Phyton-International Journal of Experimental Botany
DOI:10.32604/phyton.2020.08918
Article

Tech Science Press

Multi-strain Inoculation with PGPR Producing ACC Deaminase is More Effective Than Single-strain Inoculation to Improve Wheat (*Triticum aestivum*) Growth and Yield

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Received: 24 October 2019; Accepted: 18 December 2019

Abstract: Rhizosphere bacteria that colonize plant roots and confer beneficial effects are referred as plant growth promoting rhizobacteria (PGPR). Among all PGPR, some rhizobacteria have an ability to produce ACC deaminase enzyme. This enzyme catalyzes stress ACC into a-ketobutyrate and ammonia instead of letting it to be converted to ethylene. Ethylene level rises in plants under stress conditions i.e., drought, salinity, poor soil fertility etc. As poor soil fertility is a big hurdle to achieve the optimum yield of crops, inoculation of ACC deaminase PGPR can overcome this problem to some extent. The aim of the current study was to examine the influence of multi-strain and single-strain inoculation of different ACC deaminase producing PGPR on wheat growth and yield. There were three PGPR strains, *Enterobacter cloacae, Serratia ficaria and Burkholderia phytofirmans* which were used as consortia and single-strain inoculations. The results showed that inoculation of *E. cloacae* + *S. ficaria* + *B. phytofirmans* significantly increased plant height (63%), spike length (61%), number of spikelets spike-1 (61%), number of grains spike^{-1} (131%), 1000 grains weight (33%), grains yield (71%), straw yield (71%) and biological yield (68%) of wheat as compared to control. A significant improvement in N (37 and 200%), P (46 and 166%) and K (39 and 61%) of seeds and shoot respectively, validated the efficacious and more effective role of multi-strain (*E. cloacae* + *S. ficaria* + *B. phytofirmans*) inoculation over control. It is obviously concluded that multi-strain ACC deaminase producing PGPR inoculation is a better approach as compared to single-strain inoculation for the improvement in growth and yield of wheat.

Keywords: Ethylene; growth attributes; nutrients; consortium; rhizobacteria

1 Introduction

Plant growth is directly linked with nutrients and water holding potential of soil [1]. High volatilization of ammonia, leaching of nitrate [2] and immobilization of phosphorus and potassium decrease the fertility level of soils [3]. Poor soil fertility status is a main hurdle to achieve the target of getting maximum yield from crops. Furthermore, intensive cultivation of exhaustive crops has depleted the nutrient concentrations in the soils [4]. Low availability of nutrients is associated with poor growth, development of plants [5–6].

Ethylene (C_2H_4) is a plant-signaling molecule which involves in the germination of seeds, root development and elongation, flower senescence, leaf abscission and fruit ripening. It is produced in a two-step process i.e., (1) enzymatic conversion of S-adenosyle methionine (SAM) to 1-amino cyclopropane-1-carboxylic acid (ACC); (2) conversion of ACC to ethylene, which is catalyzed by ACC-oxidase [7]. However, synthesis of endogenous ethylene level is significantly enhanced upon exposure of plants to abiotic stresses including low soil fertility [8–9].

Currently, various strategies are in use to maximize the yield of crops under nutritional stress using chemical fertilizers, green manures, farmyard manures and bio-fertilizers etc. In this context, the combined use of bio-fertilizers and inorganic fertilizers is more effective and environmental friendly approach [10–11]. It has been observed that rhizosphere is a zone of intense microbial activity immediately adjacent to the plant root and nutrient rich environment due to release of sugars, amino acids, organic acids, plant hormones and enzymes [12]. The plant growth promoting rhizobacteria (PGPR) can secrete multiple metabolites and enzymes that confer beneficial effects on plants growth [13–14].

In addition, the PGPR containing ACC deaminase have been found to be effective enough in enhancing the resistance against abiotic stresses and to promote the health of plants [15–16]. ACC deaminase is a polymeric enzyme which is dependent on pyridoxal 5-phosphate (PLP) [17]. It is established that the PGPR containing ACC deaminase can reduce the accumulation of ethylene by breaking cyclo-propanoid amino acid ACC (ethylene precursor), into intermediate compounds, ammonia and α-ketobutyrate [8]. The ACC deaminase producing PGPR enhance the uptake of N, P and K too especially in cereal crops like wheat and maize [18–19].

Across the globe, wheat is consumed as a staple food. It is widely cultivated due to its high nutritional value and capability to grow under a vast range of climatic conditions. Wheat shares 44% of daily human intake of iron (Fe) and 25% of zinc (Zn) [20]. In Pakistan, wheat is cultivated over an area of 8,740 thousand hectares with the production of 25,195 million tonnes. However, the yield of wheat is yet below the demand of exponentially increasing population [21]. That's why current experiment was conducted with the aim to examine the influence of single and multi-strain inoculation of ACC deaminase producing PGPR along with chemical fertilizers on growth and yield of wheat. It is hypothesized that multi-strain inoculation of ACC-deaminase producing PGPR would be a better approach to improve the growth and yield of wheat comparative to single-strain inoculation.

2 Materials and Methodology

2.1 Experimental Site

A field study was conducted in the experimental area of the Department of the Soil Science, Bahauddin Zakariya University Multan, Pakistan. For determination of soil texture, hydrometer method was used [22]. The pH and EC of soil were noted by making soil paste with 1:1 and 1:10 soil-water ratio respectively on pH and EC meters accordingly. The available P was found out through the protocol described by Olsen's [23], Extractable K was determined by following Nadeem et al. [24] and Walkley [25] protocol was followed for soil organic matter determination. The pre-experimental soil characteristics are provided in Tab. 1.

Table 1: Physio-chemical characterization of soil

Parameters	Unit	Values
Physical Analysis		
Sand	%	35
Silt	%	35
Clay	%	30
Saturation percentage	%	47
Textural class	Clay loam	
Chemical Analysis		
pH*s*		8.60
EC*e*	dS m^{-1}	2.30
TDS	mg kg^{-1}	1.15
OM	%	0.40
Total nitrogen	%	0.05
Available phosphorus	mg kg^{-1}	5.6
Extractable potassium	mg kg^{-1}	130

2.2 Treatments

There were eight treatments with three replications. The treatments included (1) un-inoculated control, (2) *Enterobacter cloacae* (3) *Serratia ficaria*, (4) *Burkholderia phytofirmans*, (5) *Enterobacter cloacae* + *Serratia ficaria*, (6) *Enterobacter cloacae* + *Burkholderia phytofirmans*, (7) *Serratia ficaria* + *Burkholderia phytofirmans* and (8) *Enterobacter cloacae* + *Serratia ficaria* + *Burkholderia phytofirmans*. The NPK fertilizers as 120, 100 and 60 kg ha^{-1} respectively, were applied along with every treatment including control.

2.3 Bacterial Inoculation

Peat was used as a standard carrier for bacterial inoculation. Peat was collected from a local nursery and sterilized at 121°C for 21 min. For seed inoculation, the DF media was prepared without agar [26]. Uniform cell density, 10^8-10^9 CFU mL^{-1} was achieved prior to seed inoculation in case of every single and multi-strain inoculation.

2.4 PGPR Strains

The PGPR strains, *Enterobacter cloacae* (W6), *Serratia ficaria* (W10) and *Burkholderia phytofirmans* (PsJN) were obtained from Laboratory of Soil Microbiology and Biochemistry, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad, Pakistan. All the strains were positive in ACC deaminase activity.

2.5 Wheat Variety and Sowing

Wheat cultivar used in the trial was AAS-2008 (a developed variety of Wheat for lower Punjab). Sowing was done by using dibbler (recommended 9 inch row to row distance) for sowing of seeds of Wheat. Each plot size was 12 m^2 (4 m × 3 m). Recommended row to row (R × R) distance i.e., 9 inch was maintained.

2.6 Irrigation and Fertilizer Application

Recommended number of irrigation (4-5) was applied throughout the research period in this field experiment, as per requirement. The NPK Fertilizers as urea, DAP and SOP were added at the rate of 120, 60 and 60 kg ha^{-1} as recommended fertilizer doses [40].

2.7 Harvesting

Plants were harvested at the time of maturity. Plant height was measured at physiological maturity stage. Five tillers were chosen at random from each replication. Length of five random selected spikes was measured from each replication. Number of spikelet $spike^{-1}$ was also counted from the five randomly selected spike. Randomly harvested tillers were threshed separately. After separating grains from straw, number of grains per spike was counted. Five samples having 100 grains were taken from each plot and weighed. Then the weight of 100 grains was converted into 1000-grain weight by multiplication. Five plants were selected on random basis and the weight of whole produce of a plant was noted. Biological yield was calculated and adjusted to kg ha^{-1} unit by multiplying the one meter square biological yield with 10,000 value. With the help of one meter square (1 m^2) quadrate/frame, plants were harvested and sun dried for three days. Then they were threshed manually and after separating the grains and straws, grains were weighed and adjusted to kg ha^{-1} unit by multiplying the one meter square grain yield by 10,000 value.

$$1\ m^2 \times 10,000 = \text{kg ha}^{-1}$$

Straw yield was calculated as:

$$\text{Straw yield} = \text{Biological yield} - \text{Grain yield}$$

2.8 Nutrients Analyses

For determination of nitrogen, potassium and phosphorus contents in grain and straw samples wet digestion was done as described by Wolf [27]. The potash was determined with flamephotometer (Jenway PFP-7), using standard curve [24]. Phosphorus was determined by spectrophotometer using standard curve [28]. Nitrogen was determined by Kjeldahl's method described by Jackson [29].

2.9 Statistical Analyses

Collected data from treatments on various parameters were arranged and analyzed by ANOVA (analysis of variance) and their means were compared with Tukey's test at 0.05% probability [30].

3 Results

3.1 Growth Attributes

Results revealed that single and multi-strains inoculation significantly affected wheat plant height, spike length, number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$. Inoculation of *E. cloacae* × *S. ficaria* × *B. phytofirmans* remained significantly better over control for plant height, spike length, number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$. No significant change was observed among each other where *E. cloacae* + *S. ficaria* and *S. ficaria* + *B. phytofirmans* were used for plant height and spike length but *E. cloacae* + *B. phytofirmans* performed significantly better over *S. ficaria* + *B. phytofirmans* for number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$ (Tab. 2). It was also observed that sole *E. cloacae, S. ficaria* and *B. phytofirmans* differed significantly over control for plant height, spike length, number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$. Among sole inoculation, *B. phytofirmans* remained significantly better from *E. cloacae* and *S. ficaria* for number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$. Maximum increases of 62, 61, 61 and 131 % were noted in plant height, spike length, number of spikelets $spike^{-1}$ and number of grains $spike^{-1}$ respectively over control where *E. cloacae* + *S. ficaria* + *B. phytofirmans* was applied.

Table 2: Effect of single and multistrains inoculation of ACC deaminase producing PGPR on growth attributes of wheat

Treatments	Plant Height (cm)	Spike Length (cm)	Number of spikelets spike^{-1}	Number of grains spike^{-1}
Control	55.00 E	8.67 E	14.67 F	25.67 H
E. cloacae	66.33 CD	11.00 CD	18.00 E	46.00 F
S. ficaria	62.00 DE	9.33 DE	19.33 D	42.67 G
B. phytofirmans	66.67 CD	11.33 BCD	20.00 C	48.33 E
E. cloacae* + *S. ficaria	73.00 BC	11.67 BC	20.00 C	50.00 C
E. cloacae* + *B. phytofirmans	77.67 B	12.67 AB	21.67 B	54.33 B
S. ficaria* + *B. phytofirmans	75.00 B	11.67 BC	20.67 C	49.00 D
E. cloacae* + m *S. ficaria* × *B. phytofirmans	89.33 A	14.00 A	23.67 A	59.33 A

Different letters exhibit significant deference ($p \leq 0.05$) between treatments.

3.2 Yield Attributes

Multi-strain bacterization produced highly significant effects on 1000-grain weight of wheat, grain yield, straw yield and biological yield. Inoculation with *E. cloacae* + *S. ficaria* + *B. phytofirmans* remained significantly best among all the treatments regarding improvement in 1000-grains weight, grain, straw and biological yield. Inoculation with *E. cloacae* × *B. phytofirmans* remained significantly better over *E. cloacae* + *S. ficaria* for 1000-grains weight, grain, straw and biological yield (Tab. 3). In addition, no significant change was noted among effects of *E. cloacae* + *B. phytofirmans* and *S. ficaria* + *B. phytofirmans* regarding 1000-grain weight, grain, straw and biological yield. Single inoculation with *E. cloacae, S. ficaria* and *B. phytofirmans* also remained significantly better from control for 1000-grain, weight, grain, straw and biological yield. Maximum increases of 33, 71, 71 and 68% were noted in 1000-grains weight, grain, straw and biological yield respectively over control where *E. cloacae* + *S. ficaria* + *B. phytofirmans* was applied.

Table 3: Effect of single and multistrains inoculation of ACC deaminase producing PGPR on yield attributes of wheat

Treatments	1000 grains weight (g)	Grain Yield (Mg ha^{-1})	Straw Yield (Mg ha^{-1})	Biological Yield (Mg ha^{-1})
Control	45 F	2.21 F	6.62 F	8.83 E
E. cloacae	46 E	2.94 CD	8.81 CD	11.7 C
S. ficaria	51 D	2.51 EF	7.54 EF	10.1 DE
B. phytofirmans	51 D	2.82 DE	8.46 DE	11.3 CD
E. cloacae* + *S. ficaria	52 CD	2.78 DE	8.33 DE	11.1 CD
E. cloacae* + *B. phytofirmans	54 B	3.35 B	10.1 B	13.4 AB
S. ficaria* + *B. phytofirmans	53 BC	3.32 BC	9.97 BC	13.3 B
E. cloacae* + *S. ficaria* + *B. phytofirmans	60 A	3.77 A	11.3 A	14.8 A

Different letters exhibit significant deference ($p \leq 0.05$) between treatments.

3.3 Nutrients Concentration in Seeds and Shoot

Multi-strain PGPR inoculation significantly affected N, P and K concentration in shoot and seeds of wheat. The *E. cloacae* + *S. ficaria* + *B. phytofirmans* proved significantly better among all the treatments regarding N, P and K concentration in shoot and seeds. The *E. cloacae* + *B. phytofirmans* remained significantly better over *S. ficaria* + *B. phytofirmans* and exhibited significant increase in N, P and K concentration in shoot and seeds. The *S. ficaria* + *B. phytofirmans* remained better than *E. cloacae* + *S. ficaria* with respect to N concentration in seed and K in shoot. However, both the *S. ficaria* + *B. phytofirmans* and *E. cloacae* + *S. ficaria* remained statistically alike for N in shoot, K in seeds and P in seed and shoot (Tab. 4). Inoculation with *E. cloacae, S. ficaria* and *B. phytofirmans* in separate also proved significantly better from control for N, P and K concentration in shoot and seeds of wheat. Maximum increases of 37, 200, 57, 167, 28 and 38% were noted in N in seeds, N in shoot, P in seeds, P in shoot, K in seeds and K in shoot respectively over control where *E. cloacae* + *S. ficaria* + *B. phytofirmans* was applied.

Table 4: Effect of single and multistrains inoculation of ACC deaminase producing PGPR on nutrients concentration of wheat seed and shoot

Treatments	N in seeds	N in shoot	P in seeds	P in shoot (%)	K in seeds	K in shoot
Control	1.35 G	0.05 E	0.30 G	0.06 E	1.80 F	0.49 G
E. cloacae	1.53 E	0.07 DE	0.37 E	0.09 D	2.20 D	0.58 EF
S. ficaria	1.44 F	0.07 DE	0.31 F	0.10 CD	2.10 E	0.57 F
B. phytofirmans	1.53 E	0.08 D	0.41 D	0.11 C	2.30 C	0.59 DE
E. cloacae* + *S. ficaria	1.64 D	0.10 C	0.43 C	0.12 C	2.30 C	0.60 D
E. cloacae* + *B. phytofirmans	1.72 B	0.14 B	0.45 B	0.15 B	2.40 B	0.69 B
S. ficaria* + *B. phytofirmans	1.65 C	0.11 C	0.43 C	0.12 C	2.30 C	0.62 C
E. cloacae* + *S. ficaria* + *B. phytofirmans	1.85 A	0.15 A	0.47 A	0.16 A	2.50 A	0.79 A

Different letters exhibit significant deference ($p \leq 0.05$) between treatments

4 Discussion

In the current study, the multi-strain PGPR inoculation significantly increased wheat plant height, spike length, number of spikelets spike^{-1} and number of grains spike^{-1} as compared to the control and even single-strain inoculation in most of the cases. The improvement in plant height might be due to reduction in stress ethylene level by sole consortium of ACC deaminase producing PGPR. Mayak et al. [31] stated that elevated 1-aminocyclopropane-1-carboxylic acid (ACC), especially under limited availability of water and nutrients in plant, increases ethylene concentration in root and shoot of plants. The plant roots and seeds exude the ACC into rhizosphere that is converted by ACC-deaminase into NH_3 and α-ketobutyrate thus decreasing ethylene level. Reduction in ethylene results in better roots elongation that might have facilitated plant to take in water and nutrients by exploring more soil volume [8]. In addition to ACC-deaminase activity, the improvement in wheat growth might also be favoured due to production of other growth-improving organic entities. Xie et al. [32] also noted IAA as an allied factor, playing a role in crops growth improvement. High IAA synthesis by PGPR increases surface area and length of adventitious and lateral roots in plants that help in nutrients uptake [33]. In addition, roots exudates (organic acids, phytosiderophores, sugars, vitamins, amino acids, nucleosides and mucilage) attract the PGPR for

colonization in roots with an improvement in the uptake of water and solubilization of immobilized (P and K) nutrients [34–35]. The multi-strains PGPR *E. cloacae* + *S. ficaria* + *B. phytofirmans* inoculation in specific, distinctly counteracted the adverse effects of drought in wheat in terms of improvement in 1000-grain weight, grain yield, straw yield and biological yield. This increase in yield of wheat might be due to an improvement in N, P and K uptake facilitated by the PGPR consortium. Plants growth is largely dependent on nutrients and H_2O holding potential of soil [10,37]. According to Hassan et al. [38], better uptake of N, P and K nutrients with PGPR application play a vital role in the development of shoot and root dry weight [36]. The probability of secretion of acid phosphatases enzyme may another important factor that would have helped in growth promotion through consortium [39].

5 Conclusion

Finally, the conclusion may be drawn out of the present study that multi-strain inoculation with the PGPR possessing an ACC-deaminase activity is more effective than single-strain inoculation to improve wheat growth and yield.

Conflicts of Interest: The authors declare no conflicts interests.

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