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 bZIP11/1 from group S1, are regulated transcriptionally and/or post-transcriptionally by sugar and energy status (Mair et al. 2015; Matioli 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 (16h light/8h 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 SnRK1a1 in the background of a SnRK1a2 knockout mutant (WiscDsLox320B03) (snrk1a1i/1a2) utilizing the pOpOff2 system (Wielopolska et al. 2005). WT seedlings and two independent snrk1a1i/1a2 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 the snrk1a1i/1a2 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 mutants 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.
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,
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 roll1-2, 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 roll1 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 (Matioli 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

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