**Arabidopsis thaliana AtTFIIB1 gene displays alternative splicing under different abiotic stresses**

J.A. MIRANDA-RÍOS1,+, J.A. RAMÍREZ-TRUJILLO1,+, D.J. JAIME-GALLARDO1, N.S. HERNÁNDEZ-BUENO1, M. RAMÍREZ-YÁÑEZ3, G. ITURRIAGA3, and R. SUÁREZ-RODRÍGUEZ1,*

1 Laboratorio de Fisiología Molecular de Plantas, Centro de Investigación en Biotecnología, Universidad Autónoma del Estado de Morelos, 622 09 Cuernavaca, Morelos, México
2 Programa de Genómica Funcional de Eucariontes, Centro de Ciencias Genómicas, Universidad Nacional Autónoma de México, 622 10 Cuernavaca, Morelos, México
3 Tecnológico Nacional de México, Instituto Tecnológico de Roque, 381 10 Roque Celaya, Guanajuato, México

*Corresponding author: E-mail: rsuarez@uaem.mx

**Abstract**

In *Arabidopsis*, there are 14 TFIIB-like proteins that have been phylogenetically categorized into the TFIIB, BRF, and Rn7/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 *cis*-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.

**Received** 11 September 2020, **last revision** 26 March 2021, **accepted** 30 March 2021.

**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.

**Acknowledgments:** This work was supported by grants from the Programa Iberoamericano de Ciencia y Tecnología para el Desarrollo-Spain (107PIC0312) and Fondo Mixto Morelos, Consejo Nacional de Ciencia y Tecnología-México (120159) to Paul Gaytán and Eugenio López for oligonucleotide synthesis. We thank Lorena Chávez, Simón Guzmán, and Jorge Ramírez for technical assistance in the microarray determinations, and also to David Brown for editing the English text of a draft of this manuscript.

*These authors contributed equally.

**Conflict of interest:** The authors declare that they have no conflict of interest.
**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 and have been shown to regulate downstream gene regulation, such as gene expression in response to developmental and environmental stimuli are widespread. For example, in Arabidopsis thaliana, the isogenic mutant SUA7 transcripts differing in size near 100 nucleotides when cells are exposed to heat stress (Hoopes et al. 2000). Furthermore, TFIIIB 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 TFIIIB) 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, TFIIIB 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 tii14 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 ABRP3 (plant-specific TFIIIB-related protein) coding region. The tii14 mutant displayed insensitivity 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 TFIIIB1, BRP1, BRP2, BRP3, and BRP4 are differentially expressed under different developmental stages, in response to stress conditions, and that the TFIIIB1 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(photon) 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 °C 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 °C for 1 h.
before proceeding to RNA extraction. For cold stress, two-week-old seedlings were incubated at 0 °C 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 ground 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
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.
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. 2B). 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. 2C). 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. 2D). The BRP4 showed an increase in
The TFIIIB1 maintained similar expression patterns in normal growth conditions (Fig. 1) as well as under dehydration (Fig. 2A). 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 TFIIIB1 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. 3B-D).

To determine if the expression of the alternative isoform of TFIIIB1 is induced specifically only under dehydration stress conditions, qPCR analysis under abiotic stress conditions mentioned above was taken (Fig. 3A and 4A). The results demonstrated that the expression of the alternative splicing product of TFIIIB1 also takes place under heat stress (32 °C for 1 h) (Fig. 3A) and salinity (100, 200, 300, 400, and 500 mM NaCl for 1 h); both TFIIIB1 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. 4A). This TFIIIB1 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 TFIIIB1 transcript (Fig. 5A). The sequencing results showed that both fragments belong to AtTFIIIB1, but the larger isoform was the product of an alternative splicing process by retention of the third intron of 93 nucleotides (Fig. 5B, Fig. 1 Suppl.). The cDNAs sequences alignment 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. 5B).

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 TFIIIB1 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 TFIIIB1 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 (Grüngberg and Hahn 2013). The sequential recruitment order to PIC formation starts with TFIIID (TBP and TAFs multiprotein complex) in TATA box, TFIIA, TFIB, TFIIJ, RNA pol II, TFIIIE, 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-
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. 2B).
under dehydration, it increased its expression in seedlings (Fig. 3B), although, under salinity stress, the BRP1 expression was downregulated (Fig. 4B).

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. 2C), as well as in seedlings under low and high temperature, dehydration (Fig. 3C), and salinity (Fig. 4). 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. 2D) as well as in seedlings under cold, heat, and dehydration (Fig. 3D). However, its expression was downregulated in seedlings under salinity with a slight increase in 500 mM NaCl (Fig. 4B).

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. 2E). Although BRP4 expression in seedlings was restricted to flower tissue, it showed upregulation under cold, heat, and dehydration (Fig. 3E) as well as an induction under salinity (Fig. 4B).

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, Nytkò et al. 2013). Stop codons that are located distant from the mRNA 3’ termini or more than 50 nucleotides upstream of the 30-exon-exon junction are recognized as substrates for NMD (Horí 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, *AtTFIIB1*, 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*) binding domain, generating a siPEP or miP with a regulatory role under abiotic stress conditions. Previous reports of TFIIB1 in yeast, citruses, 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.

**References**

Agarwal, P.K., Gupa, K., Lopato, S., Agarwal, P.: Dehydration responsive element binding transcription factors and their applications for the engineering of stress tolerance. - J. exp. Bot. 68: 2135-2148, 2017.

Cavel, E., Pillot, M., Pontier, D., Lahmy, S., Bies-Etheve, N., Vega, D., Grimanelli, D., Lagrange, T.: A plant-specific transcription factor IIB-related protein, pBRP2, is involved in endosperm growth control. - PLoS ONE 6: e17216, 2011.

Filichkin, S.A., Priest, H.D., Givan, S.A., Shen, R., Bryant, D.W., Fox, S.E., Wong, W.K., Mockler, T.C.: Genome-wide mapping of alternative splicing in *Arabidopsis thaliana*. - Genome Res. 20: 45-58, 2010.

Fiol, D.F., Kültz, D.: Rapid hypersomotic conduction of two tilapia (*Oreochromis mossambicus*) transcription factors in gill cells. - Proc. nat. Acad. Sci. USA 102: 927-932, 2005.

Franco-Zorrila, J.M., López-Vidriero, I., Carrasco, J.L., Godoy, M., Vera, P., Solano, R.: DNA-binding specificities of plant transcription factors and their potential to define target genes. - Proc. nat. Acad. Sci. USA 111: 2367-2372, 2014.

Fu, Y., Bannach, O., Chen, H., Teune, J.H., Schmitz, A., Steger, G., Xiong, L., Barbazuk, W.B.: Alternative splicing of anciently exonized 5S rRNA regulates plant transcription factor TFIIIA. - Genome Res. 19: 913-921, 2009.

Grünerberg, S., Hahn, S.: Structural insights into transcription initiation by RNA polymerase II. - Trends biochem. Sci. 38: 603-611, 2013.

He, M., He, C.-Q., Ding, N.-Z.: Abiotic stresses: general defenses of land plants and chances for engineering multistress tolerance. - Front. Plant Sci. 9: 1771, 2018.

Hernández-Campuzano, B., Van Dijck, P., Avonce, N., Iturriaga, G., Suárez, R.: [A mutant of *Arabidopsis thaliana* by T-DNA insertion is insensitive to sugars and tolerant to abiotic stress.]. - Rev. iberoameric. Cienc. 1: 89-101, 2014. [In Spanish]

Hoang, X.L.T., Nhi, D.N.H., Thu, N.B.A., Thao, N.P., Tran, L.P.: Transcription factors and their roles in signal transduction in plants under abiotic stresses. - Curr. Genomics 18: 483-497, 2017.

Hoopes, B.C., Bowers, G.D., Di Visconte, M.J.: The two *Saccharomyces cerevisiae* SUA7 (TFIIB) transcripts differ at the 3′-end and respond differently to stress. - Nucl. Acids. Res. 28: 4435-4443, 2000.

Horí, K., Watanabe, Y.: Context analysis of termination codons in mRNA that are recognized by plant NMD. - Plant Cell Physiol. 7: 1072-1078, 2007.

Keller, M., Hu, Y., Mesilovic, A., Fragkostefanakis, S., Schleiff, E., Simm, S.: Alternative splicing in tomato pollen in response to heat stress. - DNA Res. 24: 205-217, 2017.

Knutson, B.A.: Emergence and expansion of TFIIB-like factors in the plant kingdom. - Gene 526: 30-38, 2013.

Kostrewa, D., Zeller, M.E., Armache, K.J., Seizl, M., Leike, K., Thomm, M., Cramer, P.: RNA polymerase II–TFIIB structure and mechanism of transcription initiation. - Nature 462: 323-330, 2009.

Lagrange, T., Hakimi, M.A., Pontier, D., Courtiès, F., Alcaraz, J.P., Grunwald, D., Lam, E., Leber-Mache, S.: Transcription factor IIB (TFIIB)-related protein (pBRP), a plant-specific...
member of the TFIIB-related protein family. - Mol. cell. Biol. 9: 3274-3286, 2003.
Lareau, L.F., Brooks, A.N., Soergel, D.A., Meng, Q., Brenner, S.E.: The coupling of alternative splicing and nonsense-mediated mRNA decay. - Adv. exp. Med. Biol. 623: 190-211, 2007.
Layat, E., Cotterell, S., Vaillant, I., Yukawa, Y., Tutois, S., Tourmente. S.: Transcript levels, alternative splicing and proteolytic cleavage of TFIIIA control 5S rRNA accumulation during Arabidopsis thaliana development. - Plant J. 71: 35-44, 2012.
Lindemose, S., O'Shea, C., Jensen, K.M., Skriver, K.: Structure, function and networks of transcription factors involved in abiotic stress responses. - Int. J. mol. Sci. 14: 5842-5878, 2013.
Liu, X., Bushnell, D.A., Wang, D., Calero, G., Kornberg, R.D.: Structure of an RNA polymerase II–TFIIB complex and the transcription initiation mechanism. - Science 327: 206-209, 2010.
Magnani, E., De Klein, N., Nam, H.-I., Kim, J.-G., Pham, K., Fiume, E., Mudgett, M.B., Rhee, S.Y.: A comprehensive analysis of micro proteins reveals their potentially widespread mechanism of transcriptional regulation. - Plant Physiol. 165: 149-159, 2014.
Mizoi, J., Shinozaki, K., Yamaguchi-Shinozaki K.: AP2/ERF family transcription factors in plant abiotic stress responses. - Biochim. biophys. Acta 1819: 86-96, 2012.
Nakashima, K., Yamaguchi-Shinozaki, K., Shinozaki, K.: The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. - Front. Plant Sci. 5: 1-7, 2014.
Ni, J.Z., Grate, L., Donohue, J.P., Preston, C., Nobida, N., O'Brien, G., Shiue, L., Clark, T.A., Blume, J.E., Ares, M., Jr.: Ultraconserved elements are associated with homeostatic control of splicing regulators by alternative splicing and nonsense-mediated decay. - Genes Dev. 6: 708-718, 2007.
Niu, Q.-K., Liang, Y., Zhou, J.-J., Dou, X.-Y., Gao, S.-C., Chen, L.-Q., Zhang, X.-Q., Ye, D.: Polen-expressed transcription factor 2 encodes a novel plant-specific TFIIB-related protein that is required for pollen germination and embryogenesis in Arabidopsis. - Mol. Plants 6: 1091-1108, 2013.
Nyikó, T., Kerényi, F., Szabadkai, L., Benkovics, A.H., Major, P., Sonkoly, B., Mérai, Z., Barta, E., Niemiec, E., Kurfé, J., Silhavy, D.: Plant nonsense-mediated mRNA decay is controlled by different autoregulatory circuits and can be induced by an EJC-like complex. - Nucl. Acids Res. 41: 6715-6728, 2013.