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Characterization of Nodule-Specific cDNA Clones from *Sesbania rostrata* and Expression of the Corresponding Genes During the Initial Stages of Stem Nodules and Root Nodules Formation

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We have constructed a *Sesbania rostrata* stem nodule-specific cDNA library. By screening with heterologous probes from pea and soybean, we have isolated several nodulin cDNA clones. On the basis of nucleotide and amino acid sequence homology, two nearly full-length cDNA clones coding for two different leghemoglobin-like proteins have been identified. The inserts of two other clones reveal a high degree of amino acid sequence homology (81% and 72%) to the early nodulin Enod2 from soybean; the characteristic heptapeptide repeat units PPHEKPP and PPYEKPP of the soybean Enod2 are conserved in the proteins encoded by these *Sesbania* cDNA clones. The time course of Enod2 and leghemoglobin mRNA appearance during the formation of stem nodules and root nodules on *S. rostrata* was analyzed by northern blot hybridization. Significant differences were found for the initiation of mRNA accumulation of these nodulins between *S. rostrata* and soybean.

Additional keywords: nodulin mRNA accumulation, *Rhizobium*-legume symbiosis.

Nodule development can be divided into at least three stages (Vincent 1980): preinfection, infection and nodule formation, and nodule function. The preinfection stage comprises the recognition of a host legume by the respective rhizobia species leading to the attachment of the bacteria to root hairs and root hair curling. During the next stage infection threads are formed through which the bacteria can enter the roots; simultaneously, cortical cells of the root dedifferentiate and form meristems. When infection threads reach such meristematic cells, bacteria are released into the cytoplasm and develop into bacteroids. Finally, further differentiation of infected and noninfected nodule cells during the last stage results in a nitrogen-fixing nodule.

In the course of nodulation a specific set of host genes is expressed. The developmental regulation of these so-called nodulin genes is under investigation for several legumes, especially soybean, pea, and alfalfa (for review see Verma *et al.* 1986 and Govers *et al.* 1987a). Most of the nodulin genes characterized so far are induced during the switch from nodule formation to nodule function, like leghemoglobin, glutamine-synthetase, and uricase genes. Recently, however, cDNA clones for an early nodulin, Enod2, were found in soybean and pea; the corresponding gene is induced during nodule formation (Franssen *et al.* 1987; Gloudemans *et al.* 1987; Govers *et al.* 1987b). The expression level of this early nodulin gene is identical after infection of the host plant with fix$^+$ or fix$^-$ rhizobia, which carry deletions in nif-genes proving that nitrogen fixation in a functional nodule is not necessary for its induction. Additionally, Enod2 mRNA could be detected in empty soybean nodules induced by rhizobia that cannot penetrate plant cells. This indicates that Enod2 is not involved in the infection process but in nodule morphogenesis (Franssen *et al.* 1987).

Sesbania rostrata is one of the few legumes that form nodules not only on roots but also on the stem. The formation of stem nodules is induced upon crack entry of strain ORS 571 of *Azorhizobium caulinodans* at root primordia on the stem (Tsien *et al.* 1983). So far, the expression pattern of no specific nodulin gene besides leghemoglobin has been investigated during the formation of nodules on the stem and on the roots of *S. rostrata*, and time points earlier than 7 days after inoculation have not been analyzed for leghemoglobin either. This leaves open the questions as to whether nodulin genes are coordinately expressed after infection of the two different organs and whether the development of nodules on one part of the plant affects nodule morphogenesis on the other part.

In this report we describe the isolation and characterization of cDNA clones for an early and a late nodulin from *S. rostrata*, namely Enod2 and leghemoglobin, respectively. These cDNAs were used to probe the initiation of Enod2 and leghemoglobin mRNA accumulation during the first 10 days of nodule formation on stems and roots.

MATERIALS AND METHODS

Plant material. Mature stem nodules from *S. rostrata* used for construction of a cDNA library were kindly provided by A. Szalay. To analyze the time course of nodulation, seeds from *S. rostrata* were sterilized with 0.1% (w/v) $HgCl_2$ in 95% (v/v) ethanol for 15 min, followed by concentrated sulphuric acid for 30 min and then germination in sterile soil under a 16-hr light/8-hr dark cycle at 28° C. Twenty-one days after sowing, plants were infected with strain ORS 571 of *A. caulinodans*. Stems were painted with a brush that was first dipped into a bacterial lawn on an agar plate (2 days after streaking and growth at 28° C);

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simultaneously, roots were infected by watering the soil with a solution containing strain ORS 571 of *A. caulinodans* at a concentration of approximately 4×10^8 per milliliter. At 4, 7, and 10 days after inoculation root nodules and stem nodules were harvested as well as roots, stems, and leaves from uninfected plants grown for the same time as controls. Nodules from the 4-day time point of nodulation were isolated under a binocular microscope by peeling the stem epidermis.

RNA isolation. Total RNA from nodules used for cDNA synthesis was prepared according to the guanidinium thiocyanate method as described previously (Nagy *et al.* 1988). Poly(A)$^+$ RNA was selected by chromatography on oligo(dT) cellulose (Maniatis *et al.* 1982).

For northern blot experiments total RNA was extracted according to Nagy *et al.* (1988), with 2 mM aurin tricarboxylic acid as a RNase inhibitor.

cDNA synthesis and cloning. cDNA was synthesized from 2 μg of poly(A)$^+$ RNA from stem nodules of *S. rostrata* as template with a cDNA synthesis kit from Boehringer, Mannheim, used as recommended by the manufacturer. After methylase treatment and addition of *Eco*RI linkers, cDNAs were fractionated on a Biogel A-50m (Bio-Rad, Richmond, CA) column. Two fractions were obtained, one containing cDNAs shorter than 700 bp and the other with cDNAs longer than 700 bp. These two cDNA fractions were used to generate two separate cDNA libraries in lambda gt11 as vector and strain Y1088 of *E. coli* as host. The library with the longer cDNA inserts consisted of 1.7×10^5 independent clones and was screened for Enod2-like clones. The library with the shorter cDNA inserts consisted of 1.2×10^4 independent clones and was screened for leghemoglobin clones.

Plaque screening. Screening for Enod2-specific cDNA clones was done with an oligodeoxynucleotide comprising 60 nt of the coding region of the soybean Enod2 cDNA sequence (Franssen *et al.* 1987). Leghemoglobin cDNA clones were searched with a 500-nt-long antisense RNA probe transcribed from a pea leghemoglobin gene fragment. Approximately 50,000 pfu were grown per plate with a diameter of 13 cm and blotted to nitrocellulose filters (Schleicher and Schuell), as recommended by the manufacturer. Afterwards, the filters were baked for 2 hr at 80° C under vacuum. The filters were prehybridized for 4–8 hr at 50° C in a solution containing 6× SSC (1× SSC corresponds to 150 mM NaCl, 15 mM Na-citrate, pH 7.0), 5× Denhardt's solution (1× Denhardt's solution corresponds to 0.02% [w/v] each of Ficoll, polyvinylpyrolidone, and bovine serum albumin), 0.1% (w/v) SDS, and 0.1 mg/ml of denatured salmon sperm DNA. Subsequently, the filters were hybridized for 24 hr at 50° C in a solution containing 6× SSC, 1× Denhardt's solution, 0.1% (w/v) SDS, 0.05% (w/v) Na-PP$_i$, 0.1 mg/ml of denatured salmon sperm DNA, 10% (w/v) dextran sulphate, and 5×10^6 dpm/ml of 5′ terminally labeled Enod2-specific oligodeoxynucleotide. When radioactively labeled leghemoglobin-specific antisense RNA was used as probe, filters were first prehybridized for 4–8 hr at 37° C in a solution of 50% (v/v) formamide, 5× Denhardt's solution, 0.5% (w/v) SDS, 0.05% (w/v) Na-PP$_i$, 1 mM EDTA, and 0.2 mg/ml of denatured salmon sperm DNA. Hybridization was then carried out for 24 hr at 37° C in a solution of the same composition but with 10^7 dpm/ml of radioactive probe. In all cases the final stringent washing step was done in 0.1× SSC, 0.1% (w/v) SDS for 15 min at 55° C.

Phages from hybridizing plaques were eluted from the agar into SM-buffer (500 mM Tris-HCl, pH 7.5, 100 mM $MgSO_4$, 0.01% [w/v] gelatin) and rescreened three more times to obtain single plaques. Phages from purified single plaques were amplified, and DNA was isolated as follows (according to the method of R. Ott): a 13-cm plate with 5×10^4–10^5 pfu was overlayed with 10 ml of SM-buffer and shaken overnight at 4° C. The liquid was then incubated with 0.1 ml of DNase I (1 mg/ml) at 37° C for 30 min. Afterwards, 0.4 ml of 20% (w/v) SDS and 0.5 ml of 0.5 M EDTA, pH 8.0, were added and the solution was incubated at 70° C for 20 min. Two phenol extractions and one chloroform extraction followed this procedure. The DNA in the aqueous phase was precipitated with ethanol and resuspended in 0.5 ml of TE. Finally, the solution was treated with 1 μl of DNase-free RNase (10 mg/ml) at 37° C for 15 min. Inserts were cut out from the phage vector with *Eco*RI and subcloned into pEMBL12(+).

DNA sequencing. After subcloning into pEMBL12(+), cDNA inserts were sequenced by the dideoxy method (Sanger *et al.* 1977). DNA sequence data were analyzed with the help of the commercially available programs DNASIS and PROSIS from Hitachi.

Northern blot hybridization. Twenty μg of total RNA was electrophoresed on glyoxal-agarose gels, blotted to nitrocellulose, and hybridized with radioactively labeled cDNA inserts (uniformly labeled with the random primed DNA labeling kit from Boehringer, Mannheim; 10^6 dpm/ml of hybridization solution) as described elsewhere (Nagy *et al.* 1988).

Southern blot hybridization. Total genomic DNA (25 μg) was digested to completion and electrophoresed on 0.6% (w/v) agarose gels. After denaturation and renaturation, the DNA was transferred to nitrocellulose membranes (Schleicher and Schuell). Prehybridization was in 5× SSC, 5× Denhardt's solution, 0.1% (w/v) SDS, 0.1% (w/v) Na-PP$_i$, 0.1 mg/ml of tRNA, for 4–12 hr at 65° C. Hybridization was carried out in the same buffer but with 10% (w/v) dextran sulphate and random primed DNA probe at 10^6 dpm/ml overnight at 65° C. The stringent washing step was done in 0.1× SSC, 0.1% (w/v) SDS at 55° C for 15 min.

RESULTS AND DISCUSSION

Construction and screening of a stem nodule-specific cDNA library. Two cDNA libraries were constructed in lambda gt11 starting with 2 μg of poly(A)$^+$ RNA from stem nodules of *S. rostrata* as template for first strand cDNA synthesis. By screening with a pea leghemoglobin-specific riboprobe, we detected a large number (0.5–1%) of hybridizing clones in the library containing cDNA inserts shorter than 700 bp. Use of a soybean Enod2-specific oligodeoxynucleotide as probe resulted in six hybridizing clones in the library containing cDNA inserts longer than 700 bp. After Southern analysis of these clones (data not shown), the inserts of two putative leghemoglobin and three putative Enod2 cDNAs were subcloned into pEM8L12(+) and sequenced.

Structural analysis of cDNA clones coding for leghemoglobins. A comparison of the DNA sequence (coding strands) of the 625-bp-long insert of clone LbSr1 and of the

553-bp-long insert of clone LbSr6 is shown in Figure 1. In an overlap of 549 bp, the DNA sequences of these two inserts are 73% identical. This homology between LbSr1 and LbSr6 cDNA sequences is low compared with the sequence homology between soybean leghemoglobin cDNA sequences, which reaches more than 90%, indicating a higher divergence of leghemoglobin genes in *S. rostrata*. The insert of clone LbSr1 codes for an open reading frame of 148 amino acids that is 70% homologous to the deduced amino acid sequence of leghemoglobin lbc$_2$ from soybean (Fig.2a). On the DNA sequence level the homology between LbSr1 from *S. rostrata* and lbc$_2$ from soybean is 70% (data not shown). No higher DNA or amino acid sequence homology of LbSr1 to other published cDNA sequences from soybean leghemoglobin genes (Hyldig-Nielsen *et al.* 1982: Wiborg *et al.* 1982) can be found. The insert of clone LbSr6 encodes a protein that is at least 146 amino acids long and 68% identical with the deduced amino acid sequence of leghemoglobin lba from soybean (Fig.2b). The DNA sequence of the LbSr6 insert is 70% homologous to the soybean lba cDNA sequence (data not shown). Again, no higher homologies to cDNA or amino acid sequences of other soybean leghemoglobin genes can be found. By anion-exchange chromatography, leghemoglobins from stem nodules and root nodules of *S. rostrata* could be divided into seven major components (Bogusz *et al.* 1987). Comparison of the amino terminal amino acid sequence of these components with the deduced amino acid sequence of our leghemoglobin cDNA clones reveals that LbSr1 most likely corresponds to component LbII and LbSr6 to component LbIV. However, one mismatch was found between LbSr1 and LbII, and three mismatches were identified between LbSr6 and LbIV. These differences could arise if components LbII and LbIV are composed of subgroups with minor amino acid sequence heterogeneities that cannot be resolved on anion-exchange columns. The sequence comparison clearly suggests that LbSr1 and LbSr6 represent nearly full-length cDNA clones because both contain the amino terminus of leghemoglobins. The insert length of LbSr1 and LbSr6 also supports this assumption (mature leghemoglobin mRNA from *S. rostrata* is about 700 nt long).

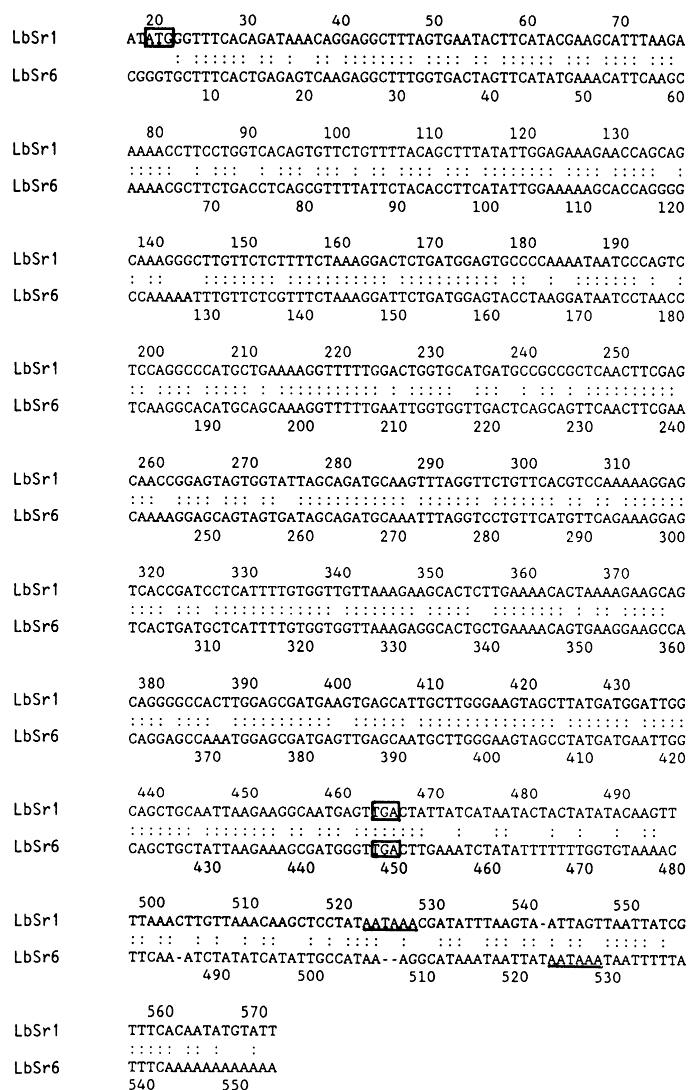


Fig. 1. Comparison of cDNA sequences of *Sesbania rostrata* coding for leghemoglobins. The 3′ terminal 555 nucleotides of the coding strand of clone LbSr1 (total length of the cDNA insert: 625 bp) and the coding sequence of clone LbSr6 are aligned to give maximum homology. Nucleotides that are identical in both cDNAs are indicated by a colon between the lines. The start codon ATG in clone LbSr1 as well as the termination codon TGA are framed. The putative polyadenylation signal AATAAA is underlined in both clones. Numbering starts with the first nucleotide of the cDNA insert as 1 in both cases.

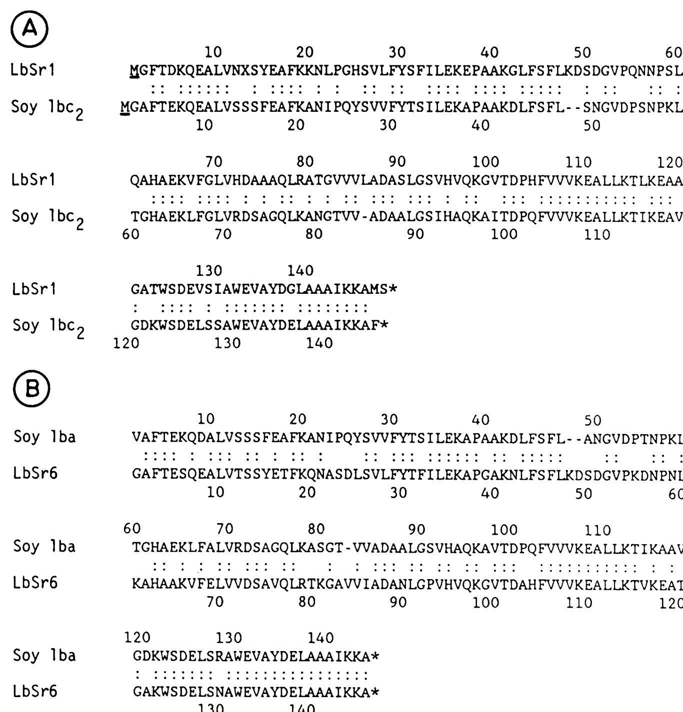


Fig. 2. Comparison of leghemoglobin amino acid sequences from soybean (Hyldig-Nielsen *et al.* 1982: Wiborg *et al.* 1982) and *S. rostrata*. The deduced amino acid sequences of clone LbSr1 from *S. rostrata* and lbc$_2$ from soybean (**A**) and of LbSr6 from *S. rostrata* and lba from soybean (**B**) are aligned for maximum homology. The one-letter code for amino acids is used. Identical amino acids in each of the two different sequences are indicated by a colon between the lines. The initiating methionine (M) is underlined, and the termination signal in the cDNA sequence is represented by an asterisk in each of the sequences. Numbering starts with the initiating methionine in the cases where the complete coding region is covered by the cDNA sequences (LbSr1, Soy lbc$_2$, and Soy lba) and with the first amino acid of the longest open reading frame in LbSr6 as 1. X at position 13 of LbSr1 very likely represents threonine.

Structural analysis of cDNA clones coding for Enod2-like proteins. A maximum of 90% homology was found between the 575-bp-long insert of clone Enod2-3A and the 470-bp-long insert of clone Enod2-3B, as shown for the coding strands in Figure 3. The 5′ terminal 267 bp (Enod2-3A) and 192 bp (Enod2-3B) encode an open reading frame of 89 and 64 amino acid residues, respectively (Figs. 3 and 4). The deduced amino acid sequence of Enod2-3A from *S. rostrata* is 81% homologous to the deduced amino acid sequence of Enod2 from soybean (Fig. 4a). The amino acid sequence homology between Enod2-3B from *S. rostrata* and Enod2 from soybean reaches 72% (Fig.4b). Significantly, the characteristic heptapeptide repeat units PPHEKPP and PPYEKPP of Enod2 from soybean (Franssen *et al.* 1987) are conserved in both Enod2-like proteins encoded by the cDNA inserts of *S. rostrata* with minor variations. All variations within these repeat units can be deduced from single base exchanges; for example, the CAT (histidine; H) to TAT (tyrosine; Y) transition. Other (hydroxy)proline-rich proteins like extensins from carrot and soybean (Chen and Varner 1985: Cassab *et al.* 1985) are also composed of repeat units that have, however, a different structure. We do not know whether one of our Enod2 cDNA clones corresponds to the methionine-free Enod2 found in soybean (Franssen *et al.* 1987).

The homology between the two Enod2 cDNA inserts from *S. rostrata* is higher in the 3′ terminal noncoding region than in the coding region (Fig. 3). This is somewhat unusual because in most gene families, for instance, the pea rbcS-family (Fluhr *et al.* 1986), homology is higher in the protein coding sequences. This might suggest a regulatory function for the noncoding sequences of Enod2-3A and Enod2-3B from *S. rostrata*. However, no particularly high homology to the soybean Enod2 cDNA can be found in this region (data not shown).

The isolation of two different Enod2 cDNA clones indicates the existence of at least two active Enod2 genes or alleles in the genome of *S. rostrata*. By Southern analysis we could detect only a single hybridizing band after digestion of the genomic DNA with the restriction enzymes *Eco*RI, *Hin*dIII, and *Eco*RV (Fig. 5, lanes 1–3). This suggests that either the genes are clustered or are located in separate

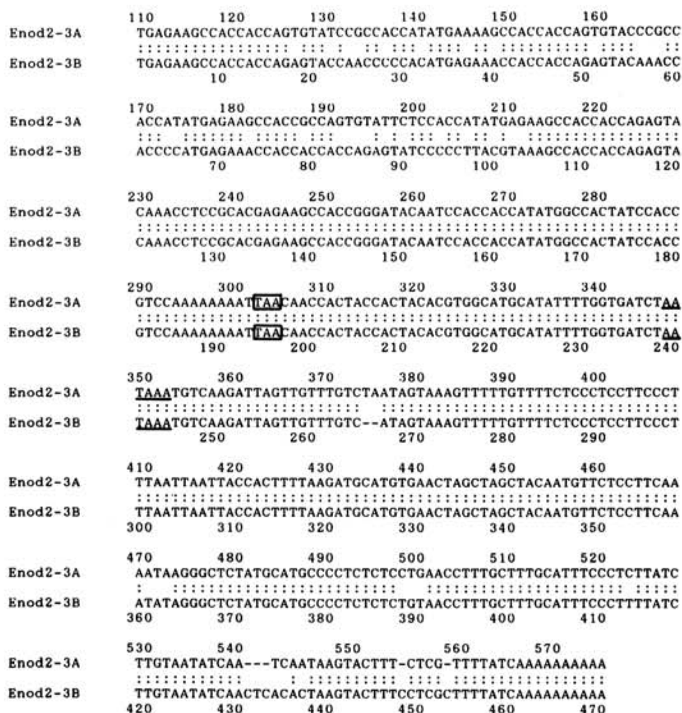


Fig. 3. Comparison of cDNA sequences from *S. rostrata* coding for Enod2-like proteins. The 3′ terminal 467 nucleotides of the coding strand of clone Enod2-3A (total length of the cDNA insert: 575 bp) are aligned with the coding strand of the Enod2-3B insert to give maximum homology. Identical nucleotides are indicated by a colon between the lines. The termination codon TAA is framed, and the putative polyadenylation signal AATAAA is underlined in both cases. Numbering begins with the first nucleotide of the cDNA insert in both clones as 1.

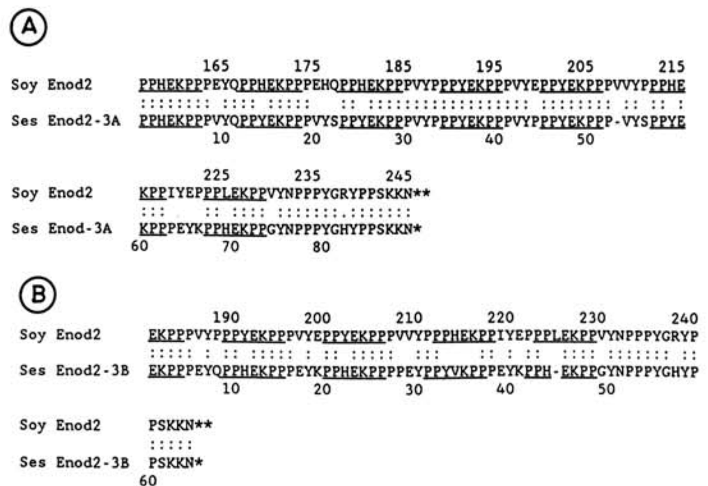


Fig. 4. Comparison of Enod2 amino acid sequences from soybean (Franssen *et al.* 1987) and *S. rostrata*. The deduced amino acid sequences of cDNA clone Enod2-3A (**A**) and of clone Enod2-3B (**B**) from *S. rostrata* are aligned with the deduced amino acid sequence of a soybean Enod2 cDNA clone (Soy Enod2) to give maximum homology. Identical amino acids are indicated by a colon between the lines. The one-letter code is used for the amino acids. The characteristic heptapeptide repeat units and their derivatives are underlined. Asterisks represent stop codons in the cDNA sequence. Numbering starts with the initiating methionine in the soybean protein and with the first amino acid of the longest open reading frame for the proteins of *S. rostrata*.

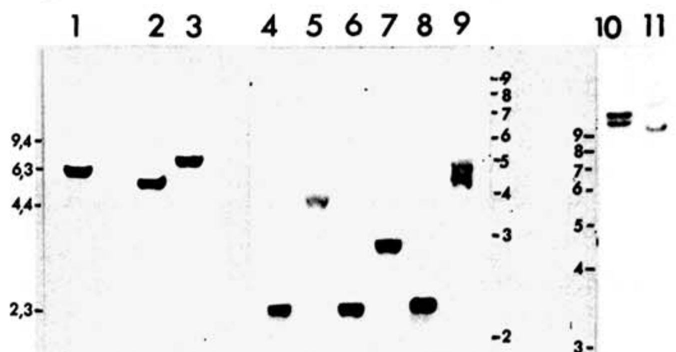


Fig. 5. Genomic organization of genes coding for Enod2 in *S. rostrata*. Genomic DNA of *S. rostrata* (25 μg per lane) was digested, run on 0.6% (w/v) agarose gels, blotted onto nitrocellulose paper, and hybridized with a mixture of the two Enod2 cDNAs. Lane 1, *Eco*RI; lane 2, *Hin*dIII; lane 3, *Eco*RV: lane 4, *Hin*dIII + *Nhe*I; lane 5, *Hin*dIII + *Sph*I; lane 6, *Eco*RI + *Nhe*I; lane 7, *Eco*RI + *Sph*I; lane 8, *Nhe*I; lane 9, *Sph*I; lane 10, *Xba*I; lane 11, *Bgl*II. Lambda/*Hin*dIII fragments were used as size markers.

regions with extended sequence homology. Both cDNA clones contain *Nhe*I and *Sph*I sites at the very 3′ end of the coding region. Double digests with either one of these enzymes and *Eco*RI or *Hin*dIII (Fig. 5, lanes 4–7) give single bands and therefore make it very unlikely that the two genes are clustered. Digests with the enzymes *Bgl*II and *Xba*I give at least two bands (Fig. 5, lanes 10–11). Taken together, these results suggest that Enod2-3A and Enod2-3B are flanked by highly homologous stretches of DNA. It should be noted that *S. rostrata* is a wild species and consequently may be heterozygous for Enod2. If that is the case, the two Enod2 clones could represent alleles of a single genetic locus.

The sequence of the third putative Enod2 cDNA clone from *S. rostrata* did not show any significant homology either to the Enod2 cDNA sequence from soybean or to the sequences of clones Enod2-3A and Enod2-3B from *S. rostrata* (data not shown). The heptapeptide repeat units PPHEKPP or PPYEKPP are not present in the deduced amino acid sequence of this *Sesbania* cDNA clone. Probably, it was detected because it has a relatively high G/C content (59%) like the oligonucleotide used for screening the cDNA library.

Enod2 and leghemoglobin transcript levels during nodulation of stems and roots. Inserts from characterized cDNA clones of *S. rostrata* were used as probes to analyze the time course of Enod2 and leghemoglobin mRNA accumulation during the first 10 days of nodule formation on stems and roots. Hybridization of an Enod2-specific probe with total RNA from stem nodules reveals that Enod2 mRNA can be detected for the first time 4 days after inoculation (Fig. 6a). Thereafter, the transcript level steadily increases until 10 days after inoculation. As a control, uninoculated stem material was collected at the same time points, and no Enod2 mRNA could be found in these samples (data not shown). The accumulation of Enod2 mRNA during the development of root nodules (Fig. 6b) coincides with that observed in stem nodules. Provided that the different inoculation procedures applied in our studies and in the investigation of soybean as described earlier (sowing and inoculation at the same time) (Gloudemans *et al.* 1987) lead to an identical infection process, nodules from *S. rostrata* accumulate Enod2 mRNA 3 days earlier than nodules from soybean (4 days after inoculation as compared with 7 days after sowing and inoculation). However, a relevant comparison of the Enod2 mRNA accumulation patterns in *S. rostrata* and soybean requires cytological proof that both have reached similar stages of nodule development at these time points. Finally, further investigations must answer the question whether Enod2 mRNA can be found in empty nodules from *S. rostrata* induced by bacteria that cannot penetrate plant cells. This was shown for nodules from soybean, indicating that Enod2 is not involved in the infection process but in nodule morphogenesis (Franssen *et al.* 1987).

The initiation of leghemoglobin mRNA accumulation was analyzed with the insert of the cDNA clone LbSrl of *S. rostrata* as probe for northern hybridization of total RNA from different stages of nodule formation. The first faint leghemoglobin-specific signal is detectable 4 days after inoculation (Fig. 7). Throughout the analyzed period of nodulation, the leghemoglobin mRNA level increases. No leghemoglobin mRNA can be found in uninoculated stems or roots at the measured time points of nodulation (data not shown). In soybean, alfalfa, pea, and common bean leghemoglobin, mRNA is first detectable much later during nodulation, namely 9–11 days after a similar inoculation procedure as applied in our investigations (Sengupta-Gopalan *et al.* 1986: Lullien *et al.* 1987; Govers *et al.* 1987b; Campos *et al.* 1987). Additionally, the lag phase between Enod2 and leghemoglobin mRNA accumulation is shorter in nodules of *S. rostrata* (about 1 day) than in soybean nodules (3–4 days) (Gloudemans *et al.* 1987). These results suggest that nodules from *S. rostrata* mature faster than nodules from other legumes. However, in a previous publication, the first measurable leghemoglobin mRNA level in stem and root nodules from *S. rostrata* was found 9 days after inoculation (de Lajudie and Huguet 1988). A possible reason for this discrepancy could be the different methods of inoculation (spraying stems instead of painting stems; no simultaneous inoculation of stems and roots). A difference in the initiation of leghemoglobin mRNA accumulation between separate and simultaneous inoculation of stems and roots could indicate that nodule development on

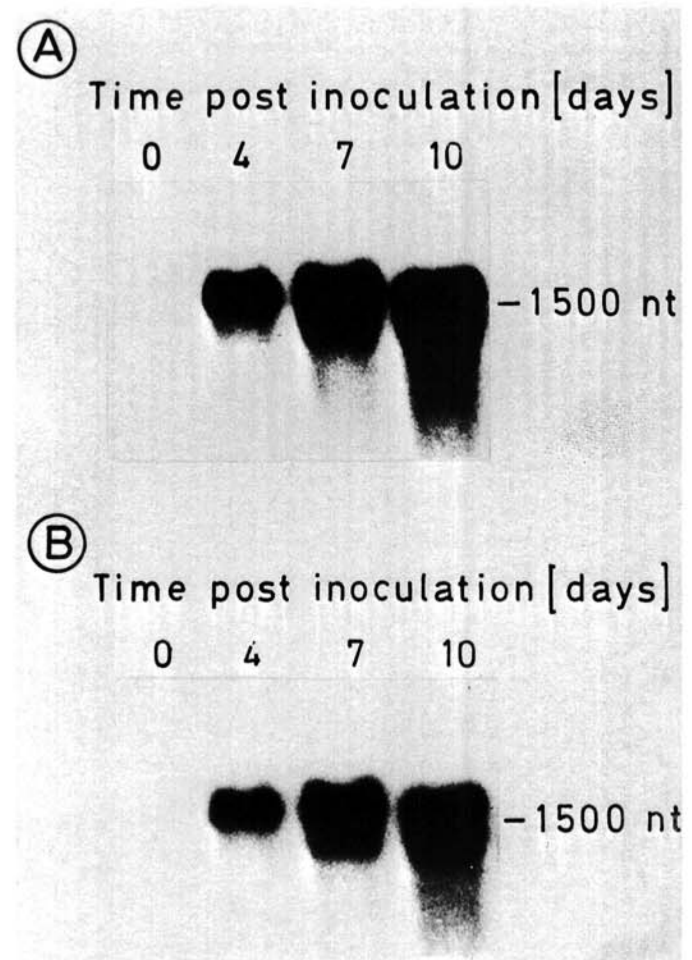
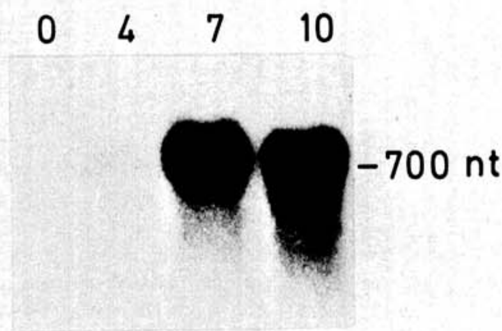


Fig. 6. Initiation of Enod2 mRNA accumulation. Twenty μg of total RNA from stem nodules (**A**) or root nodules (**B**) of *S. rostrata* harvested 4, 7, or 10 days after inoculation (as indicated) was hybridized with a homologous Enod2-specific probe on a northern blot. As a control, in each panel RNA from uninoculated stems or roots of 21-day-old plants (time point 0) were run in parallel. The length of the hybridizing mRNA (indicated by an arrow) was estimated according to lambda/*Hin*dIII and phiX174/*Hae*III DNA fragments as size markers.



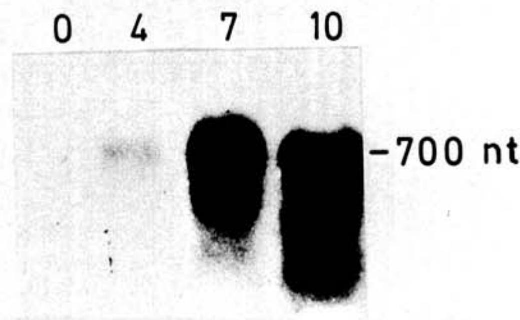


Fig. 7. Initiation of leghemoglobin mRNA accumulation. Details are as described in Figure 6, except that the insert of cDNA clone LbSr1 was used as a leghemoglobin-specific probe for northern hybridization.

one organ of the plant affects nodule morphogenesis on the other organ.

Leghemoglobin mRNA(s) from *S. rostrata* have a length of about 700 nt; this corresponds to the length of leghemoglobin mRNA from other legumes. We are not able to discriminate between the expression of individual members of the leghemoglobin gene family with the probe used in our northern hybridization experiments. The hybridizing RNA molecules shorter than 700 nt in 10-day-old root nodules (Fig. 7b) could derive from additional leghemoglobin mRNAs specifically accumuluting 10 days after inoculation or from specific or unspecific degradation of the longer mRNA species. We are in favor of the latter explanation, because after several trials, we were not able to exclude unspecific mRNA degredation in our samples from late time points postinoculation (10 days and later).

In general, our observation of identical patterns of Enod2 and leghemoglobin mRNA accumulation in root nodules and stem nodules from *S. rostrata* support the idea that both types of nodules are formed and function in a similar way.

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