISDR1A (S. lycopersicum SHORT-CHAIN ALCOHOL DEHYDROGENASE/REDUCTASE 1A), SISDR1B and SISDR1C were significantly upregulated by SITAF1. Other ABA synthesis genes, such as SINCED1 (S. lycopersicum 9-CIS-EPOXYCAROTENOID DIOXYGENASE 1), SINCED2 and SINCED3 as well as Sitis (which encodes an aldehyde oxidase), were slightly induced by SITAF1. Finally, we quantified the ABA level in 8-d-old seedlings of wild-type and SITAF1 transgenic plants. The ABA level was significantly higher in pTAF1:TAF1-GFP (Fig. 8) However, the ABA level remained unchanged between SITAF1-kd and CR lines compared with wild-type (data not shown) suggesting that regulation of ABA by SITAF1 may be redundant with other control mechanisms. Additionally, the expression of SIAOX1a (S. lycopersicum ALTERNATIVE OXIDASE 1a) was considerably enhanced by SITAF1.

SITAF1 potential direct target genes

To identify potential target genes of SITAF1, we first attempted to identify its consensus binding motifs. SITAF1 is phylogenetically clustered into a stress-responsive SNAC group (Nuruzzaman et al., 2010), which bind a (C/T)ACG core motif (Fujita et al., 2004; Tran et al., 2004; Olsen et al., 2005; Xue et al., 2006; A. Wu et al., 2012; Garapati et al., 2015). To determine the DNA-binding sequences of SITAF1, a diverse set of the (C/T)ACG motif-containing sequences, including the high-affinity binding sites of TaNAC69 from wheat (Triticum aestivum), and AtJUB1, ATAF1 and ANAC019 from Arabidopsis, were used as probes for measuring the potential DNA-binding activity of SITAF1 towards these probes (Table S4). Similar to TaNAC69, SITAF1 has two types of binding sites (BS-I and BS-II). BS-I has a sequence of CGT(A/G)-5N(T/C)ACG(C/T/G)(A/C/T)(A/T/G)(C/T/G)(T/C), which contains two ((C/T)ACG or CGT(A/G)) core motifs and a spacer of five or six nucleotides. BS-II contains only one (C/T)ACG core motif with a sequence of (C/T)ACGN(C/A/T)(T/A)N(C/T)/A. However, the sequence flanking the left side of the core motif appears to be important for its binding activity (Table S4; Xue et al., 2006).

Next, we searched for its consensus binding motifs in the promoters (1 kb) of SITAF1 early responsive genes. Among those, nine genes (SlH87, SlJUB1, SlJUB2, SIRED10 (S. lycopersicum EARLY RESPONSE TO DEHYDRATION 10), SISDR1A, SlJ2A, SlAREB1, SlSREB29B (S. lycopersicum RESPONSIVE TO DESiccation 29B) and SlAOX1a) harbour an SITAF1 binding site in their promoters (Fig. 6b). Importantly, after 4 h of NaCl (200 mM) treatment, expression of all potential direct target genes of SITAF1 was elevated in pTAF1:TAF1-GFP-L1 seedlings compared with wild-type, but reduced in Cr-taf1-L18 plants (Fig. 6c). As expected, expression of the genes was intermediary in the SITAF1-kd seedlings (Fig. 6d).

To determine binding of SITAF1 to the promoters of the potential direct target genes we employed an electrophoretic mobility shift assay (EMSA). As depicted in Fig. 6d, SITAF1 binds and physically interacts with a 40-bp promoter fragment (harbouring SITAF1 BS) of all the potential direct target genes.

Discussion

We investigated the role of NAC transcription factor SITAF1 for the response to salt stress in tomato and discovered its involvement in the regulation of key processes underlying the tolerance to salinity stress; to summarise the role of SITAF1 in this process, we provide a model in Fig. 7. Expression of SITAF1 is highly upregulated by dehydration, exposure to hydrogen peroxide (H2O2), salt stress and by treatment of plants with ABA, a phytohormone integrating stress signals with growth and developmental programmes (Fig. 1). We observed that SITAF1-knockout (CR-taf1) and SITAF1-knockdown (kd) lines exhibited enhanced sensitivity to salt stress, while an increased expression of SITAF1 in overexpressors conferred increased tolerance to salinity stress. Furthermore, proline, a compatible solute involved in osmotic adjustment, accumulated to higher levels in pTAF1:TAF1-GFP plants than wild-type during salinity stress, while a reduction in proline content was observed in SITAF1-kd lines. Proline levels often increase in plants during drought and salt stress, and proline contributes to osmotic adjustment under stress conditions (Verbruggen and Hermans, 2008; Szabados and Savouré, 2010).

The TCA cycle plays a central role in energy metabolism. A decline in the content of the TCA cycle intermediates is frequently observed in glycophytes under salt stress (Gong et al., 2005; Sanchez et al., 2008; Zhao et al., 2014; Richter et al., 2019). In all genotypes tested here (i.e. wild-type, SITAF1-kd and pTAF1:TAF1-GFP), we observed a slight increase in citric acid after salt stress (Table S2). Malate and fumarate decreased in all genotypes, however the level of reduction was more pronounced in SITAF1-kd, but less prominent in pTAF1:TAF1-GFP plants (Fig. 5). While a change in the flux mode with a concomitant reduction of oxaloacetate-derived aspartate and 2-oxoglutamate-derived glutamine, indicating a reduction in the flux of carbon skeletons from the TCA cycle to amino acids, appears to be common in all lines, the negative effect of salt stress appeared to be less pronounced in pTAF1:TAF1-GFP plants, in accordance with their higher salinity tolerance compared with wild-type.
In the longer term, salinity leads to ion toxicity. Plants have evolved mechanisms to alleviate the toxic effects of Na\(^+\) by regulating Na\(^+\) transport from root to shoot, exclusion of Na\(^+\) from the cytoplasm, or sequestration of salt ions in vacuoles (Yamaguchi and Blumwald, 2005; Munns and Tester, 2008; Deinlein et al., 2014). We observed a higher accumulation of Na\(^+\) in leaves.
of SlTAF1-kd than wild-type plants while, by contrast, pTAF1: TAF1-GFP plants accumulated significantly less Na\(^+\) in leaves. These data suggested that SlTAF1 contributes to the lower accumulation of Na\(^+\) in the plant’s aerial parts, consistent with its role in improving salt tolerance.

In Arabidopsis, the class I HKT sodium transporter AtHKT1;1 retrieves Na\(^+\) ions from the xylem transpiration stream, thereby preventing its transport to shoots (Davenport et al., 2007; Moller et al., 2009). The function of HKT1 appears to be conserved in dicotyledonous plants such as tomato. Asins et al. (2013) identified quantitative trait loci (QTL) involved in the regulation of shoot Na\(^+\) homeostasis, harbouring closely linked SIHK1;1 and SIHK1;2 sodium transporter genes. These two genes are expressed in xylem parenchyma and phloem cells suggesting a role for retrieving Na\(^+\) from the xylem transpiration stream and possibly loading it into phloem sieve tubes. Moreover, silencing of SIHK1;2 led to a higher Na\(^+\)/K\(^+\) ratio in leaves and salt-hypersensitivity (Jaime-Perez et al., 2017). Here, shoot and root tissues of pTAF1:TAF1-GFP plants showed higher transcript abundance of SIHK1;1 and SIHK1;2 after 2 d of salt treatment; conversely, transcript levels of both genes were significantly reduced in SlTAF1-kd plants. Taken together, the above data suggest that SlTAF1 is involved in controlling the retrieval of Na\(^+\) from the xylem transpiration stream via regulating the expression of SIHK1;1 and SIHK1;2 under saline conditions.

Recently, Shkolnik et al. (2019) showed that an ABA-responsive element (ABRE) in the promoter of AtHKT1;1 is required for its enhanced expression in response to salt and ABA treatment. SIHK1;1 and SIHK1;2 promoters do not contain SlTAF1 binding sites and can, therefore, not be directly bound by SlTAF1. It is therefore likely that SlTAF1 enhances the expression of SIHK1;1 in an ABA-dependent manner during salinity stress.

During salinity stress, Na\(^+\) moves from roots to shoots through the xylem transpiration stream and accumulates in aerial parts of the plant which leads to toxicity. Optimising transpiration by controlling stomatal aperture is amongst the main determinants for reducing the rate of Na\(^+\) transport to shoots, thereby leading to salt acclimation over time (Shabala, 2013; Campos et al., 2016). Here, we observed higher stomatal conductance and stomatal pore area in response to salt stress in SlTAF1-kd than wild-type plants, while the opposite trend was detected in pTAF1:TAF1-GFP-L1 (Fig. 4). ABA plays a vital role for stomatal closure and regulating water loss via transpiration (Raghavendra et al., 2010). Expression of SlTAF1 is induced by ABA treatment (Fig. 1c). Moreover, SlTAF1 is involved in ABA...
biochemical pathways for the production of LEA proteins, which play a crucial role in stress tolerance.

**SlTAF1 Enhances Expression of ABA Biosynthesis Genes**

SlTAF1 enhances expression of ABA biosynthesis enzymes, such as SlNCED1, which is positively regulated by ABA signal. The transcriptional upregulation of SlNCED1 is correlated with increased ABA levels in transgenic plants expressing SlTAF1 (Table S3). Electrophoretic mobility shift assays (EMSA) revealed that SlTAF1 physically interacts with the promoters of these genes (Fig. S8) suggesting that SlTAF1 is involved in regulating ABA biosynthesis.

**Salt Stress Tolerance**

The overexpression of SlTAF1 in Arabidopsis seedlings resulted in elevated drought tolerance (Mishra et al., 2012). Indeed, the ABA level was higher in pTAF1:TAF1-GFP plants compared with wild-type (Fig. S8) suggesting that SlTAF1 is an activator of its own expression. Among SlSDR1s, however, only SlSDR1A is a positive regulator of drought stress tolerance in tomato. SlSDR1A overexpression reduces 

**ABA Biosynthesis**

ABA biosynthesis is positively affected by SlTAF1 through the transcriptional upregulation of ABA biosynthesis genes and signalling, and defence-related components. SlTAF1 enhances expression of SlERD10, SlRD29B, and SlAOX1a, which encode alternative oxidase enzymes, in a Cis-acting ABREs (Fig. S9), suggesting that the induction of SlTAF1 by ABA occurs via ABRE-binding TFs; this, however, remains to be demonstrated.

**Conclusion**

In summary, our study demonstrates that SlTAF1 regulates various responses to salt stress at the molecular, physiological, and developmental levels and plays an important role for salinity tolerance.
Acknowledgements

The CRISPR/Cas9 cloning cassettes for tomato were kindly provided by Professor Dr Yuval Eshed, Weizmann Institute of Science, Rehovot, Israel. We thank Dr Karin Koehl and her team for plant care and setting up the hydroponic culture system. We thank Franziska Brueckner for performing ion measurements, and Dr Arun Sampathkumar for helping with microscopy. We very much thank the anonymous reviewers for their critical comments on the manuscript that helped to improve it. We thank the University of Potsdam and the Max Planck Institute of Molecular Plant Physiology for supporting our research. Vikas Devkar received a fellowship from the Indian Council of Agricultural Research (ICAR), New Delhi, India. Research by VT and MS is funded from European Regional Development Fund-Project ‘Centre for Experimental Plant Biology’ (no. CZ.02.1.01/ 0.0/0.0/16_019/0000738). The authors declare no competing financial interests.

Author contributions

SB and BM-R conceived the study; SB designed the research and supervised the work; VD generated the transgenic lines, performed salt treatment experiments, determined plant phenotypes, performed qRT-PCR analyses and contributed to the design of the research; VD and VPT jointly performed the EMSA experiments and confocal microscopy studies; G-PX performed the binding site selection assays; VT and MS performed the ABA measurements; JV performed primary metabolite profiling under the supervision of ARF; RH performed the ion measurements; SB wrote the manuscript with contributions from VD and BM-R; all authors read and commented on the manuscript.

ORCID

Salma Balazadeh 1 https://orcid.org/0000-0002-5789-4071
Vikas Devkar 1 https://orcid.org/0000-0002-0649-3227
Alisdair R. Fernie 1 https://orcid.org/0000-0001-9000-355X
Rainer Hoeferli 1 https://orcid.org/0000-0001-8590-9800
Bernd Mueller-Roeber 1 https://orcid.org/0000-0002-1410-464X
Miroslav Strnad 1 https://orcid.org/0000-0002-2806-794X
Venkatesh P. Thirumalaikumar 1 https://orcid.org/0000-0002-2005-1460
Veronika Turečková 1 https://orcid.org/0000-0001-8519-805X
José G. Vallarino 1 https://orcid.org/0000-0002-0374-8706
Gang-Ping Xue 1 https://orcid.org/0000-0002-1135-1768

References

Asins MJ, Villalta I, Aly MM, Olias R, Alvarez DEMP, Huertas R, Li J, Jaime-Perez N, Haro R, Raga V et al. 2013. Two closely linked tomato HKT coding genes are positional candidates for the major tomato QTL involved in Na+/K+ homeostasis. Plant, Cell & Environment 36: 1171–1191.

Balazadeh S, Siddiqui H, Allu AD, Matallana-Ramirez LP, Caldana C, Mehrnia M, Zanor MI, Kohler B, Mueller-Roeber B. 2010. A gene regulatory network controlled by the NAC transcription factor ANAC092/AtNAC2/ORE1 during salt-promoted senescence. The Plant Journal 62: 250–264.

Belhaj K, Chaparro-Garcia A, Kamoun S, Nekrasov V. 2013. Plant genome editing made easy: targeted mutagenesis in model and crop plants using the CRISPR/Cas system. Plant Methods 9: 39.

Belver A, Olias R, Huertas R, Rodriguez-Rosales MP. 2012. Involvement of SISOS2 in tomato salt tolerance. Bioengineered 3: 298–302.

Brooks C, Nekrasov V, Lippman ZB, Van Eck J. 2014. Efficient gene editing in tomato in the first generation using the clustered regularly interspaced short palindromic repeats/CRISPR-associated9 system. Plant Physiology 166: 1292–1297.

Campos JF, Cara B, Perez-Martin F, Pineda B, Egea I, Flores FB, Fernandez-Garcia N, Capel J, Moreno V, Angosto T et al. 2016. The tomato mutant arsl (altered response to salt stress) identifies an R1-type MYB transcription factor involved in stomatal closure under salt acclimation. Plant Biotechnology Journal 14: 1345–1356.

Chen JQ, Meng XP, Zhang Y, Xia M, Wang XP. 2008. Over-expression of OsDREB genes lead to enhanced drought tolerance in rice. Biotechnology Letters 30: 2191–2198.

Davenport RJ, Munoz-Mayor A, Jha D, Essah PA, Rus A, Tester M. 2007. The Na+ transporter AtHKT1;1 controls retrieval of Na+ from the xylem in Arabidopsis. Plant, Cell & Environment 30: 497–507.

Davenport U, Stephan AB, Horie T, Luo W, Xu G, Schroeder JI. 2014. Plant salt-tolerance mechanisms. Trends in Plant Science 19: 371–379.

Du M, Zhai Q, Deng L, Li S, Li H, Yan L, Huang Z, Wang B, Jiang H, Huang T et al. 2014. Closely related NAC transcription factors of tomato differentially regulate stomatal closure and reopening during pathogen attack. Plant Cell 26: 3167–3184.

Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. 2003. OsDREB genes in rice, Oryza sativa L. encode transcription activators that function in drought- and cold-responsive gene expression. The Plant Journal 33: 751–763.

Ebrahimian-Motlagh S, Ribone PA, Thirumalaikumar VP, Allu AD, Chan RL, Mueller-Roeber B, Balazadeh S. 2017. JUNGBRUNNEN1 confers drought tolerance downstream of the HD-Zip I transcription factor AtHB13. Frontiers in Plant Science 8: 2118.

Ernst HA, Olsen AN, Larsen S, Lo Leggio L. 2004. Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors. EMBO Reports 5: 297–303.

Fraciante I, Carli P, Ercolano MR, Pernice R, Di Matteo A, Fogliano V, Pellegriti N. 2007. Antioxidant nutritional quality of tomato. Molecular Nutrition and Food Research 51: 609–617.

Fujita M, Fujita Y, Maruyama K, Seki M, Hiratsu K, Ohme-Takagi M, Tran LS, Yamaguchi-Shinozaki K, Shinozaki K. 2004. A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. The Plant Journal 39: 863–876.

Gálvez FJ, Baghour M, Hao G, Cagnac O, Rodriguez-Rosales MP, Venema K. 2012. Expression of LeNHX isoforms in response to salt stress in salt-sensitive and salt-tolerant tomato species. Plant Physiology and Biochemistry 51: 109–115.

Garapati P, Xue GP, Munne-Bosch S, Balazadeh S. 2015. Transcription factor ATAF1 in Arabidopsis promotes senescence by direct regulation of key chloroplast maintenance and senescence transcriptional cascades. Plant Physiology 168: 1122–1139.

Gharsallah C, Fakhfakh H, Grubb D, Gorseane F. 2016. Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. AOB Plants 8: plw055.

Golldack D, Luking I, Yang O. 2011. Plant tolerance to drought and salinity: stress regulating transcription factors and their functional significance in the cellulose biosynthesis in the plant. Journal of Plant Physiology 168 (8): 764–775.

Gonzalez-Guzman M, Apostolova N, Belles JM, Barrero JM, Piqueras P, Ponce MR, Micol JL, Serrano R, Rodriguez PL. 2002. The short-chain alcohol dehydrogenase ABA2 catalyzes the conversion of xanthoxin to abscisic aldehyde. Plant Cell 14: 1833–1846.

© 2019 Max Planck Institute of Molecular Plant Physiology
New Phytologist © 2019 New Phytologist Trust
www.newphytologist.com
Research

Huertas R, Olias R, Eljakaoui Z, Galvez FJ, Li J, De Morales PA, Belver A, Hu WH, Yan XH, He Y, Ye XL. 2018. Jensen MK, Rung JH, Gregersen PL, Gjetting T, Fuglsang AT, Hansen M, Jensen MK, Hagedorn PH, Joehnk N, Lyngkjaer MF, Collinge DB, Lyngkjaer MF. 2008. Huertas R, Rubio L, Cagnac O, Garcia-Sanchez MJ, Alche Jde D, Venema K, Fernandez JA, Rodriguez-Rosales MS. 2013. Jaime-Perez N, Pineda B, Garcia-Sogo B, Atares A, Athman A, Byrt CS, Olias Lata C, Prasad M. 2011. Hong Y, Zhang H, Huang L, Li D, Song F. 2016. Graether SP, Boddington KF. 2014. Klaring HP, Zude M. 2009. Rodriguez-Rosales MP. 2012. Kikuchi S. 2010. Liu Y, Sun J, Wu Y. 2016. Arabidopsis ATAF1 enhances the tolerance to salt stress and ABA in transgenic rice. Journal of Plant Research 129: 955–962. Luedemann A, Strassburg K, Erban A, Kopka J. 2008. TagFinder for the quantitative analysis of gas chromatography-mass spectrometry (GC-MS)-based metabolite profiling experiments. Bioinformatics 24: 732–737. Mao C, Ding J, Zhang B, Xi D, Ming F. 2018. ONaNAC2 positively affects salt-induced cell death and binds to the OsAAP3 and OsCOX1 promoters. The Plant Journal 94: 454–468. Massareto IL, Albalaidejo I, Purgato E, Flores FB, Plascencia F, Egea-Fernandez JM, Bolarin MC, Egea I. 2018. Recovering tomato landraces to simultaneously improve fruit yield and nutritional quality against salt stress. Frontiers in Plant Science 9: 1778. Maathuis FJM. 2014. Sodium in plants: perception, signaling, and regulation of sodium fluxes. Journal of Experimental Botany 65: 849–858. Mishra KB, Iannaccone R, Petrozza A, Mishra A, Armentano N, La Vecchia G, Triticik M, Cellini F, Nedbal L. 2012. Engineered drought tolerance in tomato plants is reflected in chlorophyll fluorescence emission. Plant Science 182: 79–88. Mittler R, Vanderauwera S, Gollery M, Van Breusegem F. 2004. Reactive oxygen gene network of plants. Trens in Plant Science 9: 490–498. Moller IS, Gilliham M, Moreno V, Perez-Rodriguez P, Riano-Pachon DM, Correa IG, Rensing SA, Kersten B, Mueller-Roemer B. 2010. PlantTFDB: updated content and new features of the NAC transcription factor family database. Nucleic Acids Research 38: D822–827. Petrov V, Hille J, Mueller-Roemer B, Gechev TS. 2015. ROS-mediated abiotic stress-induced programmed cell death in plants. Frontiers in Plant Science 6: 69. Puranik S, Sahu PP, Srivastava PS, Prasad M. 2012. NAC proteins: regulation and role in stress tolerance. Trends in Plant Science 17: 369–381. Raghavendra AS, Gunogunta VK, Christmann A, Grill E. 2010. ABA perception and signaling. Trends in Plant Science 15: 395–401. Richter JA, Behr JH, Erban A, Kopka J, Zörb C. 2019. Ion-dependent metabolic responses of Vicia faba L. to salt stress. Plant, Cell, & Environment 42: 295–309.
Rohrmann J, Toghe T, Alba R, Osorio S, Caldana C, McQuinn R, Arvidsson S, van der Merwe MJ, Riano-Pachón DM, Mueller-Roeber B et al. 2011. Combined transcription factor profiling, microarray analysis and metabolite profiling reveals the transcriptional control of metabolic shifts occurring during tomato fruit development. The Plant Journal 68: 999–1013.

Romani F, Ribone PA, Capella M, Miguel VN, Chan RL. 2016. A matter of quantity: Common features in the drought response of transgenic plants overexpressing HD-Zip I transcription factors. Plant Science 251: 139–154.

Szabados L, Savoury Tran LS, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyama K, Fujita Tak H, Negi S, Ganapathi TR. 2017. New Phytologist/C211 Wang LL, Hu ZL, Zhu MK, Zhu ZG, Hu JT, Qanmber G, Chen GP. 2017. New Phytologist/S212 Shkolnik D, Finkler A, Pasmanik-Chor M, Fromm H. 2019. Arabidopsis thaliana dehydration stress 1

Shinozaki Y, Nicolas P, Fernandez-Pozo N, Ma Q, Evanich DJ, Shi Y, Xu Y, Shabala S. 2009. Plant Physiology Mueller-Roeber B, Balazadeh S. 2018. Plant Biotechnology

Yamaguchi-Shinozaki K, Nakashima K. 2010. Plant Science Yamaguchi-Shinozaki K, Shinozaki K. 2018. Plant Physiology

2019 Max Planck Institute of Molecular Plant Physiology

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.
Fig. S6 Effect of salt stress on Na\(^+\) and K\(^+\) ions in tomato roots.

Fig. S7 Involvement of SlTAF1 in ABA-mediated stomatal closure.

Fig. S8 Higher accumulation of ABA in pTAF1:TAF1-GFP-L1 than wild-type plants.

Fig. S9 Schematic presentation of the positions of ABRE elements in the promoter of SlTAF1.

Methods S1 Supporting methods.

Table S1 Oligonucleotide sequences.

Table S2 Primary metabolite profiles of wild-type and SlTAF1 transgenic plants.

Table S3 Expression of SlTAF1 and stress-associated genes in tomato seedlings.

Table S4 DNA-binding specificity of SlTAF1.

Please note: Wiley Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the New Phytologist Central Office.