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Potential use of rhizobia as promoters of nodulation, growth and N uptake by lentil

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Abstract

Nodulation, growth, N content and its uptake by lentil grown on silt loam soil were examined during November 2006 to May 2007. Seeds were inoculated with four rhizobial strains viz. strain-638, strain-640, strain-2, mixed strains (Strain 638, 640 and 2) and sown in pots following completely randomized design with four replications. Urea @ 45 kg ha^{-1} and a control treatment were included to compare the effectiveness of the rhizobial strains. The results indicated that rhizobia significantly influenced most of the parameters studied except leaf area, pod number and dry matter weight. The highest value for all the parameters was recorded from plants inoculated with strain-638. A maximum to 77% increased nodulation over control was calculated from plants inoculated with the same strain. Different varieties had significant influence on all the parameters except leaf area. The highest value for most of the parameters was found in Binamasur-1 except plant height, nodule dry weight and stover N content which were obtained from Binamasur-3. Interaction effects of different rhizobial strains and varieties also showed significant variation only in stover N content and its uptake by lentil. Positive correlations were observed between dry matter weight, grain N content, nodule number and plant height. The overall results suggested that rhizobial strains can be used for producing higher yield without any nitrogenous fertilizer in lentil and Binamasur-1 can be cultivated inoculating with strain-638 for the maximum nodulation, N fixation and dry matter production.

Keywords: Rhizobial strains, Lentil, Nodulation and N uptake

Introduction

Atmosphere contains 78.08% N_2, which is practically unavailable to plants. Leguminous crops can assimilate atmospheric N_2 with the help of *Rhizobium*. Successful *Rhizobium*-legume symbiosis can increase the incorporation of biological N fixation into soil ecosystems (Vance, 2001). This association between legume and *Rhizobium* may reduce about 70 million tons of atmospheric N to ammonia per annum which amounts to about 40 % of all biologically fixed N per year (Burns and Hardy, 1975). Legumes play a vital role in developing new strategic approaches to ensure sustainable increase in agricultural productivity without harming the environment (Hardarson *et a1.,*1987). Inoculation with highly effective and exotic strain of *Rhizobium* is a well known practice but its use may not always result in higher yields due to predominance of less effective and indigenous strains of rhizobia in soil. Success of an inoculant strains depends on its effectiveness and ability to compete for nodulation and nature of soil. The use of any legume in crop rotation is one of the principal ways of supplying additional N for the non-leguminous crops. The amount of N_2 fixed by a lentil crop has been reported to be around 103 to 105 kg ha^{-1} yr^{-1} in Egypt and USA and nearly 80 percent requirement of N of the crop was met by symbiotic N_2 fixation and no additional N fertilizer was needed (Khan *et al.,* 2000). Lentil (*Lens culinaris* L.) is one of the most important pulse crops in Bangladesh and its productivity can be increased with the inoculation of rhizobial strains. For augmenting the efficiency of biological N_2 fixation, the native, efficient and competitive strains are required for seed inoculation. Effectiveness of the symbiotic N_2 fixation depends upon the relationship between host, efficient rhizobial strains and ecological factors. The aim of this present work was to evaluate the effects of different rhizobial strains inoculation on nodulation, growth, N content and its uptake by lentil varieties.

Materials and Methods

To examine the response of lentil to rhizobial inoculation, a pot experiment was carried out at Bangladesh Institute of Nuclear Agriculture (BINA) during November 2006 to May 2007. Silt loam soil (0-15 cm) was collected from the farm of Bangladesh Agricultural University, Mymensingh and was air dried, ground and sieved with 2 mm sieve. The soil has the following properties: pH 6.8, organic matter 1.55%, total N 0.12%, available P 33.88 $\mu g\ g^{-1}$, exchangeable K 0.16 cmol kg^{-1}, available S 10.66 $\mu g\ g^{-1}$ and cation exchange capacity 12.50 cmol kg^{-1}. The soil was analyzed following the standard methods of analysis (Page *et al.,* 1982). This was a two-factor experiment (rhizobial strains and variety). Four strains viz. strain-638, strain-640, strain-2, mixed strains (Strain 638, 640 and 2) and three varieties viz. Binamasur-1, Binamasur-2 and Binamasur-3 were used in this experiment. A control and urea @ 45 kg ha^{-1} treatments were also included for comparison. The experiment was laid out following completely randomized design with four replications. Triple superphosphate, muriate of potash and gypsum were applied according to the fertilizer recommendation guide (BARC, 2005). Seeds were surface sterilized to avoid fungal infection by soaking the seeds with 1% sodium hyphochloride for one minute. After sterilization, the seeds were soaked in rhizobial culture for two hours and 8-10 seeds were sown in each pot. Intercultural operations were done to ensure normal growth of the crops as and when necessary. Adequate soil moisture content was maintained by daily addition of distilled water. The grain and stover N content was determined by micro Kjeldahl method as described by Jackson (1973). Analysis of variance was done with the help of computer package MSTAT developed by Russel (1986) and the mean differences of the treatments were adjudged by LSD test.

Results and Discussion

Different rhizobial strains inoculation significantly influenced the number of nodules $plant^{-1}$. The highest number of nodules (76.6) was counted and recorded from the plant inoculated with strain-638 which was significantly different from the number of nodules produced by other strains and urea treated plants including control. Identical number of nodules $plant^{-1}$ was counted in plants when they were inoculated with strain-640, strain-2 and mixed strains (638, 640 and 2). The lowest number of nodules (43.2) was recorded in plants which was neither inoculated by any rhizobial strains nor fertilized by urea. It was also observed that inoculated rhizobial strains increased the nodulation from 26-77% over control. Maximum increase in nodules $plant^{-1}$ (77%) was calculated from the plants inoculated with the strain-638 (Table 1). Different varieties of lentil also showed significant variation with respect to number of nodules $plant^{-1}$ (Table 2). The highest (83.9) and lowest (38.7) number of nodules were recorded from Binamasur-1 and Binamasur-2, respectively. The interaction effect of different rhizobial strains and variety on the number of nodules $plant^{-1}$ was not significant (Table 3). The highest number of nodules (107) was counted from Binamasur-1 that was inoculated with strain-638 and the lowest (27.5) from the treatment combination Binamasur-2 and control, respectively. The nodulation capacity of different rhizobial strains varied from soil to soil and plant to plant. The lower number of nodules in both control and urea treated plants might be due to non-inoculation with rhizobial strains. Moreover, it is also reported that direct application of N fertilizers generally delayed or inhibited nodulation and N_2 fixation. Because of this adverse effect, N fertilization usually was not recommended for leguminous crops (Schulze, 2004). Higher nodulation might be due to the effective symbiosis between legume and rhizobial strains. Thus, the finding of this experiment is in agreement with those of Slattery *et al.* (2004) who reported that *Rhizobium leguminosarum* bv *viciae* was responsible for effective nodulation of faba bean, lentils and field pea. Egamberdiyeva *et al.* (2004) also investigated the effect of inoculation with *Bradyrhizobium japonicum* S-2492 on soybean nodulation. They observed positive effects on nodule number. Hanson and Gan (2003) also observed an average of more than 72% increase in nodule number plant^{-1} over control in lentil by rhizobial inoculation. This large increase in nodule number clearly showed that rhizobial inoculation was essential for increasing nodulation that in turn leads to higher yields.

Table 1. Effects of rhizobial strains on the growth, yield attributes, N content and its uptake by lentil

Rhizobial strains	Nodules plant^{-1} (No.)	Nodule wt. plant^{-1} (mg)	Increased nodules over control (%)	Plant height (cm)	Leaf area plant^{-1} (cm^2)	Pod plant^{-1} (No.)	Dry matter wt. at 50 DAS (g)	N content (%)		N uptake (g pot^{-1})	
								Grain	Stover	Grain	Stover
Control	43.2	19.75	-	31.5	104	112.3	0.69	3.89	1.86	0.90	0.41
Strain-638	76.6	31.00	77	37.4	116	151.3	0.96	4.46	2.29	1.58	0.80
Strain-640	63.7	28.33	47	35.2	106	125.3	0.87	4.22	2.23	1.22	0.61
Strain-2	56.7	23.75	31	33.6	136	117.3	0.80	4.16	2.09	1.00	0.48
Mixed	54.8	22.33	26	35.0	111	121.4	0.81	4.15	1.98	1.03	0.45
Urea	48.6	22.08	12	33.9	111	118.1	0.80	4.04	2.09	1.00	0.50
LSD	12.32	6.01	-	2.3	31	27.10	0.17	0.11	0.06	0.62	0.09

Table 2. Effects of varieties on the growth, yield attributes, N content and its uptake by lentil

Variety	Nodules plant^{-1} (No.)	Nodule dry wt. plant^{-1} (mg)	Plant height (cm)	Leaf area plant^{-1} (cm^2)	Pod plant^{-1} (No.)	Dry matter wt. at 50 DAS (g)	N content (%)		N uptake (g pot^{-1})	
							Grain	Stover	Grain	Stover
Binamasur-1	83.9	25.33	29.7	125	213.5	0.96	4.32	1.82	1.79	0.82
Binamasur-2	38.7	18.16	35.5	107	81.4	0.82	4.24	2.30	0.82	0.44
Binamasur-3	49.1	30.12	38.2	110	77.9	0.69	3.91	2.15	0.75	0.36
LSD	8.71	4.25	1.65	22	19.17	0.12	0.08	0.04	0.44	0.06

Table 3. Interaction effects of different rhizobial strains and varieties on the growth, nodulation, yield attributes, N content and its uptake by lentil

Interaction variety x treatment		Nodule plant^{-1} (No.)	Nodule dry wt. plant^{-1} (mg)	Plant height (cm)	Leaf area plant^{-1} (cm^2)	Pod plant^{-1} (No.)	Dry matter wt. at 50 DAS (g)	N content (%)		N uptake (g pot^{-1})	
								Grain	Stover	Grain	Stover
V_1	T_1	64.0	19.50	27.0	104	195.3	0.81	3.59	1.59	1.40	0.61
V_1	T_2	107.0	33.25	32.3	121	253.8	1.10	4.28	2.13	2.61	1.29
V_1	T_3	96.3	31.00	31.5	109	211.3	1.06	3.94	1.99	1.98	0.99
V_1	T_4	88.3	26.00	28.9	112	212.0	0.94	3.81	1.71	1.53	0.69
V_1	T_5	82.5	22.50	29.8	97	201.0	0.96	3.97	1.72	1.65	0.64
V_1	T_6	65.5	19.75	28.8	100	207.8	0.87	3.85	1.77	1.54	0.71
V_2	T_1	27.5	12.75	31.3	148	70.8	0.63	4.03	2.01	0.67	0.33
V_2	T_2	50.8	22.50	38.3	127	105.3	0.99	4.40	2.50	1.05	0.59
V_2	T_3	43.3	21.25	35.8	94	80.5	0.87	4.25	2.41	0.87	0.44
V_2	T_4	35.5	16.50	35.5	156	77.3	0.82	4.36	2.39	0.82	0.44
V_2	T_5	37.3	18.75	36.3	118	81.0	0.71	4.24	2.17	0.74	0.38
V_2	T_6	37.8	17.25	35.8	115	73.8	0.90	4.14	2.32	0.81	0.48
V_3	T_1	38.0	27.00	36.3	83	71.0	0.62	4.05	1.99	0.62	0.30
V_3	T_2	72.0	37.25	41.8	99	94.8	0.80	4.70	2.23	1.08	0.51
V_3	T_3	51.5	32.75	38.3	115	84.3	0.67	4.47	2.29	0.81	0.41
V_3	T_4	46.3	28.75	36.5	88	62.5	0.63	4.32	2.16	0.66	0.33
V_3	T_5	44.5	25.75	39.0	118	82.3	0.77	4.24	2.04	0.70	0.33
V_3	T_6	42.5	29.25	37.3	114	72.8	0.63	4.14	2.17	0.63	0.33
LSD		11.7	10.41	4.8	54	46.9	0.30	0.20	1.33	1.08	0.16

Legend : V_1= Binamasur-1, V_2= Binamasur-2, V_3= Binamasur-3, T_1= Control, T_2= Strain-638, T_3= Strain -640, T_4= Strain –2, T_5 =Mixed strain (638,640 & 2) and T_6= Urea

The nodule weight plant^{-1} significantly differed due to different rhizobial inoculation. The highest weight (31.0 mg) was recorded from the plant inoculated with strain-638 which was identical with the plant inoculated with strain-640 (Table 1). The lowest weight (19.75 mg) was obtained from the plant that was non inoculated. The response of different varieties with respect to nodule weight was significant. The highest (30.12 mg) and lowest (18.16 mg) weight were recorded from Binamasur-3 and Binamasur-2, respectively (Table 2). Nodule weight was not significantly influenced due to the interaction effects of different rhizobial strains and variety. Maximum nodule weight (33.25 mg) was obtained from Binamasur-1 which was inoculated with strain-638 and minimum (12.75 mg) from the uninoculated Binamasur-2 (Table 3). Higher number of nodule might be responsible for higher nodule weight in plants inoculated with rhizobial strains. A similar finding was also reported by Kantar *et al.* (2003) who found that bacterial inoculations significantly increased nodule dry weight compared to control in chickpea. Hanson and Gan (2003) also observed that inoculation with rhizobia clearly increased in nodule dry mass.

Significant variation in plant height was observed due to different rhizobial strains inoculation in lentil (Table 1). The tallest plant (37.4 cm) was observed when the seeds were inoculated with strain-638 which was identical with the plants inoculated with strain-640 and mixed strains (638, 640 and 2). The shortest plant (31.5 cm) was observed when the seeds were not inoculated with any rhizobial strains. Different varieties significantly influenced the plant height of lentil. The tallest plant (38.2 cm) was observed in Binamasur-3 and the shortest (29.7 cm) from Binamasur-1 (Table 2). No significant variation in plant height was found due to the interaction effects of different rhizobial strains and variety (Table 3). The variation in plant height might be due to the fact that rhizobial inoculation increased N uptake by the plant which in turn was responsible for higher growth and development of the plant. A similar finding was reported by Solaiman (1999) who found that *Bradyrhizobium japonicum* inoculation on lentil significantly increased plant height as compared to control. Plant height was positively correlated with dry matter weight (r=0.97).

Leaf area is one of the most important indexes of photosynthetic efficiency of the plant. Leaf area was not significantly influenced due to different rhizobial strains inoculation. The highest leaf area (136 cm^2) was measured from the plant inoculated with strain-2 and the lowest (104 cm^2) from the non inoculated control (Table 1). Different varieties of lentil showed no significant variation with respect to leaf area. The highest (125 cm^2) and lowest (107 cm^2) leaf area were recorded from Binamasur-1 and Binamasur-2, respectively. The interaction effect of different rhizobial strains and variety on leaf area was not significant (Table 3). The highest leaf area (156 cm^2) was recorded from Binamasur-2 that was inoculated with strain-2 and the lowest (83 cm^2) from the treatment combination Binamasur-3 and control, respectively.

Different rhizobial strains inoculation did not show any significant difference on the number of pod plant^{-1} of lentil. Maximum number of pod (151.3) was counted from the plant inoculated with strain-638 and minimum (112.3) from the control (Table 1). Number of pod was significantly influenced by the different varieties of lentil. The maximum (213.5) and minimum (77.9) number of pod were recorded from Binamasur-1 and Binamasur-3, respectively (Table 2). Different rhizobial strains and variety did not differ significantly on the number of pod. Maximum number of pod (253.8) was counted in Binamasur-1 which was inoculated with rhizobial strain-638 and minimum (62.5) from Binamasur-1 which was inoculated with strain-2 (Table 3). Rhizobial strains increased N uptake by lentil which could be responsible for higher pod production compared to control. A similar finding was also reported by Kantar *et al.* (2003) who found that bacterial inoculations significantly increased pod number compared to control in chickpea.

Total dry matter weight plant^{-1} of lentil at 50 DAS was not significantly differed due to different rhizobial strains inoculation. The highest value (0.96 g) was recorded from the plant inoculated with strain-638 and the lowest (0.69 g) from the control plant (Table 1). The response of different varieties with respect to total dry matter weight was significant. The highest (0.96 g) and lowest (0.69 g) total dry matter weights were produced by Binamasur-1 and Binamasur-3, respectively (Table 2). Total dry matter weight was not significantly influenced due to the interaction effects of different rhizobial strains and variety. Maximum dry matter weight (1.10 g) was obtained from Binamasur-1 which was inoculated with strain-638 and minimum (0.62 g) from the treatment combination Binamasur-3 and control (Table 3). A similar finding was also reported by Icgen (2002) who found that bacterial inoculations significantly increased the total biomass of soybean compared to control. Dry matter weight was positively correlated with nodule plant^{-1}(r=0.65).

The results indicated that the grain N content of lentil was significantly affected due to different rhizobial strains inoculation. The highest grain N content (4.46%) was recorded from the plant inoculated with strain- 638 which differed significantly from N content obtained from the plant inoculated with other rhizobial strains, urea treated plant including control. The lowest grain N content (3.89%) was determined from the plant that was neither inoculated nor fertilized with urea (Table 1). The grain N content was significantly influenced by different varieties of lentil. The highest grain N content (4.32%) was determined from Binamasur-1 which was identical with the grain N content of Binamasur-2. The lowest grain N content (3.91%) was recorded from Binamasur-3 (Table 2). Grain N content of lentil showed no significant variation due to the interaction effects of different rhizobial strains inoculation and varieties. The highest grain N content (4.7%) was obtained from Binamasur-3 which was inoculated with strain-638 and the lowest (3.59%) from the treatment combination Binamasur-1 and control (Table 3). The rhizobial strains increased the atmospheric N_2 fixation. This N is easily available for which N assimilation rate of the plant is increased. This might be one of the reasons of higher N content of the inoculated plants compared to control and urea.

The lower N content of urea treated plants might be due to the fact that fertilized N inhibited the N fixation by the rhizobial strains which could be easily lost from the soil by various means and ways. The findings of this experiment was in agreement with those of Kantar *et al.* (2003) who reported that bacterial inoculation increased total N content (%) significantly compared to control in chickpea. Icgen (2002) also observed a similar finding who reported that standard strains of *Rhizobium* increased 3.5-fold higher total N content of soybean compared to control. Grain N content was positively correlated with dry matter weight (r=0.96) and nodule plant^{-1} (r=0.98). Different rhizobial strains inoculation also significantly affected the stover N content of lentil. The highest stover N content (2.29%) was determined from the plant inoculated with strain-638 and the lowest (1.86 %) from the control (Table 1). The results indicated that stover N content significantly differed among different varieties of lentil. The highest (2.30%) and lowest (1.82%) stover N content were recorded from Binamasur-2 and Binamasur-1, respectively (Table 2). Significant variation was observed in stover N content of lentil due to the interaction effects of different rhizobial strains inoculation and varieties. The highest stover N content (2.50%) was obtained from Binamasur-2 that was inoculated with strain-638 and the lowest (1.59%) from Binamasur-1 that was non inoculated (Table 3).

The result reflected that the grain N uptake by lentil was significantly influenced due to different rhizobial strains inoculation. The highest grain N uptake (1.58 g pot^{-1}) was calculated from the plant inoculated with strain-638 which was statistically identical with the grain N uptake by the plant inoculated with strain-640, strain-2, mixed strains and urea treated plants. The lowest grain N uptake (0.90 g pot^{-1}) was calculated from the non-inoculated plant (Table 1). The response of different varieties showed significant variation in grain N uptake by lentil. The highest grain N uptake (1.79 g pot^{-1}) was recorded from the Binamasur-1 and the lowest (0.75 g pot^{-1}) from Binamasur-3 (Table 2). Interaction effects of different rhizobial strains and varieties with respect to grain N uptake by lentil showed no significant variation. The highest value (2.61 g pot^{-1}) was calculated from Binamasur-1 that was inoculated with strain-638 and the lowest (0.62 g pot^{-1}) from Binamasur-3 and control treatment combination (Table 3). The results revealed that the stover N uptake by lentil was significantly influenced due to different rhizobial strains inoculation as well as varieties. Maximum stover N uptake (0.80 g pot^{-1}) was recorded from the plant inoculated with strain-638 and minimum (0.41 g pot^{-1}) from the non inoculated plant (Table 1). Different rhizobial strains and varieties also interacted significantly on the stover N uptake by lentil (Table 3).

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