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PLANT GENE EXPRESSION DURING EFFECTIVE AND INEFFECTIVE NODULE DEVELOPMENT OF THE TROPICAL STEM-NODULATED LEGUME SESBANIA ROSTRATA

P. de Lajudie[1] and T. Huguet
Laboratoire de Biologie Moléculaire des Relations Plantes-Microorganismes, INRA-CNRS, BP 27, Auzeville, 31326 CASTANET-TOLOSAN CEDEX, FRANCE.
(1) Permanent Address : Laboratoire de Biologie des Sols, ORSTOM, BP1386 DAKAR, SENEGAL.

Sesbania rostrata is a tropical legume which develops nitrogen-fixing nodules on both stems and roots when infected by a specific Rhizobium (Dreyfus and Dommergues, 1981). This fixation is very efficient (Dommergues et al., 1985), and Sesbania rostrata has a real potential in agriculture as a green manure (Dreyfus et al., 1985 ; Rinaudo and Moudiongui, 1986). In agreement with what is described in other nitrogen-fixing symbioses i.e. root-nodulated plants like Glycine max (Legocki and Verma, 1980), Pisum sativum (Bisseling et al., 1983), Phaseolus vulgaris (Cullimore et al., 1983) and Medicago sativa (Lang-Unnasch and Ausubel, 1985), we have found plant genes specifically activated in stem and root nodules of Sesbania rostrata.

We compared two-dimensional (2D) polyacrylamide gel electrophoresis patterns of in vitro translation products of poly A$^+$-mRNA purified from uninfected roots and stems and from root and stem nodules induced by wild type Rhizobium sp. strain ORS571 (Dreyfus et al., 1983). 200-300 polypeptides could routinely be distinguished in each pattern, their molecular weights ranging from 10 000 to more than 100 000 daltons. The figure shows the 2D-patterns of the different tissues. Most of the polypeptides are present in all tissues. However at least 30-40 polypeptides exhibit different intensities in stems, roots, stem nodules and root nodules, reflecting tissue-related plant gene expression. Among these we found, according to nomenclature proposed by Van Kammen (1984), at least 7 "nodulins" (see figure : spots 28, 26, Lb1, Lb2, Lb3, Lb4, Lb5) and 9 "nodule-stimulated polypeptides" (see figure : spots 66, 54, 53, 52, 51, 38, 37, 25, 22) common to both root and stem nodules. In addition, whilst 44 is a genuine "root nodulin" (since it is not present in uninfected roots), it is a "stem nodule-stimulated polypeptide" (since there are trace amounts in uninfected stems). In the same way, 39 is a "stem nodulin" and a "root nodule-stimulated" polypeptide. In addition, by comparing uninfected stems to stem nodules, we found 3 "stem nodulins" (38, 33, 33') and 3 "stem nodule-stimulated" polypeptides (32, 32', 31), which are either not present or not stimulated in root nodules ; on the other hand, by comparison of root nodules with uninfected roots, there are 4 "root nodulins" which are not stimulated in stem nodules.

We used an antiserum raised against leghemoglobin purified from Sesbania rostrata stem nodules to immunoprecipitate the corresponding in vitro translation products. None of the uninfected root and stem poly A$^+$ RNA translation products reacted with the antiserum. On the other hand, five abundant polypeptides of molecular weights below 17kdal (belonging to the class of common root and stem nodulins mentioned above) were immunoprecipitated from both stem and root nodules. According to their decreasing isoelectric points, we named them Lb1 to Lb5 (see figure). In mature stem and root nodules, Lb2 and Lb5 are major polypeptides, Lb3 and Lb4 moderately abundant and Lb1 is a minor species.

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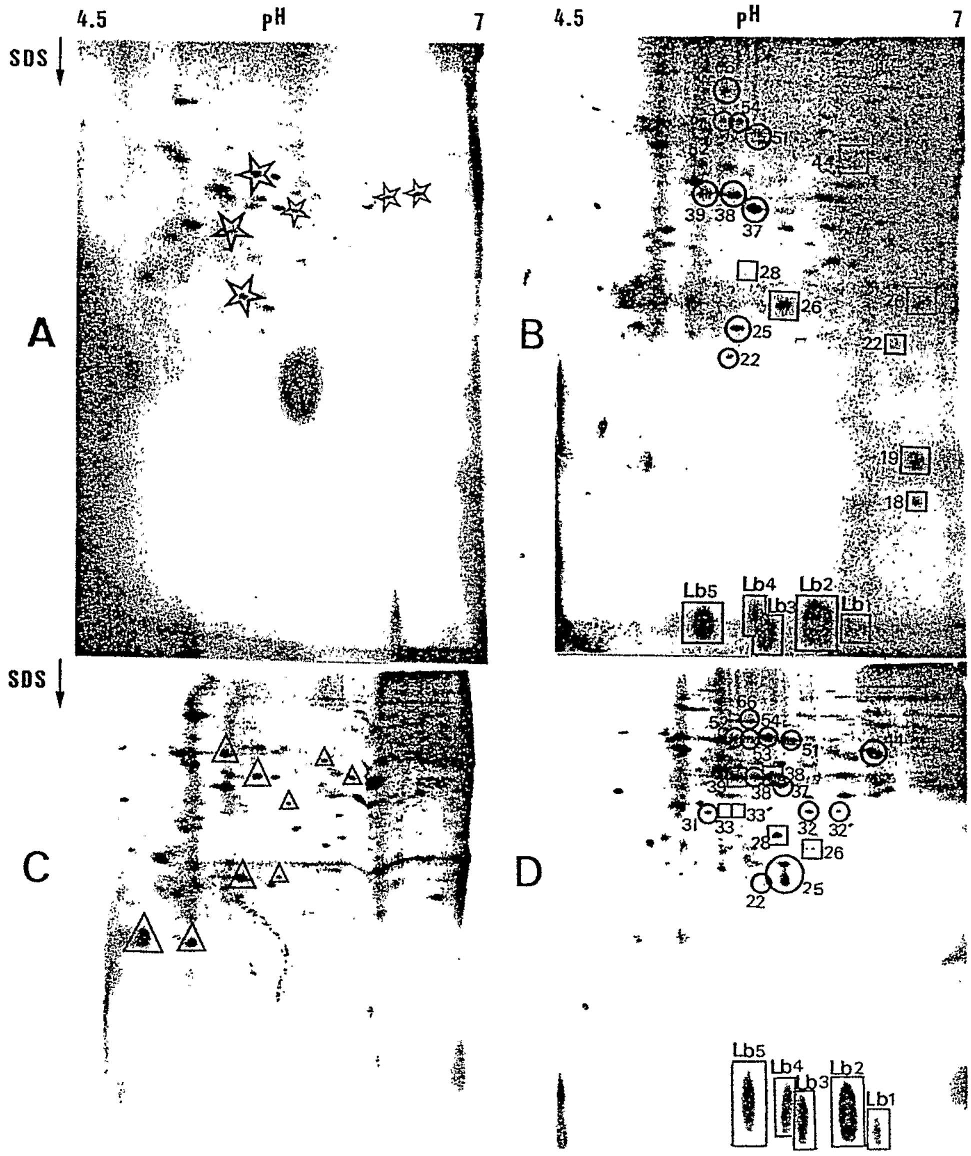

Differential plant gene expression in stem and root nodules compared to uninfected stems and roots. Fluorographs of two-dimensional polyacrylamide gel of in vitro translation products from poly A^+-mRNA isolated from :
(A) 15 days-old uninfected roots (B) effective root nodules induced by wild type Rhizobium sp. strain ORS571, 6 weeks after sowing (C) 15 days-old uninfected stems (D) 32 days-old effective stem nodules induced by wild type Rhizobium sp. strain ORS571.
Numbers indicate the polypeptides approximative molecular weights (Kdal).
□ nodule-specific polypeptide ○ nodule-stimulated polypeptide
☆ root-stimulated polypeptide △ stem-stimulated polypeptide

We studied plant gene expression during stem nodule development. Leghemoglobin components can first clearly be detected at day 12 after inoculation, concomitantly with the majority of the other nodule-stimulated polypeptides and their intensities increase throughout our observation period (32 days after stem inoculation). Moreover their relative intensities vary during the same period : at day 12, Lb2, Lb3, Lb5 have more or less the same intensities while Lb4 is major and Lb1 is not clearly visible ; after day 16, Lb2 and Lb5 become majoritary, Lb3 and Lb4 are moderately abundant and Lb1 appears but remains minoritary. Apart from leghemoglobin, other nodule-stimulated genes are activated at different stages of development : some are stimulated early, like 44 and 38, detectable at day 6, or 28 and 25 detectable at day 7. Some polypeptides reach their maximum intensity and then remain more or less at the same level : this is the case for 38, 28 (plateau reached at day 12), for 31 (plateau reached at day 14), and for 54, 53, 51, 39, 32 and 32' (maximum intensity from day 18). Some others are transiently activated : 44 is very intense during the first stages and then decreases gradually ; 33, 33', 25 and 22 reach their maximum expression between days 12-16, then decrease. All these observations indicate that plant genes are sequentially expressed during stem nodule development in _Sesbania rostrata_ like in pea (Bisseling _et al._, 1983) and soybean (Fuller and Verma, 1984).

We also analysed ineffective stem nodules induced by a nif^- mutant _Rhizobium_ sp. strain 5740 (Elmerich _et al._, 1982). The resulting pattern is comparable with that of the effective stem nodules, except for the five leghemoglobin components which appear less abundant (data not shown). This indicates that in _S. rostrata_, like in other described systems (Fuller and Verma, 1984 ; Govers _et al._, 1985 ; Lang-Unnasch and Ausubel, 1985), leghemoglobin genes are activated in ineffective nodules induced by nif^- _Rhizobium_ sp. mutants, but are expressed at a reduced level compared to efficient nodules.

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