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AM Pande
Microbiology Research Laboratory, Rajasthan Aryans College Washim (M.S.), India 444505

Kulkarni NS
Microbiology Research Laboratory, Rajasthan Aryans College Washim (M.S.), India 444505

MG Bodhankar
Bharti Vidyapeeth University, Pune (M.S.) India 411030

Correspondence
Kulkarni NS
Microbiology Research Laboratory, Rajasthan Aryans College Washim (M.S.), India 444505

Effect of PGPR with ACC- Deaminase activity on growth performance of wheat cultivated under stress conditions

AM Pande, Kulkarni NS, MG Bodhankar

Abstract
In India, the Vidarbha region of Maharashtra is occupied with wide range of land under hyper salinity condition and drought. Plant growth and total agro productivity is adversely affected by the environmental stress condition. The crop plant with the tolerance to stress condition is an emergent regional need, to conserve the decrease in crop yield potential. Hence, the research is focused on the effect of PGPR with ACC Deaminase activity on growth performance of wheat cultivated under stress conditions. The wheat *c.v.lok-1* treated separately with six strains of *PGPR* containing ACC Deaminase and cultivated in randomized block design in an artificially generated saline plots with water stress, the plant growth response was evaluated by comparing with un-inoculated control, at Germination, Root-Shoot Growth, & Seedling Vigor up to grand period up to 45 DAS. The results revealed that all the six isolates exhibited a significant increase in Germination; Root-Shoot Length & Vigor Index over uninoculated control. The study indicates the possible use of PGPR containing ACC deaminase to conserve losses, in wheat growth and total agro productivity in environmental stress condition.

Keywords: PGPR, ACC Deaminase, Ethylene, Wheat.

1. Introduction
Ethylene is also known as a stress hormone, because of its involvement in evoking physiological responses in plants exposed to a variety of different stresses including salt stress (Wang *et al*., 1990; Abeles 1993; Morgan & Drew 1997) [22, 2, 15]. It is believed that stress stimulates 1-aminocyclo-propane 1-carboxylic acid (ACC) synthesis, an immediate precursor of ethylene (Wang & Adam, 1982) [21]. Thus salinity can increase rates of ethylene biosynthesis via elevated levels of ACC (El Beltagy *et al*., 1997) [6], which may lead to physiological changes in plant tissues. So any check on this accelerated ethylene production in plants can improve growth under salt stress. Salinity decreased germination percent, root length, callus size, coleoptile length and seedling growth (Lallu and Dixit, 2005; Ganndha *et. al.,* 2005; Bera *et. al.,* 2006 and Agnihotri *et. al.,* 2006) [10, 7, 4, 1]. Ethylene production has been often associated with reduced growth and premature senescence, and may therefore be an indicator of plant susceptibility to stresses such as drought and heat (Morgan & Drew, 1997; Wang *et al*., 1990) [15, 22]. It has been observed that plants that are inoculated with PGPR containing ACC deaminase are dramatically more resistant to the deleterious effects of stress ethylene that is synthesized as a consequence of stressful conditions such as, drought, and high salt contents (Mayak *et al.,*2004(a); Mayak *et al.,*2004(b) [11, 12] In most of these cases, it has been reported that the PGPR containing ACC deaminase significantly lowered the level of ACC in the stressed plants, thereby limiting the amount of stress ethylene synthesis and hence damage to the plant. During stress conditions, ethylene concentrations increase resulting in plant growth inhibition, especially root growth. The inhibition of root growth impairs the ability of plants to capture water and nutrients resulting in reduced yield (Kulkarni & Phalke, 2009) [9]. ACC deaminase are an enzyme that catalyzes ACC into *alpha ketobutyrate* and ammonia (Glick *et al*., 1998) [8]. This enzyme is found in some microorganisms and thus enables them to grow on minimal salts media containing ACC as the sole nitrogen source. Ethylene is synthesized from ACC by using the enzyme ACC oxidase and derived from S–adenosylmethionine by using enzyme ACC synthase (Shaharoona *et al*., 2006) [17]. Therefore, the use of plant growth promoting bacteria containing ACC deaminase prove useful in developing strategies to facilitate plant growth in stress conditions.

2. Materials and Method

A pot experiment was carried out in Microbiology Research Laboratory, Rajasthan Aryans College Washim (M.S.) INDIA. Six isolates (KP5 KP6, KP11, KP14, KP22 andKP31) of plant growth promoting rhizobacteria containing ACC-Deaminase activity were isolated from wheat rhizosphere. Rhizosphere soils were collected from wheat farms. Rhizobacteria were isolated by dilution plate technique using salt minimal DF media (Dworkin and Foster, 1958) [5] containing ACC as a sole nitrogen source (enrichment technique). Further all the cultures were sub cultured on fresh plates. All the Six isolated cultures showing prolific growth were selected for the pot trials and were stored at 4±1 °C and maintained by transferring on fresh medium weekly. Inoculum was prepared in minimal salt media containing ACC (TCI make Japan) as sole nitrogen source. Each isolate was inoculated in 150 ml conical flask containing 60 ml DF salt minimal medium and incubated at 28±1 °C for 48hrs. An optical density of 0.5 recorded at lambda 535 nm was achieved by dilution with sterilized water to maintain uniform cell density of 10^8- 10^9cfu / ml. Experimentation was conducted to determine the effect of PGPR with ACC deaminase activity on growth performance of wheat as test crop cultivated under stress conditions. The certified seeds were purchased from local market and treated separately with six different PGPR isolates having ACC-Deaminase activity.Twenty uniformly inoculum treated seeds were sown in pots having 20 kg soil collected from saline belt at Telhara tehsil of Akola district. The physicochemical characters of saline soil were analysed by adopting methods recommended by (Muhammad Ashraf *et. al* 2011) [16]. Seeds without seed treatment were used and sown as control. Three pots for each isolate were prepared. The germination percent was recorded after a weak, further the plants were thinned to allow ten uniform seedlings in each pot. Pots were arranged in greenhouse under stress conditions without giving any agriculture practise. The plants were observed for the root length, shoot length and Seedling Vigorat 45 DAS. Samples were analysed for growth performance using standard methods comparing with un-inoculated control. The data were subjected to statistical analysis using Computer software SPSS19.0.Version.

3. Result and Discussion

3.1 The physicochemical characteristics of saline soil

The physicochemical characteristics of collected saline soil samples were determined during the present investigation reviled that the pH was (8.5), Electrical conductivity (EC) 5.0 mmhos/$m^{2,}$ Exchangeable Sodium (ESP) 8%and Cation Exchange Capacity (CEP) 12 Cmo/kg. Whereas the Salinity parameters such as extractable cations (Na Ca, K, Mg, and Cl), was recorded to be70 $mgkg^{-1}$, 2963$mgkg^{-1}$, 587$mgkg^{-1}$, 735$mgkg^{-1}$and 88 $mgkg^{-1}$ respectively. The finding was found to be significantly higher in EC, ESP, CEC, and salinity parameter as compared to the properties on soil from non-saline regions.

3.2 Growth parameters

Effect of PGPR treatment having ACC-Deaminase activity on wheat under artificial drought and salt affected soil conditions, has been investigated at pot culture level, the results reviled the improvement in wheat growth parameters significantly. The seed treatment of PGPR having ACC-Deaminase activity significantly improve the germination per cent, root - shoot length and seedling vigor index of wheat over the un-inoculated control. The Maximum germination was recorded by strainKP-31(93.84%) followed by KP-22(92.57%), KP-11(90.12%), KP-14(89.56%), KP-6(88.81%) and the minimum was by KP-5(87.89%) comparatively the lowest germination (77.50%) was recorded in uninoculated control. In case of root elongation, maximum root length was recorded by strain KP-14(9.92 cm) followed by KP-31(7.30cm), KP-11(7.30cm), KP-22(7.20cm), KP-6(6.38cm) and the minimum was by KP-5(5.83cm)comparatively the lowest root length (4.70cm) was recorded in uninoculated control. Whereas the in shoot growth the Maximum shoot length was recorded by strain KP-22(11.62 cm) followed by KP-31(11.54cm), KP-14(10.83cm), KP-11(10.658cm), KP-6(9.50cm) and the minimum was by KP-5(9.25cm) comparatively the lowest shoot length (8.84cm) was recorded in uninoculated control. The Maximum seedling vigor index was recorded was by strain KP-31(1090.2) followed by KP-22(1082.8), KP-14(977.8), KP-11(967.0), KP-6(850.0) and the minimum was by KP-5(818.8) comparatively the lowest seedling vigor index (689.8) was recorded in uninoculated control.

The PGPR having ACC-Deaminase was found to be capable of promoting root, shoot growth, and vigor of wheat seedlings. This growth promotion might to be attributed to the decreased ethylene levels due to inoculation with ACC-Deaminase containing rhizobacteria as, production of ethylene is accelerated during seed germination which may have inhibitory effects on seed germination and root growth in absence of AAC-Deaminase activity (Glick *et al*., 1998) [8]. These finding imply that the inoculation with rhizobacteria containing ACC-deaminase results in increased development of seed germination and longer roots, which subsequently affects shoot growth and it yields positively. This contention is strongly supported by the work carried and reported by several other researchers (Glick *et al*., 1998; Mayak *et al*., 1999; Wang *et al*., 2000; Belimove *et al*., 2002; Shaharoona *et al*., 2003, 2007; Zafarul- Hye *et al*., 2007) [8, 13, 3, 18, 19]. Hence, it may be concluded that the inoculation with PGPR having ACC-Deaminase activity is effective in promoting plant growth under drought and salt stress by lowering the ethylene level and significantly increased root length, shoot length, germination per cent and seed vigor index. These findings may imply that PGPR having ACC Deaminase activity could prove to be effective inoculants for improving growth of wheat yields.

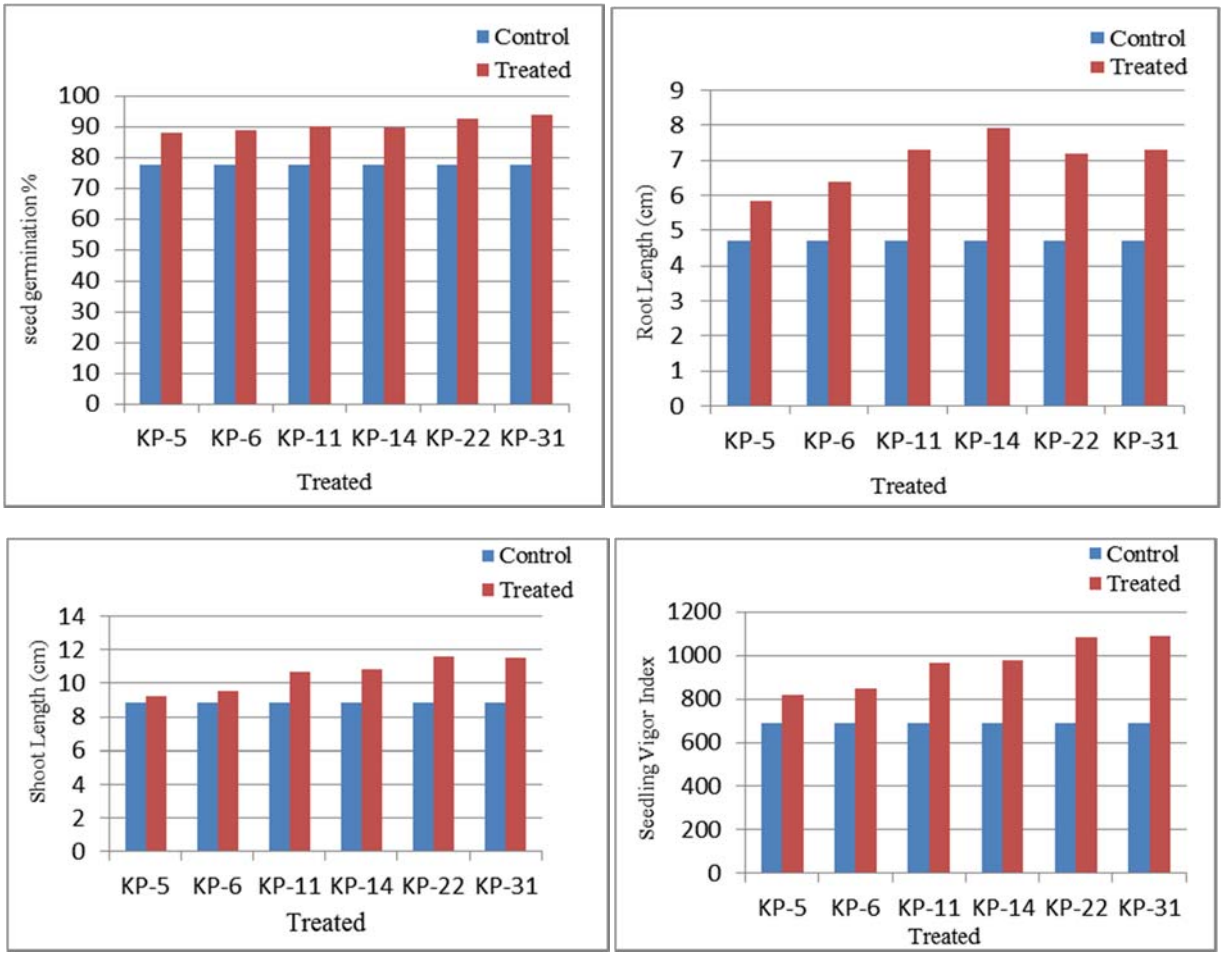
Table 1: Physiochemical characters of soil collected from slain belt of Vidarbha region.

Sr. No	Characters	Findings
1.	P^H	8.5
2.	EC	5.0 mmhos/m^2
3.	ESP	8 %
4.	CEC	12Cmo/kg
5.	Water Extractable Sodium,	70 $mgkg^{-1}$
6.	Water Extractable Calcium,	2963 $mgkg^{-1}$
7.	Water Extractable Potassium,	587 $mgkg^{-1}$
8.	Water Extractable Magnesium,	735 $mgkg^{-1}$
9.	Water Extractable Chloride.	88 $mgkg^{-1}$

EC = Electrical conductivity (dS/cm);
ESP = Exchangeable Sodium Percentage
CEP = cation exchange capacity (Cmo/kg)

Table 2: Effect of PGPR with ACC Deaminase activity on growth parameters of wheat at 45 DAS

S.NO.	Seed Treatment	Mean Growth Parameters Treated			
		Seed Germination (%)	Root length (cm)	Shoot Length (cm)	Seedling Vigor Index
1	KP-5	87.89	5.83	9.25	818.8
2	KP-6	88.81	6.38	9.50	850.0
3	KP-11	90.12	7.30	10.65	967.0
4	KP-14	89.56	7.92	10.83	977.8
5	KP-22	92.57	7.20	11.62	1082.8
6	KP-31	93.84	7.30	11.54	1090.2
7	control	77.50	4.70	8.84	689.8
F-Test		Sig	Sig	Sig	Sig
S.E.(m)		0.9	0.02	0.13	6.9
C.D.@0.5%		4.5	0.12	0.65	34.62



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