Long-distance transport of sucrose in source leaves promotes sink root growth by the EIN3-SUC2 module

Chen Tong, Cong Li, Xiao-Ying Cao, Xu-Dong Sun, Qin-Xin Bao, Xin-Rong Mu, Chang-Yue Liu, Gary J. Loake, Hu-hui Chen, Lai-Sheng Meng

1 School of Life Science, Jiangsu Normal University, Xuzhou, Jiangsu, People’s Republic of China, 2 Public Technical Service Center, Kunming Institute of Zoology, Chinese Academy of Sciences, Kunming, Yunnan, People’s Republic of China, 3 Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, Yunnan, People’s Republic of China, 4 Jiangsu Normal University - Edinburgh University, Centre for Transformative Biotechnology of Medicinal and Food Plants, Jiangsu Normal University, Xuzhou, People’s Republic of China, China, 5 Institute of Molecular Plant Sciences, School of Biological Sciences, Edinburgh University, King’s Buildings, Edinburgh, United Kingdom, 6 School of Life Science, Nanjing Agricultural University, Nanjing, Jiangsu, People’s Republic of China

☯ These authors contributed equally to this work.
* G.Loake@ed.ac.uk (GJL); chenhuh@njau.edu.cn (HHC); menglsh@jsnu.edu.cn (LSM)

Abstract

In most plants, sucrose, a major storage sugar, is transported into sink organs to support their growth. This key physiological process is dependent on the function of sucrose transporters. Sucrose export from source tissues is predominantly controlled through the activity of SUCROSE TRANSPORTER 2 (SUC2), required for the loading of sucrose into the phloem of Arabidopsis plants. However, how SUC2 activity is controlled to support root growth remains unclear. Glucose is perceived via the function of HEXOKINASE 1 (HXK1), the only known nuclear glucose sensor. HXK1 negatively regulates the stability of ETHYLENE-INSENSITIVE3 (EIN3), a key ethylene/glucose interaction component. Here we show that HXK1 functions upstream of EIN3 in the regulation of root sink growth mediated by glucose signaling. Furthermore, the transcription factor EIN3 directly inhibits SUC2 activity by binding to the SUC2 promoter, regulating glucose signaling linked to root sink growth. We demonstrate that these molecular components form a HXK1-EIN3-SUC2 module integral to the control of root sink growth. Also, we demonstrate that with increasing age, the HKX1-EIN3-SUC2 module promotes sucrose phloem loading in source tissues thereby elevating sucrose levels in sink roots. As a result, glucose signaling mediated-sink root growth is facilitated. Our findings thus establish a direct molecular link between the HKX1-EIN3-SUC2 module, the source-to-sink transport of sucrose and root growth.

Author summary

In Arabidopsis and most crops, sucrose transporters are positioned in the vascular bundles of leaf blades where they have crucial roles in balancing source and sink activities.
However, little molecular detail is currently available regarding the modulation of sucrose transporters activity. Here, we demonstrate that the transcriptional regulator, \textit{EIN3}, functions downstream of \textit{HEXOKINASE 1 (HXK1)}, which acts upstream of \textit{SUC2} in the regulation of root sink growth mediated by glucose signaling. Further, \textit{EIN3} directly represses \textit{SUC2} function by negatively regulating \textit{SUC2} transcription. We further demonstrate that these components form the HXK1-\textit{EIN3-SUC2} module to facilitate sucrose phloem loading in source tissues thereby elevating sucrose content in sink roots.

\section*{Introduction}

Sugars produced from photosynthesis affect plant growth and development throughout the life cycle, from germination to senescence. Sucrose is a major photosynthetic product and the predominant transported sugar \cite{1}. The loading of sucrose into the phloem is essential for \textit{Arabidopsis} growth, development and reproduction. The sucrose transporter \textit{SUC2} in \textit{Arabidopsis} is a phloem-specific SUC (SUCROSE TRANSPORTER), which is specifically expressed in companion cells \cite{2}. \textit{SUC2} has a central role in the loading of sucrose into the phloem and is essential for high-performance sucrose transport from source to sink organs in \textit{Arabidopsis} \cite{2–4}. The \textit{suc2} mutant exhibits stunted growth, delayed development and sterility. Further, these mutant plants accumulate massive amounts of starch and sucrose in the leaf blades and transport of sugar to sink organs, including roots, the shoot apex and inflorescences is blocked \cite{3}. Therefore, \textit{SUC2}, is a key sucrose transporter that mediates sucrose phloem transport and its loss-of-function leads to seriously delayed growth and thereby accumulated sucrose in source tissues. A previous report has shown that growth of sink roots may be promoted by exogenously provided glucose acting through either signaling or metabolism \cite{5}. However, whether and how plant root growth is mediated by the loading of sucrose into the phloem is largely unknown.

In plants, sucrose cannot be directly utilized, rather it is irreversibly hydrolyzed by invertase to the hexoses, glucose and fructose \cite{3}. Glucose is perceived and transduced through two principal mechanisms: indirect sensing via a variety of energy and metabolite sensors including [SNF1-RELATED PROTEIN KINASE1 (SnRK1) and target of rapamycin (TOR)] or direct sensing through glucose sensors \cite{6}. Glucose is indirectly sensed by the cellular energy sensor, SnRK1 (SNF1-RELATED KINASE1), which in turn directly interacts with and subsequently phosphorylates and promotes the degradation of a key ethylene signaling component, ETHYLENE-INSENSITIVE3 (EIN3), a plant-specific transcription factor \cite{6}. On the other hand, the direct glucose sensor, Hexokinase1 (HXK1), has both signaling and enzymatic functions \cite{7–8} and is the only known nuclear glucose sensor \cite{7}. Glucose indirectly and negatively controls the stability of the EIN3 transcriptional regulator via HXK1, whereas the mechanistic link between glucose sensing and EIN3 degradation has not to date been demonstrated \cite{9}. EIN3 stability rapidly increases with ethylene treatment, while in the absence of ethylene signaling, EIN3 is targeted via the SCF\textsubscript{EBF1/EBF2} and subsequently degraded via the 26S proteasome \cite{10–11}. Through binding to specific promoter element termed an EIN3 binding site (EBS), EIN3 modulates the expression of numerous downstream target genes.

In this study, we show that glucose decreases the stability of EIN3 by the activity of a glucose sensor \textit{HEXOKINASE 1}, which in turn elevates \textit{SUC2} function within source tissues, enhancing the loading of sucrose into the phloem in these tissues and thereby promoting the growth of sink organs, such as roots.
Results

**EIN3 and EIL1, acting downstream of HXK1, negatively regulate glucose signaling mediated-root growth**

Previous data has shown that root growth may be promoted through glucose [5]. Glucose is directly perceived by the function of HXK1, the only known nuclear glucose sensor. Therefore, we explored if HXK1 was involved in the regulation of root growth. Whereas the HXK1/GIN2 loss-of-function mutant, gin2-1, is in the Ler background, another loss-of-function mutant, hxk1-3, is in the Col-0 background. When *Arabidopsis* seedlings were grown on solid MS medium with 1% glucose, both the *hxk1*-3 and *gin2*-1 mutants exhibited a shorter primary root length, compared to wild-type seedlings (Fig 1A and 1B and S1 Fig). By contrast, corresponding seedlings expressing a cauliflower mosaic virus 35S (35S): *HXK1* transgene showed elongated primary root length compared to wild-type (Fig 1A and 1B). These findings indicate that *HXK1* promotes root growth.

Glucose indirectly and negatively controls the stability of the EIN3 transcriptional regulator through HXK1 function [7]. Therefore, we determined if EIN3/EIL1 is involved in the regulation of root growth. We employed *Arabidopsis* plants expressing a cauliflower mosaic virus 35S (35S): EIN3 transgene (S2A Fig) and loss-of-function mutations in either *EIN3* or the most closely related *EIN3* paralog, *EIN3-like 1 (EIL1)* [12], to investigate this pos1. When 35S:EIN3, *eil1*-3, *ein3/eil1, ein3-1* and wild-type seedlings were grown on solid MS medium without sugars for 5 days, their primary root length was not significantly different (S3 Fig). However, when these seedlings were grown on solid MS medium with 1% glucose for 5 days, the 35S: EIN3 line showed a shortened primary root length, compared to wild-type (Fig 1C and 1D and S4B and S4D Fig). In contrast, *eil1*-3, *ein3/eil1* and *ein3-1* seedlings showed elongated roots.

Fig 1. HXK1 is upstream of EIN3/EIL1 to positively regulate primary root growth. A, The image illustrating 5-day-old *Arabidopsis* 35S: HXK1, wild-type and *hxk1*-3 seedlings grown on MS medium with 1% glucose. Bar = 1.0 cm. B, Bar graph illustrating the differences in primary root length in (A). C, The image illustrating 5-day-old 35S:EIN3, *eil1*-3, *ein3/eil1, ein3-1* and wild-type seedlings grown on MS medium with 1% glucose. Bar = 1.0 cm. D, Bar graph illustrating the differences in primary root length in (C). E, The image illustrating 5-day-old *hxk1*-3, *ein3-1* and *hxk1/ein3* seedlings grown on MS medium with 1% glucose. Bar = 1.0 cm. F, Bar graph illustrating the differences in primary root length in (E). Error bars represent SD (n = 12). Student’s *t* test ([*P* < 0.01; [*P* < 0.05]).

https://doi.org/10.1371/journal.pgen.1010424.g001
relative to wild-type (Fig 1C and 1D and S4A and S4C Fig). These findings indicate that \textit{EIN3} inhibits root growth.

To determine whether \textit{EIN3} acts downstream of \textit{HXK1} in the regulation of root growth, we crossed \textit{hxk1-3} with \textit{ein3-1} plants. The \textit{ein3-1} line generated elongated primary roots, compared with wild-type plants (Fig 1E and 1F). Further, \textit{hxk1/ein3} mutant plants also generated elongated primary roots, similar to that of the \textit{ein3-1} mutant (Fig 1E and 1F). These findings indicate that \textit{EIN3} acts downstream of \textit{HXK1} in the regulation of root growth.

To investigate the link between glucose signaling and \textit{EIN3} dependent signaling, we generated a \textit{EIN3pro:EIN3-GFP/hxk1-3} line. A decrease of \textit{EIN3pro:EIN3-GFP} signaling in wild-type, but not in the \textit{hxk1-3} line was apparent post glucose application (Fig 2). Thus, increased GFP fluorescence was generated in the \textit{hxk1-3} relative to that than in the wild-type background. These findings indicate that glucose decreases \textit{EIN3} stability via the glucose signaling sensor, HXK1.

Taken together, our results show that \textit{HXK1} positively regulates and \textit{EIN3} negatively regulate primary root growth. Further, \textit{EIN3} act downstream of \textit{HXK1} in the regulation of glucose signaling that mediates root growth.

**\textbf{EIN3 negatively regulates glucose signaling mediated-expression of \textit{SUC2}}**

Chromatin immunoprecipitation sequence (ChIP-Seq) analysis of \textit{EIN3} [13] indicates that \textit{EIN3} may directly regulate expression of the \textit{Sucrose Transporter 4} (\textit{SUC4}) [14]. Further, \textit{SUC2}, but not \textit{SUC4}, has been proposed to function as a major transporter of sucrose loading into the phloem in \textit{Arabidopsis} [1]. Therefore, we determined if \textit{SUC2} is a downstream target gene of \textit{EIN3}.

We first performed quantitative (q)PCR experiments to monitor the transcript abundance of \textit{SUC2} in wild-type, \textit{ein3-1} and \textit{ein3/eil1} seedlings. When seedlings were grown on MS medium with 1% glucose for 5 days, the expression of \textit{SUC2} was significantly elevated in \textit{ein3-1} and \textit{ein3/eil1} plants relative to wild-type (Fig 3A). These qPCR results are consistent with previously reported data [15]. These findings indicate that \textit{EIN3} might repress \textit{SUC2} expression. Further, we monitored \textit{SUC2} expression over time in response to exogenously supplied glucose. The expression of \textit{SUC2} in wild-type, but not \textit{ein3/eil1} seedlings, was increased post
glucose application (Fig 3B), indicating that glucose signaling promoted 
SUC2 expression is partially dependent on EIN3 and EIL1.

To monitor EIN3-dependent transcriptional activity during *Arabidopsis* root growth, we employed a transgenic line containing the β-glucuronidase (*GUS* reporter gene) driven by five tandem repeats of the EBS driven by a minimal 35S promoter (5 × EIN3 (EIN3 binding site)-GUS) [16]. GUS staining intensity in roots decreased with increasing exogenous glucose (Fig 3C–3E), suggesting that glucose decreases the transcriptional activity of EIN3.

Together, these findings suggest that with increasing age, glucose accumulation decreases the transcriptional activity of EIN3, which may in turn elevate glucose signaling mediated-expression of SUC2.

### EIN3 directly binds to the promoter of SUC2 to repress SUC2 expression

Investigation of the DNA sequence comprising the promoter of SUC2 revealed a few putative EIN3 binding sites (EBSs) [16] (Fig 4A). To explore if EIN3 can interact with any of these EBSs, we employed a transgenic *Arabidopsis* line possessing a 35S-EIN3-GFP transgene in an ein3/eil1 genetic background (35S:EIN3-GFP/wild-type crossed with ein3/eil1 and subsequently 35S:EIN3-GFP/ein3/eil1 seedlings were identified by qPCR). We subsequently utilized...
chromatin immunoprecipitation (ChIP) analysis to determine whether the transcription factor EIN3 directly binds to the EBSs in the promoter sequence of \textit{SUC2}. Indeed, in the absence of glucose, EIN3 directly bound to one \textit{SUC2} promoter sequence (S2) but not to three other \textit{SUC2} promoter sequences (S1, S3 and S4) (Figs) (Fig 4B). Further, glucose treatment decreased the amount of amplified S2 sequence of the \textit{SUC2} promoter after an anti-GFP ChIP (Fig 4C), which may result from glucose decreasing EIN3 transcriptional activity (Fig 3C–3E).

To confirm EIN3 could directly bind to the S2 EBS within the promoter of \textit{SUC2} in vitro, electrophoretic mobility shift assay (EMSA) experiments were performed. Indeed, EIN3 bound exclusively to the labeled S2 EBS element \textit{in vitro} (Fig 4D). Excess unlabeled competitor DNA abolished this binding ability, implying EIN3 binding specificity (Fig 4D). Moreover, EIN3 binding to the labeled S2 EBS \textit{in vitro} was not abolished by the mS2 (mutant S2) DNA sequence \textit{in vitro} (Fig 4E). Further, transient expression data in \textit{N. benthamiana} showed that co-expressions of EIN3 decreased the expression of the reporter gene \textit{PGreenSUC2pro}: \textit{Luciferase (LUC)} (Fig 4F and 4G).

Taken together, these data indicate that EIN3 directly binds to the \textit{SUC2} promoter to suppress \textit{SUC2} expression.
SUC2 acts downstream of EIN3 to positively regulate root growth

Since EIN3 negatively regulates root growth (Fig 1) and EIN3 directly inhibits the expression of SUC2 (Figs 3 and 4), we therefore determined if SUC2 positively regulates root growth.

In short days, Arabidopsis plants expressing a cauliflower mosaic virus 35S (35S):SUC2 transgene (S2B Fig) exhibited elongated primary root length, compared to wild-type seedlings (Fig 5A and 5B and S5A and S5C Fig). By contrast, SUC2 loss-of-function homozygous suc2-5 (suc2-5 is a null allele) mutant seedlings exhibited severely retarded growth and development [17]. We observed that seedlings of suc2-5 showed a shortened primary root length, compared with wild-type (Fig 5C and 5D and S5B and S5D Fig). These findings indicate that SUC2 promotes root growth.

To determine whether EIN3 genetically acts upstream of SUC2 in the regulation of root growth, we crossed suc2-5 with ein3-1 seedlings. The ein3-1 line exhibited elongated primary roots, whereas suc2-5 seedlings produced a shortened primary root, compared with wild-type (Fig 5E and 5F). Further, ein3/suc2 mutant seedlings generated a shortened primary root, which was similar to that of the suc2-5 mutant (Fig 5E and 5F).

Together, these findings indicate that SUC2 genetically acts downstream of EIN3 to positively regulate root growth.

Sucrose phloem loading and accumulation in source and sink organs are regulated by an EIN3-SUC2 module

In Arabidopsis, SUC2 encodes a proton/sucrose symporter localized throughout collection and transport phloem within the vasculature and is thus essential for effective transport of sucrose.
from source to sink organs [4]. Therefore, we determined if the EIN3-SUC2 module mediates the loading of sucrose into the phloem.

A \[14C\] sucrose phloem loading assay showed that the rate of sucrose phloem loading in wild-type, ein3/eil1, suc2-5/+ , 35S:EIN3 and 35S:SUC2 vegetative leaves. These seedlings were grown in soil for 15 days under short days and normal light conditions, and vegetative leaves were immersed in the sucrose phloem loading buffer with \[14C\] sucrose and incubated for 3 hours. The amount of \[14C\] sucrose phloem loading was assayed via a scintillation counter. FW, Fresh weight. B, Levels of vegetative leaf sucrose (source tissue) in 9-, 11- and 13-day-old ein3/eil1, suc2-5/+ and wild-type plants grown in soil at exposure to light for 4 hours. Sucrose per gram fresh weight. C, Levels of root sucrose (sink tissue) in 9-, 11- and 13-day-old ein3/eil1, suc2-5/+ and wild-type plants grown in soil at exposure to light for 8 hours. Sucrose per gram fresh weight. Error bars represent SD (n = 3). Student’s t test (’’ P < 0.05; ’’’ P < 0.01). D and E, The expression of vegetative leaves in 5×EBS (EIN3 binding site)-GUS seedlings. Parafin section of leaf blades. Arrows indicate sieve tubes and companion cells in phloems in D. Bars = 100 μm in D and E. E is amplified in the pane of D.

https://doi.org/10.1371/journal.pgen.1010424.g006
To confirm sucrose phloem loading was linked to the EIN3-SUC2 module, we assayed the sucrose level of vegetative leaves (source organs) and roots (sink organs) in 9, 11 and 13 day-old wild-type, su2-5/+ and ein3/ei1 seedlings following exposure to light for 4 and 8 hours, respectively. In all seedlings, sucrose accumulation in both source and sink organs increased with increasing age (Fig 6B and 6C), suggesting total sucrose levels increase with tissue age under these conditions. Further, following exposure to light for 4 hours, sucrose accumulation over time was lower in the ein3/ei1 vegetative leaves and was higher in the su2-5/+ line relative to wild-type (Fig 6B), because the phloem loading rate of sucrose in vegetative leaves over time was higher in the ein3/ei1 line and lower in su2-5/+ plants relative to in wild-type (Fig 6A). As a result, following exposure to light for 8 hours, sucrose accumulation over time was higher in ein3/ei1 roots and was lower in the su2-5/+ line relative to wild-type (Fig 6C), which lead to elongated roots in ein3/ei1 seedlings and shortened roots in su2-5/+ mutant seedlings (Fig 5).

Together, these findings indicate that the EIN3-SUC2 module regulates sucrose accumulation in both vegetative leaves (source organs) and roots (sink organs) by mediating the loading of sucrose into the phloem. As a result, sink growth in roots is promoted by sucrose transport.

Discussion

Plants acquire their carbon from atmospheric carbon dioxide. During daytime plant sugar phosphates are largely generated via photosynthetic assimilation and are further metabolized to support plant growth and development [1, 2, 3]. Excess photoassimilates are stored mostly in a form of starch in Arabidopsis and at relatively low levels of sucrose, both starch and sucrose are in part metabolized via their turnover (synthesis and breakdown) during the day and are stored at night [1, 2, 3]. Under advantageous environmental conditions, enhanced photosynthesis, will in turn enhance sucrose phloem loading to maintain the balance between source and sink [1]. In whole-plant carbon partitioning, it is noteworthy that sucrose phloem loading in source leaf blades is a step.

SUC2 has a central role in the phloem loading of sucrose and is essential for high-performance sucrose transport from source to sink organs in Arabidopsis and presumably SUC2 homologs in most crop plants undertake similar key roles [2–4]. Recently, posttranscriptional modulation of SUC2 in coordination with a change in plant biomass has also been reported [18]. Also, transcriptional modulation of phloem-loading of SUC in sugar beet in response to exogenously fed sucrose has been investigated [19], however, the associated regulatory pathway remained unclear. In this context, signal cues and associated signaling components that directly regulate SUC2 transcription and SUC2 activity have not been identified.

Our findings found that EIN3, acting the downstream of HXK1, functions upstream of SUC2 in the modulation of glucose signaling mediated-root growth (Figs 1 and 5). Further, the transcription factor EIN3 directly binds to the SUC2 promoter to inhibit SUC2 expression (Fig 4) to suppress root growth (Figs 1 and 5). These components thus form the HXK1-|EIN3-| SUC2 module to promote sucrose phloem loading in source tissues and thereby raise sucrose levels in sink roots (Fig 6). As a result, glucose signaling mediated-root growth is facilitated (Figs 2 and 7). Further, EIN3 transcriptionally suppresses SUC2 activities, providing insight for a previous observation that down-regulation of sucrose phloem loading is linked to transcriptional regulation of SUC transporters [14]. Diverse environmental stresses also result in a change in SUC transcriptional levels [14]. However, increases in sucrose phloem loading may also result from post-transcriptional regulation [14], as a recent report has revealed posttranscriptional regulation of SUC2 in response to high-light acclimation [18].
In this study, HXK1 is a central factor, and this protein has both signaling and enzymatic functions [7–8]. HXK1 is a key integration point of various plant hormone or external stimuli that modulate plant growth and metabolism [7–8]. Further, HXK1 signaling and enzymatic activities directly or indirectly affect many genes associated with photosynthesis, including key components of the photosynthetic machinery as well as RUBISCO and PAL [7]. Therefore, the HXK1-EIN3-SUC2 module may regulate the formation of major photosynthetic products, sucrose, and sucrose transport from source leaves to sink roots (Fig 7). The resulting HXK1-EIN3-SUC2 module may promote sink root growth by the interaction of glucose and various plant hormone or external stimuli, as glucose and auxin signaling interaction control Arabidopsis sink root growth [5].

In the case of Beta vulgaris (Bv) SUC1 (a sugar beet leaf sucrose symporter), sucrose negatively and specifically regulates both sucrose transport activity and steady-state mRNA levels [19]. By contrast, in this work, with increasing age, sucrose/glucose accumulation was increased (Fig 6B and 6C), which in turn decreased EIN3 transcriptional activity (Fig 3C–3E) by decreasing EIN3 levels [9, 21] thereby raising Arabidopsis thaliana SUC2 (AtSUC2) dependent sucrose transport (Figs 6B and 6E and 7). Since Solanum tuberosum SUC4 (StSUC4) may play a role as a suppressor of StSUC1 [20], BvSUC1 and AtSUC2 may have opposite functions in the regulation of sucrose phloem loading.

However, when glucose accumulation in source leaves reaches a given threshold level of excess glucose, ethylene production is stimulated [15] and thus the level of EIN3 increases [9, 21], which in turn directly decreases SUC2 transport activity (Figs 4 and 7 and S6 Fig).

**Fig 7.** Model illustrating how glucose and ethylene signaling regulates sink root growth of Arabidopsis plants by controlling sucrose phloem loading. Sink root growth is promoted by the glucose signaling-HXK1-EIN3-SUC2 pathway, whereas sink root growth is inhibited by the ethylene signaling-EIN3-SUC2 pathway. The ethylene-EIN3 is ethylene canonical signal transduction pathway, which comprises the ethylene receptors ETR1, CTR1, EIN2, EIN3, and EIL1. The HXK1-EIN3 comprises that the regulation is via regulation of ubiquitination and proteolysis. Arrows and bars represent positive and negative regulation, respectively.

https://doi.org/10.1371/journal.pgen.1010424.g007
Consequently, sink root growth is delayed, as ethylene inhibits root growth [22]. Therefore, we speculate that when glucose accumulation reaches a given threshold level, excess glucose stimulates ethylene production, which in turn inhibits root growth by its canonical signal transduction pathway suppressing SUC2 activity (Fig 7). Thus, excess levels of glucose consequently inhibit root growth via increased ethylene levels. Indeed, following a 3.5% glucose application, ein3/eil1 plants had shortened root length (S7A and S7B Fig) and enhanced ethylene production (S7C Fig), relative to wild-type seedlings.

It has long been known that light promotes root growth. Light decreases EIN3 levels, whereas darkness elevates the accumulation of this transcriptional regulator [23]. When EIN3 levels are elevated [23] under darkness, SUC2 transcripts are reduced [24]. Thus, darkness raises EIN3 protein levels and consequently decreases SUC2 expression (Fig 3). As a result, this reduces sucrose phloem loading and thus enhances sucrose accumulation in source leaves. The resulting sucrose in sink roots is decreased, which in turn reduces root growth (S8 Fig).

Ethylene promotes hypocotyl growth [25], however, glucose and ethylene also have antagonistic functions in the regulation of plant growth [9]: hypocotyl length is decreased with an increasing sugar concentration [26]. Under basal conditions, HXK1 positively regulates hypocotyl growth [7]. However, under these conditions, EIN3 and SUC2 do not impact hypocotyl growth (S9 Fig). These findings suggest that the EIN3-SUC2 pathway may not be involved in the regulation of hypocotyl growth.

Materials and methods

Plant materials and growth conditions

The 5 × EBS-GUS/wild-type [16], ein3-1 and ein3/eil1 [21] mutants, 35S:SUC2 and suc2-5 [17] and 35S:EIN3 transgenic line [21] were all described previously.

The ein3/suc2 mutant was obtained from F2 plants (ein3-1/suc2-5) that had elongated hypocotyls on solid MS medium with 6.0 μm ACC [21] and have increased anthocyanin accumulation in the petiole [17]. The hxk1/ein3 mutant was obtained from F2 plants (hxk1-3/ein3-1) that had elongated hypocotyls on solid MS medium with 6.0 μm ACC [21] and have elongated primary roots grown on solid MS medium with 6% glucose [7]. Agrobacterium tumefaciens with pCB2004EIN3pro::EIN3-GFP were transformed into wild-type and hxk1-3 plants. These plants were further characterized by using qPCR. Transgenic plants were generated using the Agrobacterium tumefaciens-mediated floral dip method [22].

For simultaneous germination, seeds were treated with vernalization of 4˚C overnight and then sown on solid MS medium supplemented with 1% sucrose, pH 5.8 and 0.8% agar [22]. Seedlings grown on agar were maintained in a growth room under 16/8 h of light/dark cycle with cool white fluorescent light at 21 ± 2˚C for long-day conditions [27]. Unless otherwise noted, plants grown in soil were maintained in a controlled environment growth chamber under short-day conditions (10h light/14 h dark) and normal light conditions (130 μmol quanta PAR m⁻² s⁻¹) or high light conditions (230 μmol quanta PAR m⁻² s⁻¹) at 22˚C, as has been described by ref [28].

In all experiments, wild-type is the Col-0 background. Three biological replicates were performed with similar results, and error bars represent SD.

Assay of sugar metabolites

Plant Sucrose and glucose Assay Kit (Beijing Solarbio Science & Technology Co., Ltd, Cat#BC2465; http://www.solarbio.com/goods-9298.html) was used for assay of sugar metabolites. 0.1g mature leaf blades were ground into homogenate at 23˚C and then 0.5 ml extraction buffer added and centrifuged after extensive grinding and finally kept at 80˚C for 10 min and
shaken 3–5 times. After cooling these extracts were centrifuged at 4,000 g for 10 min, the supernatant was transferred to a fresh tube and 2 mg reagent 5 added to decolorize at 80 °C for 30 min. Then 0.5 ml extraction buffer was added, mixed and centrifuged at 4,000 g for 10 min. The supernatant was transferred to a fresh tube for visible light analysis.

Three centrifugal tubes per sample were used with 25 μL of sample and then standard product (reagent 1) and water were added, respectively. Fifteen μL of reagent 2 was added, mixed and boiled at 100 °C for 5 minutes. Subsequently, 175 μL of reagent 3 and 50 μL of reagent 4 were added, respectively. They were reacted in boiling water for 10 min, followed by measuring the light absorption value at 480 nm after cooling. The sucrose content was calculated accordingly, with respect to the sample standard absorption value and sample’s weight.

**Phloem loading of sucrose**

Nine-day-old seedlings grown on solid MS medium with sugars or without were used to assay phloem loading of sucrose. Following incubation in pretreatment solution (pH 5.7; liquid MS medium) for 40–50 mins, the cotyledons were incubated in the phloem loading buffer (pH 5.7; MS medium with 0.1–0.2% Sucrose) with [14C] Sucrose (0.50 μCi mL⁻¹) and then were cultivated for 2–3 hours. Following three washes in desorption solution (pH 5.7; liquid MS medium and 1% Sucrose), these seedlings were put in scintillation vials and 3.0 mL of scintillation cocktail was added. The amount of [14C] phloem loading of sucrose was analyzed on a scintillation counter and expressed as cpm mg⁻¹ fresh weight.

**Confocal laser scanning microscope for GFP imaging**

Subcellular localization of EIN3pro:EIN3-Green Fluorescent Protein (GFP) fusion proteins was examined in the epidermis of 5-day-old transgenic cotyledons harbouring EIN3pro:EIN3:GFP.

These cotyledons were observed with a confocal laser scanning microscope (LSM 900; Zeiss) for GFP. All images were gained in the multitrack pattern and analyzed by using LSMImage-examiner software (Zeiss). The data were exported as eight-bit TIFF files and generated by using Adobe Photoshop 5.5 (Adobe Systems).

**Ethylene assay**

These experiments were completed on 8-day-old wild-type and ein3/eil1 plants by using a gas chromatograph equipped with a flame ionization detector [Echrom Technologies (Shanghai) Ltd Co], as was described [15].

**Plasmid constructs**

For generating pCB2004EIN3pro::EIN3-GFP plasmids, the relevant primers are F-ggg gac aag ttt gta caa aaa agc agg ct AAT GTG TGG AAA CAT GGA TTT CT, R-ggg gac cac tttg tac aag aaa gct ggg t TTAGAACCATATGGGATA CAT; F-ggg gac aag ttt gta caa aaa agc agg ct cat att tgc atc tct cta tta gt; R-ggg gac cac tttg tac aag aaa gct ggg t atc gta ttt tta atc act cct aaa c were used.

**Quantitative PCR**

Total RNA was extracted from tissues indicated in the figures by the TRIZOL reagent (Invitrogen), as has been described by refs [27–29]. SYBR green was used to monitor the kinetics of PCR product in real-time RT-PCR, as has been described by ref [27]. Primer pairs of SUC2
(5'-ATC CAA TGG AGA AAG CTG CAA A-3' and 5'-ACC ACA GAG CCA AAT CAG GAA-3') were utilized. Primer pairs of EIN3 have been described by ref [29].

**GUS assay and histochemical analysis of GUS activity**

GUS assays were described previously [30–31]. In details, by using a mix buffer (0.4 mM of K3Fe(CN)6/K4Fe(CN)6, 1 mM X-gluc, 60 mM NaPO4 buffer, and 0.1% (v/v) Triton X-100), Transgenic plants were stained, followed by incubating at 37°C for 6–10 h. With GUS staining, chlorophyll was removed by using 30, 60, 70, 90 and 100% ethanol about 40 min for every process. Samples were observed by using stereoscopic microscope (SZX16, OLYMPUS). Histochemical analysis of GUS activity was undertaken as described previously [16]. In details, stained seedlings were transferred to microfuge tubes, which had a solution of 10 mM EDTA, 100 mM Na phosphate buffer, 2 mM potassium ferricyanide, 0.1% Triton X-100, pH 7.0, 1 mg/mL 5-bromo-4-chloro-3-indolyl-b- D -glucuronide and 2mM potassium ferrocyanide at 36–38°C over-night. These stained seedlings were cleared with an ethanol series.

**Transactivation assay**

To examine EIN3 activity, we performed the below experiments. To generate proSUC2-LUC, the promoter was PCR amplified with primers proSUC2-F and proSUC2-R (ProSUC2-F- ttctgcaagccggggatatcactaggtagttgtaatgccgccggtatcactaggtagttgtaatgc; ProSUC2-R- tgtttttggcgtcttccatggatttgacaaacaagaaaagtaagaa) for the gnomic DNA of Arabidopsis and inserted into the cloning site of the pGreen0800-LUC vector.

The MproSUC2-LUC construct containing mutations in the S2 sequence of the SUC2 promoter was generated using overlap extension PCR with primers (pGreen-SUC2pF: ttctgcaagccggggatatcactaggtagttgtaatgccgccggtatcactaggtagttgtaatgc, R-M-EMSA-SUC2: acactatatgaaatattgaaaCCatattaatCCCaaatataaataaatagttg, pGreen-SUC2pR: tgtttttggcgtcttccatggatttgacaaacaagaaaagtaagaa, M-EMSA-SUC2: caccattagttatatattGGGatattaatGcGtttcaatttacaaggtt) and inserted into pGreen0800-LUC vector. Two fragments of MproSUC2 were combined into a integrated fragments of MproSUC2 by using primers (pGreen-SUC2pF: ttctgcaagccggggatatcactaggtagttgtaatgc, pGreen-SUC2pR: tgtttttggcgtcttccatggatttgacaaacaagaaaagtaagaa).

The Agrobacterium with the effector or reporter constructs were coincubated for 3 hour and then infiltrated into 20-day-old N. Benthamiana leaf blades. Leaf blades of these seedlings were incubated under middle light conditions (160 μmol quanta PAR m⁻² s⁻¹) for 2–4 days after infiltration. The firefly LUC activities were photographed after spraying with 1 mM luciferin. To determine the dualluciferase, activities of firefly luciferase and Renilla luciferase were measured, as has been previously described [32]. For LUC images, confocal laser scanning microscopy was performed by using an Epi-fluorescence microscopy (Olympus 80i, Olympus Corporation, Japan). The leaves were infiltrated with luciferin solution, and imaged by using a Tanon 5200S Luminescent Imaging Workstation.

**Protein expression and purification**

The plasmid pGEX-5X-1 for EIN3 was utilized. The coding sequence of EIN3 was amplified by the primer pair (5'-GGATCC ATGATGTTTA ATGAGATGGG -3' and 5'-CTCGAGTGGTCTGTTTGGGAT-3') and cloned into the BamH1 and XhoI restriction sites of pGEX-5X-1. Recombinant glutathione S-transferase binding protein (GST)-tagged EIN3 was extracted from transformed E. coli (Rosetta2) after 10 h of incubation at 16°C following induction with 10 μM isopropyl-β-D-1-thiogalactopyranoside. These recombinant proteins were purified using GST-agarose affinity.
ChIP-PCR

The transgenic lines containing 35S:EIN3-GFP/ein3/eil1 were utilized. ChIP was performed with seedlings [28]. Leaf blades were incubated in buffer (1.0 mM PMSF, 0.5 M sucrose, 1 mM EDTA, 10–12 mM Tris, pH 8.0, and 1% formaldehyde) under vacuum for 15–20 min for crosslinking the chromatin. Then, 0.1 M Gly was placed in the mixture, incubating for an additional 5 min for terminating the reaction. Leaf blades were placed and ground in liquid nitrogen and re-suspended to lysis buffer (150 mM NaCl, 1 mM EDTA, 0.1% SDS, 0.1% deoxycholate, 50 mM HEPES, pH 7.5, 1% Triton X-100, 10 mM Na-butyrate, 1 mM PMSF, and 13 complete protease inhibitor [Roche]). Chromatin was sheared to about 200 to 500 bp fragments via sonication followed by centrifugation. At 4°C, supernatants were precleared under protein G agarose beads for 1–1.5 h. Input material (supernatant containing chromatin) was used for immunoprecipitation with anti-GFP antibody. Anti-GFP antibody bound to EIN3-FLAG or GFP-chromatin complexes was incubated with protein G agarose beads for 1–1.5 h at 4–6°C and then washed several times and eluted with elution buffer. Input and immunoprecipitated chromatin were uncross-linked for 6 h at 6°C with 5 M NaCl. Immunoprecipitated chromatin and input were used for PCR analysis. The ChIP DNA products were analyzed using two pairs of primers that were synthesized to amplify ~200 bp DNA fragments in the promoter region of SUC2. Primer sequences (CHIP-SUC2-1F: atgcatgcataatgcacaccatttatgt, CHIP-SUC2-1R: actgcattttcacactcctccactgt; CHIP-SUC1-1F: Acagtgggagaggtgaaaatg cag CHIP-SUC1-1R: gcgagtggcactagaataattttcggtgat; SUC2 3F: GTTTTTTCGTCTTTTCATGGGCG SUC2 3R: ACTATGAGGAGACGTATG; CHIP-SUC2-4F: actttgcatgttgcctgagat CHIP-- SUC2-4R: tgcattttcatcataatcacttttga) were used.

Electrophoretic Mobility Shift Assay (EMSA)

The electrophoresis mobility shift assay (EMSA) was performed by using the LightShift Chemiluminescent EMSA Kit (Pierce, 20148) according to the manufacturer’s instructions. The biotin-labeled SUC2-S2 DNA fragments and its mutated derivatives (EMSA-SUC2: caccattatgtttatatcataatcattcataatctcctacagtgt R-EMSA-SUC2: S-acacttagaattaattgaaatgtatatgatcattcatgattgttggcctgagat agaggaatcataaatggtg; SUC2 3F: GTTTTTTCGTCTTTTCATGGGCG SUC2 3R: ACTATGAGGAGACGTATG; CHIP-SUC2-4F: actttgcatgttgcctgagat CHIP-- SUC2-4R: tgcattttcatcataatcacttttga) were synthesized, annealed and used as probes. The corresponding biotin unlabeled DNA fragments were utilized as competitor sequences in the assay. The probes were incubated with EIN3 fusion protein at room temperature for 20 mins in a binding buffer (56 concentrations: 50 mM HEPES-KOH [pH 7.5], 375 mM KCl, 6.25 mM MgCl, 1 mM DTT, 0.5 mg/mL BSA, Glycerol 25%). Each 20 ml binding reaction contained 25 fmol Biotin-probe, 6 mg protein, and 1 mg Poly (dIdC) to minimize non-specific interactions. The reaction products were analyzed by 6.5% native polyacrylamide gel electrophoresis. This experimental process has been described previously [30,33].

Supporting information

S1 Fig. The gin2-1 mutant had shortened primary roots. (PPTX)

S2 Fig. 35S:EIN3 and 35S:SUC2 seedlings were identified by using qPCR. (PPTX)
S3 Fig. Primary root length of 35S:EIN3, eil1-3, ein3/eil1, wild-type (Col-0), and ein3-1 seedlings under no sugar conditions. (PPTX)

S4 Fig. EIN3/EIL1 negatively regulates primary root growth. (PPTX)

S5 Fig. SUC2 positively regulates primary root growth. (PPTX)

S6 Fig. Sucrose phloem loading of 35S:SUC2 mature leaves with ACC treatment. (PPTX)

S7 Fig. The ein3/eil1 root growth under excess glucose levels. (PPTX)

S8 Fig. The ein3/eil1 mutant has shortened primary root length in the dark. (PPTX)

S9 Fig. The HXK1-EIN3-SUC2 pathway may be involved in the regulation of hypocotyl growth. (PPTX)

Acknowledgments

The hxxk1-3 seeds were obtained from the ABRC (Ohio State University). The 35S:EIN3, ein3/eil1, eil1-3 and ein3-1 seeds were kindly provided by Prof H. W. Guo (South University of Science and Technology of China, China). pGreen0800-LUC vector and 5×EBS:GUS seeds were kindly provided by Prof Ziqiang Zhu (Nanjing normal university, Nanjing, China). The 35S:SUC2 and suc2-5 (SALK_087046) seeds were kindly provided by Prof D Liu (Tsinghua University, China). The gin2-1 (CS6383) and 35S:HXK1 seeds were kindly provided by Prof R Scott Poethig (University of Pennsylvania, Philadelphia, United States).

Author Contributions

Conceptualization: Lai-Sheng Meng.

Data curation: Qin-Xin Bao.

Formal analysis: Chen Tong, Lai-Sheng Meng.

Funding acquisition: Hu-hui Chen.

Investigation: Chen Tong, Cong Li, Xiao-Ying Cao, Xu-Dong Sun, Qin-Xin Bao, Xin-Rong Mu, Chang-Yue Liu, Lai-Sheng Meng.

Project administration: Lai-Sheng Meng.

Resources: Xiao-Ying Cao.

Software: Xiao-Ying Cao, Xu-Dong Sun.

Supervision: Lai-Sheng Meng.

Writing – original draft: Lai-Sheng Meng.

Writing – review & editing: Gary J. Loake, Lai-Sheng Meng.
References

1. Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, et al. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science. 2012; 335: 207–211. https://doi.org/10.1126/science.1213351 PMID: 22157085

2. Stadler R, Sauer N. The Arabidopsis thaliana AtSUC2 Gene is Specifically Expressed in Companion Cells. Plant Biology. 2015; 109(4): 299–306.

3. Gottwald JR, Krysan PJ, Young JC, Evert RF, Sussman MR. Genetic evidence for the in planta role of phloem-specific plasma membrane sucrose transporters. Proceedings of the National Academy of Sciences. 2000; 97: 13979–13984. https://doi.org/10.1073/pnas.250473797 PMID: 11087840

4. Srivastava AC, Ganesan S, Iismaïl IO, Ayre BG. Functional Characterization of the Arabidopsis AtSUC2 Sucrose/H+ Symporter by Tissue-Specific Complementation Reveals an Essential Role in Phloem Loading But Not in Long-Distance Transport. Plant Physiology. 2008; 148(1): 200–211.

5. Mishra BS, Singh M, Aggrawal P, Laxmi A. Glucose and Auxin Signaling Interaction in Controlling Arabidopsis thaliana Seedlings Root Growth and Development. PLoS ONE. 2009; 4(2).

6. Kim GD, Cho YH & Yoo SD (2017). Regulatory Functions of Cellular Energy Sensor SNF1-Related Kinase1 for Leaf Senescence Delay through ETHYLENE-INSENSITIVE3 Repression. Scientific Reports | 7: 3193 | https://doi.org/10.1038/s41598-017-03506-1 PMID: 28600557

7. Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, et al. Role of the Arabidopsis glucose sensor HXK1 in nutrient, light, and hormonal signalling. Science. 2003; 300: 332–336.

8. Cho YH, Yoo SD, Sheen J. Regulatory Functions of Nuclear Hexokinase1 Complex in Glucose Signaling. Cell. 2006; 127: 579–589. https://doi.org/10.1016/j.cell.2006.09.028 PMID: 17081979

9. Yanagisawa S, Yoo SD, Sheen J. Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. Nature. 2003; 425: 521–525. https://doi.org/10.1038/nature01984 PMID: 14523448

10. Guo H, Ecker JR. Plant responses to ethylene gas are mediated by SCF(EBF1/EBF2)-dependent proteolysis of EIN3 transcription factor. Cell. 2003; 115: 667–677. https://doi.org/10.1016/s0092-8674(03)00969-3 PMID: 14675532

11. Grava S, Potuschak T, Yanagisawa S, Parmentier Y, Lechner E, Genschik P, et al. EIN3-dependent regulation of plant ethylene hormone signaling by two Arabidopsis F box proteins: EBF1 and EBF2. Cell. 2003; 115: 679–689.

12. Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Ecker JR. Activation of the ethylene gas response pathway in Arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. Cell. 1997; 89: 1133–1144.

13. Chang KN, Zhong S, Weirauch MT, Hon G, Pelizzola M, Li H, et al. Temporal transcriptional response to ethylene gas drives growth hormone cross-regulation in Arabidopsis. eLife. 2013; 2.

14. Xu Q, Chen S, Ren Y. Regulation of Sucrose Transporters and Phloem Loading in Response to Environmental Cues. Plant physiology. 2018; 176:930–945. https://doi.org/10.1104/pp.17.01088 PMID: 29158330

15. Jeong SW, Das PK, Jeoung SC, Song JY, Lee HK, Kim YK, et al. Ethylene suppression of sugar-induced anthocyanin pigmentation in Arabidopsis. Plant Physiology. 2010; 154(3): 1514–1531.

16. Li Z, Peng J, Wen X, Guo H, et al. ETHYLENE-INSENSITIVE3 Is a Senescence-Associated Gene That Accelerates Age-Dependent Leaf Senescence by Directly Repressing miR164 Transcription in Arabidopsis. The Plant Cell. 2013; 25: 3311–3328.

17. Lei M, Liu Y, Zhang B, Zhao Y, Wang X, Zhou Y, et al. Genetic and genomic evidence that sucrose is a global regulator of plant responses to phosphate starvation in Arabidopsis. Plant Physiology. 2011; 156: 1116–1130.

18. Xu Q, Yin S, Ma Y, Song M, Liesche J. Carbon export from leaves is controlled via ubiquitination and phosphorylation of sucrose transporter SUC2. Proceedings of the National Academy of Sciences. 2020; 117(11): 201912754. https://doi.org/10.1073/pnas.1912754117 PMID: 32123097

19. Vaughn MW, Harrington GN, Bush DR. Sucrose-mediated transcriptional regulation of sucrose sympporter activity in the phloem. Proceedings of the National Academy of Sciences. 2002; 99(16): 10876–10880. https://doi.org/10.1073/pnas.172198599 PMID: 12149483

20. Riesmeier JW, Frommer. WB. Einfluß der U¨ber exression von Saccharo setransportern auf das Blu¨h- verhalten von Pflanzen. German Patent. 1994; No. P 44 39 748.8.

21. An F, Zhao Q, Ji Y, Li W, Jiang Z, Yu X, et al. Ethylene-induced stabilization of ETHYLENE INSENSITIVE3 and EIN3-LIKE1 is mediated by proteasomal degradation of EIN3 binding F-box 1 and 2 that requires EIN2 in Arabidopsis. The Plant Cell. 2017; 22(7): 2384–2401.

22. Ma B, Yin C-C, He S-J, Lu X, Zhang W-K, et al. (2014) Ethylene-Induced Inhibition of Root Growth Requires Abscisic Acid Function in Rice (Oryza sativa L.) Seedlings. PLoS Genet 10(10): e1004701. https://doi.org/10.1371/journal.pgen.1004701 PMID: 25330236
23. Zhong S., Zhao M., Shi T., Shi H., An F., Zhao Q., and Guo H. (2009). EIN3/EIL1 cooperate with PIF1 to prevent photo-oxidation and to promote greening of Arabidopsis seedlings. Proc. Natl. Acad. Sci. USA 106: 21431–21436. https://doi.org/10.1073/pnas.0907670106 PMID: 19948955

24. Dijkwel et al (1997). Sucrose Control of Phytochrome A Signaling in Arabidopsis. The Plant Cell, 9, 583–595. https://doi.org/10.1105/tpc.9.4.583 PMID: 9144963

25. Yu Y, Wang J, Zhang Z, Quan R, Zhang H, et al. (2013) Ethylene Promotes Hypocotyl Growth and HY5 Degradation by Enhancing the Movement of COP1 to the Nucleus in the Light. PLoS Genet 9(12): e1004025. https://doi.org/10.1371/journal.pgen.1004025 PMID: 24348273

26. Kühn C., Franceschi V.R., Schulz A., Lemoine R., and Frommer W.B. (1997). Macromolecular Trafficking Indicated by Localization and Turnover of Sucrose Transporters in Enucleate Sieve Elements. Science 275: 1298–1300. https://doi.org/10.1126/science.275.5304.1298 PMID: 9036853

27. Meng LS, Yao SQ. Transcription co-activator Arabidopsis ANGIUSTIFOLIA3 (AN3) regulates water-use efficiency and drought tolerance by modulating stomatal density and improving root architecture by the transrepression of YODA (YDA). Plant Biotechnology Journal. 2015; 13: 893–902.

28. Meng LS, Wei ZQ, Cao XY, Tong C, Lv MJ, Yu F, et al. Cytosolic Invertase Mediated Root Growth Is Feedback-Regulated by A Glucose-Dependent Signaling Loop. Plant Physiology. 2020; 84, 895–908. https://doi.org/10.1104/pp.20.00778 PMID: 32820066

29. Meng LS, Xu MK, Wan W, Yu F, Li C, Wang JY, et al. Sucrose Signaling Regulates Anthocyanin Biosynthesis Through a MAPK Cascade in Arabidopsis thaliana. Genetics. 2018; 210(2): 607–619.

30. Meng LS, Wang YB, Yao SQ, Liu A. Arabidopsis AINTEGUMENTA (ANT) Mediates Salt Tolerance by Trans-repressing SCABP8. Journal of Cell Science. 2015; 128: 2919–2927.

31. Meng LS, Li YQ, Liu MQ, Jiang JH. The Arabidopsis ANGIUSTIFOLIA3- YODA Gene Cascade Induces Anthocyanin Accumulation by Regulating Sucrose Levels. Frontiers in Plant Science. 2016; 7: 1728.

32. Li J, Li G, Gao S, Martinez C, He G, Zhou Z, et al. Arabidopsis transcription factor ELONGATED HYPO-COTYL5 plays a role in the feedback regulation of phytochrome A signaling. The Plant Cell. 2010; 22: 3634–3649.

33. Meng LS. The ARF2-ANT-COR15A gene cascade regulates ABA signaling-mediated resistance of large seeds to drought in Arabidopsis. Journal of Cell Science. 2015; 128: 3922–3932.