

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

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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, Ca²⁺ 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.

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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 envi-

ronmental 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-

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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 (HXX)-dependent and the other HXX-independent pathway. The HXX-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 HXX-dependent and HXX-independent sugar signalling pathways. The evidences in the favour of HXX dependent signalling came from the observations that those sugar analogues that can be phosphorylated by HXX 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 HXX blocked the severe repression caused by 2-DG. These observations indicated that HXX 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 HXX. The evidence for HXX-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 HXX, 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 HXX-independent sugar signalling pathways (Roitsch 1999).

Three glucose signal transduction pathways in plants have been suggested (Xiao *et al* 2000). These are AtHXX1-dependent pathway in which gene expression was correlated with the AtHXX1-mediated signalling function. Second was glycolysis-dependent pathway that was influenced by catalytic activity of both AtHXX1 and heterologous yeast HXX2. Third is the HXX-independent pathway in which gene expression is independent of AtHXX1. However, by using two independent knockout mutations for HXX1 isoform, it was concluded that G-6-P metabolism is uncoupled from HXX1-dependent signalling (Moore *et al* 2003). A mechanism for glucose sensing by HXX1, wherein a change in conformation by substrate binding initiates a signalling cascade, has been proposed (Harrington and Bush 2003). It still remains unclear if HXX senses glucose in a linear concentration dependent manner or it is flux sensor. Hexose sensing and signalling functions are, however, dependent on sub-cellular localization, translocation and interactions with down stream effectors of HXX (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, Ca²⁺ 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-

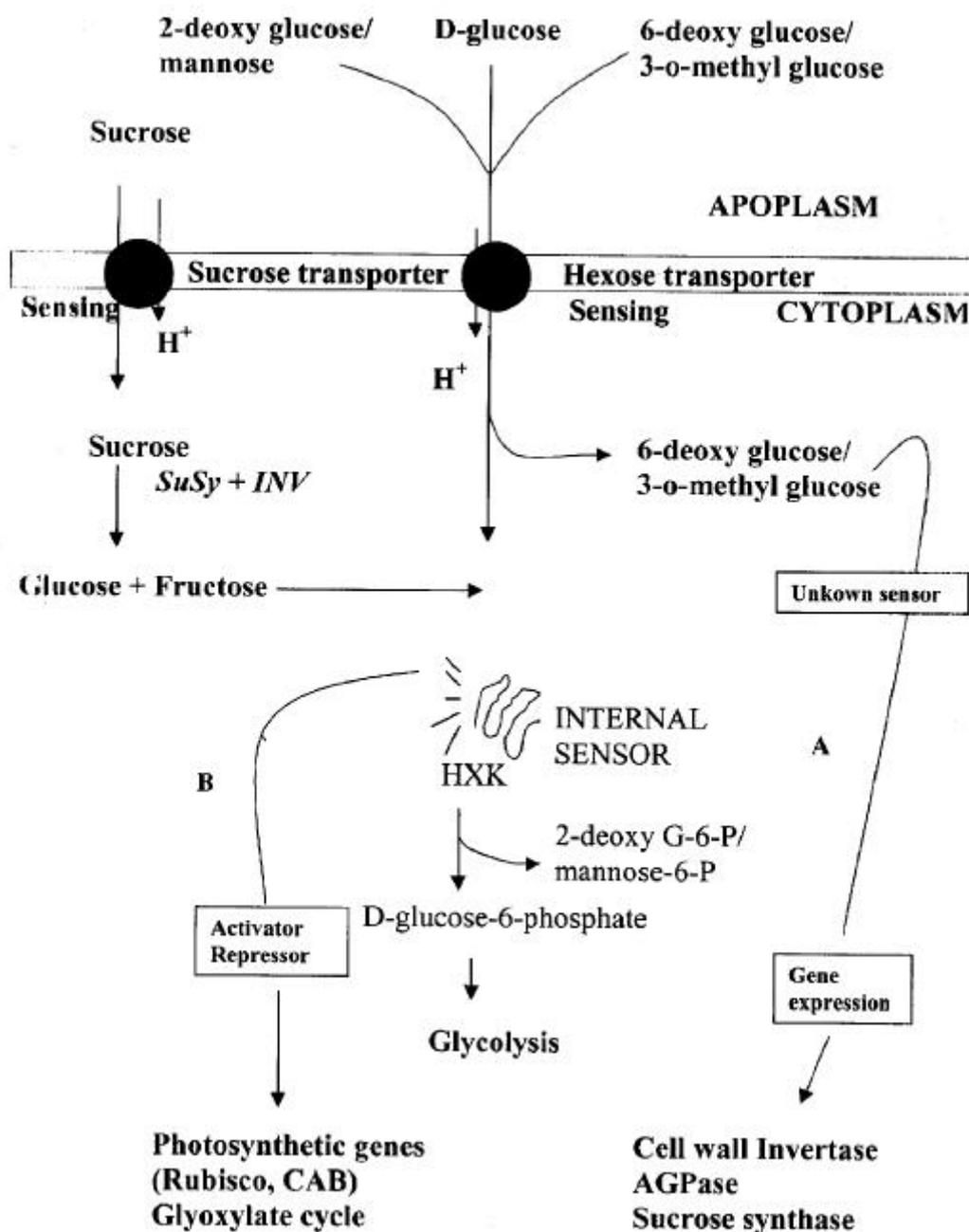


Figure 1. Sugar sensing and signal transduction pathways in higher plants based on Lalonde *et al* (1999) and Rolland *et al* (2002). A, hexokinase-independent pathway; B, hexokinase-dependent pathway, CAB, chlorophyll A binding protein, HXK, hexokinase; AGPase, ADP glucose pyrophosphorylase; SuSy, sucrose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

nas-Huertero *et al* 2000). ABI5 transcript also accumulates in response to stress (Arroyo *et al* 2003).

Sugar-mediated signalling in sweet potato and *Arabidopsis* 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 induced (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 ad-

dition 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

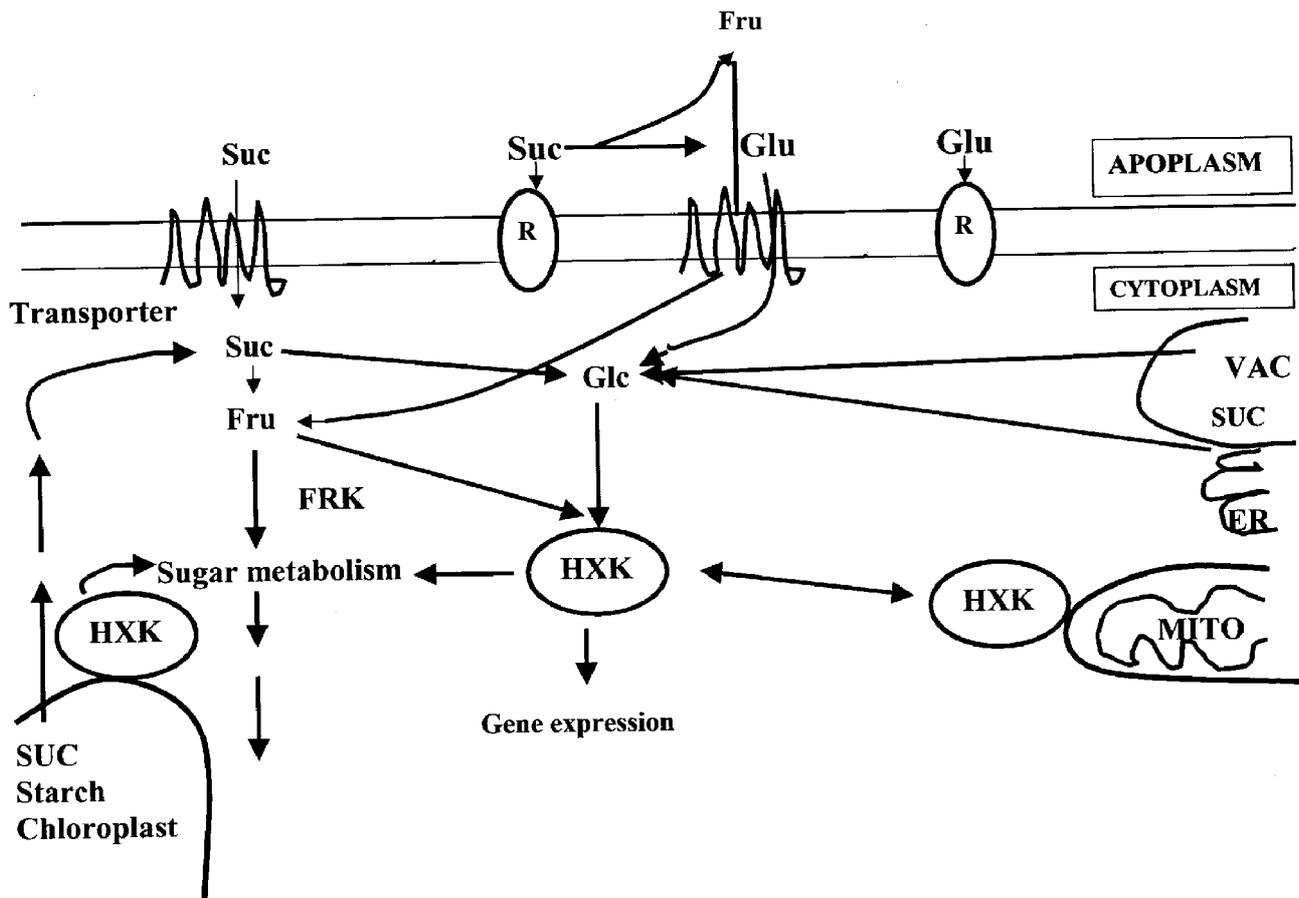


Figure 2. The possible sites for sugar signal sensing by hexokinase based on Rolland *et al* (2002). HXK, hexokinase; Glc, glucose; Suc, sucrose; Vac, vacuole; ER, endoplasmic reticulum; MITO, mitochondria; Fru, fructose; and FRK, fructokinase.

(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 α -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, α -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). α -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 α -amylase genes in the presence of gibberellic acid (GA). Induction of α -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 β -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 sugar-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* β -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 β -amylase expression (*hba1*, high level β -amylase) and low level β -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 palantinos, which is not taken up by the cells, exerts the same response in potato tubers as sucrose. Application of palantinos 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).

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.

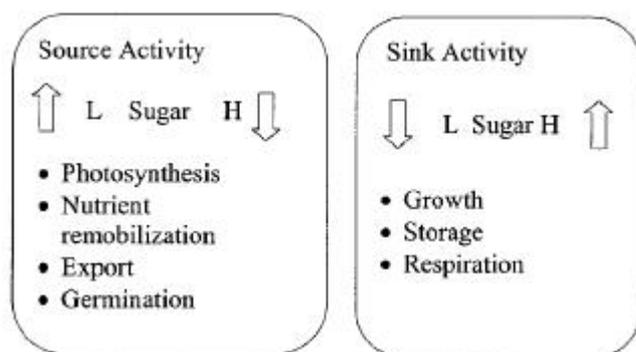


Figure 3. Differential effects of source-sink activities depending upon the availability of nutrients based on available information in literature. \uparrow , induction; \downarrow , repression; L, low sugar content; and H, high sugar content.

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-

motors 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-

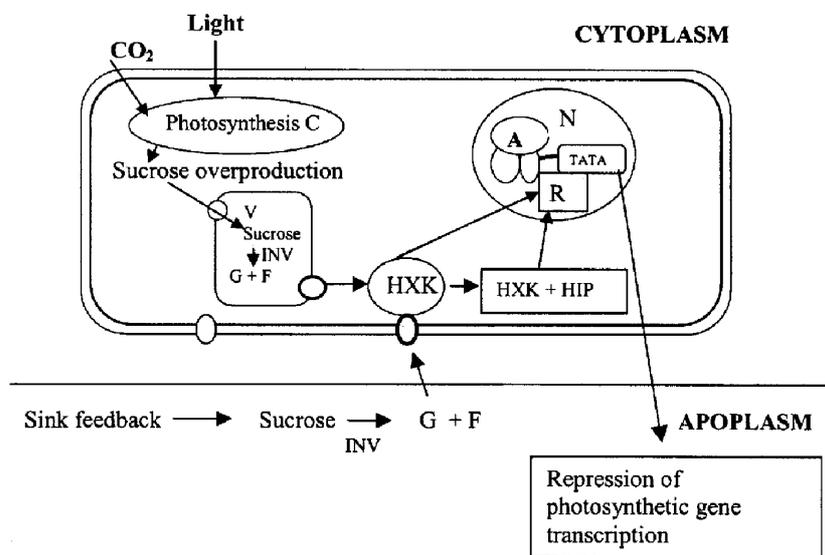


Figure 4. Model showing repression of photosynthetic gene transcription by sugars in higher plants based on Smeekens (1998), Sturm and Jang (1999), Barker *et al* (2000) and Rolland *et al* (2002). A, transcriptional activator; C, chloroplast; N, nucleus; V, vacuole; INV, invertase; R, transcriptional 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 signaling 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 endosperm 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* (Yokohama *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 targeting 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 (TPS1) 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 promoter 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 (*a-Gal*) transcripts were reduced in antisense lines compared with wild type whereas sense plants had increased accumulation of *a-Gal* mRNA. Down-regulating *a-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 do not 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 targeted 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 en-

hanced tolerance against different kinds of stresses has been summarized in table 1.

11. Crosstalk between stress signalling pathways

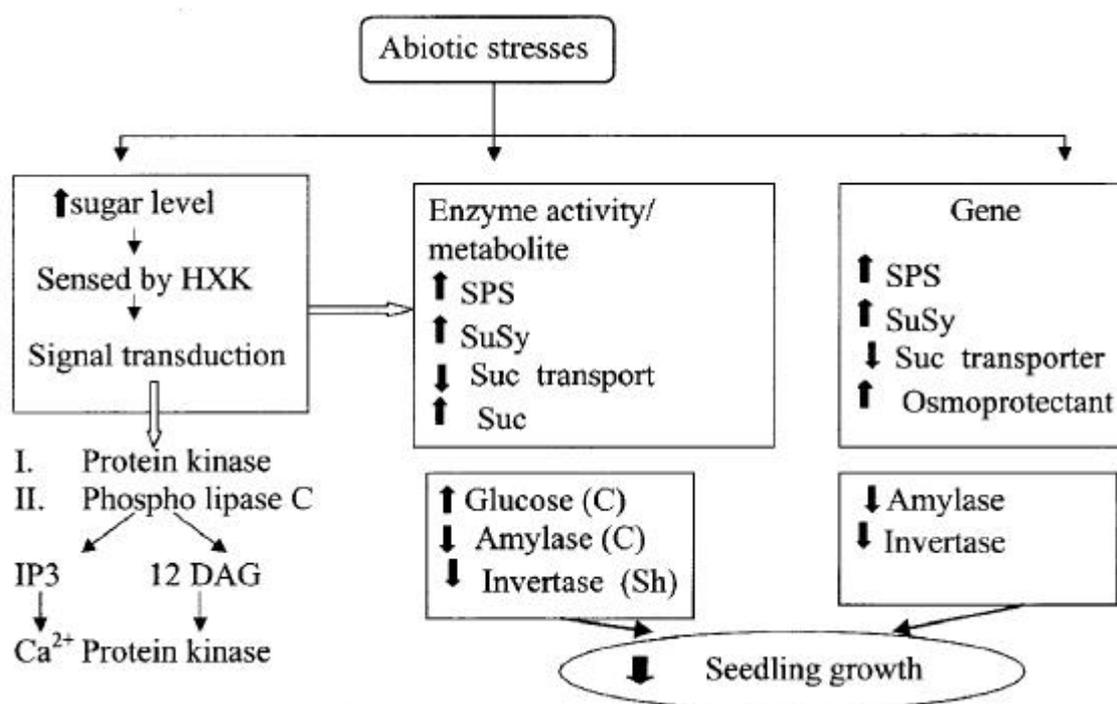
A number of genes have been reported to be up-regulated under water stress. Many of these belonged to enzymes of carbohydrate metabolism like glyceraldehyde-3-phosphate dehydrogenase, SPS, SuSy and PEP carboxylase. Many of the genes corresponding to enzymes involved during sugar signalling cascades were 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 plantagineum* 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	<i>SACB</i>	Tobacco	Drought	Pilon-Smits <i>et al</i> 1995
	<i>SACB</i>	Sugarbeet	Drought	Pilon-Smits <i>et al</i> 1999
Mannitol	<i>Mt1D</i>	<i>Arabidopsis</i>	Salt	Thomas <i>et al</i> 1995
	<i>Mt1D</i>	Tobacco	Salt	Tarczynski <i>et al</i> 1992; Tarczynski <i>et al</i> 1993; Karkas <i>et al</i> 1997
Sorbitol	<i>Mt1D</i>	Wheat	Drought and salt	Abebe <i>et al</i> 2003
	<i>S6PDH</i>	Persimmon	Salt	Gao <i>et al</i> 2001
Trehalose	<i>TPSI</i>	Tobacco	Drought	Zhao <i>et al</i> 2000
	<i>TPSI</i>	Potato	Drought	Yeo <i>et al</i> 2000
	<i>Ots A, Ots B</i>	Tobacco	Drought	Pilon-Smits <i>et al</i> 1998
	<i>Ots A, Ots B</i>	Rice	Drought and salt	Garg <i>et al</i> 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 biphosphatases (two genes)
Betaine aldehyde dehydrogenase (involved in glycine betaine biosynthesis)	<i>b</i> -glucosidase (three genes)
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.

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