Bacteria belonging to Rhizobiaceae display the ability to infect and nodulate their leguminous host plants. The obvious biological activity of Rhizobium in the formed nodules is its ability to fix atmospheric nitrogen into ammonia, which is subsequently assimilated into amino acids as nutrients for the host plants. Infection and nodulation only occur with host-specific combinations of the rhizobial and host plant species. For example, the specific hosts for R. leguminosarum bv. trifolii and Bradyrhizobium japonicum are clover and soybean, respectively. A whole series of reactions resulting in nodulation and nitrogen fixation are controlled by nod and nif genes, respectively, in the bacterial symbiont.

Agrobacterium is a phytopathogen that causes crown gall disease on a wide variety of dicotyledonous plants. It can also induce tumors on certain monocots and gymnosperms. The infection process of A. tumefaciens is rather complex, involving a number of chemical signal substances elicited by both the pathogen and host plant. Agrobacterium infection is commenced when the bacterial virulent (vir) genes located on its Ti plasmid are induced and expressed by certain phenolic compounds secreted from the wounded site of the plant. Agrobacteria adsorb to the plant cells and transfer part of their Ti plasmid (pTi), called the transferring (T)-DNA, to the chromosome DNA of the host plant cell (Watson et al., 1975).

The taxonomical relationship between root nodule bacteria (Rhizobium) and Agrobacterium is known to be very close (Sawada et al., 1993). The first successful transfer of nodulating ability in Agrobacterium was achieved by transfer of the symbiotic plasmid (pSym) from R. leguminosarum bv. trifolii to a pTi-cured A. tumefaciens strain (Hooykaas et al., 1982). We hypothesized that the extension of the host range of Rhizobium would occur when rhizobial nodulation and nitrogen-fixing abilities were transferred to Agrobacterium. In this experiment, the transfer of the symbiotic plasmid (pRT4Sa) of R. leguminosarum bv. trifolii 4S (Nod+, Fix+) to A. tumefaciens A136 (Ti-plasmid-cured strain) was performed. We report here the pos-
K2HPO4, 0.22 g; MgSO4, 0.1 g; CaCl2, 0.44 g; FeCl3, sodium succinate, 1.35 g; sodium glutamate, 1.1 g; medium (Sherwood, 1970) was prepared as follows: (TY) medium (Beringer, 1974) were used. For selection of host range exhibited by isolated transconjugants.

Materials and Methods

Bacterial strains and plasmids. Strains are shown in Table 1. R. leguminosarum bv. trifolii 4S is a wild-type strain containing 3 megaplasmids (pRT4Sa, pRT4Sb, and pRT4Sc). pRT4Sa (315 kb) is the symbiotic plasmid (Higashi et al., 1983). Strain 4S can nodulate clovers but not alfalfa or vetch. A. tumefaciens A136 is an avirulent, Ti-plasmid-cured derivative of strain C58 (Watson et al., 1975). The 17.75 kb nod gene region of strain 4S was cloned into the broad host range plasmid (Higashi et al., 1983). Strain 4S can

Table 1. Bacterial strains and plasmids.

<table>
<thead>
<tr>
<th>Strains and plasmids</th>
<th>Relevant characteristics</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhizobium leguminosarum bv. trifolii</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4S</td>
<td>Wild type, Hac+, Inf+, Nod+, Fix-</td>
<td>Higashi and Abe (1980)</td>
</tr>
<tr>
<td>4S5</td>
<td>4S carrying pRT4Sa::Tn5-mob, Hac-, Inf-, Nod-, Fix-, Km'</td>
<td>This study</td>
</tr>
<tr>
<td>H1</td>
<td>pSym-cured derivative of 4S, Hac-, Inf-, Nod-, Fix-</td>
<td>Higashi et al. (1983)</td>
</tr>
<tr>
<td>H1R1</td>
<td>H1 carrying pRT4Sa::Tn5-mob, Hac-, Inf-, Nod-, Fix-</td>
<td>This study</td>
</tr>
<tr>
<td>Agrobacterium tumefaciens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A136</td>
<td>pTi-cured derivative of C58, Rif', Onc-</td>
<td>This study</td>
</tr>
<tr>
<td>AT4S-series</td>
<td>A136 carrying pRT4Sa::Tn5 from 4S, Hac', Inf', Nod', Rif', Km'</td>
<td>Watson et al. (1975)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S17-1(pSUP5011)</td>
<td>Donor of Tn5-mob, Km', Nm', Sm' thi, pro</td>
<td>Simon (1984)</td>
</tr>
<tr>
<td>MM294(pRK2013)</td>
<td>Helper for conjugation, Km', thi, pro</td>
<td>Figurski and Helinski (1979)</td>
</tr>
<tr>
<td>HB101(pC4S8)</td>
<td>HB101 carrying pC4S8, Tc'</td>
<td>This study</td>
</tr>
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<td>Plasmid</td>
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<tr>
<td>pRT4Sa</td>
<td>pSym of 4S, 315 kb</td>
<td>Higashi et al. (1983)</td>
</tr>
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<td>pRT4Sa::Tn5-mob</td>
<td>pRT4Sa containing Tn5-mob, Km'</td>
<td>This study</td>
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<tr>
<td>pRK2013</td>
<td>Helper plasmid, Km'</td>
<td>Figurski and Helinski (1979)</td>
</tr>
<tr>
<td>pC4S8</td>
<td>pLAFR1 carrying 17.75 kb nod region of pRT4Sa, Tc'</td>
<td>This study</td>
</tr>
<tr>
<td>pLAFR1</td>
<td>Wide host range cosmid</td>
<td>Friedman et al. (1982)</td>
</tr>
</tbody>
</table>

Hac, root hair curling; Inf, infection thread formation; Nod, nodulation; Fix, nitrogen fixation; Onc, oncogeny; Km, kanamycin; Rif, rifampicin; Nm, neomycin; Sm, streptomycin; Tc, tetracycline; Ap, ampicillin.

with appropriate antibiotics, kanamycin (20 µg/ml) for E. coli and rifampicin (50 µg/ml) for Agrobacterium. Rhizobial strains containing Tn5-mob were cultured in media supplemented with kanamycin (100 µg/ml). Agrobacterium transconjugants were cultured on LB medium containing both rifampicin (50 µg/ml) and kanamycin (100 µg/ml).

Bacterial mating procedure. Two mating procedures are shown in Fig. 1. The first mating was performed between E. coli S17-1(pSUP5011) (Simon, 1984) and R. leguminosarum bv. trifolii 4S. Both strains (10^8 cells each) were mixed in a 1 ml suspension in an Eppendorf tube and incubated for 8 h at 27°C. Transconjugants were screened on minimal medium plates containing kanamycin (100 µg/ml) (Jagadish and Szalay, 1984). Kanamycin-resistant colonies were picked up randomly. Each colony was tested for nodulation on white clover and confirmed for Tn5-mob insertion on pRT4Sa by Southern hybridization. One strain was selected and named strain 4S5 for use in subsequent transformation. The second mating was performed among E. coli MM294 (pRK2013 containing the tra gene from pRK2) (Figurski and Helinski, 1979), Tn5-mob-inserted Rhizobium strain 4S5 and pTi-cured Agrobacterium strain A136 (ca. 10^9 cells/ml of each). Each bacterial culture was mixed in 100 µl at a ratio of 1:2:2 by volume. After centrifugation at 10,000 x g for 1 min, the cell pellet was rinsed and suspended in 200 µl of sterilized distilled water. Fifty microliters of this bacterial suspension was transferred to a membrane filter (pore size 0.45 µm) on a TY agar plate without antibiotics.
and cultured overnight at 27°C. Proliferated cells on the membrane filter were suspended in 1 ml sterilized distilled water, spread on LB plates containing kanamycin at 100 µg/ml and rifampicin at 50 µg/ml, and cultured for 4–5 days at 27°C. The proliferated colonies were mixed and inoculated on clover seedlings. After 30 days of incubation, bacteria were reisolated from formed nodules on antibiotic-containing LB plates as reported previously (Uchiumi et al., 1995). The reisolated strains were referred to as the AT4S series.

**DNA isolation and analyses.** Total DNAs were isolated from *Rhizobium* and *Agrobacterium* by the modified method of Casse et al. (1979). Plasmid DNA from *E. coli* was isolated according to the method of Birnboim (1983). For Southern hybridization, total DNAs of *Rhizobium* and *Agrobacterium* were digested with EcoRI or HindIII for the detection of *nod* genes and Tn5-mob, respectively. After electrophoresis in 0.7% agarose, plasmid DNAs and digested DNA fragments were blotted on nylon 66 filters (NYTRAN NY13N, Schleicher & Schuell, pore size 0.45 µm) (Reed and Mann, 1985) hybridized with probes for the Tn5-mob- and *nod*-gene regions of strain 4S (pC4S8). For each DNA probe, Tn5-mob was extracted from pSUP5011 by HindIII digestion, and the *nod* gene was extracted from pC4S8 by EcoRI digestion. Hybridized profiles were visualized with DNA labeling and detection kits (digoxigenin-DIG ELISA and DIG Luminescent, Boehringer Mannheim).

**Random amplified polymorphic DNA (RAPD) method and *nodC* detection of *Rhizobium*, *Agrobacterium* and *Agrobacterium* transconjugants by PCR.** Two different PCR oligonucleotide primers were used for the RAPD method (Dye et al., 1995). The sequences were 5’GGTGCGGGGA3’ for RAPD1 and 5’GTTTCGCTCC3’ for RAPD2. The reactions were performed in 50 µl volumes, using 50 ng of bacterial template DNA, 50 pmol of primer nucleotide, and 2.5 U TaKaRa Taq polymerase (Takara Shuzo Co. Ltd., Ohtsu, Japan). The reaction conditions were as follows: 4 min at 95°C for the first denaturation, 1 min at 95°C, 1 min at 37°C, 2 min at 72°C for 40 cycles, and 5 min at 72°C for the final extension. For *nodC* detection, the sequences of the amplification primers were as follows: 251F, AGCGGTCAGAATCGGATCaAA, and 566R, AGCCATTGACTGATGATGAA, reported by Ueda et al. (1995); and PCR cycles were 1 min at 94°C for the first denaturation, 30 s at 94°C, 30 s at 37°C, 2 min at 60°C for 5 cycles, 30 s at 94°C, 30 s at 50°C, 30 s at 72°C for 40 cycles, and 5 min at 72°C for the final extension. The amplifications were performed with a GeneAmp PCR System 2400 (Perkin-Elmer Co., USA). The PCR products were analyzed by 1.0% agarose gel electrophoresis.

**Nodulation and nitrogen fixation test.** Infection and nodulation tests of the transconjugants were examined on white clover (*Trifolium repens* L. cv. Ladino), alfalfa (*Medicago sativa* L. cv. Common), hairy vetch (*Vicia hirsuta* (L.) S. F. Gray), Mung bean (*Vigna mungo* L.) and soybean (*Glycine max* (L.) Merrill cv. Peking) (Fahraeus, 1957; Higashi and Abe, 1980). The nitrogen fixation ability of nodules formed on white clover and alfalfa was detected by acetylene-reducing activity (ARA) as described previously (Hardy et al., 1968). Infection threads formed in clover root hairs were observed by light microscopy.

**Scanning electron microscopy.** Nodules were longitudinally and/or cross-sectioned at the central position, fixed overnight at 4°C with 2.5% glutaraldehyde in 50 mM Na-cacodylate buffer (pH 6.5), and dehydrated with an ethanol series (more than 30 min each). After dehydration in absolute ethanol, nodules were treated twice with tert-butanol (2-methyl-2-propanol) and ethanol (1:1 v/v) followed by 100% tert-butanol, and then frozen overnight in a refrigerator. Specimens were dried under reduced pressure, coated with platinum at 15 nm thickness, and examined by a field-emission scanning electron microscope (4100H, Hitachi Co., Ltd., Tokyo, Japan).
Results

In the first step of mating (Fig. 1), insertion of the Tn5-mob from pSUP5011 into the Sym plasmid pRt4Sa of strain 4S was performed. Total DNA extracted from a kanamycin-resistant transconjugant (4S5) was hybridized with the Tn5-mob probe, indicating the presence of the Tn5-mob insertion in the pRt4Sa (Fig. 2a, b). The transconjugant 4S5 maintained nodulation and nitrogen fixation abilities on white clover (Table 2).

The transfer of pRt4Sa::Tn5-mob (Km') from strain 4S5 to Agrobacterium tumefaciens A136 (Rif') using triparental mating with E. coli MM294 (pRK2013) (Fig. 1) resulted in Agrobacterium transconjugants at a transferring frequency of about 10^-6 (per input donor cell). Transconjugant strains were inoculated to white clover seedlings and reisolated from formed nodules to screen the symbiotic plasmid (pRt4Sa)-transferred Agrobacterium. The transferred plasmid was recognized by agarose gel electrophoresis and Southern hybridization using the nod genes of strain 4S as the probe (Fig. 3a, b, and c). In strain AT4Sa, plasmids pRt4Sa::Tn5-mob and pRt4Sa could not be detected (Figs. 2a and 3a). However, the existence of both Tn5-mob and nod genes in strain AT4Sa was confirmed on the total DNA blotted filters by Southern hybridization, as well as other AT4S-series strains (Figs. 2b, 3b, and 3c). RAPD analyses of Rhizobium, Agrobacterium and Agrobacterium transconjugant strains (AT4S-series) indicated that strains H1 and 4S5 exhibited similar band patterns to Rhizobium strain 4S, whereas strains AT4Sa, AT4SB and AT4SG exhibited common band patterns originating from Agrobacterium strain A136 (Fig. 4). The PCR analysis using primers for nodC detection confirmed that all tested AT4S-series strains contained the same fragment size (350 bp) of DNA as found in strains 4S and 4S5 (Fig. 5).

The AT4S-series strains were tested for nodule induction and N2 fixation (acetylene reduction) on white clover (Table 2). Nodules were visualized within 6 to 7 days after inoculation with the transconjugant and wild-type strains (Fig. 6A). Strains AT4Sa, AT4SB, AT4SD, AT4SE, and AT4SG induced two to three times more nodules on white clover than did wild-type strain 4S. The nodulation frequency with AT4S-series strain inoculation was more than 90% as well as strain 4S at 10 to 15 days after inoculation (data not shown). Interestingly, strain AT4Sa also nodulated hairy vetch (V. hirsuta) at the 4th week after inoculation, and strain AT4SG nodulated alfalfa (M. sativa) at the 3rd week after inoculation (Fig. 6B). Nodules on these al-

Table 2. Induction of nodules and their acetylene reduction activity on white clover inoculation with Rhizobium and Agrobacterium. a

<table>
<thead>
<tr>
<th>Activity</th>
<th>4S</th>
<th>4S5</th>
<th>H1</th>
<th>H1R1</th>
<th>AT4Sa</th>
<th>AT4SB</th>
<th>AT4SD</th>
<th>AT4SE</th>
<th>AT4SG</th>
<th>A136</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodulation</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>++++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Acetylene reduction</td>
<td>0.19</td>
<td>0.25</td>
<td>0</td>
<td>0.37</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

a Rhizobium strains; 4S, 4S5, H1, H1R1, Agrobacterium strain; A136. Agrobacterium-transconjugant strains; AT4Sa, AT4SB, AT4SD, AT4SE, AT4SG.

b For nodulation: Total 20–50 seedlings were used. +, <5 nodules/plant; ++, 5<10 nodules/plant; ++++, 10< nodules/plant.

c Acetylene reduction (nm/plant/h) was measured at 50 days after inoculation.
Ternate hosts can emerge within 7–10 days after inoculation with the compatible symbiont, *Rhizobium* (Table 3). Figure 6A shows the nodules formed on white clover by inoculation with transconjugant strain AT4SB. A complete infection thread was formed in the curled root hair cell of white clover at the 7th day after inoculation with transconjugant strain AT4SG (Fig. 6C). The clover nodules formed after inoculation with strain 4S and the AT4S strain series contained club-shaped bacteroid cells (Fig. 7).

The nodules formed on alfalfa roots inoculated with transconjugant strain AT4SG were also examined by SEM. These nodules contained bacteria exhibiting the characteristic Y-shaped morphological change of bacteria as was found in alfalfa nodules induced by *R. meliloti* strain 1021 (Fig. 8).

Triparental mating between strain AT4Sa (donor) and strain H1 (recipient) was also performed. One of the resulting transconjugant strains derived from this cross was referred to as strain H1R1. The retraced

---

**Fig. 3.** Southern hybridization profiles of isolated plasmids (a) and EcoRI-digested total DNA (b, c) from *Rhizobium* and *Agrobacterium* transconjugants with *nod* genes as the probe.

Lanes 1–4, *Rhizobium* strains: 1, 4S; 2, 4S5; 3, H1; 4, H1R1. Lane 5, *Agrobacterium tumefaciens* A136. Lanes 6–8, *Agrobacterium* transconjugants: 6, AT4Sa; 7, AT4SB; 8, AT4SG. Lanes 2, 4, 7, 8 show *nod* genes on a pRt4Sa (Sym plasmid) indicated by arrowheads, but Lane 6 did not exhibit a clear band and hybridized pattern at the pRt4Sa position (a). Several hybridized band profiles were shown on the EcoRI-digested total DNA fragments (b, c). AT4Sa (Lane 6) exhibited very faint hybridized patterns, indicated by stars.

**Fig. 4.** RAPD analysis of *Rhizobium* and *Agrobacterium* transconjugants.

Primer 1 (a) and 2 (b) are listed in MATERIALS AND METHODS. Lanes 1–4, *Rhizobium* strains: 1, 4S; 2, 4S5; 3, H1; 4, H1R1. Lane 5, *Agrobacterium tumefaciens* A136. Lanes 6–9, *Agrobacterium* transconjugants: 6, AT4Sa; 7, AT4SB; 8, AT4SG; 9, AT4SGα (strain reisolated from an alfalfa nodule formed by AT4SG inoculation).
pRt4Sa::Tn5-mob from strain AT4Sa to H1 was confirmed by the existence of plasmid profiles of pRt4Sa and Southern hybridization probed with Tn5-mob (Fig. 2) and nod genes (Fig. 3). The RAPD pattern of strain H1R1 also indicated the same profiles as in Rhizobium strains 4S, H1 and 4S5 (Fig. 4b). The white clover nodules formed by strain H1R1 had levels of nitrogen fixation activity which were similar or even higher than in nodules induced by wild-type strain 4S (Table 2). This result is proof that Agrobacterium-transconjugant strain AT4Sa possess functional nod, nif and fix genes derived from pRt4Sa.

Discussion

The exchange of infection signals between rhizobia and host plants is well known (Higashi, 1993). Nod factors (lipochitin-oligosaccharides) are synthesized and secreted from the Rhizobium cell as a result of nod-gene expression. These products have been recognized as major mediators of host specificity in root hair deformation and nodule primordia induction (Spaink, 1995). In this experiment, typical morphological changes of root hair, such as curling, branching and infection thread formation in the root hair cell, and nodule formation were also confirmed with AT4S-series inoculated clover root. These observations proclaim that nod-gene expression of Agrobacterium-transconjugant cells and plant signal response may occur as normally as wild-type strain 4S.

Truchet et al. (1984) and Wong et al. (1983) have reported the transfer of rhizobial Sym plasmid to A. tumefaciens, and tested their symbiotic performance in the appropriate host plant. However, no nitrogen-fixing nodules have been obtained in either case; the results have shown atypical bacterial penetration with-out infection threads and no intracellular bacteria in formed nodules. In contrast, detailed microscopical studies revealed that infection threads were formed in...
Fig. 7. Scanning electron micrographs of clover nodules induced by _Rhizobium_ strain 4S (A, B, C) and _Agrobacterium_ transconjugant strain AT4SG (D, E, F).

nvb, nodule vascular bundle; rvb, root vascular bundle; bd, bacteroid; ap, amyloplast; it, infection thread. Scale bars in µm.
Fig. 8. Scanning electron micrographs of alfalfa nodules induced by *Rhizobium melloti* 1021 (A, B, C) and *Agrobacterium* transconjugant strain AT4SG (D, E, F).

nm, nodule meristem; nvb, nodule vascular bundle; bd, bacteroid. Scale bars in μm.
root hairs of white clover inoculated with pTi-cured Agrobacterium transconjugants containing pSym from R. leguminosarum bv. trifolii (Dazzo, unpublished data).

We confirmed, in this report, the mobilization of megaplasmid (pRt4Sa, 315 kb) by insertion of the Tn5-mob gene, and the transferred plasmid was conservatively maintained in the recipient Agrobacterium cell accompanying normal infection thread and nodule formation abilities (Fig. 6). Hirsch et al. (1984) reported that Agrobacterium transconjugants isolated by transfer of the symbiotic plasmid from R. meliloti induced nodule structures on alfalfa roots, but neither peribacteroid membrane nor bacteroid differentiation were observed. In the root nodule cells of clover and alfalfa induced by strain AT4SG, typical morphological changes and enlargement of the symbiotic bacteria, such as club and Y shapes, were observed (Figs. 7, 8). This suggests that the rhizobial nodulation genes on pRt4Sa could be normally expressed in an Agrobacterium cell background and that bacteroid differentiation has taken place.

The extended host range of strain AT4SG was confirmed by Southern hybridization (Figs. 2, 3), while in the RAPD pattern of the reisolated bacteria from the alfalfa nodule formed by strain AT4SG, indicated as strain AT4SGu, no typical difference could be identified in the major band pattern as strains AT4SB and AT4SG (Fig. 4). There is no clear answer why only two strains, AT4Sa and AT4SG, expressed this host range extension (Table 3). The creation of a wide host-range strains of root nodule bacteria by using rhizobial Sym plasmid transfer to Agrobacterium was also attempted by several research groups. Hooykaas et al. (1982) reported that characteristic spherical root nodules were formed on bean by the transconjugated strains of R. trifolii or A. tumefaciens introduced to the Sym plasmid of R. phaseoli, but no nitrogen fixation could be detected. However, Martinez et al. (1987) reported that the Agrobacterium transconjugants harboring the 410 kb plasmid of R. phaseoli formed effective nodules on Phaseolus vulgaris and Leucaena leucocephala. They showed that the 410 kb plasmids of R. phaseoli were expressed in A. tumefaciens cell background and directed the formation of effective nodules. Novikova and Safronova (1992) had also reported the transfer of pSym of R. galegae to A. radiobacter, where the transconjugants formed effective nodules on Medicago sativa.

At present, no nitrogen-fixing activity has been found with the nodules induced by the AT4S-series strains on either clover or alfalfa (Table 2), although bacteroid differentiation in the nodule cells was observed in both hosts (Figs. 7, 8). In the strain AT4Sa, transferred plasmid (pRT4Sa) could not be detected (Fig. 3a), but the existence of a nod-gene region derived from strain 4S was confirmed by Southern hybridization of EcoRI-digested total DNA. Moreover, the transfer of pRT4Sa::Tn5-mob from strain AT4Sa to pSym-cured strain H1 has been confirmed as a plasmid band in the retraced strain H1R1 (Fig. 3a). For the moment, there is no evidence on how to present the pRT4Sa::Tn5-mob in the strain AT4Sa, whereas the retraced strain H1R1 displayed normal nodulation and nitrogen-fixing ability (Table 2). We predict that there must be some regulation system to express the nitrogen fixation genes, which may only be activated in certain Rhizobium cell backgrounds. The retraced transconjugants exemplified by strain H1R1 have a bright prospect of further analyses on the early stage of the infection process and nitrogen-fixing mechanisms induced by nod- or nif-gene expressions.

We are grateful to Prof. Dr. Frank B. Dazzo, Michigan State University, USA, for his critical reading and helpful advice regarding this manuscript. A part of this work was supported by a grant for the “Research for the Future” program, JSPS-RFTF96L00601, The Japan Society for the Promotion of Science.

Table 3. Nodulation on five kinds of leguminous plant with Rhizobium and Agrobacterium-transconjugant inoculation.

<table>
<thead>
<tr>
<th>Plants</th>
<th>4S</th>
<th>4S5</th>
<th>AT4Sa</th>
<th>AT4SB</th>
<th>AT4SG</th>
<th>A136</th>
<th>H1</th>
<th>H1R1</th>
<th>Cont.</th>
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</thead>
<tbody>
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<td>Trifolium repens</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Medicago sativa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.d.</td>
<td>-</td>
</tr>
<tr>
<td>Vicia hirsuta</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.d.</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Vigna mungo</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.d.</td>
<td>-</td>
<td></td>
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<tr>
<td>Glycine max</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>n.d.</td>
<td>-</td>
<td></td>
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</tbody>
</table>

For nodulation, 20 seedlings of T. repens and M. sativa, 10 seedlings of V. hirsuta, V. mungo and G. max were tested. n.d., not determined.

References


Casse, F., Boucher, C., Julliot, J. S., Michel, M., and Denarie, J.

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