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Research Paper

Biochemical Responses of Lentil (*Lens culinaris* Medik.) to Differential *Rhizobium* Inoculation and Starter Doses of Nitrogen

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ABSTRACT

Lentil production has always been important as it is the one of the most important *rabi* crops in India, but the low productivity of lentil in the country is mainly due to poor cultivation practices, infertile soil and low input of resources. Nitrogen is the largest nutrient required by plants. It is the most supplied plant nutrients as well as the most commonly deficient nutrient as its availability to plant has certain limit. Being a legume crop lentil has inherent capacity to fix atmospheric nitrogen to fulfil its nitrogen requirements through biological nitrogen fixation. Nitrogen fixation in legumes is governed by several factors like *Rhizobial* strains as well N availability in the soil. This study was undertaken to evaluate the response of Lentil crop to differential *Rhizobium* and starter nitrogen doses. Biochemical parameters like amounts of photosynthetic pigments chlorophyll 'a', chlorophyll 'b', carotenoids and nitrate reductase activity were recorded during different growth stages and the results are presented and discussed in this paper.

***Key words*: Lentil, Rhizobium, Nitrogen, Growth, Chlorophyll, Amino acids nitrate reductase**

Lentil (*Lens culinaris* Medik.) is one of the mankind's oldest and early domesticated food legumes. In developing countries like India, lentil constitutes a major source of dietary protein. In our country where food consumption exceeds production and poor and marginal farmers cannot afford costly chemical fertilizer, leguminous crops like lentil having capacity to fix sufficient nitrogen, high calorific values of food and forage utility have made special relevance and increasingly attractive. It can fix 46-192 kg N ha^{-1} [1-2]. However, in order to encourage the bacterial activity for biological nitrogen fixation, the application of starter doze of nitrogen is recommended during the early growing season [3]. Nitrogen fixation in legumes is governed by several factors like rhizobial strains as well N availability in the soil. Presence of nitrogen (N) plays significant roles in symbiotic nitrogen fixation through their effects on nodulation and fixation process [4]. If the availability of nitrogen in soil is sufficient, the crop favours fertilizer nitrogen uptake instead of nitrogen fixation [5-6]. Continuous and excess application of chemical fertilizer has negative impact on soil health and environment. It also degrades the soil fertility by disturbing the population of many beneficial microorganism which otherwise maintain the soil fertility. Rhizobium inoculation to enhance the plant nitrogen fixation capacity and to meet the nutrient requirement of leguminous plant by replacing inorganic nitrogen application would provide an alternative potential to enhance production on a sustainable basis [7]. Keeping in view the importance of nitrogen requirement and rhizobium inoculation on the growth and development of lentil the following investigation was undertaken.

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MATERIALS AND METHODS

The investigation was carried out as pot culture experiment in Department of Plant Physiology, Institute of Agricultural Sciences, Banaras Hindu University, Varanasi during the *Rabi* season of 2017-18. The lentil variety HUL-57 was procured from the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, Banaras Hindu University. *Rhizobium leguminosarum* strain was procured from the department of Microbiology, Indian Agricultural Research Institute, New Delhi. The strain was sub-cultured and maintained in Yeast Mannitol (YEM) growth medium (Broth) at Microbiology Laboratory, in the Department of Genetics and Plant Breeding, Institute of Agricultural Sciences, BHU. YEM broth contains mannitol as a carbon source and yeast extract as a source of both nitrogen and growth factors for bacteria. Lentil seeds were surface sterilized in 3% Sodium Hypochlorite (NaOCl) solution for 5 minutes to eliminate possible seed borne microorganisms and rinsed three times with sterile distilled water. Lentil seeds were inoculated by slurry method. The slurry composed of charcoal which provides carbonaceous nutrition to bacteria. It also contains Calcium Carbonate ($CaCO_3$) which provides stability and avoids rupture of bacterial membrane. Sugar is also used as a sticking agent. Recommended rate of liquid Rhizobial strain is 2.8 mL kg^{-1} seed. Based on these seeds were separately inoculated for full and half dose treatments respectively. The inoculated seeds were dried in shade for 3 hours. About 5-6 inoculated seeds were sown in each pot at 3-4 cm depth. Two healthy plants were maintained after emergence. The study included the following treatments T_0: Control – Rhizobium Zero (R_0) + Nitrogen zero (N_0), T_1: Rhizobium Zero (R_0) + Nitrogen half (N_H), T_2: Rhizobium Zero (R_0) + Nitrogen full (N_F), T_3: Rhizobium Half (R_H) + Nitrogen zero (N_0), T_4: Rhizobium Half (R_H) + Nitrogen half (N_H), T_5: Rhizobium Half (R_H) + Nitrogen full (N_F), T_6: Rhizobium full (R_F) + Nitrogen zero (N_0), T_7: Rhizobium full (R_F) + Nitrogen half (N_H) and T_8: Rhizobium full (R_F) + Nitrogen full (N_F). The observations were recorded at 30, 60 and 90 days after sowing. All the samples for biochemical estimation were taken between 10 am to 11 am in the morning. Leaf samples were collected from all the leaves and mixed for taking the required quantity of representative sample for estimation.

RESULTS AND DISCUSSION

Leaf chlorophyll content (Table 1) increased with increase in *Rhizobium* application. However maximum increase in chlorophyll "a", chlorophyll "b" and carotenoids content in lentil leaves was found highest in plants inoculated with full dose of Rhizobium and half dose of nitrogen application [8-12]. The increase in chlorophyll content can be attributed to greater availability of nitrogen through BNF compared to uninoculated and unfertilized (N). The chlorophyll synthesis depends on N nutrition, as it is one of the constituent elements of chlorophyll molecule that contains 4N in tetrapyrrole ring [13]. Deficiency of nitrogen in lentil cause significant reduction in chlorophyll "a" content [14].

Rhizobium inoculation increases total soluble sugar (TSS) content in lentil leaves [15]. Maximum increase in TSS content (Table 1) was found in full dose of *Rhizobium* inoculation couple with half dose of nitrogen. TSS content increased with nitrogen up to certain extent and start decreasing with increase in nitrogen level [16].

The fee amino acid content in leaves of lentil was found to be non-significant with respect to treatments. The accumulation of free amino acid in response to Rhizobium inoculation is wide spread among legume crop which in turn results in more protein synthesis and more protein content. Under limited availability of nitrogen free amino acid content in lentil leaves decline which could be a resultant effect of insufficient availability of nitrogen for synthesis of amino acid [17]. Increased nitrogen also promotes enzymatic activity which regulates various metabolic functions. The soluble protein content lentil was found to be increased with *Rhizobium* inoculation. It showed that the maximum increased in soluble protein content occurred with half dose of *Rhizobium* with half dose of nitrogen [18-20]. Proteins are the building block of plant body. Proteins are required for cell growth, enzymes and cell membranes. Increase in protein content due to rhizobium inoculation can be accounted to larger fixation of nitrogen and greater assimilation of NO_3 into amino acid for biosynthesis of proteins.

Table 1 Effect of *Rhizobium* inoculation under differential starter doses of nitrogen on photosynthetic pigments of lentil leaves at three different growth stages

Treatments	Chlorophyll 'a' content (mg g^{-1} FW)	Chlorophyll 'b' content (mg g^{-1} FW)	Carotenoids content (mg g^{-1} FW)	Total soluble sugar content (mg g^{-1} FW)	Free amino acid content (mg g^{-1} FW)	Total soluble protein content (mg g^{-1} FW)
T_0 (R_0N_0)	0.92	0.78	0.88	16.92	7.04	12.47
T_1 (R_0N_H)	1.26	0.87	0.95	17.33	7.45	16.23
T_2 (R_0N_F)	1.52	0.92	1.02	18.48	7.71	17.50
T_3 (R_HN_0)	1.48	0.88	1.49	17.76	7.06	22.74
T_4 (R_HN_H)	1.50	1.00	1.41	19.65	8.23	21.88
T_5 (R_HN_F)	1.52	1.07	1.43	20.59	8.56	19.04
T_6 (R_FN_0)	1.56	1.12	1.57	20.66	8.07	28.44
T_7 (R_FN_H)	1.56	1.14	1.61	20.78	8.26	30.67
T_8 (R_FN_F)	1.55	1.09	1.00	20.91	8.96	18.92
SE.m.±	0.08	0.07	0.41	0.89	0.38	0.60
CD (1%)	0.25	0.22	0.14	2.65	NS	2.45

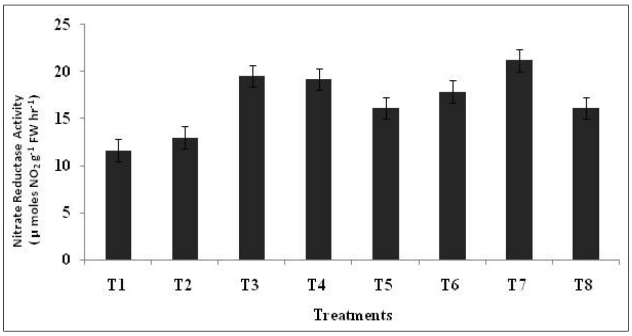

Fig 1 Effect of *Rhizobium* inoculation under differential starter doses of nitrogen on Nitrate Reductase Activity (µ moles NO_2 g^{-1} FW hr^{-1}) of lentil leaves

The application of *Rhizobium* and nitrogen influences nitrate reductase (NR) activity which was found to increase with increasing the dose of inoculation (Fig 1). The maximum NRA was recorded by plant with full dose of *Rhizobium* inoculation with half recommended dose of inorganic N. Similar reports have been suggested by [21-23]. The increase in NRA can also be attributed to de novo synthesis of m-RNA and enzyme in addition to post translational regulation by dephosphorylation and phosphorylation. Nitrate reductase activity decreased with addition of nitrogen fertilizer. This finding is in agreement with [24] who studied effect of N-fertilization and *Rhizobium* inoculation on in vivo nitrate reductase activity and nitrogen content of black gram. The decrease in NR activity with application of nitrogen may also be attributed to the fact that application of N causes reduction in formation of nodules which results in inhibition of N_2 fixation, as roots do not allow bacterial infection.

CONCLUSIONS

Leguminous crops have a major role in fixing atmospheric nitrogen and also enriching the soil for long term sustenance of soil health when compared to inorganic nitrogen fertilizers. Lentil is a resource deprived crop which is usually grown on nutrient deficient marginal soils without integrated nutrient management methods. In order to improve the lentil crop growth in such soils the study of *Rhizobium* inoculation was taken up along with in different doses, full and half recommended inorganic nitrogen fertilization. The study revealed that full dose *Rhizobium* inoculation when combined with half the dose of inorganic nitrogen fertilizer resulted in improved biochemical parameters studied such as amounts of photosynthetic pigments, total soluble sugars, total soluble proteins and improvements in nitrate reductase activity. The study indicates that use of *Rhizobium* inoculation even for the leguminous lentil crop is beneficial when it is grown under marginal or low fertile soils where rhizosphere soil microbial population is severely affected due to excess use of inorganic fertilizers. Further, it is also essential to substantiate the role of enzymes of nitrogen and carbon metabolism for the improvement of biochemical parameters studied.

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