# Low Glucose Uncouples Hexokinase1-Dependent Sugar Signaling from Stress and Defense Hormone Abscisic Acid and $C_2H_4$ Responses in Arabidopsis[1][C][W]

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All living organisms operate a variety of metabolic pathways that enable them to be self-sustainable with basic nutrients. To maintain the balance of metabolite and energy levels, organisms have developed sophisticated sensing and signaling mechanisms that underlie the physiological responses to cell metabolite fluctuations.

In plants including Arabidopsis (*Arabidopsis thaliana*), Glc has a regulatory role in many important plant developmental processes such as germination, seedling development, root, stem, and shoot growth, photosynthesis, carbon and nitrogen metabolism, flowering, and senescence (Rolland et al., 2006). Hexokinase (HXK) is an evolutionarily conserved Glc sensor in many organisms. In plants, it has dual functions in Glc metabolism as well as Glc sensing and signaling that modulate many physiological processes by integrating nutrient, light, and hormone signals (Moore et al., 2003; Rolland et al., 2006). The HXK1 loss-of-function *glucose insensitive2-1* (*gin2-1*) and *gin2-2* have been isolated through a mutant screen based on a high Glc (6%) repression assay, displaying inhibition of cotyledon expansion, chlorophyll accumulation, and shoot growth at the early stage of seedling development (Moore et al., 2003). Biochemical and physiological characterization of the mutants revealed that HXK1 can modulate both growth-promoting and growth-inhibiting responses depending on the growth conditions. The role of HXK1 in sugar signaling is independent of its metabolic function, as catalytically inactive mutations of HXK1 (S177A and G104D) complement *gin2-1* and restore Glc-dependent phenotypes (Moore et al., 2003).

As mentioned above, Arabidopsis seedling establishment is prominently influenced by high Glc in the presence of Murashige and Skoog (MS) medium (Fig. 1A). The Glc-dependent phenotypes are not caused by osmotic effects of the high sugar level in the medium since an equal concentration of mannitol cannot mimic the same sugar effect (Fig. 1C). Again, complementation with wild-type HXK1 or the catalytically inactive mutants HXK1S177A or G104D restore wild-type responses in *gin2* (Fig. 1A).

Genetic analysis of the Glc response has demonstrated complex interactions among Glc and other plant hormones such as abscisic acid (ABA) and ethylene (Zhou et al., 1998; Arenas-Huertero et al., 2000; Cheng et al., 2002; Arroyo et al., 2003; Leon and Sheen, 2003; Rook and Bevan, 2003; Lin et al., 2007). For example, both *gin1*, an allele of *aba deficient2* (*aba2*) in the ABA pathway, and *gin4*, an allele of *constitutive triple response1* (*ctr1*) in the ethylene pathway, exhibit the insensitive phenotype in the presence of high Glc (Fig. 1B). Similar to *gin2*, both *gin1* (*gin1-3*) and *ctr1* (*ctr1-1*) develop green cotyledons on high Glc. Since GIN1/ABA2 encodes a short-chain dehydrogenase/reductase involved in ABA biosynthesis and CTR1/GIN4 is a negative regulator of ethylene signaling, high Glc signaling seems to require ABA biosynthesis and is antagonized by ethylene signaling.

Although sugar repression assays based on seedling phenotypes have been widely applied in genetic screens, the use of high concentrations of sugar has raised concerns about physiological relevance, significance, and specificity (Leon and Sheen, 2003; Rook and Bevan, 2003). The high osmolarity caused by high concentration of Glc and the high concentration of nitrate in the MS medium appear to complicate the sugar responses (Moore et al., 2003; Cho et al., 2006). To evaluate Glc responses that are more physiologically relevant, we have further tested seedling growth responses at a lower Glc concentration (2%). Seedling

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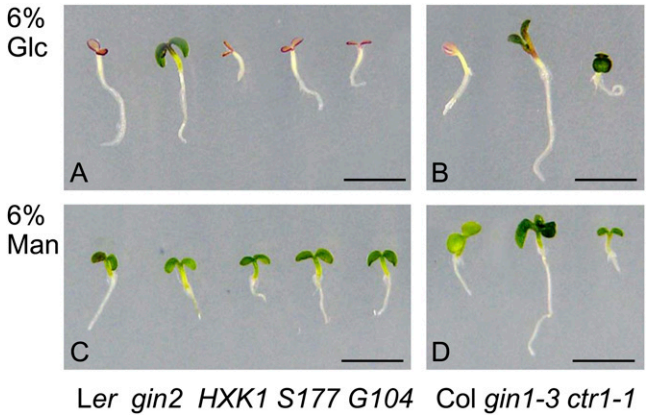


**Figure 1.** Glc-insensitive phenotype of *gin2* displayed on a high 6% Glc MS medium. A, The *gin2* mutant (5 d) shows insensitivity to Glc-mediated developmental arrest on a 6% Glc plate (1× MS) under constant light. B, The *gin1* and *ctr1* mutants show an insensitive phenotype to 6% Glc (1× MS). C and D, Similar seedling growth was observed on 6% mannitol (Man) MS medium. Scale bars, 5 mm. [See online article for color version of this figure.]

developmental arrest, typically manifested by inhibition of chloroplast differentiation and chlorophyll accumulation, has been observed similarly on 2% Glc without MS medium (Fig. 2A). As in 6% Glc MS medium, *gin2* still shows Glc insensitivity, and wild-type HXK1 or mutant HXK1 complements *gin2* (Figs. 1A and 2A). These responses are not induced by 2% mannitol, supporting sugar signaling specificity (Fig. 2C). The results indicate that the high Glc requirement to observe the seedling developmental arrest is due to the high nitrate content in MS, which counteracts sugar signaling in plants (Stitt, 1999; Stitt and Krapp, 1999; Moore et al., 2003; Cho et al., 2007). As expected, the low Glc-dependent seedling phenotypes were not observed in the presence of nitrate as $KNO_3$ and $NH_4NO_3$ in the growth medium (Supplemental Fig. S1).

Interestingly, ABA and ethylene signaling appears not to interfere with Glc signaling any longer at a low concentration. For example, both *gin1/aba2* and *ctr1/gin4* show Glc sensitivity on 2% Glc without MS medium (Fig. 2, A and B). These observations implicate that the early seedling developmental arrest is genuinely governed by HXK1-dependent/specific Glc signaling (Figs. 1 and 2). Thus, high Glc concentration may cause HXK1-independent signaling that can interact with plant stress/defense hormone signaling and complicate the sugar response (Leon and Sheen, 2003; Rook and Bevan, 2003; Rolland et al., 2006). These results suggest that sugar signaling specificity can be uncoupled from cross talk with other stress/defense hormone responses in *gin* mutants for the early seedling developmental arrest phenotype.

To gain molecular insight into the low Glc signaling response, we have examined the expression of marker genes for Glc signaling (*CHLOROPHYLL A/B BINDING PROTEIN2* [*CAB2*]), ABA (*ABA INSENSITIVE4* [*ABI4*] and *ABI5*), and ethylene signaling (*ETHYLENE RESPONSE FACTOR1* [*ERF1*]; Finkelstein et al., 1998; Finkelstein and Lynch, 2000; Yanagisawa et al., 2003). Gene expression has been monitored using quantitative reverse transcriptase-dependent PCR in 3-d-old wild-type (Landsberg *erecta* or Columbia-0), *gin2*, *gin1*, and *ctr1* seedlings growing on 2% Glc medium without MS (Supplemental Table S1). Transcript levels were normalized to those of seedlings growing on 2% mannitol medium without MS that served as an osmotic control for the experiment. *CAB2* expression is reduced by low Glc in wild type but not in *gin2*, supporting dependency of Glc signaling on HXK1 function (Fig. 3A). *ERF1* is repressed in wild type by low Glc, but enhanced in *gin2* (Fig. 3B). Both *CAB2* and *ERF1* expression is suppressed in *gin1* and *ctr1* (Fig. 3, A and B). *ABI4* and *ABI5* expression is greatly enhanced in wild type (Fig. 3, C and D) by Glc, but significantly reduced in *gin2*. Furthermore, *ABI4* and *ABI5* expression is suppressed in the presence of low Glc in *gin1* and *ctr1* (Fig. 3, C and D). Since *ABI4* and *ABI5* expression is induced in *gin2* by high Glc (data not shown), it appears that the high Glc MS medium condition activates additional signaling pathways to the HXK1-dependent Glc signaling. Based on the analysis in *gin1* and *ctr1* at the low Glc condition, the *ABI4* and *ABI5* gene expression regulation appears to be still under the regulation of ABA and ethylene signaling.

Here, we have shown that high 6% Glc modulates early seedling development through HXK1 function in the presence of MS. The high Glc signaling interacts positively with ABA, but negatively with ethylene signaling. Low 2% Glc is also sufficiently potent to induce HXK1-dependent seedling developmental arrest. However, ABA synthesis and ethylene signaling seem to be dispensable in low Glc repression despite that the regulation of *ABI4* and *ABI5* expression is still GIN1 or CTR1 dependent. Taken together, it becomes clear that HXK1-dependent Glc signaling uncoupled

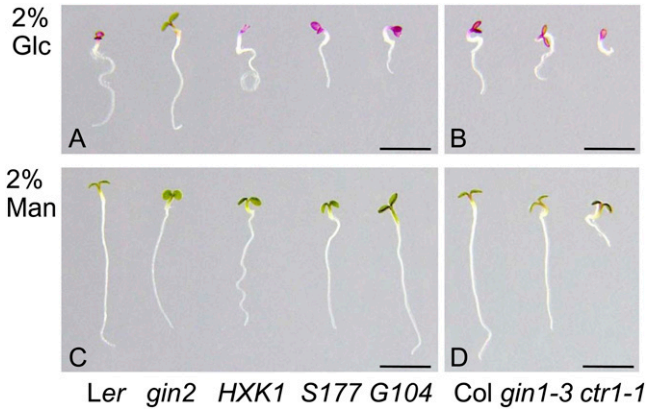


**Figure 2.** Glc-insensitive phenotype of *gin2* displayed on a low 2% Glc medium. A, The *gin2* mutant (3 d) shows Glc insensitivity on a 2% Glc plate (without MS). B, The *gin1* and *ctr1* mutants show Glc-sensitive phenotype on 2% Glc medium (without MS). C and D, Similar seedling growth observed on 2% mannitol (Man; without MS). Scale bars, 5 mm.

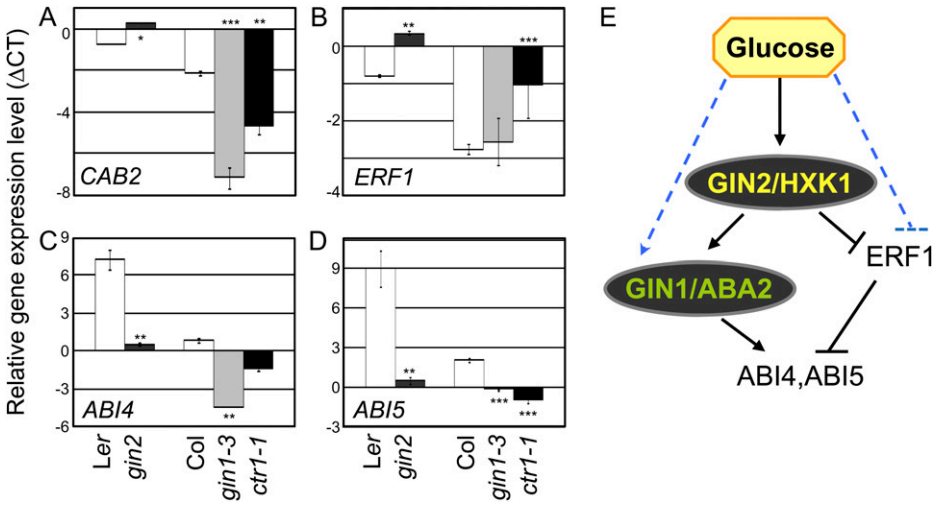


**Figure 3.** Low Glc-dependent gene regulation. A, *gin2* mutants are insensitive to Glc repression of *CAB2* (At1g29920). B, *ERF1* (At3g23240) expression is repressed by low Glc signaling in wild type (Landsberg *erecta*, Columbia), *gin1*, and *ctr1*, but not in *gin2*. C and D, *ABI4* (At2g40220) and *ABI5* (At2g36270) expression is induced by low Glc signaling in wild type, but not in *gin1*, *gin2*, and *ctr1*. Values are normalized based on those obtained from seedlings grown on mannitol and means of triplicate measurements with error bars are shown. The experiments were repeated twice with similar results. Asterisks over bars indicate differences between wild type and mutants with statistical significance at * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ (*t* test). E, A model of HXK1-dependent (solid line) pathways in high and low Glc signaling and HXK1-independent (dashed line) pathways in high Glc signaling. [See online article for color version of this figure.]

from other plant stress and defense hormone signaling has a central regulatory role in early seedling establishment (Fig. 3E).

Further characterization of sugar response mutants for low Glc repression would provide new insights into sugar sensing and signaling events underlying the physiological responses with respect to internal and external environmental changes that perturb cellular metabolic balances in higher plants. As plant biomass improvement becomes an important task of plant science, understanding of sugar sensing and signaling that promotes as well as limits plant growth and development in different conditions will provide valuable information contributing to plant-based environmental restoration and biorenewable energy production.

## Supplemental Data

The following materials are available in the online version of this article.

**Supplemental Figure S1.** Low Glc-insensitive phenotype of Arabidopsis seedlings in the presence of nitrogen.

**Supplemental Table S1.** Oligonucleotide sequences for quantitative reverse transcriptase-dependent PCR.

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