Research Article

Efficacy of different Rhizobium Strains on Nodulation and Seed Yield in Mungbean (*Vegna radiata* L.) Cultivar "Inqalab Mung"

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Abstract | Studies on bio-fertilizers that support the biological N fixation in legume crops is economical and environmentally sound activity in crops production. Response of mungbean cultivar (Inqalab Mung) inoculated with four strains of rhizobium (TAL-634, TAL-169, NARC-BK and NARC-M) was evaluated during two consecutive years 2015 and 2016 at the Agricultural Research Institute, Rata Kulachi, Dera Ismail Khan (31° 49/ N latitude and 70° 55/ East longitude), Khyber Pakhtunkhwa, Pakistan. The current experiments were carried out in a randomized complete block design with split plot arrangements replicated four times. The effects of different Rhizobium strains were found significant on yield parameters of the crop. Inoculation with rhizobium strain NARC-BK strain showed the maximum and significantly (p<0.05) higher number of branches, pod clusters plant^{-1}, pods plant^{-1} and pods cluster^{-1}, pod length, 1000-seed weight, number of seed pod^{-1} (by 37, 18, 53, 31, 14, 7 and 5%, respectively). Rhizobium strains TAL-169 exceeded NARC-BK with 6% increase only in root length. Only NARC-BK could produce the 5.85 nodules after 15 days after sowing whilst strains in number of nodules were in the order; NARC-BK > TAL-634 > TAL-169 > NARC-M after 30 days (104, 40, 20 and 1% increase over the control, respectively) and 45 days (456, 118, 44 and 14% increase over the control, respectively). Nodules dry weight plant^{-1} (52.7 mg), seed yield (1269 kg ha^{-1}) and harvest index (43%) were maximum with NARC-BK. Seasons had significant effect on number of branches, pod clusters, and pods plant^{-1},1000-seed weight, root length, nodule dry weight plant^{-1}, seed yield and harvest index with reduction by 5, 2.4, 8, 1.6, 3, 6, 2 and 2% respectively, in 2016 as compared to 2015. Number of nodules plant^{-1} showed 21, 16 and 7% reduction during 2016 over the 2015 after the 15, 30 and 45 days of sowing. Interaction effect of inoculum strains and the environment was significant only on number of pod plant^{-1}. The study concluded that NARC-BK is the best rhizobia strain for fetching maximum seed yield and its contributing parameters in mungbean crop irrespective of variable seasonal effect in Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

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Introduction

Pulses are an imperative part of profitable and sustainable agricultural system. Mungbean is one of the important pulse crop grown throughout Pakistan (Ali et al., 2000). Its seeds are rich in protein, fats, carbohydrates, calcium and phosphorus (24.2, 1.3,60.4, 0.12 and 0.34%, respectively) and is a rich source of vitamin-A due to which it occupies importance as an alternate of meat protein when consumed as food with cereals (Ahmad et al., 2013).Besides being important for poor man's diet protein, mung bean crops fix atmospheric nitrogen and improve nitrogen economy of soil (Ahmad et al., 2013). In Pakistan, mungbean is cultivated as a minor crop and according to an estimate, 127.4 thousand hectares area under cultivation with production of 98.7 thousand tones (Anon, 2014-15). A number of factors are responsible for its low yield including soil's nutritional and nodulation insufficiencies (Ali et al., 2012) resulting in inadequate biological nitrogen fixation.

Under the conditions of low native suitable rhizobium population, biological nitrogen fixation is triggered in leguminous crop plants through suitable micro-organisms applied to the crop seed, root or soil in the form of live formulates that colonize the indo or ecto-rhizosphere, fix N from the atmosphere and increase its availability to the host plant. (Morel et al., 2012). The legume potential to harness the nitrogen fixing bacteria association is, therefore, an issue of ecological and economical interests (Zahran, 2009). The fixation of atmospheric N not only fulfils crop's requirements for N but also lefts N rich organic matter for residual soil fertility. However, its full potential of nitrogen fixation and soil fertility improvement can only be harnessed if the crop is inoculated with suitable rhizobia micro-organisms in order to maximize the crop yield (Aslam et al., 2010). Such inoculation of the crop or soil with suitable inoculant is more effective with respect to crop response of nitrogen fixation if the soil is lacking suitable bacterial strain for association with the crop grown.

No doubt, nitrogen fixing potential of microbes is a matter of universal importance, however, different cultivars of the same crop might have different genetic potential for association with nitrogen fixing microbes and to form effective nodules and alternatively, different rhizobia strains should be assessed for their potential to colonize the rhizosphere, form association and effective nodules on crop roots for nitrogen fixation of different cultivars of the same crop without any deleterious effects on non-target crops grown alternatively (Mansoor et al., 2016). Nodulation potential of mungbean has been reported poor in Pakistan (Mehboob et al., 2008). Two important factors affect nitrogen fixation potential i.e. nodulation and its effectiveness (Zahir et al., 2003; Zahir et al., 2010). After considerable research, it was learnt that these two targets in nitrogen fixing activity can be achieved if suitable rhizobium partners of the crop are searched out and recommended for different varieties of the crop under different agro-ecological conditions of the country for increased biological and seed yield (Raza et al., 2004; Ahmed et al., 2006) Similarly, research on the starter dose of inorganic N for obtaining effective nodulation and nitrogen fixation of mungbean has been reported (Biswas et al., 2000). Since, the ineffective nodulation due to the absence of its symbiotic relationship with rhizobium in the area is one of the possible reasons of improper nodulation of the mungbean. Therefore, study of suitable rhizobium strain for maximizing symbiosis with mungbean for getting maximum yield under Dera Ismail Khan climate was the important target of this study.

Materials and Methods

The experiment consisted of a two years field trial at the Agricultural Research Institute, Rata Kulachi, Dera Ismail Khan (31° 49 N, 70° 55 E), Khyber Pakhtunkhwa, Pakistan, during the crop seasons 2015 and 2016 in a randomized complete block design with split plot arrangements replicated four times. Fertilizer strains were evaluated in sub-plots whilst environments (at two different years 2015 and 2016) were considered as the main plot factors. Pre sowing soil sample from the site was obtained at 0-30cm for assessing soil according to procedures described in Ryan et al. (1996). Soils of the study site were alkaline (pH=7.8) and moderately calcareous (12.5%), low in organic matter (1.1%), mineral N (10.5 mg kg^{-1}) and P (3.5 mg kg^{-1}) and marginal in K (111 mg kg^{-1}) contents. Treatments in the experiment included a control (S0) and four rhizobium strains; TAL-634 (S1), TAL-169 (S2), NARC-BK (S3) and NARC-M (S4). Rhizobium strains and mungbean seed of the mungbean cultivar "Inqalab Mung" for the study were obtained from National Agriculture Research Center, Islamabad. Seeds were inoculated with the inoculums of four selected strains just before sowing. Crop was sown on 15th May, 2015 and 10th May,2016 with plants 10 cm and rows 30 cm apart. Plot size was 8*4 m^2 accommodating 26 rows with row length of 4 m (Ahmad et al., 2004). Nitrogen and phosphorus fertilizers as 20 and 50 kg ha^{-1}basal dose were mixed with soil using urea and single super phosphate, respectively, at the time of sowing (Mansoor et al., 2017). Weeds were controlled manually. Crop was irrigated on need basis. Rest of the management was uniform in all treatments.

Data on various growth and yield parameters of the crop were recorded according to the procedure described by Murtaza et al. (2014). Data on branches plant^{-1}, clusters plant^{-1}, pods plant^{-1}, pod length, plant height were collected on ten plants randomly selected at maturity from each treatment and averaged. Data on pods cluster^{-1} were obtained as; pods plant^{-1}/ cluster plant^{-1}. 1000 seed were counted randomly from each treatment seed yield and averaged. Seed pod^{-1} werefrom randomly selected 20 pods. Five -lants from each treatment were uprooted carefully with spadeon 15, 30 and 45 day after sowing (DAS). Soil from the roots were gently washed with water and nodules plant^{-1} werecounted, root length were measured at all three intervals. Nodules plant^{-1} were collected, dried in an oven at 70 ^{0}C for 48 hours and weighed for nodules dry weight. The biological and seed yields were determined from harvested crop from one m^{-2} are. Harvest index was calculated (Saleem et al., 2011).

$$Harvest\ Index\% = \frac{Economic\ yield}{Biological\ yield} \times 100$$

Statistical analysis

Data were subjected to genotype by environment analysis based on combined data over the two crop seasons to determine variance amongst treatments, crop seasons and their interaction (Gomez and Gomez, 1984). Least Significant Difference (LSD) test was used to compare and separate the means of different categories using STATIX-8.1 computer software.

Results and Discussion

Nitrogen, is a macro-nutrient required in higher amounts by the all crops since it is essential for structural, genetic and functional role in plant cell as well as its major contribution to plant growth and development for higher gain yield (Bambara and Ndakidemi, 2010; Hawkefor, 2014; Leghari et al., 2016). Legumes are, however, provided with natural potential of atmospheric nitrogen fixation if make association with suitable microbial partner, the rhizobia. Mungbean, also needs suitable rhizobia for association to fix atmospheric nitrogen for increased growth and productivity (Salahuddin et al., 2009).

Data indicated that inoculation with rhizobium strains significantly ($p < 0.05$) affected the plant height, branches plant^{-1}, root length and nodule dry weight plant^{-1} (Table 1). Mean data combined over seasons indicated that these parameters were maximum and significantly highest (by 37 and 12% and 6 fold increase over the control, respectively) among the treatments when inoculated with rhizobium strain NARC-BK while the minimum number for these parameters were recorded in the inoculation control. However plant height reduced by 5% in the NARC-BK treatment over the control. Inoculation with TAL-634 competed to the NARC-BK in these with 32 and 6% and 1.6 fold increase in the branches plant^{-1}, root length and nodule dry weight plant^{-1}, respectively, but remained significantly inferior to it except plant height and significantly higher to rest of the treatments. These results confirm that performance of inoculum to improve growth and yield of the target crop is based on capability of the inoculum and its compatibility with the crop. TAL-634 significant lag behind to NARC-BK except in plant height (Table 1) would mean the onset of N_2-Fixation with NARC-BK might be delayed but is more effective than TAL 634 and other inoculums so that much of its assimilation of N_2 is used in reproductive growth and yield parameters. The Maximum root length of 23.6 cm was recorded with inoculation of rhizobium strain TAL-169 followed by NARC-BK strains (22.4 cm) both showing 18 and 12% increase in root length over the control, respectively. Increase in root length with Tal-634 and NARC-M over the control was 6 and 3%, respectively. These results were in accordance with the finding of Ahmad et al. (2006) who reported maximum root length at maturity stage with the seed inoculation of rhizobium strains as compared to fertilizer and control treatments. These results further confirm the suitability of NARC-BK strains for mungbean. Faramawy (2014) also reported that mungbean vegetative growth (plant height, stem diameter and number of branches) and pod production (pod number plant^{-1} and pod weight plant^{-1}) were significantly increased with the inoculation with *Bradyrhizobium japonicum*, *Azotobacter chroococcum*, *Bacillus megatherium* (PDB) and vasicular arbuscular mycorrhizae.

Change in plant height, branches plant^{-1}, root length and nodule dry weight plant^{-1} were significant over seasons (Table 1). Results showed reduction in branches plant^{-1}, root length and nodule dry weight plant^{-1} by 5, 3 and 6%, respectively, whlst plant height increased by 2% in 2016 as compared to 2015. Reduction in growth parameters during 2016 could be due to harsher environmental conditions for the crop during 2016 where temperature was higher and humidity was lower as compared to 2015 (Figure 1). Interaction effect of inoculum strains and the environment was significant only for plant height whilst their interaction on number of branches plant^{-1}, root length and nodule dry weight plant^{-1} were not significant. This means that both the inoculum and the environment were capable to affect the morphological parameters individually as well as interactively. Salahuddin et al. (2009), Hossain et al. (2011) and Faramawy (2014) reported that different bio-fertilizer treatments and their interactions significantly improved various growth, yield and quality parameters in mungbean crop.

Table 1: *Morphological attributes and nodule dry weight plant^{-1} of mungbean crop combined over two seasons as affected by different rhizobium strains.*

Treat-ments	Plant height (cm)	Branches plant^{-1}	Root length (cm)	Nod. dry wt plant^{-1} (mg)
Control	65.6 b	3.55 e	20.05 e	7.70 e
TAL-634	66.15 a	4.7 b	21.35 c	20.4 b
TAL-169	64.5 c	4.4 c	23.6 a	13.35 c
NARC-BK	62.4 d	4.85 a	22.40 b	52.7 a
NARC-M	64.45 c	4.05 d	20.75 d	11.9 d
LSD ($_{p<0.05}$)	0.19	0.13	0.31	0.90
Years				
2015	64.1 b	4.42 a	21.98 a	21.84 a
2016	65. 14 a	4.20 b	21.28 b	20.58 b
LSD ($_{p<0.05}$)	0.39	0.09	0.24	1.51
Year* Rhizobium	**	ns	ns	ns

Nod: nodule; Wt.: weight; Mean followed by different letters in each column are differ from each other significantly; NS: non-significant.

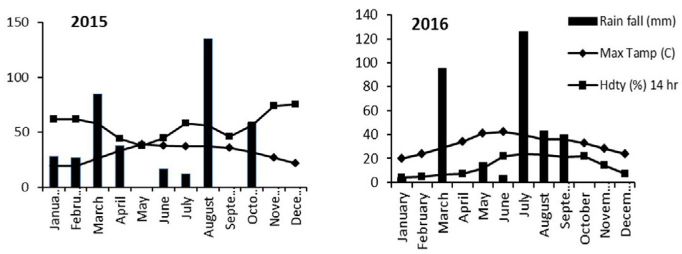

Figure 1: *Agro-meteorological / rainfall data recorded for 2015 and 2016 at Arid Zone Research Center, PARC, D.I. Khan.*

Analysis further revealed that inoculation with rhizobium strains significantly ($p < 0.05$) affected pod length, pod clusters plant^{-1}, pods plant^{-1} and pods cluster^{-1} (Table 2). Mean data combined over seasons indicated that these parameters were maximum and significantly highest (by 14, 18, 53 and 31% increase over the control, respectively) among the treatments when inoculated with rhizobium strain NARC-BK while the minimum number for these parameters were recorded in the inoculation control. Inoculation with TAL-634 competed to the NARC-BK in these with 8, 11, 48 and 30% increase in the aforesaid parameters, respectively, but remained significantly inferior to it and significantly higher to rest of the treatments. These results confirm that performance of inoculum to improve growth and yield of the target crop is based on capability of the inoculum and its compatibility with the crop. Previous results revealed that inoculation with suitable micro-organisms increased the number of nodules, pod bearing branches and number of pods over the uninoculated control (Ali et al., 2000; Chattha et al., 2017) perhaps due to enhanced nitrogen fixation by the legume crop due to inoculation with rhizobium that enhanced vegetative growth and yield parameters. This is further supported by our data (Figure 2) indicating highly significant correlation ($r^2 = 0.78$) between the number of nodules and pod length. Higher number of pods plant^{-1} after inoculation might be due to increased growth and more accumulation of assimilate resulting from sufficient N acquisition because of symbiotic N_2-fixation (Yoseph and Shanko, 2017). The study further supported by Aslam et al. (2010) and Muhammad et al. (2006) reported that number of pods per plant of mungbean were significantly increased by N fertilizers in combination with rhizobium strains.

Table 2: *Reproductive characteristics of mungbean crop combined over two seasons as affected by different rhizobium strains.*

Treatments	Pod Length (cm)	Pods clust. plant^{-1}	Pods plant^{-1}	Pods cluster^{-1}
Control	6.85 d	17.85 e	43.1 e	2.40 e
TAL-634	7.41 b	19.85 b	63,65 b	3.12 b
TAL-169	7.35 bc	19.30 c	57.7 c	2.98 c
NARC-BK	7.80 a	21.10 a	65.85 a	3.15 a
NARC-M	7.09 cd	18.45 d	48.6 d	2.9 d
LSD ($_{p<0.05}$)	0.28	0.25	0.19	0.02
Years				
2015	7.298	19.54 a	57.84 a	2.92
2016	7.3	19.08 b	53.72 b	2.9
LSD ($_{p<0.05}$)	ns	0.20	0.14	ns
Year*Rhizobium	ns	ns	**	ns

Clust.: clusters; Mean followed by different letters in each column are different from each other significantly; ns: non-significant.

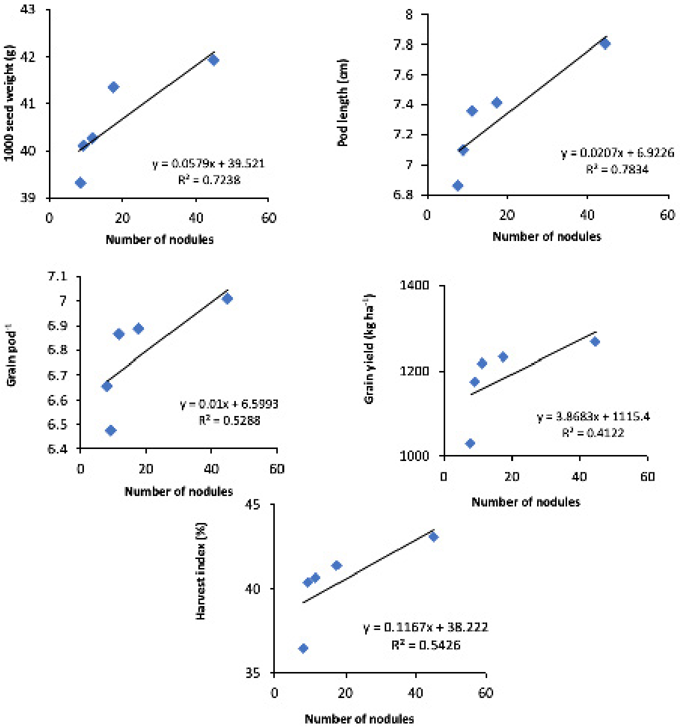

Figure 2: *Correlation between number of nodules plant^{-1} and various growth and yield parameters.*

Change in pod clusters plant^{-1}and pods plant^{-1} were significant over the seasons and showed reduction by 2.4 and 8%, respectively, in 2016 as compared to 2015 whilst the pod length and number of pods cluster^{-1} remained statistically unchanged (Table 2). Reduction in growth and yield parameters during 2016 could be due to harsher environmental conditions for the crop during 2016 where temperature was higher and humidity was lower as compared to 2015 (Figure 1).

Interaction effect of inoculum strains and the environment was significant only for number of pod plant^{-1} whilst their interaction on number of pod length, pods cluster plant^{-1} and pods cluster^{-1} were not significant (Table 2). This means that both the inoculum and the environment was capable to affect the growth and yield parameters individually as well as interactively. Salahuddin et al. (2009), Hossain et al. (2011) and Faramawy (2014) reported that different bio-fertilizer treatments and their interactions significantly improved various growth, yield and quality parameters in mungbean crop.

Inoculation with Rhizobium strains significantly (p< 0.05) incread number of nodules plant^{-1} at the 15, 30 and 45 days intervals during (Figure 3). Data averaged over two seasons revealed that 15 days after sowing (DAS) only one strain NARC-BK could produce the 5.85 nodules while no nodule was observed in rest of the rhizobium strains and the control (Figure 3a). The number of nodules plan^{-1}after 30 and 45 days after sowing was in the order, NARC-BK > TAL-634 > TAL-169> NARC-M > Control showing 104, 40, 20 and 1% increase over the control after 30 days of sowing and 456, 118, 44 and 14% increase over the control after 45 days of sowing. The strain NARC-M could not succeed to develop symbiotic relationship for efficient nodule formation during first 30 days. Furthermore, the nodulation potency of NARC-BK strain proved at all three intervals with best symbiotic relationship with mungbean crop. The results are supported by Aslam et al. (2010) who stated that inoculation of chickpea with rhizobium strain significantly increased nodule plant^{-1} by 27% as compared to control treatment. Number of nodules plant^{-1} over the years was significant (p<0.05) at all three intervals. Number of nodules plant^{-1} showed 21, 16 and 7% reduction during 2016 over the 2015 crops season at after the 15, 30 and 45 days of sowing (Figure 3b). Again, the effect of harsh climatic conditions with high temperature and low humidity during 2016 (Figure 1) is evident on these results. There was no interaction between the crop rhizobium strains and crop seasons to affect the number of nodules plant^{-1}

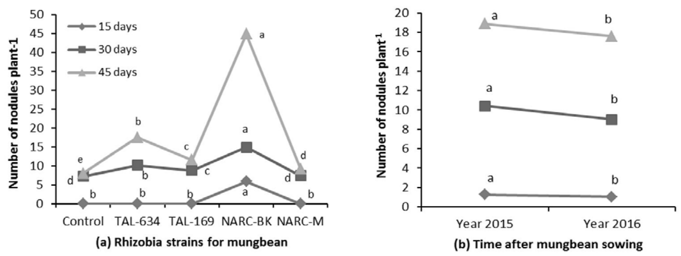

Figure 3: *Number of nodules plant^{-1} in (a) amongst different Rhizobia strains (b) between two years as affected by time interval after sowing. Means in a data series having different letters are significantly different at p<0.05.*

Analysis further revealed that number of seed pod^{-1}, 100-seed weight, seed yield and harvest index of the crop (Table 3) were significantly (p< 0.05) affected by inoculation with various rhizobium strains. The treatment inoculated with strain NARC-BK showed the maximum 5% increase in seed pod^{-1}, 7% increase in 1000 seed weight, 23% increase in seed yield and 18% increase in harvest index over the control. TAL-634 competed but still remained significantly inferior in all these parameters to NARC-BK (Table 3). This means the onset of N_2-Fixation with NARC-BK is delayed but is more effective than TAL 634 and other inoculums so that much of its assimilation of N_2 is used in reproductive growth and yield parameters.

This is furthermore, clear from significant correlation between the number of nodules plant^{-1} with 1000 seed weight (r^2=0.72), number of nodules plant^{-1} with grains pod^{-1} (r^2 = 0.53), number of nodules plant^{-1} with seed yield (r^2 = 0.41) and number of nodule plant^{-1} with harvest index (r^2= 0.54) (Figure 2). Qureshi et al. (2017) reported that rhizobium inoculation significantly enhanced yield and related traits. Our study further support the studies done by Aslam et al. (2010) and Muhammad et al. (2006) who reported that number of pods per plant, number of seeds per plant, 1000-seed weight and seed yield of mungbean were significantly increased by N fertilizers in combination with rhizobium strains. The results are also in accordance with Saleem et al. (2011) who reported that plots treated with amendments along with seed inoculation of rhizobium have high harvest index as compared to control.

Table 3: *Yield characteristics of mung bean crop combined over two seasons as affected by different rhizobium strains.*

Treatments	Seed pod^{-1}	1000 seed wt. (g)	Seed yield (kg ha^{-1})	Harvest index (%)
Control	6.66 bc	39.29 e	1032 d	36.45 d
TAL-634	6.89 ab	41.35 b	1232 b	41.30 b
TAL-169	6.87 ab	40.25 c	1220 b	40.60 c
NARC-BK	7.01 a	41.9 a	1270 a	43.05 a
NARC-M	6.48 c	40.1 d	1176 c	40.35 c
LSD ($_{p<0.05}$)	0.27	0.14	20.7	0.51
Years				
2015	6.74	40.92 a	1200	40.72 a
2016	6.82	40.24 b	1172	39.98 b
LSD ($_{p<0.05}$)	ns	0.26	ns (30)	0.45
Year*Rhizobium	*	**	ns	ns

Mean followed by different letters in each column are different from each other significantly; ns: non-significant.

Seasons effect was significant on 1000 seed weight and harvest index only whereas its was nearly significant on seed yield and non-significant on seed pot^{-1} (Table 3). The 1000 seed weight and harvest index were 1.6 and 2% whilst seed yield was 2.3% lower in 2016 as compared to 2015. Like other parameters, variation in temperature and humidity between the seasons as depicted in Figure 1 might be responsible for this change. Interaction between the rhizobium strains and crop seasons was significant on seed pod^{-1} and 1000 seed weight. Higher temperature initially might improve plant growth but extremely high temperature during the entire crop season during 2016 might have affected negatively the capability of inoculum to fix N_2 to its potential and its translocation into the seed which resulted in reduced seed pod^{-1}, 1000 seed weight.

Conclusions and Recommendations

The study concluded that, irrespective of seasonal variation between the two years, NARC-BK manifested itself as the best amongst different rhizobia strain tested for fetching maximum seed mung bean seed yield and its contributing parameters in District Dera Ismail Khan, Khyber Pakhtunkhwa, Pakistan.

Novelty Statement

Ineffective nodulation in legumes indicate either the absence or low native population of suitable rhizobium strain to trigger biological nitrogen fixation, which, in the area is one of the possible reasons of low mungbean seed yield. This study strived for searching and recommending suitable rhizobium strain for maximizing symbiosis with and biological nitrogen fixation by the mungbean crop for getting maximum yield under agro-climatic conditions of Dera Ismail Khan District, Khyber Pakhtunkhwa.

Author's Contribution

Rafiq-ur-Rehman conducted the experiment. Dr. Zahoor Ahmad was the supervisor. Dr. Muhammad Mansoor was co-supervisor and helped in field activities. Dr. Wiqar Ahmad helped in write up of the paper and Dr. Shah Masaud helped in data collection and statistical analysis.

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