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EVALUATION OF *AZOTOBACTER VINELANDII* STRAIN SRIAZ3 BIOINOCULATION ALONG WITH N-SOURCE ON YIELD, YIELD ATTRIBUTES AND NUTRIENT UPTAKE OF RICE (*ORYZA SATIVA* L.) CULTIVAR

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Abstract

A field study with Rice (*Oryza sativa* L. cv. Pyari) was conducted in Agronomy Research Plot, OUAT, Bhubaneswar with eight (8) treatments and three (3) replications i.e. T_1- Control, T_2- N_1 (50% N), T_3- N_2 (75% N), T_4- N_3(100% N), T_5- *A. vinelandii* strain SRIAz3, T_6 - *A. vinelandii* strain SRIAz3 + N_1, T_7 - *A. vinelandii* strain SRIAz3 + N_2 and T_8 - *A. vinelandii* strain SRIAz3 + N_3. After harvest of the crop yield, yield attributes [as panicle length (in cm), number of grains per panicle, test weight (in gm), grain yield (t ha^{-1}), straw yield (t ha^{-1}), grain: straw ratio, and harvest index were recorded] and nutrient analysis (concentration and uptake of N, P and K) was performed. Plots treated with T7 found to have highest grain: straw ratio as well as harvest index i.e. 0.90 and 0.47 respectively at the same time T_7 treated plots were having highest yield in terms of both grain and straw i.e. 3.87 and 4.32 t ha^{-1}. Again T_7 treated plants performed maximum with respect to concentration (1.47% and 0.38% respectively) and uptake (56.97 and 14.73 kg ha^{-1} respectively) of N and K but on a contrary maximum performance in case of P concentration (0.22) and uptake (7.92 kg ha^{-1}) was found in plants treated with T_8. Application of PGPR and N fertilizers increased the N efficiency by increasing N content and N uptake in plants which reveals the fact that balanced application of both organic and inorganic sources of nutrients in various plant available forms is unavoidable in yield improvement of crops.

Key words: *A. vinelandii*, rice, harvest index, yield and uptake.

Introduction

Following photosynthesis, nitrogen fixation is the second most important process in crop production. Photosynthesis captures sunlight and produces energy and nitrogen fixation uses nitrogen gas to form ammonium. Nitrogen fixation can provide for free up to 300-400 kg N $ha^{-1}yr^{-1}$. The atmosphere comprises of ~78% nitrogen as an inert gas, N_2, which is unavailable to plants. Above every hectare of ground there are ~80000 tons of this unavailable nitrogen. In order to be converted to available form it needs to be fixed through either the industrial process (Haber Bosh Process) or through biological nitrogen fixation (BNF). Without these nitrogen-fixers, life on this planet would probably disappear within a relatively short period of time (Benson, 2001). This process of biological nitrogen fixation (BNF) accounts for 65% of the nitrogen currently utilized in agriculture, which eighty percent comes from symbiotic associations and the rest from free-living or associative systems. These include: (a) Symbiotic nitrogen fixing (N_2-fixing) forms, viz. Rhizobium, the obligate symbionts in leguminous plants and *Frankia* in non-leguminous trees, and (b) Non-symbiotic (freeliving, associative or endophytic) N_2-fixing forms such as *cyanobacteria, Azospirillum, Azotobacter, Acetobacter diazotrophicus, Azoarcus,* etc (Tilak *et al.*, 2005; Rifat *et al.*, 2010).

Azotobacter represents the main group of heterotrophic freeliving nitrogen-fixing bacteria. *Azotobacter* spp. are most specifically noted for their nitrogen fixing ability but they have also been noted for their ability to produce different growth hormones (IAA and other auxins, such as gibberllins and cytokinins), vitamins and siderophores. *Azotobacter* is capable of converting nitrogen to ammonia, which in turn is taken up by the plants (Kamil *et al*., 2008). Biofertilizers can fix atmospheric N through the process of biological nitrogen fixation (BNF), solubilize plant nutrients like phosphates, and stimulate plant growth through synthesis of growth promoting substances. *Azotobacter* represents the main group of heterotrophic freeliving nitrogen-fixing bacteria principally inhabiting neutral or alkaline soils. The occurrence of this organism has been reported from the rhizosphere of a number of crop plants such as rice, maize, sugarcane, bajra, vegetables and plantation crops (Arun, 2007). They derive food from the organic matter present in the soil and root exudates and fix atmospheric N. As chemical fertilizers degrade soil

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environment and reduce fertilizer use efficiency, biological nitrogen fixation (BNF) by *Azotobacter* sp. which have the potency to supplement 19-47 % of total N requirement, i.e., 0.4-0.9 t ha^{-1} (7-20 %) in rice (Choudhury and Kennedy, 2004), would be a wiser alternative. Microbial inoculum not only increased the nutritional assimilation of plant (total N, P and K), but also improved soil properties, such as organic matter content and total N in soil (Wua *et al*., 2004).

Materials and Methods

The present study entitled "*Evaluation of Azotobacter vinelandii strain SRIAz3 bioinoculation along with N-source on various yield related attributes and nutrient uptake of rice cultivar"* was carried out as a field trial conducted in the Agronomy Research Plot, OUAT, Bhubaneswar located at $20^{0}26'$N latitude and $85^{0}80'$E longitude and altitude of 30 m above mean sea level, which is 60km away from Bay of Bengal with Rice (*Oryza sativa* L.) variety Pyari as test crop with a total of eight treatments each replicated three times in a randomised block design mentioned as follows; T_1 - Control, T_2 - N_1 (50% N), T_3 - N_2 (75% N), T_4 - N_3 (100% N), T_5 - *A. vinelandii* strain SRIAz3, T_6 - *A. vinelandii* strain SRIAz3 + N_1, T_7 - *A. vinelandii* strain SRIAz3 + N_2 and T_8 - *A. vinelandii* strain SRIAz3 + N_3. Vermicompost was applied @ 2 t ha^{-1} to the respective plots at the time of final land preparation. The test crop received N-P_2O_5-K_2O @ 80-40-40 kg ha^{-1} in the form of urea, DAP and MOP respectively. After the harvest of crop by threshing following ripening and yellowing 80% grains the grains long with stovers were prepared for nutrient analysis and yield attributes s follows.

Effect of *Azatobacter* bioinoculation along with N source on yield and yield attributes of rice

The panicles from the randomly selected and tagged ten hills for taking biometric observations at harvest were used and various yield attributing observations as panicle length (in cm), number of grains per panicle, test weight (in gm), grain yield (t ha^{-1}), straw yield (t ha^{-1}), grain: straw ratio, and harvest index were recorded.

Panicle length from ten tillers selected from randomly labeled plants in each plot was recorded from base to the tip of the panicle. The mean value was calculated and expressed in centimeter. Ten panicles were selected randomly from ten labeled hills and then grains were separated and counted. The mean value was worked out and recorded as number of grains per panicle. One thousand grains were counted from randomly selected ten hills per treatment and their weight was recorded and expressed in grams as test weight. The grains were separated by threshing separately from each plot and were dried under sun for 3 days. Later winnowed and cleaned and then weight of the grains per plot was recorded. From the plot values, the grain yield per ha was computed and expressed in t ha^{-1}. Straw from each plot was dried under sun for 10 days, weight was recorded on complete drying and expressed in t ha^{-1}.

Grain: straw ratio

Grain: straw ratio of each treatment was calculated by dividing the grain weight with the corresponding straw weight.

Harvest index

The harvest index was worked out from grain, straw and chaff yields using the formula.

Harvesting index = Economic yield (t ha^{-1})/Biological yield (t ha^{-1})

Here the economic yield is the grain yield and biological yield is the total biomass (grain + straw + chaff) yield.

Effect of *Azatobacter* bioinoculation along with N source on nutrient concentration along with their uptake in rice

To estimate the nutrient uptake as affected by already mentioned treatments the grain and straw samples were analyzed for determination of N, P and K concentration following standard protocols.

The N content of grain and straw sample was determined by Kjeldehl method. NH_3 gas was extracted in a Kel-plus auto analyzer and the distillate was titrated against a standard 0.02N H_2SO_4 for N estimation. The P content of grain and straw was determined by Vanadomolybdo-phosphoric and yellow colour method after di-acid digestion. P estimation was done by spectrophotometer as described by Jackson, 1967.The K content of grain and straw was determined by di-acid digestion and subsequent filtration. K concentration was estimated by flame photometer as described by Jackson, 1967. N, P and K uptake by grain and straw of rice was estimated by multiplying N, P and K content of it's grain and straw with their respective yields.

Statistical analysis

Statistical analysis were performed by the software R version 3.2.2 and were tested with Duncan's new multiple range test at 5% critical range using the package "agricolae" and the graphs were constructed by using the software Graph Pad Prism 6.0. The values are the means of three replicates.

Result

Effect of *Azatobacter* bioinoculation and N sources on yield and yield attributes of rice

Data on grain and straw yield revealed (Table 1) that the plots treated with T_7 (*A. vinelandii strain* SRIAz3 + N_2) produced highest yield i.e. 3.87 and 4.32 t ha^{-1} respectively among all the treated plots followed by T_8 (*A. vinelandii strain* SRIAz3 + N_3) and T_6 (*A. vinelandii strain* SRIAz3 + N_1). Control maintained lowest grain and straw yield i.e. 1.71 and 2.57 t ha^{-1} respectively (Figure 1).

Grain: Straw ratio and harvest index followed similar pattern and found highest in T_7 (*A. vinelandii strain* SRIAz3 + N_2) i.e. 0.90 and 0.47 respectively.

Effect of *Azatobacter* bioinoculation and N sources on nutrient concentration along with their uptake in rice

The grain N, P and K concentration ranged between 1.05-1.47, 0.16-0.22 and 0.31-0.38% respectively (Table 2). Maximum N concentration of grain (1.47 %) was recorded in T_7 (*A. vinelandii strain* SRIAz3 + 75% N), while P concentration was maximum (0.22%) in treatment T_8 (*A. vinelandii strain* SRIAz3 + 100% N). However, the K concentration in grain was maximum (0.38%) on T_7 (*A. vinelandii strain* SRIAz3 + 75% N) as well as T_8 (*A. vinelandii strain* SRIAz3 + 100% N).The grain N, P and K uptake ranged between 18.01 – 56.97, 2.74 – 7.92 and 5.84 – 14.73 kg ha^{-1} respectively (Table 3). Maximum N and K uptake in grain i.e. 56.97 and 14.73 kg ha^{-1} in the treatment T_7 (*A. vinelandii strain* SRIAz3 + 75% N), whereas P uptake was maximum (7.92 kg ha^{-1}) in plots treated with T_8 (*A. vinelandii strain* SRIAz3 + 100% N). Again, the treatment T_7 (*A. vinelandii strain* SRIAz3 + 75% N) recorded maximum straw N (32.96 kg ha^{-1}), P (5.61 kg ha^{-1}) and K (67.39 kg ha^{-1}) uptakes closely followed by treatment T_8 (*A. vinelandii strain* SRIAz3 + 100% N).

Ranges of straw N, P and K concentrations (0.48- 0.76, 0.09- 0.13 and 1.02- 1.56 %) with their respective uptakes (12.35 – 32.96, 2.31 – 5.61 and 26.25 – 67.39 kg ha^{-1}) were revealed from further datas.

Discussion

Grain yield, harvest index and grain: straw ratios were significantly influenced by combined effect of *A. vinelandii* strain SRIAz3 and 75% N compared over other treatments. However, no signifcant difference was obtained in the straw yield among the bioinoculated and uninoculted plots except the plots receiving 50% N and control. Wua *et al*. (2005) achieved similar results. According to Wani *et al*. (2013) *Azotobacter* genus synthesizes auxins, cytokinins, and GA-like substances and these growth materials are the primary substances controlling the enhanced growth. These hormonal substances, which originate from the rhizosphere or root surface, affect the growth of the closely associated higher plants.

Biofertilizers (also known as bioinoculants), the organic preparations containing microorganisms are beneficial to agricultural production in terms of nutrient supply particularly with respect to N, P and K (Wani *et al*., 2013). Concentration of N in grain and N, P, K in straw was significantly influenced by the combined effect of *A. vinelandii* strain SRIAz3 and 75% N, while no significant difference was obtained in K concentration of grain. P contration in grain was significantly affected by integarted application of *A. vinelandii* strain SRIAz3 with 100% N. In case of straw no significant diiference was recorded in the plots receiving combined application of *A. vinelandii* strain SRIAz3 and doses (50, 75 and 100 %) of N, however, these treatments showed significantly higher N concentration in straw compared to the uninoculated and control.

Nutrient (N and K) uptake in grain and straw were significantly influenced by application of *A. vinelandii* strain SRIAz3 and 75% N compared to other treatments. However, integrating the nitrgen fixing strain with various doses (50, 75 and 100%) of N didn't influenced the the N uptake in straw. Kundu and Gaur (1984) reported that, inoculation effect of *A. chroococcum, P. striata* and *A. awamorii* on yield and nutrient uptake in rice green house condition appreciably increased the yield and uptake of nutrients with or without chemical fertilizers. Application of PGPR and N fertilizers increased the N efficiency by increasing N content and N uptake in plants. Abbasi *et al*., 2011 also opined that application of PGPR with N fertilizer increased the fertilizer N efficiency by increasing N content and N uptake in plants.

Conclusion

Application of *Azotobacter vinelandii* strain SRIAz3 isolated from SRI field (OUAT, Bhubaneswar) as bioinoculant along with N sources significantly enhances various attributes related yield along with uptake of applied nutrients which further contributed to a significant increase in yield. Which reveals the importance of balanced application of both organic and inorganic sources of nutrients in various plant available forms in yield improvement of crops.

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Table 1: Effect of bioinoculation and N sources on yield and yield attributing parameters

Treatments	Grain Yield (t ha^{-1})	Straw Yield (t ha^{-1})	Grain: Straw ratio	Harvest Index
Control	1.71^{d}	2.57^{c}	0.67	0.40
N1 (50% N)	2.56^{c}	3.55^{b}	0.72	0.42
N2 (75% N)	3.01^{bc}	3.92^{ab}	0.77	0.43
N3 (100% N)	3.36^{ab}	4.04^{ab}	0.83	0.45
A. vinelandii strain SRIAz3	2.55^{c}	3.72^{ab}	0.68	0.41
A. vinelandii strain SRIAz3 + N1	3.44^{ab}	4.20^{a}	0.82	0.45
A. vinelandii strain SRIAz3 + N2	3.87^{a}	4.32^{a}	0.90	0.47
A. vinelandii strain SRIAz3 + N3	3.60^{ab}	4.26^{a}	0.84	0.46
CV (%)	12.65	8.32	16.12	11.79

*Means averaged over three replicates represented by the same letter in columns are not significantly different ($p<0.05$).

Table 2: Effect of bioinoculation and N sources on nutrient concentration

Treatments	Grain nutrient concentration (%)			Straw nutrient concentration (%)		
	N	P	K	N	P	K
Control	1.05^{e}	0.16^{c}	0.34^{a}	0.48^{c}	0.09^{d}	1.02^{e}
N1 (50% N)	1.13^{de}	0.17^{bc}	0.31^{a}	0.65^{ab}	0.09^{d}	1.22^{d}
N2 (75% N)	1.21^{cd}	0.17^{bc}	0.31^{a}	0.68^{ab}	0.10^{cd}	1.32^{cd}
N3 (100% N)	1.28^{bc}	0.18^{abc}	0.31^{a}	0.69^{ab}	0.10^{cd}	1.37^{c}
A. vinelandii strain SRIAz3	1.20^{cd}	0.20^{abc}	0.35^{a}	0.54^{bc}	0.10^{cd}	1.40^{bc}
A. vinelandii strain SRIAz3 + N1	1.33^{b}	0.21^{ab}	0.35^{a}	0.71^{a}	0.11^{bc}	1.52^{a}
A. vinelandii strain SRIAz3 + N2	1.47^{a}	0.20^{abc}	0.38^{a}	0.76^{a}	0.13^{a}	1.56^{a}
A. vinelandii strain SRIAz3 + N3	1.35^{b}	0.22^{a}	0.38^{a}	0.72^{a}	0.12^{ab}	1.51^{ab}
CV (%)	5.09	11.73	16.38	12.64	6.24	4.94

*Means averaged over three replicates represented by the same letter in columns are not significantly different ($p<0.05$).

Table 3: Effect of bioinoculation and N sources on nutrient uptake

Treatments	Grain uptake (kg ha^{-1})			Straw uptake (kg ha^{-1})		
	N	P	K	N	P	K
Control	18.01^{e}	2.74^{d}	5.84^{e}	12.35^{d}	2.31^{f}	26.25^{e}
N1 (50% N)	28.96^{d}	4.35^{c}	7.94^{de}	23.12^{bc}	3.20^{e}	43.31^{d}
N2 (75% N)	36.41^{cd}	5.23^{c}	9.33^{cde}	26.60^{abc}	3.91^{cde}	51.64^{cd}
N3 (100% N)	43.08^{bc}	6.05^{bc}	10.43^{bcd}	27.87^{abc}	4.04^{cd}	55.33^{bc}
A. vinelandii strain SRIAz3	30.57^{d}	5.09^{c}	8.91^{cde}	20.08^{c}	3.72d^{e}	52.07^{cd}
A. vinelandii strain SRIAz3 + N1	45.82^{bc}	7.23^{ab}	12.06^{abc}	29.71^{ab}	4.62^{bc}	63.89^{ab}
A. vinelandii strain SRIAz3 + N2	56.97^{a}	7.75^{a}	14.73^{a}	32.96^{a}	5.61^{a}	67.39^{a}
A. vinelandii strain SRIAz3 + N3	48.60^{ab}	7.92^{a}	13.68^{ab}	30.74^{ab}	5.11^{ab}	64.38^{ab}
CV (%)	16.25	16.31	19.64	17.51	9.98	10.70

*Means averaged over three replicates represented by the same letter in columns are not significantly different ($p<0.05$).

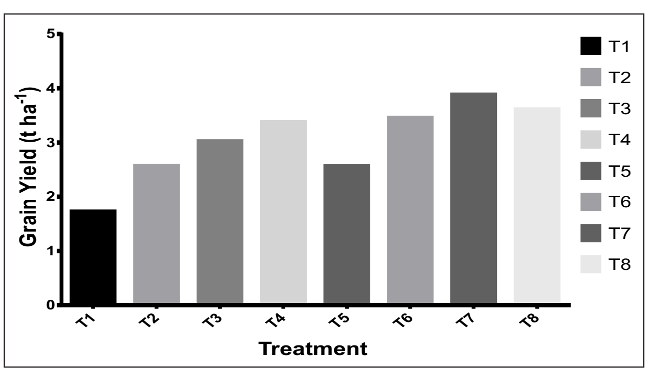

Figure 1: Effect of Bioinoculation and N sources on Grain yield (t ha^{-1})