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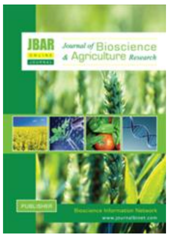

Effects of Bradyrhizobium on growth nodulation and nitrogen uptake by akashmoni

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ABSTRACT

Bacteria were isolated and characterized from the root nodules of Akashmoni (Acacia nilotica) to study their effects on growth parameters, nodulation and N uptake by sapling of Akashmoni. Five isolates were obtained from Akashmoni and were identified them as Bradyrhizobium on the basis of their colony, morphological and biochemical characteristics. A pot experiments were conducted by using 6 treatments, comprising 5 isolates of Bradyrhizobium and an uninoculated control to evaluate the performance of the isolates. The experiment was laid out in a Completely Randomized Design (CRD) with 3 replications. Statistically higher values for all the parameters were obtained from Bradyrhizobium inoculation over uninoculated control (T_1) except leaf number plant^{-1} at 30 DAS. The highest value of plant height (cm plant^{-1}) and leaf number plant^{-1} at 60 DAS were recorded as 30.33 cm plant^{-1} and 12.67 plant^{-1}, respectively due to the treatment T_3 (AN-R-2). The highest nodule numbers plant^{-1} and dry weight of nodule (mg plant^{-1}) were also noted as 134.7 plant^{-1} and 76.6 mg plant^{-1} for the same treatment. The highest root, shoot and total dry matter yields of Akashmoni were also found for the same treatment T_3 (AN-R-2). The total dry matter yield of Akashmoni was increased by 149, 198, 144, 55 and 82 % over control due to T_2 (AN-R-1), T_3 (AN-R-2), T_4 (AN-R-3), T_5 (AN-R-4) and T_6 (AN-R-5), respectively. The total N in shoot showed a similar pattern and corresponding increase in N uptake by shoots were 191, 396, 285, 80 and 242 %, respectively. Considering all the parameters the isolate T_3 (AN-R-2) showed the best performance.

Key Words: *Akashmoni, Bradyrhizobium, Nitrogen uptake*

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I. Introduction

Biological Nitrogen Fixation (BNF) by tree legumes is important in relation to global environment. After Rio de Janerio Earth Summit in 1992 organized by United Nations, attention is focused on the global environmental problem. Heavy use of nitrogenous fertilizer is responsible for the ground water

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and surface water pollution through nitrate ions and destruction of ozone layer by gaseous oxides of nitrogen. But, biological nitrogen fixation is friendly to environment and it may reduce the use of nitrogen as chemical fertilizer. But no bacterial biofertilizer has yet been developed for the tree legumes in Bangladesh whereas many countries of the world have been producing and using such bacterial biofertilizers for the tree legumes successfully. So, it is necessary to develop bacterial biofertilizer for tree legumes to reclaim environmental and soil hazards and to achieve sustainable agriculture.

In the context of afforestation of marginal or denuded land, nodulated nitrogen fixing tree legumes have a special advantage over other tree species. A wide range of leguminous N_2-fixing trees are utilized in forestry, agroforestry and land reclamation (Dommergues, 1993 and National Academy of Sciences, 1977). These species are grown for wood production and for pulp, fuel and wind breaks, as browse for domestic grazing animals and to improve soil fertility for interplanted and / or subsequent crops. In Third world countries, fertilizer nitrogen is applied for cultivation of only a few cash crops and main dietary crops like rice and wheat. But management of quick growing and high yielding trees for timber and fuel on a zero input land should be a target for the government as well as for the privately fostered agencies, especially at the Nursery level. Microbial technologies hold great promise in the operation of producing healthy saplings at nursery level in a scientific way by inoculating nitrogen fixing bacteria. Artificially prepared inoculants added to soil often aid in the sound establishment of tree stands on degraded land. Keeping the above points in view, the present study was undertaken to isolate *Bradyrhizobium* spp. from the root nodules of Akashmoni (*Acacia nilotica*) for selecting effective strains to produce inoculants and to study their effects on growth parameters, nodulation, dry matter production of and total nitrogen uptake by Akashmoni.

II. Materials and Methods

Isolation and characterization of *Bradyrhizobium*

Mature and large sized nodules were collected from saplings of Akashmoni grown in a commercial nursery near to the Bangladesh Agricultural University campus. Nodules were washed in tap water to remove gross soil contamination. The nodules were immersed briefly (5 seconds) into 95 % ethanol. After that, nodules were put into the petridish containing 0.1 %-acidified mercuric chloride and left for 4 minutes. Then, nodules were rinsed in five changes of sterile water. Nodules were then transferred to a sterile petri plate and crushed individually with fine pointed sterile forceps to produce turbid suspension. One loopful of the suspension was streaked out on yeast-mannitol agar plates(K_2HPO_4 0.5g, $MgSO_4$ 0.2g, NaCl 0.1g, Mannitol 10g, Yeast water 100ml, Distilled water 900ml). The plates were then incubated at 28^0C and checked for typical growth colonies of *Bradyrhizobium* along the streak lines. Well-isolated single colonies were picked off and rest reaked on clean plates to obtain pure cultures. The form, margin, surface, diameter, optical characteristics, consistency and pigmentation of the colonies were studied on YMA media according to Harry and Paul (1972). Shape of the bacteria was examined by direct staining with crystal violet solution. Gram reaction was examined by modified Hucker and Conn's method (1923). Motility was examined by hanging drop method. Congo red dye absorption, Starch utilization and pH test was done.

Pot study

This was a pot study carried out in the Net house of the Department of Soil Science, Bangladesh Agricultural University, Mymensingh during September to November 2002 to study the effects of *Bradyrhizobium* inoculation on Akashmoni. The experiment was laid out in a Complete Randomized Design (CRD) with 6 treatments with 3 replications. The treatments were: T_1 (Control), T_2 (AN-R-1), T_3 (AN-R-2), T_4 (AN-R-3), T_5 (AN-R-4) and T_6 (AN-R-5). Measured quantity of 0.5 kg soil and sands mixture was used in each pot. The total numbers of soil and sands mixture filled pots were 18. The experimental soil was collected from BAU Horticulture Farm which belongs to Sonatola series of Old Brahmmaputra Floodplain (AEZ-9) and sands were collected from the adjacent riverside of the river Old Brahmmaputra. Air-dried soil was ground to pass through a 2 mm sieve and the sands were also passed through the 2 mm sieve. Soil and sands were mixed at 1:1 ratio. Soil and sands mixture was sterilized in an autoclave at 121^0 C and 15 PSI for 20 minutes. The pots were also sterilized.

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Plant analysis

The shoot and root samples were ground by a grinding mill to pass through a 20-mesh sieve and stored in paper bags in a desiccator. Total N content in plant samples were determined by Kjeldahl method. Catalyst mixture (K_2SO_4: $CuSO_4.5H_2O$: Se = 10: 1: 0.1) and 30% H_2O_2 and conc. H_2SO_4 were used for digesting plant samples. Estimation of N in the digest was done by distillation with 40% NaOH followed by titration of the distillate trapped in H_3BO_3 with 0.01 N H_2SO_4 (Page *et al.,* 1989).

Statistical analysis

The collected data were analyzed statistically to test the significance and F-test was done to compare the treatment effects. The mean comparisons of the treatments were evaluated by DMRT (Duncun's Multiple Range Test) (Gomez, and Gomez, 1984).

III. Results and Discussion

Isolation and characterization of Akashmoni isolates

Five *Bradyrhizobial* isolates were obtained from the root nodules of Akashmoni on YMA plates. They were designated as AN-R-1, AN-R-2, AN-R-3, AN-R-4 and AN-R-5. Like Akashmoni, 5 the colonies of *Bradyrhizobium* isolates from Akashmoni root nodules appeared on Yeast Mannitol Agar (YMA) media within 4-5 days of inoculation. The colony characteristics of rhizobial isolates of Akashmoni did not differ vary widely (Table 01). The isolates produced circular creamy white colonies on YMA media with entire margin. Diameter of the colonies of Akashmoni isolates varied between 1.2 mm to 1.8 mm. Their surface was smooth and the optical characteristics of the colonies were translucent with viscous consistency (Table 01). Results in Table 02 indicate that all the isolates under present study were motile, rod shaped and Gram negative. The rhizobial cells appeared as transparent bodies in a grey background studied with negrosine. Vincent *et al.* (1980) stated that *Bradyrhizobium* was gram negative, rod shaped and generally motile. Three biochemical tests e.g. congo red dye absorption, starch utilization and pH test were done in the laboratory to study the biochemical characteristics of different Akashmoni isolates. Results in Table 02 indicate that the isolates AN-R-2 and AN-R-3 absorbed congo red dye moderately but AN-R-1, AN-R-4 and AN-R-5 absorbed the dye slightly. In general, the rhizobia absorb the dye weakly whereas many other bacteria take up the dye strongly (Vincent, 1970). Results in Table 2 also indicate that all the isolates failed to utilize the starch and all of them were alkali producer. This result has been supported by Odee *et al.* (2002). It appeared from above observation that the isolates were slow growing *Rhizobia* i.e. *Bradyrhizobia* (Bernet *et al.,* 1985).

Table 01 Morphological characteristics of Akashmoni (*Acacia nilotica*) isolates

Host	Isolate No.	Shape	Gram reaction	Motility	Background staining
Akashmoni (*Acacia nilotica*)	AN-R-1	Rod	-	+	Transparent
	AN-R-2	Rod	-	+	Do
	AN-R-3	Rod	-	+	Do
	AN-R-4	Rod	-	+	Do
	AN-R-5	Rod	-	+	Do

- = Negative + = Poorly motile ++ = Moderately motile

Table 02 Biochemical characteristics of Akashmoni (*Acacia nilotica*) isolates

Host	Isolate No.	Congo red dye absorption	Starch utilization	pH test
Akashmoni (*Acacia nilotica*)	AN-R-1	(+)	-	Alkali producer
	AN-R-2	(++)	-	Do
	AN-R-3	(++)	-	Do
	AN-R-4	(+)	-	Do
	AN-R-5	(+)	-	Do

- = Negative; (+) = Slight ; (++) = Medium; (+++) = High

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Response of Akashmoni to *Bradyrhizobium* isolates

Plant height: Plant height of Akashmoni both at 30 and 60 DAS was influenced significantly by the treatments (Table 03). Plant height ranged from 7.1 cm to 12.0 cm at 30 DAS (Table 03). The highest plant height of 12.0 cm was obtained with the treatment T_3 (AN-R-2) which differed significantly from the rest of the treatments. The treatment effects of T_5 (AN-R-4), T_6 (AN-R-5), T_2 (AN-R-1) and T_4 (AN-R-3) were statistically identical but significantly different from that of the treatment T_1 (control). At 60 DAS, plant height ranged from 12.3 cm to 30.3 cm. The tallest plant was obtained with the treatment T_3 (AN-R-2) which was statistically significant over all other treatments. The treatment T_4 (AN-R-3) was statistically identical to the treatments T_5 (AN-R-4), and T_2 (AN-R-1) which were statistically superior to T_6 (AN-R-5). Treatment T_6 (AN-R-5) was statistically superior to T_1 (control) in terms of plant height.

Leaf Number: The treatments had no significant effects on the leaf number at 30 DAS (Table 03). Numerically, the highest leaf number was 5.7 noted in the treatment AN-R-1 (T_2) and AN-R-4 (T_5) and the lowest 4.3 was observed in uninoculated control (T_1). At 60 DAS, there was significant difference among the treatments (Table 03). All the isolates gave significantly higher number of leaves over control (T_1). The highest number of leaves plant^{-1} was 12.7 recorded in AN-R-2 (T_3) and the lowest 8.3 in control (T_1). The treatments T_3 (AN-R-2), T_4 (AN-R-3), T_2 (AN-R-1) and T_5 (AN-R-4) were statistically similar but superior to the control (T_1). The better leaf number for isolate AN-R-2 (T_3) may be due to the higher rate of N uptake.

Table 03. Effects of *Bradyrhizobium* isolates on plant height (cm plant^{-1}) and leaf number plant^{1} of Akashmoni (*Acacia nilotica*) at 30 and 60 DAS

Treatment	Plant height (cm plant^{-1})		Leaf number plant^{-1}	
	30 DAS	60 DAS	30 DAS	60 DAS
T_1 = Control	7.1c	12.3d	4.3	8.3c
T_2 = AN-R-1	8.6b	23.1b	5.7	10.7ab
T_3= AN-R-2	12.0a	30.3a	5.0	12.7a
T_4 =AN-R-3	8.2bc	25.7b	5.3	11.3ab
T_5 = AN-R-4	9.0b	23.5b	5.7	10.7ab
T_6 = AN-R-5	8.7b	18.4c	4.7	9.7bc
C.V. (%)	8.53	8.12	15.29	10.23
S_X	0.4393	1.049	NS	0.6237

In a column, figures having similar letter do not differ significantly (as per DMRT at 5 % level of significance).

Nodule number plant^{-1}: There was significant effect of inoculation with different *Bradyrhizobium* isolates in producing the number of nodules plant^{-1} (Table 04). The results showed that all the isolates gave significantly higher number of nodules on root plant^{-1} over control (T_1). The number of root nodules plant^{-1} ranged from 8.7 noted in unioculated control (T_1) to 134.7 recorded due to inoculation with AN-R-2 (T_3). The isolate AN-R-2 (T_3) gave significantly higher number of root nodules over all other isolates. Again, inoculation with AN-R-4 (T_5) and AN-R-1 (T_2) recorded statistically identical results but the effect of AN-R-4 (T_5) was superior to that of AN-R-3 (T_4) and AN-R-5 (T_6). Besides, there was no statistical difference between the treatment effects of AN-R-1 (T_2) and AN-R-3 (T_4) as well as AN-R-5 (T_6). Martin *et al.* (1997) stated that grown saplings of *Acacia mangium,* inoculated with *Bradyrhizobium* spp. developed a very high number of small nodules distributed all along the root system, resulting in an increase in nitrogen and chlorophyll content in plant tissues.

Nodule weight: Results show that nodule weight of *Acacia nilotica* was significantly influenced by inoculation with *Bradyrhizobium* isolates (Table 04). The results showed that all the isolates gave significantly higher dry weight of nodules plant^{-1} over control (T_1). The highest dry weight of nodule 76.6 mg plant^{-1} recorded in the isolate AN-R-2 (T_3) and the lowest (8.7 mg plant^{-1}) in control (T_1). The effect of inoculation with AN-R-3 (T_4), AN-R-1 (T_2) and AN-R-4 (T_5) were statistically identical but the isolate AN-R-3 (T_4) was superior to the effect of the isolate AN-R-5 (T_6).

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Table 04. Effects of *Bradyrhizobium* isolates on nodulation of Akashmoni (*Acacia nilotica*) at 60 DAS

Treatment	Root nodule number plant^{-1}	Dry weight of nodule (mg plant^{-1})
T_1 = Control	8.7d	3.4d
T_2 = AN-R-1	111.0bc	47.9bc
T_3= AN-R-2	134.7a	72.6a
T_4 =AN-R-3	106.0c	54.1b
T_5 = AN-R-4	121.0b	44.7bc
T_6 = AN-R-5	102.0c	36.9c
C.V. (%)	6.73	17.57
S_X	3.78	4.456

Dry matter yield

Dry matter yield of root: The effects of inoculation on dry matter yields of root were highly significant (Table 05). Data in Table 05 show that all the isolates recorded significantly higher root dry matter yield over control (T_1). The dry weight of root ranged from 86.4 to 401.1 mg plant^{-1}. The highest dry weight of root was recorded in the isolate AN-R-2 (T_3) followed by the isolates AN-R-1 (T_2), AN-R-3 (T_4), AN-R-5 (T_6) and AN-R-4 (T_5) and the lowest in control (T_1). The better root dry matter yield of the isolate AN-R-2 (T_3) might be due to high rate of nodulation and N_2-fixation.

Dry matter yield of shoot: There was a significant effect of inoculation with different *Bradyrhizobium* isolates on dry matter yield of shoot of the sapling *Acacia nilotica* (Table 05). Results indicate that all inoculant effects were superior over uninoculated control (T_1). The highest dry matter yield of shoot (1127.0 mg plant^{-1}) was recorded for the isolate AN-R-2 (T_3) and the lowest (427.0 mg plant^{-1}) was noted in the uninoculated control (T_1). Amongst the isolates, the lowest dry matter yield was 623.3 mg plant^{-1} for the isolate AN-R-4 (T_5) which was statistically similar to 680.0 mg plant^{-1} noted in the isolate AN-R-5 (T_6) but they both were superior over control (T_1). Again, the isolate AN-R-3 (T_4) and AN-R-1 (T_2) were statistically similar but superior to the isolates AN-R-5 (T_6) as well as AN-R-4 (T_5). The better growth of the shoot due to inoculation with the isolate AN-R-2 was due to high nodulation and N_2-fixation.

Total dry matter yield: The effect of isolates on the total dry matter yield of *Acacia nilotica* was significant. Results in Table 05 show that the effect of all the isolates recorded significantly higher total dry matter yield over uninoculated control (T_1). The isolate AN-R-2 (T_3) gave significantly higher total dry matter yield plant^{-1} over all the treatments. The effect of the isolates AN-R-1 (T_2) and AN-R-3 (T_4) were statistically identical but superior to the effect of isolates AN-R-5 (T_6) and AN-R-4 (T_5) in producing total dry matter yield (mg plant^{-1}). There was no statistical difference between the isolate effects of AN-R-5 (T_6) and AN-R-4 (T_5). The highest total dry matter yield of the isolate (1528.0 mg plant^{-1}) was recorded for the isolate AN-R-2 (T_3) and the lowest (513.3 mg plant^{-1}) for the control (T_1). The percent total dry matter increase over control ranged from 55 noted in the isolate AN-R-4 (T_5) to 198.0 recorded in AN-R-2 (T_3). The percent total dry matter increase over control was 198, 149, 144, 82 and 55 due to inoculation with the isolates AN-R-2 (T_3), AN-R-1 (T_2), AN-R-3 (T_4), AN-R-5 (T_6) and AN-R-4 (T_5), respectively. The highest value of percent total dry matter increase over control (T_1) was almost double which indicated the efficacy of the isolate. The better total dry matter yield of the isolate AN-R-2 (T_3) was due to high nodulation and N_2-fixation. Haque *et al.* (1997) and Parveen *et al.* (1997) also found similar result. Thamizhchelvan *et al.* (1991) isolated and selected effective *Rhizobium* isolates in terms of dry matter accumulation and total nitrogen content from 5 tree legume species. Results reported in Table 04 and 05 indicate that the total dry matter yield (mg plant^{-1}) was positively correlated with the number of nodules on root plant^{-1} (r = 0.7652**).

Nitrogen Content of shoot

Results reported in Table 06 indicate that nitrogen content of shoot of the sapling of *Acacia nilotica* was significantly influenced by the inoculation. The isolate AN-R-5 (T_6) gave significantly higher N content of shoot over all other treatments (Table 05). The highest N content of shoot (3.9 %) was recorded for the isolate AN-R-5 (T_6) and the lowest 1.8 % noted for the uninoculated control (T_1). Balasundaram (1987) also found the significant effect of inoculation. Della-Cruz *et al.* (1988) showed

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that dual inoculation of rhizobia plus arbuscular mycorrhizal fungi (AMF) significantly increased dry weight, nitrogen and phosphorus content of the shoot, increased root nodulation and improved the height and diameter of tree legumes.

Total N uptake by shoot

Analysis of variance showed significant effect of isolates on total N uptake by shoot of the sapling of *Acacia nilotica.* Results in Table 06 indicate that the isolate AN-R-2 (T_3) recorded significantly higher total N uptake by shoot than rest of the treatments. The isolates AN-R-3 (T_4) and AN-R-4 (T_5) were statistically similar to each other but superior to all other treatments in producing N uptake by shoot. The N uptake by shoot observed in AN-R-1 (T_2) was significantly higher over AN-R-4 (T_5) but inferior to AN-R-2 (T_3), AN-R-3 (T_4) and AN-R-5 (T_6). The percent N uptake increase over control ranged from 80.0 in AN-R-4 (T_5) to 396.0 recorded in AN-R-2 (T_3) which was also uptake the highest amount of nitrogen. The percent N uptake was increased by 396, 285, 242, 191and 80 over the control (T_1) due to inoculation of the isolates AN-R-2 (T_3), AN-R-3 (T_4), AN-R-1 (T_2) and AN-R-4 (T_5), respectively. The better percent N uptake increase over control by the isolate AN-R-2 (T_3) was due to high rate of nodulation and N_2-fixation. This indicated the efficacy of the isolate. Ovalle *et al.* (1996) conducted an experiment using three N_2-fixing tree legumes (*Acacia cavan, Prosopis alba* and *P. chilensis*) and reported that each tree fixed on an average 4.91 kg N year^{-1}. Results reported in Table 4 and 6 indicate that the N uptake by shoot (mg plant^{-1}) was positively correlated with the number of nodules on root plant^{-1} (r = 0.7169**).

Table 05. Effects of *Bradyrhizobium* isolates on dry matter yield (mg plant^{-1}) of Akashmoni (*Acacia nilotica*) at 60 DAS

Treatment	Dry matter yield (mg plant^{-1})		Total dry matter yield (mg plant^{-1})	% Dry matter increase over control
	Shoot	Root		
T_1 = Control	427.0d	86.4f	513.3d	--
T_2 = AN-R-1	930.0b	348.9b	1279.0b	149
T_3= AN-R-2	1127.0a	401.1a	1528.0a	198
T_4 =AN-R-3	953.3b	296.5c	1250.0b	144
T_5 = AN-R-4	623.3c	172.9e	796.3c	55
T_6 = AN-R-5	680.0c	251.8d	931.8c	82
C.V. (%)	9.27	6.26	8.41	--
S_X	42.29	9.38	50.97	--

Table 06. Effects of *Bradyrhizobium* isolates on N content of shoot (%) and total N in shoot (mg plant^{-1}) of Akashmoni (*Acacia nilotica*) at 60 DAS

Treatment	N content of shoot (%)	N uptake by shoot (mg plant^{-1})	% N uptake increase over control
T_1 = Control	1.8f	7.8e	--
T_2 = AN-R-1	2.4d	22.7c	191
T_3= AN-R-2	3.4b	38.7a	396
T_4 =AN-R-3	3.2c	30.0b	285
T_5 = AN-R-4	2.2e	14.0d	80
T_6 = AN-R-5	3.9a	26.7b	242
C.V. (%)	3.67	9.56	--
S_X	0.06055	1.286	--

In a column, figures having similar letter do not differ significantly (as per DMRT at 5 % level of significance).

IV. Conclusion

Considering all the growth parameters, nodulation, dry matter yield, N content of shoot and total N uptake by shoot, it may be inferred that the isolate AN-R-2 was the best. At the same time, other isolates were also found promising. Further studies are necessary to establish the promising strains of *Bradyrhizobium* for cultivation of *A. nilotica*.

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