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Short Communication

Sugar-responsive transcription factor bZIP3 affects leaf shape in Arabidopsis plants

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Abstract Sugars are essential for plant metabolism, growth and development. Plants must therefore manage their growth and developmental processes in response to sugar availability. Sugar signaling pathways constitute a complicated molecular network and are associated with global transcriptional regulation. However, the molecular mechanisms underlying sugar signaling remain largely unclear. This study reports that the protein basic-region leucine zipper 3 (bZIP3) is a novel sugar-responsive transcription factor in Arabidopsis plants. The expression of *bZIP3* was rapidly repressed by sugar. Genetic analysis indicated that *bZIP3* expression was modulated by the SNF1-RELATED KINASE 1 (SnRK1) pathway. Moreover, transgenic plants overexpressing *bZIP3* and dominant repressor form *bZIP3-SRDX* showed aberrant shaped cotyledons with hyponastic bending. These findings suggest that bZIP3 plays a role in plant responses to sugars and is also associated with leaf development.

Key words: Arabidopsis, leaf development, SnRK1, sugar signaling.

Sugars play fundamental roles in plants, not only as metabolites but also as signaling molecules that modulate plant metabolism, growth, and development (Rolland et al. 2006; Smeekens et al. 2010). Thus, plants have evolved systems in which their growth and developmental processes are tightly controlled by cellular sugar availability (Eveland and Jackson 2012; León and Sheen 2003). HEXOKINASE1 (HXK1) is a glucose sensor, mediating photosynthetic and glucose-related regulation of transcription (Moore et al. 2003; Xiao et al. 2000). SNF1-RELATED KINASE 1 (SnRK1) and TARGET OF RAPAMYCIN (TOR) kinases function as master regulators of plant responses to cellular energy status associated with deprivation of nutrients, including sugars, thereby regulating growth and development (Li and Sheen 2016; Smeekens et al. 2010). SnRK1 acts at two different levels, modulating the activity of key metabolic enzymes and massively reprogramming transcription (Baena-González and Sheen 2008; Emanuelle et al. 2016). Transcription factors play crucial roles in many biological processes, including sugar signal transduction. The basic-region leucine zipper (bZIP) transcription factors contain a basic region that binds to DNA and a leucine zipper domain for dimerization. The *Arabidopsis thaliana* genome encompasses 75 genes for putative bZIP transcription factors, which can be subdivided into 10 groups (Jakoby et al. 2002). Groups C and S1 bZIP transcription factors have been reported to be partially responsible for transcriptional regulation in SnRK1-mediated signaling in response to low energy stress (Baena-González et al. 2007; Mair et al. 2015). The functions of some of these bZIPs, such as bZIP63 from group C and bZIP1/11 from group S1, are regulated transcriptionally and/or post-transcriptionally by sugar and energy status (Mair et al. 2015; Matiolli et al. 2011; Wiese et al. 2004). Nevertheless, the detailed mechanisms by which sugar signals mediate plant growth and development remain unclear. To understand these mechanisms, it is necessary to identify key transcription factors in sugar signaling and to determine their function. This study reports our identification of a novel sugar-responsive transcription factor bZIP3.

Arabidopsis thaliana ecotype Columbia-0 (Col-0) plants, designated wild type (WT), were grown on MS medium containing various sugar concentrations under long day growth conditions (16 h light/ 8 h dark) at 22°C after seeds were surface-sterilized and incubated for 2 days at 4°C in the dark. To generate transgenic plants overexpressing *bZIP3*, the full-length *bZIP3* coding sequence in Col-0 cDNA was amplified using the primers

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listed in Supplementary Table S1. The amplified cDNA fragment was cloned into the pENTR/D-TOPO vector (Invitrogen, Massachusetts, USA) and transferred to pDEST_35S_HSPH binary vectors (Oshima et al. 2011), according to the manufacturer's instructions (Invitrogen). The constructed vector was introduced into *Agrobacterium tumefaciens* GV3101 (pMP90) by electroporation, followed by Arabidopsis transformation using the floral dip method (Clough and Bent 1998). For gene expression analysis, total RNA was extracted from Arabidopsis seedlings as described (Aoyama et al. 2017), followed by quantitative RT-PCR (qRT-PCR) using SYBR premix EX Taq (TaKaRa) and gene-specific primers (Supplementary Table S1), with Mx3000P (Agilent Technologies, California, USA) according to the manufacturer's protocol.

To explore novel transcription factors mediating sugar signaling, the transcriptome profile of Arabidopsis transcription factors was searched with Genevestigator, an available microarray database (https://www.genevestigator.com). The *bZIP3* (*At5g15830*) gene was identified as a candidate sugar-responsive transcription factor in Arabidopsis. According to the database, *bZIP3* expression was down-regulated by glucose treatment. Sugar-responsive *bZIP3* expression was assessed by qRT-PCR analysis. Briefly, WT Arabidopsis seedlings were grown for 8 days on the sugar-free medium and then transiently treated with 0 mM or 200 mM glucose at 0 h after light on (Zeitgeber time 0). Seedlings were collected at 1 h, 4 h, 8 h, and 24 h after treatment, and their total RNA was extracted for qRT-PCR analysis. *bZIP3* expression showed a diurnal pattern, with a significant reduction at 1 h after treatment with glucose (Figure 1A). The level of expression of *bZIP3* was also reduced by sucrose and fructose and, to a lesser extent, by osmotic stress treatment with mannitol (Figure 1B), confirming that *bZIP3* expression is regulated by sugar availability. RT-PCR analysis of the tissue-specific expression pattern of *bZIP3* showed that *bZIP3* is broadly expressed in all plant tissues, especially in older leaves and roots (Figure 2).

To understand the upstream regulation of sugar-responsive *bZIP3* expression, we assessed the involvement of the SnRK1 signaling pathway. Because complete loss-of-function mutants of SnRK1 exhibit embryonic lethality (Baena-González et al. 2007), we generated an inducible RNAi knockdown mutant of *SnRK1α1* in the background of a *SnRK1α2* knockout mutant (WiscDsLox320B03) (*snrk1α1i*/*1α2*) utilizing the pOpOff2 system (Wielopolska et al. 2005). WT seedlings and two independent *snrk1α1i*/*1α2* lines (#2 and #3) were grown for 7 days on sugar-free medium, in the presence or absence of dexamethasone (DEX). RT-PCR analysis showed that expression of *bZIP3* and *DORMANCY ASSOCIATED GENE 2* (*DRM2*), a target marker gene of SnRK1 (Baena-González et al. 2007), in

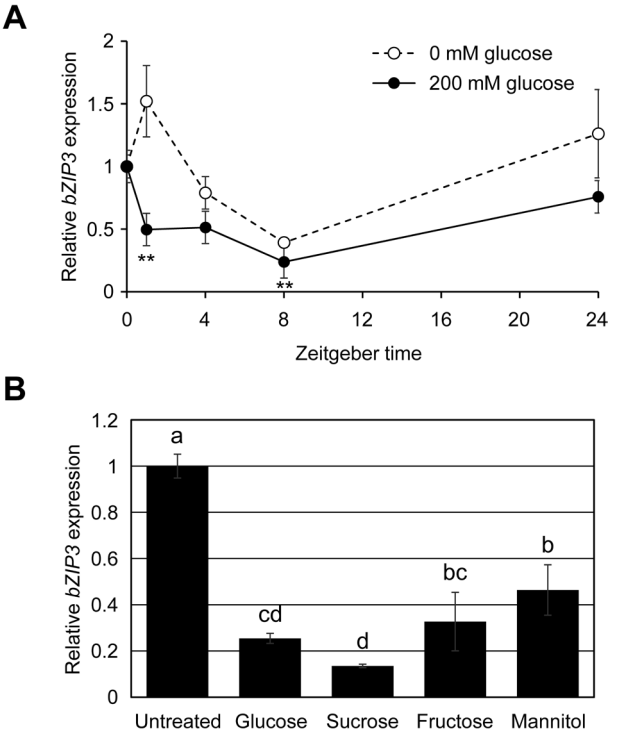

Figure 1. Sugar effect on *bZIP3* expression. (A) Expression of *bZIP3* in response to glucose over time, calculated as relative to *bZIP3* expression level in the sample collected at Zeitgeber time 0 without glucose treatment and normalized to *IPP2* levels. Results are reported as means±SD (n=4), and significant differences between samples incubated in the presence and absence of glucose were determined at each point by Student's t-tests (* $p<0.05$, ** $p<0.01$). (B) Expression of *bZIP3* in response to various sugars. Seedlings were incubated with 200 mM glucose, sucrose, fructose or mannitol for 1 h and *bZIP3* expression was assayed by qRT-PCR. Levels of expression were calculated relative to that of untreated seedlings. Results are reported as means±SD (n=3). Letters indicate significant differences ($p<0.05$), as determined by one-way ANOVA with Turkey's *post hoc* test.

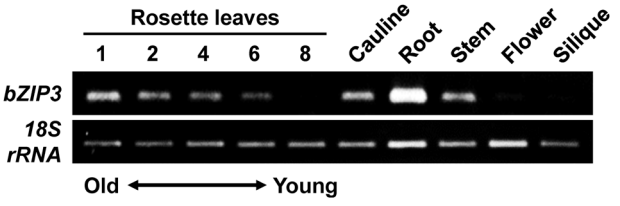

Figure 2. Semi-quantitative RT-PCR analysis of *bZIP3* gene expression in tissues of 5-week-old WT Arabidopsis plants. *18S rRNA* was used as an internal control.

the *snrk1α1i*/*1α2* mutant were dramatically repressed in the presence of DEX (Figure 3), suggesting that sugar-responsive *bZIP3* expression is mediated via SnRK1.

To clarify the physiological function of bZIP3 in plants, we investigated the phenotypes of loss-of-function mutant and transgenic plants overexpressing *bZIP3* under the control of the CaMV 35S promoter (*bZIP3* OX). A null mutant line of *bZIP3* (*bzip3-1*, SAIL_261_F01) was identified in a T-DNA insertion population provided by the Arabidopsis Biological Resource Center

(Figure 4A). *bZIP3* OX lines were generated using the pDEST_35S_HSPH binary vector (Oshima et al. 2011) (Figure 4B). WT, *bzip3-1* and *bZIP3* OX seedlings were grown for 7 days on MS medium containing 1% sucrose. *bZIP3* OX showed abnormal cotyledons with hyponastic bending and uneven surfaces (Figure 4D). In contrast, these characteristics were not observed in *bzip3-1* and WT cotyledons, both of which exhibited epinasty (Figure 4D). Because some single knock-out mutants of a transcription factor do not exhibit markedly different phenotypes because of genetic redundancy, we also generated transgenic plants expressing bZIP3 fused with the Superman repression domain X (SRDX) (*bZIP3-SRDX*), a fusion that converts a transcription factor to a strong repressor dominantly suppressing the target genes (Hiratsu et al. 2003). *bZIP3-SRDX* transgenic lines were created using a pDEST_35S_SRDX_HSPH binary vector (Oshima et al. 2011), and the expression of *bZIP3-SRDX* was confirmed by qRT-PCR analysis (Figure 4C). The *bZIP3-SRDX* seedlings had abnormally shaped cotyledons, similar to those of the *bZIP3* OX seedlings (Figure 4D). It should be examined whether bZIP3 functions as transcriptional repressor because both of the *bZIP3* OX and *bZIP3-SRDX* exhibited hyponastic bending of cotyledons. Leaf shape is determined by various factors at several stages of development, including leaf initiation, outgrowth, expansion, and maturation (Moon and Hake 2011; Sinha 1999). After leaf initiation, the polarity at three axes, the proximal/distal, adaxial/abaxial and medial/lateral axes,

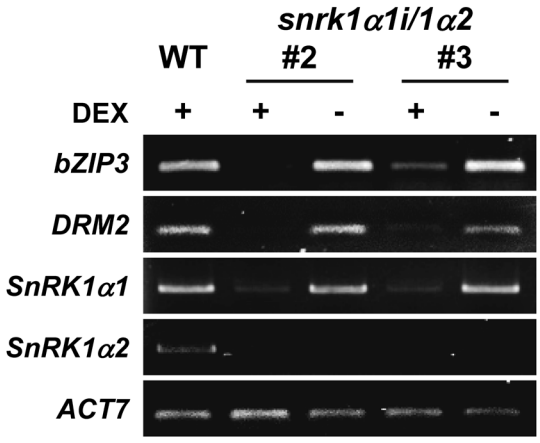

Figure 3. *bZIP3* expression level in *snrk1α* mutants. WT and two lines (#2 and #3) of *snrk1α1i/1α2* seedlings were grown for 7 days on sugar-free half MS medium. Total RNA was isolated and *bZIP3* expression analyzed by RT-PCR. *ACT7* was used as a reference gene for the expression of all genes assayed and *DRM2* was used as a marker for SnRK1 function.

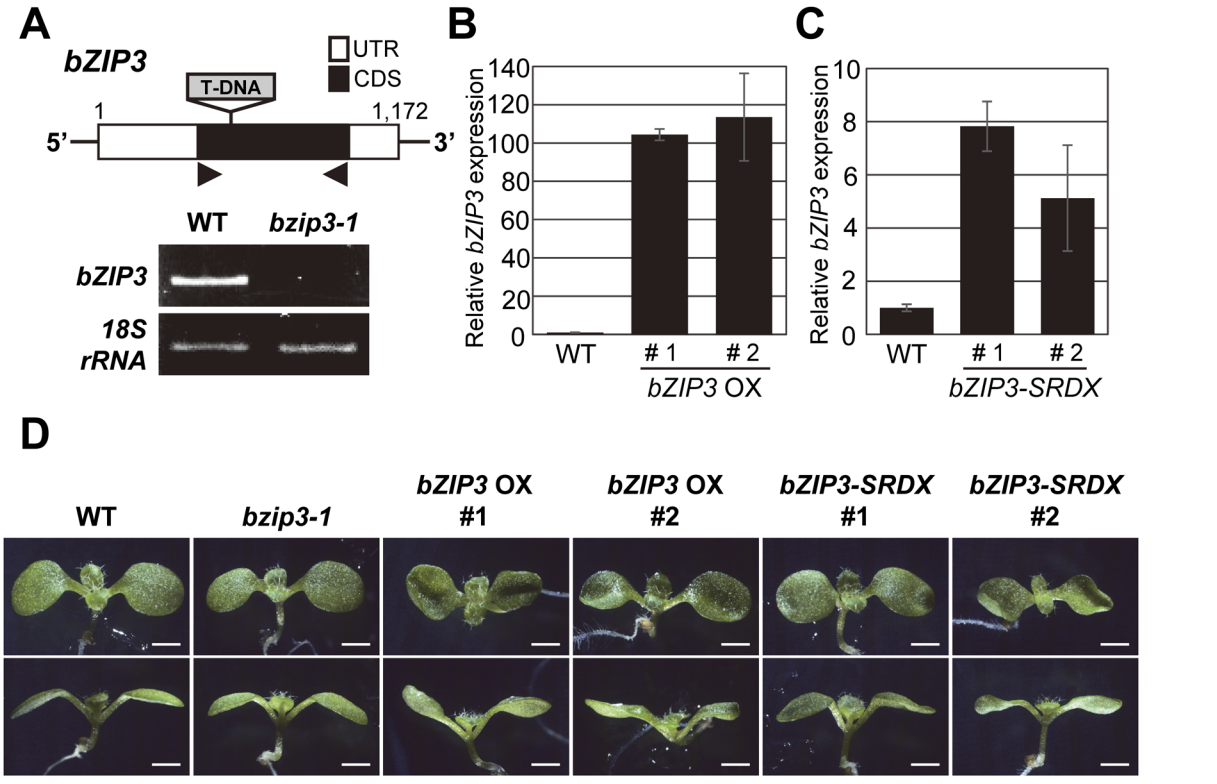

Figure 4. Shape of cotyledons in *bZIP3* OX and *bZIP3-SRDX* plants. (A) Gene structure of *bZIP3* and the location of the T-DNA insertion in the *bzip3-1* mutant. The positions of the primers used for RT-PCR are indicated by arrowheads. (B) and (C) qRT-PCR analysis of *bZIP3* OX and *bZIP3-SRDX* plants. Total RNA was extracted from seedlings of indicated plants grown on MS medium. *18S rRNA* was used as an internal control for RT-PCR and qRT-PCR analyses. Relative expression levels were compared with that of WT seedlings. Results are reported as means±SD ($n=3$). (D) Seedlings of WT, *bzip3-1*, *bZIP3* OX and *bZIP3-SRDX* grown for 7 days on MS medium containing 1% sucrose. The photographs show the top (upper panels) and side (lower panels) views of each cotyledon. Bars=1 mm.

contribute to the shaping of the leaf (Moon and Hake 2011; Sinha 1999). Although little has been reported on the relationship between sugars and leaf morphogenesis, some mutants have aberrant cotyledons, similar to *bZIP3* OX and *bZIP3-SRDX* seedlings. A loss of function mutant of *UDP-L-RHAMNOSE SYNTHASE* (*RHM1*), called *rol1-2*, showed hyponastic growth and aberrant pavement cells in cotyledons (Ringli et al. 2008). These phenotypes were caused by an alteration in the flavonol conjugation profile through auxin-induced or auxin-independent processes in Arabidopsis (Ringli et al. 2008). Rhamnose is an important component of pectin, and *rol1* mutants exhibited a modification in pectin structure (Diet et al. 2006). Recently, *bZIP3* was identified as a gene that could putatively increase enzymatic saccharification efficiency (Ohtani et al. 2017), suggesting that bZIP3 is involved in altering secondary cell wall properties. In contrast, a previous study (Matiolli et al. 2011) suggested that the sugar-responsive *bZIP3* expression pattern is similar to that of *bZIP63*, which mediates energy starvation signaling to modulate central metabolism and leaf senescence (Mair et al. 2015). Further investigations of bZIP3 function may provide new insights into the interplay between sugar signaling and plant development, including the regulation of leaf shape.

References

Aoyama S, Terada S, Sanagi M, Hasegawa Y, Lu Y, Morita Y, Chiba Y, Sato T, Yamaguchi J (2017) Membrane-localized ubiquitin ligase ATL15 functions in sugar-responsive growth regulation in Arabidopsis. *Biochem Biophys Res Commun* 491: 33–39

Baena-González E, Rolland F, Thevelein JM, Sheen J (2007) A central integrator of transcription networks in plant stress and energy signalling. *Nature* 448: 938–942

Baena-González E, Sheen J (2008) Convergent energy and stress signaling. *Trends Plant Sci* 13: 474–482

Clough SJ, Bent AF (1998) Floral dip: A simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* 16: 735–743

Diet A, Link B, Seifert GJ, Schellenberg B, Wagner U, Pauly M, Reiter WD, Ringli C (2006) The *Arabidopsis* root hair cell wall formation mutant *lrx1* is suppressed by mutations in the *RHM1* gene encoding a UDP-L-rhamnose synthase. *Plant Cell* 18: 1630–1641

Emanuelle S, Doblin MS, Stapleton DI, Bacic A, Gooley PR (2016) Molecular insights into the enigmatic metabolic regulator, SnRK1. *Trends Plant Sci* 21: 341–353

Eveland AL, Jackson DP (2012) Sugars, signalling, and plant development. *J Exp Bot* 63: 3367–3377

Hiratsu K, Matsui K, Koyama T, Ohme-Takagi M (2003) Dominant repression of target genes by chimeric repressors that include the EAR motif, a repression domain, in *Arabidopsis*. *Plant J* 34: 733–739

Jakoby M, Weisshaar B, Dröge-Laser W, Vicente-Carbajosa J, Tiedemann J, Kroj T, Parcy F (2002) bZIP transcription factors in *Arabidopsis*. *Trends Plant Sci* 7: 106–111

León P, Sheen J (2003) Sugar and hormone connections. *Trends Plant Sci* 8: 110–116

Li L, Sheen J (2016) Dynamic and diverse sugar signaling. *Curr Opin Plant Biol* 33: 116–125

Mair A, Pedrotti L, Wurzinger B, Anrather D, Simeunovic A, Weiste C, Valerio C, Dietrich K, Kirchler T, Nägele T, et al. (2015) SnRK1-triggered switch of bZIP63 dimerization mediates the low-energy response in plants. *eLife* 4: e05828

Matiolli CC, Tomaz JP, Duarte GT, Prado FM, Del Bem LE, Silveira AB, Gauer L, Corrêa LG, Drumond RD, Viana AJ, et al. (2011) The Arabidopsis bZIP gene AtbZIP63 is a sensitive integrator of transient abscisic acid and glucose signals. *Plant Physiol* 157: 692–705

Moon J, Hake S (2011) How a leaf gets its shape. *Curr Opin Plant Biol* 14: 24–30

Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the *Arabidopsis* glucose sensor HXK1 in nutrient, light, and hormonal signaling. *Science* 300: 332–336

Ohtani M, Ramachandran V, Tokumoto T, Takebayashi A, Ihara A, Matsumoto T, Hiroyama R, Nishikubo N, Demura T (2017) Identification of novel factors that increase enzymatic saccharification efficiency in Arabidopsis wood cells. *Plant Biotechnol* 34: 203–206

Oshima Y, Mitsuda N, Nakata M, Nakagawa T, Nagaya S, Kato K, Ohme-Takagi M (2011) Novel vector systems to accelerate functional analysis of transcription factors using chimeric repressor gene-silencing technology (CRES-T). *Plant Biotechnol* 28: 201–210

Ringli C, Bigler L, Kuhn BM, Leiber RM, Diet A, Santelia D, Frey B, Pollmann S, Klein M (2008) The modified flavonol glycosylation profile in the *Arabidopsis rol1* mutants results in alterations in plant growth and cell shape formation. *Plant Cell* 20: 1470–1481

Rolland F, Baena-González E, Sheen J (2006) Sugar sensing and signaling in plants: Conserved and novel mechanisms. *Annu Rev Plant Biol* 57: 675–709

Sinha N (1999) Leaf development in angiosperms. *Annu Rev Plant Physiol Plant Mol Biol* 50: 419–446

Smeekens S, Ma J, Hanson J, Rolland F (2010) Sugar signals and molecular networks controlling plant growth. *Curr Opin Plant Biol* 13: 274–279

Wielopolska A, Townley H, Moore I, Waterhouse P, Helliwell C (2005) A high-throughput inducible RNAi vector for plants. *Plant Biotechnol J* 3: 583–590

Wiese A, Elzinga N, Wobbes B, Smeekens S (2004) A conserved upstream open reading frame mediates sucrose-induced repression of translation. *Plant Cell* 16: 1717–1729

Xiao W, Sheen J, Jang JC (2000) The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Mol Biol* 44: 451–461