CrossMark

Expression of the Arabidopsis *ABF4* gene in potato increases tuber yield, improves tuber quality and enhances salt and drought tolerance

María Noelia Muñiz García[1] · Juan Ignacio Cortelezzi[1] · Marina Fumagalli[1] · Daniela A. Capiati[1,2]

Abstract

Key message In this study we show that expression of the Arabidopsis *ABF4* gene in potato increases tuber yield under normal and abiotic stress conditions, improves storage capability and processing quality of the tubers, and enhances salt and drought tolerance.

Abstract Potato is the third most important food crop in the world. Potato plants are susceptible to salinity and drought, which negatively affect crop yield, tuber quality and market value. The development of new varieties with higher yields and increased tolerance to adverse environmental conditions is a main objective in potato breeding. In addition, tubers suffer from undesirable sprouting during storage that leads to major quality losses; therefore, the control of tuber sprouting is of considerable economic importance. ABF (ABRE-binding factor) proteins are bZIP transcription factors that regulate abscisic acid signaling during abiotic stress. ABF proteins also play an important role in the tuberization induction. We developed transgenic potato plants constitutively expressing the Arabidopsis *ABF4* gene (35S::ABF4). In this study, we evaluated the performance of 35S::ABF4 plants grown in soil, determining different parameters related to tuber yield, tuber quality (carbohydrates content and sprouting behavior) and tolerance to salt and drought stress. Besides enhancing salt stress and drought tolerance, constitutive expression of *ABF4* increases tuber yield under normal and stress conditions, enhances storage capability and improves the processing quality of the tubers.

Keywords Potato · ABF · bZIP · Tuber yield · Sprouting · Salt stress · Drought

Introduction

Potato (*Solanum tuberosum* L.) is the world's third most consumed food crop (Birch et al. 2012). Potato production is expected to decrease in the coming decades in different parts of the world, due to climate change (Hijmans 2003). Furthermore, potato plants are vulnerable to high salinity and drought, which negatively affect crop yield, tuber quality and market value (Vasquez Robinet et al. 2008). According to the Intergovernmental Panel on Climate Change (IPCC), the global climate deterioration will inexorably lead to an increased frequency of drought (Easterling et al. 2000; Ipcc 2008). Worldwide, about 20% of the agricultural irrigated lands are affected by high salinity. The salinized areas are increasing rapidly as a consequence of different factors, including low precipitation, irrigation with saline water, and improper agricultural practices (Yeo 1999). To overcome this problem, the availability of new potato varieties with higher yields, and less sensitive to adverse environmental cues is critical. Introducing exogenous genes, or altering the expression of endogenous genes by transgenic technology, is currently the most commonly used strategy to increase tuber yield, by improving tuberization behavior of commercial potato varieties under changing environmental conditions (Dutt et al. 2017).

The economic importance of potato plants resides in their capacity to produce tubers. The potato tuber is a source of

✉ Daniela A. Capiati
dcapiati@dna.uba.ar; dcapiati@gmail.com

1   Institute of Genetic Engineering and Molecular Biology "Dr. Héctor Torres" (INGEBI), National Scientific and Technical Research Council (CONICET), Vuelta de Obligado 2490, C1428ADN Buenos Aires, Argentina

2   Biochemistry Department, School of Exact and Natural Sciences, University of Buenos Aires, Buenos Aires, Argentina

valuable nutrients (Camire et al. 2009) that need to be preserved between harvesting and consumption. Tubers suffer from undesirable sprouting during storage that leads to major quality losses (Sonnewald and Sonnewald 2014), therefore, besides engineering tolerance to adverse environmental conditions, the control of tuber sprouting is also an important objective in potato breeding. New strategies are required, since current cold storage techniques or application of sprout inhibitors are expensive and often problematic (Sonnewald 2001). An alternative approach is the development of transgenic potatoes with delayed tuber dormancy. Dormancy break and sprout growth are controlled by phytohormones including abscisic acid (ABA), gibberellic acid (GA) and cytokinins (Destefano-Beltrán et al. 2006; Hartmann et al. 2011). Modulation of phytohormones content or responsiveness are possible targets for biotechnology.

Plant adaptation to adverse environmental conditions involves changes in genome-wide gene expression patterns. Numerous transcription factors (TFs) that respond to abiotic stresses have been identified, including bZIP, WRKY, AP2/EREBPs and bHLHs (Hirayama and Shinozaki 2010). ABF (ABRE-binding factor) proteins, also referred to as AREB (ABA-response element binding factor) belong to the group A of bZIP TF. ABFs are key regulators of ABA signaling during abiotic stress (Choi et al. 2000; Uno et al. 2000); they bind to the ABA-responsive element (ABRE), the major *cis*-acting regulatory sequence that controls ABA-dependent gene expression (Hirayama and Shinozaki 2010). Besides *Arabidopsis thaliana*, ABF TFs have been described in several plant species, such as barley (*Hordeum vulgare*; Casaretto and Ho 2005), wheat (*Triticcum aestivum*; Kobayashi et al. 2008), rice (*Oryza sativa*; Hossain et al. 2010a, b), tomato (*Solanum lycopersicum*; Hsieh et al. 2010; Yáñez et al. 2009), trifoliate orange (*Poncirus trifoliate*; Huang et al. 2010; Zhang et al. 2015), grapevine (*Vitis vinifera*; Boneh et al. 2012) and cotton (*Gossypium hirsutum*, Liang et al. 2016; Kerr et al. 2017). Despite their role in stress signaling, ABF TFs have been implicated in other physiological processes, such as sugar signaling (Kang et al. 2002; Kim et al. 2004), tomato fruit development (Bastías et al. 2011, 2014), response to pathogens (Orellana et al. 2010) and leaf senescence (Gao et al. 2016).

In previous studies, we obtained evidence suggesting that ABF TFs may play key roles in potato tuberization induction. We identified and characterized an ABF TF in potato, named StABF1. *StABF1* expression is increased during tuber development, and by tuber-inducing conditions; moreover, StABF1 phosphorylation is stimulated by tuber-inducing conditions, while GA inhibits tuber development and StABF1 phosphorylation, suggesting that StABF1 may act as a positive regulator of tuberization (Muñiz García et al. 2012). To confirm this hypothesis, we attempted to overexpress the *StABF1* gene in potato, however, we have so far been unable to generate these transgenic plants. Therefore, we developed potato plants expressing the Arabidopsis *ABF2* or *ABF4* genes under the control of the 35S promoter from Cauliflower Mosaic Virus (35S::ABF2 and 35S::ABF4, respectively). ABF2 and ABF4 are the closest orthologs to StABF1 (sharing 57% and 50% amino acid sequence identity, respectively). Both ABF2 and ABF4 have been involved in sugar signaling and metabolism (Kang et al. 2002; Kim et al. 2004), which are crucial for tuberization induction. Stolons from 35S::ABF4 and 35S::ABF2 plants exhibit an enhanced tuberization induction in vitro, however, only *ABF4* expression significantly increases the number of the tubers obtained from cultured stolons (Muñiz García et al. 2014).

Our previous results suggest that *ABF4* is a good candidate to improve potato crop yield. Due to the role of ABF TFs in abiotic stress responses, *ABF4* might also enhance salt and drought tolerance. In addition, ABF4 regulates the expression of ABA and GA metabolic genes in stolons cultured in vitro (Muñiz García et al. 2014); consequently, this transcription factor could be useful to regulate dormancy and sprouting time. The aim of the present study was to assess the performance of the 35S::ABF4 plants grown in soil, determining different parameters related to tuber yield, tuber quality (carbohydrates content and sprouting behavior) and tolerance to salt and drought stress.

Materials and methods

Plant material and growth conditions

Transgenic potato plants (*Solanum tuberosum* cv. Spunta) expressing the Arabidopsis *ABF4* gene under the control of the cauliflower mosaic virus 35S promoter (35S::ABF4) were generated by Agrobacterium-mediated transformation (Muñiz García et al. 2014). Regenerated plants carrying no plasmid but obtained by the same regeneration method were used as controls (wild type) for phenotypic analysis. No phenotypic differences were observed between the regeneration controls and transformed vector controls (Supplementary Fig. S1), confirming that the phenotype observed in 35S::ABF4 plants is specifically due to the expression of *ABF4*. Unless otherwise specified, plants were obtained from seed tubers; for each experiment, the size of the seed tubers was uniform (5–15 g/tuber; unless otherwise specified), with no significant differences in the tuber weight. Tubers were cultivated in 1 L pots with commercial soil mixture (Grow Mix Multipro, Terrafertil Argentina), placing 1 tuber per pot. Plants were cultivated in a greenhouse maintained between 22 and 24 °C, under a 16-h light/8-h dark cycle.

Dry matter content

Dry matter content was determined by oven-drying finely chopped tubers at 80 °C till constant weight (16 h). Dry matter (%) was calculated as follows: $100 \times$ dry weight/fresh weight.

Starch, reducing sugars, glucose and sucrose content

Tubers were chopped, dried at 80 °C till constant weight and ground to a fine powder. Starch was determined as described in Stritzler et al. (2017), except that glucose was determined by the glucose oxidase method using the Glycemia kit purchased from Wiener Lab, Argentina. Soluble sugars were extracted from dried tuber samples with 80% (v/v) ethanol in a 70 °C water bath for 90 min. Reducing sugars content was determined by the Nelson-Somogy method (Somogyi 1952; Nelson 1994). Glucose was determined using the Glycemia kit (Wiener Lab). Sucrose was determined by the glucose oxidase/invertase method described in Teixeira et al. (2012), except that glucose was determined as described above, and sucrose content was calculated from the difference in glucose content after and before invertase treatment.

Sprouting behavior

Tubers obtained from wild-type and 35S::ABF4 plants grown in a greenhouse under non-stress condition were harvested and placed in darkness at 22 °C for 20 weeks. A sprouted tuber was defined as a tuber presenting at least one sprout equal or longer than 3 mm. The sprout/tuber weight ratio was determined by weighing the sprouts of each tuber and dividing them by total tuber weight, thereby minimizing the effect of tuber size on sprout weight. Alternatively, the percentage of sprouted tubers was determined after 30 weeks of storage at 4 °C in darkness.

Measurement of leaf water loss

To measure water loss by air drying, leaflets from 4 weeks-old wild-type and transgenic potato plants cultivated in the greenhouse were detached and placed at 22 °C. The fresh weights were measured at different time points (each 30 min during 5 h, and 24 h after the cutting). Water loss was calculated as the percentage of initial fresh weight lost at each time point.

Relative water content (RWC)

RWC was determined in detached leaflets with their corresponding petiole from 4 weeks-old wild-type and transgenic potato plants, as described in Capiati et al. (2006).

Leaflets were placed in individual containers with water for 48 h before treatment. Leaflets were then subjected to salt stress (250 mM NaCl) or dehydration (deprived of water) for 1 or 4 h. Controls without treatment were carried out in each determination.

RWC was determined according to the formula:

$$RWC\ (\%) = 100\ (FW - DW)/(TW - DW)$$

Fresh weight (FW) was measured at the end of the stress period. Turgor weight (TW) was determined by subjecting leaflets to rehydration in water for 24 h, after stress treatment. Dry weight (DW) was obtained after drying the samples at 60 °C for 16 h.

Survey of agronomic characteristics under stress conditions

Salt and drought stress were performed; well-watered plants were used as controls. For salt stress, plants were grown under well-watered conditions for 3 weeks, then, plants were irrigated with 100 mM NaCl (200 ml per 1 L pot, three times a week) for 7 weeks; the average soil conductivity after 5 weeks of NaCl application was 9.97 mS/cm. For drought treatment, plants were grown under well-watered conditions for 3 weeks, drought stress was applied by withholding water for 5 weeks, followed by 2 weeks of re-watering. Following stress application, different parameters were determined, such as tuber weight, tuber yield, proline and chlorophyll content, photosynthesis and survival rate.

Proline content

Proline content was determined in leaves according to the method of Bates et al. (1973) with minor modifications. The samples (50 mg) were ground to a fine powder in liquid nitrogen, resuspended in 200 μL of distilled water, incubated in a boiling bath for 30 min and centrifuged 10 min at $18,000 \times g$. 100 μL of the supernatant were mixed with 100 μL of 0.2 M sodium citrate buffer (pH 4.6) and reacted with 400 μL ninyhidrin 1% (p/v) prepared in acetic acid:water (3:2), for 45 min at 100 °C. The reaction was stopped in an ice bath. The reaction mixture was extracted with 800 μL of toluene, mixed vigorously and centrifuged 10 min at $18,000 \times g$. The absorbance was read at 515 nm. The proline concentration was determined from a standard curve.

Protein oxidation

Carbonyl determination was carried out in leaves by the dinitrophenylhydrazine (DNPH) method, as described by Levine et al. (1994). Three replicates and their respective blanks were used for each determination. Samples were

incubated with 1% (w/v) streptomycin sulphate for 20 min to remove the nucleic acids. After centrifuging at 5000×*g* 20 min, supernatants (200 μL) were mixed with 300 μL of 10 mM DNPH in 2.5 M HCl; the blanks were incubated in 2.5 M HCl. After incubating 1 h at room temperature, proteins were precipitated with 275 μL of trichloroacetic acid (TCA) 50% (w/v), and the pellets were washed with 600 μL of TCA 10% (w/v). The pellets were then washed three times with ethanol:ethylacetate (1:1) and dissolved with 6 M guanidine hydrochloride in 20 mM potassium phosphate buffer (pH 2.3). The absorbance at 370 nm was measured. Protein recovery was determined for each sample by measuring the absorbance of the blanks at 280 nm. Carbonyl content was calculated using the molar absorption coefficient for aliphatic hydrazones ($0.022 \, \text{nmol}^{-1} \, \text{mL}$).

Gas exchange measurements

Gas exchange measurements were carried out using a portable gas exchange system (Li6800; Li-Cor Inc., Lincoln, NE, USA). Measurements were performed on the youngest fully expanded leaves. Leaf temperature was set at 25 °C; irradiance was fixed at $1000 \, \mu\text{mol/m}^{-2} \, \text{s}^{-1}$. The photosynthesis and electron transport rate, stomatal conductance and transpiration rate were calculated with the software provided by the manufacturer.

Chlorophyll content

Chlorophyll content was determined by the method described by Arnon (1949). Fresh leaf samples were homogenized in acetone 80% (v/v) (1 mL per 10 mg of tissue) followed by centrifugation at 3000×*g* for 5 min. The absorbance at 663 and 646 nm was determined. Chlorophylls content were calculated using the following formulae:

$$\text{Chlorophyll a (mg/L)} = \left(12.21 \times A_{663}\right) - \left(2.81 \times A_{646}\right)$$

$$\text{Chlorophyll b (mg/L)} = \left(20.13 \times A_{646}\right) - \left(5.03 \times A_{663}\right)$$

Statistical analysis

Statistical analysis was carried out using the Student's *t* test. A p value < 0.05 was considered statistically significantly.

Results

35S::ABF4 plants exhibit improved tuber yields under normal conditions

35S::ABF4 plants were obtained as described in Muñiz García et al. (2014). All the transgenic lines analyzed in vitro showed an increased tuberization capacity as compared to wild-type controls (Muñiz García et al. 2014; Supplementary Fig. S2). Four lines (4.1, 4.2, 4.3 and 4.4) were selected for preliminary characterization. Plants were transfer ex vitro to soil, and we observed that 35S::ABF4 lines showed normal vegetative growth (Fig. 1a) and presented higher tuber yields than the wild-type controls (not shown). Tubers were collected from the ex vitro plants and planted to evaluate the tuberization capacity under normal (non-stress) conditions; tuber yields were higher in all 35S::ABF4 lines (Fig. 1b), with no differences in the average tuber weight between transgenic and wild-type plants (Fig. 1c). No differences in the number of stolons per plant were observed between 35S::ABF4 and wild-type controls (except for line 4.4 that presented less stolons per plant) (Fig. 1d), however the percentage of tuberizing stolons and the number of tubers per plant were higher in all transgenic lines (Fig. 1e, f). This preliminary characterization was carried out with plants obtained from very small seed tubers (1–2 g each); these plants were much smaller than those developed from larger tubers (used in the subsequent experiments; Figs. 2, 3, 4, 5, 6, 7, 8, 9), and consequently had significantly lower yields.

Only two 35S::ABF4 lines (4.2 and 4.3) presented a single transgene copy (Muñiz García et al. 2014); these lines were selected for further analysis. Wild-type plants and lines 4.2 and 4.3 obtained from seed tubers (5–15 g each) were cultivated under normal conditions. No significant growth alterations were observed between 35S::ABF4 and wild-type plants (Supplementary Fig. S3). As expected, these plants were larger than those developed from small seed tubers (Supplementary Fig. S3a; Fig. 1a), and therefore produced larger tubers, more tubers per plant and higher yields (Fig. 2). Again, the tuberization capacity of the 35S::ABF4 lines was higher with respect to wild-type controls (Fig. 2).

It is important to note that the experiments of tuber yields under normal or stress conditions (using seed tubers of 5–15 g) were performed in 1 L pots, due to the limited space in the greenhouse; the yields obtained using this pot size ranged between 21 and 33 g tuber/plant. When plants were cultivated in 3 L pots, the tuber yields were significantly higher: 70.9 ± 5.8 for wild type and 87.4 ± 2.1 for line 4.3 ($p < 0.05$, line 4.3 vs. wild type); the number of tubers per plant was 2.3 ± 0.3 for wild type and 3.4 ± 0.2 for line 4.3 ($p < 0.05$, line 4.3 vs. wild type).

Characteristics of 35S::ABF4 tubers developed under normal conditions

Tubers from 35S::ABF4 plants showed an elongated shape (Fig. 3a, b). Dry matter and starch content were higher in transgenic tubers (Fig. 3c, d). The content of total reducing sugars and glucose were lower in 35S::ABF4 tubers, with no differences in the sucrose content, as compared to wild-type controls (Fig. 3e).
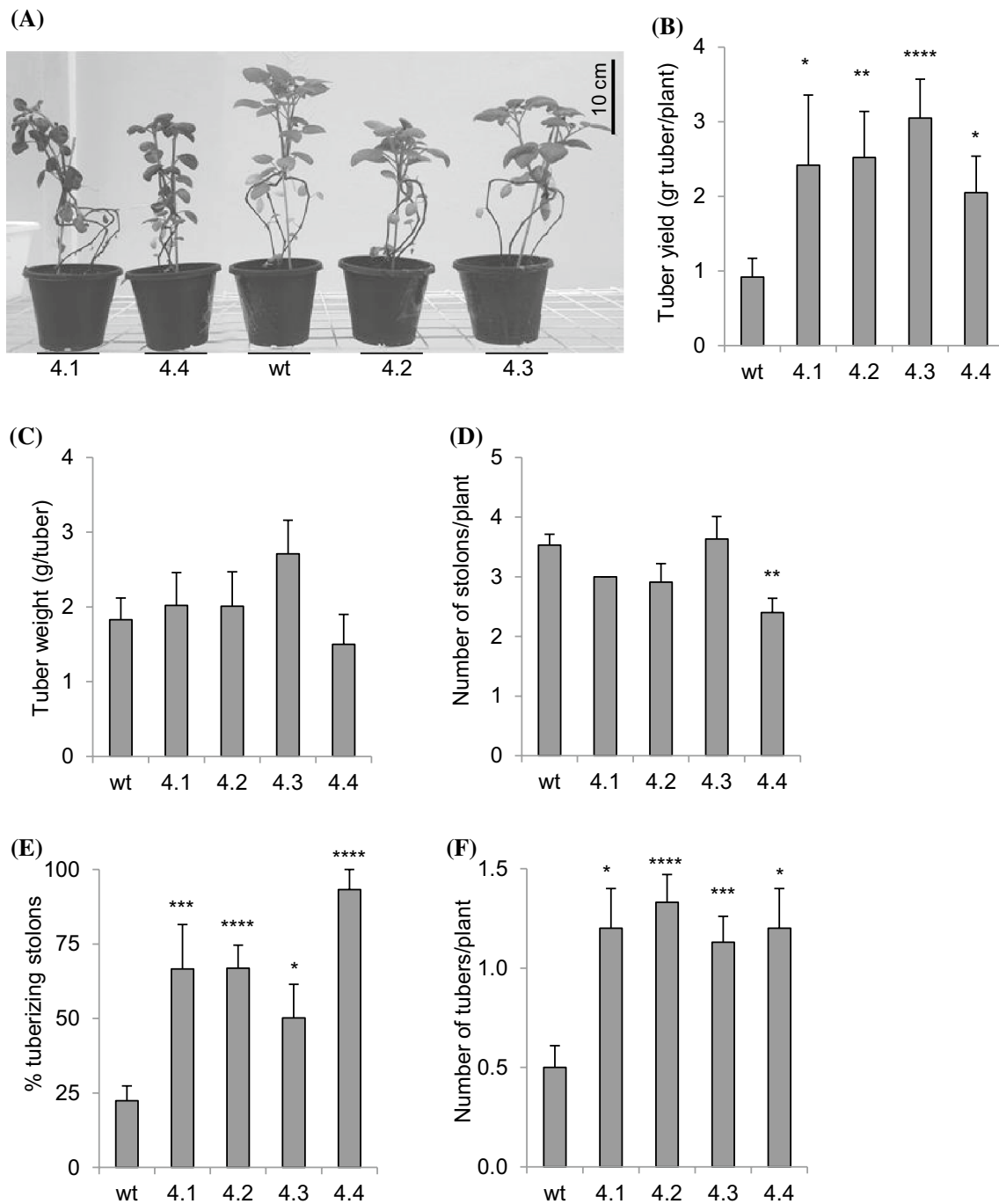
Fig. 1 Preliminary characterization of 35S::ABF4 plants. **a** Representative image of wild-type (wt) and transgenic plants (4.1, 4.2, 4.3 and 4.4) 4 weeks after ex vitro transfer. **b–f** Tuberization of plants grown from small tubers (1–2 g each) obtained from ex vitro plants grown under normal (non-stress) conditions. Tubers were harvested 10 weeks after planting, and the tuber yield, tuber weight, number of stolons per plant, percentage of tuberizing stolons (stolons presenting at least one tuber or subapical swelling), and number of tubers per plant were determined. The data shown in the bar graphs are the mean $\pm$ SEM of ten plants per line. The asterisks indicate statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$, with respect to wt

The sprouting behavior of 35S::ABF4 tubers during storage was analyzed. The percentage of sprouted tubers after 20 weeks of storage at 22 °C was lower in transgenic lines than in the wild-type controls (Fig. 4a); moreover, the sprouts of 35S::ABF4 tubers were shorter (Fig. 4b), and the sprout weight and sprout/tuber weight ratio were significantly lower in transgenic tubers (Fig. 4c, d). No significant differences in the number of sprouts per tuber were observed
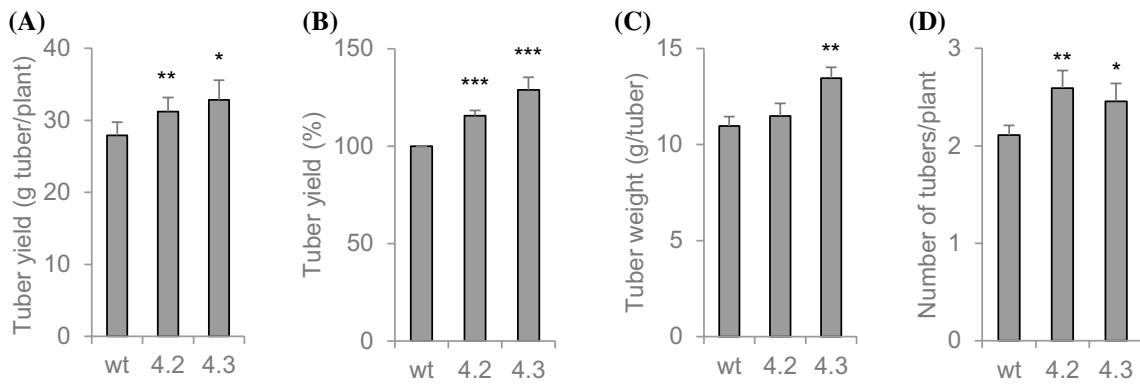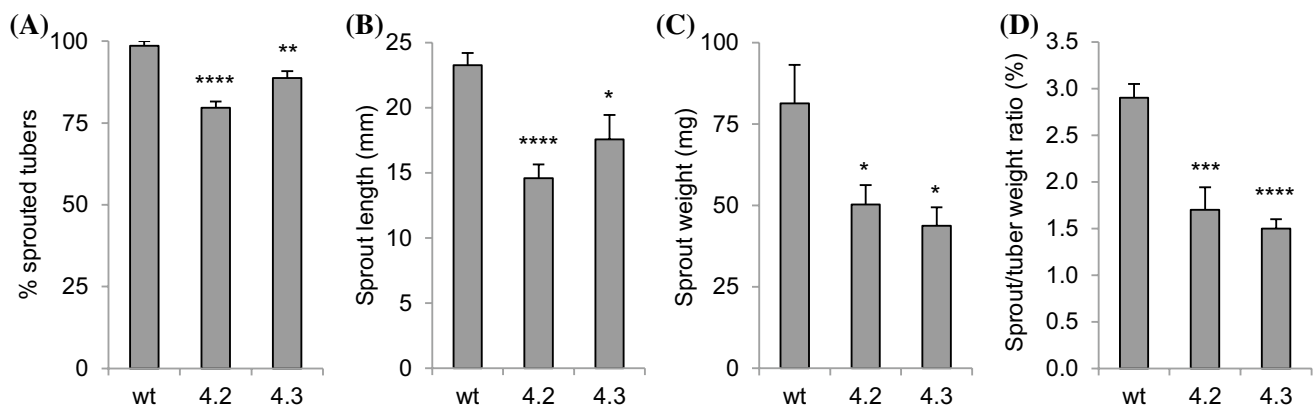
Fig. 2 Tuberization of 35S::ABF4 plants under normal conditions. Wild-type (wt) and transgenic plants (4.2 and 4.3) obtained from seed tubers (5–15 g each) were grown under non-stress conditions; tubers were harvested after plant senescence (approximately 10 weeks). **a** Tuber yield. **b** Percentage of tuber yield of 35S::ABF4 plants with respect to wild-type controls. **c** Average tuber weight. **d** Number of tubers obtained per plant. Data are the mean ± SEM of five independent experiments, each consisting of ten plants per line; experiments were carried out over a period of 3 years. The asterisks indicate statistical significance: *p < 0.05, **p < 0.01, ***p < 0.005, with respect to wt
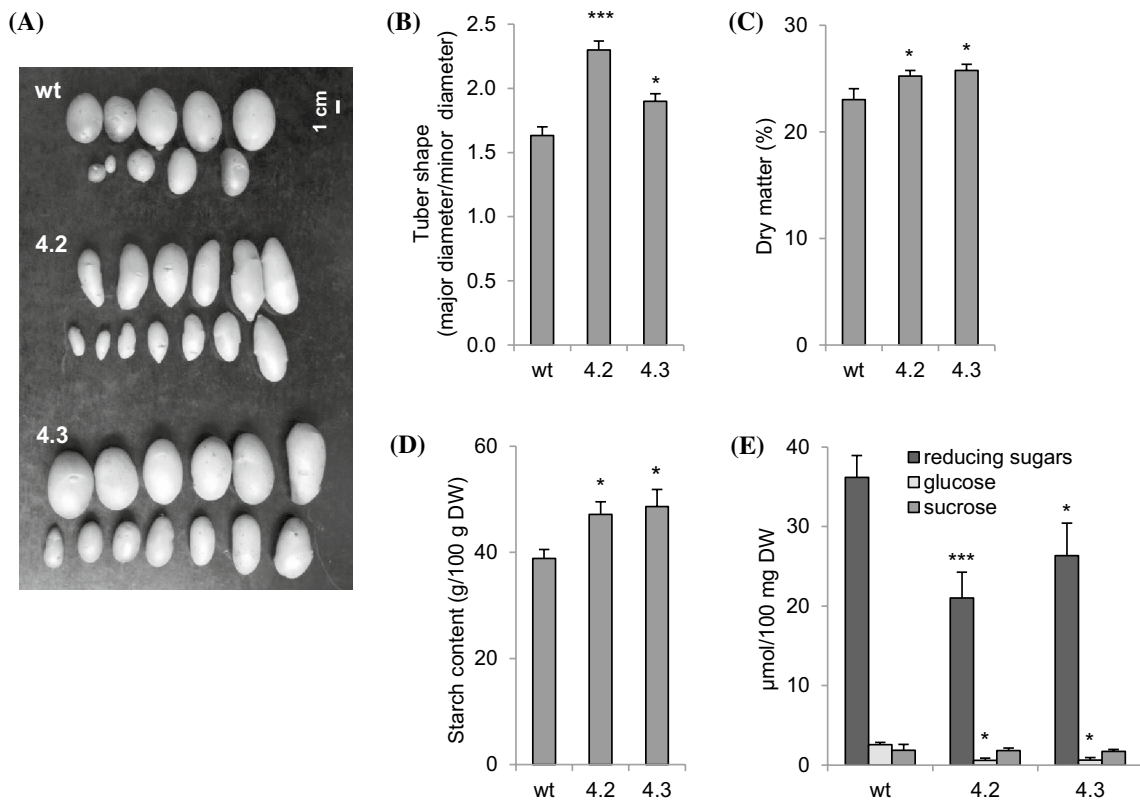
Fig. 3 Characteristics of 35S::ABF4 tubers developed under normal conditions. Wild-type (wt) and transgenic plants (4.2 and 4.3) obtained from seed tubers were grown under non-stress conditions; tubers were harvested after plant senescence (approximately 10 weeks). **a** Representative image of the tubers. **b** Tuber shape, defined as the ratio between the major and minor diameter; data are the mean ± SEM of three independent experiments, each consisting of 20–30 tubers per line. **c–e** Dry matter, starch content, and soluble sugars content; the data are presented as the mean ± SEM from 10 to 15 tubers per line. The asterisks indicate statistical significance: *p < 0.05, ***p < 0.005, with respect to wt. *DW* dry weight

between 35S::ABF4 and wild-type plants (1.3 ± 0.1 sprouts/ tuber for wild type, 1.1 ± 0.1 for line 4.2 and 1.0 ± 0.1 for line 4.3). Noteworthy, all of the transgenic and wild-type tubers were able to develop plants, when placed in soil after 20 weeks of storage at 22 °C; all tubers reached 100% emer- gence 5 days after planting. The sprouting behavior was also determined after 30 weeks of storage at 4 °C; under these conditions, the percentage of sprouted tubers was signifi- cantly lower in 35S::ABF4 lines than in wild-type tubers: 68.0 ± 15.4% for wild type, 0.0 ± 0.0 and 25.0 ± 0.0% for line 4.2 and 4.3, respectively (p < 0.05, for lines 4.2 and 4.3 vs. wild type).

## 35S::ABF4 plants show enhanced tolerance to salt and drought stress

Expression of *ABF4* in potato plants resulted in reduced water loss; the leaves of 35S::ABF4 plants lost water more slowly than wild-type controls (Fig. 5a, b; Supplementary Fig. S4). Relative water content (RWC) was determined in transgenic and wild-type leaves after exposure to 250 mM NaCl or dehydration; as shown in Fig. 5c, under control con- ditions no differences were observed between 35S::ABF4 lines and wild type, however, after 4 h of salt stress, and 1 or 4 h of dehydration, RWC values of transgenic leaves were higher than those of wild type. There were no differences in the stomatal conductance or transpiration rates between 35S::ABF4 and wild-type plants under well-watered condi- tions (Fig. 5d, e). Under salt stress, both parameters were decreased in 35S::ABF4 and wild-type plants, however, the transgenic lines showed lower stomatal conductance and transpiration rates than wild-type controls (Fig. 5d, e).

Proline content was determined to evaluate the perfor- mance of 35S::ABF4 plants under stress. Proline act as an osmoprotectant and is accumulated in response to salin- ity and drought; it has also been described to function as a radical scavenger and antioxidant (Liang et al. 2013). Proline content significantly increased after exposure to salt stress or drought in both, 35S::ABF4 and wild-type plants (Fig. 6a); transgenic lines exhibited higher pro- line contents than wild type, either under well-watered conditions or during salt or drought stress (Fig. 6a). No significant difference in the protein oxidation level was observed between transgenic and wild-type plants, under well-watered or stress conditions (Fig. 6b).

The photosynthesis rate was determined in 35S::ABF4 plants under well-watered and salt stress conditions. As expected, photosynthesis and electron transport rate were significantly reduced by salt stress, but no differences were observed between transgenic and wild-type plants under normal or stress conditions (Fig. 7a, b).

35S::ABF4 plants exhibited higher survival rates than wild-type controls under salt stress (Fig. 8a, b), however, under drought stress, transgenic and wild-type plants showed similar survival rates (Fig. 8c). Chlorophyll con- tent was determined as an indicator for stress-induced leaf senescence (Fang et al. 1998). There were no sig- nificant differences in chlorophyll a or b content between 35S::ABF4 and wild-type plants under well-watered or drought conditions, however the content of both, chloro- phyll a and b resulted higher in transgenic lines after salt stress (Fig. 8d). No differences were observed in the ratio of chlorophyll a to chlorophyll b between 35S::ABF4 and wild-type plants under well-watered or stress conditions (Fig. 8e).
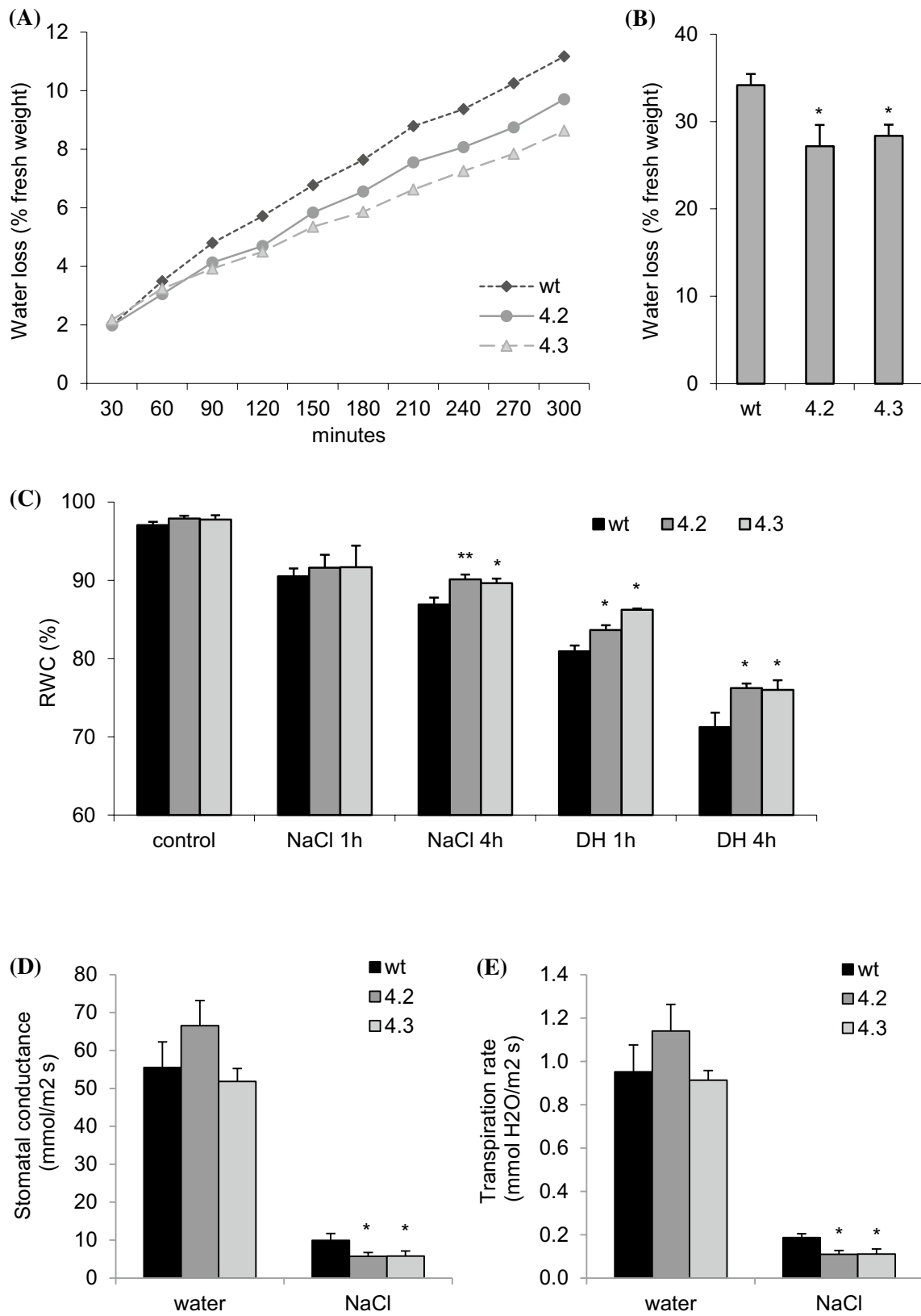
◀**Fig. 5** Water loss, RWC, stomatal conductance and transpiration rate of 35S::ABF4 plants. **a** Water loss of detached leaflets from wild-type (wt) and 35S::ABF4 (4.2 and 4.3) plants. Quantitative data of four independent experiments, each consisting of five leaflets from different plants per condition, are displayed in the graph. The results are shown in a line graph for better visual comparison (statistical analysis is shown in Fig. S4). **b** Water loss after 24 h of air drying. Quantitative data of four independent experiments (mean ± SEM), each consisting of ten leaflets from different plants per condition, are displayed in the bar graph. **c** RWC of wild-type and 35S::ABF4 detached leaflets after exposure to NaCl 250 mM or dehydration (DH) for 1 or 4 h. Quantitative data of five independent experiments (mean ± SEM), each consisting of 10–15 leaflets from different plants per condition, are displayed in the bar graph. **d** and **e** Stomatal conductance and transpiration rate of wild-type and 35S::ABF4 plants. Plants were grown in greenhouse under well-watered conditions (water) or subjected to salt stress (NaCl) as described in "Materials and methods". Stomatal conductance and transpiration rate were determined by gas exchange measurements; data of two independent experiments (mean ± SEM), each consisting of ten plants per condition, are displayed in the bar graphs. The asterisks indicate statistical significance: *$p < 0.05$, **$p < 0.01$, with respect to wt

35S::ABF4 plants exhibit improved tuber yields under salt and drought stress

Under salt stress, 35S::ABF4 lines showed higher tuber yields than wild-type plants, with an increase in productivity of 68.8 and 52.6% for lines 4.2 and 4.3, respectively (Fig. 9a, b). No differences in the average tuber weight were observed between transgenic and wild-type plants grown in saline conditions (Fig. 9c), however, the number of tubers obtained per plant was higher in 35S::ABF4 lines (Fig. 9d). Accordingly, the percentage of tuberizing stolons was higher in 35S::ABF4 plants under salt stress (Supplementary Fig. S5a), with no differences in the number of stolons per plant with respect to wild-type controls (Supplementary Fig. S5b).

Under drought stress, 35S::ABF4 plants showed higher tuber yields than wild-type controls, with an increase in productivity of 51.3 and 60.4% for lines 4.2 and 4.3, respectively (Fig. 9e, f). The average tuber weight was higher for 35S::ABF4 lines under drought stress (Fig. 9g), however, no differences were detected in the number of tubers produced per plant between transgenic and wild-type plants (Fig. 9h).

A drastic reduction in tuber yield in both wild-type and transgenic plants occurred under the salinity and drought conditions applied (Fig. 9a, e). When a milder drought stress was applied, a less decrease in productivity was observed, however, the 35S::ABF4 plants showed 69–85% higher yields than wild-type plants (Supplementary Fig. S6). This result indicates that the absolute values of yield depend on the severity of the stress, however the differences in tuber yield between wild-type and transgenic plants remain essentially constant.

The characteristics of the tubers developed under drought stress were determined. Tubers from 35S::ABF4 showed an elongated shape (Supplementary Fig. S7a). Relative to the wild-type control, the dry matter and starch content were higher in transgenic tubers, but this trend was not statistically significant (Supplementary Fig. S7b and c). The reducing sugars content was lower in 35S::ABF4 tubers, although this difference was only significant for line 4.2 (Supplementary Fig. S7d). Overall, the differences in starch and sugars contents between 35S::ABF4 and wild-type tubers developed under drought stress were less marked than the differences observed when tubers were obtained under normal conditions. Drought stress drastically decreased the reducing sugars content and slightly reduced the starch content in both, wild-type and transgenic tubers, with respect to the tubers developed under normal conditions (Fig. 3d, e).

Discussion

In a previous study we showed that constitutive expression of the Arabidopsis *ABF4* gene in potato plants enhances tuberization in cultured stolons, and determined that ABF4 might act a mediator of the ABA-GA crosstalk during tuberization induction. These previous results were obtained using an in vitro tuberization system, in which isolated stolons are cultured in darkness on medium supplemented with 8% sucrose (Muñiz García et al. 2014). Differently, in whole plants, tuber development depends on the photoassimilates generated in the leaves, a process that is influenced by the photosynthetic rate, stomatal aperture, total leaf area, leaf chlorophyll, etc. Moreover, tuberization is controlled by signals that are exported from leaves in response to environmental and hormonal factors, perceived and integrated in the aerial part of the plant (Suárez-López 2013). Based on our previous results in vitro, we hypothesized that *ABF4* might be a good candidate to improve potato crop yield, however, considering the complexity of the entire tuberization process, in soil experiments with whole plants are required to confirm this hypothesis. In this study we evaluated the performance of the 35S::ABF4 plants grown in soil, under greenhouse conditions, determining the tuber yield, carbohydrate components and sprouting behavior of the tubers, and the tolerance to salt and drought stress.

Transgenic expression of ABF TFs in Arabidopsis and other species has been shown to increase tolerance to abiotic stress, however, these improvements are often associated with growth retardation (Kang et al. 2002; Kim et al. 2004; Kerr et al. 2017). Interestingly, 35S::ABF4 plants exhibit normal growth and development (Fig. 1; Fig. S3). Other reports also describe improvements in stress tolerance of agricultural plants that ectopically express ABF genes, with no effects on growth (Oh et al. 2005; Orellana et al. 2010).

Under non-stress conditions, 35S::ABF4 lines present higher tuber yields than wild-type plants, due to an increase
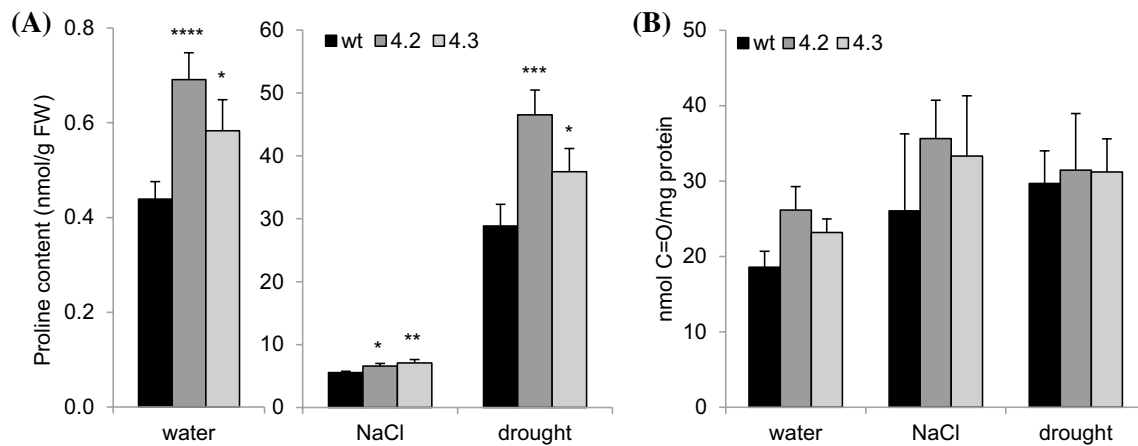
Fig. 6 Proline content and protein oxidation of 35S::ABF4 plants. Wild-type (wt) and transgenic plants (4.2 and 4.3) were grown under well-watered conditions (water) or subjected to salt stress (NaCl) or drought as described in "Materials and methods". Proline content (**a**) and protein oxidation (**b**) were determined in leaves. The data shown in the bar graphs are the mean ± SEM of 12 plants per condition for proline determination and six for protein oxidation. The asterisks indicate statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$, with respect to wt. *FW* fresh weight
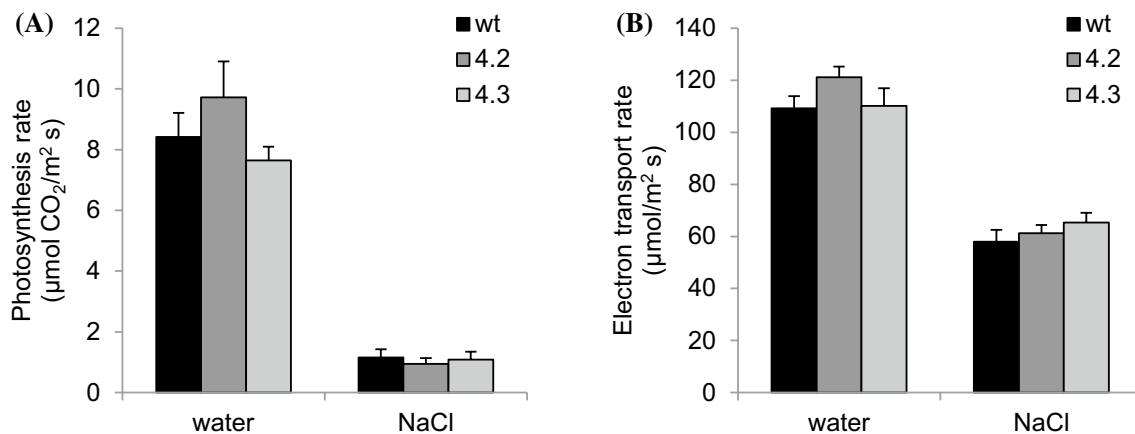
Fig. 7 Photosynthesis (**a**) and electron transport rate (**b**) of 35S::ABF4 plants. Wild-type (wt) and transgenic plants (4.2 and 4.3) were grown under well-watered conditions (water) or subjected to salt stress (NaCl) as described in "Materials and methods". Photosynthesis and electron transport rate were determined by gas exchange measurements; data of two independent experiments (mean ± SEM), each consisting of ten plants per condition, are displayed in the bar graphs

in the number of tubers produced per plant, rather than an increased tuber weight (Figs. 1, 2). This result confirms that ABF4 can improve potato productivity, as suggested by the evidence obtained in our previous study using an in vitro tuberization system (Muñiz García et al. 2014).

35S::ABF4 tubers exhibit a more elongated shape than wild-type controls (Fig. 3) which might be due to the mechanism of action of ABF4. During tuberization induction, a decrease in the bioactive GA levels, caused by the up-regulation of *StGA2ox1* (that codes for the GA inactivating enzyme), occurs in the stolon subapical region (Kloosterman et al. 2007). This causes a change in the plane of cell division that leads to lateral cell expansion and division (Shibaoka 1994; Fujino et al. 1995; Xu et al. 1998). We demonstrated

previously that ABF4 increases *StGA2ox1* expression in stolons during tuberization induction (Muñiz García et al. 2014). A possible reason for the elongated shape could be a reduction of bioactive GA levels occurring all over the stolon, instead of being restricted to the subapical region, as a consequence of the constitutive expression of *ABF4* that causes the up-regulation of *StGA2ox1* all along the stolon length.

35S::ABF4 tubers have higher dry matter and starch content, and lower content of total reducing sugars and glucose (Fig. 3). These are desirable traits in commercial potato tubers. High dry matter and starch content increases chip yield, crispy consistency, and reduces oil absorption during frying (Rommens et al. 2010). Low reducing sugars levels
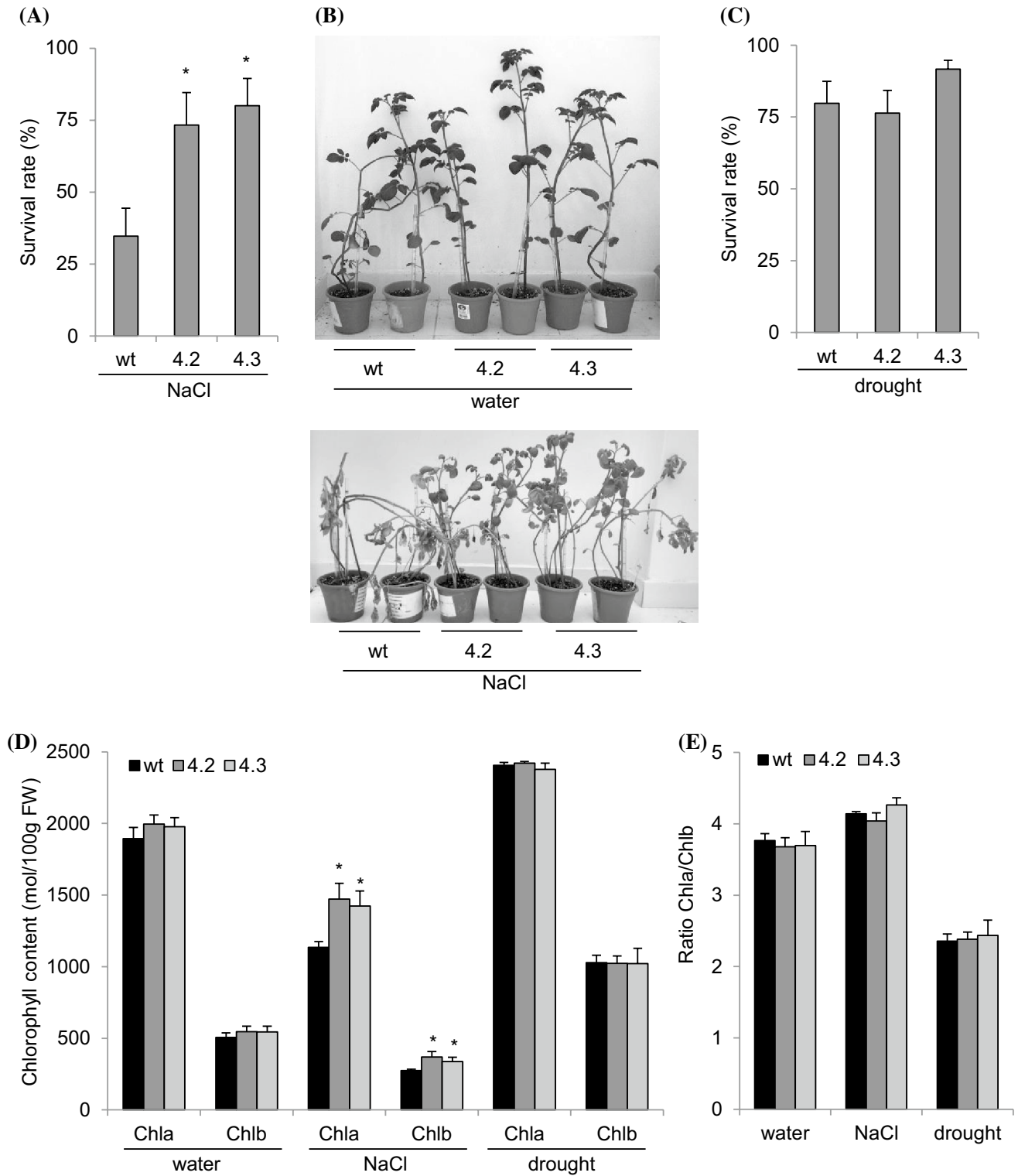
Fig. 8 Survival rate and chlorophyll content of 35S::ABF4 plants under salt stress and drought. Wild-type (wt) and transgenic plants (4.2 and 4.3) were grown under well-watered conditions (water) or subjected to salt stress (NaCl) or drought as described in "Materials and methods". **a** Survival rate under salt stress. **b** Representative image of plants 4 weeks after application of salt stress. **c** Survival rate under drought stress. Data are the mean ± SEM of five independ-ent experiments, each consisting of ten plants per line; experiments were carried out over a period of 3 years. **d** and **e** Chlorophyll a and b content, and ratio between chlorophyll a and chlorophyll b. The data shown in the bar graphs are the mean ± SEM of ten plants per con-dition. The asterisks indicate statistical significance: *p < 0.05, with respect to wt. *FW* fresh weight
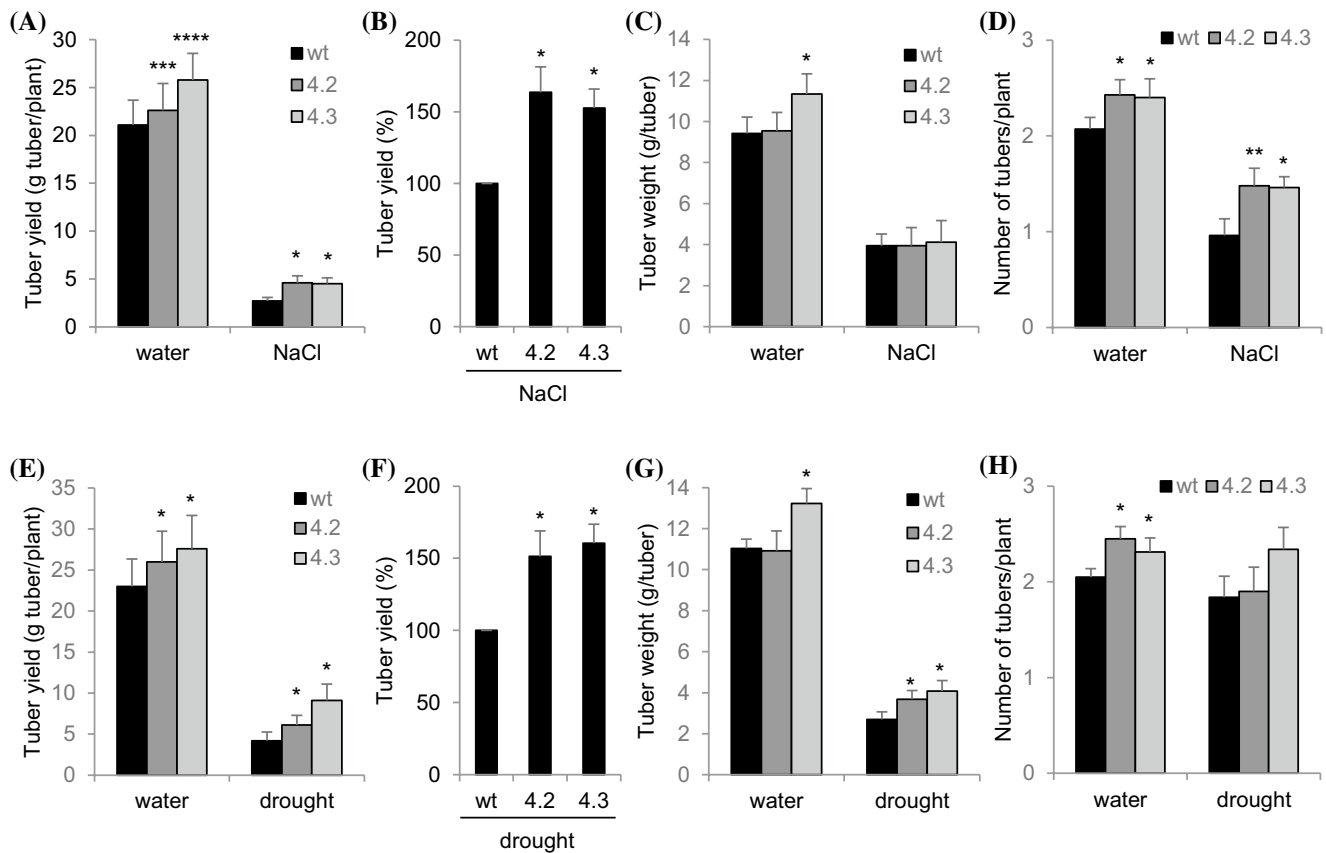
Fig. 9 Tuberization of 35S::ABF4 plants under salt stress or drought conditions. Wild-type (wt) and transgenic plants (4.2 and 4.3) obtained from seed tubers (5–15 g each) were grown under well-watered conditions (water) or subjected to salt stress (NaCl; **a–d**) or drought (**e–h**) as described in "Materials and methods". **a** and **e** Tuber yield. **b** and **f** Percentage of tuber yield under salt stress of 35S::ABF4 plants with respect to wild-type plants. **c** and **g** Average tuber weight. **d** and **h** Number of tubers obtained per plant. Data are the mean ± SEM of five independent experiments, each consisting of ten plants per line; experiments were carried out over a period of 3 years. The asterisks indicate statistical significance: *$p < 0.05$, **$p < 0.01$, ***$p < 0.005$, ****$p < 0.001$, with respect to wt

are required to reduce darkening of processed products, which negatively affects consumer acceptance (Wang-Pruski and Nowak 2004). Expression of the Arabidopsis *ABF4* gene in potato increased starch content by 23–25% on dry matter basis; the estimated increase on fresh weight basis was about 33–40%. Total reducing sugars and glucose were decreased by 27–42% and 80%, respectively (dry matter basis). These differences represent an improvement in the processing quality of 35S::ABF4 tubers. The mechanism by which ABF4 regulates starch and sugars content in potato tubers remains unknown. It has been demonstrated that SlAREB1 alters the content of sugar derivatives in tomato fruits, probably by regulating the transcript levels of genes related to carbohydrate metabolism (Bastías et al. 2014). It would be interesting to determine the expression of enzyme-encoding genes involved in primary carbohydrate metabolic pathways in 35S::ABF4 tubers.

Potato tubers, particularly Spunta variety, are mostly consumed fresh, thus long-term storage after harvest is required. Sprouting contributes to the loss of quality causing

mobilization of storage compounds, mainly starch and proteins, and shrinkage due to water loss (Sonnewald and Sonnewald 2014). During storage at room temperature (22 °C), sprouting and sprout growth is delayed in 35S::ABF4 tubers (Fig. 4); the sprout/tuber weight ratio was reduced by 41–48%, with respect to wild-type controls. Sprouting is also delayed in transgenic tubers during storage at 4 °C. These results clearly indicate that 35S::ABF4 tubers have longer storage capabilities under the conditions used in this work, however more studies are required to determine the sprouting behavior under different storage conditions, and the mobilization of storage compounds. Notably, the expression of *ABF4* has no effect on the sprouting or emergence of plants when tubers are planted after the dormancy period, which would have been a great disadvantage.

The mechanism involved in the retardation of sprouting and inhibition of sprout growth in 35S::ABF4 tubers is possibly related to the role of ABF4 in the regulation of ABA and GA metabolic genes expression. ABA is required for the maintenance of tuber dormancy (Suttle and Hultstrand

1994). ABA levels are high in dormant tubers and decrease during storage; this changes in ABA content are controlled by the expression of the biosynthetic genes *zeaxanthin epoxidase* (*ZEP*) and *9-cisepoxycarotenoid dioxygenase* (*NCED*), and genes coding for the catabolic *ABA 8´-hydroxylase* (*CYP707A*) (Destefano-Beltran et al. 2006). Although the function of GA in dormancy regulation remains controversial, the evidence obtained so far indicates a role for GA in sprout growth promotion rather than in dormancy release (Hartmann et al. 2011). We have previously demonstrated that heterologous expression of *ABF4* leads to an increase in *StZEP* and *StNCED1* transcript levels, accompanied by an increase in the expression of *StGA2ox1* in stolons (Muñiz García et al. 2014). It is possible that the same regulation occurs in mature 35S::ABF4 tubers, resulting in higher ABA content that increases dormancy level and delays sprouting, and lower bioactive GA content that retards sprout growth.

ABF TFs regulate the expression of several abiotic stress-responsive genes (Choi et al. 2000; Uno et al. 2000), which can mitigate the effect of stress leading to adjustment of the cellular milieu and plant tolerance (Hirayama and Shinozaki 2010). In addition, ABF proteins are known to regulate ABA-mediated stomatal closure, contributing to plant stress adaptation by reducing transpiration levels (Kang et al. 2002). According to these previously described functions, 35S::ABF4 plants show an increased capacity to conserve water under normal and stress conditions (Fig. 5). This effect is due to a reduced stomatal conductance and transpiration rates under stress (Fig. 5), and to an increase in the content of the osmoprotectant proline under normal and stress conditions (Fig. 6).

It has been shown that over-expression of exogenous and endogenous ABF homologs in cotton plants substantially increases dehydration resilience, but also induces stomatal closure and reduces photosynthetic activity (Kerr et al. 2017). Although the expression of *ABF4* in potato plants leads to a reduced stomatal conductance under salt stress, photosynthesis is not affected in the transgenic lines, under neither salt stress nor well-watered conditions (Fig. 7); this is consistent with the fact that 35S::ABF4 plants show normal vegetative growth.

35S::ABF4 plants are certainly more tolerant to soil salinity than wild-type controls, showing higher survival rates, and higher chlorophyll content during stress, indicative of a reduced stress-induced leaf senescence (Fig. 8). Consequently, 35S::ABF4 lines have higher yields than wild-type plants under salt stress (Fig. 9). Under drought stress, no differences were observed in the survival rate or chlorophyll content between 35S::ABF4 and wild-type plants (Fig. 8), however, transgenic lines exhibit higher yields (Fig. 9), indicating that ABF4 contributes to improve the performance under drought as well as under salt stress. Potato plants resulted more tolerant to drought than to salinity under the experimental conditions used in this study, as shown by the survival rates (79.8% for drought and 35.0% for salt stress, in wild-type plants). Therefore, it is possible that the drought stress applied was not severe enough to reveal differences in the survival rates between transgenic and wild-type plants.

Interestingly, under salt stress, 35S::ABF4 plants show an increased number of tubers per plant with no changes in the average tuber weight with respect to wild-type controls, while, under drought stress, transgenic plants produce larger tubers with no changes in the number of tubers per plant (Fig. 9). These results suggest that ABF4 acts through different mechanisms in response to either salt or drought stress.

Although salt and drought stress applied in this study severely affect tuber yields, 35S::ABF4 plants have 50–85% more yield than wild-type controls under these conditions (Fig. 9; Fig. S6), suggesting that this transgenic crop could have a clear advantage over wild-type plants in regions affected by salinity or drought. Field trials under drought or on saline soils are required to determine the economic significance of these results, however, it is logical to speculate that regardless of the absolute values of yield, which depend on the severity of the stress, the productivity of the 35S::ABF4 lines will be significantly higher than that of wild-type plants.

Considerable effort has been directed toward genetic improvement of different traits in potato plants. To date, 47 potato transformation events have been approved for use as food or as food ingredients in different countries (ISAAA's GM Approval Database; http://www.isaaa.org/gmapproval database/), and several genetically modified potatoes are currently being developed or going through the regulatory approval process for the commercial release (ISAAA Brief 52-2016). Regarding salinity and drought tolerance, the earliest approaches were based on the transgene expression of genes encoding enzymes or proteins, which directly function to protect cells from stress, such as *trehalose-6-phosphate synthase, betaine aldehyde dehydrogenase* and *mannitol 1-phosphate dehydrogenase* (Stiller et al. 2008; Zhang et al. 2011; Rahnama et al. 2011; Eltayeb et al. 2011; Bayat et al. 2010; Upadhyaya et al. 2011). Two interesting recent approaches consist of the over-expression of the *non-specific lipid transfer protein1* gene (*StnsLTP1;* Gangadhar et al. 2016), or the annexin-coding *STANN1* gene (Szalonek et al. 2015), which improve tolerance to abiotic stress by enhancing the activation of antioxidative defense mechanisms. Due to the complexity of abiotic stress responses, which are regulated by multiple genes, in the past few years TFs were evaluated as tools to engineer salinity and drought tolerance. The TFs introduced in transgenic potato plants to date belong to the MYB family (Shin et al. 2011; Cheng et al. 2013), the DREB subfamily of AP2/EREBP TFs (Celebi-Toprak et al. 2005; Movahedi et al. 2012; Bouaziz et al. 2012, 2013), and the group S of bZIP TFs (*Capsicum annuum CaBZ1* gene;

Moon et al. 2015). In this work, we evaluated the Arabidopsis *ABF4* gene as a potential tool for potato crop improvement. The ABF4 TF has the advantage that the expression of a single gene modifies different important characteristics in potato plants. Besides enhancing salt stress and drought tolerance, constitutive expression of *ABF4* increases tuber yield under normal and stress conditions, enhances storage capability and improves the processing quality of the tubers. Thus, this study provides valuable information to improve potato crop productivity and tuber quality using molecular breeding technology.

Author contributions MNMG and JIC: characterization of the phenotype of 35S::ABF4 plants. MF: maintenance of the plants in greenhouse; planting and harvesting tubers; application of stress treatments. DAC: design, direction and coordination of the study; manuscript writing.

References

Arnon DI (1949) Copper enzymes in isolated chloroplasts. Polyphenol oxidase in *Beta vulgaris*. Plant Physiol 24:1–15. https://doi.org/10.1104/pp.24.1.1

Bastías A, López-Climent M, Valcárcel M, Rosello S, Gómez-Cadenas A, Casaretto JA (2011) Modulation of organic acids and sugar content in tomato fruits by an abscisic acid-regulated transcription factor. Physiol Plant 141:215–226. https://doi.org/10.1111/j.1399-3054.2010.01435.x

Bastías A, Yañez M, Osorio S, Arbona V, Gómez-Cadenas A, Fernie AR, Casaretto JA (2014) The transcription factor AREB1 regulates primary metabolic pathways in tomato fruit. J Exp Bot 65:2351–2363. https://doi.org/10.1093/jxb/eru114

Bates LS, Waldren RP, Teare I (1973) Rapid determination of free proline for water stress studies. Plant Soil 39:205–207. https://doi.org/10.1007/BF00018060

Bayat F, Shiran B, Belyaev DV, Yur'eva NO, Sobol'kova GI, Alizadeh H, Khodambashi M, Babakov V (2010) Potato plants bearing a vacuolar Na^+/H^+ antiporter HvNHX2 from barley are characterized by improved salt tolerance. Russ J Plant Physiol 57:696. https://doi.org/10.1134/S1021443710050134

Birch PRJ, Bryan G, Fenton B, Gilroy EM, Hein I, Jones JT, Prashar A, Taylor MA, Torrance L, Toth IK (2012) Crops that feed the world 8: potato: are the trends of increased global production sustainable. Food Secur 4:477–508. https://doi.org/10.1007/s12571-012-0220-1

Boneh U, Biton I, Schwartz A, Ben-Ari G (2012) Characterization of the ABA signal transduction pathway in *Vitis vinifera*. Plant Sci 187:89–96. https://doi.org/10.1016/j.plantsci.2012.01.015

Bouaziz D, Pirrello J, Ben Amor H, Hammami A, Charfeddine M, Dhieb A, Bouzayen M, Gargouri-Bouzid R (2012) Ectopic expression of dehydration responsive element binding proteins (StDREB2) confers higher tolerance to salt stress in potato. Plant Physiol Biochem 60:98–108. https://doi.org/10.1016/j.plaphy.2012.07.029

Bouaziz D, Pirrello J, Charfeddine M, Hammami A, Jbir R, Dhieb A, Bouzayen M, Gargouri-Bouzid R (2013) Overexpression of StDREB1 transcription factor increases tolerance to salt in transgenic potato plants. Mol Biotechnol 54:803–817. https://doi.org/10.1007/s12033-012-9628-2

Camire ME, Kubow S, Donnelly DJ (2009) Potatoes and human health. Crit Rev Food Sci Nutr 49:823–840. https://doi.org/10.1080/10408390903041996

Capiati DA, País SM, Téllez-Iñón MT (2006) Wounding increases salt tolerance in tomato plants: evidence on the participation of calmodulin-like activities in cross-tolerance signalling. J Exp Bot 57:2391–2400. https://doi.org/10.1093/jxb/erj212

Casaretto JA, Ho TH (2005) Transcriptional regulation by abscisic acid in barley (*Hordeum vulgare* L.) seeds involves autoregulation of the transcription factor HvABI5. Plant Mol Biol 57:21–34. https://doi.org/10.1007/s11103-004-6520-x

Celebi-Toprak F, Behnam B, Serrano G, Kasuga M, Yamaguchi-Shinozaki K, Naka H, Watanabe J, Yamanaka S, Watanabe KN (2005) Tolerance to salt stress of the transgenic tetrasomic tetraploid potato, *Solanum tuberosum* cv. Desiree appears to be induced by the *DREB1A* gene and *rd29A* promoter of *Arabidopsis thaliana*. Breed Sci 55:311–319. https://doi.org/10.1270/jsbbs.55.311

Cheng YJ, Kim MD, Deng XP, Kwak SS, Chen W (2013) Enhanced salt stress tolerance in transgenic potato plants expressing IbMYB1, a sweet potato transcription factor. J Microbiol Biotechnol 23:1737–1746. https://doi.org/10.4014/jmb.1307.07024

Choi H, Hong J, Ha J, Kang J, Kim SY (2000) ABFs, a family of ABA-responsive element binding factors. J Biol Chem 275:1723–1730. https://doi.org/10.1074/jbc.275.31723

Destefano-Beltran L, Knauber D, Huckle L, Suttle JC (2006) Effects of postharvest storage and dormancy status on ABA content, metabolism, and expression of genes involved in ABA biosynthesis and metabolism in potato tuber tissues. Plant Mol Biol 61:687–697. https://doi.org/10.1007/s11103-006-0042-7

Dutt S, Manjul AS, Raigond P, Singh B, Siddappa S, Bhardwaj V, Kawar PG, Patil VU, Kardile HB (2017) Key players associated with tuberization in potato: potential candidates for genetic engineering. Crit Rev Biotechnol 37:942–957. https://doi.org/10.1080/07388551.2016.1274876

Easterling DR, Meehl GA, Parmesan C, Changnon SA, Karl TR, Mearns LO (2000) Climate extremes: observations, modeling, and impacts. Science 289:2068–2074. https://doi.org/10.1126/science.289.5487.2068

Eltayeb AE, Yamamoto S, Eltayeb Habora ME, Yin L, Tsujimoto H, Tanaka K (2011) Transgenic potato overexpressing Arabidopsis cytosolic AtDHAR1 showed higher tolerance to herbicide, drought and salt stresses. Breed Sci 61:3–10. https://doi.org/10.1270/jsbbs.61.3

Fang Z, Bouwkamp JC, Solomos T (1998) Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of *Phaseolus vulgaris* L. J Exp Bot 49:503–510. https://doi.org/10.1093/jxb/49.320.503

Fujino K, Koda Y, Kikuta Y (1995) Reorientation of cortical microtubules in the sub-apical region during tuberization in single-node stem segments of potato in culture. Plant Cell Physiol 36:891–895. https://doi.org/10.1093/oxfordjournals.pcp.a078835

Gangadhar BH, Sajeesh K, Venkatesh J, Baskar V, Abhinandan K, Yu JW, Prasad R, Mishra RK (2016) Enhanced tolerance of transgenic potato plants over-expressing non-specific lipid transfer protein-1 (StnsLTP1) against multiple abiotic stresses. Front Plant Sci 7:1228. https://doi.org/10.3389/fpls.2016.01228

Gao S, Gao J, Zhu X, Song Y, Li Z, Ren G, Zhou X, Kuai B (2016) ABF2, ABF3, and ABF4 promote ABA-mediated chlorophyll degradation and leaf senescence by transcriptional activation of chlorophyll catabolic genes and senescence-associated genes in

Arabidopsis. Mol Plant 9:1272–1285. https://doi.org/10.1016/j.molp.2016.06.006

Hartmann A, Senning M, Hedden P, Sonnewald U, Sonnewald S (2011) Reactivation of meristem activity and sprout growth in potato tubers require both cytokinin and gibberellin. Plant Physiol 155:776–796. https://doi.org/10.1104/pp.110.168252

Hijmans RJ (2003) The effect of climate change on global potato production. Am J Pot Res 80:271–280. https://doi.org/10.1007/BF02855363

Hirayama T, Shinozaki K (2010) Research on plant abiotic stress responses in the post-genome era: past, present and future. Plant J 61:1041–1052. https://doi.org/10.1111/j.1365-313X.2010.04124.x

Hossain MA, Cho JI, Han M, Ahn CH, Jeon JS, An G, Park PB (2010a) The ABRE-binding bZIP transcription factor OsABF2 is a positive regulator of abiotic stress and ABA signalling in rice. J Plant Physiol 167:1512–1520. https://doi.org/10.1016/j.jplph.2010.05.008

Hossain MA, Lee Y, Cho JI, Ahn CH, Lee SK, Jeon JS, Kang H, Lee CH, An G, Park PB (2010b) The bZIP transcription factor OsABF1 is an ABA responsive element binding factor that enhances abiotic stress signalling in rice. Plant Mol Biol 72:557–566. https://doi.org/10.1007/s11103-009-9592-9

Hsieh TH, Li CW, Su RC, Cheng CP, Tsai YC, Chan MT (2010) A tomato bZIP transcription factor, SlAREB, is involved in water deficit and salt stress response. Planta 231:1459–1473. https://doi.org/10.1007/s00425-010-1147-4

Huang XS, Liu JH, Chen XJ (2010) Overexpression of PtrABF gene, a bZIP transcription factor isolated from *Poncirus trifoliata*, enhances dehydration and drought tolerance in tobacco via scavenging ROS and modulating expression of stress-responsive genes. BMC Plant Biol 10:230. https://doi.org/10.1186/1471-2229-10-230

Ipcc (2008) Climate change and water. In: Bates BC, Kundzewicz ZW, Palutikof J, Wu S (eds.) Technical paper of the intergovernmental panel on climate change, IPCC Secretariat, Geneva

ISAAA (2016) Global Status of Commercialized Biotech/GM Crops: 2016. ISAAA Brief No. 52. ISAAA, Ithaca. ISBN: 978-1-892456-66-4

Kang J, Choi H, Im M, Kim SY (2002) Arabidopsis basic leucine zipper proteins mediate stress-responsive abscisic acid signaling. Plant Cell 14:343–357. https://doi.org/10.1105/tpc.010362

Kerr TC, Abdel-Mageed H, Aleman L, Lee J, Payton P, Cryer D, Allen RD (2017) Ectopic expression of two AREB/ABF orthologs increases drought tolerance in cotton (*Gossypium hirsutum*). Plant Cell Environ. https://doi.org/10.1111/pce.12906

Kim S, Kang JY, Cho DI, Park JH, Kim SY (2004) ABF2, an ABRE binding bZIP factor, is an essential component of glucose signaling and its overexpression affects multiple stress tolerance. Plant J 40:75–87. https://doi.org/10.1111/j.1365-313X.2004.02192.x

Kloosterman B, Navarro C, Bijsterbosch G, Lange T, Prat S, Visser RG, Bachem CW (2007) StGA2ox1 is induced prior to stolon swelling and controls GA levels during potato tuber development. Plant J 52:362–373. https://doi.org/10.1111/j.1365-313X.2007.03245.x

Kobayashi F, Maeta E, Terashima A, Takumi S (2008) Positive role of a wheat HvABI5 ortholog in abiotic stress response of seedlings. Physiol Plant 134:74–86. https://doi.org/10.1111/j.1399-3054.2008.01107.x

Levine RL, Willians JA, Stadtman ER, Shacter E (1994) Carbonyl assays for determination of oxidatively modified proteins. Methods Enzymol 233:346–363. https://doi.org/10.1016/S0076-6879(94)33040-9

Liang X, Zhang L, Natarajan SK, Becker DF (2013) Proline mechanisms of stress survival. Antioxid Redox Signal 19:998–1011. https://doi.org/10.1089/ars.2012.5074

Liang C, Meng Z, Meng Z, Malik W, Yan R, Lwin KM, Lin F, Wang Y, Sun G, Zhou T, Zhu T, Li J, Jin S, Guo S, Zhang R (2016) GhABF2, a bZIP transcription factor, confers drought and salinity tolerance in cotton (*Gossypium hirsutum* L.). Sci Rep 6:35040. https://doi.org/10.1038/srep35040

Moon SJ, Han SY, Kim DY, Yoon IS, Shin D, Byun MO, Kwon HB, Kim BG (2015) Ectopic expression of a hot pepper bZIP-like transcription factor in potato enhances drought tolerance without decreasing tuber yield. Plant Mol Biol 89:421–431. https://doi.org/10.1007/s11103-015-0378-y

Movahedi S, Tabatabaei BS, Alizade H, Ghobadi C, Yamchi A, Khaksar G (2012) Constitutive expression of Arabidopsis *DREB1B* in transgenic potato enhances drought and freezing tolerance. Biol Plant 56:37. https://doi.org/10.1007/s10535-012-0013-6

Muñiz García MN, Giammaria V, Grandellis C, Téllez-Iñón MT, Ulloa RM, Capiati DA (2012) Characterization of StABF1, a stress-responsive bZIP transcription factor from *Solanum tuberosum* L. that is phosphorylated by StCDPK2 in vitro. Planta 235:761–778. https://doi.org/10.1007/s00425-011-1540-7

Muñiz García MN, Stritzler M, Capiati DA (2014) Heterologous expression of Arabidopsis ABF4 gene in potato enhances tuberization through ABA-GA crosstalk regulation. Planta 239:615–631. https://doi.org/10.1007/s00425-013-2001-2

Nelson N (1994) A photometric adaptation of the Somogyi method for the determination of glucose. J Biol Chem 153:375–380

Oh SJ, Song SI, Kim YS, Jang HJ, Kim SY, Kim M, Kim YK, Nahm BH, Kim JK (2005) Arabidopsis CBF3/DREB1A and ABF3 in transgenic rice increased tolerance to abiotic stress without stunting growth. Plant Physiol 138:341–351. https://doi.org/10.1104/pp.104.059147

Orellana S, Yañez M, Espinoza A, Verdugo I, González E, Ruiz-Lara S, Casaretto JA (2010) The transcription factor SlAREB1 confers drought, salt stress tolerance and regulates biotic and abiotic stress-related genes in tomato. Plant Cell Environ 33:2191–2208. https://doi.org/10.1111/j.1365-3040.2010.02220.x

Rahnama H, Vakilian H, Fahimi H, Ghareyazie B (2011) Enhanced salt stress tolerance in transgenic potato plants (*Solanum tuberosum* L.) expressing a bacterial *mtl*D gene. Acta Physiol Plant 33:1521–1532. https://doi.org/10.1007/s11738-010-0690-8

Rommens CM, Shakya R, Heap M, Fessenden K (2010) Tastier and healthier alternatives to French Fries. J Food Sci 75:109–115. https://doi.org/10.1111/j.1750-3841.2010.01588.x

Shibaoka H (1994) Plant hormone-induced changes in the orientation of cortical microtubules: alterations in the cross-linking between microtubules and the plasma membrane. Annu Rev Plant Physiol Plant Mol Biol 45:527–544. https://doi.org/10.1146/annurev.pp.45.060194.002523

Shin D, Moon SJ, Han S, Kim BG, Park SR, Lee SK, Yoon HJ, Lee HE, Kwon HB, Baek D, Yi BY, Byun MO (2011) Expression of StMYB1R-1, a novel potato single MYB-like domain transcription factor, increases drought tolerance. Plant Physiol 155:421–432. https://doi.org/10.1104/pp.110.163634

Somogyi M (1952) Notes on sugar determination. J Biol Chem 195:19–23

Sonnewald U (2001) Control of potato tuber sprouting. Trends Plant Sci 6:333–335. https://doi.org/10.1016/S1360-1385(01)02020-9

Sonnewald S, Sonnewald U (2014) Regulation of potato tuber sprouting. Planta 239:27–38. https://doi.org/10.1007/s00425-013-1968-z

Stiller I, Dulai S, Kondrák M, Tarnai R, Szabó L, Toldi O, Bánfalvi Z (2008) Effects of drought on water content and photosynthetic parameters in potato plants expressing the trehalose-6-phosphate synthase gene of *Saccharomyces cerevisiae*. Planta 227:299–308. https://doi.org/10.1007/s00425-007-0617-9

Stritzler M, Muñiz García MN, Schlesinger M, Cortelezzi JI, Capiati DA (2017) The plasma membrane H⁺-ATPase gene family in

Solanum tuberosum L. Role of PHA1 in tuberization. J Exp Bot 68:4821–4837. https://doi.org/10.1093/jxb/erx284

Suárez-López P (2013) A critical appraisal of phloem-mobile signals involved in tuber induction. Front Plant Sci 4:253

Suttle JC, Hultstrand J (1994) Role of endogenous abscisic acid in potato microtuber dormancy. Plant Physiol 105:891–896. https://doi.org/10.1104/pp.105.3.891

Szalonek M, Sierpien B, Rymaszewski W, Gieczewska K, Garstka M, Lichocka M, Sass L, Paul K, Vass I, Vankova R, Dobrev P, Szczesny P, Marczewski W, Krusiewicz D, Strzelczyk-Zyta D, Hennig J, Konopka-Postupolska D (2015) Potato annexin STANN1 promotes drought tolerance and mitigates light stress in transgenic *Solanum tuberosum* L. plants. PLoS ONE 10:e0132683. https://doi.org/10.1371/journal.pone.0132683

Teixeira AI, Ribeiro LF, Rezende ST, Barros EG, Moreira MA (2012) Development of a method to quantify sucrose in soybean grains. Food Chem 130:1134–1136. https://doi.org/10.1016/j.foodchem.2011.07.128

Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. Proc Natl Acad Sci USA 97:11632–11637. https://doi.org/10.1073/pnas.190309197

Upadhyaya CP, Venkatesh J, Gururani MA, Asnin L, Sharma K, Ajappala H, Park SW (2011) Transgenic potato overproducing L-ascorbic acid resisted an increase in methylglyoxal under salinity stress via maintaining higher reduced glutathione level and glyoxalase enzyme activity. Biotechnol Lett 33:2297–2307. https://doi.org/10.1007/s10529-011-0684-7

Vasquez Robinet C, Mane SP, Ulanov AV, Watkinson JI, Stromberg VK, De Koeyer D, Schafleitner R, Willmot DB, Bonierbale M, Bohnert HJ, Grene R (2008) Physiological and molecular adaptations to drought in Andean potato genotypes. J Exp Bot 59:2109–2123. https://doi.org/10.1093/jxb/ern073

Wang-Pruski G, Nowak J (2004) Potato after-cooking darkening. Am J Pot Res 81:7–16. https://doi.org/10.1007/BF02853831

Xu X, Vreugdenhil D, van Lammeren AMM (1998) Cell division and cell enlargement during potato tuber formation. J Exp Bot 49:573–582. https://doi.org/10.1093/jxb/49.320.573

Yáñez M, Cáceres S, Orellana S, Bastías A, Verdugo I, Ruiz-Lara S, Casaretto JA (2009) An abiotic stress-responsive bZIP transcription factor from wild and cultivated tomatoes regulates stress-related genes. Plant Cell Rep 28:1497–1507. https://doi.org/10.1007/s00299-009-0749-4

Yeo AR (1999) Predicting the interaction between the effects of salinity and climate change on crop plants. Sci Hortic 78:159–174. https://doi.org/10.1016/S0304-4238(98)00193-9

Zhang N, Si HJ, Wen G, Du HH, Liu BL, Wang D (2011) Enhanced drought and salinity tolerance in transgenic potato plants with a BADH gene from spinach. Plant Biotechnol Rep 5:71–77. https://doi.org/10.1007/s11816-010-0160-1

Zhang Q, Wang M, Hu J, Wang W, Fu X, Liu JH (2015) PtrABF of *Poncirus trifoliata* functions in dehydration tolerance by reducing stomatal density and maintaining reactive oxygen species homeostasis. J Exp Bot 66:5911–5927. https://doi.org/10.1093/jxb/erv301