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COMPARATIVE EFFECTIVENESS OF ACC-DEAMINASE AND/OR N FIXING RHIZOBACTERIA IN RICE (*Oryza sativa* L.)

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Abstract

Plant growth promoting rhizobacteria (PGPR) colonize rhizosphere and enhance growth in crop plants, by producing various regulatory substances. Since PGPR employ diverse array of mechanisms and exact mechanism and role are still to be established. Therefor experiment was designed 1) to identify and 2) to select potential rhizobacterial strains based on their impact on the growth, physiological and enzymatic activities of rice. A CRD pot experiment with six PGPR isolates; two containing only ACC-deaminase activity (ACC1 and ACC2), 2 isolates containing N fixing activity (*Azotobacter* and RN1) and 2 PGPR isolates (AN1 and AN2) containing both abilities, was conducted under controlled environment. Root infusion with all the selected PGPR isolates improved plants responses than control (CK) conversely, the AN1 isolate with both the ACC- deaminase activity and N fixing ability was found to be the most efficient. AN1 increased the root length by 3.8%, shoot length by 3.4% and other parameters over control. Macronutrient uptake e.g. N (5.0%, 5.4%), P (4.5%, 3.6%) and Mg (4.0%, 3.0%), plant micronutrient uptake e.g. Zn (5.6%, 8.1%), Cu (5.0%, 5.1%) and Fe (4.7%, 5.6%) and plants antioxidant enzymes activities were also augmented up to 5.5% by rhizobacterial inoculation. Overall PGPR isolates enriched both plant growth and physiology by mainly by improving nutrient availability, uptake and by alleviating ethylene stress due to its ACC cleaving ability in the rhizosphere. Therefor application of rhizobacterial strains as biofertilizers is viable and ecological technique to facilitate crop production on sustainable basis.

Key words: ACC-deaminase, antioxidant enzymes, growth attribute, PGPR, rice seedlings

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1. Introduction

The species of bacteria which specifically inhabit root zone of the plants and potentially stimulate plant growth and are renowned as plants growth promoting rhizobacteria (PGPR) (Ahmed and Kibret, 2014; Mihalache et al., 2016). PGPR are non-symbiotic rhizospheric bacteria which inhabit roots promote plant growth (Nadeem et al., 2006). They and support plants development and growth both directly and indirect means. It is well established that these bacterial strains improve growth conditions through production of phytohormones; auxin, cytokinins, gibberelins and also by increased bioavailability of the essential nutrients (Hassan et al., 2015). These bacterial strains are also believed to eradicate plants stress by improving soil conditions through synthesis of various organic acids (Ahemad and Malik, 2011). When ornamentals, forest trees, vegetables, and agricultural crops are infested with rhizobacterial isolates exhibit numerous positive effects on plant growth attributes and productivity e.g. enhanced rate of germination, plant potency, plant height, plant weight, antioxidant enzymes activity, and nodulation in legumes etc. (Sahran and Nehra, 2011). Sustainable agricultural vision can

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only be achieved by reducing dependency upon inorganic fertilizers and inducing disease resistance, tolerance towards salt, drought, heavy metals stress and better nutritional value. These traits in crop plants can be accomplished through application of the beneficial soil micro flora (Hayat et al., 2010). However annual consumption of approximately 153.98 M tons of chemical fertilizers is to obtain higher yields of crop plants (Naveed et al., 2008). Although fertilizers application instantly improves yields conversely there are several negative impacts on soil health, overall quality of environment and ultimately these are toxic to all forms therefor some self-propagating eco-friendly life alternatives are imperative. Therefore, the application of these growth promoting rhizobacterial isolates can not only reduce dependency and demand of chemical fertilizers but also protects and improves environmental health over time on sustainable basis (Hassan et al., 2015).

Plants in nature have adapted mechanisms to withstand and survive harsh conditions. Several environmental stresses trigger reactive oxygen species [O], H_2O_2 in plant cells and these can cause severe injuries to plant systems (Hassan et al., 2013). Plants have therefor adopted a competent antioxidant mechanism for capturing these ROS and hence safeguard cellular mechanisms from any injury (Aehmad, 2012). Plants produce diverse enzymes to cope the severity of the harsh biotic and abiotic factors; these enzymes include peroxidases, glutathione S-transferase, catalase, phenylalanine ammonia-lyase (Glick, 2012), and collectively known as antioxidant enzymes. Chen et al. (2000) inoculated the cucumber seedlings with PGPR and observed a high level of antioxidant enzymes, upon the attack of soil born pathogen *Pythium aphanidermatum*.

There are several studies indicating a linear correlation between PGPR infusion with roots and enhanced activities of wide range of antioxidant enzymes e.g. catalase under stress rice and wheat (Young et al., 1995). Catalase reduces oxidative stress in plants by converting H_2O_2 in water and oxygen (Zhen et al. 2009). Singh at el., (2016) also reported that PGPR infusion remarkably improved 15-85% yield of wheat seedlings by improving their salinity tolerance. It was reflected in the antioxidant enzymes activity of the wheat seedlings inoculated with the selected PGPR isolates than control. Hence inoculation of agricultural crops with suitable and competent beneficial isolates has one more benefit of increasing the plants resistance and survival thus ultimately improved disease management that can otherwise cause harmful effects and may halt plant growth.

Ethylene is well established for its role in breaking seed dormancy and stimulating seed germination (Khalid et al., 2006), however its role as stress regulating hormone has also been well established and in higher concentrations it has growth inhibiting effect on plants (Ma et al., 2014).

Ethylene induces stress responses through interacting and regulating other hormonal pathways Ma et al. (2014) proposed ethylene induced inhibition of root growth in rice was through ABA. PGPR infusion significantly reduces ethylene induced stress in plants Zahir et al. (2008) proposed that it is owing to the enzyme ACC-deaminase. ACC is precursor of ethylene which is produced in roots and is then transferred to the shoots and sight of growth inhibition. If ACC levels are controlled and decreased ultimately it results in lower levels of ethylene and hence improves growth of plants (Khan et al., 2015). PGPR having ACC-Deaminase lowers ethylene by breaking down and converting its precursor ACC into 2-oxobutanoate and NH_3 (Arshad et al., 2007). IAA produced by rhizobacteria interferes with the plants physiology, changes plants auxin pool, and thereby is identified as a phyto-stimulation factor (Spaepen and Vanderleyden, 2011). Therefor it can be proposed that PGPR isolates producing IAA can vigorously improve plants growth and physiology.

Mia et al. (2010) also reported PGPR infusion improved growth attributes of musa plantlets and this was owing to the fact that PGPR improve mineral nutrient contents of the shoots as well as roots. Çakmakçi et al. (2007) suggested that these rhizospheric bacteria modify soil characteristics and make media more suitable for mineral uptake through production of rhizospheric enzymes. Therefore the use of PGPR as a biofertilizer can be a viable aepproach moreover these PGPR strains must be proficient, persistent and able colonize the rhizospheric soils (Glick, 2012).

Therefore, isolation and identification of efficient PGPR strains is imperative to incorporate them in sustainable agricultural practices (Hassan et al., 2014). The experiment was carried out to attest following hypothesis (1) Identification and isolation of potent PGPR strains, (2) to establish relative effectiveness of these isolates in improving the growth and physiological attributes of rice seedlings. Three rhizobacterial isolates 1) comprising ACC-deaminase activity (ACC1, ACC2), 2) exhibiting N-fixing ability (*Azotobacter*, RN1) and 3) having both the attributes (AN1, AN2).

2. Material and methods

2.1. Physical chemical analysis

The field soil was collected from the rhizosphere (5 cm depth) of tomato using an augur. The soil was then brought back to the laboratory where it was sorted for any roots left in it and processed for further analysis. Soil pH and EC were calculated using method of Hassan et al. (2013) and other physicochemical properties of the soil (Table 1) were measured following methods of US Salinity Laboratory Staff (1954).

2.2. Isolation of PGPR

Rhizospheric soil collected from the field under tomato cultivation was used to isolate desired strains. The rhizospheric soil was obtained by juddering of the tomato roots. Enrichment technique and dilution plate techniques by Wollum II (1982) were employed for isolation of two rhizobacterial strains.

The rhizobacterial strains exhibiting both N fixing and ACC-deaminase activity were further selected by growing isolates on cross nutrient media i.e. isolate with ACC-deaminase activity were incubated in agar mannitol media and other strain was incubated on minimal salt media. The isolates "AN1 and AN2" grew on both media hence they have both attributes.

Table 1. Properties of the soil used

Particulars	***Values***
Texture	Loamy sand
pH	7.96
EC (µS cm^{-1})	643
P	6.12
Lime (%)	19.33
Organic matter (%)	0.64
K	129.5
Na	92.9
SAR	8.7
Cu	0.08
Cr	0.07
Fe	0.96
Mn	0.09
Ni	0.16
Zn	1.32

Essential nutrients were measured in (mg/kg)

2.3. Preparation of inocula

Autoclaved, modified mannitol agar media and minimal salt media were used for incubation of isolates comprising N fixing and ACC-Deaminase respectively. For preparation of the inocula selected rhizobacterial isolates were grown in 250 ml sterilized flasks. The isolates were incubated for two consecutive days at 28°C on orbital shaker at 100 rpm to maintain consistency of media and bacterial populations. Optical density of the broth was continuously measured to maintain uniform bacterial population of during inoculation.

2.4. Characterization of isolates

Davis and Whitbread (1989) method was followed for root colonization under precisely gnotobiotic conditions. Sterilized sand filled glass jars were placed in growth chamber four seeds per jar were sown and conditions were maintained at 16 hrs day light, 70% humidity and 18°C for seven continuous days. Rhizobacterial strains were obtained from the roots of seven days old seedlings by excising roots and briskly shaking them in phosphate buffer. NBRIP P growth medium was used to determine P solubilization potential of these selected isolates. After incubation of 7 days at 28°C P solubilizing index (PSI) was calculated according to Premono's formula (1996).

ACC deaminase was assayed through methods described by Honma and Shimomura (1978). Spectrophotometer was used to estimate auxin production of bacterial strains (Table 2) following Sarwar et al. (1992).

2.5. Determination of plant physical and physiological parameters

Plant physical parameters were measured to establish growth attributes and responses therefor after harvesting shoot and root lengths, the fresh and dry weight was determined. Chl. a, b and carotenoids were separately (Arnon, 1949). Plant N (N) was estimated according to the methods of Van-Schouwengerg and Walinge (1973), the plants P by Buresh's et al. (1982). The plants micronutrients were estimated by spectroscopy after dry ashing the plant tissues (Chapman and Pratt, 1961). For antioxidants enzyme activity; GST was calculated by the assay of Habig et al. (1974). Ascorbate peroxidase was estimated photometrically by following the method of Azada and Nakano (1987) whereas Cakmak and Horst's assay (1991) was applied to plot catalase activity by using spectrophotometer.

2.6. Experimental design

Six PGPR isolates; two containing only ACC-deaminase activity (ACC1 and ACC2), 2 isolates containing N fixing activity (*Azotobacter* and RN1) and 2 PGPR isolates (AN1 and AN2) containing both abilities were selected for incubation experiment.

Table 2. Representation of PGPRs used in experiment

Isolates	***ACC-deaminase activity (µmol)***	***N fixation***	***P solubilization***	***IAA (mg L^{-1})***		***CFU g^{-1} root***	***PSI***
				With L-TRP	***Without L-TRP***		
ACC1	18.58	-ve	+ve	17.8	9.57	3.2×10^7	1.43±0.13
ACC2	13.83	-ve	-ve	10.1	7.01	2.2×10^7	-
AN1	9.89	+ve	+ve	30.1	7.16	11.7×10^7	3.85±0.16
AN2	7.11	+ve	+ve	18.9	5.45	8.2×10^7	2.12±0.14
Azotobacter	0.0003	+ve	-ve	13.8	8.71	7.2×10^7	-
RN1	0.0001	+ve	+ve	8.96	4.69	5.6×10^7	1.14±0.11

-ve = Absent, +ve = Present

Germinated seeds (variety Basmati 515) were inoculated by dipping in the broth containing selected PGPR isolates. For control treatment seeds were soaked in autoclaved glass jars bearing '0.03 M $MgSO_4$' to eliminate any microbe. Experiment was carried under controlled conditions in CRD and four replicates for each treatment. Growing seedlings were provided nutrients with sterilized half strength and N free Hoagland solution for 28 days after germination. Rice plantlets were harvested after 28 days and growth and physiological attributes were recorded.

2.7. Statistical analysis

The data were organized using Microsoft Excel 2010 and presented as means ±standard errors (SEs). Statistically results were tested by Statistix 8.1 (Statistix, USA). Significant differences among treatments were accomplished through least significant difference test ($p < 0.05$). Then Origin pro 8.5 was used for graphical presentation.

3. Results

3.1. Root and shoot length

PGPR isolates having N fixing and/ or ACC-deaminase activity exhibited growth promoting effects and resulted in increased root and shoot lengths in rice seedlings ranging from 1.5-% to 3.8-% and 1.4-% to 3.4 % (Fig. 1). However supreme results were recorded for AN1 isolate i.e. 3.8-% and 3.4-% increase than to control (CK).

Similarly, AN2 was the second most influencing isolate in terms of growth promotion in rice seedlings and caused 3.2-% and 2.9-% enhanced root and shoot length as compared to CK. *Azotobacter*, RN1, ACC1 and ACC2 caused 2.6- and 2.3-%, 2.3- and 2.0-%, 1.8- and 1.6-% and 1.5- and 1.4-% increases respectively in comparison to control (CK).

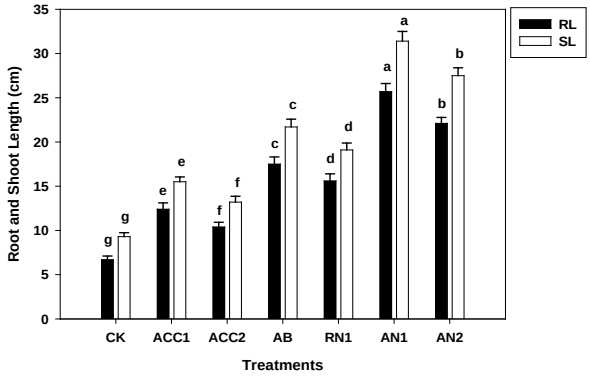

Fig. 1. Comparative root and shoot length of rice under inoculation of different PGPR isolates and results are indicating AN1 is most effective isolate, parameters with similar letter do not differ significantly

3.2. Biomass of seedlings

The significantly improved fresh and dry weights of rice seedlings were observed when these were inoculated with the selected rhizobacterial isolates (Fig. 2). Data recorded upon harvesting presented that inoculation with all PGPR isolates potentially promoted fresh and dry weight of rice seedlings ranging from 1.4% to 3.3% and 1.5% to 4.5% than untreated set of seedlings (CK). Although all isolates were recorded to significantly increase biomass production of seedlings however the AN1 isolate was found to be the most promising, caused an increase of 3.3% and 4.5% respectively. The AN2 was the next most effective in promoting biomass production up to 2.9 and 4.3% than control. The order of effectiveness in biomass production was found to be *Azotobacter* > RN1> ACC1> ACC2 causing approximately 2.4% and 2.8%, 2.1% and 2.4%, 1.8% and 2.0 % and 1.0% and 1.2% increases in fresh and dry weights respectively.

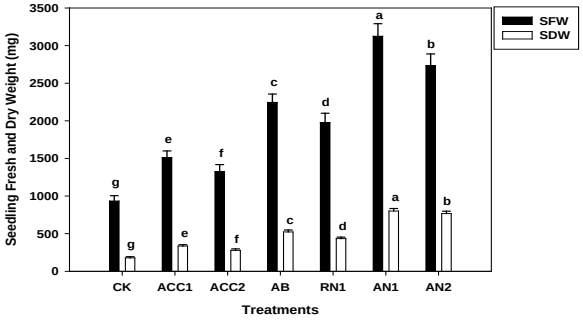

Fig. 2. Comparative biomass production in rice seedlings under different rhizobacterial isolates and results are indicating AN1 is most effective isolate, parameters with similar letter do not differ significantly

3.3. Carotenoid and chlorophyll in plant leaves

The photosynthetic pigments were significantly ($P < 0.05$) improved in rice seedlings when inoculated with the selected PGPR isolates ranging values for chl a, b and carotenoid contents, 1.4 to 4.2 %, 1.2 to 3.6 % and 2.0 to 5.1 % (Fig. 3). The most effective isolate was AN1 that promoted 4.2-%, 3.6-% and 5.1-% increased production of these essential pigments in treated rice seedlings than control. The isolates AN2 > *Azotobacter* > RN1 > ACC1 > ACC2 that resulted in about 3.7-%, 3.0-% and 4.6 %, 2.8-%, 2.4-% and 3.8-%, 2.5-%, 2.1-% and 3.5-%, 1.7-%, 1.6-% and 2.3-% and 1.4-%, 1.2-% and 2.0-% orderly increase compared to uninoculated control.

3.4. Macronutrient contents in shoot

Nutrient unavailability is not always due to their shortage in rhizosphere but these are

physiologically unavailable due to several environmental stresses thus resulting in poor growth and health of plants (Ahmad and Kibret, 2012). Jha and Subramanian (2013) showed improved physiology and nutrient uptake in rice paddies under salinity stress when inoculated with ACC-deaminase producing PGPR isolates. Similar results were observed in this study when rice seedlings showed comparatively improved nutrient. Shoots were assayed for their macronutrients content in response to the PGPR inoculation and results showed significant role of rhizobacterial isolates in increased uptake of plants primary macronutrient including N, P, K, Ca, and Mg (Fig. 4). Among all AN1 isolate showed the highest N (5.0%), P (4.5%), K (4.3%), Ca (3.7%) and Mg (3.0%) contents in shoots. The other isolates also showed similar results in the descending order of nutrient uptake AN2 > *Azotobacter* > RN1> ACC1 > ACC2 in the plant shoots than control (CK).

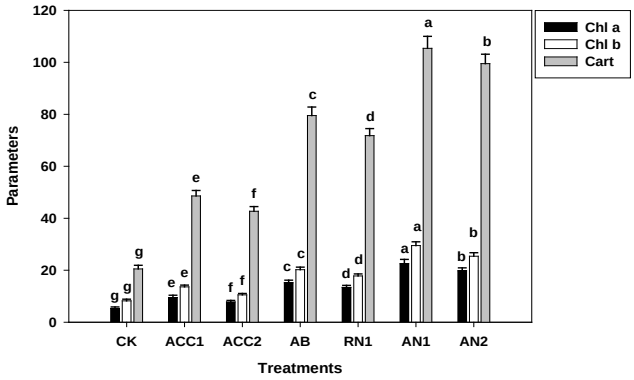

Fig. 3. Chl. a, b and carotenoid content of rice under different PGPR inoculations and results are indicating AN1 is most effective isolate, parameters with similar letter do not differ significantly

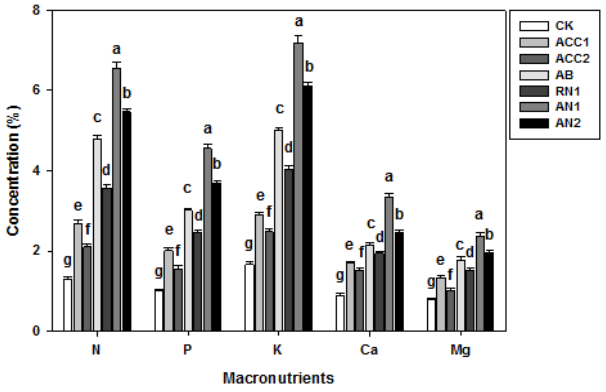

Fig. 4. Relative effects of different PGPR isolates on macronutrient contents in shoot of rice and results are indicating AN1 is most effective isolate, parameters with similar letter do not differ significantly

3.5. Macronutrient uptake by root

Root infused PGPR isolates also significantly improved macronutrient content of the roots. Increased plants ionic uptake in roots (Fig. 5) was also showing linear trends with shoots i.e. AN1> AN2 > *Azotobacter* > RN1> ACC1 > ACC2. The highest N (5.4-%), P (3.6-%), K (3.4-%) Ca (3.5-%) and Mg (4.0-%) contents were recorded for AN1 inoculated treatments.

Other isolates AN2, *Azotobacter*, RN1, ACC1 and ACC2 also significantly augmented the N (4.1-%, 3.3-%, 2.8-%, 2.3-% and 2.0-%), P (3.2-%, 2.7-%, 2.5-%, 2.2-% and 2.0-%), K (3.0-%, 2.2-%, 1.7-%, 1.5-% and 1.4-%), Ca (3.1-%, 2.6-%, 2.2-%, 1.8-% and 1.5-%) and Mg (3.5-%, 3.3-%, 2.7-%, 2.3-% and 1.4-%) in the plant roots over control.

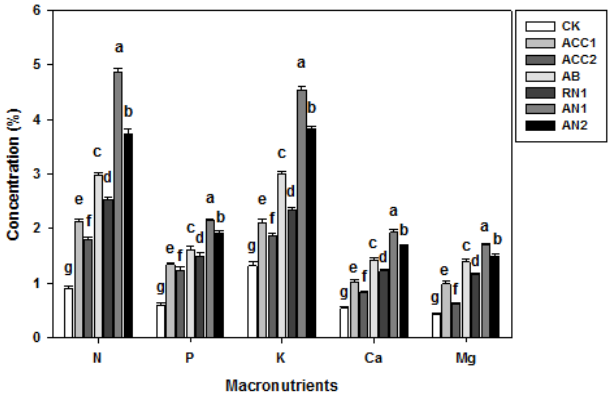

Fig. 5. Relative effects of different PGPR isolates on macronutrients levels in roots of rice seedlings and results are indicating AN1 is most effective isolate, parameters with similar letter do not differ significantly

3.6. Micronutrient uptake by shoot

PGPR inoculation improves micronutrient uptake in plants and this was exhibited by all the selected rhizobacterial isolates when infused with plant roots than control (Fig. 6). AN1 was again found to be the most effective in triggering the micronutrient uptake and resultant beneficial ions content in the plant shoots with Zn (5.6%), Cu (5.0%), Fe (4.7%) and Mn (4.2%) over control. The other isolates AN2, *Azotobacter*, RN1, ACC1 and ACC2 also increased the Zn (4.9-%, 4.0-%, 3.3-%, 2.5-% and 1.8-%), Cu (4.2-%, 3.1-%, 2.5-%, 1.9-% and 1.4-%), Fe (3.9-%, 3.2-%, 2.7-%, 2.1-% and 1.6-%) and Mn (3.6-%, 3.1-%, 2.6-%, 2.2-% and 1.8-%) contents in shoots compared to control (CK).

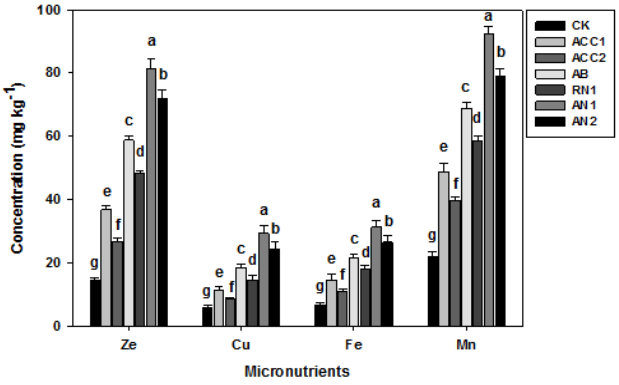

Fig. 6. Relative micronutrient contents in shoots of rice seedlings in response to different PGPR isolates. Parameters with similar letter do not differ significantly.

3.7. Micronutrient contents in root

Roots were also assayed for their micronutrients content and results indicated that inoculated treatments had increased micronutrient contents than control (Fig. 7). Among all isolates the maximum Zn (8.0%), Cu (5.1%), Fe (5.6%) and Mn (4.7%) in roots were found in AN1. AN2, *Azotobacter*, RN1, ACC1 and ACC2 also tremendously increased the Zn (6.9%, 5.4%, 4.2%, 3.0% and 1.8%), Cu (3.9%, 3.1%, 2.3%, 1.6% and 1.2%), Fe (4.6%, 3.8%, 2.9%, 2.2% and 1.4%) and Mn (4.0%, 3.4%, 2.7%, 2.4% and 1.8%) contents in the rice seedling roots compared to control (CK).

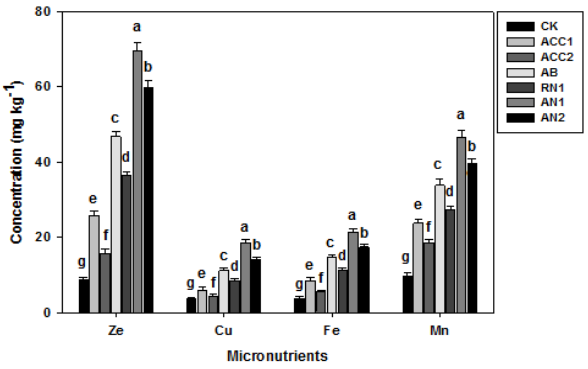

Fig. 7. Relative micronutrients uptake in roots of rice seedlings in response to different PGPR isolates and results are indicating AN1 is most effective isolate, parameters with similar letter do not differ significantly

3.8. GST, Peroxidase (Pox) and Catalase (Cata) enzymes activity

The enhanced activity of antioxidant enzymes reflects improved responses to the stress and hence better survival. PGPR are well established to improve stress mechanism i.e. antioxidant enzyme production. In this study results revealed that inoculating the rice seedlings with PGPR isolates potentially augmented the GST activity, Pox and Cata enzymes activities as well (Fig. 8). GST, Pox and Cata enzymes activities were potentially improved by all PGRB isolates however AN1 strains were the most efficient and caused maximum increase in the enzymes activity is 6.7%, 6.1% and 7.1% for GST, Pox and Catalase respectively than control.

The effectiveness of the isolates AN2 ≃ *Azotobacter* ≃ RN1 was found to be approximately overlapping with 6.0%, 4.8% and 6.2%, 3.8%, 3.7%, 3.9% and 3.2%, 3.2% and 3.0% improved in the GST, Pox and Cata enzymes activities over uninoculated control. Whereas, ACC1 and ACC2 were recorded relatively less efficient than other strains however they still triggered 2.0%, 2.2% and 2.4% and 1.5%, 1.8% and 1.8-% more enzyme activity comparing with other rhizobacterial isolates correspondingly.

3.9. Physiognomies of isolates

The selected rhizobacterial isolates used in this study were also studied for their pertinent physiognomies (Table 2). The relative effectiveness and efficiencies of the six PGPR isolates were established from results of various parameters and AN1 was found to be the most effective with maximum root colonizing activity i.e. 11.7×10^7. The relative effectiveness of these isolates in the descending order was AN1> AN2 > *Azotobacter* > RN1 > ACC1> ACC2 and again highest auxin (30.1 mg L^{-1}) production was recorded for AN1 when provided with L-TRP.

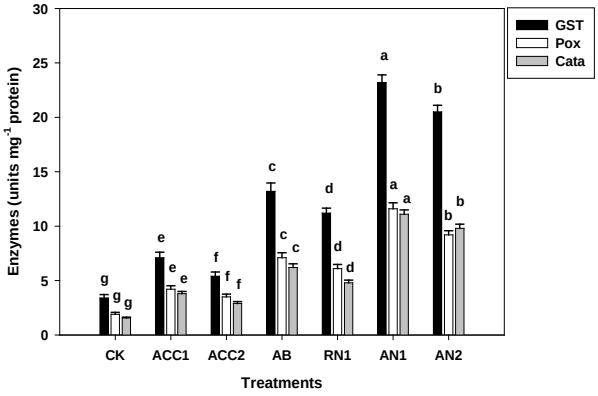

Fig. 8. Relative antioxidant enzymes activities in rice seedlings in response to different PGPR isolates and results are indicating AN1 is most effective isolate, parameters with similar letter do not differ significantly

4. Discussion

The results suggested positive correlation between inoculation and growth responses of inoculated rice seedlings. Significantly improved growth attributes were recorded for the inoculation of rice seedling with rhizobacterial isolate containing both abilities i.e. ACC hydrolyzing and N fixation than control. The isolates were efficient in the descending manner AN1 > AN2 > *Azotobacter* > RN1 > ACC1 > ACC2 while AN1 being the most effective isolate in improving productivity of rice (Figs. 1-2).

Generally, rhizobacteria promote plant growth directly by supplementing uptake of essential nutrients e.g. nitrogen, phosphorus and by controlling phytohormones or indirectly by diminishing the injurious effects of several stresses on plants (Glick, 2012). Aehmad and Kibert (2014) however discovered that ACC-deaminase production by PGPRs ameliorated vigor and growth attributes in tomato by hydrolyzing ACC thereby negative effect of ethylene was alleviated and eventually root and shoot elongation and biomass production were supremely enhanced in the PGPR inoculated tomato. Similarly, Hassan et al. (2015) reported 3.5 and 3.2 times more root and shoot elongation and 3.41 and 3.91 folds increase in fresh and dry weight of wheat under ACC-Deaminase and N fixing inoculum than control. In this study AN1 isolate having both abilities was found to be the most effective with 4.5% and 3.3% higher fresh and dry weight while 3.4% and

3.8% more root and shoot elongation than control. Therefor the reason of the AN1 being the most effective isolate with maximum root colonization and potential to produce more auxin (Table 2). This differential effectiveness of these isolates in promoting growth may be owing to their differential potentials of root colonization and phytohormones production and ability to eliminate ACC in rhizosphere (Aehmad and Kibert, 2014).

PGPR application improves plants physiology by both direct and indirect ways primarily by decomposing ACC and thus reducing otherwise inhibitory effects of ethylene (Hassan et al., 2013). Mg is essential macronutrient in plant growth and physiology it is also proposed that enhanced Mg uptake and availability as a result of inoculation with rhizobacterial isolates significantly improved chlorophyll content of rice seedlings under examination. These results are also in line with the improved nutrient uptake e.g. Fe (4.7%) and Mn (4.2%) in response to AN1 inoculation. The other isolates were effective in the order AN2 > *Azotobacter* > RN1 > ACC1 > ACC2. Hassan et al., (2014) showed 10.7% increase in chlorophyll production in tomato in response to the TAN1 isolate with activities over control. Mia et al. (2010) also suggested that treatment with rhizobacterial strains Sp7 and UPMB10 potentially increased pigment production. Similarly, Hassan et al. (2015) examined significantly higher chl a, b and carotenoid contents of wheat seedlings under inoculation of PGPR exhibiting both abilities.

PGPR inoculation improves uptake and availability of essential nutrients including both micronutrient and macronutrients uptake in rice seedlings and this was exhibited by all the selected rhizobacterial isolates when infused with plant roots compared to the control (Fig. 6). Nonetheless AN1 isolate was again found to be the most effective in triggering the nutrient uptake and resultantly improved beneficial ions content of the seedlings. For instance, AN1 caused 8.0% increased Zn uptake in the roots of rice seedlings compared to control. Sahran and Nehra (2011) showed that crop plants with PGPR showed enhanced mineral uptake. Mia et al. (2010) also reported PGPR infusion improved growth attributes of musa plantlets and this was owing to the fact that PGPR improve mineral nutrient contents of the shoots as well as roots. Çakmakçi et al. (2007) suggested that these rhizospheric bacteria modify soil characteristics and make media more suitable for mineral uptake through production of rhizospheric enzymes. Similarly, Abbasi et al. (2011) suggested application of PGPR producing ACC-Deaminase brought about 58% higher nutrient content of the wheat seedlings and this uptake was even higher when ACC-Deaminase producing rhizobacteria was co-applied with N fixing isolates.

In this study, results revealed that inoculating the rice seedlings with rhizobacterial isolates significantly augmented GST, Pox and Cata enzymes activities as well (Fig. 8). GST, Pox and Cata enzymes activities were potentially improved by all PGRB isolates however AN1 strains were the most efficient and caused maximum increase i.e. 6.8-%, 6.1% and 7.1% for GST, Pox and Catalase over control. In the study of Chen et al. (2000) at Oswego, New York, reported a significant rise in the plant enzymes activity i.e. peroxidases, phenoloxidase and phenylalanine ammonialyase in the cucumber plant after the inoculation with PGPR. Stefan et al. (2013) plotted higher values for the antioxidant enzymes activity runner bean after the inoculation with PGPR isolates. Hassan et al. (2015) showed significantly high antioxidants activity of the tomato seedlings inoculated with the PGPR producing both the N fixing and ACC-Deaminase activities over control. Overall PGPR isolates enriched both plant growth and physiology by mainly by improving nutrient availability, uptake and by alleviating ethylene stress due to its ACC cleaving ability in the rhizosphere.

5. Conclusions

Infusion of root colonizing isolates has promising effects on growth and physiology of the plants. In this experiment all the isolates tend to improve growth attributes many folds than the uninoculated control however AN1 with both the abilities of N fixing and ACC-Deaminase stood out to be the most effective and efficient.

The relative effectiveness of these isolates in the descending order was AN1 > AN2 > *Azobacter* > RN1> ACC1> ACC2 Since these root colonizing bacteria exhibit a wide range of mechanisms to promote plants responses further thorough research is indispensable to understanding the mechanisms employed by the PGPR in alleviating stress and promoting growth.

However, it is established that inoculation of crops with rhizobacterial strains is a viable and ecological technique to facilitate crop production on sustainable basis, by minimizing need for chemical fertilizers and hazards associated with them.

References

Abbasi M., Sharif S., Kazmi M., Sultan T., Aslam M., (2011), Isolation of plant growth promoting rhizobacteria from wheat rhizosphere and their effect on improving growth, yield and nutrient uptake of plants, *Plant Biosystems*, **145**, 159-168.

Ahemad M., (2012), Implications of bacterial resistance against heavy metals in bioremediation: a review, *Institute of Integrative Omics and Applied Biotechnology*, **3**, 39-46.

Ahemad M., Kibret M., (2014), Mechanisms and applications of plant growth promoting rhizobacteria: current perspective, *Journal of King Saud University-Science*, **26**, 1-20.

Ahemad M., Malik A., (2011), Bioaccumulation of heavy metals by zinc resistant bacteria isolated from agricultural soils irrigated with wastewater, *Bacteriology Journal*, **2**, 12-21.

Arnon D.I., (1949), Copper induced enzyme in isolated chloroplasts; polyphenol oxidase in *Beta vulgaris*, *Plant Physiology*, **24**, 1-15.

Arshad M., Saleem M., Hussain S., (2007), Perspectives of bacterial ACC deaminase in phytoremediation, *Trends in Biotechnology*, **25**, 356-362.

Buresh R.J., Austin E.R., Craswell E.T., (1982), Analytical methods in N-15 research, *Fertilizer Research*, **3**, 37-62.

Çakmakçi R., Dönmez F., Aydm A., Şahin F., (2006), Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions, *Soil Biology and Biochemistry*, **38**, 1482-1487.

Cakmak I., Horst W.J., (1991), Effect of aluminium on lipid peroxidation, superoxidase dismutase, catalase and peroxidase activities in root tips of soybean (*Glycine max*), *Physiologia Plantarum*, **83**, 463-468.

Chapman H.D., Pratt P.F., (1961), *Methods of Analysis for Soils, Plants and Water*, University of California, Berkeley, CA, USA.

Chen J., Abawi G.S., Zucherman B.M., (2000), Efficacy of *Bacillus thuringiensis*, *Paecilomyces marquandii* and *Streptomyces costaricanus* with organic amendment against *Meloidogyne hapla* infecting lettuce, *Journal of Nematololgy*, **32**, 70-77.

Dworken M., Foster J., (1958), Experiment with some microorganisms which utilize ethane and hydrogen, *Journal of Bacteriology*, **75**, 92-601.

Davies K.G., Whitbread R., (1989), A comparison of method for measuring the colonization of root system by fluorescent pseudomonads, *Plant and Soil*, **116**, 239-246.

Glick B.R., (2012), Plant growth-promoting bacteria: mechanisms and applications, *Scientifica*, **2012**, http://dx.doi.org/10.6064/2012/963401.

Habig W.H., Pabst M.J., Jakoby W.B., (1974), Glutathion S-Transferases, the first enzymatic step in mercapturic acid formation, *The Journal of Biological Chemistry*, **249**, 7130-7139.

Hassan W., Akmal M., Muhammad I., Younas M., Zahaid K.R.., Ali F., (2013), Response of soil microbial biomass and enzymes activity to cadmium (Cd) toxicity under different soil textures and incubation times, *Australian Journal of Crop Science*, **7**, 674-680.

Hassan W., David J., Bashir F., (2014), ACC-deaminase and/or nitrogen-fixing rhizobacteria and growth response of tomato (Lycopersicon pimpinellfolium Mill.), *Journal of Plant Interactions*, **9**, 869-882.

Hassan W., Hussain M., Bashir S., Shah A., Bano R., David J., (2015), ACC-deaminase and/or nitrogen fixing rhizobacteria and growth of wheat (Triticum aestivum L.), *Journal of Soil Science and Plant Nutrition*, **15**, 232-248.

Hayat R., Ali S., Amara U., Khalid R., Ahmed I., (2010), Soil beneficial bacteria and their role in plant growth promotion: a review, *Annals of Microbiology*, **60**, 579-598.

Honma M., Shimomura T., (1978), Metabolism of 1-aminocyclopropane-1-carboxylic acid, *Agricultural and Biological Chemistry*, **42**, 1825-1831.

Hussain M.I., Asghar H.N., Arshad M., Shahbaz M., (2013), Screening of multi-traits rhizobacteria to improve maize growth under axenic conditions, *Journal of Animal and Plant Science*, **23**, 514-520.

Jha Y., Subramanian R., (2013), Paddy plants inoculated with PGPR show better growth physiology and nutrient content under saline condition, *Chilean Journal of Agricultural Research*, **73**, 213-219.

Singh R.P., Jha P.N., (2016), The multifarious PGPR Serratia marcescens CDP-13 augments induced systemic resistance and enhanced salinity tolerance of wheat (*Triticum aestivum* L.), *PloS One*, **11**, e0155026.

Khalid A., Akhtar M.J., Mahmood M.H., Arshad M., (2006), Effect of substrate-dependent microbial ethylene production on plant growth, *Microbiology*, **75**, 231-236.

Khan M.I.R., Nazir F., Asgher M., Per T.S., Khan N.A., (2015), Selenium and sulfur influence ethylene formation and alleviate cadmium-induced oxidative stress by improving proline and glutathione production in wheat, *Journal of Plant Physiology*, **173**, 9-18.

Lal L., (2002), *Phosphatic Biofertilizers*, In: *Phosphate Mineralizing and Solubilizing Microorganisms*, Agrotech Pub. Academy, Udaipur, India, 224-236.

Laslo E., Gyorgy E., Mara G., Tamas E., Abraham B., Lanyi S., (2012), Screening of plant growth promoting rhizobacteria as potential microbial inoculants, *Crop Protection*, **40**, 43-48.

Mayak S., Tirosh T., Glick B.R., (2004), Plant growth-promoting bacteria confer resistance in tomato plants to salt stress, *Plant Physiology and Biochemistry*, **42**, 565-572.

Memon K.S., Rashid A., Puno H.K., (1992), *Phosphorus Deficiency Diagnosis and P Soil Test Calibration in Pakistan*, Proc. Phosphorous Decision Support Sys. College Station, TX, 125-147.

Mia M.A.B., Shamsuddin Z.H., Wahab Z., Marziah M., (2010), Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth and N incorporation of tissue-cultured *Musa plantlets* under N-free hydroponics condition, *Australian Journal of Crop Science*, **4**, 85-90.

Mihalache G., Zamfirache M.M., Hamburda S., Stoleru V., Munteanu N., Stefan M., (2016), Synergistic effect of Pseudomonas lini and Bacillus pumilus on runner bean growth enhancement, *Environmental Engineering and Management Journal*, **15**, 1823-1831.

Nadeem S.M., Zahir Z.A., Naveed M., Arshad M., Shahzad S.M., (2006), Variation in growth and ion uptake of maize due to inoculation with plant growth promoting rhizobacteria under salt stress, *Soil and Environment*, **25**, 78-84.

Nakano Y., Azada K., (1987), Purification of ascorbate peroxidase in spinach chloroplasts: its inactivation in ascorbate depleted medium and reactivation by monodehydroascorbate radical, *Plant Cell Physiology*, **28**, 131-140.

Naveed M., Zahir Z.A., Khalid M., Asghar H.N., Akhtar M.J., Arshad M., (2008), Rhizobacteria containing ACC-deaminase for improving growth and yield of wheat under fertilized conditions, *Pakistan Journal of Botany*, **40**, 1231-1241.

Premono H.M.E., Moawad A.M., Vlek P.L.G., (1996), Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere, *Indonesian Journal of Crop Science*, **11**, 13-23.

Raymond J., Siefert J.L., Staples C.R., Blankenship R.E., (2004), The natural history of N fixation, *Molecular Biology and Evolution*, **21**, 541-554.

Regan D.L., (1988), *Other Micro-Algae*, In: *Microalgal Biotechnology*, Borowitzka M.A., Borowitzka L.J. (Eds.), Cambridge University Press, Cambridge, 135-150.

Sahran B.S., Nehra V., (2011), Plant growth promoting rhizobacteria: a critical review, *Life Science and Medicine Research*, **2011**, LSMR-21.

Saikia R., Kumar R., Arora D.K, Gogoi D.K., Azad P., (2006), *Pseudomonas aeruginosa* inducing rice resistance against *Rhizoctonia solani*: production of salicylic acid and peroxidases, *Folia Microbiology*, **51**, 375-380.

Saleem M., Arshad M., Hussain S., Bhatti A.S., (2007), Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture, *Journal of Industrial Microbiology and Biotechnology*, **34**, 635-648.

Sarwar M., Arshad M., Martins D.A., Frankenberger Jr., (1992), Tryptophan-dependent biosynthesis of auxins in soil, *Plant Soil*, **147**, 207-215.

Spaepen S., Vanderleyden J., (2011), Auxin and plant-microbe interactions, *Cold Spring Harbor Perspectives in Biology*, **3**, 1-13.

Stefan M., Munteanu N., Stoleru V., Mihasan M., (2013), Effects of inoculation with plant growth promoting rhizobacteria on photosynthesis, antioxidant status and yield of runner bean, *Romanian Biotechnological Letters*, **18**, 8132-8143.

US Salinity Lab Staff, (1954), Diagnosis and improvement of saline and alkali soils. USDA Hand Book No 60. pp. 160. Washington DC, USA.

Van-Schouwenberg J.C.H., Walinge I., (1973), *Methods of Analysis for Plant Material*, Wageningen Agricultural University, The Netherlands.

Wollum II A.G., (1982), *Cultural Methods for Soil Microorganisms*, In: *Methods of Soil Analysis*. *Part 2: Chemical and Microbiological Properties*, Page A.L. (Ed.), Agronomy No. 9, ASA Madison, WI, 781-802.

Young S.A., Guo A., Guikema J.A., White F., Leach I.E., (1995), Rice cationic peroxidase accumulation in xylem vessels during incompatible interaction with *Xanthomonas oryzae*, *Plant Physiology*, **107**, 1333-1341.

Zahir Z.A., Munir A., Asghar H.N., Shaharoona B., Arshad, M., (2008), Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of pea (*Pisum sativum*) under drought conditions, *Journal of Microbiology and Biotechnology*, **18**, 958-963.