A Supernodulation and Nitrate-Tolerant Symbiotic (nts) Soybean Mutant

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ABSTRACT

The nodulation characteristics of soybean (Glycine max) mutant nts382 are described. The mutant nodulated significantly more than the parent cultivar Bragg in the presence and absence of several combined nitrogen sources (KNO₃, urea, NH₄Cl, and NH₄NO₃). The number of nodules on the tap root and on lateral roots was increased in the mutant line. In the presence of KNO₃ and urea, nitrogenase activity was considerably higher in nts382 than in Bragg. Mutant plants were generally smaller than wild-type plants. Although nts382 is a supernodulator, inoculation with Rhizobium japonicum was necessary to induce nodule formation and both trial strains CB1809 (= USDA136) and USDA110 elicited the mutant phenotype. Segregation of M₂ progeny derived from a M₁ wild-type plant indicated that the mutant character is inherited as a Mendelian recessive. The mutant is discussed in the context of regulation of nodule formation and of hypotheses that have been proposed to explain nitrate inhibition of nodulation.

Nodule formation in legumes is tightly regulated. Indeed, symbiotic development is subject to both external factors and internal (or 'autoregulation') control mechanisms. Several environmental conditions, such as light intensity (photosynthate- or non-photosynthate mediated), temperature, pH, and soil moisture, influence nodulation (19). However, under optimum conditions for plant growth, exogenous nitrate represents a major environmental factor controlling the extent of symbiosis (5, 13). Small amounts of nitrate have been demonstrated to stimulate nodulation. Above these minute concentrations, however, nodule fresh weight is inversely related to the level of nitrate in the growth medium (20). To a lesser extent, other sources of combined nitrogen have also been shown to inhibit nodulation (7, 8, 10, 24, 27). The extent of this inhibition does vary considerably with the legume species, the form of combined nitrogen administered, and the experimental system. Some sources of combined nitrogen, for example urea or NH₄⁺, cause acidification of the growth medium (14), and the inhibitory effects of these nitrogen sources may be mediated indirectly through a reduction in pH rather than through the nitrogen status of the plant. To circumvent this complication, Vigue et al. (30) controlled pH fluctuations by the inclusion of a pH-buffering carboxy resin in the pots (17). In this system using soybeans, nitrate but not urea suppressed nodule fresh weight per plant at the range of concentrations tested. This was partially explained by reduced uptake of nitrogen in urea-fed plants. In contrast to the effect of nitrate and urea on nodule mass, rates of acetylence reduction per unit of nodule mass were similar for nitrate and urea treatments (30).

In the absence of externally supplied combined nitrogen, nodulation is tightly regulated with the number of infections greatly exceeding the final number of mature nodules (2). Interruption of invasion is related to the effectiveness of the host-Rhizobium association (21) as well as to other internal factors not directly related to the nitrogen status of the plant (23). Generally, ineffective strains of Rhizobium form more nodules, especially after the initial stages of nodule formation (21). Nutman also showed that excision of effective (but not ineffective) red clover nodules resulted in a transient increase in the number of nodules subsequently formed (22). In fact, removal of the nodule meristem of effective nodules was sufficient to stimulate subsequent nodule development. The effect of nodule meristem excision on nitrogenase activity was not considered, but the argument that an inhibitory factor emanating from the growing point, and not the bacterial tissue, of the nodule was supported by the finding that excision of the main root tip had the same effect as nodule meristem removal. Clear evidence for internal regulation or autoregulation independent of nitrogen fixation and in the early stages of nodule initiation was reported by Pierce and Bauer (23). Using the spot inoculation technique (26), they showed that inoculation of soybean roots with R. japonicum several hours prior to a second inoculation substantially reduced nodulation by the second inoculation. In a split-root system for soybeans, Kossak and Bohlool (16) showed that autoregulation prior to nodule appearance and nitrogenase activity was not restricted to root tissue immediately adjacent to the inoculated area, since prior inoculation of one side of a split-root suppressed nodulation on the other side (16). Studies by Calvert et al. (4) characterized this rapid regulatory phenomenon (23) further and showed that suppression of nodulation due to prior inoculation is mediated through suppression of nodule emergence rather than by inhibition of root hair infection. Clearly, the pending fruition of an infection is subject to internal (or auto-) regulation that exists both prior and subsequent to nitrogen fixation.

Recently, we isolated 15 independent soybean mutants that continued to nodulate in the presence of nitrate (6). These lines were designated nts (nitrate-tolerant-symbiosis) mutants. Previously, only four nodulation mutants of soybean had been discovered and all of these naturally occurring mutants are characterized by decreased nitrogenase activity. Soybean plants homzygous for the recessive mutation nts are resistant to nodulation; however, the blockage can be partially circumvented by inoculation with high R. japonicum cell densities (18). R₄, R₅, and R₆ are strain-specific dominant mutations that condition ineffective nodulation (3, 28, 29).

In this paper we report the nodulation characteristics of mutant line nts382. The effects of various nitrogen sources on nodulation, nitrogen fixation, and plant growth are described, as well

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as the influence of two R. japonicum strains on the expression of the supernodulation phenotype. Data are presented that indicate that the mutant character is inherited as a Mendelian recessive.

MATERIALS AND METHODS

Soybean (Glycine max [L.] Merr.) cv Bragg was used in this study. Mutant line nts382 was selected for increased nodulation in the presence of high nitrate concentrations. The isolation procedure has been described in detail (6) and is summarized in Figure 1. M₃ plants of mutant line nts382 were used here. Plants were cultured in either pots of river sand or in Leonard jars (9).

In experiments testing the effect of various nitrogen sources on nodulation and N₂ (C₂H₂) fixation, plants were cultured in 20 cm diameter pots of sand. Three Bragg or nts382 seeds were planted 1 cm below the surface and inoculated with Rhizobium japonicum strain CB1809 (= USDA136). The pots were reino-
culated at day 4. The nutrient solution was as used by Herridge (12), except that all nutrients other than CaCl₂ and the nitrogen source were administered at quarter strength for the first 2 weeks. KNO₃ (2.75 and 5.5 mm), urea (5.5 mm, i.e. 11 mM N), NH₄Cl (5.5 mm), NH₄NO₃ (5.5 mm, i.e. 11 mM N) and KCl (as control) were added to the nutrient solution as required. The pots were watered daily with 700 ml of nutrient solution, which is sufficient to flush out residual nutrients from the previous watering.

M₄ family 382 was one of 15 families that segregated for the nts phenotype (Fig. 1). Both nts variants and wild-type (non-nts) siblings were saved to produce M₅ families (i.e. families derived from single M₄ plants). Those M₅ families derived from wild-type M₄ plants were screened for segregation of the nts character (Fig. 1). Seeds were planted in 25 cm diameter pots of river sand (12 seeds per pot). The pots were inoculated with R. japonicum strain USDA110 at day 0 and day 4. Nutrients were administered as described above, except that all pots received 1.4 L of nutrient solution 3 times a week. After 7 weeks of growth, the plants were screened for the nts character.

To control access of R. japonicum strains to the roots (9), Bragg and nts382 were cultured in Leonard jars. Seeds were surface sterilized by rinsing in 95% ethanol followed by immersion of the seeds for 10 min in 3% NaOCl. After several rinses in sterile distilled H₂O, the seeds were transferred to water agar and germinated at 28°C in the dark. When the radical was approximately 0.5 to 1 cm long (2-3 d after sterilization), the seedlings were transferred to Leonard jars (N-free or supplemented with KNO₃ or urea). The jars either remained uninoculated or were inoculated with R. japonicum strains CB1809 or USDA110.

The plants were cultured in a temperature-controlled glass-
house (mean maximum temperature = 26.9°C; mean minimum temperature = 14.8°C) at a latitude of 37° 17’S. Incandescent bulbs supplemented natural light such that the photoperiod was 16 h. At harvest, the plants were measured for nodule number, nodule fresh weight, nitrogenase activity, and plant fresh weight.

Acetylene reduction was used to estimate nitrogen fixation on intact plants (11). Plants were incubated in 1040 ml air-tight jars with 2 to 3 ml of distilled H₂O (to prevent desiccation) at 25 to 27°C. The atmosphere in the jars was 6% acetylene in air. Rates were calculated from integrator units obtained from a Hewlett-Packard Integrator-Recorder 3390 coupled to a HP5590A flame ionization gas chromatograph. Samples were taken at 40 min and rates were determined to be linear over that period. Genotype and combined nitrogen effects were statistically tested by analysis of variance using the general statistical program Genstat (1). The LSD was computed when the F statistic was significant (0.05 level of significant). Chi-square analysis was used to statistically test segregation ratios. It was necessary to include Yates correction term in chi-square calculations due to the size of the expected classes (25).

RESULTS

Effect of Combined Nitrogen on Nodulation, Nitrogenase Activity, and Growth. Bragg and nts382 were inoculated with R. japonicum CB1809 and cultured for 4 weeks in the presence and absence of various combined nitrogen sources. The nitrogen-free treatment received 5.5 mm KCl as a control.

Nodule Number. Under all the conditions tested, 4-week-old nts382 plants had considerably more nodules than wild-type Bragg plants (Fig. 2). In the parent cultivar, all nitrogen sources reduced nodule number (Figs. 2 and 3). In contrast, mutant line nts382 grown on KNO₃ or urea had increased nodule number per plant over that of the KCl controls (Fig. 2). When data for nodule number are expressed per plant fresh weight, nodule number in nts382 was unaffected by increasing KNO₃ concentration (Fig. 3). Urea (5.5 mm), on the other hand, caused a small reduction in nodule number per plant biomass in the mutant. The relative degree of inhibition of nodule formation by urea was greater in Bragg than in nts382 (Fig. 3). Mutant line nts382 formed nodules on 5.5 mm NH₄Cl and NH₄NO₃, which totally prevented nodule formation in Bragg (Figs. 2 and 3). Ammonium chloride inhibited growth to a larger extent than did ammonium nitrate (Table 1); however, NH₄NO₃ was more inhibitory than NH₄Cl on nodule formation in nts382 (Fig. 3).

Nitrogenase (Acetylene Reduction) Activity per Plant Biomass. In Bragg, supplementing the nutrient media with nitrate or urea caused a substantial reduction in nitrogenase activity measurable 4 weeks after planting (Fig. 4). In contrast, 2.75 mm KNO₃ significantly stimulated acetylene reduction per gram plant fresh weight in nts382. Nitrogenase activity for nts382 plants cultured on 5.5 mm KNO₃ was not significantly different from the nitrogen-free mutant plants (Fig. 4). For both Bragg and nts382, urea was more inhibitory than equimolar KNO₃ concentrations. Regardless of the combined nitrogen supply, nts382 had higher nitrogenase activity than Bragg, but the difference under N-free conditions was not significant at the 0.05 level of significance. Nitrogenase activity per plant fresh weight in 4-week-old nts382 plants cultured on 2.75 and 5.5 mm KNO₃ was significantly higher than for Bragg plants cultured in the absence of nitrogen.
the podfill a trates significant. It were produced healthy vigorous plants (Table I). Regardless controls, weeks of culture, growth (Fig. 4). (Fig. 4).

Visual Characteristics of Nodulation by nts382. Figure 5 illustrates a sample of plants that contributed to the data presented above. The photographs indicated that prolific nodulation in nts382 may occur at the expense of root growth. Mutant line nts382 continued to nodulate prolifically throughout development. Figure 6 shows the tap root of a nts382 plant harvested at the podfill stage of development.

Plant Fresh Weight. Nitrate (both concentrations) and urea treatments produced healthy vigorous plants and stimulated growth of Bragg and nts382 over that of the respective KCl controls (Table I). Regardless of genotype, plants cultured on 5.5 mM NH₄Cl and NH₄NO₃ were unhealthy and stunted. Ammonium nitrate produced no effect on plant fresh weight after 4 weeks of culture, whereas NH₄Cl inhibited fresh weight accumulation in both Bragg and nts382. Plant fresh weight was consistently higher in Bragg than in nts382 (Table I).

Axenic Culture in Leonard Jars. The observations obtained from pots inoculated with R. japonicum strain CB1809 and described above were confirmed in Leonard jars supplemented with KNO₃ and urea. Both R. japonicum stains tested, namely CB1809 and USDA110, elicited the nts phenotype in mutant line 382. Uninoculated controls of nts382 and Bragg did not nodulate.

Nodulation Pattern. Table II shows the tap root nodulation pattern of N₂-dependent nts382 and Bragg plants. Tap root length was less in nodulated nts382 plants than in Bragg plants cultured under identical conditions (Table II). The tap root nodulation interval is defined as the distance between the uppermost and lowermost nodule on the tap root. This parameter was larger in nts382, both in absolute terms (2 times that of Bragg) and when expressed as a percentage of tap root length (3 times that of Bragg). Nodule density (nodules/cm) was also increased in nts382 (Table II). Nodule density, expressed on the total tap root length was 9 times higher in nts382 than in Bragg. Within the nodulation interval, the nodule density was 2.5 times higher in the mutant line (Table II). A similar contrast in nodulation pattern was observed on lateral roots and, furthermore, on nts382 plants cultured on various nitrogen sources (Fig. 5).

Inheritance of the nts382 Character. Following mutagenesis on M₁ seeds, resultant M₂ plants were grown through to produce M₃ seeds. Seeds from each M₁ plant were grouped together to give M₂ families. Mutant line nts382 was selected from a M₂ family that segregated for the mutant phenotype (Fig. 1). From 17 plants in M₂ family 382, two expressed the nts phenotype and
The remaining 15 plants expressed the wild-type phenotype and were indistinguishable from the parent cultivar Bragg for nodule formation in the absence of nitrate. In the M₃ screen, plants were harvested 6 weeks after planting and culture on 5 mM KNO₃. The nodule per plant for M₂ nts segregants was 146 ± 71 (± SD) and for M₂ wild-type (non-nts) segregants it was 26 ± 11. Bragg plants cultured under identical conditions had 19 ± 7 nodules per plant.

M₃ progeny derived from M₂ nts plants all expressed the mutant character indicating that these M₂ nts plants were homozygous for the mutation. Similarly, all M₄ progeny (used in experiments described above) had the nts phenotype.

M₂ wild-type (non-nts) segregants were also repotted and grown through to seed. The seeds derived from each M₂ plant were grouped together to give M₃ families. One of six such M₃ families segregated 14 nts plants:40 wild-type plants. This ratio closely approximates 1:3, the expected segregation ratio for a recessive character in progeny derived from a self-fertilized heterozygous wild-type plant (chi-square was equal to 0.00 and was not significant).

Mutant nts plants in the segregating M₃ family qualitatively had a similar phenotype to the original M₂ nts selections, and to the M₃ and M₄ progeny derived therefrom. Nodule number per plant for these nitrate-grown nts segregants was 789 ± 181 (± SD), whereas wild-type segregants had 32 ± 19 nodules per plant. Furthermore, plant fresh weight was lower in nts segregants; 8.23 ± 2.33 (± SD) g per plant compared to 15.19 ± 3.20 g per plant for wild-type segregants.

After 7 weeks culture on 5 mM KNO₃, nts segregants had 23 times the nodule fresh weight per plant biomass of wild-type segregants (Table III). Increased nodule formation in nts plants was observed on the tap root as well as on the lateral roots (Table III); this trend was consistent with the nodule pattern shown in Figure 5 for nts382 grown under a variety of combined nitrogen regimes. As a result of the increased nodule mass, nitrate-grown nts segregants had 7 times the nitrogenase (acetylene reduction) activity per plant biomass of nitrate-grown wild-type segregants (Table III).

**DISCUSSION**

Mutant line nts382 was one of several nts (nitrate-tolerant-symbiosis) mutants selected from an M₂ population of parent cultivar Bragg for increased nodule formation in the presence of nitrate (6). Individual M₂ families (resulting from a single mutagenized M₁ seed) were originally screened. M₂ family 382 segregated for the nts character (Fig. 1), as did all of the M₂ nts mutant families.
The plants thereof had the and Methods.

Table I. Plant Fresh Weight of Bragg and nts382 Plants Cultured for 4 Weeks on Various Nitrogen Sources

<table>
<thead>
<tr>
<th>Nitrogen source</th>
<th>Plant Fresh Wt*</th>
<th>Bragg</th>
<th>nts382</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 mm (5.5 mm KCl)</td>
<td>3.24 (100)*</td>
<td>1.96 (100)*</td>
<td></td>
</tr>
<tr>
<td>2.75 mm KNO₃</td>
<td>5.61 (173)</td>
<td>2.37 (121)</td>
<td></td>
</tr>
<tr>
<td>5.5 mm KNO₃</td>
<td>8.05 (248)</td>
<td>3.68 (188)</td>
<td></td>
</tr>
<tr>
<td>5.5 mm urea</td>
<td>4.80 (148)</td>
<td>3.51 (179)</td>
<td></td>
</tr>
<tr>
<td>5.5 mm NH₄NO₃</td>
<td>2.12 (65)</td>
<td>1.27 (65)</td>
<td></td>
</tr>
<tr>
<td>5.5 mm NH₄Cl</td>
<td>3.04 (94)</td>
<td>2.18 (111)</td>
<td></td>
</tr>
</tbody>
</table>

* LSD₀.₀₅ = 1.56 for Bragg and 1.08 for nts382.  
* Plant fresh weight as percent of 5.5 mm KCl control.

This observation indicated that each of the mutant families arose from independent mutation events and that the mutants were a result of the mutagenesis program (6). M₂ nts382 selections were homozygous for the mutation, since all M₂ progeny derived thereof had the nts phenotype, as did all M₃ progeny, including the plants used in some experiments described here. M₃ progeny

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Fig. 6. Nodulation of nts382 at the podfill stage of development. a, The tap root of a nts382 plant that had been cultured in a 25-cm pot of vermiculite. This plant had received slow-release nitrogen fertilizer throughout growth. b, Close-up of Figure 6a.
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Table II. Tap Root Nodulation Pattern of N₂-Dependent nts382 and Bragg Plants

<table>
<thead>
<tr>
<th>Parameter</th>
<th>nts382*</th>
<th>Bragg</th>
<th>LSD₀.₀₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root length (cm)</td>
<td>16.6</td>
<td>28.1</td>
<td>3.1</td>
</tr>
<tr>
<td>Nodulation interval (cm)</td>
<td>14.4</td>
<td>7.6</td>
<td>2.1</td>
</tr>
<tr>
<td>Nodulation interval (% root length)</td>
<td>86.5</td>
<td>27.1</td>
<td>6.7</td>
</tr>
<tr>
<td>Nodule density on root length (nodules-cm⁻¹)</td>
<td>4.32 (2.0)*</td>
<td>0.47 (0.67)</td>
<td>—(0.15)</td>
</tr>
<tr>
<td>Nodule density on nodulation interval (nodules-cm⁻¹)</td>
<td>5.07</td>
<td>2.10</td>
<td>0.99</td>
</tr>
</tbody>
</table>

* Each entry in the table for nts382 represents the mean of seven plants.  
* Each entry in the table for Bragg represents the mean of 28 plants.  
* Raw data required square-root transformation to satisfy assumptions for an analysis of variance; means and LSD of transformed data are shown in parentheses.

Table III. Nodulation and Nitrogenase (Acetylene Reduction) Activity in KNO₃-Grown nts and Wild-Type Segregants from a nts382 M₃ Family

This family was derived from a wild-type M₂ plant. The segregation ratio was 14 nts:40 wild type, which approximates 1:3 (chi-square = 0.00 and was not significant). Plants were inoculated with R. japonicum USDA110 and harvested after 7 weeks growth on 5 mm KNO₃. Data are expressed per g plant fresh weight.

<table>
<thead>
<tr>
<th>Symbiotic Parameter</th>
<th>nts382*</th>
<th>Wild-type</th>
<th>LSD₀.₀₅</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nodule number-gram plant fresh wt⁻¹</td>
<td>9.6 (2.22)*</td>
<td>0.7 (0.45)</td>
<td>(0.20)</td>
</tr>
<tr>
<td>on tap root</td>
<td>89.2 (9.4)*</td>
<td>15 (1.1)</td>
<td>(0.4)</td>
</tr>
<tr>
<td>mg nodule fresh wt-g plant fresh wt⁻¹</td>
<td>145.3 (12.0)*</td>
<td>6.2 (2.5)</td>
<td>(0.5)</td>
</tr>
<tr>
<td>nmol C₂H₄g-plant fresh wt⁻¹min⁻¹</td>
<td>8.0 (2.05)*</td>
<td>1.1 (0.00)</td>
<td>(0.76)</td>
</tr>
</tbody>
</table>

* Each entry in the table is the mean of 14 and 18 plants, for nts and wild-type, respectively, except that acetylene reduction data are the means of seven nts and nine wild-type plants.  
* Raw data required either log, (c) or square-root (d) transformation to satisfy assumptions for an analysis of variance; means and LSD of transformed data are shown in parentheses.

of a wild-type M₂ plant segregated 3 wild type:1 mutant, indicating that the nts382 character is inherited as a Mendelian recessive.

Regardless of the presence or absence of combined nitrogen, nts382 nodulated more than the parent cultivar Bragg. This trend was consistent over a range of combined nitrogen sources that caused varying degrees of inhibition of nodulation in Bragg. For example, under conducive conditions for nodulation, 4-week-old N₂-dependent nts382 plants had 9 times the nodule number of N₂-dependent Bragg plants. Similarly, under conditions that totally prevented nodulation in Bragg (5.5 mm NH₄Cl or NH₄NO₃), nts382 plants were still nodulated. The mutant line also had increased nodulation in Leonard jars, in deep soil pots (soil obtained from soybean field at Breeza, NSW, Australia) and in Georgia (USA) fieldplots. Nodule initiation and nodule growth are coordinated in nts382, and nts382 plants have a considerably larger nodule mass than wild-type plants (6; Table III). We use the term supernodulator to describe nts382, since this soybean...
genotype has an increased nodule mass under a wide range of environmental conditions.

Although nts382 is a supernodulator, it is not a constitutive nodulator, since it still requires the inducer (i.e. *R. japonicum*) to be present. The two strains of *R. japonicum* used, namely CB1809 and USDA110, elicited the nts phenotype in the mutant line. Both these strains form an effective symbiosis with the parent cultivar. A wider spectrum of fast- and slow-growing *R. japonicum* strains, that vary in their ability to nodulate the parent cultivar, were tested on nts382 to ascertain whether the nts character confers a change in the promiscuity of the host plant. Those strains that nodulated Bragg elicited the mutant phenotype on nts382, and strains that were nod- on the parent cultivar were also unable to induce nodule formation on nts382.

In Bragg, all nitrogen sources caused a significant reduction in the stimulatory parameters that were measured (Figs. 2–5). In contrast, enriching the nutrient solution with KNO3 did not inhibit any symbiotic parameters in nts382 and was in some cases stimulatory (Figs. 2 and 4). Urea in the nutrient solution stimulated nodule number per plant but significantly inhibited nodule number and nitrogenase activity per gram plant fresh weight in the mutant line. Consistent with the comparative effects of KNO3 and urea on nts382, urea was also more severe on symbiotic development in Bragg (Figs. 2–4). This is different to what Vigue et al. (30) found with soybean cultivar Steele. Using a carboxyl buffer to refine the culture medium (17), they demonstrated that nitrate was more inhibitory on nodulation than was urea. To minimize the drop in pH associated with urea and ammonium utilization (14), the pots in our experiments were flushed daily with 700 ml of nutrient solution. This procedure, without a specific buffering agent, produced healthy vigorous urea-fed plants that had significantly stimulated fresh weights in Bragg and nts382 (Table I). Nevertheless, it is unclear whether pH fluctuations played a role in the inhibition of the symbiosis by urea, NH4Cl, and NH4NO3. This, however, is not a contentious issue, since the salient feature of nts382 illustrated by these experiments is that it consistently nodulated prolifically in comparison to parent cultivar Bragg, regardless of the environmental conditions imposed by the provision of various nitrogen sources.

Since prolific nodulation by nts382 was not confined to culture on nitrate, it is unlikely that increased nodulation on nitrate resulted from an inability to utilize nitrate. KNO3 enhanced the growth of nts382 plants (Table I) and, furthermore, nts382 has the same nitrate reductase activity as Bragg (6). Indeed, nts382 is a mutant in the regulation of nodule initiation and nodule Table IV. Symbiotic Parameters of nts382 Expressed as a Proportion of Bragg

<table>
<thead>
<tr>
<th>Nitrogen Source</th>
<th>Symbiotic Parameter*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Nodules/plant*</td>
</tr>
<tr>
<td></td>
<td>Nodules/g plant fresh wt*</td>
</tr>
<tr>
<td></td>
<td>Nitrogenase activity#</td>
</tr>
<tr>
<td>0 mm (5.5 mm KCl)</td>
<td>9</td>
</tr>
<tr>
<td>2.75 mm KNO3</td>
<td>18</td>
</tr>
<tr>
<td>5.5 mm KNO3</td>
<td>31</td>
</tr>
<tr>
<td>5.5 mm urea</td>
<td>65</td>
</tr>
<tr>
<td>5.5 mm NH4Cl*</td>
<td>∞</td>
</tr>
<tr>
<td>5.5 mm NH4NO3*</td>
<td>∞</td>
</tr>
</tbody>
</table>

*nts382 + Bragg. h.e.d. Data from Figures 2, 3, and 4, respectively. *NH4Cl and NH4NO3 totally prevented nodule formation in Bragg.

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