The Arabidopsis GSQ5/DOG1 Cvi allele is induced by the ABA-mediated sugar signalling pathway, and enhances sugar sensitivity by stimulating ABI4 expression

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Summary

ABI4 encodes an AP2 family transcription factor that is a central regulator in sugar responsive gene expression in plants. Sugar-induced ABI4 regulates plant genes essential for photosynthesis, and carbon, nitrogen and lipid metabolism. ABI4 activity is induced via the ABA-mediated sugar signalling pathway, which is initiated by the glucose sensing protein hexokinase. Natural variation in sugar sensitivity was used to identify new loci involved in sugar signalling. Five quantitative trait loci (QTLs) for glucose sensitivity (GSQ1–GSQ5) were identified in a Ler/Cvi recombinant inbred line (RIL) population. The GSQ3, GSQ4 and GSQ5 loci are positioned in regions not previously associated with known sugar-sensing genes. GSQ5 was fine mapped and cloned using a candidate-gene approach. The GSQ5 locus was shown to encode the DELAY OF GERMINATION 1 (DOG1) gene. DOG1 was previously identified as a major locus in seed dormancy control. Glucose addition induced the expression of the GSQ5/DOG1 Cvi allele, whereas the Ler and Col alleles did not respond to glucose. Positive feedback was observed between the ABA-mediated sugar signalling pathway and the GSQ5/DOG1 Cvi allele. Expression of the GSQ5/DOG1 Cvi allele requires the ABA-mediated sugar signalling pathway, of which ABI4 is an important component. In addition, sugar induction of ABI4 was promoted by the GSQ5/DOG1 Cvi allele.

Keywords: sugar signalling, ABI4, natural variation, QTL cloning, DOG1.

Introduction

Sugars play pivotal roles as signalling molecules in plants. Changes in sugar levels affect gene expression, photosynthesis, nutrient allocation, cell division, defence responses and various developmental processes, including seed development, germination, flowering and senescence (Gibson, 2005; Rolland et al., 2006). Sugar signalling in plants occurs through multiple signal transduction pathways (Smeekens, 2000). In Arabidopsis, a hexokinase-dependent pathway was proposed that senses glucose (Glc) via the sensor protein hexokinase1 (HXK1) (Jang et al., 1997). HXK1 has separate catalytic and sensory functions, showing that glucose metabolism is not involved in hexokinase signalling (Moore et al., 2003). Arabidopsis HXK1 is part of a nuclear complex that includes vacuolar H+-ATP B1 (VHA-B1) and the 19S regulatory particle of the proteasome subunit (RPT5B), and this complex directly binds to promoter elements of glucose-responsive genes (Cho et al., 2006). The presence of a sucrose-specific sensing pathway was proposed based on observations that several effects of sucrose can not, or can only partially, be mimicked by the sucrose breakdown products glucose and fructose, or by other sugars. For example, sucrose induces the transcription of genes for anthocyanin biosynthesis (Solfanelli et al., 2006; Teng et al., 2005), and represses AtbZIP11 mRNA translation (Rook et al., 1998; Wiese et al., 2004). A sucrose-specific sensor protein has not been identified in plants so far.

Different mutant screening strategies in Arabidopsis were used to reveal the components of sugar-signalling
pathways. One such mutant screen is based on sugar-induced early seedling developmental arrest, which was used to identify, e.g. glucose-insensitive (gin; Zhou et al., 1998) and sugar-insensitive (sis) (Laby et al., 2000) mutants. Other mutant screens employed the aberrant expression of a sugar-regulated promoter fused to reporter genes. These screens were used to identify mutants such as sucrose uncoupled (sun; Dijkwel et al., 1997), low beta-amylase (iba; Mita et al., 1997), impaired sucrose induction (isi; Rook et al., 2001) and reduced sugar response (rsr; Martin et al., 1997). Gene cloning and functional analysis of a number of mutants suggested that glucose is sensed via the ABA-mediated sugar signalling pathway. In this model, glucose triggers signalling through the sensor protein HXK1, leading to increased ABA levels and enhanced expression of effector genes like ABA-INSENSITIVE 4 (ABI4) (Arenas-Huertero et al., 2000; Cheng et al., 2002; Moore et al., 2003).

ABI4 encodes a member of the DREB subfamily of ERF/AP2 transcription factors (Finkelstein et al., 1998; Sakuma et al., 2002), and is an important element in sugar signalling. ABI4 was identified in gin (Arenas-Huertero et al., 2000), sis (Laby et al., 2000), sun (Huijser et al., 2000) and isi (Rook et al., 2001) screens, indicating its central importance in sugar-induced seedling developmental arrest, sugar repression of the plastocyanin (PC) gene and sugar induction of the ADP-glucose pyrophosphorylase large subunit 3 (ApL3) gene. ABI4 overexpression leads to increased glucose sensitivity (Finkelstein et al., 2002), whereas abi4 mutants show reduced sugar sensitivity. In abi4, sugar feedback inhibition of photosynthesis is reduced (Van Oosten et al., 1997). The ABI4 protein was demonstrated to bind to the S-box in the ribulose-1,5-bisphosphate carboxylase small subunit (RBCC) promoter. This binding is essential for sugar and ABA repression of RBCC transcription, and probably other photosynthesis genes with a similar S-box as well, such as PC and chlorophyll a/b-binding protein (CAB1; Acevedo-Hernandez et al., 2005). Recently, more functions of ABI4 have been revealed. For example, ABI4 regulates lipid mobilization in the embryo (Penfield et al., 2006), ABI4 mediates the effect of trehalose on starch mobilization and growth, independently of the ABA-mediated sugar signalling pathway (Ramon et al., 2007), and ABI4 is essential in plastid-to-nucleus retrograde signalling (Koussetzky et al., 2007). Arabidopsis accessions show substantial natural occurring variation, which constitutes an important resource for the functional analysis of the genome (Alonso-Blanco and Koornneef, 2000; Koornneef et al., 2004). Quantitative trait loci (QTLs) can readily be detected in recombinant inbred line (RIL) populations using molecular marker technology and statistical analysis. Genomic regions responsible for allelic variation leading to particular phenotypes can be identified through QTL analysis. The genes involved can then be cloned by a map-based cloning approach, using populations derived from near-isogenic lines (NILs) spanning the QTL interval. FLC, FRI and EDI (early day-length insensitive) are examples of the successful identification and functional analysis of genes using natural variation as a genetic resource, instead of mutants in specific wild-type strains (Koornneef et al., 2004).

We studied natural variation in sugar sensing in different Arabidopsis accessions. Seedling establishment in the Cape Verde Islands (Cvi) accession is more sensitive to glucose (Glc) than in the Landsberg erecta (Ler) accession. Using QTL analysis in the Ler/Cvi RIL population (Alonso-Blanco et al., 1998), five QTLs for Glc sensitivity were identified. Three of these QTLs were located in genomic regions not previously associated with sugar sensitivity. A major Glc sensitivity QTL (GLUCOSE SENSING QTL 5, GSQ5), of which the Cvi allele increases Glc sensitivity, was found to be identical to DELAY OF GERMINATION 1 (DOG1), involved in controlling seed dormancy (Bentsink et al., 2006). A positive feedback loop between the GSQ5/DOG1 Cvi allele and the ABA-mediated sugar signalling pathway was uncovered.

Results

Natural variation in Glc sensitivity of Arabidopsis Ler and Cvi accessions

Seeds of Ler and Cvi accessions were plated on half-strength MS with increasing concentrations of Glc to investigate natural variation in sugar sensitivity. Following stratification, seedling greening was scored after 7 days growth under continuous light at 22°C. In these experiments, Cvi was more sensitive to Glc than was Ler. The difference in sugar sensitivity between the Cvi and Ler accessions was most significant on media supplemented with 6% Glc. At this concentration, 65% of Ler seedlings showed greening versus 3% of Cvi seedlings (Figure 1a and c). This is not an osmotic effect, as seedlings grown on 6% sorbitol (Sor) show normal greening (Figure 1b).

The available Ler/Cvi RIL population (Alonso-Blanco et al., 1998) was used to obtain quantitative data for glucose sensitivity for subsequent QTL mapping. Seeds of this RIL population were plated on half-strength MS containing 6% Glc. The percentage of green seedlings of each RIL was scored after 7 days of growth. Two independent experiments were performed, each one in duplicate, and the mean of green seedling percentages were calculated. A range of Glc sensitivities was observed in the RIL population, with transgression beyond the parental values (Figure 1c).

Identification of Glc sensitivity QTLs in the Ler/Cvi RIL population

A total of five QTLs for Glc sensitivity were detected. These were named GLUCOSE SENSING QTL 1 (GSQ1) to GSQ5. These QTLs were located on chromosome 1 (GSQ1and
GSQ2, 3 (GSQ3), 4 (GSQ4) and 5 (GSQ5), respectively. The additive effects of these QTLs accounted for 40.8% of the total variance. The Cvi alleles of GSQ4 and GSQ5 increase the glucose sensitivity, whereas the Cvi alleles of GSQ1, GSQ2 and GSQ3 decrease the glucose sensitivity (Figure 2a). Two-way interaction ANOVA analysis did not detect any significant interaction among these QTLs.

Integration of the amplified fragment length polymorphism (AFLP) map into the physical map by in silico AFLP analysis (Peters et al., 2001) makes it possible to compare the positions of QTLs detected in this study with known genes involved in sugar sensing. ABA3 (Rolland et al., 2002), AXR1 (Moore et al., 2003) and RTP5B (Cho et al., 2006) were co-located with GSQ1, and ABA3 was near the peak of this QTL. VHA-B1 (Cho et al., 2006) is co-located with GSQ2, and TPS1 (Afonce et al., 2004) is located on the edge of the two-logarithm of odds (LOD)-support interval of this QTL. ABA1 (Arenas-Huertero et al., 2000) and HYS1 (Yoshida et al., 2002) are located on the edge of the two-LOD-support interval of GSQ5, but far away from the peak of the QTLs. Other QTLs did not co-locate with known sugar-sensing genes (Figure 2a).

Three NILs (Keurentjes et al., 2006) were tested to confirm the QTL results from the RIL population. LCN1-2, LCN3-1 and LCN5-14 have the GSQ1, GSQ3 and GSQ5 target genomic regions of Cvi integrated into the Ler background, respectively (Figure 2b). On 5.5% and 6.0% Glc, the percentages of green seedlings of LCN3-1 and LCN1-2 were higher than that of the Ler parent, showing that these lines were less sensitive to glucose than Ler. The percentage of green seedlings was lower in LCN5-14 than in Ler, showing that LCN5-14 was more sensitive to Glc (Figure 2c). The seedlings of the three NILs show normal greening when growing on 6% Sor (data not shown). These results fully confirmed the GSQ1, GSQ3 and GSQ5 QTLs.

GSQ5 is identical to DOG1

The GSQ5 phenotype was independently confirmed using another NIL line: NIL DOG17-1 (Figure 3a,b). NIL DOG17-1 covers the peak region of GSQ5, but the introgressed Cvi fragment is smaller than in LCN5-14 (Alonso-Blanco et al., 2003). F1 seedlings derived from the reciprocal crosses between Ler and NIL DOG17-1 showed the same Glc supersensitive phenotypes as NIL DOG17-1 (Figure 3c), indicating that the GSQ5 Cvi allele is dominant.

Homozygous sub-NILs of NIL DOG17-1 that were selected from the Ler × NIL DOG17-1 F2 progeny, by using molecular markers, were used for fine mapping of GSQ5. The genotypes of these sub-NILs are shown in Figure 3a. All of the tested sub-NILs showed the same Glc-supersensitive phenotype as NIL DOG17-1 (Figure 3b). This mapped GSQ5 to a region of 298 kb, between marker cK2N11a and MPL12a. In this 288-kb region, a major QTL for seed dormancy, DOG1, has been identified in the Ler/Cvi RIL population (Alonso-Blanco et al., 2003), and the cloning of the gene was described previously (Bentsink et al., 2006). The Cvi allele of DOG1 as found in the NIL DOG17-1 line shows a slightly ABA-hypersensitive phenotype (Bentsink et al., 2006). Thus, DOG1 might be the candidate gene for GSQ5.

To test this hypothesis, the Glc sensitivity of the dog1 mutant was investigated. This dog1 mutant was derived from mutating the DOG1 Cvi allele in the NIL DOG17-1 background (Bentsink et al., 2006). In addition, the Glc sensitivity of two independently generated transgenic Ler lines (R3-2-3 and R3-1-6), homozygous for a 5.6-Kb genomic fragment of the DOG1 Cvi allele (Bentsink et al., 2006), was also tested. Osmotic stress did not affect seedling greening in the dog1 mutant and the two transgenic lines (data not shown). In the dog1 mutant the Glc-supersensitive phenotype was lost, when compared with its NIL DOG17-1 wild-type allele. Conversely, both R3-2-3 and R3-1-6 DOG1 Cvi transgenic lines showed a Glc-supersensitive phenotype (Figure 3d). These results show that DOG1 and GSQ5 are encoded by the same gene.

Sugar sensitivity of different GSQ5/DOG1 alleles

Different GSQ5/DOG1 alleles have different effects on seed dormancy (Bentsink et al., 2006). The Cvi allele shows a strong dormancy phenotype, whereas Ler and Col alleles have weak dormancy phenotypes. A loss-of-dormancy phenotype is observed in the mutated dog1 allele in the NIL DOG17-1 and Col backgrounds (Bentsink et al., 2006). Interestingly, the effects of the different GSQ5/DOG1 alleles differed in sugar sensitivity and seed dormancy. The
GSQ5/DOG1 Cvi allele showed a sugar-supersensitive phenotype and a strong dormancy phenotype. However, the non-dormant dog1 mutant (in the NIL DOG17-1 background) showed a sugar sensitivity that was comparable with Ler (Figure 3e), instead of the expected opposite sugar-insensitive phenotype. Similarly, the non-dormant gsq5/dog1 knock-out lines in the Col background, SALK_000867 and SM_3_20808 (Bentsink et al., 2006), did not show a strong sugar-insensitive phenotype either (Figure 3f). These results indicate that the Ler and Col GSQ5/DOG1 alleles do not have a significant effect on sugar sensitivity, compared with their loss-of-function alleles.

GSQ5/DOG1 gene expression in response to Glc was investigated in NIL DOG17-1, Ler and Col. Glc treatment of Ler/Cvi recombinant inbred line (RIL) population. The GSQ loci are indicated by bars representing the two-LOD-support interval, with arrowheads indicating whether Cvi increases or decreases glucose (Glc) sensitivity. The LOD score peak is indicated with an arrowhead next to the interval bars. The LOD score range and percentage of explained variance are indicated with a grey shaded arrowhead and bars, respectively. Known genes involved in sugar sensing are shown in bold/italic.

(b) Genotypes of LCN1-2, LCN3-1 and LCN5-14 near-isogenic lines (NILs). The solid black bars represent the Cvi genomic segments in the Ler background.

(c) Sugar sensitivity of NILs. Seedlings were grown on half-strength MS with 5, 5.5, 6 and 6.5% Glc for 7 days at 22°C under continuous light.
NIL DOG17-1 led to an approximately twofold induction of GSQ5/DOG1, compared with control Sor treatment. Glc treatment slightly repressed GSQ5/DOG1 in Ler (Figure 3g) and in Col (data not shown). These results show that Glc induced the expression of the GSQ5/DOG1 Cvi allele, but not the Ler and Col alleles.

The GSQ5/DOG1 Cvi allele enhances Glc induction of AB14

The observation that NIL DOG17-1 is slightly hypersensitive to ABA during germination (Bentsink et al., 2006) suggests that the glucose-hypersensitive phenotype of NIL DOG17-1 might similarly result from increased ABA sensitivity. AB14 is induced by the ABA-mediated sugar signalling pathway, and its expression was monitored in NIL DOG17-1. AB14 gene expression was induced over threefold more by Glc in NIL DOG17-1 than in Ler (Figure 4a). As previously reported, Glc-induced AB14 expression leads to the repression of photosynthesis genes such as CAB1, RBCS and PC (Acevedo-Hernandez et al., 2005; Arenas-Huertero et al., 2000; Huijser et al., 2000). These three genes were more strongly repressed in Glc-treated NIL DOG17-1 when compared with Ler (Figure 4b–d). These results suggest that the GSQ5/DOG1 Cvi allele enhances the Glc induction of AB14 in the ABA-mediated sugar signalling pathway.

Glc induction of the GSQ5/DOG1 Cvi allele requires the ABA-mediated sugar signalling pathway

To investigate the interaction between the ABA-mediated sugar signalling pathway and Glc induction of the GSQ5/DOG1 Cvi allele, the GSQ5/DOG1 Cvi allele was combined with aba1-1 (Bentsink et al., 2006), hxk1/gin2 and abi4-1, respectively. These three combinations showed sugar-insensitive phenotypes on 6% Glc (Figure 5d,f,i), similar to the parental lines aba1-1 (Figure 5c), hxk1/gin2 (Figure 5e) and abi4-1 (Figure 5h), respectively. In contrast, the NIL DOG17-1 line showed a severe Glc-arrested phenotype (Figure 5b). The Ler and Col wild types showed the developmental-arrest phenotype as reported previously (Figure 5a,g). These results indicate that the ABA-mediated...
Promotion of ABI4 expression by GSQ5/DOG1

Figure 4. The GSQ5/DOG1 Cvi allele enhances ABI4 sugar induction. Expression level of ABI4 (a), CAB1 (b), RBSC (c) and PC (d) in Ler and near-isogenic line (NIL) DOG17-1 grown at 22°C under continuous light for 7 days in 5% glucose (Glc) or 5% sorbitol (Sor). Total RNA was isolated from the seedlings and used for qRT-PCR experiments. Values represent the average of two technical repeats from a representative experiment. Bars indicate standard errors. Similar results were obtained in two independent experiments, where RNA levels were qRT-PCR quantified in duplicate.

Figure 5. The ABA-mediated sugar signalling pathway induces the GSQ5/DOG1 Cvi allele. Sugar sensitivity of Ler (a), near-isogenic line (NIL) DOG17-1 (b), aba1-1 (c), GSQ5/DOG1-Cvi x aba1-1 (d), gin2-1 (e), GSQ5/DOG1-Cvi x gin2-1 (f), Col (g), ab4-1 (h), GSQ5/DOG1-Cvi x ab4-1 (i) grown in continuous light in the presence of 6% glucose (Glc) for 7 days at 22°C. (j) Expression level of GSQ5/DOG1 in NIL DOG17-1, GSQ5/DOG1-Cvi x aba1-1, GSQ5/DOG1-Cvi x gin2-1, GSQ5/DOG1-Cvi x ab4-1 grown in 6% Glc or 6% sorbitol (Sor). Total RNA was isolated from the seedlings and used for qRT-PCR experiments. Values represent the average of two technical repeats from a representative experiment. Bars indicate standard errors. Similar results were obtained in two independent experiments, where RNA levels were qRT-PCR quantified in duplicate.

Sugar signalling pathway is required for the role of the GSQ5/DOG1 Cvi allele in determining sugar sensitivity. Moreover, the sugar-insensitive phenotype displayed by mutations in the ABA-mediated sugar-signalling pathway could not be rescued by the GSQ5/DOG1 Cvi allele. The GSQ5/DOG1 Cvi allele was not induced in any of the combined lines (GSQ5/DOG1 Cvi combined with aba1-1, hxt1/gin2 or ab4-1; Figure 5j), showing the essential role of the ABA-mediated sugar signalling pathway in the sugar induction of the GSQ5/DOG1 Cvi allele. In conclusion, Glc induction of the GSQ5/DOG1 Cvi allele requires the ABA-mediated sugar signalling pathway.

Discussion

Mechanisms of sugar sensing and signalling in plants have been uncovered using mutant selection and gene identification strategies. Mutant selection procedures often relied
on relatively high sugar levels to induce seedling growth arrest. Seedlings are sensitive to sugar-induced arrest during an approximately 3-day time window following germination. In this time window embryonic marker genes such as ABI3, ABI5 and several LEA genes can be induced by sugars, suggesting that the germinated seedling reverts to an ‘embryonic state’ in the presence of high sugar levels, resulting in a growth-arrested state (Dekkers et al., 2008). Possibly elevated sugar conditions mimic the late stages of seed development, and are incompatible with seedling growth. Osmotic agents do not induce the embryonic marker genes and do not show this seedling growth arrest.

The plant Glc sensor HXK1 (Moore et al., 2003) was identified using high sugar level selection. HXK1 was shown to be involved in ABA-mediated sugar signalling (Gibson, 2004; Leon and Sheen, 2003; Rolland et al., 2002). Several other genes involved in sugar signalling were identified, including a plant-specific Arm repeat protein (Rook et al., 2006), and genes involved in vitamin B6 synthesis (Wagner et al., 2006) and nonsense RNA decay (Yoine et al., 2006). The function of these genes in sugar signalling is mostly unknown. Natural variation analysis can break through the narrow genetic backgrounds of the wild-type accessions used in laboratory-generated mutants, and provide new resources for the identification of genes and functional alleles (Alonso-Blanco and Koornneef, 2000). In this study, five QTLs for sugar sensing were identified. Three of these were positioned in regions that do not harbour known sugar-sensitivity genes. One of these three loci, GSQ5, was cloned and was shown to be identical to the DOG1 gene, encoded by At5g45830. The gsq5/dog1 mutant derived from NIL DOG1-1 and T-DNA insertion knock-out lines of gsq5/dog1 show a normal sugar-sensitivity phenotype, similar to that of Col and Ler. These GSQ5/DOG1 alleles do not seem to affect the response to sugars. Therefore, a mutant approach with Col and Ler accessions would not have identified GSQ5/DOG1 as an important gene in sugar signalling.

The expression of the GSQ5/DOG1 Cvi allele, but not that of the Ler and Col alleles, was induced by Glc. Polymorphisms between Ler and Cvi alleles, and Col and Cvi alleles of GSQ5/DOG1, were observed in both the cis-regulatory region and the protein-coding region (Bentsink et al., 2006). Results obtained by these authors suggested that the amino-acid substitutions present in the coding region of the different GSQ5/DOG1 alleles probably do not have functional consequences (Bentsink et al., 2006). Likewise, the enhanced sugar-induced gene expression of the GSQ5/DOG1 Cvi allele might be responsible for its sugar-super-sensitive phenotype.

ABA biosynthesis is required for both seed dormancy and sugar sensing. Of the identified ABA-insensitive mutants, only abi3 shows a loss of both seed dormancy (Ooms et al., 1993) and sugar signalling (Yuan and Wysocka-Diller, 2006; Dekkers et al., 2008), other ABIIs seem to be involved in either seed dormancy or in sugar signalling. The GSQ5/DOG1 Cvi allele enhances both seed dormancy (Bentsink et al., 2006) and sugar sensitivity (Figure 3). However, distinct mechanisms are probably involved, because the important ABA-mediated sugar signalling gene ABI4 does not seem to play a role in seed dormancy (Cadman et al., 2006), and Ler and Col alleles of GSQ5/DOG1 do not affect sugar sensing, but instead confer weak dormancy.

Glucose induction of the GSQ5/DOG1 Cvi allele depends on the ABA-mediated sugar signalling pathway (Figure 5j). Moreover, the ABA-mediated sugar signalling pathway is essential for the effect of the GSQ5/DOG1 Cvi allele on sugar sensitivity (Figure 5c,e,h). The ABI4 regulatory gene is an important downstream component of the ABA-mediated sugar signalling pathway, and the GSQ5/DOG1 Cvi allele somehow increases ABI4 sugar sensitivity. In the sugar–ABA signalling pathway, sugar, ABA and their complex interactions are essential (Arenas-Huertero et al., 2000; Cheng et al., 2002; Moore et al., 2003; Zhou et al., 1998), and ABI4 is induced by the synergistic action of sugar and ABA (Cheng et al., 2002), with sugar being the more likely direct signal (Arroyo et al., 2003). ABA synthesis is essential for sugar induction of ABI4, although ABA is not the only activator of ABI4 (Arroyo et al., 2003; Cheng et al., 2002). The Cvi allele of GSQ5/DOG1 shows a slightly ABA-hyper-sensitive phenotype in seed germination, whereas the ABA sensitivity of the GSQ5/DOG1 Ler allele is similar to the gsq5/dog1 mutant (Bentsink et al., 2006). These results suggest that GSQ5/DOG1 acts in ABA responses rather than in sugar responses, but this assumption must be confirmed. Our results indicated that the induction of GSQ5/DOG1 Cvi requires sugar signalling, and ABA synthesis and signalling. However, more information is needed to reveal whether glucose or ABA is the direct signal for GSQ5/DOG1 Cvi induction.

Enhanced sugar induction of ABI4 was observed in the GSQ5/DOG1 Cvi NIL. The stronger sugar repression of photosynthesis genes in NIL DOG1-1 confirmed this observation. Interestingly, the GSQ5/DOG1 Cvi allele did not enhance sugar-induced ABA1 and ABA2 expression (data not show). These observations point to an additional positive feedback loop between the ABA-mediated sugar signalling pathway and the GSQ5/DOG1 Cvi allele. On the one hand, sugars induce the expression of the GSQ5/DOG1 Cvi allele via the ABA-mediated sugar signalling pathway. On the other hand, the sugar-induced GSQ5/DOG1 Cvi allele enhances the sugar induction of ABI4, thereby stimulating the ABA-mediated sugar signalling pathway. As a result, the expression of photosynthesis genes and seedling development are severely repressed. In Col or Ler, this loop does not exist because GSQ5/DOG1 alleles in these accessions are not sugar induced.
Among Arabidopsis ecotypes, a huge but unexplained variation in GSQ5/DOG1 expression was observed (Bentsink et al., 2006). Moreover, GSQ5/DOG1 is subject to alternative splicing, with several splicing variants being present in the mRNA pool (Bentsink et al., 2006). Possibly, GSQ5/DOG1 has a much broader function that is not limited to the control of dormancy. Moreover, the relationship between DOG1 and ABI4 can differ between seed dormancy and seedling stages. GSQ5/DOG1 belongs to a plant-specific gene family with at least four members (At4g18660, At4g18680, At4g18690 and At4g18650; Bentsink et al., 2006). No information is available on the function of other members of the GSQ5/DOG1 gene family.

The ecological significance of the observed differences in sugar responsiveness of GSQ5/DOG1, and for that matter ABI4, is unknown. The GSQ5/DOG1 Cvi allele enhances the sugar response of the important ABI4 gene. ABI4 controls important processes such as resource capture and mobilization, and these processes must affect plant competitiveness. So far, such studies have not been performed.

**Experimental procedures**

**Plant material**

The RIL population used for QTL analysis was derived from a cross between Ler and Cvi. These RILs were previously characterized genetically using AFLP and cleaved amplified polymorphic sequence (CAPS) markers (Alonso-Blanco et al., 1998).

Three NILs (Keurentjes et al., 2006), LCN1-2, LCN3-1 and LCN5-14, which carry single introgression fragments from Cvi in a Ler genetic background, were used to confirm GSQ1, GSQ3 and GSQ5 sugar-sensitivity phenotypes. NIL DOG1-7 (Bentsink et al., 2006) and its homozygous sub-NILs (NIL8-10, NIL11-3, NIL1-5, NIL10-9 and NIL8-3) were used for fine mapping of GSQ5. Markers for genotyping these lines were as described by Bentsink et al. (2006). Two independent transgenic Ler lines harbouring the GSQ5/DOG1 Cvi allele (R3-1-6, R3-2-3), the doq1 mutant and two homozygous T-DNA insertion lines in DOG1 (SALK_000867 and SM_3_20808) were as described by Bentsink et al. (2006).

The GSQ5/DOG1 Cvi allele combined with aba1-1 was also as described by Bentsink et al. (2006). The GSQ5/DOG1 Cvi allele was combined with gin2 and abi4 mutations by crossing NIL DOG1-7 and gin2-1 (in the Ler background) or abi4-1 (in the Col background). The combination lines were selected using molecular markers linked to the GSQ5/DOG1 Cvi allele (Bentsink et al., 2006), and CAPS markers for gin2 (forward, 5′-CTTGGCTTAGGCGTTTTCTG-3′; reverse, 5′-GCATGGTCTGCTTCCAAAAT-3′; restriction enzyme, BsgI and abi4; Brocard-Gifford et al., 2003) in the F2 population.

**Analysing sugar sensitivity**

Surface sterilization of seeds was performed as described by Teng et al. (2005). After sterilization, seeds were stratified in 0.1% agarose for 4 days at 4°C in the dark, followed by plating on half-strength MS medium, pH 5.8, solidified with 0.8% plant agar (Duchefa, http://www.duchefa.com). The different concentrations of Glc and Sor were added as indicated. Plates were incubated at 22°C under continuous fluorescent light (100 μmol/m²/s) for 7 days, and then the percentages of green seedlings were investigated. In some experiments, seed coats were removed before stratification to break the seed dormancy.

**QTL analyses**

The mean of green seedling percentages of four independent duplicate analyses of the RIL population in the Glc-sensitivity assay was transformed by the angular transformation (± arcsin). This transformed dataset was used for QTL mapping. QTL analyses were performed as described by Teng et al. (2005).

**Real-time RT-PCR**

Total RNA was isolated using the RNeasy mini plant kit (Qiagen, http://www.qiagen.com). The quality and the quantity of the RNA were analysed by electrophoresis on agarose and UV spectrometry, respectively. Total RNA was DNase treated (Fermentas, http://www.fermentas.com) to remove genomic DNA, and a 1-μg aliquot was reverse transcribed using M-MLV (Promega, http://www.promega.com) according to the manufacturer’s instructions. Real-time PCR was performed using an ABI Prism 7000 sequence detector and Cybergreen™ chemistry (ABI, http://www.appliedbiosystems.com). Expression levels were calculated relative to actin2 (At3g18780) levels using the Q-gene method that takes the relative efficiencies of the different primer pairs into account ( Muller et al., 2002). The standard errors of two technical repeats were calculated according to equation 3 of Simon (2003). Primers were designed to be genespecific by the CATMA consortium (http://www.CATMA.org).

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