Research Article

Isolation of Nodule Associated Bacteria for Promotion of Lentil Growth

Muhammad Rasheed*, Tayyaba Naseer, Asma Hassan, Fayaz ul Hassan, Rifat Hayat, Ghulam Jilani, Samman Gul Vaseer and Muhammad Bilal Ali

Department of Agronomy, Pir Mehr Ali Sah Arid Agriculture University, Rawalpindi, Pakistan.

Abstract | Rhizobia have natural ability to fix nitrogen in legume plant through symbiotic association of legume-*rhizobium* by forming nodules on the roots of legume plants. To ensure an optimum *rhizobial* population in the rhizosphere is necessary that improves the nodulation, N_2 fixation and growth of legume crops. The experiment was conducted to access the effect of isolated bacteria from nodules on growth and nodulation of the lentil cultivars. Different cultivars were used in this experiment; Markaz 2009, Masoor 2009 and NIA 2005 treatments were used viz BS_0 (control), (Pseudomonas stutzeri), BS_2 (Lysinibacillus pakistanensis) and BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h). Application of BS_3 enhanced the plant height (31.423 cm), leaf area plant^{-1} (15.459 cm^2), leaf area index (1.9323) and leaf area duration (110.84). Among the different lentil cultivars, NIA 2005 gave the better response regarding these traits. The maximum fresh weight plant^{-1} (7.2560 g) and dry weight plant^{-1} (0.6047 g) was recorded at treatment BS_3 and among the different cultivars, NIA 2005 depicted better response than other two cultivars. Crop growth rate showed maximum value (3.3977 gm^{-2}day^{-1}) at treatment BS_3 and among the cultivars, NIA 2005 gave highest crop growth rate than others. Net assimilation rate increased due to increase in dry matter of plant and crop growth rate. Various *rhizobial* strains showed nodule number (23.233), nodule size (0.1022 cm) and nodule weight (20.034 mg) of different lentil cultivars. Among the treatments, BS_3 gave maximum of these traits while the NIA 2005 exhibit postive response on nodulation. *Rhizobacteria* isolated from root nodules improved the nodulation and capacity growth of lentil cultivars by producing plant growth regulators and nutrients uptake.

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***Correspondence** | Muhammad Rasheed, Department of Agronomy, Pir Mehr Ali Sah Arid Agriculture University, Rawalpindi, Pakistan; **Email:** rasheed786@uaar.edu.pk
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Introduction

Lentil (*Lens culinaris* Medik) has the higher protein percentage (24%) than other pulses used as food and feed. In addition to serve as human nutrition, it improves and maintains the soil fertility due to its biological nitrogen fixation (BNF) ability. Lentil seed being high in protein or carbohydrate content justifies as best supplement to cereal diets. Nutritional value of lentil is less in spite of containing more protein contents because lentil seed crop is insufficient in cystine and methionine (Rashid, Gonzalez, Young and Wink, 2014).

Lentil is a worldwide leguminous crop of substantial economic and agricultural significance. It is a crop of rainfed area, mainly sown in developing countries, in soils having low nutrient level or low fertility. The

most important attribute of the lentil is distinctive ability for (N_2) fixation symbiotically and become the source of nitrogen (N_2) in agro ecological system. To fix (N_2) symbiotically with growth promoting rhizobacteria, pulses are peculiar because they are known to promote the status of nitrogen in the soil by reducing the atmospheric nitrogen into ammonia (NH_3) and ultimately increase the soil fertility and crop productivity (Riah, Bena, Heulinb, Lajudieb and Laguerre, 2014).

Integrated nutrient management lowers the biospheric hazardous by using the microbial inoculation and reduces the use of inorganic fertilizers Singh and Singh (2018). It also improves the lentil growth and yield by contribution in improvement of nutritional value and the removal of pathogens. Seed inoculation improves the availability N_2 fixation and applied nutrients especially phosohrous (P) and enhances the uptake of nutrient efficiently. Dihydrogen (H_2) oxidizing rhizobacteria suppressed the growth and number of the pathogenic microorganisms and increases root and shoot biomass from 40-55 % compared to control (Abdellatif et al., 2017). It also has augmented the nodulation and growth of lentil plants. The effect of PGPR on lentil crop growth and nodulation in co-inoculation with *Rhizobacteria* increases the lentil nodulation and production by facilitating 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity and synthesizing indole acetic acid Caamano et al. (2018).

Inoculatiom of plant growth promoting *Rhizobacteria* (PGPR) from the lentil root nodule scrutinizes the plant growth promoting capabilities remarkably increases the root and shoot properties, nodulation, nutrient accumulation and uptake, improves the soil properties by increasing electrical conductivity (EC), total N, organic matter content, available Potassium (K) and P Zafar et al. (2012). PGPR also used as biofertilizer to increase the growth and productivity of the plant. Seed inoculation with different *Rhizobial* culture along with application of herbicide (pendimethalin) improved the plant growth and reduced the weed competition significantly (Singh et al., 2018) while plant growth and yield of the lentil crop increased drastically by inoculating with *rhizobium* which could be due the production of plant hormones and suppression of pathogenic organisms due to antibiotics effects of these produced hormones (Chandrakar, Nagre, Ransing and Singh, 2016). Isolation technique standardized for examine and stimulate the *Rhizobium* involvement to BNF capacity and also lowers the use of synthetic fertilizers. Bacterial strains appear in different colour, texture and morphology on agar plates which further can be used for their physiological, biochemical and morphological traits along with their capabilities for contributing in lentil plant healthier growth in harsh conditions (Varun et al., 2017).

Crop yield enhances by bacteria (endophytes) which are used as biofertilizers. Endophytic bacteria (Rhizobial and non Rhizobial) have capability to promote plant growth activities by nutrients such as potassium and P solubilisation, ACC deaminase, indole acetic acid (IAA), production of siderophores and plays a vital role in N_2 fixation Batra et al. (2018). The compatibility and antagonistic effect between *Rhizobium* and Trichoderma species as some strains of Trichoderma contributes in *Rhizobium* growth and some of them prevent it. Seed inoculation of different Trichoderma isolates and *Rhizobium* showed significant results on lentil crop individually and in combination as well. It improves the root nodulation that ultimately enhances the total biomass and growth of lentil crop with augmenting the organic agriculture production (Das et al., 2017).

The minimum average yield of the lentil crop might be due to an insufficiency of adapted high yielding crops (genetic growth potential and environmental adaptability), absence of agronomic management, without application of fertilizer plantation on marginal lands. Deficiency of native *rhizobial* symbionts of the lentil cultivars in the soil that is due to incessant cereal mono cropping adopted in dry lands. Therefore, a study had been hypothesised to access the effect of nodule associated bacteria on growth and nodulation of lentil. Objectives of the study was to isolate the bacteria from lentil nodules and to access their impact on nodulation and growth of the lentil cultivars.

Materials and Methods

Two experiments were conducted firstly in the laboratory to isolate the nodule bacteria secondly the effect of Rhizobium was on lentil in the controlled conditions. Extensive survey of lentil crop was undertaken for taking lentil crop root nodules from Chakwal and Attock areas of Pothwar through the coordination of Barani Agriculture Research Institute

(BARI) and GRS research scientists. The spade was used to excavate the lentil root nodules. Nodules were brought to laboratory in ice containing container and stored in freezer at 40 ^{0}C. Isolation of the nodules of lentil from roots and then unbroken and healthy nodules were collected for further isolation purpose. For separation from roots, the side of nodule of root was cut about 0.5 cm. Then dip the healthy nodules for 6-12 seconds in 95% ethanol for surface sterilization, then 4-5 times washing with sterilized water to get more sterilized nodules, and then about 4-5 minutes dipping in 3% hydrogen peroxide (H_2O_2). Yeast Extract Mannitol Agar (YMA) media was prepared comprising of yeast extract (3g), mannitol (10g), KH_2PO_4 (0.25), K_2HPO_4 (0.25), NaCl (0.1g), $MgSO_4.7H_2O$ (0.1g) and Agar (5g) and then autoclave it.

The nodules were crushed and a little quantity of suspension of nodules streaked on YMA media containing petri plates with the help of sterilized streaking loop. These plates were covered with parafilm and placed for 48-54 hours in incubator at about 28°C. After 2-3 days the grown colonies were picked individually and streak it at a separate YMA media for obtaining purified culture (Carter and Gregorich, 2007). The isolated strains were identified using modern polymerase chain reaction (PCR) techniques and DNA sequence analysis. For this purpose, nearly complete 16S rRNA gene sequences of the strains were obtained after PCR amplification of the genes by using universal forward 9F (5-GAGTTTGATCCTGGCTCAG-3) and reverse primers: 1510R (5-GGCTACCTTGTTACGA-3). Amplified PCR products of 16S rRNA genes were sanitized using PCR purification kit (QIAGEN) according to the standard protocol suggested by the creator (Khalid et al., 2015). The purified PCR products were sequenced by using reverse and universal forward primers. A greenhouse experiment was conducted under complete randomized design with factorial arrangements, with 3 replicates. Three different cultivars were used in this experiment; Markaz 2009, Masoor 2009 and NIA 2005. Four different treatments were used viz BS_0 (control), BS_1 (Pseudomonas stutzeri), BS_2 (Lysinibacillus pakistanensis) and BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) Soil was analysed before time of sowing for the measurement of pH, and Electrical conductivity (EC) was 8.05 and 0.17 dSm^{-1} respectively. Lentil crop parameters data was recorded during study including height of the plant, Leaf area $plant^{-1}$ (LAP), Fresh weight $plant^{-1}$, Dry matter $plant^{-1}$, leaf area index, leaf area duration, crop growth rate (g m^{-2} day^{-1}), net assimilation rate (g m^{-2} day^{-1}) and nodule parameters. Data collected about growth and nodulation components was statistically analyzed by using software Statistics 8.1 and least significance difference test at 5% of probability (P) level was used to evaluate the comparison among means of all the parameters.

Statistical analyses

Data collected about growth and nodulation components was statistically analysed by using software Statistics 8.1 and least Significance difference test at 5% of probability level.

Results and Discussion

Isolation of bacteria from lentil nodules

Three bacterial strains were isolated from lentil nodules and these were sampled from Bari District Chakwal. Designated as BS_1, BS_2 and BS_3 and preserve in the cold cabinet at 4^{0}C.

Identification of bacterial isolates through 16srrna gene sequencing

Identification, similarity percentage and their accession number of 16SrRNA gene sequencing of isolated lentil nodule strains given in the Table 1. All three bacterial strains isolated from lentil nodules were identified using 16SrRNA gene sequencing with universal primer. Sequencing were carried out commercially from Macrogen Korea. The strains belong to Pseudomonas and Lysinibacillus genus. L29 and L31 were identified as Pseudomonas stutzeri whereas L30 were identified as Lysinibacillus pakistanensis. These two genus are proven plant growth promoting *Rhizobacteria* that facilitate crop growth through various direct and indirect mechanisms.

Phylogenetic trees of isolated bacterial strains

Phylogenetic trees of isolated bacterial strains were constructed by using MEGA 4 computer programme. Relationship of lentil bacterial strains with their nearly related species described (Figure 1 and 2).

Vegetative growth

The highest plant height (31.423cm) was observed in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) which was followed by the treatment BS_1

Table 1: *Identification of lentil nodules bacteria through 16SrRNA gene sequencing.*

Strain ID	Source of Isolation	Species	Similarity %	Taxonomy	Completeness %	DDBJ accession of 16SrRNA gene sequencing
L-29	Lentil nodules	Pseudomonas stutzeri	99.37	Bacteria;Proteobacteria;Gammaproteobacteria;Pseudomonadales;Pseudomonasaceae;Pseudomonas	97.6	ATCC 17588(T)
L-30	Lentil nodules	Lysinibacillus pakistanensis	98.53	Bacteria; Firmicutes; Bacilli; Bacillales; Planococcaceae; Lysinibacillus	97.2	JCM 18776(T)
L-31	Lentil nodules	Pseudomonas stutzeri	99.37	Bacteria; Proteobacteria; Gammaproteobacteria; Pseudomonadales; Pseudomonasaceae; Pseudomonas	97.6	ATCC 17588(T)

Table 2: *Effect of various rhizobial strains on plant height (cm) of three lentil cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0 (control)	23.673 N.S	26.580 N.S	29.280 N.S	26.511 D
BS_1 (Pseudomonas stutzeri)	27.857 N.S	29.550 N.S	32.267 N.S	29.891 B
BS_2 (Lysinibacillus pakistanensis)	26.193 N.S	27.370 N.S	30.040 N.S	27.868 C
BS_3 (Pseudomonas stutzeri) + P	29.353 N.S	30.977 N.S	33.940 N.S	31.423 A
Mean	26.769 C	28.619 B	31.382 A	

Probability level = ≤0.05 LSD $_{(0.05)}$ = 1.6587.

Table 3: *Effect of various strains on leaf area plant^{-1} of three lentil cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0 (Control)	11.492 N.S	12.520 N.S	13.894 N.S	12.635 D
BS_1 (Pseudomonas stutzeri)	12.877 N.S	14.318 N.S	16.396 N.S	14.530 B
BS_2 (Lysinibacillus pakistanensis)	12.058 N.S	13.309 N.S	15.237 N.S	13.534 C
BS_3 (Pseudomonas stutzeri)+ P	13.563 N.S	14.935 N.S	17.879 N.S	15.459 A
Mean	12.497 C	13.770 B	15.851 A	

Probability level = ≤0.05; LSD $_{(0.05)}$ = 0.9561.

(Pseudomonas stutzeri) was (29.891cm). However, the least significant plant height (26.511cm) was recorded in treatment BS_0 (control). There was a significant difference among the different cultivars and each cultivar had statistically significant difference with the other. Data from Table 2 showed the maximum plant height (31.382cm) in cultivar V_3 (NIA 2005) which was followed by the cultivar Masoor 2009 with (28.619cm). The least significant plant height (26.769cm) was observed in Cultivar Markaz 2009. Overall there was no significant effect was observed between the interaction of lentil cultivars and the *Rhizobial* strains (Table 2). Minimum result found in control treatment due to no *Rhizobial* strain was applied while maximum plant height was due to increased soil fertility and nutrient uptake that ultimately enhanced plant growth. These results are in line with the findings of (Singh et al., 2018; Huang and Erickson, 2007). Plant growth promoting rhizobacteria might had improved plant height and productivity by producing phytohormones. Plant height increased due to inoculation of rhizobial strains in lentil cultivars. Similar results have been reported by (Gull, Hafeez, Saleem and Malik, 2004).

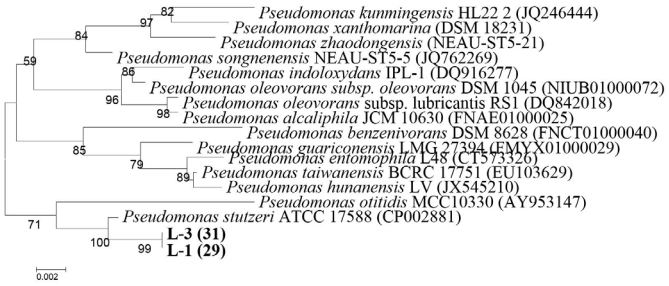

Figure 1: *Phylogenetic tree depicting relationship of strains L-1 (29) and L-3 (31) with their closely related species.*

Among different treatments, highly significant effect on leaf area plant^{-1} was recorded in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) with (15.459cm^2) and it was followed by treatment BS_1 (Pseudomonas stutzeri) with (14.530cm^2) while the

minimum leaf area plant^{-1} was (12.635cm^2) obtained in treatment BS_0 (control). There was also a significant difference among different lentil cultivars and each cultivar had statistically significant difference with the other. Data from the Table 3 showed that the maximum leaf area plant^{-1} (15.851cm^2) was observed in V_3 (NIA 2005) which was followed by Masoor 2009 with leaf area plant^{-1} (13.770cm^2) while the minimum leaf area plant^{-1} (12.497cm^2) was recorded by Markaz 2009. It revealed that interactive effect of lentil cultivars and bacterial strains was statistically non-significant (Table 3). The interactive effect of lentil cultivars and bacterial strains was statistically non-significant. It could be due to the reason that no bacterial strain was applied in BS_0 (control) which resulted in the minimum leaf area plant^{-1}. These results are in line with the findings of (Saghafi, Ghorbanpour and Lajayer, 2018). The variation in leaf area plant^{-1} among these different lentil cultivars were obtained due to their environmental adaptability and genetic growth potential.

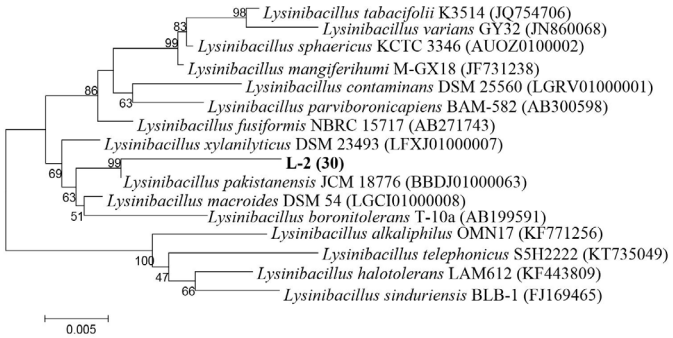

Figure 2: *Phylogenetic tree depicting relationship of strain L-2 (30) with their closely related species.*

Leaf area index

The highest leaf area index (1.9323) was recorded in treatment BS_3 (Pseudomonas stutzeri+ P_2O_5@40kg/h) which was followed by treatment BS_1 (Pseudomonas stutzeri) with leaf area index (1.8163). The least leaf area index (1.5794) was observed in BS_0 (control). There was also a statistically significant effect shown among various cultivars, data from the Table 4 presented that the highest leaf area index (1.9814) was observed in V_3 (NIA 2005) it was followed by Masoor 2009 with leaf area index (1.7213) and the least significant effect produced by Markaz 2009 with (1.5622) lead area index. The results showed there was a non-significant difference between the lentil cultivars and different bacterial strains. However, there was a significant effect shown by the treatments on the mean value of leaf area index (Table 4). The variation in leaf area index among the lentil cultivars might be attributed to their response to environment and their genetic potential. Similar results were also found by Rasheed et al. (2010) who reported that leaf area index significantly due to genetic variability among lentil cultivars. Among different treatments, the highest leaf area duration (110.84) was recorded in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) followed by treatment BS_1 (Pseudomonas stutzeri) with the leaf area duration (104.28) while the least leaf area duration (91.79) was encountered by treatment BS_0 (control). Results from the Table 5 also shown the significant difference among the various lentil cultivars. The highest leaf area duration (113.11) was recorded in NIA 2005 which is followed by Masoor 2009 with leaf area duration (99.35) and the least significant leaf area duration (90.55) was recorded by Markaz 2009. Data clearly showed that the interactive effect of different lentil cultivars and the *Rhizobial* strains was statistically non-significant but there was a statistically significant difference among the means of lentil cultivars and *Rhizobial* strains (Table 5). The least significant effect on leaf area duration was due to no *Rhizobial* strains applied.

Fresh weight

Lentil cultivar which was V_3 (NIA 2005) produced maximum fresh weight plant^{-1} (7.2560g) at treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) while the minimum fresh weight plant^{-1} (5.5080g) was produced at treatment BS_0. Other two cultivars V_1 (Markaz 2009) and V_2 (Masoor 2009) also showed the similar trend for the same level of treatments. There was a significant difference among the treatments on mean value of fresh weight plant^{-1}. Among these treatments, the highest fresh weight plant^{-1} (5.9800g) was recorded in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h), it was followed by treatment BS_1 (Pseudomonas stutzeri) with fresh weight plant^{-1} (5.4320g). The highest fresh weight plant^{-1} (6.4170g) was noted in cultivar V_3 (NIA 2009) which was followed by V_2 with fresh weight plant^{-1} (5.0850g) and the minimum fresh weight plant^{-1} (4.2050g) was encountered in V_1 (Markaz 2009). This data showed that the interactive effect between lentil cultivars and the various *Rhizobial* strains was highly statistically significant (Table 6). The least fresh weight plant^{-1} was produced in treatment BS_0 (control). As there was no application of *Rhizobial* strain in the control treatment so it resulted in minimum fresh weight plant^{-1}. The genetic growth potential in cultivar V_3 (NIA 2005) made it to produce more fresh weight plant^{-1}.

Table 4: *Effect of various strains on leaf area index of three lentil cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0 (Cotrol)	1.4365 N.S	1.5650 N.S	1.7367 N.S	1.5794 D
BS_1 (Pseudomonas stutzeri)	1.6096 N.S	1.7898 N.S	2.0495 N.S	1.8163 B
BS_2 (Lysinibacillus pakistanensis)	1.5072 N.S	1.6636 N.S	1.9046 N.S	1.6918 C
BS_3 (Pseudomonas stutzeri) + P	1.6953 N.S	1.8668 N.S	2.2348 N.S	1.9323 A
Mean	1.5622 C	1.7213 B	1.9814 A	

Probability level = ≤0.05; $LSD_{(0.05)}$ = 0.1195.

Table 5: *Effect of various strains on leaf area duration of three lentil cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0 (Control)	82.93 N.S	91.12 N.S	101.32 N.S	91.79 D
BS_1 (Pseudomonas stutzeri)	93.52 N.S	103.11 N.S	116.22 N.S	104.28 B
BS_2 (Lysinibacillus pakistanensis)	87.45 N.S	95.88 N.S	107.98 N.S	97.10 C
BS_3 (Pseudomonas stutzeri) + P	98.29 N.S	107.28 N.S	126.95 N.S	110.84 A
Mean	90.55 C	99.35 B	113.11 A	

Probability level = ≤0.05; $LSD_{(0.05)}$ = 7.0074.

Table 6: *Effect of various strains on fresh weight plant^{-1} of three lentil cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0 (Control)	3.6360 g	4.6400 ef	5.5080 d	4.5947 D
BS_1 (Pseudomonas stutzeri)	4.5240 f	4.9560 e	6.8160 b	5.4320 B
BS_2 (Lysinibacillus pakistanensis)	3.9200 g	4.8000 ef	6.0880 c	4.9360 C
BS_3 (Pseudomonas stutzeri) + P	4.7400 ef	5.9440 c	7.2560 a	5.9800 A
Mean	4.2050 C	5.0850 B	6.4170 A	

Probability level = ≤0.05; $LSD_{(0.05)}$= 0.3292.

Table 7: *Effect of various strains on dry matter plant^{-1} of three lentil cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0 (Control)	0.3030 g	0.3867 ef	0.4590 d	0.3829 D
BS_1 (Pseudomonas stutzeri)	0.3770 f	0.4130 e	0.5680 b	0.4527 B
BS_2 (Lysinibacillus pakistanensis)	0.3267 g	0.4000 ef	0.5073 c	0.4113 C
BS_3 (Pseudomonas stutzeri) + P	0.3950 ef	0.4953 c	0.6047 a	0.4983 A
Mean	0.3504 C	0.4238 B	0.5347 A	

Probability level = ≤0.05; $LSD_{(0.05)}$= 0.0274 .

Dry matter

The lentil cultivar (NIA 2005) produced maximum dry matter plant^{-1} (0.6047g) at treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) it was followed by treatment BS_1 (Pseudomonas stutzeri) with (0.5680g) and minimum dry matter plant^{-1} (0.4590g) was obtained at treatment BS_0 (control). The cultivar V_2 (Masoor 2009) attained higher dry matter plant^{-1} (0.4953g) at treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) followed by treatment BS_1 (Pseudomonas stutzeri) with (0.4130g) and the least dry matter plant^{-1} was recorded in treatment BS_0 (Control) with (0.3867g). The cultivar V_1 produced higher dry matter plant^{-1} 0.3950 g at treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) followed by treatment BS_1 (Pseudomonas stutzeri) with (0.3770g) while the least dry matter plant^{-1} was encountered in treatment BS_0 (control). There was a significant difference among the treatments on mean value of dry matter plant^{-1}. Among these treatments, the highest dry matter plant^{-1} (0.4983g) was recorded in treatment BS_3 (Pseudomonas stutzeri+ P_2O_5@40kg/h) followed by treatment BS_1 (Pseudomonas stutzeri) with (0.4527g) and the least significant dry matter

plant^{-1} (0.3829g) was obtained in treatment BS_0 (control). Data in Table 7 showed the significant difference among the cultivars on mean value of dry matter plant^{-1}. The highest dry matter plant^{-1} 0.5347 g produced in cultivar NIA 2005 followed by Masoor 2009 with dry matter plant^{-1} 0.4238 g. The least dry matter plant^{-1} 0.3504 g was observed in Markaz 2009. The interactive effect between lentil cultivars and the various *Rhizobial* strains was significant (Table 7). As there was no application of *Rhizobial* strain in the control treatment, that's why it resulted in minimum dry matter plant^{-1}. Saghafi et al. (2018) reported the same results that rhizobacteria increased the dry matter compared to control.

Crop growth and net assimilation rate

The highest crop growth rate (3.3977 gm^{-2}day^{-1}) was observed in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) which was followed by the treatment BS_1 (Pseudomonas stutzeri) with (3.0901 gm^{-2}day^{-1}) while the minimum crop growth rate (2.4228 gm^{-2}day^{-1}) was attained in treatment BS_0 (control). The highest crop growth rate (3.6597 gm^{-2}day^{-1}) was obtained by the cultivar NIA 2005 followed by the Masoor 2009 with (3.3807 gm^{-2}day^{-1}) and the least significant (1.7476 gm^{-2}day^{-1}) effect was produced in Markaz 2009 (Table 8). It mainly depends on the value of net assimilation rate and leaf area index of the plant. As there was no application of *Rhizobial* strain in the control treatment so it resulted in minimum crop growth rate. Higher crop growth rate might be due to more production of nodules and good utilization of phosphorus as *Rhizobial* strain improves the availability of phosphorus and nitrogen to the plants and ultimately increase the crop growth rate. The crop growth rate increased by the availability of certain nutrients in legumes (Khan and Azam, 2002).

Among these treatments, the highest net assimilation rate (2.0560gm^{-2}day^{-1}) was produced by the treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) followed by the treatment BS_1 (Pseudomonas stutzeri) with net assimilation rate (1.9674gm^{-2}day^{-1}) and least net assimilation rate was (1.7583gm^{-2}day^{-1}) at treatment BS_0 (control). Among these cultivars, the highest net assimilation rate (2.3085gm^{-2}day^{-1}) was encountered by the NIA 2005 followed by the Masoor 2009 with (2.1217gm^{-2}day^{-1}). The least net assimilation rate (1.3355 gm^{-2}day^{-1}) was produced by the cultivar Markaz 2009. There was statistically significant difference among the treatment and cultivar means of net assimilation rate (Table 9). It mainly depends on the dry matter and leaf area duration of the plant. The minimum net assimilation rate might be due to no application of *Rhizobial* strain in control treatment. There is a direct relationship between the net assimilation rate and the dry matter of the plant, if dry matter of the plant is more then there would be more net assimilation rate. Similar results were also reported by Khan and Azam (2002).

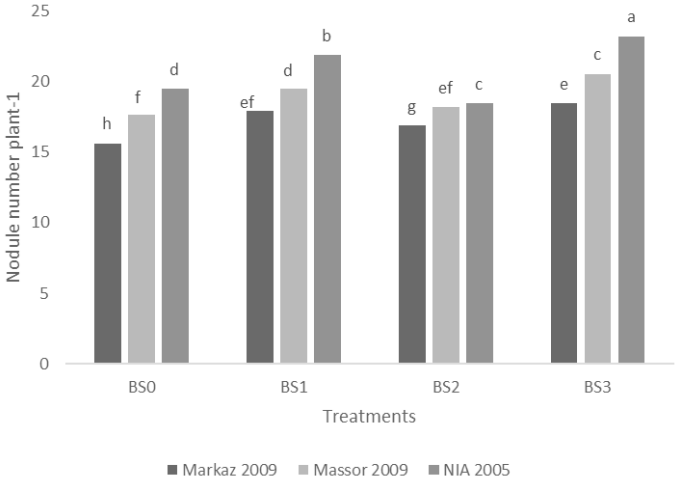

Figure 3: *Effect of various strains on nodule number plant^{-1} of three lentil cultivars.*

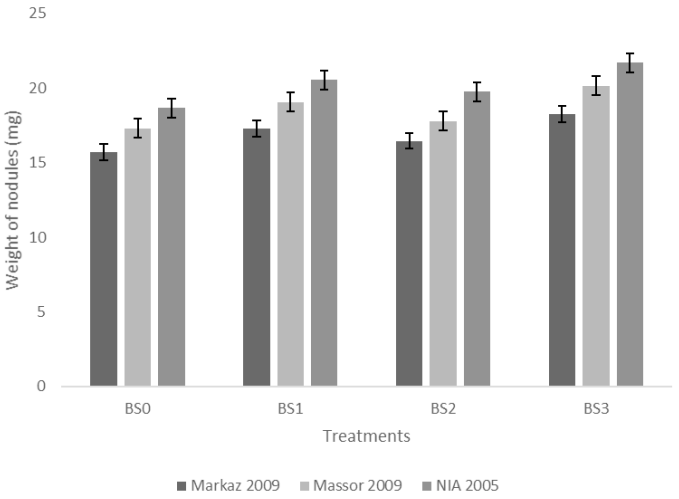

Figure 4: *Effect of various strains on weight of nodules of three lentil cultivars.*

Nodule classification

Number, weight, size and grading of nodules: The highest number of nodules plant^{-1} 23.233 was produced by the cultivar V_3 (NIA 2005) at treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) which was followed by the treatment BS_1 (Pseudomonas stutzeri) with 21.900 number of nodules while the least number of nodules plant^{-1} 19.533 was recorded at treatment BS_0 (control). Rest of the cultivars showed the exactly same trend with the same level of treatments.

Table 8: *Effect of various strains on crop growth rate (gm^{-2} day^{-1}) of three lentil cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0 (Control)	1.4961 N.S	2.9110 N.S	2.8612 N.S	2.4228 C
BS_1 (Pseudomonas stutzeri)	1.8114 N.S	3.4911 N.S	3.9677 N.S	3.0901 B
BS_2 (Lysinibacillus pakistanensis)	1.6209 N.S	3.2852 N.S	3.5147 N.S	2.8070 B
BS_3 (Pseudomonas stutzeri) + P	2.0621 N.S	3.8355 N.S	4.2953 N.S	3.3977 A
Mean	1.7476 C	3.3807 B	3.6597 A	

Probability level = ≤0.05; $LSD_{(0.05)}$ = 0.5306.

Table 9: *Effect of Various strains on Net Assimilation Rate (gm^{-2} day^{-1}) of Three Lentil Cultivars.*

Treatments	V1 (Markaz 2009)	V2 (Masoor 2009)	V3 (NIA 2005)	Mean
BS_0(Control)	1.2304 N.S	1.8578 N.S	2.1866 N.S	1.7583 B
BS_1 (Pseudomonas stutzeri)	1.3433 N.S	2.2477 N.S	2.3113 N.S	1.9674 A
BS_2 (Lysinibacillus pakistanensis)	1.2812 N.S	2,1342 N.S	2.3025 N.S	1.9059 AB
BS_3 (Pseudomonas stutzeri) + P	1.4869 N.S	2.2472 N.S	2.4339 N.S	2.0560 A
Mean	1.3355 C	2.1217 B	2.3085 A	

Probability level = ≤0.05; $LSD_{(0.05)}$ = 0.3215.

There was highly significant difference among the different treatment levels. Among these treatments, the highest number of nodules $plant^{-1}$ 20.767 was observed in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) which was followed by the treatment BS_1 (Pseudomonas stutzeri) with 19.789 while the least significant number of nodules was observed in treatment BS_0 (control). There was also a significant difference noted among different cultivars. The cultivar NIA 2005 produced highest number of nodules $plant^{-1}$ 20.783 it was followed by the Masoor 2009 with 19.00 nodules while minimum number of nodules $plant^{-1}$ was encountered in Markaz 2009.

The interactive effect between the cultivars and the *Rhizobial* strain was highly significant statistically (Figure 3). Tagore et al. (2013) found the promotive effect of strains on number on nodules $plant^{-1}$. These results are also in line with the findings of Saini and khanna (2012) increased number of nodules attributed to the ability of *Rhizobial* strains. Among these treatments, maximum nodule weight $plant^{-1}$ 20.034 mg was observed in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) which was followed by the treatment BS_1 (Pseudomonas stutzeri) with nodule weight 18.971 mg and the least significant nodule weight $plant^{-1}$ 17.232 was noted in treatment BS_0 (control). Maximum nodule weight that was 20.170 mg found in cultivar NIA 2005 it was followed by the cultivar V_2 (Masoor 2009) with 18.577 mg while the least significant nodule weight 16.935mg was recorded in cultivar V_1 (Markaz 2009). The interaction between *Rhizobial* strains and different cultivars was statistically non-significant. However, there was a significant difference among the different treatments and cultivars regarding nodule weight (Figure 4). Increased in nodule weight by *Rhizobial* inoculation. Among these treatments, highest nodule size 0.1022 cm was found in treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) which was followed by the treatment BS_1 (Pseudomonas stutzeri) with 0.0844 cm (Singh et al. 2010). The least significant nodule size 0.0567 cm was observed in treatment BS_0 (control). Among these cultivars, maximum nodule size 0.1042 cm was recorded in cultivar NIA 2005 which was followed by the cultivar V_2 (Masoor 2009) with 0.0758 cm while the least significant nodule size 0.0533 cm was noted in cultivar V_1 (Markaz 2009). Data showed the interactive effect between different cultivars and the *Rhizobial* strains was non-significant. However, there was statistically significant difference among the treatment and cultivar means (Figure 5). The least significant nodule size was observed in BS_0 (control) this could be due to the reason that no *Rhizobial* strain was applied in control treatment. It was mentioned before that nodule grading was determined on the basis of nodule numbers by using the rating criterion. Among the treatments, maximum number of nodules was produced at treatment BS_3 (Pseudomonas stutzeri + P_2O_5@40kg/h) that was represented with grade 3 while among the cultivars, maximum number of nodules was produced by the cultivar V_3 (NIA 2005) represented with grade 3. The interactive effect between treatment means and the cultivars means

was highly significant. There was also a significant difference among means of treatment (Figure 6). According to Rupela (1999), grade 3 presented much better nodulation with 20-40 numbers of nodules.

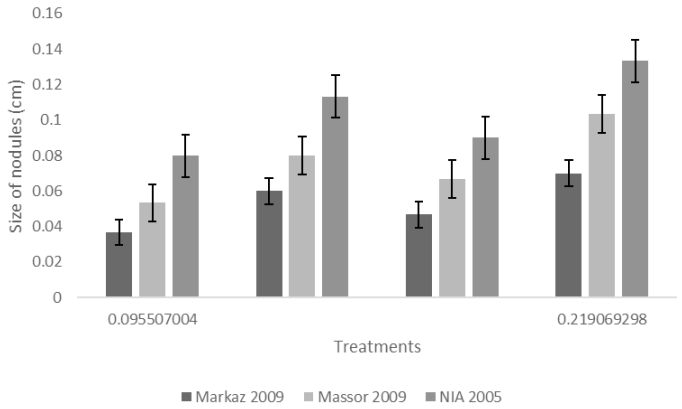

Figure 5: *Effect of various strains on size of nodules of three lentil cultivars.*

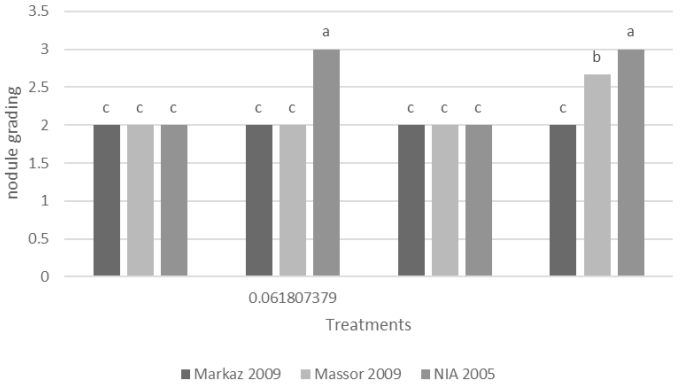

Figure 6: *Effect of various strains on nodule grading of three lentil cultivars.*

Conclusions and Recommendations

Rhizobacteria isolated from root nodules significantly improved the nodulation and production of lentil cultivars by producing plant growth regulators such as cytokinins, indole acetic acid and gibberellins and increasing nutrients (especially nitrogen and phosphorus) uptake. Inoculation of PGRP and phosphorus application remarkably increased the root and shoot properties, nodulation, nutrient accumulation and uptake.

Author's Contribution

Muhmmad Rasheed conceived research idea and overall management, Tayyaba Naseer conducted experiment, Asma Hassan prepared draft, Fayaz ul Hassan suggested to improve work and Rifat Hayat helped in data analyses, Ghulam Jilani provided technical support and Muhmmad Bilal Ali wrote review of literature.

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