

Review article.

## **Sucrose Synthase and Nodule Nitrogen Fixation under Drought and Other Environmental Stresses**

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### **Abstract**

Nitrogen fixation in legume root nodules is greatly influenced by their water status. The decline of nitrogen fixation under water stress was originally related to a photosynthate shortage, but it was then shown that the decline in nitrogen fixation preceded the decrease in photosynthesis. For some time it was thought that an increase in the oxygen diffusion resistance in nodules could account for the diminished nitrogen fixation. However, recent research has supported the idea of a restricted metabolic capability of nodules when they are subjected to drought. The evidence presented here supports the hypothesis that reduced metabolic capacity is due to a decline in sucrose synthase activity, leading to an accumulation of sucrose in nodules and a depletion of substrates for bacteroid respiration. It is not yet clear whether this leads to a closure of the oxygen diffusion barrier in order to balance the oxygen flux and avoid nitrogenase damage or if both processes are concomitant. The likely involvement of sucrose synthase in the response of nodules to environ-

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mental stresses other than drought is addressed. Future prospects for research on nodule sucrose synthase and its implications for nitrogen fixation are presented.

Keywords: Carbon metabolism, drought, environmental stresses, nitrogen fixation, nodules, sucrose synthase

## 1. Introduction

Water availability, nitrogen availability and temperature are the major factors controlling the distribution of vegetation over the earth's surface. Also, crop yields are, arguably, more dependent on an adequate supply of water than on any other single factor (Kramer and Boyer, 1997). Even plants with an optimum water supply experience transient water shortage periods, where water absorption cannot compensate for water loss by transpiration; a situation that largely depends on environmental factors such as temperature, relative humidity and wind speed. In addition, many other environmental stresses, such as cold, salt and high temperature, have a water-stress component. Hsiao (1973) summarised the sequence of events that occurs when water stress develops, with cellular growth being the most sensitive response, followed by a wide range of biochemical and physiological events as water potentials become more negative. Events occurring later on during the water stress period or at very negative water potentials, are often indirect responses to earlier events rather than direct responses to water stress itself. This picture is further complicated because the sensitivity of some responses is highly dependent on plant species. Furthermore, in the last few years, drought has also been related to the induction of oxidative stress (Morán et al., 1994; Iturbe-Ormaetxe et al., 1998). Intense research on the molecular basis of plant responses to drought has also been carried out over the last few years, but this is outside the scope of this review and other reviews can be found elsewhere (Bray, 1993; 1997; Ingram and Bartels, 1996; Shinozaki and Yamaguchi-Shinozaki, 1997).

Biological nitrogen fixation (BNF) in angiosperms occurs in legumes in symbiosis with the prokaryotic genera *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium*, *Azorhizobium* and *Allorhizobium* (collectively termed as rhizobia); several woody species, in symbiosis with the actinomycetes *Frankia* (actinorhizal plants) and in the genus *Gunnera* in symbiosis with the cyanobacterium *Nostoc*. BNF is commonly overlooked in most overviews on water stress effects on plant functioning. Therefore, this paper will consider BNF in legume/rhizobial associations with particular reference to responses to water and related environmental stresses. Nevertheless, during the publication process of this paper, a review on symbiotic nitrogen fixation responses to drought has been published (Serraj et al., 1999a).

BNF is commonly described as a very sensitive process to a wide range of environmental stresses (Sprent et al., 1988), such as acidic soils (Coll et al., 1989), salinity (Velageleti and Marsh, 1989) and, also, water stress (Kirda et al., 1989). Recently, however, the idea of nitrogen-fixing plants being more sensitive to environmental stresses than those given fertiliser nitrate has been challenged. For example, symbiotic pea is more tolerant to certain herbicides than nitrate-dependent plants (González et al., 1996) and nitrogen fixing plants of both pea (Frechilla, 1994) and lucerne (Antolín et al., 1995) are more tolerant to mild drought than nitrate-reducing plants. Obviously, these comparisons largely depend on the genetic capability of the plant in terms of nitrate reduction and the extent of the adverse effect of drought not only on nitrogen fixation, but also on nitrate transport and nitrate reduction in the particular species.

Moreover, it should be noted that the negative effect of these environmental stresses on BNF is the sum of three different responses: effects on the infection of legumes by rhizobia, effects on nodule growth and development and, finally, direct effects on nodule functioning. Assessment of the latter is complicated by the fact that a large number of studies have been performed with the traditional closed acetylene reduction assay, that has been shown not to give an adequate estimation of nitrogen fixation (Minchin et al., 1983; 1986). Additional sources of disparity among different studies are the above mentioned genotypic differences within species and, also, the different modes of drought application, particularly whether water stress is obtained just by dehydration or by means of an osmoticum, such as polyethylene glycol additions. In the latter case, the imposition of abrupt water stresses may have different physiological consequences compared with those arising from a natural drought (Frechilla et al., 1993).

## 2. Nodule Functioning Responses to Drought

Sprent (1971), using detached nodules, showed that nitrogen fixation in legume root nodules is dependent on their water status and Pankhurst and Sprent (1975) found that increasing  $pO_2$  from 10 to 100 kPa completely restored BNF and respiration in detached soybean nodules. However, the interpretation of such results is complicated because of nodule detachment. Huang et al. (1975a) discounted reduced respiration as the primary reason for the decrease in acetylene reduction activity of water stressed soybean root nodule. The same authors concluded that a lack of assimilates resulting from the reduction in shoot photosynthesis was the primary cause (Huang et al., 1975b). Finn and Brun (1980) also found a parallel decline in nitrogenase and photosynthesis suggesting that inhibition of nodule activity was not caused solely by the

decrease in photosynthesis, but also by changes in photosynthate pools. However, Durand et al. (1987) and Djekoun and Planchon (1991) showed that BNF is more sensitive than photosynthesis to moderate water deprivation: BNF decreases steadily throughout the water deficiency period whilst photosynthesis decreases only slightly for the first days under drought; although, later on in the water shortage period, photosynthesis tends to drop dramatically. Indeed, a severe water stress appears to allow the maintenance of some BNF, whilst photosynthesis disappears almost completely (Djekoun and Planchon, 1991).

The mechanism by which BNF is inhibited by water stress has been explained by a variable oxygen diffusion barrier (Minchin et al., 1983; Sheehy et al., 1983; Hunt and Layzell, 1993). Thus, the current model for nodule response to many environmental stresses, including drought, is that the stress triggers the closure of the oxygen diffusion barrier. This is thought to provoke a decrease in the availability of oxygen which leads to reduced bacteroid respiration and, therefore, a decline in nitrogen fixation. The mode of operation of such a barrier at the cellular and molecular levels remains unknown, although there is now considerable evidence for its existence (cf. Witty and Minchin, 1998). Its operation in the nodule cortex has often been related to changes in the gaseous path of oxygen from the atmosphere to the infected region (Witty et al., 1986). Hypotheses for such changes include variation in osmolite concentration, leading to alteration in cell shape and blockage of intracellular cell spaces (Hunt et al., 1990), the presence of a glycoprotein located in the intercellular spaces of the nodule cortex (James et al., 1991), and respiratory consumption of oxygen in the endodermis or inner cortex, associated with the presence of high levels of antioxidants (Dalton et al., 1998). Recently, the occurrence of osmocontractile cells in the inner cortex, similar to those found in stomata and the pulvinus, has been suggested to control oxygen conductance in nodules by reversible exchange of intercellular water (Serraj et al., 1995; Drevon et al., 1998). This latter suggestion finds experimental support in the fact that a putative aquaporin, found in the tonoplast of the pulvinus motor cells, is particularly abundant in the tonoplast of the inner cortical cells of the nodules (Serraj et al., 1998). An attempt has recently been made to integrate these various proposed components of the barrier within a temporal framework; termed the rolling wave hypothesis (Minchin, 1997).

Whilst it is clear that water stress causes an increase in oxygen resistance in nodules (Durand et al., 1987), it is unclear whether such changes are ultimately responsible for the decline in BNF. If nitrogen fixation is only limited by the internal oxygen concentration of the nodules (eg. Layzell et al., 1990), it should be fully recoverable by elevating rhizosphere  $pO_2$ . However, it has been recently shown that BNF in water stressed nodules cannot be restored simply by increasing oxygen concentration (Guerin et al., 1990; Diaz del Castillo et al.,

1994). Also, Purcell and Sinclair (1995) showed that nitrogen fixation was inhibited by water-stress imposed by PEG-6000 before changes in nodule permeability could be detected. However, in another paper, Serraj and Sinclair (1996) found that nitrogen fixation and nodule permeability decreased concomitantly.

These results lead to the suggestion of a reduced metabolic capability of nodules subjected to water stress. Our objective in this review is to summarise recent research on sucrose synthase activity in nodules and show how a decline in this activity may, in turn, lead to a reduced nitrogen fixation (Fig. 1). For this purpose, an update of nodule functioning is outlined.

### 3. Carbon Metabolism in Nodules

Optimal nodule functioning depends on an appropriate combination of carbon metabolism, which provides energetic substrates and carbon skeletons for N assimilation, nitrogen metabolism for assimilation of fixed N, and the flux of oxygen, which functions as the terminal electron acceptor in the electron transport chain of plant mitochondria and bacteroids, but whose concentration must be low enough to avoid nitrogenase damage.

Nodule nitrogen fixation depends on the supply of sucrose delivered from the phloem, as in other sink tissues in many plants (Avigad, 1982; Hawker, 1985). This sucrose may be hydrolysed by either sucrose synthase (SS) or alkaline invertase (AI), and follow the glycolytic pathway to provide energy and carbon skeletons for bacteroid respiration and ammonia assimilation (Fig. 2). AI hydrolyses sucrose irreversibly to produce glucose and fructose, whilst SS catalyses the readily reversible reaction:



The enzyme is thought to function primarily in the direction of sucrose cleavage in plant sink tissues supplied with ample sucrose substrate and with a high demand for carbon in biosynthetic and respiratory pathways (Winter et al., 1997).

Based on enzyme kinetic data, Morel and Copeland (1984, 1985) concluded that AI seemed to have a predominant role in providing hexoses from sucrose, with SS activity being related to the production of UDP-glucose for the formation of other nucleotide sugars and polysaccharides, such as starch and cellulose. Moreover, Copeland et al. (1989) found no SS activity in the central region of soybean nodules and only a small amount in the cortex, which seemed to dismiss a key role for this enzyme in the metabolism of sucrose within nodules. However, Kouchi et al. (1988), Gordon (1991) and Gordon et al. (1992)

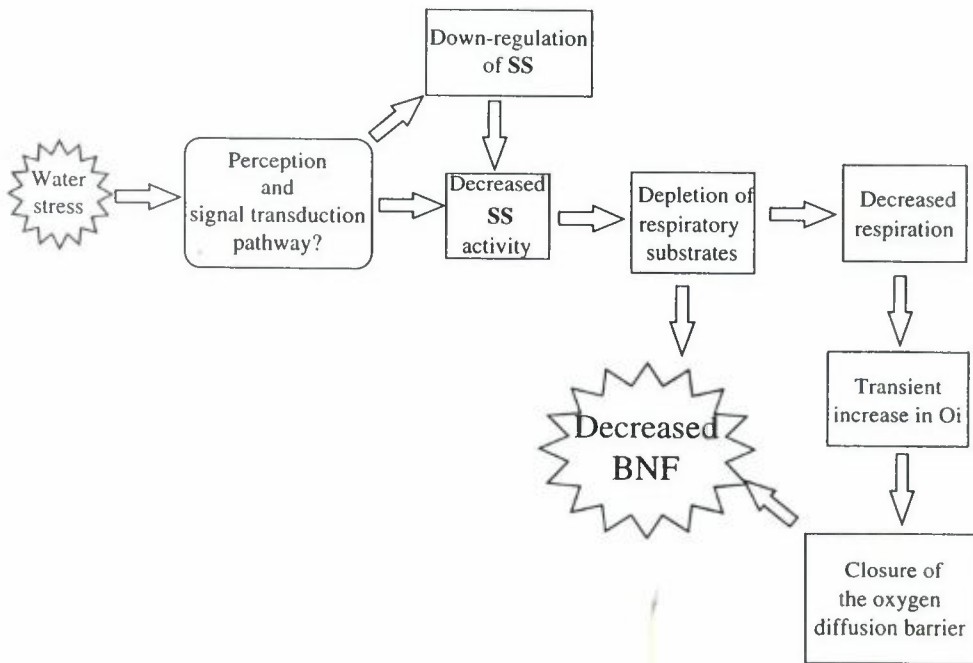


Figure 1. Scheme representing the possible sequence of events leading to a decreased nitrogen fixation in nodules subjected to water stress. The signal transduction pathway linking water stress perception and sucrose synthase activity remains largely unknown. See text for full details.

found substantial SS activity in the central region of soybean nodules and it is now clear that high levels of SS activity are present in the nodules of all legumes so far examined (cf. Gordon and James, 1997). It now appears from work with pea mutants containing only 10% of wild-type SS activity, that SS is essential for normal nodule development and function. Plants with this low level of SS were not able to fix nitrogen (Gordon et al., 1999).

Hexoses formed either by SS or AI are hydrolysed through the glycolytic pathway to obtain phosphoenolpyruvate. Phosphoenolpyruvate carboxylase (PEPC), a cytosolic enzyme which is particularly abundant in nodules (up to 1–2% soluble protein), then catalyses the combination of respiratory  $\text{CO}_2$  (as  $\text{HCO}_3^-$ ) with phosphoenolpyruvate to produce oxaloacetate, which is readily converted into malate by malate dehydrogenase (MDH). This malate can be either used as a source of carbon and energy for bacteroid consumption, or enter the mitochondria and be oxidised in the TCA cycle and contribute to ammonia assimilation in the GS/GOGAT cycle. Dicarboxylic acids, like malate, but not other putative substrates such as  $\alpha$ -ketoglutarate, glutamate, pyruvate and arabinose, are the likely candidates to support nitrogen fixation in nodules. The

latter are poorly transported across the peribacteroid membrane, whilst this membrane is permeable to malate and succinate because of the presence of a dicarboxylate carrier with a high affinity for malate ( $K_m = 2$  mM) and succinate ( $K_m = 15$  mM) (Jording et al., 1994; Udvardi and Day, 1997 and references therein).

As mentioned above, in addition to this pathway, SS activity may be also involved in starch formation. In other plant heterotrophic tissues, SS plays a key role in the sucrose-starch conversion (Pozueta-Romero et al., 1999), through conversion of UDP-glucose to ADP-glucose (see Kleczkowski, 1996, and references therein). However, information is not yet available on the occurrence of a cytosolic ADP-glucose pyrophosphorylase or the ADP-glucose carrier activity of the plastid adenylate translocator in legume nodules. Nevertheless, a transport capacity of pea root amyloplasts has been demonstrated for glucose-1-phosphate, glucose-6-phosphate (Borchert et al., 1993), ATP and ADP-glucose (Schunemann et al., 1993). How much of the UDP-glucose flux obtained from SS activity is allocated to feed the glycolytic pathway or starch formation in nodules (Fig. 2) remains to be determined.

#### 4. Sucrose Synthase and Water Stress in Determinate Nodules

Studies of stress physiology have played an important role in developing an understanding of the functioning of different plant systems. Thus, in order to find primary responses to water stress in soybean nodules, González et al. (1995) produced a progressive depletion of soil moisture by withholding water and, therefore, plants experienced a gradual and mild water stress. They then monitored enzyme activities related to carbon (SS, AI, UDPGG, PEPC and MDH) and nitrogen metabolism (GS and GOGAT) in nodules as well as the levels of leghaemoglobin (Lb), nitrogenase components and nodule metabolites (glucose, fructose, sucrose, starch, amino acids, ureides and proline). Under these conditions, SS was the only enzyme whose activity declined, coincident with BNF (Fig. 3). The decline in activity was directly related to SS protein levels, as determined by Western blot analysis. Furthermore, a time-course study of drought effects on soybean nodules also demonstrated that SS mRNA declines dramatically within 24 h and this effect is reflected in SS activity within 48 h from the onset of water-stress (Gordon et al., 1997).

Sucrose levels increased in these nodules, showing that there was no shoot limitation for BNF and suggesting that the decrease in SS activity leads to an accumulation of sucrose (Fig. 4). Recently, Ramos et al. (1999) have shown, in a drought tolerant cultivar of bean, that sucrose synthase is also down regulated in the short-term, producing a parallel increase in nodule sucrose content. This would lead to a depletion of substrates for bacteroid respiration and would,

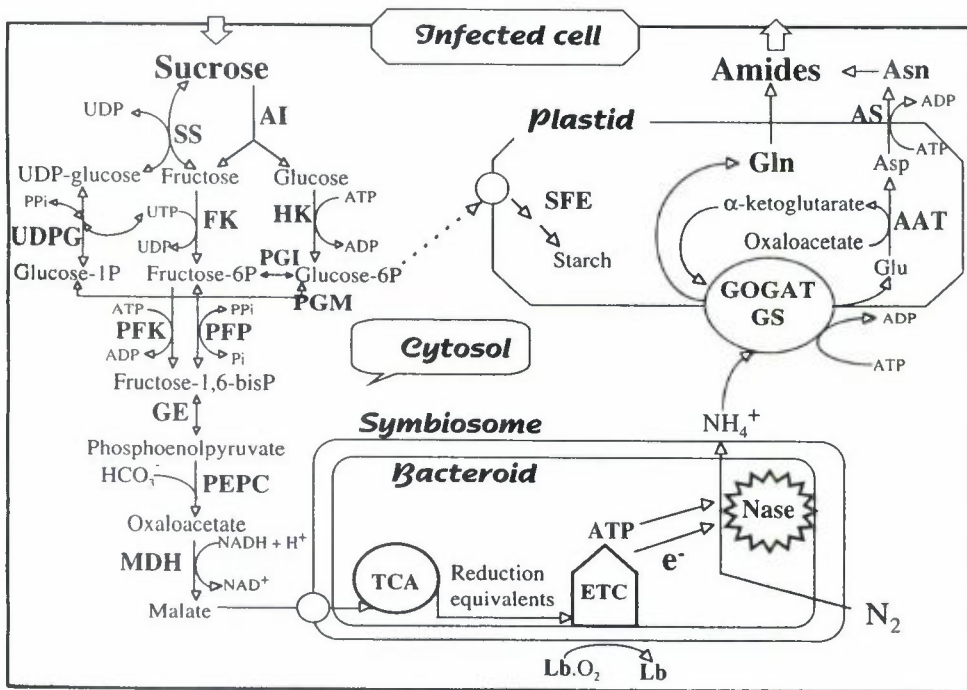


Figure 2. General overview of the carbon and nitrogen metabolism in infected cells of amide exporting legumes. Abbreviations: AAT, aspartate aminotransferase; AI, alkaline invertase; AS, Asn synthetase; ETC, electron transport chain; FK, fructokinase; GE, glycolytic enzymes (aldolase, triose phosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase; phosphoglycerate mutase, enolase); GS/GOGAT, Gln synthetase/Glu synthase cycle; HK, hexokinase; Lb, leghaemoglobin; MDH, malate dehydrogenase; Nase, nitrogenase; PEPC, phosphoenolpyruvate carboxylase; PFK, phosphofructokinase; PFP, fructose-6-phosphate:pyrophosphate phosphotransferase; PGI, phosphoglucose isomerase; PGM, phosphoglucomutase; SFE, starch-forming enzymes (PGM, ADP-glucose pyrophosphorylase, starch synthase); SS, sucrose synthase; TCA, tricarboxylic acid cycle; UDPG, UDP-glucose pyrophosphorylase. o denotes membrane carriers, such as the symbiosome dicarboxylate carrier and the putative hexoses phosphate carrier or adenylate translocator in plastids.

in turn, induce a transient accumulation of oxygen in the infected region, leading to a closure of the oxygen diffusion barrier. Both the depletion of respiratory substrates and the concomitant closure of the oxygen diffusion barrier would cause the observed decline in BNF (Fig. 1).



## 5. Is the Role of Sucrose Synthase also Crucial in Indeterminate Nodules under Water Stress?

Despite the fact that carbon metabolism is apparently the same in determinate and indeterminate nodules (Gordon and James, 1997), Sinclair and Serraj (1995) found greater sensitivity to water stress in ureide exporting legumes, with determinate nodules, as compared to amide exporters, with indeterminate nodules. Differences could occur between determinate and indeterminate nodules because of different vascular bundle arrangements (generally anastomosed in soybean *versus* free-ended in pea) and other anatomical features (see Brown and Walsh, 1994). However, the most important differences are probably in the characteristics of the nitrogen compounds exported, the biochemical machinery to synthesise nitrogen-export products and, therefore, the chemical interchanges between cortical cells.

Other workers have suggested that early responses to water stress in indeterminate nodules are due to a protease-induced reduction in Lb content, which would limit oxygen supply to the bacteroids and affect respiration and energy production (Guerin et al., 1991; Irigoyen et al., 1992). It has also been argued that water stress could lead to the occurrence of transient anaerobic conditions within indeterminate nodules and this oxygen limitation would lead to an enhanced anaerobic metabolism, involving alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) (Irigoyen et al., 1992).

However, in a study of mild drought stress in pea (González et al., 1998), SS activity was also correlated with nodule water potential, while Lb content was not affected and there was no enhancement of anaerobically inducible enzymes (such as PDC and ADH). As with determinate nodules (González et al., 1995; Gordon et al., 1997), the decrease in SS activity was due to a decline in SS protein, as monitored by immunoblotting. Data drawn from three independent experiments, involving three species, indicates a correlation between nitrogen fixation and nodule SS activity (Fig. 3).

## 6. A Role for Sucrose Synthase in the Response to Other Stresses

The key role of SS in nodule metabolism under drought could also be relevant under other environmental stresses. Thus, salinity has been reported to produce an accumulation of sugars and a depletion of malate in pea nodules (Delgado et al., 1993) and recently it has been shown that salinity actually caused a decrease in SS activity in lupine nodules (Fernández-Pascual et al., 1996).

SS activity and transcript levels both decline significantly in nodules of soybean plants subjected to dark (Gordon et al., 1993), which suggests that SS gene expression can be controlled by environmental factors and, in turn, regulate

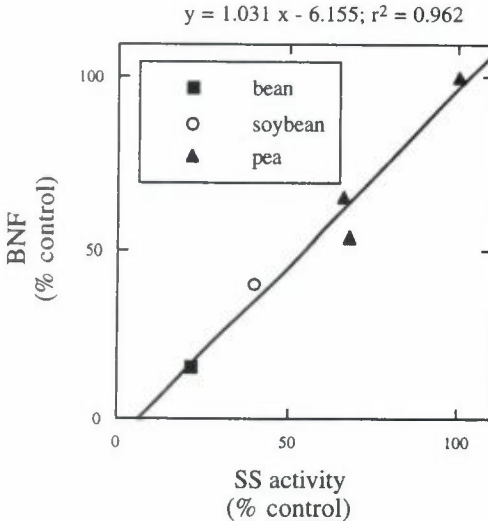


Figure 3. Relationship between nitrogen fixation, determined as acetylene reduction with a flow-through system and expressed as percentage of control nodules ( $\mu\text{mol C}_2\text{H}_4 \text{ g}^{-1} \text{ nodule DW min}^{-1}$ ), and sucrose synthase activity, also expressed as percentage of control nodules ( $\mu\text{mol product mg}^{-1} \text{ nodule protein min}^{-1}$ ), of nodules subjected to water stress. Data are redrawn from González et al. (1998), Gordon et al. (1997) and Ramos et al. (1999).

the carbon flux through nodules and, therefore, nitrogen fixation. Indeed, the likely involvement of SS in the response of soybean nodules to a wide range of abiotic factors has been further confirmed by Gordon et al. (1997); where it is shown that nitrate, salt and drought all cause a decline in SS activity that is directly correlated with nitrogen fixation. As mentioned earlier, SS mRNA declined dramatically within 24 h of withholding water and this effect was reflected in lower SS activity within 48 h from the onset of water-stress. In conflict with this hypothesis, however, time-course studies of nitrate effects on soybean nodules show nitrogenase activity decreasing within 18 h and SS mRNA virtually disappearing within 24 h, but SS activity did not start to decrease until 3 d (Gordon, Skøt and Minchin, unpublished data). Furthermore, in *Arabidopsis thaliana*, where SS is encoded by two genes *Sus1* and *Sus2* (Déjardin et al., 1999), *Sus1* gene expression is correlated with osmotic potential rather than any particular sugar, and therefore, was also correlated with cold, salt and water-stress. However, these changes in gene expression were not correlated with SS activity (Déjardin et al., 1998).

## 7. Sucrose Synthase versus Alkaline Invertase

Despite the kinetic properties of the enzymes involved in sucrose cleavage (Table 1) and the secondary role for SS suggested by Morell and Copeland (1985), sucrose levels in nodules are commonly in the range 2 to 55 mM (Kouchi and Yoneyama, 1984; Streeter, 1987; González et al., 1995; Gordon et al., 1997), although they can reach 130 mM in water stressed pea nodules (González et al., 1998). Thus, assuming similar amounts of AI and SS in nodules (Gordon, 1991; González et al., 1995; Gordon and James, 1997), SS activity could be responsible for from 37% (lower sucrose levels) to 59% (higher sucrose levels) of the sucrose flux in nodules.

In soybean nodules, the supply of 1-fluorosucrose, which is an extremely poor substrate for AI, but can be cleaved by SS (Hitz et al., 1985), suggests that, at least in young nodules, SS is almost entirely responsible for sucrose metabolism and even represents 60% of sucrose metabolism in older nodules (Harrison, 1995), despite the predicted catalytic constants. Moreover, studies using mutant peas, in which SS activity, but not AI, is greatly reduced, clearly demonstrate that SS is essential for N<sub>2</sub> fixation (Gordon et al., 1999). The extent of sugar import into many plant organs has been correlated with SS activity (Claussen, 1983; Sung et al., 1988; Nguyen-Quoc et al., 1990) and additional evidence for the crucial role of SS for sink strength in the potato tuber has been recently produced using transgenic tubers with SS activity reduced by antisense inhibition (Zrenner et al., 1995). Therefore, SS appears to be the key enzyme of sucrose hydrolysis in nodules and many other heterotrophic tissues (Heldt, 1997).

The SS route is apparently favoured in many instances, possibly because it only requires half the net energy of the alternate pathway through invertase (Black et al., 1987). This has also been suggested for nodules by Gordon (1992), who estimated the net production or utilisation of ATP depending on whether

Table 1. Kinetic properties of the enzymes involved in sucrose cleavage in legume nodules

	Sucrose synthase	Alkaline invertase
V (U mg <sup>-1</sup> protein h <sup>-1</sup> )	13.3 ± 2.0	8.0 ± 1.0
K <sub>m</sub> (sucrose) (mM)	31.3 ± 7.1	10.2 ± 1.1
K <sub>i</sub> (sucrose) (mM)	31.9 ± 13.1	nd
K <sub>m</sub> (UDP) (μM)	5.0 ± 2.0	na
K <sub>i</sub> (UDP) (μM)	5.0 ± 2.0	na

nd: not determined; na: not applicable. Data are from Morell and Copeland (1984; 1985).

sucrose is metabolised by SS or AI. Although calculations depend on which is the actual pathway involved in malate formation (see Fig. 2), it appears that in every case metabolism via SS is energetically more favourable than AI. Although the amount of ATP appears to be trivial, this may be crucially important in nodule infected cells, where ATP production during mitochondrial oxidative phosphorylation is severely impaired due to limited oxygen availability. Indeed it has been suggested that in stress conditions the P/O ratio may be even more adverse (de Lima et al., 1994; Kuzma et al., 1995; 1999).

## 8. Biochemical and Molecular Regulation of Sucrose Synthase

There is still little biochemical and molecular information on nodule SS as compared to other plant systems, such as cereal endosperms and potato tubers. At the biochemical level, it is known that nodule SS is a homotetrameric enzyme, which consists of four subunits with a molecular weight of approximately 92 kDa (Morell and Copeland, 1985; Küster et al., 1993). In general, it is thought that the regulation of SS activity involves the control of the steady state level of enzyme protein (Nguyen-Quoc et al., 1990; Koch et al., 1992) and the concentration of hexose sugars, which inhibit the cleavage reaction *in vitro* (Doehlert, 1987). However, it has been suggested that disulfides may reversibly inhibit SS in the cleavage direction (Pontis et al., 1981) and Thummler and Verma (1987) have suggested that SS activity may be regulated by free heme in nodules.

In addition, it has recently been shown that SS can be phosphorylated in maize leaves (Huber et al., 1996) and soybean nodules (Zhang and Chollet, 1997), and it has been proposed that SS activity could be regulated *in vivo* by phosphorylation, leading to a reversible modification of the affinity for sucrose (Huber et al., 1996). However, a reassessment of the phosphorylation effects on SS activity with the phenylglycoside arbutin shows that phosphorylation has little effect on its activity, but decreases its surface hydrophobicity, which may promote changes in its membrane association (Winter et al., 1997). Thus, the phosphorylation of the membrane-associated enzyme causes release from the membrane, whilst dephosphorylation of the soluble enzyme promotes membrane-association. It is tempting to speculate that phosphorylation may have effects not on the regulation of catalytic activity, but on the physiological role being played by the enzyme. For example, membrane-bound forms would readily supply UDP-glucose for cellulose synthesis, and the occurrence of a SS-glucan synthase complex has been postulated (Amor et al., 1995). Furthermore, very recently, it has been shown that the cytosolic phosphorylated SS is either free in the cytosol or possibly associated with the cytoskeleton (Winter et al., 1998). Binding to actin could

function to regulate the activity or provide a medium for coordination of different enzymes in the same pathway, but the physiological significance of such binding remains unknown.

At the molecular level, it is known that in monocots, there are two forms of SS (SS1 and SS2), encoded by two genes (*Sh1* and *Sus1*, respectively), whilst it has been thought until recently that there is a single copy gene in dicots, including legumes (Küster et al., 1993). Both monocot SS forms show a marked biochemical resemblance. Their kinetic constants and nucleotide specificities are very similar and their protein structures closely related (Echt and Chourey, 1985). However, there are some differences in the regulation of their expression by carbohydrate levels (Koch, 1996). Thus, in maize, the *Sh1* gene is expressed maximally when the supply of metabolizable sugars is limited, and the corresponding mRNA persists during carbohydrate starvation stress in root tips, and is enhanced at key sites and times during reproductive development (Koch et al., 1992). In contrast, the *Sus1* gene responds positively to abundant carbohydrate supply and is expressed in a broad range of importing tissues (Koch, 1996).

For nodules, Küster et al. (1993) isolated the first full length cDNA encoding a nodule enhanced SS from *Vicia faba*, which was expressed 10–20 fold more strongly in nodules than roots, and recently the full length cDNA of soybean nodules has also been cloned (Zhang et al. 1997). Very recently it has emerged that several dicots species also have more than one SS gene. *Solanum tuberosum* has two genes (Fu and Park, 1995) and in *Pisum sativum* there appear to be two in the seed coat; *PsSucS1* (Déjardin et al., 1997) and *PsSucS2* (Buchner et al., 1998), which are only 70% identical to each other on the amino acid level. For peas, *PsSucS1* appears to be the nodule enhanced gene. The *Medicago truncatula* nodule gene *MtSucS1* has homologies to both monocot SS genes: 76% to *Sh1* and 75% to *Sus1* (Hohnjec et al., 1999). Therefore, it is difficult to use genetic homology to predict nodule SS behaviour in response to carbohydrate content.

## 9. A Reassessment of the Physiological Regulation of Sucrose Synthase

SS may be regulated by a number of physiological factors in nodules and a linear, inverse correlation between SS activity and sucrose concentration can be found in nodules subjected to drought (Fig. 4). However, despite the carbohydrate-modulated regulation of SS found in plants (Koch, 1996), SS inhibition in nodules appears to be the cause of sucrose accumulation and not the reverse: time-course analysis of SS activity and sucrose accumulation in water stressed pea nodules shows that the decline in SS activity precedes any change in sucrose levels (see Figs. 3A and 5A in González et al., 1998).

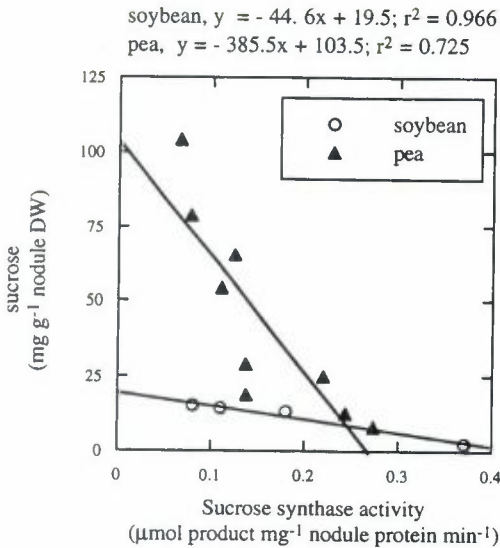


Figure 4. Linear correlation between sucrose synthase activity and sucrose accumulation in nodules subjected to water stress. Data are redrawn from González et al. (1995) and González et al. (1998).

In other plant systems, SS is known to be induced by low oxygen environments (Taliercio and Chourey, 1989; Ricard et al., 1991; Xue et al., 1991). However, in nodules, oxygen levels are already extremely low and decline still further during stress. This further decline does not enhance expression of SS but is correlated with a decline in SS transcription. It is possible that SS gene expression in nodules is regulated by oxygen in different ways to those quoted above. This appears to be the case for dissimilatory nitrate reductase in free living and symbiotic bacteria. Thus, activities that are expressed in anaerobic or microaerobic conditions, such as those related to denitrification, are fully expressed at 15  $\mu\text{M}$  oxygen in free-living denitrifiers (Körner and Zumft, 1989), with no further increase at lower oxygen concentrations. However, in bacteroids of lucerne nodules, dissimilatory nitrate reductase activity can be detected at an internal  $\text{O}_2$  concentration ( $\text{O}_i$ ) of 40 nM, but activity is enhanced by circumstances that further decrease  $\text{O}_i$  (Arrese-Igor et al., 1992, 1993).

Another possibility, following the suggestion made by Déjardin et al. (1998), is that SS activity may be regulated by water potential. It is known that SS activity in nodules is strongly correlated with nodule water potential during drought (Fig. 5) and that SS activity increases again if plants are re-supplied with water. However the regulatory mechanism linking water potential and gene expression is unknown at present.

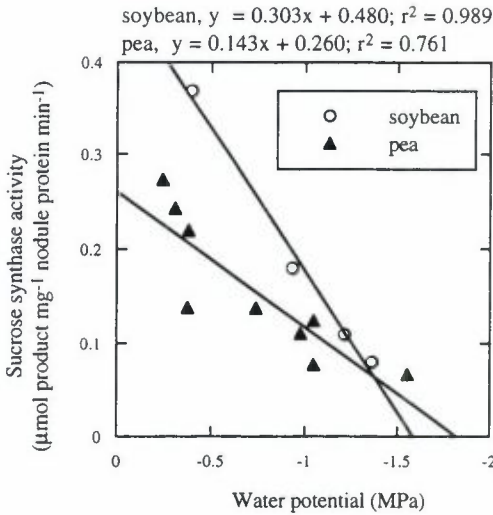


Figure 5. Sucrose synthase activity dependence on  $\Psi_w$  in nodules. Data are redrawn from González et al. (1995) and González et al. (1998).

Another putative compound to be involved in the signal transduction pathway linking water-stress and the down-regulation of SS is the plant hormone abscisic acid (ABA). ABA is the major transduction signal operating during drought stress in plants. However, experiments with exogenously supplied ABA to pea nodules show that SS activity is insensitive to ABA, whilst other common responses to water-stress in nodules, such as reduced Lb content, are triggered by ABA (E. M. González, L. Gálvez and C. Arrese-Igor, unpublished results).

## 10. Is Sucrose Synthase Activity Regulated by the Whole Plant?

Although the accumulation of sucrose under water stress could be considered as an osmo-protection mechanism for nodule functioning, as proposed for other tissues under water stress (Koch et al., 1992), it is clear that, in nodules, the depletion of carbon substrates for bacteroids is critical for BNF activity (Fig. 2). Therefore, the down-regulation of nodule SS activity may be more related to a whole-plant adjustment to water stress.

In the last few years the efficiency of nitrogen fixation in nodules has been suggested to depend on the translocation of export compounds (Streeter, 1993) and, therefore, in one way or another on the nitrogen status of the plant

(Hartwig and Nösberger, 1994), with nodule activity being regulated by feedback mechanisms involving phloem nitrogen (Parsons et al., 1993). It has recently been suggested that such a regulation would depend on levels of phloem glutamine (Neo and Layzell, 1997) or asparagine (Serraj et al., 1999b). Such a feedback regulation would also benefit the whole plant carbon balance. Soybean nodules may require a carbon flux of up to  $100 \mu\text{mol sucrose g}^{-1} \text{DW h}^{-1}$  (Walsh et al., 1989), which represent a consumption up to 40–50% of daily photosynthetic products (Gordon et al., 1987). From this consumption, approximately 50% is lost as respired  $\text{CO}_2$  (Ryle et al., 1985; 1986; Gordon et al., 1987). Therefore, when an environmental stress severely impairs the overall plant performance, the down-regulation of SS would decrease nodular sink strength and prevent nodules from becoming an excessive burden on a diminishing plant carbohydrate pool. This scenario may be an evolutionary mechanism developed to aid plant survival under stress.

## 11. Conclusions and Future Prospects

The arguments and papers reviewed here suggest that SS is essential for the metabolism of sucrose in nodules and, in addition, that SS may be a key component in the perception and response to stress, including drought. As such, the regulation of SS gene expression and of SS enzyme activity in the context of stress deserves considerably more research attention.

1. It is not yet clear whether the down-regulation of the SS gene (and as a result the decline in SS content and activity) is the cause or the consequence of the decline in nitrogen fixation under water stress conditions. The results reviewed in this paper provide evidence of a likely regulation of nodule functioning through SS gene expression and SS activity (see Fig. 1), but the possibility still remains that the primary response is a decrease in internal  $\text{O}_2$  levels as a consequence of the closure of the oxygen diffusion barrier. However, experimental evidence suggests that nitrogen fixation cannot be enhanced by exposure to high external  $\text{O}_2$ , which implies reduced metabolic potential. Furthermore, down-regulation of the SS gene as a result of low  $\text{O}_2$  is contrary to the known expression patterns of the SS gene in other plant systems (see earlier discussion). In addition, down regulation of SS during drought does not coincide with an enhancement in the activities of anaerobic enzymes in nodules, which is likely to occur if there is a prolonged period of lower than normal internal oxygen concentrations. The evaluation of SS gene expression in nodules as a means by which the plant regulates BNF, requires the urgent isolation and characterisation of the nodule SS promoter.

2. There is also a gap between the coincident decline in SS activity and BNF and the conclusive demonstration of a causative relationship. Experimental



evidence on the concentration of key metabolites is still lacking. Thus, analysis of hexose phosphates and organic acids levels should help to demonstrate a real physiological connection between SS activity and BNF. Preliminary data suggest that the down-regulation of SS during drought is followed by a depletion of organic acids in nodules (L. Gálvez, E.M. González and C. Arrese-Igor, unpublished results), which would give further support to the model shown in Fig. 1.

Further research on the expression and activity of SS in nodules should focus on the signal transduction pathway linking water-stress perception and the decline in SS activity (Fig. 1). This issue remains largely unknown and only indirect evidence seems to discard the involvement of sucrose (section 9) or ABA (section 9). An analysis of the SS promoter and the elucidation of its regulation by factors such as O<sub>2</sub>, nitrogenous signal molecules (section 10), carbohydrates (other than sucrose) or osmotic potential (section 9) may help to solve this question. The evidence so far provided by pea mutants (in which SS is present at less than 10% of normal levels (Gordon et al., 1999)) or antisense *Lotus japonicus* (in which SS is down-regulated (Skøt et al., 1997)) stresses the importance of SS in normal nodule functioning and development. Future work to alter the levels of SS in nodules (particularly up-regulation), and, in addition, to characterise the SS promoter, would provide further valuable insights into the role of SS in nodule functioning. Plants with additional nodule SS activity could be used to test the hypotheses that enhanced activity could lead to an improvement in nitrogen fixation during normal growth or when the plant is exposed to environmental stresses. The recent isolation and sequencing of a number of nodule SS genes (EMBL accession numbers AF079851; AJ001071; AJ012080; AJ131943; AJ131964; Déjardin et al., 1997; Craig et al., 1999; Hohnjec et al., 1999) now make these ideas feasible within the very near future.

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### Note

Whilst this paper was in press, it has been reported that nodules initiated by a *Bradyrhizobium japonicum* strain which accumulated high amounts of

trehalose also showed a higher sucrose synthase activity [Muller, J., Boller, T., and Wiemkem, A. 1998. Trehalose affects sucrose synthase and invertase activities in soybean (*Glycine max* [L.] Merr.) *Journal of Plant Physiology* 153: 255–257.] This result could be relevant to the discussion of carbohydrates influencing SS expression (see sections 9 and 11).

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