Plant Physiol. (1997) 114: 575–582

Negative Regulation in the Expression of a Sugar-Inducible Gene in *Arabidopsis thaliana*[1]

A Recessive Mutation Causing Enhanced Expression of a Gene for β-Amylase

Satoru Mita[2], Hideyuki Hirano, and Kenzo Nakamura*

Laboratory of Biochemistry, School of Agricultural Sciences, Nagoya University, Chikusa, Nagoya 464–01, Japan (S.M., H.H., K.N.); and Division of Developmental Biology, National Institute for Basic Biology, Okazaki 444, Japan (H.H., K.N.)

Expression of a β-amylase gene of *Arabidopsis thaliana* (*ATβ-Amy*) is regulated by sugars. We identified a mutant, *hba1*, in which the level of expression of *ATβ-Amy* in leaves of plants that had been grown in a medium with 2% sucrose was significantly higher than that in wild-type plants. Higher than wild-type levels of β-amylase in *hba1* plants depended on the presence of 1 to 2% sucrose or 1% glucose in the medium, whereas leaves of mutant plants grown with higher levels of sugars had β-amylase activities similar to those in leaves of wild-type plants. The *hba1* phenotype was recessive and did not affect levels of sugars and starch in leaves. It is proposed that expression of *ATβ-Amy* is regulated by a combination of both positive and negative factors, dependent on the level of sugars, and that *HBA1* might function to maintain low-level expression of *ATβ-Amy* until the level of sugars reaches some high level. Results of crosses of *hba1* plants with transgenic plants that harbored an *ATβ-Amy:GUS* transgene with 1587 bp of the 5′-upstream region suggested that *HBA1* affects expression of *ATβ-Amy* in *trans*. The *hba1* plants also had growth defects and elevated levels of anthocyanin in their petioles. However, sugar-regulated changes in levels of several mRNAs other than β-amylase mRNA were unaffected in *hba1* plants, suggesting that only a subset of sugar-regulated genes is under the control of *HBA1*.

The interorgan transport and availability of Suc or of other transported sugars is likely to be an important factor in the control of growth and development of plants. A variety of genes are known to be regulated, either positively or negatively, depending on the gene, by the levels of sugars in the sugar-importing as well as in the sugar-exporting sites of the plant body (Rocha-Sosa et al., 1989; Hattori et al., 1990; Johnson and Ryan, 1990; Müller-Röber et al., 1990; Sheen, 1990; Nakamura et al., 1991; Tsukaya et al., 1991; Yu et al., 1991; Koch et al., 1992; Yang et al., 1993; Graham et al., 1994). A signal related to carbohydrate metabolism, rather than the Suc molecule itself or an osmotic signal, seems to be involved in sugar-regulated expression of these genes. However, the underlying mechanisms and, in particular, the mechanisms of transduction to the cell nucleus of signals related to carbohydrate metabolism are poorly understood.

In yeast (Rose et al., 1991; Trumbly, 1992; Özcan and Johnston, 1995) and in mammals (Epstein et al., 1992; German, 1993), specific isoforms of hexokinase play a major role in sensing Glc and controlling the expression of Glc-sensitive genes. Hexokinase also plays a role in sensing sugars and leading to the repression of genes for photosynthetic proteins in plant cells (Jang and Sheen, 1994). It has also been suggested that sugar-inducible gene expression in plants might be caused by a reduction in the cellular level of free phosphate (Sadka et al., 1994). The sugar-inducible expression of at least some plant genes requires the continuous dephosphorylation of proteins (Takeda et al., 1994), signaling by Ca^{2+} ions (Ohto et al., 1995), and protein kinase activity (Ohto and Nakamura, 1995). In leaves of tobacco, sugars apparently regulate levels of several CDPKs that are associated with the plasma membrane (Ohto and Nakamura, 1995).

Genetic studies potentially provide a powerful tool with which to dissect the complex regulatory pathways that are involved in sugar regulation of genes in various parts of the plant body. Furthermore, mutants affecting regulation by sugars should reveal their physiological roles in the growth and development of plants under various environmental conditions. Results of extensive genetic studies on repression and induction by Glc of gene expression in yeast (Trumbly, 1992; Özcan and Johnston, 1995) indicate that multiple signal transduction pathways transmit the Glc signal to many Glc-sensitive genes either separately or with intergene interactions. These studies have identified components such as transcription factors, protein kinases, and sugar transporters, in addition to hexokinase, that are involved in the regulation.

[1] This work was supported in part by Grants-in-Aid for Scientific Research on Priority Areas ("The Molecular Basis of Flexible Organ Plans in Plants," nos. 06278101 and 06278102) from the Ministry of Education, Science and Culture of Japan.

[2] Present address: Laboratory of Postharvest Physiology, Department of Biological Sciences, Faculty of Agriculture, Shizuoka University, 836 Ohya, Shizuoka 422, Japan.

* Corresponding author; e-mail kenzo@nuagr1.agr.nagoya-u.ac.jp; fax 81–52–789–4094.

Abbreviations: CDPK, calcium-dependent protein kinase; CHI, chalcone flavanone isomerase; CHS, chalcone synthase; Col-0, Columbia; DFR, dihydroflavonol 4-reductase; WS, Wassilewskija.

Sugar-inducible expression of a single-copy gene for β-amylase in leaves of *Arabidopsis thaliana* (*ATβ-Amy*; Mita et al., 1995) can be easily assayed by the starch-iodine assay. We previously identified the *lba1* and *lba2* mutants of Arabidopsis, in which the sugar-induced levels of β-amylase are severely reduced (Mita et al., 1997). Both *lba1* and *lba2* are recessive mutations in *trans*-acting genes, and the sugar-inducible accumulation of anthocyanin was also affected in *lba1* plants. We report here the isolation and characterization of another type of Arabidopsis mutant, designated *hba1*, in which a high level of expression of *ATβ-Amy* was observed in leaves of plants grown on medium with 2% Suc.

MATERIALS AND METHODS

Plant Materials and Treatment with Sugars

The Col-0 ecotype of *Arabidopsis thaliana* (L.) Heynh. was used as the wild-type strain. Transgenic plants with the *ATβ-Amy:GUS* transgene, prepared from the WS ecotype, were described previously (Mita et al., 1995). Seeds were sterilized in a solution of NaClO for 5 min, washed five times in sterile water, and chilled at 4°C overnight. They were then sown on gellan gum plates that contained Murashige and Skoog salts (pH 5.8), 100 mg L^{-1} myo-inositol, 10 mg L^{-1} thiamine-HCl, 1 mg L^{-1} nicotinic acid, 1 mg L^{-1} pyridoxine HCl, and various concentrations of Suc or Glc, and incubated at 22°C under continuous fluorescent light at an intensity of 70 μmol m^{-2} s^{-1}. For treatment with Suc, mature leaves were excised from 3-week-old plants. The cut edges of their petioles were immersed in a sterile solution of sugar in water and they were incubated in this way under continuous light at 22°C (Mita et al., 1995). Leaves immediately after harvest from the plants (nontreated leaves) served as controls.

Screening for Mutants

Mutants with altered patterns of Suc-inducible expression of *ATβ-Amy* were selected by screening M_2 plants descended from ethyl methanesulfonate-treated seeds that had been grown on medium with 2% Suc as described previously (Mita et al., 1997). Individual plants with abnormally high levels of amylase activity in nontreated leaves were self-pollinated, and a similar analysis was repeated with more than eight M_3 plants per line. Lines in which nontreated leaves of all of the M_3 plants had elevated levels of amylase activity were selected. These lines were backcrossed at least twice to the Col-0 wild type.

Extraction of Protein and Enzyme Assays

Extraction of proteins, assays for total amylase activity and GUS activity, and activity staining of the band of β-amylase on polyacrylamide gels that had been prepared with soluble starch were performed as described previously (Mita et al., 1995).

Isolation and Analysis of RNA

Total RNA was extracted from leaves and northern hybridization was performed with ^{32}P-labeled fragments of the cDNAs for β-amylase, CHS, CHI, DFR, and CDPK, and α-tubulin of *A. thaliana* as described previously (Mita et al., 1995, 1997).

Quantitation of Starch, Sugars, and Anthocyanin

For the quantitation of starch, Suc, Glc, and Fru, leaves were harvested, weighed, and ground in liquid nitrogen. Soluble sugars were extracted three times in 80% (v/v) ethanol at 70°C for 30 min. The extracts were cooled and centrifuged at 16,000*g* for 20 min, and the resultant supernatants were passed through a C_{18} Sep-Pak cartridge (Millipore). Each eluate was evaporated to dryness. The residue was dissolved in water and assayed enzymatically by the standard methods for Suc, Glc, and Fru (Stitt et al., 1989). The amount of starch in the ethanol-insoluble fraction was determined as described previously (Nakamura et al., 1991). Anthocyanin was quantitated as described previously (Mita et al., 1997).

RESULTS

Expression of *ATβ-Amy* in Wild-Type and *hba1* Plants

We screened about 7000 M_2 plants that were descended from ethyl methanesulfonate-treated seeds of *A. thaliana* ecotype Col-0 for altered levels of amylase activity in rosette leaves with and without feeding the excised leaves with Suc (Mita et al., 1995). In addition to mutants in which the sugar-induced level of β-amylase activity was significantly lower than that in wild-type plants (Mita et al., 1997), we identified a mutant in which the levels of amylase activity in leaves immediately after harvest from the plants (nontreated leaves) were significantly higher than those in the wild-type plants. This trait was inherited after backcrossing with the Col-0 wild type, and the mutation in this line was designated *hba1* (for high level of beta-amylase activity).

Seeds of the Col-0 wild-type and homozygous *hba1* mutant plants were germinated and grown on Murashige and Skoog plates that contained 2% Suc for 3 weeks. Mature leaves were detached and the cut edges of their petioles were soaked in a 6% solution of Suc under continuous light for 2 d. Extracts prepared from nontreated leaves served as controls, since extracts from leaves that had been detached and fed with water gave levels of β-amylase activity similar to those of nontreated leaves (Mita et al., 1995). Amylase activity in leaves of wild-type plants increased significantly after being fed with Suc as a result of increases in levels of β-amylase protein and of the mRNA for β-amylase (Fig. 1; Mita et al., 1995). The level of amylase activity in nontreated leaves of *hba1* plants was about six times that in nontreated leaves of the wild type, and was similar to that in leaves of wild-type plants after they had been fed with Suc (Fig. 1). Feeding of leaves from the *hba1* plants with 6% Suc resulted in a slight increase in amylase activity. The high levels of amylase activity in nontreated

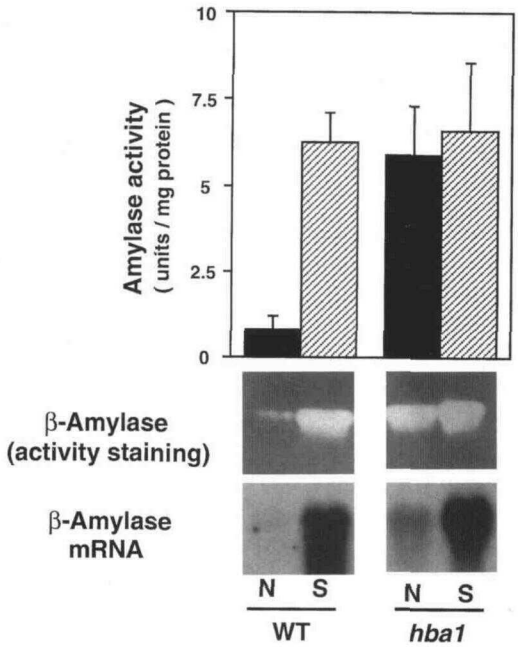

Figure 1. Expression of *ATβ-Amy* in leaves of wild-type and *hba1* plants. Rosette leaves were detached from 3-week-old Col-0 wild-type (WT) and *hba1* mutant plants (*hba1*) that had been grown on Murashige and Skoog medium with 2% Suc, and their petiole parts were dipped in a 6% solution of Suc for 2 d under continuous light (S). Some of the leaves were used for the extraction of protein. β-amylase activity was assayed by measuring the total amylolytic activity or by activity staining of the band of β-amylase on a polyacrylamide gel that contained soluble starch. One unit of amylase activity corresponds to the amount of enzyme that releases 1 μM of reducing sugar per minute under the conditions of the assay. For activity staining of β-amylase, proteins (7 μg) were fractionated by gel electrophoresis. Total RNA was isolated from the remaining leaves and analyzed for the level of β-amylase mRNA by northern hybridization. Twenty micrograms of total RNA was loaded in each lane. N, Nontreated leaves.

leaves of *hba1* plants were associated with elevated levels of the β-amylase protein, as revealed by activity staining of proteins that had been separated in a polyacrylamide gel prepared with soluble starch (Fig. 1). In addition, the levels of mRNA for β-amylase in nontreated leaves of *hba1* plants were also higher than those in wild-type leaves (Fig. 1). In contrast to amylase activity and β-amylase protein, the level of mRNA for β-amylase in leaves of *hba1* mutant plants increased after feeding of leaves with 6% Suc (Fig. 1). It is suggested that posttranscriptional control, in addition to transcriptional control, is involved in the regulation of the level of β-amylase protein.

Expression of *ATβ-Amy* in *hba1* Plants in Response to Sugars

We examined the levels of amylase activity in leaves of wild-type and *hba1* plants that had been grown on medium with various concentrations of Suc for 3 weeks. Although the level of amylase activity in leaves varied among individual plants and from experiment to experiment, the tendencies were always similar. The results of one typical experiment are shown in Figure 2A. The levels of amylase activity in leaves of both wild-type and *hba1* plants rose with increasing concentration of Suc in the medium (Fig. 2A). The higher levels of amylase activity were due to increased levels of β-amylase protein (data not shown). Amylase activities in leaves of *hba1* plants were significantly higher than those in wild-type plants when the concentration of Suc in the medium was 1 or 2%. However, leaves of plants that were grown with 5% Suc had high levels of amylase activity that did not differ significantly between *hba1* plants and wild-type plants. By contrast, when seeds were germinated on vermiculite and grown autotrophically in pots for 3 weeks, the amylase activity of leaves was very low both in *hba1* plants and wild-type plants (Fig. 2A). Higher than wild-type levels of amylase activity in leaves of *hba1* plants were also observed in plants when grown on medium with 1% Glc, but not on medium with 3% Glc (Fig. 2B). These results suggest that the high level of β-amylase activity in *hba1* mutant plants was not due to constitutive expression of *ATβ-Amy* and that it occurred specifically at intermediate levels of sugars.

When *hba1* plants that had been grown for 20 d on

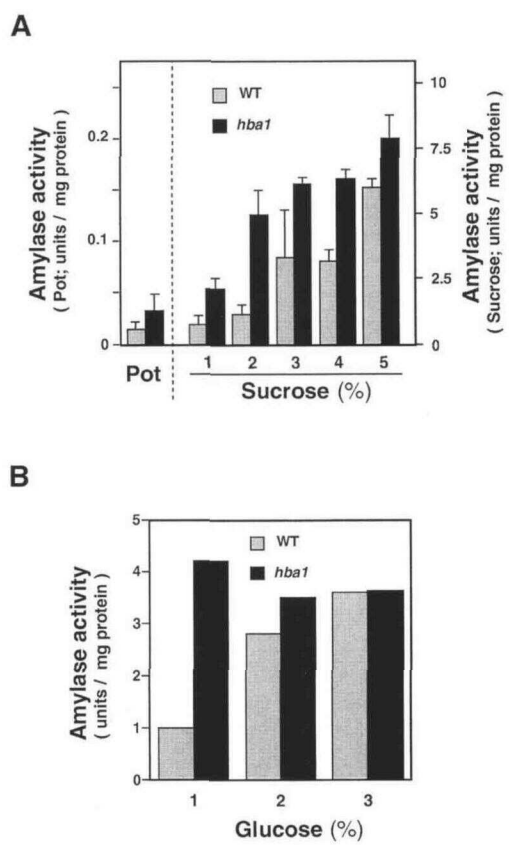

Figure 2. Amylase activity in leaves of *hba1* mutant plants that had been grown with various concentrations of sugars. Col-0 wild-type plants and *hba1* mutant plants were grown on Murashige and Skoog medium that contained various concentrations of Suc (A) or Glc (B). Proteins were extracted from rosette leaves of 3-week-old plants and amylase activity was determined. Amylase activity in leaves of plants that had been grown autotrophically in vermiculite was also assayed (Pot).

medium with 2% Suc were grown for 20 additional d under the same conditions on fresh plates, the specific activity of amylase decreased from about 6 units mg^{-1} protein to about 2 units mg^{-1} protein (Fig. 3). Among these bolted plants, amylase activities in bracts of *hba1* plants were about three times higher than in bracts of the wild-type plants (Fig. 3). Flower buds of both the wild-type and *hba1* plants had high levels of β-amylase. These changes in the site of high levels of β-amylase probably resulted from repartitioning of excess carbon in the plant body during plant growth. The levels of amylase activity (Fig. 3), β-amylase protein, and mRNA for β-amylase (data not shown) in roots were not affected by the *hba1* mutation under these growth conditions.

Levels of Sugars and Starch in Leaves of Wild-Type and *hba1* Plants

We compared the levels of sugars in leaves of wild-type and *hba1* mutant plants that had been grown on medium with 2% Suc. As shown in Figure 4A, levels of Suc, Glc, and Fru in leaves of *hba1* plants were similar to those observed in leaves of wild-type plants. In addition, the levels of sugars that accumulated in leaves that had been excised from *hba1* plants and fed with 6% Suc for 2 d were similar to those observed in leaves excised from wild-type plants and fed similarly (data not shown).

The Suc-induced expression of *ATβ-Amy* in excised leaves of Arabidopsis occurs concomitantly with the accumulation of large amounts of starch (Mita et al., 1995). Several mutants of Arabidopsis that are defective in starch metabolism have elevated levels of β-amylase in their leaves under certain growth conditions (Caspar et al., 1989). As shown in Figure 4B, the levels of starch in leaves of *hba1* plants that had been grown on medium with 2% Suc were similar to those of wild-type plants. Furthermore, the mutation also had no effect on the levels of starch that accumulated in leaves that had been excised and fed with 6% Suc for 2 d. These results suggested that the high-level expression of *ATβ-Amy* in leaves of *hba1* plants was not due to an increased uptake of sugars or to the altered metabolism of starch. The results further support the hypothesis that the sugar-induced accumulation of β-amylase and that of starch are regulated separately (Ohto et al., 1992; Takeda et al., 1995; Mita et al., 1997).

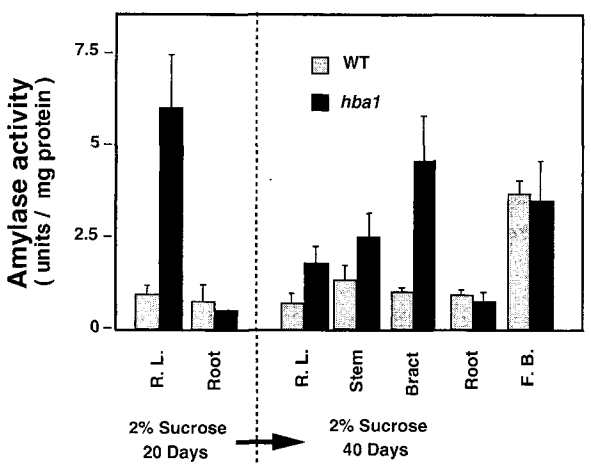

Figure 3. Levels of amylase activity in various organs of *hba1* mutant plants. Col-0 wild-type plants and *hba1* mutant plants were grown on Murashige and Skoog medium that contained 2% Suc. The amylase activities in various organs of 20-d-old plants (20 Days) or 40-d-old mature plants (40 Days) were assayed. R.L., Rosette leaves; F.B., flower buds.

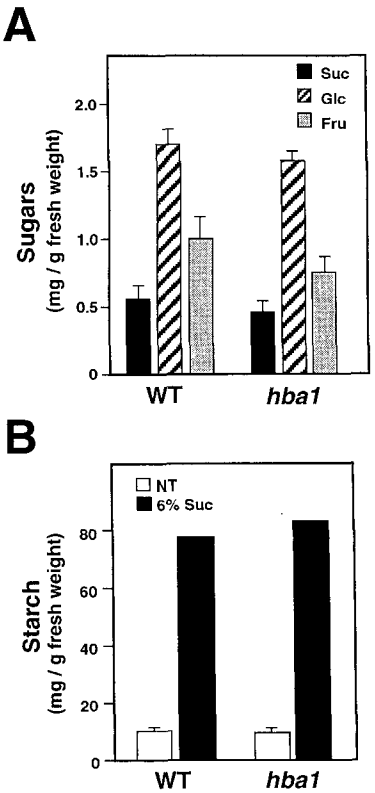

Figure 4. Levels of sugars and starch in leaves. Rosette leaves from 3-week-old Col-0 wild type (WT) and *hba1* mutant plants (*hba1*) that had been grown on Murashige and Skoog medium with 2% Suc were analyzed for contents of Suc, Glc, and Fru (A), or starch (B). Means of results of two independent experiments are shown with SD.

The Phenotype of *hba1* Plants

In Arabidopsis the accumulation of anthocyanin in leaves is regulated by sugars (Tsukaya et al., 1991; Mita et al., 1995). The *lba1* mutation, associated with reduced levels of Suc-induced expression of *ATβ-Amy*, has pleiotropic effects on the sugar-inducible accumulation of anthocyanin, and mutant plants also have characteristic growth defects (Mita et al., 1997). The *hba1* mutant plants grew poorly on medium with 1% Suc, as compared with the wild type, and some of them died before bolting. In addition, *hba1* mutant plants grown with 2% Suc accumulated higher levels of anthocyanin in their petioles compared with the wild-type plants, although the level of anthocyanin fluctuated from experiment to experiment (Fig. 5). The level of anthocyanin in the petioles of *hba1* plants decreased when the concentration of Suc in the medium was decreased to 1% (Fig. 5), whereas high-level accumulation of anthocya-

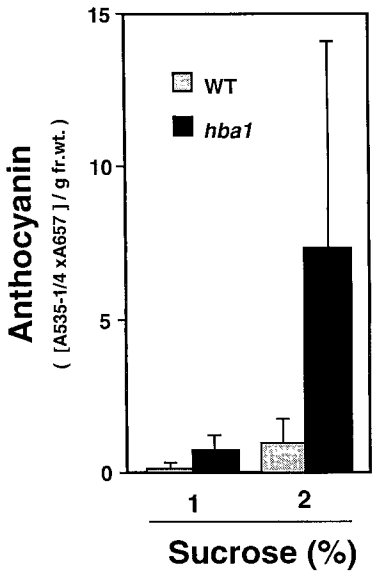

Figure 5. Anthocyanin levels in petioles of wild-type and *hba1* plants. Col-0 wild-type (WT) and *hba1* plants (*hba1*) were grown on medium that contained 1% or 2% Suc for 3 weeks and the amount of anthocyanin in petioles was determined. Means of results of three independent experiments are shown with SD.

nin was observed when the concentration of Suc in the medium was increased to 5% (data not shown).

We made reciprocal crosses between *hba1* mutant plants and the Col-0 wild type (WT) as follows: *hba1* (f) × WT (m) and WT × *hba1*. All of the F_1 plants from both crosses grew normally and had low levels of amylase activity that were similar to those observed with the Col-0 wild type (Fig. 6).

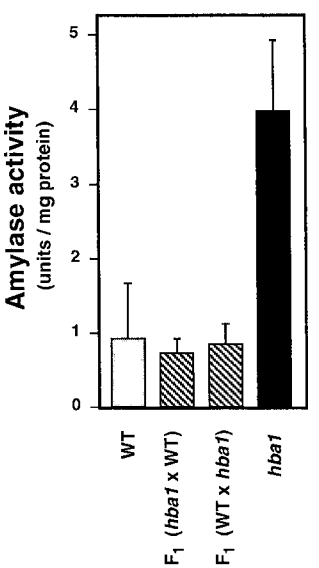

Figure 6. Genetic analysis of the *hba1* mutation. The amylase activity in leaves of F_1 plants from reciprocal crosses between the Col-0 wild-type (WT) and *hba1* mutant plants (*hba1*) and grown on medium with 2% Suc was determined. The number of plants assayed were as follows; WT, 10; *hba1*, 12; F_1 plants from *hba1* × WT, 16; F_1 plants from WT × *hba1*, 8.

In the F_2 generation from the *hba1* × WT cross, wild-type and mutant plants segregated in a ratio of 3.8:1 (107 wild type and 28 mutants), consistent with a ratio of 3:1 (x^2 = 1.29, $P > 0.05$). The ratio of wild-type to mutant plants in the F_2 generation from the WT x *hba1* cross was 5:1 (110 wild type and 22 mutant, $x^2 = 4.89$, $P < 0.05$). From these results it appears that *hba1* is a single recessive mutation. The deviation from a ratio of 3:1 in the cross with *hba1* mutant plants as males might have resulted from the reduced viability of pollen with the *hba1* mutation.

The *hba1* Mutation Affects Expression of *ATβ-Amy* in *trans*

We crossed homozygous *hba1* mutant plants with a transgenic line that carried an *ATβ-Amy:GUS* fusion gene containing 1587 bp of DNA 5′ to transcription start site of *ATβ-Amy* (Mita et al., 1995). The transgenic line was prepared using the WS ecotype, and T3 lines that were homozygous for the transgene were used. F_1 plants that were heterozygous for both the *hba1* mutation and the *ATβ-Amy:GUS* transgene were self-pollinated. Hygromycin-resistant F_2 progeny that carried the *ATβ-Amy* transgene were grown on medium with 2% Suc for 3 weeks, and then leaf extracts were assayed for activities of both amylase and GUS. As shown in Figure 7, all but one of the progeny with high levels of amylase activity (*hba1*/*hba1*) also had higher levels of GUS activity than those found in progeny with normal levels of amylase activity (*hba1*/*HBA1* and *HBA1*/*HBA1*). Similar results were obtained with other F_1 plants from a different cross. These results suggest that *hba1* is not a mutation in *ATβ-Amy* and that the *HBA1* gene influences the expression of *ATβ-Amy* at the level of its transcription, acting in *trans* in a negative manner. Since the *ATβ-Amy:*

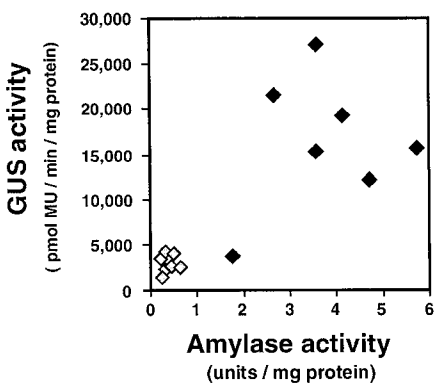

Figure 7. Effects of the *hba1* mutation on expression of the *ATβ-Amy:GUS* transgene. A homozygous *hba1* plant was crossed with the transgenic line of ecotype WS that carried the *ATβ-Amy:GUS* transgene. F_1 plants that were heterozygous for both the *hba1* mutation and the transgene were self-pollinated. Leaves of 3-week-old F_2 plants that had been grown on medium with 2% Suc were used for extraction of proteins, and the activities of endogenous amylase and GUS in the extracts were determined. Data obtained from 14 individual F_2 plants, derived from one particular heterozygous F_1 plant, of which 7 were wild type (shaded diamonds) and 7 had the *hba1* phenotype (black diamonds) are shown. Similar results were obtained with different heterozygous F_1 plants.

GUS fusion gene contained the 5′-untranslated and the N-terminal coding regions from *ATβ-Amy*, in addition to the 5′-upstream sequence, we cannot exclude the possibility that the *hba1* mutation might affect initiation of translation or the stability of mRNA in *trans*.

Expression of Other Sugar-Modulated Genes in *hba1* Plants

Suc fed to excised leaves of Arabidopsis results in the accumulation of anthocyanin and in increased levels of mRNAs for CHS, CHI, and DFR (Tsukaya et al., 1991; Mita et al., 1997). In contrast to the level of mRNA for β-amylase, the levels of mRNAs for CHS, DFR, and CHI were not significantly affected by the *hba1* mutation in nontreated and in Suc-treated leaves (Fig. 8) and also in petioles (data not shown). The levels of mRNAs for CDPKs, which are encoded by a multigene family (Urao et al., 1994), and α-tubulin in leaves were unaffected either by feeding Suc or by the *hba1* mutation (Fig. 8). These results suggest that the *hba1* mutation did not affect the sugar-modulated expression of genes in general but affected only a subset of sugar-modulated genes.

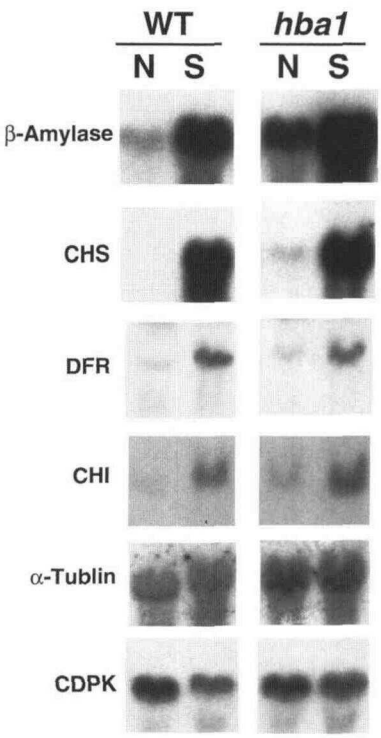

Figure 8. Expression of other sugar-modulated genes in leaves of *hba1* mutant plants. Rosette leaves, detached from 3-week-old wild-type (WT) and *hba1* plants (*hba1*), were treated with a 6% solution of Suc for 24 h under continuous light. Total RNA was isolated and analyzed for levels of mRNAs for β-amylase, CHS, CHI, DFR, α-tubulin, and CDPK by northern hybridization (S). Twenty micrograms of total RNA was loaded in each lane. N, Nontreated leaves from the same batch of plants.

DISCUSSION

The *hba1* mutant isolated in this study showed increased levels of expression of *ATβ-Amy* in leaves of plants that had been grown with medium that contained 2% Suc (Fig. 1). This contrasts with the behavior of *lba1* and *lba2* mutant plants, in which levels of sugar-induced expression of *ATβ-Amy* in leaves are lower than in the wild type (Mita et al., 1997). The *hba1* mutation had a similar effect on expression of the *ATβ-Amy:GUS* transgene (Fig. 7), a result that suggests that high-level expression of *ATβ-Amy* in leaves of *hba1* mutant plants was not due to a mutation in the structural gene and that the mutation affects expression of *ATβ-Amy* in *trans*. The high level of β-amylase in leaves of *hba1* plants did not seem to be due to increased uptake and transport of sugars or to defects in the metabolism of starch (Fig. 4). Furthermore, northern hybridization (Fig. 8) indicated that the *hba1* mutation did not affect the expression of sugar-responsive genes in general. In common with *LBA1* and *LBA2* (Mita et al., 1997), these results suggest that *HBA1* might encode some downstream component of the signal transduction pathway that transmits the sugar signal(s) to subsets of sugar-responsive genes.

The level of expression of *ATβ-Amy* in leaves of *hba1* mutant plants was not constitutively high, but was dependent on the concentration of sugars in the medium. Higher than wild-type levels of β-amylase occurred specifically in the presence of "intermediate" or "low" levels of sugars (Fig. 2). Since *hba1* is recessive (Fig. 6), the product of the *HBA1* gene appears to affect expression of *ATβ-Amy* in a negative manner in the absence of high levels of sugars. We propose that the level of expression of *ATβ-Amy* is regulated by a combination of positive and negative regulators, depending on the level of sugars, and, moreover, that the product of *HBA1* might have a role in maintaining expression of *ATβ-Amy* at a low level until the level of sugars is sufficiently high. At higher levels of sugars, either the function of the *HBA1* product is diminished or positive regulatory mechanisms predominate. In leaves of *lba1* and *lba2* mutant plants, sugar-responsive expression of *ATβ-Amy* is not abolished per se. However, the level of expression of *ATβ-Amy* in response to high levels of sugars, such as Suc at 3% and higher, is significantly reduced compared with that in the wild type (Mita et al., 1997). The products of *LBA1* and *LBA2* genes might be involved in a positive regulatory mechanism that operates primarily in response to "high" levels of sugars.

The regulation by sugars of the expression of many plant genes does not necessarily exhibit the same dependence on sugar concentration in each case. In leaf-petiole cuttings of sweet potato, expression of genes for sporamin and β-amylase showed a similar dependency on Suc concentration, but other proteins were induced at lower Suc concentrations (Hattori et al., 1991). In the yeast *Saccharomyces cerevisiae*, expression of four *HXT* genes for hexose transporters is induced by Glc in each case, but expression of these *HXT* genes shows unique responses that depend on the concentration of Glc in the range between 0.1 and 4%. Genetic analysis has revealed the involvement of at least

three distinct signal transduction pathways in the induction of expression of these *HXT* genes (Özcan and Johnston, 1995). It was shown that some of the regulatory components involved in the induction by Glc of these genes were also involved in the repression by Glc of other genes. Whether mutations that affect the sugar-inducible expression of *ATβ-Amy* also affect repression by sugars of gene expression remains to be analyzed. Similar to *lba1* plants (Mita et al., 1997), *hba1* plants showed anomalous accumulation of anthocyanin. The petioles of *hba1* plants contained increased levels of anthocyanin when plants were grown with intermediate levels of Suc (Fig. 5). These results suggest that sugar-induced accumulation of β-amylase and of anthocyanin share common regulatory mechanisms, at least in part. The physiological function of β-amylase in plants, which is probably located outside the chloroplasts, is not well understood (Lin et al., 1988; Lizotte et al., 1990; Nakamura et al., 1991; Mita et al., 1995). The β-amylase in phloem sieve elements has been suggested to have a role in preventing the accumulation of starch after sugar loading (Wang et al., 1995). In leaves of sweet potato, the sugar-induced accumulation of β-amylase occurs in mesophyll cells in addition to vascular tissue (Takeda et al., 1995). It occurs concomitantly with the accumulation of starch and sporamin, a tuber storage protein with homology to wound-inducible trypsin inhibitors (Hattori et al., 1989; Bradshaw et al., 1990). Furthermore, accumulation of sporamin and β-amylase was also induced by polygalacturonic acid and by ABA (Ohto et al., 1992; Takeda et al., 1995). β-Amylase might have some defensive role under metabolic stress due to high levels of sugars.

In spite of increased accumulation of anthocyanin, the levels of mRNAs for CHS, DFR, and CHI were not significantly affected in leaves (Fig. 8) and in petioles (data not shown) of *hba1* plants, even though slightly enhanced levels of the mRNAs for CHS and CHI were noticed in some experiments. The sugar-inducible changes in the levels of mRNAs for CHS, DFR, and CHI were also not significantly affected by the *lba1* mutation (Mita et al., 1997). It is not known at present whether increased accumulation of anthocyanin in *hba1* plants is due to increased activities of these enzymes, to increased expression of genes other than genes for CHS, CHI, and DFR that are involved in flavonoid synthesis, or to some other mechanism.

The *hba1* plants grew poorly on medium with 1% or lower levels of Suc, although they grew normally on vermiculite or when the concentration of Suc in the medium was increased to 3%. The *lba1* mutant plants also grew poorly on medium with low concentrations of sugars and their leaves are yellowish green and contained less chlorophyll (Mita et al., 1997). The growth defects and the yellowish green phenotype of *lba1* plants can be largely suppressed by higher concentrations of Suc or growth in vermiculite. It is possible that these abnormal features are due to the effects of the *hba1* and *lba1* mutations on sugar-regulated genes that play important roles in growth and development rather than to effects on the expression of *ATβ-Amy* or on the accumulation of anthocyanin. The effects of these mutations on the expression of *ATβ-Amy* could be an indirect effect of an altered growth response of mutant plants to sugars.

Received August 5, 1996; accepted February 22, 1997.

LITERATURE CITED

Bradshaw HD, Hollick JB, Parsons TJ, Clarke HR, Gordon MP (1990) Systemically wound-responsive genes in poplar trees encode proteins similar to sweet potato sporamins and legume Kunitz trypsin inhibitors. Plant Mol Biol **14:** 51–59

Caspar T, Lin TP, Monroe J, Bernhard W, Spilatro S, Preiss J, Somerville C (1989) Altered regulation of β-amylase activity in mutants of *Arabidopsis* with lesions in starch metabolism. Proc Natl Acad Sci USA **86:** 5830–5833

Epstein PN, Boschero AC, Atwater I, Cai X, Overbeek PA (1992) Expression of yeast hexokinase in pancreatic β cells of transgenic mice reduces blood glucose, enhances insulin secretion, and decreases diabetes. Proc Natl Acad Sci USA **89:** 12038–12042

German MS (1993) Glucose sensing in pancreatic islet beta cells: the key role of glucokinase and the glycolytic intermediates. Proc Natl Acad Sci USA **90:** 1782–1785

Graham IA, Denby KJ, Leaver CJ (1994) Carbon catabolite repression regulates glyoxylate cycle gene expression in cucumber. Plant Cell **6:** 761–772

Hattori T, Fukumoto H, Nakagawa S, Nakamura K (1991) Sucrose-induced expression of genes coding for tuberous root storage protein, sporamin, of sweet potato in leaves and petioles. Plant Cell Physiol **32:** 79–86

Hattori T, Nakagawa S, Nakamura K (1990) High-level expression of tuberous root storage protein genes of sweet potato in stems of plantlets grown *in vitro* on sucrose medium. Plant Mol Biol **14:** 595–604

Hattori T, Yoshida N, Nakamura K (1989) Structural relationship among the members of a multigene family coding for the sweet potato tuberous root storage protein. Plant Mol Biol **13:** 563–572

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

Johnson R, Ryan CA (1990) Wound-inducible potato inhibitor II genes: enhancement of expression by sucrose. Plant Mol Biol **14:** 527–536

Koch KE, Nolte KD, Duke ER, McCarty DR, Avigne WT (1992) Sugar levels modulate differential expression of maize sucrose synthase genes. Plant Cell **4:** 59–69

Lin TP, Spilatro SR, Preiss J (1988) Subcellular localization and characterization of amylases in *Arabidopsis* leaf. Plant Physiol **88:** 251–259

Lizzotte PA, Henson CA, Duke SH (1990) Purification and characterization of pea epicotyl β-amylase. Plant Physiol **92:** 615–621

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

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

Müller-Röber BT, Kossmann J, Hannah LC, Willmitzer L, Sonnewald U (1990) One of two different ADP-glucose pyrophosphorylase genes from potato responds strongly to elevated levels of sucrose. Mol Gen Genet **224:** 136–146

Nakamura K, Ohto M, Yoshida N, Nakamura K (1991) Sucrose-induced accumulation of β-amylase occurs concomitant with the accumulation of starch and sporamin in leaf-petiole cuttings of sweet potato. Plant Physiol **96:** 902–909

Ohto M, Hayashi K, Isobe M, Nakamura K (1995) Involvement of Ca^{2+}- signalling in the sugar-inducible expression of genes coding for sporamin and β-amylase of sweet potato. Plant J **7:** 297–307

Ohto M, Nakamura K (1995) Sugar-induced increase of calcium-dependent protein kinases associated with the plasma membrane in leaf tissues of tobacco. Plant Physiol **109:** 973–981

Ohto M, Nakamura-Kito K, Nakamura K (1992) Induction of expression of genes coding for sporamin and β-amylase by polygalacturonic acid in leaf-petiole cuttings of sweet potato. Plant Physiol **99:** 422–427

Özcan S, Johnston M (1995) Three different regulatory mechanisms enable yeast hexose transporter (*HXT*) genes to be induced by different levels of glucose. Mol Cell Biol **15:** 1564–1572

Rocha-Sosa M, Sonnewald U, Frommer W, Stratmann M, Schell J, Willmitzer L (1989) Both developmental and metabolic signals activate the promoter of a class I patatin gene. EMBO J **8:** 23–29

Rose M, Albig W, Entian KD (1991) Glucose repression in *Saccharomyces cerevisiae* is directly associated with hexose phosphorylation by hexokinase PI and PII. Eur J Biochem **199:** 511–518

Sadka A, DeWald DB, May GD, Park WD, Mullet JE (1994) Phosphate modulates transcription of soybean *vspB* and other sugar inducible genes. Plant Cell **6:** 737–749

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

Stitt M, Lilley RMC, Gerhardt R, Heldt HW (1989) Metabolite levels in specific cells and subcellular compartments of plant leaves. Methods Enzymol **174:** 518–552

Takeda S, Kowyama Y, Takeuchi Y, Matsuoka K, Nishimura M, Nakamura K (1995) Spatial patterns of sucrose-inducible and polygalacturonic acid-inducible expression of genes that encode sporamin and β-amylase in sweet potato. Plant Cell Physiol **36:** 321–333

Takeda S, Mano S, Ohto M, Nakamura K (1994) Inhibitors of protein phosphatases 1 and 2A block the sugar-inducible gene expression in plants. Plant Physiol **106:** 567–574

Trumbly RJ (1992) Glucose repression in the yeast *Saccharomyces cerevisiae*. Mol Microbiol **6:** 15–21

Tsukaya H, Ohsima T, Naito S, Chino M, Komeda Y (1991) Sugar-dependent expression of the *CHS-A* gene for chalcone synthase from petunia in transgenic *Arabidopsis*. Plant Physiol **97:** 1414–1421

Urao K, Katagiri T, Mizoguchi T, Yamaguchi-Shinozaki K, Hayashida N, Shinozaki K (1994) An *Arabidopsis thaliana* cDNA encoding Ca^{2+}-dependent protein kinase. Plant Physiol **105:** 1461–1462

Wang Q, Monroe J, Sjölund RD (1995) Identification and characterization of a phloem-specific β-amylase. Plant Physiol **109:** 743–750

Yang Y, Kwon H-B, Peng H-P, Shin M-C (1993) Stress responses and metabolic regulation of glyceraldehyde-3-phosphate dehydrogenase genes in *Arabidopsis*. Plant Physiol **101:** 209–216

Yu S-M, Kuo Y-H, Sheu G, Sheu Y-J, Liu L-F (1991) Metabolic derepression of α-amylase gene expression in suspension-cultured cells of rice. J Biol Chem **266:** 21131–21137