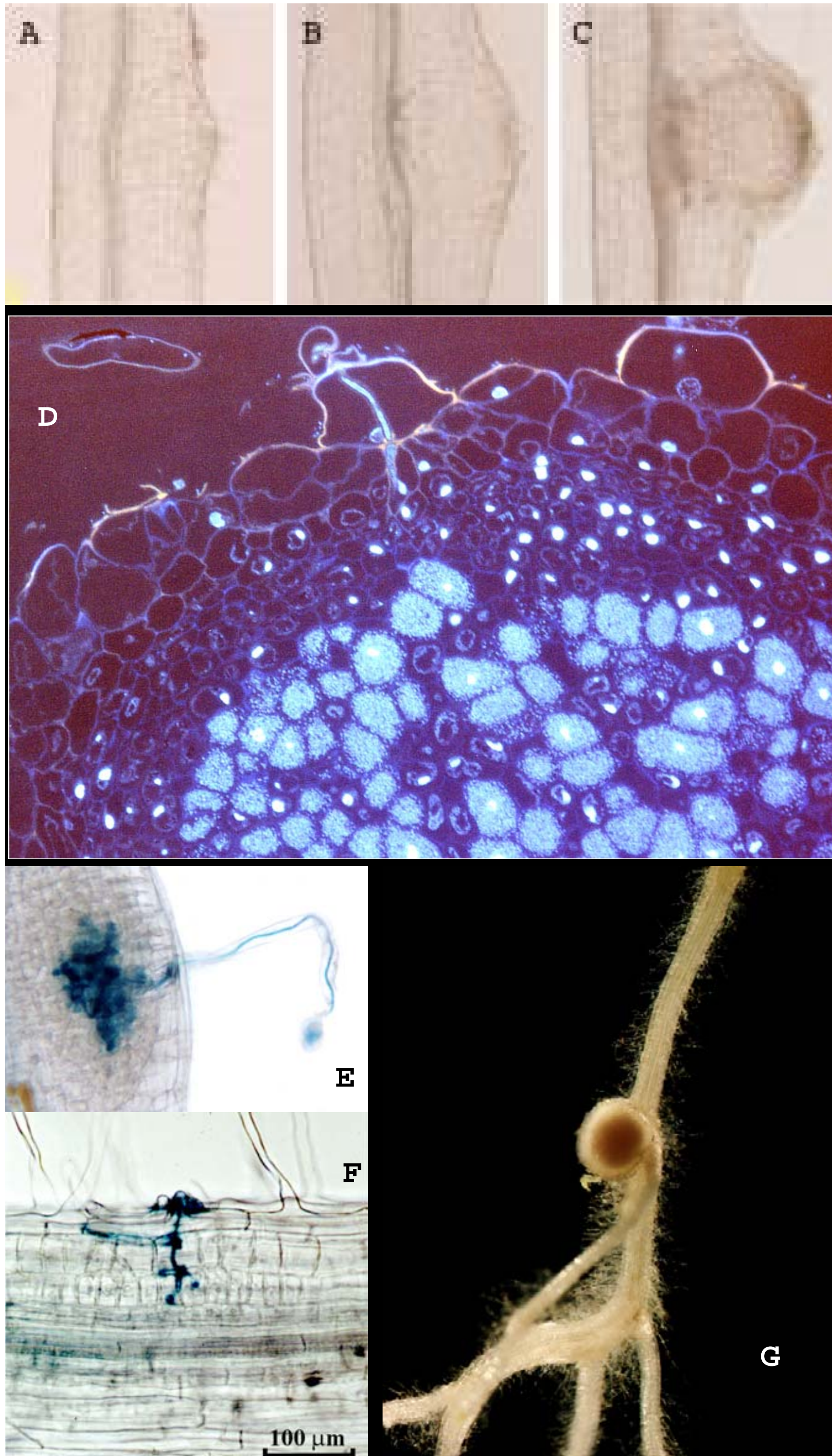
COLOUR PLATES

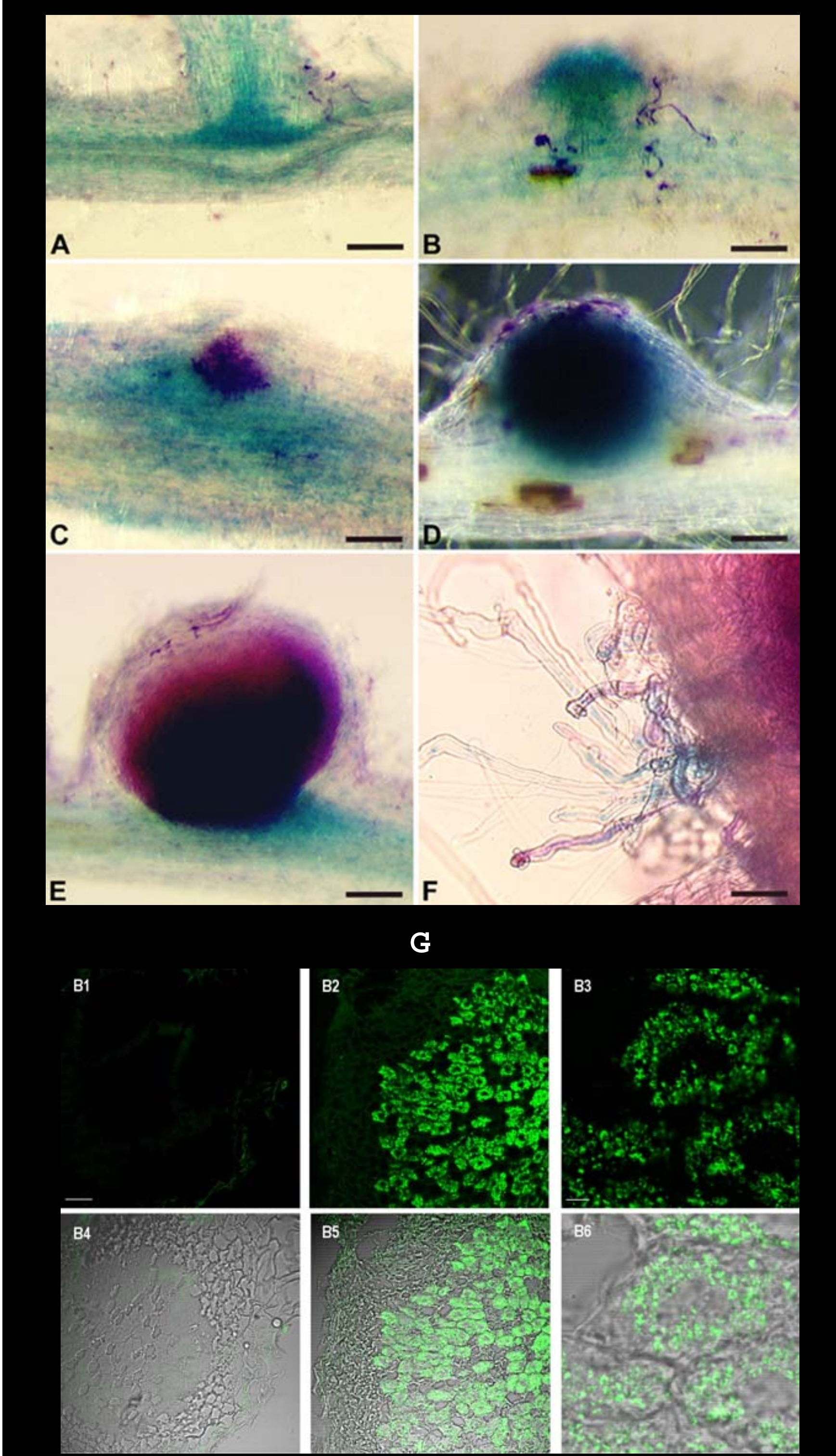
Colour plate 1.

Colour plate 1. Different plant organs of Lotus japonicus *plants. a-d) Developmental stages of* Lotus japonicus *flowers relevant to hand pollination (reproduced with permission from Jiang and Gresshoff, 1997). Flowers in a and b are too young and inappropriate for fertilisation as female flower. The correct age of the flower is shown in c: optimal flowers are 6-7 mm in length and the standard petal is still fused, they show no curvature of the style as well as complete extension of the anther filament and ripening of the pollen. The flower in d is too old, pollen has been released from the anther and self-pollination has already taken place. e) Flowers from Gifu (yellow) and Filicaulis (red) ecotypes. f) Seedpod containing up to 20 seeds. g) Trifoliate leaf. h) 2 months old* Lotus japonicus *plant. See chapter 1.1.*



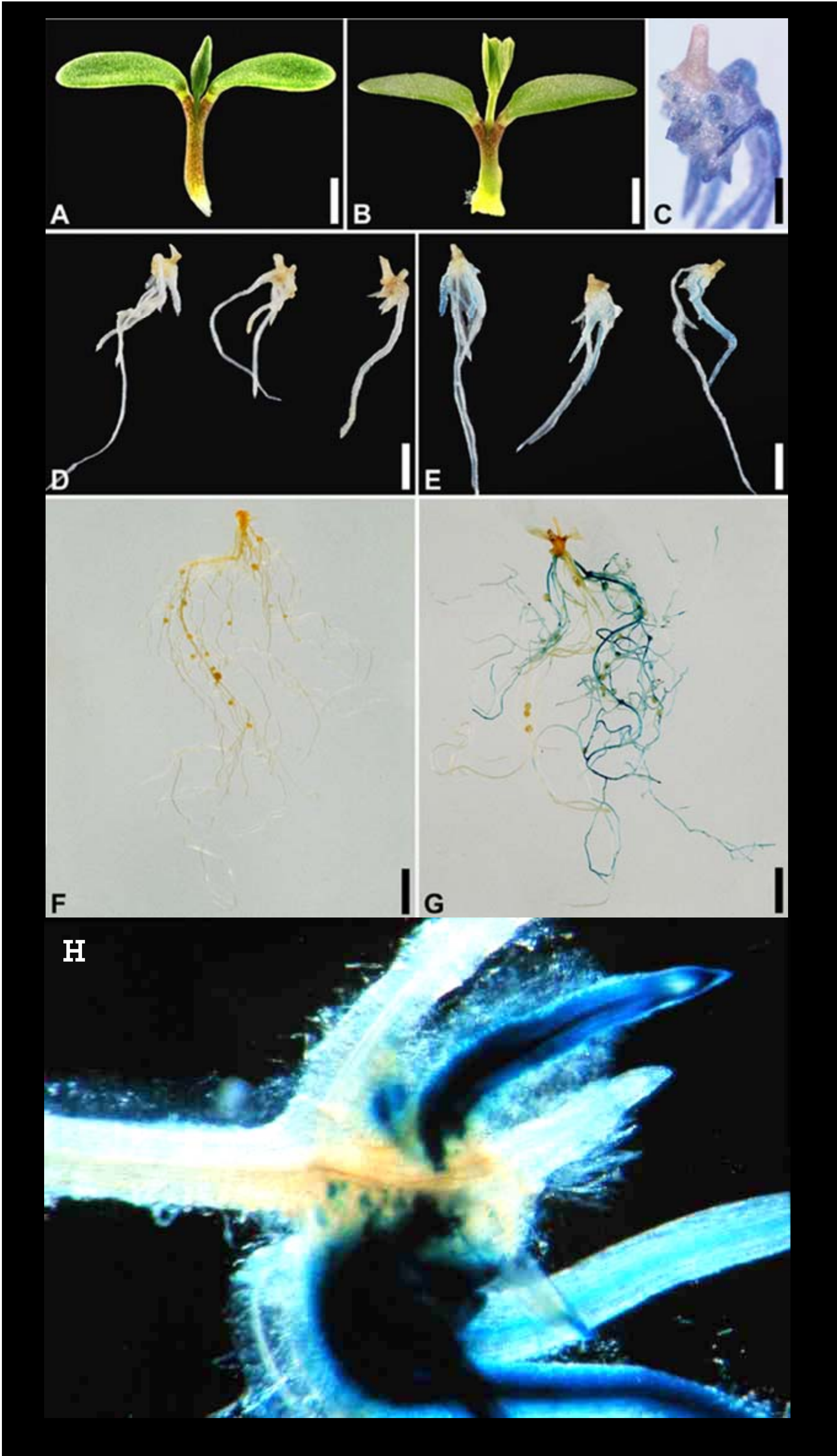
Colour plate 2.

Colour plate 2. Formation of nodules in Lotus japonicus *plants infected with* Mesorhizobium loti. *A-C) Cleared tissues showing successive stages of nodule development. A: Cortical cell division, B: Bump, C: Developing nodule. D)* Lotus japonicus *nodule 2 weeks after infection with* Mesorhizobium loti. *DAPI staining. E) Brightfield micrograph of* L japonicus *nodule 10 days after inoculation with* M loti *strain NZP2253 carrying a hemA:LacZ reporter gene fusion. Roots were stain for β-galactosidase activity, cleared, and examined using brightfield microscopy. F) Brightfield micrograph of* L japonicus *roots showing the progression of the IT towards the developing nodule primordium. L japonicus roots were inoculated with* M loti *strain NZP2253 carrying a hemA:LacZ reporter gene fusion, stained for β-galactosidase activity, and examined using brightfield microscopy. G) Fully mature nitrogen-fixing nodule formed on* L japonicus *roots. See chapter 2.1.*



Colour plate 3.

Colour plate 3. A-F) Double staining of L. japonicus *hairy roots expressing MsENOD12B-gusA (X-gluc as substrate, blue precipitate) during nodulation induced by* M. loti *R7A constitutively expressing β-gal (M-gal as substrate, red precipitate). Bar in panels A, B, D, and E=125 µm. Bar in panel C=95 µm. Bar in panel F=30 µm (See chapter 3.1). G) Immunolocalisation of MnSOD in* Lotus japonicus *nodules by fluorescent detection of MnSOD primary antibodies. Images represent longitudinal sections of 13 days post inoculation (dpi) nodules. Alexa488-conjugated anti-rabbit immunoglobulin Gs were used as secondary antibodies. B1, B2, and B3, are the confocal microscope images showing the signal in the infected cells of the central tissue (background subtracted). B4, B5, and B6, are overlays of B1, B2, and B3, and the corresponding transmitted light images. B3 and B6 are a high magnification of the central tissue. No signal was observed in the controls (B1, B4). Bars = 30 µm (B1, B2, B4, and B5) and 3 µm (B3 and B6) (See chapter 3.2).*



Colour plate 4.

Colour plate 4. A-G) Hairy roots induced by A. rhizogenes *LBA 1334 in* L japonicus *seedlings. Panel A: there is no swelling observed at the base of the hypocotyl of a Lotus seedling that has not been infected with LBA 1334 4 days after sectioning of the main root. In contrast, a slight swelling and root hairs appears in the area of wounding in a seedling 4 days after sectioning of the main root at the base of the hypocotyl and subsequent infection with LBA 1334 (panel B). Panels C, E, and G show GUS expression as blue precipitate using 5-bromo-4chloro-3-indolyl-β-glucoronic acid as substrate in roots transformed with CaMV35S-gus:intron cloned in various binary vectors. GUS histological staining was performed as described in Scarpella et al (2000). Panel C shows that all emerging hairy roots of a Lotus seedling were tranformed with LBA1334 carrying GUS as reporter gene cloned in pCAMBIA1301. Note that GUS is also expressed in loci that have not yet differentiated into hairy roots. Depending on the binary vector used, 5-10% of the transformed plants will show GUS expression in all emerging roots, the rest of the transformed plants wil present a combination of GUS-stained and non-stained roots. Staining was performed 7 days after transfer of the seedling to HRE medium. Panel E shows blue roots of seedlings transformed with LBA1334 carrying gus in a pPZP derivative and panel D, roots emerging in control seedlings, infected with LBA 1334, that do not show blue staining. GUS histological staining was performed 10 days after transfer to HRE. As many as 20 roots can be initiated, however only a few (2-10 roots) grow rapidly and produce lateral roots at this stage of development. Panel F shows the results of GUS staining of a control plant tranformed with LBA 1334 carrying pBin19 and panel G, the staining of a plant tranformed with LBA 1334 carrying pBin19 CaMV35S-gus:intron and showing GUS activity in nodulated hairy roots. Note that in both cases nodules are concentrated on the most developed root, which in 60-70 % of the cases, is transformed. Predominance of 1 or 2 roots is apparent after 2-3 weeks growth in HRE, clay particles or Jensen-agar plates. Staining of plants in panels F and G was performed 5 weeks after inoculation with* M. loti *strain R7A and growth of roots in clay particles. Bars in panels A, B, C= 1mm; in panels D, E= 4.5 mm; in panels F, G= 10 mm (see chapter 6.2). H) GUS staining obtained in hairy roots co-transformed with the T-DNA vector carrying the 35S-gusAint cassette (see chapter 6.1).*