Review

Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants

ANIL K GUPTA* and NARINDER KAUR

Department of Biochemistry and Chemistry, Punjab Agricultural University, Ludhiana 141 004, India

**Corresponding author (Fax, 91-161-2400945; Email, anilkgupta@satyam.net.in)*

Sucrose is required for plant growth and development. The sugar status of plant cells is sensed by sensor proteins. The signal generated by signal transduction cascades, which could involve mitogen-activated protein kinases, protein phosphatases, Ca^{2+} and calmodulins, results in appropriate gene expression. A variety of genes are either induced or repressed depending upon the status of soluble sugars. Abiotic stresses to plants result in major alterations in sugar status and hence affect the expression of various genes by down- and up-regulating their expression. Hexokinase-dependent and hexokinase-independent pathways are involved in sugar sensing. Sucrose also acts as a signal molecule as it affects the activity of a proton-sucrose symporter. The sucrose transporter acts as a sucrose sensor and is involved in phloem loading. Fructokinase may represent an additional sensor that bypasses hexokinase phosphorylation especially when sucrose synthase is dominant. Mutants isolated on the basis of response of germination and seedling growth to sugars and reporter-based screening protocols are being used to study the response of altered sugar status on gene expression. Common *cis*-acting elements in sugar signalling pathways have been identified. Transgenic plants with elevated levels of sugars/sugar alcohols like fructans, raffinose series oligosaccharides, trehalose and mannitol are tolerant to different stresses but have usually impaired growth. Efforts need to be made to have transgenic plants in which abiotic stress responsive genes are expressed only at the time of adverse environmental conditions instead of being constitutively synthesized.

[Gupta A K and Kaur N 2005 Sugar signalling and gene expression in relation to carbohydrate metabolism under abiotic stresses in plants; *J. Biosci.* **30** 761–776]

1. Introduction

Many environmental stresses like drought, cold and salinity lead to major alternations in carbohydrate metabolism (Hare *et al* 1998; Thomashow *et al* 1999; Wanner and Junttila 1999; Kaur *et al* 2000) and the sugar signalling pathways interact with stress pathways to modulate metabolism. Indirectly, the sugars play an important role during plant growth and development under abiotic stresses by regulating carbohydrate metabolism. A large number of stress responsive genes have been reported to be induced by glucose, indicating the role of sugars in environmental responses (Price *et al* 2004). The regulation of 31 genes corresponding to enzymes of carbohydrate metabolism in *Arabidopsis* under cold, drought and salt stresses (Seki *et al* 2002), differential regulation of carbohydrate content and enzymes of carbohydrate metabolism (Castrillo 1992; Pelleschi *et al* 1997) and role of sugars as signalling molecules in gene expression under abiotic stresses (Ho *et al* 2001) prompted us to write this review.

Sugars produced during photosynthesis are the substrates of carbon and energy metabolism and are used in the biosynthesis of polysaccharides like starch and cellu-

Keywords. Abiotic stresses; carbohydrate metabolism; gene expression; sugar sensing; sugar signalling.

Abbreviations used: CDPK, Calcium dependent protein kinase; 2-DG, 2-deoxy glucose; 6-DG, 6-deoxy glucose; GA, gibberellic acid; G-6-P, glucose-6-phosphate; HSF, heat shock transcription factor; HXK, hexokinase; 3-o-MG, 3-oxy methyl glucose; PC, plastocyanin; RFO, raffinose family oligosaccharides; SNF, sucrose nonfermenting; SPS, sucrose phosphate synthase; SRS, sugar response sequence; SuSy, sucrose synthase; TPS, trehalose-6-phosphate synthase.

lose in plants. The sugar status of plant cells is sensed by sensor proteins. Sugar sensing is the interaction between a sugar molecule and a sensor protein in such a way that a signal is generated. The signal then initiates signal transduction cascades that result in cellular responses such as altered gene expression and enzymatic activities. Sugars as signalling molecules affect the plants at all stages of growth starting from seed germination to seed development. Sugars, like hormones, can act as primary messengers and regulate signals that control the expression of various genes involved in sugar metabolism.

2. Sugar sensing and signalling

Two systems for hexose sensing have been suggested. One is hexokinase (HXK)-dependent and the other HXK-independent pathway. The HXK-dependent system requires the phosphorylation of sugars while the independent one senses sugars as such (Smeekens 2000). Sugars also act as regulatory signals that control the expression of various genes involved in many processes (Koch 1996; Jang and Sheen 1997; Smeekens 1998; Lalonde *et al* 1999; Roitsch 1999). Plants have both HXK-dependent and HXK-independent sugar signalling pathways. The evidences in the favour of HXK dependent signalling came from the observations that those sugar analogues that can be phosphorylated by HXK were able to trigger repression of photosynthetic genes (Jang and Sheen 1994). Secondly, further metabolism of sugar phosphates was not necessary to cause repression because 2-deoxy glucose (2-DG) and 2-deoxy mannose that cannot be metabolized after phosphorylation could also cause severe repression. These findings suggested that sugar signalling pathways donot overlap with downstream glucose metabolic pathways. The possibility of glucose being converted to other derivatives that could trigger repression without undergoing phosphorylation was also ruled out (Jang and Sheen 1994). Glucose-6-phosphate (G-6-P) was shown to act as repression signal (Brun *et al* 1993). Direct delivery of sugar phosphates into maize cells via electroporation did not trigger repression of photosynthetic genes (Jang and Sheen 1997). Based on the intracellular concentration of G-6-P which did not increase upon treatment with glucose, it was suggested that glucose is a direct signal. Furthermore, mannoheptulose, a competitive inhibitor of HXK blocked the severe repression caused by 2-DG. These observations indicated that HXK is the sensor in mediating the repression signal. It was further supported by the fact that 3-oxy-methyl-glucose (3-o-MG) could not relieve the repression caused by 2-DG because, 3-o-MG cannot be phosphorylated by HXK. The evidence for HXK-independent signalling pathways came from the observations that glucose analogue 6-deoxy glucose (6-DG), which can be transported across the plasma membrane but cannot be phosphorylated by HXK, activated the expression of genes encoding cell wall invertase (CIN), sucrose synthase (SuSy) and phenylalanine ammonia lyase (Roitsch *et al* 1995; Godt and Roitsch 1997; Ehness *et al* 1997) as shown in figure 1. Similarly 3-o-MG which cannot be phosphorylated but activated patatin class-1 promoter, thereby suggested the existence of HXK-independent sugar signalling pathways (Roitsch 1999).

Three glucose signal transduction pathways in plants have been suggested (Xiao *et al* 2000). These are AtHXK1-dependent pathway in which gene expression was correlated with the AtHXK1-mediated signalling function. Second was glycolysis-dependent pathway that was influenced by catalytic activity of both AtHXK1 and heterologus yeast HXK2. Third is the HXK-independent pathway in which gene expression is independent of AtHXK1. However, by using two independent knockout mutations for HXK1 isoform, it was concluded that G-6-P metabolism is uncoupled from HXK1-dependent signalling (Moore *et al* 2003). A mechanism for glucose sensing by HXK1, wherein a change in conformation by substrate binding initiates a signalling cascade, has been proposed (Harrington and Bush 2003). It still remains unclear if HXK senses glucose in a linear concentration dependent manner or it is flux sensor. Hexose sensing and signalling functions are, however, dependent on subcellular localization, translocation and interactions with down stream effectors of HXK (Rolland *et al* 2002). The possible sites for sugar signalling are shown in figure 2. Multiple glucose signal transduction pathways that control diverse genes and processes are intimately linked to developmental stages and environmental conditions (Xiao *et al* 2000).

A signalling function for sucrose was also suggested as it repressed m-RNA levels and transport activity of the proton-sucrose symporter (Barker *et al* 2000). The dual function of sugars as a nutrient and a signalling molecule complicates the analysis of mechanisms involved in signal transduction pathways (Rolland *et al* 2001).

3. Signal transduction cascades

Very little is known about the effect that sugars have on expression of genes involved in sugar signalling cascade. The sugar sensors feed information (sugar signalling) into signal transduction cascades that result in various types of plant responses. The signal transduction cascades involve mitogen-activated protein kinases, protein phosphatases, Ca^{2+} and calmodulin (Barker *et al* 2000). It has been reported that a putative sugar signalling component (AtSR2) that belongs to the SNF 1-related protein family is induced by sucrose, glucose and fructose (Chi-

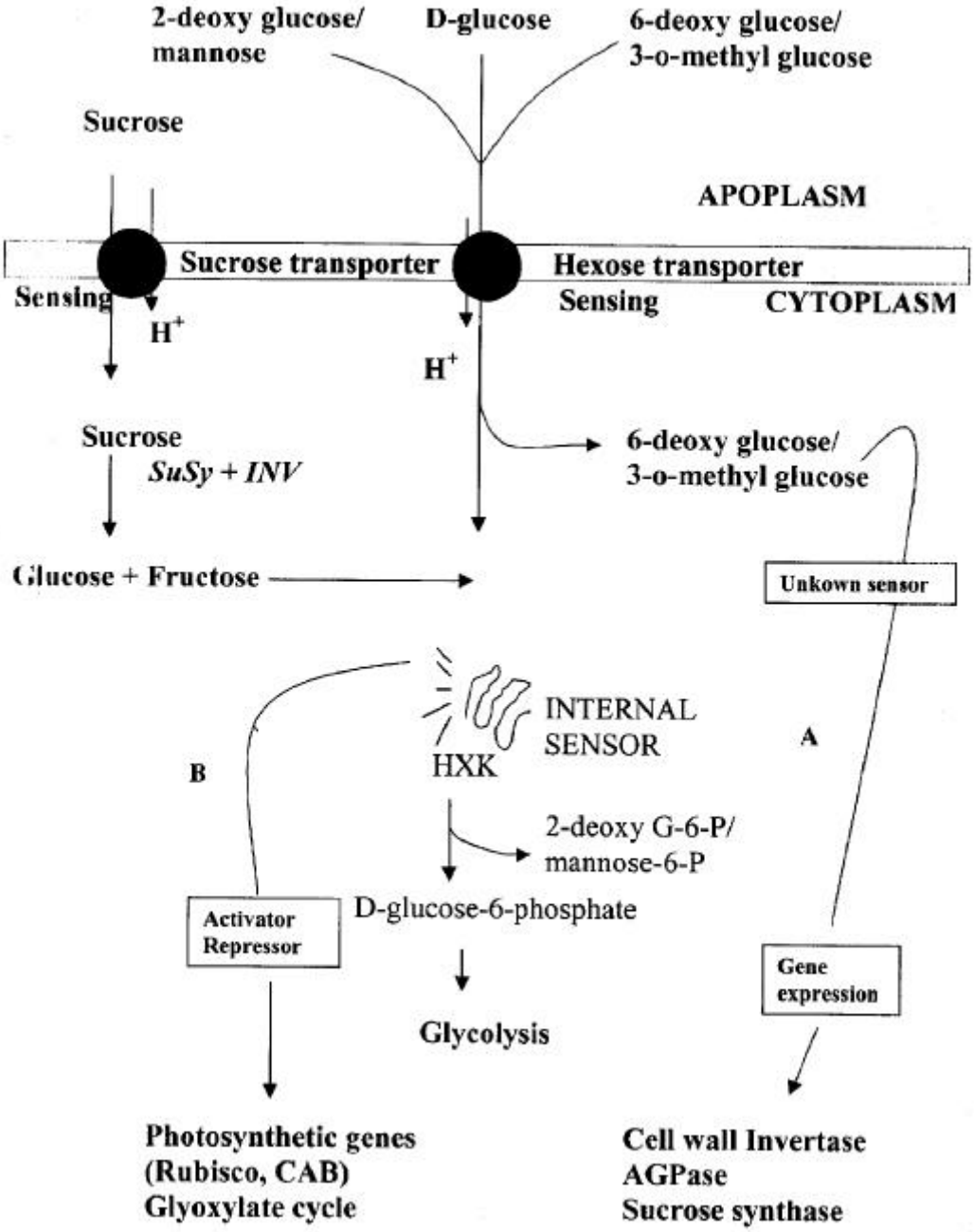

Figure 1. Sugar sensing and signal transduction pathways in higher plants based on Lalonde *et al* (1999) and Rolland *et al* (2002). A, hexokinase-independent pathway; B, hexokinase-dependent pathway, CAB, chlorophyll A binding protein, HXK, hexokinase; AGPase, ADP glucose pyrophosphorylase; SuSy, sucrose synthase; and INV, invertase.

kano *et al* 2001). Expression level of several genes involved in ABA biosynthesis and in the glucose post germination response is also modulated by glucose (Cheng *et al* 2002). During the characterization of gin 6 mutant, (abscisic acid insensitive 4) ABI4 transcript accumulated when plants were grown in the presence of glucose, suggesting a possible regulation of this gene by sugars (Arenas-Huertero *et al* 2000). ABI5 transcript also accumulates in response to stress (Arroyo *et al* 2003).

Sugar-mediated signalling in sweet potato and *Arabiodopsis* genes encoding *b*-amylase and the small unit of ADPglucose pyrophosphorylase (AGPase) has been demonstrated (Mita *et al* 1995; Ohto *et al* 1995). Specific inhibitors of protein-Ser/Thr phosphatases have been re-

ported to block the sugar induction of these genes in sweet potato as well as reporter gene expression in *b*-amylase *promotor-iudA (Amy-Gus)* fusion genes in tobacco. Inhibitor of Ser/Thr protein kinase, staurosporine inhibited sugar induction of the *Amy-Gus* gene in tobacco (Ohto and Nakamura 1995). Sugar-induced calcium-dependent (calmodulin domain) Ser/Thr protein kinase (CDPK), associated with the plasma membrane in leaf tissues of tobacco, has also been reported (Ohto and Nakamura 1995).

In *Chenopodium rubrum* cell culture system, source-specific *RUBISCO* gene was repressed by sugars whereas the sink specific *CIN* gene and the pathogen induced *PAL* gene were sugar inducted (Ehness *et al* 1997). These genes were found to be coordinately regulated by glucose in an HXK independent pathway. Four different protein phosphatase inhibitors were able to mimic the glucose-mediated regulation of these genes (Smeekens 2000). Thus protein dephosphorylation is involved in transducing the sugar signal. Sucrose nonfermenting (SNF) kinase in addition to coupling sugar perception to altered gene expression has also been found to control the activity of enzymes leading to changes in metabolism (Smeekens 2000). The phosphorylation of proteins by SNF-1-like and other kinases followed by binding of 14-3-3 proteins results in rapid adaptation of enzymatic activities and metabolic pathways to changing condition. A family consisting at least ten *14-3-3* genes has been cloned from *Arabidopsis* (Wu *et al* 1997).

A single enzyme can be a target for different protein kinases that regulate its activity in opposite direction e.g. sucrose phosphate synthase (SPS) can be both activated and repressed by a site-specific protein phosphorylation (Toroser and Huber 1997). Diurnal regulation of SPS is controlled by ser 158 phosphorylation through activity of SNF like kinase that leads to its inactivation. This control is overridden by stress induced activation via a calcium dependent protein kinase (CDPK) mediated Ser-424 phosphorylation (Toroser and Huber 1997). The SPS and SuSy enzymes can also be phosphorylated by CDPK

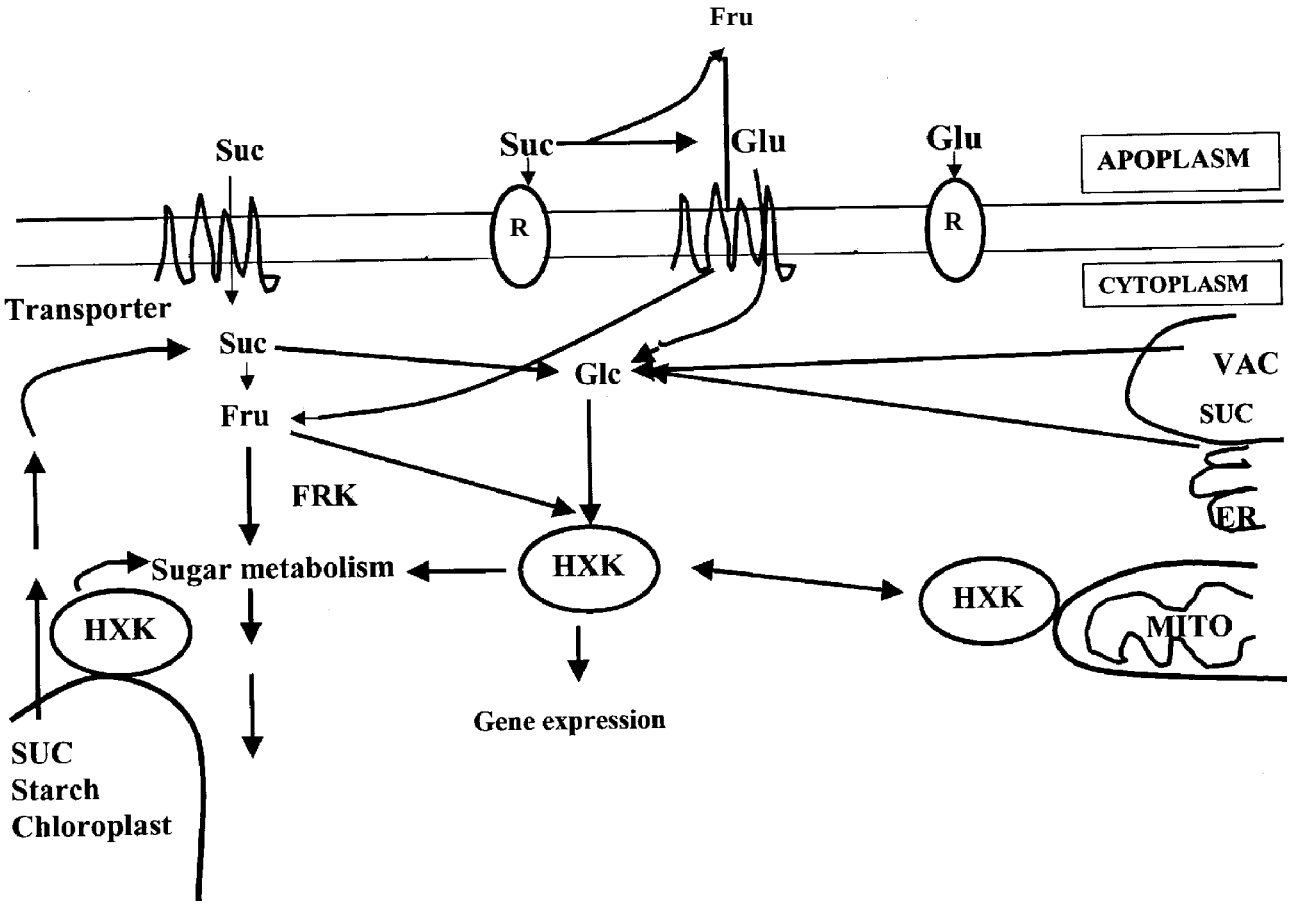

Figure 2. The possible sites for sugar signal sensing by hexokinase based on Rolland *et al* (2002). HXK, hexokinase; Glc, glucose; Suc, sucrose; Vac, vacuole; ER, endoplasmic reticulum; MITO, mitochondria; Fru, fructose; and FRK, fructokinase.

(Huber *et al* 1996). Phosphorylation of SuSy selectively activates the sucrose cleavage reaction (Huber *et al* 1996). Various studies have shown that transcriptional regulation is not the only response to sugars, the sucrose regulated *ATB2* gene is controlled at translation (Rook *et al* 1998) and modulating mRNA stability is a major control element for cereal *a*-amylase gene expression (Chan and Yu 1998a).

Translation of mRNA of *Arabidopsis* basic leucine zipper gene (ATB2) is repressed specifically by sucrose. Glucose and fructose individually and together were ineffective in this repression. The ATB2 mRNA has a complex leader containing small open reading frames. Deletion of this leader abolishes sucrose repression, which shows that a sucrose-specific signal controls translation (Rook *et al* 1998). Repression of mRNA levels and transport activity of proton-sucrose symporter by sucrose has been shown in excised sugar beet leaves (Chiou and Bush 1998).

Role of sugar sensing has been established during germination in rice. When glucose level exceeded the demand, *a*-amylase gene expression was down regulated by a process that involves sugar sensing. For *Ramy3D* gene it was found that HXK was most likely involved in transmitting the glucose signal. The HXK substrate 2-DG induced signalling was inhibited by HXK inhibitor glucosamine. 3-o-MG and 6-DG were not effective in signalling (Umemura *et al* 1998). *a*-amylase expression was inhibited in the barley seed germination by hexoses that are substrates for HXK but not by other hexoses (Perata *et al* 1997)

Sugars affect the expression of *a*-amylase genes in the presence of gibberellic acid (GA). Induction of *a*-amylase in the scutellum and aleurone layer of germinating barley seeds and de-embryonated cowpea cotyledons has been found to be repressed by sugars (Morita *et al* 1998; Kaur *et al* 2005). The sugar and GA responsive elements in the promoter of the *Ramy* gene appear to overlap, which indicate that the two signal transduction pathways communicate at a point upstream of the promoter elements (Morita *et al* 1998).

The role of sugars during different stages of seed development has been demonstrated on the expression of genes encoding sucrose metabolizing enzymes and hexose and sucrose transporters (Weber *et al* 1997; Tegeder *et al* 1999; Loreti *et al* 2001). However, sugars must act in concert with other factors and phytohormones. HXK, a hexose sensor, is able to determine the flux of hexoses entering glycolysis, while sucrose transporters act as disaccharide sensors and may sense the apoplastic sugar concentration and/or the flux of sugars crossing the plasma membrane (Lalonde *et al* 1999) as shown in figure 1. Fructokinase may represent an additional sensor that bypass HXK phosphorylation, particularly if sucrose degradation occurs via SuSy (Pego and Smeekens 2000).

4. Mutants for studying sugar sensing and signalling

Sugar response mutants have been isolated based on the effects caused by high or low sugar levels during germination or early seedling growth (Rolland *et al* 2002). Other mutants have been selected by screening transgenic plants with altered expression of sugar-inducible promoters. Reporter-based screening protocols in which promoters of sugar induced or sugar repressed genes are linked to reporters like *b*-glucuronidase (*GUS*) or luciferase (*LUC*) genes are being used for isolating relevant mutants. These constructs are introduced into plants and used as tools to select sugar-unresponsive and suger-hyperresponsive mutants. The plastocyanin (PC) gene of *Arabidopsis* can be repressed by sugars (Dijkwel *et al* 1996) and a seedling carrying a PC-promoter luciferase reporter gene construct is similarly repressed by sugars. Mutants defective in sucrose repression were identified on the basis of normal luminescence when grown on sucrose (Dijkwel *et al* 1997).

A similar strategy was used to select for mutants in sugar induction. The patatin class-1 (B33) promoter is induced by sugars and signalling mutants were selected by using transgenic *Arabidopsis* plants harbouring the Pat (B33)-indA constructs (Martin *et al* 1997). In this way, reduced sugar response (rsr) mutants were identified in which sucrose-induced expression of patatin is perturbed.

The *Arabidopsis b*-amylase gene is induced by sugars and mutants that display either an increased or a reduced sugar sensitivity have been isolated in amylase activity screens (Mita *et al* 1997a,b). A mutant showing elevated *b*-amylase expression (hba1, high level *b*-amylase) and low level *b*-amylase (lba) independent of the presence of sugars in the medium was isolated (Mita *et al* 1997b). Mutant seedlings that develop more or less normally in the presence of 6% glucose have been isolated and named glucose insensitive (gin) mutants (Zhou *et al* 1998).

5. Sugars and regulation of carbohydrate metabolism under environmental stresses

Sugar sensing and signalling are involved in the control of growth and development during the entire plant life cycle starting from germination (Gazzarrini and McCourt 2001; Eastmond and Graham 2001). High sugar accumulation during early seedling development may reflect undesirable growth conditions at a crucial developmental period (Lopez-Molina *et al* 2001) resulting in reversible developmental arrest that acts as a protection mechanism. Sugars regulate growth activities by modulation of gene expression and enzymes activities in both carbohydrate exporting (source) and importing (sink) tissues. This ensures optimal synthesis and use of carbon and energy

resources (Stitt and Krappe 1999; Coruzzi and Bush 2001). In general low sugar status enhances photosynthesis, reserve mobilization and export, whereas the abundant presence of sugars promotes growth and carbohydrate storage (Koch 1996) as shown in figure 3. Accumulation of sugars in source tissues downregulates photosynthesis thus maintaining homeostasis. The differential source sink effects allow the adaptation of carbon metabolism to changing environmental conditions and to the availability of other nutrients (Rolland *et al* 2002). The effect of abiotic stresses on metabolic events in source and sink tissues has been summarised in figure 3.

Independent glucose and disaccharide sensing processes modulating α-amylase in barley embryos have been reported (Loreti *et al* 2000). Fructose moiety in the non-metabolizable disaccharide like palatinose, turanose and fluoro sucrose plays an important role in modulating the expression of α-amylase (Loreti *et al* 2000). Sucrose derivatives though had no effect on *RbcS* expression but resulted in transient induction of extra cellular invertase; and transient activation of MAP kinases (Sinha *et al* 2002). In contrast the metabolisable sugars resulted in repression of *RbcS*; induction of extracellular invertase; and failed to activate MAP kinase activity. The differential effects were also reported in transcript stability of α-amylase (Loreti *et al* 2000). The existence of an extracellular sugar-sensing mechanism has been proposed, since palantinose, which is not taken up by the cells, exerts the same response in potato tubers as sucrose. Application of palatinose to discs of potatoes increased the invertase activity which resulted in a shift in favour of starch synthesis (Fernie *et al* 2001).

Down-regulation of photosynthetic enzymes by changes in metabolite pool size has been suggested (Foyer 1988). A model for sugar repression of photosynthetic gene transcription in higher plants is represented in figure 4. Increased soluble acid invertase activity on dehydration has been reported in maize leaves (Pelleschi *et al* 1997). It was correlated with higher hexose content. Increased invertase activity was also reported in mature bean leaves (Castrillo 1992) and in pigeonpea (Keller and Ludlow 1993). The observed changes in source leaf in response to water deficit differed from those in sink organs. Cell wall invertase activity was inhibited under water stress in maize ovaries (Zinselmeier *et al* 1995). The upregulation of extracellular invertase was suggested to be a common response to various biotic and abiotic stress related stimuli like pathogen infection and salt stress (Roitsch *et al* 2003). A marked accumulation of hexoses was correlated with an increase of vacuolar invertase activity in mature maize leaves under drought but it did not affect the cell wall invertase activity (Pelleschi *et al* 1999). In vegetative sink and source organs of water stressed maize plants, organ specific induction of acid invertase was correlated with an increase in *Ivr2* gene transcripts and in the vacuolar invertase proteins (Kim *et al* 2000; Roitsch *et al* 2003).

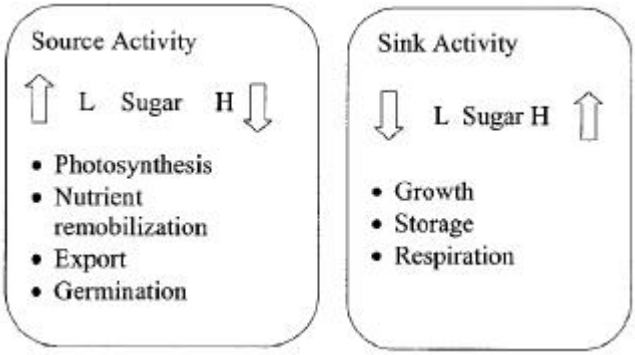

Figure 3. Differential effects of source-sink activities depending upon the availability of nutrients based on available information in literature. ⇧, induction; ⇩, repression; L, low sugar content; and H, high sugar content.

Another important component of carbohydrate metabolism in source leaves is SPS. A decrease in SPS activity in leaves of plants subjected to drought/mild water stress has been reported (Castrillo 1992; Pelleschi *et al* 1997).

The changes in enzyme activities resulted in increased sucrose content and accumulation of soluble carbohydrates which could be due to decreased export or reduced demand of sink organs under stress (Daie 1988; Gupta *et al* 1993a,b; Kaur *et al* 1998). The signal for these changes in metabolism could be induced by alterations in the balance between source and sink organs. An artificial increase in leaf carbohydrate content modified the gene expression for enzymes of photosynthetic metabolism (Krapp and Stitt 1995). Simple sugars, such as sucrose and glucose, are efficient modulators of gene expression (Koch *et al* 1992). Sugar-regulated genes have been identified and the functions of the encoded polypeptides range from an involvement in plant metabolism to light perception and cell cycle control (Jang and Sheen 1997; Lalonde *et al* 1999). In many plants, the genes for sucrose synthase and invertase are subjected to sugar regulation (Ehness *et al* 1997). Some genes required for carbon metabolism are regulated by glucose repression (Jang and Sheen 1997; Smeekens and Rook 1997). The plant cells have independent sensors for sucrose and glucose. Cells sense changes in the ratio between sucrose and glucose and feed this information into markedly different signal transduction pathways.

6. Effect of sugars on gene expression

No common or conserved *cis*-acting element for the sugar-regulated expression has been reported in the pro-

moters of sugar-regulated plant genes (Lu *et al* 1998; Kim and Guiltinan 1999; Sheen 1999). However, genes under the common sugar signalling pathways may share common *cis*-acting elements to which a transcription factor binds coordinately. Kim and Guiltinan (1999) have reported that a subset of sugar-regulated genes share conserved motifs in their promoter.

Expression of genes for sporamin (*spo*) and *b*-amylase (*b-Amy*) is inducible in vegetative tissues such as stem, leaf and petiole by high levels of sucrose or other metabolizable sugars (Nakamura *et al* 1991; Hattori *et al* 1991). Sugar inducible expression of these genes responds similarly to various inhibitors of the signal transduction components (Ohto *et al* 1995). These results suggest that expression of *b-Amy* and *spo* is regulated by similar mechanisms. Analysis of the expression of a fusion gene, which consisted of the 5 upstream sequence of the gene for *b*-amylase and in the light is regulated at the level of transcription. Analysis of a short-inducible promoter derived from *b-Amy* indicated that TGGACGG element plays an important role in sugar inducible expression of both of the truncated promoters of *spo* and *b-Amy* (Maeo *et al* 2001).

The activation of gene expression by sugars has been studied with the promoters of genes encoding patatin, *b*-amylase and vegetative storage protein (Ishiguro and Nakamura 1994; Sadaka *et al* 1994; Martin *et al* 1997). Both positive and negative *cis*-elements were found. A conserved sucrose responsive element (SURE) and its binding factor have been identified (Ishiguro and Nakamura 1994). Glucose repression of rice *a*-amylase gene promoters has revealed multiple *cis*-elements important for sugar-regulated gene expression (Chan and Yu 1998b; Hwang *et al* 1998; Lu *et al* 1998; Morita *et al* 1998). Hexokinase is proposed to be the sensor mediating the sugar repression of *a*-amylase gene (Umemura *et al* 1998). In the promoter of a rice *a*-amylase gene *aAmy3*, major sugar response sequence (SRS) was located between 186 and 82 base pairs upstream of transcriptional site (Lu *et al* 1998). The SRS converted a sugar-insensitive rice actin gene promoter into a sugar-sensitive promoter in a dose-dependent manner. Three essential motifs: namely the GC box, G Box and TATCCA element within the SRS have been identified. Sequences containing either the GC box plus G box, or the TATCCA element, each mediated sugar response. The TATCCA element is also an important component of gibberellin response complex of the *a*-amylase gene in germinating cereal grains, suggesting that regulation of *a*-amylase gene expression by sugar and hormonal signal may share common regulatory mechanism (Lu *et al* 1998).

Specific regulatory elements involved in glucose repression have also been identified in the promoters of bean *RBCS2* (Urwin and Jenkins 1997) and cucumber malate genes (Sarah *et al* 1996). Sucrose-specific induction of gene expression has been reported for patatin promoter and phloem-specific rolC promoter (Jefferson *et al* 1990; Kim *et al* 1994; Yokohama *et al* 1997). Glu-

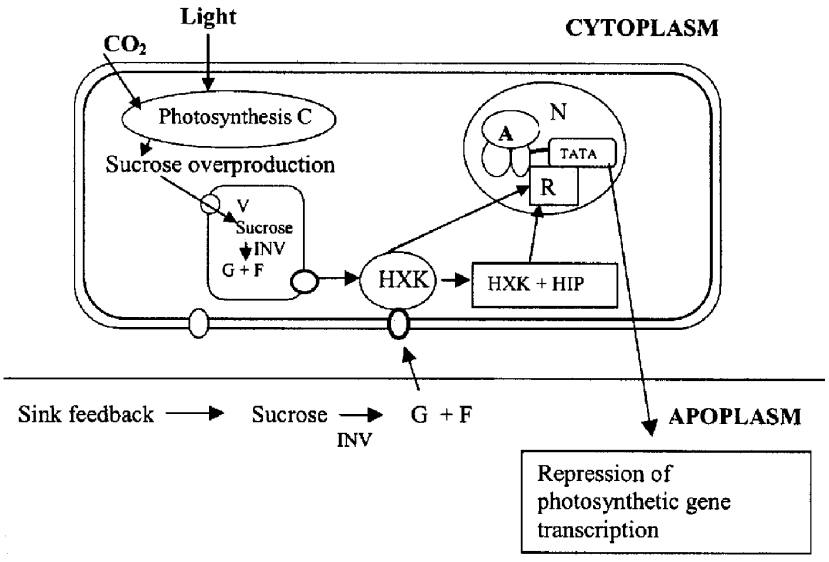

Figure 4. Model showing repression of photosynthetic gene transcription by sugars in higher plants based on Smeekens (1998), Sturm and Jang (1999), Barker *et al* (2000) and Rolland *et al* (2002). A, transcriptional activator; C, chloroplast; N, nucleus; V, vacuole; INV, invertase, R, transcriptional repressor; HXK, hexokinase; HIP, hexokinase interacting protein; G, glucose; and F, fructose.

cose analogue 3-o-MG is an effective inducer of the patatin promoter (Martin *et al* 1997). Sucrose responsive elements are found in patatin class promoter (Kim *et al* 1994). Sucrose-responsive sequences are also found in several sucrose-inducible sucrose synthase genes (Fu *et al* 1995). A gene coding for a DNA-binding protein, which recognizes the SP8 motif in sweet potato sporamin and potato *b*-amylase gene promoter, SPF1, has been cloned. It encodes a negative regulator that is not transcribed in presence of sucrose (Ishiguro and Nakamura 1994).

The core sequence of WRKY-binding element (W-box) is found in promoters of wheat, barley and oat *a-Amy2* gene (Rushton *et al* 1995). W-boxes are also found in many *Arabidopsis* genes involved in plant defense (Du and Chen 2000; Maleck *et al* 2000). G-box motif (CACGTC) is found on several sugar-regulated promoters and is involved in the transcriptional control of phytochrome-mediated control of gene expression (Martinez-Garcia *et al* 2000). B-box motif is similar to the CCACGTGG ABA-responsive element (Pla *et al* 1993). *b*-amylase transcript is induced by ABA (Ohto *et al* 1992) and induction of *b*-phaseolin promoter by exogenous ABA in tobacco embryo is modulated by external sucrose (Bustos *et al* 1998). It appears that sugar signalling may converge in the transcriptional control of W- and G-boxes in diverse promoters (Rolland *et al* 2002).

A computer analysis of the promoter of grape VvHT1 (Hexatransporter 1) revealed the presence of several sugar boxes. Glucose and sucrose doubled the *b*-glucuronidase activity conferred by the VvHT1 promoter whereas fructose has no effect. Possibly VvHT1 promoter activity may be stimulated by two independent signalling i.e. hexose as well sucrose pathway (Atanssova *et al* 2003).

Sugars can also regulate the gene expression by affecting the mRNA stability through specific 3′ untranslated sequences (Chan and Yu 1998a). Sugars can enhance the stability of transcripts encoding rice alcohol dehydrogenase and glyceraldehyde-3-phosphate dehydrogenase (Ho *et al* 2001).

A proton-coupled sucrose symporter mediates phloem loading. Antisense expression of the *SUT1* in transgenic plants inhibits assimilate partitioning leading to elevated levels of soluble carbohydrates and starch in their leaves, reduced sucrose transport activity and impaired growth (Riesmeier *et al* 1994; Kühn *et al* 1996). Sucrose symporter is regulated by changing levels of sucrose in the leaf and sucrose dependent transduction pathway is an important regulatory step in resource allocation (Chiou and Bush 1998). Sucrose transporters are the key proteins in carbon partitioning and are localized at strategic position, thus being an important target for regulation. Sucrose transport and metabolism are highly regulated at the transcriptional and post transcriptional levels (Kühn *et al* 1999). A correlative evidence for the role of sucrose transporters in sucrose accumulation and starch biosynthesis in barley endosperum has been proposed (Weschke *et al* 2000).

SUT genes have been identified in number of plants (Smeekens 1998). *SUT* genes encode highly hydrophobic proteins. They consist of 12 membrane-spanning domains and are distantly related to hexose transporter family found in yeast and plants (Ward *et al* 1998; Rentsch *et al* 1998). Studies conducted in tobacco, potato and tomato by using immunolocalization techniques have shown that sucrose transporter (SUT1) is involved in phloem loading and are present in plasma membrane of sieve elements (Kühn *et al* 1997). Sucrose controls the expression of number of genes. Examples include an *Arabidopsis* zipper gene, *ATB2*, *RolC* promoter from *Agrobacterium* and *SUT1* (Yokohamo *et al* 1997; Chiou and Bush 1998). Sucrose obviously has two functions one as a source of carbon and other affecting expression of some important genes.

7. Fructans in stress tolerance

Fructans are polyfructose molecules that are produced by many plants and bacteria and furthermore these may play a role in adaptation to osmotic stress due to their high soluble nature (Gupta and Kaur 2000). Meer *et al* (1994) modified non-fructan storing potato plants by introducing the microbial fructosyl transferase gene. Constructs were created in which fructosyl transferase gene of *Bacillus subtilis* (*SacB*) was fused to vacuolar targetting sequence of yeast carboxypeptidase Y (*cpy*) gene. These constructs were placed under the control of the constitutive cauliflower mosaic virus 35S promoter and introduced into potato tissue. The regenerated potato plants accumulated fructans (Meer *et al* 1994). Drought treatment resulted in 33% more fresh weight in transformed fructan-accumulating plants than in the control plants (Pilon-Smits *et al* 1995).

A cDNA from *Helianthus tuberosus* which encodes 1-sucrose: sucrose fructosyl transferase (1-SST) was cloned into plasmid pPG5 harbouring the bialaphos resistance gene (*pat*). The resulting plasmid pSST507 containing both *1-sst* and *pat* sequences was used to transform stomatal guard cell protoplast of sugarbeet. The plants regenerated from transformed protoplasts stored low molecular weight fructans in their tap roots (Sevenier *et al* 1998). Such fructan-storing transgenic sugar beet plants performed better compared to control plants during dehydration stress (Pilon-Smits *et al* 1999). The molecular mechanisms behind how fructans protect against abiotic stresses are unclear. However, on the basis of some *in vitro* experiments, it was proposed that fructans could

protect lipid bilayers from stress by virtue of the ability of fructans to interact with phospholipids (Damel *et al* 1998). Fructans are also inserted in bilayers and thereby protect the lipid bilayers from undergoing phase transition (Vereyken *et al* 2001). It appears that during cold stress, phase transition of lipids is responsible for most of the damage caused from increased membrane permeability. Further it was shown that lipid bilayer vesicles became less leaky upon freeze-drying treatment in presence of fructans (Hincha *et al* 2000).

8. Trehalose in stress tolerance

Trehalose, a non-reducing disaccharide of glucose, is known as a reserve metabolite in yeast and fungi. Trehalose has been shown to stabilize proteins and membrane lipids. Tobacco plants were transformed with the gene for trehalose-6-phosphate synthase (TPSI) driven by the promoter of the *rbcs* gene from *Arabidopsis*. The TPS1 positive plants accumulated trehalose and exhibited an improved drought tolerance (Holmstrom *et al* 1996). When tobacco was transformed with *otsA* and *otsB* genes from *Escherichia coli* which encode trehalose-6-phosphate synthase and trehalose-6-phosphate phosphatase, the leaves of the transgenic plants had a better photosynthetic efficiency and a higher dry weight accumulation under drought stress than controls (Pilon-Smits *et al* 1998). Expression of the yeast gene for TPS1 in tobacco, driven by the drought-inducible promoter of *RD29* and by 35S promotor of CaMV in potato led to improved tolerance in these transgenic plants (Yeo *et al* 2000; Zhao *et al* 2000).

The regulated overexpression of *E. coli* trehalose biosynthetic genes (*otsA* and *otsB*) as a fusion gene for manipulating stress tolerance in rice has been reported (Garg *et al* 2002). The fusion gene has the advantage of necessitating only a single transformation event and a higher net catalytic efficiency for trehalose formation (Garg *et al* 2002).

9. Raffinose oligosaccharides in stress tolerance

Raffinose family oligosaccharides (RFO) are α-galactosyl derivatives of sucrose. These are raffinose, stachyose and verbascose and form components of the carbohydrate reserves of seeds. These rank second to sucrose in abundance as soluble carbohydrates. RFOs may play a role in desiccation tolerance during seed maturation. Their role in protecting plants under water-deficit stress has been reported by Taji *et al* (2002). Drought, high salinity and cold-treated *Arabidopsis* plants accumulate a large amount of raffinose and galactinol, but not stachyose. However, raffinose and galactinol were not detected in unstressed plants, suggesting their role in tolerance to stresses (Taji *et al* 2002). Transgenic plants constitutively expressing galactinol synthase 2 (*GolS2*) exhibited improved stress tolerance (Taji *et al* 2002). The *SIP1* gene encodes a protein of unknown function that has 81% similarity to a putative cucumis raffinose synthase (Anderson and Kohorn 2001). *Arabidopsis* mutants with inactive *SIP1* had reduced drought tolerance (Anderson and Kohorn 2001). Galactinol synthase (GolS) catalyses the first step in the biosynthesis of RFOs and plays a key regulatory role in carbon partitioning between sucrose and RFOs (Saravitz *et al* 1987). Three stress-responsive galactinol synthase (*GolS*) genes (*AtGolS* 1, 2 and 3) among seven *Arabidopsis GolS* genes were identified (Minorsky 2003). GolS catalyses the first step in the biosynthesis of RFO from UDP-galactose. GolS activity in seeds increased by cold and desiccation (Liu *et al* 1998; Dowrie *et al* 2003). Expression of *GolS* genes was induced by cold stress in *Arabidopsis* and *Ajuga reptans* plants (Liu *et al* 1998; Sprenger and Keller 2000). *AtGolS1* and *AtGolS2* were induced by drought and high salinity stresses. The over expression of *AtGolS2* caused an increase in endogenous galactinol and raffinose and reduced transpiration thus acting as osmoprotectants during drought stress in plants. It was established that an unidentified gene belonging to a group of ABA-independent, desiccation stress-inducible genes isolated from rice (*Oryza sativa*) encodes the rice homolog, the *GolS* gene (Liu *et al* 1998). Utilization of raffinose series oligosaccharides was delayed under water deficit stress in chickpea seedlings (Gupta *et al* 1993b).

The acquisition of tolerance to heat stress is correlated with induction of heat shock protein expression. In *Arabidopsis*, 21 different heat shock transcription factors (HSF) genes have been identified (Nover *et al* 2001). *GolS1* is one of the genes that is heat-inducible in wild type *Arabidopsis* but showed constitutive m-RNA levels in transgenic plants expressing heat shock factor 3 (*HSF3*) gene (Panikulangara *et al* 2004). The conclusion that *GolS1* is a true target of HSF regulation was confirmed by the investigation of transgenic plants carrying *GolS*-promoter *GUS*-reporter constructs. It appears that HSFs may be involved in drought- and salinity-induced *GolS1/GolS2* expression whereas the expression of cold-inducible *GolS3* is regulated by DREB1/CBF (Panikulangara *et al* 2004).

The tomato (*Lycopersicon esculentum*) *Lea-Gal* gene under the control of *Figwort mosaic virus* promoter was introduced into petunia in the sense and antisense orientations using *Agrobacterium tumefaciens*-mediated transformation (Pennycooke *et al* 2003). RNA gel blots confirmed that α-galactosidase (α-*Gal*) transcripts were reduced in antisense lines compared with wild type whereas sense plants had increased accumulation of α-*Gal* mRNA. Down-regulating α-*Gal* in petunia resulted

in an increase in freezing tolerance at the whole-plant level whereas over expression of the *a-Gal* gene caused a decrease in endogenous raffinose and impaired freezing tolerance (Pennycooke *et al* 2003).

10. Sugar alcohols in stress tolerance

The studies on the role of sugar alcohols in tolerance to salt stress in terrestrial species are mainly correlative. Their levels show increase in higher plants, for example, mannitol in celery (Stoop and Pharr 1994) and glucitol in *Plantago* (Briens and Larher 1983) under salt stress. In celery, salt treatments increased mannitol and mannitol-6-phosphate reductase (Everard *et al* 1994). Arabidopsis and tobacco plants, which donot usually contain mannitol, with *mt1D* gene for with mannitol-1-phosphate dehydrogenase from *E. coli*, produced mannitol at all stages of leaf development and showed considerable resistance to salt stress though the level of mannitol was very low (Tarczynski *et al* 1992). Wheat was transformed with *mt1D* gene of *E. coli*. The ecotopic expression of *mt1D* gene for biosynthesis of mannitol in wheat improved tolerance to water stress and salinity (Abebe *et al* 2003).

Tobacco plants were transformed with a construct in which the *mt1D* gene was targetted to chloroplast. The resulting transgenic plants accumulated mannitol which resulted in their enhanced resistance to oxidative stress (Shen *et al* 1997). Similarly seeds of transgenic *mt1D* expressing *Arabidopsis* plants were able to germinate in medium supplemented with 400 mM NaCl, where control seeds ceased to germinate at 100 mM NaCl (Thomas *et al* 1995). Similarly in mannitol accumulating transgenic plants, 150 mM NaCl had no effect on growth where it reduced biomass of wild plants by 44% (Karakas *et al* 1997). The available information on expression of certain sugars and sugar alcohols in transgenics leading to enhanced tolerance against different kinds of stresses has been summarized in table 1.

11. Crosstalk between stress signalling pathways

A number of genes have been reported to be up-regulated under water stress. Many of these belonged to enzymes of carbohydrate metabolism like glyceraldehyde-3-phosphate dehydrogenase, SPS, SuSy and PEP carboxylase. Many of the genes corresponding to enzymes involved during sugar signalling cascades were also up-regulated (Ingram and Bartels 1996). These findings indicate that enzymes of sugar metabolism are critical in stress tolerance. Increasing sucrose synthesis and SPS activity is not only a drought response of desiccation tolerant plants such as *Craterostigma plantigineum* but also of plants that cannot withstand extreme drying such as spinach (Quick *et al* 1989). Seki *et al* (2002) monitored the expression profiles of 7000 *Arabidopsis* genes under drought cold or high salinity stress using a full length cDNA-micro array. In total 277 drought and 194 high salinity inducible genes were identified. Out of these total genes identified under cold, drought and salinity stresses, 20 genes belonging to enzymes of carbohydrate metabolism were up-regulated and 11 down-regulated (table 2). The number of cold stress-inducible genes were much lesser in comparison to drought and salinity thereby showing that water stress is the most severe limiting factor of plant growth. The role of sugar metabolism under abiotic stress is very important in modulating plant development as sugars are involved in signal transduction pathways as discussed earlier in this review. The induction of genes of regulatory protein like protein kinases, phosphatases and calmodulin-binding proteins which are thought to function in further regulating functional genes under stress conditions further support the role of sugars in signal transduction cascades. The existence of crosstalk between drought

Table 1. Transgenic plants engineered to produce different sugars/sugar alcohols for enhanced tolerance to stress.

Sugar/sugar alcohol	Gene	Host plant	Enhanced tolerance	References
Fructan	*SACB*	Tobacco	Drought	Pilon-Smits *et al* 1995
	SACB	Sugarbeet	Drought	Pilon-Smits *et al* 1999
Mannitol	*Mt1D*	Arabidopsis	Salt	Thomas *et al* 1995
	Mt1D	Tobacco	Salt	Tarczynski *et al* 1992; Tarczynski *et al* 1993; Karkas *et al* 1997
	Mt1D	Wheat	Drought and salt	Abebe *et al* 2003
Sorbitol	*S6PDH*	Persimmon	Salt	Gao *et al* 2001
Trehalose	*TPS1*	Tobacco	Drought	Zhao *et al* 2000
	TPS1	Potato	Drought	Yeo *et al* 2000
	Ots A, Ots B	Tobacco	Drought	Pilon-Smits *et al* 1998
	Ots A, Ots B	Rice	Drought and salt	Garg *et al* 2002

Table 2. The genes of enzymes involved in carbohydrate metabolism that are up- and down-regulated under various abiotic stresses in *Arabidopsis* (Seki *et al* 2002).

Genes up-regulated	Genes down-regulated
Cytosolic glyceraldehyde-3-phosphate dehydrogenase	Phospho ribulokinase (one gene)
Sucrose phosphate synthase	Glyceraldehyde-3-phosphate dehydrogenase (three genes)
Sucrose synthase	Aldolases (two genes)
Phosphoenol pyruvate carboxylase	Fructose bisphosphatases (two genes)
Betaine aldehyde dehydrogenase	*b*-glucosidase (three genes)
(involved in glycine betaine biosynthesis)	
Δ-pyrroline-5-carboxylase synthase (proline biosynthesis)	
S-adenosyl-L-methionine synthetase	
Lipoxygenase	
Ca^{2+}-dependent, calmodulin independent protein kinase	
Protein kinase	
Phosphatidyl inositol specific phospholipase C	
Cytosolic ascorbate peroxidase	
Cytosolic copper zinc superoxide dismutase	
Glutathione S-transferase	
L-isoaspartyl methyl transferase	

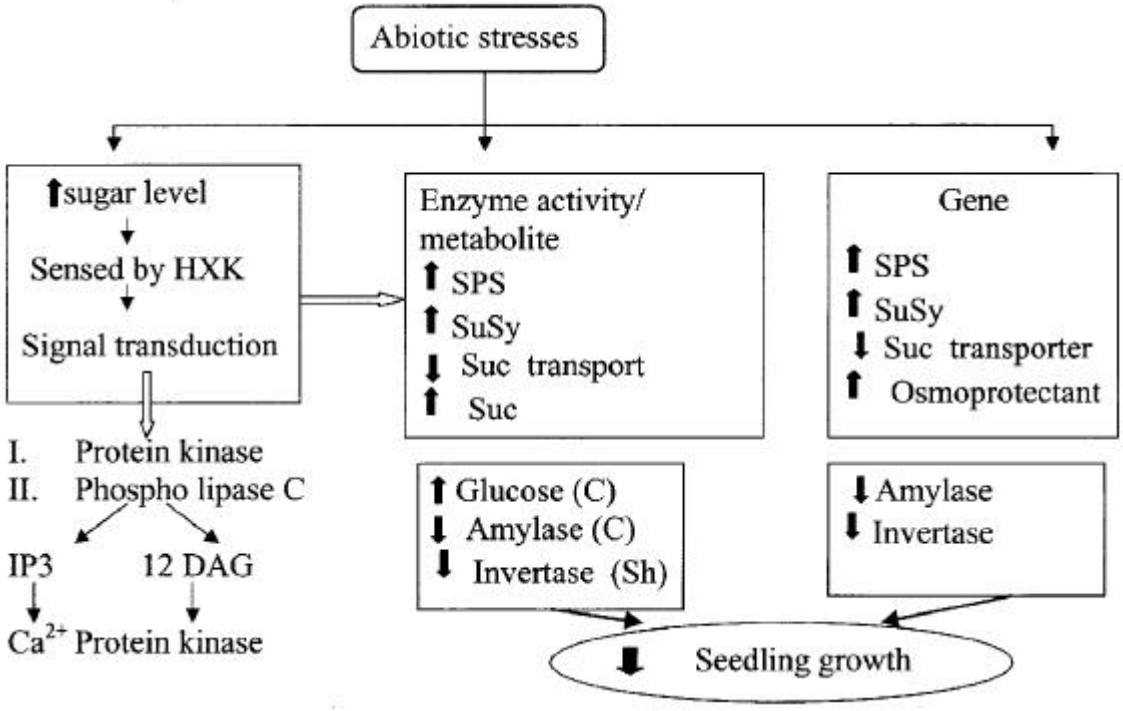

Figure 5. Crosstalk among sugar signal transduction, enzyme activities and gene regulation based on Kaur *et al* (1998), Umemura *et al* (1998), Lalonde *et al* (1999), Seki *et al* (2002) and Price *et al* (2004). ⬆, induction; ⬇, repression; C, cotyledons; Sh, shoot; IP3, inositol triphosphate; DAG, diacyl glycerol; SuSy, sucrose synthase; SPS, sucrose phosphate synthase; Suc, sucrose; and HXK, hexokinase.

and high salinity stress signalling processes in plants is shown in figure 5.

12. Conclusions

Sugars have dual role in plants. They are involved in various metabolic events and also regulate various genes especially those involved in photosynthesis, sucrose metabolism and synthesis of osmoprotectants. Sugars like fructans, raffinose series oligosaccharides and trehalose also act as protectants against abiotic stresses. Transgenic plants expressing these sugars have more tolerance to abiotic stresses, but a number of times they have reduced

growth and lower yield. Future efforts should be in two directions. One based on traditional wisdom to identify germ plasm which has inbuilt high content of abiotic stress tolerant markers and can be straightforward included in the breeding programme of evolving abiotic stress-tolerance crops. The other to have transgenics which express the desired characters only at the time of exposure to abiotic stresses so that they can give good yield along with capability to tolerate stresses.

References

Abebe T, Guenzi A C, Martin B and Cashman J C 2003 Tolerance of mannitol-accumulating transgenic wheat to water stress and salinity; *Plant Physiol*. **131** 1748–1755

Anderson C M and Kohorn B D 2001 Inactivation of *Arabidopsis SIP1* leads to reduced levels of sugars and drought tolerance; *J. Plant Physiol*. **158** 1215–1219

Arenas-Huertero F, Arroyo A, Zhou L, Sheen T and Leon P 2000 Analysis of *Arabidopsis* glucose insensitive mutants, gin 5 and gin 6, reveals a central role of the plant hormone ABA in the regulation of plant vegetative development by sugars; *Genes Dev*. **14** 2085–2086

Arroyo A, Bossi F, Finkelstein R R and 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

Atanssova R, Leterrier M, Gaillard C, Agasse A, Sagot F, Coutosa-Thevenot P and Delrot S 2003 Sugar-regulated expression of a putative hexose transport gene in grape; *Plant Physiol*. **131** 326–334

Barker L, Kuhn C, Weise A, Schulz A, Gebhardt C, Hirner B, Hellman H, Schulze W, Ward J M and Frommer W B 2000 *SUT2*, a putative sucrose sensor in sieve elements; *Plant Cell* **12** 1153–1164

Briens M and Larher F 1983 Sorbitol accumulation in plantaginaceae: further evidence for functions in stress tolerance; *Z. Pflanzenphysiol*. **110** 447–458

Brun T, Roche E, Kim K-H and Prentik M 1993 Glucose regulates acetyl CoA carboxylase gene expression in a pancreatic *b*-cell line (INS-1); *J. Biol. Chem*. **268** 18905–18911

Bustos M M, Iyer M and Gagliardi S J 1998 Induction of a *b*-phaseolin promoter by exogenous abscisic acid in tobacco: Developmental regulation and modulation by external sucrose and Ca^{2+} ions; *Plant Mol. Biol*. **37** 265–274

Castrillo M 1992 Sucrose metabolism in bean plants under water deficit; *J. Exp. Bot*. **43** 1557–1561

Chan M T and Yu S M 1998a The 3 untranslated region of a rice *a*-amylase gene mediates sugar-dependent abundance of mRNA; *Plant J*. **15** 685–695

Chan M T and Yu S M 1998b The 3 untranslated region of a rice *a*-amylase gene functions as a sugar-dependent mRNA stability determinant; *Proc. Natl. Acad. Sci. USA* **95** 6543–6547

Cheng W H, Endo A, Zhou I, Penney J, Chen H C, Arroyo A, Leon P, Nambara E, Asami T and Seo S 2002 A unique short-chain dehydrogenase/reductase in *Arabidopsis* abscisic acid biosynthesis and glucose signalling; *Plant Cell* **14** 2723–2743

Chikano H, Ogawa M, Ikeda Y, Koizumi N, Kusano T and Sano H 2001 Two novel genes encoding SNF-1 related protein kinase from *Arabidopsis thaliana*: differential accumulation of AtSR1 and AtSR2 transcripts in response to cytokinins and sugars and phosphorylation of synthase by AtSR2; *Mol. Gen. Genet*. **264** 674–681

Chiou T J and Bush D R 1998 Sucrose is a signal molecule in assimilate partitioning; *Proc. Natl. Acad. Sci. USA* **95** 4784–4788

Coruzzi G M and Bush D R 2001 Nitrogen and carbon nutrient and metabolite signaling in plants; *Plant Physiol*. **125** 61–64

Daie J 1988 Mechanism of drought-induced alterations in assimilate partitioning and transport in crops; *CRC Crit. Rev. Plant Sci*. **7** 117–137

Demel R A, Dorrepaal E, Ebskamp M J M, Smeekens S and de Kruijff B 1998 Fructans interact strongly with model membranes; *Biochim. Biophys. Acta* **1375** 36–42

Dijkwel P P, Huijser C, Weisbeek P J, Chua N-H and Smeekens S C M 1997 Sucrose control of phytochrome A signaling in *Arabidopsis*; *Plant Cell* **9** 583–595

Dijkwel P P, Kock P, Bezemer R, Weisbeek P and Smeekens S C M 1996 Sucrose represses the developmentally controlled transient activation of the plastocyanin gene in *Arabidopsis thaliana* seedlings; *Plant Physiol*. **110** 455–463

Dowrie B, Gurusinghe S, Dahal P, Thacker R R, Synhder J C, Nonogaki H, Yim K, Fukanaga K, Alvarado V and Bradford K J 2003 Expression of a *galactinol synthase* gene in tomato seeds is up-regulated before maturation desiccation and again after imbibition whenever radicle protrusion is prevented; *Plant Physiol*. **131** 1347–1359

Du L and Chen Z 2000 Identification of genes encoding receptor-like protein kinases as possible target of pathogen- and salicylic acid-induced WRKY DNA-binding proteins in *Arabidopsis; Plant J*. **24** 837–847

Eastmond P J and Graham I A 2001 Re-examining the role of the glyoxylate cycle in oilseeds; *Trends Plant Sci*. **6** 72–78

Ehness R, Ecker M, Godt D E and Roitsch T H 1997 Glucose and stress independently regulate source and sink metabolism and defence mechanisms via signal transduction pathways involving protein phosphorylation; *Plant Cell* **9** 1825–1841

Everard J D, Gucci R, Kann S C, Flore J A and Loescher W H 1994 Gas exchange in the leaves of celery (*Apium graveolens* L.) at various levels of root zone salinity; *Plant Physiol*. **106** 281–292

Fernie A R, Roessner U and Geigenberger P 2001 The sucrose analogue palatinose leads to a stimulation of sucrose degradation and starch synthesis when supplied to discs of growing potato tubers; *Plant Physiol*. **125** 1967–1977

Foyer C H 1988 Feed back inhibition of photosynthesis through source-sink regulation in leaves; *Plant Physiol. Biochem*. **26** 483–492

Fu H, Kim S Y and Park W D 1995 High level tuber expression and sucrose-inducibility of a potato *Sus4* sucrose synthase gene requires 5 and 3 flanking sequences and the leader intron; *Plant Cell* **7** 1387–1394

Gao M, Tao R, Miura K, Dandekar A M and Sugiura A 2001 Transformation of Japanese persimmon (*Diospyros kaki* Thunb) with apple cDNA encoding NADP-dependent sorbitol-6-phosphate dehydrogenase; *Plant Sci*. **160** 837–845

Garg A K, Kim J-K, Owens T G, Ranwala A P, Choi Y D, Kochian L V and Wu R J 2002 Trehalose accumulation in rice plants confers high tolerance levels to different abiotic stresses; *Proc. Natl. Acad. Sci. USA* **99** 15898–15903

Gazzarrini S and McCourt P 2001 Genetic interactions between

ABA, ethylene and sugar signalling pathways; *Curr. Opin. Plant Biol.* **4** 387–391

Godt D E and Roitsch T 1997 Differential regulation of a tomato invertase gene family suggests an important function of extracellular isoenzymes in establishing and maintaining sink metabolism; *Plant Physiol.* **115** 273–282

Gupta A K and Kaur N 2000 Fructan metabolism in Jerusalem artichoke and chicory; in *Carbohydrate reserves in plants – Synthesis and regulation* (eds) A K Gupta and N Kaur (The Netherlands: Elsevier Science) pp 223–248

Gupta A K, Singh J, Kaur N and Singh R 1993a Effect of polyethylene glycol-induced water stress on uptake, interconversion and transport of sugars in chickpea seedlings; *Plant Physiol. Biochem.* **31** 743–747

Gupta A K, Singh J, Kaur N and Singh R 1993b Effect of polyethylene glycol-induced water stress on germination and reserve carbohydrate metabolism in chickpea cultivars differing in tolerance to water deficit; *Plant Physiol. Biochem.* **31** 369–378

Hare P D, Cress W A and van Staden J 1998 Dissecting the roles of osmolyte accumulation during stress; *Plant Cell Environ.* **21** 535–554

Harrington N and Bush D R 2003 The bifunctional role of hexokinase in metabolism and glucose signaling; *Plant Cell* **15** 2493–2496

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

Hincha D K, Hellwege E M, Heyer A G and Crowe J H 2000 Plant Fructans stabilize phosphatidylcholine liposomes during freeze-drying; *Eur. J. Biochem.* **267** 535–540

Ho S-L, Chao Y-C, Tong W-F and Yu S-M 2001 Sugar coordinately and differentially regulates growth- and stress-regulated gene expression via a complex signal transduction network and multiple control mechanisms; *Plant Physiol.* **125** 877–890

Ho Y, Tang W, Swain J D, Green A L, Jack T P and Gan S 2001 Networking senescence-regulating pathways by using *Arabidopsis* enhancer trap lines; *Plant Physiol.* **126** 707–716

Holmstrom K O, Mantyla E, Welin B, Mandal A, Palva E T, Tunnela O E and Londesborough J 1996 Drought tolerance in tobacco; *Nature* (*London*) **379** 683–684

Huber S C, Huber J L, Liao P C, Gage D A and McMichael R W Jr 1996 Phosphorylation of serine-158 of maize leaf synthase. Occurrence *in vivo* and possible regulatory significance; *Plant Physiol.* **112** 793–802

Hwang Y S, Karrer E E, Thomas B R, Chen L and Rodriguez R L 1998 Three *cis*-elements required for rice *a*-amylase *Amy3D* expression during sugar starvation; *Plant Mol. Biol.* **36** 331–341

Ingram J and Bartels D 1996 The molecular basis of dehydration tolerance in Plants; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47** 377–403

Ishiguro S and Nakamura K 1994 Characterization of a cDNA encoding a novel DNA-binding protein, SPF1, that recognizes SP8 sequences in the 5 upstream region of genes coding for sporamin and *b*-amylase from sweet potato; *Mol. Gen. Genet.* **28** 563–571

Jang J C and Sheen J 1994 Sugar sensing in higher plants; *Plant Cell* **6** 1665–1679

Jang J-C and Sheen J 1997 Sugar sensing in higher plants; *Plant Cell* **9** 5–19

Jefferson R, goldsbrough A and Bevan M 1990 Transcriptional regulation of a *patatin-1* gene in potato; *Plant Mol. Biol.* **14** 995–1006

Karakas B, Ozias-Akins P, Stushnoff C, Suefferheld M and Rieger M 1997 Salinity and drought tolerance of mannitol-accumulating transgenic tobacco; *Plant Cell Environ.* **20** 609–616

Kaur P, Gupta A K and Kaur N 2005 Embryo is not required for initiation of *a*-amylase activity in germinating cowpea seeds; *Indian J. Biochem. Biophys.* **42** 161–165

Kaur S, Gupta A K and Kaur N 2000 Effect of GA_3, kinetin and indole acetic acid on carbohydrate metabolism in chickpea seedlings germinating under water stress; *Plant Growth Regul.* **30** 61–70

Kaur S, Gupta A K and Kaur N 1998 Gibberellin A_3 reverses the effect of salt stress in chickpea (*Cicer arietinum* L.) seedlings by enhancing the amylase activity and mobilization of starch in cotyledons; *Plant Growth Regul.* **26** 85–90

Keller F and Ludlow M M 1993 Carbohydrate metabolism in drought stressed leaves of pigeon pea (*Cajanus cajan*); *J. Exp. Bot.* **44** 1351–1359

Kim J Y, Mahe A, Brangeon J and Prioul J L 2000 A maize vacuolar invertase IVR2 is induced by water stress, organ tissue specificity and diurnal modulation of expression; *Plant Physiol.* **124** 71–84

Kim K N and Guiltinam M J 1999 Identification of *cis*-acting elements important for expression of the starch-branching enzyme 1 gene in maize endosperm; *Plant Physiol.* **121** 225–236

Kim S Y, May G D and Park W D 1994 Nuclear protein factors binding to class 1 patatin promoter region are tuber-specific and sucrose inducible; *Plant Mol. Biol.* **26** 603–615

Koch K E 1996 Carbohydrate modulated gene expression in plants; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **47** 509–540

Koch K E, Nolte K D, Duke E R, McCarty D R and Avigne W T 1992 Sugar levels modulate differential expression of maize sucrose synthase genes; *Plant Cell* **4** 59–69

Krapp A and Stitt M 1995 An evaluation of direct and indirect mechanisms for the sink-regulation of photosynthesis in spinach: changes in gas exchange, carbohydrates, metabolites, enzyme activities and steady state transcript levels after cold-girdling source leaves; *Planta* **195** 313–323

Kühn C, Barker L, Burkle L and Frommer W B 1999 Up date on sucrose transport in higher plants; *J. Exp. Bot.* **50** 935–953

Kühn C, Franceschi V R, Schulz A, Lemoine R and Frommer W B 1997 Localization and turnover of sucrose transporters in enucleate sieve elements indicate macromolecular trafficking; *Science* **275** 1298–1300

Kühn C, Quick W P, Schulz A, Riesmeier J W, Sonnewald V and Frommer W B 1996 Companion cell-specific in inhibition of the potato sucrose transporter *SUT1*; *Plant Cell Environ.* **19** 1115–1123

Lalonde S, Boles E, Hellmann H, Barker L, Patrick J W, Frommer W B and Wood J M 1999 The dual action of sugar carriers: Transport and sugar sensing; *Plant Cell* **11** 707–726

Liu J J, Krenz D C, Galvez A F and DeLumen B O 1998 Galactinol synthase (GS) increased enzyme activity and levels of mRNA due to cold and desiccation; *Plant Sci.* **134** 11–20

Lopez-Molina L, Mongrand S and Chua N H 2001 A post-germination developmental arrest checkpoint is mediated by abscisic acid and requires the AB 15 transcription factor in *Arabidopsis*; *Proc. Natl. Acad. Sci. USA* **98** 4782–4787

Loreti E, Alpi A and Perata P 2000 Glucose and disaccharide

sensing mechanisms modulate the expression of *a*-amylase in barley embryos; *Plant Physiol*. **123** 939–948

Loreti E, Bellis L D, Alpi A and Perata P 2001 Why and how do plant cells sense sugars; *Ann. Bot*. **88** 803–812

Lu C A, Lim E K and Yu S M 1998 Sugar response sequence in the promoter of a rice *a*-amylase gene serves as a transcriptional enhancer; *J. Biol. Chem*. **273** 10120–10131

Maeo K, Tomiya T, Hayashi K, Akaike M, Morikami A, Ishiguro S and Nakamura K 2001 Suger-responsive elements in the promoter of a gene for *b*-amylase of sweet potato; *Plant Mol. Biol*. **46** 627–637

Maleck K, Levine A, Euglem T, Morgan A, Schmid J, Lawton K A, Dangi J L and Dietrich R A 2000 The transcriptome of *Arabidopsis thaliana* during systemic acquired resistance; *Nat. Genet*. **26** 403–410

Martin T, Hellman H, Schmidt R, Willmitzer L and Frommer W B 1997 Identification of mutants in metabolically regulated gene expression; *Plant J*. **11** 53–62

Martinez-Garcia J F, Huq E and Quali P H 2000 Direct targeting of light signals to a promoter element-bound transcriptional factor; *Science* **288** 859–863

Meer I M, Ebskamp M J M, Visser R G F, Weisbeek P J and Smeekens S C M 1994 Fructan as a new carbohydrate sink in transgenic potato plants; *Plant Cell* **6** 561–570

Minorsky P V 2003 The hot and the classic; *Plant Physiol*. **131** 1159–1160

Mita S, Hirano H and Nakamura K 1997a Negative regulation in the expression of a sugar-inducible gene in *Arabidopsis thaliana*; a recessive mutation causing enhanced expression of a gene for *b*-amylase; *Plant Physiol*. **114** 575–582

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

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

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

Morita A, Umemura T, Kuroyanagi M, Futsuhara Y, Perata P and Yamaguchi J 1998 Functional dissection of a sugar-repressed *a*-amylase gene (*Ramy1A*) promotor in rice embryos; *FEBS Lett*. **423** 81–85

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

Nover L, Bharti K, Doring P, Mishra S K, Ganguli A and Scharf K D 2001 *Arabidopsis* and the heat stress transcription factor world. How many heat stress transcription factors do we need?; *Cell Stress Chaperones* **6** 177–189

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

Ohto M and Nakamura K 1995 Sugar-induced increase of calcium dependent protein kinases associataed with the plasma membrane in leaf tissues of tobacoo; *Plant Physiol*. **109** 973–981

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

Panikulangara T J, Eggers-Schumacher G, Wunderlich M, Stransky H and Schöffl F 2004 *Galactinol synthase1*. A novel heat shock factor target gene responsible for heat-induced synthesis of raffinose family oligosaccharides in *Arabidopsis*; *Plant Physiol*. **136** 3148–3158

Pego J and Smeekens S C M 2000 Plant fructokinases: a sweet family get-together; *Trends Plant Sci*. **5** 531–536

Pelleschi S, Guy S, Kim J Y, Pointe C, Mahe A, Barthens L, Leonardi A and Prioul J L 1999 *IVR2*, a candidate gene for a QTL of vacuolar invertase activity in maize leaves. Gene-specific repression under water stress; *Plant Mol. Biol*. **39** 373–380

Pelleschi S, Rocher J-P and Prioul J-L 1997 Effect of water restriction on carbohydrate metabolism and photosynthesis in mature maize leaves; *Plant Cell Environ*. **20** 493–503

Pennycooke J C, Jones M L and Stushnoff C 2003 Down-regulating *a*-galactosidase enhances freezing tolerance in transgenic potato; *Plant Physiol*. **133** 1–9

Perata R T N, Williamson J D, Conkling M A and Pharr D M 1997 Sugar repression of mannitol dehydrogenase activity in celery cells; *Plant Physiol*. **114** 307–314

Pilon-Smits E A H, Ebskamp M J M, Paul M J, Jeuken M J W, Weisbeek P J and Smeekens S C M 1995 Improved performance of transgenic fructan accumulating tobacco under drought stress; *Plant Physiol*. **107** 125–130

Pilon-Smits E A H, Terry N, Sears T, Kim H and Van Dun K 1999 Enhanced drought resistance in fructan-producing sugar beet; *Plant Physiol. Biochem*. **37** 313–317

Pilon-Smits E A H, Terry N, Sears T, Kim H, Zayed A, Hwang S, van Dun K, Voogd E, Verwoerd T C, Krutwagen R W and Goodijin O J M 1998 Trehalose producing transgenic tobacco plants show improved growth performance under drought stress; *J. Plant Physiol*. **152** 525–532

Pla M, Vilardell J, Guiltinan M J, Marotte W R, Niogret M F, Quatrano R S and Pages M 1993 The *cis*-regulatory element CCACGTGG is involved in ABA and water-stress responses of the maize gene *rap28*; *Plant Mol. Biol*. **21** 259–266

Price J, Laxmi A, Martin S K and Jang J-C 2004 Global transcription profiling reveals multiple sugar signal transduction mechanisms in *Arabidopsis*; *Plant Cell* **16** 2128–2150

Quick P, Siegl G, Neuhaus E, Feil R and Stitt M 1989 Short term water stress leads to a stimulation of sucrose synthesis by activating sucrose phosphate synthase; *Planta* **177** 535–546

Rentsch D, Boorer K and Frommer W B 1998 Molecular biology of sucrose, amino acid and oligopeptide transporters at the plasma membrane of plant cells; *J. Membr. Biol*. **162** 177–190

Riesmeier J W, Willmitzer L and Frommer W B 1994 Evidence for an essential role of sucrose transporter in phloem loading and assimilate partitioning; *EMBO J*. **13** 1–7

Roitsch T 1999 Source-sink regulation by sugar and stress; *Curr. Opin. Plant Biol*. **2** 198–206

Roitsch T, Balibrea H E, Hofmann M, Proels R and Sinha A K 2003 The upregulation of extracellular invertase was suggested to be a common response to various biotic and abiotic stress related stimuli like pathogen infection and salt stress; *J. Exp. Bot*. **54** 513–524

Roitsch T, Bittner M and Godt D E 1995 Induction of apoplastic invertase of *Chenopodium rubrum* by D-glucose and a glucose analogue and tissue-specific expression suggest a role in sink-source regulation; *Plant Physiol*. **108** 285–294

Rolland F, Moore B and Sheen J 2002 Sugar sensing and signaling in plants; *Plant Cell* S185–S205

Rolland F, Winderickx J and Thevelein J M 2001 Glucose sensing mechanisms in eukaryotic cells; *Trends Biochem. Sci*. **26** 310–317

Rook F, Gerrits N, Kortsee A, van Kampe M and Borrias M 1998 Sucrose-specific signaling represses translation of the *Arabidopsis ATB2 bZ1* transcription factor gene; *Plant J*. **15** 253–263

Rushton P J, Macdonald H, Huttly A K, Lazarus C M and Hooley R 1995 Members of a new family of DNA-binding proteins bind to a conserved cis-element in the promoters of *a-Amy2* genes; *Plant Mol. Biol*. **29** 691–702

Sadaka A, DeWald D B, May G D, Park W D and Mullet J E 1994 Phosphate modulates transcription of soybean *VspB* and other sugar inducible genes; *Plant cell* **6** 737–749

Sarah C J, Graham I A, Reynolds S J, Leaver C J and Smith S M 1996 Distinct *cis*-acting elements direct the germination and sugar responses of the cucumber malate synthase genes; *Mol. Gen. Genet*. **250** 153–161

Saravitz D M, Pharr D M and Carter T E 1987 Galactinol synthase activity and soluble sugars in developing seeds of four soybean genotypes; *Plant Physiol*. **83** 185–189

Seki M, Narusaki M, Ishida J, Nanjo T, Fujita M, Oone Y, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Taji T, Shinozaki K Y, Carninci P, Kawai J, Hayashizaki K Y and Shinozaki K 2002 Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high salinity stresses using a full length cDNA micro array; *Plant J*. **31** 279–292

Sevenier R, Hall R D, Meer I M, Hakkert H J C, Tunen A J and Koops A J 1998 High level fructan accumulation in a transgenic sugar beet; *Nature Biotechnol*. **16** 843–846

Sheen J 1999 C4 gene expression; *Annu. Rev. Plant Physiol. Plant Mol. Biol*. **50** 187–217

Shen B, Jensen R G and Bohnert H J 1997 Increased resistance to oxidative stress in transgenic plants by targeting mannitol biosynthesis to chloroplasts; *Plant Physiol*. **113** 1177–1183

Sinha A K, Romer U, Kockenberger W, Hofmann M, Elling L and Roitsch T 2002 Metabolism and non-metabolizable sugars activate different signal transduction pathways in tomato; *Plant Physiol*. **128** 1480–1489

Smeekens S 1998 Sugar regulation of gene expression in plants; *Curr. Opin. Plant Biol*. **1** 230–234

Smeekens S 2000 Sugar induced signal transduction in plants; *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52** 49–81

Smeekens S and Rook F 1997 Sugar sensing and sugar mediated signal transduction in plants; *Plant Physiol*. **115** 7–13

Sprenger N and Keller F 2000 Allocation of raffinose family oligosaccharides to transport and storage pools in *Ajuga reptans*: the role of two distinct galactinol synthases; *Plant J*. **21** 249–258

Stitt M and Krappe A 1999 The interaction between elevated carbon dioxide and nitrogen nutrition. The physiological and molecular background; *Plant Cell Environ*. **22** 583–621

Stoop J M H and Pharr D M 1994 Growth substrate and nutrient salt environment after mannitol to hexose partitioning in celery petioles; *J. Am. Soc. Hortic. Sci*. **119** 237–242

Sturm A and Jang G-Q 1999 The sucrose-cleaving enzymes of plants are crucial for development, growth and carbon partitioning; *Trends Plant Sci*. **4** 401–407

Taji T, Qhsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K and Shinozaki K 2002 Important roles of drought-and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*; *Plant J*. **29** 417–426

Tarczynski M C, Jensen R G and Bohnert H J 1992 Expression of a bacterial with *mtlD* gene in transgenic tobacco leads to production and accumulation of mannitol; *Proc. Natl. Acad. Sci. USA* **89** 2600–2604

Tarczynski M C, Jensen R G and Bohnert H J 1993 Stress protection of transgenic plants by production of osmolyte mannitol; *Science* **259** 508–510

Tegeder M, Wang X D, Frommer W B, Offler C E and Patric J W 1999 Sucrose transport into developing seeds of *Pisum sativum* L; *Plant J*. **18** 151–161

Thomas J C, Sepathi M, Arendall B and Bohnert H J 1995 Enhancement of seed germination in high salinity by engineering mannitol expression in *Arabidopsis thaliana*; *Plant Cell. Environ*. **18** 801–806

Thomashow M F 1999 Plant cold accumulation: freezing tolerance genes and regulatory mechanisms; *Annu. Rev. Plant. Physiol. Plant Mol. Biol.* **50** 571–599

Toroser D and Huber S C 1997 Protein phosphorylation as a mechanism for osmotic stress activation of sucrose-phosphate synthase in spinach leaves; *Plant Physiol*. **114** 947–955

Umemura T, Perata P, Futsuhara Y and Yamaguchi J 1998 Sugar sensing and *a*-amylase gene expression in rice embryos; *Planta* **204** 420–428

Urwin N A R and Jenkins G I 1997 A sucrose repression element in the *Phaseolus vulgaris rbcS2* gene promoter resembles responsible for sugar stimulation of plant and mammalian genes; *Plant Mol. Biol*. **35** 929–942

Vereyken I J, Chupin V, Demel R A, Smeekens S and de Kruijff B 2001 Fructans insert between the headgroups of phospholipids; *Biochim. Biophys. Acta* **1510** 307–320

Wanner L A and Junttila O 1999 Cold-induced freezing tolerance in *Arabidopsis*; *Plant Physiol*. **120** 391–399

Ward J, Kühn C, Tegeder M and Frommer W B 1998 Sucrose transport in plants; *Int. Rev. Cytol*. **178** 41–47

Weber H, Borisjuk L, Heim U, Sauter N and Wobus U 1997 A role for sugar transporters during seed development, molecular characterization of a hexose and a sucrose carrier in fava bean seeds; *Plant Cell* **9** 895–908

Weschke W, Paritz R, Saner N, Wang Q, Neubohn B, Weber H and Wobus U 2000 Sucrose transport into barley seeds: Molecular characterization of two transporters and implications for seed development and starch accumulation; *Plant J*. **21** 455–467

Wu K, Rooney M F and Ferl R J 1997 The *Arabidopsis* 14-3-3 multigene family; *Plant Physiol*. **114** 1421–1431

Xiao W, Sheen J and Jang J-C 2000 The role of hexokinase in plant sugar signal transduction and growth and development; *Plant Mol. Biol*. **44** 451–461

Yeo E T, Kwon H B, Han S E, Lee J T, Ryn J C and Byun M O 2000 Genetic engineering of drought-resistant potato plants by introduction of the trehalose-6-phosphate synthase (*TPS1*) gene from *Saccharomyces cerevisae*; *Mol. Cells* **10** 263–268

Yokohama R, Hirosa T, Fuju N, Aspuria E T, Kats A and Uchimiya H 1997 The rolC promotor of *Agrobacterium rhizogenes* Ri plasmid is activated by sucrose in transgenic tobacco plants; *Mol. Gen. Genet*. **244** 15–22

Zhao H W, Chen Y J, Hu Y L, Gao Y and Lin Z P 2000 Construction of a trehalose-6-phosphate synthase gene driven by drought-responsive promotor and expression of drought-resistance in transgenic tobacco; *Acta Bot. Sinica* **42** 616–619

Zhou L, Jang J C, Jones T L and Sheen J 1998 Glucose and ethylene signal transduction cross talk revealed by an *Arabidopsis* glucose-insensitive mutant; *Proc. Natl. Acad. Sci. USA* **95** 10294–10299

Zinselmeier C, Westgate M E, Schussler J R and Jones R J 1995 Low water potential disrupts carbohydrate metabolism in maize (*Zea mays* L.) ovaries; *Plant Physiol.* **107** 385–391

MS received 29 March 2005; accepted 12 August 2005

*e*Publication: 26 October 2005

Corresponding editor: DEEPAK PENTAL