



## BOTANICAL BRIEFING

### Why and How Do Plant Cells Sense Sugars?

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The ability to sense sugars is crucial for the modulation of gene expression in plants. Despite the importance of this phenomenon, our knowledge of sugar sensing in plants is scant. Several valuable hypotheses have been put forward based on the extensive knowledge of sugar sensing in yeast. In recent years, tests of these hypotheses have shown that hexokinase and sucrose-non-fermenting- (SNF-) related proteins appear to be involved in sugar sensing and transduction, not only in yeast but also in higher plants. However, even if plants share with yeast some elements involved in sugar sensing, several aspects of sugar perception are likely to be peculiar to higher plants. Plants should be able to sense not only glucose but also other hexoses, such as fructose and disaccharides (sucrose, maltose and others). In this Botanical Briefing we outline recent discoveries in this field, with emphasis on arabidopsis and cereals. The use of transgenic plants and mutants to identify sugar sensor(s) and elements in the signalling pathways and their cross-talk with the hormonal signalling is discussed.

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**Key words:** Abscisic acid, *Arabidopsis thaliana*, cereals, hexokinase, sugar sensing.

#### INTRODUCTION

Sugars play a central role in plant life: they are produced by photosynthesis, transported to sink tissues, channelled to respiration or converted into storage compounds (lipids, starch, sucrose, fructans) which are eventually broken down into their constituent carbohydrates. It is conceivable that regulation of the metabolic processes involved is dependent upon the concentration of sugars, and therefore that plants should be able to sense these concentrations within a cell. However, sensing intracellular sugars provides rather incomplete information about the metabolic status of a plant cell, since it ignores the concentration of sugars in the apoplast and, perhaps more importantly, the flux of sugars between apoplast and cytosol. We may therefore speculate that an efficient sugar sensing machinery should include: (1) a sensor able to perceive the concentration of apoplastic sugars; (2) a sensor able to respond to the flux of carbohydrates entering the cell; and (3) a means of sensing sugars in the cytosol. A cytosolic sensor might respond to the flux of intracellular sugars into metabolism rather than their steady state concentration. Furthermore, the 'complete' sugar-sensing network should be able to provide information on sucrose (the major transported sugar), other disaccharides (e.g. maltose) and hexoses (e.g. glucose and fructose), which are present in the cytosol or moving to a variety of cell compartments. The possible complexity of the sugar-sensing regulatory web makes it difficult to dissect, and

we will describe here some of the approaches, tools and results obtained in plants, highlighting the advantages and possible related pitfalls of each.

#### SUGAR SENSING IN YEAST AS A MODEL FOR SUGAR SENSING IN PLANTS

Knowledge of sugar sensing in plants is limited, although this process has been dissected in detail for micro-organisms (Johnston, 1999). The pathway of sugar sensing/transduction in yeast provides an array of hypotheses that may be usefully applied to sugar sensing in plants. For example, in yeast, glucose is able to trigger repression/induction of several genes, and the same holds true for plants. The glucose-signalling pathway in yeast has been reviewed in detail in several recent articles (Jang and Sheen, 1997; Smeekens and Rook, 1997; Yu, 1999; Koch *et al.*, 2000). The pathway includes extracellular sensing at the level of a sugar transporter/receptor, intracellular sensing through the action of a hexokinase (HXK), a crucial role for the sucrose-non-fermenting-1 (SNF1) kinase complex, phosphatases, and several other protein complexes such as DNA-binding proteins. Hexokinase plays a role as the glycolytic enzyme channelling glucose into glycolysis ( $\text{Glc} + \text{ATP} \rightarrow \text{Glc6P} + \text{ADP}$ ), and there are several indications (see below) that HXK is involved in sensing glucose not only in yeast, but also in plants. SNF1-like protein kinases have also been identified in plants and there is evidence of their possible role in sugar modulation of plant genes. However a number of differences between sugar sensing in yeast and in plants are to be expected; some of these differences are described in the following sections.

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## TOOLS TO STUDY SUGAR SENSING IN PLANTS

### *Sugar analogues*

Among plant carbohydrates, hexoses such as glucose and fructose predominate together with disaccharides such as sucrose and, in some tissues, maltose; each might be sensed to transduce a specific signalling pathway. It is reasonable to ask which of these sugars is sensed. Does sensing of sugars depend upon their metabolism? It is not easy to answer these questions, since sugars fed to a plant to test their effects on gene expression are rapidly interconverted: sucrose is broken down into glucose and fructose, while hexose-feeding leads to sucrose synthesis (Loreti *et al.*, 2000). The use of non-metabolizable sugars can, in principle, overcome problems related to interconversions. Moreover, since some compounds differ from their naturally occurring analogues in their uptake kinetics, this allows discrimination between intracellular and extracellular sensing. Yet other analogues are known to be partly metabolized, and this allows the role of enzymes as sugar sensors to be evaluated. However, there are also possible pitfalls in the use of these compounds: (1) some analogues can be toxic, resulting in non-specific gene repression, independent of their possible sensing; (2) some analogues which are believed not to be metabolized may be metabolized, at least in some plant systems; and (3) some analogues are taken up by some plant systems but not by others, making interpretation of data complex when uptake of the analogue is crucial for testing an hypothesis. In spite of these problems, the use of analogues is still contributing to the dissection of sugar sensing in plants.

Sugar analogues commonly used include (see also Table 1):

**Mannose:** a glucose epimer, known to be phosphorylated by hexokinases to mannose-6P, which is poorly metabolized (but mannose-6P is metabolized in some plant systems; Stoop and Pharr, 1993). At low concentrations mannose is able to modulate some sugar-regulated processes, even though it is also toxic to several plant systems. Mannose has been used to demonstrate that metabolism of hexoses beyond their phosphorylated counterparts is not needed to trigger sugar-repression of genes (Graham *et al.*, 1994; Jang and Sheen, 1994). Mannose has also been used to screen for sugar-insensitive arabidopsis mutants (see below).

**2-Deoxyglucose:** a glucose analogue, phosphorylated by hexokinases to 2-deoxyglucose-6P, which is poorly metabolized (although there is recent experimental evidence of its metabolism into 2-deoxy-sucrose; Klein and Stitt, 1998). 2-Deoxyglucose is toxic at low concentrations, and provides similar clues as mannose.

**6-Deoxyglucose:** a glucose analogue transported inside the cell but not a substrate for HXK. Since 6-deoxyglucose can trigger gene induction, these genes are regulated by a sensing pathway independent of HXK (Roitsh *et al.*, 1995). Other genes are insensitive to 6-deoxyglucose but responsive to 2-deoxyglucose, suggesting that phosphorylation is needed for sensing (Jang and Sheen, 1994).

**3-O-Methylglucose:** a glucose analogue transported inside the cell but not a substrate for HXK (and not transported in some plant systems; Komor *et al.*, 1985). In

some plant systems, 3-O-methylglucose does not modulate sugar-regulated genes, indicating that transport of the hexose is not sufficient to trigger gene modulation (Jang and Sheen, 1994). However, in other plant systems, 3-O-methylglucose triggers induction of sugar-regulated genes, suggesting that HXK is not the sugar sensor (Martin *et al.*, 1997).

**Sucrose analogues:** turanose and palatinose are disaccharides including a glucose moiety linked to fructose 1→6, and 1→3 respectively (sucrose is Glc[1→2]Fru). These disaccharides are not metabolized in barley embryos (Loreti *et al.*, 2000), but data are not available for other species.

**Other disaccharides:** these include, galactobiose (Gal[1→3]Gal); cellobiose ( $\beta$ Glc[1→4]Glc); lactulose ( $\beta$ Gal[1→4]Fru); lactose ( $\beta$ Gal[1→4]Glc); leucrose (Glc[1→5]Fru); isomaltose (Glc[1→6]Glc); gentiobiose ( $\beta$ Glc[1→6]Glc) and melibiose (Gal[1→6]Glc). Some of these disaccharides (cellobiose, leucrose, isomaltose, gentiobiose) are metabolized in isolated barley embryos (Loreti *et al.*, 2000), while the others are useful tools to study disaccharide sensing.

**Trehalose, Glc[1→1]Glc:** this disaccharide can be metabolized by plants, but in the presence of validamycin A its metabolism is arrested, allowing the evaluation of its possible direct sensing (Wingler *et al.*, 2000).

### *Transgenic plants*

The use of transgenic plants has allowed tests of whether sugar sensing in plants has similarities with the well-characterized yeast model where HXK plays a role as a sugar sensor (see Jang and Sheen, 1997). Several lines of experimental evidence had suggested that this glycolytic enzyme plays the same role in plants (see also below) as in yeast, but the most convincing evidence arises from experiments performed using transgenic arabidopsis expressing sense- or antisense-HXK genes (Jang *et al.*, 1997). Antisense-HXK plants are insensitive to high glucose concentrations, while plants overexpressing HXK are hypersensitive to sugars, giving strong support to the possible role of HXK as a sugar sensor in plants (Jang *et al.*, 1997) even though the role of HXK in signalling the sugar status is still under discussion (Halford *et al.*, 1999). Another component of the yeast signalling pathway is the protein kinase SNF1 (Celenza and Carlson, 1986). SNF1-related proteins have been identified in plants, and their role in sugar sensing has been tested by using potato plants expressing an antisense SNF1-related protein kinase. Results indicated that the SNF1-related protein plays a role in transducing the sugar signal, triggering the induction of sucrose synthase in potato leaves (Purcell *et al.*, 1998).

The use of transgenic plants has not only provided a means to test whether components of the yeast sugar-signalling pathway are involved in plant sugar sensing, but also to identify plant-specific systems. Rook *et al.* (1998) took advantage of transgenic arabidopsis expressing ATB2 promoter-GUS fusions (ATB2 is a bZIP transcription factor) to discover sucrose specific regulation of ATB2 translation, providing evidence for one of the few sucrose-specific plant systems. Transgenic arabidopsis plants

TABLE 1. *Sugar analogues as tools to study sugar sensing*

Sugar	Uptake	Direct phosphorylation by HXK	Metabolization (glycolysis)	Putative sensor								
				Extracellular hexose sensor	Extracellular disaccharide sensor	Hexose transporter	Disaccharide transporter	Intracellular hexose sensor	Hexokinase	Post-HXK sensor	Intracellular disaccharide sensor	
Glucose	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
2-Deoxyglucose	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
6-Deoxyglucose	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓
3-O-methylglucose	✓			✓	✓	✓	✓	✓	✓	✓	✓	✓
Mannose	✓	✓		✓	✓	✓	✓	✓	✓	✓	✓	✓
Sucrose	✓		✓									
Palatinose									✓			
Turanose									✓			

For each sugar, its ability to cross the plasma membrane (uptake), its phosphorylation by HXK, and the possibility of further metabolism through glycolysis is indicated. The deduced putative sensor able to perceive the sugar, based on its metabolic properties, is also indicated. The sugars reported are all able to trigger induction or repression of a variety of genes (see text for references).

harbouring constructs comprising the promoter of sugar-regulated genes controlling a reporter gene (GUS or LUC) have also been used as tools to screen sugar-sensing mutants: transgenic plants have been mutagenized, and the resulting mutants screened using non-destructive methods to identify mutants unable to respond to sugars (Dijkwel *et al.*, 1997; Martin *et al.*, 1997).

#### PLANT MODEL SYSTEMS TO STUDY SUGAR SENSING

The ability of plants to sense the presence of sugars has been reported for a large number of species, including arabidopsis, cereals, *Chenopodium*, potato, sweet potato, tobacco, cucumber, carrot, *Vicia*, sugar beet, celery, cassava, soybean, petunia, ivy, rubber tree, spinach, and many others. Protoplasts, cell cultures, leaves, embryos, aleurone, cotyledons, roots, stems, tubers, petioles and flowers have all been the subject of experiments performed in the light, in the dark, by feeding sugars to detached tissues or to intact plants. The overwhelming conclusion is that it is virtually impossible to generalize between plant systems. We will describe results from two model systems that have provided significant information about sugar sensing in plants, namely arabidopsis and cereals at the germination stage.

##### *Arabidopsis sugar sensing mutants*

To identify components of the sugar-signalling machinery in plants, several groups have screened and selected mutants—almost exclusively arabidopsis—with altered sugar sensing. Several mutants have been selected using a germination medium containing high sugar concentrations, since wild-type arabidopsis shows slow germination under such conditions (Fig. 1A–F). In an alternative approach, a ‘marker’ gene, which is sugar inducible/repressible and, ideally, whose expression is easy to assay, has been used. Mita *et al.* (1997) screened arabidopsis mutants for plants unable to respond to  $\beta$ -amylase induction ( $\beta$ -amylase is induced by sugars in arabidopsis leaves, and its enzyme activity is easily assayed). Selecting for low  $\beta$ -amylase in leaves treated with sucrose allowed the isolation of a mutant named *lba1* (low beta amylase). The *lba1* mutant is unable to respond to sucrose, glucose and fructose, not only as far as  $\beta$ -amylase induction is concerned, but also other sugar-regulated processes, such as the accumulation of anthocyanins. Interestingly, the Landsberg *erecta* ecotype of arabidopsis is also a low  $\beta$ -amylase mutant (named *lba2*): *lba1* and *lba2* are, however, recessive traits unlinked to each other (Mita *et al.*, 1997). The same group has also isolated an arabidopsis mutant with enhanced expression of  $\beta$ -amylase (*hba* = high beta-amylase), even at low sugar levels, suggesting the existence of a negative regulation of sugar induction of  $\beta$ -amylase (Mita *et al.*, 1997). Other groups have developed an alternative approach for the selection of mutants, using reporter genes such as GUS or LUC under the control of a sugar-modulated promoter. Martin *et al.* (1997) selected *rsr* mutants (reduced sucrose response) from mutagenized transgenic arabidopsis expressing the chimeric gene patatin-GUS (the patatin gene is sugar induced), while

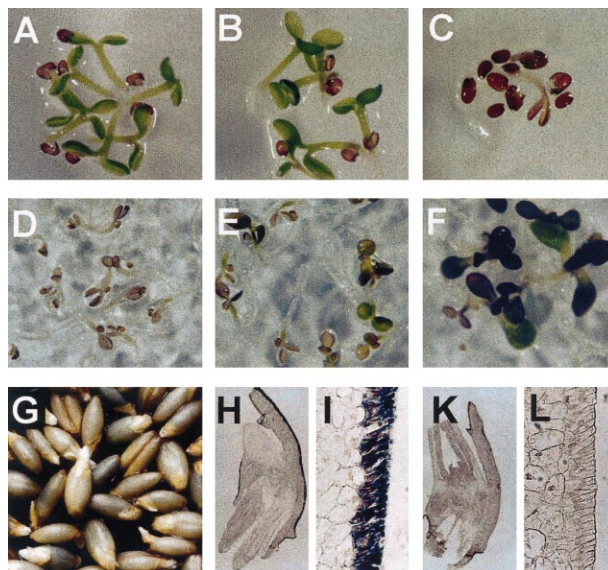


FIG. 1. Plant model systems used to study sugar sensing. A–C, Effect of glucose on arabidopsis seed germination: glucose represses seed germination while mannitol has no effect. A, Control seeds germinating on Murashige-Skoog medium in the light. B, Seeds germinating on mannitol (333 mM). C, Seeds germinating on glucose (333 mM). D–F, Sugar sensing transgenics and mutants: glucose affects the germination of wild-type seeds, but has limited effects on antisense-HXK transgenic seeds or on the *gin1* mutant. D, Wild-type seeds germinating on 333 mM glucose. E, Transgenic arabidopsis seeds harbouring an antisense-HXK construct germinating on 333 mM glucose. F, Germination of the *gin1* mutant on 333 mM glucose. G–L, Glucose repression of  $\alpha$ -amylase in barley embryos. G, Germinating barley grains. H, *In situ* hybridization of an  $\alpha$ -amylase probe reveals expression of  $\alpha$ -amylase in the scutellar epithelium of barley embryos. I, Magnified view of the  $\alpha$ -amylase hybridization signal in the scutellar epithelium. K, *In situ* hybridization of an  $\alpha$ -amylase probe reveals the absence of  $\alpha$ -amylase expression in the scutellar epithelium of barley embryos treated with 25 mM glucose. L, Magnified view of the scutellar epithelium of glucose-treated barley embryos, showing the absence of  $\alpha$ -amylase hybridization signal.

Dijkwel *et al.* (1997) used a mutagenized population of transgenic arabidopsis carrying a plastocyanin promoter-luciferase fusion gene (plastocyanin is repressed by sugars) to isolate several *sun* mutants (sucrose uncoupled).

##### *Cereals*

Cereal grains store a massive amount of starch in the endosperm. The first step for the mobilization of these reserves is the degradation of the starch, mediated by the action of a set of enzymes including  $\alpha$ -amylases. The induction of  $\alpha$ -amylases is triggered by gibberellins and is repressed by abscisic acid (ABA): sugars also play an important role in the regulation of the expression of some  $\alpha$ -amylase genes. In rice,  $\alpha$ -amylases are encoded by at least 11 genes, not all regulated by gibberellins (Mitsui and Itoh, 1997). Indeed, the rice  $\alpha$ -amylase *Ramy3D* and *Ramy3E* genes are known to be modulated by sugar availability in germinating rice grains, while *Ramy1A* is under hormonal control. *Ramy1A*, *Ramy3D* and *Ramy3E* differ in their localization of expression, *Ramy1A* and *Ramy3E* being expressed in both embryo (scutellar epithelium) and

aleurone, while *Ramy3D* is expressed exclusively in the embryo (Hwang *et al.*, 1999). Little is known about sugar modulation of  $\alpha$ -amylase in rice aleurone, but  $\alpha$ -amylase genes are not sugar repressed in barley aleurone, suggesting that the aleurone tissue does not have competence for sugar modulation of these genes. On the other hand, sugars repress  $\alpha$ -amylase genes in the embryo, with *Ramy3D* and *Ramy3E* being strongly repressed if germination is carried out in the presence of 100–300 mM glucose (Yu *et al.*, 1996). Interestingly, *Ramy1A* has also been shown to be sugar regulated in rice embryos (Morita *et al.*, 1998), suggesting cross-talk between gibberellin and sugar signalling. Indeed, the induction of  $\alpha$ -amylase in barley embryos is triggered by gibberellins but repressed in the presence of sugars (Perata *et al.*, 1997; see Fig. 1G–L). Sugar repression of  $\alpha$ -amylase in cereals seems to involve both transcriptional and post-transcriptional regulation, as elegantly demonstrated by Yu and co-workers (Sheu *et al.*, 1996; Yu, 1999).

Sugars may trigger an important functional switch in the physiology of the epithelium of cereal grains, from the initial secretion of  $\alpha$ -amylases, to its role in absorbing and utilizing endosperm degradation products (Okamoto *et al.*, 1980; Fincher, 1989). Indeed, Karrer and Rodriguez (1992) have demonstrated the coordinated expression of starch-degrading enzyme(s) ( $\alpha$ -amylases, repressed by sugars as germination proceeds) and sucrose metabolizing enzymes (sucrose synthase, induced by sugars as germination proceeds). A possible scenario is described here. After the initial steps of starch degradation have occurred, glucose derived from starch represses  $\alpha$ -amylase genes in the scutellar epithelium but not in the aleurone (Perata *et al.*, 1997). Meanwhile, the sucrose synthesizing enzyme (sucrose phosphate synthase) is induced, possibly by gibberellins, in the scutellum (Chávez-Bárceñas *et al.*, 2000), where sucrose synthesis takes place (Nomura *et al.*, 1969). Sucrose induces the companion-cell-specific sucrose transporter (*OsSUT1*; Matsukura *et al.*, 2000), allowing an efficient sucrose transport to the developing roots and shoot. Thereafter, sugars induce sucrose degrading enzymes such as sucrose synthase (Karrer and Rodriguez, 1992), allowing utilization of sucrose transported to the developing seedling.

Genes encoding sucrose-degrading enzymes in maize are also modulated by sugar (Koch, 1996). In maize, this is true for sucrose synthase genes as well as for invertase genes (Koch *et al.*, 1992; Xu *et al.*, 1996). The two sucrose synthase genes *Sh1* and *Sus1* are differentially regulated by sugars: the *Sh1* gene being induced by low sugar availability while the *Sus1* gene is expressed at higher concentrations if excised root tips are incubated in high glucose (Koch *et al.*, 1992). A similar pattern of expression is observed with the invertase gene family: *Ivr2* being sugar enhanced, whereas *Ivr1* is induced by low sugar concentrations and repressed by higher sugar availability (Xu *et al.*, 1996). Sugar regulation of sucrose-degrading enzymes may have consequences for sugar sensing (Koch, 1996). Sucrose can be a signal itself, but the two hexoses produced by its hydrolysis can be sensed by hexose sensor(s). We can speculate that sucrose can be sensed by a translocator as it crosses the plasma membrane or, following hydrolysis by sucrose degrading enzymes in the cytosol, sensed by cytosolic hexose sensor(s). Whether

sucrose is degraded by invertases or sucrose synthases makes a difference in the number of phosphorylatable hexoses produced, since invertases hydrolyse sucrose into glucose + fructose, while sucrose synthases produce fructose + UDP-glucose. Since HXK-based sugar sensor(s) perceive phosphorylatable sugars only, the action of invertases amplifies the metabolic signal (see also Koch, 1996).

## MULTIPLE SUGAR SENSING MECHANISMS IN PLANTS

### *Hexokinases as sugar sensors*

The possible involvement of plant HXKs as sugar sensors has been investigated using various approaches, described in several review articles, and is the subject of debate among scientists (see Halford *et al.*, 1999; Halford and Purcell, 1999; Moore and Sheen, 1999; Pego *et al.*, 1999). Here, we will only summarize some evidence on the role of HXK as a sugar sensor. The use of glucose analogues demonstrated that only analogues that are substrates for HXKs were able to modulate some sugar-regulated genes. Indeed, mannose and 2-deoxyglucose repress sugar-regulated genes, while 6-deoxyglucose and 3-*O*-methylglucose are ineffective (Graham *et al.*, 1994; Jang and Sheen, 1994). The phosphorylated sugars do not represent a signal for the plant, as demonstrated by the inability of glucose 6-phosphate introduced into protoplasts via electroporation, to elicit the same effect as glucose. Additionally, inhibitors of HXK activity (mannoheptulose, glucosamine) can reverse the effect of glucose (Jang and Sheen, 1994; Umemura *et al.*, 1998; Pego *et al.*, 1999). It appears, therefore, that the sugar phosphorylation step rather than the phosphorylated sugar represents a signal for the plant. Transgenic arabidopsis plants harbouring antisense HXK genes are glucose insensitive, while over-expression of HXK results in glucose hypersensitivity (Jang *et al.*, 1997). However, the role of HXK as a sugar sensor is still controversial (see Halford *et al.*, 1999). A complex set of enzymes with different affinity and specificity characteristics is able to phosphorylate not only glucose (glucokinases), but also fructose (fructokinases) or both glucose and fructose (HXK). This complexity strongly differentiates plants from yeast, and further knowledge of plant HXKs would help to elucidate the role of this protein in sugar sensing.

In arabidopsis, sugar-phosphorylating activity is higher toward fructose than glucose. Indeed, fructokinases are particularly active, whereas HXKs, showing a reduced affinity for fructose, are present at relatively low levels (S. Gonzali, L. De Bellis, A. Alpi, unpubl. res.). *Arabidopsis thaliana* (AtHXKs) hexokinases possess different catalytic properties from yeast-HXK, the main difference being the higher phosphorylating activity of yeast-HXK toward fructose. It follows that transgenic plants over-expressing AtHXKs or yeast-HXKs (Jang and Sheen, 1997) show different glucose-phosphorylation/fructose-phosphorylation ratios, which could influence the overall sugar-sensing process in these plants. Further biochemical studies of the sugar status and hexose affinities of plant HXKs could

unveil unexpected clues concerning the role of HXKs in sugar sensing.

Remarkably, an arabidopsis *mig* (mannose-insensitive germination) mutant turned out to be a fructokinase mutant, suggesting that fructokinases, and not just HXKs, could be involved in sugar sensing (Pego and Smeekens, 2000). However, fructokinases are normally unable to phosphorylate mannose (Gonzali et al., 2001), and the link between the mutation and the phenotype is intriguing. The subcellular localization of plant HXKs is also not described in detail. The assumption that HXKs acting as sugar sensors are exclusively cytosolic is not compatible with results showing that transgenic tobacco plants expressing a yeast invertase in the apoplast or in the vacuole are able to sense the high hexose level due to increased sucrose breakdown, while hexoses are not sensed if generated in the cytosol by a yeast invertase targeted to the cytosol (Herbers et al., 1996). Results obtained by Herbers et al. (1996) suggest that sensing may occur in the endomembrane system, where HXKs are unlikely to be localized.

It is reasonable to hypothesize that sugar sensing in plants depends upon HXK-dependent and independent pathways (Xiao et al., 2000), at least as far as hexose sensing is concerned. Some genes are not repressed by sugar in HXK-antisense arabidopsis plants (HXK-dependent sensing pathway), while other genes are derepressed—in arabidopsis plants over-expressing not only arabidopsis HXK but also yeast HXK. Since yeast HXK does not play a role as a sugar sensor in plants (Jang et al., 1997), this result is suggestive of the existence of a glycolysis-dependent sensing pathway detecting the level of an unknown metabolite downstream of HXK (Xiao et al., 2000). Other genes are not affected by HXKs, and their expression is independent of the overexpression or antisense repression of HXKs (Xiao et al., 2000). Sensing of disaccharide requires specific sensor(s).

#### Disaccharide sensing

It is not easy to study the direct sensing of sucrose as a specific signalling molecule. The rapid metabolism of sucrose into glucose and fructose molecules is possibly sensed by hexose sensor(s). Furthermore, since most plant tissues can readily synthesize sucrose when fed with hexoses, it is not possible to attribute the effects of hexoses to their direct sensing, since sucrose sensing could possibly occur. Experiments dealing with the effect of sucrose on genes whose expression is not affected by hexoses help in unveiling sucrose sensing. Sucrose sensing has been demonstrated for the modulation of the patatin promoter (Wenzler et al., 1989; Jefferson et al., 1990), of the *rolC* promoter in transgenic tobacco (Yokoyama et al., 1994), and of proton-sucrose symporter activity in sugar beet (Chiou and Bush, 1998). Interestingly, sucrose represses translation of a transcription factor in arabidopsis (Rook et al., 1998). In these experiments the authors could separate the effects of sucrose from those related to its metabolism into glucose and fructose, since the effect of these hexoses was either absent or less pronounced when compared to those of sucrose. Loreti et al. (2000) recently described disaccharide

sensing in barley embryos. The expression of  $\alpha$ -amylase in the scutellar epithelium is repressed not only by glucose and sucrose, but also by lactulose ( $\beta$ Gal[1 $\rightarrow$ 4]Fru), palatinose (Glc[1 $\rightarrow$ 6]Fru), and turanose (Glc[1 $\rightarrow$ 3]Fru), which are not metabolized but able to repress  $\alpha$ -amylase. Melibiose, a Gal[1 $\rightarrow$ 6]Glc disaccharide that is also not metabolized, is unable to repress  $\alpha$ -amylase. Structure-function analysis suggests that a fructose moiety is needed for disaccharide sensing. Disrupting the fructosyl moiety of lactulose and palatinose, or replacing the fructose moiety of  $\beta$ Gal[1 $\rightarrow$ 4]-Fru with glucose or galactose results in molecules unable to repress  $\alpha$ -amylase. Comparison of the molecular requirements for sucrose transport with those for disaccharide-sensing suggests that these sugars are perceived, possibly at the plasma membrane, independently from sucrose transport (Loreti et al., 2000).

The ability to sense sucrose analogues such as palatinose and turanose has also been demonstrated in tobacco leaves (Sonnewald and Herbers, 1999). The ability to sense disaccharides not containing fructose has been demonstrated in arabidopsis using trehalose (Wingler et al., 2000). Trehalose is able to induce an ADP-glucose pyrophosphorylase gene (*Apl3*) in arabidopsis, whereas it is unable to induce the  $\beta$ -amylase gene. Since palatinose and turanose are unable to induce the *Apl3* gene in arabidopsis (E. Loreti and P. Perata, unpubl. res.), it is tempting to speculate that distinct sensors sense trehalose and sucrose analogues.

#### Is ABA needed to mediate sugar sensing?

The recent characterization of sugar-insensitive arabidopsis mutants has revealed that ABA is involved in the transduction of sugar signals: several of these mutants are either ABA-insensitive or unable to produce ABA. ABA may indeed mediate the sugar signalling, and this is supported by experiments with glucose (388 mM) resulting in increased ABA content in arabidopsis seedlings, while the *gin5* mutant is unable to increase its ABA content under the same conditions (Arenas-Huertero et al., 2000).

One of the sugar insensitive arabidopsis mutants (*sun6*) has been characterized and found to be identical to *ABI4* (*ABSCISIC ACID INSENSITIVE-4*; Huijser et al., 2000). The *sun6* mutant therefore not only exhibits a glucose insensitive phenotype (*gin* phenotype), but also an ABA insensitive (*abi*) phenotype. Furthermore, *sun6* also shows a mannose-insensitive germination (*mig* phenotype). These results were supported by the finding that other sugar insensitive mutants (*gin6*: Arenas-Huertero et al., 2000; and *sis5*: Laby et al., 2000) are allelic to *abi4*. Other sugar insensitive mutants are allelic to genes playing a role in ABA synthesis, namely the *gin1* (allelic to *aba2*; data from J. Sheen's group, reported in Gibson, 2000), and the sucrose insensitive mutant *sis4* (allelic to *aba2*; Gibson, 2000). The interesting *prl1* mutant, altered in the response to several plant hormones (including ABA), is glucose hypersensitive. At the same time, it shows derepression of sugar-repressed genes, indicating that the *prl1* mutation simultaneously affects the phenotype of arabidopsis (sugar hypersensitive phenotype when germinated on 175 mM glucose) and influences a negative regulator counteracting

the activity of elements involved in sugar repression on genes (Németh *et al.*, 1998).

As described above, many sugar insensitive mutants are also either ABA-insensitive (*sis5*, *sun6*, *gin6* = *abi4*) or ABA synthesis mutants (*sis4*, *gin1* = *aba2*). However, not all the ABA-insensitive mutants display a sugar-insensitive phenotype. Indeed, the *abi1*, *abi2* and *abi3* mutants are not sugar insensitive, while the *abi5* mutant shows a moderate glucose and mannose-insensitive phenotype (Huijser *et al.*, 2000; Laby *et al.*, 2000), indicating that a specific ABA-related transduction pathway mediates sugar sensing. Interestingly, a glucose-hypersensitive mutant (*pr11*) is also supersensitive to ABA and other hormones (Németh *et al.*, 1998). It is not, however, easy to connect ABA with sugar signalling, and a number of hypotheses can be put forward (see also Gibson, 2000). High glucose (333–388 mM) treatment results in enhanced ABA synthesis (Arenas-Huertero *et al.*, 2000), which may be responsible for the slow germination of arabidopsis seeds. Indeed, some sugar-insensitive mutants are unable to synthesize ABA, and show normal germination, but sugar sensitivity can be restored by ABA treatment (Arenas-Huertero *et al.*, 2000). This effect of high glucose does not appear to be related to the osmotic stress connected to the use of high glucose concentrations, since a similar mannitol concentration is unable to trigger the same developmental arrest as glucose (Arenas-Huertero *et al.*, 2000). Furthermore, even if some sugar-insensitive mutants show an osmotolerant phenotype, tolerance to osmotic stress does not appear to be sufficient for conferring sugar tolerance, since arabidopsis mutants showing an osmotolerant phenotype (e.g. *abi2-1*) are not sugar insensitive (Laby *et al.*, 2000). Additionally, mutants showing a glucose-insensitive phenotype are also mannose-insensitive, and mannose is able to repress germination when used at concentrations as low as 1–5 mM, a level unable to cause an osmotic stress. However, it is still possible that arabidopsis mutants selected using high glucose concentrations as a selective medium are, at least in part, stress-related mutants. Indeed 333–388 mM glucose exerts some osmotic stress, and mannose 1–5 mM is rather toxic to plant tissues (Graham *et al.*, 1994), even though the effects of mannose in arabidopsis cannot be ascribed to phosphate starvation (Pego *et al.*, 1999). Evidence for overlapping stress and glucose transduction pathways have indeed been reported (Ehness *et al.*, 1997). Stress conditions may elicit the production of stress-related hormones (ABA and ethylene). It is worth noting that the involvement of ethylene in sugar sensing has been demonstrated by experiments showing that ethylene mutants (*eto1-1*, ethylene overproducing; *ctr1-1*, constitutive triple response) are glucose insensitive whereas an ethylene-insensitive mutant (*etr1-1*) is glucose hypersensitive (Zhou *et al.*, 1998). *etr1-1 gin1-1* double mutants are glucose insensitive, indicating that *GIN1* acts downstream of *ETR1* (Zhou *et al.*, 1998). Remarkably, Gibson (2000) reported that the *gin1* mutant had been identified as allelic to *aba2* (data from the laboratory of J. Sheen), and thus was unable to synthesize ABA, while the *etr1* mutant is an ethylene-insensitive mutant. An interaction between ethylene and ABA has recently been discovered, showing that the *etr1* mutant is also ABA

supersensitive (Beaudoin *et al.*, 2000). It is therefore possible to hypothesize that the *etr1* mutant is glucose hypersensitive as a consequence of glucose-induced ABA synthesis linked to ABA supersensitivity due to the *etr1* mutation. The double mutant *etr1-1 gin1-1* is glucose-insensitive despite being ABA-supersensitive, as a consequence of its inability to synthesize ABA in response to glucose.

Overall, the data currently available indicate that many of the sugar-sensing mutants are altered in their ability to produce or transduce ABA signals (Fig. 2). All these arabidopsis mutants were selected using high sugar concentrations (up to 333–388 mM glucose), and a possible glucose-mediated osmotic effect activating the ABA signalling pathway cannot be excluded, even if, as reported above, osmotic stress does not appear to be sufficient to explain the involvement of ABA in sugar tolerance in arabidopsis.

There appears to be a difference between sugar sensing in arabidopsis and that in cereals. Experimental evidence tends to exclude a role of ABA in sugar repression of  $\alpha$ -amylase in cereals. Glucose treatment (90 mM, a concentration able to trigger a significant repression of  $\alpha$ -amylase genes) results in a decreased ABA content in rice embryos (Toyofuku *et al.*, 2000), but not in barley embryos (Perata *et al.*, 1997; Toyofuku *et al.*, 2000). Interestingly, glucose represses the ABA-induced *Rab16A* gene in both rice and barley embryos (Toyofuku *et al.*, 2000), and the overall results indicate that glucose interferes with ABA signal transduction, while ABA does not mediate the effects of glucose. The promoter regions of the *Rab16A* (ABA-induced and sugar repressed) and *Ramy3D* (sugar repressed, but unaffected by ABA) genes share some homologies (discussed in Toyofuku *et al.*, 2000) suggesting that interaction between the two signalling pathways in cereals is possible; at present this hypothesis awaits confirmation.

## CONCLUSION

Plant cells can modulate their metabolic status by sensing sugars. A simplified view is that a high sugar concentration suggests a good metabolic status, while low sugar indicates possible imminent starvation. The reality is more complex: 'high' or 'low' sugar is not itself a good indicator of the real availability of sugars, since soluble carbohydrates have to be sensed in sub-cellular compartments and, ideally, rather than sensing the presence of sugars the plant should sense the 'flux' of sugars (e.g. the flux of sugars crossing the plasma membrane). We can hypothesize that sugars could be sensed at various levels, such as: (1) sugar concentration in the apoplast; (2) sugar flux crossing the plasma membrane; (3) intracellular sugar level; and (4) sugar flux into glycolysis. Furthermore, the possible sensing of flux from/to the vacuole or plastids cannot be ruled out. Depending on the specificity of sensors, the scenario becomes even more complex (see Fig. 3), since we can imagine distinct hexose and disaccharide sensors. In this context, HXK can be considered as a hexose sensor able to determine the flux of hexoses entering glycolysis, while sucrose transporter(s), possibly acting as disaccharide sensors, may sense the apoplastic sugar concentration and/

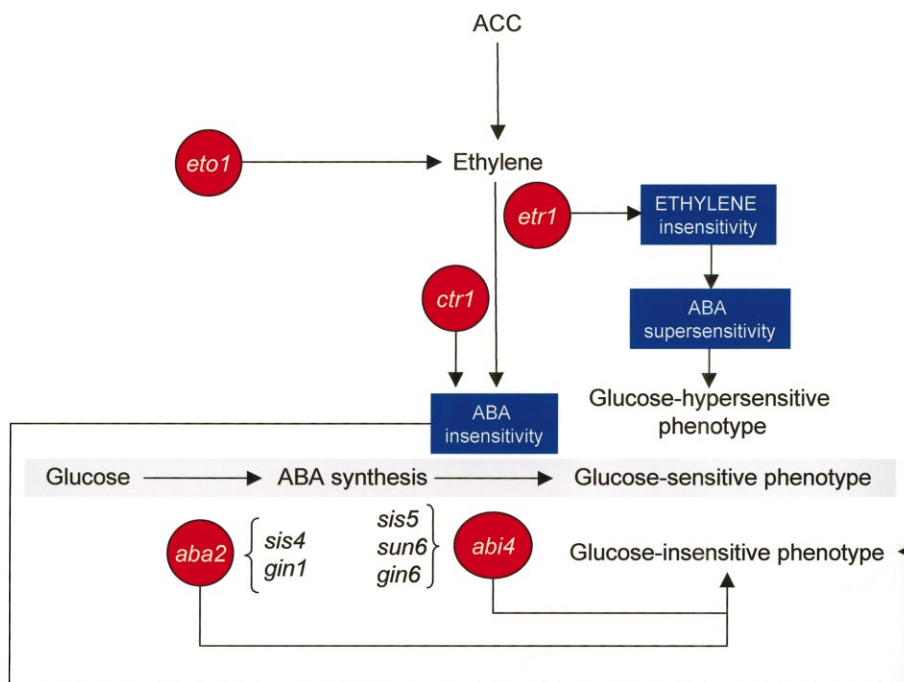


FIG. 2. Hypothetical model of sugar-hormones cross-talk in the transduction of sugar signals (see text for references). Glucose treatment induces ABA synthesis, and ABA affects the phenotype of germinating arabidopsis seedlings (glucose-sensitive phenotype). Supporting this scheme, the *aba2* (= *sis4*, *gin1*) mutant shows a glucose-insensitive phenotype in the presence of high glucose concentrations. Sensitivity to ABA also plays a role in sugar sensitivity, and the *abi4* (= *sis5*, *sun6*, *gin6*) mutant is indeed glucose-insensitive. 1-Aminocyclopropano-1-carboxylic acid (ACC) treatment confers a glucose-insensitive phenotype to glucose-treated arabidopsis seedlings, possibly because ethylene induces ABA-insensitivity in arabidopsis. This is also supported by the glucose-insensitivity of the *ctr1* and *eto1* mutant. Additionally, the *etr1* mutant is ethylene insensitive and, as a consequence, ABA supersensitive, resulting in a glucose-hypersensitive phenotype.

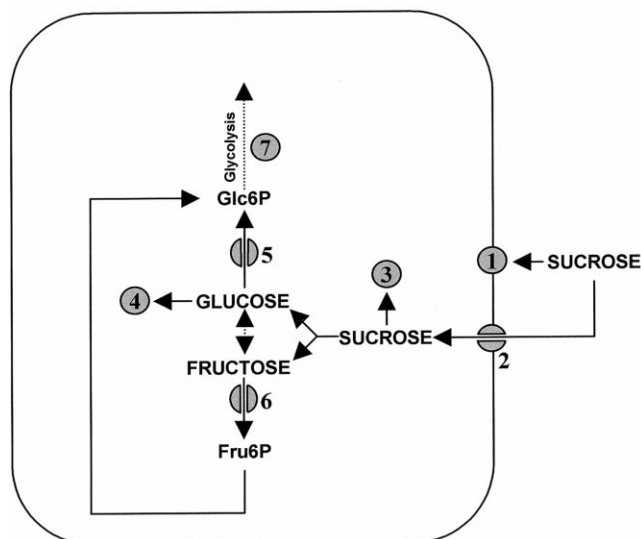


FIG. 3. Multiple sugar sensing in plants (see text for references). Sucrose in the apoplast is possibly sensed by a sucrose sensor (1) not acting as a transporter (sensing of apoplastic sucrose) or (2) by a sucrose transporter acting as a sensor (sensing of sucrose flux into the cell). Intracellular sucrose is sensed inside the cell (3), or after its breakdown into its constituent hexoses by a hexose sensor (4) distinct from HXK (intracellular hexose sensing). HXK acting as a hexose sensor (5) may sense the flux of sugars entering glycolysis, and the same may apply to a fructokinase sensor (6), while a glycolysis-dependent sensing pathway (7) senses the flux into metabolism.

or the flux of sugars crossing the plasma membrane (Lalonde *et al.*, 1999). Fructokinase may represent an additional sensor that bypasses HXK-phosphorylation, particularly if sucrose degradation occurs via sucrose synthase (Pego and Smeekens, 2000). The level of complexity depicted suggests that despite the successful description of some sugar sensing mechanisms in recent years, more efforts are needed to obtain a complete picture of sugar sensing in plants.

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