ASSESSMENT OF SYMBIOTIC NITROGEN FIXING ABILITY OF CULTIVATED LEGUMES IN TWO CROPPING SEASONS: CENTRAL HIMALAYA

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Abstract:

Symbiotic nitrogen fixation by *rhizobium*-legume association may be an important source of nitrogen for soil in Himalayan region, though it has hardly been estimated. Assessment of nitrogenase activity and effect of legume crop cultivation on soil chemical properties reveals that Himalayan legume crops possess great potential for nitrogen fixation and soil fertility improvement. Though their nitrogen fixing and soil fertility enhancing efficiency is influenced by change in plant phenology and cropping pattern, yet their planned incorporation into farming system, can orient hill farming towards sustainability. Of the various crops studied, *Glycine max* and *Vigna angularis* were found most promising in Himalayan region for maintaining soil fertility.

Key words: Central Himalayan agro-ecosystem, cultivated legumes, Nitrogenase activity, soil fertility enhancer, organic agriculture

Introduction:

Like many mountain countries, the Indian Himalayan region is characterized by a complex mosaic of distinct agro-ecosystems, differentiated by their climatic, edaphic, and geological characters, vegetation and cropping patterns, crop rotations and other features. Owing to diverse topography and climatic conditions, the Himalayan Mountains are bestowed with an amazing diversity of crops. To maintain and conserve this diversity, the farming communities of this region practice low-input agriculture with a significant concern for agricultural sustainability[1], but a greater challenge in sustainable management of agriculture is infertile, fragile, rainfed agricultural land which includes about 85 % of total farming system of the region. The infertile land requires more nitrogen for proper plant growth and better yield and thus the demand for soil nitrogen in Himalayan agriculture is increasing day-by-day. This is not a localized scenario but the demand for nitrogen in world agriculture is increasing at the rate of increasing world population i.e. about 2% per annum[2].

Legume crops fix atmospheric nitrogen and hence can be considered as a viable source of nitrogen in Central Himalayan perspective, where landholding is small and traditional agriculture is entirely practiced on rainfed terraces (Fig1a) under low-input system. By providing a biological source of nitrogen, legume can play a key role in sustainable production and management of hill agriculture and in maintaining soil fertility of nutrient poor soil of resource deficient farmers of the region who cannot afford or do not have access to nitrogen fertilizers[3-5]. Also, since atmospheric nitrogen is a renewable resource, biological nitrogen fixation (BNF) by legume crops is an ecologically viable source of nitrogen in agricultural system[6], which increases food production without compromising for food quality as compared to inorganic fertilizers which provide an unsustainable nitrogen source to the crop and soil.

Thus, legume crops have great relevance as soil fertility maintainer in Central Himalayan prospect. They can fix 40-48 million tones of N/year[7-8] and hence, can contribute in retaining a low-input sustainable farming system. They can lead to crop productivity enhancement also[9-11]. But despite of huge significance, fewer efforts have been made so far to promote and increase the area under cultivation of these crops particularly in Central Himalayan region. Though they have been an important component of Himalayan agriculture (Fig 1b, 1c, 1d) since centuries, a recent survey showed that the area as well as production of Himalayan legume and other traditional crops has declined to greater extent [12].

Present study is an effort (i) to document prominent Himalayan cultivated legume crops and their altitudinal distribution (ii) to assess nodulation behavior and nitrogenase activity of some prominent cultivated legumes (ii) to evaluate the effect of legume crop cultivation on soil chemical properties (%NPK) and (iii) to discuss and suggest strategies to incorporate legumes essentially into cropping system so as to develop a sustainable and organic farming system. Assessment of nitrogenase activity is an important criterion to evaluate the nitrogen fixing potential of any crop under a specific environmental condition and thus the study will benefit planners, agriculturists and environmentalist to execute plans and strategies for improving and maintaining soil fertility of agricultural terrains of Central Himalayan region. The study will also help to draw scientist, policy planners and government interest towards making effort for conservation, management, research and extension work intended for leguminous crops in Himalayan region.

Material and methods

Present study was carried out in the Central Himalaya (Uttarakhand), situated between $20^0$31'9" to 31^0 26'5" N & $77^0$35'5" to $80^0$6' E [12] with particular emphasis on Mandakini valley. An extensive survey in 10 selected villages of the valley all falling in Rudraprayag district was carried out to collect information on prominent cultivated legume crops, their distribution, utilization pattern, agronomic practices, indigenous uses etc. To assess nitrogenase activity, field experiment was conducted for two years from 2005 to 2006. A total of 12 legume crops (Table1), of which eight (8) grown during kharif season viz *Cajanus cajan* (Pigeon pea), *Glycine max* (Black soyabean), *Glycine max* (White soyabean), *Macrotyloma uniflorum* (Horsegram), *Phaseolus vulgaris* (Kidney bean), *Vigna angularis* (Adzuki bean), *Vigna mungo* (Black gram), *Vigna unguiculata* (Cow pea), and four in rabi season viz *Lens culinaris* (Lentil), *Pisum arvense* (Wild pea), *Vicia faba* (Broad bean) and *Pisum sativum* (Pea) were selected for the experiment. The kharif season crop duration is from May to November and rabi season crop duration is from October to March. The agricultural field used for the experiment had been managed organically previously and the preceding crops in the experimental plots selected for kharif and rabi season experiment were *Hordeum vulgare* (Jau) and *Oriza sativa* (Paddy) respectively. The crops were sown following traditional farming system in which legumes are mixed with non-legume crops. Kharif season legume crops were mixed with Finger millet (*Eleusine coracana*) whereas rabi season crops were mixed with wheat (*Triticum aestivum*). The field trial for both the season was arranged in a completely randomized block design with 3 replicates for each crop. The plot size was kept 2X2 mts. No fertilizers or organic manure was applied at any stage of the plant growth. No inoculation treatment was given to any crop as these crops were in cultivation in the experimental plot previously. Data related to number of nodules/plant, weight of nodules/plant and nitrogenase activity/plant/hr was taken at the time of flowering. For this five plants per plot were gently uprooted, the roots were then tenderly shaken and cleaned with brush to remove the attached soil particles. The number of nodules/plant, fresh wt. of nodules/plant and nitrogenase activity/plant were assessed following the methodology given by Hardy et al.[13].

To assess the effect of associated non-legume under mixed cropping and plant growth and phenological changes on nodule nitrogenase activity and to evaluate the effect of legume crop cultivation on soil NPK (%) content, three legume crops viz Horsegram (*Macrotyloma uniflorum*), Adzuki bean (*Vigna angularis*) and Black soyabean (*Glycine max*) were selected. The experimental treatment comprised mono crop stands of said legumes and mixed crop stands of these legumes with Finger millet (*Eleusine coracana*). The experimental set up was completely randomized block design with three replicates for each treatment and the plot size was 3X4cm. To assess variation in nitrogenase activity, nodules were collected, counted, weighed and assayed at an interval of 15 days till maturity following same procedure as for above. For soil analysis soil samples were taken from each plot at two depths viz 0-15cm and 15-30cm before sowing and after final harvest. Total soil NPK (%) was estimated following the methodology of Anderson and Ingrame [14].

Results and Discussions

Symbiotic Nitrogen Fixation in Himalayan cultivated legumes

Nodule number, their fresh weight and nitrogenase activity (µmoles of ethylene produced/plant/hr) were assessed in all the selected legume crops at the time of flowering in rainfed farming system (Table1). As *Glycine max* is well known for its high potential to obtain its N requirement by symbiotic nitrogen fixation[15], here also among various kharif season legume crops, *Glycine max* varieties viz Black soyabean and White soyabean have shown highest nitrogenase activity of 6.46 and 6.35 µmoles of ethylene produced/plant/hr respectively. Among, other kharif legumes, Adzuki bean (5.28), Cow pea (4.82), Horsegram (4.76) and Kidney bean (3.09) have shown significant nitrogen fixing potential under rainfed agro-ecosystem. Among rabi season crops Lentil (1.94) exhibited highest nitrogenase activity. It is therefore recommended to increase the area under cultivation of these crops in Himalayan region to increase soil fertility so that yield of other associated and subsequent crops can be improved organically.

Effect of mixed cropping on nitrogenase activity

Substantial effect of associated non-legume crop under mixed cropping on nodule number, fresh weight and nitrogenase activity of legume crop has been observed (Figure 2a, 2b, 2c, 2d, 2e, 2f). The results indicate that the legume's root nodule nitrogenase activity was always higher under mixed cropping as compared to mono cropping and it may be due to the strain induced by non-legume on legume crop for nitrogen demand. Plant phenological changes also affect nitrogenase activity and under both mixed and mono cropping, the nitrogenase activity/plant increases with plant growth till flowering i.e. upto 90 days and decreases afterwards till maturity. At the time of final harvest (at 150 days) no nodules were seen and thus nitrogenase activity was not found in any crop. Similar results were reported by various workers in Pea [16-19], soyabean [20-22] and common bean [16]. The results suggest 90 days as the most appropriate time for estimation of nitrogenase activity in these Himalayan crops. The decline in nitrogenase activity after flowering is may be due to the stress induced to the plant due to initiation of reproductive stage [23] or due to pod formation which are a strong sink for photo assimilation than nodules [20, 21]. Though nitrogenase activity decreases after flowering under both mixed and mono cropping but the decline is more drastic under mixed cropping as compared to mono cropping and it may be due to low soil moisture content under mixed cropping. Dense canopy cover of legume under mono cropping led soil to retain soil moisture content for long time and hence cause slow decline in nitrogenase activity. However, under mixed cropping, the climbing support provided by non-legume to legume crop led sunlight to reach directly on the ground which causes low soil moisture content and decreased nodular activity.

Effect of legume crop cultivation on soil chemical properties

Legume crop cultivation also effect soil chemical properties (Table 2). As compared to soil %N before sowing, an increase in soil %N was observed in all the crop stands (mono and mixed) and soil depths studied, at the time of final harvest, but the results were significantly ($p=0.05$) high at both the depths viz 0-15 and 15-30 cm under mono cropping and at 0-15 cm depth only under mixed cropping. The results suggest more pronounced effect of crop on top soil compared to sub-soil.

Among various mono crop stands studied, maximum increase in soil %N was found under Black soyabean cultivation followed by Adzuki bean and Horsegram, at both the depths, but the difference is significantly high at 0-15cm depth only. Similarly under mixed cropping also maximum increase in soil %N was observed under Black soyabean +Finger millet combination at both the depths. Similar results were obtained by Shivram and Ahlawat[24] under Pegionpea/blackgram intercropping system. Simpson et al.[25] reported annual increment of soil N between 25-100kg N/ha in sub-terranean clover-based pastures. However, Dalal [26], Reeves et al.[27] and Strong et al.[28] were unable to find any consistent effect of prior legume crop cultivation on level of total soil nitrogen. Holford[29] found that 2-3 years of Lucerne cultivation was required to increase levels of total soil N significantly. Though significant increase in available soil phosphorus was reported by Singh and Faroda[30] and Shivram and Ahlawat[24] under various legume based cropping systems, but here in all the stands studied, decrease in %P and %K content was recorded at the time of final harvest, though the values were not significantly different from values at before sowing.

Priority interventions for successful incorporation of legumes into farming system

Many successful stories of using legumes for rehabilitation of degraded land and improving soil fertility of agricultural and pasture land has been noticed from Australia, United States and New Zealand[31]. Similarly in Central Himalayan region managed cultivation of legumes followed by research, development and implementation activities can overwhelm the problem of soil infertility and low crop productivity. To essentially incorporate legume into farming system, awareness among farmers about the potential of legume crops as a source of nitrogen has to be raised. For this, awareness programmes are need to be organized among villager/farmers about the harmful effects of inorganic fertilizers and continuous cereal crop cultivation on one hand and the benefits of legume crop cultivation on soil fertility improvement, productivity enhancement of associated and subsequent crops, pests management, control of soil erosion and plant disease[27] on the other hand. There is also a strong need to enhance farmers interest towards traditional mountain crops and farming system since all these legume crops are traditionally cultivated from centuries, however during recent past due to various socioeconomic changes like weather uncertainties, changed food habits, cultivation of HYV and introduced cash crops like potato, kidney bean, *Amaranthus* etc, the area under cultivation of traditional crops including legumes has reduced to a greater extent. Maikhuri et al.[12] reported 72-95% decline in traditional crop cultivation including legume with in a period of two decades (1974-1994). Further, in-depth research is need to be done for screening the compatible nitrogen fixing crops/species at various agro-climatic zones of the Himalayan region to include best ones in the farming system, which are ecologically, economically and organically viable for short and long term basis. These species will definitely be useful either in isolation or in combination for soil fertility and crop yield improvement. In addition, breeding programmes to develop new crop lines that are suitable for Himalayan region and can derive higher % of nitrogen from the atmosphere are need to be prioritized. Selection of rhizobia strains that can serve as inoculants, and incorporation of improved management practices that increases nitrogen fixation will further help to achieve the desire goal.

Conclusion

The study reveals great potential of Himalayan cultivated legumes for nitrogen fixation and soil fertility improvement. Though their nitrogen fixing and soil fertility enhancing efficiency is very much influenced by change in plant phenology and cropping pattern and other environmental factors, yet their planned incorporation into farming system, as an integral part of agriculture can provide ecological stability to fragile rainfed agriculture land of Himalayan region and can orient hill farming towards sustainability. The foundation of any agricultural system depends solely on its sustainability. So, biological nitrogen fixation, particularly by cultivated legumes can help in maintaining a productive and sustainable agriculture system in Central Himalayan region. The use of legume crops in farming system is an eco-friendly and profitable approach to arrest the decline in soil fertility. Though legume crop cultivation in Himalayan region have emerged over centuries of cultural and biological evolution and has been an important component of traditional agriculture, but during recent past, a shift from traditional to modern and cash crop oriented agriculture has been observed in Himalayan region which results in huge loss in agricultural diversity including legumes[32-34]. Many promising avenues for introducing legume crops into cropping systems and for enhancement of the contributions from nitrogen fixation to soil fertility improvement has been proposed, yet it is difficult to find best way of incorporating these findings into large scale in the Himalayan region and thus need more studies to conclude some promising results.

There is widespread interest today in reinstating ecological rational into agricultural production and in making major adjustments in conventional agriculture to make it environmentally, socially and economically viable and compatible. One focus is on input substitution i.e. replacing costly and degrading agro-chemical and high input technologies for more environmentally sound low external input techniques. This approach does not address the ecological cause of the environmental problems in modern agriculture/ cash crop oriented agriculture which is deeply rooted in the non-legume monoculture structure prevalent in large stock production system.

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Table1: Altitudinal range, number of nodules/plant, fresh weight of nodules (g/plant) and nitrogenase activity/plant of some prominent cultivated legume crops in Central Himalayan agro-ecosystem

- ± indicates SD values

Botanical name (English name)	**Vernacular name**	**Altitudinal range (m asl)**	**No. of nodules /plant**	**Fresh wt. of nodules/plant (g/plant)**	**Nitrogenase activity (μmoles of ethylene produced /plant/hr)**
KHARIF SEASON CROPS (May-November)					
Cajanus cajan (L.)Huth (Pigeon pea)	Tor	500-1650	48 ±5.03	1.77 ±0.14	1.13 ±0.25
Glycine max (L.) Merrill (White soya bean)	Safedbhatt	700-1700	92 ±7.58	1.75 ±0.14	6.35 ±1.05
Glycine max (L.) Merrill (Black soya bean)	Kalabhatt	1000-1500	99.6 ±12.42	1.58 ±0.20	6.46 ±0.59
Macrotyloma uniflorum (Lam.) Verdc. (Horsegram)	Gehet	500-2000	74 ±11.98	0.9 ±0.16	4.76 ±0.41
Phaseolus vulgaris L. (Kidney bean)	Rajma	1500-2500	44 ±8.2	0.53 ±0.06	3.09 ±0.07
Vigna angularis (Willd.)Ohwi and Ohashi (Adzuki bean)	Rains	1000-2250	75.2 ±10.57	0.84 ±0.05	5.28 ±0.26
Vigna mungo (L.) Hepper (Black gram)	Urd or Kalidal	500-1750	38 ±5.19	1.2 ±0.15	2.8 ±0.13
Vigna unguiculata (L.) Walpers (Cow pea)	Sonta	500-1750	70±12. 23	0.73±0.04	4.82±0.46
RABI SEASON CROPS (October-March)					
Lens culinaris Medikus (Lentil)	Masoor	500-1500	31 ±6.08	0.28 ±0.07	1.94 ±0.09
Pisum arvense L. (Pea)	Kong	2200-2642	19 ±6.5	0.10 ±0.01	1.22 ±0.15
Pisum sativum L. (Pea)	Matar	500-2643	31.3 ±10.0	0.26 ±0.06	1.07 ±0.01
Vicia faba L. (Broad bean)	Shivchana	500-1500	44.6 ±6.6	0.39 ±0.03	1.24 ±0.05

Table2: Soil nitrogen (%), phosphorus (%) and potassium (%) content before and at final harvest in Horsegram, Adzuki bean and Black soyabean grown under mono and mixed cropping in Central Himalaya.

Parameter	Soil depth (cm)	Before sowing	At Final Harvest					
			Horsegram mono cropped	Horsegram +Finger millet	Adzuki Bean mono cropped	Adzuki bean +Finger millet	Black soyabean mono cropped	Black soyabean +Finger millet
%N	0-15	0.34* ±0.02	0.55* ±0.01	0.41* ±0.01	0.66* ±0.03	0.44* ±0.01	0.78* ±0.02	0.45* ±0.02
%N	15-30	0.30* ±0.03	0.38* ±0.02	0.34 ±0.02	0.41* ±0.01	0.35 ±0.02	0.41* ±0.02	0.36 ±0.02
%P	0-15	0.033 ±0.003	0.030 ±0.003	0.029 ±0.003	0.030 ±0.002	0.028 ±0.004	0.03 ±0.002	0.030 ±0.003
%P	15-30	0.028 ±0.003	0.026 ±0.002	0.026 ±0.003	0.028 ±0.004	0.026 ±0.003	0.027 ±0.003	0.026 ±0.004
%K	0-15	1.48 ±0.03	1.47 ±0.03	1.46 ±0.04	1.45 ±0.04	1.44 ±0.03	1.45 ±0.03	1.44 ±0.03
%K	15-30	1.43 ±0.03	1.42 ±0.03	1.41 ±0.02	1.40 ±0.03	1.40 ±0.03	1.41 ±0.04	1.40 ±0.04

*The values are significantly different (p=0.05) from the corresponding values at before sowing

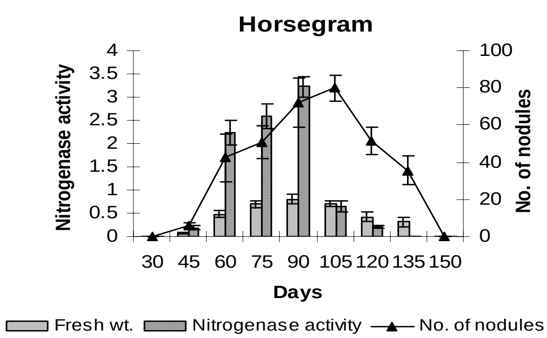
- ± indicates SD value

Figure 1a: Central Himalayan rainfed agro-ecosystem

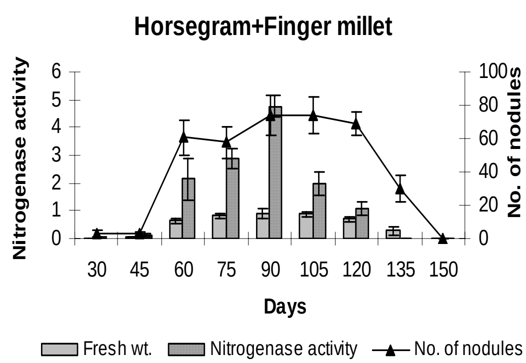
Figure 1b: Legume cultivation in rainfed agriculture with finger millet

Figure 1c: Legume cultivation on the bunds of paddy field

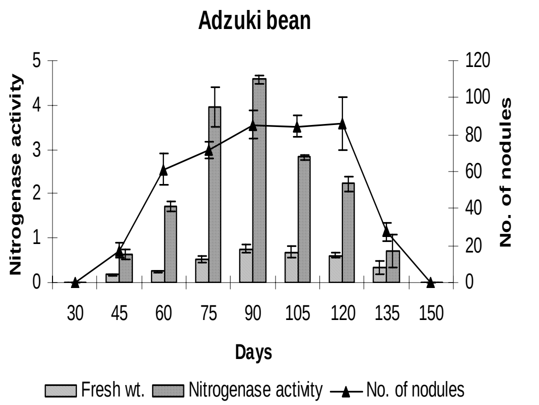
Figure 1d: Adzuki bean climbing on Finger millet



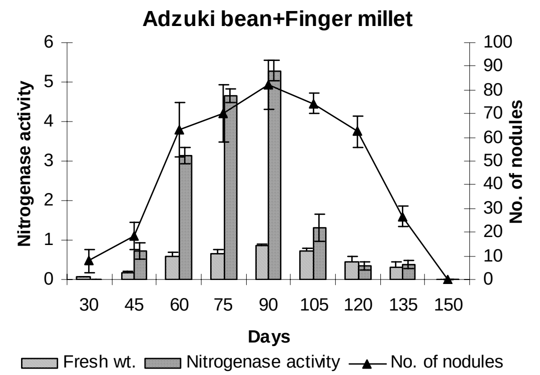
2a: Horsegram mono cropped



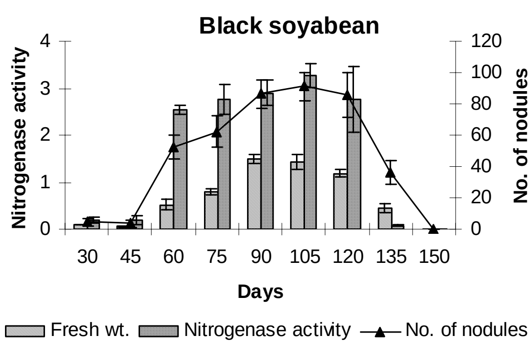
2b: Horsegram+ Finger millet mixed cropped



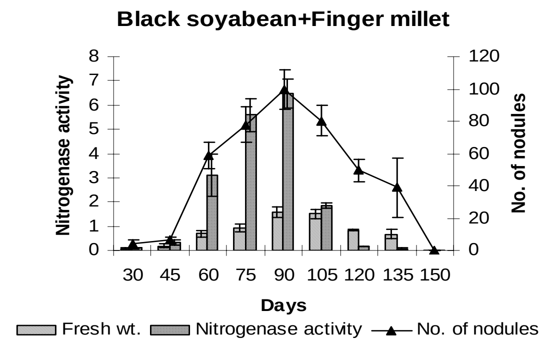
2c: Adzuki bean mono cropped



2d: Adzuki bean +Finger millet mixed cropped



2e: Black soyabean mono cropped



2f: Black soyabean +Finger millet mixed cropped

Figure 2a, 2b, 2c, 2d, 2e, 2f: Fortnightly variation in nodule fresh weight/plant (gm/plant), number of nodules/plant and nitrogenase activity/plant (µmoles of ethylene produced/plant/hr) in Horsegram, Adzuki bean and Black Soya bean under mono and mixed cropping in Central Himalayan agro-ecosystem.

Objective

- To test the symbiotic effectiveness of the rhizobial isolates collected in the first phase of the project on the respective host plants
- To classify the legume nodulating bacteria on the basis of selective media

Symbiotic Effectiveness of cowpea *Bradyrhizobium* strains that nodulates Mungbean (*Vignata radiata*)

Strain No.	Nodule Number	Plant Dry weight (mg)	Total Nitrogen Conten (mg)	Nitrogenase activity
Control (Uninoculated)	0.00	136.2	12.4	0.00
BGBD-2	35.7	328.0	37.3	5.6
BGBD-3	25.9	267.3	29.0	5.1
BGBD-6	41.8	345.2	42.0	6.4
BGBD-7	35.0	309.0	32.9	4.9
BGBD-9	27.3	280.1	32.1	4.3
BGBD-10	47.0	350.2	43.8	7.5
BGBD-13	32.9	309.8	31.0	6.1
BGBD-14	21.8	237.0	28.6	4.3

Symbiotic Effectiveness of cowpea *Bradyrhizobium* strains that nodulates Tor (*Cajanus cajan*)

Starin No.	Nodule Number	Plant Dry weight (mg)	Total Nitrogen content (mg)	Nitrogenase Activity
Control (Uninoculated)	0.00	151.2	10.6	0.00
BGBD-16	18.9	315.9	23.8	6.9
BGBD-17	28.7	278.0	27.9	7.8
BGBD-18	38.0	354.0	32.7	9.6
BGBD-20	15.0	285.4	20.7	5.4

Symbiotic Effectiveness of *Rhizobium leguminasrum bv phaseoli* strains on Rajma (*Phaseolus vulgaris* L.)

Strain No.	Nodule Number	Plant Dry weight (mg)	Total Nitrogen Content (mg)	Nitrogenase Acivity
Control (Uninoculated)	0.00	218.9	40.4	0.00
BGBD-21	25.9	335.8	66.8	9.23
BGBD-22	31.4	367.0	76.9	13.8
BGBD-23	28.9	349.2	79.4	10.8
BGBD-26	45.6	418.7	90.6	16.8
BGBD-28	34.7	340.8	77.6	11.9

Symbiotic Effectiveness of *Rhizobium* strains that nodulate Gahat (*Macrotyloma uniflorium*)

Strain No.	Nodule Number	Plant dry weight (mg)	Total Nitrogen Content (mg)	Nitrogenase activity
Control (Uninoculated)	0.00	124.8	12.5	0.00
BGBD-44	21.9	239.3	28.4	5.9
BGBD-45	28.6	256.6	35.9	7.2
BGBD-46	16.0	207.6	20.5	4.7

Growth performance of the BGBD nodule Isolates

- A selective media known as BAc media was used to differentiate Burkholderia from other legume nodulating bacteria. It contains the following (in g/l)

Azelic acid : 2.0

L-Citrulline : 0.2

K2HPO4 : 0.4

KH2PO4 : 0.4

MgSO4 : 0.2

pH : 5.7

Observation: Out of the 64 isolates tested, 38 showed growth on the Azelic acid medium that indicates that a very high number of legume nodulating bacteria are either Burkholderia or very close to that. Although it is claimed by the authors that this is very specific media for Burkholderia, a molecular study is required to come on conclusion.