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The *sugar-insensitive1* (*sis1*) Mutant of *Arabidopsis* Is Allelic to *ctr1*

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Soluble sugar levels affect a diverse array of plant developmental processes. For example, exposure to high levels of glucose or sucrose inhibits early seedling development of *Arabidopsis thaliana* (L.) Heynh. Media-shift experiments indicate that Arabidopsis seedlings lose their sensitivity to the inhibitory effects of high sugar levels on early development within approximately two days after the start of imbibition. The *sugar-insensitive1* (*sis1*) mutant of Arabidopsis was isolated by screening for plants that are insensitive to the inhibitory effects of high concentrations of sucrose on early seedling development. The *sis1* mutant also displays glucose and mannose resistant phenotypes and has an osmo-tolerant phenotype during early seedling development. The *sis1* mutant is resistant to the negative effects of paclobutrazol, an inhibitor of gibberellin biosynthesis, on seed germination. Characterization of the *sis1* mutant revealed that it is allelic to *ctr1,* a previously identified mutant with a constitutive response to ethylene. © 2001 Academic Press

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Organisms ranging from bacteria to humans have the ability to sense and respond to available sugar levels. For example, carbon catabolite repression, a process in which glucose inhibits expression of genes required to metabolize alternative energy sources, has been extensively characterized in bacteria (1) and fungi (2–4). Plants, like other organisms, need to respond to sugar levels. Although the effects of soluble sugar levels on plant development and metabolism remain largely uncharacterized, several plant processes have been shown to be affected by soluble sugar levels (5–10). For example, supplying exogenous sucrose or glucose to aerial portions of the plant allows Arabidopsis to flower in complete darkness (11). In addition, soluble sugar levels regulate the expression of a variety of plant genes (9, 12, 13).

Plants are likely to have several signal transduction pathways for sugar responses, as relatively simple organisms, such as the yeast *Saccharomyces cerevisiae*, respond to sugar via as many as four signal transduction pathways (14). Direct evidence for the existence of multiple plant sugar-response pathways comes from experiments indicating the presence of both hexokinase-dependent (15–18) and hexokinase-independent (18–23) pathways for sugar response in plants. To date, very few components of plant sugar-response pathways have been identified. Components that have been identified include SNF1-like protein kinases (24, 25), the PRL1 protein (26–28), and hexokinases (15–18), although the role of hexokinases in sugar sensing remains controversial (29).

Genetic approaches are being used to determine which plant processes are affected by soluble sugar levels, as well as to identify components of the signal transduction pathways by which these effects are mediated. Although several groups of sugar-response mutants have been identified (21, 27, 30–36), very few of the affected genes from these mutants have been cloned. Exceptions include the *PRL1* gene, which encodes a protein that interacts with SNF1-like protein kinases in a yeast two-hybrid screen (26, 27). In addition, the *sis5* (30), *sun6* (31), and *gin6* (32) mutants contain defects in the *ABI4* gene, which encodes a protein with significant sequence similarity to transcriptional regulators (37).

Elucidation of plant sugar-response pathways is complicated by the fact that many developmental and physiological processes are likely to be regulated by a complex web of environmental, metabolic, and hormonal factors (5). Consistent with this hypothesis are recent findings that several mutants with defective responses to sugar also exhibit alterations in phytohormone response or metabolism. For example, the *prl1* mutant, which is hypersensitive to the inhibitory effects of sucrose and glucose on early seedling development, is also hypersensitive to auxin, abscisic acid,

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cytokinin, and ethylene (27, 28). In addition, several mutants that are insensitive to the inhibitory effects of sucrose and/or glucose on early seedling development also display defects in phytohormone response or metabolism. The *sis4* (30) mutants are allelic to the abscisic acid biosynthesis mutant *aba2-1* (38), and the *sis5* (30), *sun6* (31), and *gin6* (32) mutants are allelic to the abscisic acid insensitive mutant *abi4-1* (39). In addition, the *ctr1-1* (40) and *eto1-1* (41) mutants, which were identified on the basis of exhibiting ethylene constitutive response and overproduction phenotypes, respectively, are also resistant to glucose (33). Whether the connections between sugar and phytohormone response pathways are relatively direct or indirect remains to be determined (5).

Here we report the identification and characterization of the *sugar insensitive1*, or *sis1*, mutant. The *sis1* mutant is allelic to the *ctr1-1* mutant, which exhibits a constitutive response to ethylene (40). In addition to being insensitive to sucrose and glucose, the *sis1* mutant is resistant to mannose and displays an osmotolerant phenotype during early seedling development. The *sis1* mutant can also germinate on media containing paclobutrazol, an inhibitor of gibberellin biosynthesis (42).

MATERIALS AND METHODS

Materials and growth conditions. Wild-type and M2 seeds of *Arabidopsis thaliana* var. Columbia were obtained from Dr. Chris Somerville (Carnegie Institute, Palo Alto, CA). The M2 seeds were harvested from M1 plants generated by ethylmethane sulfonate mutagenesis of seeds. The *ctr1-1*, *eto1-1*, *etr1-1*, and *ein4* mutants were obtained from the Arabidopsis Biological Resource Center at Ohio State University. Unless otherwise indicated, plants were grown on minimal Arabidopsis media (43) under continuous fluorescent light at 22 to 25°C. Aminoethoxyvinylglycine and 1-aminocyclopropane-1-carboxylic acid were obtained from Sigma (St. Louis, MO). Paclobutrazol was obtained from Chem Service, Inc. (West Chester, PA).

Mutant screen and sugar sensitivity assays. To screen for sugar-insensitive mutants, approximately 28,000 M2 seeds derived from ethylmethane sulfonate mutagenized populations were surface-sterilized and sown on solid minimal Arabidopsis media (43) supplemented with 0.3 M sucrose. The sowing density was approximately 1000 seeds per 100 mm petri plate. The plates were incubated at approximately 22°C and 50 to 65 μmol photons m^{-2} s^{-1} continuous light for 14 days prior to scoring. Plants with relatively normal shoot systems were transplanted to soil, grown to maturity, and re-assayed in the following generation. Sugar, sorbitol, betaine, and NaCl sensitivity assays were performed by sowing surface-sterilized seeds on solid minimal Arabidopsis media supplemented with the additives indicated in each experiment. The assays were conducted as described above, except that the light intensity was 90 to 120 μmol photons m^{-2} s^{-1} and 50 to 100 seeds were scored in a typical assay.

Hypocotyl elongation assays. Seeds were sown on solid minimal Arabidopsis media with 0.03 M glucose, with or without 10 μM aminoethoxyvinylglycine. The plates were incubated at 4°C in the dark for 3 days, placed at room temperature in the light for 1 h, and then left in the dark at room temperature for five more days prior to measuring hypocotyl lengths.

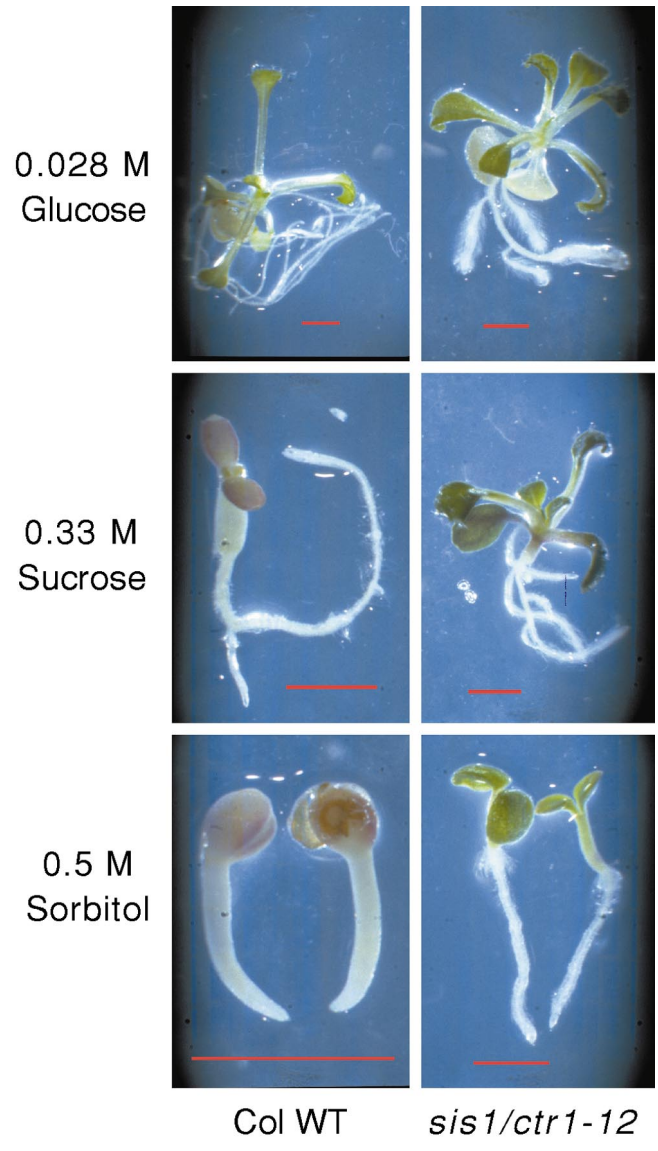

FIG. 1. Growth of wild-type and *sis1/ctr1-12* seedlings on high concentrations of sucrose and sorbitol. Columbia wild-type (Col WT) and *sis1/ctr1-12* seedlings were grown on the indicated media under 90–120 μmol photons m^{-2} s^{-1} continuous light for 16 days prior to photographing. Red bars = 1.5 mm. Photographs of Columbia wild-type plants were previously published (30) and are reprinted with permission from Blackwell Science Ltd.

DNA sequencing. Oligonucleotides complementary to the *CTR1* gene were used to amplify genomic DNA from Columbia wild-type and *sis1/ctr1-12* plants via PCR. DNA fragments were separated on agarose gels and purified using the Qiaex 2 Gel Extraction Kit (Qiagen Inc., Santa Clarita, CA). Sequencing was performed by a commercial facility (Lone Star Labs Inc., Houston, TX). The entire coding region of the *CTR1* gene, plus approximately 120 bp of 5′ sequences and 300 bp of 3′ sequences, was sequenced from the *sis1/ctr1-12* mutant on one DNA strand. Where the sequence of the *CTR1* gene from the *sis1/ctr1-12* mutant differed from the wild-type *CTR1* sequence (GenBank Accession No. AL162506), the *CTR1* genes from both the *sis1/ctr1-12* mutant and its wild-type Columbia parent were sequenced on both DNA strands.

Paclobutrazol sensitivity assays. Seeds were surface sterilized and sown on solid minimal Arabidopsis media supplemented with paclobutrazol (stock solution = 0.12 M in 100% ethanol) to a final concentration of 0.12 mM. The plates were incubated in the dark at 4°C for 3 days and then shifted to 21°C and 45 to 60 μmol photons m^{-2} s^{-1} continuous light. Seed germination (defined as emergence of any part of the seedling from the seed coat) was scored 3 and 4 days after shifting to 21°C.

Chlorophyll and anthocyanin assays. To assay chlorophyll levels, seeds were surface sterilized and sown on solid minimal Arabidopsis media supplemented with 0.03 or 0.15 M sucrose. Plants were grown under 60 to 80 μmol photons m^{-2} s^{-1} continuous fluorescent light for 2 weeks prior to assaying. Chlorophyll levels were measured as described (44). Whole shoot systems from three plants were used for each assay. To measure anthocyanin levels, seeds were surface sterilized and grown on solid minimal media with 0.03 M sucrose for 2 weeks. The seedlings were then transferred to solid minimal media with 0.03 or 0.18 M sucrose and grown for an additional week under 60 to 80 μmol photons m^{-2} s^{-1} continuous light prior to assaying. Anthocyanin assays were performed as described (45). Whole shoot systems from three plants were used for each assay. Results were scaled relative to the anthocyanin levels found in wild-type plants grown on media containing 0.18 M sucrose.

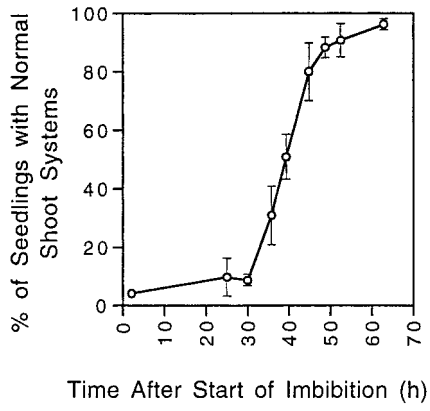

FIG. 2. Time course of sensitivity to 0.3 M sucrose. Wild-type Arabidopsis seeds were sown on media lacking sugar and then transferred to media with 0.3 M sucrose at the indicated times. Plants were grown for a total of 10 days prior to scoring. Results are means ± SD (n = 3). This experiment was repeated, with similar results.

RESULTS

Exogenous sucrose inhibits development during a narrow temporal window. High concentrations of exogenous sucrose or glucose inhibit the development of wild-type Arabidopsis seedlings (30, 31, 33). For example, when seeds of *Arabidopsis thaliana* var. Columbia are sown on solid minimal Arabidopsis media (43) containing 0.3 to 0.33 M sucrose, the majority of the seeds germinate (defined as the emergence of any part of the seedling from the seed coat), but only a few percent develop into seedlings with relatively normal shoot systems. As illustrated in Fig. 1, within 2 weeks, approximately 98% of the seedlings arrest development without forming true leaves or green, expanded cotyledons.

Wild-type Arabidopsis seedlings lose susceptibility to the inhibitory effects of high sucrose concentrations on early development within approximately 48 h of the start of imbibition. This conclusion is based on media-shift experiments in which wild-type Arabidopsis seeds are sown on media lacking sugar and then transferred to media with 0.3 M sucrose at different times after the start of imbibition. A high percentage of seeds/seedlings transferred to 0.3 M sucrose less than 30 h after the start of imbibition fail to develop normal shoot systems. In contrast, the majority of seedlings transferred to 0.3 M sucrose more than 48 h after the start of imbibition develop relatively normally (Fig. 2). Sugar-mediated inhibition of seedling development is reversible, as seedlings grown for 14 days on 0.3 M sucrose and then shifted to media without sucrose begin to form green, expanded cotyledons within 24 h.

Isolation of sugar-insensitive1sis1) mutant. A mutant screen was undertaken to identify plants that are insensitive to the developmental block imposed by high sugar concentrations. Approximately 28,000 M2 seeds derived from EMS-mutagenized *Arabidopsis thaliana* var. Columbia were sown on solid Arabidopsis minimal media (43) with 0.3 M sucrose. After 2 weeks, seedlings that had formed relatively normal shoot systems were transferred to soil and grown to maturity. Seeds from these plants were then rescreened for the ability to form normal shoot systems on 0.3 to 0.33 M sucrose. A high percentage of the seeds of one plant were found to develop into seedlings with normal shoot systems on 0.33 M sucrose (Fig. 1). This mutant was named *sugar-insensitive1* (*sis1*).

The sis1 mutant is allelic to the ethylene-response mutant, ctr1. Plants carrying the *sis1* mutation form compact rosettes and have very short roots with an abundance of root hairs, even when grown on soil or on media containing a low concentration of exogenous sugar (Fig. 1). These phenotypes are similar to those seen in a previously identified mutant, the *ctr1* mutant, which displays a constitutive response to ethylene (40). As defects in ethylene response and metabolism have previously been reported to alter sugar response (33), the *sis1* mutant was screened for ethylene overproduction and constitutive response phenotypes. When etiolated wild-type, *ctr1-1* (40), *eto1-1* (41), and *sis1* plants are grown in the presence and absence of aminoethoxyvinylglycine, an inhibitor of ethylene biosynthesis (41), the *sis1* mutant closely resembles the *ctr1-1* mutant (Table 1).

To determine whether *sis1* is allelic to *ctr1-1*, complementation analyses were performed. Crosses were made between *sis1* and *ctr1-1* plants and the resulting progeny scored for both constitutive ethylene response and *sis* phenotypes. These experiments indicate that the *ctr1-1* mutant fails to complement either the *sis* or constitutive ethylene response phenotypes of the *sis1*

TABLE 1

Complementation Analysis

Plant line	% Expanded cotyledons on 0.3 M glucose[a]	Hypocotyl length (cm)[b]	
		0 μM AVG[c]	10 μM AVG[c]
Col WT[d]	3	1.7 ± 0.1	1.6 ± 0.2
eto1-1[e]	ND[f]	0.8 ± 0.1	1.9 ± 0.3
ctr1-1	33	0.7 ± 0.1	0.64 ± 0.05
sis1/ctr1-12	36	0.8 ± 0.1	0.7 ± 0.2
sis1/ctr1-12 × *ctr1-1*, F1	56	0.8 ± 0.1	0.8 ± 0.1

[a] Seeds/seedlings were grown for 14 days prior to scoring.
[b] Hypocotyl lengths were scored after 5 days of growth in the dark. Results are means ± SD (n = 4–5).
[c] AVG = aminoethoxyvinylglycine.
[d] Col WT = Columbia wild-type.
[e] The *eto1-1* mutant was assayed in an independent experiment.
[f] ND = not determined.

mutant (Table 1). The *CTR1* gene has been isolated and found to encode a protein with significant sequence similarity to members of the Raf family of serine/threonine protein kinases (40). Sequencing of the *CTR1* gene from the *sis1* mutant revealed the presence of a point mutation that alters codon 764 from GGT (encodes Gly) in the wild-type to GAT (encodes Asp) in the mutant. The substitution of this Gly residue, which is highly conserved in the Raf family of serine/threonine protein kinases (46), by an Asp residue is likely to result in a significant decrease in CTR1 activity. In light of the convincing evidence that the *sis1* mutation lies in the *CTR1* gene, the *sis1* mutant has been re-named *ctr1-12*.

Characterization of the sis1/ctr1-12 mutant. The *sis1/ctr1-12* mutant was identified on the basis of its resistance to high concentrations of sucrose. As high concentrations of glucose and sucrose appear to exert similar effects on wild-type plants (30), it was of interest to determine whether the *sis1/ctr1-12* mutant is also insensitive to glucose. As shown in Fig. 3, the *sis1/ctr1-12* mutant displays significant resistance to the inhibitory effects of 0.3 M glucose on seed germination, cotyledon expansion, and formation of true leaves.

The *sis1/ctr1-12* mutant was also tested for osmotolerance. As 70 to 80% of wild-type *Arabidopsis thaliana* var. Columbia seedlings are able to develop shoot systems with expanded cotyledons and true leaves on 0.3 M sorbitol (30), it is advantageous to use higher concentrations of sorbitol to assay for osmo-tolerance during early seedling development. These experiments indicate that the *sis1/ctr1-12* mutant is resistant to the inhibitory effects of 0.4 M (Fig. 3) and 0.5 M (Fig. 1) sorbitol on cotyledon expansion and true leaf formation. The *sis1/ctr1-12* mutant is also resistant to high concentrations of other osmolytes during very early seedling development. For example, 34% of *sis1/ctr1-12* seedlings, but only 4% of wild-type seedlings, form expanded cotyledons when grown on media containing 0.2 M NaCl. In addition, 62% of mutant seedlings, but only 20% of wild-type seedlings, form expanded cotyledons when grown on media containing 0.45 M betaine. The effects of high osmolyte concentrations on later stages of development were not assayed in these experiments.

The *sis1/ctr1-12* mutant was also examined for resistance to mannose. Mannose is a glucose analog that has been postulated to affect sugar-regulated gene expression (16, 17) and seed germination (47) through a hexokinase-mediated signaling pathway. The *sis1/ctr1-12* mutation confers significant resistance to mannose (Fig. 3). As the concentrations of mannose used in these experiments are quite low (1.7 mM), the mutant's mannose-insensitive phenotype is not due to its osmotolerant phenotype.

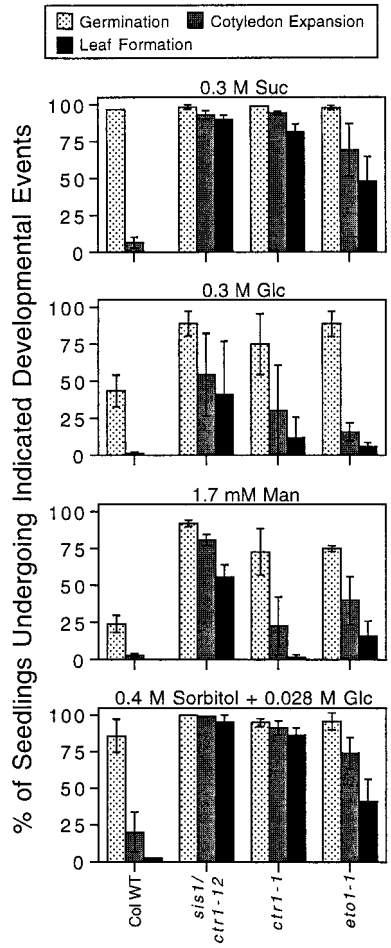

FIG. 3. Sensitivity of mutant and Columbia wild-type (Col WT) plants to sucrose (Suc), glucose (Glc), mannose (Man), and sorbitol. Seeds were grown on the indicated media under 90–120 μmol photons m^{-2} s^{-1} continuous light for 13 days prior to scoring. Results are means ± SD (n = 3, except n = 2 for Col WT).

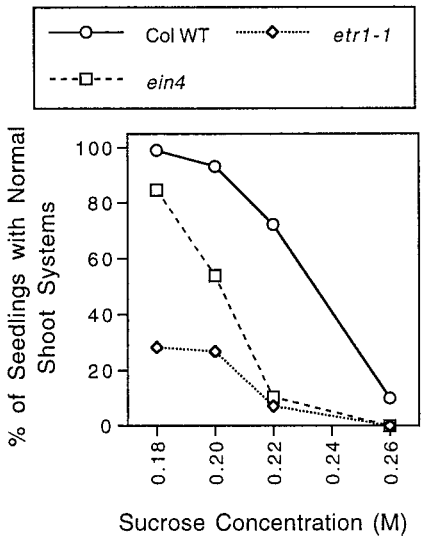

FIG. 4. Increased sensitivity of *etr1-1* and *ein4* to exogenous sucrose. Columbia wild-type (Col WT), *etr1-1* and *ein4* seeds were grown on the indicated media for 19 days prior to scoring.

Other mutants with defects in ethylene response or metabolism were also tested for altered sensitivity to several sugars as well as to osmotic stress. The *ctr1-1* and *eto1-1* mutants have previously been shown to confer a *glucose-insensitive* (*gin*) phenotype (33). Results presented in Fig. 3 indicate that the *ctr1-1* (40) and *eto1-1* (41) mutants are resistant to the inhibitory effects of sucrose, glucose, sorbitol, and mannose on seed germination, cotyledon expansion, and true leaf formation. The results presented in Fig. 3 also indicate that *ctr1-1*, *eto1-1*, and *sis1/ctr1-12* mutants, unlike wild-type plants, exhibit a significant degree of resistance to 0.3 M sucrose within 2 h of the start of imbibition, as seeds were sown on the indicated media less than 2 h after the start of imbibition.

An ethylene-insensitive mutant, *etr1-1* (48), has previously been shown to exhibit increased sensitivity to glucose (33). The response of the *etr1-1* mutant, as well as an additional ethylene-insensitive mutant designated *ein4* (49), to exogenous sucrose was examined. As shown in Fig. 4, the *etr1-1* and *ein4* mutants exhibit significantly increased sensitivity to the inhibitory effects of exogenous sucrose on early shoot development. For example, the majority of wild-type seedlings are able to form relatively normal shoot systems on media containing 0.22 M sucrose, whereas only 7–10% of *etr1-1* and *ein4* seedlings grown on this media exhibit normal morphology.

In addition to inhibiting early seedling development, sugar levels have been postulated to affect a range of developmental and physiological processes (5–10). Therefore, it was of interest to determine whether the *sis1/ctr1-12* mutation alters all sugar responses or just a subset. As testing all possible sugar responses is not practical, two sugar effects that have been documented in several studies and that can be reliably and quantitatively assayed were chosen for further study. These effects are the decrease in chlorophyll levels (50) and the increase in anthocyanin levels (51) seen in response to increasing sugar concentrations. As shown in Fig. 5A, *sis1/ctr1-12* and wild-type plants have similar responses to the effects of exogenous sucrose on chlorophyll levels. In contrast to previously reported studies, both wild-type and mutant plants consistently exhibited increased chlorophyll levels at higher sugar concentrations in multiple independent experiments conducted as part of this study. The reason for the different response of chlorophyll levels to sugar concentration seen in this study and in other studies remains to be determined. Possible explanations include differences in growth conditions (e.g., light intensity, media composition), in the method of supplying sugar to the plants (through the roots in this study, through cut petioles in other studies) and the fact that very young seedlings were assayed in this study. As shown in Fig. 5B, *sis1/ctr1-12* plants also have a wild-type response to the effects of sugar levels on anthocyanin accumulation. These results indicate that the *sis1/ctr1-12* mutation does not affect all sugar responses.

In addition to screening the *sis1/ctr1-12* mutant for other sugar-response phenotypes, it was of interest to test for a defective response to paclobutrazol. Paclobutrazol is an inhibitor of gibberellin biosynthesis (42) that has a negative effect on seed germination (52). Most of the *sis* mutants isolated in independent mutant screens are resistant to paclobutrazol (5, 30). Therefore, it was of interest to determine whether the *sis1/ctr1-12* mutant and related mutants (i.e., *ctr1-1* and *eto1-1*) are also resistant to paclobutrazol. Wild-

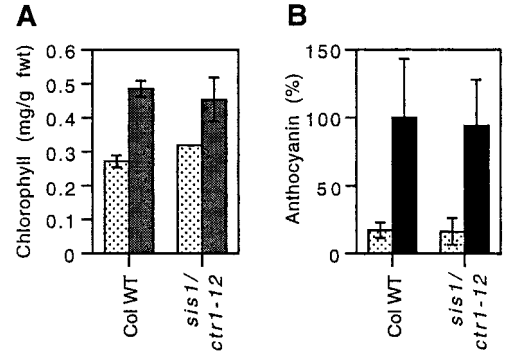

FIG. 5. Effect of sucrose concentration on chlorophyll and anthocyanin levels. (A) Chlorophyll levels in whole shoot systems of mutant and Columbia wild-type (Col WT) seedlings grown on 0.03 M sucrose (▨) or 0.15 M sucrose (■). Results are means ± SD (n = 3). This experiment was repeated twice, with similar results. (B) Anthocyanin levels in whole shoot systems of seedlings grown on 0.03 M sucrose (▨) or 0.18 M sucrose (■). Anthocyanin levels were divided by sample fresh weights (fwt) and scaled relative to the value obtained for wild-type seedlings grown on 0.18 M sucrose. Results are means ± SD (n = 8–9). This experiment was repeated twice, with similar results.

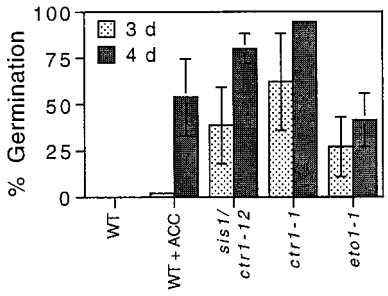

FIG. 6. Germination on paclobutrazol. Mutant and Columbia wild-type (WT) seeds were sown on minimal media with 0.12 mM paclobutrazol and, where indicated, 50 μM 1-aminocyclopropane-1-carboxylic acid (ACC). The seeds were incubated in the dark for 3 days at 4°C, then shifted to 44–60 μmol photons m^{-2} s^{-1} continuous light at 21°C. Percent seed germination was scored 3 and 4 days (d) after seeds were shifted to continuous light. Results are means ± SD (n = 3, except n = 2 for WT and WT + ACC).

type and mutant seeds were sown on media containing paclobutrazol. As shown in Fig. 6, a high percentage of the seeds from each mutant, but not of wild-type seeds, germinate on paclobutrazol, indicating that the *ctr1-1*, *eto1-1*, and *sis1/ctr1-12* mutations cause a paclobutrazol-resistant phenotype. In addition, exogenous 1-aminocyclopropane-1-carboxylic acid (an ethylene biosynthetic precursor) also confers resistance to the negative effects of paclobutrazol on seed germination (Fig. 6).

DISCUSSION

Although soluble sugar levels have been suggested to play an important role in the regulation of a number of plant metabolic and developmental processes, little is known about the mechanisms by which plants sense and respond to sugar. To address this problem, a screen was conducted to identify Arabidopsis mutants that are defective in their ability to sense and/or respond to sugar. The basis of this screen was the sugar-mediated inhibition of early seedling development. Although the molecular mechanism by which this inhibition occurs remains to be elucidated, the finding that seedlings lose their susceptibility to high sugar concentrations within approximately 48 h of the start of imbibition aids in developing possible models. For example, the timing with which Arabidopsis seedlings become resistant to high sugar concentrations is similar to the timing with which *Brassica napus*, a close relative of Arabidopsis, undergoes a dramatic metabolic shift (53). During the first 2 days after the start of imbibition, breakdown of *B. napus* seed storage lipids proceeds while the CO_2-evolving reactions of the tricarboxylic acid cycle are bypassed. After 2 days, the activity of decarboxylative enzymes increases, promoting carbon flux through the entire tricarboxylic acid cycle (53). Interestingly, the expression of two genes that allow the decarboxylative steps of the tricarboxylic acid cycle to be bypassed has been shown to be negatively regulated by sugar levels (16). So, sensitivity to high sugar concentrations may be related to the bypass of the CO_2-evolving reactions of the tricarboxylic acid cycle. Seedlings may then lose their susceptibility to high sugar concentrations once they undergo the metabolic shift that results in activation of the entire tricarboxylic acid cycle. However, additional experiments will be required to distinguish between this model and other possible models.

The *sis1/ctr1-12* mutant was identified on the basis of its sucrose-insensitive phenotype. Additional experiments revealed that the *sis1/ctr1-12*, *ctr1-1*, and *eto1-1* mutations also confer resistance to glucose, mannose, and sorbitol. The finding that these mutants are resistant to both sucrose and glucose is perhaps not surprising, as glucose and sucrose are readily interconvertible within cells. As the concentrations of glucose and sucrose used in these experiments are quite high (typically 0.3–0.33 M), the *sis1/ctr1-12*, *ctr1-1*, and *eto1-1* mutations were tested for an osmo-tolerant phenotype. Experiments conducted as part of another study indicate that concentrations of sorbitol in the same molar range used to assay glucose and sucrose sensitivity are insufficient to cause a significant inhibition of cotyledon expansion and true leaf formation, even in wild-type Arabidopsis plants (30). Therefore, it is necessary to use higher (e.g., 0.4 M) concentrations of sorbitol to assay for osmo-tolerance. These experiments indicate that the *sis1/ctr1-12*, *ctr1-1*, and *eto1-1* mutants are resistant to high concentrations of sorbitol. Additional experiments indicate that the *sis1/ctr1-12* mutant (the *ctr1-1* and *eto1-1* mutants were not included in these experiments) is also resistant to high concentrations of NaCl and betaine during early seedling development. Although the molecular basis of this osmo-tolerant phenotype is currently unknown, one possibility is that the mutants might accumulate abnormally high levels of intracellular sugars, as a result of being insensitive to sugar. Higher intra-cellular sugar levels could then have an osmo-protectant effect. Additional experiments will be required to determine the mechanism by which the *ctr1* and *eto1* mutations confer osmo-tolerant phenotypes during early seedling development, as well as to determine whether these mutations result in osmo-tolerant phenotypes at later developmental stages.

Although the *sis1/ctr1-12*, *ctr1-1*, and *eto1-1* mutants have an osmo-tolerant phenotype, this osmo-tolerant phenotype is insufficient to explain the mutants' sugar-insensitive phenotype. This conclusion is based on the finding that the mutants are also insensitive to mannose at concentrations (typically 1.5 to 4 mM) that are too low to exert osmotic stress. Mannose is a glucose analog that, like glucose, has been postulated to affect

sugar-regulated gene expression (16, 17) and seed germination (47) through a hexokinase-mediated signaling pathway.

Characterization of the *sis1*/*ctr1-12* mutant revealed that it is allelic to the constitutive ethylene response mutant, *ctr1-1* (40). Previously, a model was proposed that postulates the existence of "cross-talk" between the sugar and ethylene response pathways. This model is based on the finding that a glucose-insensitive mutant of Arabidopsis, the *gin1* mutant, shows phenotypic similarities to mutants that overproduce ethylene or that have a constitutive response to ethylene (33). The authors of that study also showed that the *eto1-1* and *ctr1-1* mutants exhibit reduced sensitivity, whereas the *etr1-1* mutant exhibits increased sensitivity, to high concentrations of glucose. In addition, the *prl1* mutant of Arabidopsis exhibits increased sensitivity to both glucose and ethylene (27, 28). The results presented here also indicate that alterations in ethylene metabolism or response can affect sugar response. However, how direct the connection between the ethylene and sugar response pathways is remains to be elucidated. A convergence between the glucose and ethylene signal transduction pathways, as was postulated by Zhou and his colleagues, is one possibility (33).

Alternatively, the connection between the ethylene and sugar response pathways may be more indirect. Different phytohormones are believed to act relatively independently in the control of some processes. For example, brassinosteroids and auxin are postulated to affect stem elongation via different signal transduction pathways (54). In addition, results presented here indicate that mutations in the *CTR1* and *ETO1* genes, as well as application of the ethylene biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid, can alleviate the need for gibberellin during seed germination. Previously, exogenous ethylene was shown to promote germination of mutant seeds defective in gibberellin biosynthesis (55). These results indicate that increased ethylene levels, as well as mutations that cause a constitutive ethylene response, can alleviate the need for gibberellin during seed germination. However, these results do not necessarily imply a direct connection between the ethylene and gibberellin response pathways during regulation of seed germination. Similarly, ethylene and sugar could affect early seedling development via relatively independent signal transduction pathways. Further definition of the relationship between sugar and phytohormone response pathways will likely require much greater knowledge regarding the components of these pathways then is currently available (5).

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