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RESEARCH ARTICLE

ACC-deaminase and/or nitrogen fixing rhizobacteria and growth of wheat (*Triticum Aestivum* L.)

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Abstract

Plant growth promoting rhizobacteria (PGPR) are beneficial bacteria, which can enhance the growth of the plants, when applied to crops. A pot experiment was conducted to examine the effect of six PGPR isolates on the growth of wheat. Inoculation with rhizobacterial isolates increased the all measured physical, chemical and enzymatic growth parameters compared to control (CK). However, the WAN1 isolate had the highest effect, and significantly ($P < 0.05$) increased the root length (3.51-fold), shoot length (3.22-fold), seedling fresh (3.41-fold) and dry (3.91-fold) weight, chlorophyll a (3.90-fold), chlorophyll b (3.51-fold), carotenoid contents (7.23-fold), plant macronutrient uptake i.e. N (7.20-fold, 6.71-fold), P (7.41-fold, 5.01-fold), K (5.51-fold, 3.91-fold), Ca (6.40-fold, 5.21-fold) and Mg (5.82-fold, 7.11-fold) in shoot and root, plant micronutrient uptake i.e. Zn (6.40-fold, 9.11-fold), Cu (7.31-fold, 7.02-fold), Fe (6.41-fold, 7.52-fold) and Mn (4.57-fold, 5.21-fold) in shoot and root and plant antioxidant enzymes i.e. glutathione S-transferase (7.51-fold), peroxidase (5.21-fold) and catalase (5.01-fold) respectively. Our results revealed that inoculation of agricultural crops with PGPR is a very useful approach to increase the plant growth. The ACC (1-aminocyclopropane-1-carboxylate) enrichment technique is an efficient approach to select promising PGPR. The PGPR containing dual abilities i.e. both ACC-deaminase and nitrogen fixing ability are more effective than PGPR possessing either ACC-deaminase or nitrogen fixing activity alone for growth promotion of crops.

Keywords: PGPR, ACC-deaminase, Ethylene and auxin, wheat

1. Introduction

Soil microorganisms are vital to agro-ecosystem health via their roles in organic matter decomposition, nutrient cycling and their associations with other organisms and plants i.e. symbiosis (Hassan *et al.,* 2013a; Glick, 2012). In agricultural soils, microorganisms are known to influence profoundly the status of soil fertility and health, and hence crop production (Ahmed and Kibret, 2014; Hassan *et al.,* 2013a). Different bacterial genera are vital components of soils, as it has been estimated that 80–90% of the processes in soil are reactions mediated by soil microbes (Hassan and David, 2014). They are involved in various biotic activities of the soil ecosystem to make it dynamic for nutrient turn over and ultimately sustainable crop production (Ahmed and Kibret, 2014).

The large active groups of bacteria that inhabiting the rhizosphere benefit the crop plants in several ways, collectively called as plant growth promoting rhizobacteria (PGPR) (Hassan *et al.,* 2014a). Interactions between plants and microorganisms in the rhizosphere can clearly affect growth and development of the crop plants and yield (Hassan *et al.,* 2014a; Ahmed and Kibret, 2014). The PGPR can stimulate plant growth indirectly by inhibiting other deleterious microbes, root pathogens, biological control of root crop diseases, by production of antibiotics and siderophores (Ahmed and Kibret, 2014; Hayat *et al.,* 2010). Directly, PGPR provide the plant with a compound that is synthesized by the bacterium or facilitating the uptake of the nutrients (Ahmed and Kibret, 2014; Glick, 2012). Therefore, inoculation of agricultural crops with PGPR result in multiple positive effects on plant e.g. increases in the plant growth and vigor, increment in the chlorophyll, protein, proline and antioxidant enzymes activity and overall biomass of the host plant (Glick, 2012; Hayat *et al.,* 2010). Currently, the biological approaches for improving crop production are gaining strong attention among the agricultural scientists and researchers following integrated plant nutrient management system.

The PGPR synthesize different phytohormones, including auxins, cytokinins, and gibberellins, which can enhance various stages of plant growth; and synthesize enzymes, including phosphatase, catalase, that can modulate plant growth and development, and strengthen their immune system (Glick, 2012). The secretion of a wide range of chemical compounds also modifies the chemical and physical properties of the soil (Ahmed and Kibret, 2014; Glick, 2012). Phytohormone auxin, (indole-3-acetic acid/IAA) is a class of phytohormones which are involved in the regulation of growth and development throughout the life cycle of plants (Ahmed and Kibret, 2014; Etesami *et al.,* 2014). Similarly, Ethylene is also a vital phytohormone, which plays significant roles in the germination and growth and development of the seeds and crop plants (Bhattacharyya and Jha, 2012). Ethylene, produced endogenously by approximately all plants, however, apart from being a plant growth regulator, in excessive amount, ethylene has also been established as a stress hormone (Bhattacharyya and Jha, 2012). Several PGPR are actively involved in the synthesis of auxins in pure culture as well as in soil, and hence can play significant roles in the growth and development of crop plants (Glick, 2012). Alike, the PGPR containing ACC (1-aminocyclopropane-1-carboxylate) deaminase activity is helpful in lowering the ethylene levels by converting ACC into NH_3 and α-ketobutyrate in plants, and support the plants for vigorous growth and development (Ahmed and Kibret, 2014).

Wheat (*Triticum* spp.) is one of the most important cereal crops in the world (Schalchli *et al.,* 2012). Globally, it is the most important human staple food grain and ranks second in total production as a cereal crop after maize (FAOSTAT, 2006). In order to meet the demands of the increasing world's population, the use of chemical fertilizers increased significantly (FAOSTAT, 2006). It has been estimated that the total annual fertilizer use has risen from 37 M t of N, P and K in 1961-65 to 161 M t in 2005-09 (FAOSTAT, 2009). The excessive use of chemical fertilizers, drastically increase the pollution in both natural and man made ecosystems (Hassan *et al.,* 2014b). Therefore, it is important to find out other self-propagating and eco-friendly sources, which can replace the chemical fertilizers entirely or partially (Hassan *et al.,* 2014b). In this regard, the use of PGPR as a bio-fertilizer is a viable approach. Keeping in view all these challenges, the current study was conducted with the objectives: (1) to isolate and screen the most effective multi-traits PGPR strains for wheat (2) to examine the physical, chemical and enzymatic responses of wheat plant to three different rhizobacterial strains, strain 1: containing ACC-deaminase activity (WACC1, WACC2), strain 2: having nitrogen fixing ability (WRN1, WRN5)

and strain 3: having both ACC-deaminase activity and nitrogen fixing ability (WAN1, WAN2).

2. Materials and Methods

2.1. Physico-chemical analysis

The soil was collected at 0-5 cm depth with the help of an augur. The samples were air-dried and passed through 2-mm sieve for further use in the incubation study. Soil texture, pH, EC (electrical conductivity), available P, N, extractable K and organic matter (OM) and saturation percentage were determined by the methods of Hassan, 2013, Hassan *et al.,* (2013 b, c, d) and Hassan *et al.*, (2014 c, d, e). The soil was sandy loam (sand 60.3%, clay 25.5% and silt 14.2%) in texture, and alkaline in nature with saturation percentage 41.5%, pH 7.98, EC 1.71, OM 0.41%, available P, N and K 8.81, 3.67 and 423 mg kg^{-1} respectively.

2.2. Isolation of PGPR

For isolation of bacterial strains, gently rhizosphere soil was collected from tomato crop. Rhizobacteria were isolated by dilution plate technique (Wollum-II, 1982) using Dworkin and Foster (DF) salt minimal media (Dworkin and Foster, 1958). For PGPR containing ACC-deaminase, ACC was the sole nitrogen source. Whereas, modified mannitol agar media was used to isolate PGPR possessing nitrogen fixing activity (Enrichment Technique).

2.3. Isolation of rhizobacteria containing dual abilities

Isolated rhizobacteria were further grown on both (DF salt minimal and modified mannitol agar) media to isolate the rhizobacteria containing dual abilities i.e. nitrogen fixing and ACC-deaminase activity. The isolates having ACC-deaminase activity was grown on modified mannitol agar media used for N fixing isolates and the rhizobacteria possessing N fixing activity was grown on media used for isolates having ACC-deaminase activity. The isolates WAN1 and WAN2 grew on both media and proved that having both abilities.

2.4. Preparation of inocula

Autoclaved 250 ml flasks with DF salt minimal media were used for the preparation of inocula, for the rhizobacterial isolates containing ACC-deaminase activity and modified mannitol agar media for the rhizobacterial isolates having nitrogen fixing activity. The autoclaved flasks were incubated at 28 ± 1°C for 48 hours in the orbital shaking incubator at 100 rpm. Optical density was measured carefully to maintain the uniform population of bacteria in the broth at the time of inoculation.

2.5. Characterization of isolates

Root colonization was done by the method of Davis and Whitbread, (1989). For this, inoculated seeds were sown in glass jars containing sterilized sand, and the jars were kept in a growth chamber (18 °C, 70 % relative humidity, 16 hours daylight). After 7 days of germination, the roots were cut off, dipped in phosphate buffer, and were shaken vigorously to remove the bacteria. After 2 days of incubation at 28 °C the number of colonies (CFU/cm) was calculated.

For the determination of phosphorus solubilizing activity National Botanical Research Institute's Phosphate growth medium (NBRIP) was used. Rhizobacterial strains were cultured and a loop full of each culture was placed on the plates (five per plate) and incubated at 28°C for a week. A clearing zone around the colonies after a week was calculated as a positive for phosphate solubilization

The formula of Premono *et al*., (1996) was used for the determination of phosphate solubilizing index (PSI) of these rhizobacterial isolates.

$$PSI = \frac{\text{Colony diameter + Halozone diameter}}{\text{Colony diameter}}$$

The ACC-deaminase activity was determined by the modified method of Honma and Shimomura, (1978) which measures the amount of α-ketobutyrate when the enzyme ACC-deaminase cleaves ACC.

The auxin produced by rhizobacteria as indole acetic acid (IAA) in the presence and absence of L-tryptophan (L-TRP) was essayed by the method of Sarwar *et al*., (1992). Some pertinent characteristics of the rhizobacterial isolates are shown in Table 1.

Table 1. Characterization of rhizobacterial strains

PGPR isolates	ACC-deaminase activity (µmol α-ketobutyrate g-biomass 1/2 h^{-1})	Nitrogen fixing activity	Phosphorous solubilization	IAA production mg L^{-1}		Root colonization CFU g^{-1} root	Phosphate solubilizing index (PSI)
				With L-TRP	Without L-TRP		
WACC1	20.5	-ve	+ve	18.8	11.5	3.5×10^7	1.73±0.13
WACC2	17.8	-ve	-ve	10.1	7.12	2.1×10^7	-
WAN1	10.2	+ve	+ve	28.8	8.22	12.4×10^7	4.18±0.16
WAN2	8.11	+ve	+ve	23.5	5.15	9.7×10^7	2.81±0.14
WRN1	0.0006	+ve	-ve	14.3	9.71	7.3×10^7	-
WRN5	0.0004	+ve	+ve	7.96	4.29	4.9×10^7	1.11±0.11

-ve = Absent

+ve = Present

2.6. Determination of plant physical parameters

The fresh and dry weight of the shoots and roots were determined with the help of an analytical balance. For dry weight plants were oven dried at 70 °C.

2.7. Determination of chlorophyll and carotenoids

The method of Arnon, (1949) was used for the determination of chlorophyll and carotenoid contents in the acetone extract (80% v/v) at 663, 645 and 480 nm for chlorophyll a, b and carotenoid, respectively.

2.8. Macro and micronutrients in plant

The H_2SO_4 titration method was used for the plant nitrogen (N) determination (Van-Schouwengerg and Walinge, 1973). For the measurement of the plant phosphorous (P) the method of Buresh *et al*., (1982) was followed. The plant potassium (K) was determined by using the dry ashing method (Chapman and Pratt, 1961). The micronutrients i.e. Ca^{+2}, Mg^{+2}, Mn^{+2}, Zn^{+2}, Cu^{+2} and Fe^{+3} were determined by atomic absorption spectroscopy after dry ashing (Chapman and Pratt, 1961).

2.9. Plant enzymes activity

For enzymes activity analyses plant samples were prepared by homogenizing 0.5 g of frozen leaf material in 3 ml of cold solution containing 50×10^{-3} M Na phosphate buffer (pH 7.8), 1×10^{-3} M Ethylenediaminetetraacetic acid and 2% (w/v) Polyvinylpolypyrrolidone. The homogenate was centrifuged at 0 °C for 40 minutes at 13000 g. The glutathione S-transferase (GST) was essayed by the method of Habig *et al.,* (1974). The method of Nakano and Azada, (1987) was used for the spectrophotometric determination of peroxidase (POX) activity. Whereas, the spectrophotometric determination of catalase (Cata) activity was done by the method of Cakmak and Horst, (1991).

2.10. Experimental design

Two PGPR isolates (WACC1 and WACC2) containing ACC-deaminase activity, two PGPR isolates (WRN1 and WRN5) having nitrogen fixing activity and two PGPR isolates (WAN1 and WAN2) possessing both ACC-deaminase and nitrogen fixing activity were selected for incubation experiment. The inoculum for trial was prepared by growing the selected PGPR isolates. For inoculation of treatments, germinated seeds (variety Uqab-2000) were dipped in broth having selected ACC-deaminase and/or nitrogen fixing isolates. For control treatments, the wheat seeds were dipped in sterilized flasks containing 0.03 M $MgSO_4$. Four inoculated seeds were sown in each pot having 500 g soil/pot. Sterilized ½ strength nitrogen free Hoagland solution was used to provide nutrients to growing seedling. There were four replications for each treatment. Pots were arranged in complete randomized design (CRD) in a growth room under axenic conditions. After 28 days of germination, wheat plants were harvested and parameters regarding root and shoot length, seedling fresh and dry weight, chlorophyll a, b and carotenoid contents, macro and micro nutrients in plant shoot and root and antioxidant enzymes glutathione S-transferase, peroxidase and catalase activities were recorded.

2.11. Statistical analysis

The data were statistically analyzed by Statistix 8.1 statistical package (Statistix, USA). Parametric statistics of ANOVA analysis was carried out to estimate the effect of rhizobacterial isolate inoculation on plant growth. Mean separations were achieved using a least significant difference (LSD) test at $p < 0.05$.

3. Results

3.1. Root and shoot length

The inoculation of wheat seedlings with rhizobacterial isolates containing nitrogen fixing and/or ACC-deaminase activity increased the root and shoot length as compared to uninoculated control. The root and shoot length ranged from 1.51-fold to 3.51-fold and 1.40 to 3.22 fold under normal growth conditions (Figure 1). The isolate WAN1 showed maximum increase in the root and shoot length that was 3.51-fold and 3.22-fold respectively as compared to uninoculated control. Conversely, WAN2 was the next effective isolate in promoting root and shoot length (3.1-fold and 2.91-fold) as compared to uninoculated control. The other isolates namely, WRN1, WRN5, WACC1 and WACC2 caused 2.51-fold, 2.21-fold, 2.22-fold, 1.91-fold, 1.81-fold, 1.61-fold and 1.51-fold and 1.40-fold increase in root and shoot length as compared to uninoculated control.

3.2. Fresh and dry weight of seedlings

The effect of inoculation with either ACC-deaminase and/or nitrogen fixing activity possessing rhizobacteria on the fresh and dry weight of seedling is shown in Figure 2. Data showed that fresh and dry weight of seedlings increased significantly ($P < 0.05$) as a result of inoculation, ranged from 1.51 to 3.41 fold and 1.60 to 3.91 fold. All rhizobacterial isolates caused significant ($P < 0.05$) increase in the fresh weight of seedlings. Nevertheless the WAN1 was the most efficient isolate, caused an increase of 3.41-fold and 3.91-fold. The WAN2 was the second best isolate, caused an increase of 3.11-fold and 3.41-fold. The next effective isolates were WRN1, WRN5, WACC1 and WACC2 that resulted in about 2.61-fold and 2.92-fold, 2.41-fold and 2.61-fold, 1.81-fold and 2.21-fold and 1.51-fold and 1.60-fold increase respectively.

3.3. Chlorophyll a, b and carotenoid contents

The inoculation with either ACC-deaminase and/or nitrogen fixing activity containing rhizobacteria significantly ($P < 0.05$) increased the chlorophyll a, b and carotenoid contents. The increase in the chlorophyll a, b and carotenoid contents ranged from 1.63 to 3.91 fold, 1.61 to 3.51 fold and 1.81 to 7.21 fold (Figure 3). Isolate WAN1 was found to be the most effective that caused 3.91-fold, 3.51-fold and 7.21-fold increase in chlorophyll a, b and carotenoid contents over uninoculated control respectively. The next effective isolates were WAN2, WRN1, WRN5, WACC1 and WACC2 that resulted in about 3.41-fold, 3.20-fold, 6.01-fold, 3.01-fold, 2.61-fold, 4.91-fold, 2.61-fold, 2.30-fold, 3.41-fold, 1.91-fold, 1.90-fold, 2.62-fold, and 1.63-fold, 1.60-fold and 1.81-fold increase in chlorophyll a, b and carotenoid contents respectively, compared to uninoculated control.

3.4. Macronutrient contents in shoot and root

Data showed that inoculation with rhizobacteria containing either ACC-deaminase and/or nitrogen fixing activity increased the plant ionic uptake e.g. macronutrient contents (N, P, K, Ca, and Mg) in shoot and root (Figure 4). Among all isolates the maximum N (7.11-fold and 6.71-fold), P (7.41-fold and 4.90-fold), K (5.51-fold and 3.90-fold), Ca (6.40-fold and 5.21-fold) and Mg (5.80-fold and 7.01-fold) contents in plant shoot and root were found in WAN1. The other isolates namely WAN2, WRN1, WRN5, WACC1 and WACC2 also significantly ($P < 0.05$) increased the N (5.81-fold, 5.11-fold, 3.62-fold, 2.61-fold and 1.90-fold), P (5.81-fold, 4.62-fold, 3.61-fold, 2.80-fold and 2.01-fold), K (4.61-fold, 3.71-fold, 2.91-fold, 2.03-fold and 1.61-fold), Ca (4.40-fold, 3.71-fold, 3.30-fold, 2.71-fold and 2.31-fold) and Mg (4.51-fold, 4.01-fold, 3.21-fold, 2.61-fold and 1.71-fold) in the plant shoot compared to control. Similarly, the other isolates namely WAN2, WRN1, WRN5, WACC1 and WACC2 also significantly ($P < 0.05$) increased the N (5.11-fold, 4.01-folds, 3.31-fold, 2.71-fold and 2.30-fold), P (4.30-fold, 3.60-fold, 3.31-fold, 2.91-fold and 2.61-fold), K (3.21-fold, 2.50-fold, 1.91-fold, 1.72-fold and 1.41-fold), Ca (4.41-fold, 3.61-fold, 3.01-fold, 2.41-fold and 1.80-fold) and Mg (6.11-fold, 5.61-fold, 4.50-fold, 3.70-fold and 1.91-fold) in the plant root compared to control.

3.5. Micronutrient contents in shoot and root

Data showed that inoculation with rhizobacteria containing either ACC-deaminase and/or nitrogen fixing activity increased the plant ionic uptake e.g. micronutrient contents (Zn, Cu, Fe and Mn) in shoot and root (Figure 5). Among all isolates the maximum Zn (6.41-fold and 9.11-fold), Cu (7.31-fold and 7.01-

fold), Fe (6.40-fold and 7.51-fold) and Mn (4.50-fold and 5.20-fold) contents in plant shoot and roots were found in treatments inoculated with WAN1. The other isolates namely WAN2, WRN1, WRN5, WACC1 and WACC2 also significantly ($P < 0.05$) increased the Zn (5.60-fold, 4.61-fold, 3.70-fold, 2.81-fold and 1.90-fold), Cu (6.01-fold, 4.41-fold, 3.30-fold, 2.51-fold and 1.70-fold), Fe (5.31-fold, 4.20-fold, 3.41-fold, 2.70-fold and 1.90-fold) and Mn (3.90-fold, 3.31-fold, 2.81-fold, 2.41-fold and 1.90-fold) contents in the plant shoot compared to control. Similarly, the other isolates namely WAN2, WRN1, WRN5, WACC1 and WACC2 also significantly ($p < 0.05$) increased the Zn (7.80-fold, 6.10-fold, 4.71-fold, 3.31-fold and 1.91-fold), Cu (5.20-fold, 4.01-fold, 2.91-fold, 1.90-fold and 1.31-fold), Fe (6.01-fold, 5.01-fold, 3.80-fold, 2.71-fold and 1.60-fold) and Mn (4.41-fold, 3.71-fold, 3.01-fold, 2.60-fold and 2.01-fold) contents in the plant root compared to control.

3.6. Glutathione S-transferase, peroxidase and catalase activity

The inoculation with rhizobacteria containing either ACC-deaminase and/or nitrogen fixing activity significantly ($P < 0.05$) increased the GST, POX and Cata activity (Figure 6). All rhizobacterial isolates showed increase in the GST, POX and Cata activity. Nevertheless the isolate WAN1 was the most effective and showed highest increase in the GST, POX and Cata activity i.e. 7.51-fold, 5.31-fold and 5.01-fold. The WAN2, WRN1 and WRN5 were the next best effective rhizobacterial isolates and caused an increase of 5.51-fold, 4.21-fold and 4.01-fold, 4.10-fold, 3.20-fold and 3.41-fold, 3.01-fold, 2.60-fold and 2.81-fold in the GST, POX and Cata activity respectively. Whereas, WACC1 and WACC2 showed minimum increase in the GST, POX and Cata activity comparing with other rhizobacterial isolates i.e. 2.20-fold, 2.01-fold and 2.21-fold, and 1.41-fold, 1.50-fold and 1.48-fold correspondingly.

3.7. Characteristics of isolates

Some pertinent characteristics of rhizobial isolates used in the experiment are given in Table 1. It was observed that, among all isolates, WAN1 had maximum root colonization activity i.e. 11.7×10^7 and produced more auxin (30.1 mg l^{-1}) in the presence of L-TRP. Conversely, WACC1 had maximum ACC-deaminase activity i.e. 18.58 µmol α-ketobutyrate g-biomass 1/2 h^{-1}.

4. Discussion

Inoculation with rhizobacterial isolate containing ACC-deaminase and/or nitrogen fixing activity significantly ($P < 0.05$) increased the root and shoot length, and fresh and dry weight of the seedlings compared to uninoculated control. Comparing the effect of rhizobacterial isolates, the WAN1 showed maximum root and shoot length, and fresh and dry weight of the seedlings than other isolates and uninoculated control (Figures 1-2). The effect of other rhizobacterial isolates was in the order WAN2 > WRN1 > WRN5 > WACC1 > WACC2. It has been reported that certain microorganisms contain an enzyme ACC-deaminase that hydrolyzed the ACC into NH_3 and α-ketobutyrate, and reduced the inhibitory effects of ethylene for vigorous growth of plants i.e. root-shoot length and fresh and dry weights (Mayak *et al*., 2004). Shaharoona *et al*., (2003) investigated the effect of ACC-deaminase possessing rhizobacteria on the growth of maize seedlings, and found significant increase in the root and shoot lengths and fresh and dry weights of maize seedlings over uninoculated control. The reason of the WAN1 being the best rhizobacterial isolate was, having both nitrogen fixing and ACC-

deaminase activities and maximum root colonization ability. It was found that rhizobacterial isolate having both nitrogen fixing and ACC-deaminase activities showed more promising effects on agricultural plants, and increase the plant growth, root and shoot length, and fresh and dry weights due to its maximum root colonization activity, and ability to minimize the endogenous levels of ethylene synthesis (Glick, 2012; Shaharoona *et al*., 2003).

The data of chlorophyll a, b and carotenoid contents showed that among all experimental isolates again WAN1 showed significant ($P < 0.05$) increase in chlorophyll a, b and carotenoid contents compared to other rhizobacterial isolates and uninoculated control (Figure 3). The overall effect of other isolates was in the order of WAN2 > WRN1 > WRN5 > WACC1 > WACC2. Yet again isolate having both ACC-deaminase and nitrogen fixing activities i.e. WAN1 proved to be the best than isolates either having nitrogen fixing activity or ACC-deaminase activity alone. Stefan *et al*., (2013) found that the PGPR strains (S4 and S7), alone or in combination, significantly increased the chlorophyll and carotenoid contents of the runner bean. Mia *et al*., (2010) revealed that inoculation with rhizobacterial strains (Sp7 and UPMB10) significantly increased the growth attributes e.g. leaf area, chlorophyll and carotenoid contents.

The significant increase ($P < 0.05$) in the plant ionic uptake e.g. macronutrient (N, P, K, Ca and Mg) and micronutrients (Zn, Cu, Fe and Mn) in the shoot and root was observed after inoculation with rhizobacterial isolates (Figures 4-5). Among the rhizobacterial isolates WAN1 was the most effective isolate and caused highest increase in the macro and micronutrient contents of the plant shoot and root. The efficacy of other rhizobacterial isolates was in the following order WAN2 > WRN1 > WRN5 > WACC1 > WACC2. Sahran and Nehra, (2011) concluded that inoculation with PGPR significantly increased the plant mineral (N, P, K, Ca, Mg) consumption. Hayat *et al*., (2010) concluded that growth promoting rhizobacteria increase the mobility and availability of plant nutrients in the soil, and as a result increase the nutrient uptake of the plants. Nadeem *et al*., (2006) concluded that inoculation with PGPR significantly increase the plant ionic consumption i.e. N, P and K in the shoot and root of the maize plant.

The data of plant enzymes activity showed (Figure 6) that inoculation with rhizobacterial isolates considerably ($P < 0.05$) enhanced the antioxidant plant enzymes activity i.e. glutathione S-transferase, peroxidase and catalase activities. All rhizobacterial isolates had positive effect on plant enzymes activity, however the WAN1 was found to be the best. The effect of other rhizobacterial isolates was in the order WAN2 > WRN1 > WRN5 > WACC1 > WACC2. Stefan *et al*., (2013) observed that the PGPR strains (S4 and S7), alone or in combination, considerably increased the activity of superoxide dismutase and peroxidase enzymes. Mia *et al*., (2010) found that rhizobacterial inoculation (Sp7 and UPMB10) significantly increased the nitrogen uptake in plants which ultimately increased the formation of protein and enzyme for better physiological activities. Hayat *et al*., (2010) stated that PGPR increase the plant vigor and decrease the biotic and abiotic stresses by producing different anti-oxidant enzymes.

Results of isolates characteristics clearly revealed that among all isolates, WAN1 had maximum root colonization activity and produced more auxin in the presence of L-TRP, whereas, WACC1 had maximum ACC-deaminase activity among all rhizobacterial isolates (Table 1). The difference in plant growth promotion by different rhizobacterial isolates may be due to the differences in their efficiency of colonizing the germinating roots, production of plant hormones (e.g. auxin) and ability to hydrolyze the

ACC in plant roots (Hassan *et al*., 2014). It has been reported that traits e.g. production of IAA, auxin, phosphate solubilization and chitin production by PGPR are helpful for better nutrient mobilization and availability for plants, as a result inoculation with rhizobacteria containing ACC-deaminase could result in the development of much better germination of seeds and longer roots, which subsequently affects over all growth of the host plant (Zafarul-Hye *et al*., 2007). Phosphate solubilization, dinitrogen fixation, ACC-deaminase and antifungal activity, IAA and siderophore biosynthesis characteristics of PGPR are responsible for the plant growth promotion and high yield (Ahmed and Kibret, 2014).

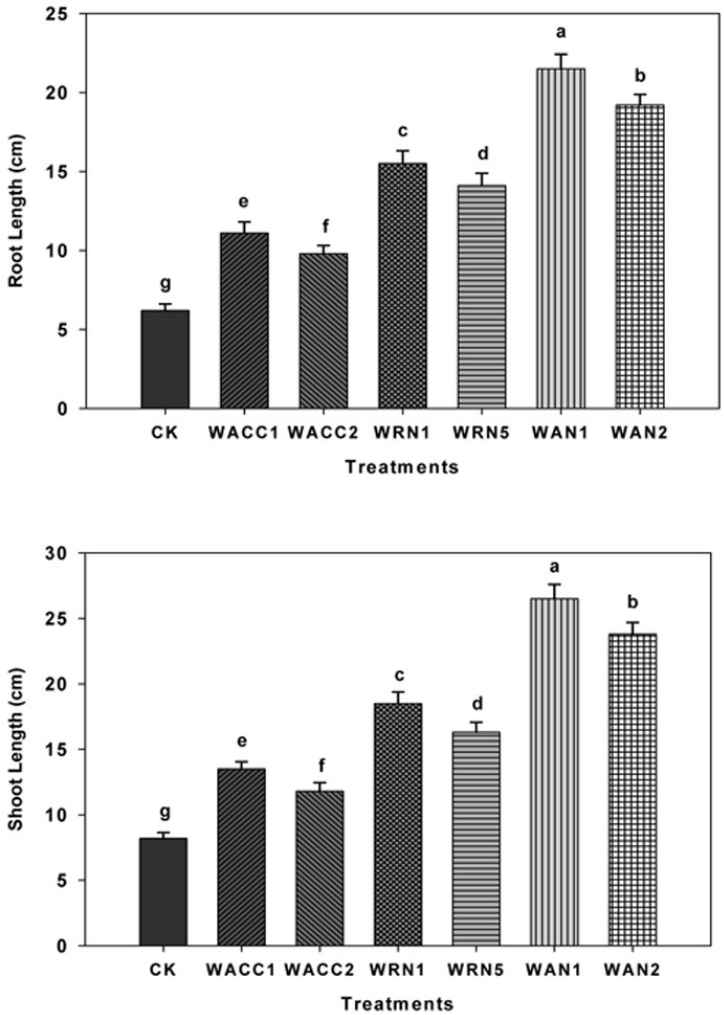

Figure 1. Comparative effectiveness of ACC-deaminase and/or nitrogen fixing rhizobacteria on root and shoot length of wheat. Different letters (a-g) on bars indicate significant differences of mean values for root length. Bars represent standard errors.

CK = Control

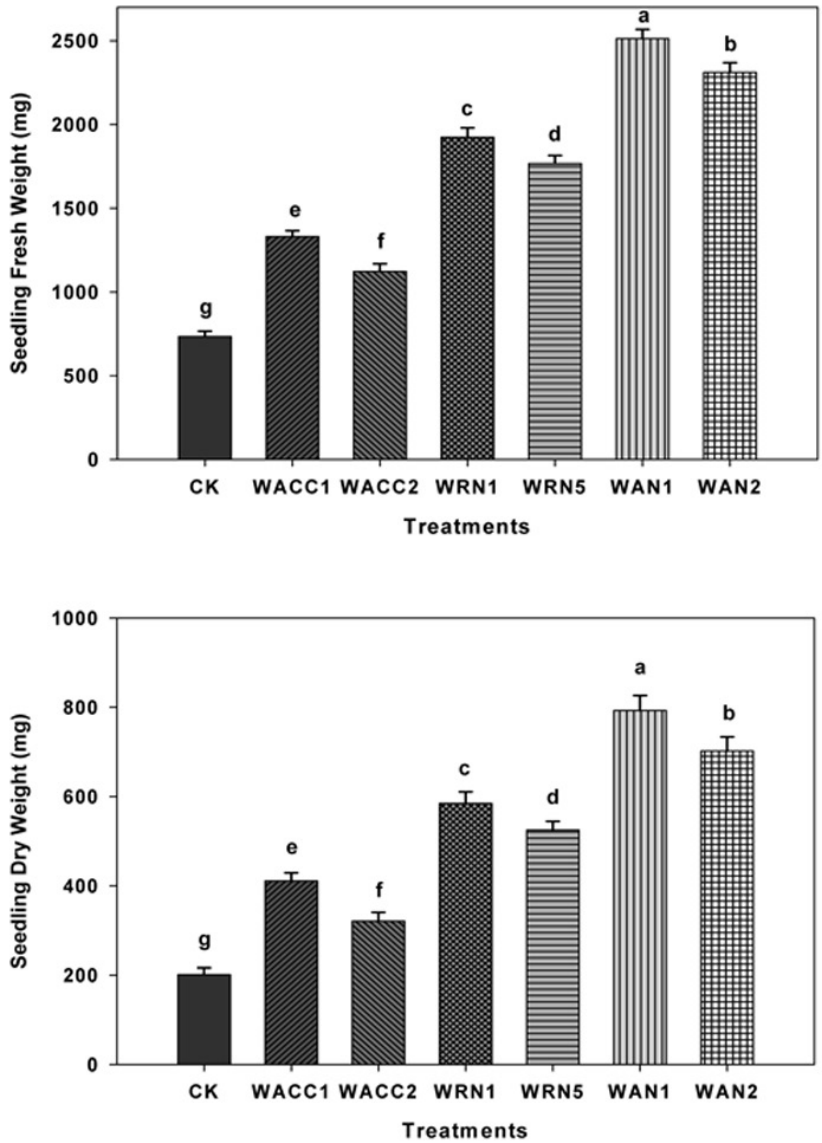

Figure 2. Comparative effectiveness of ACC-deaminase and/or nitrogen fixing rhizobacteria on seedling fresh and dry weight of wheat. Different letters (a-g) on bars indicate significant differences of mean values for seedling fresh weight. Bars represent standard errors.

CK = Control

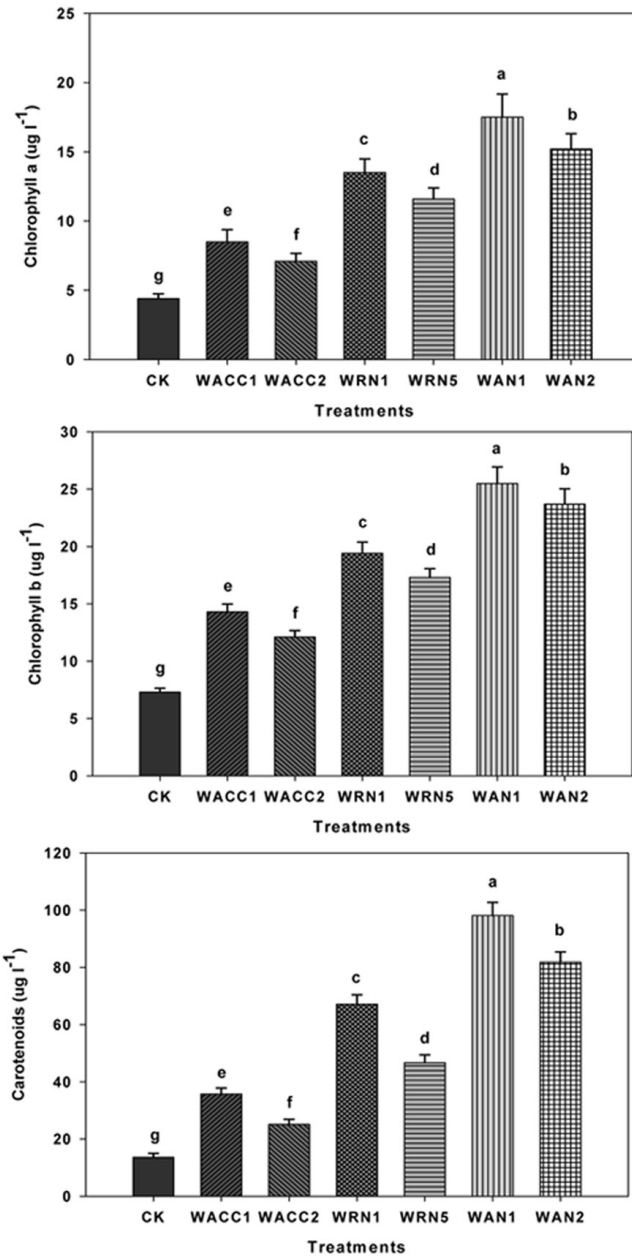

Figure 3. Comparative effectiveness of ACC-deaminase and/or nitrogen fixing rhizobacteria on chlorophyll a, b and carotenoid contents of wheat. Different letters (a-g) on bars indicate significant differences of mean values for chlorophyll a contents. Bars represent standard errors.

CK = Control

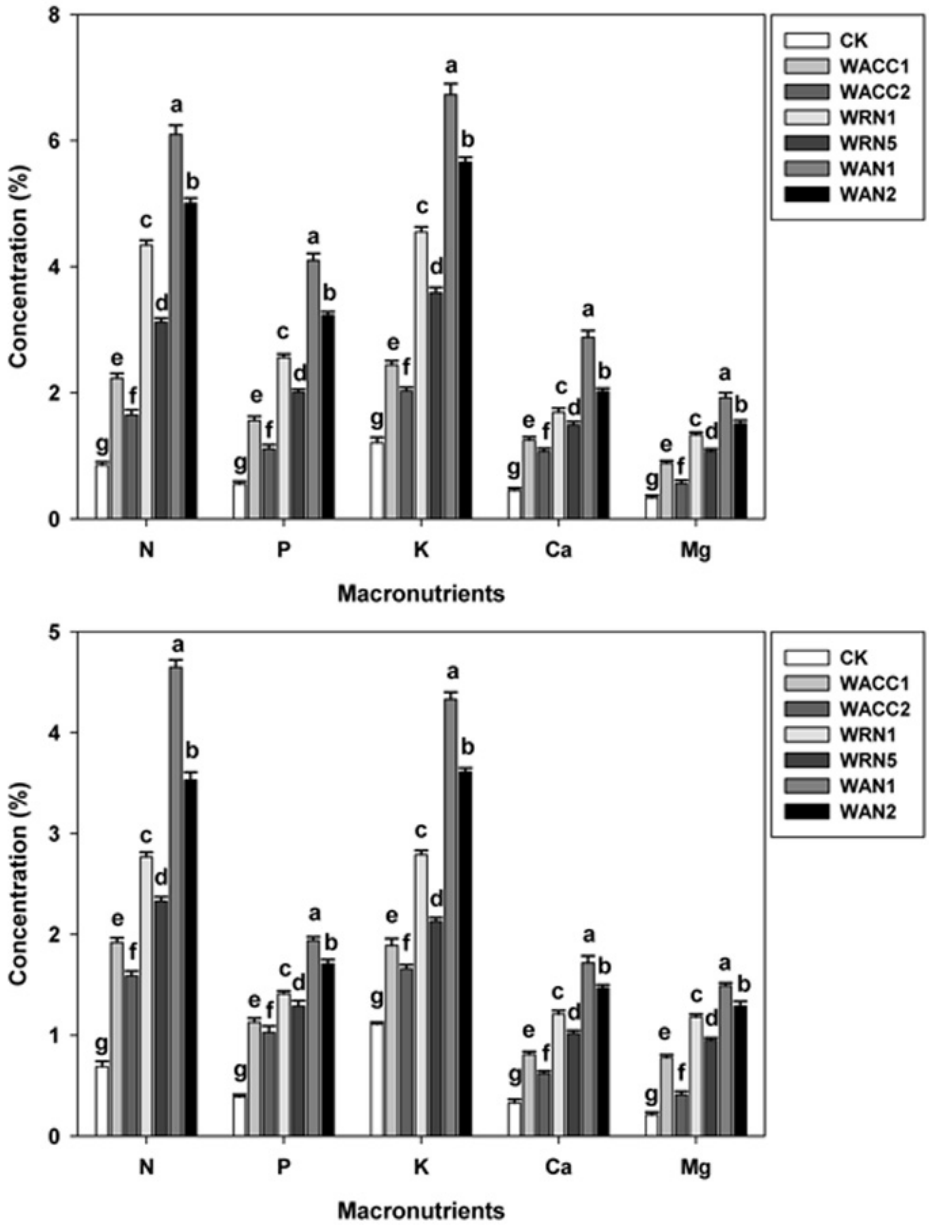

Figure 4. Comparative effectiveness of ACC-deaminase and/or nitrogen fixing rhizobacteria on macronutrient contents (N, P, K, Ca, and Mg) in shoot and root of wheat. Different letters (a-g) on bars indicate significant differences of mean values for macronutrient contents. Bars represent standard errors.

CK = Control

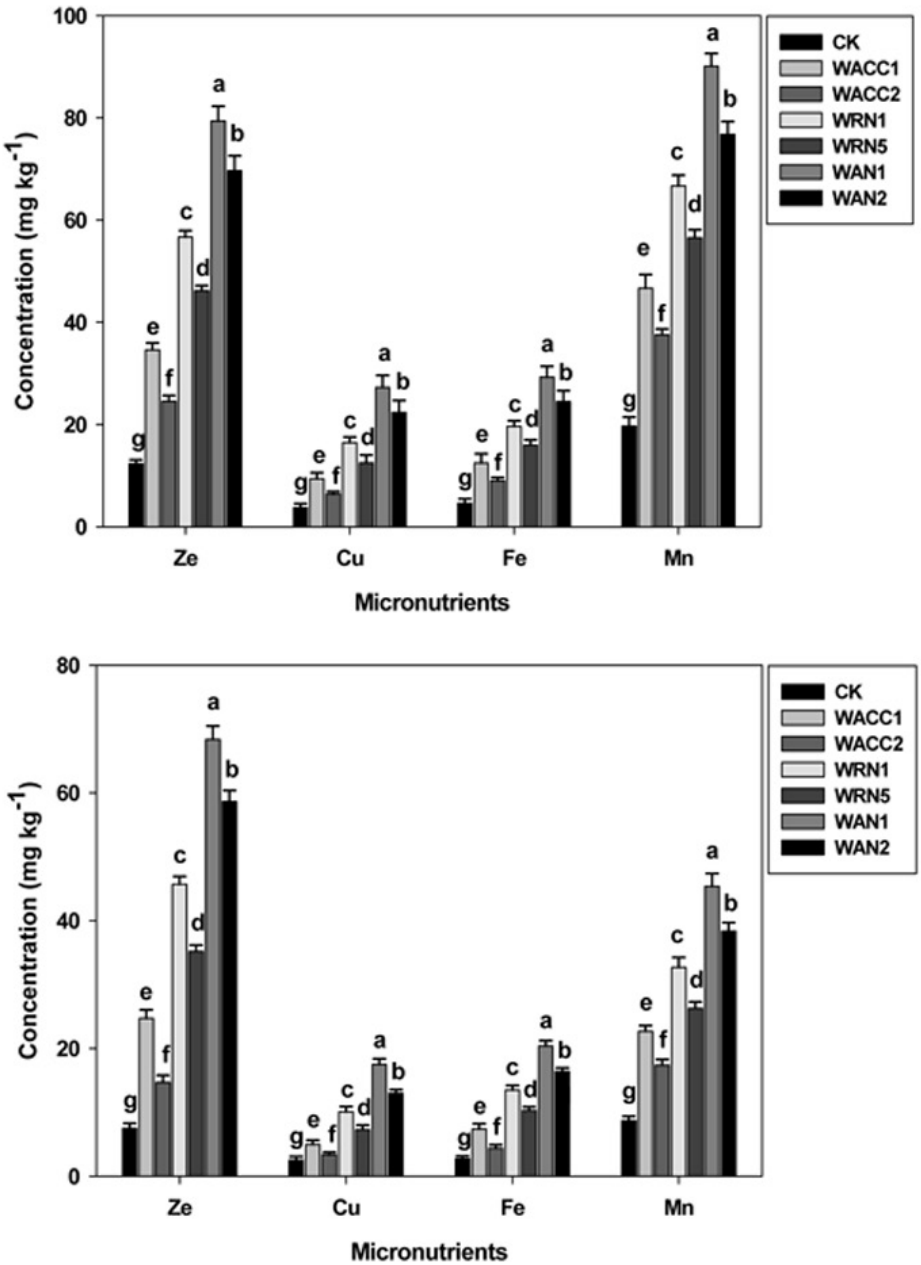

Figure 5. Comparative effectiveness of ACC-deaminase and/or nitrogen fixing rhizobacteria on micronutrient contents (Zn, Cu, Fe and Mn) in shoot and root of wheat. Different letters (a-g) on bars indicate significant differences of mean values for micronutrient contents. Bars represent standard errors.

CK = Control

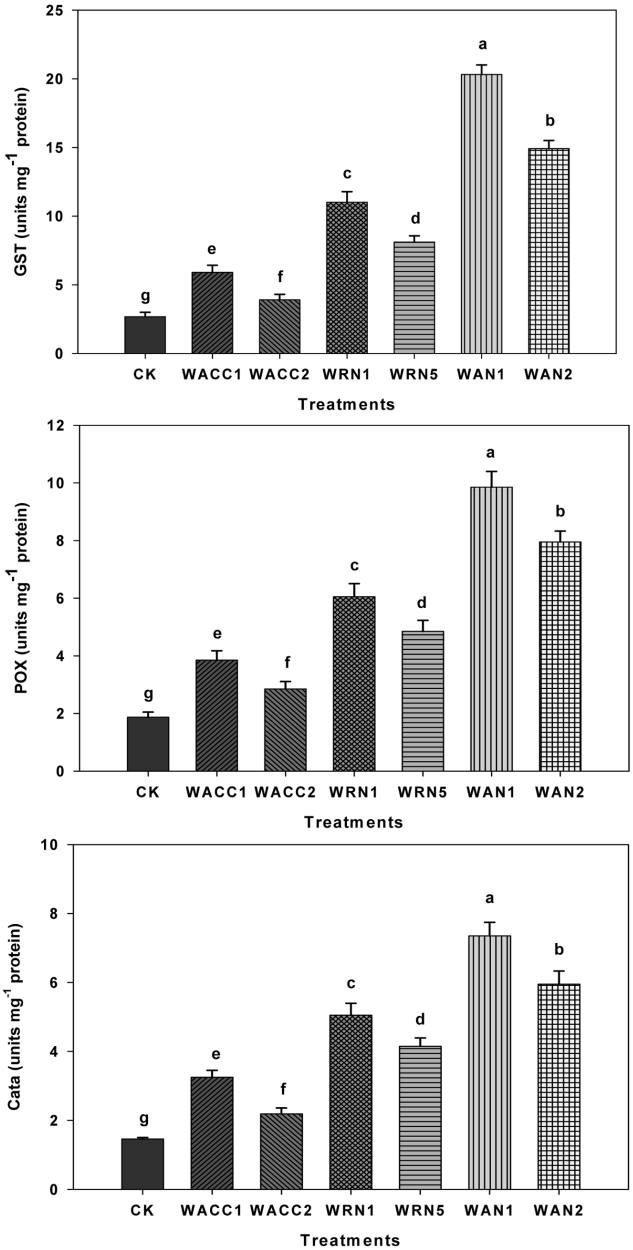

Figure 6. Comparative effectiveness of ACC-deaminase and/or nitrogen fixing rhizobacteria on enzymes GST, POX and Cata activity. Different letters (a-g) on bars indicate significant differences of mean values for glutathione S-transferase activity. Bars represent standard errors.

CK = Control; GST = glutathione S-transferase; POX = peroxidase; Cata = Catalase

5. Conclusions

Inoculation of agricultural crops with rhizobacterial strains is a very viable and eco-friendly approach to increase crop production on sustainable basis. The plant-beneficial rhizobacteria may decrease the global dependence on hazardous agricultural chemicals which destabilize the agro-ecosystems. PGPR protect the plants from the deleterious effects of biotic and abiotic stresses by producing phytohormones and antioxidant enzymes and increase the plant growth by increasing the nutrients availability and uptake. The ACC enrichment technique is an effective and efficient approach to select most promising PGPR. Rhizobacteria containing both ACC-deaminase and nitrogen fixing activity are more effective than rhizobacteria containing either ACC-deaminase or nitrogen fixing activity alone for growth promotion of agricultural crops.

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