Transgenic Tomato Plants Ectopically Expressing *BrRZFP1* Gene Encoding C3HC4-type RING Zinc Finger Protein

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ABSTRACT C3HC4-type RING zinc finger proteins are known to be essential in the regulation of plant processes, including responses to abiotic stress. In order to explore the potential of the *BrRZFP1* gene to enhance tolerance toward multiple stresses in different host plant genomes, we generated transgenic tomato (*Solanum lycopersicum* L. cv. Goldenbell) plants. The tomato plants overexpressing *BrRZFP1* acquired a higher tolerance to drought stress. However, the transgenic plants did not appear to be more cold tolerant than the WT, in any tested condition. The data obtained indicate that the specificity and the degree of *BrRZFP1* activity depend on the host genomic background. In physiological assessment of salt stress tolerance, transgenic plants showed more dry matter accumulation and maintained significantly higher levels of leaf chlorophyll content along with increasing levels of salt stress than the wild type plants. This study shows that *BrRZFP1* is a candidate gene in the engineering of crops for enhanced drought and salt stress tolerance.

Keywords *BrRZFP1* gene, Abiotic stresses, Transgenic plants, Tomato

INTRODUCTION

The rapidly growing world population has made it necessary to limit the losses of crop productivity due to plant responses to environmental stress conditions, such as elevated or low temperatures, drought, salinity, poor soil nutrition, radiation, oxidative stress, and heavy metals. These abiotic factors activate an array of signaling pathways that ultimately lead to plant adaptation to stress, either through post-transcriptional or post-translational regulation (Mazzucotelli *et al.* 2008). Alternative splicing, degradation or accumulation of stress-related transcripts define the plant response to stresses at the mRNA level, whereas protein phosphorylation and dephosphorylation, ubiquitination, and sumoylation are some of the adaptive responses at the protein level (Mazzucotelli *et al.* 2008). To develop plant cultivars inherently resistant to abiotic stress would help secure crop production. However, progress in genetic improvement of crops for abiotic-limiting environments is

slow and more limited (Evenson and Gollin 2003), due to the lack of knowledge about the physiological processes limiting growth under abiotic stress conditions, poor understanding of abiotic stress tolerance mechanism, and lack of efficient techniques for screening breeding materials for abiotic stress tolerance (Khush 2001).

In recent years, transcription factors are known to be essential regulators of plant cellular and physiological responses to environmental stimuli. Several plant transcription factors, regulating the biotic and abiotic stress response, have been identified and the function of some of them (i.e. CBFs/DREBs, ZFPs and SCOF-1) in freezing and cold tolerance has been demonstrated (Xiong *et al.* 2002; Tran *et al.* 2007; Cong *et al.* 2008; Zheng *et al.* 2009; Golldack *et al.* 2011). Zinc finger proteins (ZFPs) are one of the best studied transcription factor families, playing important roles in various cellular functions, including transcriptional activation, regulation of apoptosis and protein folding and assembly (Laity *et al.* 2001). The zinc-binding motifs of

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ZFPs vary widely in structure, as well as function, which can range from DNA or RNA binding to protein–protein interactions and membrane association (Laity *et al.* 2001). One group of ZFPs contains the RING-finger motif, which consists of 40–60 amino acid residues that bind two atoms of zinc, and can be further divided into two general classes: RING-H2 (C3H2C3) and RING-HC (C3HC4) ZFPs (Freemont *et al.* 1991; Freemont 1993). Since their initial identification, many genes encoding RING finger proteins have been isolated from a variety of organisms, including animals, plants and viruses, and have been shown to possess a diverse range of biological functions (Saurin *et al.* 1996; Kam *et al.* 2007; Ma *et al.* 2009). Plants must respond to various abiotic stresses, such as high salinity, high and low temperatures and drought, in order to survive. These adverse environmental stresses limit crop growth and productivity, and also induce expression of a variety of genes, including ZFPs (Xiong *et al.* 2002; Bartels and Sunkar 2005; Islam *et al.* 2009). Previous studies of RING zinc finger proteins from various plants show that they are linked to a range of environmental stress processes, e.g. drought and other abiotic stresses, as well as disease resistance (Wang *et al.* 2006; Cheung *et al.* 2007; Kam *et al.* 2007; Zhang *et al.* 2007; Yang *et al.* 2008; Islam *et al.* 2009). Additionally, ZFPs function in the formation, development or signaling processes linked to stress processes, e.g. light perception, peroxisome formation and during seed and root development (Pepper and Chory 1997; Xu and Li 2003; Chen and Ni 2006; Wang *et al.* 2006; Prestele *et al.* 2010). It is important to note that RING proteins can also function as E3 ubiquitin ligase proteins, which also have a strong role in many of the same stress-related processes. The aim of this work was to investigate the possibility to utilize the *BrRZFPI* gene to enhance tolerance towards multiple environmental stresses in tomato (*Solanum lycopersicum* L.), one of the major agricultural commodities, characterized by a high sensitivity to stresses. Namely, we analyzed transformed tomato plants for tolerance to chilling, drought and resistance to pathogens. We found that *BrRZFPI* is able to improve the tolerance to some of the assayed drought stress, but not to chilling. In addition, to test if the constitutive expression of *BrRZFPI* modifies the tomato fruit quality, we analyzed

the soluble solids content (SSC), skin color, lycopene synthesis and ethylene production.

MATERIALS AND METHODS

Plant materials

Tomato plants (*Solanum lycopersicum* L. Mill. cv. Goldenbell) were grown in a controlled environment chamber at 25°C, with a 16 h/8 h photoperiod, a light intensity of 120 $\mu\text{mol m}^{-2} \text{s}^{-1}$ and 70% relative humidity. For transformation, the tomato seeds were surface-sterilized by immersion for 1 min in 70% ethanol, followed by 5 min in 2.5% NaOCl supplied with 0.1% Tween and washed several times with sterilized distilled water. The seeds were cultured for two days in distilled water and then transferred to Magenta boxes containing 50 mL of 1 x Murashige and Skoog (MS) salts (Murashige and Skoog 1962), Gamborg B5 vitamins (Gamborg *et al.*, 1968), 2% sucrose and 0.8% agar, pH 5.8. The cultures were maintained in a growth chamber. Eleven days after germination, the cotyledon sections, with the distal and proximal ends cut off, were used for *Agrobacterium*-mediated transformation.

Construction of plant transformation vector

The 1,041 bp open reading frame (ORF) of the *BrRZFPI* cDNA was amplified by polymerase chain reaction (PCR) using the forward primer *BrRZFPIF1* (5'-ATGCCTTCT-TCTGGAGATCCC-3') and the reverse primer *BrRZFPIR1* (5'-TTAAACAAATGGCATAGTTTTAC-3'). The amplified product was purified and cloned into the pMD-18T vector (Takara, Korea), and digested with *Bam*HI and *Sac*I. Subsequently the resulting fragment was inserted into the *Bam*HI and *Sac*I-predigested modified expression vector pCAMBIA2300. The resulting vector, pCAMBIA:: *BrRZPI*, contained the *BrRZPI* gene driven by the CaMV35S promoter and terminated by nopaline synthase terminator (Nos). The pCAMBIA:: *BrRZPI* was introduced into *A. tumefaciens* strain EHA105 by the freeze-thaw transformation method. Single transformed colony was transferred into liquid bacterial culture medium (LB medium with 50 mg/L rifampicin, 25 mg/L streptomycin and 50 mg/L kanamycin) and shaken at 240 rpm at 28°C for two days. The

suspension was then mixed with sterile glycerol (1 : 1 v/v) and stored at -70°C until use.

Tomato transformation

Cotyledons from the 2-week-old tomato seedlings grown in the growth chamber were used for cocultivation with *A. tumefaciens* to generate transgenic plants, according to the method of Van Roekel *et al.* (1993).

Total RNA extraction and RT-PCR analysis

Total RNA was extracted by using TRIzol1 reagent (Invitrogen) from tomato plants grown for four weeks in the greenhouse. Material from the control and treated plants was always collected at 9 a.m., in order to avoid variability in the gene expression due to the circadian clock regulation. The plant samples were immediately frozen in liquid nitrogen. Five micrograms of DNase treated RNA were retrotranscribed to first-strand cDNA with the Super Script™ First-Strand Synthesis System for RT-PCR kit (Invitrogen). PCR reactions were then carried out with the following gene-specific primers: *BrRZFPI*: (F) 5'-ATGC-CTTCTTCTGGAGATCCC-3', (R) 5'-CGACGTTGAA-TCTCAAGTC AC-3'; Tomato Actin (F) 5'-GTGACAATGGA AACTGGAATG-3', (R) 5'-AGACGGAGGATAGCGTGAGG-3'. The samples were subjected to 30 amplification cycles under the following annealing conditions: 58°C for *BrRZFPI* and 65°C for Actin DNA. The amplification products were separated by electrophoresis on 1% agarose gels.

Chilling treatment

The leaves from 4-week-old plants were layered on the surface of the MS agar medium. The plates were placed in a cabinet for photo inhibition treatments consisting of an irradiance of 750 $\mu\text{mol m}^{-2} \text{s}^{-1}$, provided by a 440-W lamp, at a temperature of 4°C for 5 h and 30 min. The chlorophyll fluorescence emission, from the upper surface of the leaves, was measured with a plant efficiency analyser (Hansatech Instruments, Norfolk, UK), according to Tarantino *et al.* (1999). Before measurements, the leaves were dark-adapted for 30 min at room temperature. Each experiment was replicated three times and in each experiment the leaves of one plant were assayed three times at different time intervals.

Drought treatment

To evaluate the drought tolerance of the transgenic tomato lines, the relative water content (RWC) of the transgenic and WT plants leaves was measured. Ten 4-week-old plants for each genotype grown individually in 15 cm pots were placed at random in a climatic chamber at 24 °C, 16 h/8 h light/dark regime, 60/70% humidity. The earth of the vases was abundantly watered and left to percolate for 24 h. Subsequently, the plants were left dry. Since then (t_0), for every four days, eight leaves of each genotype were randomly collected and weighed (fresh weight, FW). Afterward, the leaves were kept, to become impregnated, on Petri dishes for 24 h lined with filter paper saturated with demineralized water. They were then weighed (turgid weight, TW) and kept at 70°C for 48 h, to determine the dry weight (DW). The RWC was calculated as a percentage of $(FW - DW)/(TW - DW)$. For *in vivo* assays, 4-week-old WT and transgenic T₁ plants were grown in 15 cm pots and placed at random in a greenhouse (24°C, 16 h/8 h light/dark regime, 60/70% humidity). The soil contained in the pots was abundantly watered and, subsequently, plants were left dry without watering for 28 days. Then, the plants were watered for 7 days and plant survival was evaluated. We have performed two experiments: in the first one we tested WT, AT1 and AT4 plants; in the second one we used WT, AT2 and AT3 lines. In each experiment, 20 plants/genotype were assayed.

Soluble sugars, proline and amino acid content

Soluble sugars and proline contents were determined on frozen leaves from the same plants used for *in vivo* drought assays: leaves were detached from WT and transgenic 4-week-old plants grown under normal conditions and from the same plants after 28 days of water deficit. Three individual plants for each genotype were tested. Plant material was extracted using 0.6N perchloric acid, and the supernatant collected after centrifugation at 16000 x g for 15 min. Sucrose, glucose and fructose were quantified using the Boehringer Mannheim kit and the reduction of NADP was monitored at 340 nm. Standard curves of sucrose, glucose and fructose were prepared. On the same extracts, proline concentration was determined as described previously (Mattana *et al.* 2005). Proline content was

calculated on the basis of a standard curve of L-Pro. The amino acid content was determined using the method of Moore (1968).

Soluble solids content in fruits

Juice was squeezed from ripe tomatoes and collected onto a digital Attago N1 refractometer. The soluble solids content (SSC) was measured at 20°C and the results were expressed as degrees Brix (°Brix).

Lycopene measurement

The lycopene standard was purchased from Sigma. A stock solution, dissolving 5 mg lycopene in 10 mL chloroform, was prepared and sonicated for 20 min. Owing to the lycopene instability, the standard purity was checked by a spectrophotometer, with $E_{472\text{nm}}^{1\%} = 3450$ in hexane. From the stock solution, a 1:250 dilution was prepared in hexane, to a final concentration of 2 mg/mL, and absorbance was measured at 472 nm, using a 1 cm path length quartz cuvette after blanking with hexane. The purity was calculated as follows: % Purity = $(\text{Abs} \times 10000 / 3450 \times C) \times 100\%$, where Abs = absorbance units, and C = standard concentration in mg/mL. The spectrophotometric purity was then used to correct the concentration of the chromatographic calibration solutions. From the 500 mg/mL stock solution in chloroform, standard 1:10, 1:25, 1:50, 1:100 and 1:250 dilutions were prepared with chloroform and methanol (1:1), to create a five point linearity curve for HPLC analysis. To avoid oxidation during the sample preparation, the whole laboratory procedures were conducted under dim light. Each sample was analyzed in duplicates, with triplicate injections for HPLC. Twenty milliliters of 2N HCl/L-ascorbic acid solution were added to 50 mg of sample, into a 50 mL glass centrifuge tube wrapped with aluminum foil. The samples were then mixed at room temperature for 30 min on a mechanical orbital shaker and, after an addition of 10 mL of 0.1% (w/v) Butylated Hydroxytoluene (BHT)-stabilized toluene, an additional 10 min-shaking was carried out. After centrifugation, the samples were placed in a refrigerator maintained at 2–6°C for 60 min. One milliliter aliquot of the toluene upper layer was 1:25 diluted with methanol and tetrahydrofuran

(THF) (60:40), and immediately tested with HPLC analysis. A Shimadzu LC-10ADvp, SIL-10ADvp high-performance liquid chromatograph equipment, with SPD-10Avp and RF-10Axl detectors, was used for sample analyses; the HPLC pumps, auto sampler and detectors were controlled via Class vp 3.4 software. The analytical column was a Luna RP C18 (4.6 mm x 250 mm, particle size = 5 mm), provided with a guard column obtained from Chemtek Analytica. The column temperature was maintained at 30°C. The lycopene isomer separation was carried out at a 1.2 mL/min flow rate, using an isocratic elution with a mobile phase mixture of 90% methanol and 10% THF (O'Neil and Schwartz 1992). The injection volume was 10 mL and the 472 nm wavelength was used for absorbance detection of the lycopene. The data were analyzed using the Statistical Package for the Social Sciences (SPSS) 10.0-window program (1999). One-way variance analysis (ANOVA) was employed to identify significant differences between samples at $p \leq 0.01$ significance level.

Fruit color measurement

Color measurements of tomatoes were made using a portable CR-400 tristimulus colorimeter (Minolta Chroma Meter CR 400, Osaka, Japan) and Spectra-Match software, set to L*, a*, b* mode. The colorimeter has a beam diameter of 8 mm, three response detectors set at 0 viewing angle and a CIE standard illuminant C with diffuse illumination. Each fruit was measured twice, at two different sites on opposite sides of the equatorial region. A total of 10 tomatoes were assayed, both from WT and each transformed line, and three color readings were taken from each sample. L* indicates the lightness, ranging from black (0) to white (100); a* specifies the amount of red and green tonality, on a green (-) to red (+) axis; and b* indicates the amount of blue and yellow tonality, on a blue (-) to yellow (+) axis. The results were expressed as the ratio of red to green color (a^*/b^*), hue angle value [$h = \arctan(b^*/a^*)$, where 0° = red purple; 90° = yellow; 180° = bluish-green and 270° = blue], saturation (or chroma) [$C = (a^{*2} + b^{*2})^{1/2}$] and tomato color index [$\text{TCI} = a^*/L^*(a^{*2} + b^{*2})^{1/2}$] (Shewfelt et al., 1988).

RESULTS

Development of transgenic plants

The leaf explants were transferred to fresh selection medium MS2 after two days of co-cultivation on MS1 medium. One week later, some explants had grown larger and thicker, followed by shoots regeneration which gradually appeared from the edges of these explants. After one month, 138 shoots of 2–3 cm in length were cut off from the base of explants and placed on rooting medium MS3. After two weeks, 30 kanamycin-resistant plants developed normal roots on MS3. These 30 plants were transplanted to perlite for further molecular analysis and functional tests. Later, some plants were transferred to soil in greenhouse for seed production. The presence of transgenes in putative transgenic plants was first analyzed by PCR with specific primers to *BrRZFPI* gene. Among 30 transformants, 16 were found to be positive for the *BrRZFPI* gene through PCR analysis (data not shown). Subsequently, to further confirm transgenic status of the *BrRZFPI*-positive plants and to determine the copy number of insertions in independent transgenic lines, the genomic DNA was digested with *Bgl*II and hybridized with labelled 1.4 kb *BrRZFPI* gene probe. Southern analysis

confirmed the integration of the *BrRZFPI* gene in the genome of some transgenic lines, including AT04, AT06, AT09, AT11 and AT17. The number of the *BrRZFPI* gene insertions in transgenic lines varied from 1 to 3, while the wild type plant did not show any hybridization signal (Fig. 1).

Analysis of transgenic T₁ progenies

T₁ progenies derived from the independent primary transformants (AT04, AT09 and AT17) expressing *BrRZFPI* gene were analyzed for the segregation patterns of the induced *BrRZFPI* gene by PCR analysis. The *BrRZFPI* gene in AT04 line except in the other two lines segregated at a ratio of 3 : 1 (Table 1), indicating the integration of the transgenes into tomato genomes in Mendelian manner.

Evaluation of *BrRZFPI*-expressing plants for cold tolerance

To assess cold stress damage on *BrRZFPI*-expressing plants, the Photosystem II (PSII) stability was measured to reflect the level of cellular damage after cold treatments. The ratio between the variable fluorescence (F_v) and the maximum fluorescence (F_m) was used to estimate the quantum yield of the PSII photochemistry. The leaves from control and transformed plants were exposed to an excess

Fig. 1. Molecular analysis of *BrRZFPI* gene in transgenic tomato plants. (A) Southern hybridization, (B) RT-PCR analysis.

of light energy ($750 \mu\text{mol m}^{-2} \text{s}^{-1}$) at low temperature (4°C). Fv/Fm values were measured at different times during treatment. The *BrRZFP1*-transformed plants exhibited values of Fv/Fm comparable to that of the WT, indicating that the *BrRZFP1* expression did not affect the PSII efficiency (Table 2). The cold treatment at high light intensity caused a marked inhibition of the PSII for both the WT and transgenic plants, as indicated by a decrease in the Fv/Fm values from 0.8 up to 0.5.

Evaluation of *BrRZFP1*-expressing plants for drought tolerance

To evaluate the resistance of transgenic lines to drought stress, 4-week-old plants were not watered for 16 days and RWC was measured every 4 days. The RWC of leaves in transgenic plants remained high during water deficit treatments. By contrast, a marked reduction in the water content was observed in the WT plants (Fig. 2). For the survival rate test, the water deficit was extended to 28 days

Fig. 2. Bioassay of relative water content (RWC) in T₁ transgenic tomato lines for drought stress tolerance. WT; wild type plant, AT04-AT17; transgenic lines. Data represent the means of ten replicates per line per treatment $\pm$ standard error.

Table 1. Segregation of *BrRZFP1* gene T₁ progenies of transgenic tomato lines.

Line	Total assays	Expected segregation ratio	PCR for the <i>BrRZFP1</i> gene		
			<i>BrRZFP1</i> +	<i>BrRZFP1</i> -	Chi-square
AT04	60	3 : 1	43	17	0.35
AT09	60	3 : 1	51	9	3.20
AT17	60	3 : 1	53	7	5.68

Table 2. Photosystem II inhibition by cold and high light treatment. Photoinhibition (evaluated as Fv/Fm) induced in detached leaves from 4-week old transgenic and WT tomato plants treated at 4°C and $750 \mu\text{mol m}^{-2} \text{s}^{-1}$.

	Photoinhibition (Fv/Fm)		
	1h	3h	6h
WT	0.83	0.62	0.52
AT04	0.81	0.67	0.53
AT06	0.84	0.63	0.51
AT09	0.82	0.68	0.54
AT11	0.80	0.64	0.52
AT17	0.81	0.65	0.51

and then the plants were re-watered for 7 days. This set up was performed twice. In the first experiment, the survival rate was 9/20, 15/20 and 14/20 for the WT and transgenic lines AT04 and AT17, respectively. In the second experiment, the survival rate was 7/20, 15/20 and 11/20 for the WT and transgenic lines AT04 and AT17, respectively. All results observed indicate that transgenic lines are more resistant to drought stress than WT plants and that there is no significant different behavior between transgenic plants expressing the *BrRZFPI* gene driven by a constitutive or an inducible promoter. Since in *Arabidopsis* the overexpression of *BrRZFPI* increases the level of osmoprotectant compounds (Jung *et al.* 2013), we also measured the content of soluble sugars and proline in leaves of WT and 4-week-old transgenic tomato plants under normal and water deficit conditions. In normal growth conditions, the concentrations of sucrose, fructose and glucose were constitutively higher in transgenic lines with respect to WT (Table 3). In response to water deficit conditions, the soluble sugar content increased in all

genotypes. In particular, values in WT were in all cases lower than those of transgenic plants, and AT04 line achieved the same levels of the AT17 line. The proline content in transformed tomato is not much higher than in WT plants under normal conditions; however, there is an increase in response to water stress treatment in both AT04 and AT17 with respect to WT plants (Table 3).

Tomato fruit analysis

To test if the use of the constitutive overexpression of the *BrRZFPI* transcription factor modified the tomato fruit quality, we performed the analysis reported in Table 4. °Brix is a measure of the total soluble solid percentage (chiefly fructose and glucose) increasing in turn with fruit color and ripeness: its value was higher in the transgenic fruits than in the WT ones. Also the lycopene content, in the transgenic lines freshly harvested ripe tomatoes, was slightly higher than in the WT samples.

Table 3. Soluble sugar and proline content in WT and transgenic tomato lines before and after 28 days of dehydration stress.

	Glucose ($\mu\text{g}/\text{mg}$ Fw)		Fructose ($\mu\text{g}/\text{mg}$ Fw)		Sugar ($\mu\text{g}/\text{mg}$ Fw)		Proline/ total amino acid%	
	Before	After	Before	After	Before	After	Before	After
WT	0.91	1.43	1.03	2.27	0.34	0.42	1.92	5.64
AT04	1.21	2.96	1.21	3.77	0.98	1.84	2.30	6.89
AT06	1.31	2.98	1.89	3.88	0.91	1.86	2.12	7.27
AT09	1.51	3.10	2.70	3.76	0.99	1.98	2.83	7.32
AT11	1.43	3.01	2.04	4.42	1.23	2.01	2.69	7.21
AT17	1.69	2.92	2.57	3.97	1.19	2.11	2.48	7.10

Each value is the mean ($n = 5$ individual plants).

Table 4. Main fruit traits of wild type and transgenic tomato lines.

	Weight (g)	°Brix	Lycopene ($\mu\text{g}/\text{g}$)	L	a	b
WT	14.2	5.8	19.28	40.47	22.71	18.46
AT04	16.3	5.9	24.32	39.66	20.86	19.88
AT06	15.8	6.3	27.38	38.76	21.34	20.21
AT09	14.9	6.5	24.88	39.44	22.74	19.86
AT11\	13.8	6.9	32.44	38.27	29.66	18.44
AT17	12.9	6.3	27.68	36.99	24.58	19.01

DISCUSSION

Transcription factors have been shown to play important roles in signal transduction and gene expression under plant stress responses to salt, cold and drought (Chen *et al.* 2002; Shinozaki *et al.* 2003; Bartels and Sunkar 2005; Yang *et al.* 2008). A better understanding of stress-regulated transcription factors through cloning and characterization will be highly valuable in improving plant stress tolerance (Wang *et al.* 2003). The C3HC4-type RING zinc finger proteins play important roles in a variety of plant processes, including regulation of growth and development, protein–protein interactions and signaling networks (Freemont 1993; Borden *et al.* 1995; Tsuge *et al.* 2001; Wang *et al.* 2006). In addition, these transcription factors have been linked to abiotic stress processes for cold and salt (Lee *et al.* 2001; Xiong *et al.* 2002; Mukhopadhyay *et al.* 2004; Huang *et al.* 2011). Importantly, several studies have also linked RING domain containing proteins to E3 ubiquitin ligase activity and these same abiotic stress-regulated processes (Lyzenga and Stone 2011), e.g. zinc finger proteins such as *ZFP1* from *Artemisia desertorum* (Yang *et al.* 2008), so additional experimentation is required to determine if *BrRZFP1* may have an E3 ligase role. To further study these stress-related processes, the first C3HC4-type RING zinc finger protein, *BrRZFP1*, from cold tolerant *B. rapa* was cloned and examined. We found that *BrRZFP1* is expressed over a range of plant tissues, but at very high levels in roots during early development of germinating seedlings. This is the first report of *BrRZFP1* involvement in stress responses, following previous transcriptome analyses of *B. rapa* for abiotic stress responses genes (Lee *et al.* 2008; Yu *et al.* 2012). Interestingly, while several transcription factors were found to be stress-regulated in these studies, few zinc finger protein were among those identified. We are unsure why *BrRZFP1* was not identified in these prior transcriptome analyses, possibly due to the incomplete nature of microarrays or genome sequence used. Nevertheless, it demonstrates that direct experimentation on genes of interest is an essential process. Like *Arabidopsis* (Jung *et al.* 2013), tomato transgenic plants overexpressing *BrRZFP1* acquired a higher tolerance to drought stress. It is known that, under drought stress

conditions, plants accumulate several compatible solutes, such as soluble sugars and proline, as a mechanism to improve tolerance to water deficit (Bonhert and Jensen 1996). Following drought treatment, an increase in the level of these osmolytes was observed in both WT and transgenic tomato plants, but levels accumulated by transgenics were always higher than those observed in WT. As far as free sugars are concerned, their content in *BrRZFP1* constitutively expressing plants was higher than in WT plants also under normal growth conditions. These results indicate that the improvement of drought tolerance in *BrRZFP1* transgenics depends, at least partially, on the changes in the accumulated levels of compatible solutes. Contrary to the results obtained in *Arabidopsis*, the tomato *BrRZFP1* transgenic plants did not appear to be more cold tolerant than the WT, in any tested condition. The different behavior between *Arabidopsis* and tomato could lie in the different selective pressure in response to different environments colonized by the two species. The tomato is a tropical plant and selection pressures could have caused the loss of the target genes in the cold pathway controlled by *BrRZFP1*. The results of Huang *et al.* (2008) point to the same direction, demonstrating that the tomato *ZFP* region involved in freezing tolerance differs considerably from that of *Arabidopsis*: it is much smaller in size and potentially less diverse in function. Owing to the dietary relevance of tomato fruits, we tested the effect of the *ZFP1* transcription factor constitutive overexpression on the quality of transgenic tomato fruits. The results indicate a slight increase both in the lycopene and soluble solids content in transgenic plants. The other tested fruit characteristics were not significantly different between transgenic and control plants. In conclusion, the obtained data show that the *BrRZFP1* stress-response machinery is only partially conserved between tomato and *Arabidopsis*. The specificity and the degree of *BrRZFP1* activity depend on the host genomic background. This fact may represent a constraint in the use of transcriptional factors in the improvement of crop plants. On the other hand the use of heterologous transcription factors represents a good strategy to minimize possible mechanisms of transgene cosuppression during crop transformation.

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