Supporting Online Materials

Materials and methods

Mutant selection and Characterization. Glucose insensitive (*gin*) mutants (L*er* ecotype) were screened from 67,100 M2 seeds mutagenized by EMS as previously described (*S1*). Wild type (WT) but not *gin2* mutant seedlings showed typical developmental arrest on 6% glucose MS medium. For glucose repression assays, seedlings were grown on 6% glucose (Glc) or 6% mannitol (Man) MS medium for 5 days under constant light or on agar plates without or with 2% glucose (G) or 20 mM KNO_3 (N). Endogenous HXK1 proteins in the WT, *gin2-1* and *gin2-2* extracts and HXK proteins in the WT and in transgenic *35S::HXK1* and *35S::HXK2* were detected by protein blot analysis using an anti-HXK antibody (*S2*). Higher amounts of protein were loaded to detect endogenous HXK proteins. Protein extracts from the *35S::HXK1* transgenic plants were diluted 10-fold before sample loading.

WT and *gin2-1* plants were also grown on soil at 23ºC for 18 to 25 days with different light intensity under a 16 h photoperiod. Low light (LL) is 80 μmol/m^2/s and high light (HL) is 240 μmol/m^2/s. WT and *gin2-1* flowering plants were grown on soil at 23ºC for 30 days with the indicated light intensity. Leaf cross sections were carried as described (*S3*). For low nutrient and low light assays, seedlings were grown on 0.2% Glc and 1/10 MS medium for 8 days under constant dim light (15 μmol/m^2/s).

For gene expression analysis, seedlings were grown similarly on medium for 6 days before treating with 2% Glc for 6 h. Polymerase chain reaction (PCR) was carried out with cDNA made with 25 ng total RNA using oligo-dT and SuperScript II Reverse transcriptase (Invitrogen). The PCR primers are for

CAB(At3g27690):
5'ATGGCCACTTCAGCAATCCAA3'/5'CACAACTTGACACGCCCATAT3',
sedoheptulose bisphosphatase (*SBP*, At3g55800):
5'ATGGAGACCAGCATCGCGTG3'/5'CTTCCACTGGACCTCCCAT3',
carbonic anhydrase(*CAA*, At5g14740):
5'TGAATACGCTGTCTTGCACC3'/5'TGTGATGGTGGTGGTAGCGA3', and
ubiquitin4 (*UBQ*, At5g20620):
5'GTGGTGCTAAGAAGAGGAAGA3'/5'TCAAGCTTCAACTTCTTCTTT3'.
The *UBQ* primers were included in each PCR as an internal control. The number of PCR cycles was calibrated for each pair of primers: 23 cycles for *CAB*, *SBP* and *UBQ*, and 20 cycles for *CAA*. The expression of *RBCS* at different time points of light and glucose treatment in WT and *gin2-1* was analyzed by using seedlings grown in liquid medium and the Affymetrix GeneChips (*S4*).

For auxin and cytokinin response assays, hypocotyls of 11-day-old seedlings were transferred to callus induction medium (0.5 mg/L 2,4-dichlorophenoxylacetic acid, 0.05 mg/L 6-benzylaminopurine, 0.05 mg/L kinetin) for 4 days and then cultured for 1 month on medium containing 0.2 □□ indole-3-acetic acid (IAA) to test auxin responses or on medium containing 0.86 □M IAA + 4.9 □M 2-isopentenylaminopurine (2-IP) for cytokinin responses.

For ethylene treatment, seedlings were grown for 8 days under light on 6% Glc MS medium, without (Control) or with 5 □M 2-IP or 50 □M 1-aminocyclopropane-1-carboxylic acid (ACC), the ethylene precursor (*S5*).

Analysis of sugar phosphorylation activities and metabolites. Leaves were extracted with buffer plus 0.05% Triton X-100, desalted, and assayed for hexose phosphorylation activities (*S6*) with 1 mM glucose (GK) or 1 mM fructose (FK). Leaf glucose (Glc), fructose (Fru), sucrose, glucose 6-phosphate (G6P), and fructose 6-phosphate (F6P) were measured spectrophotometrically from ethanol (sugar) or perchloric acid (sugar-phosphates) extracts (*S7*). Recovery of sugar-phosphates was > 95%. Values are means ± 1 SD (n = 4-6).

Protoplast transient expression assays. Transiently expressed (5 h) HXK1 and HXK2 proteins with the HA epitope tag were detected by immunoprecipitation using an anti-HA (*S8*) or anti-HXK1 antibody (*S2*). For gene expression analysis, maize mesophyll protoplasts were co-transfected with glucose responsive reporter genes *RBCS-CAT* or *PPDK-CAT* (*S9*) and control DNA or constructs expressing HXK1 or mutants (S177A and G104D). An internal control *UBQ-GUS* was included in each sample (*S9*). Protoplasts were incubated for 6 h with indicated glucose concentrations. Gene expression levels with 0.5 mM or 5 mM Glc were determined with duplicate samples. The expression of *UBQ-GUS* was not affected by glucose. The experiments were repeated six times with similar results. For glucose phosphorylation activity assay after imunoprecipitation, *Arabidopsis* mesophyll protoplasts (20,000/sample) were transfected with constructs expressing HA-tagged HXK1, S177A or G104D as previously described (*S8*). Proteins were expressed for 5 h in replicate samples and then protoplasts were harvested. Proteins were bound to anti-HA antibody and precipitated after binding to protein A-agarose. Beads were washed 3 times, resuspended in assay buffer, and incubated while mixing at 25C for 2 h. Activity was monitored, after gently pelleting the beads, by periodic measuring of A_{340} of the supernatant (*S7*). After activity assay, beads were collected,

resuspended in SDS buffer, and proteins were immunoblotted using a polyclonal antibody to HXK1 (*S2*).

Figures

fig. S1. Glucose repression of chlorophyll accumulation in seedlings.

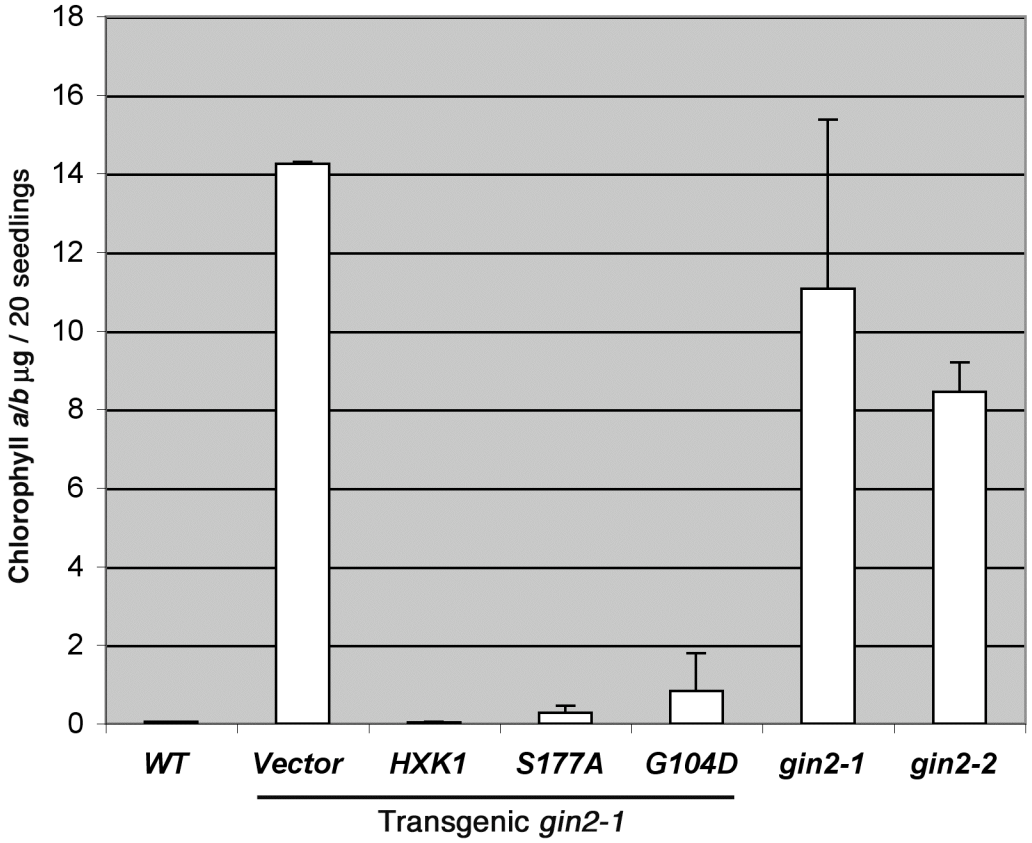

fig. S2. Glucose repression of *RBCS* expression in WT and *gin2-1* seedlings.

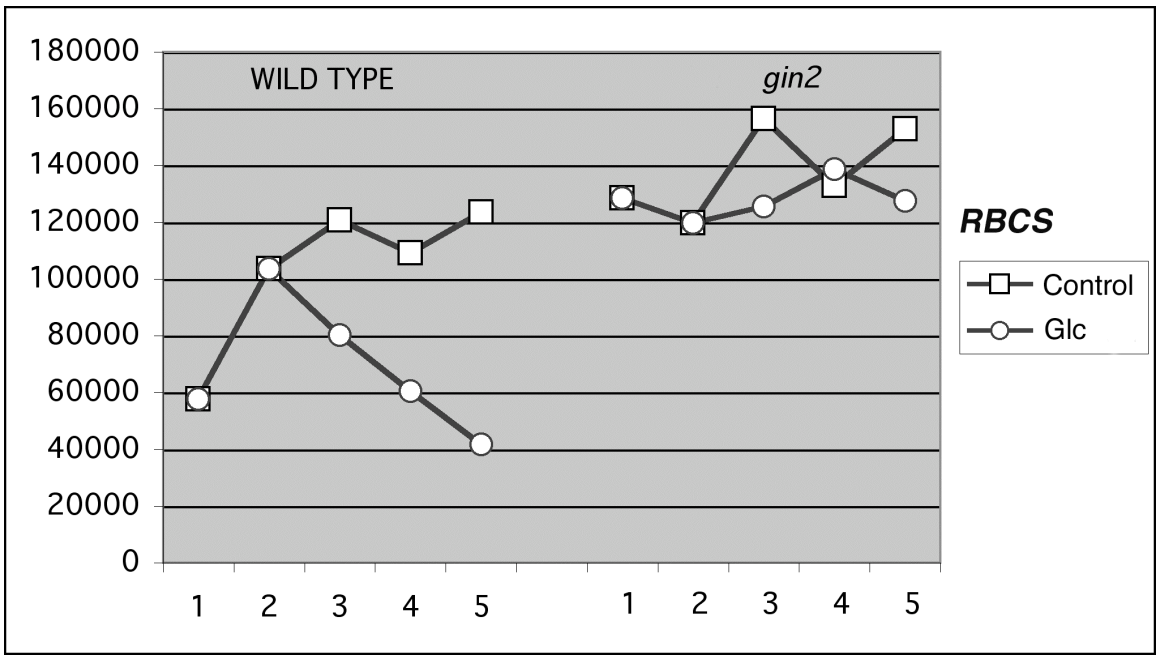

fig. S3. Glucose phosphorylation activity for wild type and mutant HXK1 proteins.

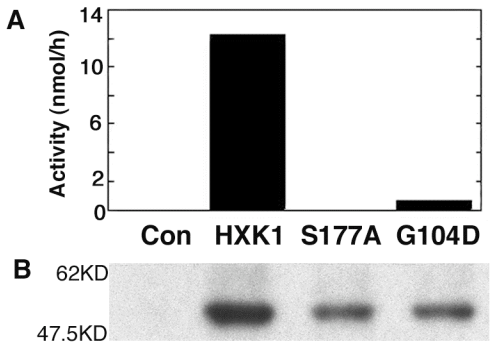

Figure Legends

fig. S1. Glucose repression of chlorophyll accumulation in seedlings. WT, various *gin2-1* transgenic lines, and *gin2* mutants were grown on plates with 7% glucose MS medium for 14 days. Chlorophyll was extracted from 20 seedlings per sample and measured (*S10*). The *gin2-1*

transgenic lines were transformed with empty vector (Vector) as a control, WT HXK1, and two HXK1 mutants (S177A, G104D) in a binary vector with the 35S promoter (*S8*).

fig. S2. Glucose repression of *RBCS* in WT and *gin2-1* seedlings.

WT and *gin2-1* seedlings were grown in liquid culture (1/10 MS and 0.2% Glc) under constant low light for 10 days and in the dark for 12 h before sampling. RNA samples were collected in the dark (1) or after 2h (2), 5h (3), 8h (5) of light without (control) or with 2% Glc. Relative transcript expression of the *RBCS* gene obtained from Affymetrix GeneChips (*S4*) is shown.

fig. S3. Glucose phosphorylation activity for wild type and mutant HXK1 proteins.

(A) Glucose phosphorylation activity in control protoplasts (Con) and in protoplasts expressing wild type (HXK1) and mutants (S177A and G104D). No endogenous activity was detected after immunoprecipitation. The experiment was repeated twice with the same result. (B). Protein expression levels of HXK1, S177A and G104D. Protein blot was probed with the anti-HXK1 antibody (*S2*).

Tables

table S1. Genetic analysis of *gin2-1* and *gin2-2*.

For *gin2-1* x WT backcross (F2):	WT	*gin2* (green)
Line 1:	64	19
Line 2:	80	21
Line 3:	79	29
Line 4:	95	28
Line 5:	91	34
Total:	**409**	**131**

For *gin2-2* x WT backcross (F2):	WT	*gin2* (green)
Line 1:	82	25
Line 2:	71	24
Line 3:	88	28
Line 4:	94	20
Line 5:	61	16
Line 6:	50	19
Total:	**446**	**132**

F2 seeds from multiple *gin2-1* or *gin2-2* backcross lines were plated on 2% glucose agar medium without MS. Seedlings with mutant (green) and WT phenotype were counted.

table S2. Phenotypic analysis of WT and *gin2-1* plants.

Phenotypes	WT	*gin2-1*
Fresh weight of rosettes at 18 days	184 mg (n=8)	27 mg (n=8)
First internode length at 30 days	71 mm (n=14)	19 mm (n=14)
Dry seed weight per plant	18.5 mg (n=8)	3.1 mg (n=8)
Hypocotyl length (low nutrient and light)	7 mm (n=8)	2 mm (n=8)

References and Notes

S1. L. Zhou, e. al., *Proc. Natl. Acad. Sci. USA* **95**, 10294 (1998).

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S4. F. Rolland, J. Sheen, (unpublished).

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S6. D. C. Doehlert, *Plant Physiol*. **89**, 1042 (1989).

S7. M. Stitt, R. McC. Lilley, R. Gerhardt, H. W. Heldt., *Methods of Enzymology* **174**, 518 (1989).

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