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Phenetic Characterization of Nitrogen Fixing *Azotobacter* from Rhizospheric Soil of Southern Rajasthan

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Abstract

The present research was conducted to characterize the indigenous plant growth promoting (PGP) *Azotobacter* strains isolated from plant root interface of semi-arid regions of Rajasthan (India) and to study their potential to be used as bio-fertilizers. A total of 172 *Azotobacter* strains were isolated, purified and based on the morphological test i.e. gram staining, pigmentation, cyst formation, fluorescence etc, broadly classified as *Azotobacter*. Further the secluded strains were examined for biochemical analysis and plant growth promoting characters. All the isolates showed different biochemical characteristics and significant PGP traits. IAA activity of the *Azotobacter* strains ranges from 54.5-6000 µg/mL. Ammonia, HCN and siderophore was produced by 92.4%, 78.4% and 80.23% of the total isolates respectively. Solubilization of phosphate was observed in 97.6% of the total isolates. These strains were also characterized for qualitative and quantitative N_2 fixation abilities and the result indicated that 114 strains showed positive results on nitrogen free malate agar medium (NFMM) containing bromothymol blue (BTB) and able to produce 18.93-475.6 N-moles C_2H_4 mg protein^{-1} h^{-1} of acetylene reduced by *Azotobacter* strains. *In vitro* pot studies revealed that the selected native *Azotobacter* strains having high ARA results significantly increase the plant growth characters. Shoot length, root length, root number and chlorophyll content and leaf number increases by 45.62%, 17.60%, 97.49%, 49.69% and 27.83% respectively in pot inoculated with AZO23-3 as compared to control. These effective strains can further be utilized for development of effective microbial formulations.

Keywords:- Biochemical Test, Cyst, Pigmentation, Antibiotic sensitivity, Acetylene Reductase Assay, Pot study

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INTRODUCTION

Excessive use of inorganic chemical-based fertilizers is not only cost intensive but also creates the problem of soil, agriculture and environmental management strategies. Organic farming is the most important component for improving plant, soil health and to overcome the uses of chemical fertilizers[1]. To search an alternative approach to usage of chemical fertilizers, the use of bio-fertilizer is gaining momentum in agriculture. Biofertilizers are organic fertilizers, which are composed of beneficial living microbial inoculants. These microorganisms promotes growth of plants and the healthy structure of soils by converting nutritionally nitrogen, phosphorus, potassium and many essential elements from unavailable to available form for plants[2]. Bio-inoculates also entail organic fertilizers and low input agricultural tool which manifest in an available form due to the symbiosis of micro-organisms and their association with plants[3].

One of important bio-inoculants is *Azotobacter* which is gram negative, aerobic and free-living N_2-fixing bacteria non-symbiotically fixes the atmospheric nitrogen, degrades cellulose, phosphates and most importantly it degrades lignin in trace amounts[4,5]. It is oval or spherical in shape and capable for forming thick-walled cysts under favorable condition[6]. *Azotobacter* strains binds with the beyond reach atmospheric nitrogen and liberate it in the form of ammonium ions which is utilized by plants to fix in the soil with an average of 20 kg N/ha per year. Moreover, *Azotobacter* is also able to multiply at 4.8-8.5 pH range and fixes atmospheric N_2 and the optimum pH range is 7.0-7.5[7]. The other beneficial effects of *Azotobacter* is that it helps in increasing the overall nitrogenase activity of soil and also posses keratinolytic activity[8,9]. The genus *Azotobacter* is an adjunct to *Pseudomonadaceae* family which is a subclass of γ-Proteobacteria. This subclass is comprehend of seven species: *Azotobacter vinelandii*, *A. chroococcum*, *A. salinestris*, *A. nigricans*, *A. beijerinckii*, *A. paspali*, and *A.armeniacus*[10]. *Azotobacter* survives in soil for longer duration due to the formation of cyst. *Azotobacter* also stimulates plant growth by facilitating the plants to uptake essential nutrients from the surrounding and they also produces phytohormones like auxins, gibberellins, cytokinins[11]. Therefore considering the above properties, *Azotobacter* sp. is used as a beneficial biological agent, extensively seek for a various crops like wheat, rice, sorghum, sugarcane, maize, sorghum, and maize[12]. To develop commercial biofertilizer the initial steps are isolation and characterization of native *Azotobacter* strains. Consequently, this study was designed to isolate and characterize *Azotobacter* for their various plant growth promoting characteristics and N_2 fixing attributes.

MATERIAL AND METHODS

Isolation of *Azotobacter* sp

The *Azotobacter* sp. was isolated on Nitrogen free Jensen's medium by serial dilution and spread plate technique[13]. The serial soil dilution of soil samples were done upto 10^{-6} and afterwards spread on Jenson medium plates. The inoculated petriplates were kept in invert position for incubation at 28±2°C for about 48 h. The *Azotobacter* colonies were large, ovoid, pleomorphic in shape and were purified on *Azotobacter* medium[14] and preserved in glycerol stocks.

Biochemical analysis of *Azotobacter s*p

Pure strains of *Azotobacter* sp. were isolated from soil samples and were characterized according to procedure mentioned in Bergey's Manual of Systematic Bacteriology[15]. The cyst formation was induced according to the procedure of Socolofsky and Wyss[16] and the staining of cysts were done by the method of Vela and Wyss[17]. Under biochemical characteristics, starch hydrolysis, H_2S production, TSI test, Nitrate reduction, Urease, Catalase and Oxidase reaction were studied. Carbon source *i.e.*, glucose, mannitol, lactose and sucrose utilization were also assayed. Antibiotic sensitivity of *Azotobacter* isolates were determined by disk agar diffusion method[18] using standard concentration of antibiotic discs of Ampicillin, Rifampicin, Kanamycin, Chloramphenicol and Penicillin.

PGP activitiesIAA Production

Quantification measurement of IAA production was followed by the method as described by Gordon and Weber[19]. The produced IAA concentration in the culture was measured by plotting the standard graph of IAA obtained in the range of 10-100µg/ ml.

NH_3 Production

Ammonia production was tested for *Azotobacter* strains as described by Cappuccino and Sherman[20]. Overnight grown bacterial culture was inoculated in 10 ml peptone broth and incubated at 35±0.1°C for 48 h in shaking postion. 0.5 mL of Nessler's reagent was added after incubation.

HCN Production

Azotobacter culture was streaked on nutrient agar medium, which containedglycine (4.4g per liter) and in the same petriplate a 0.5% picric acid solution (in 2% sodium carbonate) soaked Whatman filter paper was placed inside the lid. These plates were sealed with parafilm and incubated at 25 ± 2°C for 4 days and the color change of filter paper was observed (Castric, 1977)[21].

Siderophore Production

Siderophore production was assayed on the Chrome azurol S agar medium as described by Schwyn and Neilands[22].

Phosphate solubilizing activity

The potential to solubilize insoluble phosphates on the modified Sperber's medium was determined by measuring clear zone around the colonies[23].

Estimation of Nitrogen production and fixation

Qualitative estimation

For qualitative estimation of nitrogen production Nitrogen free Malate media, containing with bromothymol blue (BTB) as an indicator, was used for detection of nitrogen fixing activity. Plates were inoculated with purified isolates and incubated at 37ºC up to 24 h. Those isolates producing blue colored zone on Nitrogen free Malate media were considered nitrogen fixers and they were further tested by quantification of nitrogen fixation by Kjeldahl and acetylene reduction activity (ARA) method.

Nitrogen Production

Total nitrogen fixation by the *Azotobacter* isolates in the growing medium was quantified by the method[24].

Total Nitrogen was calculated and expressed as % (percentage) using the formula:

Quantitative estimation by ARA

Quantification of nitrogen fixation was carried out by ARA assay following the method of Stewart et al.[25] Briefly, five ml of modified Ashby's medium[26] were inoculated with *Azotobacter* and incubated at 30°C for 24 h. Further, the cotton plugs of the tubes were replaced with rubber stopper and 5 ml head space was injected with 10% (v/v) acetylene and incubated at 30°C for 2 h. The C_2H_4 production was measured using gas chromatography and the ARA values were recorded as N moles C_2H_4 mg protein^{-1} h^{-1}.

***In vitro* studies on the effect of selected *Azotobacter* on growth of maize seedling**

The pot experiment with selected *Azotobacter* strains having high nitrogenase activities were conducted in plastic pots filled with sterile perlite/vermiculite/coco-peat mixture in triplicate as per CRD design on maize (FEM-2 variety)[27]. Various plant growth parameters like leaf number, chlorophyll content of leaf, average shoot length, root length and root numbers, were analyzed.

RESULT AND DISCUSSION

Isolation and characterization of the *Azotobacter* strains

Total of 172 *Azotobacter* strains were isolated from different rhizospheric soil samples on *Azotobacter* selected media. The phenetic characteristics of *Azotobacter* strains are summarized (supplementary sheet Table 1). Most of the bacterial colonies isolated were circular (even) in shape, whitish in colour and the size ranges between 1.0-4.0 mm in size Jimenez et al.[28], reported that most *Azotobacter* isolates showed circular form, entire margin with whitish (cream color), smooth, irregular, shining, 3-8mm diameter colonies. All the bacterial isolates were gram negative. Out of 172 isolates, 22 produced water soluble yellow pigment, 10 produced brown pigment, 2 isolates produced red pigment. Out of 172 isolates, 27 showed a green fluorescence, 3 presented a yellow fluorescence and 5 displayed a red fluorescence. Only two isolates did not form cyst, remaining 170 isolates formed cyst. Shaikh and Mohd. Shakir[29] performed cyst formation test on 39 *Azotobacter vinelandii,* and found that 38 out of 39 *Azotobacter* produced cyst.

All the bacterial isolates were characterized by detecting their biochemical characteristics. Results of the investigation results indicated that out of 172 isolates, 129 isolates were found to hydrolyze starch; 146 isolates were

Table 1. Qualitative and quantitative estimation of nitrogen fixation by *Azotobacter* strains

S.No.	Isolates	N_2 Fixed (PPM)	Nmoles C_2H_4 mg protein^{-1} hr^{-1}
1.	AZO1-1	48.95	59.30159
2.	AZO1-2	48.95	87.79894
3.	AZO1-4	80.68	62.86772
4.	AZO2-2	35.36	56.95238
5.	AZO2-3	123.73	57.48148
6.	AZO2-11	42.16	49.24868
7.	AZO2-12	395.65	433.0931
8.	AZO2-13	42.16	34.08466
9.	AZO2-15	42.16	201.3968
10.	AZO3-6	60.28	27.30159
11.	AZO3-7	80.68	59.66138
12.	AZO4-5	55.75	50.74074
13.	AZO4-7	28.56	84.87831
14.	AZO4-11	17.23	125.1534
15.	AZO5-2	67.08	22.46561
16.	AZO6-1	37.62	30.7619
17.	AZO6-2	89.74	43.82011
18.	AZO6-3	64.82	76.8254
19.	AZO7-1	53.49	41.60847
20.	AZO8.2	64.82	399.7778
21.	AZ010-1	85.21	36.10582
22.	AZO11-1	48.95	22.67725
23.	AZO11-2	37.62	55.49206
24.	AZO12-2	58.02	22.35979
25.	AZO13-1	53.49	62.16931
26.	AZO13-2	60.28	29.8836
27.	AZO14-1	151.22	53.33333
28.	AZO17-1	112.4	51.78836
29.	AZO17-4	53.49	53.67196
30.	AZO17-7	64.82	55.24868
31.	AZO17-8	80.68	78.13757
32.	AZO17-10	67.08	56.78307
33.	AZO17-11	78.41	133.1217
34.	AZO17-12	139.59	41.01587
35.	AZO19-1	80.68	124.6243
36.	AZO19-2	44.42	43.60847
37.	AZO20-1	123.73	290.0106
38.	AZO20-2	46.69	372.836
39.	AZO20-3	46.69	107.9577
40.	AZO21-1	105.6	121.8201
41.	AZO22-1	39.89	304.6772
42.	AZO23-2	193.98	86.61376
43.	AZO23-3	35.36	475.6402
44.	AZO23-6	114.67	48.2963
45.	AZO23-7	51.22	39.84127
46.	AZO25-1	68	54.93122
47.	AZO25-1	56.4	54.93122
48.	AZO26-1	79.32	80.06349
49.	AZO30-1	98.2	55.34392
50.	AZO30-2	178	164.328
51.	AZO31-1	191.2	23.86243
52.	AZO32-1	102.4	39.89418
53.	AZO33-1	100	158.3175
54.	AZO34-2	198	60.07407
55.	AZO35-1	77.22	51.59788
56.	AZO35-2	121.1	62.69841
57.	AZO36-2	150.1	41.01587
58.	AZO37-1	139.2	59.64021
59.	AZO38-1	67	58.68783
60.	AZO39-1	89.2	92.07407
61.	AZO39-2	56.89	37.10053
62.	AZO40-1	56.22	88.49735
63.	AZO40-2	120	132.4021
64.	AZO41-1	122.4	72.79365
65.	AZ041-2	78.05	80.57143
66.	AZO42-1	67	175.3228
67.	AZO43-1	89.22	46
68.	AZO44-1	108	48.07407
69.	AZO45-1	78	38.66667
70.	AZO45-2	178	43.51323
71.	AZO46-1	152.1	34.19048
72.	AZO46-2	98.2	65.15344
73.	AZO47-1	111.1	128.0476
74.	AZO47-2	156	74.26455
75.	AZO48-1	89	58.71958
76.	AZO49-2	102.3	98.67725
77.	AZO51-1	101	24.56085
78.	AZO52-1	39.89	93.74603
79.	AZO52-4	103.34	123.164
80.	AZ052-5	62.55	36.31746
81.	AZO52-6	58.02	93.56614
82.	AZO56-1	189	53.14286
83.	AZ056-2	152	45.95767
84.	AZO4.3	70	191.7778
85.	AZO4.6	63	35.28042
86.	AZO5.4	79	116.0317
87.	AZO6.4	57	42.6455
88.	AZO8.3	71	178.6349
89.	AZO8.4	48	145.3122
90.	AZO11.5	53	61.19577
91.	AZO19.4	49	33.33333
92.	AZO52.7	64	53.68254
93.	AZO52.8	80	70.44444
94.	AZO52.9	66	21.01587
95.	AZO52.10	36	72.77249
96.	AZO DP-5	55	18.93122
97.	AZO DP-6	99	69.12169
98.	AZOS-4	70	148.0106
99.	AZOS-5	85	29.50265
100.	AZO AV	69	42.19048
101.	AZO5 M	78	106.3386
102.	AZO5 AI	83	95.66138
103.	AZO5AII	61	29.25926

104.	AZO6PG1	51	111.873
105.	AZO15M	89	41.93651
106.	AZO7AII	75	77.38624
107.	AZO4 A	68	24.56085
108.	Azo12A	80	55.64021
109.	AZO6A1	78	337.7037
110.	AZO6AII	77	50.2963
111.	AZO7M	44	33.62963
112.	AZOMAAM	55	89.54497
113.	AZO15AI	49	107.3968
114.	AZO2W	95	50.58201

found to produce H_2S; 163 isolates hydrolyze urea; 151 isolates gave positive reaction for oxidase test; 153 isolates reduces nitrate and 117 isolates gave positive reaction for TSI test. All the isolates gave positive reaction for catalase activity. The results of biochemical properties of *Azotobacter* species observed by Tejera et al.[30] were in close agreement with the results observed in the present study. These findings clearly indicated the distribution of *Azotobacter* species in different soil ecosystem. Ahmad et al.[31] studied the biochemical tests in *Azotobacter* species and reported that *Azotobacter* are positive for Catalase, and Starch hydrolysis. Similarly, Shrivastava[32] also reported all the isolates were positive for catalase. Most of the *Azotobacter* isolates utilize sucrose, lactose, mannitol and glucose as carbon source used in the present study. Out of 172 isolates, 18 isolates utilize 4 sugars; 13 isolates utilize 3 sugars; 42 isolates utilize 2 sugars and 78 isolates utilize 1 sugar as carbon source.

For antibiotic resistance, 172 isolates of *Azotobacter* strains were tested by the antibiotic disk method on Jenson medium plates. Table 2 in supplementary table sheet shows the resistance pattern of 172 *Azotobacter* isolates against Ampicillin, Rifampicin, Kanamycin, Chloramphenicol and Penicillin antibiotics discs. Multiple antibiotic resistances were seen in a majority of the isolates. Out of 172 strain 105 isolates showed sensitivity and 87 shows resistance against Ampicillin. Only 3 strains showed resistance and 169 were sensitive for Rifampicin. 122 isolates were sensitive and 50 are resistant for Kanamycin. Only one strain out of 172 shows resistance for Chloramphenicol. 85 *Azotobacter* strains were found resistant to penicillin while 87 were sensitive to penicillin. Bhaduri et al.[32] studied among 18 isolates of Asymbiotic N_2-fixers (*Azotobacter*) and symbiotic N_2-fixers (*Rhizobium* spp for the antibiotic resistance and susceptibility of against different antibiotics including Chloramphenicol and Rifampicin. The antibiotics ampicillin, chloramphenicol, kanamycin, penicillin and rifampicin resistance tested on 117 isolates of *A. chroococcum* were tested by the antibiotic disc method by Sindhu et al.[33].

Plant growth promoting (PGP) activities

Pink color appearance in bacterial culture after addition of orthophosphoric acid and Salkowski reagent to supernatant in LB broth confirmed that bacterial strains are positive for IAA production. In the present study IAA activity ranges from 54.5-6000µg/mL. Maximum IAA production 6000µg/ml was observed for isolate AZO 17-6 followed by 1500µg/mL for isolates AZO1-1, AZO1-3, AZO2-8, AZO4-11, AZO17-2, AZO17-3, AZO17-4, AZO22-2 and AZO22-4. Minimum IAA production 54.5µg/mL was observed for isolate AZO 53-1. Ahmad et al.[35] reported that *Azotobacter* spp. produced 38.82 µg/mL IAA in culture medium supplemented with Tryptophan at the rate of 5mg/

Table 2. *In vitro* studies on the effect of *Azotobacter* strains on growth and yield of maize seedling

S.No.	Treatment	Average Shoot Length (cm)	Average Root Length(cm)	Average Root Number	Average Leaf number	Total Chlorophyll (µg/ml)
1	Control	13.7±0.12	34.6±0.66	9.96±0.58	6±1.0	35.74±0.95
2	AZO2-12	18.75±0.20	39.7±0.95	18.93±1.15	6.67±0.58	51.57±1.90
3	AZO8.2	17.12±0.50	37.37±0.96	13.67±0.58	6.67±1.53	46.76±1.49
4	AZO22-1	15.59±0.38	36.75±1.05	14.67±0.58	6.0±0.0	39.91±1.20
5	AZO23-3	19.95±0.51	40.69±1.03	19.67±0.58	7.67±0.58	53.50±1.15
6	AZO6A1	16.56±0.52	37.12±0.98	15.33±1.53	6.0±0.58	45.72±1.27

Value ± SD; Data are recorded after 30 days of germination

mL. Kannapiran and Ramkumar[36] have reported that the amount of IAA produced by *Azotobacter chroococcum* and *Azotobacter beijerinckii* were 23.6 and 17.6 µg/mL respectively. Deshmukh and Vidhale[37] isolated four *Azotobacter* species from the rhizospheric soil in the alluvial valley of Purna river and its tributaries occupy parts of Amravati and found to produce high level (9.2 to 40.0mg/mL) of IAA. According to Mali and Bodhankar[38], 25 isolates of *Azotobacter chroococcum* isolated from the rhizosphere soil of groundnut were tested for production of IAA in 55 µg/mL.

The production of ammonia is an essential PGPR trait that can influence the plants growth indirectly. Estimation of ammonia production examined by the development of faint yellow to dark brown color after addition of Nessler's reagent to overnight grown bacterial culture in peptone broth indicated 92.4% ammonia production by the bacterial isolates the. These results are similar to those of Ahmad et al.[39], who revealed the production of ammonia commonly detected in the *Azotobacter* isolates. HCN production was indicated by the change in yellow to brown to red color of the filter paper strips. Out of 172 isolates, 78.4% isolates gave positive reaction for HCN production. For siderophore production, formation of yellow orange halo around the colony on CAS agar medium showed that 80.2% of 172 isolate produce siderophore. Altaf and Malik[40] examined the 15 *Azotobacter* sp. for various PGPR activity and found that 33% and 80% of the isolates showed HCN and siderophore production respectively. Solubilization of phosphorous was indicated by observing for the zone of clearance around the bacterial colony in the plates. Out of 172 isolates, 97.6% isolates solubilize phosphate producing clear zone around the colony. The results observed were in close agreement with the previous works of Farajzadeh et al.[41].

Estimation of Nitrogen production and fixation

Azotobacter strains were preliminary screened for nitrogen fixation on nitrogen free Malate agar medium containing Bromothymol Blue (BTB) as an indicator. Out of 172 bacterial isolates, 114 isolates produced blue color zone on medium indicating fixation of nitrogen by them. Further, these 114 *Azotobacter* isolates were quantified for nitrogen production by Kjeldahl method. Nitrogen production ranges from 17.23-395.6 ppm. Maximum nitrogen production 395.6 ppm was observed for isolate AZO2-12 followed by 198 ppm for isolate AZO34-2. Minimum nitrogen production 17.23 ppm was observed for isolate AZO4-11.

All the 114 *Azotobacter* isolates were screened for acetylene reduction ability (ARA). The comparison of nitrogenase activity of all efficient nitrogen-fixing isolates is shown in Table 1. All the isolates showed nitrogenase activity ranging from 18.93-475.6 Nmoles C_2H_4 mg protein^{-1} hr^{-1}. Maximum nitrogenase activity was observed for isolate AZO23-3 *i.e* 475.64 Nmoles C_2H_4 mg protein^{-1} h^{-1} and of AZO DP-5 was observed to be the least *i.e.* 18.93 Nmoles C_2H_4 mg protein^{-1} h^{-1}. In present study the obtained ARA ranges were significantly higher than other observations and corroborated with Rodelas et al.[42] who reported the ARA results of pure cultures of strains were between 9.70 to 257.73 nmol C_2H_4 h^{-1} vial^{-1}. According to Tejera et al.[28] *A. chroococcum* isolates from soil showed ARA production within ranged between 79.6 to 329.5 nmol C_2H_4 h^{-1} culture.

Nitrogen fixation potentiality of the selected isolates of *Azotobacter* sp was estimated by Akhter et al.[43], ranging from 04.95 to 10.55 mg N/g substrate. Kizilkya[44] reported that the *Azotobacter* fix nitrogen in the range of 3.50 to 29.35 µg N mL^{-1} for medium culture. Variation in efficiency of *Azotobacter* may be due to difference in strains and different growth conditions being used in different studies[45].

Studies of *In vitro* effect of *Azotobacter* on growth and yield of maize seedling

The experiment of pot culture was conducted in plastic pots filled with sterile planting mixture. Plant growth promoting activity of *Azotobacter* isolates were studied on FEM-2 variety of Maize. Cultivable seeds were treated with *Azotobacter* inoculant thorough seed bacterization method and sown. Five *Azotobacter* strains with higher ARA values were selected for pot studies. Pot experiment data were recorded after 30 days of germination were summarized in Table 2.

O Maize seedlings raised from bacterized seeds with selected *Azotobacter* isolates, observed higher growth in treated ones as compared with absolute control. This indicates the positive effect

of *Azotobacter* strains on maize plantlet. Leaf number, total chlorophyll content, Shoot length, root number, root length, have been significantly increased in the maize plantlet inoculated with the selected *Azotobacter* strains as compared to uninoculated control.

Shoot length, root length, root number and chlorophyll content increases by 45.62%, 17.60%, 97.49% and 49.69% respectively in pot inoculated with AZO23-3 followed by AZO2-12 (36.86%, 14.74%, 90.06% and 44.29%, respectively). Leaf number increases by 27.83% in pot inoculated with AZO23-3 followed by AZO2-12 and AZO8.2 (11.17%). All the *Azotobacter* strains significantly influenced the observed parameters as compared to uninoculated control and contributed to plant growth. Similar finding was observed by Mahato and Neupane[46] who reported *Azotobacter* seed bacterization in maize promote the growth of treated plants and find increased growth in root and shoot lengths. Perdomo et al.[47] reported *Azotobacter* bacterial inoculation in cotton positively influenced plant growth parameters and reduced 50% nitrogen fertilization doses. These findings advocated the application of *Azotobacter* for improvement of plant growth due to their intrinsic abilities of fixing atmospheric nitrogen and expressing plant growth-promoting substances.

The plant growth promoting traits and the nitrogen fixation by the local *Azotobacter* strains is a very critical for the selection of such strains for biofertilizer formulations in order to replace the ineffective strains. The results indicated that inoculation by multi PGP *Azotobacter* strains significantly improved the plant growth under *in vitro* condition and may be used for commercial production. Hence, the dedicated field studies are required to confirm the efficacy of these *Azotobacter* strains.

SUPPLEMENTARY INFORMATION

Supplementary information accompanies this article at https://doi.org/10.22207/JPAM.15.1.40

Additional file: Additional Table S1- S2.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

DJ conceived and designed the experiments; GK, AAB, SC performed laboratory experiments; DJ, SKK, RHM and DR wrote the manuscript. All authors read and approved the final manuscript.

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DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript and also in the Supplementary Files.

ETHICS STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors.

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