^{15}N aided Studies on Legume Nitrogen Fixation in Tista Meander Floodplain Soils of Bangladesh

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Abstract
Nitrogen fixations in different legumes were estimated by using ^{15}N isotope dilution technique, at the BNF laboratory of Soil Science Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. Field experiments were conducted at BINA sub-station farm, Rangpur, in the Tista Meander Floodplain soils of Bangladesh, during the year of 2004-05 and 2005-06. Three different legumes (such as – lentil, mungbean and soybean) and two inoculation systems (with and without inoculant) were randomly assigned in a split-plot design with 3 replications. For quantification the amount of biological N fixation by different legumes, crops were grown in the isotope treated micro plots {treated with 10.48% ^{15}Na.e. labelled $(NH_4)_2SO_4$ fertilizer} and a non nodulated crop (wheat) was included in a separate plot as reference.

Quantity of total nitrogen (TN) and Nitrogen derived from atmosphere (Ndfa) in different legumes were significantly higher in inoculated plots comparatively than un-inoculated ones. When the legume seeds were properly treated with inoculant's before sowing, the highest amount of TN quantified in soybean (189.63 kg/ha) which was followed by lentil (83.52 kg/ha) and mungbean (70.16 kg/ha). The similar trend was also noticed in case of total atmospheric nitrogen fixation by different legumes and soybean crops fixed the highest (84.18% of its N_2 amounting to 159.62 kg N/ha), which was followed by lentil (82.82% of its N_2 amounting 69.13 kg N/ha) and mungbean (% 80.73 of its N amounting 56.64 kg N/ha). Due to inoculation the total net N inputs added to the soil system for succeeding crops was comparatively higher than un-inoculated situation and among the different legumes the maximum net N inputs was observed as 50.95 kg N/ha from inoculated soybean.

Key words
^{15}N tracer, legumes and net N inputs.

Introduction
Managing nitrogen inputs in crop production system to achieve economic and environmental sustainability is a major challenge facing agriculture. Relying less on commercial fertilizer N and more on biological N_2 fixation by legumes has been suggested as a way to meet this challenge (Keeney 1982). Declining soil fertility, particularly N is recognized as a major threat to continued rice/cereal cropping in Bangladesh soil, specially, Tista Meander Floodplain. It is widely believed that legumes improve soil fertility because of their N_2-fixing ability. Variation exists in legumes for the amount of N fixed and for the proportion of plant N derived from biological N fixation. We need to identify legumes and genotypes that yield more and derive a large part of their nitrogen requirement from fixation (Wani *et al.* 1995). This study was therefore, conducted to quantify the amount of N fixed by three important legumes viz. lentil, mungbean and soybean with and without inoculant condition and also estimate the total net N inputs in soil system.

Materials and Methods
Field experiments were conducted during 2004-05 and 2005-06 at Bangladesh Institute of Nuclear Agriculture (BINA) Rangpur sub-station farm, located at $25^0 43'$ N latitude and $89^0 16'$ E longitude in the north-west part of Bangladesh. The monthly average rainfall was 237mm and the minimum and maximum temperature of the study area were, 20.55 and 29.55^0C, respectively. The soil of the experimental site was silt loam (19% clay, 51% silt and 30% sand) in Tista Meander Floodplain, having pH 5.7 (in water) with C 1.5%, total N 0.07%, Olsen's P 15.5ppm and exchangeable K 0.10cmol/kg. Three different legumes, such as – lentil (*Lens culinaris* Medik; cv. Binamusur 1), mungbean (*Vigna sinensis*; cv. Binamoog 4) and soybean (*Glycine max* L.; cv. Sohag) were tested with and without rhizobial inoculant following split-plot design with three replications. For quantification of biological nitrogen fixation potentials, ^{15}N labelled ammonium sulphate with 10.48% a.e. @ 20kg N/ha was uniformly sprayed to 1.00 square meter area of the legume plots. The reference crop wheat (*Triticum aestivum*) received 100kg N/ha in the isotopic subplot. The size of the

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each main plot was 6m X 5m and in each plot contains two ^{15}N micro plots (1m x 1m), which were assigned for isotopic study. For $^{15}N/^{14}N$ ratio analysis was performed using NOI-7 emission spectrophotometer (Martina 2002) and ^{15}N related calculations were done by using the equations from Hardarson and Danso 1990 and Toomsan *et al*. 1995. Analysis of variance was performed on the data and means were classified following Duncan's New Multiple Range Test ($P<0.05$).

Results

Total nitrogen content in different legumes

Among the different tested legumes, the highest total amount of N (average of 2 years result) was estimated as 189.63 kg N/ha in the treatment T_6 (inoculated soybean) which was significantly differed with the other treatments and the lowest amount of 41.04 kg N/ha total N recorded in un-inoculated mungbean (Figure 1). Due to inoculation, on average, the total N uptake increased as 77.36, 70.96 and 98.38% in lentil, mungbean and soybean plant, comparatively than un-inoculated legumes.

Quantification of nitrogen derived from atmosphere (Ndfa) in legume

Ndfa in legume seed

Through ^{15}N study, the quantity of Ndfa in legume seed was estimated from the different types of legumes grown with and without inoculant in Tista Meander Floodplains at Rangpur (Table - 1). Among the different treatment combinations, it was observed that inoculated soybean seed fixed the maximum amount of nitrogen from the atmosphere (94.30 and 96.71 kg N/ha during the year of 2004-05 and 2005-06) which was statistically different with the amount of Ndfa fixed by inoculated lentil (41.38 and 42.36 kg/ha) and mungbean (31.00 and 35.05 kg/ha). The minimum amount of seed Ndfa estimated as 14.50 and 15.00 kg N/ha (during 2004-05 and 2005-06) from un-inoculated mungbean. Due to inoculation, on average, the seed Ndfa increased as 138, 123 and 177% in lentil, mungbean and soybean respectively. The highest quantity of seed Ndfa (95.51 kg/ha) was recorded from the inoculated soybean and the lowest amount of 14.75 kg/ha seed Ndfa was observed from un-inoculated mungbean plot.

Ndfa in legume stover

Interaction effects of different types of legumes with and without inoculant significantly affected on the quantity of stover Ndfa in both years. Among the different treatments, the inoculated soybean stover showed the highest N fixation as 67.45 and 60.78 kg N/ha during 2004-05 and 2005-06 and the lowest values were observed as 11.15 and 11.20 kg N/ha from un-inoculated mungbean. Application of rhizobial inoculant significantly increases the amount of biological N fixation from the air in the stover of different legumes and due to inoculation the percent increase of Ndfa in legume stover over un-inoculated legumes were estimated as 105, 111 and 282% in lentil, mungbean and soybean respectively. The average highest amount of stover Ndfa (64.12 kgN/ha) was recorded from the treatment combination of inoculated soybean and the lowest amount was (11.18 kgN/ha) observed from the treatment un-inoculated mungbean.

Total Ndfa in legume

Considering the amount of total Ndfa, it was observed that the interaction effect of different legumes with and without inoculant significantly varied on the fixation of total Ndfa and during the year of 2004-05 and 2005-06, the maximum amount of total nitrogen (161.74 and 157.49 kg/ha) fixed from air by inoculated soybean, which was followed by inoculated lentil (70.56 and 67.70 kg/ha) and inoculated mungbean (56.64 and 56.69 kg/ha). The minimum total Ndfa was accounted from un-inoculated mungbean (25.65 and 26.19 kg/ha during 2004-05 and 2005-06). Due to application of rhizobial inoculant, the amount of total Ndfa increased as 123, 118 and 212% in lentil, mungbean and soybean, over un-inoculated situation. On average, the inoculated soybean fixed the highest amount of total Ndfa (84.18% of its N_2 amounting to 159.62 kg N/ha), which was followed by the quantity of total Ndfa in inoculated lentil (82.82% of its N_2 amounting 69.13 kg N/ha) and inoculated mungbean (% 80.73 of its N amounting 56.64 kg N/ha).

Different sources of estimated legume nitrogen

From our study, the total legume nitrogen and their proportional quantity derived from different sources such as - Ndfa, Ndfs (nitrogen derived from soil) and Ndff (nitrogen derived from fertilizer) were also estimated by using ^{15}N tracer technique (Figure 1). From the figure, it was clear that the maximum amount of N came from atmosphere source. When the different legume seeds were treated with rhizobial inoculant, the highest amount of Ndfa quantified in soybean (84.18% of its N_2 amounting 159.62 kg N/ha), which was followed by the amount of Ndfa in lentil (82.82%; and 69.13 kg N/ha) and mungbean (80.73%; and 56.64 kg N/ha). The minimum amount of 25.92 kg N/ha was noticed from un-inoculated mungbean.

The legume plant received a considerable amount of N from soil in un-inoculated situation, though some inefficient natural rhizobia were present in soil. Among the different treatment combinations, the highest amount of total Ndfs was recorded in un-inoculated soybean plots (34.29 kg N/ha), whereas, the lowest value of total Ndfs was noted in inoculated mungbean as 10.44 kg N/ha. The mean total Ndfs of un-inoculated soybean plot was about more than three fold higher than the total Ndfs found from inoculated mungbean. From the study it was realized that due to inoculation a considerable amount (on average) of total N saved from the soil, which expressed as 47.93% in inoculated soybean, 10.62% in inoculated mungbean and 11.25% in inoculated lentil, respectively.

Considering the nitrogen from fertilizer source, it was revealed that all legume plants received more nitrogen from fertilizer sources in un-inoculated situations comparatively than in inoculated ones. Among the different treatment combinations, significantly highest total Ndff was recorded as 10.17 kg N/ha from un-inoculated soybean, whereas, the lowest total Ndff was found as 3.08 kg N/ha from inoculated mungbean. From the mean data, it was observed that, in all legumes, a considerable amount of N saved from fertilizer sources due to inoculation, which expressed as 32.64, 11.14 and 10.47% in soybean, lentil and mungbean respectively.

Table 1. Ndfa (Nitrogen derived from atmosphere, kg/ha) of different legumes with and without inoculant grown at Tista Meander Floodplain soil, Rangpur.

Treatments / Year	2004-05 Seed	Stover	Total	2005-06 Seed	Stover	Total
Lentil (un-inoculated)	16.87d	14.22d	31.08e	18.31d	12.35e	30.67e
Lentil (inoculated)	41.38b	29.18b	70.56b	42.36b	25.30b	67.70b
Mungbean(un-inoculated)	14.50d	11.15e	25.65f	15.00d	11.20e	26.19f
Mungbean(inoculated)	31.00c	25.63c	56.64c	35.05c	21.59c	56.69c
Soybean (un-inoculated)	33.63c	16.22d	49.85d	35.08c	17.34d	52.42d
Soybean (inoculated)	94.3a	67.45a	161.74a	96.71a	60.78a	157.49a
CV (%)	8.60	3.8	6.39	4.54	5.13	3.42

Note: Values in a column under a factor/ interaction treatment having same letter do not differ significantly at 5% level of probability.

Contribution of legumes to N supply to the system

In legume-rice rotation, different legumes contribute to N supply to the system which causes a significant and positive yield effect on subsequent rice. From our experiment the contribution of different legumes was studied through calculation of the net N inputs to the system from atmospheric nitrogen fixation. The net N inputs to the system from nitrogen fixation have been calculated as the fixed nitrogen returned to the soil in the stover minus the soil nitrogen removed in the grain and the details of the experimental results from both locations and years are presented (Figure 2). Due to application of rhizobial inoculant, the net N inputs to the system showed highly significant variations and the higher amount of net N was added to soil system from the inoculated legumes whereas, in un-inoculated situation, the net N inputs showed a negative result. The maximum amount of net N was recorded (50.95 kg N/ha) from the treatment inoculated soybean which was followed by inoculated lentil (20.94 kg N/ha) and inoculated mungbean (17.88 kg N/ha). Without inoculant all the legumes comparatively added the minimum net N to the system and specially soybean showed a negative result (- 5.36 kg N/ha).

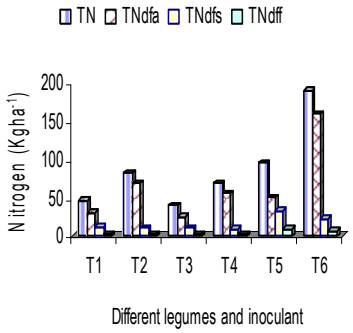

Figure 1. Total N, Ndfa, Ndfs, and Ndff of legumes as affected by inoculant (average of 2 years result data).

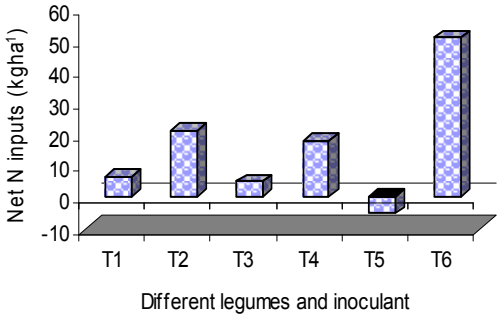

Figure 2. Net N inputs added to the soil system as affected by different legumes and inoculant (average of 2 years result data).

Note: T_1 = Lentil un-inoculated T_2 = Lentil inoculated
T_3 = Mungbean un-inoculated T_4 = Mungbean inoculated
T_5 = Soybean un-inoculated T_6 = Soybean inoculated

Conclusions

Quantity of total N (TN) and Nitrogen derived from atmosphere (Ndfa) in different legumes were significantly higher in inoculated plots comparatively than un-inoculated ones. The highest amount of total N was estimated as 189.63 kg N/ha in inoculated soybean and due to inoculation, the total N uptake increased as 77.36, 70.96 and 98.38% in lentil, mungbean and soybean plant, comparatively than un-inoculated legumes. Soybean crops fixed the highest amount of atmospheric N (84.18% of its N_2 amounting to 159.62 kg N/ha), which was followed by lentil (82.82% of its N_2 amounting 69.13 kg N/ha) and mungbean (% 80.73 of its N amounting 56.64 kg N/ha). The total net N inputs added to the soil system for succeeding crops was comparatively higher in inoculated plots than un-inoculated ones and the maximum net N inputs was observed as 50.95 kg N/ha from inoculated soybean.

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