

Sugar effects on early seedling development in *Arabidopsis*

Sara Rognoni · Sheng Teng · Laura Arru ·
Sjef C. M. Smeeckens · Pierdomenico Perata

Received: 5 March 2007 / Accepted: 8 May 2007 / Published online: 16 June 2007
© Springer Science+Business Media B.V. 2007

Abstract Sugars affect a broad variety of processes, from growth and development to gene expression. Although it has already been shown that sugars act as signaling molecules, little is known about the mechanisms by which plants respond to them. Much progress has been made on understanding sugar sensing and signaling thanks to the analysis of mutants with abnormal sugar response. Some of the genetic strategies applied are based on the inhibitory effect of sugar on post-germinative development of *Arabidopsis thaliana*. High concentrations of exogenous sugars delay germination and arrest early growth, preventing seedlings from expanding cotyledons and developing true leaves and an extensive root system. The characterization of several *Arabidopsis*

mutants identified for their altered sugar sensitivity has disclosed a network in which sugars and plant hormones cooperate to control seedling development. Remarkably, many mutations turned out to be novel alleles of hormone-related genes, mainly ABA and ethylene. The aspects described above, emphasizing the connections between sugar and plant hormones revealed by mutants derived in seedling-based screens, are reviewed in this paper.

Keywords *Arabidopsis thaliana* · Seedling development · Hormones · Mutants · Sugar sensing

Introduction

Information concerning the sugar status of plant cells is of great importance during all stages of the plant life cycle as the availability or lack of sugars triggers many metabolic and developmental responses. A number of physiological, biochemical and molecular approaches have been used to study sugar responses in plants. Mutants are among these important tools, used to analyse the physiological function of complex signaling systems. Furthermore, mutants allow the study of functional interaction between genes. The presence of high concentrations of soluble sugars in the medium arrests seedling development, and screens taking advantage of this phenotype have been applied by many laboratories, leading to the selection of a relatively large number of mutants

S. Rognoni (✉) · L. Arru
Department of Agricultural Sciences, University
of Modena and Reggio Emilia, via Kennedy 17,
Reggio Emilia 42100, Italy
e-mail: rognoni.sara@unimore.it

S. Teng
National Center for Gene Research, Chinese Academy
of Sciences, 500 Cao Bao Road, Shanghai 200233,
P.R. China

S. C. M. Smeeckens
Molecular Plant Physiology, University of Utrecht,
Padualaan 8, Utrecht 3584CH, The Netherlands

P. Perata
Plant and Crop Physiology Laboratory, Scuola Superiore
Sant'Anna, Via Mariscoglio 34, Pisa 56124, Italy

either insensitive or over-sensitive to sugars in terms of arrest of the post-germinative development. However, the use of relatively high sugar concentrations has raised concerns, since this approach may lead to the selection of mutants resistant to osmotic stress rather than altered in their ability to sense sugars. Indeed, the identification of several sugar-insensitive mutants allelic to mutants affected in abscisic acid synthesis and signal transduction has reinforced this view. Nevertheless, careful analysis of several sugar sensing mutants reveals that the developmental arrest triggered by sugars is largely independent of the osmotic component arising from the treatment with exogenous sugars. Furthermore, recent studies also highlighted that the sugar sensing processes are uncoupled from the role of sugars as nutrients. In this review we describe the evidence supporting the role of sugars as signaling molecules altering the developmental program at the seedlings stage, integrated in a complex web of environmental, metabolic, and hormonal factors.

Germination and early seedling development

Germination encompasses those events beginning with the uptake of water by the quiescent dry seed and ending with the radicle emergence, and subsequent growth is generally defined as seedling development. After germination, a plant's success depends on its ability to grow autotrophically, and in this transition time plants live as heterotrophs until the photosynthetic apparatus becomes competent. During normal post-germinative growth of Arabidopsis seedlings the majority of storage reserves are mobilized in the 3–4 days after imbibition when the radicle and cotyledons are just emerging from the seed coat (Eastmond and Graham 2001). In oilseeds, such as Arabidopsis, after the onset of germination there is extensive conversion of the lipids, stored as triacylglycerides within oil bodies in the cotyledons, to soluble carbohydrate (Canvin and Beevers 1961). Unlike lipids, sucrose can be transported and then used to support growth and respiration (Bewley and Black 1985) before the seedlings develop the capacity to photosynthesize (reviewed in Baker et al. 2006).

Progression through the phases of increased metabolic activity and initiation of growth is strictly

regulated by both environmental and hormonal signals until seedlings become committed to growth. For example, light promotes seed germination in many species and its effects are thought to be mediated, at least in part, by a combination of increased synthesis and perception of gibberellins and decreased ABA levels (Bentsink and Koornneef 2002; Yang et al. 1995; Koornneef and Karssen 1994). On the other hand, germination is strongly inhibited by ABA and high solute concentrations or limited water availability (Bewley et al. 1985). Like germination, seedling establishment is a crucial period in which quick responses to a range of factors are essential for survival of the developing plant. It has been suggested that this period defines a checkpoint during which internal and external conditions are monitored (Lopez-Molina et al. 2001). Shortly after stratification (imbibition at low temperatures), already germinated Arabidopsis seeds are still able to prevent adult growth if external environmental circumstances suddenly become adverse (Dekkers et al. 2004; Lopez-Molina et al. 2001).

Plants post-embryonic growth and early development display a remarkable flexibility, which resides in the plant's capacity to integrate and respond to developmental, metabolic, and environmental signals. This plasticity is the result of a complex signaling network that relates plant hormones, physical factors, and nutrients, including carbohydrates. Sugars are indeed key players in many processes, as they are energy sources and structural components for the plant cell. Mechanisms by which plants determine the carbohydrate availability, namely sugar sensing, and regulate sugar responses (sugar signaling), are fundamental throughout plant life. Already many studies of the carbohydrate regulation systems in plants have shown that sugars themselves are often the signal molecules. Therefore, in addition to their metabolic role, sugars have now been recognized to also have a hormone-like function (reviewed in Rolland et al. 2006; Leon and Sheen 2003; Smeekens 2000).

Effects of sugars on early development

Glucose and other sugars have been shown to affect seed germination and early seedling development. In experimental conditions, the presence of high glucose concentration is sensed during germination and

results in seedling developmental arrest (Jang and Sheen 1997). Sugar effects on these processes are complicated because they appear to exert a positive effect in some assays but a negative one in others. Furthermore, it has been demonstrated that sugars influence these developmental processes via more than one pathway (reviewed in Rolland et al. 2006).

Germination of seeds on media containing low (30 mM) up to high (300 mM) concentration of sugars is not prevented but significantly delayed (Dekkers et al. 2004; Price et al. 2003; To et al. 2002; Ullah et al. 2002). By contrast, equimolar concentrations of sorbitol or mannitol do not exert a similar influence, indicating that the effects of sugar concentrations on germination rates are not solely due to osmotic stress (Dekkers et al. 2004; Price et al. 2003). This sugar-induced delay of germination appears to involve the hormone abscisic acid (ABA), since ABA deficient mutants show reduced sensitivity to glucose during germination (Dekkers et al. 2004). The decrease of ABA levels in seeds germinating on sugar-supplemented media is delayed as well, suggesting that glucose-induced germination delay is due to a slower rate of endogenous ABA removal (Price et al. 2003). However, this sugar effect is temporally restricted: glucose exposure must occur within the first two days from the beginning of germination to be effective (Dekkers and Smeekens 2007).

Given sufficient time, the majority of wild type *Arabidopsis* seeds germinate in the presence of high concentration of glucose or sucrose. However most of these seedlings fail to form expanded cotyledons, true leaves or extensive root systems (Gibson et al. 2001; Arenas-Huertero et al. 2000; Laby et al. 2000; Nemeth et al. 1998; Zhou et al. 1998; Jang et al. 1997). Although glucose concentrations lower than 110 mM promotes growth, seedlings germinated on higher glucose media are shorter and their hypocotyls length is inversely proportional to glucose concentration (Jang et al. 1997). In addition, sugar affects chlorophyll levels and expression of photosynthetic genes. Seedlings grown on such medium fail to develop chloroplasts (To et al. 2003). The etiolated cotyledons accumulate red/purple anthocyanins pigments (Baier et al. 2004; Nemeth et al. 1998; Mita et al. 1997), due to the induction of their biosynthetic pathway (Solfanelli et al. 2006). Moreover *Arabidopsis* seedlings grown on media containing exogenous sugars do not mobilize the majority of their seed

storage lipids (Martin et al. 2002; To et al. 2002). This developmental arrest imposed by sugars presumably signals a feedback regulation in the seed, which contains the source of carbon metabolites for seedling growth. Under natural conditions, adverse environmental factors may limit early seedling growth and sugar consumption after germination, resulting in sugar accumulation and developmental arrest (Cheng et al. 2002).

Screens based on sugar-induced arrest of early development

The finding that glucose and sucrose can act as negative regulators of seedling development has provided efficient screens for identifying mutants defective in sugar sensing or signaling. Similar genetic screens based on aberrant growth on ABA-containing media have permitted the isolation of mutants defective in ABA responses at germination or seedling growth (Lopez-Molina and Chua 2000; Koornneef et al. 1984; Koornneef et al. 1982). It is expected that mutants that are able to continue early development when grown on high concentration of sugars are likely to be affected in sugar perception.

By means of this easily detectable phenotype (Fig. 1), many mutants with altered sugar responses have been isolated and characterized by different groups (Table 1). Plants showing *glucose insensitive* (*gin*; Jang et al. 1997; Zhou et al. 1998) or *sugar insensitive* (*sis*; Gibson et al. 2001; Laby et al. 2000) phenotypes, as well as *glucose oversensitive* (*glo*; Rolland et al. 2002) or *sucrose super sensitive* (*sss*; Pego et al. 2000) phenotypes (Fig. 1), represent a valuable tool in unravelling sugar-response pathways. Among others, the *pleiotropic regulatory locus1*



Fig. 1 7 day old *Arabidopsis* seedlings grown on 330 mM glucose displaying wild type (on the left), *gin* (in the middle) and *glo* (on the right) phenotype

Table 1 Genetic screens based on developmental arrest induced by sugar (GLC, glucose; SUC, sucrose; TF, transcription factor; TUR, turanose)

	Screening conditions	References	Mutants	Gene
<i>gin</i> <i>Glucose Insensitive</i>	Seedling development on 330 mM GLC	Zhou et al. 1998; Arenas-Huertero et al. 2000; Laby et al. 2000; Moore et al. 2003	<i>gin1/aba2/sis4</i> <i>gin2</i> <i>gin4/sis1</i> <i>gin5/aba3</i> <i>gin6/sis5/abi4</i>	<i>ABA2</i> , SDR1/xanthoxin oxidase <i>AtHKK1</i> , hexokinase1 <i>CTR1</i> , RAF-like protein kinase <i>ABA3</i> , Mo-cofactor sulfurase <i>ABI4</i> , Apetala2 TF
<i>glo</i> <i>Glucose OverSensitive</i>	Seedling growth arrest on 220 mM GLC	Zhou et al. 1998; Rolland et al. 2002; Yaganisawa et al. 2003	<i>ein2</i> <i>ein3</i>	<i>EIN2</i> (ethylene insensitive) <i>EIN3</i> (ethylene insensitive), TF
<i>gss</i> <i>Glucose SuperSensitive</i>	Seedling growth arrest on 56 mM GLC	Pego et al. 2000		
<i>sig</i> <i>Sucrose Insensitive Growth</i>	Seedling development on 350 mM SUC	Pego et al. 2000		
<i>sis</i> <i>Sugar Insensitive</i>	Seedling development on 300 mM SUC	Laby et al. 2000; Gibson et al. 2001	<i>sis1/gin4</i> <i>sis4/aba2</i> <i>sis5/gin6/abi4</i>	<i>CTR1</i> , RAF-like protein kinase <i>ABA2</i> , SDR1/xanthoxin oxidase <i>ABI4</i> , Apetala2 TF
<i>sss</i> <i>Sucrose SuperSensitive</i>	Non-germination on 350 mM SUC	Pego et al. 2000		
<i>prl1</i> <i>Pleiotropic Regulatory Locus1</i>	Growth arrest on 175 mM GLC or SUC	Nemeth et al. 1998	<i>prl1</i>	<i>PRL1</i> , nuclear WD ^a protein
<i>tin</i> <i>Turanose Insensitive</i>	Seedling development on 90 mM TUR	Gonzali et al. 2005	<i>tin</i>	chimeric <i>WOX5</i> ^b (TIN)

^a WD, protein made up of highly conserved repeating units usually ending with Trp–Asp (Neer et al. 1994)

^b *WOX5*, *WUSCHEL*-related homeobox gene (Haecker et al. 2004)

(Nemeth et al. 1998) mutant displays enhanced sensitivity to sucrose and glucose; the mutation was shown to affect the *PRL1* gene, which encodes a WD-protein that potentially interacts with multiple signaling components, as supported by the observation that *prl1* responses to several plant hormones is changed (Nemeth et al. 1998). Another example is *gin2*, the null mutant of the glucose sensor *hexokinase1* (*AtHKK1*). Analysis of *gin2/hxk1* mutants has provided compelling evidence for a separate signaling function of *HKK1* independent of its metabolic activity (Moore et al. 2003). Recent findings have shown that *HKK1* forms a glucose signaling complex core with VHA-B1 and RPT5B that directly modulates transcription of target genes independently

from glucose metabolism (Cho et al. 2006). The *HKK*-dependent sugar signaling pathway was already known to be responsible for the regulation of several genes including photosynthetic genes, nitrate reductase, and others (Jang and Sheen 1997; Sheen et al. 1999). Sugars can also positively regulate the expression of more genes through *HKK*-independent pathways (Xiao et al. 2000; Roitsch 1999; Sheen et al. 1999; Koch 1996). Chen et al. (2003) identified a RGS-like (regulator of G-protein signaling) protein in Arabidopsis, as a critical modulator of plant cell proliferation (Chen and Jones 2004; Chen et al. 2003). Remarkably, *Atrgs1* mutants are less sensitive to high concentrations of glucose, while the germination of *AtRGS1*-overexpressors is hypersensitive to

glucose (Chen et al. 2006). It was suggested that *AtRGS1* is involved in the regulation of seedling development responses to sugar. Moreover, *AtRGS1* interacts with the α -subunit of the heterotrimeric G-protein GPA1 (Chen et al. 2003). *gpa1* mutants display hypersensitivity to ABA-mediated sugar effects (Ullah et al. 2002), consistent with the hypothesis that sugar operates *via* ABA. *AtRGS1* likely functions in a *HXK*-independent glucose signaling pathway since sugar metabolism and phosphorylation by *HXK* are not required for *AtRGS1*-mediated signaling, as suggested by experiments using different mono- and disaccharide sugars, or sugar analogues as well (Chen and Jones 2004).

The general term ‘sugar-response mutants’ refers to mutants that display sugar-induced changes in growth and development different from wild type, as well as to mutants with a modified sugar responsiveness at the level of gene expression (Rook and Bevan 2003). The latter include screens based on transgenic lines containing fusions of sugar-responsive promoters to reporter genes or selection markers. For instance, *sucrose uncoupled* mutants contain a luciferase reporter gene driven by the plastocyanin promoter (Dijkwel et al. 1997); the *impaired sucrose induction (isi)* mutants contain the *ApL3* promoter fused to a negative selection marker, the bacterial cytochrome *P₄₅₀* gene (Rook et al. 2001); the *reduced sucrose response (rsr)* mutants contain the potato patatin promoter combined with the β -glucuronidase gene (Martin et al. 1997). These distinct approaches based on the effects of high sugar media on seedling development or on changes in the activity/expression of sugar-responsive genes, yielded overlapping mutants.

Sugar-signaling affecting seedling development is independent of carbohydrates metabolism

Seedling developmental arrest by high sugar levels can be seen as a regression of metabolism from a state in which seed reserves are mobilized to support seedling establishment to a storage-dominated metabolic state (Gazzarrini and McCourt 2001; Rook et al. 2001). Although exogenous sucrose decreases the rate at which storage lipid is broken down in young seedlings (Eastmond et al. 2000), feeding sugars to young *Arabidopsis* seedlings has little impact on the

levels of expression of either fatty acid β -oxidation or glyoxylate cycle genes (Rylott et al. 2001; Hooks et al. 1999). Yet, the control of gene expression observed with non-metabolizable or partially metabolizable sugar analogues clearly suggests the involvement of specific signal sensing and transduction mechanisms that do not require sugar catabolism. Glycolytic intermediates downstream of glucose, including its immediate phosphorylated product glucose-6-phosphate, do not repress the expression of photosynthetic genes, as glucose does (Jang and Sheen 1997). On the other hand, the glucose epimer mannose and the glucose analog 2-deoxyglucose, which are phosphorylated by hexokinase but not metabolized in the glycolytic pathway, can both trigger the repression signal (Jang and Sheen 1994; Jang et al. 1997; Jang and Sheen 1997). The sugar’s dual function as a nutrient and a signaling molecule significantly complicates analysis of the mechanisms involved. To uncouple the two functions Moore et al. (2003) generated two *hxx1* mutants in which phosphorylation activity is abolished, but interestingly, these catalytically inactive HXK1 proteins still have a signaling function in repressing promoters of photosynthesis genes, similarly to the wild type HXK1 (Moore et al. 2003). Moreover, the transformation of *gin2-1/hxx1* transgenic plants with the two catalytically inactive *HXK1* alleles, enabled the glucose sensitivity phenotype of the transgenic seedlings to be rescued, rendering them susceptible again to a glucose-induced developmental block (Moore et al. 2003). Altogether these results indicate that downstream carbohydrate metabolism is dispensable.

Does the osmotic component play a role in the sugar-dependent seedling developmental arrest?

Genetic screens based on high sugar media have an obvious osmotic component that raises the question whether the observed developmental response is due to an indirect osmotic effect rather than a direct sugar signal. High concentrations in the medium may select mutants able to resist high concentration of any solutes in the medium, not exclusively sugars. Adequate osmotic controls are required to guarantee that mutants identified by high sugar screens are altered in the responsiveness to sugars and not to osmotic potentials in general. This is the case with

the *sis1*, *sis2*, *sis4*, and *sis5* mutants, which display osmo-tolerant phenotypes during early seedling development when grown on elevated sorbitol levels (Gibson 2000; Laby et al. 2000). Nonetheless, there are observations that suggest that the responses to sugars and to osmotic stress are mediated by distinct mechanisms.

Only high sugar levels, and not other osmotica, can stop seedling development (Leon and Sheen 2003; Arenas-Huertero et al. 2000; Zhou et al. 1998). The developmental differences between wild type plants and sugar-response mutants are not observed when they are grown in the presence of an osmoticum at the same concentration. Mannitol and sorbitol are reduced forms of glucose and are ordinarily employed as appropriate controls to assay for osmo-tolerance. They are not efficiently metabolized by plants, causing a constant osmotic stress.

Glucose effects on seedling growth and differentiation seem to be mediated, at least partly, by an increase in ABA biosynthesis (Leon and Sheen 2003; Cheng et al. 2002; Arenas-Huertero et al. 2000). Notwithstanding the well known role of ABA in salt and osmotic-stress responses (Finkelstein et al. 2002; Gazzarrini and McCourt 2001), glucose and stress signaling are distinct in their regulation by ABA. Many genes important for ABA biosynthesis in Arabidopsis, such as the *ABA1/LOS6* (zeaxanthin epoxidase, ZEP), *NCED3* (9-cis-epoxy-carotenoid dioxygenase), *AAO3* (Arabidopsis aldehyde oxidase-3), and *ABA3/LOS5* are induced by ABA (Cheng et al. 2002; Finkelstein and Rock 2002). Among these ABA biosynthesis genes, *ABA1/LOS6*, *AAO3*, and *ABA3/LOS5*, are also up-regulated by glucose. The *ABA2* transcript encodes the enzyme responsible for the conversion of xanthoxal to ABA-aldehyde in the major ABA biosynthesis pathway; however *ABA2* is not regulated by ABA, salt, or osmoticum but is induced by glucose (Cheng et al. 2002; Gonzalez-Guzman et al. 2002). By contrast, *NCED3* expression, which is highly activated by stress conditions and encodes a rate-limiting enzyme for ABA biosynthesis (Iuchi et al. 2001), is not increased by glucose, thus discriminating glucose signals from stress signals that activated *NCED3* (Cheng et al. 2002).

Glucose and osmotic stress signaling pathways involve overlapping but different mechanisms. Sugars control the post-germination growth via ABA-signaling transcription factors, *ABI3*, *ABI4*,

and *ABI5* (Leon and Sheen 2003; Cheng et al. 2002; Arenas-Huertero et al. 2000), whose transcripts respond to osmotica, ABA, and glucose. Cheng et al. (2002) demonstrated that *ABI3*, *ABI4*, and *ABI5* are dramatically induced by glucose, but not by mannitol. In particular *ABI4* and *ABI5* genes are also responsive to low concentration of 2-deoxyglucose, which is a non-metabolizable glucose analogue. 2-deoxyglucose is able to elicit the sugar signal, while exerting a negligible osmotic stress when compared to the generally higher levels of glucose used (Arroyo et al. 2003). Additionally, the glucose-regulation of *ABI4* and *ABI5* is altered in ABA mutants with wild type sugar response (Arroyo et al. 2003).

Finally, sugar signaling modulates additional plant hormone signaling, including those related to ethylene, auxin, and cytokinin, again separating it from the pathways involved in osmotic, salt, and drought stresses (Rolland et al. 2006; Cheng et al. 2002; Sheen et al. 1999; Zhou et al. 1998).

The role of abscisic acid in the sugar-dependent developmental arrest of seedling growth

Characterization of the sugar response mutants has revealed that many of them are also defective in phytohormone metabolism or response (Rolland et al. 2006; Gibson 2005; Leon and Sheen 2003; Finkelstein and Gibson 2002), disclosing a tight interplay between sugar and hormones pathways (Fig. 2). Among the phytohormones, ABA has been found to be of major importance in the response to sugars. In fact genetic screens based on continued seedling development on high sugar media have generated a large number of mutants allelic to known *ABA synthesis (aba)* genes. The central role for ABA in plant sugar signaling is demonstrated by the characterization of, for instance, *gin5*, which is allelic to *ABA3* (Rolland et al. 2002). The *ABA3* gene encodes a molybdenum cofactor (Mo–Co) sulfurase, necessary in the last step of ABA biosynthesis (Xiong et al. 2001). Furthermore, both *sis4* and *gin1* mutants are allelic to *aba2* (Cheng et al. 2002; Laby et al. 2000), which is deficient in a short-chain dehydrogenase/reductase (*SDR1*) that converts xanthoxin to abscisic aldehyde on the way to ABA (Gibson 2005; Cheng et al. 2002; Gonzalez-Guzman et al. 2002). Notably, the expression of several genes involved in ABA

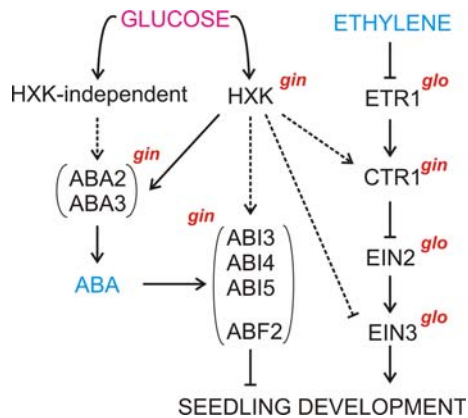


Fig. 2 Scheme of interactions between sugar and the hormones ABA and ethylene (adapted from Rolland et al. 2006); the sugar-responsive phenotype of the mutant plants is indicated in red above the name of the genes involved in these signaling cascades

biosynthesis is up-regulated by glucose, and in particular *ABA2* transcript levels are induced by glucose, but not by osmotic stress (Cheng et al. 2002; Gonzalez-Guzman et al. 2002).

Several mutant alleles of *ABA insensitive (abi)* genes have also been picked up in sugar-response screens. *gin6* and *sis5* are both alleles of *ABI4* (Arenas-Huertero et al. 2000; Laby et al. 2000), which encodes a transcription factor belonging to the *Apeta12* family (Finkelstein et al. 1998). *ABI4* takes part in the *HXK*-dependent signaling pathway that regulates photosynthetic genes, such as *chlorophyll a/b binding protein (CAB1)*, *small subunit of ribulose-1,5-bisphosphate carboxylase (RBSC)* and *plastocyanin (PC)* (Jang and Sheen 1994; Sheen et al. 1999). Moreover, expression of *ABI4* itself is induced by glucose, especially during a restricted time-window of seedling development (Arroyo et al. 2003; Cheng et al. 2002). Similarly, another transcription factor involved in ABA signaling, the leucine zipper *ABI5* is up-regulated by glucose (Arroyo et al. 2003; Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000). *ABI5* is also controlled by ABA, drought, and salt (Brocard-Gifford et al. 2003; Lopez-Molina et al. 2001), and is present only shortly after seed germination. During this interval it was proposed to have a key role in monitoring environmental conditions (Lopez-Molina et al. 2001). The role of *ABI5* in sugar signaling is supported by the sugar-insensitive phenotype of the *abi5* mutant (Arenas-Huertero et al.

2000; Brocard et al. 2002; Laby et al. 2000). One additional *abi* mutant involved in sugar signaling is *abi3*. *ABI3* is a transcriptional factor acting upstream of *ABI5* (Lopez-Molina et al. 2002) and is essential for late embryo maturation (McCourt 1999). *ABI3* expression is strongly induced by ABA and glucose, and some *ABI3* alleles seem to be implicated specifically in the pathway controlling sugar-induced seedling developmental arrest (Dekkers 2006; Nambara et al. 2002). However, results from a combination of loss-of-function alleles and double mutants between *ABI3*, *ABI4* and *ABI5* suggest that these three factors interact in complex ways to determine overall ABA seed sensitivity and downstream in the hexokinase mediated sugar signaling pathway (Soderman et al. 2000). Another member of the subset of ABA response loci affecting sugar signaling is *ABI8* (Brocard-Gifford et al. 2004). The *abi8* mutant needs glucose supplemented in the medium to grow and it is resistant to the inhibitory effects of high glucose concentrations, thus *ABI8* appears to be involved in glucose response (Brocard-Gifford et al. 2004). Genetic analyses place *ABI8* possibly in a separate pathway from that of *ABI* transcription factors in regulating ABA effects on sugar response; yet, *ABI8* acts downstream of *EIN2* and *EIN3* in the ethylene signaling pathway linked to the *HXK1*-mediated glucose response (Brocard-Gifford et al. 2004; Yanagisawa et al. 2003).

Not all of the *abi* mutants display abnormal responses to sugars, indicating the existence of multiple pathways for glucose and ABA signaling (Leon and Sheen 2003; Finkelstein and Rock 2002; Arenas-Huertero et al. 2000). Still, many mutants selected for their abnormal response to ABA reveal altered sugar sensitivity. Recently *ABF2* was added to the ABA-sugar web (Kim et al. 2004). The basic leucine zipper *ABF2* belongs to a small subfamily of *ABRE*-binding proteins named *ABFs* or *AREBs* (Choi et al. 2000; Uno et al. 2000) and is highly homologous to *ABI5* (Finkelstein and Lynch 2000; Lopez-Molina and Chua 2000). *ABF2*-overexpressing plants show glucose hypersensitivity and retarded growth in standard conditions. On the other hand, the *abf2* mutant seedlings grow faster than wild type seedlings and display reduced sensitivity to 165 mM glucose (Kim et al. 2004). For these reasons, *ABF2* appears to participate in the regulation of seedling growth and glucose responses (Kim et al. 2004).

It has been suggested that sugar responses are directly mediated by ABA, via the induction of its biosynthesis, and by the activation of some ABA signaling genes (Arenas-Huertero et al. 2000). This proposal arises from the observation that ABA biosynthesis mutants are insensitive to high glucose concentrations (Rook et al. 2001; Arenas-Huertero et al. 2000; Laby et al. 2000), as well as the observation that pre-treatment with the ABA synthesis inhibitor fluridone blocks the glucose inhibitory effects (Ullah et al. 2002). An alternative model, in which ABA modulates the response to the sugar signal through a separate ABA signaling pathway, has been proposed as well (Rook et al. 2001). In this view, seedling developmental arrest on high-sugar media is the consequence of an osmotically induced storage state, prompted by an increase in ABA levels as a result of osmotic effects. The glucose-dependent arrest of seedling growth is a complicated trait, and clearly ABA accumulation driven by glucose contributes to this phenotype.

Adding more complexity, glucose and ABA interactions vary depending on their concentrations. Low concentrations of glucose allow the germination of wild type seeds on otherwise inhibitory ABA concentrations (Finkelstein and Lynch 2000; Garcarrubio et al. 1997). These findings are in contrast with the inhibitory effect reported for elevated concentrations of sugars. It has been suggested that exogenous sugars allow germination by overcoming a nutritional deficiency caused by the inhibition of reserve mobilization by exogenous ABA (Garcarrubio et al. 1997). Though, the optimal glucose concentration for promotion of seed germination is too low to be consistent with a purely nutritional effect (Finkelstein and Lynch 2000). Although low concentrations of sugar or peptone can overcome exogenous ABA inhibition of radicle emergence up to high concentrations of ABA, subsequent seedling growth and greening remain blocked (Finkelstein and Lynch 2000; Garcarrubio et al. 1997). Characterization of the effects of exogenous glucose on the seed germination rates of different ABA-metabolic and ABA-response mutants has, however, suggested that glucose retards seed germination via a pathway that does not involve the ABA-response pathway components *ABI2*, *ABI4* and *ABI5* (Dekkers et al. 2004; Brocard-Gifford et al. 2003; Price et al. 2003).

Sugar responses cross talk with other hormonal signaling pathways

Ethylene

Ethylene clearly interacts with sugar signals in controlling seedling development. Some sugar-insensitive mutants have small and dark-green leaves, resembling the ethylene constitutive response mutant *ctr1* (Leon and Sheen 2003; Kieber et al. 1993). In fact, *sis1* and *gin4* have been shown to be mutant alleles of the *CONSTITUTIVE TRIPLE-RESPONSE1* gene, a negative regulator of ethylene signaling (Cheng et al. 2002; Gibson et al. 2001; Kieber et al. 1993). Further support came from the finding that *ethylene overproducer1* (*eto1*) and *ctr1* mutants can overcome the glucose-induced developmental arrest, whereas the ethylene-resistant *etr1* and ethylene-insensitive *ein2* and *ein3* mutants exhibit glucose hypersensitivity (glo phenotype; Yanagisawa et al. 2003; Zhou et al. 1998). Moreover, epistatic analysis of *gin1* puts the *GIN1/ABA2* gene in between the ethylene receptor *ETR1* and the glucose sensor *HXK1*, perhaps as a mediator of the opposite roles of glucose and ethylene in seedling development (Zhou et al. 1998). A molecular link between glucose and ethylene signaling is provided by the fact that glucose and ethylene antagonistically regulate protein stability of *EIN3*, a central transcriptional regulator in ethylene signaling, through the plant glucose sensor hexokinase (Yanagisawa et al. 2003; Moore et al. 2003; Solano et al. 1998; Chao et al. 1997).

Ethylene effects on seedling growth may, however, be explained by reports that ethylene is a negative regulator of ABA action (Beaudoin et al. 2000; Ghassemian et al. 2000). Based on the elevated ABA levels found in the *ein2* mutant, it is likely that ethylene signaling partially represses the biosynthesis of ABA (Leon and Sheen 2003; Cheng et al. 2002; Ghassemian et al. 2000), so promoting germination and seedling development. In addition, *CTR1* operates as a negative regulator blocking the ethylene cascade (Kieber et al. 1993), and its presence should allow the increase in ABA levels required during glucose response (Arenas-Huertero et al. 2000; Cheng et al. 2002), which again could result in a positive feedback loop of the glucose signaling. Further investigation of glucose and ethylene signaling might uncover separate branching pathways

responsible for downstream responses and elucidate interactions between ethylene and glucose, as well as other plant hormones.

Auxin, cytokinins and brassinosteroids

The *HXK1* glucose-signaling pathway interplays closely with the signaling pathways regulated by auxin and cytokinin (Moore et al. 2003). A defect in auxin-induced cell proliferation and root formation was observed in the *gin2/hxk1* mutants, even though no difference in endogenous auxin levels was detected between wild type and *gin2/hxk1* hypocotyls (Moore et al. 2003). The *gin2/hxk1* lack of auxin response, also when applied exogenously, points towards a deficiency in auxin signaling and/or uptake (Moore et al. 2003). In accordance with this observation, seedling growth of the auxin-resistant mutants *transport inhibitor response1* and *auxin resistant axr1* and *axr2* (Gray et al. 2001) is insensitive to growth inhibition by glucose (Rolland et al. 2006).

Additional evidence suggesting that sugar signaling is somehow connected to auxin response, comes from the identification of a *turanose insensitive mutant (tin; Gonzali et al. 2005)*. Turanose, a non-metabolizable sucrose analogue that profoundly reduces seedling growth, has been used to dissect the sucrose-signaling pathway in plants (Gonzali et al. 2005). Turanose insensitivity is associated with altered auxin homeostasis, as suggested by the analysis of the *tin* mutant (Gonzali et al. 2005).

Sugar and cytokinin response pathways also interact closely. For example, the *gin2* mutant shows delayed leaf senescence and hypersensitivity of hypocotyl explants to cytokinin mediated shoot induction (Moore et al. 2003). Cytokinin treatment also eliminates seedling developmental arrest induced by 330 mM glucose in MS medium (Zhou et al. 1998). Moreover, plants transformed with cytokinin histidine kinase (*CKII*) and the transcription factor *ARR2*, both involved in cytokinin signal transduction, can overcome the glucose repression response and are thus glucose insensitive (Moore et al. 2003; Hwang and Sheen 2001). However, the antagonistic relation between cytokinin and sugars might be a secondary effect of ethylene biosynthesis promoted by cytokinin, that antagonizes glucose signaling (Moore et al. 2003; Zhou et al. 1998). Further confirmation of sugar and cytokinin acting antagonistically arises

from the analysis of a cytokinin receptor mutant, *ahk3*, which shows cytokinin resistance and enhanced sucrose sensitivity (Franco-Zorrilla et al. 2005). Moreover, also the cytokinin-resistant *cnr1* mutant was found to be hypersensitive to sugars as well and, additionally, shows an altered auxin response (Laxmi et al. 2006).

Sugar-mediated pathways are also linked to brassinosteroids. The *brassinosteroid, light and sugar1* (Laxmi et al. 2004) mutant selected in a screen for photomorphogenic mutants is impaired in brassinosteroid responses as well as in sugar signaling (Kim et al. 2004; Laxmi et al. 2004). *bls1* sugar hypersensitivity is rescued by application of brassinosteroid and, likely, brassinosteroid deficiency results in impaired sugar responses. Moreover brassinosteroids response is related to several hormones, such as ABA and ethylene, that participate in sugar signaling, and that are probably responsible of the augmented sugar sensitivity observed in the *bls1* mutant.

Conclusions and future perspectives

Monitoring endogenous sugars levels is a vital regulatory function for coordinating nutrient metabolism and hormone synthesis and signaling. Genetic screens have been most useful in identifying genes implicated in different signal transduction pathways. Although designed for specific components involved in a single pathway, these screens have often selected mutations conferring changes in the sensitivity to more than one stimulus. This has been most notable in genetic screens for sugar-response mutants. The premise of these screens was to identify mutants able to germinate and grow on media containing sugar levels that normally inhibit growth and development. Many mutations recovered are novel alleles of hormone-related genes, mainly ABA and ethylene. Consistent with this, mutants isolated for their altered response to these hormones are often somehow affected in their sugar perception as well.

Rapid progress has been made in sugar sensing research taking physiological assays that are influenced by sugars to isolate sugar responsive mutants. As high sugar concentration are used, it is a pertinent to ask whether the developmental arrest can be attributed to an osmotic and/or metabolic effect. Several line of evidence point to separate, although

partially overlapping, pathways for sugar and osmoticum/ABA signals. Besides other data suggest that sugar sensing and signaling are uncoupled from the nutrient function of sugars.

Our understanding of sugar response has benefited from mutants isolated in seedling-based screens. However this strategy allow the identification of genes functional in sugar sensing and signaling only at this particular stage, while genes involved in sugar responses, but mainly expressed in mature plants, will be missed (Rook and Bevan 2003).

How sugars influence developmental responses and yet, at the same time, how they relate to many hormones regulating the same process is an important question. Hitherto, only a few of the mutants with increased or reduced susceptibility to sugar-induced early growth arrest were unique to sugar-response screens. This highlights the need for more unambiguous screening strategies that are able to discriminate between the primary sugar-signaling cascade and secondary pathways consequently activated. Better-designed mutant screens able to identify components more directly involved in sugar signaling will be most useful in unravelling sugar-mediated systems that control gene expression and plant development.

References

- Arenas-Huertero F, Arroyo A, Zhou L, Sheen J, Leon P (2000) Analysis of Arabidopsis glucose insensitive mutants, *gin5* and *gin6*, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugar. *Genes Dev* 14:2085–2096
- Arroyo A, Bossi F, Finkelstein RR, Leon P (2003) Three Genes that affect sugar sensing (*Abscisic Acid Insensitive 4*, *Abscisic Acid Insensitive 5*, and *Constitutive Triple Response 1*) are differentially regulated by glucose in Arabidopsis. *Plant Physiol* 133:231–242
- Baier M, Hemmann G, Holman R, Corke F, Card R, Smith C, Rook F, Bevan MW (2004) Characterization of mutants in Arabidopsis showing increased sugar-specific gene expression, growth, and developmental responses. *Plant Physiol* 134:81–91
- Baker A, Graham IA, Holdsworth M, Smith SM, Theodoulou FL (2006) Chewing the fat: [beta]-oxidation in signalling and development. *Trends Plant Sci* 11:124–132
- Beaudoin N, Serizet C, Gosti F, Giraudat J (2000) Interactions between abscisic acid and ethylene signaling cascades. *Plant Cell* 12:1103–1116
- Bentsink L, Koornneef M (2002) Seed dormancy and germination. In: Somerville CR, Meyerowitz EM (eds) *The Arabidopsis book*. Rockville, MD, USA
- Bewley JD, Black M (1985) *Seeds: physiology of development and germination*. New York, USA
- Brocard IM, Lynch TJ, Finkelstein RR (2002) Regulation and role of the Arabidopsis *Abscisic Acid-Insensitive 5* gene in abscisic acid, sugar, and stress response. *Plant Physiol* 129:1533–1543
- Brocard-Gifford I, Lynch TJ, Garcia ME, Malhotra B, Finkelstein RR (2004) The Arabidopsis thaliana ABSCISIC ACID-INSENSITIVE8 locus encodes a novel protein mediating abscisic acid and sugar responses essential for growth. *Plant Cell* 16:406–421
- Brocard-Gifford IM, Lynch TJ, Finkelstein RR (2003) Regulatory networks in seeds integrating developmental, abscisic acid, sugar, and light signaling. *Plant Physiol* 131:78–92
- Canvin DT, Beevers H (1961) Sucrose synthesis from acetate in the germinating castor bean: kinetics and Pathway. *J Biol Chem* 236:988–995
- Chao Q, Rothenberg M, Solano R, Roman G, Terzaghi W, Joseph R (1997) Activation of the ethylene gas response pathway in arabidopsis by the nuclear protein ETHYLENE-INSENSITIVE3 and related proteins. *Cell* 89:1133–1144
- Chen JG, Jones AM (2004) AtRGS1 function in Arabidopsis thaliana. In: David PS (ed) *Methods in enzymology regulators of g-protein signaling*. Part A. Academic Press
- Chen JG, Willard FS, Huang J, Liang J, Chasse SA, Jones AM, Siderovski DP (2003) A seven-transmembrane RGS protein that modulates plant cell proliferation. *Science* 301:1728–1731
- Chen Y, Ji F, Xie H, Liang J, Zhang J (2006) The regulator of G-Protein signaling proteins involved in sugar and abscisic acid signaling in arabidopsis seed germination. *Plant Physiol* 140:302–310
- Cheng WH, Endo A, Zhou L, Penney J, Chen HC, Arroyo A, Leon P, Nambara E, Asami T, Seo M, Koshiba T, Sheen J (2002) A unique short-chain dehydrogenase/reductase in arabidopsis glucose signaling and abscisic acid biosynthesis and functions. *Plant Cell* 14:2723–2743
- Cho YH, Yoo SD, Sheen J (2006) Regulatory functions of nuclear hexokinase1 complex in glucose signaling. *Cell* 127:579–589
- Choi Hi, Hong Jh, Ha Jo, Kang Jy, Kim SY (2000) ABFs, a family of ABA-responsive element binding factors. *J Biol Chem* 275:1723–1730
- Dekkers BJW (2006) Sugar signaling during germination and early seedling establishment in Arabidopsis. Ph.D. Dissertation, Utrecht University
- Dekkers BJW, Schuurmans AMJ, Smeekens SCM (2004) Glucose delays seed germination in *Arabidopsis thaliana*. *Planta* V218:579–588
- Dekkers BJW, Smeekens S (2007) Sugar and abscisic acid regulation of germination and transition to seedling growth. In: Bradford K, Nonogaki H (eds) *Seed development, dormancy and germination*. Blackwell Publishing, Oxford, UK
- Dijkwel PP, Huijser C, Weisbeek PJ, Chua NH, Smeekens SCM (1997) Sucrose control of phytochrome a signaling in arabidopsis. *Plant Cell* 9:583–595
- Eastmond PJ, Germain V, Lange PR, Bryce JH, Smith SM, Graham IA (2000) Postgerminative growth and lipid

- catabolism in oilseeds lacking the glyoxylate cycle. *PNAS* 97:5669–5674
- Eastmond PJ, Graham IA (2001) Re-examining the role of the glyoxylate cycle in oilseeds. *Trends Plant Sci* 6:72–78
- Finkelstein RR, Gibson SI (2002) ABA and sugar interactions regulating development: cross-talk or voices in a crowd? *Curr Opin Plant Biol* 5:26–32
- Finkelstein RR, Gampala SSL, Rock CD (2002) Abscisic acid signaling in seeds and seedlings. *Plant Cell* 14:S15–S45
- Finkelstein RR, Lynch TJ (2000) Abscisic acid inhibition of radicle emergence but not seedling growth is suppressed by sugars. *Plant Physiol* 122:1179–1186
- Finkelstein RR, Rock CD (2002) Abscisic acid biosynthesis and response. In: Somerville CR, Meyerowitz EM (eds) *The Arabidopsis book*. Rockville, MD, USA
- Finkelstein RR, Wang ML, Lynch TJ, Rao S, Goodman HM (1998) The Arabidopsis abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. *Plant Cell* 10:1043–1054
- Franco-Zorrilla JM, Martin AC, Leyva A, Paz-Ares J (2005) Interaction between phosphate-starvation, sugar, and cytokinin signaling in Arabidopsis and the roles of cytokinin receptors *CRE1/AHK4* and *AHK3*. *Plant Physiol* 138:847–857
- Garcarrubio A, Legaria JP, Covarrubias AA (1997) Abscisic acid inhibits germination of mature Arabidopsis seeds by limiting the availability of energy and nutrients. *Planta* 203:182–187
- Gazzarrini S, McCourt P (2001) Genetic interactions between ABA, ethylene and sugar signaling pathways. *Curr Opin Plant Biol* 4:387–391
- Ghassemian M, Nambara E, Cutler S, Kawaide H, Kamiya Y, McCourt P (2000) Regulation of abscisic acid signaling by the ethylene response pathway in Arabidopsis. *Plant Cell* 12:1117–1126
- Gibson SI (2000) Plant sugar-response pathways. Part of a complex regulatory web. *Plant Physiol* 124:1532–1539
- Gibson SI (2005) Control of plant development and gene expression by sugar signaling. *Curr Opin Plant Biol* 8:93–102
- Gibson SI, Laby RJ, Kim D (2001) The *sugar-insensitive1* (*sis1*) mutant of Arabidopsis is allelic to *ctr1*. *Biochem Biophys Res Commun* 280:196–203
- Gonzalez-Guzman M, Apostolova N, Belles JM, Barrero JM, Piqueras P, Ponce MR, Micol JL, Serrano R, Rodriguez PL (2002) The short-chain alcohol dehydrogenase *ABA2* catalyzes the conversion of xanthoxin to abscisic aldehyde. *Plant Cell* 14:1833–1846
- Gonzali S, Novi G, Loreti E, Paolicchi F, Poggi A, Alpi A, Perata P (2005) A turanose-insensitive mutant suggests a role for *WOX5* in auxin homeostasis in *Arabidopsis thaliana*. *Plant J* 44:633–645
- Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCFTIR1-dependent degradation of AUX/IAA proteins. *Nature* 414:271–276
- Haecker A, Gross-Hardt R, Geiges B, Sarkar A, Breuninger H, Herrmann M, Laux T (2004) Expression dynamics of *WOX* genes mark cell fate decisions during early embryonic patterning in Arabidopsis thaliana. *Development* 131:657–668
- Hooks MA, Kellas F, Graham IA (1999) Long-chain acyl-CoA oxidases of Arabidopsis. *Plant J* 20:1–13
- Hwang I, Sheen J (2001) Two-component circuitry in Arabidopsis cytokinin signal transduction. *Nature* 413:383–389
- Iuchi S, Kobayashi M, Taji T, Naramoto M, Seki M, Kato T, Tabata S, Kakubari Y, Yamaguchi-Shinozaki K, Shinozaki K (2001) Regulation of drought tolerance by gene manipulation of 9-cis-epoxycarotenoid dioxygenase, a key enzyme in abscisic acid biosynthesis in Arabidopsis. *Plant J* 27:325–333
- Jang JC, Leon P, Zhou L, Sheen J (1997) Hexokinase as a sugar sensor in higher plants. *Plant Cell* 9:5–19
- Jang JC, Sheen J (1994) Sugar sensing in higher plants. *Plant Cell* 6:1665–1679
- Jang JC, Sheen J (1997) Sugar sensing in higher plants. *Trends Plant Sci* 2:208–214
- Kieber JJ, Rothenberg M, Roman G, Feldmann KA, Ecker JR (1993) *CTR1*, a negative regulator of the ethylene response pathway in Arabidopsis, encodes a member of the Raf family of protein kinases. *Cell* 72:427–441
- Kim S, Kang JY, Cho DI, Park JH, Kim SY (2004) ABF2, an ABRE-binding bZIP factor, is an essential component of glucose signaling and its overexpression affects multiple stress tolerance. *Plant J* 40:75–87
- Koch KE (1996) Carbohydrate-modulated gene expression in plants. *Annu Rev Plant Physiol Plant Mol Biol* 47:509–540
- Koornneef M, Jorna ML, Brinkhorst-van der Swan LC, Karsen CM (1982) The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of Arabidopsis thaliana (L.) heynh. *TAG Theor Appl Genet* V61:385–393
- Koornneef M, Karssen CM (1994) Seed dormancy and germination. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, USA
- Koornneef M, Reuling G, Karssen CM (1984) The isolation and characterization of abscisic acid-insensitive mutants of Arabidopsis thaliana. *Physiol Plant* 61:377–383
- Laby RJ, Kincaid MS, Kim D, Gibson SI (2000) The Arabidopsis sugar-insensitive mutants *sis4* and *sis5* are defective in abscisic acid synthesis and response. *Plant J* 23:587–596
- Laxmi A, Paul LK, Peters JL, Khurana JP (2004) Arabidopsis constitutive photomorphogenic mutant, *bls1*, displays altered brassinosteroid response and sugar sensitivity. *Plant Mol Biol* V56:185–201
- Laxmi A, Paul LK, Raychaudhuri A, Peters JL, Khurana JP (2006) Arabidopsis cytokinin-resistant mutant, *cnr1*, displays altered auxin responses and sugar sensitivity. *Plant Mol Biol* V62:409–425
- Leon P, Sheen J (2003) Sugar and hormone connections. *Trends Plant Sci* 8:110–116
- Lopez-Molina L, Chua NH (2000) A null mutation in a bZIP factor confers ABA-insensitivity in *Arabidopsis thaliana*. *Plant Cell Physiol* 41:541–547
- Lopez-Molina L, Mongrand S, Chua NH (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the *ABI5* transcription factor in Arabidopsis. *PNAS* 98:4782–4787

- Lopez-Molina L, Mongrand S, McLachlin DT, Chait BT, Chua NH (2002) ABI5 acts downstream of ABI3 to execute an ABA-dependent growth arrest during germination. *Plant J* 32:317–328
- Martin T, Hellmann H, Schmidt R, Willmitzer L, Frommer WB (1997) Identification of mutants in metabolically regulated gene expression. *Plant J* 11:53–62
- Martin T, Oswald O, Graham IA (2002) Arabidopsis seedling growth, storage lipid mobilization, and photosynthetic gene expression are regulated by carbon:nitrogen availability. *Plant Physiol* 128:472–481
- McCourt P (1999) Genetic analysis of hormone signaling. *Annu Rev Plant Physiol Plant Mol Biol* 50:219–243
- Mita S, Murano N, Akaike M, Nakamura K (1997) Mutants of Arabidopsis thaliana with pleiotropic effects on the expression of the gene for beta-amylase and on the accumulation of anthocyanin that are inducible by sugars. *Plant J* 11:841–851
- Moore B, Zhou L, Rolland F, Hall Q, Cheng WH, Liu YX, Hwang I, Jones T, Sheen J (2003) Role of the Arabidopsis glucose sensor hxx1 in nutrient, light, and hormonal signaling. *Science* 300:332–336
- Nambara E, Suzuki M, Abrams S, McCarty DR, Kamiya Y, McCourt P (2002) A screen for genes that function in abscisic acid signaling in Arabidopsis thaliana. *Genetics* 161:1247–1255
- Neer EJ, Schmidt CJ, Nambudripad R, Smith TF (1994) The ancient regulatory-protein family of WD-repeat proteins. *Nature* 371:297–300
- Nemeth K, Salchert K, Putnoky P, Bhalerao R, Koncz-Kalman Z, Stankovic-Stangeland B, Bako L, Mathur J, Okresz L, Stabel S, Geigenberger P, Stitt M, Redei GP, Schell J, Koncz C (1998) Pleiotropic control of glucose and hormone responses by PRL1, a nuclear WD protein, in Arabidopsis. *Genes Dev* 12:3059–3073
- Pego JV, Kortstee AJ, Huijser C, Smeekens SCM (2000) Photosynthesis, sugars and the regulation of gene expression. *J Exp Bot* 51:407–416
- Price J, Li TC, Kang SG, Na JK, Jang JC (2003) Mechanisms of glucose signaling during germination of Arabidopsis. *Plant Physiol* 132:1424–1438
- Roitsch T (1999) Source-sink regulation by sugar and stress. *Curr Opin Plant Biol* 2:198–206
- Rolland F, Baena-Gonzalez E, Sheen J (2006) Sugar sensing and signaling in plants: conserved and novel mechanisms. *Annu Rev Plant Biol* 57:675–709
- Rolland F, Moore B, Sheen J (2002) Sugar sensing and signaling in plants. *Plant Cell* 14:S185–S205
- Rook F, Bevan MW (2003) Genetic approaches to understanding sugar-response pathways. *J Exp Bot* 54:495–501
- Rook F, Corke F, Card R, Munz G, Smith C, Bevan MW (2001) Impaired sucrose-induction mutants reveal the modulation of sugar-induced starch biosynthetic gene expression by abscisic acid signalling. *Plant J* 26:421–433
- Rylott EL, Hooks MA, Graham IA (2001) Co-ordinate regulation of genes involved in storage lipid mobilization in *Arabidopsis thaliana*. *Biochem Soc Trans* 29:283–287
- Sheen J, Zhou L, Jang JC (1999) Sugars as signaling molecules. *Curr Opin Plant Biol* 2:410–418
- Smeekens S (2000) Sugar-induced signal transduction in plants. *Annu Rev Plant Physiol Plant Mol Biol* 51:49–81
- Soderman EM, Brocard IM, Lynch TJ, Finkelstein RR (2000) Regulation and function of the Arabidopsis ABA-insensitive4 gene in seed and abscisic acid response signaling networks. *Plant Physiol* 124:1752–1765
- Solano R, Stepanova A, Chao Q, Ecker JR (1998) Nuclear events in ethylene signaling: a transcriptional cascade mediated by ETHYLENE-INSENSITIVE3 and ETHYLENE-RESPONSE-FACTOR1. *Genes Dev* 12:3703–3714
- Solfanelli C, Poggi A, Loreti E, Alpi A, Perata P (2006) Sucrose-specific induction of the anthocyanin biosynthetic pathway in Arabidopsis. *Plant Physiol* 140:637–646
- To J, Reiter WD, Gibson S (2002) Mobilization of seed storage lipid by Arabidopsis seedlings is retarded in the presence of exogenous sugars. *BMC Plant Biology* 2:4
- To JPC, Reiter WD, Gibson SI (2003) Chloroplast biogenesis by Arabidopsis seedlings is impaired in the presence of exogenous glucose. *Physiol Plant* 118:456–463
- Ullah H, Chen JG, Wang S, Jones AM (2002) Role of a heterotrimeric G Protein in regulation of Arabidopsis seed germination. *Plant Physiol* 129:897–907
- Uno Y, Furihata T, Abe H, Yoshida R, Shinozaki K, Yamaguchi-Shinozaki K (2000) Arabidopsis basic leucine zipper transcription factors involved in an abscisic acid-dependent signal transduction pathway under drought and high-salinity conditions. *PNAS* 97:11632–11637
- Xiao W, Sheen J, Jang J-C (2000) The role of hexokinase in plant sugar signal transduction and growth and development. *Plant Mol Biol* 44:451–461
- Xiong L, Ishitani M, Lee H, Zhu JK (2001) The Arabidopsis *LOS5/ABA3* locus encodes a molybdenum cofactor sulfurylase and modulates cold stress- and osmotic stress-responsive gene expression. *Plant Cell* 13:2063–2083
- Yanagisawa S, Yoo SD, Sheen J (2003) Differential regulation of EIN3 stability by glucose and ethylene signalling in plants. *Nature* 425:521–525
- Yang YY, Nagatani A, Zhao YJ, Kang BJ, Kendrick RE, Kamiya Y (1995) Effects of gibberellins on seed germination of phytochrome-deficient mutants of *Arabidopsis thaliana*. *Plant Cell Physiol* 36:1205–1211
- Zhou L, Jang JC, Jones TL, Sheen J (1998) Glucose and ethylene signal transduction crosstalk revealed by an Arabidopsis glucose-insensitive mutant. *PNAS* 95:10294–10299