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Effect of Plant Growth Promoting Endophytic Bacteria *Gluconacetobacter diazotrophicus,* on Germination Attributes and Seedling Growth of Rice Varieties under *In vitro* Conditions

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The production of rice mainly depends on nitrogen fertilizer. Extensive applications of chemical nitrogen fertilizer have adverse effects on plant health and the environment. The use of plant growth promoting microorganisms can increase the soil fertility and productivity of rice and also reduce the negative impact of chemical nitrogen fertilizer. *Gluconacetobacter diazotrophicus PAL5* is a gram-negative endophytic bacterium that plays an important role in nitrogen fixation and plant growth promotion. The present investigation was to evaluate the effect of inoculation with *G. diazotrophicus* on seed germination indices of fifteen rice varieties (*Oryza sativa* L.) and seedling growth under in vitro conditions. Among the fifteen rice varieties inoculated with *G. diazotrophicus*, the highest increase in germination indices was observed in five varieties, *viz.* BPT5204, TN1, ISM, IR64, and Dhan53, which showed an increase in germination percentage (4.26-10%), Mean germination time (3.8-5.8%), germination speed 10.5- 26.8%), synchronization index (22.74-43.60%), standard deviation of germination rate (2.10-9.70%) and coefficient of variance of germination (11.80-18.36%) in comparison with the control on agar medium after 5 days. Furthermore, inoculation resulted in a significant increase in growth parameters (viz., root length, shoot length, root dry weight, shoot dry weight, seedling vigour index- I and seedling vigour index-II) over uninoculated control seeds in five rice varieties in comparison with the remaining rice varieties. The results thus reveal endophytic *G. diazotrophicus* not only increased the germination percentage but also enhanced the seedling growth parameters of rice varieties while eliciting a genotype-specific response in rice genotype.

Keywords: Endophyte; Gluconacetobacter diazotrophicus; germination indices; plant growth promoting bacteria; rice varieties; seedling growth.

1. INTRODUCTION

Rice is indeed a staple food crop for over 3.5 billion populations worldwide by 2025 [1]. In India, rice is cultivated on about 46 million hectares with a yearly production of 158 million tonnes [2]. Nitrogen (N) is the major limiting nutrient for rice production [3] and synthetic chemical nitrogen fertilizers have become increasingly prevalent in rice cultivation during the past few decades. For the cultivation of rice, 16% of N fertilizer was used worldwide [4,5]. The continuous use of chemical nitrogen fertilizers for sustainable rice production results in several problems, including declining soil fertility, soil pollution, and environmental degradation. Only 20-50% of applied nitrogen was utilized by the rice crop, indicating that 60- 80% of applied N remains surplus in the crop field and prone to losses [6] Therefore, Plant Growth-Promoting Bacteria (PGPBs) that can fix atmospheric N and N smart rice cultivars are alternatives to chemical nitrogen fertilizers.

Certain PGPBs (rhizospheric and endophytic) can fix atmosphere nitrogen, by entrapping N_2 and converting it into NH_3, a form that is readily utilized by the plants via Biological Nitrogen fixation (BNF). In rice cultivation, 50 to 70% of the nitrogen requirement has been reported to be provided by these bacteria *via* the BNF process [7]. Rice is known to establish genotype variable associations with certain diazotrophic bacteria such as *Herbaspirillum seropedicae* resulting in plant dependent BNF [8]. Several PGPBs are directly associated with plant roots and can exist within root tissues (endophytes). These bacteria synthesize hormones such as auxins and gibberellins which promote plant development [8], increase host biomass [10] provide pathogen defence [11,12,13], and enhance host plants tolerance to environmental stresses [14,15]. *G. diazotrophicus*, is an endophytic diazotrophic nitrogen-fixing aero-tolerant bacterium [16]. In association with the plant, it produces the plant growth hormones including auxins, gibberellins, cytokinins, abscisic acid, salicylic acid, gluconacin, and solubilization of macro and micronutrients like P and Zn [17]. Additionally, it has biocontrol properties against phytopathogens like *Colletotrichum falcatum, Xanthomonas albilineans*, and nematode *Meloidogyne incognita* [18].

To optimize the potential agricultural benefits from interactions with beneficial diazotrophs, a better understanding of the processes by which rice regulates its performance may be useful [19,20,21]. With this background, the present investigation studies the compatibility of different rice varieties to *G. diazotrophicus* bacteria inoculation in terms of germination indices and seedling growth parameters.

2. MATERIALS AND METHODS

2.1 Rice Varieties and Bacterial Strain

A set of 15 different rice varieties that possess the potential to be cultivated in diverse ecosystems were selected for evaluation and the seeds of the varieties were obtained from ICAR-Indian Institute of Rice Research, Hyderabad (Table 1). The nitrogen-fixing bacterium *Gluconacetobacter diazotrophicus* PAL5 (MTCC 1224) was procured from CSIR - Institute of Microbial Technology, Chandigarh.

2.2 *In vitro* Germination Indices and Seedling Growth Parameters

Seeds of different rice varieties were surface sterilized by using 70% ethanol for 1 min, and 0.1% $HgCl_2$ for 1 min followed by rinsing with sterile distilled water six times. *G. diazotrophicus* bacterial inoculum was prepared by dissolving the culture pellet in phosphate buffer saline such that cell concentration was adjusted optical density (O.D) at 600 nm to 1 (1x 10^8 CFU/ml). Seeds were soaked in bacterial inoculum for 24 hours at 28^{o}C. After incubation, seeds were transferred to sterile petri dishes containing water agar (0.6%, w/v) and incubated at the 28^{o}C in dark and allowed to germinate for 7 days. Each petri dish contained 25 seeds of a variety in 3 replications per variety were maintained. Observations on the number of seeds germinated each day were recorded. Morphological traits *viz.* root length (cm), shoot length (cm), root fresh weight (gm), shoot fresh weight (gm), root dry weight (gm), and shoot dry weight (gm) of seedling of both control and inoculated rice varieties were also recorded on the seventh day of germination [22]. Germination percentage and seedling vigour index were calculated by using the following formulas [23].

Germination percentage = (Number of seeds germinated / Total number of seeds) X 100

Seedling Vigour Index I (SVI- I) = (Mean of root length + Shoot length) X Germination percentage (cm).

Seedling Vigour Index II (SVI-II) = (Mean of root dry weight + Shoot dry weight) X Germination percentage (gm).

2.3 Statistical Analysis

Germination data of fifteen rice varieties were analyzed using the GerminaQuant software [24] for germination and related attributes. All germination indices and morphological data were analyzed by using a statistical package [25] by performing a t-test (two pair-wise means) for statistically significant differences between the treatment means. Principle component analysis was performed with software, Microsoft Excel for Windows 2010 add-in with XLSTAT Version 2010.5.05 [26].

Table 1. List of rice varieties used to screen for interaction with *G. diazotrophicus*

S. No	Rice Varieties	Ecosystem	Area of cultivation
1	Drr Dhan53	Irrigated	All India
2	IR64	Irrigated	All India
3	RNR15048	Irrigated	Telangana
4	Sonasali	Irrigated	All India
5	Tellahamasa	Irrigated	Andhra Pradesh, Telangana
6	TN1	Irrigated	All India
7	Vikas	Irrigated	All India
8	BPT5204	Rainfed shallow lowlands	Andhra Pradesh, Karnataka
9	Improved Samba Mahsuri (ISM)	Rainfed shallow lowlands	Andhra Pradesh, Telangana, Tamil Nadu, Karnataka, Maharashtra, Bihar
10	Rasi	Rainfed upland	Andhra Pradesh, Orissa, Madhya Pradesh, Maharashtra, Tamil Nadu
11	Ravi	Rainfed upland	Andhra Pradesh
12	Salivahana	Rainfed low lands	West Bengal
13	Nidhijaldhi	Deep water flooding areas	Uttar Pradesh
14	Swarna	Shallow low lands	Andhra Pradesh, Telangana, Karnataka, Tamil Nadu, and Kerala
15	Drr Dhan 39	Saline or alkaline-tolerant	Orissa, Kerala, Gujarat

3. RESULTS AND DISCUSSION

3.1 *In vitro* Seed Germination Indices

Inoculation with *G. diazotrophicus* significantly improved germination and germination indices (germination percentage, mean germination time, germination speed, synchronization index, standard deviation of germination, and coefficient of variance of germination) of selected rice varieties in comparison to control after 7 days under in vitro conditions (Table 2, Fig. 1).

There was a significant increase in the germination percentage of rice varieties inoculated with *G. diazotrophicus* than control (*P*=0.003). Germination percentage in inoculated rice varieties ranged from 84± 2.28 to 100± 1.87 whereas 80± 1.75 to 92± 1.34 was observed in uninoculated control rice varieties. Among the 15 rice varieties inoculated with *G. diazotrophicus*, germination percentages of BPT5204 (100± 1.87), TN1 (100± 1.87, IR64 (100± 1.87), ISM (98± 1.35), and Dhan53 (94± 0.31) were showing higher relative performance in appraisal with the control and lower relative performance observed in Nidhijaldhi (90± 0.73).

The mean germination rate in inoculated rice varieties varied from 2.00± 0.09 to 3.37± 0.17 and 2.00± 0.13 to 3.74± 0.032 in control varieties (*P*=0.042). There was a reduction in the mean germination rate in rice varieties inoculated with *G. diazotrophicus* in comparison with the control exhibiting the improvement of the germination of seeds due to inoculation. The highest relative performance of mean germination rate with the control among the inoculated 15 rice varieties was observed in BPT5204 (5.8%), TN1 (5.8%), IR64 (5.1%), ISM (4.1%), and Dhan53 (3.8%) and minimum in Nidhijaldhi (2.4%). Similarly, the germination speed of inoculated rice varieties ranged from 29.65± 3.88 to 50.00± 1.91 and 26.70± 3.73 to 50.00± 2.29 in control varieties indicating seeds of rice varieties treated with *G. diazotrophicus* took less time for germination when equated to the control (*P*=0.067) signifying enhancement of germination speed of rice seeds due to inoculation with *G. diazotrophicus*.

The synchronization index of inoculated rice varieties ranged from 0.38± 0.09 to 1.00± 0.08, whereas in control 0.37± 0.07 to 1.00± 0.09 (*P*=0.13). Maximum relative performance with control of synchronization index was observed in inoculated rice varieties BPT5204, TN1, IR64, ISM, and Dhan53 (1.00± 0.08) among the fifteen rice varieties, signifying inoculated rice seeds germinated at the same time. A significant increase in the standard deviation of germination was observed in response to the *G. diazotrophicus* inoculation (*P*= 0.0001). The standard deviation of germination varied from 1.02± 0.02 to 1.23± 0.03 in inoculated rice varieties and 1.05± 0.02 to 1.06± 0.02 in control. Higher relative performance with the control among the rice varieties was observed in BPT5204, TN1, IR64, ISM, and IR64, indicating the uniformity of germination and higher germination in the inoculated varieties. A similar trend was observed in the coefficient of variance of germination (*P*= 0.0009). An increase in the coefficient of variance of germination in inoculated varieties signifies an increase in the number of germinated seeds with a decrease of time in appraisal with control. Our results indicate that there was a significant increase in comparable execution in germination and related attributes in all rice varieties treated with the *G. diazotrophicus* in comparison with the control. Among fifteen inoculated rice varieties BPT5204, TN1, IR64, ISM, and Dhan53 showed a high relative effectiveness of germination indices compared to the control.

Similarly to this study, the response of rice seeds to microbial inoculation with PGPB *Bacillus sp. KS-54* increased the final germination and lowered the mean germination time in the controlled conditions and also increased the values of the emergence index indicating fast and synchronized germination in the field conditions [27]. Comparable results were reported in different tomato genotypes treated with *G. diazotrophicus* enhanced the germination percentage and also depended upon genotype [28,29].

3.2 Effect of *G. diazotrophicus* on Seedling Growth in Different rice Varieties

There was a significant effect of *G. diazotrophicus* inoculation on the seedling growth in rice varieties. A significant increase in the root length (6.9- 43%), shoot length (1.5- 31.5%), root dry weight (4.76-23.08%) and shoot dry weight (4.27-27.86%) of rice varieties was observed in comparison with the control (*P*= 0.001). Among all inoculated rice varieties, five *viz.* BPT5204, TN1, IR64, ISM, and Dhan53 were observed to have relatively high performance in contrast with the control. Whereas seedling vigour index I and seedling vigour index II increased from 16.1 to

Table 2. Influence of *Gluconacetobacter diazotrophicus* inoculation on germination indices of rice varieties

S.No	Varieties	Germination rate (%)		Mean Germination rate (days)		Germination speed (%)		Synchronization Index		Standard deviation of germination (days)		Coefficient of variance of germination (%)	
		Control	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**
1	BPT5204 (BP)	90± 0.83	100± 1.87	3.74± 0.032	3.02± 0.17	40.00± 0.29	50.00± 1.91	0.48± 0.04	1.00± 0.08	1.05± 0.02	1.02± 0.02	51.17± 1.33	56.24± 2.28
2	DRR Dhan39 (DH39)	88± 0.31	92± 0.21	2.71± 0.05	2.65± 0.07	29.05± 3.13	29.65± 3.88	0.57± 0.02	0.53± 0.05	1.14± 0.00	1.12± 0.01	46.72± 0.19	47.26± 0.03
3	DRR Dhan53 (DH53)	86± 0.21	94± 0.31	2.54± 0.01	2.27± 0.03	39.32± 0.47	44.16± 0.24	0.62± 0.01	0.80± 0.02	1.06± 0.02	1.02± 0.02	51.03± 1.29	53.13± 1.48
4	IR64 (IR)	92± 1.34	100± 1.87	2.50± 0.01	2.00± 0.09	26.70± 3.73	33.11± 2.90	0.50± 0.03	0.73± 0.00	1.06± 0.02	1.02± 0.02	50.03± 1.04	52.06± 1.20
5	ISM (IS)	90± 0.83	98± 1.35	3.24± 0.19	2.86± 0.13	36.88± 1.10	37.69± 1.60	0.74± 0.02	0.92± 0.05	1.26± 0.03	1.24± 0.04	30.39± 4.02	30.42± 4.36
6	Nidhijaldhi (ND)	92± 1.34	90± 0.73	2.07± 0.12	2.61± 0.06	48.38± 1.87	38.34± 1.41	0.87± 0.06	0.39± 0.09	1.25± 0.03	1.23± 0.03	34.64± 2.92	34.73± 3.25
7	Rasi (RS)	86± 0.21	86± 1.76	2.08± 0.11	2.30± 0.02	48.17± 1.82	43.57± 0.08	0.86± 0.05	0.56± 0.04	1.17± 0.01	1.13± 0.01	41.42± 1.18	41.62± 1.48
8	Ravi (RV)	82± 1.24	84± 2.28	2.00± 0.13	2.00± 0.09	50.00± 2.29	50.00± 1.91	0.43± 0.05	0.38± 0.09	1.14± 0.00	1.12± 0.00	47.72± 0.44	48.00± 0.16
9	RNR15048 (RNR)	86± 0.21	92± 0.21	3.44± 0.24	3.37± 0.26	36.88± 1.10	37.69± 1.60	1.00± 0.09	1.00± 0.08	1.09± 0.01	1.06± 0.01	50.01± 1.03	51.85± 1.15
10	Salivahana (SA)	80± 1.75	88± 1.24	2.42± 0.02	2.15± 0.06	30.85± 2.66	34.97± 2.37	0.42± 0.06	0.59± 0.03	1.12± 0.00	1.07± 0.01	50.41± 1.13	51.47± 1.05
11	Sonali (SO)	84± 0.72	92± 0.21	2.19± 0.08	2.05± 0.08	45.60± 1.15	48.84± 1.58	0.37± 0.07	0.53± 0.05	1.19± 0.01	1.15± 0.01	38.38± 1.96	38.50± 2.28
12	Swarna (SW)	90± 0.83	92± 0.21	2.07± 0.12	2.18± 0.05	48.22± 1.83	45.83± 0.72	0.86± 0.05	0.69± 0.01	1.14± 0.00	1.09± 0.00	49.98± 1.02	51.10± 0.96
13	Tellahamasa (TH)	92± 1.34	100± 1.87	2.15± 0.09	2.04± 0.08	46.46± 1.38	48.98± 1.62	0.77± 0.03	0.91± 0.05	1.06± 0.02	1.02± 0.02	51.12± 1.32	54.21± 1.76
14	TN1 (TN)	92± 1.34	100± 1.87	2.50± 0.01	2.00± 0.09	40.00± 0.29	50.00± 1.91	0.48± 0.04	1.00± 0.08	1.14± 0.00	1.09± 0.00	47.01± 0.26	48.00± 0.16
15	Vikas (VI)	90± 0.83	96± 0.83	2.15± 0.09	2.09± 0.07	46.16± 1.30	47.94± 1.32	0.71± 0.02	0.84± 0.03	1.09± 0.01	1.05± 0.01	50.02± 1.04	52.03± 1.20
	t- stat	**5.61**		**1.85**		**1.59**		**1.15**		**11.99**		**3.8**	
	***P* (≤0.05)**	**0.0032**		**0.042**		**0.067**		**0.13**		**0.0001**		**0.0009**	

Each value denotes mean ± SE for 3 replicates

Table 3. Effect of *G. diazotrophicus* inoculation on seedling growth and vigour in different rice varieties

Varieties	Root length (cm)		Shoot length (cm)		Root dry weight (gm)		Shoot dry weight(gm)		Seedling vigour-I (cm)		Seedling vigour-II (gm)	
	Control	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**	**Control**	**G. diazotrophicus**
Dhan39	5.00± 1.38	6.76± 1.23	7.39± 0.21	8.28± 0.20	0.007± 0.00	0.009± 0.00	0.043± 0.00	0.052± 0.00	1090.32± 26.54	1383.68± 34.24	5.19± 0.22	5.83± 0.10
Dhaan53	5.50± 0.92	7.50± 0.62	6.01± 0.76	6.90± 0.63	0.010± 0.00	0.012± 0.00	0.010± 0.01	0.012± 0.01	1035.90± 40.60	1411.20± 27.13	3.06± 0.33	4.35± 0.28
IR64	6.99± 0.45	9.77± 1.26	6.68± 0.29	7.80± 0.09	0.040± 0.01	0.051± 0.001	0.040± 0.00	0.050± 0.00	1148.28± 11.57	1616.44± 25.91	1.68± 0.69	2.27± 0.82
ISM	6.50± 0.00	9.16± 0.75	5.10± 1.40	6.17± 1.07	0.019± 0.00	0.024± 0.00	0.016± 0.00	0.021± 0.00	997.60± 50.50	1441.02± 19.42	6.86± 0.66	9.44± 1.03
Nidhijaldhi	7.52± 0.94	8.04± 0.17	7.70± 0.43	7.81± 0.08	0.010± 0.00	0.011± 0.00	0.036± 0.00	0.038± 0.00	1308.92± 29.94	1378.95± 35.46	3.59± 0.19	4.22± 0.31
Rasi	6.88± 0.35	8.32± 0.06	6.58± 0.36	7.25± 0.42	0.036± 0.00	0.043± 0.01	0.021± 0.00	0.025± 0.00	1211.40± 4.74	1432.44± 21.64	4.59± 0.07	6.16± 0.19
Ravi	7.82± 1.21	8.90± 0.54	9.10± 1.42	9.36± 0.84	0.013± 0.00	0.014± 0.00	0.060± 0.01	0.063± 0.01	1387.44± 50.22	1533.84± 4.56	4.63± 0.08	5.67± 0.06
RNR15048	6.82± 0.29	8.15± 0.08	7.35± 0.18	7.87± 0.05	0.010± 0.00	0.011± 0.00	0.030± 0.00	0.034± 0.00	1275.30± 21.25	1537.92± 5.62	4.27± 0.02	5.36± 0.02
Salivahana	6.55± 0.05	7.87± 0.31	7.82± 0.51	8.40± 0.27	0.007± 0.00	0.009± 0.00	0.040± 0.00	0.047± 0.00	1322.04± 33.33	1627.00± 28.63	4.27± 0.02	4.85± 0.15
Sonali	7.37± 0.80	8.56± 0.26	7.90± 0.57	8.13± 0.11	0.011± 0.00	0.012± 0.00	0.048± 0.00	0.051± 0.00	1404.84± 54.72	1669.00± 39.49	5.20± 0.23	6.75± 0.34
Swarna	3.90± 2.39	5.16± 2.55	5.00± 1.47	5.57± 1.43	0.039± 0.01	0.046± 0.001	0.018± 0.00	0.022± 0.00	818.80± 96.69	965.70± 142.25	5.09± 0.20	5.94± 0.13
Tellahamasa	7.80± 1.19	10.33± 1.72	7.80± 0.50	8.69± 0.44	0.008± 0.00	0.010± 0.00	0.047± 0.00	0.057± 0.00	1435.20± 62.56	1902.00± 99.70	6.72± 0.62	7.71± 0.59
TN1	6.00± 0.46	8.51± 0.21	10.00± 2.05	12.87± 2.95	0.024± 0.00	0.031± 0.00	0.027± 0.00	0.037± 0.00	1280.00± 22.47	1881.44± 94.38	3.98± 0.09	5.38± 0.02
Vikas	6.93± 0.39	8.30± 0.04	6.80± 0.20	7.30± 0.39	0.009± 0.00	0.010± 0.00	0.033± 0.00	0.038± 0.00	1180.78± 3.17	1435.20± 20.93	3.44± 0.23	4.19± 0.32
t- stat	**9.69**		**4.84**		**4.54**		**7.33**		**8.17**		**8.18**	
***P* (≤0.05)**	**0.0002**		**0.0001**		**0.0020**		**0.0005**		**0.0001**		**0.0007**	

Each value denotes mean ± SE for 3 replicates

52.9% and 18.7 to 48.6% respectively in rice varieties and the highest increase in BPT5204 (SVI-I 52.9%, SVI-II 48.5%), TN1 (SVI-I 36.6%, SVI-II 34.3%), IR64 (SVI-I 40.0%, SVI-II 37%), ISM (SVI-I 44.4%, SVI-II 42.7%) and Dhan53 (SVI-I 36%, SVI-II 34%) perceived among the remaining rice varieties (Fig. 1, Table 3).

Our study revealed that inoculation of *G. diazotrophicus* increased seedling growth parameters in all rice varieties to the control. Enhancement in seedling growth as observed in the study was also recorded in red rice inoculated with *G. diazotrophicus*, wherein root and shoot length were increased under drought and stressed conditions compared to the control [30]. At a moderate level of drought and nitrogen stress in maize, *G. diazotrophicus* treated plants exhibited higher shoot and root lengths, either individually or in combination with N deficit [31,32]. Similarly, *G. diazotrophicus* individually or in combination with other phosphate solubilization bacteria increased the plant height, root length, dry weight, grain yield, and phosphorus content of rice compared to the control under greenhouse conditions [33]. Tomato treated with *G. diazotrophicus* enhanced the plant growth, dry weight, root length surface, and number of leaves [29].

3.3 Principal Component Analysis of Germination Indices and Growth Parameters of Rice Varieties Inoculated with *G. diazotrophicus*

A principal component analysis (PCA) was carried out for germination indices and growth parameters of rice varieties inoculated with *G. diazotrophicus* and in uninoculated control (Table 4).

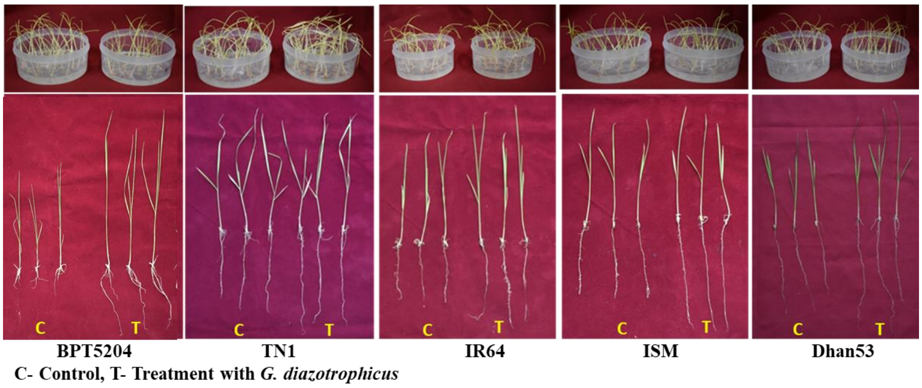

Fig. 1. Growth promotion of rice varieties in response to *G. diazotrophicus* inoculation under in vitro conditions

Table 4. Variable loading scores and variation proportion of each principal component of germination indices and plant growth parameters in rice varieties inoculated with *G. diazotrophicus*

Parameters	PC_1	PC_2	PC_3	PC_4	PC_5
Germination percentage (GRP)	0.44	-0.50	**0.51**	0.13	0.28
Mean germination time (MGT)	-0.51	-0.18	0.20	**0.72**	-0.01
Germination speed (GSP)	0.39	-0.01	0.29	-0.56	**-0.58**
Synchronization index (SYN)	0.19	-0.51	**0.68**	0.06	-0.20
Germination standard deviation (SDG)	-0.26	**0.86**	0.35	-0.04	0.09
Coefficient of variation (CVG)	0.17	**-0.80**	-0.48	-0.02	-0.20
Root length (RL)	**0.84**	0.04	0.19	0.19	0.17
Shoot length (SL)	**0.76**	0.17	-0.27	0.03	0.05
Root dry weight (RDW)	-0.11	-0.28	0.25	-0.60	**0.64**
Shoot dry weight (SDW)	**0.66**	0.38	-0.48	0.06	0.10
Seedling vigour index- I (SV-I)	**0.95**	0.01	0.04	0.20	0.07
Seedling vigour index- II (SV-II)	0.30	0.45	**0.55**	0.10	-0.17
Eigenvalue	3.45	2.37	1.90	1.31	0.99
Variability (%)	28.79	19.76	15.83	10.89	8.27
Cumulative %	28.79	48.54	64.37	75.27	83.53

The PCA on 12 parameters revealed five principal components (PCs) with eigenvalues > 1, which captured 75.27% of the cumulative variability across the rice varieties (Table 4, Fig. 2a). PC_1 accounted for 28.79% of the total variability, consisting mostly of plant traits such as RL, SL, SDW, and SV-I. PC_2 accounted for 19.76% of the total variation primarily for SDG. PC_3 (15.83%) variability consisted of GRP, SYN and SV-II. Variability of 10.89% for PC_4 (MGT), and 8.27% for PC_5 (GSP, and RDW) were recorded respectively.

The first component explained most of the total variability of the included data. The variables with the highest weight value in this component were those related to germination percentage, germination indices, root length, shoot length, and seed vigour index of rice varieties inoculated with *G. diazotrophicus* which are all positively correlated. Variables with a negative correlation were those related to mean germination time, standard deviation of germination, and root dry weight of control rice varieties. Whereas in the PC_2 component, high and positively correlated with the standard deviation of germination of rice varieties inoculated with *G. diazotrophicus*. High and negatively correlated weight value was observed with CVG in the control rice varieties.

Rice varieties treated with the *G. diazotrophicus* fell in the right quadrants with more variables representing higher plant growth and germination, highlighting plant growth promotion of the *G. diazotrophicus*. The control treatments were at the left quadrant with few variables indicating low plant growth parameters and germination (Fig. 2b). Five rice varieties treated with *G. diazotrophicus*, BPT5204, TN1, ISM, IR64, and Dhan53 were observed to fall in the right quadrants of the scatter plot of PCA indicating that these rice varieties have a higher and positive correlation with the germination indices and plant growth parameters.

G. diazotrophicus individually or in combination with the *Rhizobium sp* in the presence of insoluble silicate sources improved the morpho-physiological and molecular attributes of rice under heat stress conditions [34]. In red rice a positive correlation between the morphological growth parameters was reported due to inoculation with *G. diazotrophicus* [30]. In rice cultivars, BPT5204 and ISM seeds treated with *G. diazotrophicus* improved the germination, seedling vigour index, and plant growth under in vitro and in vivo conditions [35]. *G. diazotrophicus,* endophytically colonizes and enhances the growth parameters viz. plant height, number of tillers, biomass, and nitrogen content of rice [36,37]. In rice, bacterial endophytes modulate the plant growth during germination and early seedling development [38]. Thus our study revealed that rice varieties inoculated with *G. diazotrophicus* increased seed germination and related attributes, and also growth parameters of seedlings, may be linked with the production of plant growth hormones or unknown metabolites and their interaction with rice root by the PGPB [39].

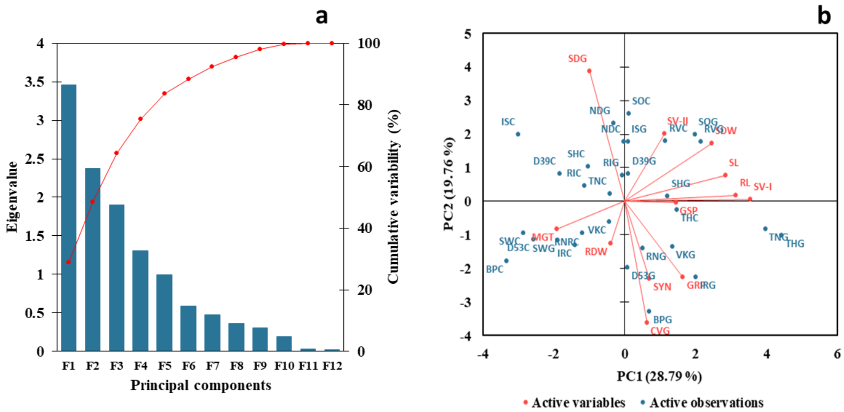

Fig. 2. a. Principal component analysis scree plot for different components. b. Scatter plots of the first two principal components (PCs) from principal component analysis (PCA) for germination indices and growth parameters in different rice varieties inoculated with *G. diazotrophicus* and control. Abbreviations of variables mentioned in the already mentioned in the Tables 1 and 3. C- Control, G- *Gluconacetobacter diazotrophicus*

In the present study, the seed germination indices and seedling growth of different rice varieties in response to plant growth-promoting bacteria *Gluconacetobacter diazotrophicus* under *in vitro* conditions were evaluated. Among the fifteen rice varieties treated with the *G. diazotrophicus*, five varieties i.e., BPT5204, TN1, ISM, IR64, and Dhan53 showed higher germination indices like germination percentage, mean germination rate, germination speed, synchronization index, the standard deviation of germination and also seedling growth parameters viz., root length, shoot length, root dry weight, shoot dry weight, vigour index- I and vigour index- - II equated with control. Though we used a limited number of rice varieties in the present study, it enables the selection of rice varieties compatible with *G. diazotrophicus* for which endophytic bacteria can be used as a biofertilizer in the rice field leading to higher growth promotion.

4. CONCLUSION

Rice crop productivity majorly depends on nitrogen fertilizer. The use of plant growth-promoting nitrogen-fixing bacteria and compatible genotypes to the PGPB is one of the alternatives for sustainable rice production. The results obtained in this study demonstrated that with *Gluconacetobacter diazotrophicus* inoculation enhanced germination and seedling growth of all fifteen rice varieties, the extent of beneficial response to inoculation varied genotypes.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Farooq A, Farooq N, Akbar H, Hassan ZU, Gheewala SH. A critical review of climate change impact at a global scale on cereal crop production. Agronomy. 2023;13(1):162.
2. Statista. Rice - statistics & facts Statista; 2023. Available:https://www.statista.com/topics/1443/rice
3. Ladha JK, Reddy PM. Nitrogen fixation in rice systems: State of knowledge and prospects. Plant and Soil. 2003;252:151-167.
4. Alam MS, Khanam M, Rahman MM. Environment-friendly nitrogen management practices in wetland paddy cultivation. Frontiers in Sustainable Food Systems. 2023;7:1020570
5. Heffer P, Gruere A, Roberts T. 19th Assessment of fertilizer use by crop at the global level. Paris, France. November; 2017.
6. Chivenge P, Sharma S, Bunquin MA and Hellin. Improving nitrogen use efficiency—A key for sustainable rice production systems. Frontiers in Sustainable Food Systems. 2021;5:737412.
7. Reddy INBL, Kim BK, Yoon IS, Kim KH, Kwon TR. Salt tolerance in rice: Focus on mechanisms and approaches. Rice Science. 2017;24:123–144.
8. James EK, Gyaneshwar P, Mathan N, Barraquio WL, Reddy PM, Iannetta PPM, Olivares FL, Ladha JK. Infection and colonization of rice seedlings by the plant growth-promoting bacterium *Herbaspirillum seropedicae* Z67. Molecular Plant-microbe Interactions. 2002;15:894–906.
9. Krome K, Rosenberg K, Dickler C, Kreuzer K, Ludwig-Müller J, Ullrich-Eberius C, Scheu S, Bonkowski M. Soil bacteria and protozoa affect root branching via effects on the auxin and cytokinin balance in plants. Plant and Soil. 2009;328:191–201.
10. Oliveira ALM, Canuto EL, Urquiaga S, Reis VM, Baldani JI. Yield of micro-propagated sugarcane varieties in different soil types following inoculation with diazotrophic bacteria. Plant and Soil. 2006;284:23–32.
11. Arencibia A, Vinagre F, Estevez Y, Bernal A, Perez J, Cavalcante JJ, Santana I, Hemerly AS Gluconoacetobacter diazotrophicus elicitate a sugarcane defence response against a pathogenic bacteria *Xanthomonas albilineans*. Plant Signal and Behavior. 2006;1:265–273
12. Shittu HO, Castroverde DC, Nazar RN, Robb J Plantendophyte interplay protects tomato against a virulent Verticillium. Planta. 2008;229:415–26
13. Shittu HO, Shakir AS, Nazar RN, Robb J. Endophyte induced verticillium protection in tomato is range-restricted. Plant Signal Behaviour. 2009: 4:160–1
14. Chowdhury SM, Nagarajan T, Tripathi R, Mishra MN, Le Rudulier D, Tripathi AK. Strain-specific salt tolerance and osmoregulatory mechanisms in *Azospirillum brasilense*.

FEMS Microbiology Letters. 2007;267:72–79.
15. Pereyra MA, Zalazar CA, Barassi CA. Root phospholipids in Azospirillum- inoculated wheat seedlings exposed to water stress. Plant Physiology and Biochemistry. 2006; 44:873–879
16. Meneses CHSG, Rouws LFM, Araújo JLS, Vidal MS, Baldani J. Exopolysaccharide production is required for biofilm formation and plant colonization by the nitrogen-fixing endophyte Gluconacetobacter diazotrophicus. Molecular Plant-Microbe Interaction. 2011;24(12):1448–1458.
17. Vessey, J.K. Plant Growth Promoting Rhizobacteria as Biofertilizers. Plant and Soil. 2003; 255:571-586.
18. Chawla, Manisha Phour, Sunita Suneja, Seema Sangwaan, Sneh Goyal. Gluconacetobacter diazotrophicus: An overview. Research in Environment and Life Sciences. 2014;7: 1-10.
19. Weyens N, Van Der Lelie D, Taghavi S, Newman L, Vangronsveld J. Exploiting plant-microbe partnerships to improve biomass production and remediation. Trends Biotechnology. 2000; 27:591–8
20. Gardner JB, Drinkwater LE. The fate of nitrogen in grain cropping systems: a meta-analysis of 15N field experiments. Ecological Application. 2009;19:2167–84.
21. Mohanty SR, Bodelier L, Floris V, Conrad R. Differential effects of nitrogenous fertilizers on methane-consuming microbes in rice field and forest soils. Applied and Environmental Microbiology. 2006;72: 1346–54.
22. Shende ST, Apte RG, Singh T Influence of Azotobacter on germination of rice and cotton seeds. Current Science. 1977; 46(19): 675–676.
23. Abdul- Baki AA, Anderson JD. In: Physiological and biochemical deterioration of seeds. Kozlowski, T.T. (ed.). Seed biology. Academic Press, New York. 1973;2:283-315.
24. Lozano-Isla, Flavio, Benites-Alfaro, Omar E, Pompelli, Marcelo F. GerminaR: An R package for germination analysis with the interactive web application “GerminaQuant for R”. Ecological Research, 2019;34(2): 339–346.
25. Statistix 8.1. User’s Manual. Analytical Software, Tallahassee; 2003.
26. Addinsoft. XLSTAT statistical and data analysis solution, Long Island, NY, USA; 2019. Available:https://www.xlstat.com
27. Javed T, Afzal I, Mauro RP. Seed coating in direct-seeded rice: An innovative and sustainable approach to enhance grain yield and weed management under submerged conditions. Sustainability. 2021;13:21900
28. Restrepo GM, Ceballos N, Valencia LF. Plant growth promotion by Gluconacetobacter diazotrophicus and its interaction with genotype and phosphorus availability in tomato seedlings. Organic Agriculture. 2021;11: 601–614.
29. Botta AL, Santacecilia A, Ercole C, Cacchio P, Del Gallo M*. In vitro* and *in vivo* inoculation of four endophytic bacteria on Lycopersicon esculentum. New Biotechnology. 2013;30:666–674.
30. Silva R, Filgueiras L, Santos B, Coelho M, Silva M, Estrada-Bonilla G, Vidal M, Baldani JI, Meneses C. *Gluconacetobacter diazotrophicus* Changes: The Molecular Mechanisms of Root Development in Oryza sativa L. Growing Under Water Stress. International Journal of Molecular Sciences. 2022; 21(1):333.
31. Tufail MA, Touceda-González M, Pertot I, Ehlers R-U. *Gluconacetobacter diazotrophicus* Pal5 Enhances Plant Robustness Status under the Combination of Moderate Drought and Low Nitrogen Stress in Zea mays L. Microorganisms. 2021;9(4):870.
32. Sandhya V, Ali SZ, Grover M, Reddy G, Venkateswaralu B. Effect of plant growth promoting Pseudomonas spp. on compatible solutes antioxidant status and plant growth of maize under drought stress. Plant Growth Regulators. 2010; 62:21–30.
33. Stephen J, Shabanamol S, Rishad K.S. Growth enhancement of rice (*Oryza sativa*) by phosphate solubilizing *Gluconacetobacter sp*. (MTCC 8368) and Burkholderia sp. (MTCC 8369) under greenhouse conditions. Biotechnology, 2015;3(5):831–837.
34. Chaganti C, Phule AS, Chandran LP, Sonth B, Kavuru VPB, Govindannagari R and Sundaram RM. Silicate solubilizing and plant growth promoting bacteria interact with biogenic silica to impart heat stress tolerance in rice by modulating physiology and gene expression. Frontier in Microbiology. 2023;14:1168415
35. Bandeppa S, Phule AS, Rajani G, Babu KVP, Barbadikar K M, Babu MBB P,

Mandal PK, Sundaram RM, and Latha PC. Effect of Nitrogen-fixing Bacteria on Germination, Seedling Vigour and Growth of Two Rice (*Oryza sativa* L.) Cultivars. International Journal of Plant & Soil Science. 2022; 34(16): 94–106.

36. Muthukumarasamy R, Cleenwerck I, Revathi G, Vadivelu M, Janssens D, Hoste B, Gum KU, Ki-Do P, Son CY, Sa T, Caballero-Mellado J. Natural association of *Gluconacetobacter diazotrophicus* and diazotrophic Acetobacter peroxydans with wetland rice. Systemic Applied Microbiology. 2005;28(3):277-286. 28.
37. Govindarajan M, Balandreau J, Kwon SW, Weon HY, Lakshminarasimhan C. Effects of inoculation of Burkholderia vietnamensis and related endophytic diazotrophic bacteria on grain yield of rice. Microbial Ecology. 2008;55:2-37.
38. Walitang DI, Kim K, Madhaiyan M. et al. Characterizing endophytic competence and plant growth promotion of bacterial endophytes inhabiting the seed endosphere of Rice. BMC Microbiology. 2017;17:209.z
39. Dal Cortivo C, Barion G, Visioli G, Mattarozzi M, Mosca G, Vamerali T. Increased root growth and nitrogen accumulation in common wheat following PGPR inoculation: Assessment of plant-microbe interactions by ESEM. Agriculture, Ecosystem & Environment. 2017;247:396-408.

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