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Accumulation of soluble carbohydrates, trehalase and sucrose synthase in effective (Fix^+) and ineffective (Fix^-) nodules of soybean cultivars that differentially nodulate with *Bradyrhizobium japonicum*

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Abstract. Roots of soybeans have the ability to form symbioses with nitrogen-fixing rhizobial bacteria to form nitrogen-fixing (Fix^+) nodules, thus allowing the plant to grow in the absence of mineral nitrogen. Several soybean cultivars from China nodulated normally with *Bradyrhizobium japonicum* USDA110 s*pc4*, but developed only a few nodules with 61-A-101, another *B. japonicum* strain. When soybeans were infected with *Rhizobium* sp. NGR234, ineffective (Fix^-) nodules that do not fix nitrogen were formed. Plants infected with NGRΩ*nodD2*, a mutant strain overproducing lipo-chitooligosaccharidic nodulation signals (Nod factors), showed significantly higher numbers of ineffective nodules. Nodules from the different plant–microsymbiont combinations were characterized with respect to their accumulation of soluble carbohydrates and their induction of trehalase and sucrose synthase. These two plant enzymes are known to be nodule-stimulated proteins. Pool sizes of soluble carbohydrates in nodules showed strain-specific alterations in sucrose and trehalose, whereas *myo*-inositol and pinitol were affected in a more cultivar-specific way. Immunoblots with nodulin-specific antiserum indicated that sucrose synthase is induced in Fix^+ nodules, but undetectable in Fix^- nodules, indicating a strain-specific induction profile. Trehalase activity in nodules showed a similar strain-specific induction profile. High enzyme activity was measured for nodules harboring the *Bradyrhizobium* strains, whereas ineffective nodules containing NGR234 exhibited activities in the range of uninfected roots. Nodules induced by NGRΩ*nodD*2 showed increased trehalase activity. A similar induction of trehalase was observed when uninfected roots were treated with Nod factors purified from NGR234. The data obtained are discussed in the context of carbohydrate allocation in nodules and the question of how rhizobial bacteria influence the carbohydrate metabolism of their host plant is addressed.

Introduction

On roots of legumes, rhizobia may induce formation of nodules, highly specialized root organs, in which atmospheric dinitrogen is reduced to ammonia (effective nodules with nitrogen-fixing bacteroids, Fix^+). It is well established that the nodule symbiosis depends on genotype–genotype relationships between the plant and the microsymbiont. The first steps of the interaction are determined by rhizobial signals, namely the lipo-chitooligomeric Nod factors and various exopolysaccharides (Perret *et al.* 2000). Recent findings also indicate a role for effector molecules secreted by the bacterial type III secretion-system, which has been identified in certain rhizobial strains (Marie *et al.* 2001).

Nodulation of soybean, the most important legume crop worldwide, depends on a variety of genetic determinants of both the host and the microsymbiont (Qian *et al.* 1996). Recent results indicate, for instance, that *Sinorhizobium fredii* strains with defective inositol dehydrogenase have competitive disadvantages compared with their respective wild-types (Jiang *et al.* 2001). Similar observations were made for *Rhizobium leguminosarum* bv. *viciae* mutants that cannot use *myo*-inositol as a carbon source (Fry *et al.* 2001).

Abbreviations used: Fix^+, effective nodules; Fix^-, ineffective nodules; fw, fresh weight; nkat, nanokatal (nanomole substrate transformed per second); NNP, normal nodulation potential; pkat, picokatal; RNP, restricted nodulation potential.

10.1071/FP03002 1445-4408/03/090965

This is interesting as *myo*-inositol is one of the more abundant, non-structural carbohydrates in soybean nodules. Conversely in uninfected roots, pinitol, a methylated derivative of inositol, is more abundant than *myo*-inositol (see Müller *et al.* 1994*a*). A similar shift in the metabolic profile from *myo*-inositol to pinitol can be observed in nodules of poorly nodulating soybean mutants (Müller *et al.* 1995*a*) and in ineffective (Fix^-) soybean nodules colonized by bradyrhizobia unable to fix nitrogen (Müller *et al.* 1994*a*).

A major factor in the effectiveness of nodules is the correct allocation of sucrose to the nodule (for review see Vance and Heichel 1991) and its catabolism by sucrose synthase (EC 2.4.1.13). This key enzyme is induced in nodules (Morell and Copeland 1985; Thummler and Verma 1987; Gordon *et al.* 1992) and down-regulated in response to stresses that inhibit nitrogen fixation (e.g. Gonzalez *et al.* 1995; Müller *et al.* 1996). The resulting catabolites, fructose and UDPglucose, are used to fuel the energy-consuming process of nitrogen fixation and provide substrates for the biosynthesis of other carbohydrates. Of particular interest in this perspective are trehalose, a non-reducing disaccharide accumulated by bacteroids of some rhizobial strains, and the induction of the trehalose-cleaving plant enzyme trehalase (EC 3.2.1.28) in nodules (Müller *et al.* 1995*b*; Farias-Rodriguez *et al.* 1998; Aeschbacher *et al.* 1999).

In previous studies (Xie *et al.* 1996, 1999) Chinese soybean cultivars have been described as forming nodules upon inoculation with *Bradyrhizobium japonicum* 61-A-101. Certain soybean cultivars were poorly nodulated by this strain, whereas they retained the capacity to establish symbiosis with *B. japonicum* USDA110 s*pc4*. This strain nodulates soybean very effectively (Ravuri and Hume 1992) and accumulates more than five times less trehalose than strain 61-A-101 in nodules, thus, drawing less assimilate from the plant host (Müller *et al.* 1994*a*, 1998). In contrast to the bradyrhizobial strains, soybean nodules induced by the broad host-range *Rhizobium* sp. NGR234 are ineffective (Pueppke and Broughton 1999).

Here we investigated the accumulation of soluble carbohydrates and the induction profile of sucrose synthase and trehalase in Fix^+ and Fix^- nodules of Chinese soybean cultivars with different symbiotic potential. Plants were inoculated with 61-A101, USDA110 s*pc4*, NGR234 as well as with strain NGRΩ*nodD*2, a derivative of NGR234, which overproduces Nod factors (Fellay *et al.* 1998). We present general nodulation parameters and show that carbohydrate pools, sucrose synthase and trehalase depend on both the plant genotype and the rhizobial strains harbored by the plant.

Materials and methods

Biological materials

Seeds of soybean cultivars [*Glycine max* (L.) Merr.] were kindly provided by the Chinese Academy of Agricultural Science (Beijing, People's Republic of China). For this study the following cultivars were selected: 'Da Hei Qi', 'Da Zi Hua', 'Dong Da Li', 'Du Lu Huang', 'An Tu Bai Hua Lu Da Dou' and 'Gong Jiao 6308-1'. *Glycine max* cv. 'Maple Arrow' was obtained from Semences UFA (Busigny, Switzerland). All seeds were surface-sterilized with 30% H_20_2 for 20 min, followed by washing with sterile tap-water and incubation at 27°C on 1% water agar plates for germination (Staehelin *et al.* 1992). *Bradyrhizobium japonicum* 61-A-101 (Stripf and Werner 1978) and USDA110 s*pc*4 (Regensburger and Hennecke 1983) were grown to stationary phase in 20E-medium (Werner *et al.* 1975) at 27°C on a rotary shaker at 140 rpm. *Rhizobium* sp. NGR234 (Stanley and Cervantes 1991; Pueppke and Broughton 1999) and its mutant strain NGRΩ*nodD*2 (Fellay *et al.* 1998) were raised as described by Fellay *et al.* (1998).

Establishment of the symbiotic interactions

Soybean seedlings were transferred to sterilized Leonard jars filled with perlite and vermiculite (1 : 1) and a nutrient solution supplemented with 1 mM KNO_3 as described (Stripf and Werner 1978). In previous studies, 1 mM KNO_3 was sufficient to sustain growth of Fix^- nodules without impairing nodulation by Fix^+ strains (Staehelin *et al.* 1992; Müller *et al.* 1994*b*). After one week, plantlets were inoculated with 5 mL bacterial cultures (see Müller *et al.* 1996) or left uninoculated. Plants were cultivated in a phytotron (14-h day at a photon flux of 300 μmol m^{-2} s^{-1} and 26°C, 10-h night at 20°C) and harvested after four weeks of co-cultivation.

Analysis of non-structural carbohydrates

Pool sizes of soluble carbohydrates were measured by gas chromatography as described previously (Müller *et al.* 1994*b*, 1996).

Extraction of soluble proteins, trehalase assay and immunoblotting

Frozen roots or nodule samples were ground in morpholinoethanesulfonic acid (K^+) buffer (pH 6.3, 0.1 M) containing EDTA and phenylmethylsulfonyl fluoride (2 mM each). The buffer : sample ratio was 1 mL g^{-1} fresh weight (fw) for roots or 2–3 mL g^{-1} fw for nodules. The crude extracts were centrifuged at 20000 *g* for 10 min. Supernatants (0.1 mL) were diluted with 0.9 mL of saturated ammonium sulfate and kept on ice for at least 3 h. Proteins were precipitated by centrifugation (10000 *g*, 10 min) followed by resuspension in morpholinoethanesulfonic acid (K^+) buffer (pH 6.3, 50 mM; total volume 0.1 mL). Trehalase was assayed according to Müller *et al.* (1995*c*) by incubating aliquots of resuspended proteins in morpholinoethanesulfonic acid (K^+) buffer (pH 6.3, 50 mM) containing 10 mM trehalose at 37°C for 15–60 min. Reactions were terminated by boiling and the glucose generated was determined with glucose oxidase–peroxidase (Boehringer Mannheim GmbH, Mannheim, Germany). Soluble proteins were assayed according to Bradford (1976). Soluble nodule proteins were separated on SDS–PAGE gels in which the lower separating gel contained 10% (w/v) acrylamide in 0.375 M Tris–HCl, pH 8.8, 0.1% (w/v) SDS, and the upper stacking gel contained 3% acrylamide in 0.125 M Tris–HCl, pH 6.8 and 0.1% SDS. Immunoblotting (western blotting) was performed as described by Müller *et al.* (1994*b*) using commercial soybean milk instead of skim milk for blocking. The protein concentration of the soybean milk was adjusted to 3% w/v by dilution in Tris-buffered saline (Tris pH 7.5, 50 mM, NaCl 150 mM). The antiserum was raised as described and recognizes leghaemoglobin and nodule-specific sucrose synthase (Müller *et al.* 1994*b*).

Treatment with Nod factors

Roots of soybeans (cv. 'Maple Arrow') were treated with acetylated and sulfated Nod factors purified from strain NGR234 (Price *et al.* 1992) as described earlier (Xie *et al.* 1995, 1998). These Nod factors are biologically active on soybean roots (Schmid *et al.* 1994; Xie *et al.* 1995, 1999).

Results

We have previously reported that a total of 92 soybean varieties commonly grown in the People's Republic of China were tested for nodulation deficiency after infection with *B. japonicum* 61-A-101. Several cultivars formed only a few nodules with this strain, indicating a restricted nodule potential (RNP cultivars), but nodulated normally with *B. japonicum* USDA110 s*pc4* (Xie *et al.* 1999). For this study, we used four of these RNP cultivars ('Da Hei Qi', 'Da Zi Hua', 'Dong Da Li', and 'Du Lu Huang') and compared them with three cultivars with normal nodulation potential (NNP), which included 'An Tu Bai-Hua Lu Da Dou', 'Gong Jiao-6308-1' and the Canadian cultivar 'Maple Arrow'.

Plants of each cultivar were inoculated with 61-A-101, USDA110 s*pc4*, *Rhizobium* sp. NGR234 and with the mutant strain NGRΩ*nodD2*, which overproduces Nod factors. Nodule number and biomass were similar within NNP and RNP cultivars, but differed between these groups in a strain-specific manner. Therefore, these results were grouped according to NNP and RNP cultivars instead of listing each cultivar separately. The NNP cultivars were strongly nodulated by both tested *Bradyrhizobium* strains (> 50 nodules per plant). Strain-specific differences were found for the RNP cultivars, which formed less than five nodules with 61-A-101. Strain USDA110 s*pc4* restored nodulation on all RNP cultivars tested. Strain NGR234 induced approximately 55 ineffective nodules per plant on NNP cultivars, while RNP cultivars formed less than 30 nodules with this strain. When the Nod-factor-overproducing mutant strain NGRΩ*nodD2* was used as an inoculum, NNP cultivars formed significantly more nodules than with the wild-type, on average, 136 nodules per plant. RNP cultivars also formed a slightly higher numbers of nodules, approximately 50 (ANOVA followed by LSD test; Fig. 1*A*).

Nodule biomass was higher than 0.4 g per plant in NNP cultivars colonized by 61-A-101 or USDA110 s*pc4*. In RNP cultivars infected with USDA110 s*pc4*, nodule biomass (average 0.39 g) was significantly lower than in NNP cultivars (average 0.55 g). Nodules of RNP cultivars colonized by 61-A-101 had an average biomass of 0.11 g per plant. This value was significantly lower than nodule biomasses of NNP cultivars colonized by each *B. japonicum* and significantly lower than the biomass of nodules of RNP cultivars colonized by USDA110 s*pc4*. NNP cultivars inoculated with the NGR234 formed about 0.2 g of Fix^- nodules per plant. This value was nearly 10 times lower in RNP cultivars. When the Nod-factor-overproducing mutant NGRΩ*nodD2* was used, biomass did not significantly differ between NNP and RNP cultivars (ANOVA followed by LSD test; Fig. 1*B*).

In most cases, nodules induced by 61-A-101 and USDA110 s*pc4* were effective, as determined by the acetylene reduction method. In nodules of all selected cultivars colonized by USDA110 s*pc4*, nitrogenase activity did not show pronounced differences. In contrast, nitrogenase activity of nodules colonized by 61-A-101 varied strongly within RNP cultivars, from less than 0.01 nkat g^{-1} fw ('Da Zi Hua') to 2 nkat g^{-1} fw ('Du Lu Huang'). The NGR strains only occasionally induced bacteria-containing nodules and these were characterized by absent or very low acetylene-reducing activity in all cultivars (data not shown). Upon microscopic investigation, most of these ineffective nodules harboring NGR234 or NGRΩ*nodD2* looked like 'empty nodules' free of bacteria or 'pseudonodules', which sometimes reached the size of the bacteria-containing nodules.

Accumulation of soluble carbohydrates in nodules

Uninfected roots of NNP and RNP cultivars contained *ca* 1% sucrose on a dry weight basis (% dw). In both types of cultivars, effective nodules colonized by 61-A-101 contained *ca* 1.3–1.6% dw sucrose. These values were not significantly

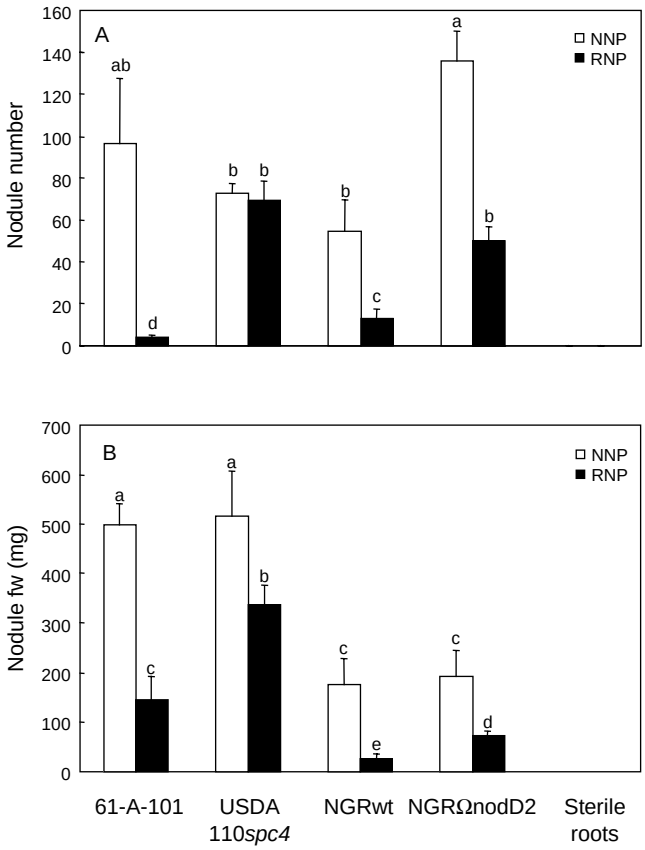

Fig. 1. Number (*A*) and biomass (*B*) of nodules. Soybean cultivars had a normal nodulation potential (NNP) or a reduced nodulation potential (RNP) with respect to *B. japonicum* 61-A-101. Plants were infected with strain 61-A-101, *B. japonicum* USDA110 s*pc4*, *Rhizobium* sp. NGR234 or with its mutant NGRΩ*nodD2*. Mean values ± s.e. are given for three NNP cultivars and four RNP cultivars respectively. Values topped by the same letters are not significantly different ($P<0.05$; ANOVA followed by Student–Newman–Keuls test).

increased compared with roots. High concentrations of sucrose (up to 3% dw) were found, however, in extracts from nodules of both types of cultivars colonized by USDA110 s*pc4*. In contrast to effective nodules, sucrose contents of ineffective nodules colonized by NGR234 were, in general, lower than in uninfected roots and showed significant differences between NNP and RNP cultivars. In nodules from RNP cultivars, sucrose concentration was twice as high as in nodules from NNP cultivars. Nodules from RNP cultivars colonized by NGRΩ*nodD2* exhibited sucrose pool sizes in the same range as uninfected roots (Fig. 2*A*; ANOVA followed by Student–Newman–Keuls test).

The bacterial disaccharide trehalose was found in nodules in a strain-dependent manner. In nodules of all tested cultivars colonized by 61-A-101, trehalose contents were in the same range as sucrose contents (*ca* 1% dw), thus, confirming earlier results obtained with cv. 'Maple Arrow' (Müller *et al.* 1998). Trehalose contents of nodules colonized by USDA110 s*pc4* were significantly lower (*ca* 0.2% dw). Trehalose was completely absent in NGR234- or NGRΩ*nodD2*-harboring ineffective nodules of all seven cultivars. Where present, trehalose contents were not significantly different between NNP and RNP cultivars (Fig. 2*B*; ANOVA followed by Student–Newman–Keuls test).

Nodule contents of pinitol and *myo*-inositol, a direct precursor of pinitol biosynthesis, are shown in Figs 3*A* and *B*, respectively. Pinitol was always a major carbohydrate in uninfected roots (*ca* 1% dw) and in the pseudonodules of NGR234. Conversely, uninfected roots contained very small pools of *myo*-inositol. The contents of pinitol and *myo*-inositol in nodules were found to be dependent on both cultivars and bacterial strains. Nodules from RNP cultivars harboring 61-A-101 had more than three times more pinitol than nodules from NNP cultivars colonized by this strain, while *myo*-inositol pool sizes were reciprocally affected. In nodules colonized by USDA110 s*pc4*, pinitol and *myo*-

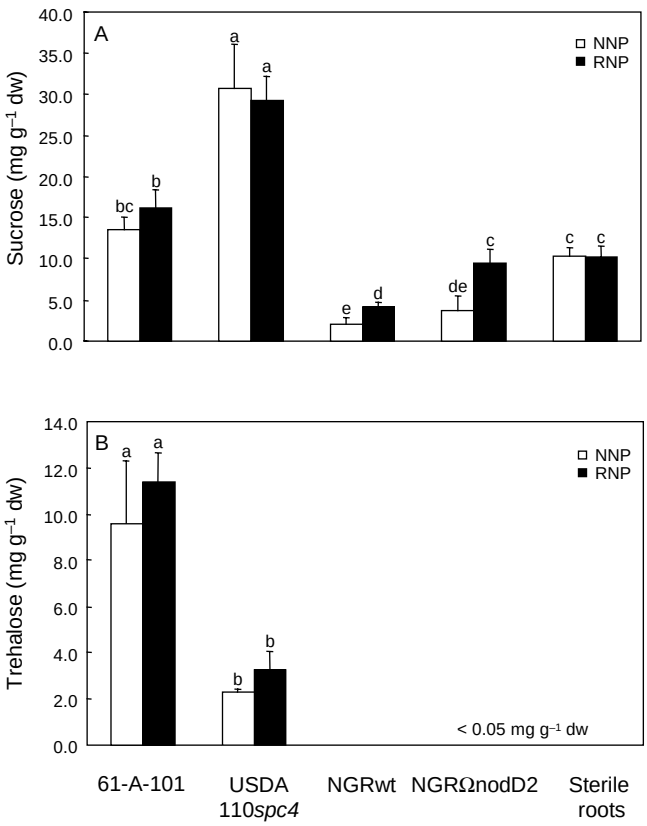

Fig. 2. Sucrose (*A*) and trehalose (*B*) pool sizes of nodules. Soybean cultivars had a normal nodulation potential (NNP) or a reduced nodulation potential (RNP) with respect to strain 61-A-101. Plants were left uninfected (sterile roots) or infected with 61-A-101, USDA110 s*pc4*, NGR234 and its mutant NGRΩ*nodD2*. Carbohydrates were analysed by gas chromatography. Mean values ± s.e. are given for three NNP cultivars and four RNP cultivars respectively. Values topped by the same letters are not significantly different (P<0.05; ANOVA followed by Student–Newman–Keuls test).

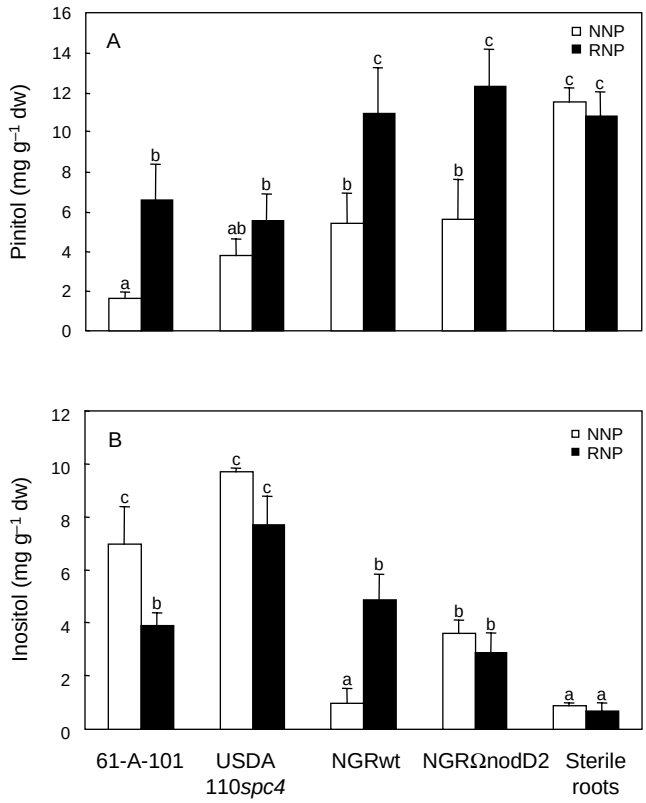

Fig. 3. Content of cyclitols in nodules. Soybean cultivars had a normal nodulation potential (NNP) or a reduced nodulation potential (RNP) with respect to strain 61-A-101. Plants were left uninfected (sterile roots) or infected with 61-A-101, USDA110 s*pc4*, NGR234 and its mutant NGRΩ*nodD2*. Cyclitol (pinitol and *myo*-inositol) contents were analysed by gas chromatography. Mean values ± s.e. are given for three NNP cultivars and four RNP cultivars respectively. Values topped by the same letters are not significantly different (P<0.05; ANOVA followed by Student–Newman–Keuls test). (*A*) Pinitol; (*B*) *myo*-inositol.

inositol pool sizes did not significantly differ between NNP and RNP cultivars. Differences between NNP and RNP cultivars were also observed upon analyses of ineffective nodules induced by NGR234. Here, pinitol contents were twice as high in nodules from RNP cultivars as in nodules from NNP cultivars, thus reaching levels found in uninfected roots (1% dw).

Induction of sucrose synthase

An antiserum against leghaemoglobin (14 kDa) and nodule-specific sucrose synthase (major band at 90 kDa) was used to examine the induction of the plant enzyme sucrose synthase in the different nodule types. In all effective nodules induced by the *Bradyrhizobium* strains, a strong signal corresponding to leghaemoglobin was detected. Sucrose synthase was induced in nodules of all cultivars colonized by 61-A-101 and in nodules of NNP cultivars harboring USDA110 s*pc4*. Interestingly, RNP cultivars like 'Dong Da Li' had a weaker sucrose synthase signal when inoculated with strain USDA110 s*pc4* in comparison with strain 61-A-101. Sucrose synthase was almost or completely undetectable in nodules induced by NGR234 or NGRΩ*nodD2* (Fig. 4). Similar findings were obtained with proteins from other NNP and RNP cultivars (blots not shown).

Induction of trehalase activity

The different nodule types were further studied by determining their hydrolytic activities of the plant enzyme trehalase. As shown in Fig. 5, a strain-specific induction was observed. Trehalase was strongly induced in Fix^+ nodules containing the *Bradyrhizobium* strains (12–14 nkat g fw^{-1} for both strains and all cultivars) compared with uninfected roots (less than 0.1 nkat g fw^{-1}). Fix^- nodules induced by the NGR strains had trehalase activities in the range of uninfected roots. Interestingly, in nodules induced by the Nod-factor-overproducing NGRΩ*nodD2*, trehalase activity was more than ten times higher (Fig. 5).

To demonstrate that Nod factors could, indeed, be responsible for the high stimulation of trehalase activity,

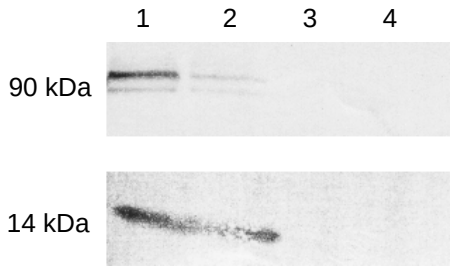

Fig. 4. Induction of nodule-specific sucrose synthase. Nodules of an RNP cultivar ('Dong Da Li') were induced by 61-A-101 (lane 1), USDA110 s*pc4* (lane 2), NGR234 (lane 3) and NGRΩ*nodD2* (lane 4). After SDS–gel electrophoresis, the proteins (15 µg per lane) were blotted onto a nitrocellulose membrane and treated with the antiserum (sucrose synthase, double band at 90 kDa; leghaemoglobin, 14 kDa).

Table 1. Trehalase activity in soybean roots treated with Nod factors

Soybeans (cv. 'Maple Arrow') were axenically grown in Leonard jars supplemented with 5 mM KNO_3 and treated with 0.1 µM Nod factors purified from strain NGR234 or left untreated. Plants were harvested after four weeks and trehalase activity was measured as described. Mean values ± s.e. are given for *n* independent plants. Values followed by the same superscript letters are not significantly different (*P* as indicated; ANOVA followed by Student–Newman–Keuls test)

Treatment	Trehalase activity (nkat g^{-1} protein)	(pkat g^{-1} fw)
Control ($n = 6$)	51 ± 6^a	39 ± 6^a
Acetylated Nod factor ($n = 6$)	181 ± 27^b	116 ± 8^b
Sulfated Nod factor ($n = 3$)	212 ± 36^b	141 ± 21^b
P	<0.005	<0.0005

uninfected roots from soybean (cultivar 'Maple Arrow') were treated with Nod factors (0.1 µM) purified from NGR234 and plants harvested after four weeks. Upon treatment with both sulfated and acetylated Nod factors, a 4-fold stimulation of root trehalase activity both with protein and with biomass as reference could be observed. This stimulation was highly significant (ANOVA followed by Student–Newman–Keuls test; Table 1).

Discussion

Accumulation of carbohydrates in soybean nodules depended on both the host plant genotype and the microsymbiont. Strain-specific alterations were found for the pool sizes of sucrose and trehalose, whereas the contents of cyclitols were affected in a more cultivar-specific manner. RNP cultivars seem to accumulate more pinitol than NNP

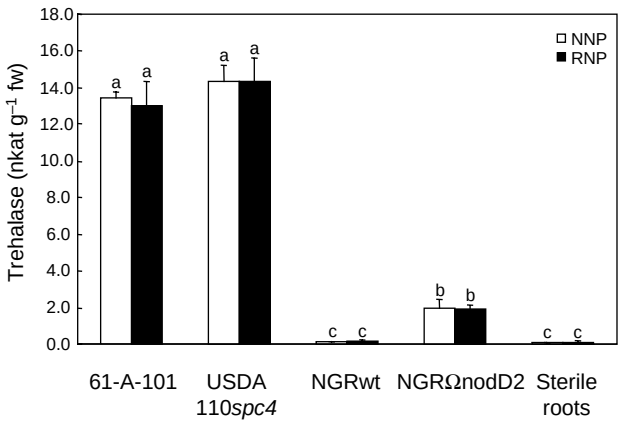

Fig. 5. Trehalase activity of nodules. Soybean cultivars had a normal nodulation potential (NNP) or a reduced nodulation potential (RNP) with respect to strain 61-A-101. Three NNP cultivars and four RNP cultivars were left uninfected (sterile roots) or infected with 61-A-101, USDA110 s*pc4*, NGR234 and NGRΩ*nodD2*. Mean values ± s.e. are given for three NNP cultivars and four RNP cultivars respectively. Values topped by the same letters are not significantly different ($P<0.05$; ANOVA followed by Student–Newman–Keuls test).

cultivars in effective nodules infected with 61-A101 and in ineffective nodules harboring NGR234, but not in effective nodules induced by USDA110 s*pc4*. Similarly, levels of *myo*-inositol differed between RNP and NNP cultivars when plants were infected with strain 61-A-101 or NGR234. Hence, the accumulation of cyclitols in soybean nodules is significantly influenced by the plant genotype, indicating a more 'root-like' carbohydrate profile.

Sucrose synthase was induced in nodules in a strain-specific manner. Recently published studies have shown that externally applied trehalose induced sucrose synthase activity in uninfected soybean roots (Müller *et al.* 1998). Thus, trehalose-producing bacteroids could, indeed, affect assimilate sink properties in the surrounding tissue. This is consistent with our observation that the nodule-specific sucrose synthase, which is responsible for sucrose breakdown, is higher in nodules induced by 61-A-101 in comparison with USDA110 s*pc4* (Fig. 4; see also Müller *et al.* 1998). Consequently, the nodules induced by 61-A-101 generally showed a low ratio of sucrose to trehalose, when compared with those of strain USDA110 s*pc4*. By comparing more than 90 different soybean nodule types, a significant, negative correlation between sucrose and trehalose pool sizes has been observed (Fig. 2 and data not shown). It seems likely therefore, that the strain-specific accumulation of trehalose reduces the sucrose pool of the host, indicating a certain competition for carbohydrates between the bacteroids and the host.

In this perspective, the induction of trehalase could be a possible way for the plant to limit these sink-inducing effects of trehalose in nodules. The trehalose-free pseudonodules induced by NGR234 (Fig. 5) exhibited low trehalase activity, suggesting a correlation between trehalose and trehalase in nodules. It was postulated that trehalose, possibly released from the bacteroids, induced trehalase activity in the host cytoplasm (Mellor 1992). Experiments have shown, however, that trehalase activity is not induced directly by trehalose, but regulated by auxins (Müller *et al.* 1995*c*). The present work suggests that Nod factors are positive regulators of trehalase activity. Nod factors have been shown to inhibit auxin transport (Mathesius *et al.* 1998) and affect carbohydrate partitioning (Xie *et al.* 1998). Hence, the stimulating effect of Nod factors on trehalase activity in soybean roots could be related to changes in auxin levels. Further studies are required to understand the mechanism by which phytohormones, Nod factors and unknown metabolites of rhizobial origin control carbohydrate metabolism during nodule symbiosis.

Acknowledgments

We are indebted to Prof. D Werner (University of Marburg, Germany) and to Prof. H Hennecke (ETH Zürich, Switzerland) for providing us bacterial strains. This work was supported by the Swiss National Foundation and a grant from the Roche Foundation to JM.

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Manuscript received 6 January 2003, received in revised form 5 May 2003, accepted 8 August 2003