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RESEARCH ARTICLE OPEN ACCESS

Endophyte Bacteria Alter Physiological Traits and Promote Growth of Rice (*Oryza sativa* L.) in Aluminium Toxic and Phosphorus Deficient Acid *Inceptisols*

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Abstract

Endophytic bacteria are known to impart biotic and abiotic stress tolerance under stressful environments. More than 70% arable area in North-Eastern Himalaya is occupied by acidic soil. The aim of this research was to test the extent of endurance achieved by suitable. Endophytic bacterial inoculation for enhancing crop fitness and also to quantify the realistic benefits for crop growth and performance with bacterial inoculation grown in acidic soil. To achieve this, the strain 22WE, isolated from the wild rice variety *Zizania latifolia,* was selected because of its growth promoting properties. 16S rDNA sequence of the isolate confirmed its identification as *Serratia nematodiphila.* This study assessed whether endophyte inoculation can provide the habitat-fitness benefits to rice crops in acidic soil. Further, Kharif rice (var. CAUR3) plants were inoculated with the endophyte selected strain of *Serratia nematodiphila.* Endophyte inoculated rice plants had significantly (P<0.05) higher root surface area, root volume, root and shoot biomass contents than that of uninoculated plants grown under aluminium (Al) toxic and phosphorus (P) nutrient conditions. The DAB staining assay exhibited that endophyte inoculation confers advantage to rice plants in terms of reduced reactive oxygen against Al-induced stress. The interaction effects between endophyte inoculation and Al levels was significant (P<0.05) for the malondialdehyde and cell membrane stability values. The current study showed that toxic effect of an approximate 100ppm Al incurred under any acid soil can be compensated due to the endophyte inoculation.

Keywords: Acid *Inceptisols*, DAB assay, Endophyte, Lipid peroxidation, Rice, Root architecture

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INTRODUCTION

Acid soils constitute approximately 40-50% of the world's arable land (Kochain *et al.*, 2005) and in India about 33% of the cultivated land (Mandal., 1997). A large portion of extremely acidic soil around 65% is part of the North Eastern (NE) region of India (Sharma and Singh., 2002). Rice is the principal food grain crop of the people of NE India, occupying 3.51 million hectares which accounts for more than 80% of the total cultivated region. The total rice production of NE region is much low (Roy *et al.*, 2015). Al toxicity is a major threat for production of crops on acid soils (Silva., 2012). Earth's crust has abundance of Al and at soil pH below 5.0, mineral Al solubilises into trivalent Al^{3+} which has toxic effects on plants (Panda *et al.*, 2009). Root growth is inhibited by phytotoxic-Al (Al^{3+}) and consequently hinders the uptake of water and nutrient in plants (Rahman *et al.*, 2018). Adoption of altered root architecture due to varied plant hormone synthesis, generation of higher reactive oxygen species, higher peroxidation of fatty acid and increased osmolytes like proline in rice plants grown under aluminium toxicity attributes to aluminium resistance (Guo *et al.*, 2012). To increase soil pH and reduce Al toxicity farmers are advised to apply lime but due to the buffering capacity of soil the effect of such amendments are not much beneficial (Zheng and Yang., 2005). On the other hand, P is easily fixed by Al and Fe present in acidic soil making P unavailable for root uptake (Adnan *et al.*, 2018). Thus, P deficiency is regarded as another significant constraint in acidic soil for crop cultivation. Although P fertilizer is applied to increase crop production it is expensive and has detrimental environmental affects.

In this context beneficial bacteria (both rhizospheric bacteria and endophytes) can possibly improve plant development in a wide scope of climatic and edaphic stress conditions (Glick., 2012). Beneficial bacteria (rhizobacteria and endophyte) can colonize the surface or inside various plant tissue parts and rhizosphere zone, where they either exhibit functions which benefits the host or live commensally. Soil is a complex microbial habitat where plant seeds typically fall and germinate when they receive environmental signals. As seeds begin to germinate, seed endophytes form part of the seedling bacterial community and colonize various plant tissues as the plant begins to grow. Hence endophyte bacteria can be characterised as those bacteria that colonize inside tissues of the plant and usually results to be beneficial to the host. (Hardoim *et al.*, 2016). There is considerable evidence that endophytes are involved in enhanced nutrient acquisition, control plant disease, produce plant growth hormone and provide habitat-adaptive fitness to the host plant (Redman *et al.*, 2011; Mitter et al, 2013; Prashar *et al.*, 2014). Endophytes isolated from various plants parts produce indole acetic acid, cytokinins, gibberellins, siderophores, supply essential vitamins to host plant and solubilise insoluble phosphates which enhances plant growth (Etminani and Harighi; 2018, Jha *et al.,* 2012). Inoculating plants with bacteria having plant growth promoting properties to enhance plant growth and productivity is seemingly useful practice adopted. The exploitation of endophytes in mitigating environmental stress on plants has been acquiring new intrigue (Ryan *et al.*, 2007; Bulgarelli *et al.*, 2013) and it is now well established that endophytes can improve growth of plants under various abiotic stress conditions (Khare *et al.*, 2018).

So, it was hypothesized that the habitat-fitness of rice crop in Al toxic and P deficient acid soils is independent of endophyte colonization benefits. In order to test our hypothesis we examined the influence of endophyte inoculation on root morphology and a few important physiological stress indicators of rice plant (var CAUR3) grown along a toxicity gradient of Al in an acid *Inceptisols*. We also studied the interaction of endophyte on stress physiology, growth and development of rice in a microcosm set-up which received a gradient of P doses.

MATERIALS AND METHODS

Reference Strain

Serratia nematodiphila (22WE) isolated from the roots (surface sterilized) of wild rice variety *Zizania latifolia*, collected from the natural habitat of North-East India was the reference strain used in our study. The endophyte was isolated in nutrient agar medium at 30°C.

Genetic characterization of the strain

The endophyte was identified by 16S rDNA gene sequences. Lysozyme method as

depicted by Sultana *et al.*, 2019 was used to extract the genomic DNA of the isolate. The pure culture colonies of the endophyte were incubated for 24 hrs in 5ml Luria Bertani (LB) broth at 30°C and shaking speed of 150 rpm. Two milliliter aliquots from this broth were centrifuged. The cell pellet of the culture isolates obtained after centrifugation was subjected to lysis by addition of 10µl lysozyme (1mg/ml, Hi Media, India) followed by addition of 90µl sterile water. The sample was then incubated for 30 minutes and 5 minutes at 30°C and 95°C respectively. For the PCR amplification genomic DNA in the supernantant was used. 16S rDNA of the endophyte genomic DNA was amplified by using universal bacterial specific primers:-

27F (5-AGAGTTTGATCCTGGTCAG-3)

1492R (5-GGTTACCTTGTTACGACTT-3) (Lane *et al.*, 1991).

Amplification was done in a Gradient Master Cycler 5331 (Eppendorf Make, Germany) using standard PCR conditions and PCR mixtures (Dey *et al.*, 2018). GenElute PCR clean-up kit (Sigma–Aldrich, USA) was used for the purification of amplified products and sequenced by First BASE Laboratories, Malaysia. The 16S rRNA sequence obtained was looked for similarity in the GenBank reference genome database using BLASTn. Eztaxon website was used to confirm the strain designated. The sequences of the isolate are available in GenBank with accession number. The GenBank accession number of the strain is MH014960.

Surface sterilization and inoculation of seeds

The inoculum was prepared by incubation of *Serratia nematodiphila* in LB broth for 24 h. The pellet of the bacterial suspensions was obtained by centrifugation at 2,400 g for 12 mins at 25°C. The pellet was suspended in sterile distilled water and the cell density of the bacterial suspension was adjusted to 10^8 cells mL^{-1} by OD measurement at 600 nm. For surface sterilization of the seeds of the CAUR3 variety, seeds were first immersed in 70% ethanol for 1 min followed by immersion in 2% sodium hypochlorite for 5 mins and then finally in 70% ethanol for 30 secs. The seeds were then rinsed in sterile water four times and then immersed in bacterial suspension of $10x10^8$ cells mL^{-1} for 2 hrs. After that the inoculated seeds were kept on sterile tissue paper for about 45 mins to dry and allowed to germinate for 2 days.

Plant growth promoting (PGP) properties of the endophyte

Estimation of IAA

Free IAA-like substances, was determined according to Thakuria *et al.*, (2004). Nutrient Broth amended with 0.2% L-tryptophan was prepared where the endophyte suspension of the pure isolate (100µl) was allowed to grow for 48 hrs by incubation at the temperatute of 32°C and shaking at the speed of 100 rpm. 1 ml of the supernatant obtained after centrifugation of the broth at 10,000 g for 12 mins at 4°C was mixed with 4ml Salkowski's reagent (0.5 M ferric chloride ($FeCl_3$ and 35% perchloric acid ($HClO_4$). After 20 mins free IAA-like substances was determined using spectrophotometer by observing the absorbance at 530 nm and from the standard curve prepared using different concentration of serially diluted IAA (Sigma–Aldrich, USA).

Cellulolytic Activity

The cellulose degrading effectiveness of the endophyte strain was determined by incubating the strain in CMC agar plates for a period of 48 hrs (Verma *et al.*, 2001). The plates were stained with 1% Congo Red and counter stained with 1 molar NaCl. The zone formed around the colony indicates the cellulolytic activity of the strain.

Mineral phosphate solubilisation

Pikovskaya's medium was used for the determination of the solubilization of phosphate by the endophyte. The broth was inoculated with the bacterium and incubated at 30°C for 72 h and shaking with the speed of 150 rpm. The clear supernatant of the culture obtained after centrifugation (4000 rpm for 1 min) was used for the estimation of the soluble phosphorus by stannous chloride blue colour method as described by Brayet and Kurtz (1945).

Determination of 1-aminocyclopropane-1-carboxylate (ACC) deminase activity

To determine ACC deaminase activity endophyte isolate was grown in DF (Dworking and Foster) broth having a concentration of 0.5 mol^{-1} ACC following Penrose and Glick., (2003) method. Enzymatic cleavage of ACC produced α-ketobutyrate which was determined from the standard curve of a series of known concentration (between 0.1 and 1.0 mol) of α-ketobutyrate

(Sigma–Aldrich, USA) colorimetrically using a spectrophotometer (OD 540 nm). ACC deaminase activity was determined in micromoles of α–ketobutyrate per milligram of protein every hour.

Microcosm experiments

Two microcosm experiments, with acid *Inceptisols* (pH 4.80; soil organic carbon 1.67%; exchangeable acidity, Al and calcium plus magnesium 0.98, 0.3 and 0.7 meq per 100g soil and soil available P – 3.84 mg kg^{-1} respectively) was taken in pot culture filled with air dried sieved (2mm) and sterilized soil. Pot diameter was 30 cm and bulk density maintained at 1.36 g cc^{-1}. Four Al treatments (control, 100, 200 and 300 ppm Al kg^{-1} soil as $AlCl_3$) under two conditions i.e. with and without endophyte inoculation were imposed in one microcosm Four levels of P as fertilizer treatment (control, 30kg/ha, 60kh/ha/, 90kg/ha soil as KH_2PO_4) under the two conditions i.e. with and without endophyte inoculation in another microcosm were imposed.

Each treatment contained 6 replicate pots. Endophyte treated and untreated seedlings of the *Kharif* rice (var. CAUR3) were transplanted in pot soils according to treatments. In each pot, three plants of rice, of the CAUR3 variety were maintained throughout the period of the experiment. The important morphological and physiological characters sensitive to Al toxicity and the impact of the endophyte were assessed 35 days after transplantation (DAT). Similarly morphological and physiological characters and the impact of endophyte on the plants grown under P nutrition condition were also assessed after 35 days DAT. For each parameter, influence of factors (with and without endophyte or Al levels and with and without endophyte or P levels) and interactions between endophyte and Al levels and interactions between endophyte and P levels were determined by 2-way factorial CRD using SPSS v. 21.0.

Determination of Root characteristics

Representative plant samples were collected from each pot under different treatments at active growth stage (35DAT) of rice plant for determination of important root morphological parameters such as root volume (RV), root length (RL), root surface area (RSA) and root diameter (RD) which were determined by using root scanner (EPSON PERFECTION V700 PHOTO). The interpretation of the scanned image was made by professional and user friendly WinRHIZO software. It is a non-statistical method used for measuring different root parameters.

Estimation of root and shoot biomass

Roots and shoots were collected and their fresh weight was determined in a precision electronic balance and then died in a hot air oven (60°C) to a constant weight. Dry root and shoot weight was measured and expressed in gram per plant.

Evaluation of stress responsive physiological traits

Representative plant samples were collected at 35 DAT from each pot under different Al treatments for determination of important and key aluminium stress responsive physiological traits *viz*. chlorophyll content (Chl a, b, carotenoid), cell membrane integrity, DAB staining and lipid peroxidation. The same physiological traits were also assayed for the rice plants grown in different P levels.

Determination of leaf chlorophyll content

Around 0.5g leaves were taken for the estimation of leaf pigments (chlorophyll-a, chlorophyll-b and carotenoids). Fresh leaves were homogenized using acetone (80%). The supernatant obtained after centrifugation of the homogenized leaf samples were taken in a 100ml volumetric flask. After adjusting the supernatant in the volumetric flask to 100 ml with 80% acetone leaf pigments were analyzed calorimetrically. The chlorophyll pigments were expressed in mg/g tissue (Nayek *et al.*, 2014).

Chlorophyll a (C_a) = [12.7(A663) – 2.69(A645)] X(V/1000)X(1/W)

Chlorophyll b (C_b) = [22.9 (A645) – 4.68(A663)] X(V/1000) X (1/W)

Carotenoids = ($100A_{470}$ -1.82C_a-85.02C_b)/198

Where,

A = Absorbance measured at the specific wave lengths

V = Final volume of chlorophyll extracted in acetone (80%)

W = Weight of fresh tissues

Cell membrane integrity

The cell membrane integrity of fresh leaves of the rice plants (35 DAT) was analyzed by Sullivan's method (Sullivan and Ross, 1979). Finely cut leaf pieces weighing 0.5 g were immersed in

50 ml of deionised water and incubated under laboratory conditions for 3h. At the end of 3h, initial electrical conductivity (C_1) was measured using conductivity meter (Elico co.). Then the beaker containing deionized water with leaf pieces was allowed to boil for around 30 mins and the final electrical conductivity (C_2) was estimated. Cell membrane integrity was computed and expressed as follows:

$$CMI\ (\%) = [1-(C_1/C_2)] \times 100$$

Measurement of melondialdehyde accumulation by TBARS assay

The melondialdehyde was determined by the reaction described by Yagi (1987). Leaf tissues were homogenized in trichloroacetic acid and centrifuged. About 1ml of solution containing trichloro acetic -acid and thiobarbituric acid was added to 0.5ml of supernatant and centrifuged. The supernatant was taken and its absorbance was measured using spectrophotometer at 532 nm. Aborbance was further measured at 600nm to correct non specific turbity by subtracting the absorbance value at 600mn from the absorbance value at 532 nm.

DAB staining assay

To detect hydrogen peroxide (H_2O_2) produced on the leaves due to stress imposition, DAB (3, 3'-diaminobenzidine) staining assay was performed. DAB is oxidized by H_2O_2 and generate brown precipitate product (Christensen *et al.*, 1997).

RESULTS AND DISCUSSION

Roots usually suffer greater exposure to Al stress than shoots. In the present investigation, detailed analysis of root morphological characters, RL RSA, RV and RD with the range of Al concentrations 100ppm 200ppm and 300ppm kg^{-1} soil showed that there is a decrease in RL, RV, RSA while RD showed an increase in both treated and untreated plants and these values clearly corroborated with the root architecture depicted in the (Fig. 1. and Fig. 2). But in the endophyte inoculated rice plants there was a significant (0.05) increase in the RSA, RV and RL and decrease in the RD compared to that of uninoculated plants. It was further shown that high aluminium toxicity (Al concentrate of 300ppm kg^{-1} soil) had extremely

Table. 1 Plant growth promoting activities *Serratia nematophila*

Cellulase Activity zone diameter in mm	P-Solubilization $Ca_3(PO4)_4$ ($\mu gml^{-1}h^{-1}$)	Na-Phytate ($\mu gml^{-1}h^{-1}$)	ACC Deaminase ($\mu gml^{-1}h^{-1}$)	IAA like substances ($\mu gml^{-1}h^{-1}$)
18	169.5	2.97	51.3	623.0

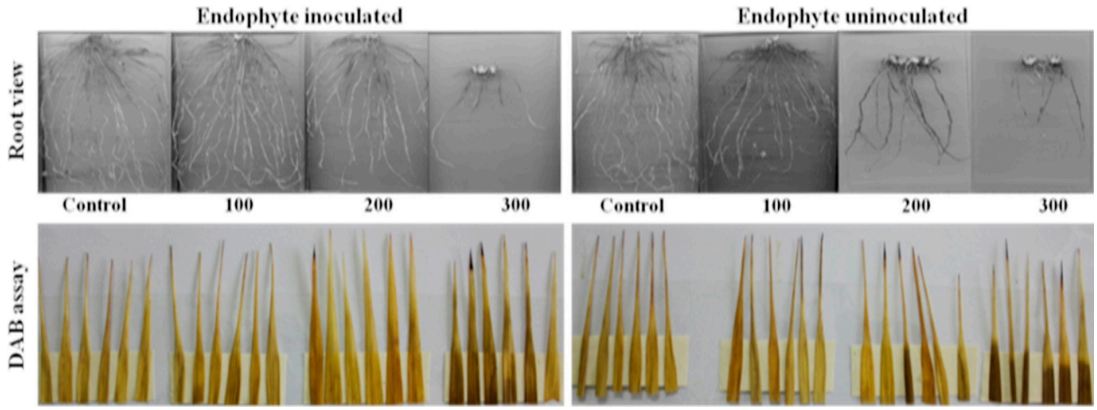

Fig.1. Influence of endophyte colonization and Al levels on root morphological features and H_2O_2 activity in fully expanded 3rd leaf determined by DAB assay at 35 DAT of Kharif (CAUR3) grown in an acid inceptisols.

toxic affect on the rice roots which caused drastic reduction in their growth for both endophyte inoculated and uninoculated plants.

P deficiency can result in the modification of root architecture. In beans and Arabidopsis P deficiency resulted in the decrease of primary root system but increase in the growth of lateral roots while in maize there was reduction in lateral root elongation (Lynch and Brown, 2001, Ticconi *et al.*, 2004). In our study there was also signinificant difference (P<0.05) in the RL, and RV between the inoculated and uninoculated plants. The RSA, RV and RD was highest in P dose 60 kg/ha and RL highest in the plants under under control in both inoculated and uninoculated plants.High dose of P had negative effect on the root morphological features. RSA, RV and RD was greater in endophyte inoculated plants than the uninoculated plants in all the P doses and root length lesser in the inoculated plants. (Fig. 3 and Fig. 4).

Al-toxicity causes oxidative stress which results in the alteration of cell wall properties, is a well established phenomenon (Yamamoto *et al.*, 2001). Al can act as catalyst in the production of reactive oxygen species (ROS) which is known to oxidize various cellular units (lipids, proteins, enzymes, and nucleic acids) which causes cell death. The final product of lipid peroxidation, malondialdehyde (MDA) is an indicator of the degree of membrane peroxidation (Wu *et al.*,

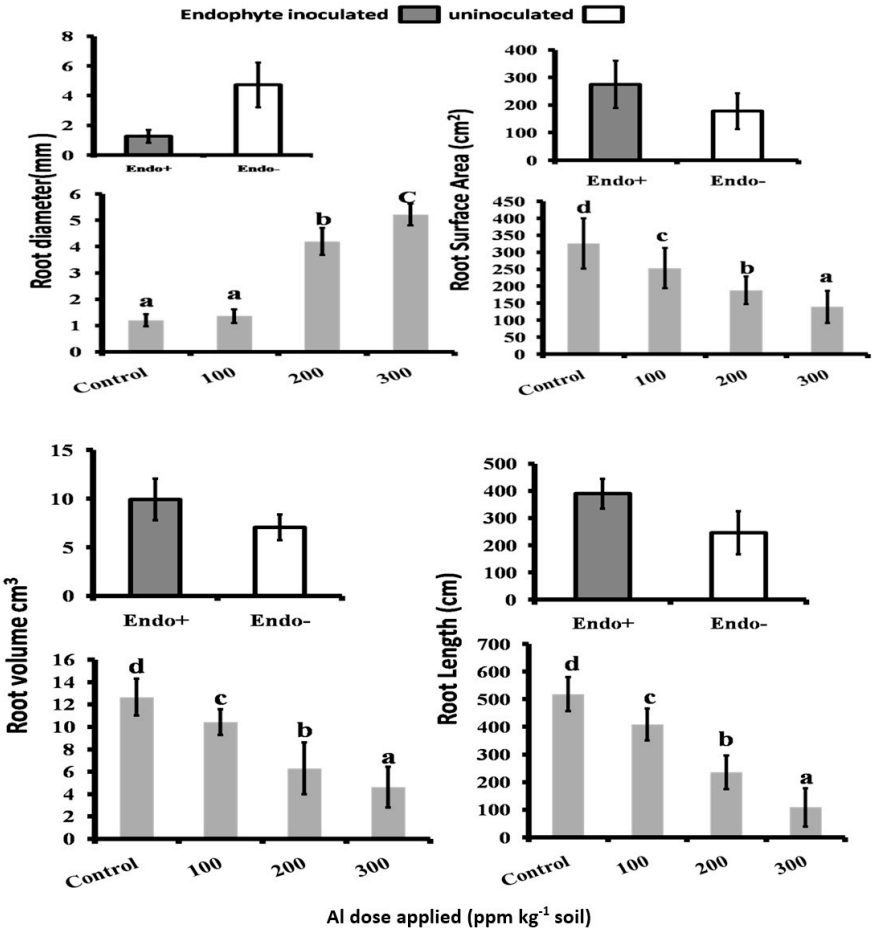

Fig. 2. Root morphological features: Root Diameter (RD), Root Surface Area (RSA), Root Volume (RV) and Root Lenght (RL) of the rice crop(CAUR3 var) grown under different Al-levels and with and without endophyte inoculation. Statististical Analysis was performed by Two Way ANOVA followed by Turkey's post hoc comparision with three replications using SPSS v.21 (SPSS Inc., Chicago, IL, USA).

2014). In our study MDA content increased with increasing Al concentration in both endophyte inoculated and uninoculated plants. Plants grown under Al concentration 300 ppm kg^{-1} soil showed highest MDA content and there was not much difference in the MDA content of the plants grown under Al concentration 300 ppm kg^{-1} soil for both endophyte inoculated and uninoculated. However endophyte inoculated plants showed modification in the amount of lipid peroxidation at control, 100ppm, 200ppm levels of Al and there was significantly (P<0.05) lesser amount of MDA in endophyte inoculated plants than uninoculated plants (Table 2).

It is established that plants adopt different mechanisms to circumvent the harmful effects of low P levels in soil. P deficiency in plants can cause photo-oxidative stress which is known to cause an increase in ROS production which can damage lipids, proteins and nucleic acids in chloroplast. (Hernandez and Bosh., 2015).We studied the MDA content in the plants grown under different P doses. Our results indicated that the MDA content in the control was highest for both the inoculated and uninoculated plants and lowest in the plant grown under P dose 60kg/ha soil (Table 3). MDA content of endophyte inoculated and uninoculated plants showed no significant difference.

H_2O_2 is a typical ROS which is generated in the cells under normal as well as under stress conditions (Sharma *et al.*, 2012). DAB staining assay used to visualize the H_2O_2 in the leaves showed that with the increasing Al concentration the DAB staining enhanced as shown in Fig. 1. But the extent of H_2O_2 production in leaves of endophyte inoculated plants was much lesser than that of uninoculated plants in all Al levels (Fig. 1) except at 300 ppm kg^{-1} soil concentration of Al, the leaves of both endophyte inoculated and uninoculated plants could be not be visually distinguished by DAB staining.

The H_2O_2 production in the endophyte uninoculated plants in the control was more in the control than the inoculated plants (Fig. 3) in case of the P treated plants. At P doses 30kg/ha, 60kg/ha and 90kg/ha soil DAB assay could not show any visual difference among the leaves of both inoculated and uninoculated rice plants (Fig. 3).

It has been reported that abiotic stress damage selective permeability of cell membrane and thus the cell cannot mantain its internal composition (Bajji *et al.*, 2002). CMS (Cell Membrane Stability) study which measures the percentage cell membrane leakage showed that with increasing Al concentration percentage leakage increased for both the endophyte inoculated and uninoculated plants (Table.2.) The CMS values was significantly lesser (P<0.05) in endophyte inoculated plants than the uninoculated plants (Table 2).

Similarly the CMS value in the inoculated plants grown under the different P doses was significantly less than the uninoculated plants (Table.3). The CMS value was lowest in the plants grown under 60kg/ha followed be 30kg/ha, 90kg /ha soil and control in both inoculated and uninoculated plants.

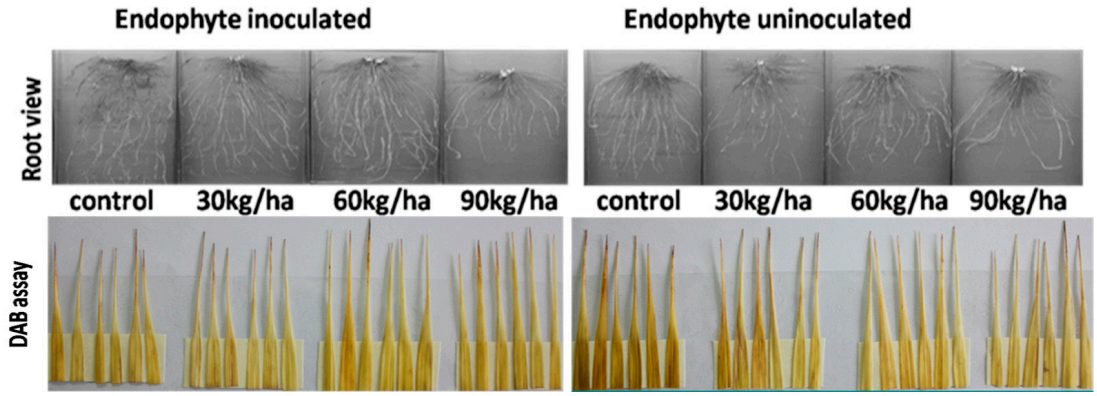

Fig.3. Influence of endophyte inoculation and P levels on root morphological features and H_2O_2 activity in fully expanded 3rd leaf tips determined by DAB assay at 35 DAT of *Kharif* rice (CAUR3) grown in an acid *Inceptisols*

It is documented that Al stress can result in the decrease of photosynthetic rate in plants which leads to chlorosis and necrosis of leaves (Thornton *et al.*, 1986). In our study tested chlorophyll content, both chlorophyll a and b decreased with increasing aluminium concentration. The endophyte inoculated plants produced higher chlorophyll a and b content as shown in Table. 2. Carotenoid is another leaf pigment which aid in the light absorbtion for photosynthesis and also protects chlorophyll from any photo damage. (Armstrong and Hearst, 1996). Carotenoid has also been reported to have antioxidant properties (Stahl and Sies, 2003). In our study there was significant (P<0.05) increase in the carotenoid content of uninoculated plants than the inoculated plants.

P deficiency has detrimental effect on photosynthetic characteristics in rice as reported by Xu *et al.*, 2007. We studied the chla, chlb and carotenoid in the leaves grown under the different P levels for both the endophyte inoculated and uninoculated rice plants. Chla content was significantly different among the inoculated and

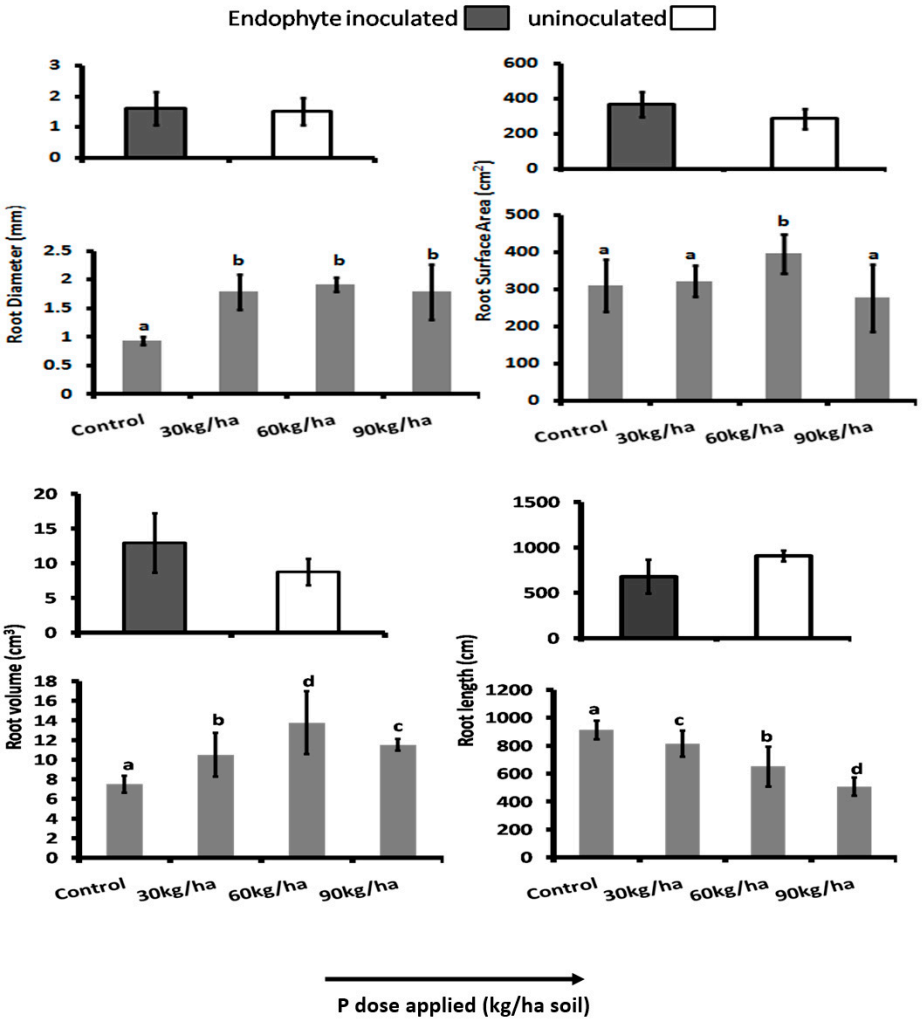

Fig. 4: Root morphological features: Root Diameter (RD) Root Surface Area (RSA), Root Volume (RV) and Root Length (RL) of the rice crop (CAUR3 var) grown under different P-doses and with and without inoculation. Statististical Analysis was performed by Two Way ANOVA followed by Turkey's post hoc comparision with three replications using SPSS v.21 (SPSS Inc., Chicago, IL, USA).

Table 2. Effect of endophyte inoculation and Al levels on leaf pigmentation, plant stress, and root and shoot biomass in rice .

Treatment	Al levels	Chl-a (mg g-1 fresh wt.)	Chl-b (mg g-1 fresh wt.)	CMS (%)	MDA (µM g-1 leaf tissue)	Caro (mg g-1 fresh wt.)	DRW (g plant-1)	DSW (g plant-1)
Endophyte inoculated	Control	1.51±0.04	0.31±0.04	3.84±0.19	20.7±2.9	0.08±0.003	0.42±0.03	1.47±0.14
	100ppm kg-1 soil	1.47±0.01	0.36±0.02	4.37±0.23	27.5±1.7	0.09±0.005	0.32±0.02	1.28±0.03
	200ppmkg-1soil	1.30±0.03	0.42±0.02	5.58±0.20	33.2±2.6	0.12±0.008	0.28±0.01	0.83±0.14
	300ppmkg-1 soil	1.26±0.02	0.48±0.03	5.73±0.18	34.6±1.9	0.15±0.014	0.24±0.04	0.78±0.04
Endophyte uninoculated	Control	1.39±0.04	0.39±0.02	5.64±0.23	25.9±2.4	0.10±0.007	0.34±0.01	1.27±0.07
	100ppmkg-1soil	1.31±0.03	0.42±0.02	6.80±0.19	33.4±2.5	0.11±0.005	0.28±0.01	1.03±0.08
	200ppmkg-1soil	1.26±0.06	0.52±0.03	7.04±0.52	37.8±2.6	0.14±0.014	0.21±0.03	0.75±0.06
	300ppm-1soil	1.08±0.17	0.62±0.02	8.54±0.46	50.3±4.5	0.14±0.007	0.16±0.01	0.54±0.02
Level of significance								
Endophyte		***	***	***	***	***	***	***
Al level (ppm kg-1 soil)		**	***	***	***	***	**	**
Endophyte x Al (ppm kg-1 soil)		**	ns	**	**	**	ns	ns

Values are means ± SD; Each mean represents average of 3 replicate analyses. Level of significance was determined by performing Univariate 2-Factorial Completely Randomized Design within the General Linear Model using SPSS v.21.

Chl-a: Chlorophyll-a ,Chl-b: Chlorophyll -b contents in the flag leaf (mg g-1 fresh wt.); CMS: cell membrane stability in the flag leaf (expressed as % cell content leakage); Caro: Carotenoid content in the flag leaf (mg g-1 fresh wt.); lipid peroxidation expressed as µM MDA g-1 leaf tissue; DRW: dry root weight (g plant-1); DSW: dry shoot weight (g plant-1).(*-significant and ns-nonsignificant).

Table 3. Effect of endophyte inoculation and P levels on leaf pigmentation, plant stress, and root and shoot biomass in rice.

Treatment	P levels fresh wt.)	Chl-a(mg g^{-1} fresh wt.)	Chl-b(mg g^{-1} fresh wt.)	CMS %	MDA(µM g^{-1} leaf tissue)	Caro(mg g^{-1} fresh wt.)	DRW (g plant-1)	DSW (g plant-1)
Endophyte inoculated	Control	1.12±0.01	0.85±0.47	2.89±0.22	7.20±0.27	2.10±0.16	0.80±0.12	2.68±0.10
	30(kg/ha soil)	1.26±0.05	0.94±0.54	0.66±0.03	5.27±1.27	1.84±0.05	1.23±0.60	2.30±0.14
	60(kg/ha soil)	1.19±0.05	0.76±0.47	0.24±0.02	4.63±1.34	1.05±0.08	1.89±0.10	2.07±0.15
	90(kg/ha soil)	1.07±0.11	0.90±0.53	2.45±0.01	6.63±0.12	2.15±0.13	0.76±0.02	1.88±0.12
Endophyte uninoculated	Control	0.91±0.08	0.25±0.00	6.39±0.22	11.27±3.94	1.63±0.23	0.68±.08	2.30±0.15
	30(kg/ha soil)	1.10±0.08	0.33±0.07	2.08±0.03	7.60±1.34	1.37±0.25	0.80±0.02	1.57±0.09
	60(kg/ha soil)	1.19±0.08	0.24±0.08	1.97±0.73	5.82±1.17	0.97±0.25	0.84±0.04	1.17±0.07
	90(kg/ha soil)	0.77±0.04	0.27±0.06	3.95±0.61	8.69±1.06	2.51±0.63	0.62±0.03	0.92±0.01
Level of significance								
Endophyte	***	**	***	**	**	***	***	
P level (kg ha-1soil)	***	ns	***	**	***	***	***	
Endophyte x P (kg ha-1 soil)**		ns	***	ns	ns	**	**	

Values are means ± SD; Each mean represents average of 3 replicate analyses. Level of significance was determined by performing Univariate 2-Factorial Completely Randomized Design within the General Linear Model using SPSS v.21.

Chl-a: Chlorophyll a, Chl-b: Chlorophyll -b contents in the flag leaf (mg g-1 fresh wt.); CMS: cell membrane stability in the flag leaf (expressed as % cell content leakage); Caro: Carotenoid content in the flag leaf (µg g-1 fresh wt.); lipid peroxidation expressed as µM MDA g-1 leaf tissue; DRW: dry root weight (g plant-1); DSW: dry shoot weight (g plant-1).(*-significant and ns-nonsignificant).

uninoculated plants (Table.3). The inoculated plants had significantly (*P<0.05*) more chla content than uninoculated plants. However in the inoculated and uninoculated plants chlb and carotenoid content had no significant difference.

Both root and shoot dry weight had greater values in the inoculated plants than the uninoculated plants at low and moderate Al concentration and hence showed greater root and shoot biomass in control, 100ppm and 200 pmm kg^{-1} soil. The root growth reduced to a great extent with the increasing Al levels. Interestingly, the endophyte inoculated plants of 100 ppm Al kg^{-1} soil level and the uninoculated control plants produced comparable root biomass (Fig. 1 and Table 2).

The endophyte inoculated plants under different P doses showed significantly higher (P<0.05) root and shoot biomass as evident from the dry root and shoot values shown in Table 2. The dry root and was highest among the plants grown under P-dose 60 kg/ha soil followed by 30kg/ha, control and 90 kg/ha soil in both inoculated and uninoculated plants (Fig. 3 and Table 3) and shoot weight highest among the control in both inoculated and uninoculated plants (Table 3)

This study clearly demonstrated that inoculation of efficient endophytes could exhibit fitness benefits to the host plant against Al toxicity as evident from improved root architecture, lowering stress impact (reduced CMS, MDA as well as H_2O_2) and incremental increase in shoot and root biomass in endophyte inoculated plants. Efficient endophyte could provide plant physiological advantage, growth and development under P-deficient acid soil as the rice plants grown in acidic soil under control having P deficiency showed better root-shoot biomass, better root morphological features and lower stress indicator-CMS values. The inoculated plants grown under increased P dose (30kg/ha, 60kg/ha, 90kg/ha soil) also showed better physiological and growth parameters which indicates the efficiency of endophytes to improve growth parameters of plants along with fertilizer. It is found that the strain could dissolve insoluble inorganic phosphate, amended Pikovskaya's broth (pH 6.8)} and organic phosphate Na-phytate amended Pikovskaya's broth (pH 4.6), produce indole-acetic-acid like substances, cellulose degrading activity and ACC deaminase activity which are widely recognized as plant growth promoting (PGP) mechanisms of beneficial microbes (Table 1). It has also been documented that IAA can help root and shoot development under stress conditions (Shi, 2004). Microbial phosphate solubilisation is a promising tool as biofertilizer as they mediate the solubilisation of inorganic phosphate and mineralization of organic phosphate which makes hosphorous available to the plants and enhance plant growth (Gaur *et al.*, 1972). ACC is a well known plant growth promoting enzyme. This enzyme cleaves ACC, the immediate precursor of ethylene to ammonia and α-ketobutyrate (Honma and Shimomura1978). As a result ethylene levels in plants are lowered which enhances plant growth as high ethylene levels in plants reduces their growth or can even cause plant death (Glick *et al.*, 2007).

These possible PGP mechanisms probably helped the endophyte inoculated rice plants to support better root and shoot development, enhanced P uptake, and thereby reducing stress factors on the plant under Al toxic acid soil and even when P was added to the soil as fertilizer. Further, the root and shoot biomass data indicated that the toxic effect of an approximate 100ppm Al could be compensated due to endophyte inoculation.

CONCLUSION

The proposed results have shown that endophyte bacteria can help plants to have better growth in abiotic stress condition. So the study has shown the significance of exploration of new endophyte population and their beneficial effect on plants under abiotic stress condition. Results from the two sets of microcosm experiments on the pattern of root endophyte interactions across Al and P concentration gradients indicated that endophyte inoculation provide fitness benefits to rice crop against Al toxicity and also benefits the crop even in P deficient soil which is supplied with P fertilizer.

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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AUTHORS' CONTRIBUTIONS

All authors listed have made a substantial, direct and intellectual contribution to the work and approved it for publication.

DATA AVAILABILITY

The dataset generated during the investigation are included in the manuscript.

ETHICS STATEMENT

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

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