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(RESEARCH ARTICLE)



Effect of different doses of nitrogen on nitrogen fixation and yield of lentil using tracer technique

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Abstract

Legumes play a predominant role in nitrogen cycling having the unique features of biological nitrogen fixation. An experiment was conducted to estimate yield, nitrogen use efficiency (NUE) and the proportion of nitrogen fixation in plant segments i.e. roots, shoots, leaves and seeds of lentil. Three treatments such as 0 kg/ha, 11.5 kg/ha and 25.3 kg/ha were used in this experiment. The highest dry matter yield obtained were 43.44 kg/ha, 2677 kg/ha, 1450 kg/ha and 1604 kg/ha at roots, shoots, leaves and seeds respectively with the treatment 11.5 kg/ha N level. ¹⁵N labelled urea was applied to wheat and lentil at 5% ¹⁵N atom excess. The total nitrogen content in lentil was found 8.39 % with an average yield of 104.5 kg N/ha whereas it was 1.95% with an average yield of 102.8 kg N/ha in case of wheat. Nitrogen derived from fertilizer in lentil was 1.173% with an average fertilizer N yield of 12.085 kg/ha whereas it was 7.538% with an average yields of 223.16 kg N/ha in wheat. Lentil fixed around 85% of its required nitrogen from atmosphere through biological fixation and a minimal amount of 14.387% of nitrogen was drawn from soil source. Nitrogen use efficiency in seed was 110.1 % in lentil and 36.22% in wheat which were much higher than the other plant segments in both cases.

Keywords: Lentil; ¹⁵N isotope; 5% Atom excess; NUE; Labeled urea

1. Introduction

Lentil (*Lens culinaris* Medik.) is one of the most important popular pulse crops in Bangladesh. Different varieties of pulses are grown throughout the country. Among the pulse crop, lentil constitutes the main source of protein and it is called poor man meat. It belongs to the leguminosae family and it is locally known as *masur*. It is thought to have originated in the Near East and then spread to Egypt, central and southern Europe, the Mediterranean basin, Ethiopia, Afghanistan, India and Pakistan, China and later to Latin America [1-2]. Lentil seeds contain 1-2% fat, 24-32% protein and minerals (iron, cobalt and iodine) and vitamin (lysine and arginine) [3-4]. These important nutritive values help to overcome malnutrition in our population. Due to the deficiency of these nutrition values, it can be hazardous for the health of the population living in the under developing countries in the world. The important feature of this legume crop is that it can fix atmospheric nitrogen by common soil bacteria (*Rhizobium* sp). These bacteria are a natural inhabitant in the nodules of this legume. Besides, its cultivation enriches soil nutrient status by adding nitrogen, carbon and organic matter which promotes sustainable crop production system [5]. Rhizobial inoculation alone is not enough for obtaining high yields of legumes because of poor nodulation and nitrogenase activity [6]. Global production of lentil was 6.3 million ton in the year 2016 [7]. In Bangladesh it is cultivated in about 154,449.49 hectares of land and total production

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of about 158228 tons of grain with an average yield of 1,073.5 kg/ha. It contributes about 38.4% of the total pulse production in the country [8]. Bangladesh is on 8th position in world ranking of lentil production which covers only 2.5% share of the total lentil produce of the world. The yield potential of lentil in Bangladesh is lower than that of the other lentil growing countries of the world like Canada, India, China etc. [9]. Low organic matter content of soil and non-judicious application of fertilizers are the main constrain for the low yield potential of lentil and other crop plants.

Nitrogen is the most essential nutrient that frequently limits the crop production. The availabilities and source of nitrogen fertilizer also affect crop yield and soil health. Having effective biological nitrogen fixations, legumes can therefore be grown without nitrogen fertilizer as atmosphere had had more than 70% nitrogen. Thus to understand the actual benefits of this plant-microbial interaction, it is essential to determine the amount of atmospheric nitrogen fixed by lentil and the nitrogen use efficiency under field condition for better crop management. A suitable method is therefore an important requirement in any study aimed at maximizing biological nitrogen fixation. A little information is available about the quantity of fixed nitrogen in lentil as influenced by a native rhizobial population. Therefore, the present study was undertaken to estimate the amount of atmospheric nitrogen fixed by lentil and nitrogen use efficiency of plants to improve the production and to develop the existing farmer's practice of N application in agricultural field.

2. Material and methods

Field experiment was carried out at regional sub-station of Bangladesh Institute of Nuclear Agriculture (BINA), Ishwardi, Pabna during the period of November-March 2017 to 2018. The experimental design followed randomized complete block design (RCBD) with four replications. The experimental plot size was 4.0m×3.0m. The experimental field was prepared by ploughing, harrowing and hand spade. Weeds, stubbles and crop residues were removed. The physiochemical prosperities of soil in the experimental field were: pH= 7.4, clay loam of soil texture, soil organic matter content of 1.61% on test, total nitrogen 0.069%, available phosphorus 12.18 cmol/kg, available potassium 0.140 cmol/kg, available sulphur 17.17 cmol/kg, cation exchange capacity (CEC) 30.2 cmol/kg. The recommended fertilizer doses of TSP were applied @160 kg/ha and MOP@110 kg/ha at the time of final land preparation.

In this study, BINA mosur-5 was selected to find out the effect of different nitrogen levels on the yield of lentil and also to estimate the amount of atmospheric nitrogen fixed using ¹⁵N. BARI wheat-30 was used as a reference crop. Seeds of lentil were sown on 24 November, 2017 and maintained a row distance 30 cm with a seed rate of 12 kg/ha. Intercultural operations were done as and when necessary. Three different concentrations of non-labeled (¹⁴N) nitrogen were applied at the rate of 0 kg/ha (T₀), 11.5 kg/ha (T₁) and 25.3 kg/ha (T₂) at the seedling stage of 15 days old seedlings in all the plots except the microplots. In the microplots, the labeled (¹⁵N) nitrogen with 5.18 atom% excess was applied at the same dose rate (T₂) but in the form of aqueous solutions. The control plot was kept free of nitrogen fertilizer. Light irrigation was applied two times within the growing period. First irrigation was applied at 42 days after planting and second was applied at pod filling stage of lentil. Weed control was done two times. Five hills from each plot were randomly selected for data collection i.e. plant height (cm), primary branch/plant, number of nodules/plant, fresh weight of nodules/plant and dry weight of nodules/plant at 50% flowering stage of the studied plant. Ten hills were randomly selected and uprooted from each plot for collecting data on yield and yield contributing characters such as on plant height (cm), number of primary branch/plant, filling pod/plant (no.), non-filling pod/plant (no.), and 1000 seeds weight (g). Data on seed yield (t/ha), straw yield (t/ha) and harvest index was taken from the microplots at the time of harvest. The collected data were analyzed statistically and means were adjudged by Duncan's Multiple Range Test (DMRT). For the estimation of ¹⁵N by using IRMS, the plant samples were collected in four distinct parts i.e. root, shoot, leaf and seed. These samples were then oven dried and grinded into fine powder form. After preparation of sample, data were analyzed using descriptive statistical method. Estimation of percent nitrogen derived from atmosphere (%Ndfa) can be made by the following equation:

$$\%Ndfa = \left(1 - \frac{\%Ndff F}{\%NdffNF}\right) \times 100$$

Where %NdffF and %NdffNF are percentage N derived from fertilizer by fixing and non-fixing plants, respectively.

The above equation is derived from the following [10-11].

$$\%NdffNF + \%NdfsNF = 100$$

$$\%NdffF + \%NdfsF + \%Ndfa = 100$$

$$\frac{\%N_{dffNF}}{\%N_{dfsNF}} = \frac{\%N_{dffF}}{\%N_{dfsF}}$$

Where $\%N_{dfsF}$ and $\%N_{dfsNF}$ are the percentage of N derived from soil in the fixing and non-fixing crops, respectively.

$$\% \text{ Ndff (Weighed average)} = \frac{\text{Total N ferti.yeild}}{\text{Total N yield}} \times 100$$

Nitrogen use efficiency of different plant segments was calculated using the following equation:

$$\text{NUE (\%)} = \frac{\text{Ndff} \left(\frac{\text{g}}{\text{m}^2} \right)}{\text{Applied N} \left(\frac{\text{g}}{\text{m}^2} \right)} \times 100$$

3. Results

Legume has the almost idiosyncratic attribute to quantify the contribution of biological nitrogen fixation (BNF) using the ^{15}N natural abundance technique that can be used without interference to the plant environment [12]. Nodule formation in roots plays the major part of this mechanism which can fix atmospheric nitrogen using rhizobium bacteria. Cultivation of lentil require lower amount of nitrogen fertilizer compared with other crops due to its own mechanism of BNF. Therefore, isotopic technique is necessary for better crop management and optimizing the art of cultivation. In this part, the yield and yield contributing traits of lentil are analyzed depending on the different fertilizer doses. The total nitrogen yield of the different plant segments and the amount of nitrogen derived from fertilizer were also estimated. Using isotopic techniques, it is also possible to determine nitrogen use efficiency. Finally, the atmospheric nitrogen fixed by the plant was also estimated.

3.1. Yield

Table-1 showed that the maximum (43.44 kg/ha) root yield was found with the treatment T1 (11.5 kg/ha N-level) and lowest value (37.65 kg/ha) was obtained at T0 (0 kg/ha N-level). In case of shoot yield the highest value (2677 kg/ha) was obtained at T1 and lowest (1720 kg/ha) was found at T2 (25.3 kg N/ha). In the amount of leaves, the maximum (1450 kg/ha) was found with T1 and lowest value (1080 kg/ha) was obtained at T0. The highest (1604 kg/ha) seed yield was recorded with T1 and the lowest value (1196 kg/ha) was obtained at T0. These data indicated that each parameter increased with the enhancement of nitrogen fertilizer at a certain level but decreased with the further increase of nitrogen. This might be due to the over doses of application of nitrogen fertilizer made toxic for the plant and also inhibited nodule formation.

Table 1 Mean comparison of yield and yield components of lentil under different N -levels

| Treatments | Root yield (kg/ha) | Shoot yield (kg/ha) | Leaf yield (kg/ha) | Seed yield (kg/ha) |
|------------|--------------------|---------------------|--------------------|--------------------|
| To | 37.65 | 1903 | 1190 | 1430 |
| T1 | 43.44 | 2677 | 1450 | 1604 |
| T2 | 38.01 | 1720 | 1080 | 1196 |
| Mean | 39.70a | 2100a | 1240a | 1410a |

This investigation is slightly different to Rabbi [13] and some other reports [14-17] who suggested that seed yield significantly increase with the increasing levels of nitrogen fertilizer. This difference may be due to the effect of genotypes of the plant studied, environmental conditions and the effect of nitrogenase enzyme. Sarker and Sing [18] reported about the significant effects of genotypes on the seed and straw yields of lentil. Significant genotype verses environment interaction was also observed for yield among a wide range of chickpea genotypes [19]. [20] Reported on the effect of locations and growing years on seed yield of field pea and lentil. Therefore, it is prerequisite to choose the appropriate pulse crops to maximize the production in a specific growth condition. All plant segments i.e. roots, shoots, leaves and seeds studied, it was observed that highest value was with the treatment of T1. Among the different plant segments studied, seed weight contained 27.78% of total dry matter compared with shoot (average of 46.36%) and leaf (25.11%). On the other hand, root showed the lowest value (0.75%) of total dry matter content. Therefore, it was found that seed yield was mostly relied on the yield of shoot. This result is somewhat different from the study of [21] and [22] where it was shown that seed yield was dependent on leaf weight. These might be due to the effect of genotypes.

3.2. Nitrogen uptake

In this study with lentil, it was found that the percent of total nitrogen uptake by root, shoot, leaf and seed were 1.295, 1.022, 1.778 and 4.295 respectively. In the amount of total N yield were 0.514 kg/ha, 21.46 kg/ha, 22.04 kg/ha and 66.56 kg/ha in the different plant segments of root, shoot, leaves and seeds accordingly. Among these plant segments, seeds showed the highest nitrogen uptake compare to other plant segments in both cases. This data also indicated that nitrogen was accumulated in seed more than (2 to 4 times) higher than other plant segments which is in accordance with the result of [23]. This table also showed that percentage of nitrogen derived from fertilizer (%Ndff) of root, shoot, leaf and seed were 0.468, 0.336, 0.219, and 0.150 respectively. Likewise the fertilizer N yield were 0.186 kg/ha, 7.061 kg/ha, 2.719 kg/ha and 2.119 kg/ha respectively. Results showed that the highest value of % Ndff was found in root. In wheat, table 3 showed the concentration of nitrogen in different plant segments i.e. root, shoot, leaf and seed were 0.411%, 0.373%, 0.936% and 1.950% whereas the total nitrogen yield were 2.92 kg/ha, 19.37 kg/ha, 17.49 kg/ha and 62.99 kg/ha respectively. These data indicated that the nitrogen concentration of shoot is less than that of other plant segments as can be expected due to the accumulation of nutrient for seed formation from shoots. This study showed that seed accumulated highest nitrogen uptake compare to other plant segments studied in both the cases. This table also showed that % Ndff of root, shoot, leaf and seed were 1.592%, 1.973%, 2.127% and 2.151%, respectively. On the other hand, the fertilizer nitrogen yield of root, shoot, leaf and seed were showed 11.30 kg/ha, 102.6 kg/ha, 39.78 kg/ha and 69.48 kg/ha respectively. In this study, it was found that the highest value of Ndff showed in seed. Ndff is less than total N content in both cases of percentage and amount of fertilizer nitrogen yield for all plant segments studied.

Comparing the fixing crop lentil with the reference crop wheat, a significant difference was found in case of total nitrogen content and nitrogen derived from fertilizer. The reference plant wheat accumulated more nitrogen in the soil applied with ¹⁵N -labelled Urea (Table 3), but the total N accumulated was far less (<5%) than that accumulated by fixing lentil plants (Table 2). It was obviously from the study that lentil fixed small proportion (up to 1.173%) from the applied labelled fertilizer of its nitrogen. In contrast, wheat derived up to 7.538% of its nitrogen from the labelled fertilizer. The fact that the fixing lentil plants had much lower ¹⁵N enrichment than the reference crop reveals that the lentil plants obtained very high proportions of their N from biological nitrogen fixation (BNF). From these data, it can be inferred that wheat meets up its nitrogen demand mainly from fertilizer and soil than lentil as wheat lacks the nitrogen fixing nodule.

Table 2 Nitrogen yield and fertilizer N yield parameters of different plant segments of lentil

| Plant segments | N contents (%) | | | N yield (Kg/ha) | | | Ndff (%) | | | Fertile N yield (kg/ha) | | |
|----------------|----------------|-------|-------|-----------------|-------|-------|----------|-------|-------|-------------------------|-------|--------|
| | Max. | Min. | Mean | Max. | Min. | Mean | Max. | Min. | Mean | Max. | Min. | Mean |
| Root | 1.511 | 0.976 | 1.295 | 0.600 | 0.387 | 0.514 | 0.578 | 0.360 | 0.468 | 0.23 | 0.143 | 0.186 |
| Shoot | 1.139 | 0.897 | 1.022 | 23.92 | 18.84 | 21.46 | 0.349 | 0.302 | 0.336 | 7.33 | 6.342 | 7.061 |
| Leaf | 2.085 | 1.469 | 1.778 | 25.85 | 18.22 | 22.04 | 0.274 | 0.187 | 0.219 | 3.40 | 2.319 | 2.719 |
| Seed | 4.688 | 4.031 | 4.295 | 66.10 | 56.84 | 60.56 | 0.171 | 0.126 | 0.150 | 2.41 | 1.777 | 2.119 |
| Total | | | 8.390 | | | 104.5 | | | 1.173 | | | 12.085 |

Table 3 Nitrogen yield and fertilizer N yield parameters of different plant segments of wheat under field condition

| Plant segments | N contents (%) | | | N yield (Kg/ha) | | | Ndff (%) | | | Fertile N yield (kg/ha) | | |
|----------------|----------------|-------|-------|-----------------|-------|-------|----------|-------|-------|-------------------------|-------|--------|
| | Max. | Min. | Mean | Max. | Min. | Mean | Max. | Min. | Mean | Max. | Min. | Mean |
| Root | 0.539 | 0.301 | 0.411 | 3.83 | 2.14 | 2.92 | 1.764 | 1.313 | 1.592 | 12.5 | 9.32 | 11.30 |
| Shoot | 0.42 | 0.284 | 0.373 | 21.84 | 14.77 | 19.37 | 2.188 | 1.873 | 1.973 | 113.8 | 97.40 | 102.6 |
| Leaf | 1.187 | 0.726 | 0.936 | 22.20 | 13.58 | 17.49 | 2.404 | 1.968 | 2.127 | 45.0 | 36.80 | 39.78 |
| Seed | 2.144 | 1.705 | 1.950 | 69.25 | 55.07 | 62.99 | 2.266 | 1.993 | 2.151 | 73.2 | 64.37 | 69.48 |
| Total | | | 3.670 | | | 102.8 | | | 7.538 | | | 223.16 |

3.3. Nitrogen use efficiency

In lentil, the maximum (110.1%) nitrogen use efficiency (NUE) was found in seed followed by other plant segments whereas the maximum (36.22%) nitrogen use efficiency (NUE) in wheat was also showed in seed followed by other plant segments (figure 1). However, lentil showed the higher proportion of nitrogen use efficiency than that of wheat plant segments in all the cases.

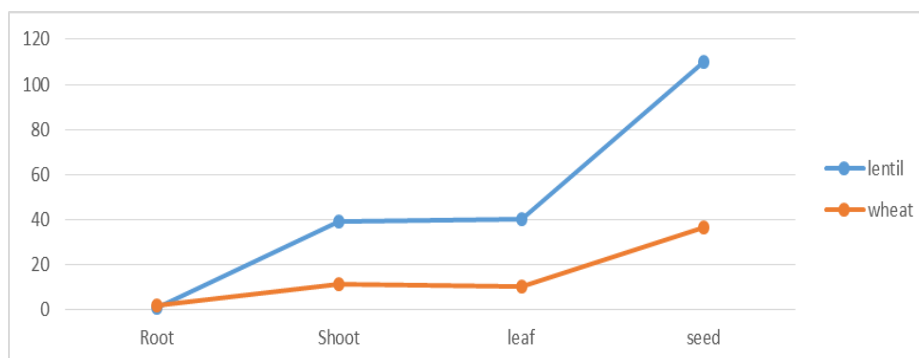


Figure 1 Comparison study of plant segments for nitrogen use efficiency (NUE) of lentil and wheat

3.4. Nitrogen fixation

Jensen *et al.* [24] reported that the amount of N fixed by the symbiotic relationship between legumes and the soil rhizobial bacteria is estimated by the relative dependence of the crop on biological nitrogen fixation for growth (i.e. %Ndfa) and the amount of N accumulated by the crop over the growing season. Significant variation in the amount of %Ndfa by various pulses species has been reported by different authors in the past. Many authors [25-27] reported that about 58-80% N is derived from the atmosphere under favourable condition. However, in this study, the maximum nitrogen obtained by lentil was up to 85% from atmospheric nitrogen through BNF which is in accordance with the earlier report [25]. The remaining proportion was up to 14% amount to 15.03 kg N/ha taken up from the soil. According to other researchers [27-29], symbiotic N fixation in lentil yields about 69 to 154 kg N/ha whereas 88.23 kg N/ha was found in this study. Those trends of %Ndfa were somewhat similar to our observations, although the amount of N fixed was relatively low in our study which may be due to the genotypic or environmental effect. Abi-Ghanem [30] also reported the genotypic background effects of biological nitrogen fixation in pulses, such as pea and lentil. Yang [31] found the lowest biological nitrogen fixation rate in Saskatchewan and concluded that it was due to the more dry conditions. Our results also indicated the significant impact of growing environment on %Ndfa and biological nitrogen fixation by lentil which is in agreement with study mentioned above.

Table 4 An overview of different sources of nitrogen accumulated by lentil under field conditions

| Plant segments | Proportion of nitrogen (%) | | | Amount of nitrogen (kg/ha) | | |
|----------------|----------------------------|-------|--------|----------------------------|-------|-------|
| | Ndff | Ndfa | Ndfs | Ndff | Ndfa | Ndfs |
| Root | 0.468 | | | 0.002 | | |
| Shoot | 0.336 | | | 0.072 | | |
| Leaf | 0.219 | | | 0.048 | | |
| Seed | 0.150 | | | 0.091 | | |
| Total | 1.173 | 84.44 | 14.387 | 12.084* | 88.23 | 15.03 |

4. Conclusion

This investigation showed the specific results of the amount of atmospheric N fixed in a particular environment and NUE of the plants in case of lentil. This study will also help to develop sustainable pulse production systems and better crop management.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare that there are no conflict of interest related to this paper.

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