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Field evaluation of native *Azotobacter* and *Azospirillum* spp. formulations for rice productivity in laterite soil

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ABSTRACT

Effects of indigenous biofertilizer formulations of six native isolates each of Azotobacter (Az.) and Azospirillum (As.) spp. of rice (var. Khandagiri and Pooja) rhizosphere, commercial formulations one each of Azotobacter and Azospirillum spp. in combination with N0 (without N), N30 (30 kg ha^{-1}) i.e. half (N/2) of recommended N and N60 (60 kg ha^{-1}) i.e. recommended N dose, and vermicompost (5 t ha^{-1}) were assessed on productivity of the drought tolerant rice var. Khandagiri in laterite soil fields of OUAT, Odisha. Compared to untreated control, rice production was increased by about 45% for N60 and 29% for N30; 52-120% and 43-109% for N60 and N30 with experimental biofertilizers combinations, respectively, whereas, 43-75% and 49-84% for commercial formulations with N30 and N60 combinations, respectively. Productivity for combined biofertilizers with N30 or N60 did not differ significantly. The experimental formulations performed better than the commercial formulations. Productivity was enhanced by about 23-92%, 21-65% and 27% by individual experimental and commercial formulations, and vermicompost, respectively. Combination of N/2 dose with the biofertilizers could reduce about half N requirement. Among different biofertilizers, the Az. vinelandii SRIAz3 and As. lipoferum CRRI1As6 formulations were most effective. Combination of these two organisms resulted in about 109% but with N/2 dose effected optimum (139-177%) rice production.

Key words: *azospirillum, azotobacter, BNF, formulation, rice, vermicompost*

Rice production depends highly on fertilizer N applications as most rice soils of the world are N deficient (Choudhury and Kennedy, 2004). By 2020, Indian rice production should increase from about 94 to 130 million tonne (MT) that would necessitate about 10 MT nitrogen excluding about 65% field loss (Sahrawat, 2000; Choudhury and Kennedy, 2004; Shrestha and Maskey, 2005; Bhattacharjee *et al*., 2008) which would be the limiting factor, especially for the N-responsive high yielding varieties (Shrestha and Maskey, 2005; Bhattacharjee *et al.*, 2008). As chemical fertilizers degrade soil environment and reduce fertilizer use efficiency of the crops, biological (mainly associative) nitrogen fixation (BNF) which would supply 19-47% of total N requirement i.e. 0.4-0.9 t/ha (7-20%) by *Azotobacter* and up to 1.8 t ha^{-1} (i.e. 22%) by *Azospirillum* spp. in rice (Choudhury and Kennedy, 2004; Shrestha and Maskey, 2005; Saikia and Jain, 2007; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010) and as plant associated N_2 fixation is not readily available for loss (Shrestha and Maskey, 2005), therefore, BNF would be the best N supplement strategy for rice.

In the flooded rice ecosystems, the *Azotobacter*, *Azospirillum, Rhizobium*, *Bacillus, Pseudomonas, Rodospirillum, Desulfovibrio, Enterobacter* spp., cyanobacteria etc. fix atmospheric nitrogen (about 80,000 t ha^{-1}), substitute 0.4-80 kg N ha^{-1} and other nutrients (Kanungo *et al*., 1997; Rao *et al*., 1998; Choudhury and Kennedy, 2004). Furthermore, the activity of N_2-fixing bacteria was reported to be generally higher in strains of cultivated rice than those of wild rice (Choudhury and Kennedy, 2004). Importance of BNF has inspired biomining and bioprospecting of the efficient diazotrophs for mass production, formulation, commercialization and field application for nitrogen supplement to different crops.

Nevertheless, rice being a monocot, associative N_2 fixing microbes like *Azotobacter* and *Azospirillum* would be the key components for *in situ* nitrogen fortification (Kader *et al*., 2000; Choudhury and Kennedy, 2004; Singh, 2006; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010). As *Azospirillum* is microaerophilic, it can function efficiently in flooded rice fields where N application is a difficult proposition. However, functionality of BNF is highly location specific and therefore, resident strains would be better suited (Choudhury and Kennedy, 2004; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010). Nevertheless, conflicting reports on enhancement of productivity by *Azotobacter* and *Azospirillum* spp. with or without various levels of N fertilizer, organic manure, compost, soil type have been recorded (Choudhury and Kennedy, 2004; Shrestha and Maskey, 2005; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010) and for last 20 years data has documented 60-70% successful field experiments with yield increase by 5-30%, especially with reduced N doses (Kanungo *et al*., 1997; Perrig *et al.*, 2007). Besides, *Azotobacter* and *Azospirillum* spp. could tolerate moderate levels of metals and other toxic compounds, and enhance N, P, Fe, Zn etc. uptake and plant growth under acidic condition (viz. laterite soils) (Govindan and Bagyaraj 1995; Rajaee *et al.,* 2007; Bashan and de-Bashan 2010) and would effectively enhanced rice production in problem soils. Unfortunately, probably no efficient and dependable BNF formulation for rice for laterite soils and eastern India has been developed to date. Therefore, native *Azotobacter* and *Azospirillum* isolates from the popular high yielding rice (*O. sativa* var. *Khandagiri* and *Pooja*) rhizosphere were selected for *in vitro* nitrogen fixation, formulated and evaluated in the fields of laterite soil along with locally available commercial biofertilizers of *Azotobacter* and *Azospirillum* spp. and compared with inorganic nitrogen sources and vermicompost using the popular high yielding drought tolerant rice (*O. sativa*) var. *Khandagiri*.

MATERIALS AND METHODS

Assessment was carried out (RBD, 3 replications) in the laterite soil fields in the Central Farm of Orissa University of Agriculture and Technology (OUAT), Bhubaneswar, Odisha, India in three cropping seasons during 2008-09 to unveil the efficient *Azotobacter* and *Azospirillum* formulations for commercial exploitation, especially in nutrition poor laterite soils. Experimental formulations of six *Azotobacter* spp. (Az) viz. SRI Az3, LTFE Az4, CRRI1 Az6, CRRI2 Az8, Majhi Az11 and Badam Az12a, and six *Azospirillum* (As) spp. viz. SRI As2, LTFE As3, CRRI1 As6, CRRI2 As7, Majhi As10 and Badam As11 rhizospheric strains of rice of system research intensification (SRI) and long term fertilizer experiment (LTFE), research fields at Majhisahi, Dhenkanal and Badamba of OUAT; Central Rice Research Institute (CRRI) field1 and field2, Cuttack, respectively, were assessed for improvement of production of drought tolerant rice (*O. sativa*) var. *Khandagiri*. Native *Azotobacter* and *Azospirillum* spp. were formulated aseptically comprising of (g/kg) charcoal powder 700, $CaCO_3$ 100, gum acacia 20 and liquid culture 180 ml containing10^9 cfu ml^{-1} i.e. final population 1.8 x 10^8 cfu g^{-1} formulation (Choudhury and Kennedy, 2004; Bhattacharjee *et al*., 2008). Commercial biofertilizer formulations of *Azotobacter* and *Azospirillum* spp. (details unknown due to IPR) were procured from the local market. The vermicompost was collected from the College of Agriculture, OUAT. The experiments were conducted in field plots (5 x 5 m sq.) of laterite soil in Central Farm of OUAT with recommended P and K (30 kg ha^{-1} each) doses and planted (20 x 20 cm spacing) with rice var. *Khandagiri* seedlings soaked for 2h in 10% (w/v) or 1.8 x 10^8 cfu ml^{-1} biofertilizer experimental or commertial formulations (Kader *et al.*, 2000; Singh, 2006; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010) individually or synergistically or in combination with N0 (fertilizer-free), N60 (recommended N 60 kg ha^{-1}), N30 (half N i.e. 30 kg ha^{-1}) doses and vermicompost (5 t ha^{-1}).

RESULTS AND DISCUSSION

The study was under taken by transplanting rice seedlings treated for 2 h with 1.8x10^8 cfu ml^{-1} bacteria which is a standard techniques among different methods viz. seed dipping in bacterial suspension for 5 min followed by drying under shade for 2–4 h, root dipping of rice seedlings in bacterial suspensions overnight before transplantation or application of bacterial suspensions to the rhizosphere of rice plants using 10^8-10^9 cells ml^{-1} (Wani, 1990; Kader *et al*., 2000; Choudhury and Kennedy, 2004; Singh, 2006; Bhattacharjee *et al*., 2008; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010) although the most efficient technique to deliver the bacteria to the plant for maximum output in terms of growth and production is not yet known (Bhatterjee *et al*., 2008). Effects of the *Azotobacter* and *Azospirillum* formulations on production of the rice var. *Khandagiri* in the laterite rice fields of OUAT are presented in table 1. Individually, the formulations showed positive impact on productivity without N supplement by about 23 and 92% (Table 1). The results supported the proposition of indifference or differential positive impact of diverse BNF strains on productivity (Kanungo *et al.*, 1998; Choudhury and Kennedy, 2004; Singh, 2006; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010). All BNF formulations and vermicompost increased rice productivity in formulations in combination with N/2 dose and 49-84% for BNF with N combination (Table 1). The experimental formulations had either comparable or better productivity than the commercial formulations (Table 1). Field evaluation by different researchers also proved that, depending on the genotype, BNFs like *Azotobacter* and *Azospirillum* spp. with recommended or reduced N would augment productivity by 0.4-2.3 t ha^{-1} (7–28%) (Kanungo *et al.*, 1997; Choudhury and Kennedy, 2004; Singh, 2006; Pedraza *et al*., 2009; Zaki *et al*., 2009). Combination of N/2 dose with the biofertilizers reduced N requirement without significantly effecting production in comparison to

Table 1. Effect of Azotobacter and *Azospirillum* spp. formulations on productivity of the rice variety Khandagiri with different N doses

Treatment	Productivity (g plant $^{-1}$) with different N level			CD
	N0 (kg ha^{-1})	N30 (kg ha^{-1})	N60 (kg ha^{-1})*	P0.05
Control	18.26	NA	NA	NA
N (60 kg/ha)*	NA	NA	26.54	NA
N/2 (30 kg/ha)	NA	23.69	NA	NA
Vermicompost (5 t/ha)	23.18	26.08	28.23	1.09
Azotobacter SRIAz3	35.15	38.22	40.21	1.07
Azotobacter LTFEAz4	29.21	30.97	33.12	1.11
Azotobacter CRRI1Az6	29.41	31.77	32.23	0.78
Azotobacter CRRI2 Az9a	28.50	31.07	33.41	0.98
Azotobacter MajhiAz11	29.71	30.61	30.99	0.77
Azotobacter BadamAz12a	32.12	34.00	36.12	1.07
Azospirillum SRIAs2	29.17	32.09	33.21	1.12
Azospirillum LTFEAs3	32.41	33.38	36.34	0.88
Azospirillum CRRI1As6	35.15	37.65	40.33	1.67
Azospirillum CRRI2As7	28.44	30.80	31.11	0.95
Azospirillum MajhiAs10	24.33	26.11	27.80	1.67
Azospirillum BadamAs11	27.42	31.54	33.78	1.78
Commercial *Azotobacter* ComAz	30.21	31.88	33.67	1.11
Commercial *Azospirillum* ComAs	22.11	26.09	27.17	1.88
CD (P=0.05)	0.98	1.10	1.06	NA

*Recommended N dose. All plots received recommended dose of P:K = 30:30 kg ha^{-1}. NA = Not applicable

combination with nitrogen (Tables 1, 2) which, however, contradicted the proposition that N application has negative effect on BNF (Shrestha and Maskey, 2005). For different treatments, improvement of productivity was by about 45% for recommended N (60 kg ha^{-1}), 29% for N/2 (30 kg ha^{-1}) dose, 54% for vermicompost with recommended N combination, 43% for vermicompost with N/2 combination, 52-120% for experimental formulations with N, 43-109% for N/2 with experimental formulation, 43-75% for commercial combination of recommended N and BNF (Table 1). The results corroborated the observations on enhancement of growth and production of different crops, including rice, either alone or in combination with recommended or reduced (N/2 or N/3) dose of inorganic fertilizers with *Azotobacter* or *Azospirillum* spp., maintenance of organic matter and available P of the post-harvest soil (Wani, 1990; Kanungo *et al.*, 1997; Kader *et al*., 2000; Kannan and Ponmurugan, 2010). Among different biofertilizers, both *Az. vinelandii*

Table 2. Effect of *Azotobacter* SRIAz3 and *Azospirillum* CRRI1As6 formulations with different nitrogen doses on productivity of rice var. *Khandagiri*

Treatment	Productivity (g plant^{-1}) with different N level			CD
	N0 (kg ha^{-1})	N30 (kg ha^{-1})	N60 (kg ha^{-1})	P0.05
Control	18.30	22.23	27.23	1.01
Azotobacter SRIAz3	19.20	37.92	40.89	2.56
Azospirillum CRRI1As6	19.28	37.55	41.86	2.78
Azotobacter SRIAz3- with *Azospirillum* CRRI1As6*	20.64	40.14	45.65	2.98
CD, P0.05	0.94	0.91	0.98	NA

All plots received recommended dose of P:K = 30:30 kg ha^{-1}. *Equal proportion of formulation mixture. NA = Not applicable

SRIAz3 and *As. lipoferum* CRRI1As6 formulations had optimum positive impact i.e. about 108% more productivity over untreated control and their combinations with N/2 dose resulted in optimum productivity, as well as, nominal improvement without N supplement (Table 1). Similar improvement of growth and productivity of rice was reported for both *As. lipoferum* and *Az. brasilense* isolates of roots and stems of rice plants (Choudhry and Knnedy, 2004). Higher productivity with combined BNFs and N fertilizer (Table 1) supported the observation on improvement of rice yield by vermicompost with or without nitrogen (Prajapati *et al*., 2009). Impact of combinations of different N levels with the *Az. vinelandii* SRIAz3 and *As. lipoferum* CRRI1As6 formulations either singly or synergistically is presented in table 2. Productivity was significantly enhanced for synergistic effect than the individual formulations either with full N (45.65, 40.89 and 41.86 g plant^{-1}, respectively) or with N/2 (40.14, 37.92 and 37.55 g plant^{-1}, respectively) doses but yield did not vary significantly between corresponding N and N/2 doses i.e. N/2 effected optimum productivity (Table 2). Synergistic effect of the two BNFs corroborated the result that combination of two *As. brasilense* strains (Pedraza *et al*., 2009) or *Azotobacter* with other BNFs (Kader *et al*., 2000) produced more than the individual strains. Contrary to optimum production for N/2 and *Az. vinelandii* SRIAz3 combination (Table 1), overall best growth and productivity of rice was observed for combined *Azotobacter* and ¾th recommended fertilizer application (Kanungo *et al*., 1997; Kader *et al*., 2000). Positive effect of BNF formulations without N supplement suggest that, probably formation and development of root branching, root hairs and primary and secondary lateral roots would increase due to the growth hormones secreted by the *Azotobacter* and *Azospirillum* spp. along with nitrogen fixation (Singh, 2006; Prajapati *et al*., 2009; Zaki *et al*., 2009; Keyo *et al*., 2011) which would enhance nutrient uptake capacity of roots (Kader *et al*., 2000; Kannan and Ponmurugan, 2010). It has been proved that associative N_2 fixation by *Azotobacter* and *Azospirillum* spp. would replenish about 19-47% N requirement of the crop i.e. would increase N accumulation by 0.4-1.8 t ha^{-1} (Kennedy and Tchan, 1992; Kanungo *et al.*, 1997; Choudhury and Kennedy, 2004; Shrestha and Maskey, 2005; Saikia and Jain, 2007; Zaki *et al*., 2009; Kannan and Ponmurugan, 2010). Tolerance to metals and acidic conditions of the BNFs would also support positive impact of the two BNFs (Govindan and Bagyaraj, 1995; Rajaee I *et al.* 2007; Bashan and de-Bashan, 2010). Thus, the results proved that root treatment with 10% (final BNF population 10^8 cfu ml^{-1}) *Az. vinelandii* SRIAz3 and *As. lipoferum* CRRI1As6 which are most common associates of rice (Choudhury and Kennedy, 2004) formulations (superior than local commercial formulations) transplanted with N/2 dose would significantly enhance rice productivity in laterite soil.

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