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# Responses of Mungbean Varieties to *Rhizobium* Inoculation in respect of Nodulation, Nitrogenase Activity, Dry Matter Yield, and Nitrogen Uptake

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**ABSTRACT: The responses of six mungbean [*Vigna radiata* (L.) Wilczek] varieties to *Rhizobium* inoculation on number and dry weight of nodules, nitrogenase activity of root nodule bacteria, dry weight of shoot and root, nitrogen content, and uptake by shoot were investigated. The mungbean varieties were BARI Mung-2, BARI Mung-3, BARI Mung-4, BARI Mung-5, BINA Moog-2, and BU Mung-1. Two-third seeds of each variety were inoculated with *Rhizobium* inoculant and the remaining one-third seeds were kept uninoculated. *Rhizobium* strains TAL 169 and TAL 441 were used for inoculation of seeds. Inoculation of seeds with *Rhizobium* strains significantly increased nodulation, nitrogenases activity, dry matter production, nitrogen content, and uptake by shoot of the crop compared to uninoculated control. There was positive correlation among the number and dry weight of nodules, nitrogenase activity, dry weight of shoot and root, nitrogen content, and uptake by shoot of the crop. It was concluded that BARI Mung-4 in association with *Rhizobium* strain TAL 169 performed best in recording nodulation, nitrogenase activity, dry matter production, and nitrogen uptake by shoot of mungbean.**

***Keywords:*** Mungbean, *Rhizobium*, nodulation, nitrogenase activity, dry matter, Nuptake

Mungbean [*Vigna radiata* (L) Wilczek] is one of the most important pulse crops in Bangladesh for its high digestibility and good flavor flatulent effects. It is an important source of different vitamins and minerals. It accounts for 5% of the total area under pulse cultivation in Bangladesh. It ranks fifth in terms of area and production but first in respect of price (Rahman & Miah, 1988). To meet up the demand of pulse in Bangladesh, there is an urgent need for increasing total production of pulses.

Nitrogen is the most deficient nutrient element in Bangladesh soils which reduces the growth and development of many crops. Most of the farmers of Bangladesh use urea fertilizer to enrich nitrogen content in soil for crop production. Mungbean can form nodules and fix atmospheric nitrogen with the help of *Rhizobium* inoculant and increase nitrogen nutrition in soil (Satter & Ahmed, 1992). Biological nitrogen fixation (BNF) technology in the form of *Rhizobium* inoculant is used in mungbean, which can be an attractive alternative of expensive urea fertilizer. Yield increases upto 10 to 37% by *Rhizobium* inoculation have been reported by Rao (1980) and Satter & Ahmed (1992). Singh & Chaubey (1971) reported a profit/cost ratio of 27/1 for inoculation with their most efficient *Rhizobium* strain. In soils where mungbean had not been grown previously, inoculation helps meet the demand for nitrogen required through biological nitrogen fixation.

A number of mungbean varieties have been released by different research organizations/universities of Bangladesh but their comparative performances in relation to nodulation, nitrogenase activity, dry matter production, nitrogen content, and uptake by shoot have not been tested. Cultivation of mungbean using *Rhizobium* inoculation is less expensive than that of chemical nitrogen fertilizer. Keeping these facts in mind the present investigation was, therefore, carried out to evaluate the responses of a number of mungbean varieties to *Rhizobium* inoculation on nodulation, nitrogenase activity, dry matter production, and nitrogen uptake.

## MATERIALS AND METHODS

The experiment was conducted at the research farm of Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh to study the performances of six mungbean varieties to *Rhizobium* inoculation on nodulation, nitrogenase activity, dry matter production, nitrogen content, and uptake by shoot. Soil samples were collected from the experimental field before sowing seeds. The soil belongs to Shallow Red Brown Terrace Soil (Salna series) under Madhupur Tract (AEZ 28) and is classified as Inceptisols. The collected soil samples were then air-dried, ground and passed through a 10 mesh sieve. The soil was of silty clay loam having pH 6.7, organic carbon 9.1 g/kg, CEC 15 $cmol^+$/kg, total N 0.7 g/kg, available P 11.05 mg/kg, exchangeable K 7.44 $cmol^+$/kg, available S 13.30 mg/kg, exchangeable Ca 6.50 $cmol^+$/kg, exchangeable Mg 3.30 $cmol^+$/kg, available Zn 2.06 mg/kg, and available B 0.38 mg/kg soil. Basal doses

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of phosphorus and potassium were applied to the soil at the rate of 27.66 kg and 33.86 kg/ha in the form of triple super phosphate and muriate of potash, respectively. No nitrogenous fertilizer was used in the experiment.

Six high yielding mungbean varieties viz. BARI Mung-2, BARI Mung-3, BARI Mung-4, BARI Mung-5, BINA Moog-2, and BU Mung-1 were used as the test crop. Two *Rhizobium* inoculants containing the fast-growing strains TAL169 and TAL441 were used for inoculation of seeds. *Rhizobium* population of the inoculant containing the strains TAL169 and TAL441 was $625\times10^6$ and $325\times10^4$ cells/ml, respectively as estimated following the method described by Miles & Misra (1938). Two-third seeds of each variety were inoculated with *Rhizobium* inoculant and the remaining one-third seeds were kept uninoculated. Inoculated seeds were air-dried before sowing. There were eighteen treatment combinations comprising six mungbean varieties and three levels of *Rhizobium* inoculation viz. uninoculated control, TAL169 and TAL441. The experiment was laid out in a split plot design with three replications. Plant samples were collected from the experimental field at preflowering and flowering stages of the crop. From each plot, five plants were carefully uprooted so that no nodule was left in the soil. The roots were washed thoroughly with water. Nitrogenase activity of the plants was assessed by measuring acetylene reducing activity (ARA) in a gas chromatograph (Shimadzu, GC-8A) fitted with a flame ionization detector and a stainless steel column (3 mm dia, 1.2 m length) as described by Hardy *et al.* (1968). The nodules from the roots of each plant were separately collected and counted. The shoot, root, and nodule materials were first air-dried and then oven-dried at 65°C for 72 hrs. The oven dry weights of shoot, root, and nodule were recorded. The oven-dried plant shoot materials were ground in a grinding machine (Wiley Cutting Mill, Model 1029-B, Yoshida Seisakusho Co. Ltd. Japan). Total nitrogen content in the shoot material was determined by ashing the plant material using salicylic acid modified Kjeldahl method following sulphuric acid digestion and then steam distillation and titration assay (Wilde *et al.* 1979). Nitrogen uptake by shoot was calculated from the data on dry matter yield and nitrogen content in shoot material of the crop. The recorded data on various characters of the crop was statistically analyzed to find out the significance of variation resulting from the treatments. The differences between the treatment means were compared by Duncan's Multiple Range Test (DMRT). Correlations between different characters were also calculated.

## RESULTS AND DISCUSSION

### Nodulation

Mungbean inoculated with *Rhizobium* strains significantly increased number and dry weight of nodules compared to that of uninoculated control plants both at preflowering and flowering stages. BARI Mung-4 in combination with *Rhizobium* strain TAL169 produced the highest number of nodules at preflowering (56.44/plant) stage (Table1). The effect of this treatment was statistically similar to BARI Mung-3 ×TAL169, BARI Mung-4×TAL441 but was superior to the rest of the treatments. However, the effect of BARI Mung-3×TAL169 was statistically similar to BARI Mung-5× TAL169, BARI Mung-3×TAL441, and BARI Mung-5× TAL441. The lowest number of nodules in uninoculated and inoculated conditions was produced by the variety BU Mung-1. The trend of nodulation at flowering stage was found more or less similar as that of preflowering stage. The highest number of nodules (66.44/plant) at this stage was produced by BARI Mung-4×TAL169. The second highest number of nodules was produced by the treatment BARI Mung-3×TAL169 but its effect was statistically similar to BARI Mung-5×TAL169, BINA Moog-2×TAL169, BARI Mung-3×TAL441, and BARI Mung-5×TAL441. Gupta *et al.* (1976) found higher number of nodules in mungbean by inoculation with effective *Rhizobium* strains. Hoque (1991) reported that the strain BAU 604 was the best strain for producing highest number of nodules of mungbean. Satter & Ahmed (1992) stated that *Bradyrhizobium* inoculation significantly increased the number of nodules of mungbean as compared to uninoculated control.

At preflowering stage, BARI mung-4 in association with TAL169 produced the maximum dry weight of nodules (33.33 mg/plant), which was statistically similar to the combined effect of BARI Mung-4×TAL441 (Table 1). BARI Mung-4×TAL169 produced 489% higher dry weight of nodules compared to BARI Mung-4 in uninoculated condition. Treatments BARI Mung-3×TAL169, BARI Mung-5×TAL 169, and BARI Mung-3×TAL441 ranked second in recording dry weight of nodules and no significant differences were found among themselves. The effects of BARI Mung-2× TAL169, BU Mung-1×TAL169, BARI Mung-2×TAL441 and BU Mung-1xTAL441 were statistically similar and produced lower dry weight of nodules. BU Mung-1 produced comparatively lower dry weight of nodules in uninoculated (3.66 mg/plant) condition. All the varieties produced significantly lower dry weight of nodules without inoculation. At flowering stage, BARI Mung-4×TAL169 produced the highest dry weight of nodule (181.70mg/plant) which was statistically superior to other treatments. The effects of BARI Mung-3×TAL169 and BARI Mung-4×TAL441 were statistically similar and ranked second in position. The performance of all the varieties in uninoculated condition were inferior to inoculated condition. Satter & Ahamed (1992) reported that *Bradyrhizobium* inoculation significantly increased nodule

**Table 1.** Interaction effect of variety and *Rhizobium* inoculation on number and dry weight of nodules, and nitrogenase activity of mungbean at preflowering and flowering stages.

| Treatment | | Nodule No./plant | | Nodules (mg/dry wt./plant) | | Nitrogenase activity (µ mole $C_2H_4$/plant/h.) | |
|---|---|---|---|---|---|---|---|
| *Rhizobium* inoculation | Variety | Preflowering stage | Flowering stage | Preflowering stage | Flowering stage | Preflowering stage | Flowering stage |
| Uninoculated control | BARI Mung-2 | 13.86l | 19.80i | 4.00g | 9.00k | 2.301e | 2.601j |
| | BARI Mung-3 | 29.33g | 37.00h | 5.33g | 14.33i | 2.543e | 4.730hi |
| | BARI Mung-4 | 37.67f | 40.00g | 5.66g | 17.00h | 2.583e | 5.780hi |
| | BARI Mung-5 | 27.33g | 29.67h | 4.66g | 12.67ij | 2.493e | 3.977ij |
| | BINA Moog-2 | 20.33h | 20.80i | 4.66g | 11.00j | 2.320e | 2.920j |
| | BU Mung-1 | 13.00l | 20.00i | 3.66g | 7.00l | 2.200e | 2.530j |
| TAL169 | BARI Mung-2 | 43.22cdef | 49.44ef | 22.67def | 163.30e | 4.298d | 163.30e |
| | BARI Mung-3 | 52.06ab | 62.06b | 28.67bc | 177.70b | 5.510bc | 177.70b |
| | BARI Mung-4 | 56.44a | 66.44a | 33.33a | 181.70a | 7.198a | 181.70a |
| | BARI Mung-5 | 47.22bc | 56.61bc | 28.33bc | 173.30c | 4.728cd | 173.30c |
| | BINA Moog-2 | 45.56cd | 56.11bcd | 24.00de | 168.30d | 4.407cd | 168.30d |
| | BU Mung-1 | 37.67f | 53.11cde | 21.00ef | 158.30f | 2.961e | 158.30f |
| TAL441 | BARI Mung-2 | 40.00def | 50.00def | 22.00ef | 158.30f | 3.060e | 15.150g |
| | BARI Mung-3 | 48.22bc | 57.89bc | 28.67bc | 174.30c | 5.167cd | 30.040cd |
| | BARI Mung-4 | 55.89a | 64.78a | 31.33ab | 177.70b | 6.161b | 34.260ab |
| | BARI Mung-5 | 46.61bc | 56.17bcd | 26.67cd | 170.00d | 4.726cd | 25.290ef |
| | BINA Moog-2 | 44.22cde | 55.56cde | 23.67de | 163.30e | 4.621cd | 23.690ef |
| | BU Mung-1 | 39.67ef | 46.67f | 18.67f | 153.30g | 2.930e | 6.530hi |

Means followed by the same letter (s) in a column are not significantly different at 5% level by DMRT

weight of mungbean as compared to uninoculated control. Rasal & Patil (1989) found that dry weight of nodules/plant of mungbean increased with increasing weight of inoculum/kg seed. Solaiman (1999a) revealed significantly higher dry weight (331.7 mg/plant) of nodules in soybean receiving inoculant+1.5 kg Mo $ha^{-1}$ as compared to uninoculated control. Table 3 shows that the number of nodules was positively correlated with dry weight of nodules of the crop.

### Nitrogenase activity of root nodule bacteria

Among the mungbean varieties, BARI Mung-4×TAL169 scored the highest nitrogenase activity of the nodule bacteria (7.198 µ mol $C_2H_4$/plant/hr) at preflowering stages and the effect was statistically superior to other treatments (Table 1). BARI Mung-4 in association with TAL169 and TAL441 recorded 179% and 139% higher nitrogenase activity over its uninoculated condition at preflowering stage, respectively. In inoculated condition the performances of BARI Mung-2 and BU Mung-1 were poor and similar to the performances of all the varieties in uninoculated condition. As like as preflowering stage, BARI Mung-4 in combination with TAL169 recorded the highest nitrogenase activity (181.70 µ mol $C_2H_2$/plant/hr) at flowering stage but the effect of this treatment was statistically similar to BARI Mung-4×TAL441. BU Mung-1 in uninoculated condition recorded the lowest nitrogenase activity than inoculated condition. Eusuf Zai *et al.* (1999) reported that nitrogenase activity of chickpea varieties significantly increased due to inoculation. Nitrogenase activity was positively correlated with the number and dry weight of nodules of the crop both at preflowering and flowering stages of the crop (Table 3).

### Dry weight of shoot

Variety and *Rhizobium* inoculation played a significant positive role in increasing dry weight of shoot of mungben at flowering stage but the effect was insignificant at preflowering stage (Table 2). At flowering stage, the highest dry weight of shoot (18.36 g/plant) was produced by BARI Mung-4 in association with TAL169 and the effect of this treatment was statistically similar to BARI Mung-3×TAL169 and BARI Mung-4×TAL441. BARI Mung-4×TAL169 recorded 90% higher dry weight of shoot over its uninocu-

**Table 2.** Interaction effect of variety and *Rhizobium* inoculation on dry weight of shoot and root, nitrogen content, and uptake by shoot at preflowering and flowering stages of mungbean.

| Treatment | | Shoot (g dry wt./plant) | | Root (mg dry wt./plant) | | Nitrogen content in shoot (%) | | Nitrogen uptake by shoot (mg/plant) | |
|---|---|---|---|---|---|---|---|---|---|
| *Rhizobium* inoculation | Variety | Preflowering stage | Flowering stage | Preflowering stage | Flowering stage | Preflowering stage | Flowering stage | Preflowering stage | Flowering stage |
| Uninoculated control | BARI Mung-2 | 2.49 | 8.77de | 190 | 448f | 1.708f | 1.80f | 80g | 213g |
| | BARI Mung-3 | 2.96 | 9.58de | 293 | 520f | 2.500e | 3.10de | 107ef | 312ef |
| | BARI Mung-4 | 3.82 | 9.68de | 320 | 703e | 2.54e | 3.12de | 120def | 328ef |
| | BARI Mung-5 | 2.58 | 9.40de | 237 | 503f | 2.32e | 2.97e | 92f | 239f |
| | BINA Moog-2 | 2.55 | 9.23de | 213 | 475f | 1.89f | 1.82f | 79g | 215g |
| | BU Mung-1 | 2.34 | 8.40e | 203 | 448f | 1.70f | 1.79f | 78g | 211g |
| TAL169 | BARI Mung-2 | 4.32 | 10.33cde | 537 | 985d | 3.38bcd | 3.56bc | 146bcde | 452bcde |
| | BARI Mung-3 | 5.30 | 17.01a | 600 | 1,200abc | 3.96a | 3.73b | 175b | 605abc |
| | BARI Mung-4 | 6.54 | 18.36a | 623 | 1,307a | 3.99a | 4.15a | 271a | 780a |
| | BARI Mung-5 | 4.78 | 12.89bc | 593 | 1,118bcd | 3.61abc | 3.65bc | 164bc | 513bcde |
| | BINA Moog-2 | 4.393 | 11.37bcde | 543 | 1,074cd | 3.59abc | 3.61bc | 157bcd | 480bcde |
| | BU Mung-1 | 3.31 | 10.64bcde | 533 | 757e | 3.21cd | 3.39bcd | 127cdef | 400cdef |
| TAL441 | BARI Mung-2 | 3.47 | 10.32cde | 323 | 970d | 3.25bcd | 3.49bc | 129cdef | 408cdef |
| | BARI Mung-3 | 3.80 | 13.58b | 447 | 1,200abc | 3.72ab | 3.67bc | 172b | 549bcd |
| | BARI Mung-4 | 6.53 | 18.18a | 513 | 1,263.ab | 3.96a | 3.76b | 189b | 644ab |
| | BARI Mung-5 | 3.88 | 11.91cd | 433 | 1,113.bcd | 3.603abc | 3.61bc | 160bcd | 511bcde |
| | BINA Moog-2 | 3.58 | 11.05cde | 367 | 987d | 3.55abcd | 3.58bc | 154bcd | 472bcde |
| | BU Mung-1 | 3.20 | 9.87cde | 347 | 703e | 3.11d | 3.32cde | 125cdef | 365def |

Means followed by the same letter (s) in a column are not significantly different at 5% level by DMRT

lated condition. The effects of BARI Mung-3xTAL441, BARI Mung-5xTAL169 and BINA Moog-2xTAL169 were statistically similar and ranked second in recording dry weight of shoot. All the varieties showed poor performance in uninoculated condition. This result agreed with that of other researchers who worked with grain legumes including mungbean (Bhuiyan *et al.*,1984; Mahmud *et al.*, 1997; Solaiman *et al.*,1999; Eusuf Zai *et al*,. 1999; Alam *et al.*,1999). Table 3 shows that the number and dry weight of nodules per plant and nitrogenase activity had a positive correlation with dry weight of shoot both at preflowering and flowering stages of the crop.

### Dry weight of root

At flowering stage maximum dry weight of root (1,307 mg/plant) was observed in BARI Mung-4 in association with *Rhizobium* strain TAL169 and the effect of this treatment was statistically similar to the interaction effect of BARI Mung-3xTAL169, BARI Mung-3xTAL441 and BARI Mung-4xTAL441 (Table 2). Lower dry weight of root was produced by all the varieties of mungbean in uninoculated condition than that of inoculated condition. Similar results were also reported by Solaiman (1999b) in mungbean, Essa & Dulaimi (1985) in soybean, Eusuf Zai *et al.* (1999) and Alam *et al.* (1999) in chickpea and Mahmud *et al.* (1997) in lentil due to *Rhizobium* inoculation.

### Nitrogen content in shoot

The shoots accumulated significant amount of nitrogen due to inoculation (Table 2). At preflowering stage, significantly higher nitrogen content was recorded by a number of treatments viz. BARI Mung-3xTAL169, BARI Mung-4xTAL169, BARI Mung-5xTAL169, BINA Moog-2xTAL169, BARI Mung-3xTAL441, BARI Mung-4xTAL441, BARI Mung-5xTAL441 and BINA Moog-2xTAL441 but the effects of these treatments were statistically similar. The performance of BU Mung-1 in recording nitrogen content in shoot was poor. At flowering stage, BARI Mung-4xTAL169 accumulated the highest amount of nitrogen (4.15%) in shoot, which was statistically superior to other treatments. The rest of the inoculated treatments were statistically similar except BU Mung-1xTAL441, which recorded lower amount of nitrogen in

**Table 3.** Relationship between different characters of mungbean recorded at preflowering and flowering stages.

| Factors | Preflowering stage | | | Flowering stage | | |
|---|---|---|---|---|---|---|
| | Nodules No. | Nodules dry weight | Shoot dry weight | Nodules No. | Nodules dry weight | Shoot dry weight |
| Nodules dry weight | 0.832 | – | – | 0.902 | – | – |
| $N_2$ase activity | 0.724 | 0.765 | 0.710 | 0.859 | 0.925 | 0.719 |
| Shoot dry weight | 0.640 | 0.739 | – | 0.567 | 0.546 | – |
| Shoot N content | 0.237 | 0.144 | – | 0.744 | 0.727 | – |
| Shoot N uptake | 0.622 | 0.610 | – | 0.628 | 0.606 | – |

n=54 r value: $P_{0.05}$=0.2184, $P_{0.01}$=0.2832

shoot. Maurya *et al.* (1987) illustrated that chickpea inoculated with *Rhizobium* strains increased nitrogen content of shoot. In the present study (Table 3) nitrogen content in shoot had a positive correlation with the number and dry weight of nodules at flowering stage of the crop.

### Nitrogen uptake by shoot

Among the varieties BARI Mung-4 in association with *Rhizobium* strain TAL169 yielded the highest nitrogen uptake by shoot (271 mg/plant) at preflowering stage, which was superior to other treatments (Table 2). The effects of BARI Mung-2×TAL169, BARI Mung-3×TAL169, BARI Mung-3×TAL441 were statistically similar to BARI Mung-5×TAL169, BINA Moog-2×TAL169 and BINA Moog-2×TAL441 and ranked second position in recording nitrogen uptake by shoot. Lower nitrogen uptake by shoot was recorded by BU Mung-1 both in inoculated and uninoculated conditions. At flowering stage BARI Mung-4 in association with strain TAL169 recorded the highest amount of nitrogen uptake (780 mg/plant), but its effect was similar to BARI Mung-3×TAL169 and BARI Mung-4×TAL441 and superior to other treatments. The effect of BARI Mung-3×TAL441 was similar to the rest of the treatments. The effects of the varieties in uninoculated condition were inferior. Solaiman (1999b) reported significantly higher nitrogen uptake by shoot of mungbean due to *Bradyrhizobium* inoculation compared to those of uninoculated control. Mahmud *et al.* (1997) observed significantly higher nitrogen uptake by shoot through inoculation in lentil. Khan *et al.* (1997) found that single application of *Rhizobium* inoculant significantly increased nitrogen uptake by shoot of chickpea compared to control. There was a strong positive correlation between nitrogen uptake by shoot and the number and dry weight of nodules per plant both at preflowering and flowering stages of the crop (Table 3).

We may conclude that inoculation of seeds with *Rhizobium* strains significantly increased nodulation, nitrogenases activity of root nodule bacteria, dry matter production, nitrogen content, and uptake by shoot of the crop compared to uninoculated control. BARI Mung-4 in association with *Rhizobium* inoculant containing the strain TAL 169 performed best in all parameters of the crop studied.

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