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Research Paper

Efficacy of PGPR on Growth and Enzyme Activity of Rice under Water Stress and Irrigated Condition

Sudha Bind*[1] and A. K. Sharma[2]

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ABSTRACT

Water stress is major abiotic stress that negatively affects the plant productivity. This study was done with the aim to evaluate the efficacy of two PGPR Strains, *Variovorax paradoxus* (RAA3) and *Pseudomonas palleroniana* (DPB16) on growth promotion of rice under water stress and irrigated condition. RAA3 and DPB16 having ACC deaminase, nitrogen fixing, phosphate, zinc, potassium solubilizing and IAA producing activity. Both bacterial strains increased all growth associated parameters, chlorophyll content and enzyme activity such as peroxidase (POD), superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) of rice under water stress and irrigated conditions as compared to the control plant. RAA3 showed better result on plant growth over the DPB16. RAA3 and DPB16 increased the shoot length (17%, 14.30%), root length (17.70%, 10.20%), shoot fresh weight (29.50%, 24.20%), root fresh weight (57.70%, 57.60%), shoot dry weight (28.50%, 27.40%), root dry weight (29.11%, 28.80%), total chlorophyll (28.71%,22.70%), SOD (27.40%, 23.80%), POD (54.80%, 31.80%), CAT (24.30%, 19.70%) and APX (39.80%, 32.20%) respectively under water stress. This study showed that PGPR having ACC deaminase activity and other plant growth promoting traits can be used as biofertilizer to enhance the plant growth through alleviation of water stress.

***Key words*:** PGPR, Water stress, ACC deaminase, Enzyme, IAA

Rice is an important staple food-crop for more than 50 % of the world's population. World population will reach up to 9 billion till the 2050, to feed this huge population of the world there is need to increase the crop productivity with same pace to assure global food security [1]. Water stress is major factor of low plant productivity worldwide and it is likely to further increase. Water stress is expected to cause the serious problem of crop productivity on 50% of the arable land by 2050 [2]. Due to continuous increase in global warming and climate change, frequency and severity of water stress is predicted to increases in crop growing regions of the world [3]. In India out of 44.6 million hectares of area under rice cultivation, about 52% of area is rainfed or under water stress.

Various strategies were adapted to increase the plant tolerance against water stress. Breeding and genetic engineering are being used for production of water stress tolerant and high yielding varieties [4] but these methods are time consuming and labour intensive and at one time only single crop is produced [5-6]. Therefore, there is need of an eco- friendly approach for plant growth under water stress. Plant-microbe interaction is very effective in plant tolerance against water stress [7]. PGPR having ability of nitrogen fixation, phosphorus solubilization, siderophore production, phytohormone production, ACC deaminase production. Due to these characteristics, PGPR can cope up with the water limiting condition thus increases the plant tolerance towards stress condition.

*Sudha Bind
sudhabind0724@gmail.com

[1-2]Department of Biological Sciences, College of Basic Sciences and Humanities, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar - 263 145, U.S. Nagar, Uttarakhand, India

MATERIALS AND METHODS

Plant material: Seeds of rice variety sahbhagi was taken and washed 3-4 times by using distilled water and surface sterilized with 4% of sodium hypoclorite and 70% of ethanol for 3 minute and 1 minute respectively. After that seeds were thoroughly washed 3-4 times with autoclaved distilled water and kept on sterilized petri plates having sterilized sheet of paper moistened with distilled water for germination and incubated at 30°C for 24-48 hours. Uniform sizes of germinated seedlings were taken for experiment.

Bacterial strains: RAA3 (*Variovorax paradoxus*) and DPB16 (*Pseudomonas palleroniana*) previously isolated by [8], were collected from Rhizosphere Biology laboratory, Department of Biological Sciences, CBSH, GBPUA&T, Pantnagar, Uttarakhand, India.

Inoculum preparation: Loopful culture of Bacterial strains were inoculated into 150 ml of flask containing 50ml of nutrient broth and incubated at 28°C for 24 hours at 120 rpm. Culture having (10^7-10^8 cfu/ml) was used for inoculation.

Glass house experiment: Sand and soil ratio was 3:1 (sand: soil) and this mixture was sterilized three times before use. After sterilization sand and soil mixture was filled in pots having capacity of 500g. Two sets were prepared one for water stress and another set for irrigated condition. Both sets were maintained at water holding capacity of sand-soil mixture (25ml) after transplanting the seedlings. After 10 days of transplanting one set is exposed to water stress by maintaining the 30% of water holding capacity. Two seedlings were planted in each pot and 1 ml of bacterial culture having (10^7-10^8 cfu/ml) was added to each seedling. Plants were grown in glass house with 28±2°C temperature, photoperiod (16/8 h day/night cycle), 400 $Em^{-2}s^{-1}$ light intensity. After 45 days plants were harvested and agronomical parameter recorded and enzyme analysis was done.

Enzyme and chlorophyll content analysis: Fresh leaf sample was taken for enzyme and chlorophyll analysis. Chlorophyll content was analyzed according to [9] and SOD [10], POD [11], APX [12] (CAT) was analyzed according to [13].

RESULTS AND DISCUSSION

Agronomical parameter

Both bacterial strains significantly enhanced the rice growth under water stress and irrigated condition as compared to the control. RAA3 showed best result among the treatment for all the agronomical parameter of rice. RAA3 and DPB16 increased Shoot length (17%, 14.3%), (23.2%, 16.5%) (Fig 1), root length (17.7%, 10.2%), (12.6%,6.3%) (Fig 2), Shoot fresh weight (29.5%, 24.2%), (38.5%, 29.8%) (Fig 3), root fresh weight (57.7%, 57.6%), (50%, 40%) (Fig 4), Shoot dry weight (28.5%, 27.4%), (58.6%, 46.9%) (Fig 5), and root dry weight (29.1%, 28.8%), (31.7%, 26.1%) (Fig 6), under water stress and irrigated condition respectively.

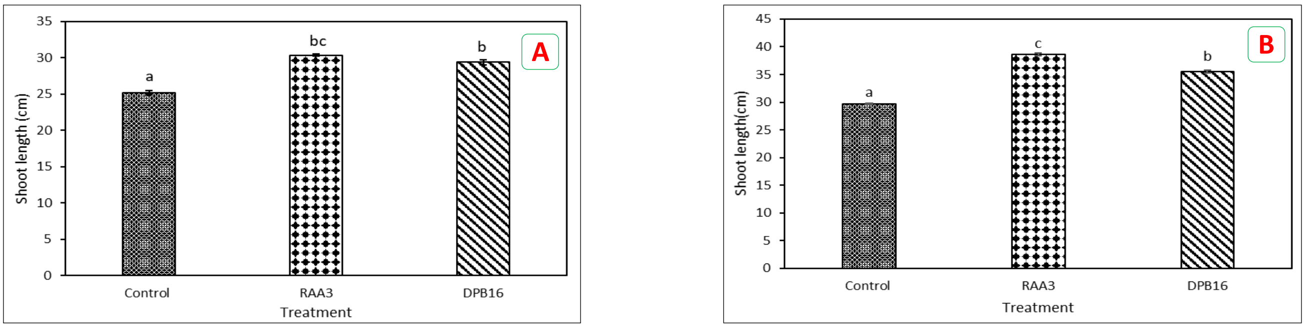

Fig 1 Effect of RAA3 and DPB16 on shoot length of rice under A. (Water stress) B. (Irrigated) condition

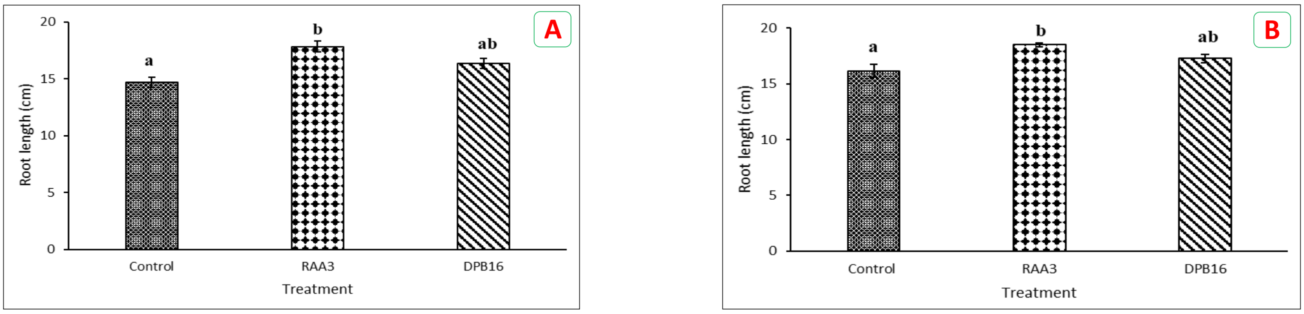

Fig 2 Effect of RAA3 and DPB16 on root length of rice under A. (Water stress) B. (Irrigated) condition

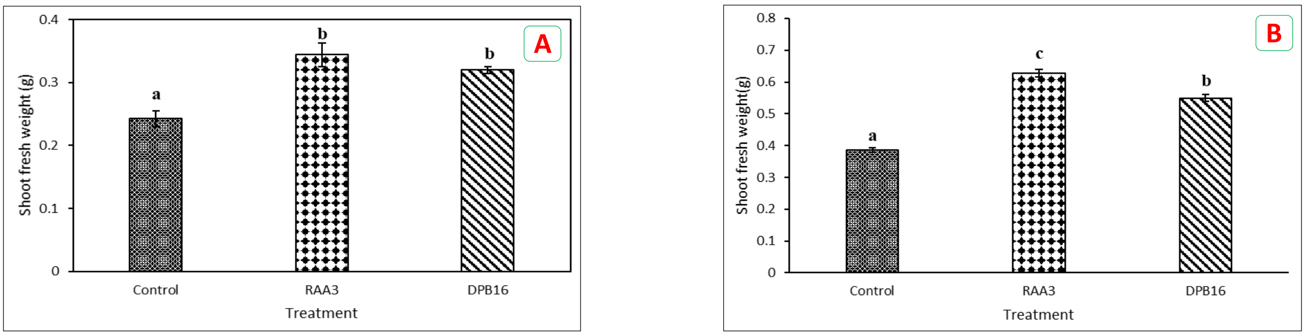

Fig 3 Effect of RAA3 and DPB16 on shoot fresh weight of rice under A. (Water stress) B. (Irrigated) condition

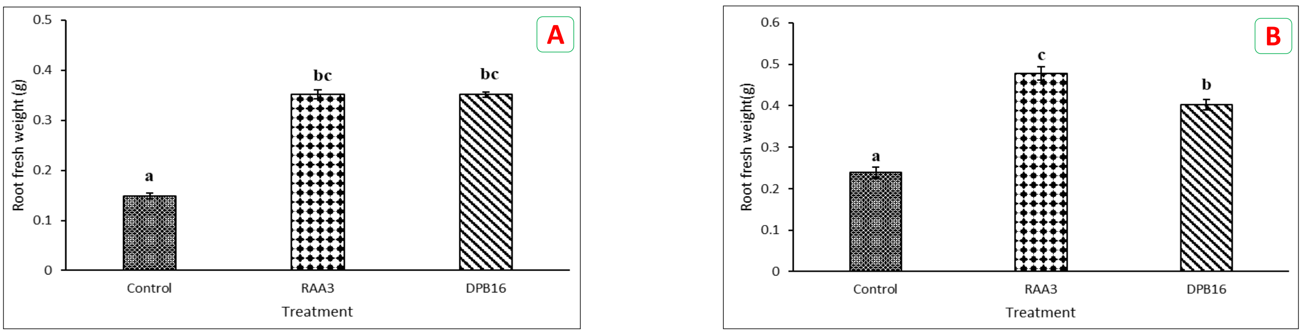

Fig 4 Effect of RAA3 and DPB16 on root fresh weight of rice under A. (Water stress) B. (Irrigated) condition

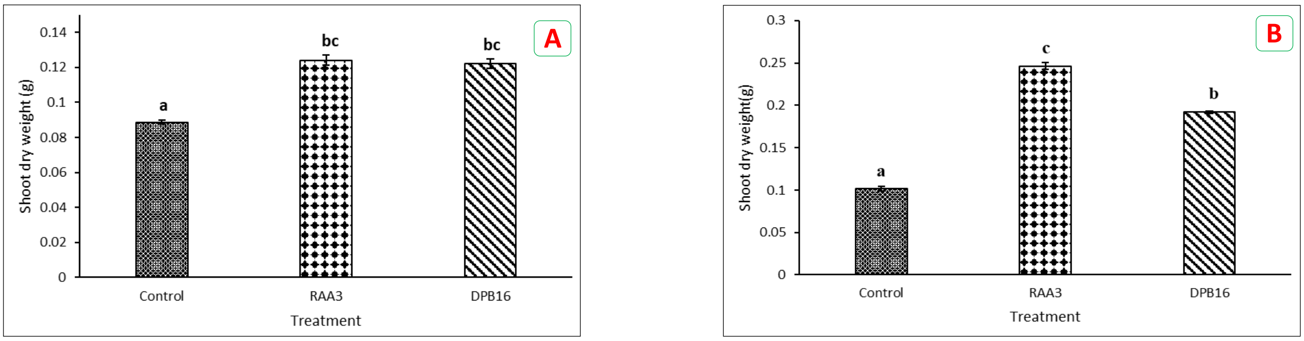

Fig 5 Effect of RAA3 and DPB16 on shoot dry weight of rice under A. (Water stress) B. (Irrigated) condition

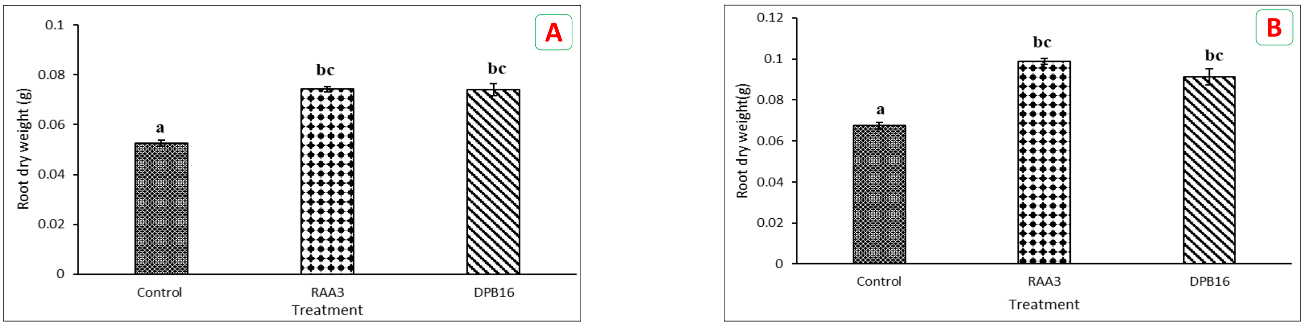

Fig 6 Effect of RAA3 and DPB16 on root dry weight of rice under A. (Water stress) B. (Irrigated) condition

Chlorophyll content and enzymatic activity

Bacteria inoculated plant exhibited significantly higher chlorophyll content and enzymatic activity. RAA3 showed maximum enhancement of chlorophyll and enzymatic content as compared to DPB16. RAA3 and DPB16 enhanced the chlorophyll content by (28.7%, 22.70) under water stress and (26.80%, 25.10%) under irrigated condition. RAA3 and DPBP16 enhanced the SOD (27.4%, 23.8%), (38.6%, 33%), POD (54.8%,31.8%), (40.50%,32%), CAT (24.3%, 19.7%), (39.4%, 34.1%) and APX (39.8%, 32.2%), (45.2%, 35.1%) activity than the control plant under water stress and irrigated condition respectively (Table 1).

Table 1 Effect of RAA3 and DPB16 on total chlorophyll content and enzymatic activity of rice under water stress and irrigated condition

Treatment	SOD (unit/mg) FW		POD (nmol/min/mg protein)		CAT (µmol/min/mg protein)		APX (nmol/min/mg protein)		Total chlorophyll (mg/g) FW	
	D	I	D	I	D	I	D	I	D	I
Control	3.01ª	1.35ª	23.86ª	3.54ª	78.1ª	44.83ª	53.15ª	29.50ª	2.73ª	3.14ª
RAA3	4.15bc	2.12bc	52.82c	5.82bc	103.30bc	74.05bc	88.44c	3.84bc	3.83bc	4.3bc
DPB16	3.95bc	1.97bc	35.04b	19.32bc	97.36bc	68.09bc	78.42bc	5.47bc	3.54bc	4.2bc
S.E.	0.12	0.062	1.54	0.75	1.54	1.64	1.52	1.44	0.09	0.040
C.V.	5.95	6.47	7.42	8.76	2.87	4.76	3.73	6.80	5.33	2.09

PGPR are group of beneficial bacteria that increases the plant growth through direct mechanism (Nitrogen fixation, solubilization of phosphate, ACC deaminase, siderophore and Phytohormone production) and indirect mechanism (Antibiotic, HCN, lytic enzyme, hydrolytic enzyme production and induced systemic resistance). Hypothesis of this study was that PGPR having multi-trait could increase the rice growth and enzymatic activity under water stress and irrigated condition. RAA3 and DPB16 possess ACC deaminase activity [8]. Bacterial strains inoculated plant exhibited significantly higher agronomical parameter than the uninoculated plant. RAA3 showed promising effect on root volume, shoot-root length, shoot-root biomass, chlorophyll content and enzymatic activity under water stress and irrigated condition. Various studies showed that PGPR inoculation enhanced the growth and yield of plant. Plants inoculated with PGPR increased shoot biomass, root biomass, reproductive yield by 45%,43%, 40% under drought stress [14]. Pea plant treated with *Variovorax paradoxus* 5C-2 increased the root biomass by 20 %, shoot biomass by 15% and increased the N, K, P uptake by 80%, 46%, 50% [15]. Inoculation of *Variovorax paradoxus* on *Arabidopsis thaliana* enhanced shoot biomass [16]. Wheat plant treated with *Variovorax paradoxus* increased the nutrient content and grain yield [8]. Pea plant inoculated with *Variovorax paradoxus* 5C-2 enhanced the shoot biomass, nodule number under water stress [17]. Finger millet when inoculated with *Variovorax paradoxus* significantly increased the SOD (17.3%), CAT (33.7%), POD (38.47%), APX (18.2%) and proline content by 41.5% under water stress condition [18]. *Pseudomonas jessenii* when inoculated *to chickpea* enhanced the nodule number, growth and yield [19]. Pea plant treated with *Pseudomonas brassicacearum* and *Pseudomonas marginalis* enhanced the root length, root density and improved the nutrient uptake [20]. Inoculation of basil plant with *Pseudomonas* sp. enhanced the plant length, chlorophyll and CAT activity under water stress [21]. According to the study of [22], treatment of maize plant with *Bacillus cereus* and *Pseudomonas fluorescence* enhanced hormone, protein, chlorophyll and proline content. Treatment of finger millet with *Pseudomonas palleroniana* enhanced the growth, foliar nutrient content and antioxidant enzymes under drought stress

[23]. Wheat plant treated with *Pseudomonas palleroniana* enhanced the fresh shoot biomass by 31.2% and root fresh biomass by 62.1%, shoot length by 20.1% and root length by 33.4% under water stress condition [24]. Eggplant when treated with *Pseudomonas palleroniana* SAW21 increased the plant height by 18.1% and shoot biomass by 20.2% under water stress [25].

CONCLUSIONS

In this study we used two PGPR strains RAA3 and DBP16 and evaluated their effect on growth and enzymatic activity of rice variety sahbhagi in glass house under water stress and irrigated condition. Our result demonstrated that both PGPR strains enhanced growth associated parameters and enzymatic activity of rice significantly under water stress and irrigated condition. Out of two bacterial strains, RAA3 showed promising effect on growth, chlorophyll content and enzymatic activity of rice under both water stress and irrigated condition. RAA3 having ACC deaminase activity reduced the ethylene level and enhanced the plant tolerance under water stress. Our findings suggested that utilization of PGPR having plant growth promoting activity can increase the growth and productivity of plants through alleviation of water stress.

LITERATURE CITED

1. Foley JA, Ramankutty N, Brauman KA, Cassidy ES, Gerber, JS, Johnston M, Zaks DP. 2011. Solutions for a cultivated planet. *Nature* 478(7369): 337-342.
2. Vinocur B, Altman A. 2005. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Curr. Opin. Biotech*. 16(2): 123-132.
3. EEA. 2011. Europe's Environment. *In*: An Assessment of Assessments. European Environment Agency, Copenhagen.
4. Ngumbi E, Kloepper J. 2016. Bacterial-ediated drought tolerance: Current and future prospects. *Applied Soil Ecology* 105: 109-125.
5. Philippot L, Raaijmakers JM, Lemanceau P, Van der Putten WH. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nat. Rev. Microbiol*. 11(11): 789-799.
6. Ullah A, Nisar M, Ali H, Hazrat A, Hayat K, Keerio AA and Khan A. 2019. Drought tolerance improvement in plants. an endophytic bacterial approach. *Appl. Microbiol. Biotechnology* 103(18): 7385-7390.
7. Paul D, Lade H. 2014. Plant-growth-promoting rhizobacteria to improve crop growth in saline soils: a review. *Agron. Sustain. Dev*. 34(4): 737-752.
8. Chandra D, Srivastava R, Gupta VV, Franco CM, Sharma AK. 2019. Evaluation of ACC-deaminase-producing rhizobacteria to alleviate water-stress impacts in wheat (*Triticum aestivum* L.) plants. *Can. Jr. Microbiol*. 65(5): 387-403.
9. Arnon DI. 1949. Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. *Plant Physiology* 24(1): 1-15.
10. Beauchamp C, Fridovich I. 1971. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. Biochem*. 44(1): 276-287.
11. Urbanek H, Kuzniak-Gebarowska E, Herka K. 1991. Elicitation of defense responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta Physiol. Plant. Poland*. 13(1): 43-50.
12. Nakano Y, Asada K. 1981. Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts. *Plant Cell Physiol*. 22(5): 867-880.
13. Beers RF, Sizer IW. 1952. A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *Jr. Biol. Chem*. 195(1): 133-140.
14. Rubin RL, Van Groenigen KJ, Hungate BA. 2017. Plant growth promoting rhizobacteria are more effective under drought: a meta-analysis. *Plant Soil* 416(1/2): 309-323.
15. Jiang F, Chen L, Belimov AA, Shaposhnikov AI, Gong F, Meng X, Dodd IC. 2012. Multiple impacts of the plant growth-promoting rhizobacterium Variovorax paradoxus 5C-2 on nutrient and ABA relations of *Pisum sativum*. *Jr. Exp. Bot*. 63(18): 6421-6430.
16. Chen L, Dodd IC, Theobald JC, Belimov AA, Davies WJ. 2013. The rhizobacterium Variovorax paradoxus 5C-2, containing ACC deaminase, promotes growth and development of *Arabidopsis thaliana* via an ethylene-dependent pathway. *Jr. Exp. Bot.* 64(6): 1565-1573.
17. Teijeiro RG, Belimov AA, Dodd IC. 2020. Microbial inoculum development for ameliorating crop drought stress: A case study of Variovorax paradoxus 5C-2. N. *Biotechnology* 56: 103-113.
18. Chandra D, Srivastava R, Glick BR, Sharma AK. 2020. Rhizobacteria producing ACC deaminase mitigate water-stress response in finger millet (Eleusine coracana (L.) Gaertn.). *3 Biotech* 10(2): 65. https://doi.org/10.1007/s13205-019-2046-4
19. Valverde A, Igual JM, Peix A, Cervantes E, Velazquez E. 2006. *Rhizobium lusitanum* sp. nov. a bacterium that nodulates *Phaseolus vulgaris*. *Int. J. Syst. Evol. Microbiol*. 56(11): 2631-2637.
20. Safronova VI, Stepanok VV, Engqvist GL, Alekseyev YV, Belimov AA. 2006. Root-associated bacteria containing 1-aminocyclopropane-1-carboxylate deaminase improve growth and nutrient uptake by pea genotypes cultivated in cadmium supplemented soil. *Biol. Fert. Soils*. 42(3): 267-272.
21. Gontia-Mishra I, Sapre S, Sharma A, Tiwari S. 2016. Amelioration of drought tolerance in wheat by the interaction of plant growth-promoting rhizobacteria. *Plant Biology* 18(6): 992-1000.
22. Khan N, Bano A. 2016. Role of plant growth promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation. *Int. Jr. Phytoremediation* 18(3): 211-221.
23. Chandra D, Srivastava R, Sharma AK 2018. Influence of IAA and ACC deaminase producing fluorescent pseudomonads in alleviating drought stress in wheat (*Triticum aestivum*). *Agric. Res*. 7(3): 290-299.
24. Chandra D, Srivastava R, Glick BR, Sharma AK. 2018. Drought-tolerant Pseudomonas spp. improve the growth performance of finger millet (*E. coracana* (L.) Gaertn.) under non-stressed and drought-stressed conditions. *Pedosphere* 28(2): 227-240.
25. Fathalla A, Sabry S. 2020. Effect of 1-aminicyclopropane 1-carboxylic acid deaminase producing fluorescent Pseudomonas on the growth of eggplant under drought stress. *Plant Arch*. 20(1): 3389-3394.