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Review

Roles of plant soluble sugars and their responses to plant cold stress

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Plants can enhance freezing tolerance by gradual exposure to low temperature known as cold acclimation. Soluble sugars have been confirmed to play an important role during the process. This is based on the fact they are most commonly detected in various species of terrestrial plants that have undergone seasonal cold acclimation. Soluble sugars exert their positive effects to protect plant cells from damage caused by cold stress through many ways including serving as osmoprotectants, nutrients as well as interacting with the lipid bilayer. In addition to these functions, more focus is on their important hormone-like functions as primary messengers in signal transduction nowadays. On the other hand, higher sugar concentrations can trigger leaf senescence, which indicates that the accumulation of soluble sugars during cold acclimation might impact on plants negatively. Further study can be done to investigate the mechanisms by which soluble sugars are involved in the plants' response to the cold signal by advanced molecular biology methodology to examine sugar-regulated gene expression under cold condition.

Key words: Plants, sugars, cold acclimation, signal transduction, biotechnology.

THE IMPACT OF SOLUBLE SUGARS ON THE COLD ACCLIMATION

Cold stress causes several dysfunctions at cellular level; these include damage to membranes, generation of reactive oxygen species, protein denaturation and accumulation of toxic products etc (Bowers, 1994; Shao et al., 2008). In nature, plants may exhibit cold tolerance due to gradual exposure to low non-freezing temperatures, and the process is known as cold acclimation (Thomashow, 1999).

The advent of molecular biology has propelled research in this area over the past two decades; today some of the events of cold signal perception, transduction and cold acclimation are defined at the molecular level (Shinozaki et al., 2000; Shinozak et al., 2001; Zhu, 2001; Thomashow, 2001; Shao et al., 2006, 2007). Cold acclimation is associated with multiple mechanisms that include changes in (a) gene expression (b) membrane composi-tion and cryobehaviour (Orvar et al., 2000; Shao et al., 2008) (c) elevation of ABA (Lang et al., 1994) (d) antioxidants (Kang and Saltveit, 2002; Shao et al., 2008) (e) accumulation of substantial amounts of compatible solutes, such as soluble sugars (Ristic et al., 2003; Wanner et al., 1999), amino acids (Lalk et al., 1985; Wanner et al., 1999; Koster, 1991; Shao et al., 2006) and glycine betaine (Kishitani et al., 1994), which are thought to play a role in protection of cells from freezing injury (Figure 1). Although so many changes occur during cold-acclimation, soluble sugars have been confirmed to play an important role based on the fact they are most commonly detected in various species of land plants that have undergone seasonal cold acclimation, including angio-sperms, gymnosperms, and lower land plants such as bryophytes (Saka and Larcher, 1987; Rutten and Santarius, 1992). It has been reported that under natural conditions, soluble sugars increase during the onset of winter when plants are subjected to low temperatures;

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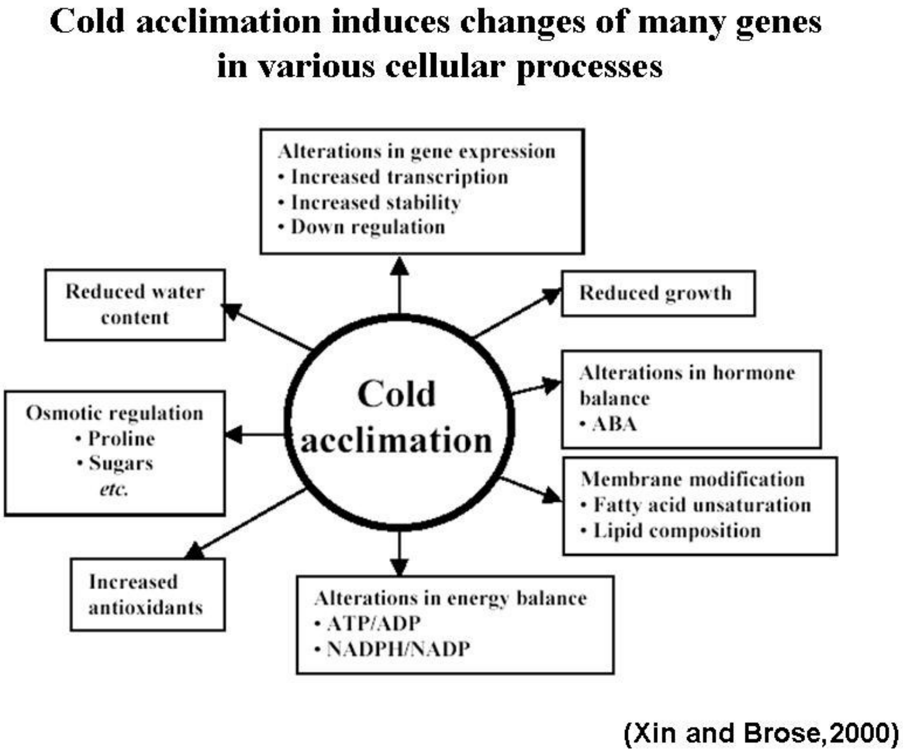
Cold acclimation induces changes of many genes in various cellular processes

(Xin and Brose,2000)

Figure 1. The well known changes during the process of cold acclimation.

conversely, soluble sugars decrease in spring when plants are deacclimating (Sakai, 1962; Siminovitch, 1981). Sugar content also increases during cold acclimation under artificial conditions (Koster and Lynch, 1992; Sasaki et al., 1996; Shao et al., 2006-2008).

DIFFERENT SUGARS INVOLVED IN THE COLD ACCLIMATION

It has been proved that many kinds of soluble sugars are included in the course of cold acclimation. For instance, European habitats which possess substantial degrees of freezing tolerance have exhibited higher degrees of freezing tolerance in winter than in summer. Increase in freezing tolerance in these mosses appeared to be associated with accumulation of soluble sugars, especially that of sucrose (Rutten and Santarius, 1992). Also, chilling of muskmelon plants led to the accumulation in the source leaves of large amounts of starch, sucrose, and hexoses, as has been reported previously for many plant species (Paul, 1991; Paul, 1990). In addition, it has been demonstrated that the levels of a few oligosaccharides increase during cold acclimation, typically in cereals and woody plants, both of which develop high degrees of freezing tolerance in winter. Galactosyl-sucrose oligosaccharides such as raffinose and stachyose are commonly found to accumulate during seasonal cold acclimation in woody plants, along with development of freezing tolerance to levels so high that some plants can even tolerate to freezing in liquid nitrogen without cryoprotectants (Sauter et al., 1996).

VARIOUS STUDIES INVOLVED IN THE EFFECT OF SOLUBLE SUGARS

Aside from the accumulation in endogenous sugars, it has been revealed that exogenous application of soluble sugars resulted in an increase in freezing tolerance in various higher plant species. There has been report that pre-incubation with glucose increased survival after cryopreservation of cabbage leaf cells (Jitsuyama et al., 1997), and pre-incubation with sucrose enhanced survival Jerusalem artichoke cells after cryopreservation (Harris et al., 2004). Moreover, improved freezing tolerance in *Arabidopsis thaliana* protoplasts occurred after the exogenous application of sucrose (Uemura et al., 2003; Li et al., 2006).

Up to now, researchers have isolated several mutant lines of *Arabidopsis* that are impaired in their freezing tolerance after cold acclimation, such as the sfr (sensitivity to freezing) mutants. These mutants have provided researchers a good insight into the relationship between the sugars and freezing resistance in the plants. The freezing tolerance in these plants before cold acclimation is unaffected which allows the freezing sensitivity of the sfr mutants to be specifically attributed to defects in the acclimation process, rather than generalized debilitation. Using electrolyte leakage assay, the quantitative examination of freezing tolerance of all the sfr mutants was achieved. The results showed all the sfr mutants actually increased their freezing tolerance during cold acclimation, but to a less extent than the wild type. The deficit was greatest in the sfr4 mutant, according to its high degree of sensitivity in survival tests on intact plants (Warren et al., 1996). Subsequently, it was detected that sfr4, alone among the mutants, showed levels of freezing tolerance similar to those of wild-type (WT) plants when grown at ambient temperatures but, unlike WT plants, underwent a reduction in its Suc and Glc contents, in contrast to the increase shown by wild type during cold acclimation (McKown et al., 1996; Uemura and Steponkus, 2003).

In addition to these mutants, there have existed many studies indicating that transgenic plants over-accumulating raffinose have higher degrees of freezing resistance than do wild-type plants (Taji et al., 2002; Pennycooke et al., 2003). Perennial ryegrass plants which were transgenic to express wheat fructosyltransferase genes accumulate increased amounts of fructan and acquire enhanced resistance on a cellular level to freeze (Hisano et al., 2004). It has also been reported that the *Arabidopsis* ecotype C24, which accumulates more raffinose than does the Columbia ecotype, shows greater freezing tolerance than that of the Columbia ecotype (Klotke et al., 2004).

THE FUNCTIONAL ROLES OF SOLUBLE SUGARS DURING COLD ACCLIMATION

All of these facts suggest critical roles for these sugars as compatible solutes in freezing tolerance. The mechanisms by which sugars act to influence freezing resistance are just beginning to be deciphered. Sucrose glucose, fructose and oligosaccharides such as raffinose as well as stachyose are commonly found in soluble sugars that accumulate along with development of freezing tolerance in higher plants. It has been suggested that these sugars not only serve as osmoprotectants but also play a role in protecting cellular membranes from damage caused by dehydration and freezing through interacting with the lipid bilayer (Anchordoguy et al., 1987; Shalaev and Shalaev, 2001; Shao et al., 2006). It is also proposed that the role of some oligosaccharides is to prevent crystallization of sucrose, thus facilitating glass formation within the cell, leading to protection of membrane phospholipids (Anchordoguy and Leopold, 1989; Crowe et al., 1988). Generally speaking, the accumulation of soluble sugars contributes to the increase in the cryostability of cellular membranes. Increased membrane cryostability is a prerequisite for freezing tolerance because freeze-induced destabilization of cellular membranes is the primary cause of injury in plants (Steponkus et al., 1993; Shao et al., 2008).

An additional role of sugars during cold acclimation is that they may function as nutrients which make plants survive the decreased temperature and dryland conditions as well as recover from freezing-stress (Eagles et al., 1993; Trunova; 1982; Shao et al., 2008).

MORE FOCUS ON THE ROLES OF SOLUBLE SUGARS AS SIGNALING MOLECULES

Although there have been many functional soluble sugars that act as documented above, nowadays, more and more attention has been put on the important hormone-like functions of soluble sugars as primary messengers in signal transduction. The pivotal role of sugars as signaling molecules is well illustrated by the variety of sugar sensing and signaling mechanisms discovered in free-living microorganisms such as bacteria and yeast (Rolland et al., 2001; Stulke and Hillen, 1999; Wang et al., 2008; Xiao et al., 2009).

Based on the biochemical, molecular, and genetic experiments, it has been concluded that soluble sugars play a central role in the control of plant metabolism, growth, and development. Moreover many researches have revealed interactions that integrate light, abiotic and biotic stress, and hormone signaling (Roitsch, 1999; Sheen, 1999; Smeekens, 2000; Gazzarrini and McCourt, 2001; Finkelstein and Gibson, 2002) and coordinate carbon and nitrogen metabolism (Stitt and Krapp, 1999; Coruzzi and Bush, 2001; Coruzzi and Zhou, 2001; Uddin et al., 2008; Wang et al., 2008; Xiao et al., 2009).

Abiotic and biotic stress stimuli, such as drought, salinity, wounding, and infection by viruses, bacteria, and fungi, can modulate source-sink activities. Because extracellular invertase, a key enzyme for hydrolyzing Suc (Sturm, 1999), is regulated by stress stimuli and hormones, it has been proposed to be a central modulator of assimilate partitioning, integrating sugar, stress, and hormone signals (Roitsch, 1999). In addition, many jasmonate-, ABA-, and stress-inducible genes are co-regulated by sugars (Reinbothe et al., 1994; Sadka et al., 1994; Shao et al., 2005-2008; Uddin et al., 2008; Wang et al., 2008).

How plants sense cold is unknown. A fall in temperature can reduce the fluidity of membranes, and this appears to be an effective direct sensor of cold in cyanobacteria. Another possibility is that, in light, photosynthetic cells may sense cold through an effect on photosystem 2 excitation pressure (Gray et al., 1997; Huner et al., 1998). Molecular studies have shown that cold acclimation in higher plants is complex involving multiple regulatory pathways (Fowler and Thomashow, 2002), furthermore while some of the signal transduction pathways interact, others are independent (Xin and Browse, 2000). Thus more than one sensor of cold is possible (Figure 2).

As mentioned above, sugar-sensing and sensing of the environment appear to be part of a complex regulatory web (Gibson, 2000). In relation to cold, high sugar supply

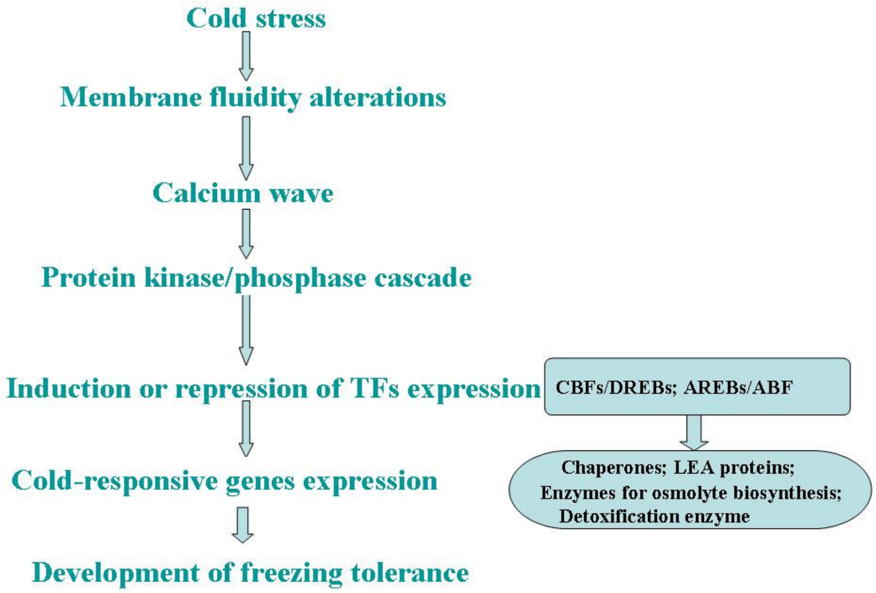

Figure 2. Sugars-involved into the events occurring in plants under cold stress (Shao et al., 2008).

induces fructan accumulation, which is also a usual response of grasses to cold (Winter, 1994; Wang et al., 2008; Xiao et al., 2009).

New insights into the mechanisms by which sugar-response pathways interact with cold response pathways have been supported by micro-array experiments examining sugar-regulated gene expression under different conditions. Based on the fact, it has been elucidated that many sugar-regulated genes are involved in the cold-induced gene expression.

Cold acclimation is accomplished by the expression of many cold-regulated genes (Thomashow, 2001; Zhu, 2001). In *Arabidopsis*, these genes are called rd (responsive to dehydration), erd (early responsive to dehydration), lti (low-temperature induced), kin (cold induced) and cor (cold-regulated). These genes are also induced by dehydration (due to water deficit or high salt) and ABA, and can be collectively called cold-responsive genes. Cold-responsive genes are regulated through C-repeat/dehydration-responsive elements (CRT/DRE) and abscisic acid (ABA)-responsive elements (cis elements), C-repeated binding factors/dehydration-responsive element binding proteins (CBFs/DREBs) and basic leucine zippers (bZIPs) (SGBF1), respectively. It has been reported that expression of key regulators of abiotic stress responses such as CBF3, COR15A, and RD29A was induced by both glucose and ABA. Constitutive expression of CBF3 in transgenic *Arabidopsis* plants induces expression of target COR (cold-regulated) genes to enhance freezing tolerance in non-acclimated plants. Expression of COR15A and RD29A is regulated by CBF3, suggesting that glucose may contribute to the regulation of cold stress tolerance (Yunhai et al., 2006; Uddin et al., 2008; Wang et al., 2008).

THE INVOLVEMENT OF SUGARS IN LEAF SENESCENCE

Soluble sugars help ensure an adequate supply of building materials and energy to carry out specific developmental programs. For example, *in-vivo* and *in-vitro* experiments suggest that in some plant species, increasing sugar levels delay seed germination and stimulate the induction of flowering and senescence. In other words, it may be beneficial for plants to adjust the timing with which nutrient-intensive events occur to supply enough materials and energy for successful completion of those events. Levels of sugars, such as sucrose, have been postulated to affect the timing at which some plant species flower (Bernier et al., 1993). Soluble sugar levels have also been shown to affect other phase changes, such as the onset of senescence (Paul and Pellny, 2003). Endogenous leaf sugar levels tend to increase during senescence. Similarly, application of exogenous sugars stimulates the early stages of senescence.

Leaf senescence is the last step in the life cycle of plants. During the process, materials used to build up leaves during vegetative growth are remobilized and transported into the developing organs (Smart, 1994; Smart et al., 1995; Williams et al., 1997).

Although senescence occurs in an age-dependent manner in many species (Noode`n, 1988), its initiation and progression can be modulated by a variety of environmental factors such as temperature, mineral deficiency, and drought conditions, as well as by internal factors such as plant growth regulators (Grbic and Bleecker, 1995; Buchanan-Wollaston, 1997; Nam, 1997; Weaver et al., 1997; Dai et al., 1999; Uddin et al., 2008; Wang et al., 2008).

Since senescence can be induced by a large number of different conditions, and several signaling pathways are involved in the regulation of senescence (Buchanan-Wollaston, 2003), it is possible that environmental signals are integrated by sugar signaling. For example, experiments on sugar regulated senescence indicate that leaf senescence can be induced by growing *Arabidopsis* plants in the presence of 2% glucose in combination with low nitrogen supply (Pourtau et al., 2004; Wingler et al., 2004). Affymetrix GeneChip data confirmed that changes in gene expression during glucose-induced senescence are characteristic of developmental senescence. Similarly, Stessman et al. (2002) found that hexoses accumulate in senescing *Arabidopsis* leaves. However, the question remains as to what causes the strong accumulation of hexoses despite the decline in photosynthetic carbon assimilation in senescing leaves. A possible source of hexoses is the breakdown of starch. In addition, Jongebloed et al. (2004) showed that phloem blockage by callose deposition could lead to an age-dependent sugar accumulation.

THE DUAL ROLES OF SUGARS POSSIBLY INVOLVED IN ENCOUNTERING COLD STRESS

During cold acclimation, soluble sugars accumulated which could theoretically have adverse impact on photosynthesis as well as induce senescence. Nevertheless, cold-acclimated leaves do not show the typical repression of photosynthesis by sugars (Strand et al., 1997; Li and Huang, 2008). On the other hand, it has been hypothesized that photosynthetic activity is regulated in a feedback manner by soluble sugars. The photosynthetic machinery might be down-regulated by sugars due to the cold-girdling petioles to prevent sugar export out of the leaf. Also, in these cases, genes related to photosynthesis were repressed, including those for chlorophyll binding protein and Rubisco (Smeekens, 2000).

However more complicated it is, there have been studies showing increased flux through the Suc biosynthetic pathway that reduces inhibition of photosynthesis in the cold (Strand et al., 2003). It was therefore conceivable that cold acclimation could interact with metabolic regulation of senescence by inhibiting the induction of senescence by sugar accumulation (Zhao et al., 2008; Shao et al., 2008; Bressan et al., 2009).

CONCLUSION AND PERSPECTIVES

Alterations in the levels of soluble sugars, such as glucose and sucrose have been shown to affect the freezing tolerance in the plants. What roles of sugars play during cold acclimation is complicated by the fact that sugars can act by affecting osmotic potentials as well as by functioning as signaling molecules. On the other hand, sugars affect developmental programs ranging from embryogenesis to senescence, which makes the research more complex. On the basis of the fact that leaves which have developed in the cold do not display the typical down-regulation of photosynthesis in response to sugar accumulation, it is possible that induction of the cold acclimation pathway have resulted in decreased sugar sensitivity and thus delayed senescence.

Further studies about the actual roles that sugars play in plants' response to cold stimuli can be done by scanning lists of sugar-regulated genes expression under cold condition using various advanced technologies such as microarray experiments. On the basis of these research investigations, we might get new insight into mechanisms by which sugar-response pathways interact with other response pathways in the cold-triggered response. At the same time, we will have a promising perspective by bioengineering plant responses to cold stress by sugar functions.

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