Research article

Overexpression of the NAC transcription factor JUNGBRUNNEN1 (JUB1) increases salinity tolerance in tomato

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A B S T R A C T

Soil salinity is a major abiotic stress affecting plant growth and yield, due to both osmotic and ionic stresses. JUNGBRUNNEN1 (JUB1) is a NAC family transcription factor that has been shown to be involved in responses to abiotic stresses, such as water deficit, osmotic, salinity, heat and oxidative stress. In Arabidopsis thaliana (Arabidopsis), JUB1 has been shown to improve plant stress tolerance by regulating H\textsubscript{2}O\textsubscript{2} levels. In the horticultural crop, Solanum lycopersicum cv. Moneymaker (tomato), overexpression of AtJUB1 has been shown to partially alleviate water deficit stress at the vegetative stage. In this study, we investigated the effect of Arabidopsis JUB1 overexpression in salinity tolerance in tomato. In hydroponically grown tomato seedlings, AtJUB1 overexpression results in higher prolines levels and improves the maintenance of water content in the plant under salinity stress. The transgenic tomato plants are more tolerant to salinity stress compared to control lines based on plant biomass. However, at the reproductive stage, we found that overexpression of AtJUB1 only provided marginal improvements in yield-related parameters, in the conditions used for the current work. The combination of improved water deficit and salinity stress tolerance conferred by AtJUB1 overexpression may be beneficial when tomato plants are grown in the field under marginal environments.

1. Introduction

Soil salinity is a major abiotic stress affecting plant growth and yield. The adverse effects of salinity occur as a result of osmotic and ionic stresses. Osmotic stress occurs immediately after salt imposition and continues for the duration of salt exposure, involving rapid signaling from root to shoot upon salt exposure and resulting in reduced cell expansion in growing tissues (Roy et al., 2014). Plants respond to osmotic stress using mechanisms involving processes such as maximizing water uptake and reducing water loss through stomatal closure (Munns and Tester, 2008). After several days of salinity stress, ions build up in photosynthetically active tissues and affect major processes such as photosynthesis, protein synthesis and energy production (Parida and Das, 2005). In addition, ionic stress results in premature leaf senescence, which reduces photosynthetically active areas available to support plant growth. Plants have evolved several mechanisms to limit the toxic effects of ionic stress; mechanisms include reducing sodium transport to the shoot and compartmentalizing it into the cell vacuole. Accumulation of compatible solutes, such as proline, sugars and amino acids is another adaptive mechanism to tolerate salinity stress (Roy et al., 2014). These solutes help the plant to adjust the osmotic pressures resulting from ion accumulation in the shoot (Munns and Tester, 2008).

Transcription factors (TFs) are key regulators in stress responses. They link stress sensing with many tolerance mechanisms by translating stress signals into changes in gene expression, that ultimately contribute to stress tolerance (Lodeyro and Carrillo, 2015). In salinity stress, for example, plants are thought to transmit the salt signal through various mechanisms, such as elevated cytosolic free Ca\textsuperscript{2+}, ROS and hormonal signaling, that affect the expression of stress related TFs (Choi et al., 2017). These TFs, subsequently, target many genes involved in stress responses to orchestrate biochemical and physiological processes critical for the stress tolerance (Lodeyro and Carrillo, 2015).

The widespread plant specific TF family, NAC (NAM, ATAF and CUC), regulates multiple processes related to plant growth, development and senescence (Souver et al., 1996; Xie et al., 2000; Guo and Gan, 2006; Kim et al., 2009; Balazadeh et al., 2011; Shahnejat-Bushehri et al., 2016). NACs have also been related to stress responses; several
NAC genes were found to be induced by biotic and abiotic stresses (Nakashima et al., 2012; Nuruzzaman et al., 2013; Shao et al., 2015; Hong et al., 2016; Wang et al., 2017). Functional characterization of NAC TFs in response to salt stress have been conducted in different plant species. For example, overexpression of wheat TaNAC29 in Arabidopsis plants improved salinity tolerance by reducing $\text{H}_2\text{O}_2$ accumulation and increasing cell membrane stability through enhancing expression of several genes encoding antioxidant enzymes (Xu et al., 2015). In rice, overexpression of SNAC1 improved the survival rate of rice seedlings grown in hydroponics and in soil (Hu et al., 2006). SNAC1 has also been shown to contribute to drought tolerance of rice at both seedling and reproductive stages by controlling stomatal closure and thus reducing the rate of water loss through transpiration (Hu et al., 2006). SNAC2 is another rice NAC TF whose expression is induced by drought, salt, cold, wounding and ascorbic acid (ABA) (Hu et al., 2008). Overexpression of SNAC2 enhanced plant germination rate and root length under high salinity (Hu et al., 2008).

JUBGBRUNNEN1 (JUB1, also known as NAC042) acts as a central regulator of plant growth (Shahnejat-Bushehri et al., 2016), longevity and stress response (Wu et al., 2012). Expression of Arabidopsis JUB1 is upregulated by abiotic stresses and confers tolerance to osmotic, salt, heat, drought and oxidative stresses (Shahnejat-Bushehri et al., 2012; Wu et al., 2012). The effect of JUB1 in stress tolerance is thought to occur through controlling $\text{H}_2\text{O}_2$ levels (Wu et al., 2012). JUB1 directly activates the expression of the transcription factor Dehydration-Responsive Element-Binding Protein 2A (DREB2A), which is an important regulator of drought and heat responses (Sakuma et al., 2006; Kant et al., 2008). DREB2A then regulates Heat-shock factor A2 (HsfA2) and thereby several Heat-Shock Protein (HSP) genes and genes encoding $\text{H}_2\text{O}_2$-scavenging enzymes (Schramm et al., 2008; Yoshida et al., 2008). With respect to growth regulation, JUB1 mediates the reduction of two growth hormones: gibberellic acid (GA) and brassinosteroids (BR). JUB1 represses the expression of several key enzymes involved in the GA and BR biosynthesis pathways (Shahnejat-Bushehri et al., 2016). In addition, JUB1 activates the expression of DELLA genes (GA1 and RGL1). DELLA proteins are transcriptional regulator proteins and regulate plant growth and stress responses. High levels of DELLA proteins mediate stress tolerance by limiting the accumulation of stress-induced reactive oxygen species (ROS) (Shahnejat-Bushehri et al., 2016).

The tomato homolog of Arabidopsis JUB1, SLJUB1, was found to be induced by different abiotic stresses, including salinity. Downregulation of SLJUB1 by virus-induced gene silencing (VIGS) in Solanum lycopersicum cv. Moneymaker (tomato) substantially decreased drought tolerance of tomato plants and, congruently, ectopic overexpression of AtJUB1 in tomato improved its tolerance to water deficit by maintaining high relative leaf water content and reducing $\text{H}_2\text{O}_2$ levels (Thirumalaikumar et al., 2018). Arabidopsis JUB1 was able to bind and directly regulate the tomato homolog of Arabidopsis DREB2A and DELLA (SIDREB1, SIDREB2 and SIDELLA). This suggests a considerable conservation in the gene regulatory networks controlled by JUB1 between Arabidopsis and tomato (Thirumalaikumar et al., 2018).

In this study, we investigate the effect of AtJUB1 in salinity tolerance in tomato. Hydroponically grown tomato plants overexpressing AtJUB1 display improved maintenance of biomass in response to salt stress compared to wild type plants, an effect associated with a higher maintenance of water within the plant. However, plants grown to maturity in soil overexpressing AtJUB1 only display marginal advantages compared to wild type plants, in the conditions used in these experiments.

2. Material and methods

2.1. Plant material

Plants of Solanum lycopersicum cv. Moneymaker (tomato) were used as wild type controls in salinity experiments. Tomato lines overexpressing AtJUB1 were previously described in (Shahnejat-Bushehri et al., 2017). In brief, tomato plants (Solanum lycopersicum, cv Moneymaker) were transformed with the 35S::AtJUB1-GFP overexpression construct described in (Wu et al., 2012).

2.2. Salinity stress in a supported hydroponic system

The salinity stress experiment was performed in a supported hydroponic system. Plants were grown in the greenhouse located at KAUST (Thuwal, Saudi Arabia) at 25°C/23°C day/night with a day length of approximately 13.5 h. Tomato seeds were surface sterilized with 20% bleach and washed thoroughly eight times with sterilized milliQ water. Seeds were stratified at 4°C for three days then germinated on agar plating consisting of ¼ Murashige and Skoog medium, 1% phyto-agar, pH 5.8. The agar plugs were placed in square pots (7 cm) filled with plastic beads and covered with water. This plant nursery was kept covered with transparent plastic covers until seeds started germinating. When the cotyledons emerged, plants were transferred from the nursery to the high-and-flow supported hydroponic system (Fig. S1) filled with growth medium. Growth medium was prepared by mixing equal amounts of three commercial media: FloraMicro, FloraGro and FloraBloom (General Hydroponics, USA). The pH was monitored throughout the experiment and maintained at 5.8–6.0 by supplementing with buffer solutions called “pH up” or “pH down” for hydroponics, supplied by Botanicare (USA). Growth solution was pumped up to the roots for 30 min, then allowed to drain for 30 min, giving an high-and-flow cycle of 60 min. This allowed the system to fully drain and roots to be well aerated before refilling. Uniformly sized seedlings at the five-leaf growth stage were selected for the salinity experiment.

Three salt concentrations were used: 0 mM NaCl (as control), 125 mM NaCl (as mild salt stress) and 200 mM NaCl (as high salt stress). To avoid osmotic shock, salt (NaCl) was applied in the following increments every 24 h: 75 mM, 125 mM, 200 mM NaCl. Calcium (in the form of CaCl$_2$) was supplemented with the salt treatments to compensate for the reduction of Ca$^{2+}$ activity due to NaCl addition (Tester & Davenport, 2003). Calcium activity and the amount of required CaCl$_2$ was calculated using GoechemEZ (Shaff et al., 2010). After 10 days of salt stress, the following measurements were made: plant biomass (fresh and dry), stem height and root length. Leaf area measurements were analyzed using the scanning software WinFOLIA (Régent Instruments Inc.). For the biochemical measurements: leaf three (as an older leaf) and leaf five (as a younger leaf) were collected. Salinity tolerance traits were assessed and analyzed according to Negrão et al. (2017).

2.3. Salinity stress in soil

To test the tomato plants under soil conditions a pot trial was performed. Tomato seeds were germinated in jiffy pellets and transferred to soil filled pots (20 cm) at the five-leaf growth stage. Salt (NaCl) was applied at the eight-leaf growth stage by adding a total volume of 1.3 L of 450 mM NaCl to the bottom of the outer pot (distributed as three doses of 400 mL, 400 mL and 500 mL over two weeks). The final concentration of NaCl in the soil was approximately 365 mM NaCl (after the soil had dried down to contain 1.6 L of water per pot). Plants were then assessed after 17 days of salt treatment.

2.4. Na and K measurements

Na and K content was measured in the roots and leaves using flame photometry. The leaf that developed during salt stress imposition (fully expanded leaf, here leaf 5) was used for Na and K measurements. Roots were rinsed twice in 10 mM MgSO$_4$ (to remove any excess NaCl from the hydroponic medium) and dried between tissue paper. Oven-dried roots and leaves were digested in 20 mL of 1% nitric acid at 85°C overnight and the concentration of Na and K were measured with the flame photometer (Sherwood Scientific Ltd., Cambridge, UK, model
2.5. Proline quantification

Proline content was determined according to the method of Bates et al. (1973) with some modifications (Vicente et al., 2004). Briefly, frozen plant material (around 100 mg) was ground to a fine powder. Sulfosalicylic acid (3%) was used for the extraction and cell debris were removed by centrifugation at 13000 rpm for 10 min. One volume of the supernatant was mixed first with one volume of freshly prepared acid ninhydrin (25 mg/mL ninhydrin in 10.44 M acetic acid and 2.4 M phosphoric acid); and then with one volume of glacial acetic acid. Samples were mixed and incubated at 99 °C for 1 h, and then the reaction was stopped by cooling the samples on ice. The proline-ninhydrin complex was extracted with two volumes of toluene. The absorbance of the organic phase was determined by UV-Spectrometer (model U-2910, Hitachi, Japan) at 520 nm, using toluene as a blank. A calibration curve was used to calculate the concentration of proline.

2.6. Determining the osmotic potential

Osmotic potential was measured on leaf 3 (old leaf) and leaf 5 (young leaf) using a vapor pressure osmometer (model 5600, Vapro, Wescor, Inc. USA). Leaves were collected in a 2 mL syringe and stored at −80 °C. Leaf sap was prepared by thawing the samples on ice and pressing the pyrolyst sxt to collect leaf sap. Only 10 μL of the leaf sap was used for analysis.

2.7. Chlorophyll quantification

Chlorophyll was extracted from leaf four according to Nayek et al. (2014) with a slight modification. Briefly, frozen plant material (approximately 50 mg) was ground to a fine powder. One mL of DMSO was added to the ground plant tissue and the mixture was sonicated for 15 min in an ultrasonic bath (model 3510, Branson, USA). The extracted mixture was centrifuged at 12,000 rpm, 4 °C for 10 min and the supernatant was collected. The same extraction procedure was repeated on the residue for a second time. Thereafter, each 2 mL supernatant was mixed with another 1 mL DMSO. The absorbance of Chlorophyll-a and Chlorophyll-b was determined by UV-spectrophotometry at 645 and 663 nm. Chlorophyll-a, Chlorophyll-b and total chlorophyll was calculated in salt and control, and then the ratio of RMR in salt to RMR in control conditions was calculated according to the following equations:

\[
\text{RMR}_{\text{salt}} = \frac{\text{DM}_{\text{salt}}}{\text{DM}_{\text{control}}} \times \frac{\text{DM}_{\text{control}}}{\text{DM}_{\text{salt}}}
\]

where DM is root dry mass.

2.9. Statistical analyses

The salinity tolerance index (ST) and relative root mass ratio were calculated according to Negrão et al. (2017), using the following equations (1)–(4):

For salinity tolerance index:

\[
\text{ST} = \frac{\text{FM}_{\text{after treatment}} - \text{FM}_{\text{before treatment}}}{\text{FM}_{\text{control}} - \text{FM}_{\text{before treatment}}}
\]  

Where FM is total plant fresh mass.

For relative root mass ratio, root mass ratio (RMR) was first calculated in salt and control, and then the ratio of RMR in salt to RMR in control conditions was calculated according to the following equations:

\[
\text{RMR}_{\text{control}} = \frac{\text{DM}_{\text{root, control}}}{\text{DM}_{\text{root+shoot,control}}}
\]

\[
\text{RMR}_{\text{salt}} = \frac{\text{DM}_{\text{root, salt}}}{\text{DM}_{\text{root+shoot, salt}}}
\]

\[
\text{RMR}_{\text{RM}} = \frac{\text{RMR}_{\text{salt}}}{\text{RMR}_{\text{control}}}
\]

3. Results

Overexpression of AtJUB1 in tomato results in increased maintenance of biomass in response to salt stress compared with wild type. We used tomato plants overexpressing AtJUB1 (referred to as AtJUB1 OE) to investigate the effect of JUB1 in salinity tolerance in tomato. These plants have been described previously by (Shahnejat-Bushehri et al., 2017) for their improved drought tolerance. After ten days of salt treatment, several physiological parameters related to salinity tolerance were assessed, including shoot and root biomass (fresh mass and dry mass), leaf area, stem height, root length, and Na and K content in the shoot and root. AtJUB1 OE plants displayed less wilting of the leaves and plants were greener than WT plants (Fig. 1A). At high salinity, the tolerance of AtJUB1 OE plants was significantly higher compared with WT plants, indicating that the AtJUB1 OE plants were better able to maintain biomass than the WT plants at high salinity levels (Fig. 1B, Supplementary Table S1). It should also be noted that it
appears that AtJUB1 OE plants do not have a further reduction in stem height as NaCl is increased from 125 to 200 mM, while there is a significant reduction in WT plants (Fig. 1C).

Under mild salinity stress, AtJUB1 OE plants displayed a higher relative root mass ratio compared to WT plants (root mass compared to total plant mass, salt relative to control) (Fig. 2A), suggesting that the AtJUB1 OE plants increase the relative allocation of biomass to the roots upon salt stress to a greater extent than under control conditions compared to the WT plants. This effect was not significant under high saline conditions. Notably, root length is decreased for both WT and AtJUB1 OE plants under salinity stress compared with control; however, there does not appear to be a significant difference between AtJUB1 OE plant and WT (Fig. 2B).

The effect of salt on parameters such as leaf area, leaf thickness and leaf elongation factor were analyzed using Winfolia software. All leaf measurements were performed on leaf five as it was the leaf that developed during salt imposition. Leaf area was marginally affected by salt treatment in both WT and AtJUB1 OE plants. Leaf thickness was not significantly increased in AtJUB1 OE plants under salt stress. AtJUB1 OE plants maintained leaf width to length ratio, while this ratio significantly decreased in WT plants under high salt treatment (Fig. S2).

3.1. AtJUB1 OE plants show altered water relations and higher proline levels during salt treatment compared with WT

Water relations are important for plant growth, including the maintenance of cellular water content and maintenance of transpiration. It has been argued in the literature that the hydraulic conductivity in the root decreases under salinity stress, thereby making it less easy for the plant to take up water and, consequently, a reduction in the water fraction can be observed (Negrão et al., 2017). Here we determined shoot water fraction in control and salt stress conditions. Under control conditions, shoot water fraction was comparable in WT and AtJUB1 OE plants and only a small reduction in water fraction was observed in response to mild salinity levels (Fig. 3A). During high salinity stress, it became apparent that AtJUB1 OE plants are better able to maintain water fraction, indicating it is better able to maintain water relations in the plant (Fig. 3A and B).

Plants synthesize compatible solutes to maintain plant water potential when salt is accumulated (Lodeyro and Carrillo, 2015). Proline is a compatible solute and its accumulation has been linked to salinity tolerance (Zhu, 2002; Hmida-Sayari et al., 2005). Under control conditions, WT and AtJUB1 OE plants had similar proline levels in young and old leaves (Fig. 4A). However, during mild and high salinity treatment, an increase in proline levels can be observed in AtJUB1 OE plants compared with WT, particularly in young leaves (Fig. 4A). This suggests that AtJUB1 OE plants are better able to maintain water relations by regulating proline accumulation. The overall osmotic potential of leaves (as measured from total leaf fluids from crushed leaves) increased with salinity stress in old and young leaves, as expected;
Fig. 3. Water content of AtJUB1 OE and WT plants. (A) Shoot water fraction of WT and AtJUB1 OE under control and salt stress conditions; (B) relative water fraction of AtJUB1 OE and wild type plants in salt relative to control. n = at least 5 plants, except WT under control conditions, where n = 4; significant differences were calculated using Student T-test, * indicates P-value < 0.05, ** indicates P-value < 0.01.

Fig. 4. Proline content and osmotic potential of AtJUB1 OE and WT plants. (A) Proline level of old leaf and young leaf WT and AtJUB1 OE plants in control and salt stress conditions; (B) Osmotic potential of old leaf and young leaf WT and AtJUB1 OE plants in control and salt stress conditions. n = 3; significant differences were calculated using Student T-test, * indicates P-value < 0.05, ** P-value < 0.01.

3.2. AtJUB1 promotes plant growth under salt stress of soil grown tomatoes

To assess whether AtJUB1 also affects salinity tolerance of tomato in soil grown plants, WT and AtJUB1 OE plants were grown in pots containing soil in the greenhouse and subjected to 350 mM NaCl treatment. Salt was imposed at the 8th leaf stage for a total of four weeks and the effect of salinity on plant growth (fresh mass and height) and fruit yield was investigated. The growth of WT plants was visibly reduced after salt stress application, while AtJUB1 OE plants maintained growth (Fig. 7A). The salinity tolerance index based on plant height and biomass showed that AtJUB1 OE plants are better able to maintain growth compared with WT (Fig. 7B and C). However, in contrast to hydroponically grown plants, the water fraction was not different between AtJUB1 OE and WT plants (Fig. 7D). All yield related parameters measured, such as total fruit number, number of flowers, and number of trusses decreased with salinity treatment in both WT and AtJUB1 OE. This decrease tends to be slightly more prominent in WT plants; however, no statistically significant differences were observed when comparing WT and AtJUB1 OE plants (Fig. 7E). In summary, overexpression of AtJUB1 in tomato plants appears to improve plant growth under salinity stress compared with WT plants, and there is a trend for positive effects on yield related parameters (however, these were not significant, at least under the conditions tested).

4. Discussion

To investigate if JUB1 is involved in salinity tolerance in tomato, we performed salt stress experiments in a hydroponics and soil setup with tomato plants overexpressing AtJUB1 (described previously by Thirumalaikumar et al., 2018). It has been shown previously that Arabidopsis overexpressing AtJUB1 had fewer senescence symptoms in response to salinity stress and was therefore more tolerant compared with WT (Wu et al., 2012). Here, we investigated if AtJUB1 overexpression also reduces senescence symptoms in tomato and if it influences biomass and fruit production in response to salinity stress. We found that AtJUB1 overexpression indeed improved the plants’ salinity tolerance based on maintenance of biomass compared with WT controls. It appears AtJUB1 overexpression also improved yield related parameters in tomato plants, although this effect was moderate and not statistically significant under the conditions tested.

Several NAC transcription factors have previously been shown to be involved in the response to salinity stress and that their overexpression affects salinity tolerance (Nuruzzaman et al., 2013). In tomatoes, for instance, it has been shown that knock-down of SlNac4 reduced the salinity tolerance of transgenic tomato plants in response to salinity stress (Zhu et al., 2014), while overexpression of the tomato gene,
**SlNac35**, in Arabidopsis and *Nicotiana benthamiana* (tobacco) appears to improve salinity tolerance based on germination and root growth compared with WT controls (Wang et al., 2016). Although many stress responsive NACs have been identified, the underlying molecular mechanisms and stress-related genes directly regulated remain to be identified.

Here we found that *AtJUB1* OE plants showed less reduction in biomass, stem height and root length under high salinity stress, while the effects of mild salinity stress were not significantly different between *AtJUB1* OE and WT plants. It is notable that the effect appears to be stronger under high salinity conditions, as opposed to mild salinity stress. A similar effect has been observed before in a durum wheat introgression line carrying a locus for the Na⁺ transporter HKT, where benefits of the HKT gene were substantial at high salinity stress, but less so at moderate stress (Munns et al., 2012). The effect of yield improvement in those durum wheat introgression lines was linked to enhanced Na⁺ exclusion from the shoot, thereby reducing the accumulation of toxic amounts of Na⁺ in the photosynthetically active tissues. To tolerate salinity stress, plants have evolved several mechanisms, such as exclusion of Na⁺ from the shoot, the compartmentalization of Na⁺ into vacuoles and stress signaling (Munns and Tester, 2008; Roy et al., 2014). We did not find differences in Na⁺ accumulation in the last fully developed leaf (leaf five) of our *AtJUB1* OE tomato plants, suggesting that Na⁺ exclusion or compartmentalization in the young leaves are not the main mechanism of salt tolerance conferred by *AtJUB1*. However, there is a possibility that Na⁺ may accumulate in the old leaves or in the stem of tomato plants.

The regulatory pathway through which *JUB1* is involved in salinity tolerance is not fully understood. A recent study by Sakuraba et al. (2017) identified PIF-4 as an upstream negative regulator of *JUB1*. PIF-4 directly binds to the *JUB1* promoter and suppresses its expression. Plants overexpressing PIF-4 were more salt sensitive, likely in part due to *JUB1* suppression by PIF-4 (Sakuraba et al., 2017). Homeodomain-leucine zipper 13 (*AtHB13*) was also identified as another upstream regulator of *JUB1* (Ebrahimian-Motlagh et al., 2017). *AtHB13* binds to the *JUB1* promoter and induces its expression under drought stress, conferring tolerance to water deficit stress likely through the DREB2A pathway. DREB2A is a key regulator in response to water deficit, heat and salt stresses (Dubouzet et al., 2003).

It has previously been described that AtJUB1 directly binds to AtDREB2A (Wu et al., 2012), and therefore is involved in the ABA-independent pathway. Interestingly, we found that *AtJUB1* OE plants had lower amounts of ABA under salt stress compared with WT. ABA is a common stress hormone, which increases in concentration upon stress
imposition to signal responses such as stomatal closure (Vishwakarma et al., 2017). The closure of stomata reduces transpiration and therefore allows plants to conserve water. In \textit{AtJUB1} OE plants, we found lower ABA levels, perhaps reflecting the higher water status (and thus lower stress levels) of the \textit{AtJUB1} OE plants. The lower ABA levels may also enable \textit{AtJUB1} OE plants to maintain transpiration and gas exchange, and, therefore, growth is better maintained compared with WT plants. It has been hypothesized that there is an interaction between the ABA-dependent signaling and ABA-independent signaling though DREB2A and AREB/ABFs (Yoshida et al., 2014). It appears there is no JUB1 binding motif in the promoter region 1 kb upstream of genes encoding for the ABA-responsive element binding factor (ABF1/2/3/4).

We found that \textit{AtJUB1} OE plants had higher proline levels under salt stress compared with WT plants. This is congruent with recent findings showing that transgenic banana overexpressing \textit{MusaNAC042} (the closest homolog to \textit{AtJUB1} in banana) had higher proline levels and are more tolerant to salinity stress (Tak et al., 2017). These transgenic banana plants also exhibited a more favorable water balance compared with WT plants (Tak et al., 2017). This is consistent with our findings and previous studies investigating drought tolerance of tomato overexpressing \textit{AtJUB1} (Thirumalaikumar et al., 2018). JUB1 binds directly to the promoters of SIDREB1, SIDREB2 and SIDELLA in tomato (Thirumalaikumar et al., 2018), which are important TFs regulating drought responses (Yamaguchi-Shinozaki and Shinozaki, 2006). However, the mechanisms through which JUB1 directly or indirectly regulates proline metabolism (e.g. activation of proline biosynthesis or reduction of degradation) remain to be investigated.

Some components of salt stress and water deficit stress are similar. For instance, both stresses can cause cellular dehydration. Several tolerance mechanisms have been identified to be important in response to both salt and/or water deficit stress, such as osmotic adjustment and maintenance of relative water content (Bartels and Sunkar, 2005). We confirmed altered water relations of plants overexpressing \textit{JUB1} and observed higher proline levels in the transgenic compared with the WT in response to salinity stress. This is congruent with previous findings that \textit{JUB1} is involved in tolerance to water deficit (Thirumalaikumar et al., 2018). The mechanism of salt tolerance in \textit{AtJUB1} OE tomatoes might be related to avoidance of water stress imposed by NaCl.

Importantly, we investigated if the improvement of salinity tolerance related to biomass in a hydroponics experiment also translates to an increase in yield and yield related parameters in soil grown plants through to maturity. It appears \textit{AtJUB1} OE has only moderate effects on improving yield parameters such as fruit number and number of trusses under salinity stress. However, it has been shown that \textit{AtJUB1} OE plants are delayed in flowering and fruit ripening compared with WT tomato plants and that several ripening-related genes are differentially regulated (Shahnejat-Bushehri et al., 2017). It also remains to be investigated if fruit quality remains comparable to wild type plants during salinity stress. Salinity has known to increase sugar levels in tomato fruit, which may be considered a positive or negative sensation to consumers. A higher sugar content, together with delayed fruit ripening may provide desirable traits for commercial growers. The trend that \textit{AtJUB1} OE tomatoes have improved yield and yield related parameters needs to be verified in future field studies.

Conflicts of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author contributions

NA performed most experiments and analyzed the data. JW and SAB
performed proline, chlorophyll and ABA analyses. NA, SA, MT and SMS conceived the project and designed experiments. NA and SMS drafted the manuscript, with contributions from all authors.

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Declarations of interest

none.

Data availability

The raw data supporting the conclusions of this manuscript will be made available by the authors, without undue reservation, to any qualified researcher.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2019.04.038.

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