

## Expression of a Gene for Uricase II (Nodulin-35) in Cotyledons of Soybean Plants

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A cDNA clone (*URcot-35*) was isolated from a soybean cotyledonary cDNA library using a cDNA clone (*URnod-35*) for nodule uricase II as a probe. *URcot-35* was a 1,170-bp cDNA with an open reading frame that encoded a protein of putative 309 amino acids with a molecular mass of 35,137 Da. The nucleotide sequence of *URcot-35* was identical to that of *URnod-35*.

Expression of the *URcot-35* gene in cotyledons was investigated by Northern dot-blot hybridization, by the reverse transcription-polymerase chain reaction with subsequent hybridization assays with the cDNA for nodule uricase II as a probe, and by immunoblotting analysis with a monoclonal antibody that was specific to nodule uricase II. The results suggested that the transcript of *URcot-35* was present in developing cotyledons and that uricase II accumulated during the pod-filling stage. This is the first report of the isolation of cDNA for uricase II from non-symbiotic tissue and the results demonstrate that uricase II in soybean cotyledons is identical to that in soybean nodules.

**Key words:** Cotyledons — Gene cloning — Gene expression — Nitrogen fixation — Nodules — Uricase.

Members of tropical tribes of legumes, such as *Glycineae*, *Phaseoleae* and *Vigneae*, have determinate round nodules, and the central part of each nodule is occupied by a mixture of infected cells and uninfected cells (Newcomb 1981). In these legumes, ammonia, a primary product of nitrogen fixation, is assimilated to ureides (allantoin and allantoic acid) by the biosynthesis of purine nucleotides de novo. A key step in the formation of allantoin is the oxidative degradation of uric acid to allantoin in a reaction catalyzed by uricase (urate oxidase; EC 1.7.3.3). Ureides are believed to be very important in the nitrogen nutrition of host plants because they are prominent nitrogenous compounds that can be translocated to the shoot portion of the

plants. To support a high rate of biosynthesis of ureides, uricase is expressed at very high levels in the uninfected cells of nodules and the protein is accumulated in peroxisomes (Schubert 1986, Van den Bosch and Newcomb 1986). The uricase protein has been reported to account for 2% of the total soluble protein in mature soybean nodules (Schubert 1986).

The expression of the gene for uricase was reported to be nodule-specific, and the enzyme protein was designated nodulin-35 (Bergmann et al. 1983). In attempts to elucidate the mechanism of the nodule-specific expression of this gene, the gene for uricase was cloned from cDNA and genomic libraries of *Glycine max* (Bergmann et al. 1983, Nguyen et al. 1985), *Phaseolus vulgaris* (Sanchez et al. 1987, Papadopoulou et al. 1995), *Vigna aconitifolia* (Lee et al. 1993) and *Canavalia lineata* (Kim and An 1993). Genomic Southern hybridization indicated that the genes for uricase in *Glycine max* and *Canavalia lineata* might be present as a small multigene family (Nguyen et al. 1985, Kim and An 1993). However, in spite of extensive research efforts, the molecular mechanism for activation of transcription of the gene for uricase is still unknown.

Although uricase II has attracted the attention of many researchers as a major nodulin in determinate nodules, the expression of uricase has also been detected in many non-nitrogen fixing plants, in particular in senescent tissues (Vincentini and Matile 1993). Even in soybean plants, weak expression of a gene for uricase was detected in a non-nitrogen fixing tissue, the germinating cotyledon (Tajima et al. 1991), and the enzyme was targeted to peroxisomes as is uricase II in nodules of the same species (Tajima et al. 1991, Damsz et al. 1994). These data suggest that the gene for uricase might be expressed at a low level in various plant cells, and that the level might increase considerably in nodules as a result of some nodule-specific mechanism for enhancing gene expression.

In the present study we analyzed the expression of the gene for uricase II in soybean cotyledons by Northern dot-blot and immunoblotting analysis from germination to pod-filling stages. To characterize transcripts of the gene in cotyledons, we isolated uricase cDNA from a cotyledonary cDNA library, and the sequence of the cDNA was compared with that of cDNAs for nodule uricases that had already been isolated from the Akisengoku (Tajima et al. 1991) and Prize (Nguyen et al. 1985) varieties of soybean.

Abbreviations: ORF, open reading frame; RT-PCR, reverse transcription-polymerase chain reaction.

The nucleotide sequences reported in this paper have been submitted to the DDBJ/EMBL/GeneBank Nucleotide Sequence Databases under the accession numbers; D86929 for cotyledon uricase (*URcot-35*); and D86930 for nodule uricase (*URnod-35*).

## Materials and Methods

**Plant material**—For preparation of germinating cotyledons, soybean seeds (*Glycine max.* cv. Akisengoku) were immersed in a 1% solution of sodium hypochlorite for 10 min with gentle stirring. After these seeds had been washed 10 times with autoclaved water, they were allowed to germinate on wet filter paper in 25°C in darkness.

For preparation of developing cotyledons, soybean seeds (*Glycine max.* cv. Akisengoku) were immersed in a suspension of *Bradyrhizobium japonicum* A1017 and planted in a field at the Experimental Farm of the Faculty of Agriculture, Kagawa University. After plants had reached the flowering stage, the pods containing developing seeds were harvested and stored at -20°C prior to experiments.

**Immunoblotting analysis**—Uricase-specific monoclonal antibodies and extracts of tissues for SDS-PAGE were prepared as described previously (Tajima et al. 1991). For staining of proteins on gels after SDS-PAGE, extracts containing 5 µg of protein were applied to each lane and after electrophoresis the gel was stained with Coomassie brilliant blue (Tajima et al. 1991). For immunoblotting analysis, the amount of protein in each lane was increased to 10 µg, and a purified preparation of uricase that contained 1 µg of protein was loaded in one lane as a control. Protein was quantitated by the method of Bradford (1976) with bovine serum albumin as the standard.

**Isolation of RNA and dot-blot hybridization**—Total RNA was extracted from cotyledons of *Glycine max.* cv. Akisengoku at various stages as reported previously (Kouchi et al. 1989). The samples of RNA were dissolved in 10 mM Tris-HCl (pH 8.0) that contained 8.7% formamide and 50 µg of RNA were dotted onto a nitrocellulose filter (Tajima et al. 1991). The membrane filter was washed with 10× SSC (1× SSC=0.15 M NaCl, 0.015 M trisodium citrate, pH 7.5) and then RNA was allowed to hybridize, with a <sup>32</sup>P-labelled probe in 50% formamide, 4.8× SSC, 5× Denhardt's solution (1× =0.02% Ficoll, 0.02% polyvinyl-pyrrolidone, 0.02% bovine serum albumin), 0.5% SDS, 50 mM HEPES (pH 7.3), and 100 µg ml<sup>-1</sup> salmon sperm DNA at 42°C for more than 16 h (Maniatis et al. 1982). The membrane was washed twice in 1× SSC and 0.5% SDS at room temperature for 15 min each, and then four times for 15 min each in 0.2× SSC and 0.1% SDS at 62°C. The DNA probe for the hybridization was prepared from a cDNA clone for nodule uricase (*URnod-35*) that had been isolated from a nodule cDNA library of soybean cv. Akisengoku (Tajima et al. 1991).

**Detection of uricase mRNA in developing soybean cotyledons by RT-PCR**—Two primers for RT-PCR were designed to amplify part of the gene for nodule uricase II on the basis of the sequence reported by Nguyen et al. (1985). The forward primer (GATGGCTAAGCAGGAAGTGGTGGAA) showed anneal to positions -1 to +24 of the gene for uricase, and the reverse primer (GACCACGATAACACTTATAACACC) showed anneal to positions +310 to +334. Total RNA (1 µg) was reverse-transcribed by Moloney murine leukaemia virus reverse transcriptase (New England Biochem, Beverly, MA, U.S.A.) in the presence of oligo(dT). The synthesized cDNA was amplified by 30 cycles of PCR (92°C for 1 min, 55°C for 1 min, and 74°C for 2 min) with Taq polymerase (Toyobo Inc., Osaka, Japan). To confirm that pods were used at the same developmental stage, pods at the third internodes of soybean plants were always harvested for preparation of RNA after it had been determined that the flowering dates were the same for each plant.

RT-PCR generated a DNA fragment of 335 bp. The ampli-

fied DNA was purified on a 1% agarose gel and was blotted onto a nylon membrane (Hybond-N; Amersham International plc, Little Chalfont, U.K.) by the conventional capillary method. The DNA fragment on the membrane was allowed to hybridize with *URnod-35* that had been labeled with an ECL kit (Amersham International plc). Conditions for hybridization and washing were the same as those described above.

**Screening the soybean cotyledonary cDNA library**—Poly(A)<sup>+</sup>-RNA was prepared from germinating cotyledons of *Glycine max.* cv. Akisengoku as described elsewhere (Kouchi et al. 1989). A cDNA library was constructed in lambdaZap II (Stratagene Inc., La Jolla, CA, U.S.A.) with a cDNA synthesis kit (Pharmacia Biotech, Uppsala, Sweden) according to the manufacturer's instructions. The library was screened with <sup>32</sup>P-labelled cDNA for nodulin-35 (*URnod-35*), isolated from soybean nodules (Tajima et al. 1991), as the probe. Positive clones were purified and the inserts were subcloned into pBluescript II (Stratagene Inc.).

**Sequencing of DNA**—A cDNA insert in pBluescript II was sequenced by the dideoxy chain-termination method (Sanger et al. 1977) with an automatic sequencer (ALF DNA Sequencer; Pharmacia-LKB, Uppsala, Sweden). Both strands were sequenced in their entirety.

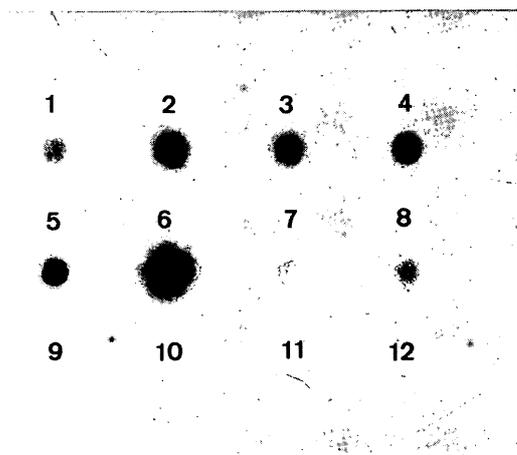
## Results

**Northern dot-blot hybridization for detection of uricase mRNA in soybean cotyledons**—For detection of the mRNA for uricase during germination and the development of cotyledon, we first performed Northern blotting analysis after electrophoresis of total RNA from developing cotyledons. With the cDNA for nodulin-35 (*URnod-35*) as probe, the RNA from developing cotyledons yielded a single hybridized band of RNA that was exactly the same size as the corresponding mRNA from nodules (data not shown). However, the hybridization signal was weak because of the low level of mRNA for uricase II in the cotyledons. Therefore, to ensure an accurate comparison of the relative abundance of the mRNA for uricase II, we performed Northern dot-blot analysis with 50 µg of total RNA in each lane, even though the assay conditions were not strictly quantitative (Fig. 1).

For preparation of developing cotyledons, we harvested green pods from two-month-old plants on the 29th day after the start of flowering. All cotyledons in the pods were collected, their diameters were measured, and they were used for extraction of RNA.

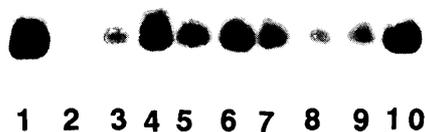
Positive signals on Northern dot-blots of RNA from developing cotyledons were observed in the seeds of almost all sizes. Low-level expression of uricase was detected even in the shells of pods. However, the level of expression of uricase mRNA in developing cotyledons appeared to be lower than that in nodules. In the case of germinating cotyledons, the signals were faint and disappeared when cotyledons were examined 24 h later.

**RT-PCR analysis of uricase mRNA during the development of soybean seeds**—To determine whether expression of the gene for uricase in developing cotyledons is



**Fig. 1** Northern dot-blot analysis of expression of the gene for uricase II (nodulin-35) during the germination and development of soybean cotyledons. For preparation of total RNA from developing cotyledons, pods were harvested from 2-month-old soybean plants 29 days after flowering. Cotyledons of various sizes in the pods were separated according to their diameter and used as samples for extraction of RNA. Results are shown for pod shells after removal of seeds (1), developing cotyledons of less than 4 mm in diameter (2), of 4–5 mm (3), of 5–6 mm (4), of 6–7 mm (5), and of more than 7 mm (6) in diameter. Fifty  $\mu\text{g}$  of total RNA were applied for each dot. Total RNA was also prepared from germinating cotyledons that were collected 2 hours (7), 1 day (8), 2 days (9), 3 days (10), 5 days (11) and 7 days (12) after the start of imbibition.

stage-specific, we examined the uricase mRNA in tissues from the beginning to the dehydration stage of seed development. Since seeds were too small at the early stage of seed development for preparation of sufficient RNA for Northern analysis, we used RT-PCR to amplify the mRNA. Figure 2 shows that RT-PCR failed to reveal the presence of uricase mRNA at the very early stage of cotyledonary development (15 days after flowering). The transcript became detectable 22 days after flowering, and its

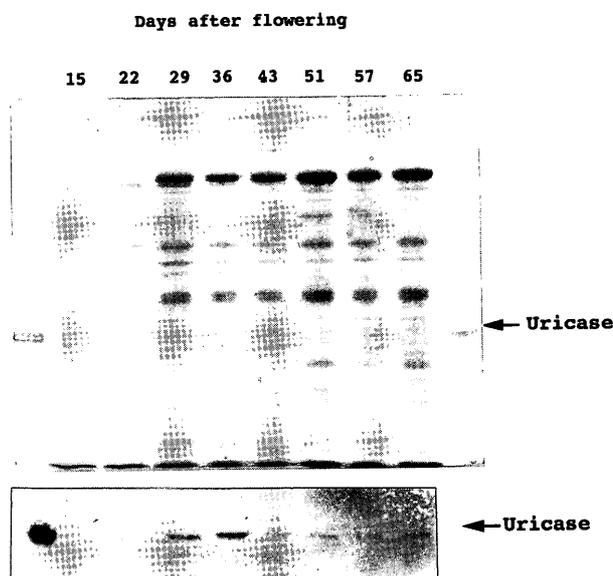


**Fig. 2** Detection of mRNA for uricase in developing cotyledons by RT-PCR. The amplified fragments in 10  $\mu\text{l}$  of the reaction mixture after RT-PCR were fractionated on a 1% agarose gel. Southern hybridization was performed using a cDNA clone for nodule uricase (*URnod-35*) as the probe. Pods were harvested 15 days (lane 2), 22 days (lane 3), 29 days (lane 4), 36 days (lane 5), 43 days (lane 6), 51 days (lane 7), 57 days (lane 8) and 65 days (lane 9) after flowering and cotyledons were isolated. Total RNA (1  $\mu\text{g}$ ) from each sample of cotyledons was subjected to RT-PCR. As a control, total RNA (0.1  $\mu\text{g}$ ) from nodules of a 2-month-old soybean plant was analyzed (lanes 1 and 10).

level was high at the middle of the pod-filling stage (29–43 days after flowering). After the cotyledons had turned yellow, the seeds started to dry out. Uricase mRNA was still detected by RT-PCR in cotyledons at the late pod-filling stage (51–57 days). Even in dried seeds (65 days after flowering), a weak signal specific for uricase mRNA remained detectable.

*Immunoblotting analysis of levels of uricase during the development of soybean seeds*—Changes in levels of uricase at various stages of the development of cotyledons were examined by SDS-PAGE with subsequent immunoblotting analysis, and the results were compared to those of RT-PCR. When extracts containing 5  $\mu\text{g}$  of protein were loaded on the gel, dense bands of protein were observed in the case of samples collected 29 to 57 days after flowering (Fig. 3), suggesting that the extensive accumulation of seed proteins began 29 days after flowering. Immunoblotting analysis also revealed the accumulation of uricase 29 and 36 days after flowering, and the results corresponded closely to those of RT-PCR (Fig. 2). A trace of uricase protein was detectable even after the seeds had become completely dehydrated.

*Isolation of a cDNA clone for a cotyledonary uricase*—For a comparison of cDNA for cotyledonary uricase with that for nodule uricase II, we constructed a cDNA library from poly(A)<sup>+</sup>-RNA of germinating soybean cotyle-



**Fig. 3** Analysis by SDS-PAGE and immunoblotting of cotyledonary proteins in seeds at various stages of development. Extracts obtained from cotyledons (15, 22, 29, 36, 43, 51, 57 and 65 days after flowering) and purified uricase from nodules were subjected to electrophoresis on a 12.5% polyacrylamide slab gel. For analysis of proteins, the gel was stained with Coomassie brilliant blue (upper panel). For immunoblotting, the proteins on the gel were transferred to a PVDF membrane (lower panel). The membrane was immunostained with antibodies against nodule uricase as described in the text.

dons. Using a cDNA clone for the nodule uricase as a probe, we isolated 24 positive clones from the library. The longest clone, which was designated ( $\lambda URcot-35$ ), contain-

ed a 1,170-kb *EcoRI* fragment, and this DNA fragment was subcloned to pBluescript II (to yield pURcot-35).

*Comparison with the gene for the nodule-specific*

			M A K Q E V V E G F K F E Q R H G K E R V R
URcot-35	1		AAAAGATGGCTAAGCAGGAAGTGGTGAAGGGTTCAAGTTCGAGCAGAGGCACGGAAAGAACCGGTGAGA
URnod-35	1		G-----
pNod-35	1	TCC-----C-----	
			V A R V W K T R Q G Q H F I V E W R V G I T L F S
URcot-35	72		GTGGCGCGGTGTGAAGACGAGGCAGGGCAGCAGCTTCATGTGGAGTGGCGGTGGGATCACTCTCTTTTCG
URnod-35	73		-----
pNod-35	76		-----
			D C V N S Y L R D D N S D I V A T D T M K N T V Y
URcot-35	147		GATTGCGTCAACTCGTACCTCCGCGATGACAACCTGACATCGTTGCTACTGATACCAGAAAACACCGGTGTAT
URnod-35	148		-----
pNod-35	151		-----
			A K A K E C S D I L S A E E F A I L L A K H F V S
URcot-35	222		GCAAAGCAAAGGAATGCTCTGACATACCTTCTGCGGAGGAGTTTGTATTCTGCTAAGCACTTTGTATCA
URnod-35	223		-----
pNod-35	226		-----
			F Y Q K V T G A I V N I V E K P W E R V T V D G Q
URcot-35	297		TTTACCAGAAGGTTACTGGTGTATTGTGAATATTGTGAAAACCATGGGAGCGTGCACGTGGATGGTCAA
URnod-35	298		-----
pNod-35	301		-----
			P H E H G F K L G S E K H T T E A I V Q K S G S L
URcot-35	372		CCTCATGAACATGGTTTCAAACCTGGGTCTGAGAAGCATACAACAGAGCGATAGTACAAAAGTCTGGTCACTT
URnod-35	373		-----
pNod-35	376		-----
			Q L T S G I E G L S V L K T T Q S G F V N F I R D
URcot-35	447		CAGTTGACTTCTGGTATTGAAGGATTGTCAGTGTGAAGACAACCCAGTCTGGTTTGTGAATTCATAAGAGAC
URnod-35	448		-----
pNod-35	451		-----
			K Y T A L P D T R E R M V A T E V T A L W R Y S Y
URcot-35	522		AAGTACACAGCACTTCCCTGATACCCGTTGAAAGGATGGTAGCAACAGAAGTAACCCGACGTGGAGGTATTCTGTAT
URnod-35	523		-----
pNod-35	526		-----
			E S L Y S L P Q K P L Y F T E K Y Q E V K K V L A
URcot-35	597		GAATCGCTGTATAGCCTCCCTCAGAAGCCGCTTTACTTTACAGAAAAGTATCAGGAAGTGA AAAAGTTCTGGCTA
URnod-35	598		-----
pNod-35	561		-----
			D T F F G P P K G G V Y S P S V Q N T L Y L M A K
URcot-35	672		GACACTTTTTTGGCCCAACAAAAGGGGAGTCTATAGCCCATCTGTTCAAACACACCTACCTGATGGCAAG
URnod-35	673		-----
pNod-35	676		-----
			A T L N R F P D I A Y V S L K L P N L H F I P V N
URcot-35	747		GCCACACTGAACAGGTTTCCCTGACATAGCTTATGTAGTCTAAAGTTGCCAAATCTTCATTTCATACCTGTCAAT
URnod-35	748		-----
pNod-35	751		-----A-----
			I S N Q D G P I V K F E D D V Y L P T D E P H G S
URcot-35	822		ATCTCAACCAGGATGGCCCTATTGTGAAGTTGAGGATGATGTACTTGCCAACGGATGAGCCACATGGGTCA
URnod-35	823		-----
pNod-35	826		-----
			I Q A S L S R L W S K L *
URcot-35	897		ATTCAAGCTAGTTTGGAGCCGCTTTGGTCAAAGCTGTAGAAACTGCAGAACTATGGTGTCTATCCCTTCTGGTG
URnod-35	898		-----
pNod-35	901		-----
			GATTGCTAGTGCAGCCTAAACTCGCAAACCTGGCCTCAGATAATGCTTTCCATTAATGTGCTTTGTAATCTGGT
URcot-35	972		-----
URnod-35	973		-----
pNod-35	976		-----
			TTTTGTTATCTTTATCTTTTGTGTTTTCATGAAGAACTATTATGCATGAATAACTTCCAGGAGGGATATTCTG
URcot-35	1047		-----
URnod-35	1048		-----
pNod-35	1046		-----G-----
URcot-35	1122		TGGGTTGCACCAATGTTAAGCTTTTTGTTTCTACTATTCCAATAAAAAA

**Fig. 4** Comparison of the nucleotide sequences of cDNA for uricase II from soybean nodules (*URnod-35*, Tajima et al. 1991; and *pNod-35*, Nguyen et al. 1985) and cotyledons (*URcot-35*). Nucleotides are numbered on the left of the sequences. A hyphen (-) indicates identical nucleotides. The predicted amino acid sequence of the longest open reading frame of the cotyledonary clone is shown in the standard single-letter code above the nucleotide sequence. The amino acids are positioned above the first nucleotide of each respective codon.

*uricase*—The nucleotide sequence of the cDNA clone for the cotyledonary uricase (*URcot-35*) was analyzed, and the cDNA clone for nodule uricase (*URnod-35*) was also sequenced to allow comparison of these cDNA clones for uricases from different tissues. The sequences were also compared to the sequence of a cDNA clone for a nodular uricase (*pNod-35*) from a different variety of soybean, Prize (Nguyen et al. 1985).

*pURcot-35* contained an ORF of 930 bp, which encoded a putative protein of 35.1 kDa. Figure 4 clearly shows that the entire nucleotide sequences of *URcot-35* and *URnod-35* were identical. A sequence of three amino acids, Ser-Lys-Leu, which is a typical signal for targeting proteins to mammalian and plant microbodies, was found at the carboxyl terminus of both putative polypeptides (Subramani 1993, Banjoko and Trelease 1995). The nucleotide sequence of *Nod-35* that had been obtained from nodules of a different variety (Prize) of soybean was also very similar to those of *URcot-35* and *URnod-35*. However, there were three nucleotide substitutions and one resultant amino acid substitution in the sequences of the cDNA and deduced protein from the Prize variety.

Our results indicated that *Glycine max* cv. Akisengoku contained identical mRNAs for uricase II in its nodules and in its cotyledonary tissues.

### Discussion

The presence of uricase II in germinating cotyledons has already been reported (Tajima et al. 1991) and its subcellular localization in peroxisomes has been demonstrated immunocytochemically (Damsz et al. 1994). In this report, we have provided evidence that identical genes for uricase II are expressed in the nodules and cotyledons of soybean plants.

Northern dot-blot assays revealed transcripts of the gene for uricase II in developing cotyledons (Fig. 1). To determine whether expression of this gene occurred throughout the development of seeds, we monitored the presence of the mRNA by RT-PCR from the beginning of seed development until the dehydration stage (Fig. 2). Although RT-PCR does not amplify transcripts quantitatively, our failure to detect uricase II mRNA at a very early stage of seed development (15 days after flowering) indicated that the expression of uricase II was negligible during the division of embryonic cells (Fig. 2), suggesting that expression of uricase II is not associated with cell division.

When seeds reached the middle of the pod-filling stage (29–43 days after flowering), the mRNA for uricase II and the protein itself were detected at high levels (Fig. 2, 3). At this developmental stage, cell division has ceased and large numbers of embryonic cells are accumulating food reserves to be used when the seed germinates (Fisher and Goldberg 1982). Figure 3 shows that the onset of accumulation of

uricase II corresponded to that of the extensive accumulation of many seed proteins.

During late embryogenesis, expression of uricase II ceased and only a small amount of uricase II was stored in dormant seeds (Fig. 2, 3) with storage proteins, trypsin inhibitors and lectins (Fisher and Goldberg 1982). Northern dot-blot analysis showed that the level of transcripts of the gene for uricase II increased one day after the soaking of seeds although the level was still very low (Fig. 1). Then the expression of the mRNA became undetectable. Northern hybridization was also performed with total RNA at this stage, and a very faint band was observed at the position that corresponded to *URnod-35* (data not shown). No differences in the expression of this gene were observed when the seeds were allowed to germinate in the light (data not shown).

Uricase II was initially detected in soybean nodules as a major nodule-specific protein, which was designated nodulin-35 (Fuller et al. 1983). The expression of nodulin-35 has been observed only in allantoin-transporting nodules and the expression of the corresponding gene has attracted the attention of many researchers (e.g., Suzuki and Verma 1991), with the subsequent detection of various genes for nodulins in many leguminous plants. However, the presence of uricase II and the corresponding mRNA in cotyledons shows that the expression of nodulin-35 is not restricted to nodules. Furthermore, Atkins et al. (1991) reported previously that legumes do not accumulate allantoin do express the gene for uricase II even when levels of ureide was very low, suggesting that the induction of this uricase might be correlated with the differentiation of interstitial cells and not with the synthesis of ureide.

In non-leguminous plants, the expression of uricase II has been reported in various tissues (Trijbels and Vogels 1966), and such expression might be essential to almost all plant cells since degradation of nucleic acids via uricase is necessary for the recycling of nitrogen to differentiating tissues. In addition, the availability of nitrogen (Theimer and Heidinger 1974), senescence (Vicentini and Matile 1993), microaerobic conditions (Xue et al. 1991), rust infection (Montalbini 1992) and phytochrome (Hong and Schopfer 1981) have been reported as factors that can induce the expression of uricase II.

Since the amounts of mRNA for uricase II in cotyledonary tissues were much lower than those in nodules, one specific characteristic of the induction of uricase II in symbiotic tissues is the high level of the accumulated protein. The physiological role of such significant expression of uricase II in nitrogen-fixing nodules of *Vigna* was examined by Lee et al. (1993). They observed that nodules that expressed an antisense RNA for uricase had lower uricase activity, and the plants exhibited symptoms of nitrogen deficiency. The data suggest that uricase II might have a physiological role in the supply of nitrogen, in addition to

its role in the metabolism of ureide. Therefore, studies of mechanisms that enhance the transcription of the gene for uricase II and the development of peroxisomes might be important in efforts to characterize nitrogen metabolism in symbiotic tissues.

In this study we isolated a full-length cDNA clone for cotyledonary uricase, *URcot-35*, and we showed that the cDNA was identical to a cDNA for nodule uricase, *URNod-35* (Fig. 4). When the sequence was compared to that of *Nod-35*, the cDNA for a nodule uricase from the Prize variety of soybean (Nguyen et al. 1985), only three nucleotide substitutions were found. There might be more than one gene for uricase in the soybean genome, and it has been suggested that the gene for uricase might form a small multigene family (Nguyen et al. 1985, Kim and An 1993). Further analysis of the expression of the gene for uricase II is necessary to determine the mechanism of the significant activation of this gene in nitrogen-fixing nodules.

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