Water-deficit effects on carbon and nitrogen metabolism of pea nodules

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Abstract

Two experiments were carried out to investigate the effects of water-deficit stress on carbon and nitrogen metabolism of Pisum sativum nodules. In the first experiment, leaf ψw was allowed to reach −1.0 MPa over a period of 14 d whilst in the second experiment −1.5 MPa was reached during the same time period. Nodule activities of phosphoenol pyruvate carboxylase, glutamine synthetase, alkaline invertase, pyruvate decarboxylase, alcohol dehydrogenase, uridine pyrophosphorylase, and malate dehydrogenase activities were not affected by water-deficit stress. In the first experiment (−1.0 MPa), sucrose synthase (SS), an enzyme which hydrolyses sucrose to support nodule metabolism, declined by 50% in activity and about 25% in content, according to Western immunoblot data. In the second experiment (−1.5 MPa), SS activity decreased by 75% together with glutamate synthase and aspartate aminotransferase which declined by 60% and 40%, respectively. Coincident with the decline of these activities, a dramatic increase in the nodule content of sucrose and a slight increase in the levels of total free amino acids were found. It has been recently suggested that the decline in SS activity and, therefore, a reduced potential to metabolize sucrose may be an important factor contributing to the overall response of soybean nodules to water stress. These results suggest that this observation may be also correct for temperate legumes with indeterminate nodules. However, in this latter case, the activity of some enzymes involved in nitrogen assimilation (glu-tamate synthase and aspartate aminotransferase) were also affected by water-deficit stress.

Key words: Pisum sativum, water stress, nitrogen metabolism, nodule metabolism, pea, sucrose synthase.

Introduction

When nitrogen-fixing legumes are subjected to water-deficit stress, it is not obvious which function of the stressed plant is actually affecting the nodule (Streeter, 1993). The effect could be due to (1) reduced photosynthesis and therefore, reduced availability of carbohydrate, (2) less water for the transport of N-products away from the nodule, (3) some direct effect on nodule gas permeability or (4) the alteration of nodule metabolic activity. There is a direct correlation between decreased nodule water potential (ψw, MPa) and the decline of nitrogenase activity (Pankhurst and Sprent, 1975), with N2 fixation and, therefore, a reduced potential to metabolize sucrose being more sensitive to water deprivation than photosynthesis (Djekoun and Planchon, 1991). It has been suggested that water stress acts directly on nodule activity by decreasing nitrogenase-linked respiration (Durand et al., 1987). Guerin et al. (1990) suggested that, in addition, metabolic potential, defined as the sum of the nodule biochemical machinery and its capacity to support nitrogen fixation at maximum rates, may also be reduced, since they were unable to overcome the inhibition by raising the concentration of oxygen. This has been supported by the work of Diaz del Castillo et al. (1994) and Diaz del Castillo and Layzell (1995).

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The main carbon source transported from the shoot into the nodules is sucrose, which may be hydrolysed by either sucrose synthase (SS) or alkaline invertase (AI). Hydrolysis by SS produces fructose and UDP-glucose, whilst AI hydrolysis produces glucose and fructose. Since, fructokinase levels in nodules are much higher than those of glucokinase (Copeland and Morell, 1985; Gordon, 1992) and UDP glucose pyrophosphorylase (UDPGPP) activity is also high (Copeland et al., 1989; Gordon, 1991), there is adequate enzymic potential in the nodule to metabolize the products of SS (Copeland and Morell, 1985). Some metabolites produced in these reactions can be used to form starch in amyloplasts, but most are transformed to phosphoenol pyruvate, which is converted to oxalacetate by phosphoenol pyruvate carboxylase (PEPC) and then to malate by malate dehydrogenase (MDH). Malate is considered to be the main carbon source to support bacteroid activity (Day and Copeland, 1992) and UDP glucose pyrophosphorylase (UDPGPP) activity is also high (Copeland et al., 1989; Gordon, 1991). It has recently been suggested that the decline in SS activity in the nodules of soybean during water-deficit stress may be responsible for the reduced metabolic potential described earlier (González et al., 1995; Gordon et al., 1997).

In temperate legumes with indeterminate nodules, nitrogen compounds are exported as amides, mostly aspartagine and glutamine, whilst in tropical/subtropical legumes with determinate nodules the main export products are ureides. The pathway of ammonium assimilation is apparently the same in amide and ureide exporting legume nodules. In both cases, nitrogen fixed in the bacteroids is exported into the host plant cytosol, where ammonia is converted to glutamine and glutamate by the enzymes glutamine synthetase (GS) and glutamate synthase (GOGAT). Glutamate and glutamine are further converted to aspartate and asparagine by aspartate aminotransferase (AAT) and asparagine synthetase (AS). In addition to this common pathway, ureides synthesis in tropical and subtropical legumes requires the oxidation of purines, which are derived from glutamine, glycine, aspartate, and ribose-5-phosphate.

Sinclair and Serraj (1995) found greater sensitivity to water stress in ureide exporting legumes, as compared to amide exporters. These authors found a correlation between nitrogen fixation sensitivity to water stress and the type of nitrogenous compounds exported from the nodules. However, most studies on water-deficit stress have been performed on ureide exporting plants, whilst less attention has been paid to the metabolism of amide exporters. With soybean, Purcell and Sinclair (1995) showed changes in nodule permeability as a consequence of reduced nodule respiration and such changes have been directly related to the export of nitrogen compounds (Walsh, 1995). If the control of nodule permeability involves the cells surrounding vascular bundles, dramatic differences could occur between determinate and indeterminate nodules, because of different vascular bundle arrangements (generally anastomosed in soybean versus free-ended in pea) and also because of the different characteristics of the nitrogen compounds exported. As outlined above, ureide and amide exporting nodules also differ in their biochemical machinery to synthesize nitrogen-export products and therefore the chemical interchange between cortical cells is likely to differ. There are also differences in the location of starch (only in uninfected cells in determinate nodules) and other anatomical features (Brown and Walsh, 1994). Thus, water-deficit effects may differ between indeterminate, amide and determinate, ureide exporting nodules.

Currently, early responses to water stress in indeterminate nodules are considered to be related to a decrease in leghaemoglobin content (Guerin et al., 1991; Irigoyen et al., 1992), rather than to the carbohydrate shortage found in soybean (González et al., 1995; Gordon et al., 1997). This apparent differential behaviour may be based on the anatomical/metabolic differences outlined above. However, a key role of SS in nodule metabolism of carbohydrates in indeterminate nodules (white clover) has recently been suggested (Gordon and James, 1997). In this paper, the hypothesis that the response to water stress in determinate nodules of pea may be due to a decrease in the nodule carbohydrate metabolism has been examined. Activities of key enzymes of carbohydrate and nitrogen metabolism are correlated with changes in the levels of carbohydrates and amino acids in pea nodules subjected to mild and severe water-deficit stress.

Materials and methods

Growth conditions and experimental procedures

Pea plants (Pisum sativum L. cv. Solara) were inoculated with Rhizobium leguminosarum bv. viciae strain NLV 8. Plants were grown in a nutrient solution lacking nitrogen (Ryle et al., 1978) in 1 dm³ pots in a controlled environment chamber (22/15 °C day/night temperature, 70% relative humidity, 500 µmol m⁻² s⁻¹ photosynthetic photon flux, 12 h photoperiod).

In the first experiment, plants were grown in vermiculite and water-deficit stress was imposed when plants were 4-weeks-old. Because of the high water holding capacity of vermiculite (0.72 cm −3 substrate) and the low transpiratory surface of these plants, the cessation of nutrient application allowed for a progressive depletion of water, resulting in a gradual water stress. Control plants were supplied with nutrients to pot capacity on a daily basis. Evaporational loss was estimated using a set of well-watered vermiculite-filled pots without plants. Each of the control and water-deficit-stressed pots was weighed every day to calculate transpiration rates in both treatments.

In a second experiment, plants were grown in a 4/3 (by vol.) mixture of vermiculite/perlite (water holding capacity, 0.41 cm⁻³ water cm⁻³ substrate) and with temperatures of 25/18 °C (day/night) and a photoperiod of 16 h. These conditions were
used to produce a faster water-deficit stress and allow a lower \( \phi_w \) to be reached than in the first experiment.

Three replicate control and water-stressed plants were sampled at days 0, 7 and 14 (experiment 1) or 0, 3, 6, 10, 12, and 14 (experiment 2). Leaf \( \phi_w \) of the second youngest fully expanded leaf was measured at midday with a pressure chamber (Scholander et al., 1965). Nodules for protein extraction and metabolite analysis were harvested, stored briefly on ice, surface-dried with tissue paper, weighed, frozen in liquid \( N_2 \), and stored at \(-80^\circ\)C. Roots and shoots were weighed after drying for 48 h at 70°C.

Gas exchange measurements and nitrogen content
In experiment 1, acetylene reduction activity (ARA) and root respiration of intact roots were measured using a flow-through gas system (Minchin et al., 1983) housed in a Fisons cabinet. Root systems were sealed into the growth pots, allowed to stabilize for 18–21 h in a gas stream of air enriched to 500 cm\(^{-3}\) CO\(_2\), then exposed to a gas stream (300 ml min\(^{-1}\)) containing 10\% (v/v) C\(_2\)H\(_2\) and 21\% (v/v) O\(_2\). Respiratory CO\(_2\) production was measured by infrared gas analysis and C\(_2\)H\(_2\) production was measured by gas chromatography using a flame ionization detector. Maximum rates of ARA were determined from the maximum rate of C\(_2\)H\(_2\) production prior to the C\(_2\)H\(_2\)-induced decline. The extent of this decline was reduced with increasing periods of exposure to water-deficit stress, but it was always possible to determine a distinct maximum for C\(_2\)H\(_2\) production. Samples were taken every minute for the first 10 min, then every 2 min for the next 20 min and then every 5 min for the last 30 min. Acetylene was kept in the gas stream for the entire period.

After steady-state conditions had been reached following exposure to C\(_2\)H\(_2\) in 21\% O\(_2\) (8.59 mol O\(_2\) m\(^{-3}\)), the O\(_2\) concentration in the gas stream was increased over the range 30–60\% (12.27 to 24.54 mol O\(_2\) m\(^{-3}\)), in steps of 10\% O\(_2\). Following each increase in O\(_2\) concentration, there was a 25–30 min equilibration period to allow new steady-state conditions to be reached.

Carbon costs of nitrogenase activity and nitrogenase-linked respiration (NLR) were determined from the slope of the linear relationship between nodulated-root respiration and C\(_2\)H\(_2\) reduction. This was based on changes which occurred in both parameters during the C\(_2\)H\(_2\)-induced decline and O\(_2\) stepping (Witty et al., 1983). These data were then used to calculate comparative resistance values (\( R \)) using an exponential curve-fitting routine for NLR against external oxygen concentration which involves a modified equation for Fick’s first law of diffusion (Minchin et al., 1992). This allows for the calculation of an additional respiration factor which, when added to NLR, represents the total flux of oxygen across the diffusion barrier (\( F \), see Minchin et al., 1992, for full details). Root respiration data obtained immediately before exposure to C\(_2\)H\(_2\) was used for calculation of the oxygen diffusion resistance in air.

In experiment 2, specific nitrogen fixation was calculated from the organic nitrogen content of the whole plant determined by Kjeldahl analysis and normalized for N-input using the average nodule dry weight of the same plants for the corresponding period.

Photosynthesis and shoot respiration was measured by infrared gas analysis using the whole plant as described by James et al. (1992).

 Extraction and assay of enzymes
Nodules (200 mg) were homogenized in a mortar and pestle with 50 mol m\(^{-3}\) MOPS, 10 mol m\(^{-3}\) DTT, 4 mol m\(^{-3}\) MgCl\(_2\), pH 7 at 0–2°C (5 cm\(^3\) g\(^{-1}\) fresh weight), and the homogenate was centrifuged for 30 min at 20 000 g, 2°C (Cresswell et al., 1992).

Samples (50 mm\(^3\)) of the supernatant were retained for phosphoenol pyruvate carboxylase (PEPC) assay, and 1 cm\(^3\) aliquots were desalted by low speed centrifugation (180 g, 1 min) through 5 cm\(^3\) columns of Bio Gel P6DG (BioRad) equilibrated with 50 mol m\(^{-3}\) MOPS pH 7 and 4 mol m\(^{-3}\) MgCl\(_2\) buffer. The desalted extract was used to assay protein (Lowry et al., 1951), leghaemoglobin (Lb, LaRue and Child, 1979) and the following enzymes: aspartate amino transferase (AAT), sucrose synthase (SS), alkaline invertase (AI), malate dehydrogenase (MDH), pyruvate dehydrogenase (PDC), glutamate synthase (GOGAT), alcohol dehydrogenase (ADH), uridine diphosphoglucose pyrophosphorylase (UDPGPP), and glutamine synthetase (GS). PEPC, AAT, SS, AI, and GS were assayed according to González et al. (1995), MDH and UDPGPP according to Gordon and Kessler (1990), PDC and ADH according to John and Greenway (1976), and GOGAT as described by Groot and Vance (1981).

Extraction of bacteroid proteins, Western immunoblotting of SS, Lb and nitrogenase components, 1 and 2, and the analyses of soluble carbohydrates and nitrogenous compounds were carried out as in González et al. (1995). Changes in the optical intensity of bands on immunoblots were assessed with a BioRad GS-700 imaging densitometer coupled to the Molecular Analyst software.

Statistical analysis
Results were examined by one-way analysis of variance. All effects discussed in this study were significant at \( P<0.05 \) in Fisher’s (protected) least significant difference (LSD) tests between means.

Results
Physiological measurements
In experiment 1, water-stressed plants did not show any decline in transpiration rates during the first 9 d as they progressively used the remaining water in the vermiculite (Fig. 1A). However, in experiment 2, transpiration rates declined after 6 d of water-deficit stress (Fig. 1B). In this latter case, vermiculite was mixed with perlite and a slightly higher temperature and longer photoperiod was used to accelerate the imposition of water-deficit stress. In both cases, the slow decline of the transpiration rates suggests that plants actually experienced a gradual and progressive water stress. Transpiration rates of control plants increased with plant growth in both experiments (data not shown).

Since an important objective of the experimental design was to ascertain primary responses to water-deficit stress, it was desirable that plants did not reach an extremely low \( \phi_w \). Throughout the first experiment, leaf \( \phi_w \) declined gradually in water-stressed plants, reaching values of \(-1.0 \) MPa (Fig. 1C), whilst in the second experiment, water potential declined to \(-1.5 \) MPa (Fig. 1D).

Mild water stress levels (experiment 1) had only a slight effect on photosynthesis per unit leaf area (Fig. 2A).
Fig. 1. Time-course of the transpiration rate in control (○) and water-stressed plants (●) of experiments 1 (A) and 2 (B) throughout the water stress period. Each point represents the mean of all plants by treatment each day. Results are means ± SE (n reduces from 18 (0 d) to 3 (14 d)).

At day 7, 1/3 of the volume lost by evapotranspiration rates was added to the water-stressed plants in experiment 2, represented as (C) and (D) represent the water deficit effects on leaf water potential in both sets of plants. Results are means ± SE (n = 3).

whilst the effect was more pronounced on respiration (Fig. 2B). Despite this small effect on photosynthesis, nitrogen fixation, expressed as acetylene reduction on a nodule dry weight basis, was reduced by 30% (Fig. 2C). An increase in nodule diffusion resistance was also observed (Fig. 2D). Respiration of the whole root system (roots and nodules) was reduced by water stress (Fig. 2E) and when this parameter was normalized on a dry weight basis, growth and maintenance respiration declined over the 14 d study period (Fig. 2F). Nitrogenase-linked respiration showed a sharp decline in parallel with the decline in nitrogen fixation (data not shown). Conversely, carbon costs of nitrogen fixation were not significantly affected by water stress with values being constant at 2.3 mol CO₂ mol⁻¹ C₂H₄ (data not shown).

In experiment 2 (severe water stress), photosynthesis rates were unchanged during the first 6 d (ψₔ = −0.4) of water deficit. However, rates declined once leaf ψₔ became more negative (Fig. 2G) with values reaching only 27% of controls at day 10 and approaching zero after 14 d. In this experiment, the effect of water stress on nitrogen fixation was monitored as the N incorporated by plants and normalized on a nodule dry weight basis. Under these conditions, specific nitrogen fixation was slightly affected by mild water stress (with a 20% decline compared with control plants at day 10), whilst at more negative water potentials (day 14), nitrogen fixation declined by 76% (Fig. 2H).

**Nodule biochemistry**

Nodule protein content, expressed on a dry weight basis was not affected in either experiment (values were 124 ± 5 mg soluble protein g⁻¹ nodule dry weight; data not shown). Also, no significant differences were observed in Lb content of nodules of mild and severely stressed plants with respect to controls, with values of 0.11 ± 0.01 mg Lb mg⁻¹ protein (data not shown). This result was further confirmed by Western blots against Lb, which did not show any obvious change in Lb levels under water deficit conditions (Fig. 4).

Water stress did not affect PEPC, AI, GS, PDC, ADH,
Fig. 2. Gas exchange parameters of control (○) and water-stressed plants (●) of experiment I: (A) net photosynthesis, (B) shoot respiration, (C) acetylene reduction activity (ARA), (D) nodule resistance, (E) total root respiration (TRR), (F) growth and maintenance respiration (GMR) of the root system; and experiment 2, (G) net photosynthesis, and (H) specific nitrogen fixation. NDW, denotes nodule dry weight and RSDW, root system (root + nodules) dry weight. Error bars denote SE (n=3) when larger than the symbol itself.
MDH, and UDPGPP whose activities were kept at control values (0.50, 0.12, 0.22, 0.06, 0.07, 12.92, and 1.6 μmol product mg−1 protein min−1, respectively. SE were less than 10% of mean values in all cases) throughout both experiments. Among the array of enzyme activities measured, sucrose synthase was the only activity which showed a reduction upon mild water-deficit stress (Fig. 3A). The decline in SS activity was directly related to SS protein levels which were reduced by about 25% under mild water stress (Fig. 4). This decline in SS occurred before any change could be detected in the nitrogenase components of the bacteroid fraction. Under more severe water-deficit stress there was a more pronounced reduction of SS activity (Fig. 3D), and GOGAT and AAT activities were also reduced (Fig. 3E, F). This contrasts with the mild water-deficit stress where GOGAT and AAT activities did not show any significant decline. However, Lb and protein content were still not affected under the more severe stress (data not shown).

Glucose and fructose concentrations did not show any response to the water deficit imposed (data not shown). However, in both experiments, water-stressed nodules showed an increase in sucrose content (Fig. 5A, E), which was consistent with the level of decline in SS activity. The starch content of control nodules tended to increase over the course of the experiments (Fig. 5B, F). In

![Fig. 3](https://example.com/fig3.png)

**Fig. 3.** Effect of water stress on SS, GOGAT and AAT activities in control (○) and water-stressed plants (●) of experiments 1 (A, B and C, respectively) and 2 (D, E and F, respectively). Results are means ± SE (n = 3).
contrast, starch levels in water-stressed nodules either remained constant (experiment 1) or declined slightly (experiment 2).

Nodule total free amino acids tended to increase throughout the mild water stress period (Fig. 5C, G). However, amino acids levels fell to control values (Fig. 5G) at the end of the severe water stress period when nitrogen metabolism had been significantly affected as shown by the reduced GOGAT and AAT activities (Fig. 3E, F). Proline accumulation in nodules (Fig. 5D, H) was related to $\psi_w$ in both experiments, with a small accumulation at mild water stress levels (Fig. 5D) and a more dramatic accumulation upon severe water stress conditions (Fig. 5H).

**Discussion**

Indeterminate nodules of pea plants subjected to progressive water stress showed a marked inhibition in their ability to reduce $\text{C}_2\text{H}_4$ (Fig. 2C) even at moderate water stress. These data were further confirmed in more severe water stress conditions when nitrogen fixation was assessed as the variation in plant nitrogen content (Fig. 2H). Previous studies with legume species representing both determinate and indeterminate nodule forms, reported a decline in total nitrogenase activity over the range of $\psi_w$ values reached in this work (Durand et al., 1987; Davey and Simpson, 1990; Guerin et al., 1990; Irigoyen et al., 1992; Djekoun and Planchon, 1991; Diaz del Castillo et al., 1994).

Such inhibition of nitrogenase activity is often correlated with an increase in resistance of the oxygen diffusion barrier of the nodule cortex (Fig. 2D). However, gas exchange studies have also suggested that closure of the oxygen diffusion barrier may only be part of the response of nodules to water deficit (Guerin et al., 1990; Hunt and Layzell, 1993; Diaz del Castillo et al., 1994). For determinate soybean nodules, Diaz del Castillo and Layzell (1995) found that internal $\text{O}_2$ concentration remained quite high in mildly stressed nodules, whilst Hunt and Layzell (1993) and Diaz del Castillo et al. (1994) found that most of the reduction in nitrogenase activity could not be restored by raising the external oxygen concentration. This implies that the nodules of water-stressed plants no longer had the metabolic capacity to support nitrogen fixation, even in the presence of sufficient oxygen. Purcell and Sinclair (1995) found that nitrogenase activity was affected prior to node permeability in an experiment using PEG 6000 to impose a severe and rapid water stress, suggesting that factors other than gas permeability could be involved in the initial decline in nitrogenase activity. However, Serraj and Sinclair (1996) reported that nodule permeability and nitrogenase activity declined simultaneously during water stress. They suggested that the initial effect was due to oxygen limitation with a secondary, metabolic, limitation occurring after 24 h.

In contrast, Guerin et al. (1991) and Irigoyen et al. (1992), suggested that the decline in nitrogen fixation in indeterminate nodules of amide exporters is due to a protease-induced reduction in Lb content which would limit oxygen supply to the bacteroids and affect respiration and energy production. It was also suggested that oxygen limitation would lead to an enhanced anaerobic metabolism, involving alcohol dehydrogenase (ADH) and pyruvate decarboxylase (PDC) (Irigoyen et al., 1992). In the experiments reported here, protease activity was not determined, but Lb and soluble protein content did not show significant differences to control values. Also, ADH and PDC activities (indicators of anaerobic metabolism) did not change throughout the water-stress period, which suggests that normal microaerobic metabolism continued, as also suggested by Diaz del Castillo et al. (1994).

At moderate water stress, the only significant decline in enzymic activity was that of sucrose synthase, with a 50% decline (Fig. 3A) which correlated with a 50% reduction in nitrogenase activity (Fig. 2C). In experiment 2, a gradual decline was observed in SS activity (Fig. 3D), even at day 6 when midday leaf $\psi_w$ had not yet declined. Correlated with the decline in SS activity was an increase in the content of sucrose (Fig. 5A, E) which indicates that the decline in $\text{N}_2$ fixation was not due to a shortage of photosynthate (Hunt and Layzell, 1993). These data suggest that the ability to metabolize sucrose may be impaired in water-stressed nodules of pea plants, as previously suggested for soybean nodules (González et al., 1995; Gordon et al., 1997).

Together with SS, the activities of GOGAT and AAT were also affected at lower $\psi_w$ (Fig. 3E, F). Reduced GOGAT activity upon water-deficit stress has previously been documented for lucerne (Becana et al., 1984) and soybean (Gordon et al., 1997). However, in this latter case the decline in GOGAT activity was not as rapid nor as extensive as the decline in SS activity and AAT activity was not affected at all. Glutamine produced by GS in the...
infected cell cytosol has to be transported to plastids for further metabolism by GOGAT, whilst the product, glutamate, must be returned to the cytosol to fuel GS activity. Since GS activity is apparently not affected by water stress, it has been suggested that glutamate, in indeterminate nodules, could be also supplied by glutamate dehydrogenase (Groat and Vance, 1981). In nodules, AAT may have a role in providing substrates for bacteroid and plant host respiration and in amino acid synthesis for transport to the shoot. This enzyme is also believed to control the redistribution of nitrogen and carbon pools between plant cell cytoplasm and other compartments and between the cytoplasm and the bacteroids (Ireland and Joy, 1985; Appels and Haaker, 1991).

In non-stressed pea nodules, amino acid and amide levels were many-fold higher that the combined contents
of amino acids and ureides in soybean nodules (compare Fig. 5C with González et al., 1995; Gordon et al., 1997). The accumulation of even higher amounts of nitrogen fixation products during water-deficit stress (Fig. 5G) is consistent with the data for ureide-exporting nodules (González et al., 1995; Gordon et al., 1997) and almost certainly reflects the balance between nitrogenase activity, utilization of nitrogen products within nodules, the control of nitrogen product export, and the import and export of water in stressed nodules. In the present experiments, transpiration rate did not decline until 6 d after the onset of water stress (Fig. 1A) suggesting that the export of amino acids should not be affected by water flux through the nodules. However, Walsh et al. (1989) showed that water imported in the phloem may be essential for the export of nitrogenous products from soybean nodules, supporting the earlier hypothesis for pea nodules of Minchin and Pate (1973). Thus, it is possible that reduced import of sucrose and water into nodules via the phloem may also reduce the export of nitrogenous products into the xylem stream.

In conclusion, carbon metabolism, in particular, sucrose synthase activity, of indeterminate pea nodules is affected by mild water deficit in a similar manner to that of determinate nodules such as soybean (González et al., 1995; Gordon et al., 1997). Gordon et al. (1997) showed that SS expression is also impaired by other stresses (salt, nitrate) in addition to water-deficit stress, and it is likely that this gene expression is a key point in the response of nodule metabolism to environmental stress. Studies carried out in different plant systems suggest that SS genes are affected by oxygen levels and carbohydrate concentration (Ricard et al., 1991; Koch, 1996), however, these regulatory conditions do not match with those inside legume nodules and it is still unclear which factors cause a decrease in SS gene expression within nodules. It is also unclear as to whether the decline in SS content and activity is the cause or the consequence of the decline in nitrogen fixation under water-stress conditions. The data presented here do not solve this question, but they clearly suggest that the effect of water stress on SS does not differ between ureide and amide exporting nodules. However, in addition to this common response, a more severe water stress resulted in nitrogen metabolism being affected in indeterminate pea nodules at the levels of GOGAT and AAT activity. It can be concluded that SS plays a key role in the regulation of nitrogen fixation in indeterminate nodules, but that the low metabolic capacity of water-stressed indeterminate nodules is also caused by the modulation of other enzyme activities.

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