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Glucose and ethylene signal transduction crosstalk revealed by an *Arabidopsis* glucose-insensitive mutant

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ABSTRACT Glucose is an essential signaling molecule that controls plant development and gene expression through largely unknown mechanisms. To initiate the dissection of the glucose signal transduction pathway in plants by using a genetic approach, we have identified an *Arabidopsis* mutant, *gin1* (glucose-insensitive), in which glucose repression of cotyledon greening and expansion, shoot development, floral transition, and gene expression is impaired. Genetic analysis indicates that *GIN1* acts downstream of the sensor hexokinase in the glucose signaling pathway. Surprisingly, *gin1* insensitivity to glucose repression of cotyledon and shoot development is phenocopied by ethylene precursor treatment of wild-type plants or by constitutive ethylene biosynthesis and constitutive ethylene signaling mutants. In contrast, the ethylene insensitive mutant *etr1-1* exhibits glucose hypersensitivity. Epistasis analysis places *GIN1* downstream of the ethylene receptor, *ETR1*, and defines a new branch of ethylene signaling pathway that is uncoupled from the triple response induced by ethylene. The isolation and characterization of *gin1* reveal an unexpected convergence between the glucose and the ethylene signal transduction pathways. *GIN1* may function to balance the control of plant development in response to metabolic and hormonal stimuli that act antagonistically.

Glucose has profound effects on gene expression, metabolism, and development in microorganisms, animals, and plants (1–9). Although the glucose signal transduction pathways are well characterized in unicellular microorganisms, relatively little is known about the molecular basis of glucose responses in multicellular eukaryotes. In higher plants, glucose has been implicated to be the primary sugar signal that controls many aspects of plant development, including germination, hypocotyl elongation, cotyledon greening and expansion, primary and lateral root growth, true leaf development, floral transition, and the onset of senescence. At the molecular level, the expression of a broad spectrum of genes is either repressed or induced by glucose (4–9). Recently, hexokinase (HXK), the enzyme that catalyzes the phosphorylation of hexose sugars at the first step of the glycolytic pathway, has been shown to be the glucose sensor in plants (9–12). Studies in transgenic *Arabidopsis* plants with elevated or reduced *Arabidopsis thaliana* HXK levels or with a heterologous yeast HXK provide supporting evidence that HXK is a bifunctional enzyme with catalytic and regulatory activities, and glucose signaling may be uncoupled from glucose metabolism in plants (12). However, the downstream components in the glucose-signaling pathway are mostly unknown. Although interactions between sugar and light or hormonal signaling pathways have been suggested (13–23), the mechanisms underlying the crosstalk between glucose and other signaling pathways remain obscure.

We report here the phenotypic, molecular, and genetic analyses of a recessive *Arabidopsis* mutant (glucose-insensitive, *gin1*) that is defective in many glucose-specific responses, including cotyledon greening and expansion, shoot development, floral transition, and gene expression. Genetic analysis indicates that *gin1* is epistatic to HXK in the glucose-signaling pathway. Interestingly, we discovered that *gin1* insensitivity to glucose repression of cotyledon and shoot development can be phenocopied by ethylene precursor treatment of wild-type plants or by constitutive ethylene mutants, whereas the ethylene insensitive mutant exhibits glucose hypersensitivity. Further characterizations suggest that *GIN1* may act downstream of the glucose sensor and ethylene receptor to mediate the opposing roles that glucose and ethylene play in controlling developmental processes throughout the plant life cycle.

MATERIALS AND METHODS

Isolation of the *gin1* Mutant. Two *A. thaliana* ecotypes Wassilewskija (Ws) and Landsberg *erecta* (Ler) were the strains used for mutant isolation. T4 pools of T-DNA tagged lines or M2 populations from ethyl methanesulfonate (EMS)-mutagenized seeds were screened. Seeds were surface-sterilized and germinated on MS medium [Murashige and Skoog (MS) basal salts with B5 vitamins and 0.7% Phytoagar (pH 5.8)] containing 7% glucose under continuous fluorescent light (60 μE m^{-2} s^{-1}) at 24°C. The use of 7% instead of 6% glucose eliminated false positives in the high density screen (2000 seedlings/150-mm plate). Putative glucose insensitive mutants that developed true leaves were transferred to pots containing Metro-Mix 200 (Scotts-Sierra, Marysville, OH) and grown under continuous light (60 μE m^{-2} s^{-1}) for seed collection.

Genetic Analysis. The *gin1-1* mutant was backcrossed to the wild type (Ws-0), and the F1 seedlings were allowed to self-pollinate. F1 and F2 seedlings were scored for glucose sensitivity and greenhouse phenotype. The crosses between *gin1-1* and *gin1-2* were performed for complementation test. For mapping of the *GIN1* locus, homozygous *gin1-1* plants in the Ws background were crossed to wild-type plants of Columbia (Col-0) and Ler background. From the segregating F2 generation, 850 homozygous *gin1-1* mutants were selected for mapping with simple sequence length polymorphism markers (24).

RNA Analysis. For analysis of genes involved in photosynthesis, total RNA isolation and RNA blot analysis were performed as described (12).

Glucose Uptake Assay. Glucose uptake assays of seedlings and protoplasts were performed as described (25, 26). Seedlings were grown in liquid MS medium with 2% glucose for 3 days. For each sample, five seedlings were incubated in MS

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Abbreviations: HXK, hexokinase; *gin*, glucose insensitive; MS medium, Murashige and Skoog medium; EMS, ethyl methanesulfonate; ACC, 1-aminocyclopropane-1-carboxylic acid; *eto*, ethylene overproduction; *ctr*, constitutive triple response; *etr*, ethylene insensitive.

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medium and 2 μCi [^{14}C]glucose (1 Ci = 37 GBq) in the presence of 1 mM unlabeled glucose for the indicated times. Uptake was terminated by washing in ice-cold buffer (5 mM Mes, pH 6.0) twice. The accumulated radioactivity was determined by liquid scintillation counting. Protoplasts were isolated as described (27). Approximately 2 × 10^6 protoplasts were added to the absorption solution (0.4 M mannitol/10 mM $CaCl_2$/5 mM Mes, pH 6.0/2 μCi [^{14}C]glucose/1 mM unlabeled glucose) and samples were taken at different time points. Protoplasts were washed in the absorption solution without glucose twice and glucose uptake was determined as above. Rates of glucose uptake were expressed as nmol per 10^4 protoplasts at various time points.

Immunoblot Analysis. *Arabidopsis* plants were grown in liquid culture (half-strength MS basal salts, B5 vitamins, and 1% sucrose) for 2 weeks. Rosette tissues (100 mg) were ground in 500 μl 2× SDS/PAGE loading gel buffer (0.125 M Tris, pH 6.8/4% SDS/20% glycerol/0.78% DTT) and centrifuged. Equal amount of protein from each sample were separated on 10% SDS/PAGE and electrophoretically transferred to polvinylidene difluoride membrane. Immunodetection of HXK was performed as per the manufacturer's instructions (New England BioLabs, Phototope-Star Western Blot Detection Kit) as described (12).

RESULTS

Isolation and Genetic Analysis of the *gin1* Mutant. Analysis of the glucose signal transduction pathway in higher plants has been facilitated by the discovery that light-grown *Arabidopsis* seedlings exhibit a dramatic developmental arrest specifically induced by elevated glucose levels (9, 12). In wild-type *Arabidopsis* plants, the greening and expansion of cotyledons and the initiation of true leaf development are normal when plants are grown on standard culture medium with MS salts and 2% glucose. However, these processes are completely suppressed when the glucose level is raised to 6% (12) (Fig. 1*A*). It has been shown that the developmental arrest induced by glucose can be exaggerated or eliminated by increasing or decreasing the levels of the specific glucose sensor (AtHXK) in transgenic *Arabidopsis* plants (12). More significantly, this glucose-dependent developmental arrest is uncoupled from glucose metabolism through the glycolytic pathway and tricarboxylic acid (TCA) cycle, as increasing the total hexose phosphorylation activity by overexpressing the heterologous yeast HXK2 in *Arabidopsis* did not confer plants more sensitive to the glucose-induced developmental arrest (12). Taking advantage of this striking and reproducible glucose response in early seedling development, we screened for *Arabidopsis* mutants displaying *gin* phenotypes. The putative *gin* mutants are likely to affect glucose signaling but not glucose metabolism through the glycolytic pathway and TCA cycle.

A total of 80,000 seeds derived from the 6,500 independent T-DNA tagged lines generated by Kenneth Feldmann, Univ. of Arizona (ecotype Ws) and 30,000 EMS mutagenized M2 seeds (derived from 3,500 M1) of Ler were screened. Two *gin1* alleles were found. *gin1-1* was obtained from T-DNA pools and *gin1-2* was from EMS pools. Genetic analysis indicated that the mutant phenotype was caused by a single recessive mutation (Table 1). Because both alleles showed similar phenotypes on glucose plates and in greenhouse, the studies in this paper were performed with the *gin1-1* allele. *gin1-1* developed green and expanded cotyledons in the presence of MS salts and 6% glucose (Fig. 1 *A* and *B*). However, the *gin1-1* mutant exhibited morphology similar to the wild-type in the presence of 6% mannitol (Fig. 1*A*), suggesting that the *gin1-1* mutation specifically affects responses to glucose but not to osmotic stress. The *gin1-1* mutant but not the wild-type seedlings went on to develop true leaves and grow normally in the presence of 6% glucose (Fig. 1*C*). In the greenhouse, *gin1-1* was shorter in

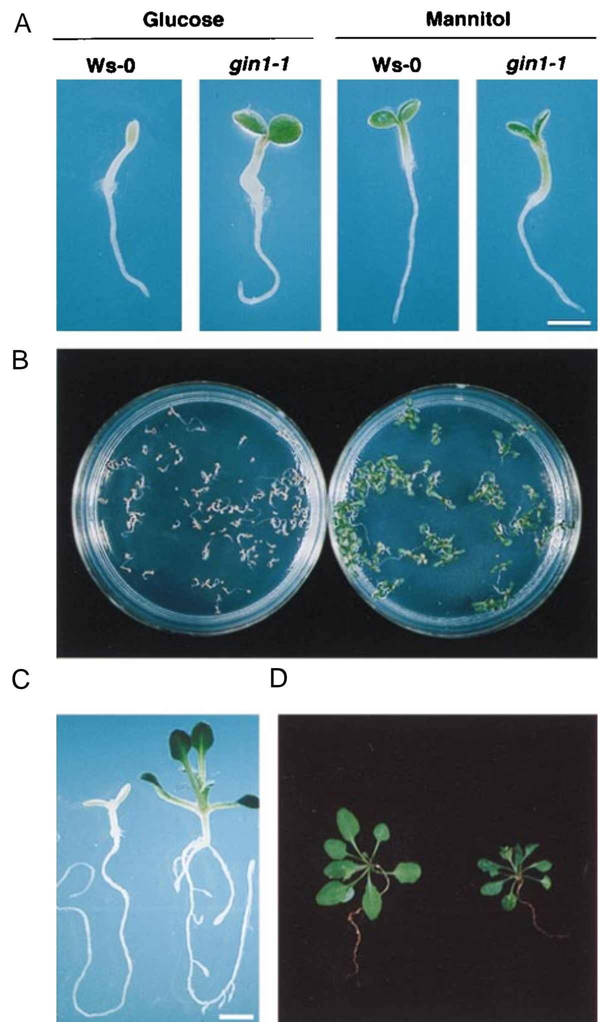

FIG. 1. Phenotypes of *gin1-1* plants. (*A*) Cotyledon development. *gin1-1* and wild-type (Ws-0) seeds were germinated and grown on MS medium containing 6% glucose or mannitol for 4 days in the light (60 μE m^{-2} s^{-1}). (Bar = 1.3 mm.) (*B*) The *gin1-1* mutant population is uniformly insensitive to glucose. Approximately 150 seeds of wild type (*Left*) and *gin1-1* (*Right*) were germinated and grown on MS medium containing 6% glucose for 4 days in the light. (*C*) True leaf development. Wild-type (*Left*) and *gin1-1* (*Right*) seedlings were grown as in *B* for 10 days. (Bar = 1.9 mm.) (*D*) Greenhouse phenotype. Both wild-type (left) and *gin1-1* (right) plants were grown in Metro-Mix 200 under continuous light (60 μE m^{-2} s^{-1}) for 3 weeks.

stature, with smaller and darker-green rosettes, compared with wild type (Fig. 1*D*). These aberrant features of *gin1-1* in the absence of exogenous glucose indicate that the gene product defined by *GIN1* may play an essential role in the normal growth and development of *Arabidopsis*.

To map the *GIN1* locus, *gin1-1* in the Ws background was crossed to wild-type Col and Ler plants. From the F2 popu-

Table 1. Genetic analysis of the *gin1* mutant

Cross	Type	Total	Mutant*	Wild Type	χ^2†
GIN1/GIN1 ×	F1	51	0	51	
gin 1-1/gin 1-1	F2	352	84	268	0.24‡
GIN1/GIN1 ×	F1	55	0	55	
gin 1-2/gin 1-2	F2	689	165	524	0.41‡
gin1-1/gin1-1 × *gin 1-2/gin 1-2*	F1	55	55	0	

*Mutant phenotypes include the glucose insensitivity on MS medium containing 6% glucose and greenhouse phenotypes.
†The χ^2 is given for the ratio of 3:1 (wild type/mutant).
‡Not significant at $P = 0.05$.

lation, 850 *gin1* homozygous plants were selected and genomic DNA was extracted from each plant. Initial mapping with simple sequence length polymorphism markers from each of the five chromosomes of *Arabidopsis* indicated that the *gin1* mutation was tightly linked to nga280 and nga128 on chromosome 1 (only 9 recombinants were detected among the 850 plants, which is equivalent of 1700 chromosomes). Together with the analysis of the flanking markers using these recombinants (data not shown), *GIN1* is located ≈0.6 cM on the centromeric side of nga280/nga128. *GIN1* appears to define a new locus because other sucrose mutants (*sun*, *rsr, lba*) are mapped at distinct chromosome locations (20–22).

***gin1* Is Insensitive to the Glucose Repression of Genes Involved in Photosynthesis.** The expression of many genes involved in photosynthesis has been shown to be repressed by sugars in diverse plant species (4, 6–9, 13, 28). During early postembryonic growth of *Arabidopsis*, the transient activation of several genes important for photosynthesis is light independent but sugar repressible (19, 29). To avoid the morphological complications induced by light, we examined the specific effect of glucose on the expression of the chlorophyll *a*/*b*-binding protein (*CAB1*), plastocyanin (*PC*), and the 33-kDa oxygen-evolving polypeptide (*OE33*) genes (19, 29, 30) in dark-grown wild-type and *gin1-1* plants. There was no difference in gene expression between the wild-type and *gin1-1* plants in the absence of exogenous glucose. However, the presence of 4% glucose significantly reduced the expression of these three genes in wild type, but not in *gin1-1* (Fig. 2*A*). This result suggests that *GIN1* may play a regulatory role in glucose repression of multiple genes involved in photosynthesis.

Flowering Time in *gin1* Is Not Delayed by Glucose. Sugars are known to affect flowering time in plants, although the mechanism is poorly understood (31, 32). We tested the effect of exogenous glucose on the time required for bolting of the inflorescence in *gin1-1* and wild type. Seeds were first germinated in the presence of 2% glucose for 3 days before transfer to plantcons with 2% or 6% glucose-MS medium. Under constant white fluorescence light (LD), wild-type plants grown on 6% glucose medium bolted 16 days later than plants grown on 2% glucose medium. Under short day (SD) condition (8 hr light/16 hr dark), plants grown on high glucose bolted 30 days later than the plants grown on low glucose (Fig. 2*B*). The delay of flowering by high levels of glucose was accompanied by an overt increase of rosette leaf number from 8 (2%) to 20 (6%) under SD condition. In contrast, *gin1-1* did not show a significant delay in bolting time when grown on 6% glucose plates under either conditions (LD or SD) (Fig. 2 *B* and *C*). The insensitivity of *gin1-1* to the glucose-dependent delay of flowering was even detectable when both wild-type and mutant plants were grown on 2% glucose under SD conditions (Fig. 2*B*). Higher levels of glucose caused further delay of flowering in wild-type plants. Our study illustrates that the delay of flowering by glucose is mediated at least in part by *GIN1*.

***gin1* Is Not Defective in Glucose Uptake.** To determine whether deficiency in glucose uptake can account for the *gin1* phenotypes, we compared the uptake rates of glucose by using seedlings or protoplasts of both mutant and wild type. The rate of glucose uptake in *gin1-1* was similar to that in wild type under both assay conditions (Fig. 3*A*). It is unlikely that the insensitivity of *gin1-1* to glucose is caused by a deficiency in glucose transport.

***gin1* Is Epistatic to HXK in the Glucose Signaling Pathway.** We have previously shown that reducing HXK levels by overexpression of the antisense *HXK* genes in *Arabidopsis* diminished glucose sensitivity (12). Thus, it was important to determine whether *gin1* is a *HXK* mutant. The protein level of HXK, determined by immunoblot analysis, was identical in wild type and *gin1-1* (Fig. 3*B*). *gin1-1* also had wild-type levels of *HXK* mRNA and total glucose phosphorylation activity

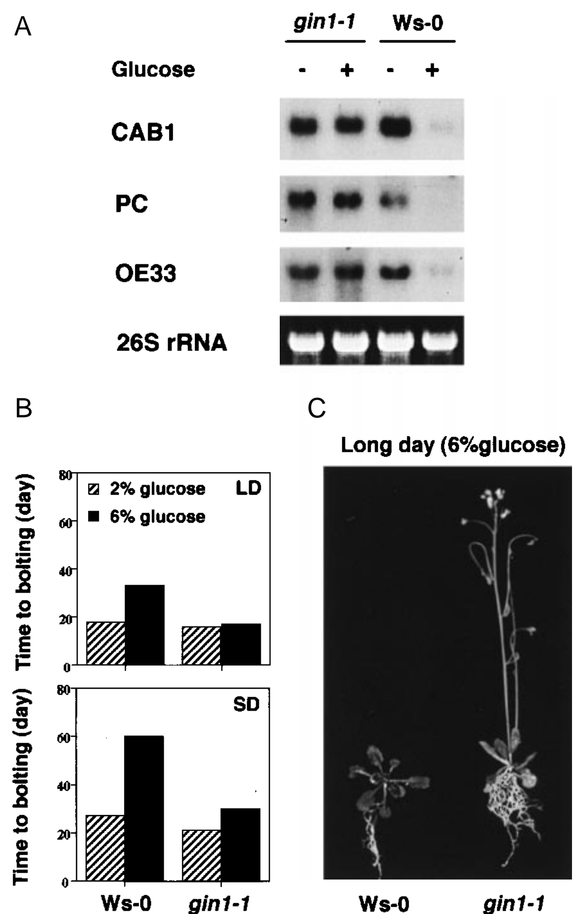

FIG. 2. Reduced glucose sensitivity in *gin1-1*. (*A*) Glucose repression of photosynthetic genes. Total RNA was isolated from *gin1-1* and wild-type (Ws-0) seedlings grown on MS medium containing 0 (−) or 4% glucose (+) in the dark for 3 days. RNA blot analysis was performed by using 5 μg of RNA per lane. Ribosomal RNA stained with ethidium bromide was used as a loading control. (*B*) Glucose effect on flowering. Lighting was continuous (60 $\mu E\ m^{-2}\ s^{-1}$) for LD, or day/night cycles (8 hr/16 hr) for SD treatment. Bolting time was determined by the appearance of an inflorescence stem. Data were obtained from 10 replicates. Standard deviation was zero as all plants bolted within the same day under each condition. (*C*) Flowering is not delayed by glucose in *gin1-1*. *gin1-1* and wild-type (Ws-0) plants were grown on MS medium with 6% glucose for 4 weeks under continuous light.

(data not shown). Furthermore, *gin1-1* was mapped to chromosome 1, whereas the two known *Arabidopsis HXK* genes are located on chromosomes 2 and 4 (12). The results indicate that *gin1-1* is unlikely to be a mutant of *HXK* or regulator of *HXK* expression and activity.

To determine whether *GIN1* plays a role in the glucose signaling pathway mediated by HXK, we generated a "double mutant" carrying *gin1-1* and a transgene overexpressing an *Arabidopsis HXK* gene (35S-*AtHXK1*). The 35S-*AtHXK1* transgenic plant have a high level of HXK and is hypersensitive to glucose, as described (12) (Fig. 3 *B* and *C*). Although the double mutant 35S-*AtHXK gin1-1* plants had a high level of HXK protein (Fig. 3*B*), it showed the same glucose insensitive phenotype as *gin1-1* grown on MS medium with 6% glucose (Fig. 3*C*). Consistently, 35S-*AtHXK1 gin1-1* plants were similar to *gin1-1* phenotypically, with small and dark-green rosettes when grown in the greenhouse (data not shown). These results suggest that *GIN1* likely acts downstream of *HXK* in the *HXK*-mediated glucose signal transduction pathway.

Ethylene Plays a Role in the Glucose Insensitivity of *gin1*. The phenotypes of *gin1-1* provided an important clue to *GIN1*

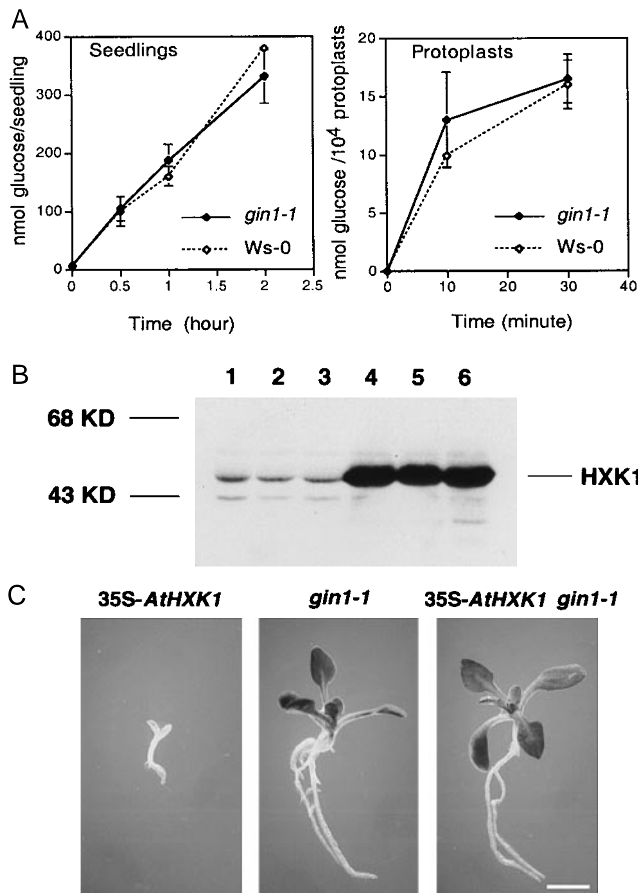

FIG. 3. *GIN1* as a positive regulator in the glucose signaling pathway mediated by *HXK*. (*A*) Glucose uptake. The rates of glucose uptake in the seedlings and protoplasts of *gin1-1* (filled symbols) and wild-type (Ws-0) (opened symbols) plants was determined (10). Values are the means of three measurements (each with duplicated samples). Error bars indicate the standard deviation. (*B*) Immunoblot analysis of HXK. HXK levels were determined in wild-type Ws-0 (lane 1), *gin1-1* (lane 2), wild-type Bensheim (lane 3), 35S-*AtHXK1* transgenic plant (Bensheim ecotype) (lane 4), and two independent lines of 35S-*AtHXK1 gin1-1* (lanes 5 and 6). Proteins were extracted from shoots of 14-day-old seedlings grown in liquid culture (11). (*C*) 35S-*AtHXK1 gin1-1* can overcome the glucose induced developmental arrest. 35S-*AtHXK1, gin1-1*, and 35S-*AtHXK1 gin1-1* seedlings were grown on MS medium with 6% glucose. (Bar = 1.9 mm.)

function. For example, seeds of *gin1-1* germinate faster (data not shown), and *gin1-1* plants have smaller, darker-green rosettes (Fig. 1*C*) which are phenotypes induced by ethylene in the wild type and are found in constitutive ethylene response mutants (33–43). We were interested in understanding whether this phenotypic convergence was indicative of a relationship between the glucose and the ethylene signaling pathways. Wild-type seeds (Ws-0 and Col-0) were germinated on MS medium with 6% glucose. To enhance ethylene production from the seedlings, the immediate ethylene precursor, 1-aminocyclopropane-1-carboxylic acid (ACC), which is readily converted to ethylene in plants (38), was added into the glucose plates. As shown in Fig. 4*A*, wild-type seedlings treated with ACC could overcome the developmental arrest normally induced by glucose. This result reveals an unconventional finding that ethylene may antagonize glucose signals to promote cotyledon and shoot development in wild-type seedlings.

Ethylene Mutants Show Altered Glucose Sensitivity. To further test the involvement of ethylene in glucose signaling, we analyzed the glucose sensitivity of three classes of ethylene mutants: the ethylene overproduction mutant *eto1-1*, the constitutive ethylene triple response mutant *ctr1-1*, and the ethylene-insensitive mutant *etr1-1* (35–43). Both *eto1-1* and *ctr1-1* overcame the glucose-induced developmental arrest without ACC treatment, whereas *etr1-1* remained glucose sensitive even in the presence of ACC (Fig. 4*A*). These data provide the most compelling evidence that glucose sensitivity is tightly linked to the ethylene signaling pathway.

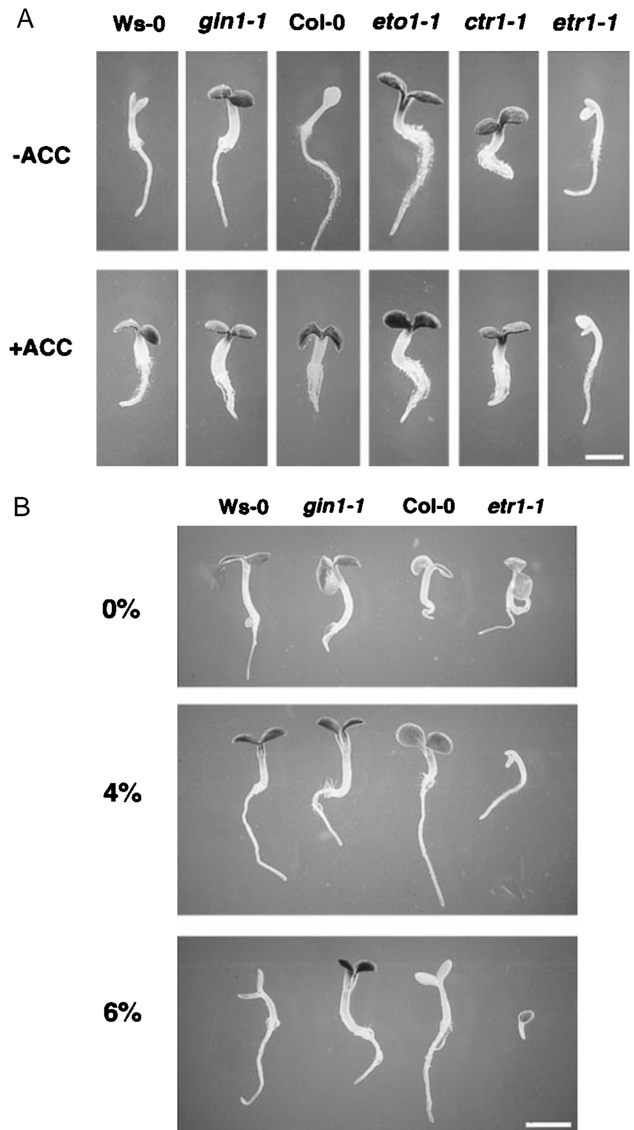

FIG. 4. Crosstalk between ethylene and glucose signaling. (*A*) ACC treatment can phenocopy *gin1* phenotype. Glucose-induced developmental arrest was examined in wild-type seedlings, *gin1-1*, and ethylene mutants on MS medium containing 6% glucose in the absence (*Upper*) or presence (*Lower*) of 50 μM of ACC. Wild-type (Ws-0) and *gin1-1* seedlings were 4 days old and all others were 5 days old. (Bar = 1.3 mm.) (*B*) *etr1-1* is hypersensitive to glucose. Wild-type, *gin1-1*, and *etr1-1* seeds were germinated and grown on MS medium containing 0%, 4% or 6% glucose for 4 days in the light (60 μE m^{-2} s^{-1}). (Bar = 1.3 mm.)

The *etr1-1* Mutant Displays Glucose Hypersensitivity. Interestingly, *etr1-1* appeared to be more sensitive to glucose than the wild type (Fig. 4*A*). We carried out a comparison between the *etr1-1* and wild-type plants at different concentrations of glucose. No overt difference in cotyledon development was found in the absence of exogenous glucose (Fig. 4*B*). However, in the presence of 3–4% glucose, *etr1-1* already displayed the developmental arrest that could only be found in the wild-type seedlings in the presence of 6% glucose (Fig. 4*B*). At an earlier stage, the germination of *etr1-1* seeds was severely

retarded in the presence of 6% glucose (Fig. 4*B*). These results provide further supporting evidence that both ethylene and glucose play important, possibly opposite, roles in germination and the development of cotyledons and true leaves.

***GIN1* Acts Downstream of *ETR1*.** To examine further the role of *GIN1* in the ethylene signal transduction pathway, the *etr1-1 gin1-1* double mutant was generated and analyzed. The dominant *etr1-1* mutation in the ethylene receptor confers ethylene insensitivity (35, 37), and its presence in the double mutant was confirmed by DNA sequencing (data not shown). Unlike *etr1-1*, the *etr1-1 gin1-1* seedlings could overcome the developmental arrest induced by 6% glucose (Fig. 5*A*), indicating that *GIN1* or the target of *GIN1* acts downstream of *ETR1* in the ethylene signaling pathway. In the greenhouse, the double mutant also showed *gin1-1*, but not *etr1-1*, phenotypes. For instance, *etr1-1 gin1-1* plants were dark-green with small rosettes (<50% of *etr1-1*), and flowered 2 weeks earlier than *etr1-1* under constant light (Fig. 5*B*). These results provide further evidence that *gin1-1* represents a rare constitutive ethylene signaling mutant and *GIN1* may function to mediate the opposing roles of glucose and ethylene in controlling developmental processes throughout the plant life cycle.

Surprisingly, the typical ethylene-induced triple response, including shortening and radial swelling of the hypocotyl, inhibition of root elongation, and exaggerated curvature of the apical hook in dark-grown seedlings, was not observed in *gin1-1* grown in the absence of ACC in the dark (Fig. 5*C*). As only a portion of the ethylene regulatory circuits appeared to be perturbed in *gin1-1*, we expected that *gin1-1* would show some ethylene phenotypes not found in the *etr1-1 gin1-1* double mutant. When treated with ACC, *gin1-1* showed additional ethylene responses, such as inhibition of root elongation and stimulation of root hair formation (44), whereas *etr1-1 gin1-1* seedlings did not exhibit more ethylene phenotypes in the presence of ACC (Fig. 5*D*). Thus, *GIN1* plays a role in a branch of ethylene signaling pathway that can be uncoupled from the ethylene signaling pathway controlling root hair initiation and the triple response exhibited in the other constitutive ethylene signaling mutant *ctr1-1* (43). Mutations in the *GIN1*-specific pathway cannot be recovered from the previous ethylene mutant selection based on the triple response.

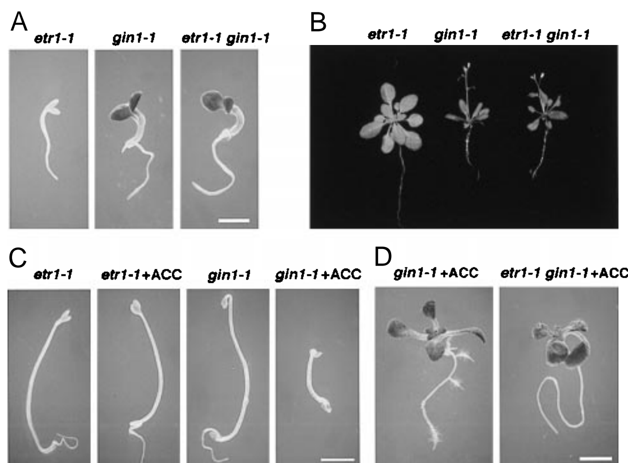

FIG. 5. Phenotypes of the *etr1-1 gin1-1* double mutant. (*A*) *gin1-1* is epistatic to *etr1-1. etr1-1, gin1-1*, and *etr1-1 gin1-1* seedlings were grown on MS medium containing 6% glucose for 5 days under constant light. (Bar = 1.3 mm.) (*B*) Greenhouse phenotype of *etr1-1 gin1-1* Four-week-old *etr1-1, gin1-1,* and *etr1-1 gin1-1* plants grown in the greenhouse are shown. (*C*) The *GIN1* pathway is uncoupled from the ethylene triple response. *etr1-1* and *gin1-1* seedlings were grown on MS medium containing 1% sucrose in the absence or presence of 50 μM ACC for 3 days in the dark. (Bar = 1.6 mm.) (*D*) The *GIN1* pathway is genetically separable from the ethylene root hair response. *gin1-1 and etr1-1 gin1-1* seedlings from *A* were transferred to MS medium containing 1% sucrose and 50 μM ACC for an additional 2 days. (Bar = 1.9 mm.)

DISCUSSION

In addition to its roles as a major energy source and structural/storage component, glucose has recently been shown to act as a regulatory molecule in higher plants (4–9). Through genetic and phenotypic analyses of a glucose insensitive mutant (*gin1*) in *Arabidopsis*, we have shown that *GIN1* defines a positive regulator that plays a pivotal role in the glucose repression of germination, cotyledon greening and expansion, true leaf development, floral transition, and gene expression (Fig. 6). The physiological importance of glucose regulation is manifested by the pleiotropic phenotypes of the *gin1* mutant grown in the absence of exogenous glucose. By using distinct strategies and sucrose-dependent gene expression, other types of sugar mutants (e.g., *sun*, *rsr, lba, hba*) also have been isolated from *Arabidopsis* (19–23). The genetic, phenotypic, and molecular characterization of these distinct mutants will offer a new opportunity to elucidate the complex signaling networks controlled by different sugar signals in higher plants.

HXK as the glucose sensor appears to be evolutionarily conserved in bacteria, yeast, mammals, and higher plants. However, in multicellular eukaryotes, little is known about the downstream components in the glucose signal transduction pathways. Based on the analysis of transgenic *Arabidopsis* plants carrying distinct *HXK* genes, it has been proposed that there may be HXK-dependent pathways and HXK-independent pathways in plants (9) (Fig. 6). The cross between HXK overexpressor lines and *gin1-1* showed that the double mutant gives totally *gin1* phenotype, both on high glucose plate and in soil. This result indicates that *gin1* is the first glucose signaling mutant that defines a positive regulator acting downstream of the HXK-dependent signaling pathway (Fig. 6).

Most surprisingly, *GIN1* seems to execute, in a glucose signal-dependent manner, some of its functions by antagonizing a branch of the ethylene signal transduction pathway that is uncoupled from the typical ethylene triple response. Thus, only a branch of the ethylene signaling pathway is altered in *gin1-1*. The *GIN1* pathway is genetically separable from the

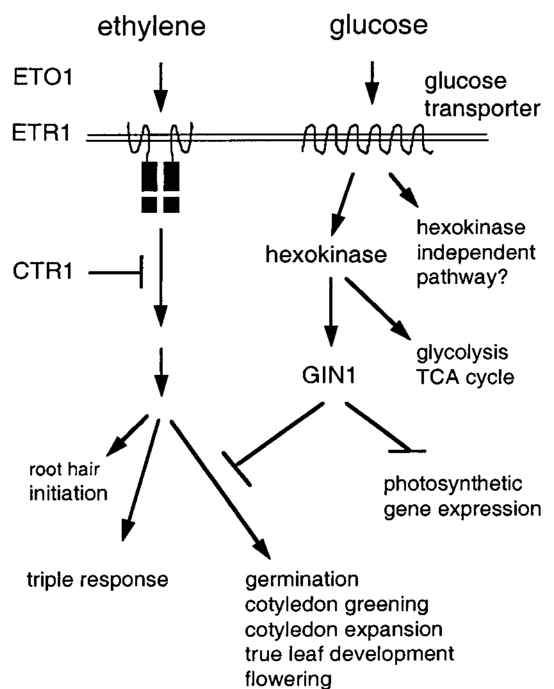

FIG. 6. Model for glucose and ethylene signaling.

pathway conferring other ethylene responses (Fig. 6). The role of ethylene in photosynthetic gene expression is unclear and will require further investigation (Fig. 6). Ethylene is best known as a plant growth regulator that controls fruit-ripening and senescence (33–43). However, recent studies have added numerous physiological roles of ethylene in cell elongation and expansion, root hair development, pathogen defense, wounding, and nodulation (44–47). The characterization of *gin1* mutant has revealed an unexpected interplay between the glucose and ethylene signaling pathways in coordinated regulation of many developmental transitions in *Arabidopsis*. Besides environmental and hormonal signals, the perception and transmission of metabolic signals also contribute to the regulation of plant growth and development through intertwined signaling networks in higher plants.

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