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Anabaena as a Substitute for Urea–Nitrogen during Rice Cultivation in Wet fields of Barh Bihar India.

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Abstract

The nitrogen–fixing cyanobacterium Anabaena oryzae was found growing luxuriantly alongwith rice cultivars in the wet fields of Barh in Patna district of Bihar during rice growing seasons. However, they possessed few heterocysts possibly due to the persistent supplementation of synthetic chemical nitrogen fertilizers in the fields to enhance rice yield and such continuous application might have resulted in induction of chemical imbalance and nitrogen eutrophication in the paddy field soils. The cyanobacterium was isolated to unialgal state from the local paddy fields and cultured in mass using the standard trough method (Sahu et.al.,2012). Algalization of wet paddy fields with A. oryzae pellets at the rate of 10 Kg ha^{-1} resulted in 20 to 30 percent increase in yield components of the Lalat variety of rice over those of the field soils unsupplemented with Urea - N. The yield was also better in algalized soil than in soil supplemented with 30 Kg ha^{-1} Urea–Nitrogen. However, supplementation with a combined dose of 10 Kg ha^{-1} A. oryzae and 30 Kg ha^{-1} Urea–Nitrogen did not produce much significant alteration in the yield components of rice over their individual applications. The cyanobacterial population produces heterocysts at higher frequencies only on field soil which was not supplemented with Urea - Nitrogen. The results indicated that the cyanobacterium A. oryzae is an efficient source of natural bio-N-fertilizer and can serve as a substitute for chemical-N-fertilizer during rice cultivation. It can sustain better rice yield alongwith preserving the natural chemical balance of the paddy field soil coupled with the reduction in cost of rice production which is otherwise incurred in the procurement of industrially synthesized chemical fertilizers. The experiments were carried out in the paddy fields of Barh employing the methodology adoptedby Venkataraman (1992).

***Key words**: Nitrogen-fixing, Anabaena, bio-N-fertilizer, Substitute, Urea-N.*

Introduction

Nitrogen is an important element for plant growth and its availability in sufficient amount boosts the productivity of plants per unit area. The input of fertilizers, the chief source of nitrogen, thus becomes a basic requirement of modern intensive rice farming in order to fulfil the increased demand of nutrients to sustain their high yield. However, increased cost of fertilizers is becoming an economical constrains for the poor farmers who are in majority in India and hence, looking for alternative cheaper means of improving available nitrogen in the soil becomes crucial (Menamo and Woldge, 2016). Secondly, the continuous application of chemical fertilizers in the crop fields not only induces ecological and biochemical imbalance on the soil but also causes serious threat to human life. To overcome this problem the concepts of replacing chemical fertilizers by eco-friendly plant growth promoting Rhizobacteria, Mycorrhizal fungi, Cyanobacteria and many other microscopic organisms is gaining momentum. The approach has been successfully practiced in crop fields in many countries like China Uganda, Bangladesh and India. The innovative view of farm production attracts the growing demand of biological based organic fertilizers exclusively alternative to industrially manipulated agrochemicals (Raja, 2013) The practices have led to improved nutrient uptake, plant growth and plant tolerance to abiotic and biotic stresses (Bhardwaj et.al., 2014).

The contribution of Blue-Green Algae (cyanobacteria) as an alternative source of nitrogen supplement, particularly in the rice-fields, has been realized (Dey, 1939; Singh, 1961; Horne, 1971). It gave stimulus to the agricultural scientists to employ the algalization technology to improve rice yield and the results were encouraging (Venkataraman, 1979, 1992; Kaushik, 2000; Anand et.al., 2015). Pereira et.al.,(2009) have shown that indigenous isolates of cyanobacteria are more effective contributors to improved rice yield in comparison to their imported counterparts. Unfortunately, such studies have not properly been undertaken with the cyanobacterial flora growing in the rice fields of Bihar. The present investigation was, thus, undertaken to screen a few cyanobacterial species growing luxuriantly in the paddy fields of Barh (Patna), Bihar, during rice growing season for their efficiency to serve as the potential source of bio-N-fertilizer in the local rice fields.

Materials and Methods

Anabaenaoryzae, a filamentous; heterocystous and nitrogen fixing cyanobacterium , was found growing abundantly in the paddy fields of Barh during rainy season . The alga was collected from the paddy field , isolated to unialgal condition and then grown in Chu 10 (M) medium (Chu,1942) under standard cultural conditions (temperature 28 ± 2^0 C, light intensity 2500 Lux with a light /

dark cycle of 16h / 8h). Algal pellets for algalization in the paddy field were prepared from stock cultures using "*Trough Method"* (Sahu et.al., 2012). The Lalat variety of rice commonly cultivated in this locality was used as the test crop for screening the efficiency of *A. oryzae* as bio-N-fertilizer in place of chemical nitrogen fertilizer during rice cultivation in the selected paddy fields of Barh in Bihar.

The paddy field was divided into small plots of $5m^2$in size. The field soil was added with superphosphate at the rate of 70 kg $ha^{-1}$and 1 kg $ha^{-1}$of B.H.C. powder. It was further supplemented with 30 kg $ha^{-1}$of urea nitrogen and/or 10 kg ha^{-1} of *A. oryzae*. Prior to application of algal inoculums, the prepared soil surface was over layered with water up to 10 cm. in height adjusted to pH 7.5. The algal pellets were collected from stock culture, washed with distilled water, homogenized with the help of glass beads, washed again with double distilled water and then sprinkled over the surface of the plot water. Twenty days old rice seedlings of uniform health and size, obtained from a local farmer, were transplanted in experimental plots at the rate of two seedlings per hill and the hill to hill distance was maintained at 6 inches Venkataraman (1979).

The treatments were arranged following a randomized block design with three replicates for each treatment. The seedlings were allowed to grow under natural environmental conditions. Water was gently added in the plots at intervals to maintain its level and compensate the water loss due to evaporation from the surface. The plots in which the field soil was not supplemented with urea-N were designated as N^-whereas those supplemented with urea –N were referred to as N^+. Similarly the plots inoculated with *A. oryzae* were termed as, *Ao^+*whereas those uninoculated with the above algal species were named as Ao^- .The agronomic parameters such as height of the rice plants, number of panicle per plant, number of grains per panicle and weight of 1000 grains were recorded following random selection of ten hills from each of the three replicates of each treatment at the time of harvesting.

Results

The effect of algalization of the field soil with or without nitrogenous fertilizer on the growth and yield of *Oryzasativa* var. Lalat are presented in tables- 1 and 2

Table-1- Effect of cyanobacterialization on the growth and grain yield of Lalat variety of rice.

Soil treatment	Plant height cm.	Number of panicles per plant	Number of grains per panicle	Weight of 1000 grains gm.
N^-Ao^-	19.89 ± 1.16	5.0± 0.63	5.5± 0.79	4.37± 0.14
N^+Ao^-	50.47± 1.12	12.0 ± 0.84	11.8± 1.09	19.68± 0.25
N^-Ao^+	53.79± 0.82	12.8± 0.86	12.5± 0.94	19.87±0.16
N^+Ao^+	50.86± 1.00	12.1± 0.75	11.8± 0.75	19.77±0.21

Table-2- Heterocyst frequencies of *A. oryzae* growing in plots containing soil with or without urea-N.

Cyanobacterial species	Heterocyst Frequency	
	N^- plot	N^+ plot
A.Oryzae	5.6± 0.32	0.53± 0.22

The seedlings grew and produced grain containing panicles in all the experimental plots including the control in which the soil was neither supplemented with urea- N nor inoculated with any cyanobacterial inoculum. In these plots the rice plants attained an average height of 19. 89cms. The number of panicles per plant was 5.0 and the number of mature grains in a panicle was 5.5. The weight of 1000 grains was only 4.37 gm. However in plots supplemented with urea –N but not algal inoculum the mature plants attained an average height of 50.47cms. The number of panicles per plant was 12.0 and the number of grains per panicle was 11.8. The weight of 1000 grains in these plants was recorded to be 19.68 gm. The algalization of plot soil with *A. oryzae* in the absence of urea-N resulted in the attainment of plant height up to 53.79 cm. The number of panicles per plant was 12.8 and the number of grains per panicle was 12.5. The weight of 1000 grains was found to be 19.87gm. But algalization with *A. oryzae* in the presence of urea –N the plant height, number of panicles per plant, number of grains per panicle and weight of 1000 grains were recorded to be 50.86cm, 12.1, 11.8 and 19.77gm. respectively.

The heterocysts were formed with almost normal frequencies in the cyanobacterial species on plots where the soil was not supplemented with urea-N. But the heterocysts were formed rarely and with significantly reduced frequencies on the plots where the soils were added with both algal inoculum and urea –N.

Discussion

The present experimental results confirm the significant role played by nitrogen in the regulation of yield potential of rice as its Lalat variety has exhibited about two to three times higher growth and about five times increase in grain weight when grown on soil supplemented with urea–N than its growth and yield on soil without urea-N supplementation. The findings are in agreement with the observations of Patnaik and Rao (1979) where the rice plant has recorded five times more yield on soil with nitrogen fertilization than on soil without nitrogen fertilization. Nitrogen is one of the most important limiting factors which control the yield of rice during rice cultivation and hence the farmers are compelled to employ nitrogenous fertilizers in the paddy fields to sustain better rice yield. Although certain growth and yield of the Lalat variety has been obtained on nitrogen unsupplemented soil during the present investigations, it might be the result of pre- existence of traces of nitrogen in the test soil. Application of*A.oryzae* in the test soil, unsupplemented with urea – N, was found effective in enhancing the growth and yield of the Lalat variety of rice over those of nitrogen deficient soil. The results indicate the efficiency of this cyanobacterial species in promoting the growth and yield of rice in soil with negligible nitrogen fertilization. Similar observations were earlier made by Singh (1961), Subrahmanyan and manna (1966), Begum et.al., (2011). Roy (2013) had also indicated that cyanobacterial species of *Nostoc, Anabaena, Aulosira* and *Scytonema* have the potentiality to act as the source of N- fertilizers. Venkataraman (1981), Mian and Stewart (1985), Ladha et.al. (1987) have shown using N^{15}/N^{14} tracer technique that nitrogen from cyanobacteria is directly transferred to rice plants and around 40% of the cyanobacterial nitrogen has been found to be recovered by rice plants. The rice yield following treatment with cyanobacteria has been reported to be comparable to one following application of 30kg ha^{-1} chemical nitrogen fertilizer (Singh and Singh, 1989). In the present experimental screening as well the application of 10 kg ha^{-1} ofcyanobacterial inoculums in the test soil has given either equal or even slightly better yield of rice than with employment of 30 kg ha^{-1} of urea nitrogen as fertilizer.

The observation of either insignificant or very little increase in growth and yield of the Lalat variety of rice on soil supplemented with a combined dose of 30kg ha^{-1} urea-N and 10 kg ha^{-1} algal inoculums than on soil supplemented with only urea-N or the algal inoculums might be due to nitrogen as a depression in the presence of Nitrogenous fertilizer as the cyanobacterial species have shown reduced heterocyst frequencies in nitrogen supplemented soils. The repressed nitrogenase activity leads to inhibition of nitrogen-fixation which might have neutralized the significance of cyanobacterialization with a view to enhancing the yield of rice. Rodgers (1982) have found that nitrogen fertilizers at concentrations as low as 0.2µm markedly repressed cyanobacterial nitrogen-fixation in crop fields. The above experimental results, thus, confirm that *A.oryzae* has the potentiality to act as bio-N-fertilizer and can be employed in paddy fields as replacement of synthetic nitrogenous fertilizers during rice cultivation in the Barh locality of Bihar.

Conclusion

The nitrogen-fixing cyanobacterium *Anabaena oryzae* naturally growing in the paddy fields of Barh in Bihar ,can serve as an eco-friendly cost-free source of bio-N-fertilizer in place of synthetic eco-hazardous costly Urea as N-fertilizer during rice cultivation with sustained rice yield.

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