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Arabidopsis thaliana AtTFIIB1 gene displays alternative splicing under different abiotic stresses

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Abstract

In *Arabidopsis,* there are 14 TFIIB-like proteins that have been phylogenetically categorized into the TFIIB, BRF, and Rrn7/TAF1B/MEE12 subfamilies. The TFIIB transcription factor (TF) subfamily plays a key role in the regulation of gene expression in eukaryotes. To identify the expression patterns of some members of the TFIIB and BRF subfamilies in *A. thaliana*, different approaches were carried out to determine the possible functions of some of these transcription factors. Through an *in silico* analysis, we identified possible c*is*-acting regulatory elements in the promoter regions that drive the expression of transcription factors, as well as we evaluated their expression by means of real-time qPCR, at different growth stages and under various stress conditions. *Cis*-acting elements analysis showed that general transcription factors possess stress-responsive elements such as W-Box (TTGACC/T type binding WRKY TFs), ARF1 (auxin response), MYB binding site promoter (auxin response and elicitors), RAV1-A (response to dehydration and salinity), and DRE elements (dehydration response) among others. The experimental results showed differential expression of *TFIIB1* and *TFIIB*. In addition, we demonstrate that in stress conditions a transcript of the TFIIB1 factor is generated as an alternative splicing product by retention of the third intron, where a premature termination codon is found. This is the first report of an alternative splicing event in a general transcription factor related to RNA pol II, which is synthesized when the plant is under abiotic stresses such as heat, dehydration, and salinity.

Keywords: abiotic stresses, alternative splicing, *Arabidopsis thaliana*, basal transcriptional factor, dehydration, heat, osmotic stress, salinity.

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Abbreviations: AS - alternative splicing; BRPs - TFIIB-related proteins; cDNA - complementary DNA; GAPDH - glyceraldehyde-3-phosphate dehydrogenase; GTFs - general transcription factors; miPs - microproteins; MS - Murashige and Skoog; nptII - neomycin phosphotransferase II; NMD - nonsense-mediated decay; PLACE - plant *cis*-acting regulatory DNA elements database; PlantCARE - plant *cis*-acting regulatory elements database; PTC - premature termination codon; RUST - regulated unproductive splicing and translation; siPEP - small interference peptide; TFs - transcription factors.

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Introduction

Plants as sessile organisms have strategies to maintain homeostasis during their life cycle and respond to adverse external stimuli such as biotic and abiotic stresses. Abiotic stresses, such as low or high temperature, deficient or excessive water, high salinity, heavy metals, and ultraviolet radiation, are hostile to plant growth and development, leading to great crop yield penalty worldwide (He *et al.* 2018). To survive, grow, and reproduce in environments that fluctuate annually and diurnally, land plants have evolved a complex system that regulates adaptation in response to stress signals. Many aspects of adaptation processes, which include developmental, physiological, and biochemical changes, are controlled by stress-responsive gene expression (Agarwal *et al.* 2017). Transcription factors play central roles in gene expression by regulating downstream gene regulation, such as *trans*-acting elements *via* specific binding to *cis*-acting elements in the promoter of target genes (Mizoi *et al.* 2012). In addition, transcription factors finely regulate signal transduction pathways in the cell to adjust and achieve optimal growth and survival under stressful environmental conditions (Yamaguchi-Shinozaki and Shinozaki 2006).

Well-established molecular and biochemical mechanisms underlying the regulation of transcription factor (TF) activities involve regulation at different stages of transcription, translation, and protein turnover (Seo *et al.* 2013). Recent studies have shown that in plant genomes post-transcriptional control of RNA metabolism is widespread. For example, in *Arabidopsis thaliana,* over 60 % of intron-containing genes undergo alternative splicing (AS). The diversity of the proteome and the expansion of the repertoire of gene/protein activities in response to developmental and environmental stimuli are provided by alternative splicing (Syed *et al.* 2012).

Plants can sense, process, and respond to environmental cues and activate related-gene expression to increase their stress tolerance (Nakashima *et al.* 2014). Many proteins interact with specific DNA sequences in the promoter regions and interact with other transcriptional regulators. It has been estimated that *A. thaliana* contains over 1 800 TFs representing more than 7 % of its proteins, and only a tenth of these have been characterized (Lindemose *et al.* 2013, Franco-Zorrilla *et al.* 2014).

The initiation of eukaryotic gene transcription requires the formation of a pre-initiation complex (PIC) on promoter regions. The PIC is composed of RNA polII, transcription factor IIB (TFIIB), and TATA box-binding proteins (TBPs) (Kostrewa *et al.* 2009). In *A. thaliana*, 14 TFIIB-like proteins have been phylogenetically categorized into the TFIIB, BRF, and Rrn7/TAF1B/MEE12 subfamilies. The TFIIB subfamily has eight members, including TFIIB1, TFIIB2, and six BRP proteins, among which BRP3 and BRP6 contain only partial TFIIB domains that are predicted to function differently from the others TFIIB (Knutson 2013).

In *Saccharomyces cerevisiae*, the isogenic mutant strains on *TFIIB* (SUA7) show deficiencies in growth at 16 and 30 ºC (Pinto *et al.* 1994); another report described the synthesis of two *SUA7* transcripts differing in size near 100 nucleotides when cells are exposed to heat stress (Hoopes *et al.* 2000). Furthermore, TFIIB is expressed in terms of adaptation to high temperatures in *Citrus clementina* (Sánchez-Ballesta *et al.* 2003). Fiol and Kültz (2005) reported an increased expression of two transcription factors (OTSF1 and TFIIB) that were rapidly and transiently induced during hyperosmotic stress in the fish *Oreochromis mossambicus*, and an alternative isoform was reported in tomato (Keller *et al.* 2017). So far, *TFIIB* and members of this gene family (BRP) recently reclassified by Knutson (2013) have been reported to be involved in the development and establishment of reproductive structures in *A. thaliana* (Cavel *et al.* 2011, Zhou *et al.* 2013, Qin *et al.* 2014). In previous work, we reported the characterization of a T-DNA *tin14* mutant of *A. thaliana* insensitive to trehalose (Hernández-Campuzano *et al.* 2014). The T-DNA insertion in this mutant is located 728 bp downstream of the *AtBRP3* (plant-specific TFIIB-related protein) coding region. The *tin14* mutant displayed insensitiveness to glucose, abscisic acid, and tolerance to osmotic stress (sorbitol), but not to salinity (Hernández-Campuzano *et al.* 2014).

In the present work, we report data showing that *TFIIB1*, *BRP1*, *BRP2*, *BRP3*, and *BRP4* are differentially expressed under different developmental stages, in response to stress conditions, and that the *TFIIB1* gene shows alternative splicing under osmotic, heat, and dehydration stress.

Materials and methods

Plants and cultivation: *Arabidopsis thaliana* L. wild-type plants used in this study were the Columbia ecotype (Col-0). Seeds were sterilized in 0.5 % sodium hypochlorite for 15 min, followed by 5 washes, and then germinated on Murashige and Skoog (MS) medium gelled with *Phytagel* with or without kanamycin in a plant culture room at a temperature of 22 °C, a 16-h photoperiod, and irradiance of 80 - 90 µmol(photons) m^{-2} s^{-1}. The seedlings were transplanted to *Metro-Mix 200* (*Grace-Sierra*, Milpitas, CA, USA).

To break dormancy and promote germination, *A. thaliana* seeds were stratified at 4 ºC in the dark for 24 h. Later the seeds were surface-sterilized, sown on MS plates or in *Magenta* boxes, and incubated under the conditions mentioned above.

Dehydration stress treatment in *Arabidopsis thaliana* adult plants: *A. thaliana* seeds previously sterilized were germinated in *Magenta* boxes and after four weeks, the plants were carefully removed and placed inside a laminar flow hood to induce dehydration for 1, 2, and 3 h. Then the different tissues such as flower, cauline leaf, and rosette leaf were removed with sterile scissors to proceed with RNA purification.

Stress treatments in *Arabidopsis thaliana* seedlings: For heat stress, two-week-old seedlings growing in conditions previously mentioned were incubated at 32 ºC for 1 h

before proceeding to RNA extraction. For cold stress, two-week-old seedlings were incubated at 0 ºC for 1 h before proceeding to RNA extraction. For salt stress, two-week-old seedlings were removed and floated on different NaCl concentrations (100, 200, 300, 400, and 500 mM) in distilled water for 1 h before proceeding to RNA extraction. For dehydration stress, two-week-old seedlings were carefully removed and placed inside a laminar flow hood to induce dehydration (1, 2, and 3 h) before proceeding to RNA extraction.

RNA extraction and real-time qPCR: Seedlings, cauline leaves, rosette leaves, and flower tissues were frozen in liquid nitrogen and grounded to a fine powder using mortar and pestle. Total RNA was isolated using *TRIzol* reagent (*Invitrogen*, Carlsbad, CA, USA) according to the manufacturer´s instructions. Integrity and concentration of the RNA were analyzed by electrophoresis on 1.5 % (m/v) agarose gel, stained with ethidium bromide, and quantified in *Nanodrop 2000* (*Thermo Scientific*, Wilmington, DE, USA) spectrophotometer.

The first cDNA strand was synthesized using 2.0 µg of total mRNA, treated with DNase I, and used for complementary DNA (cDNA) synthesis with *RevertAid H Minus M-MuLV* reverse transcriptase (both from *Thermo Scientific*) following the manufacturer´s instructions. The obtained cDNA was used for gene expression analyses. A PCR using the glyceraldehyde-3-phosphate dehydrogenase gene (*GAPDH*) (AT2G24270) was used as a control. Primers used in the present work are listed in Table 1 Suppl.

The resulting cDNAs were then diluted 10-fold and used to perform the real-time qPCR experiments using *SYBR Green qPCR Master Mix* (*Fermentas*, Waltham, Ma, USA) following the manufacturer's instructions. The reaction mix was then dispensed in a 96 well plate and analyzed using real-time thermocycler *Applied Biosystem 7300* (Foster City, CA, USA). The thermal cycler settings were as follows: 94 °C for 1 min, followed by 40 cycles of 94 °C for 20 s and 60 °C for 60 s. Relative transcriptions for each sample were obtained using the 'comparative Ct method' (Schmittgen and Livak 2008) and normalized with the geometrical mean of the *GAPDH* gene. A multiple analysis of variances (*ANOVA*) statistical test was performed to evaluate the significance of the differential expression using the mean values from three biological replicates for each condition.

Cloning of AtTFIIB1 isoforms: The RT-PCR products amplified with the primers TFIIB1Up and TFIIB1Do (Table 1 Suppl.) were cleaned-up directly from agarose gel by *QIAEX II*® gel extraction kit (*Qiagen*, Hilden, Germany) according to the manufacturer's instructions. Purified fragments were cloned in a pTZ57R/T cloning vector for sequencing according to the supplier (*Thermo Scientific*). The DNA-sequencing was performed using the *Applied Biosystems 3130xl* genetic analyzers at the Instituto de Biotecnología, UNAM, Mexico (http://www.ibt.unam.mx/sintesis/secuenciacion.html).

Data analysis: The results are expressed as means ± standard deviations (SDs). One-way analysis of variance (*ANOVA*) was used to analyze the statistical significance between groups and the $P < 0.01$ level was considered as statistically significant.

Results

To determine the profile of *cis*-regulatory elements in upstream regions of *TFIIB* genes family members in *A. thaliana*, the 5'-upstream sequences available online (http://arabidopsis.med.ohio-state.edu/AtcisDB/) were analyzed using *PLACE* and *PlantCARE* databases. Several response elements were found in the promoters of the *TFIIB* genes family, which in addition to elements involved in the

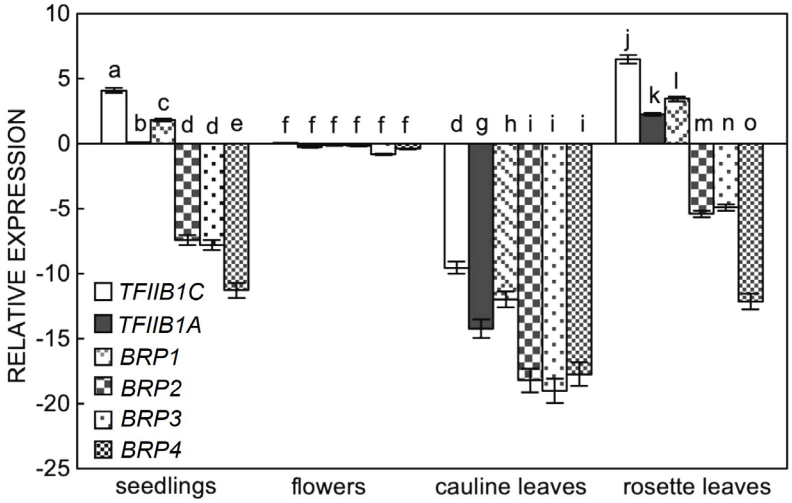

Fig. 1. Expression analysis by real-time qPCR of *TFIIB* family members under non-stressed conditions in different tissues of *A. thaliana*. Relative expressions for each sample were obtained using the comparative Ct method as mentioned in Materials and methods and normalized with the geometric mean of the *GAPDH* gene (AT2G24270). Means ± SDs, $n = 3$; *bars* with different letters are significantly different at $P < 0.01$ between any TFIIB family members.

general transcription process, contain several factors that may be associated with biotic and abiotic stress responses (Table 2 Suppl.).

To elucidate the expression of five *TFIIB* family members in *A. thaliana* at different developmental stages, a transcriptional analysis of the selected genes was performed using real-time qPCR. We selected genes whose products of genetic expression possess at least zinc ribbon and cyclin domains in the same peptide. Our results showed that *TFIIB1* and *BRP1* under normal growth conditions are constitutively expressed in seedlings, flowers, and rosette leaves. The TFIIB family members showed a slightly repressed expression in cauline leaves. On the other hand, *BRP2*, *BRP3*, and *BRP4* showed

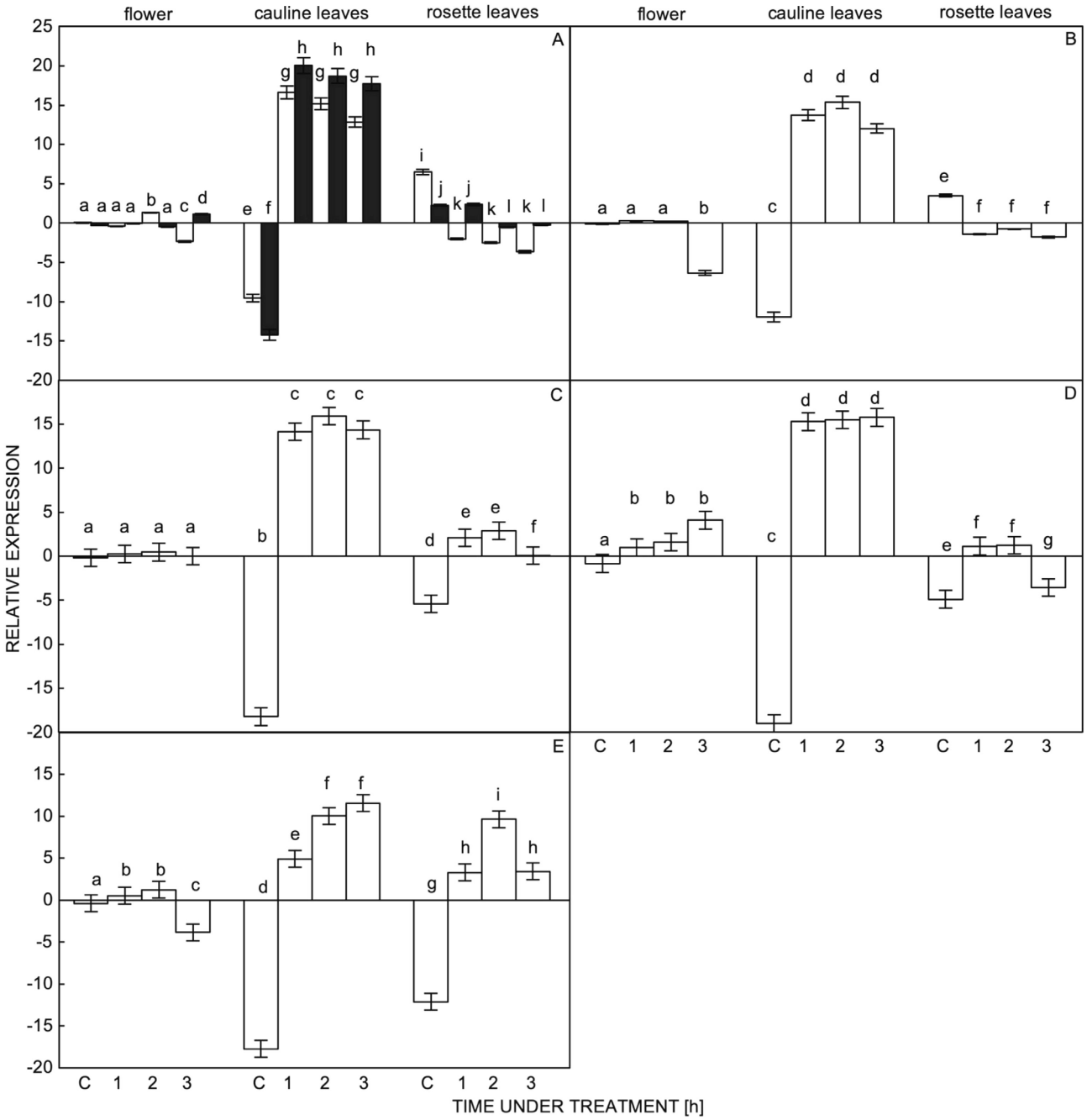

Fig. 2. Expression analysis of *TFIIB* family members under dehydration stress in different tissues of *A. thaliana*. *A* - *TFIIB1* canonical (*white bars*) and *TFIIB1* alternative splicing isoform (*black bars*), *B* - *BRP1*, *C* - *BRP2*, *D* - *BRP3*, and *E* - *BRP4*. C means growth under control conditions, 1, 2, and 3 means dehydration treatment for 1, 2, and 3 h. Relative expressions were obtained using the comparative Ct method as mentioned in Materials and methods and normalized with the geometric mean of the *GAPDH* gene. Means ± SDs, $n = 3$; *bars* with different letters are significantly different at $P < 0.01$ between TFIIB family members.

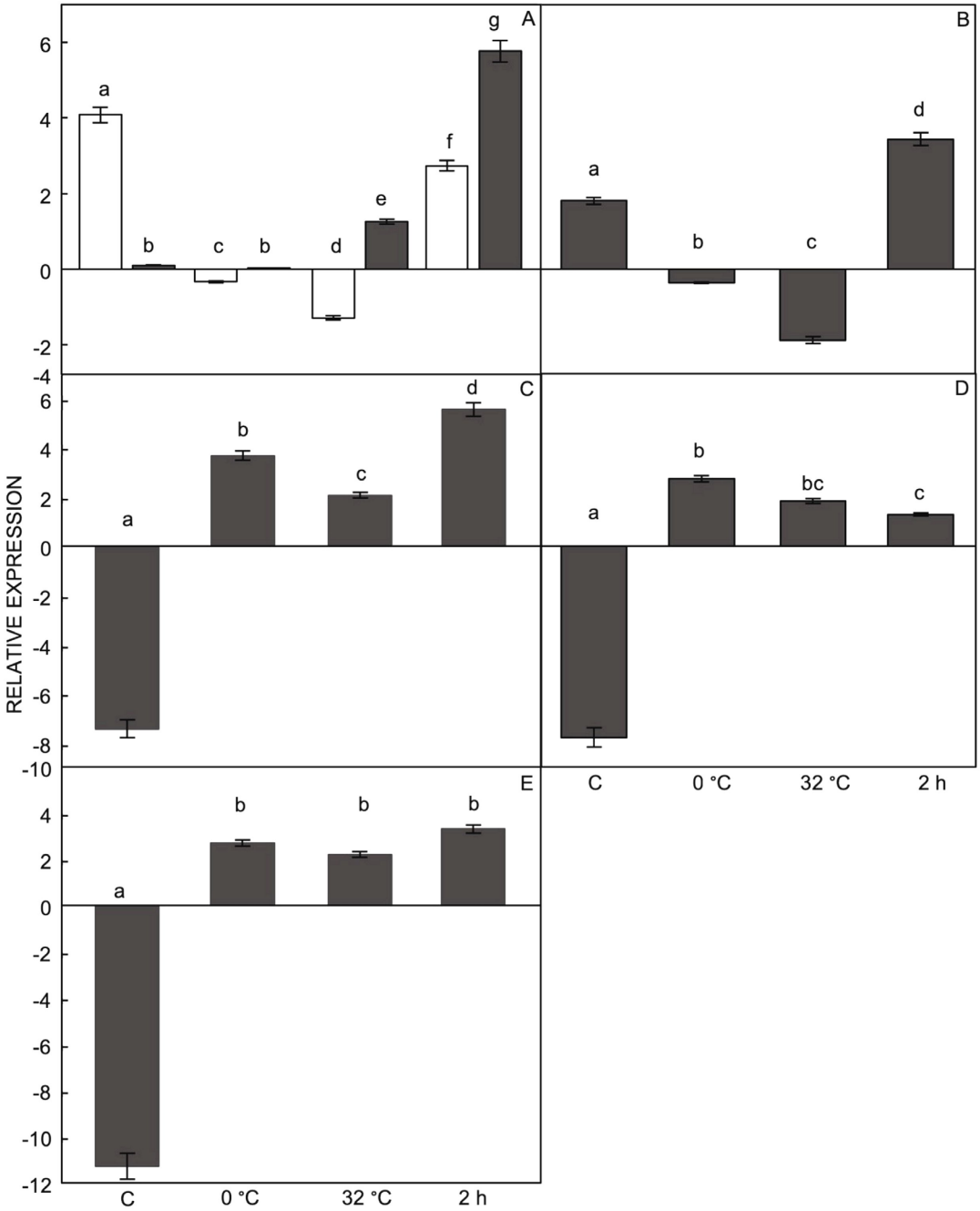

Fig. 3. Expression analysis of *TFIIB* family members under cold (0 °C), heat (32 °C), and dehydration (2 h) stress in *A. thaliana* seedlings. *A* - *TFIIB1* canonical (*white bars*) and *TFIIB1* alternative splicing isoform (*black bars*), *B* - *BRP1*, *C* - *BRP2*, *D* - *BRP3*, and E - *BRP4*. C means growth under control conditions. Relative transcriptions for each sample were obtained using the comparative Ct method as mentioned in Materials and methods and normalized with the geometric mean of the *GAPDH* gene. Means ± SDs, $n = 3$; *bars* with different letters are significantly different at $P < 0.01$ between TFIIB family members.

repression in seedlings, basal expressions in flower tissue, and repression in cauline and rosette leaves (Fig. 1).

To demonstrate the role of stress-responsive *cis*-acting elements in promoters of *TFIIB1*, *BRP1*, *BRP2*, *BRP3*, and *BRP4* genes predicted through *in silico* analysis, qPCR experiments were conducted under dehydration stress as described in Materials and methods. The *BRP1* showed basal expressions at 1 and 2 h of dehydration in flowers. However, at 3 h of stress, the *BRP1* expression was repressed in flowers, increased in cauline leaves, and repressed in rosette leaves (Fig. 2*B*). The *BRP2* did not show changes in expression in flower but showed increased expression in cauline and rosette tissues; however, it showed repression only in rosette after 3 h of dehydration stress (Fig. 2*C*). The *BRP3* showed an increased expression under dehydration after 1, 2, and 3 h in flowers, cauline, and rosette leaves, but only at 3 h of stress did the rosette show repression of expression (Fig. 2*D*). The *BRP4* showed an increase in

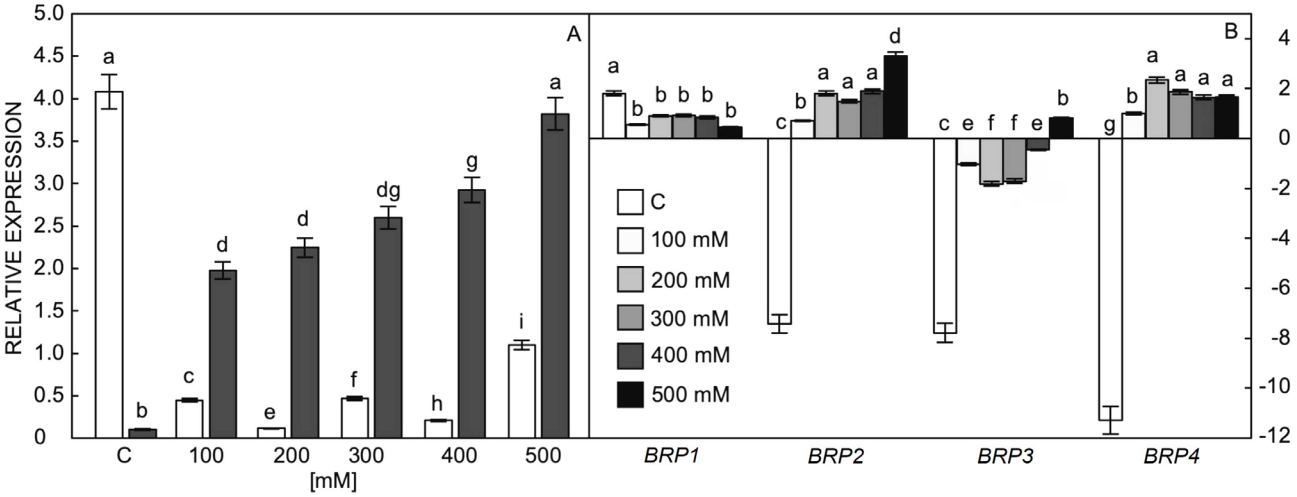

Fig. 4. Expression analysis of *TFIIB* family members in *A. thaliana* seedlings under different NaCl concentrations. *A* - Expressions of *TFIIB1* transcripts isoforms. *White bars* indicated *TFIIB1* canonical isoform while *black bars* indicated *TFIIB1* alternative isoform; *B* - *TFIIB* related protein (BRP) genes. Relative transcriptions were obtained using the comparative Ct method as mentioned in Materials and methods and normalized with the geometric mean of the *GAPDH* gene. Means ± SDs, $n = 3$; *bars* with different letters are significantly different at $P < 0.01$ between *TFIIB* family members.

expression in almost all tissues after 1, 2, and 3 h but with slight repression in flowers after 3 h of dehydration stress (Fig. 2*E*).

The *TFIIB1* maintained similar expression patterns in normal growth conditions (Fig. 1) as well as under dehydration (Fig. 2*A*). Surprisingly, an additional amplification product (93 bp larger than the canonical transcript) was synthesized in *A. thaliana* plants exposed to heat, salinity, and dehydration stresses (Fig. 1 Suppl.).

Two-week-old seedlings were subjected to different abiotic stresses such as cold (0 °C), heat (32 °C), and dehydration (2 h), and *BRP1*, *BRP2*, *BRP3*, *BRP4*, and *TFIIB1* transcripts were quantified. The *BRP1* expression decreased under cold and high temperatures, but increased under dehydration, *BRP2*, *BRP3*, and *BRP4* showed an increase under cold, heat, and dehydration (Fig. 3*B-D*).

To determine if the expression of the alternative isoform of *TFIIB1* is induced specifically only under dehydration stress conditions, qPCR analysis under abiotic stress conditions mentioned above was taken (Fig. 3*A* and 4*A*). The results demonstrated that the expression of the alternative splicing product of *TFIIB1* also takes place under heat stress (32 °C for 1 h) (Fig. 3*A*) and salinity (100, 200, 300, 400, and 500 mM NaCl for 1 h); both *TFIIB1* transcript isoforms co-existed in control as well as in salinity stress. However, the canonical isoform is the most abundant under no stress conditions, whereas the alternative isoform increases according to the NaCl concentrations (Fig. 4*A*). This *TFIIB1* isoform was absent when *A. thaliana* plants were subjected to low temperature (0 °C for 2 h) (data not shown).

To verify the identity of the alternative splicing product, we amplified it by RT-PCR, cloned, sequenced the PCR fragment, and compared it with the canonical *TFIIB1* transcript (Fig. 5*A*). The sequencing results showed that both fragments belong to *AtTFIIB1*, but the larger isoform was the product of an alternative splicing process by retention of the third intron of 93 nucleotides (Fig. 5*B*, Fig. 1 Suppl.). The cDNAs sequences analysis and *in silico* translation of both transcripts isoforms (http://www.expasy.org/) showed a 100 % identity compared to nucleic acid sequences. However, the intron retained generated an alternative isoform, which had a premature termination codon (PTC) (Fig. 5*B*).

The superimposition and structural alignment of both polypeptide chains products were analyzed using an algorithm to calculate the structural similarity of two proteins (Zhang and Skolnick 2005). The analysis showed structural similarity between these TFIIB1 isoforms, both conserved zinc ribbon domain, cyclin domain 1, but only the canonical product retained the cyclin domain 2 (Fig. 6).

All our results suggest that the transcription factor TFIIB1 and proteins related (BRP1, BRP2, BRP3, and BRP4), participate in the development and reproductive processes and may be involved in regulating mechanisms to contend with abiotic stresses such as heat, cold, dehydration, and salinity.

Discussion

Gene expression in plants is a finely regulated process, and success in homeostasis maintenance depends on several *trans*-acting elements that regulate a myriad of genes to confront the adverse conditions. Thus, many signaling pathways controlling these processes target the core transcription machinery such as RNA polymerase II (Pol II) and general transcription factors (GTFs) regulation (Grünberg and Hahn 2013). The sequential recruitment order to PIC formation starts with TFIID (TBP and TAFs multiprotein complex) in TATA box, TFIIA, TFIIB, TFIIF, RNA pol II, TFIIE, and TFIIH (Thomas and Chiang 2006, Liu *et al*. 2010, Grünberg and Hahn 2013). In *A. thaliana* 14 TFIIB-like proteins have been phylogenetically re-

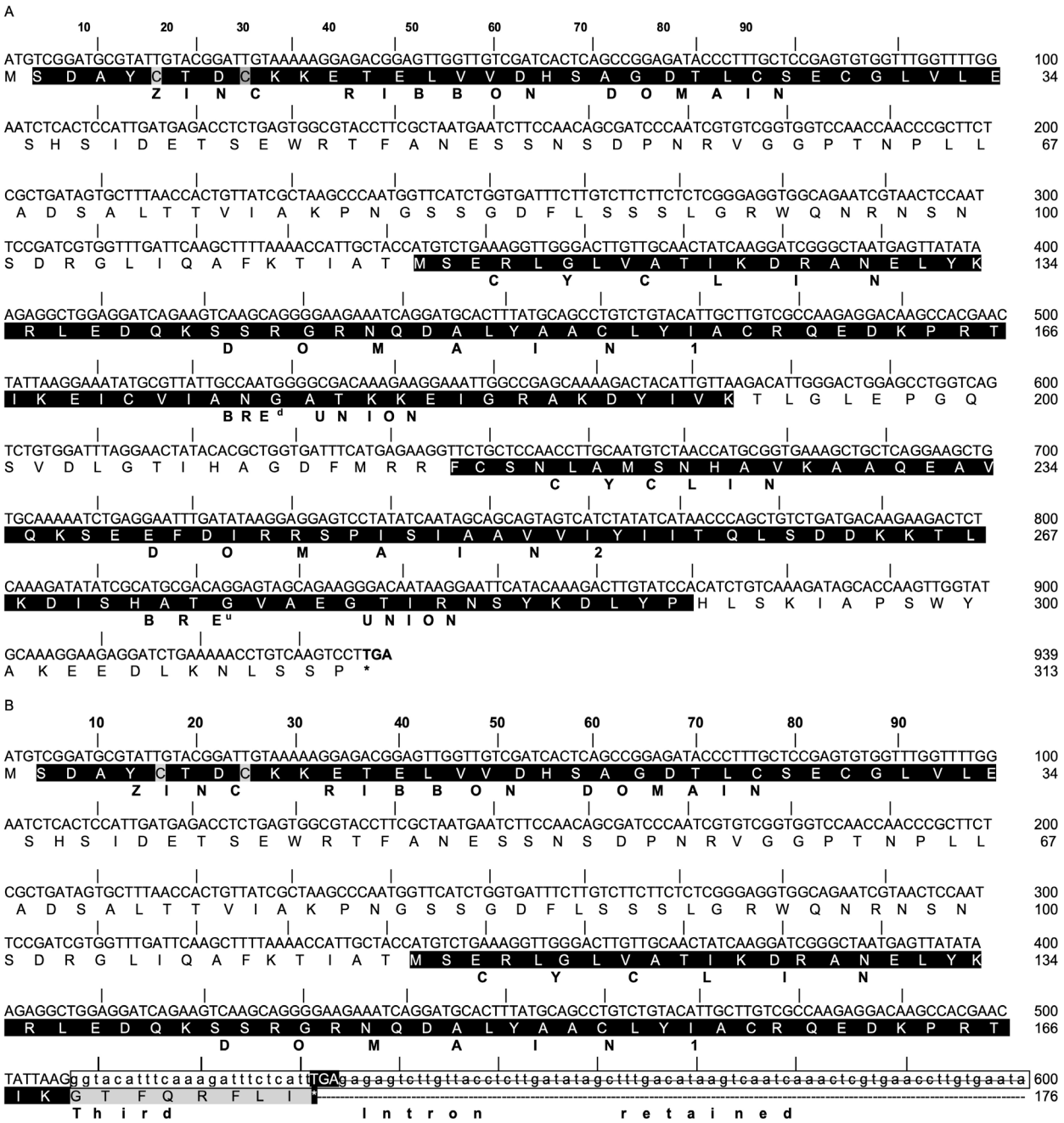

Fig. 5. Open reading frame of *A. thaliana TFIIB1*. *A* - Canonical sequence synthesized under non-stressed conditions has 939 nucleotides and encodes a polypeptide of 312 amino acid residues and *B* - sequence of the alternative *AtTFIIB1* splicing product showing the third intron retained and premature termination codon, PTC (*). The retained intron is shown in *lower case* and an *open rectangle*.

categorized into the TFIIB, BRF, and Rrn7/TAF1B/MEE12 subfamilies (Knutson 2013, Niu *et al.* 2013). The TFIIB subfamily has eight members, including TFIIB1, TFIIB2, and six BRP proteins, among which BRP3 and BRP6 contain only partial TFIIB domains and are predicted to function differently from the other TFIIBs (Knutson 2013).

In *A. thaliana*, *BRP1* (At4g36650) is expressed in all tissues and developmental stages, although with a high expression in roots and a lower in flowers and siliques. The *BRP1* is localized both in plastids and nuclei and has been reported to be involved in RNA polymerase I-dependent rRNA synthesis (Lagrange *et al.* 2003). Our results of the expression analysis in seedlings, cauline leaves, rosette leaves, and flowers indicated that *BRP1* is a constitutively expressed gene as has been reported by Lagrange *et al.* (2003). Under dehydration stress in adult plants, *BRP1* showed an upregulated expression after 1 h and remained upregulated after 2 and 3 h in cauline leaves and downregulated in rosette leaves (Fig. 2*B*). Only

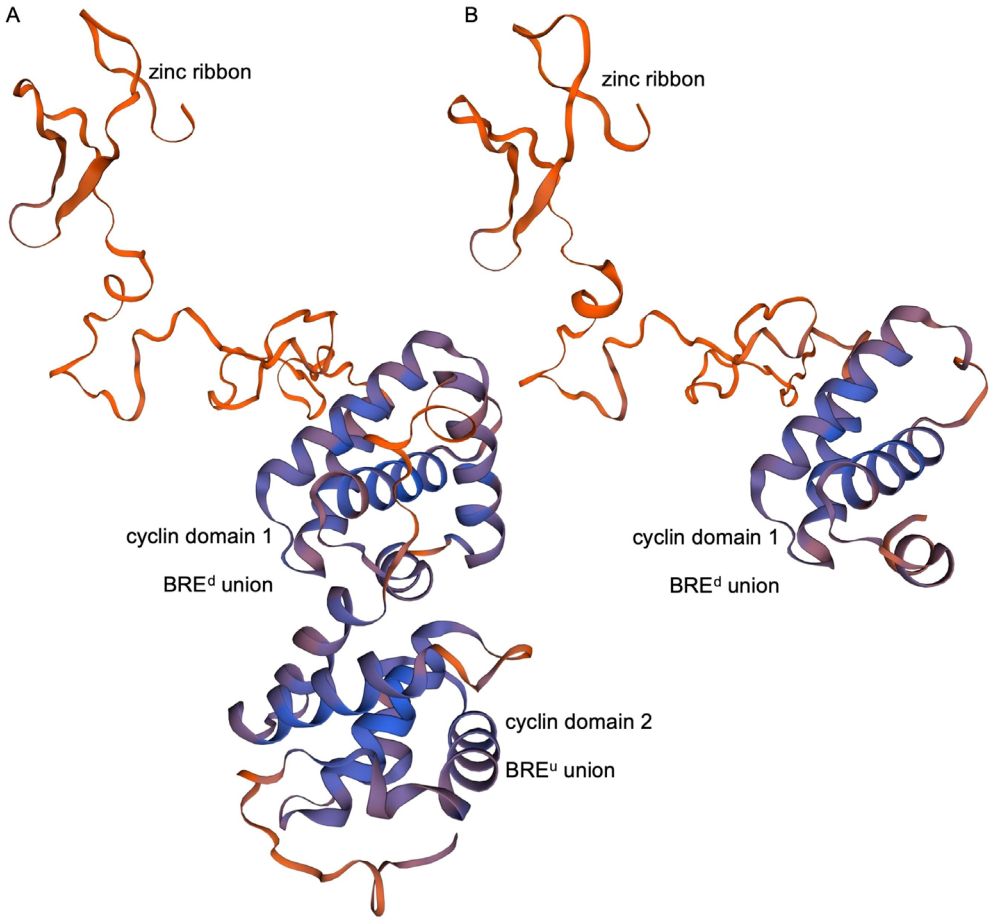

Fig. 6. Model for the structure of TFIIB1. *A* - Canonical product from *TFIIB1*, showing the complete structure of the transcription factor, such as zinc ribbon and the two cyclin domains. *B* - Model-based structure of the probable protein generated by the truncated mRNA derived from alternative splicing of the TFIIB1, the novel structure lacks the second cyclin domain, which contains the BRE^{u} union domain. BRE^{d} and BRE^{u} refers to TFIIB-recognition element downstream and upstream respectively.

under dehydration, it increased its expression in seedlings (Fig. 3*B*), although, under salinity stress, the *BRP1* expression was downregulated (Fig. 4*B*).

The expression of *BRP2* (At3g29380) is restricted to reproductive organs and seeds and is involved in the regulation of endosperm growth, and mutant lines exhibit a slower proliferation rate at the endosperm syncytial stage (Cavel *et al.* 2011). The experiments in the present work showed induction in the *BRP2* expression after 1, 2, and 3 h under dehydration stress in cauline leaves and slightly in rosette leaves (Fig. 2*C*), as well as in seedlings under low and high temperature, dehydration (Fig. 3*C*), and salinity (Fig. 4*B*).

The *BRP3* was expressed mainly in flowers (Fig. 1). Our data showed that *BRP3* (At4g10680) exhibited an evident increase in expression in floral tissue under dehydration after 1, 2, and 3 h and it was induced in cauline and rosette leaves (Fig. 2*D*) as well as in seedlings under cold, heat, and dehydration (Fig. 3*D*). However, its expression was downregulated in seedlings under salinity with a slight increase in 500 mM NaCl (Fig. 4*B*).

We showed that *BRP4* expression was restricted to flowers and exhibited an increase in the expression in flowers after 1 and 2 h but a decrease after 3 h of dehydration. The *BRP4* expression showed induction in cauline and rosette leaves after the first hour of dehydration (Fig. 2*E*). Although *BRP4* expression in seedlings was restricted to flower tissue, it showed upregulation under cold, heat, and dehydration (Fig. 3*E*) as well as an induction under salinity (Fig. 4*B*).

Zhou *et al.* (2013) mentioned that *AtTFIIB1* (At2g41630) is implicated in pollen tube growth and endosperm development. The transcript is found in all development stages of vegetative tissues such as roots, stems, leaves, flowers, flower buds, pollen grains, siliques, and seedlings but is up-regulated in flower and pollen grains. This GTF plays a special role in sexual reproduction (Zhou *et al.* 2013). Pollen germination generates a pollen tube that delivers the male gametes into an embryo sac for double fertilization, while embryogenesis enables the zygote to develop into an embryo, and those processes are polygenetic and involve the expression of several genes (Wang *et al.* 2008, Niu *et al.* 2013). The *AtTFIIB1* genomic structure is a multiexonic sequence of seven exons and six introns (Knutson 2013). Here we have shown that transcriptional and post-transcriptional regulations of RNAs were drastically altered during plant stress responses. Our results showed that *AtTFIIB1* retained the

third intron specifically under abiotic stress conditions such as heat, dehydration, and salinity. It has been widely described intron retention as the most common alternative splicing in *A. thaliana* (Hoang *et al.* 2017). The analysis *in silico* of the *AtTFIIB1* nucleotide sequence showed an encrypted premature stop codon (PTC) localized on the third intron in position 532 after +1 nucleotide in an alternative isoform transcript. Previous reports mentioned that the alternative transcripts isoforms with the PTC are part of a two-component system called RUST (regulated unproductive splicing and translation) where the alternative isoform with the PTC is recognized by a specialized system called NMD (non-sense mediated decay) which marks the mRNA with PTC for its degradation (Ni *et al.* 2007, Nyikó *et al.* 2013). Stop codons that are located distant from the mRNA 3′ termini or more than 50 nucleotides upstream of the 30-most exon-exon junction are recognized as substrates for NMD (Hori and Watanabe 2007). However, there are examples of alternative transcripts that avoid the NMD system (Lareau *et al.* 2007) that in case it is translated, generates a novel protein with possible novel functions called microprotein (miP) or small interference peptide (siPEP), which can trigger a change in the stress response (Syed *et al.* 2012). A general transcription factor, AtTFIIIA, in addition to being involved in development (Layat *et al.* 2012), has been described to be involved in abiotic stress response in *A. thaliana* and rice (Fu *et al.* 2009) when the transcript retains the third exon, which contains a premature stop codon, and when this isoform is overexpressed, the transgenic lines are more tolerant to abiotic stress. Genome-wide analysis of alternative splicing (AS) in *A. thaliana* suggested that 78 % of alternative transcripts introduced in-frame PTCs (Filichkin *et al.* 2010) provide a huge potential for the production of miPs (Syed *et al.* 2012, Magnani *et al.* 2014).

AtTFIIB1 promoter analysis shows several *cis*-elements involved in abiotic stress as well as elements involved in housekeeping and cell cycle functions. In this study, we showed an *AtTFIIB1* alternative transcript isoform by the third intron retention under abiotic stress such as heat, dehydration, and salinity. This process seems to be very strictly controlled. The intron retention provides the inclusion of PTC, and the *in silico* analysis suggests that in case it would be translated, the alternative product lacks the upstream TFIIB recognition element (BRE^u) binding domain, generating a siPEP or miP with a regulatory role under abiotic stress conditions. Previous reports of TFIIB1 in yeast, citrics, tilapia, and tomato seems to indicate that TFIIB1 may be directly involved in rapid abiotic stress response in these organisms (Pinto *et al.* 1994, Hoopes *et al.* 2000, Sánchez-Ballesta *et al.* 2003, Fiol and Kültz 2005, Keller *et al.* 2017). Our studies showed activation of *BRP1*, *BRP2*, *BRP3*, and *BRP4* in cauline leaves 1, 2, and 3 h after dehydration, repression of *BRP1* under the same conditions in rosette leaves, and activation of *BRP2*, *BRP3*, and *BRP4* in the same tissue and stress conditions. More studies are needed, such as overexpressing the canonical *TFIIB1* and the product of alternative splicing version in transgenic plants, as well as the inhibition of expression by RNAi, to corroborate our current data. The results obtained in the present work provide valuable information to gain an insight into the possible role of TFIIB1 and related proteins (BRPs) in abiotic stress tolerance in *A. thaliana* plants as well as its possible implications in several genes involved in abiotic stress response.

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