Characterization of Mutants in Arabidopsis Showing Increased Sugar-Specific Gene Expression, Growth, and Developmental Responses[1]

Margarete Baier[2,3], Georg Hemmann[3], Rachel Holman, Fiona Corke, Rod Card, Caroline Smith, Fred Rook, and Michael W. Bevan*

Department of Cell and Developmental Biology, John Innes Centre, Norwich NR4 7UH, United Kingdom

Sugars such as sucrose serve dual functions as transported carbohydrates in vascular plants and as signal molecules that regulate gene expression and plant development. Sugar-mediated signals indicate carbohydrate availability and regulate metabolism by co-coordinating sugar production and mobilization with sugar usage and storage. Analysis of mutants with altered responses to sucrose and glucose has shown that signaling pathways mediated by sugars and abscisic acid interact to regulate seedling development and gene expression. Using a novel screen for sugar-response mutants based on the activity of a luciferase reporter gene under the control of the sugar-inducible promoter of the *ApL3* gene, we have isolated *high sugar-response* (*hsr*) mutants that exhibit elevated luciferase activity and *ApL3* expression in response to low sugar concentrations. Our characterization of these *hsr* mutants suggests that they affect the regulation of sugar-induced and sugar-repressed processes controlling gene expression, growth, and development in Arabidopsis. In contrast to some other sugar-response mutants, they do not exhibit altered responses to ethylene or abscisic acid, suggesting that the *hsr* mutants may have a specifically increased sensitivity to sugars. Further characterization of the *hsr* mutants will lead to greater understanding of regulatory pathways involved in metabolite signaling.

Sugars such as Glc and Suc regulate many important cellular processes in plants (for review, see Smeekens, 2000; Rolland et al., 2002; Rook and Bevan, 2003). The expression of genes involved in photosynthate accumulation, mobilization, and storage is regulated by Glc and Suc (Koch, 1996), and by light-mediated (Neff et al., 2000) and circadian clock-mediated (Harmer et al., 2000) signaling mechanisms. This regulatory network serves to integrate the synthesis and use of carbohydrates in different tissues and organs in response to environmental changes and in response to the availability of other nutrients such as nitrogen (Coruzzi and Bush, 2001). The outputs of this regulatory network maintain an optimal dynamic carbohydrate status. For example, in conditions of high carbohydrate demand and if sufficient light energy is available, the regulatory network increases production and mobilization of photosynthate by increasing expression of genes involved in photosynthesis (Koch, 1996), and conversely, when photosynthate is not immediately required, genes involved in starch synthesis (Rook et al., 2001) are activated to maintain a balance between photosynthate supply, demand, and storage. Transport functions respond to photosynthate availability by modulation of Suc transporter gene expression and protein levels (Chiou and Bush, 1998; Vaughn et al., 2002) to integrate carbohydrate sink demand with carbohydrate source production and export.

Significant progress is being made in identifying the mechanisms controlling carbohydrate status in plants. Genetic screens in Arabidopsis have identified mutants affecting growth, development, and gene expression responses to Glc, Suc, trehalose, and Man (for review, see Rolland et al., 2002; Rook and Bevan, 2003). The *glucose-insensitive germination* (*gin*) and *sugar-insensitive* (*sis*) mutants, which exhibit normal seedling development on otherwise inhibitory concentrations of Glc, were identified in screens using the inhibition of germination and seedling establishment by high Glc and Suc concentrations (Zhou et al., 1998; Arenas-Huertero et al., 2000; Laby et al., 2000; Cheng et al., 2002; Gonzalez-Guzman et al., 2002). Similar screens have identified Glc- and Suc-supersensitive mutants that exhibit seedling developmental arrest on normally noninhibitory sugar levels (Pego et al., 1999; Rolland et al., 2002). Other screens have used the inhibition of seed germination and seedling establishment by low concentrations of Man to identify *Man-insensitive germination* (*mig*) mutants that have been proposed to affect sugar signaling by inhibiting hexokinase activity (Pego et al.,

[1] This work was supported by Syngenta (grant no. PMC19), by the Deutsche Forschungsgemeinschaft (grant no. Ba2011/1–1), and by the Biotechnology and Biological Sciences Research Council (core strategic grant to the John Innes Centre).

[2] Present address: Biochemistry and Physiology of Plants, University of Bielefeld, Universitätsstrasse 25, 33615 Bielefeld, Germany.

[3] These authors contributed equally to this work.

* Corresponding author; e-mail michael.bevan@bbsrc.ac.uk; fax 01603–450025.

Article, publication date, and citation information can be found at www.plantphysiol.org/cgi/doi/10.1104/pp.103.031674.

1999). Recently (Price et al., 2003), a Glc-sensitive step during early stages of germination has been characterized that demonstrates that low concentrations of Glc can modulate growth and development in Arabidopsis.

Mutants with altered responses to Glc and Suc have also been detected in screens for altered gene expression. The *sucrose-uncoupled* (*sun*) mutants, in which plastocyanin gene expression in seedlings is no longer repressed by sugars, were identified using a plastocyanin promoter linked to a luciferase reporter gene (Dijkwel et al., 1997). The *hba* and *lba* mutants exhibit higher or lower levels, respectively, of *At*β-Amy transcripts encoding a β-amylase gene in response to sugar (Mita et al., 1997a, 1997b), and the *ram1* mutation in a putative splice site eliminates β-amylase activity (Laby et al., 2001). We have previously described a screen using the sugar-regulated *ApL3* gene encoding a large subunit of ADP-Glc pyrophosphorylase fused to a negative selection marker. This screen identified the *impaired sucrose induction* (*isi*) mutants (Rook et al., 2001), in which the sugar-mediated increase of *ApL3* gene expression was reduced in response to Suc.

Three classes of genes contributing to altered sugar responses have been isolated in the screens described above: those involved in abscisic acid (ABA)-regulated responses, in ethylene-mediated signaling, and those involved in non-ABA or nonethylene-related processes. Two ABA-related genes have been consistently identified in several screens for sugar-response mutants. The ABA biosynthetic mutant *aba2* (Leon-Kloosterziel et al., 1996; Gonzalez-Guzman et al., 2002) was shown to be allelic to *isi4* (Rook et al., 2001), *gin1* (Cheng et al., 2002), and *sis4* (Laby et al., 2000). *sun6* (Huijser et al., 2000), *isi3* (Rook et al., 2001), *sis5* (Laby et al., 2000), and *gin6* (Rolland et al., 2002) are mutations in *ABI4*, an ABA-regulated transcription factor (Finkelstein et al., 1998). The dominant ethylene-insensitive mutant *etr1* (Bleeker et al., 1988) antagonizes the Glc-insensitive germination of *gin1* (Zhou et al., 1998), suggesting that ethylene response pathways antagonize ABA synthesis (Cheng et al., 2002). This finding was supported by Ghassemian et al. (2000), who showed that ABA levels are increased in the *ein2/era3* mutant due to ethylene negatively regulating ABA synthesis during germination and early seedling development. The *sis1* mutation, isolated in a screen for sugar-insensitive germination, is allelic to *ctr1* (Gibson et al., 2001), further confirming the key role of ethylene signaling in modulating germination by antagonizing the inhibitory effects of ABA. *gin2-1* and *gin2-2* are mutations in *AtHXK1* that cause reduced hexokinase activity, overcome developmental arrest on 6% (w/v) Glc, and cause reduced growth of plants in response to high light intensities (Jang and Sheen, 1994; Moore et al., 2003). Interestingly, catalytically inactive AtHXK1 was able to rescue these defects, suggesting that the regulatory and catalytic activities of HXK1 are separable. The isi1 and isi2 mutations, which lead to reduced expression of several sugar-regulated genes, also do not exhibit ABA-related phenotypes (Rook et al., 2001).

We wished to identify mutations causing elevated responses to lower concentrations of sugar because screens conducted in these conditions may reveal mutants exhibiting a wider range of whole-plant phenotypes that are responsive to more physiologically relevant sugar levels, and because relatively few mutants with enhanced sugar responses have been characterized to date. Our strategy to obtain this type of mutants was to mutagenize a transgenic line expressing the firefly luciferase reporter gene linked to the Suc- and Glc- regulated *ApL3* promoter (Sokolov et al., 1998; Rook et al., 2001) and to screen M2 seedlings for elevated luciferase levels on medium containing 1% (w/v) Suc.

Several *high sugar-response (hsr*) mutants have been isolated, of which four representative mutants have been characterized in this study. These recessive mutations alter responses to low levels of Suc and Glc, leading to higher expression of a variety of sugar-regulated genes, and to reduced expression of genes whose expression is suppressed by sugars. The *hsr* mutants exhibit, to varying extents, elevated starch and anthocyanin levels, and reduced chlorophyll levels in response to sugars. ABA and ethylene responses appear to be unaffected in the four *hsr* mutants examined to date. Although no major differences in the growth and development of light-grown *hsr* seedlings were seen, development of dark-grown *hsr* seedlings occurred at lower Suc or Glc concentrations. We propose that these mutants affect the sensitivity of signaling pathways controlling a variety of metabolic and developmental processes.

RESULTS

Identification of *hsr* Mutants

To screen for mutants with high sugar responses, a transgenic Arabidopsis line that expressed the firefly luciferase cDNA under the control of the *ApL3* promoter (Rook et al., 2001) was made. A 4.5-kb promoter region was fused at the ATG initiation codon of the *ApL3* gene to coding region encoding firefly luciferase (pSP LUC$^+$; Promega, Southampton, UK), and a 1.5-kb 3′ region of the *ApL3* gene was fused directly after the termination codon. Transformants containing a single locus of the *ApL3::LUC* construct were obtained. Luciferase activity in line A3L3 was very low on Suc- or Glc-free media and was inducible to high levels in an almost linear response on media containing between 1% and 3% (w/v) Suc or Glc (Fig. 1, A and B). This line was chosen for further experiments. Mutagenesis was performed as described in "Materials and Methods," and putative mutants exhibiting relatively high luciferase activity were iden-

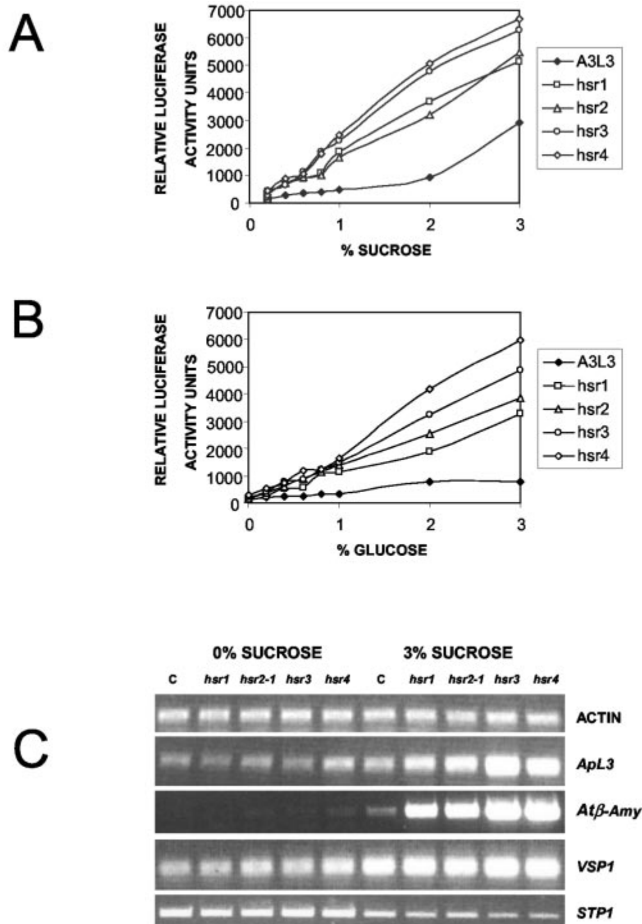

Figure 1. Gene expression in the *hsr* mutants. A and B, Luciferase activity in the *hsr* mutants. Luciferase levels were measured in 10-d-old seedlings of the A3L3 parental line and *hsr* mutants grown on media containing different concentrations of Suc (A) and Glc (B). Seedlings were sprayed with luciferin and analyzed luminometrically. Data were calculated from at least 50 seedlings. At sugar concentrations of 0.2% (w/v) or higher, *hsr* seedlings exhibited significantly higher ($P < 0.005$) levels of luciferase activity compared with the A3L3 parental line. C, Reverse transcription (RT)-PCR analysis of transcript levels in the *hsr* mutants. RT-PCR was performed on first strand cDNA made from 10-d-old seedlings grown in constant light on medium containing 0% or 3% (w/v) Suc. cDNA was standardized by reference to an actin standard. The number of amplification cycles used for each set of gene specific primers were: actin, 22; *ApL3*, 26; *Atβ*-Amy, 26; *VSP1*, 26; and *STP1*, 24. C, Control parental line A3L3.

tified in seedlings grown on media containing 1% (w/v) Suc. High luciferase activity was confirmed in 10 lines by rescreening progeny on 1% (w/v) Suc. Four representative *hsr* (*hsr1*–*hsr4*) lines expressing high and intermediate luciferase levels were selected for further analysis. Before further analysis, these lines were back-crossed at least three times to the parental A3L3 line and the mutants were reselected from the F_2 progeny based on increased luciferase activity in 7-d-old seedlings grown on media containing 1% (w/v) Suc. In subsequent phenotypic analysis, the four *hsr* mutants exhibited no significant differences when Suc or Glc were used, hence their description as *hsr* mutants.

The four *hsr* mutants were inherited as single recessive Mendelian loci, and two alleles of *hsr2* were identified, but only data for the *hsr2-1* allele is presented in this study. Mapping was carried out in crosses to *Landsberg erecta* as described in "Materials and Methods," and data were confirmed by rescreening and genotyping the progeny of recombinants. *hsr1* mapped between markers on the bacterial artificial chromosomes (BAC) T1F9 and F16M19 on chromosome 1, *hsr2* mapped at the top of chromosome 4 between markers on BAC F6N15 and T18A10, *hsr3* mapped between markers on BAC T4K22 and T8E3 on chromosome 1, and *hsr4* mapped between markers on BAC F12F1 and F13B4 on chromosome 1. The *ApL3::LUC* transgene in line A3L3 mapped to the bottom arm of chromosome 2.

Gene Expression in the *hsr* Mutants

Analysis of luciferase levels in *hsr1* through *hsr4* grown on media containing between 0% and 3% (w/v) Suc (Fig. 1A) and 0% and 3% (w/v) Glc (Fig. 1B) demonstrated significantly increased luciferase activity in the *hsr* mutants on low concentrations of both sugars compared with luciferase levels in the parental line A3L3. This demonstrated that the mutants did not have a general increase in luciferase activity but showed increased sugar responsiveness. This increased responsiveness also affected the expression of the endogenous *ApL3* gene, as shown by RT-PCR analysis of *ApL3* transcript levels in 10-d-old seedlings grown on medium containing 0% or 3% (w/v) Suc in constant light. The expression of *ApL3* was increased in all 4 *hsr* lines in seedlings grown on 3% (w/v) Suc compared with the parental line A3L3. Relatively high transcript levels were seen in the *hsr3* and *hsr4* mutants, whereas intermediate transcript levels were observed in *hsr1* and *hsr2* (Fig. 1C). These differences in *ApL3* expression levels between the *hsr* mutants were consistent with the luciferase data and were also observed in response to 3% (w/v) Glc (data not shown). To further characterize the mutants, we measured the expression of other Suc- and Glc- responsive genes in the *hsr* mutants. Figure 1C shows the results of a typical RT-PCR analysis of the expression of a selection of genes in 10-d-old seedlings. Expression of the *Atβ*-Amy gene (Mita et al., 1995) was also significantly increased in response to 3% (w/v) Suc, with the highest expression levels seen in *hsr3* and *hsr4*, similar to the pattern observed for *ApL3* expression. The expression of *VSP1*, encoding a Suc- and jasmonic acid-regulated vegetative storage protein (Berger et al., 1995), is also elevated in *hsr3* and *hsr4* in response to 3% (w/v) Suc compared with levels seen in the parental line. Expression of the high-affinity hexose transporter *AtSTP1* is down-

regulated in response to high Glc and Suc levels (Hemmann, 2000), and the expression of this gene was further down- regulated in the *hsr* mutants compared with the parental line, with the strongest reduction seen in *hsr3* and *hsr4*. Similar results were obtained from seedlings grown on 3% (w/v) Glc. These data show that, in *hsr* mutants, expression of Suc-induced genes such as *At*β-Amy and *VSP1* is enhanced in response to Suc, whereas the expression of genes repressed by Suc is further reduced in response to Suc in the *hsr* mutants.

The *hsr* Mutants Develop Normally

Several mutants with altered Glc and Suc responses exhibit a range of developmental and growth defects in light-grown plants. For example, the *hba1* mutation (Mita et al., 1997b) exhibits elevated *At*β-Amy expression in response to low Glc levels and increased anthocyanin levels (see below) similar to that seen in *hsr3* and *hsr4*. However, *hba1* grew poorly and often did not reach maturity (Mita et al., 1997b). In contrast, the *hsr* mutants grew at the same rate as the parental A3L3 line and wild-type Columbia in the greenhouse under short- and long-day conditions and were fully fertile. No changes in flowering time in short- or long-day photoperiods were observed with respect to the parental line. Only subtle phenotypic changes were observed in the four *hsr* mutants; for example, the relative lengths of the petioles and leaves were reduced (data not shown).

Plant Growth Regulator Responses

Previous screens for altered sugar responses have isolated ABA biosynthetic and response mutants (for review, see Rolland et al., 2002; Rook and Bevan, 2003). Screens for mutants defective in sugar induction of *ApL3* expression identified the *isi3* and *isi4* mutations (Rook et al., 2001) encoding the ABA response transcription factor ABI4 and the ABA2 biosynthetic gene, respectively. Ethylene responses have been implicated in sugar signaling (Zhou et al., 1998; Gibson et al., 2001), possibly by regulating ABA synthesis (Beaudoin et al., 2000; Ghassemian et al., 2000). Therefore, we predicted that some of the *hsr* mutants may exhibit ABA-hypersensitive phenotypes or reduced ethylene sensitivity. ABA responses were tested in the *hsr1* through *hsr4* mutants by measuring root elongation on media containing 5 μM ABA. At this concentration, root elongation is not maximally inhibited by ABA, and elevated sensitivity would have been detected. Root growth in the *hsr1* through *hsr4* and A3L3 lines was inhibited to an equal extent, in contrast to the ABA-insensitive mutant *abi1* (Fig. 2A), demonstrating normal ABA responses in the *hsr* mutants.

The reduction in length of dark-grown hypocotyls in response to 10 μM 1-aminocyclopropane-carboxylic acid (ACC, an ethylene precursor) was not significantly different between A3L3 and the *hsr* mutants (Fig. 2C), indicating a wild-type ethylene response in the *hsr* mutants. The ethylene-resistant mutants *ein2* (Alonso et al., 1999) and *etr1* (Bleeker et al., 1988) were not responsive to ACC in this test, as expected. Therefore, the *hsr* mutants do not display ABA or ethylene responses previously associated with altered sugar-mediated responses.

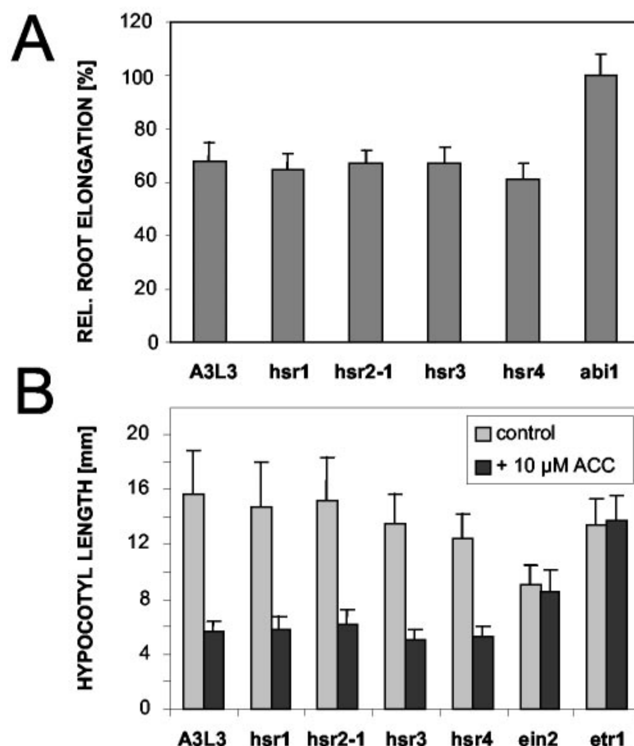

Figure 2. Growth regulator responses of the *hsr* mutants. A, Root elongation in response to 5 μM ABA. Seedlings were grown on sugar-free half-strength Murashige and Skoog medium for 4 d before transfer to vertical plates containing 5 μM ABA. Root elongation was measured after 4 d and relative root elongation was calculated relative to root growth on ABA-free plates (n = 18). The ABA-insensitive mutant *abi1* was included as a control. There were no significant differences in ABA sensitivity between the A3L3 parental line and the *hsr* mutants. B, Hypocotyl elongation in response to ACC treatment. Approximately 100 seedlings of the parental line A3L3, *hsr1*, *hsr2-1*, *hsr3*, *hsr4*, the ethylene-insensitive line *ein2*, and the ethylene-resistant line *etr1* were stratified for 4 d on Murashige and Skoog medium supplemented with 1% (w/v) Suc in the presence and absence of 10 μM ACC. Hypocotyl length was measured 6 d after transfer to 20°C in the dark. There were no significant differences between the A3L3 parental line and the *hsr* mutants.

Carbohydrate Levels and Uptake in the *hsr* Mutants

The enhanced responses of the *hsr* mutants to exogenous Suc and Glc suggest that increased cellular levels of these and related sugars may contribute to these changes. Therefore, Glc, Fru, Suc, and starch levels were measured in 10-d-old seedlings of the

mutants and the parental line grown on 1% (w/v) Suc in continuous light (Fig. 3A). These data showed no significant differences in Glc, Fru, Suc, and total sugar levels between the *hsr* mutants and the A3L3 line, although the *hsr* mutants did show a trend toward reduced Glc content. Starch levels were slightly increased in *hsr3* and *hsr4* compared with the parental line A3L3. Thus, the enhanced responses to sugars are not simply due to increased intracellular concentrations of Suc or Glc.

Altered uptake could also contribute to the enhanced responses to Suc and Glc observed in the *hsr* mutants, therefore, we measured Glc uptake in the *hsr* mutants. As Suc is cleaved by apoplastic invertase, it is not possible to distinguish between Suc and Glc uptake directly using labeled Suc (Giaquinta et al., 1983). The characterized hexose transporters in Arabidopsis (Sherson et al., 2000) transport Glc, Man, and Gal efficiently, and mutant lines are available for analysis. The uptake of ^{14}C Glc into 7-d-old seedlings of the *hsr* mutants, their parental line A3L3, a transgenic line expressing the Glc transporter STP1 (Hemmann, 2000) under the control of the 35S promoter, and its parental Wassilewskija (Ws) line was measured. There were no detectable differences in ^{14}C uptake between the A3L3 parental line and the four *hsr* mutants. The line expressing *35S:STP1* showed greatly elevated levels of ^{14}C Glc uptake, as expected (Fig. 3B). We concluded that altered Glc uptake is not responsible for the enhanced responses of the *hsr* mutants.

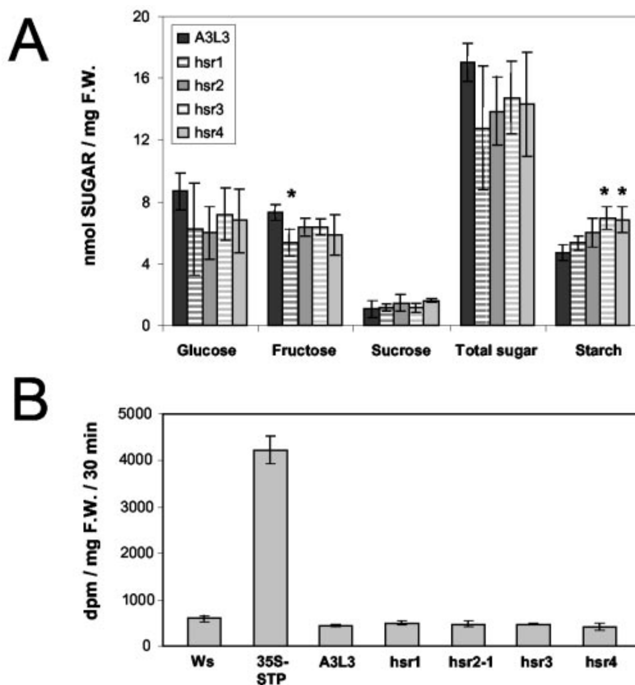

Figure 3. Carbohydrate levels and uptake in the *hsr* mutants. A, Levels of Glc, Fru, Suc, and starch were measured in 10-d-old seedlings grown on medium containing 1% (w/v) Suc in continuous light. The *hsr2-1* allele was used. Significant differences (Student's *t* test; $P < 0.05$) to A3L3 are indicated by an asterisk. B, Glc uptake in 7-d-old seedlings was measured using U-^{14}C- Glc. Ws is the ecotype transformed with the *35S:STP1* transgene overexpressing the STP1 hexose transporter. SD of three replicates is given. Independent repeats of uptake assays gave similar results. The *hsr2-1* allele was used.

Sugar Responses of the *hsr* Mutants

Several mutants implicated in Suc and Glc responses display decreased sensitivity to the inhibitory effects of high sugar concentrations on seed germination and seedling establishment (Dijkwel et al., 1996; Zhou et al., 1998; Huijser et al., 2000; Laby et al., 2000). Therefore, we tested if the *hsr* mutants displayed an increased sensitivity to sugar-mediated inhibition of seedling establishment. Seedling establishment of the *hsr* mutants was progressively reduced compared with the parental line A3L3 on increasing concentrations of Suc (Fig. 4A) or Glc (data not shown), demonstrating that elevated sugars have an opposite effect on the *hsr* mutants compared with the responses of Glc-insensitive mutants, such as *gin1* (Zhou et al., 1998).

We conducted further tests on the *hsr* mutants to determine if seedling responses to Glc were altered. Man-insensitive germination has been used to select for sugar response mutants (Pego et al., 2000). Glc-insensitive mutants such as *sun6/gin6/abi4* also show Man-insensitive phenotypes and germinate on medium containing 7.5 mM Man, a concentration that strongly inhibits germination of wild-type seedlings (Pego et al., 1999). Because we expected that the *hsr* mutants might display a Man-supersensitive phenotype, any resulting further reduction in germination would not be easily distinguished from wild-type responses. Therefore, we compared the Man inhibition of seedling growth after their establishment on Man-free medium. Growth inhibition of *hsr* seedlings by Man was measured by comparing seedling growth 5 d after transfer to medium containing 3.75 mM Man. After 5 d growth on Man, 90% of A3L3 seedlings continued to grow. In contrast, 60% of *hsr1*, 80% of *hsr2-1*, 30% of *hsr3*, and 25% of *hsr4* seedlings survived (Fig. 4B). These results confirmed that the *hsr1*, *hsr3*, and *hsr4* mutants exhibit a Man-supersensitive phenotype.

High levels of exogenous Glc and Suc antagonize the light-dependent induction of photogene expression (Krapp et al., 1993; Martin et al., 2002). To test if this response was more pronounced in the *hsr* mutants, the chlorophyll content of 10-d-old seedlings was measured after growth on different Suc concentrations. The chlorophyll content of the *hsr* mutants was more strongly reduced in *hsr* mutants compared with the parental line at Suc concentrations above 3% (w/v; Fig. 4C). This reduction could be due to reduced growth and development in the *hsr* mutants in response to elevated Suc, but this growth reduction

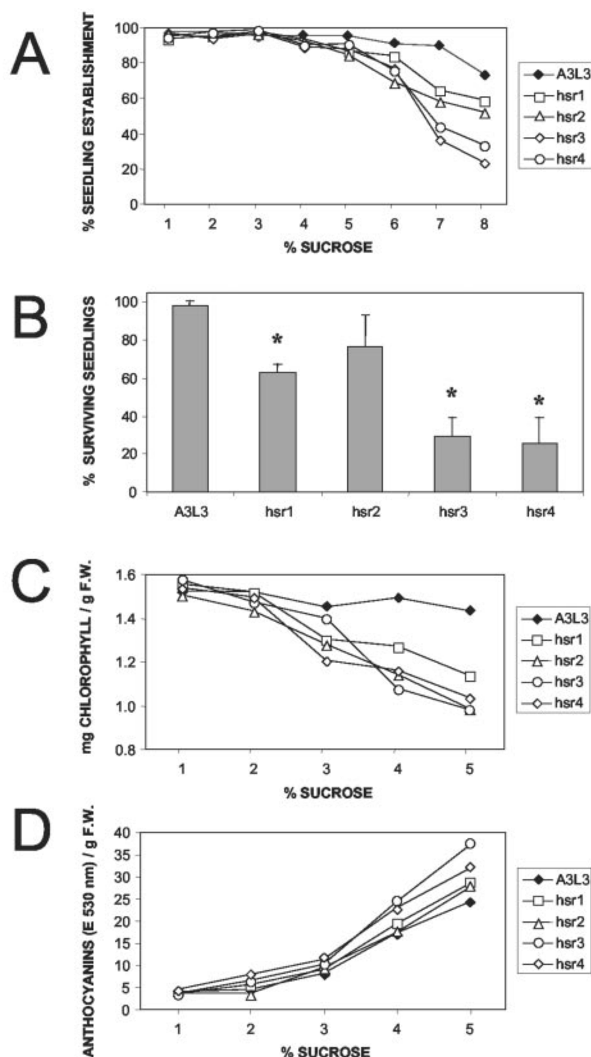

Figure 4. Phenotypes of the hsr mutants in response to sugars. A, Seedling establishment in response to different Suc concentrations was measured by plating approximately 100 seedlings on medium containing different concentrations of Suc and stratifying these for 4 d before transfer to constant light at 20°C. The percentage of seedlings exhibiting expanded green cotyledons after 5 d was measured. At ≥5% (w/v) Suc, all *hsr* mutants exhibited significantly reduced seedling establishment ($P < 0.05$) compared with the parental line A3L3. B, Seedling survival on medium containing 3.75 mM Man was tested by germinating seedlings on medium without Man for 5 d and subsequent transfer to Man-containing plates for a further 5 d. The *hsr2-1* allele was used. Significant differences (Student's *t* test; $P < 0.05$) to A3L3 are indicated by an asterisk. C, The chlorophyll content of 5-d-old seedlings was measured after growth on medium containing increasing concentrations of Suc. The results were obtained from pools of 100 seedlings for each data point. At ≥3% (w/v) Suc, all *hsr* mutants exhibited significantly reduced chlorophyll content ($P < 0.05$) compared with the parental line A3L3. The experiment was repeated three times with consistent results. The *hsr2-1* allele was used. D, The anthocyanin content of 5-d-old seedlings was measured after growth on medium containing increasing concentrations of Suc. The results were obtained from pools of 100 seedlings for each data point. At ≥5% (w/v) Suc, all *hsr* mutants exhibited significantly elevated anthocyanin levels ($P < 0.05$) compared with the parental line A3L3. The experiment was repeated three times with consistent results. The *hsr2-1* allele was used.

was only observed at Suc concentrations above 5% (w/v; Fig. 4A). Therefore, the *hsr* mutants exhibit enhanced repression of chlorophyll synthesis on media containing greater than 3% (w/v) Suc. Similar results were obtained with Glc (data not shown).

Anthocyanins accumulate when Arabidopsis is grown on high concentrations of Suc or Glc (Martin et al., 2002). Anthocyanin accumulated to higher levels in the four *hsr* mutants compared with the parental line at elevated Suc (Fig. 4D) and Glc concentrations (data not shown).

Dark Development in the *hsr* Mutants

Arabidopsis seedlings develop leaf- and flower-like organs when grown in the dark on vertical plates containing Suc (Roldan et al., 1999). This dark development or skotomorphogenesis requires direct contact between the shoot apex and the media. To assess this response for analyzing sugar response mutants, we first tested if dark development responds progressively to increasing concentrations of Suc or Glc. Wild-type Columbia seedlings were grown on medium containing different concentration of these sugars in the dark as described in "Materials and Methods," and after 3 weeks, their state of development was recorded. On medium containing 0.1% (w/v) Suc, seedlings did not develop beyond a slight opening of the cotyledonary petioles and expansion of the cotyledon (Stage 1, Fig. 5A). On medium containing 0.25% (w/v) Suc, the cotyledonary petioles were fully expanded and true leaves had just started to develop (Stage 2, Fig. 5A). At 1.0% (w/v) Suc, the first pair of true leaves had developed and a clear internode was apparent (Stage 3, Fig. 5A). The last stage of development identified was called Stage 4 (Fig. 5A), in which several pairs of true leaves had formed on medium containing 1.0% (w/v) Suc. Similar responses were observed for Glc (Fig. 5B).

Dark development was assessed in the *hsr* mutants on medium containing Glc. On 0.5% (w/v) Glc, most seedlings of the A3L3 parental line did not develop beyond Stage 2 after 3 weeks of growth in the dark (Fig. 5B). In contrast, nearly 60% of *hsr1* seedlings had developed to Stage 3, and greater than 80% of *hsr2-1*, *hsr3*, and *hsr4* seedlings had reached Stage 3. Ninety percent of Ws seedlings expressing the *35S:STP1* transgene, which exhibits increased Glc uptake (Fig. 3B), exhibited Stage 4 dark development in these conditions, in contrast to the low levels of dark development seen in Ws. These data showed that increased uptake of Glc can increase the extent of dark development. In contrast, the *hsr* mutants exhibited a greater extent of dark development than the parental line, whereas their Glc uptake (Fig. 3B) was the same as the parental line.

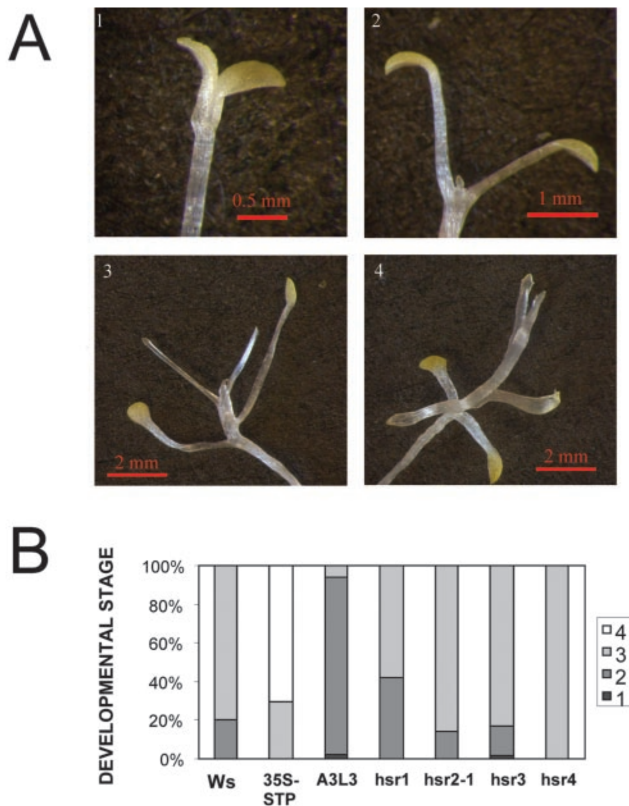

Figure 5. Dark development phenotypes of the *hsr* mutants. A, The different stages of dark development used for scoring responses to sugars are shown. Seedlings were grown on medium containing 0.1% (w/v) Suc (Stage 1), 0.25% (w/v) Suc (Stage 2), and 1.0% (w/v) Suc (Stages 3 and 4) on vertical plates in the dark at 20°C for 3 weeks. Similar results were obtained with Glc. B, The development of *hsr* mutants grown on medium containing 0.5% (w/v) Glc was scored after 3 weeks growth in the dark on vertical plates. The stages of development reached in samples of 200 seedlings were scored according Figure 5A. Ws is the parental line of the transgenic line *35S:STP1* that overexpresses the STP1 hexose transporter.

DISCUSSION

In developing seedlings, transcript levels of the large subunit of AGPase encoded by the *ApL3* gene are low but increase in response to exogenous Suc (Sokolov et al., 1998; Rook et al., 2001). We have identified *hsr* mutants that show elevated *ApL3* transcript levels in response to low concentrations of Suc or Glc. The first set of representative *hsr* mutants was selected for characterization because they showed intermediate (*hsr1* and *hsr2*) and high (*hsr3* and *hsr4*) levels of luciferase activity on low concentrations of Suc and Glc. These *hsr* mutations were fine-mapped to separate recessive loci, and our initial characterization of these mutants shows they are novel and act as negative regulators of sugar-induced and sugar-repressed processes controlling gene expression, growth, and development in Arabidopsis.

Intracellular Sugar Levels, Glc Uptake, and Growth Regulator Responses Are Unchanged in the *hsr* Mutants

Higher endogenous levels of Suc or Glc in the *hsr* mutants could explain enhanced responses of *ApL3:LUC* transgene expression to exogenous Suc and Glc concentrations between 0.2% and 3% (w/v; Fig. 1, A and B). However, Glc uptake in the *hsr* mutants was indistinguishable from the parental line (Fig. 3B), and Suc, Fru, and Glc levels in the *hsr* mutant seedlings grown on 1% (w/v) Suc were not significantly different from each other or the A3L3 parental line (Fig. 3A). These data suggest that changes in whole-plant levels of Suc, Fru, and Glc probably do not account for enhanced responses to Suc or Glc, although more subtle changes in the levels or compartmentalization of these and other key metabolites involved in signaling may contribute to the observed *hsr* phenotypes.

Because we have demonstrated that ABA can enhance sugar-mediated *ApL3* expression (Rook et al., 2001), we expected that mutants enhancing sugar-mediated *ApL3* expression, such as the *hsr* mutants, may also exhibit enhanced responses to ABA or reduced responses to ethylene. However, root elongation on 5 μM ABA (Fig. 2A), a concentration that only partly inhibited root elongation in the parental line, was not different between the parental line and the *hsr* mutants. The enhanced sugar sensitivity of the *hsr* mutants is also not due to ethylene insensitivity (Zhou et al., 1998), as hypocotyl elongation is equally sensitive to ethylene inhibition in the *hsr* mutants and the parental lines (Fig. 2B). Thus, these *hsr* mutations do not appear to have altered ABA and ethylene responses, and they do not have altered sugar levels or Glc uptake functions, suggesting they may have altered sugar-specific responses. This may be due to performing the mutant selection on low concentrations of sugar that do not induce osmotic responses such as ABA biosynthesis (Rook and Bevan, 2003).

Skotomorphogenesis Is a Sensitive Indicator of the Effects of Sugars on Growth and Development

Roldan et al. (1999) showed that dark-grown seedlings develop leaf- and flower-like structures if the shoot apex is in direct contact with Suc-containing medium. Low concentrations of Suc (1%, w/v) were sufficient to promote this skotomorphogenesis, and an increase in the rate of development was observed on media containing 3% (w/v) Suc (Roldan et al., 1999), suggesting that Suc availability at the shoot apex is required to induce and maintain development in the absence of light. We were interested in this observation because it can be used to study sugar-dependent growth and development in the absence of photosynthate production. The *hsr* mutants show increased dark development in response to Glc (Fig. 5B). A transgenic line overexpressing the *STP1*

Glc transporter (Hemmann, 2000) that has greatly enhanced Glc transport capacity (Fig. 3B) exhibited a greater degree of dark development than the parental line or the *hsr* mutants on Glc (Fig. 5B), showing that increased Glc uptake can promote dark development. However, Glc uptake in the *hsr* mutants was indistinguishable from the parental line (Fig. 3B), suggesting that the shoot apex of the *hsr* mutants may have enhanced sensitivity to Glc, or that altered partitioning of carbohydrates to the shoot meristem leads to enhanced development in the dark.

Because organ development observed during skotomorphogenesis requires cell division and is dependent on exogenous sugar, it is possible that the *HSR* genes may affect responses of the cell cycle to sugars. This interpretation is supported by the observation that sugar starvation arrests the cell cycle in a quiescent state and sugar reapplication promotes synchronous passage through G1 in Arabidopsis cell cultures (Riou-Khamlichi et al., 2000). The responsiveness of the dark development phenotype to low, physiologically relevant Glc and Suc levels suggests that it will be an important method for identifying and understanding the links between metabolite provision and perception, cell division, and growth control.

The *hsr* Mutants Affect Several Sugar-Regulated Cellular Processes

Comparison of seedling establishment, anthocyanin and chlorophyll levels, and gene expression in the *hsr* mutants with other mutants affecting sugar responses reveals a range of unique and informative phenotypes in the *hsr* mutants, and demonstrates that the screen for enhanced sugar-responsive gene expression identifies mutants affecting several cellular responses. Pego et al. (2000) gave a preliminary description of *glucose-supersensitive* (*gss*) and *sucrose-supersensitive* (*sss*) mutants that were unable to germinate or develop in the presence of noninhibitory sugar concentrations. It is possible that some of the *sss* mutants may be allelic to the *hsr* mutants described here because the *sss* mutants were screened on medium containing 12% (w/v) Suc and the *hsr* mutants characterized to date show increased sensitivity of seedling establishment to Suc concentrations above 6% (w/v) Suc (Fig. 4A). Mutants displaying the *glucose-oversensitive* (*glo*) phenotype (Sheen et al., 1999; Rolland et al., 2002) might also be expected to have some phenotypes in common with the *hsr* mutants, but these have not yet been described in detail. Anthocyanin levels in the *hsr* mutants are elevated above those measured in the parental line in response to 5% (w/v) Suc (Fig. 4D). The *hba1* mutation (Mita et al., 1997b), and the *prl1* mutation (Németh et al., 1998), which causes a wide range of light-grown phenotypes that can be phenocopied by sugar and cytokinin treatment, exhibit increased anthocyanin levels in the absence of stimuli such as light. In contrast, anthocyanin levels in the *hsr* mutants are at wild-type levels at low sugar concentrations but increase to higher levels than wild type at above 5% (w/v) Suc. Mutants with reduced ABA responses (Dijkwel et al., 1997; Zhou et al., 1998) and reduced sugar-mediated responses (F. Rook and M.W. Bevan, unpublished data) lead to reduced anthocyanin accumulation, demonstrating a reciprocal response to that seen in the *hsr* mutants. Chlorophyll levels are reduced in response to Glc and Suc (Sheen, 1990), and transcript levels of photogenes such as *CAB* and *RbcS* are reduced when Suc is applied to leaves or Suc export is disrupted (Krapp et al., 1993; Jang and Sheen, 1994). Chlorophyll levels in the *hsr* mutants were significantly reduced at high Suc concentrations compared with the parental line (Fig. 4C). As internal sugar levels are not detectably altered in the *hsr* mutants, it is possible that photogene expression and protein levels may be more responsive to sugar-mediated inhibition in the *hsr* mutants. The enhanced sensitivity of *hsr1*, *hsr3*, and *hsr4* seedlings to Man (Fig. 4B) is also a reciprocal response to that shown by *glucose-insensitive* mutants (Pego et al., 1999). The increased Man toxicity in these *hsr* mutants suggests that the *hsr* and some *gin* mutants may have opposed regulatory functions on a common pathway.

Consistently altered patterns of gene expression were observed in the *hsr* mutants that are different from patterns of gene expression seen in other mutants affecting sugar-mediated gene expression. The *hsr* mutants show enhanced expression of *At*β-Amy in response to 3% (w/v) Suc (Fig. 1C), but on 1% (w/v) Glc or Suc, expression levels were not detectable. This is in contrast to the *hba1* mutant that showed elevated *At*β-Amy expression on medium containing no or high Suc (Mita et al., 1997b). This suggests the *hsr* and *hba1* mutants affect *At*β-Amy transcript levels differently. The observation that the *hba* mutant grew poorly on Suc-containing medium further suggests the *hba* and *hsr* mutants are probably not allelic. Genes whose expression is induced or repressed by Glc or Suc were expressed at higher level in the *prl1* mutant on medium containing 3 mM Glc, thus *PRL1* also affects sugar-regulated gene expression differently from the *hsr* mutants (Németh et al., 1998). Our survey of gene expression in the *hsr* mutants suggests that *HSR* genes may encode proteins that negatively regulate the induction and repression of gene expression by sugars because *At*β-Amy, *VSP1*, and *ApL3* expression in response to Suc is enhanced, and the reduction of *STP1* gene expression is further reduced in response to Suc.

The recessive nature of the *hsr* mutations implies that the *HSR* gene products may act to repress sugar-activated and sugar-inhibited processes. Reduced function of the *HSR* gene products then leads to enhanced responses of a range of whole-plant developmental, growth, and metabolic processes to low, physiologically relevant, concentrations of exoge-

nous Suc or Glc. The observation that *hsr* mutants show opposite responses of sugar-regulated gene expression to the *isi1* and *isi2* mutants (Rook et al., 2001) in the absence of any detectable ABA-related phenotypes suggests that the *hsr* mutants may reciprocally affect sugar-mediated processes related to those affected by the *isi1* and *isi2* mutations. We do not yet know if the increased sugar responsiveness of the *hsr* mutants is due to altered partitioning of key metabolites or signaling molecules, or to altered responsiveness of signal pathways to normal levels of key metabolites or signaling molecules. The isolation and characterization of *HSR* genes will help to distinguish between these distinct hypotheses.

MATERIALS AND METHODS

Plant Material and Growth Conditions

Wild-type and transgenic Arabidopsis lines were the Colombia-0 ecotype unless otherwise specified. For in vitro growth, seeds were surface sterilized with 80% (w/v) ethanol and 20% (v/v) household bleach, washed at least five times with sterile water, stratified at 4°C for 2 d in the dark, and germinated on Murashige and Skoog medium (Duchefa Biochemie, Haarlem, The Netherlands) supplemented with 0.8% (w/v) agar, sugars, and growth regulators as indicated. Plants were grown in sterile culture in constant light or 16-h light/8-h dark cycles at 20°C and 60 μE m^{-2} s^{-1} illumination. Soil-grown plants were grown in the greenhouse under long-day conditions or in short-day growth cabinets in 8-h light/16-h dark cycles at 20°C to 25°C. The Ws lines expressing the *35S:STP1* transgene was obtained from Dr. Steve Smith (University of Edinburgh).

Construction of the Parental Line

The *ApL3::LUC* reporter gene construct was made essentially as described for the ApL3:P450 reporter gene construct (Rook et al., 2001). The luciferase-encoding *Nco*I/*Eco*RI fragment of plasmid pSP luc^{+} (Promega) was used as reporter. After cloning of the construct into pGreen0029 (Hellens et al., 2000) Arabidopsis ecotype Colombia-0 was transformed using *Agrobacterium tumefaciens* strain GV3101 (pMP90). Transformants were selected on Murashige and Skoog medium containing 1% (w/v) Glc and 50 μg mL^{-1} kanamycin and lines with single insertion sites were identified by segregation of kanamycin resistance.

Isolating and Mapping the *hsr* Mutants

From the *ApL3::LUC* transformants, line A3L3 was selected for mutagenesis as it showed clear sugar-responsive LUC activity. Approximately 20,000 seeds were treated with a 0.5% (w/v) ethyl methansulfonate (Sigma, Poole, UK) for 6 h. The mutagenized M_1 seeds were sown in 70 pools on soil. The harvested M_2 seeds were surface-sterilized and grown in microtiter plates on Murashige and Skoog medium containing 1% (w/v) Suc in continuous light. After 7 d growth, a total of 20,000 M_2 seedlings representing 3,000 M_1 plants were screened using a Victor Luminometer (Wallac/Perkin-Elmer, Boston). Five minutes after spraying with a 1 mM luciferin (Duchefa) and 0.01% (v/v) Triton X-100 solution, luciferase activity was measured for 1 s. Seedlings showing higher luminescence than the parental line grown in the same plate were selected. The progeny of putative mutants were rescreened and 10 mutants were confirmed. Four representative mutants were selected for further analysis. These were mapped from the F_2 population of crosses to Arabidopsis ecotype *Landsberg erecta* using simple sequence length polymorphic (Bell and Ecker, 1994) and cleaved-amplified polymorphic sequence (Konieczny and Ausubel, 1993) markers. About 200 chromosomes were tested to map each mutant.

RNA Isolation and RT-PCR Analysis

RNA was extracted from frozen ground plant material using TRIZOL reagent (Life Technologies, Paisley, UK) according to the manufacturer's protocol. RT and control reactions were performed as described in Baier et al. (2000). The cDNA samples were standardized on actin transcript amount using the primers Atact-S and Atact-A (Baier et al., 2000). The following gene-specific primers were derived from database entries: ApL3-S: CGTCTGAATCATGCAACC; ApL3-A: GCATTTCCTGATCTTTGTATCCTCG; β-Amy-S: CGGAGAAGGGGAAGTTTTTC; β-Amy-A: AATCTCATGCCCGTACTTCG; Vsp1-S: CGTGGTTAGAGTCCGGAGAA; Vsp1-A: TGTTGTACCCTTTCTTCACAAGG; Stp1-S: TTCTTTCAACAGCTAACCGGAATCA; and Stp1-A: GGCTAATACACTTTTTCCTTTACGACA. Each pair of primers amplified a single 500- to 600-bp PCR product. Relative transcripts levels were visualized by reducing the cycle number until the rate of PCR product generation was in the early exponential stage of amplification. Twenty-microliter samples were loaded on 1% (w/v) agarose gels in 1× Tris-acetate EDTA buffer containing 0.5 μg mL^{-1} ethidium bromide and they were electrophoresed at 100V.

Carbohydrate, Chlorophyll, and Anthocyanin Analysis

Glc, Fru, and Suc were measured sequentially in cleared supernatants of K_2CO_3-neutralized $HClO_4$ extracts of ground, frozen plant material (Galtier et al., 1995). Glc-6-P dehydrogenase-mediated NADP reduction was measured at 340 nm in a Tecan Saffire plate reader (Tecan UK, Milton Keynes, UK) in a reaction mixture containing 100 mM HEPES-KOH (pH 7.5), 5 mM $MgCl_2$, 3.2 mM NADP, 2 mM ATP, and 4 U mL^{-1} hexokinase after subsequent additions of 20 U mL^{-1} Glc-6-P dehydrogenase (Glc), 40 U mL^{-1} phosphoglucoisomerase (Fru), and 250 U mL^{-1} invertase (Suc). Starch was measured in the insoluble fraction of the $HClO_4$ homogenate after extraction of the pheophytins with 80% (w/v) acetone and washing the remaining pellet with water. Glc was released from starch by a 1-h incubation at 95°C and by a 2-h incubation at 50°C with 12 U mL^{-1} α-amylase and 2.4 U mL^{-1} amyloglucosidase in 50 mM sodium acetate buffer, pH 4.8. Glc was then measured as described above. All enzymes and substrates were purchased from Roche Diagnostics (Mannheim, Germany). Carbohydrate measurements were standardized according to the fresh weight of the tissues extracted. For chlorophyll measurements, the plant material was extracted in 80% (v/v) acetone saturated with $CaCO_3$ in the dark at −20°C. Anthocyanins were extracted in 1% (v/v) HCl in methanol. These pigments were assayed in the cleared supernatants after 5 min centrifugation at 10,000*g*. The chlorophyll content was quantified according to Arnon (1949), and anthocyanins were quantified according to Mancinelli et al. (1975).

Glc Uptake Measurements

Seedlings were grown in continuous light (60 μE m^{-2} s^{-1}) on vertical agar plates containing 1× Murashige and Skoog medium, 0.5% (w/v) MES, pH 5.7, and 0.8% (w/v) agar for 7 d. Ten milligrams of tissue (7–9 seedlings) was immersed in 0.19 mL of Murashige and Skoog medium, vacuum infiltrated using a water pump for 3 min, and left at room temperature (20°C) for 30 min to equilibrate. D-[U-^{14}C]-labeled Glc (0.1 μCi; Amersham, Buckinghamshire, UK) was added in 10 μL of water to give a final Glc concentration of 150 μM and was incubated at room temperature for 30 min. The unincorporated label was removed and the tissue was washed five times with ice-cold 1× Murashige and Skoog medium containing 1 mM unlabeled Glc. Finally, the samples were extracted twice with 1 mL of 80% (v/v) ethanol at 60°C, and the amount of label in the combined soluble extracts (in excess of 90% of the incorporated label) was determined by liquid scintillation counting.

ACKNOWLEDGMENTS

We thank John Bevan and Andrew Walsh for assistance with the mutant screen. We also thank Dr. Steve Smith (University of Edinburgh) for the *35S:STP1* line.

Received August 11, 2003; returned for revision September 16, 2003; accepted October 17, 2003.

LITERATURE CITED

Alonso JM, Hirayama T, Roman G, Nourizadeh S, Ecker JR (1999) EIN2, a bifunctional transducer of ethylene and stress responses in Arabidopsis. Proc Natl Acad Sci USA **284:** 2148–2152

Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, Léon P (2000) Analysis of Arabidopsis glucose insensitive mutants, *gin5* and *gin6*, reveal a central role of the plant hormone ABA in the regulation of plant vegetative development. Genes Dev **14:** 2085–2096

Arnon DJ (1949) Copper enzymes in isolated chloroplasts. Plant Physiol **24:** 1–15

Baier M, Noctor G, Foyer CH, Dietz KJ (2000) Antisense suppression of 2-cysteine peroxiredoxin in Arabidopsis specifically enhances the activities and expression of enzymes associated with ascorbate metabolism but not glutathione metabolism. Plant Physiol **124:** 823–832

Beaudoin N, Serizet C, Gosti F, Giraudat J (2000) Interactions between abscisic acid and ethylene signaling cascades. Plant Cell **12:** 1103–1115

Bell CJ, Ecker JR (1994) Assignment of 30 microsatellite loci to the linkage map of Arabidopsis. Genomics **19:** 137–144

Berger S, Bell E, Sadka A, Mullet JE (1995) *Arabidopsis thaliana* AtVSP is homologous to soybean VSPa and VSPb, genes encoding vegetative storage protein acid-phosphatases, and is regulated similarly by methyl jasmonate, wounding, sugars, light and phosphate. Plant Mol Biol **27:** 933–942

Bleeker AB, Estelle MA, Somerville C, Kende H (1988) Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science **241:** 1086

Cheng W-H, Endo A, Penney J, Chen H-C, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiba T et al. (2002) A unique short-chain dehydrogenase/reductase in Arabidopsis glucose signaling and abscisic acid biosynthesis and functions. Plant Cell **14:** 2723–2743

Chiou T-J, Bush DR (1998) Sucrose is a signal molecule in assimilate partitioning. Proc Natl Acad Sci USA **95:** 4784–4788

Coruzzi GM, Bush DR (2001) Nitrogen and carbon nutrient and metabolite signaling in plants. Plant Physiol **125:** 61–64

Dijkwel PP, Huijser C, Weisbeek PJ, Chua NH, Smeekens SCM (1997) Sucrose control of phytochrome A signaling in Arabidopsis. Plant Cell **9:** 583–595

Dijkwel PP, Kock PAM, Bezemer R, Weisbeek PJ, Smeekens SCM (1996) Sucrose represses the developmentally controlled transient activation of the plastocyanin gene in Arabidopsis seedlings. Plant Physiol **110:** 455–463

Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM (1998) The Arabidopsis abscisic acid response locus ABI4 encodes an APETALA2 domain protein. Plant Cell **10:** 1043–1054

Galtier N, Foyer CH, Murchie E, Alred R, Quick P, Voelker TA, Thépenier C, Lascève, G, Betsche T (1995) Effects of light and atmospheric carbon dioxide enrichment on photosynthesis and carbon partitioning in the leaves of tomato (*Lycopersicon esculentum* L.) plants overexpressing sucrose phosphate synthase. J Environ Bot **46:** 1335–1344

Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. Plant Cell **12:** 1117–1126

Giaquinta RT, Lin W, Sadler NL, Francheschi VR (1983) Pathway of phloem unloading of sucrose in corn roots. Plant Physiol **72:** 362–367

Gibson SI, Laby RJ, Kim D (2001) The sugar-insensitive1 (sis1) mutant of Arabidopsis is allelic to ctr1. Biochem Biophys Res Commun **280:** 196–203

Gonzalez-Guzman M, Apostolova N, Belles JM, Barrero JM, Piqueras P, Ponce MR, Micol JL, Serrano R, Rodriguez PL (2002) The short-chain alcohol dehydrogenase ABA2 catalyses the conversion of xanthoxin into abscisic aldehyde. Plant Cell **14:** 1833–1846

Harmer SL, Hogenesch JB, Straume M, Chang H-S, Han B, Zhu T, Wang X, Kreps JA, Kay SA (2000) Orchestrated transcription of key pathways in Arabidopsis by the circadian clock. Science **290:** 2110–2113

Hellens RP, Edwards EA, Leyland NR, Bean S, Mullineaux PM (2000) pGreen: a versitile and flexible binary Ti vector for Arabidopsis-mediated plant transformation. Plant Mol Biol **42:** 819–832

Hemmann G (2000). Functional analysis of the Arabidopsis monosaccharide transporter STP1. PhD thesis. University of Edinburgh, Edinburgh

Huijser C, Kortstee A, Pego J, Weisbeek P, Smeekens S (2000) The Arabidopsis *SUCROSE UNCOUPLED-6* gene is identical to *ABSCISIC ACID INSENSITIVE-4*: involvement of abscisic acid in sugar responses. Plant J **23:** 577–585

Jang J-C, Sheen J (1994) Sugar sensing in higher plants. Plant Cell **6:** 1665–1679

Koch KE (1996) Carbohydrate modulated gene expression in plants. Annu Rev Plant Physiol Plant Mol Biol **47:** 509–540

Konieczny A, Ausubel FM (1993) A procedure for mapping Arabidopsis mutations using codominant ecotype-specific PCR-based markers. Plant J **4:** 403–410

Krapp A, Hofmann B, Schäfer C, Stitt M (1993) Regulation of the expression of rbcS and other photogenic genes by carbohydrates: a mechanism for the "sink regulation" of photosynthesis. Plant J **3:** 817–828

Laby RJ, Kim D, Gibson SI (2001) The ram1 mutant of Arabidopsis exhibits severely decreased β-amylase activity. Plant Physiol **127:** 1798–1807

Laby RJ, Kincaid MS, Kim D, Gibson SI (2000) The Arabidopsis sugar-insensitive mutants sis4 and sis5 are defective in abscisic acid synthesis and response. Plant J **23:** 587–596

Leon-Kloosterziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE, Schwartz SH, Zeevart JA, Koornneef M (1996). Isolation and characterization of abscisic acid-deficient Arabidopsis mutants and two new loci. Plant J **10:** 655–661

Mancinelli AL, Yang C-PH, Lindquist P, Anderson OR, Rabino I (1975) Photocontrol of anthocyanin synthesis: the action of streptomycin on the synthesis of chlorophylls and anthocyanin. Plant Physiol **55:** 251–257

Martin T, Oswald O, Graham IA (2002) Arabidopsis seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon:nitrogen availability. Plant Physiol **128:** 472–481

Mita S, Hirano H, Nakamura K (1997b) Negative regulation in the expression of a sugar-inducible gene in *Arabidopsis thaliana.* Plant Physiol **114:** 575–582

Mita S, Murano N, Akaike M, Nakamura K (1997a) Mutants of *Arabidopsis thaliana* with pleiotropic effects on the expression of the gene for β-amylase and on the accumulation of anthocyanins that are inducible by sugars. Plant J **11:** 841–851

Mita S, Suzuki-Fujii K, Nakamura K (1995) Sugar-inducible expression of a gene for β-amylase in *Arabidopsis thaliana*. Plant Physiol **107:** 895–904

Moore B, Zhou L, Rolland F, Hall Q, Cheng W-H, Liu Y-X, Hwang I, Jones T, Sheen J (2003) Role of the Arabidopsis glucose sensor HXK1 in nutrient, light and hormonal signaling. Science **300:** 332–336

Neff MM, Fankhauser C, Chory J (2000) Light: an indicator of time and place. Genes Dev **14:** 257–271

Németh K, Salchert K, Putnoky P, Bhalerao R, Koncz-Kálman Z, Stankovic-Stangeland B, Bakó L, Mathur J, Ökréz L, Stabel S et al. (1998) Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in Arabidopsis. Genes Dev **12:** 3059–3073

Pego JV, Kortstee AJ, Huijser C, Smeekens SCM (2000) Photosynthesis, sugars and the regulation of gene expression. J Exp Bot **51:** 407–416

Pego JV, Weisbeek PJ, Smeekens SCM (1999) Mannose inhibits Arabidopsis germination via a hexokinase-mediated step. Plant Physiol **119:** 1017–1023

Price J, Li T-C, Kang SG, Na JK, Jang J-C (2003) Mechanisms of glucose signaling during germination in Arabidopsis. Plant Physiol **132:** 1424–1438

Riou-Khamlichi C, Menges M, Healy JMS, Murray JAH (2000) Sugar control of the plant cell cycle: differential regulation of Arabidopsis D-type cyclin gene expression. Mol Cell Biol **20:** 4513–4521

Roldan M, Gomez-Mena C, Ruiz-Garcia L, Salinas J, Martinez-Zapater JM (1999) Sucrose availability on the aerial part of the plant promotes morphogenesis and flowering in the dark. Plant J **20:** 581–590

Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in plants. Plant Cell Suppl **2002:** S185–S205

Rook F, Bevan M (2003) Genetic approaches to understanding sugar-response pathways. J Exp Bot **54:** 495–501

Rook F, Corke F, Card R, Munz G, Smith C, Bevan MW (2001) Impaired sucrose-induction mutants reveal the modulation of sugar-inducible starch biosynthetic gene expression by abscisic acid signaling. Plant J **26:** 421–433

Sheen J (1990) Metabolic repression of transcription in higher plants. Plant Cell **2:** 1027–1038

Sheen J, Zhou L, Jang JC (1999) Sugars as signaling molecules. Curr Opin Plant Biol **2:** 410–418

Sherson SM, Hemmann G, Wallace G, Forbes S, Germain V, Stadler R, Bechtold N, Sauer N, Smith S (2000) Monosaccharide/proton symporter AtSTP1 plays a major role in uptake and response of Arabidopsis seeds and seedlings to sugars. Plant J **24:** 849–857

Smeekens S (2000) Sugar-induced signal transduction in plants. Annu Rev Plant Physiol Plant Mol Biol **51:** 49–81

Sokolov LN, Déjardin A, Kleczkowski LA (1998) Sugar and light/dark exposure trigger differential regulation of ADP-glucose pyrophosphorylase genes in *Arabidopsis thaliana* (thale cress). Biochem J **336:** 681–687

Vaughn MW, Harrington GN, Bush DR (2002) Sucrose-mediated transcriptional regulation of sucrose symporter activity in the phloem. Proc Natl Acad Sci USA **99:** 10876–10880

Zhou L, Jang JC, Jones TL, Sheen J (1998) Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. Proc Natl Acad Sci USA **95:** 10294–10299