The Sucrose Synthase Gene Is Predominantly Expressed in the Root Nodule Tissue of *Vicia faba*

Helge Küster, Martin Frühling, Andreas M. Perlick, and Alfred Pühler

Lehrstuhl für Genetik, Universität Bielefeld, Postfach 100131, D-3350 Bielefeld, Germany.
Received 19 January 1993. Accepted 31 March 1993.

To analyze nodule-specific gene expression in broadbean, we have isolated and sequenced sucrose synthase (SUCS) cDNAs from a broadbean nodule-specific cDNA library. The most 5′ sequences identified from these partial cDNAs were used as a molecular probe to isolate a full-length sucrose synthase transcript sequence from a cDNA library derived from broadbean nodule mRNA. This cDNA (*VfSUCS*) contained a reading frame of 2,418 bp, coding for a protein of 806 amino acids with a deduced molecular weight of 92.5 kDa. The DNA as well as the deduced amino acid sequence displayed substantial homologies (68–95%) to other plant SUCS sequences. Northern and RNA dot blot experiments demonstrated that this gene is strongly expressed in the broadbean nodule tissue. An at least 10-fold lower *VfSUCS* expression could be detected in the uninfected root, hypocotyl, stem, and flower tissues of broadbean, whereas only traces of *VfSUCS* transcripts were recognizable in the broadbean leaf tissues. *VfSUCS* transcripts could not be detected in mature seeds of broadbean. Because of this significantly nodule-amplified type of expression, we refer to *VfSUCS* as a nodulin gene and propose to designate it *VfNOD93* (Nuf-93) for the sucrose synthase enzyme).

Additional keywords: nodule carbohydrate metabolism, nodule-enhanced sucrose synthase cDNA, symbiotic nitrogen fixation.

During the symbiotic interaction between gram-negative bacteria of the family *Rhizobiaceae* and plants of the order *Fabales* (legumes), symbiotic nitrogen fixation is carried out by differentiated bacteria (bacteroids) within specialized plant structures, the so-called root nodules. This unique plant organ is built up as a result of a coordinated bacteria-plant interaction involving differential gene expression of both symbiotic partners (Nap and Bisseling 1990a, 1990b; Brewin 1991; Sanchez *et al.* 1991; Caetano-Anollés and Gresshoff 1991; Franssen *et al.* 1992).

Whereas extensive research has uncovered many aspects of the bacterial genetics of root nodule formation and nitrogen fixation (Long 1989; Fisher and Long 1992), plant molecular biology is far from understanding the molecular and regulatory basis of root nodule genesis and function. On the other hand, a number of plant genes expressed exclusively or predominantly in the nodule have already been identified (Delauney and Verma 1988). The corresponding gene products are called nodulins (van Kammen 1984). They can be divided into early and late nodulins according to the time point of their expression. Whereas early nodulins are mainly structural proteins involved in bacterial infection and nodule organogenesis, late nodulins are involved in the nodule physiology and mostly constitute leghemoglobins or enzymes of the nodule carbohydrate and nitrogen metabolism (Verma *et al.* 1992; Verma 1988). One of the most important of these is the sucrose synthase (SUCS). This enzyme has already been characterized in soybean nodules (Morell and Copeland 1985) and recently in broadbean cotyledons (Ross and Davies 1992). In soybean, the SUCS monomers were found to be identical to the nodulin GmN-100 (Thummler and Verma 1987). Abundance and enzymatic activity of GmN-100 was shown to be 20 times greater in nodule tissue than in the roots, whereas it could not be detected in the leaves. This corresponds to findings that the amount of the respective mRNA is about 10–20 times greater in soybean nodule than in soybean root tissue, and 70 times greater in nodule than in leaf tissue of soybean (Fuller and Verma 1984). In addition, a partial GmNOD100 cDNA derived from soybean nodule mRNA as well as a SUCS cDNA sequence from mung bean seedlings have been isolated and sequenced (Thummler and Verma 1987; Arai *et al.* 1992). Apart from that, SUCS sequences have been characterized from a variety of nonlegume plants, including agronomically important species like potato (Salanoubat and Belliard 1987, 1989), maize (Werr *et al.* 1985; McCarthy *et al.* 1986), wheat (Marana *et al.* 1988), and rice (Yu *et al.* 1992) as well as *Arabidopsis thaliana* (Chopra *et al.* 1992).

Sucrose synthase (UDP-glucose: D-fructose-2-glucosyltransferase, E.C.: 2.4.1.13.) is a homotetrameric enzyme, which consists of four subunits with a molecular weight of 92–93 kDa and catalyzes the cleavage of sucrose to D-fructose and UDP-glucose (Akazawa and Okamoto 1980). This enables plant tissues to metabolize sucrose, which is considered to be the main transport intermediate to sink tissues in many plants (Avigad 1982; Hawker 1985). The cleavage products can either be metabolized or can be converted to ADP-glucose, which is necessary for starch biosynthesis. This demonstrates that SUCS is a key enzyme in many plant tissues in general.

In the root nodule, in particular, four main functions have been attributed to sucrose synthase (Thummler and Verma 1987). First, cleavage of sucrose is the initial step in the nodule carbohydrate metabolism, which is proposed

MPMI, Vol. 6, No. 4, pp. 507-514
© 1993 The American Phytopathological Society

to lead to the formation of dicarboxylic acids due to the microaerobic conditions within the nodule (Vance and Heichel 1991). These acids serve as a source of energy for the bacteroids carrying out the highly energy-dependent process of symbiotic nitrogen fixation. In addition, metabolic conversions of the cleavage products provide for substrates necessary for the fixation of ammonia released by the bacteroids. The cleavage product UDP-glucose can also be used for the plant's cell wall synthesis, which is understood to be important during the initial steps of the symbiosis. Alternatively, UDP-glucose can be converted to the ADP-glucose necessary for nodule starch synthesis.

Although the SUCS enzyme obviously plays a vital role in root nodule physiology, no full-length transcript sequence derived from the nodule tissue has been reported so far. To investigate nodule-specific gene expression in broadbean, we have constructed a nodule-specific cDNA library derived from root nodule mRNA using differential hybridization (Perlick and Pühler 1993). This library has already been used to characterize several broadbean transcripts expressed exclusively or predominantly in the nodule. Preliminary sequence analysis indicated that eight clones (clone group "VfNDS-C") contained SUCS sequences.

In this paper, we present a full-length cDNA sequence of the SUCS gene from broadbean (*Vicia faba* L.) and report on the tissue-specific type of expression of this gene.

RESULTS AND DISCUSSION

Analysis of sucrose synthase transcript sequences from a broadbean nodule-specific cDNA library.

To analyze nodule-specific gene expression in broadbean, we have constructed a nodule-specific cDNA library from nodule mRNA using differential hybridization. By cross-hybridization experiments, this library has been divided into at least 28 independent clone groups. Preliminary sequence analysis indicated that clone group "VfNDS-C" contains cDNAs homologous to plant SUCS sequences (Perlick and Pühler 1993).

The analysis of all cDNAs (Suc1 to Suc8) of this clone group revealed that none of them represented a full-length SUCS transcript sequence. Between 592 and 2,086 bp were missing, when compared to the maize SUCS coding region (data not shown). Compared to each other, the individual sequences were found to contain eight polymorphic positions, but only polymorphism 3 resulted in a change in the deduced amino acid sequence from Gly to Val for cDNA Suc4 (see Fig. 1). The cDNAs terminated at six different positions in their 3′ noncoding regions, with two clones displaying a short polyA tail (20 and 23 A-residues) 185 bases, and one clone being polyadenylated (8 A-residues) 347 bp behind the SUCS stop codon (see Fig. 1).

The cDNAs Suc1 to Suc8 (as well as the full-length clone Suc9, see below) were isolated from a nodule-specific cDNA library, which was not prepared from an inbred line, but from a commercial hybrid cultivar of broadbean. We therefore assume that the sequence differences observed are due to allelic variations rather than to the presence of multiple broadbean SUCS isogenes. Genomic Southern blot experiments (data not shown) and investigations on the protein level for the broadbean and the soybean SUCS enzyme (Ross and Davies 1992; Xue *et al.* 1991) support this assumption. Five out of eight cDNAs, although synthesized from an oligo dT primer, did not contain a polyA tail. This observation is true for most other cDNAs of our nodule-specific cDNA library as well and is not restricted to the SUCS clones. Apparently, the polyA tails have been lost before cDNA cloning, which can either

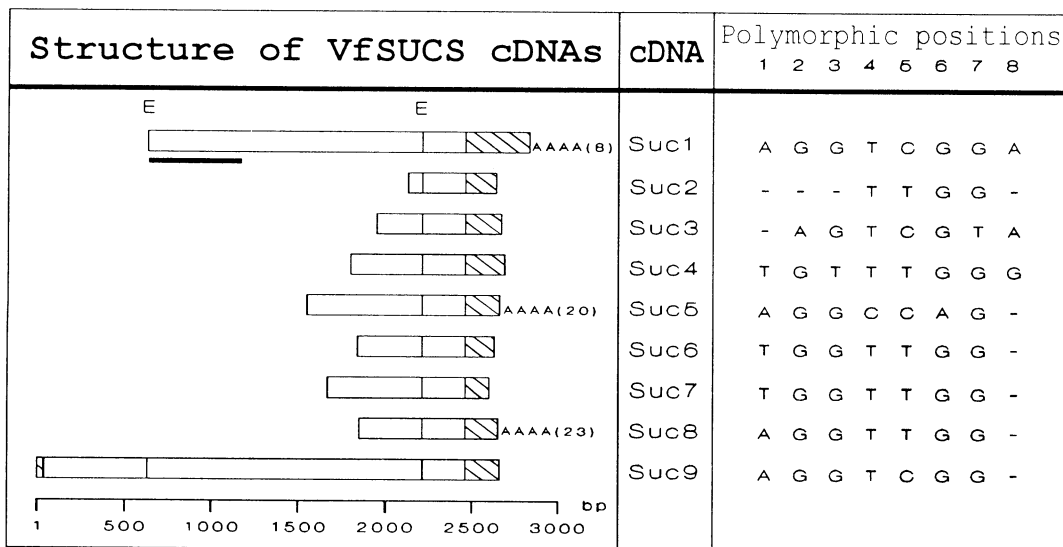

Fig. 1. Graphical representation of the structures and features of the *VfSUCS* cDNAs Suc1 to Suc9. The sequence differences of eight polymorphic positions within the DNA sequences are shown. The polymorphic positions 1–8 identified correspond to positions 1858, 2053, 2097, 2356, 2395, 2434, 2506, and 2669 of the full-length clone Suc9 (see Fig. 2). Polymorphism 3 results in a change in the deduced amino acid sequence of clone Suc4 from Gly to Val (see Fig. 2). PolyA tails identified in clones Suc1, Suc5, and Suc8 are indicated (AAAA); the respective number of A-residues is printed in brackets. The position of internal *Eco*RI sites is marked (E). An open box represents the *VfSUCS* coding region, the *VfSUCS* 5′ and 3′ nontranslated regions are indicated by hatched boxes. The extent of a 523-bp exonuclease III fragment used to screen for full-length *VfSUCS* cDNAs is marked by a black line.

```
                                *        *  M  A  T  E  R  L  T
CGTTCACCCTGTTTTCATTTTTCATAGGTGAGTAATTTGAATGGCTACTGAACGATTGAC     60
  R  V  H  S  L  R  E  R  L  D  E  T  L  T  A  N  R  N  E  I
TCGGGTTCATAGTCTGCGTGAGAGGCTTGATGAGACCTTAACTGCTAATAGGAATGAAAT    120
  L  A  L  L  S  R  I  E  A  K  G  K  G  I  L  Q  H  H  Q  V
TTTAGCTCTTCTATCAAGGATTGAAGCAAAGGGAAAGGGGATTTTGCAGCATCACCAAGT    180
  I  A  E  F  E  E  I  P  E  E  N  R  Q  K  L  T  D  G  A  F
GATTGCTGAGTTTGAAGAAATTCCTGAAGAGAATAGACAGAAGCTCACTGATGGTGCATT    240
  G  E  V  L  R  S  T  Q  E  A  I  V  L  P  P  W  V  A  L  A
TGGTGAAGTTCTCAGATCCACACAGGAAGCTATAGTTTTGCCACCATGGGTTGCACTTGC    300
  V  R  P  R  P  G  V  W  E  Y  L  R  V  N  V  H  A  L  V  V
TGTTCGTCCAAGGCCAGGTGTTTGGGAGTATCTGAGAGTGAATGTGCATGCTCTTGTTGT    360
  E  N  L  Q  P  A  E  F  L  K  F  K  E  E  L  V  D  G  S  A
TGAAAATTTGCAACCTGCTGAGTTTCTCAAATTCAAGGAAGAACTTGTTGATGGAAGTGC    420
  N  G  N  F  V  L  E  L  D  F  E  P  F  T  A  S  F  P  R  P
TAATGGTAACTTTGTGCTTGAATTGGACTTTGAACCATTTACGGCGTCTTTCCCTCGTCC    480
  T  L  N  K  S  I  G  N  G  V  Q  F  L  N  R  H  L  S  A  K
TACTCTCAACAAGTCAATTGGAAATGGTGTGCAGTTTCTCAACCGTCACCTTTCTGCTAA    540
  L  F  H  D  K  E  S  L  H  P  L  L  E  F  L  R  L  H  S  Y
ACTCTTCCATGACAAGGAGAGTTTGCATCCACTTTTGGAATTTCTCCGACTTCACAGCTA    600
  K  G  K  T  L  M  L  N  D  R  I  Q  N  P  D  S  L  Q  H  V
CAAAGGAAAGACATTGATGTTGAATGACAGAATTCAAAACCCTGATTCTCTTCAACATGT    660
  L  R  K  A  E  E  Y  L  S  T  V  D  P  E  T  P  Y  S  E  F
TCTGAGGAAAGCTGAAGAGTATCTAAGCACGGTTGATCCTGAAACTCCGTACTCGGAATT    720
  E  H  R  F  Q  E  I  G  L  E  R  G  W  G  D  S  A  E  R  V
TGAACACAGGTTCCAGGAGATTGGTTTGGAGAGAGGTTGGGGAGACAGCGCAGAGCGTGT    780
  L  E  S  I  Q  L  L  L  D  L  L  E  A  P  D  P  C  T  L  E
GCTCGAGTCCATTCAGCTTCTCTTGGATCTTCTTGAGGCTCCTGATCCTTGCACTCTTGA    840
  T  F  L  D  R  I  P  M  V  F  N  V  V  I  L  S  P  H  G  Y
GACTTTCCTTGACAGAATCCCTATGGTGTTTAATGTTGTCATTCTTTCTCCTCATGGTTA    900
  F  A  Q  D  D  V  L  G  Y  P  D  T  G  G  Q  V  V  Y  I  L
CTTTGCTCAAGATGATGTCTTGGGATACCCTGATACTGGTGGTCAGGTTGTTTACATTTT    960
  D  Q  V  R  A  L  E  S  E  M  L  N  R  I  K  K  Q  G  L  D
GGATCAAGTTCGAGCCCTCGAGAGCGAGATGCTCAATCGCATTAAGAAACAAGGCTTGGA   1020
  I  V  P  R  I  L  I  I  T  R  L  L  P  D  A  V  G  T  T  C
TATCGTTCCTCGCATTCTCATTATCACTCGTCTGCTCCCTGATGCAGTCGGAACTACTTG   1080
  G  Q  R  L  E  K  V  Y  G  T  E  H  C  H  I  L  R  V  P  F
TGGCCAACGACTTGAGAAGGTCTATGGAACCGAGCATTGTCACATTCTTCGAGTTCCCTT   1140
  R  D  Q  K  G  I  V  R  K  W  I  S  R  F  E  V  W  P  Y  L
CAGAGATCAGAAGGGAATTGTTCGCAAGTGGATCTCACGTTTCGAAGTCTGGCCATATCT   1200
  E  T  Y  T  E  D  V  A  H  E  L  A  K  E  L  Q  G  K  P  D
AGAAACCTACACCGAGGATGTTGCTCATGAGCTTGCCAAAGAGTTGCAAGGAAAACCAGA   1260
  L  I  V  G  N  Y  S  D  G  N  I  V  A  S  L  L  A  H  K  L
TCTGATTGTTGGAAACTACAGTGATGGAAACATTGTTGCTTCTTTGTTGGCACATAAACT   1320
  G  V  T  Q  C  T  I  A  H  A  L  E  K  T  K  Y  P  E  S  D
GGGTGTCACTCAGTGTACTATTGCTCATGCACTTGAGAAGACCAAGTATCCTGAATCTGA   1380
  I  Y  W  K  K  F  E  E  K  Y  H  F  S  C  Q  F  T  A  D  L
TATTTACTGGAAAAAATTTGAAGAGAAGTATCACTTCTCGTGCCAATTTACCGCTGATCT   1440
  F  A  M  N  H  T  D  F  I  I  T  S  T  F  Q  E  I  A  G  S
TTTCGCGATGAACCACACCGATTTCATCATCACAAGTACCTTCCAAGAGATTGCTGGAAG   1500
  K  D  T  V  G  Q  Y  E  S  H  T  A  F  T  L  P  G  L  Y  R
CAAGGATACTGTTGGACAGTATGAGAGTCACACTGCTTTCACTCTTCCAGGACTGTACCG   1560
  V  V  H  G  I  D  V  F  D  P  K  F  N  I  V  S  P  G  A  D
TGTTGTGCACGGTATCGATGTCTTTGATCCTAAGTTCAACATTGTGTCTCCAGGAGCTGA   1620
  Q  T  I  Y  F  P  Y  T  E  T  S  R  R  L  T  S  F  Y  P  E
TCAGACCATTTACTTCCCTTACACCGAAACTAGCCGCAGGTTGACATCGTTCTACCCTGA   1680
  I  E  E  L  L  Y  S  T  V  E  N  E  E  H  I  C  V  L  K  D
AATTGAAGAGCTTCTTTACAGCACAGTGGAAAATGAAGAGCACATATGTGTGCTCAAGGA   1740
  R  S  K  P  I  I  F  T  M  A  R  L  D  R  V  K  N  I  T  G
CCGCAGCAAGCCGATTATCTTCACCATGGCGAGGTTGGACCGTGTGAAGAACATTACAGG   1800
  L  V  E  W  Y  G  K  N  A  K  L  R  E  L  V  N  L  V  V  V
ACTAGTTGAGTGGTACGGAAAGAACGCCAAGCTACGTGAGTTGGTGAACCTTGTTGTAGT   1860
  A  G  D  R  R  K  E  S  K  D  L  E  E  K  A  E  M  K  K  M
GGCCGGAGACAGGAGGAAGGAGTCAAAGGACTTGGAAGAGAAAGCTGAGATGAAGAAGAT   1920
  Y  E  L  I  E  T  Y  K  L  N  G  Q  F  R  W  I  S  S  Q  M
GTATGAACTAATAGAGACCTACAAGTTGAACGGCCAATTCAGATGGATTTCGTCTCAGAT   1980
  N  R  V  R  N  G  E  L  Y  R  V  I  C  D  T  K  G  A  F  V
GAACCGTGTCAGAAATGGAGAGCTCTACCGTGTAATCTGCGACACAAAAGGAGCTTTCGT   2040
  Q  P  A  V  Y  E  A  F  G  L  T  V  V  E  A  M  A  T  G  L
GCAGCCTGCTGTGTACGAAGCTTTCGGTCTAACAGTCGTTGAGGCCATGGCCACTGGATT   2100
  P  T  F  A  T  L  N  G  G  P  A  E  I  I  V  H  G  K  S  G
ACCAACATTTGCAACACTCAATGGTGGACCTGCTGAGATCATTGTCCATGGAAAATCTGG   2160
  F  H  I  D  P  Y  H  G  D  R  A  A  D  L  L  V  E  F  F  E
ATTCCACATCGATCCATACCATGGCGACCGCGCTGCTGATCTCCTTGTCGAATTCTTCGA   2220
  K  V  K  A  D  P  S  H  W  D  K  I  S  L  G  G  L  Q  R  I
GAAAGTTAAGGCTGATCCATCTCACTGGGACAAGATCTCTCTCGGTGGCCTCCAACGTAT   2280
  E  E  K  Y  T  W  Q  I  Y  S  Q  R  L  L  T  L  T  G  V  Y
TGAAGAGAAGTACACATGGCAAATTTACTCTCAGAGGCTTCTTACACTCACTGGTGTCTA   2340
  G  F  W  K  H  V  S  N  L  D  R  L  E  S  R  R  Y  L  E  M
TGGTTTCTGGAAACATGTTTCTAACCTCGACCGCCTCGAGAGCCGCCGCTATCTCGAGAT   2400
  F  Y  A  L  K  Y  R  K  L  A  E  S  V  P  L  A  V  E  E  *
GTTCTATGCTCTCAAGTACCGCAAATTGGCTGAGTCTGTGCCTCTAGCTGTTGAGGAGTA   2460

ATTTGAAGAAATGAAGGAACCGGCTTTTTTTCTTTCTTATTTGGAGTCTGTTGTTTTTGA   2520

GCTTTATAAATAAAATTGTAATGATTTTGATTTTGTTGTTTGATTAAGCTTTGGATAAAG   2580

AAAATGTCAATGTCTTTTTCTTTTGCATGATTTGAAATGTGATTGGGAATATGGTCCCTT   2640

CTTCAATTTGTTGTCAATATTCGCC                                      2665
```

Fig. 2. Sequence of the full-length *VfSUCS* cDNA Suc9. The deduced amino acid sequence of the broadbean sucrose synthase is printed above the DNA sequence. The *VfSUCS* stop codon and two in-frame stop codons before the translation start site are indicated by asterisks. Nucleotides and amino acids different in cDNA1 to cDNA8 are underlined once. A putative polyadenylation signal sequence conforming to the AATAAA consensus sequence is underlined twice. The nucleotide sequence of this cDNA is available from the EMBL/GenBank/DDBJ databases under the accession number M97551.

be explained by weak hydrogen bonds and subsequent exonucleolytic digestion in the polyA region or by incomplete second-strand cDNA synthesis.

Identification and sequence analysis of a full-length sucrose synthase cDNA from broadbean nodules.

To isolate a full-length SUCS cDNA, an exonuclease III deletion fragment spanning the most 5′ 523 bp of the SUCS coding region identified so far (see Fig. 1) was used as a molecular probe to screen about 72,000 recombinant lambda gt11 phage clones from a broadbean cDNA library derived from nodule mRNA (Perlick and Pühler 1993). Phage DNA from 18 hybridizing clones was prepared, restricted with *Eco*RI, and analyzed for the presence of cDNAs about 2.7 kb in length, which is about the length of other plant SUCS transcripts. Five cDNAs could be identified, which were composed of three *Eco*RI fragments, adding up to a total length of 2.7 kb. The fragment length, as judged by agarose gel electrophoresis, was identical in each of the five clones (data not shown). Southern hybridization against SUCS probes derived from clone group "VfNDS-C" confirmed that all five cDNAs indeed contained SUCS sequences (data not shown). All *Eco*RI fragments of the cDNA Suc9 were subcloned into pSVB sequencing vectors. Sequencing from the ends of the fragments and subsequent comparisons to the maize sucrose synthase cDNA sequence *sh*l (Werr *et al.* 1985) demonstrated that clone Suc9 contained a full-length SUCS transcript sequence. To confirm the relative order of the three fragments and to exclude the possibility that small internal fragments had been missed during subcloning, primer-directed sequencing from sites adjacent to the two internal *Eco*RI sites of the clone Suc9 was carried out using the Suc9 phage DNA as a template. The presence of the terminal adapter sequences used for cDNA cloning into lambda gt11 confirmed that no terminal sequence parts had been missed during subcloning.

Figure 2 shows the complete DNA sequence of the cDNA clone Suc9. This cDNA is 2,665 bp long and contains an open reading frame of 2,418 bp, which can be translated into a protein sequence of 806 amino acids (see Fig. 2) with a predicted molecular weight of 92.5 kDa. Sequence comparisons indicated a high degree of homology to all parts of the coding region of other plant sucrose synthase genes both on the DNA and protein level (Table 1 and Fig. 3). The deduced molecular weight fitted well to biochemical estimations of the molecular weight of the sucrose synthase monomer from broadbean (Ross and Davies

Table 1. Comparison of the coding DNA and the deduced amino acid sequence of the broadbean sucrose synthase transcript to other plant sucrose synthase sequences[a]

	Homologies to the VfSUCS sequence (%)						
	TASS1	OSSS2	ZMSS1	ATSS	STSS	GMSS	VRSS
VfSUCS (DNA)	71	71	70	68	74	81	85
VfSUCS (AA)	74	75	75	68	81	92	95

[a]Sucrose synthase sequences are from broadbean (VfSUCS, this work) wheat (TASS1, Marana *et al.* 1988), rice (OSSS2, Yu *et al.* 1992), maize (ZMSS1, Werr *et al.* 1985), mouse ear cress (ATSS, Chopra *et al.* 1992), potato (STSS, Salanoubat and Belliard 1987), soybean (GMSS, Thummler *et al.* 1987) and mung bean (VRSS, Arai *et al.* 1992). The sequences from soybean and wheat are only partial sequences.

1992) and soybean (Thummler and Verma 1987). Apart from the coding region, 40 bp of the 5′ and 207 bp of the 3′ nontranslated region of the SUCS gene were present. Two in-frame stop codons in front of the reading frame (see Fig. 2) as well as the extent of homology to other SUCS genes (see Fig. 3) confirmed that the assumed start codon was correct and that clone Suc9 contained a full-length broadbean SUCS sequence (*VfSUCS*). In addition, six out of nine bases of the sequence around the translation start site (TTG<u>AATGGC</u>) identified were identical to the consensus sequence of the translation start site of dicotyledonous plants (AA^{A}/$_{C}$<u>AATGGC</u>) proposed by Elliston and Messing (1988).

In the 3′ nontranslated region of the transcript, a motif at position 2529 conformed to the consensus sequence of eukaryotic polyadenylation signal sequences ("AATAAA"), but like most other cDNAs of clone group "VfNDS-C", Suc9 itself did not contain a polyA tail. On the other hand, the length (207 bp) of the 3′ nontranslated region of the Suc9 transcript was within the range of the corresponding sequences of the *VfSUCS* clones Sucl to Suc8 (146–350 bp, Fig. 1). Here, short polyA tails could be identified at positions 185 and 347 behind the stop codon of *VfSUCS* in the three incomplete cDNA clones Sucl, Suc5, and Suc8. This showed that the polyadenylation site of the *VfSUCS* transcript could not be well defined. In addition, the polyadenylation signal sequence ("AATAAA") identified in clone Suc9 was 136 bp apart from the 3′ terminus of this cDNA (see Fig. 2), which is unusually long. On the other hand, the standard polyadenylation signal sequence "AATAAA" had not been found in a number of plant genes at all (Proudfoot 1991). We therefore conclude that additional or different signal sequences direct polyadenylation of *VfSUCS* transcripts.

```
VFSUCS    MAT----ERLTRVHSLRERLDETLTANRNEILALLSRIEAKGKGILQHHQ   46
STSUCS    MAE----RVLTRVHSLRERVDATLAAHRNEILLFLSRIESHGKGILKPHE   46
ZMSUCS1   MAAK-----LTRLHSLRERLGATFSSHPNELIALFSRYVHQGKGMLQRHQ   45
OSSUCS2   MGEAAGDRVLSRLHSVRERIGDSLSAHPNELVAVFTRLVNLGKGMLQAHQ   50
ATSUCS    MASFFDLVYHKRIGFLNKFL--SFWV----FWVCLVRYVAHGKGILQSHQ   44
          *..       .*.  ... .  ..       .   . *    ***.*. *.

VFSUCS    VIAEFEE-IPEENR-QKLTDGAFGEVLRSTQEAIVLPPWVALAVRPRPGV   94
STSUCS    LLAEFDA-IRQDDK-NKLNEHAFEELLKSTQEAIVLPPWVALAIRLRPGV   94
ZMSUCS1   LLAEFD-ALFDSDK-EKY--APFEDILRAAQEAIVLPPWVALAIRPRPGV   91
OSSUCS2   IIAEYNNAISEADR-EKLKDGAFEDVLRSAQEGIVISPWVALAIRPRPGV   99
ATSUCS    LIDEFLKTVKVDGTLEDLNKSPFMKVL---QEAIVLPPFVALAIRPRPGV   91
          ...*.    .  ..  ..     .* ..*   **.**..*.****.* ****

VFSUCS    WEYLRVNVHALVVENLQPAEFLKFKEELVDGSANGNFVLELDFEPFTASF   144
STSUCS    WEYIRVNVNALVVEELSVPEYLQFKEELVDGASNGNFVLELDFEPFTASF   144
ZMSUCS1   WDYIRVNVSELAVEELSVSEYLAFKEQLVDGQSNSNFVLELDFEPFNASF   141
OSSUCS2   WEYVRVNVSELAVELLTVPEYLQFKEQLVEEGTNNNFVLELDFEPFNASF   149
ATSUCS    REYVRVNVYELSVDHLTVSEYLRFKEELVNGHANGDYLLELHFEPFNATL   141
          ..*.**** .* *. *  .*.* ***.**..  .* ...***.****.*..

VFSUCS    PRPTLNKSIGNGVQFLNRHLSAKLFHDKESLHPLLEFLRLHSYKGKTLML   194
STSUCS    PKPTLTKSIGNGVEFLNRHLSAKMFHDKESMTPLLEFLRAHHYKGKTMML   194
ZMSUCS1   PRPSMSKSIGNGVQFLNRHLSSKLFQDKESLYPLLNFLKAHNYKGTTMML   191
OSSUCS2   PRPSLSKSIGNGVQFLNRHLSSKLFHDKESMYPLLNFLRAHNYKGMTMML   199
ATSUCS    PRPTRSSSIGNGVQLVNRHLSSIMFRNKESMEPLLEFLRTHKHDGRPMML   191
          *.*. ..******...*****. .*..***. ***.**. * ..* ..**

VFSUCS    NDRIQNPDSLQHVLRKAEEYLSTVDPETPYSEFEHRFQEIGLERGWGDSA   244
STSUCS    NDRIQNSNTLQNVLRKAEEYLIMLPPETPYFEFEHKFQEIGLEKGWGDTA   244
ZMSUCS1   NDRIQSLRGLQSSLRKAEEYLLSVPQDTPYSEFNHRFQELGLEKGWGDTA   241
OSSUCS2   NDRIRSLSALQGALRKAEEHLSGLSADTPYSEFHHRFQELGLEKGWGDCA   249
ATSUCS    NDRIQNIPILQGALARAEEFLSKLPLATPYSEFEFELQGMGFERGWGDTA   241
          ****..    **  * .*** *   .  .*** **.  .*..*.*.**** *

VFSUCS    ERVLESIQLLLDLLEAPDPCTLETFLDRIPMVFNVVILSPHGYFAQDDVL   294
STSUCS    ERVLEMVCMLLDLLEAPDSCTLEKFLGRIPMVFNVVILSPHGYFAQENVL   294
ZMSUCS1   KRVLDTLHLLLDLLEAPDPANLEKFLGTIPMMFNVVILSPHGYFAQSNVL   291
OSSUCS2   KRSQETIHLLLDLLEAPDPSTLEKFLGTIPMVFNVVIMSPHGYFAQANVL   299
ATSUCS    QKVSEMVHLLLDILQAPDPSVLETFLGRIPMVFNVVILSRYGYFAQANVL   291
          ..  . . .***.*.***.  **.**. ***.*****.*..*****..**

VFSUCS    GYPDTGGQVVYILDQVRALESEMLNRIKKQGLDIVPRILIITRLLPDAVG   344
STSUCS    GYPDTGGQVVYILDQVPALEREMLKRIKEQGLDIIPRILIVTRLLPDAVG   344
ZMSUCS1   GYPDTGGQVVYILDQVRALENEMLLRIKQQGLDITPKILIVTRLLPDAAG   341
OSSUCS2   GYPDTGGQVVYILDQVRAMENEMLLRIKQQGLNITPRILIVTRLLPDATG   349
ATSUCS    GLPDTGAQVVYILDQVRALENEMLLRIQKQGLEVIPKILIVTRLLPEAKG   341
          * ****.*********.*.*.*** **..***...*.***.*****.* *

VFSUCS    TTCGQRLEKVYGTEHCHILRVPFRDQKGIVRKWISRFEVWPYLETYTEDV   394
STSUCS    TTCGQRIEKVYGAEHSHILRVPFRTEKGIVRKWISRFEVWPYMETFIEDV   394
ZMSUCS1   TTCGQRLEKVIGTEHTDIIRVPFRNENGILRKWISRFDVWPYLETYTEDV   391
OSSUCS2   TTCGQRLEKVLGTEHTHILRVPFRTENGIVRKWISRFEVWPYLETFTDDV   399
ATSUCS    TTCNQRLERVSGTEHAHILRIPFRTEKGILRKWISRFDVWPYLETFAEDA   391
          ***.**.*.* *.** .*.*.***...**.*******.****.**. .*.

VFSUCS    AHELAKELQGKPDLIVGNYSDGNIVASLLAHKLGVTQCTIAHALEKTKYP   444
STSUCS    AKEISAELQAKPDLIIGNYSEGNLAASLLAHKLGVTQCTIAHALEKTKYP   444
ZMSUCS1   SSEIMKEMQAKPDLIIGNYSDGNLVATLLAHKLGVTQCTIAHALEKTKYP   441
OSSUCS2   AHEIAGELQANPDLIIGNYSDGNLVACLLAHKMGVTHCTIAHALEKTKYP   449
ATSUCS    SNEISAELQGVPNLIIGNYSDGNLVASLLASKLGVIQCNIAHALEKTKYP   441
          . *.  *.*. *.**.****.**..* *** *.**..*.***********

VFSUCS    ESDIYWKKFEEKYHFSCQFTADLFAMNHTDFIITSTFQEIAGSKDTVGQY   494
STSUCS    DSDIYWKKFDEKYHFSSQFTADLIAMNHTDFIITSTFQEIAGSKDTVGQY   494
ZMSUCS1   NSDIYLDKFDSQYHFSCQFTADLIAMNHTDFIITSTFQEIAGSKDTVGQY   491
OSSUCS2   NSDLYWKKFEDHYHFSCQFTTDLIAMNHADFIITSTFQEIAGNKDTVGQY   499
ATSUCS    ESDIYWRNHEDKYHFSSQFTADLIAMNNADFIITSTYQEIAGSKNNVGQY   491
          .**.*  . ...****.***.**.***..*******.*****.*..****

VFSUCS    ESHTAFTLPGLYRVVHGIDVFDPKFNIVSPGADQTIYFPYTETSRRLTSF   544
STSUCS    ESHMAFTMPGLYRVVHGINVFDPKFNIVSPGADINLYFSYSETEKRLTAF   544
ZMSUCS1   ESHIAFTLPGLYRVVHGIDVFDPKFNIVSPGADMSVYYPYTETDKRLTAF   541
OSSUCS2   ESHMAFTMPGLYRVVHGIDVFDPKFNIVSPGADMSIYFPYSESRKRLTSL   549
ATSUCS    ESHTAFTMPGLYRVVHGIDVFDPKFNIVSPGADMTIYFPYSDKERRLTAL   541
          *** ***.**********.**************  ..*..*...  .***..

VFSUCS    YPEIEELLYSTVENEEHICVLKDRSKPIIFTMARLDRVKNITGLVEWYGK   594
STSUCS    HPEIDELLYSDVENDEHLCVLKDRTKPILFTMARLDRVKNLTGLVEWYAK   594
ZMSUCS1   HPEIEELIYSDVENSEHKFVLKDKKKPIIFSMARLDRVKNMTGLVEMYGK   591
OSSUCS2   HPEIEELLYSEVDNNEHKFMLKDRNKPIIFSMARLDRVKNLTGLVELYGR   599
ATSUCS    HESIEELLFSAEQNDEHVGLLSDQSKPIIFSMARLDRVKNLTGLVECYAK   591
          .  .*.**..*.  .*.**   .*.*..***.*.*********.***** *..

VFSUCS    NAKLRELVNLVVVAG-DRRKESKDLEEKAEMKKMYELIETYKLNGQFRWI   643
STSUCS    NPRLRGLVNLVVVGG-DRRKESKDLEEQAEMKKMYELIETHNLNGQFRWI   643
ZMSUCS1   NARLRELANLVIVAG-DHGKESKDREEQAEFKKMYSLIDEYKLKGHIRWI   640
OSSUCS2   NPRLQELVNLVVVCG-DHGNPSKDKEEQAEFKKMFDLIEQYNLNGHIRWI   648
ATSUCS    NSKLRELANLVIVGGYIDENQSRDREEMAEIQKMHSLIEQYDLHGEFRWI   641
          *..*..*.***.* *     . *.* ** ** ..** .**. ..*.*..***

VFSUCS    SSQMNRVRNGELYRVICDTKGAFVQPAVYEAFGLTVVEAMATGLPTFATL   693
STSUCS    SSQMNRVRNGELYRYIADTKGAFVQPAFYEAFGLTVVEAMTCGLPTFATN   693
ZMSUCS1   SAQMNRVRNGELYRYICDTKGAFVQPAFYEAFGLTVIESMTCGLPTIATC   690
OSSUCS2   SAQMNRVRNGELYRYICDTKGAFVQPAFYEAFGLTVVESMTCGLPTFATA   698
ATSUCS    AAQMNRVRNGELYRYIADTKGVFVQPAFYEAFGLTVVESMTCALPTFATC   691
          ..************* * ****.***** ********.*.*. .***.**

VFSUCS    NGGPAEIIVHGKSGFHIDPYHGDRAADLLVEFFEKVKADPSHWDKISLGG   743
STSUCS    HGGPAEIIVHGKSGFHIDPYHGEQAADLLADFFEKCKKDPSHWETISMGG   743
ZMSUCS1   HGGPAEIIVDGVSGLHIDPYHSDKAADILVNFFDKCKADPSYWDEISQGG   740
OSSUCS2   YGGPAEIIVNGVSGFHIDPYQGDKASALLVEFFEKCQEDPSHWTKISQGG   748
ATSUCS    HGGPAEIIENGVSGFHIDPYHPDQVAGSLA-LFETCNTNPNHWVKISEGG   740
           ******* .* **.*****. .....  *. .*..   .*..* .** **

VFSUCS    LQRIEEKYTWQIYSQRLLTLTGVYGFWKHVSNLDRLESRRYLEMFYALKY   793
STSUCS    LKRIEEKYTWQIYSESLLTLAAVYGFWKHVSKLDRLEIRRYLEMFYALKY   793
ZMSUCS1   LQRIYEKYTWKLYSERLMTLTGVYGFWKYVSNLERRETRRYIEMFYALKY   790
OSSUCS2   LQRIEEKYTWKLYSERLMTLTGVYGFWKYVSNLERRETRRYLEMLYALKY   798
ATSUCS    LKRIYERYTWKKYSERLLTLAGVYAFWKHVSKLERRETRRYLEMFYSLKF   790
          *.** *.***. **..*.**..**.***.**.*.* * ***.**.*.**.

VFSUCS    RKLAESVPLAVEE         806
STSUCS    RKMAEAVPLAAE          805
ZMSUCS1   RSLASQVPLSFD          802
OSSUCS2   RTMASTVPLAVEGEPSNK    816
ATSUCS    RDLANSIPLATDEN        804
          *..*. .**. .
```

Fig. 3. Alignment of nonlegume full-length SUCS sequences with the deduced *VfSUCS* amino acid sequence. The sequences aligned are from maize (ZMSUCS 1, Werr *et al.* 1985), rice (OSSUCS2, Yu *et al.* 1992), *Arabidopsis* (ATSUCS, Chopra *et al.* 1992), potato (STSUCS, Salanoubat and Belliard 1987), and broadbean (*VFSUCS,* this work). Amino acids identical in all sequences are indicated by an asterisk, amino acids being conservatively exchanged are denoted by a period.

The *VfSUCS* sequence displays extensive homologies to other plant sucrose synthase sequences.

To analyze the *VfSUCS* transcript sequence further, comparisons of the Suc9 sequence to other plant SUCS genes were carried out. These analyses demonstrated that the *VfSUCS* transcript sequence was highly homologous to other SUCS sequences reported both on the DNA as well as on the amino acid level (see Table 1). In addition, the full-length nonlegume SUCS amino acid sequences compared were, with the exception of only a few insertions or deletions in the very 5′ and 3′ regions, colinear to the *VfSUCS* sequence (see Fig. 3) and of a very similar length ranging between 802 and 816 amino acids.

As can be expected, the degree of homology between the SUCS sequences reflected the degree of evolutionary relationship between the species individual sequences were derived from. The only major exception is the sucrose synthase from *A. thaliana*, which showed less homology to the *Vicia* sequence than did the SUCS sequences from several cereals (see Table 1 and Fig. 3).

Apart from a remarkable degree of overall homology, the alignment of plant SUCS sequences also indicated the existence of regions of considerably higher similarity (see Fig. 3). It is reasonable to assume that these parts of the sequence might be essential for the catalytic activity of SUCS enzymes in general.

The partial SUCS (nodulin N-100) sequence from soybean (Thummler and Verma 1987) showed 81% identity on the DNA and 92% identity on the amino acid level in comparison to the broadbean sequence (see Table 1). Moreover, only five out of 118 amino acids compared were completely unrelated in terms of amino acid functional similarity (see Fig. 4). This virtual identity of the primary sequence implied that the proposed downregulation of the soybean SUCS (nodulin N-100) activity via free heme in senescing nodules might also be valid for the *V. faba* enzyme.

The sucrose synthase gene from broadbean is expressed predominantly in the nodule tissue.

Analysis of the abundance of SUCS transcripts in different soybean tissues indicated that these sequences were detectable in the uninfected root tissue at a level of about 5–10% in relation to the level in nodule tissue and that the level of SUCS transcripts was about 70-fold higher in the nodule than in the leaf tissue (Fuller and Verma 1984). In our previous analysis of *VfSUCS* expression, where partial cDNAs were used as molecular probes, we found a specific SUCS expression in the nodule, but not in the root tissue (Perlick and Pühler 1993). To analyze the tissue-specific expression of the SUCS gene in broadbean in a more detailed way, Northern blot experiments using the complete *VfSUCS* cDNA sequence Suc9 as a molecular probe have now been carried out against RNA from a greater variety of broadbean tissues. The results of these experiments are shown in Figure 5.

```
VfSUCS   LPTFATLNGGPAEIIVHGKSGFHIDPYHGDRAADLLVEFF   726
         ****** ******************************|**
GmN100   LPTFATCNGGPAEIIVHGKSGFHIDPYHGDRAADLLVDFF    40

VfSUCS   EKVKADPSHWDKISLGGLQRIEEKYTWQIYSQRLLTLTGV   766
         ** * **|**||** |************************
GmN100   EKCKLDPTHWETISKAGLQRIEEKYTWQIYSQRLLTLTGV    80

VfSUCS   YGFWKHVSNLDRLESRRYLEMFYALKYRKLAESVPLAVEE   806
         ************ **************************
GmN100   YGFWKHVSNLDRRESRRYLEMFYALKYRKLAESVPLAVE    119
```

Fig. 4. Comparison of the deduced amino acid sequences of the partial nodulin N-100 transcript sequence from soybean (GmN100, Thummler and Verma 1987) and the *VfSUCS* transcript sequence Suc9 (VfSUCS, this work). Identical amino acids are indicated by asterisks, amino acids conservatively exchanged are marked by a vertical line.

These data clearly demonstrated that the *VfSUCS* gene was preferentially expressed as a 2.7-kb transcript in the broadbean nodule tissue. In contrast to our previous results (Perlick and Pühler 1993), *VfSUCS* transcripts could now be detected in the uninfected root tissue too, but at a substantially lower level than in the nodules. We assume that this could be due to different developmental and physiological stages (anaerobiosis, carbohydrate level) of the roots used for the isolation of RNA. In maize, the root tip tissue expressed SUCS genes in a manner strongly dependent on the carbohydrate supply (Koch *et al.* 1992), and the regulation of SUCS genes in soybean callus cultures was oxygen-dependent (Xue *et al.* 1991).

After longer exposition, 2.7-kb *VfSUCS* transcripts could also be detected in the hypocotyl, stem, flower, and leaf tissues, whereas no such transcripts were detectable in mature seeds of broadbean even after overexposition (data not shown).

To evaluate the relative levels of *VfSUCS* expression, RNA dot blot hybridizations against the full-length Suc9 sequence were carried out using aliquots from the same RNA preparations used for Northern blotting. As a control for the basal transcription level in the different tissues, hybridizations against a broadbean ubiquitin probe were carried out, too (see Fig. 6). The intensity of hybridizing dots was scanned (data not shown) and used to estimate

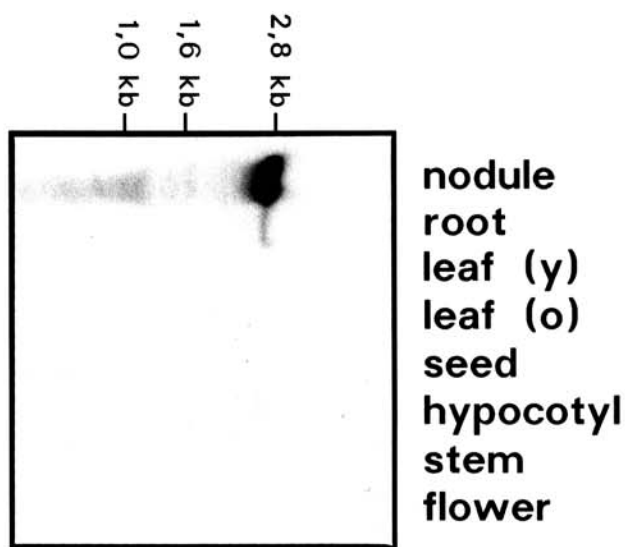

Fig. 5. Northern blot hybridization of 30 μg of total RNA from different tissues of broadbean against the full-length sucrose synthase probe Suc9. The size of RNA distance markers is indicated.

the relative levels of *VfSUCS* transcription in the tissues analyzed. The control hybridization showed that comparable amounts of ubiquitin transcripts were present in broadbean root nodule, uninfected root, hypocotyl, stem, and flower tissues (see Fig. 6). Therefore, we concluded that *VfSUCS* expression in root nodules was enhanced at least 10-fold in comparison to these tissues. In the control hybridization, a much smaller amount of ubiquitin transcripts could be detected in the leaf and seed tissues. In these cases, the amount of *VfSUCS* transcripts was comparably low, too. After overexposition, only traces of them were detectable in the leaf tissues, whereas no such transcripts could be detected in the mature seeds of broadbean. This conformed to the results obtained for SUCS expression in soybean (Fuller and Verma 1984). In comparison to the intensity of 2.7-kb *VfSUCS* transcripts detected by Northern blotting (see Fig. 5), a lower level of *VfSUCS* transcript sequences hybridizing in the dot blot had to be expected for the hypocotyl, stem, and flower tissues. We think that the differences observed could be explained by the larger amount of degraded *VfSUCS* transcripts, which was detected in the RNA from hypocotyl, stem, and flower tissues by Northern blotting (data not shown). The degraded *VfSUCS* transcript sequences added up to a higher level of total *VfSUCS* sequences in these tissues, if dot blot experiments were carried out.

From the low abundance of SUCS transcripts in tissues other than the root nodule, we concluded that the broadbean SUCS gene was expressed in a strongly nodule-amplified manner. Therefore, like the soybean GmNOD100 gene, *VfSUCS* has to be referred to as a nodulin gene. The deduced molecular weight of the *VfSUCS* monomer is 92.5-kDa, so the sucrose synthase gene has to be named *VfNOD93* according to the rules set up by van Kammen (1984) and Nap and Bisseling (1990a). The corresponding protein sequence has to be named Nuf-93.

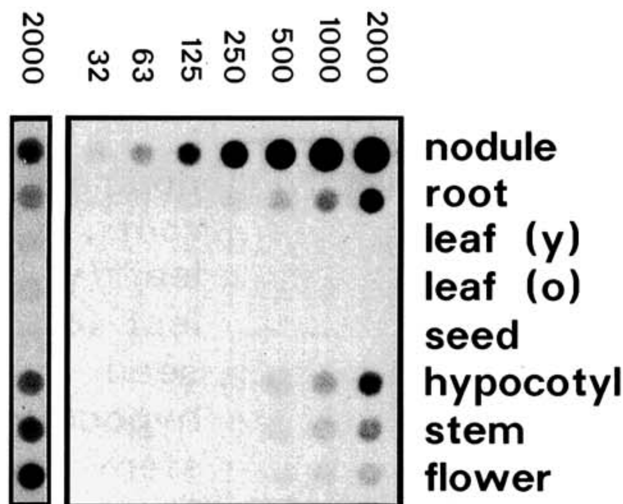

Fig. 6. Top, dot blot hybridization of different amounts of total RNA from the tissues used for Northern blotting against the full-length sucrose synthase sequence Suc9. The amount of RNA in the dots from each row is indicated in nanograms. Bottom, hybridization of the upper row against a broadbean ubiquitin probe.

Considering the evidence for the presence of only one sucrose synthase isoform in broadbean (Ross and Davies 1992), it is reasonable to assume that the *VfNOD93* transcripts identified by Northern and RNA dot blotting are derived from only one gene. Regarding this, it has to be concluded that the *VfNOD93* gene can be expressed not only in the nodules, but also in other broadbean tissues. This leads to interesting possibilities concerning the regulation of *VfNOD93* gene expression. It could well be that the gene is exclusively expressed in those parts of broadbean where sucrose supply is critical for the tissue metabolism and function (e.g., sink tissues like nodules, cotyledons, and meristematic tissue parts). This gives rise to the prospect of a regulation of *VfNOD93* gene expression via carbohydrates, as has already been demonstrated for the potato (Salanoubat and Belliard 1989) and maize (Koch *et al.* 1992) SUCS genes.

In addition, the type of expression of *VfNOD93* reported here gives further evidence that the narrow concept of nodulins as proteins exclusively found within root nodules might be misleading. We think that the *VfNOD93* type of expression much more argues in favor of a mere new combination of genes, which are already existing and functioning in other plant tissues, in a newly evolving organ like the root nodule.

In further experiments, we are going to isolate the *VfNOD93* promoter sequence to analyze the *VfNOD93* gene expression within the root nodule with respect to its possible regulation via the carbohydrate level.

MATERIALS AND METHODS

Plants, bacteria, and phage strains, plasmids, chemicals, and enzymes.

The cDNA libraries screened (Perlick and Pühler 1993) were derived from polyA$^+$ mRNA isolated from root nodules of the broadbean cultivar *V. faba* 'Kleine Thüringer' inoculated with *Rhizobium leguminosarum* VF39 (Priefer 1989). Broadbean plants were grown as described previously (Perlick and Pühler 1993). Recombinant lambda gt11 phages were grown in *E. coli* Y 1088 (Huynh *et al.* 1985). All *in vitro* DNA manipulations and cloning experiments were carried out in *E. coli* XL1Blue (Bullock *et al.* 1987). For cloning and sequencing, pSVB plasmids (Arnold and Pühler 1987) were used. Chemicals, antibiotics, enzymes, and media were purchased from Pharmacia, Freiburg, Germany; Boehringer, Mannheim, Germany; Serva, Heidelberg, Germany; and Merck, Darmstadt, Germany and used according to the manufacturer's recommendations.

Isolation of nucleic acids.

Isolation of lamdba gtll DNA was carried out as described by Sambrook *et al.* (1989). Plasmid DNA was iso-lated from 5 ml of Luria broth overnight liquid cultures of *E. coli* XL1Blue according to Priefer (1984). For sequencing purposes, pSVB cloning derivatives were prepared from *E. coli* XL1Blue grown overnight on Pennassay agar plates containing 200 μg/ml of ampicillin using the >Plasmid Mini Kit< from Diagen, Düsseldorf, Germany. Total RNA was isolated from broadbean tissues using a

protocol described by de Vries *et al.* (1982). The tissues used for RNA isolation were derived from plants 8 days old (young leaves, hypocotyl, stems), 32 days old (root nodules, uninfected roots, old leaves), or 120 days old (flowers and seeds).

Recombinant DNA techniques.

Exonuclease III digestion to generate nested deletion sequencing clones was carried out using the Double Stranded Nested Deletions Kit from Pharmacia according to the manufacturer's instructions and to Henikoff (1984). Restriction fragments used as molecular probes were isolated from agarose gels using a protocol reported by Heery *et al.* (1990). All other *in vitro* manipulations of DNA were carried out using standard protocols (Sambrook *et al.* 1989).

Plaque hybridization.

Recombinant lamdba gt11 phages were transferred onto Hybond-N nylon filters (Amersham: Braunschweig, Germany) and hybridized using procedures described by Benton and Davis (1977) and by Mason and Williams (1985). About 100 ng of probe DNA was labeled with 30 μCi of α^{32}P-dATP using the Random Primed DNA Labeling Kit from Boehringer, Mannheim, Germany. Label not incorporated was removed by gel filtration (Sephadex G50). Prehybridization (2 hr at 42° C) and hybridization (16 hr at 42° C) was carried out in a glass tube using 25 ml of the following solution: 50 ml of deionized formamide, 20 ml of of 5× Pipes (50 mM Pipes, 5 mM EDTA, 3M NaCl), 10 ml of 100× Denhardt's solution, 1 g of sodium dodecyl sulfate, 5 mg of calf thymus DNA, 5 mg of yeast RNA, H_2O to 100 ml. Stringent washes were carried out at 68° C for 15 min each (twice in 2× SSC, 0.1% [w/v] SDS; twice in 1× SSC, 0.1% [w/v] SDS; once in 0.1× SSC, 0.1% [w/v] SDS). 1× SSC is a buffered solution of 0.3 M NaCl and 0.03 M Na-citrat at pH 7.4. After a single-plaque purification step, DNA from hybridizing phages was isolated and used for Southern blotting and subcloning into pSVB sequencing vectors.

Southern hybridization.

About 2 μg of lamdba gt11 phage DNA was digested with *Eco*RI. Digests were run on 0.8% (w/v) agarose gels and transferred to Hybond-N nylon filters (Amersham) using standard vacuum blotting techniques. The DNA transferred was UV-crosslinked for 5 min and hybridized against digoxygenin-labeled probes. Labeling was carried out using the DIG DNA Labeling Kit from Boehringer according to the manufacturer's instructions. Stringent hybridizations, stringent washes, and signal detections were carried out using the DIG Luminescent Detection Kit (Boehringer).

Northern hybridization and RNA dot blotting.

Northern blotting was carried out as described previously (Perlick and Pühler 1993). About 50 ng of probe DNA was used for labeling with 30 μCi of α^{32}P-dATP using the Random Primed DNA Labeling Kit from Boehringer. Prehybridization and hybridization was carried out as described for Southern blotting, except that the hybridization solution contained no formamide and that the temperature was set to 65° C. Stringent washes were carried out at room temperature using 2× SSC, 0.1 % (w/v) SDS (once for 5 min) and at 68° C using 2× SSC, 0.1% (w/v) SDS, and 0.2× SSC, 0.1% (w/v) SDS (twice for 20 min each).

For RNA dot blots, dilution series of total RNA from nodule, leaf, mature seed, hypocotyl, stem, flower, and uninfected root tissue were prepared in a solution containing 50% (v/v) formamide, 6% (v/v) formaldehyde, and 10% 10× MOPS buffer. After denaturation for 5 min at 70° C, the RNA was transferred onto Hybond-N nylon filters soaked in 10× SSC using 500 μl of 20× SSC transfer buffer and a standard vacuum blotter. After UV-cross-linking, probe labeling, hybridizations, and washes were carried out as described for Northern blotting. Blots were stripped from probes by adding 0.1% (w/v) SDS (100° C) and allowing the solution to cool to room temperature. Subsequently, the blots were checked for the absence of radioactive probes and rehybridized using a broadbean ubiquitin cDNA sequence as a control probe. The intensity of hybridizing dots was determined using the Chromoscan3 scanner from Joyce Loebl, Gateshead, England.

Automatic sequencing.

All sequencing reactions have been carried out from double-stranded plasmid DNA templates using a nonradioactive protocol modified according to Zimmermann *et al.* (1990) together with the Autoread Sequencing Kit (Pharmacia). Sequencing gels were run on the Automatic Laser Fluorescent DNA Sequencer from Pharmacia (Ansorge *et al.* 1986, 1987) using Gibco-BRL sequencing gel mixes of standard composition. All sequences reported here have been determined from both strands.

Sequence analysis.

Nucleic acid and the derived amino acid sequences were analyzed using the ALF Manager V2.21 software (Pharmacia), the PC/Gene software package (IntelliGenetics) and the programs "analyseq" (Staden 1986), and "lfasta" (Pearson and Lipman 1988).

ACKNOWLEDGMENTS

We would like to thank Gerald Schröder for his help in performing the Northern blot experiments and Walter Arnold for the advice in nonradioactive sequencing. This work was supported by a grant from the Studienstiftung des Deutschen Volkes.

LITERATURE CITED

Akazawa, T., and Okamoto, K. 1980. Biosynthesis and metabolism of sucrose. Biochem. Plants 3:199-220.

Ansorge, W., Sproat, B. S., Stegemann, I., and Schwager, C. 1986. A nonradioactive automated method for DNA sequence determination. J. Biochem. Biophys. Methods 13:315-323.

Ansorge, W., Sproat, S., Stegemann, I., Schwager, C., and Zenke, M. 1987. Automated DNA sequencing: Ultrasensitive detection of fluorescent bands during electrophoresis. Nucleic Acids Res. 15:4593-4602.

Arai, M., Mori, H., and Imazeki, H. 1992. Expression of the gene for sucrose synthase during growth of mung bean seedlings. Plant Cell Physiol. 33:503-506.

Arnold, W., and Pühler, A. 1988. A family of high-copy-number plasmid vectors with single end-label sites for rapid nucleotide sequencing. Gene 70:171-179.

Avigad, G. 1982. Sucrose and other disaccarides. Pages 216-347 in: F. A. Loewus and W. Tanner, eds. Encyclopedia of Plant Physiology (New Ser.) Vol. 13A. Springer-Verlag, Berlin.

Benton, W. D., and Davis, R. W. 1977. Screening lamdba gt11 recombinant clones by hybridization to single plaques *in situ*. Science 196:180-182.

Brewin, N. J. 1991. Development of the legume root nodule. Annu. Rev. Cell Biol. 7:191- 226.

Bullock, W. C., Fernandez, J. M., and Short, J. M. 1987. XL1Blue: A high-efficiency plasmid transforming *recA Escherichia coli* strain with beta-galactosidase selection. BioTechniques 5:376-379.

Caetano-Annollés, G., and Gresshoff, P. M. 1991. Plant genetic control of nodulation. Annu. Rev. Microbiol. 45:345-382.

Chopra, S., Dalfevero, J., Dolferus, R., and Jacobs, M. 1992. Sucrose synthase of *Arabidopsis*: Genomic cloning and sequence characterisation. Plant Mol. Biol. 18:131-134.

Delauney, A. J., and Verma, D. P. S. 1988. Cloned nodulin genes for symbiotic nitrogen fixation. Plant Mol. Biol. Rep. 6:279-285.

de Vries, S. C., Springer, J., and Wessels, J. G. H. 1982. Diversity of abundant mRNA sequences and pattern of protein synthesis in etiolated and green pea seedlings. Planta 156:129-135.

Elliston, K., and Messing, J. 1988. The molecular architecture of plant genes: A phylogenetic perspective. Pages 21-26 in: G. Kahl, ed. The Architecture of Eucaryotic Genes. Verlag Chemie, Weinheim.

Fisher, R. F., and Long, S. R. 1992. *Rhizobium*-plant signal exchange. Nature 357:655-660.

Franssen, H. J., Yiyn, I., Yang, W. G., and Bisseling, T. 1992. Developmental aspects of the *Rhizobium*-legume symbiosis. Plant Mol. Biol. 19:89-107.

Fuller, F., and Verma, D. P. S. 1984. Appearance and accumulation of nodulin mRNAs and their relationship to the effectiveness of root nodules. Plant Mol. Biol. 3:21-28.

Hawker, J. S. 1985. Sucrose. Pages 1-51 in: P. M. Dey and R. A. Dixon, eds. Biochemistry of Storage Carbohydrates in Green Plants. Academic Press, London.

Heery, D. M., Gannon, F., and Powell, R. 1990. A simple method for subcloning DNA fragments from gel slices. Trends Genet. 6:173.

Henikoff, S. 1984. Unidirectional digestions with exonuclease III creates targeted breakpoints for DNA sequencing. Gene 28:351-359.

Huynh, T. V., Young, R. A., and Davis, R. W. 1985. Construction and screening cDNA libraries in lambda gt10 and lambda gt11. Pages 56-110 in: D. M. Glover, ed. DNA Cloning, Vol. 1: A Practical Approach. IRL Press Limited, Oxford.

Koch, K. E., Nolte, K. D., Duke, E. R., McCarthy, D. R., and Avigne, W. T. 1992. Sugar levels modulate differential expression of maize sucrose synthase genes. Plant Cell 4:59-69.

Long, S. R. 1989. *Rhizobium* genetics. Annu. Rev. Genet. 23:483-506.

Marana, C., Garcia-Olmedo, F., and Carbonero, P. 1988. Linked sucrose synthase genes in group-7 chromosomes in hexaploid wheat (*Triticum aestivum* L.). Gene 63:253-260.

Mason, P. J., and Williams, J. G. 1985. Hybridization in the analysis of recombinant DNA. Pages 113-138 in: B. D. Hames and S. J. Higgins, eds. Nucleic Acid Hybridization—A Practical Approach. IRL Press, Oxford.

McCarthy, D. R., Shaw, J. R., and Hannah, L. G. 1986. The cloning, genetic mapping, and expression of the constitutive sucrose synthase locus of maize. Proc. Natl. Acad. Sci. USA 83:9099-9103.

Morell, M., and Copeland, L. 1985. Sucrose synthase of soybean nodules. Plant Physiol. 78:149-154.

Nap, J. P., and Bisseling, T. 1990a. The roots of nodulins. Physiol. Plant. 79:404-414.

Nap, J. P., and Bisseling, T. 1990b. Developmental biology of a plant-prokaryote symbiosis: The legume root nodule. Science 250:948-954.

Pearson, W. R., and Lipman, D. J. 1988. Improved tools for biological sequence analysis. Proc. Natl. Acad. Sci. USA 85:2444-2448.

Perlick, A. M., and Pühler, A. 1993. A survey on transcripts expressed specifically in root nodules of broadbean (*Vicia faba* L.). Plant Mol. Biol. In press.

Priefer, U. 1984. Isolation of plasmid DNA. Pages 14-25 in: A. Pühler and K. N. Timmis, eds. Advanced Molecular Genetics. Springer-Verlag, Berlin.

Priefer, U. 1989. Genes involved in lipopolysaccharide production and symbiosis are clustered on the chromosome of *Rhizobium leguminosarum* biovar *viciae* VF39. J. Bacteriol. 171:6161-6168.

Proudfoot, N. 1991. Poly (A) signals. Cell 64:671-674.

Ross, H. A., and Davies, H. V. 1992. Purification and characterization of sucrose synthase from the cotyledons of *Vicia faba* L. Plant Physiol. 100:1008-1013.

Salanoubat, M., and Belliard, G. 1987. Molecular cloning and sequencing of sucrose synthase cDNA from potato (*Solanum tuberosum*): Preliminary characterization of sucrose synthase mRNA distribution. Gene 60:47-56.

Salanoubat, M., and Belliard, G. 1989. The steady-state level of potato sucrose synthase mRNA is dependent on wounding, anaerobiosis and sucrose concentration. Gene 84:181-185.

Sambrook, J., Fritsch, E. F., and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual. 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Sanchez, F., Padilla, J. E., Perez, H., and Lara, M. 1991. Control of nodulin genes in root-nodule development and metabolism. Annu. Rev. Plant Physiol. 42:507-528.

Staden, R. 1986. The current status and portability of our sequence handling software. Nucleic Acids Res. 14:217-231.

Thummler, F., and Verma, D. P. S. 1987. Nodulin-100 of soybean is the subunit of sucrose synthase regulated by the availability of free heme in nodules. J. Biol. Chem. 262:14730-14736.

van Kammen, A. 1984. Suggested nomenclature for plant genes involved in nodulation and symbiosis. Plant Mol. Biol. Rep. 2:43-45.

Vance, C. P., and Heichel, G. H. 1991. Carbon in N_2-fixation: Limitation or exquisite adaptation. Annu. Rev. Plant Physiol. Plant Mol. Biol. 42:373-392.

Verma, D. P. S. 1988. Plant genes involved in carbon and nitrogen assimilation in root nodules. Pages 43-63 in: J. E. Poulton, J. T. Romeo, and E. E. Conn, eds. Plant Nitrogen Metabolism. Plenum Press, New York.

Verma, D. P. S., Hu, C.-A. and Zhang, M. 1992. Root nodule development: Origin, function and regulation of nodulin genes. Physiol. Plant. 85:253-265.

Werr, W., Frommer, W. B., Maas, C., and Starlinger, P. 1985. Structure of the sucrose synthase gene on chromosome 9 of *Zea mays* L. EMBO J. 4:1373-1380.

Xue, Z., Larsen, K., and Jochimsen, B. U. 1991. Oxygen regulation of uricase and sucrose synthase synthesis in soybean callus tissue is exerted at the mRNA level. Plant Mol. Biol. 16:899-906.

Yin, W.-R., Wang, A.-Y., Juang, R.-H., Sung, H.-Y., and Su, J.-C. 1992. Isolation and sequence of rice sucrose synthase cDNA and genomic DNA. Plant Mol. Biol. 18:139-142.

Zimmermann, J., Voss, H., Schwager, C., Stegemann, J., Erfle, H., Stucky, K., Kristensen, T., and Ansorge, W. 1990. A simplified protocol for fast plasmid DNA sequencing. Nucleic Acids Res. 18:1067.