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COMPARATIVE PERFORMANCE OF INDIGENOUS AND EXOTIC RHIZOBIAL STRAINS ON THE GROWTH AND YIELD OF LENTIL

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Abstract

Rhizobia, the Gram negative soil bacteria, form root nodules with lentil and influence its growth and yield. We evaluated the ability of the selected rhizobial strains on growth and yield of lentil at high Ganges river floodplain soils of Bangladesh under field conditions. The field experiment included nine treatments- three indigenous rhizobial strains (BLR26, BLR175 and BLR235), two exotic strains (nifTAL638 and nifTAL640), two mixed cultures, one fertilizer-nitrogen treatment and a control. Lentil seeds were inoculated with rhizobial strains as per the treatments and planted. Inoculation of lentil with indigenous strains BLR26 and BLR175 recorded higher grain yields over all other treatments except fertilizer-nitrogen application. Inoculation of indigenous mixed strains also resulted significantly higher lentil grain yield over the exotic strains. Thus, indigenous strains BLR26 and BLR175 can be used for lentil cultivation for improving nodulation, growth and yield of lentil at high Ganges river floodplain soils of Bangladesh.

Keywords: Rhizobia; lentil; legume; nodulation, growth, yield

Introduction

Lentil (*Lens culinaris*) plays an important role in agriculture and daily diet in Bangladesh. In addition to its food value, lentil is important in cropping systems for maintaining soil fertility because of its ability to fix atmospheric nitrogen. Around 5.2% of cultivable lands are subject to legume cultivation in Bangladesh (Rahman *et al*., 2009). Among different legumes lentil is the most popular and has been cultivated since ancient times in Bangladesh. In 2010, lentil was grown on 9,199 hectares of land and the total production was 71,100 tons (FAOSTAT-Agriculture, 2010). Although lentil has been grown in Bangladesh for a long time, farmers have largely been cultivating it without proper fertilizers and management. Therefore, it is necessary to increase the yield of lentil in Bangladesh.

Nitrogen is an essential nutrient for all living organisms and necessary for high crop yield and plant quality in agriculture. Although high amount of nitrogen is present in atmosphere, only prokaryotes can convert atmospheric molecular nitrogen into a form that is available to plants. Among nitrogen fixing prokaryotes rhizobia are able to enter a mutual

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symbiosis with leguminous plants in the form of root nodules that fully or partially satisfy the nitrogen demand of the plant. The legume-rhizobia symbiosis is highly specific mutual interrelationship between the two partners. Successful rhizobia-legume symbiosis can increase the incorporation of biological nitrogen fixation (BNF) into soil ecosystems (Vance, 2001). The symbiotically fixed nitrogen can meet up 30-90% nitrogen requirement of the plant (Vessey, 2004). In addition to nitrogen fixation, rhizobia increase lentil yield and can improve crop quality and soil fertility. In this study, we evaluated the performance of three indigenous strains (BLR26 from *Rhizobium lentis*, BLR175 from *Rhizobium bangladeshense* and BLR235 from *Rhizobium binae*; Rashid *et al*., 2015) and two exotic rhizobial strains (nifTAL638 and nifTAL640) on the growth and yield of lentil in the Gangetic flood plain soils of Bangladesh.

Materials and Methods

Rhizobial strains and their isolation from nodules:

Three indigenous rhizobial strains (BLR26, BLR175 and BLR235) and two exotic strains (nifTAL638 and nifTAL640) were used in this study. The indigenous strains were isolated from lentil root nodules. Collected nodules were washed with clean water, dried on tissue paper and preserved on silica gel, and desiccated until bacterial isolation. A single nodule was crushed in 50µL of sterile water using a homogenizer. Then, a loop-full of suspension was streaked on yeast-extract mannitol agar (YEMA) plates (Vincent, 1970) and incubated at 28°C for 3-7 days. Isolated single colonies were purified by repeated streaking on YEMA and CRYEMA plates (Rashid *et al*., 2012). Single colonies were then preserved either on agar slants at 4°C, or frozen in broth with 50% glycerol at 80°C until further use. Exotic strains were collected from nifTAL, USA.

Inoculum Preparation and application:

Rhizobial strains from glycerol stocks were grown in YEM liquid media. Liquid sterile media were inoculated with a single colony of each strain. After inoculation, media were placed in an incubator shaker at 28°C for 24-36 hours. After sufficient growth (10^7 cell mL^{-1}), 25 mL of broth were injected in 75 gm of sterile peat soil to prepare 100 gm peat based rhizobial inoculum. The required lentil seeds for each plot were first coated with molasses (5% of seed weight) and then peat based inoculums (5% of seed weight) were placed on lentil seeds for proper coating the lentil seeds with rhizobial strains. After proper inoculm application, seeds were sown at experimental field.

Nodulation test:

All strains, used in this study, were tested for nodule formation with lentil (CV: Binamasour-5) at sterile conditions. Seeds from lentil (Binamasour-5) were surface-sterilized using 70% ethanol for 1 min and then 3% NaClO for 3 – 5 min. After surface sterilization, the seeds were washed six times with sterile distilled water for removing excess disinfectant from seed surface. After imbibing (4 h in sterile water), seeds were transferred aseptically to 1% water agar plates and allowed to germinate for 2 days at room temperature in the dark. Seedlings were later transferred to glass tubes (32 mm × 170 mm) containing Fåhraeus (1957) agar medium. Rhizobial cultures were grown (2 mL $plant^{-1}$) in YEM liquid medium

(circa 1 × 10^7 cells mL^{-1}) were used to inoculate 5 days old seedlings (Somasegaran and Hoben, 1994). Plants were alternately irrigated with sterile de-ionized water and Jenson's nitrogen-free seedling solutions. Plants were grown for 3 – 5 weeks in a plant growth chamber set to 25°C with 14 h light / 10 h dark cycles. Three replicates of each bacterial strain were used for nodulation test. Un-inoculated plants were served as negative controls for nodulation test. All strains except nifTAL638 produced nodules after three weeks of inoculation.

Field experiment:

The experiment was carried out at high Ganges river floodplain soils of Bangladesh at BINA sub-station Magura. Experimental farm belongs to clay soil with pH 6.7. The treatments were T_1 (control), T_2 (nifTAL638), T_3 (nifTAL640), T_4 (BLR26), T_5 (BLR175), T_6 (BLR235), T_7 (mixed-1 = nifTAL638 and nifTAL640), T_8 (mixed-2= BLR26, BLR175 and BLR235), T_9 (urea: 33 Kg ha^{-1}). Experimental field was irrigated before sowing to maintain sufficient moistures for seed germination. The land was well prepared before sowing. After land preparation, experimental plots were laid out in randomized complete block design (RCBD) with three replications. As per fertilizer recommendation guide (FRG, 2012), fertilizers like P, K, S, and Zinc were applied at the rate of 16, 20, 12 and 1 Kg ha^{-1}, respectively in all plots as basal dose and nitrogen.

A single irrigation was given after one month of sowing. Weeds were cleaned manually after 20 and 40 days of sowing. Soil samples were collected from experimental field using soil sampling Auger and preserved in a poly bag at 4°C until rhizobial population count. Rhizobial populations were determined following the protocols as described previously (Rashid *et al*., 2014). The field soil contains individual rhizobial population which was 3x $10^4 g^{-1}$.

Data on nodule weight and plant weight were recorded at about 50% flowering stage. At harvest, pod numbers $plant^{-1}$, 100-seed weight was recorded from randomly selected ten plants of each plot. Seed yields were recorded from 1 m^2 of each experimental plot and converted to kg ha^{-1}. Recorded data were analyzed using MSTAT-C software (Gomez and Gomez., 1984) and means were compared with DMRT.

Results and Discussion

Nodule weight at flowering stage: A significant variation was observed in nodules weight of lentil at flowering stage due to different treatments (Table 1). Nodule weight ranged from 13.20 to 19.93 mg $plant^{-1}$. The highest nodule weight (19.93 mg) was found with the inoculation of the strain nifTAL640 which was statistically similar to that of the strain BLR26 and the minimum value was found in the urea treatment. The treatments T_1, T_2 and T_7 recorded statistically identical nodule weight. Inoculation of lentil with the strains nifTAL638, BLR175 and BLR235 noted lower nodule weight compare to control treatment. The strains nifTAL638, BLR175, BLR235, mixed-I and mixed-II exerted similar effect on nodule weight. Exotic mixed strains showed higher nodule weight over the indigenous mixed strains.

Dry matter yield: Dry matter weight of lentil with different treatments showed significant differences (Table 1). All the treatments except T_2 (nifTAL638) recorded higher dry matter yield over control. The strain BLR26 produced the highest dry matter weight (2.96 g plant^{-1}) which was followed by the strain nifTAL640 and the lowest value was noted by the strain nifTAL638. The strain nifTAL 640 performed better compared to BLR175 and BLR235 in producing dry matter yield of lentil. Exotic mixed strain also recorded higher dry matter yield over the indigenous mixed strains. The treatments T_5, T_6, T_7 and T_8 demonstrated similar dry matter yield of lentil. Rashid *et al.*, (2009) reported that rhizobial inoculations to the lentil significantly enhance shoot dry weight than un-inoculated control.

Table 1: Effect of rhizobial strains on growth of lentil.

Treatments	At 50% flowering stage	
	Nodule weight (mg plant^{-1})	Dry matter weight (g plant^{-1})
T_1 (Control, no nitrogen)	17.43 bc	2.06 ef
T_2 (nifTAL638)	16.90 bc	2.00 f
T_3 (nifTAL640)	19.93 a	2.57 b
T_4 (BLR26)	18.83 ab	2.96 a
T_5 (BLR175)	16.16 c	2.37 cd
T_6 (BLR235)	16.43 c	2.27 d
T_7 (Mixed- I: nifTAL638 & nifTAL640)	17.37 bc	2.27 d
T_8 (Mixed-II: BLR26,BLR75 & BLR235)	16.63 c	2.23 de
T_9 (Urea:33 Kg ha^{-1})	13.20 d	2.48 bc
CV (%)	5.86	

In a column, means followed common letter (s) do not differ significantly at 5% level by DMRT. Abbreviations: Rl= *Rhizobium leguminosarum*, R= *Rhizobium*, BLR=Bangladeshi lentil rhizobia, nifTAL=Nitrogen Fixation by Tropical Agricultural Legumes.

Pod plant^{-1}: Significant variation was observed in the number of pods produced by lentil due to different treatments (Table 2). The highest number of pods (110 pods plant^{-1}) was observed in the treatment T_5 inoculated with the strain BLR26 which was followed by the treatment T_9 receiving urea fertilizer (108 pods plant^{-1}) contain the strain BLR175. Inoculation with indigenous mixed strains produced significantly higher number of pods plant^{-1} over the exotic mixed strains. Again, the control plants recorded higher number of pods compared to the treatments T_2 and T_7. The minimum pods plant^{-1} was noted in the treatment T_2 (87.14 pod plant^{-1}) inoculated with the strain nifTAL638.

Seed weight: There was no significant variation in the 100-seed weight of lentil due to different treatments (Table 2). The highest 100-seed weight (2.25 g plant^{-1}) was recorded with the nifTAL640 and the minimum value (2.11 g plant^{-1}) was recorded in the control treatment. A similar result was observed by Mckenzie *et al.*, (2001) who reported that rhizobial inoculation did not significantly enhance seed weight compared to the control.

Grain yield: Grain yield from different treatments showed significant variation (Table 2) and urea treatment produced the highest seed yield (1671 Kg ha^{-1}) which was statistically identical with that of strain BLR26 (1670 kg ha^{-1}). Inoculation of lentil with indigenous mixed strains produced higher grain yields over the exotic mixed strains. Lentil yield with

the indigenous strains BLR26 and BLR175 were higher over all other treatments except fertilizer-N treatment. The minimum yield was observed with the strain nifTAL638. Among rhizobial strains, the strain BLR26 seems to be the best for lentil inoculation. The findings of this experiment is in agreement with Gan and McDonald (2002) who reported that plants inoculated with rhizobial strains increased seed yield by an average of 35% in desi chickpea and 23% in lentil compared to control. Kantar *et al.*, (2003) also observed that bacterial inoculations significantly increased seed yield compared to control treatment.

Legumes have the ability to fix and utilize atmospheric nitrogen with the help of effective rhizobial strains. The can met up full or partial requirement of nitrogen for host plant and improve soil fertility. Effective rhizobial strains were inoculated for nodulation and growth of lentil. The results showed that inoculation of lentil with the strain BLR26 produced the highest plant dry matter yield, pods plant^{-1} and yield significantly.

Table 2: Effect of rhizobial strains on yield and yield contributing characters of lentil.

Treatments	At harvest		
	Pod plant^{-1} (no)	100- seed weight (g)	Seed yield (kg ha^{-1})
T_1 (Control, no nitrogen)	90.77 fg	2.11	1338 c
T_2 (nifTAL638)	87.14 g	2.18	1199 d
T_3 (nifTAL640)	101.50 cd	2.25	1544 ab
T_4 (BLR26)	110.00 a	2.20	1670 a
T_5 (BLR175)	103.80 bc	2.15	1636 ab
T_6 (BLR235)	94.29 ef	2.13	1512 b
T_7 (Mixed-I: nifTAL638 & nifTAL640)	88.64 fg	2.18	1284 cd
T_8 (Mixed-II: BLR26, BLR75 & BLR235)	97.22 de	2.17	1567 ab
T_9 (Urea: 33 Kg/ha)	108.00 ab	2.21	1671 a
CV (%)	3.01	4.44	4.58

In a column, means followed common letter (s) do not differ significantly at 5% level by DMRT. Abbreviations: Rl= *Rhizobium leguminosarum*, R= *Rhizobium*, BLR=Bangladeshi lentil rhizobia, nifTAL=Nitrogen Fixation by Tropical Agricultural Legumes.

This may be due to the effective symbiosis between the rhizobial strain and lentil cultivar Binamasor-5. This might be due to effective nodulation process and availability of more atmospheric nitrogen to the root zone of lentil. Similar results were also observed by Glick (2012) who concluded that bio-fertilizers which contain rhizospheric microorganisms have ability to promote and regulate plant growth directly and/or indirectly. However, enough rhizobial population was present in the field soil and subsequently; sufficient nodules were found in the control treatment. But the growth and yield of lentil in the control was lower compared to other treatments except the treatment T_2 (nifTAL638). Under field condition, rhizobia rhizobia could be mutualistics to parasites, varying dramatically in the N_2-fixing benefits provided to their hosts (Ballard *et al*., 2002; Denton *et al*., 2000; Heath and Tiffin, 2007; Thrall *et al*., 2007). Nonetheless, strains of rhizobia can vary as much as tenfold in net host benefits, even when derived from a single location (Burdon *et al*., 1999). Thus, it is possible that our experimental field soils might contained heterozygous rhizobial population

such as less effective, effective and cheater rhizobia. Initially cheater rhizobia may form false nodules with host plant but later they absorb more carbon from host to synthesis more PHB (polyhydroxy-3-butyrate) to enhance their own fitness and reproduction, which negatively compete with nitrogen fixation (Ratcliff *et al*., 2008). Thus, we did not get positive effect of symbiosis from all indigenous rhizobial strains and host lentil cultivar Binamasor-5. The performance of the exotic strains nifTAL638 was also poor compared to other strains. This might be due to lose their nodulation genes during storage (Ibañez *et al.*, 2009). Generally, rhizobial nodulation and nitrogen fixation genes exist in plasmids which are not essential part of bacterial genome and bacterial existence. To survive in adverse situation, for example during long time preservation at low temperature, bacteria release their non essential genes from genome. Therefore, it could be assumed that this strain might lose its nodulation and nitrogen fixation ability during long time preservation and subsequent sub-culturing during preservation.

The performance of indigenous rhizobial strains were better compared to exotic strains in producing lentil yield. Inoculation of lentil with the rhizobial strains BLR26 and BLR175 recorded higher grain yield of lentil over all other treatments except fertilizer-N application. Thus, the strain BLR26 or/and BLR175 may be used for lentil cultivation in the calcareous soils of Bangladesh. Further evaluations are needed to confirm the performance of this strain at field conditions of different locations.

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