A Novel Role for *Arabidopsis* CBL1 in Affecting Plant Responses to Glucose and Gibberellin during Germination and Seedling Development

Zhi-Yong Li¹,², Zhao-Shi Xu¹*, Yang Chen¹*, Guang-Yuan He², Guang-Xiao Yang², Ming Chen¹, Lian-Cheng Li³, You-Zhi Ma¹**

¹ Institute of Crop Science, Chinese Academy of Agricultural Sciences (CAAS)/National Key Facility for Crop Gene Resources and Genetic Improvement, Key Laboratory of Biology and Genetic Improvement of Triticaceae Crops, Ministry of Agriculture, Beijing, China, ² The Genetic Engineering International Cooperation Base of Chinese Ministry of Science and Technology, Chinese National Center of Plant Gene Research (Wuhan) HUST Part, College of Life Science and Technology, Huazhong University of Science & Technology (HUST), Wuhan, China

Abstract

Glucose and phytohormones such as abscisic acid (ABA), ethylene, and gibberellin (GA) coordinately regulate germination and seedling development. However, there is still inadequate evidence to link their molecular roles in affecting plant responses. Calcium acts as a second messenger in a diverse range of signal transduction pathways. As calcium sensors unique to plants, calcineurin B-like (CBL) proteins are well known to modulate abiotic stress responses. In this study, it was found that *CBL1* was induced by glucose in *Arabidopsis*. Loss-of-function mutant *cbl1* exhibited hypersensitivity to glucose and paclobutrazol, a GA biosynthetic inhibitor. Several sugar-responsive and GA biosynthetic gene expressions were altered in the *cbl1* mutant. *CBL1*protein physically interacted with AKIN1, the regulatory β subunit of the SnRK1 complex which has a central role in sugar signaling. Our results indicate a novel role for *CBL1* in modulating responses to glucose and GA signals.

References

1. Sugars modulate important processes during the almost all phases of plant development [1,2]. High sugar concentration delays seed germination and inhibits cotyledon expansion and greening, true leaf formation and root growth of *Arabidopsis* seedlings [3]. Sugar also possesses a phytohormone-like function in regulating a series of genes that control a range of biological functions, such as photosynthesis, nitrogen metabolism and carbohydrate consumption [1,4]. Sucrose, glucose and fructose are examples of sugars. Sucrose is stored in vacuoles or cleaved into glucose and fructose by invertases or UDP-glucose and fructose by sucrose synthases [5]. While sucrose is the main transported form of sugars in plants, glucose, as one of the more important sugars, has been shown to affect seed germination and early seedling development including cotyledon expansion and greening [6,7]. Though fructose has long been proposed as a possible signaling molecule, fructose signaling in plants has remained largely unexplored [8]. During the past few decades, a number of *Arabidopsis* mutants involved in sugar signaling have been identified based on the negative effects of sugar on seed germination and post-germinative growth, and some of the genes are involved in abscisic acid (ABA) and ethylene signal pathways [9,10]. For instance, the sugar insensitive mutant *sucrose uncoupled-6* (*sun6*) and glucose insensitive mutant *gin6* were found to be allelic to the mutant *abscisic acid insensitive-4* (*ABI4*), which is thought to be involved in ABA signal transduction [10–12]. The *sucrose insensitive1* (*sis1*) mutant is as allelic to *cb1*, a previously identified mutant with a constitutive response to ethylene that displays multiple phenotypes of resistance to glucose and mannose as well as the gibberellin (GA) biosynthesis inhibitor paclobutrazol (PAC) [13]. These findings suggest extensive interactions between sugar and hormone signaling pathways and raise a possibility that genes involved in sugar-hormone cross talk might be transcriptionally regulated by sugar [7,14]. And more studies have also provided significant evidence of interactions between sugar and phytohormone response as well as other metabolic pathways [7,14–16]. However, because of the dual function of sugars as nutrients and signaling molecules in plants, sugar signaling transduction is still confused.

2. Ca²⁺ acts as a second messenger in many of the diverse range of signal transduction pathways in plants. Multiple extracellular signals, such as hormones and biotic and abiotic stimuli, elicit changes in Ca²⁺ levels in the cell [17,18]. Specific Ca²⁺ signatures trigger a wide range of signal transduction pathways through various calcium sensors. All eukaryotic cells have multiple calcium sensors, and many of them have been identified. Among them, the calcineurin B-like (CBL) protein family is unique to plants. To
date, they are generally known to be involved in abiotic stress responses. Arabidopsis CBL1 functions as a positive regulator in salt or drought stress responses [19,20]. CBL9 affects plant responses to salt and mannitol as well as regulating osmotic stress-induced ABA accumulation in Arabidopsis [21]. The CBL protein family in rice is induced by various stress signals, and one of them, OsCBL5, improves salt tolerance in transgenic plants [22]. Maize ZmCBL1 was shown to modulate salt stress elicited calcium signaling and thus tolerance to salinity [23]. Although evidence is limited, previous reports have noted Ca2+ involvement in signal transduction [24,25]. Application of exogenous sugar induces accumulation of Ca2+ in the cell cytosol and increased Ca2+ may initiate Ca2+ signaling leading to the expression of sugar response genes, suggesting Ca2+ involvement in sugar signal transduction [25]. Global transcription profiling revealed that a number of genes associated with Ca2+ regulation including CaBP-22, annexin and several calcium ion binding proteins were induced by exogenous 3% glucose [15]. Sugar signalling cascade has been shown to involve mitogen-activated protein kinases, protein phosphatases, Ca2+ and calmodulin to result in various types of plant responses [26]. However, there is rare evidence to implicate a role CBLs in sugar signaling.

Here, we report the molecular and genetic characterization of CBL1 in a glucose signaling context, and its close interaction with GA signaling during seed germination and early seedling development. In this study, we found that the expression of CBL1 was mainly induced by glucose, and alteration of glucose-response gene expression in cbl1 mutant was consistent with a glucose hypersensitive phenotype. Subsequent research indicated that cbl1 did not alter its response to ABA or ethylene, whereas it was hypersensitive to PAC. Moreover, in a yeast two-hybrid screen we identified an interactor named AKINβ1, which was previously reported to be involved in sugar signaling [27]. Taken together, we suggest a novel role for CBL1 in affecting plant responses to glucose and GA signals.

Materials and Methods

Plant Materials and Growth Conditions

Seeds from Arabidopsis (Arabidopsis thaliana), ecotype Col-0, were used as wild type in this study. Both seeds of Arabidopsis (Columbia, Col-0) and T-DNA insertion lines of cbl1 were surface sterilized with 30% bleach for 10 min and extensively washed five times with sterile water. Sterile seeds were plated on 1/2 Murashige and Skoog (MS) medium plates for darkness for 3 d at 4°C, and then transferred to a growth chamber with a 16 h light period at 22°C. Screening the homozygous T-DNA insertion line (SALK_110426LP) was conducted by PCR with gene-specific primers SALK_110426LP: 5'-GGGCTACGATAGTTGAATCG-3'; SALK_110426RP: 5'-TATGATCGTCTGTTCCAGATC-3' and T-DNA border primer LBb1.3:5'-ATTGGCC-GATTTTCAAGG-3'. Homozygous mutant plants were further confirmed by RT-PCR with gene-specific primers of CBL1 (GenBank accession number: AT1G17615): 5'-AAT-GAAACTGGGGCTGATGAAACC-3' (forward) and 5'-CCCTCCGAATTGGAAGACAAAATC-3' (reverse).

RNA Extraction and Real-time PCR Analyses

Two-week-old Arabidopsis seedlings of wild-type (Col-0) plants were immersed in solutions containing 3% glucose, 3% sucrose or 3% fructose at room temperature for 6 h. Seedlings were collected and immediately frozen in liquid nitrogen. Total RNA was extracted from these plants using Trizol reagent (Takara) and treated with RNase-free DNase I (Takara). For real-time PCR, 2 μg of total RNA was used for first strand cDNA synthesis with a PrimeScript 1st Strand cDNA Synthesis kit (Takara). Quantitative expression assays were performed with the SYBR Premix Ex Tag™ kit (Takara) and an ABI 7300 according to the manufacturer’s protocols (Applied Biosystem). The PCR program was 95°C for 2 min followed by 40 cycles of denaturation for 15 s at 95°C and annealing/extension at 60°C for 1 min. Expressions of all genes were assayed in triplicate. Gene expression was calculated with the Delta-Delta cycle threshold method [28]. Relative quantitative results were calculated by normalization to UBQ10 (GenBank accession number: AT3G05320). The primer pairs used for real-time PCR are listed in Table S1.

Glucose and Mannitol Responses Assays

For germination assays, seeds of Col-0 and cbl1 mutants were surface sterilized and water imbibed in the dark for 3 d at 4°C. After stratification, 80 to 100 seeds of each genotype were sown in triplicate in petri dishes containing 1/2 MS medium, supplemented or not with 3% of glucose or 3% mannitol, before transfer to the growth chamber (16 h photoperiod). Germination (defined as the protrusion of the radicle through the seed coat), cotyledon greening, and cotyledon expansion were scored every day after transfer to the growth chamber, and cotyledon greening and expansion rates were calculated over the total of germinated seeds. For the root growth assay, the seven-day-old seedlings were placed vertically on 1/2 MS medium in the presence of zero, 3% glucose, or 3% mannitol. Root lengths were measured five days later. Average percentages were calculated with standard errors of the triplicates.

Phytohormones and Paclobutrazol Responses Assays

For the ABA or PAC responses assays, the surface-sterilized seeds were placed on 1/2 MS plates, supplemented or not supplemented with a gradient of ABA or PAC concentrations (from 0.2 μM to 1 μM). Seeds were stratified at 4°C for 3 d and germinated under a 16 h light photoperiod at 22°C. Germination, scored by radicle emergence from the seed coat, was recorded each day.

Phytohormones and Paclobutrazol Responses Assays

For germination assays, seeds of Col-0 and cbl1 mutants were surface sterilized and water imbibed in the dark for 3 d at 4°C. After stratification, 80 to 100 seeds of each genotype were sown in triplicate in petri dishes containing 1/2 MS medium, supplemented or not with 3% of glucose or 3% mannitol, before transfer to the growth chamber (16 h photoperiod). Germination (defined as the protrusion of the radicle through the seed coat), cotyledon greening, and cotyledon expansion were scored every day after transfer to the growth chamber, and cotyledon greening and expansion rates were calculated over the total of germinated seeds. For the root growth assay, the seven-day-old seedlings were placed vertically on 1/2 MS medium in the presence of zero, 3% glucose, or 3% mannitol. Root lengths were measured five days later. Average percentages were calculated with standard errors of the triplicates.

Genes Isolation and Vectors Construction

The full coding sequence of CBL1 was amplified with two primers (5'-ACTGGATCCATGCGGCTACTCC-3'; BamHI site underlined) and (5'-CTAGTGCACCCTGAGACATC-3'; SalI site underlined). For the yeast two-hybrid assays, the PCR product was cloned into the pGBKTK7 vector (Clontech) to generate the pGBKTK-CBL1 bait vector. The full coding sequence of AKINβ1 (GenBank accession number: AT5G21170) was amplified with two primers (5'-CATGGATCCATGGCGAATTCG-3'; BamHI site underlined, and 5'-TGAATTCATGGCGAATTCG-3'; SalI site underlined) and cloned into the prey vector pGADT7-Rec2 (Clontech) to generate a pGADT7-AKINβ1 vector. For the GST pull-down assay, the PCR product of CBL1 was cloned into the pGEX-4T-1 vector (Amersham) as pGEX-CBL1 in-frame to the coding sequence of glutathione S-transferase (GST), and the PCR product of AKINβ1 was cloned into pET-28a(+) vector (Novagen) to generate pET-
AKINβ1 containing a His-AKINβ1 fusion construct under control of a T7 bacterial expression promoter. All constructs were confirmed by sequencing.

Yeast Two-hybrid Screening

The Arabidopsis seedling cDNA library was constructed in a pGADT7-Rec2 vector containing a GAL4 activation domain using Matchmaker Library Construction (Clontech) and then transformed into the yeast strain AH109 (Clontech). The bait vector pGBK-T-CBL1 was transformed into yeast strain Y187 (Clontech). Yeast two-hybrid screening was performed using the MATCHMAKER two-hybrid system (Clontech). After the transformed library was induced by polyethylene glycol, cells were plated on a synthetic dropout (SD) medium that lacked Trp, Leu, Ade and His (SD-Trp-Leu-Ade-His), but was supplemented with an optimal concentration of 10 mM 3-amino-1,2,4-triazole (3-AT) to reduce any artificial interaction. Selected clones were sequenced by a T7 primer. The full sequences of the candidates were cloned from Arabidopsis cDNA and used for vector construction. These vectors were retransformed with the bait vector into the yeast strain AH109 for two-hybrid analysis. Transformants were selected by growing on SD-Trp-Leu- at 30°C for 4 d. Surviving clones were retransformed to SD-Trp-Leu-His-Ade- medium and assayed for β-galactosidase activity according to the manufacturer’s instructions (Clontech).

Protein Expression and Pull-down Assay

The recombinant vectors pGEX-CBL1 and pET-AKINβ1 were expressed in Escherichia coli strain BL21 (DE3) by induction with 0.5 mM isopropyl-1-thio-b-D-thiogalactoside (IPTG) at 22°C and purified with Glutathione Sepharose or Ni-activated His-binding resin (Amersham) according to the manufacturers’ instructions. Approximately 5 μg of GST-CBL1 fusion protein was immobilized on Glutathione-Sepharose 4B beads and incubated with Histagged AKINβ1 proteins. After incubation overnight at 4°C on a rotary incubator, the beads were washed five times with ice-cold phosphate-buffered saline, re-suspended in SDS gel-loading buffer, and loaded on SDS-PAGE (10%, w/v) gels. Protein bound to GST-CBL1 was detected by Western blotting with anti-His antibody (Amersham) and visualized using chemiluminescence following the manufacturer’s recommendations (Amersham).

Bimolecular Fluorescence Complementation (BiFC) Assay

For BiFC analysis, the full-length coding sequence of CBL1 was cloned into BamHI and XhoI sites in a PUC-pSPYcE vector [29] and fused with the N-terminal fragment of YFP to form a YFPN-CBL1 construct. The full-length coding sequence of AKINβ1 was cloned into the same restricted sites in a PUC-pSPYcE vector [29] as a fusion with the C-terminal fragment of YFP to form a YFPC-AKINβ1 construct. For transient expression, plasmids of YFPN-CBL1 and YFPC-AKINβ1 were co-transformed into Arabidopsis protoplasts following a previously described protocol [30]. Fluorescence in protoplasts was visualized by a confocal laser scanning microscope (Leica Microsystem, Heidelberg, Germany) 16 h after incubation at room temperature.

Results

CBL1 is Induced by Sugar and cbl1 Mutant Plants are Hypersensitive to Glucose

Arabidopsis CBL9 loss-of-function line cbl9 is hypersensitive to glucose [21]. CBL1 may share some overlapping functions with CBL9 due to the high similarity (~89%) in their coding regions [31–33]. Thus, whether CBL1 is involved in sugar response was remained to be examined. Analysis of 1.8 kb sequence upstream of the transcriptional start site of the CBL1 promoter [19,20] using the PLACE (www.dna.aflrc.go.jp/PLACE) and PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) databases showed that several potential cis-acting elements related to sugar response were present, such as W box, sugar response element (SURE), TATCCCA element, and SP8 motif [34–37] (Fig. 1A and Figure S1), further implying a possible role of CBL1 in sugar response. Next we tested the expression of CBL1 in response to sugars. The expression pattern showed that CBL1 was obviously induced by glucose, whereas no significant increased CBL1 mRNA levels could be observed following exposure of the plants to 3% sucrose or 3% fructose (Fig. 1B). The results indicate that the expression of CBL1 was specifically induced by glucose.

To further investigate the function of CBL1 in sugar response, seed germination and seedling development of CBL1 T-DNA insertion mutants were evaluated. The two cbl1 mutant had a different T-DNA insertion site in the genome sequence of CBL1, respectively. In cbl1-1, the T-DNA inserted in the first exon of the gene, and in cbl1-2, the T-DNA inserted in the 5’-untranslated region as previously described [20,38], and both of them did not produce a detectable amount of CBL1 transcript in a reverse transcription experiment (Fig. 1C). In the absence of glucose, there were no obvious morphological or developmental differences between wild-type (Col-0) and cbl1 plants (Fig. 1D, Control). Though the exogenous supply of 3% glucose had no obvious effect on Col-0 plants, both the expansion and greening of mutants’ cotyledons were significantly affected by the presence of glucose with cbl1 seedlings only being able to expand around 30% and green about 10% of their cotyledons (Fig. 1D). In contrast to glucose, the effect of equimolar concentrations of mannitol was similar on Col-0 and mutants for the two parameters. These findings revealed that CBL1 disruption led to glucose hypersensitive in Arabidopsis seedling development. In the presence of glucose, germination in cbl1 mutant lines was also more sensitive to glucose than that in Col-0. For example, the germination rate decreased to 83% for Col-0 plants and about 30% for cbl1 after stratification for 7 d in the presence of 3% glucose (Fig. 1E). The observed phenomenon was even more serious in mutant lines with the increase of glucose concentrations, suggesting that disruption of CBL1 in Arabidopsis enhances the sensitivity to glucose during seed germination. Moreover, root elongations were rarely arrested in Col-0 after treatment with 3% glucose for 5 d, whereas glucose clearly inhibited root growth in cbl1 mutant plants (Fig. 1F and Figure S2). These results showed that repression of CBL1 increased the inhibitory effects of glucose on germination and seedling growth.

Glucose-responsive Genes Expression is Altered in cbl1 Mutant Plants

It has been shown that high levels of sugar reduce transcription of genes for photosynthesis and nitrogen metabolism while promoting expression of genes related to carbohydrate consumption and storage [7,39]. To further examine the involvement of CBL1 in the glucose response, the expression levels of several glucose-response genes were analyzed by real-time PCR in Col-0 and cbl1 mutant in the presence or absence of glucose (Fig. 2). Among these genes, there were photosynthetic genes: CAB1 (AT1G29930) encoding a chlorophyll a/b-binding protein, the nuclear-encoded photosynthesis gene plastocyanin (PC) (AT1G76100) and RBCS, encoding the small subunit of ribulose-1,5-bisphosphate carboxylase (AT5G38410). In the absence of glucose, these genes were expressed similarly among the plants. In Col-0 seedlings 3% glucose was insufficient to repress CAB1, whereas

A Novel Role for CBL1 in Arabidopsis
A Novel Role for CBL1 in Arabidopsis

A

-1800bp

W box | SURE | TATCCA | G box | SP8

CBL1

ATG

+668

B

Relative expression level

Control | Glucose | Sucrose | Fructose

0 | 2 | 4 | 6

C

Col-0 | cbl1-1 | cbl1-2

CBL1

UBQ10

D

Col-0 | Control

Col-0 | 3% glucose

Col-0 | 3% mannitol

cbl1-1

cbl1-2

cbl1-1

cbl1-2

E

Germination rate (%)

0 | 20 | 40 | 60 | 80 | 100

Concentration of glucose (%) 0 | 1 | 2 | 3 | 6

Col-0 | cbl1-1 | cbl1-2

F

Root length (cm)

0 | 2 | 4 | 6 | 8

Control | 3% glucose | 3% mannitol

Col-0 | cbl1-1 | cbl1-2
and 3% fructose. Plants immersed in water without sugar served as a control. (C) Isolation of region of these genes by 3% glucose was much more pronounced, with both medium to media containing zero, 3% glucose, or 3% mannitol. Root lengths were measured 5 days after transfer. Data represent averages of 30 plants ± SD.

Figure 1. Expression patterns of CBL1 and glucose sensitivity of cbl1 mutant. (A) Deduced cis-acting elements present in the promoter region of CBL1. (B) CBL1 was induced by sugars. Seven-day-old Col-0 seedlings were transferred to liquid media containing 3% glucose, 3% sucrose and 3% fructose. Plants immersed in water without sugar served as a control. (C) Isolation of cbl1 T-DNA insertional mutant. T-DNA insertion sites were at 75 bp downstream and 265 bp upstream of ATG, respectively. Black boxes and solid lines denote exons and introns, respectively. No CBL1 transcript was detected by RT-PCR in mutant plants. (D) Representative growth phenotypes of Col-0 and cbl1 seeds grown in zero, 3% glucose or 3% mannitol for 7 days. Bars = 3 mm. Germination and early seedling development (cotyledon greening and expansion) rates, scored 7 d after stratification, of Col-0 and cbl1 mutant seedlings grown in control conditions or in the presence of 3% glucose or 3% mannitol (means ± SE, n = 3). (E) Germination rates of Col-0 and the mutant in the presence of different concentrations of glucose. Each point represents averages of 80 seeds ± SD. (F) Effects of glucose and mannitol on root elongation of Col-0 and mutant seedlings. Seven-day-old seedlings were transferred from regular medium to media containing zero, 3% glucose, or 3% mannitol. Root lengths were measured 5 days after transfer. Data represent averages of 30 plants ± SD.

doi:10.1371/journal.pone.0056412.g001

Identification of CBL1-interacting Proteins

CBL1 is known to be associated with a number of proteins in exerting its functional biological roles. By using the yeast two-hybrid system, a positive interactor of CBL1 was identified and sequence analysis revealed that the CBL1-interacting protein was the β regulatory subunit of the heterotrimeric complex of sucrose non-fermenting-1-related protein kinase 1 (SnRK1) named AKIN1B1. In yeast two-hybrid screening, strong growth on SD-Trp-Leu-Ade-His medium and activity of the reporter gene were observed only in yeast cells co-transformed with pGBKTT7-CBL1 and pGADT7-AKIN1B1 (Fig. 4A), indicating interaction of CBL1 and AKIN1B1 in yeast. The interaction between CBL1 and AKIN1B1 was further tested using in vitro pull-down assay with His-AKIN1B1 and glutathione S-transferase (GST)-CBL1. As shown in Figure 4B, His-AKIN1B1 also physically interacted with GST-CBL1 as shown by anti-His antibody on the resulting western blot. Moreover, the in vivo BiFC assay showed that the interaction of CBL1 and AKIN1B1 occurred on the plasma membranes of Arabidopsis protoplasts (Fig. 4C). These results demonstrate that AKIN1B1 interacts with CBL1.

Discussion

CBL1 is Involved in Sugar Response

As the important energy sources and structural storage components, sugars act to influence plant development. Also, there are increasing evidences that sugar plays important roles as a signaling molecule. For instance, glucose plays crucial roles in plant development both as an energy source and a signaling molecule, which is further associated with phytohormones [3,7]. However, factors that affect glucose and phytohormone responses remain to be fully elucidated.

cis-elements are important molecular switches involved in the transcriptional regulation of a dynamic network of gene activities controlling various biological processes. Though there is no common cis-element as a marker for the sugar signaling pathway, cis-elements involved in sugar signaling have been reported [3]. The activation of gene expression by sugars has been best studied with the promoters of genes encoding patatin, amylase, and vegetative storage protein [36,41–44]. For instance, analysis of PC and RBCS transcript levels were reduced about 2–5 folds. In the cbl1 mutants, by contrast, down-regulation of the expression of these genes by 3% glucose was much more pronounced, with both transcripts of CAB1 and PC being highly reduced. Similarly, 3% glucose exerted only a slight repression on the nitrogen metabolism gene asparagine synthetase 1 (ASV1) (AT3G47340) in Col-0 seedlings but strongly repressed its expression in the cbl1 mutant. In contrast, 3% glucose promoted expression of ADP-Glucose pyrophosphorylase, large subunit (APL) (AT4G39210), a rate limiting enzyme for starch synthesis and the anthocyanin biosynthesis gene chalcone synthase (CHS) (AT5G13930) for several folds in Col-0. And expression of the two genes by 3% glucose was much more pronounced with dozens of folds in mutant plants. These results are consistent with cbl1 mutant having a glucose-sensitive phenotype.

Suppression of CBL1 Increases Sensitivity to PAC

Previous studies demonstrated vital roles for ABA and ethylene in plant glucose responses [10,40]. It was therefore important to investigate the effect of the cbl1 mutation on ABA and ethylene signaling. A germination assay on medium containing ABA was conducted to investigate whether the glucose phenotype conferred by the CBL1 mutation is accompanied with altered sensitivity to ABA, as appears to be the case for the vast majority of the identified sugar response mutants. As shown in Fig. 3A, the exogenous application of ABA delayed germination of Col-0 and cbl1 seeds to a similar extent. This indicated that hypersensitivity to glucose in cbl1 mutant is not due to ABA sensitivity. In order to determine the response of the cbl1 mutant to ethylene, hypocotyl lengths of seedlings grown in the dark in the presence of ACC were measured on the basis that dark-grown seedlings undergo the ethylene triple response and includes shortening of the hypocotyls. As shown in Fig. 3B, the cbl1 mutation also did not alter sensitivity to ethylene precursor ACC, with the hypocotyl length decreasing to similar extents in both Col-0 and the mutant. Ethylene is known to antagonize glucose response [9]. As expected, the addition of 50 μM ACC led to a recovery in cotyledon greening in cbl1 mutant plants under high glucose concentration (Fig. 3C and D). These experiments indicated that cbl1 was not defective in responding to ABA and ethylene signaling.

GA promotes seed germination and plant growth. Further study was undertaken to investigate whether CBL1 suppression will affect plant response to GA. The results showed that cbl1 mutant was hypersensitive to PAC. A sharp decline in germination percentage of the cbl1 mutant was observed in the presence of different concentrations of PAC (Fig. 3E). For instance, in the presence of 0.5 μM PAC and 1 μM GA3 were supplied together, germination and cotyledon greening of the mutant was largely restored (Fig. 3C and D).

0.5 μM PAC and 1 μM GA3 were supplied together, germination and cotyledon greening of the mutant was largely restored (Fig. 3C and D). We supposed that hypersensitivity to PAC in cbl1 mutant plants could be due to GA-deficiency. Analysis of genes expression for GA biosynthetic and signal pathway in both Col-0 and cbl1 mutant plants was performed. Most genes selected for assay were not obviously changed in the cbl1 mutant compared to Col-0. However, expression of the key GA biosynthetic gene GA3ox1 was clearly reduced in cbl1 plants (Fig. 3F). These results suggest that CBL1 probably affects the GA response by regulating the expression of GA3ox1.

Identification of CBL1-interacting Proteins

CBL1 is known to be associated with a number of proteins in exerting its functional biological roles. By using the yeast two-hybrid system, a positive interactor of CBL1 was identified and sequence analysis revealed that the CBL1-interacting protein was the β regulatory subunit of the heterotrimeric complex of sucrose non-fermenting-1-related protein kinase 1 (SnRK1) named AKIN1B1. In yeast two-hybrid screening, strong growth on SD-Trp-Leu-Ade-His medium and activity of the reporter gene were observed only in yeast cells co-transformed with pGBKTT7-CBL1 and pGADT7-AKIN1B1 (Fig. 4A), indicating interaction of CBL1 and AKIN1B1 in yeast. The interaction between CBL1 and AKIN1B1 was further tested using in vitro pull-down assay with His-AKIN1B1 and glutathione S-transferase (GST)-CBL1. As shown in Figure 4B, His-AKIN1B1 also physically interacted with GST-CBL1 as shown by anti-His antibody on the resulting western blot. Moreover, the in vivo BiFC assay showed that the interaction of CBL1 and AKIN1B1 occurred on the plasma membranes of Arabidopsis protoplasts (Fig. 4C). These results demonstrate that AKIN1B1 interacts with CBL1.

Discussion

CBL1 is Involved in Sugar Response

As the important energy sources and structural storage components, sugars act to influence plant development. Also, there are increasing evidences that sugar plays important roles as a signaling molecule. For instance, glucose plays crucial roles in plant development both as an energy source and a signaling molecule, which is further associated with phytohormones [3,7]. However, factors that affect glucose and phytohormone responses remain to be fully elucidated.

cis-elements are important molecular switches involved in the transcriptional regulation of a dynamic network of gene activities controlling various biological processes. Though there is no common cis-element as a marker for the sugar signaling pathway, cis-elements involved in sugar signaling have been reported [3]. The activation of gene expression by sugars has been best studied with the promoters of genes encoding patatin, amylase, and vegetative storage protein [36,41–44]. For instance, analysis of
A short-inducible promoter derived from β-Amy indicated that TGGACGG element plays an important role in sugar inducible expression of both of the truncated promoters of spo and β-Amy [43]. A number of various sucrose response (repression or induction) elements (SURE) from several nuclear genes, including rbcS and SPF1, have been identified [37,44]. The investigation of rice α-amylase gene reveals four essential cis-elements that are important for high sugar starvation-induced expression: the GC-box, the G-box, the SP8 motif, and the TATCCA element [34,45]. A WRKY-type SUSIBA2 is sugar inducible and binds to the SURE and W-box [35]. The core sequence of the WRKY binding element (W-box) is found in the promoters of wheat, barley, and wild oat α-AMY2 gene [46]. As shown in Fig. 1A and Figure S1, several potential cis-acting elements respond to sugar signal, such as W-box, TATCCA element, WRKY-type SUSIBA2 and SP8 motif were present in the putative promoter of CBL1;

Figure 2. Real-time PCR analysis of expression of glucose-responsive genes in Col-0 and cbl1 mutant plants treated with zero or 3% glucose. Mean values were normalized to the transcript level of the internal control, UBQ10. CAB1, chlorophyll a/b-binding protein 1; PC, plastocyanin; ASN1, asparagine synthetase 1; CHS, chalcone synthase; APL3, ADP-glucose pyrophosphorylase, large subunit; RBCS, ribulose-1,5-bisphosphate carboxylase, small subunit.

doi:10.1371/journal.pone.0056412.g002
A Novel Role for CBL1 in *Arabidopsis*

**A**

Germination rate (%)

| ABA (μM) | Col-0 | cbl1-1 | cbl1-2 |
|----------|-------|--------|--------|
| 0        | 100   | 95     | 92     |
| 0.2      | 90    | 85     | 88     |
| 0.4      | 80    | 75     | 78     |
| 0.6      | 70    | 65     | 68     |
| 0.8      | 60    | 55     | 58     |
| 1        | 50    | 45     | 48     |

**B**

Hypocotyl length (cm)

| ACC (μM) | Col-0 | cbl1-1 | cbl1-2 |
|----------|-------|--------|--------|
| 0        | 4     | 3.5    | 3.2    |
| 1        | 3     | 2.5    | 2.2    |
| 5        | 2     | 1.5    | 1.2    |
| 10       | 1     | 0.5    | 0.2    |

**C**

Control 6% glucose 6% glucose + 50 μM ACC 0.5 μM PAC 0.5 μM GA3

Col-0

![Col-0 images]

Cbl1

![Cbl1 images]

**D**

Col-0 cbl1-1 cbl1-2

Germination greening (%)

| Condition | Col-0 | cbl1-1 | cbl1-2 |
|-----------|-------|--------|--------|
| Control   | 100   | 95     | 92     |
| 6% glucose| 90    | 85     | 88     |
| 6% glucose + 50 μM ACC | 80 | 75 | 78 |
| 0.5 μM PAC | 70 | 65 | 68 |
| 0.5 μM PAC + 1 μM GA3 | 60 | 55 | 58 |

**E**

Germination rate (%)

| PAC (μM) | Col-0 | cbl1-1 | cbl1-2 |
|----------|-------|--------|--------|
| 0        | 100   | 95     | 92     |
| 0.1      | 90    | 85     | 88     |
| 0.2      | 80    | 75     | 78     |
| 0.5      | 70    | 65     | 68     |
| 0.8      | 60    | 55     | 58     |
| 1        | 50    | 45     | 48     |

**F**

Relative expression level

| Gene      | Col-0 | cbl1-1 | cbl1-2 |
|-----------|-------|--------|--------|
| GA1       | 1     | 0.9    | 0.8    |
| SLY1      | 1.2   | 1.1    | 1      |
| K3        | 1     | 0.9    | 0.8    |
| GA3ox1    | 1.5   | 1.4    | 1.3    |
| GA3ox2    | 2     | 1.9    | 1.8    |

**G**

Glucose → CBL1 → GA → Germination and seedling growth

GA3ox1 → CBL1 → Glucose → Germination and seedling growth
suggesting CBL1 is sugar inducible gene. In Arabidopsis, CBL1 shares highest identity with CBL9 at the level of amino acid sequence among the nine CBLs [31–33]. Regarding the similarity in the amino acid sequence, expression pattern, and interactive CIPKs, we speculate that that CBL1 and CBL9 function in similar processes and share common functions [19–21,31–33,47]. Indeed, both CBL1 and CBL9 are activated by various abiotic stresses in Arabidopsis [19–21]. Particularly, using loss-of-function showed that the CBL9 mutant was hypersensitive to glucose [21]. Thus, these findings inspired us to investigate whether CBL1 is involved in sugar response.

Next we tested the expression of CBL1 in response to sugars by using real-time PCR assays. Here we found that CBL1 was obviously induced by glucose (Fig. 1B). Further study showed that the loss-of-function cbl1 mutant was hypersensitive to exogenous glucose. With the steady increase of glucose concentrations (0%–3%), seed germination and seedling development were arrested in both Col-0 and cbl1 mutant lines, but more seriously inhibited in cbl1 seedlings (Fig. 1D, E and F). Although cbl1 mutant has displayed an osmo-sensitive phenotype [19,20], this osmo-sensitive phenotype is insufficient to explain its glucose-sensitive phenotype. This conclusion is based on the finding that a developmental difference between Col-0 and mutant plants was not observed in germination and seedling growth when both genotypes were grown in the presence of 3% mannitol, which is enough to exert osmotic stress (Fig. 1D and F). This is consistent with previous studies that intermediate glucose concentrations (1.5%–3%) dramatically delay WT seed germination, similar concentrations

Figure 3. Phytohormones responses in cbl1 mutant plants. (A) Germination rates of Col-0 and mutant seeds after 5 d of incubation at 22 °C on ½ MS medium containing different concentrations of ABA. Results are presented as average values ± SE from three experiments. (B) Effect of ACC on hypocotyl elongation in Col-0 and mutant. Hypocotyl lengths were measured 5 d after stratification of seedlings grown in the dark in the presence of different concentrations of ACC. (C) Representative images of Col-0 and mutant grown in conditions indicated above the images for 7 days. Bars = 5 mm. (D) Comparison of percentage green cotyledons of Col-0 and mutant seedlings grown in control conditions or in the presence of 6% glucose supplemented or not with 50 μM ACC or in the presence of 0.5 μM PAC supplemented or not with 1 μM GA3. Data are averages of 60 plants ± SD. (E) Germination of Col-0 and mutant grown in medium with different concentrations of PAC. Each point represents averages of 80 seeds ± SD. (F) Relative expressions of GA biosynthetic and signaling genes in two-week-old seedlings of Col-0 and mutant grown on MS medium. doi:10.1371/journal.pone.0056412.g003

Figure 4. Interaction between CBL1 and AKINβ1. (A) Yeast two-hybrid interactions. Vectors were co-introduced into yeast AH109 strain in different combinations: 1, pGADT7 and pGBK7; 2, pGADT7-AKINβ1 and pGBK7; 3, pGADT7 and pGBK7-CBL1; 4, pGADT7-AKINβ1 and pGBK7-CBL1. Transformants were placed on selection medium and grown for 4 d before the β-galactosidase (β-Gal) assay. (B) In vitro GST pull-down assay. GST-CBL1 and His-AKINβ1 were expressed in E. coli and used for analysis. The presence or absence of each protein in the reaction mixture is shown as + or −, respectively. The right row shows a Coomassie Brilliant Blue-stained SDS-PAGE gel indicating captured proteins used as controls. Experiments were performed 3 times and a representative result was shown. (C) In vivo BIFC assay. The plasmids YFPN-CBL1 and YFPC-AKINβ1 were co-transformed into Arabidopsis protoplasts. The expression of CBL1 alone (YFPN-CBL1+pUC-SPYCE) was used as control. Photographs were taken with a confocal laser-scanning microscope (Leika TCS-NT). Bars = 10 μm. (D) A proposed model to illustrate biological relevance of the interaction between CBL1 and AKINβ1. In the presence of calcium, CBL1 together with certain CBL-interacting protein kinases (not only CIPK) forms a complex to modify AKINβ1, thus affecting the activity of SnRK1. Positive interaction is noted by an arrow and bars indicate repression.
doi:10.1371/journal.pone.0056412.g004
of mannitol or sorbitol have very little effect, indicating that the effect of glucose is not simply osmotic [6,48]. In addition, no significant difference between Col-0 and cbl1 mutant plants was observed in the presence of equimolar sucrose (data not shown). In contrast, loss-of-function cbl1 mutant was hypersensitive to 3% glucose (Fig. 1E and F), indicating that germination and growth arrest is glucose-specific and not due to osmotic stress. Moreover, cbl1 mutant displayed a glucose hypersensitivity phenotype at the molecular level in regulating glucose-responsive gene expression (Fig. 2). Elevated sugar levels have long been known to down-regulate the transcription of both photosynthetic genes and genes associated with nitrogen metabolism, while up-regulating the expression of genes involved in the synthesis of polysaccharides [4]. Following treatment with 3% glucose, several genes involved in photosynthesis including CAB1, PC and RBCS as well as ASN1 for nitrogen metabolism were decreased in Col-0 [49,50], but the decreases were more pronounced in cbl1 mutant seedlings (Fig. 2). Sugar-induced anthocyanin accumulation is a common phenomenon in plant species and a sugar induction of anthocyanin biosynthesis was also demonstrated in Arabidopsis seedlings [31,52]. ADP-glucose pyrophosphorylase (ADP-Glc PPase) catalyzes the first and limiting step in starch biosynthesis, which is composed of two types of subunits (small and large) [53,54]. In plants, only the large subunit of APL3 and APL4 were sugar induced [55]. As expected, real-time PCR analysis showed that transcript levels of CHS associated with anthocyanin biosynthesis and APL3 were induced by glucose in Col-0. In the cbl1 mutant, induction of the expression of these genes by 3% glucose was much more pronounced, with both transcripts being highly overinduced. Thus, the CBL1 mutation caused expression of all sugar-responsive genes to be more sensitive to exogenous glucose, suggesting that CBL1 may negatively regulate sugar-responsive gene expression by affecting a common part of the signaling pathways for both sugar-induced and sugar-repressed gene expression (Fig. 3G). Previous studies showed that repression of photosynthesis-related gene expression, such as CAB1, PC, and RBCS, was correlated with a hexokinase (HXK1)-mediated signaling function, whereas the effect of glucose on expression of CHS and ASN1 was independent of HXK1-mediated signaling [56,57]. These results suggest that CBL1 might be involved in both HXK1-dependent and HXK1-independent pathways.

CBL1 Physically Interacts with AKIN1 and Probably Opposes its Function in Sugar Signaling

Ca2+ acts as a second messenger in many of the diverse signal transduction pathways in plants. Calcium sensors, such as calmodulin (CaM), calmodulin domain protein kinases (CDPK), and CBLs, bind Ca2+ and change their conformation [31–33,58–60]. CDPKs are protein kinases, whereas CaM and CBL are small Ca2+ sensors that do not have apparent enzymatic activities. Studies have shown that CaMs associate with a number of diverse target proteins, such as NAD kinase, glucose decarboxylase and transcription factors [59]. The well known CBL-interacting proteins are a group of sucrose non-fermenting-related serine/threonine kinases (SnRK3), named CBL-interacting protein kinases (CIPK) [60–63]. For example, CBL1 and CBL9 were reported to interact and activate CIPK23 to enhance K+ uptake under low K+ condition and regulate leaf transpiration against drought stress in Arabidopsis [64,65]. Another CIPK protein of CIPK7 was also interacted with CBL1 needed for clod stress tolerance [38]. Judging from the number of CBL-mediated stimuli identified so far and the target diversity exhibited by another sensor, CaM, it is possible that CBLs associate with a variety of proteins in addition to CIPKs to mediate more diverse signals. Actually, CBL1 has also been shown to interact with other proteins besides the CIPK proteins. Activity of another interactor of CBL1 named PI-4K1 was mediated by CBL1, which was involved in growth of root hair [66]. A recent study revealed that CBL1 interacted and inactivated a specific PP2C-type phosphatase (PP2CA) for the activation of AKT1 channel with CIPK6 [67].

In this study, we confirmed that CBL1 physically interacted with AKIN1, a regulatory β-subunit of the SnRK1 complex that does not belong to the CIPK family. Based on previous studies, AtCBLs represents a multi-member protein family and the ‘specificity’ of calcium codes remains largely a mystery, raising an important question on the specificity of interaction with their targets. To assess specificity of AtCBL-AKIN1 interaction and to identify other isoform-specific interactors, we conducted systematic yeast two-hybrid experiments to screen for AKIN1 with different AtCBL isoforms. However, we did not find a physical interaction of AKIN1 with any known CBLs except for CBL1 (data not shown), indicating a specific interaction between CBL1 and AKIN1. SnRK1 belongs to a conserved family of protein kinases consisting of an α-catalytic subunit and regulatory β- and γ-subunits [68]. Arabidopsis AKIN1, being such a SnRK1 subunit, is N-myristoylated and plays an important role in specificity of recognition between the SnRK1 complex and its targets [69]. Previous studies also showed that CBL1 possessed the conserved N-myristoylation motif and CBL1-GFP fusion proteins were observed on the plasma membrane of Arabidopsis protoplasts [70]. N-myristoylation is an important modification for membrane binding ability of cytoplasmic proteins [71]. Thus, it is possible that the two proteins form a complex attached to the plasma membrane as shown in Fig. 4C. It was originally identified that overproduction of Arabidopsis AKIN1 caused developmental deficiency with less expanded cotyledons and no noticeable true leaves in the presence of glucose [27]. Thus, cbl1 displays a glucose-sensitive phenotype similar to that observed in plants with AKIN1 overexpression. Hence CBL1 and AKIN1 may exert opposite functions in sugar signaling. Interestingly, SnRK1 overexpression also showed hypersensitivity to glucose and the capacity of SnRK1 to phosphorylate its targets was shown to be regulated by the availability of the non-catalytic AKIN1 subunits [72,73]. For example, a very recent study showed that the β regulatory subunit Gbβ3 was phosphorylated by the tomato AGC Ser/Thr protein kinase Adi3, leading to suppression of SnRK1 activity [74]. Furthermore, the CBL/CIPK complexes were able to phosphorylate target proteins to regulate signal transduction [75]. Considering all the previous reported results together with the evidence presented in this study, we propose that CBL1 together with certain CIPK or other protein kinases form a complex that affects the biological function of AKIN1 through physical modification plus the interaction with AKIN1, such as phosphorylation, then affecting the activity of SnRK1 in glucose response (Fig. 4D). However, such a CBL1-interacting protein kinase remains to be verified, and more evidence is needed for the biological relevance of the interaction between CBL1 and AKIN1.

GA Deficiency in cbl1 Mutant Leads to Developmental Defective in the Presence of PAC

Phytohormones play important roles in regulation of developmental processes through close interaction with sugar signals. Previous studies showed that sugars stimulate ABA accumulation in seedling plants, and that ABA signal transduction is necessary for sugar-induced inhibition of photosynthesis and developmental arrest [76]. However, the present research demonstrated that cbl1 did not affect its response to ABA as well as ethylene (Fig. 3A and
B). This implies that CBL1 may be involved in sugar signaling by crosstalk with other phytohormones. GA is an important hormone for germination as shown by the observation that GA-deficient mutants do not germinate and germination can be induced in GA-deficient mutants by exogenous gibberellic acid (GA3) application [77]. Subsequent study showed that PAC severely repressed the germination of cbl1 mutant compared with Col-0, whereas exogenous GA3 completely rescued the defect (Fig. 3C, D and E). Thus, CBL1 appears to affect the plant response to GA during seed germination and seedling growth. Further study revealed that expression of GA3ox1 was significantly reduced in cbl1 mutant plants (Fig. 3F). GAs are derived from geranylgeranyl diphosphate though a series reactions catalyzed by ent-copalyl diphosphate synthase (CPS) and ent-kaurene synthase (KS), GA 20-oxidase (AtGA20ox), and GA 3-oxidase (AtGA3ox) in higher plants [78]. GA3ox1, encoding GA 3-oxidase 1 and catalyzing the rate-limiting step of GA biosynthesis, is essential for GA synthesis during plant growth [79]. This suggests that repression of CBL1 may lead to a deficiency in GA biosynthesis and signal response, and could be the reason why the cbl1 mutant is hypersensitive to PAC (Fig. 3G).

A previous report suggested that sugar and GA signals competed in seed germination, indicating possible crosstalk between the sugar and GA signaling pathways [80]. And glucose has been shown to inhibit seed germination of Arabidopsis [77]. Subsequent study showed that PAC severely repressed the germination of cbl1 mutant sown on glucose media or glucose containing growth media supplemented with 3% glucose or 3% mannitol. Photograph was taken after five days. Representative images were presented. Bars = 2 cm. (TIF)

**Figure S2** Effects of glucose and mannitol on seedling growth of wild-type (Col-0) and mutant (cbl1) plants. Seven-day-old seedlings were transferred to vertical plates containing growth media supplemented with zero, 3% glucose or 3% mannitol. (TIF)

**Figure S3** Effect of glucose on GA biosynthetic and signaling pathway genes expression in Col-0 and cbl1 mutant plants. Seedlings were treated without or with 3% glucose for 6 h and harvested for RNA isolation. Relative amounts of each transcript were determined by real-time PCR and normalized relative to UBQ10. All values are averages of three independent experiments. SLT1, SLEEP; RGL2, DELLA protein, RGA-LIKE 2; SPI1, SPINDLY; GID1A, GA INSENSITIVE DWARF1A; GA3ox1, GA3-oxidase. (DOC)

**Table S1** Sequence information for primers used in real-time PCR.

**References**

1. Smeekens S (2000) Sugar-induced signal transduction in plants. Annu Rev Plant Physiol Plant Mol Biol 51: 49–81.
2. Gibson NI (2005) Control of plant development and gene expression by sugar signaling. Curr Opin Plant Biol 8: 93–102.
3. Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. Annu Rev Plant Biol 57: 675–709.
4. Koch KE (1996) Carbohydrate-modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol 47: 509–540.
5. Koch K (2004) Sucrose metabolism: regulatory mechanisms and pivotal roles in sugar sensing and plant development. Curr Opin Plant Biol 7: 233–246.

6. Dekkers BJ, Schuurmans JA, Smeekens SC (2004) Glucose delays seed germination in Arabidopsis thaliana. Plants 218: 579–581.
7. Leon P, Sheen J (2003) Sugar and hormone connections. Trends Plant Sci 8: 110–116.
8. Pego JV, Smeekens S (2005) Plant fructokinases: A sweet family get-together. Trends Plant Sci 10: 531–536.
9. Zhou L, Jing J, Jones TL, Sheen J (1998) Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. Proc Natl Acad Sci U S A. 95: 10294–10299.
10. Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, Leon P (2000) Analysis of Arabidopsis glucose insensitive mutants, ga3 and ga5, reveals a central role of the interacting-proteins will be the focus of our future work to understand the role of CBL1 in sugar signaling.

**Supporting Information**

**Figure S1** Nucleotide sequences of CBL1 promoter region and putative cis-acting elements. The motifs with a significant similarity to the previously identified cis-acting elements are shown underline. The box indicates the translation initiation site. WBOX, TTTGACY; SP8, TTTGAC; SP8, ATAGA; CMSRE-1 (Carbohydrate Metabolite Signal Responsive Element 1): TGGACGG; SURE: GAGAC; G motif TACGT: G box: CACGGT; “TATCCA” element: TATCCA; “A-box”: TATCCA; Pyrimidine box: CCTTTT.

**Table S2** Sequence information for primers used in real-time PCR.

**Acknowledgments**

The vectors (PUC3pSPYNE and PUC3pSPYCE) used in the BiFC assay were kindly gifted by Dr. Yongfu Fu (Chinese Academy of Agricultural Sciences, China). We also thank Prof. Weihua Wu (China Agricultural University, China), Prof. Lizhe An (Lanzhou University, China) and Prof. Jörg Kudla (Universität Ulm, Germany) for generously providing the cbl1 mutant and RA McIntosh (Plant Breeding Institute, University of Sydney) for critically reading the manuscript.

Author Contributions

Performed mutants identification and yeast two-hybrid assays: YC. Conceived and designed the experiments: ZSY ZYM. Performed the experiments: ZYL. Analyzed the data: ZYL GXY GYH. Contributed reagents/materials/analysis tools: LCL MC. Wrote the paper: ZYL.
plant hormone ABA in the regulation of plant vegetative development by sugar. Genes Dev 14: 2083–2096.
11. Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM (1998) The Arabidopsis ABA response locus AB1 encodes an APETALA2 domain protein. Plant Cell 10: 1043–1054.
12. Huisjer G, Kortte A, Pego J, Weinbeek P, Wisman E, et al. (2000) The Arabidopsis SUCROSE UNCOUPLED-6 gene is identical to ABSICIC ACID INSENSITIVE-1 involvement of abscisic acid in sugar responses. Plant J 23: 377–385.
13. Gibson SJ, Luby RJ, Kim D (2001) The sugar-inositol (sic) mutant of Arabidopsis is allelic to AtSIC1. Biochem Biophys Res Commun 280: 196–203.
14. Yuan K, Wysocka-Diller J (2006) Phytohormone signaling pathways interact with sugars during seed germination and seedling development. J Exp Bot 57: 3359–3367.
15. Price J, Laxmi A, St Martin SK, Jagc JC (2004) Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. Plant Cell 16: 2126–2150.
16. Gibson SJ (2006) Sugar and phytohormone response pathways: navigating a signalling network. J Exp Bot 55: 255–264.
17. Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. Plant Cell 11: 691–706.
18. Harper JF (2001) Dissecting calcium oscillators in plant cells. Trends Plant Sci 6: 1–12.
19. Cheong YH, Kim KN, Pandey GK, Gupta R, Grant JJ, Li L, et al. (2004) The calcium sensor calcineurin B-like 9 modulates abscisic acid sensitivity and biosynthesis in Arabidopsis. Plant Cell 16: 1912–1924.
20. Gu ZM, Ma BJ, Jiang Y, Chen ZW, Su X, et al. (2008) Expression analysis of the Arabidopsis calcium sensor CBL1 integrates plant responses to abiotic stresses. J Integr Plant Biol 51: 513–520.
21. Pandey GK, Cheong YH, Kim KN, Grant JJ, Li L, et al. (2004) The calcium sensor calcineurin B-like 9 modulates abscisic acid sensitivity and biosynthesis in Arabidopsis. Plant Cell 16: 1912–1924.
22. Go ZM, Ba BJ, Jiang Y, Chen ZW, Su X, et al. (2008) Expression analysis of the calcineurin B-like family gene in rice (Oryza sativa L.) under environmental stresses. Gene 3359–3367.
23. Walter M, Chaban C, Schütze K, Batistic O, Weckermann K, et al. (2004) SUT2, a calcium sensor that differentially regulates salt, drought and cold responses in Arabidopsis. Plant Mol Biol 57: 815–826.
24. Barker L, Kuhn C, Weise A, Schulz A, Gebhardt C, et al. (2000) SUT2, a calcium sensor for specific signal transduction in Arabidopsis thaliana. J Biol Chem 275: 15635–15642.
25. Furuichi T, Mori IC, Takahashi K, Muto S (2001) Sugar-induced increase in leaf ADP glucose pyrophosphorylase. Plant Physiol 85: 182–187.
26. Barker L, Kuhn C, Weise A, Schulz A, Gebhardt C, et al. (2000) SUT2, a calcium sensor for specific signal transduction in Arabidopsis thaliana. J Biol Chem 275: 15635–15642.
27. Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM (1998) The Arabidopsis ABA response locus AB1 encodes an APETALA2 domain protein. Plant Cell 10: 1043–1054.
28. Huisjer G, Kortte A, Pego J, Weinbeek P, Wisman E, et al. (2000) The Arabidopsis SUCROSE UNCOUPLED-6 gene is identical to ABSICIC ACID INSENSITIVE-1 involvement of abscisic acid in sugar responses. Plant J 23: 377–385.
29. Gibson SJ, Luby RJ, Kim D (2001) The sugar-inositol (sic) mutant of Arabidopsis is allelic to AtSIC1. Biochem Biophys Res Commun 280: 196–203.
30. Yuan K, Wysocka-Diller J (2006) Phytohormone signaling pathways interact with sugars during seed germination and seedling development. J Exp Bot 57: 3359–3367.
31. Price J, Laxmi A, St Martin SK, Jagc JC (2004) Global transcription profiling reveals multiple sugar signal transduction mechanisms in Arabidopsis. Plant Cell 16: 2126–2150.
32. Sanders D, Brownlee C, Harper JF (1999) Communicating with calcium. Plant Cell 11: 691–706.
33. Kolukisaoglu U, Weinl S, Blazevic D, Batistic O, Kudla J (2004) Calcium sensors and their interacting protein kinases: Genomics of the Arabidopsis CBL1 calcium sensor family. Plant J 36: 3359–3367.
34. Huang C, Ding S, Zhang H, Du H, An L (2011) CIPK7 is involved in cold signaling by interacting with CBL1 in Arabidopsis thaliana. Plant Sci 181: 57–64.
35. Alaerts K, De Gendt N, Rojas S, Robson C, Gielen S, et al. (2006) Biochemical and molecular characterization of the Arabidopsis thaliana B-type calcium sensor CBL1. J Biol Chem 281: 32938–32947.
36. Chen MT, Yu SM (1996) The Arabidopsis CBL1 calcium sensor is required for an auxin response. Plant Mol Biol 29: 627–637.
37. Urwin NA, Jenkins GI (1997) A sucrose repression element in the Pseudomonas vulgaris sh2 gene promoter resembles elements responsible for sugar stimulation of plant and mammalian genes. Mol Gen Mol Biol 33: 929–942.
38. Huang C, Ding S, Zhang H, Du H, An L (2011) CIPK7 is involved in cold signaling by interacting with CBL1 in Arabidopsis thaliana. Plant Sci 181: 57–64.
39. Barna-González E, Rolland F, Thevelein JM, Sheen J (2007) A central integrator of transcription networks in plant stress and energy signaling. Nature 498: 938–943.
40. Finkelstein RR, Gibson SJ (2002) ABA and sugar interactions regulating development: cross-talk or voices in a crowd?Curr Opin Plant Biol 5: 26–32.
41. Martin T, Hellmann H, Schmidt R, Willmitzer L, Frommer WB (1997) Identification of mutants in metabolically regulated gene expression; Plant J 11: 53–62.
42. Sadaka A, DeWald DB, May GD, Park WD, Mullet JE (1994) Phosphate modulates transcription of soybean Vgb2 and other sugar inducible genes. Plant Cell 6: 737–749.
43. Maek, Tomyia T, Hayashi K, Akaide M, Morikami A, et al. (2001) Sugar-responsive elements in the promoter of a gene for β-amylase of sweet potato. Plant Mol Biol 46: 627–637.
44. Urwin NA, Jenkins GI (1997) A sucrose repression element in the Pseudomonas vulgaris sh2 gene promoter resembles elements responsible for sugar stimulation of plant and mammalian genes. Mol Gen Mol Biol 33: 929–942.
45. Chen MT, Yu SM (1996) The Arabidopsis CBL1 calcium sensor is required for an auxin response. Plant Mol Biol 29: 627–637.
46. D’Angelo C, Weinl S, Batistic O, Pandey GK, Cheong YH, et al. (2006) Alternative complex formation of the Ca-regulated protein kinase CIPK1 controls abscisic acid-dependent and independent stress responses in Arabidopsis. Plant J 48: 857–872.
47. Price J, Li TC, Kang SG, Na JK, Jagc JC (2003) Mechanisms of glucose signaling during germination of Arabidopsis. Plant Physiol 132: 1424–1438.
48. Moore B, Zhou L, Rolland F, Hall Q, Cheng W, et al. (2005) Role of the Arabidopsis calcineurin B-like sensor HXK1 in nutrient, light, and hormonal signaling. Science 300: 332–336.
49. Sheen J, Zhou L, Jagc JC (1999) Sugars as signaling molecules. Curr Opin Plant Biol 2: 410–418.
71. Taniguchi H (1999) Protein myristoylation in protein-lipid and protein-protein interactions. Biophys Chem 82: 129–137.
72. Jossier M, Boulé JP, Meimoun P, Arjmand A, Lessard P, et al. (2009) SnRK1 (SNF1-related kinase 1) has a central role in sugar and ABA signalling in Arabidopsis thaliana. Plant J 59: 316–328.
73. Polge C, Jossier M, Crozet P, Gisiot L, Thomas M (2008) Beta-subunits of the SnRK1 complexes share a common ancestral function together with expression and function specificities; physical interaction with nitrate reductase specifically occurs via AKINbeta1-subunit. Plant Physiol 148: 1570–1582.
74. Avila J, Gregory OG, Su D, Deeter TA, Chen S, et al. (2012) The beta-subunit of the SnRK1 complex is phosphorylated by the plant cell death suppressor Adi3. Plant Physiol 159: 1277–1290.
75. Qiu QS, Guo Y, Dietrich MA, Schumaker KS, Zhu JK (2002) Regulation of SOS1, a plasma membrane Na+/H+ exchanger in Arabidopsis thaliana, by SOS2 and SOS3. Proc Natl Acad Sci U S A. 99: 8436–8441.
76. Nagira Y, Ikegami K, Koshiha T, Ozeki Y (2006) Effect of ABA upon anthocyanin synthesis in regenerated torenia shoots. J Plant Res 119: 137–144.
77. Bentsink L, Koornneef M (2002) Seed dormancy and germination. In CR Somerville, EM Meyerowitz, eds, The Arabidopsis Book. American Society of Plant Biologists, Rockville, MD, pp: 1–17.
78. Olszewski N, Sun TP, Gubler F (2002) Gibberellin signaling: biosynthesis, catabolism, and response pathways. Plant Cell 14: S61–S80.
79. Mitchum MG, Yamaguchi S, Hanada A, Kawahara A, Yoshida Y, et al. (2006) Distinct and overlapping roles of two gibberellin 3-oxidases in Arabidopsis development. Plant J 45: 604–618.
80. Chen PW, Chiang CM, Tseng TH, Yu SM (2006) Interaction between rice MYBGA and the gibberellin response element controls tissue-specific sugar sensitivity of alpha-amylase genes. Plant Cell 18: 2326–2340.
81. Lee S, Cheng H, King KE, Wang W, He Y, et al. (2002) Gibberellin regulates Arabidopsis seed germination via RGL2, a GAI/RGA-like gene whose expression is up-regulated following imbibition. Genes Dev 16: 646–658.
82. Davière JM, de Lucas M, Prat S (2008) Transcriptional factor interaction: a central step in DELLA function. Curr Opin Genet Dev 18: 295–303.
83. Hauvermale AL, Ariizumi T, Steber CM (2012) Gibberellin signaling: A theme and variations on della repression. Plant Physiol 160: 83–92.