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ACC deaminase activity of bacterial species involved in conferring drought tolerance in sorghum (*Sorghum biolor* L.)

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Abstract
One of the important mechanisms used by many rhizobacteria to sustain plant in abiotic stress is the production of the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. In our studies the eight moisture stress tolerant (MST) bacterial isolates were used to study their ACC deaminase activity. The four MST bacterial isolates tested positive for ACC deaminase activity were isolates of L1SC8, L3SC1, L1CcC1 and L2FmA4. They were found to enhance the plant growth promoting activity of sorghum plant under moisture stress condition may be due to the reason that the bacterial enzyme ACC deaminase cleaves the plant produced ACC which is immediate precursor of ethylene in plants thereby lowers the elevated ethylene level in the plant, and thereby allow the plant to be more resistant to a wide variety of abiotic stresses.

Keywords: ACC deaminase, ethylene, drought, stress, sorghum

1. Introduction
Ethylene is a plant hormone functions as plant growth regulator at very low concentration. It is important in normal growth and development of plants. (Khalid *et al*. 2006) [1]. Besides being a plant growth regulator, ethylene also been called as a stress hormone. Normally ethylene is produced endogenously by plants at very low concentration and which is helpful in normal growth of plants. But in response to stress conditions like drought, salinity, flooding, heavy metals, ozone and pathogens, the level of ethylene is increased in plants which affect much developmental aspect of plants. The increase level of ethylene induces defoliation and inhibits root elongation that may lead to reduced crop development (Glick, 2005, Saleem *et al*., 2007; Bhattacharyya and Jha, 2012) [2, 3, 4]. 1-aminocyclopropane-1-carboxylate (ACC) is the precursor of ethylene. Some plant growth promoting rhizobacteria contains the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase which cleaves the ACC and converts it into α-ketobutyrate and ammonia. Thus it helps in lowering the ethylene level in stressed plants and facilitates normal plant growth development in stressed condition, inducing salt tolerance and drought tolerance in plants (Mayak *et al* 2004; Glick 2005) [5, 2]. Thus, plant growth promoting rhizobacteria possessing ACC deaminase when prime on seed coat may acts as a sink for ACC and maintains ethylene level in stressed plants facilitating formation of longer plant roots, which might be helpful in the uptake of water from deep soil (Reid and Renquist, 1997; Glick 2005; Dodd *et al*., 2010) [6, 2, 7]. In our studies four moisture tolerant rhizobacteria isolated from drought stress condition sorghum plant field were assess for the ACC deaminase activity.

2. Material and Methods
2.1 Sampling, isolation and screening
Total 81 Bacterial inoculants were isolated from root samples of sorghum and allied weed plants *viz., Cassia cerassia, Fimbristylis miliacea, Argemone mexicana, Chrozophoro rattleri, Fumaria parviflora* and *Euphorbia esula* surviving in sorghum field under drought condition having 11.79 to 13.38 percent soil moisture at different locations in the semi-arid region of Ahmednagar district where rainfall is less than 500mm. The soil texture was vertisols. Isolation of bacterial inoculants was done on nutrient agar medium by pour plate technique. Out of 81 isolates, ten effective moisture stress tolerant bacterial inoculants (L1SC8, L3SC1, L1CcC1, L2FmA4, L3SC4, L5SC1, L1CcC2 L1CcC3 L2FmA2 and L2FmA6 were selected

on the basis of their performance for plant growth parameter in sorghum *in vitro* condition and tested for their ACC deaminase activity.

2.2 Screening of drought stress tolerant bacterial isolates for ACC deaminase activity

All the ten drought stress tolerant bacterial isolates were inoculated and grown in 5 ml of Typticase soya broth (TSB) incubated at 28°C at 120 rpm for 24 h. After incubation, the cells were harvested by centrifugation at 3000 *g* for 5 min. The harvested pellets washed two times with sterile 0.1 M tris-HCl buffer (pH 7.5). The washed pellets again mixed in 1 ml of 0.1M tris-HCl buffer (pH 7.5) and spot inoculated on modified DF salts minimal medium containing petri plates (Dworkin and Foster, 1958) [8], supplemented with 3mM ACC as a nitrogen source. The petri plates containing DF salts minimal medium without nitrogen source i.e. ACC serve as negative control. All the plates were kept at 28°C for 72 h in incubator. Growth of isolates on ACC supplemented plates was compared to negative controls. The isolates showing growth on ACC containing DF salts minimal medium considered as positive for ACC deaminase activity. (Shaik *et al*. 2013) [9].

3. Results and Discussion

ACC deaminase activity in MST bacterial isolates

The result (Table 1) shows that the 4 moisture stress tolerant (drought tolerant) bacterial isolates out of ten were possessing ACC deaminase activity by showing growth on DF salts minimal media containing ACC as nitrogen source. These MSTB isolates might be enhancing the ACC deaminase activity of plant by lowering ethylene level during moisture stress condition to confer drought resistance in MSTB treatment plants.

The treatment of sorghum plants with these four moisture stress tolerant ACC deaminase positive bacteria isolated from semi-arid region of Ahmednagar district of western Maharashtra increased plant growth parameter in sorghum plant under drought stress condition. During plant growth under moisture stress condition, these MST bacterial isolates might be able to reduce the ethylene concentration of sorghum plants by enhancing the ACC deaminase activity and confer drought resistance in MST bacterial treated plants.

ACC (1-Aminocyclopropane-1-carboxylate) is the precursor of ethylene in plants. Lower level of ethylene is required to break the seed dormancy in many plants. But higher level of ethylene inhibits the root elongation. Many PGPR contain ACC deaminase enzyme which breakdown ACC into ammonia and alpha-ketobutyrate without converting it into ethylene. Thus ACC deaminase containing bacteria when bacterized with seeds may not elevated the ethylene level where root growth stunted and helps in formation of longer roots. By minimising deleterious effect of ethylene on plant, PGPR containing ACC deaminase helps plants to withstand in drought stress (Glick *et al.*, 1998, Glick 2005, Saleem *et al.*, 2007) [10, 2, 3].

ACC deaminase containing *Achromobacter piechaudii* ARV8 when inoculated tomato seedlings showed reduced higher level of ethylene and helps plant to withstand in water stress conditions (Mayak *et al*. 2004) [5]. Zahir *et al.* (2008) [11] found that treatment of peas with rhizobacterial isolates having ACC deaminase activity results in reducing drought inducing effect on plants by lowering ethylene level. PGPR *Bacillus licheniformis* K11 having ACC deaminase reduced the elevated ethylene concentration of pepper plants by hydrolysing ACC when exposed to drought stress and maintained the normal plant growth under drought stress conditions (Lim and Kim, 2013) [12]. Hence MST bacterial isolates contains ACC deaminase activity might be able to reduce accumulation of ACC (precursor of ethylene) in sorghum plants whose higher level have deleterious effects on root and shoot growth.

Table 2 indicate that bacterial inoculant possessing ACC deaminase activity showed more number of functional leaves i.e. green leaves compared to those having negative ACC deaminase activity except L2FmA6 bacterial inoculant. Zahir *et al.*, 2008 [11] reported that rhizobacteria positive in ACC deaminase activity significantly increased the number of leaves of pea compared to those negative in ACC deaminase activity. Also bacterial inoculant L1SC8, L3SC1 and L2FmA4 significantly increased height of sorghum plant (cm). The plant height was in range of 225.43 to 261.11 cm depending upon bacterial inoculant. The bacterial isolate L3SC1, L2FmA4 and L1SC8 were statistically superior over the untreated check for increasing the plant height under drought stress condition. Inoculation increases the plant height in sorghum plants significantly over the untreated control under drought stress condition. The cumulative effect of increase in germination %, number of leaves and plant height exhibited the increased yield of plants inoculated with bacterial strains compared to untreated control. The bacterial inoculant *S. marcescens* strain L1SC8 produce statistically significant yield over untreated control. In untreated control the yield was 22.25 q ha^1 whereas in L1SC8 treated plant the yield was 26.03 q ha^1 and followed by bacterial inoculant L2FmA4, L3SC1 and L1CcC1. The maximum increase in yield by bacterial isolates was upto 17.01 percent. The grain yield obtained from the bacterial inoculated plant was numerically more than the untreated plants.

Bresson *et al.*, (2013) [13] investigated the effects of *Phyllobacterium brassicacearum* STM196 strain A*rabidopsis thaliana* and found increase number of leaves in inoculated plant than uninoculated control to mitigate negative effect of drought stress. Inoculation increases the plant height in sorghum plants significantly over the untreated control under drought stress condition. Figueiredo and others (2008) [14] reported increase in height of *Phaseolus vulgaris* L. plants treated with PGPR than non-inoculated controls under drought. The grain yield obtained from the MST bacterial inoculated plant was numerically more than the untreated plants. Arshad *et al.*, (2008) [15] reported the decreased in grain yield when plants were exposed to drought stress at the flowering and pod formation stage, but inoculation resulted in better grain yield (up to 62% and 40% higher, respectively) than the respective uninoculated as well as nonstressed control. Shakir *et al.*, (2012) [16] found that PGPR containing ACC deaminase activity helps plants for a better crop stand that enhanced moisture and nutrient feeding volume resulting in improved yield of wheat crop from 4-14% in different trials.

Table 1: ACC deaminase activity in MST bacterial isolates

S. no.	Bacterial inoculant	ACC deaminase activity
1	L1SC8	+
2	L3SC1	+
3	L1CcC1	+
4	L2FmA4	+
5	L3SC4	-
6	L5SC1	-
7	L1CcC2	-
8	L1CcC3	-
9	L2FmA2	-
10	L2FmA6	-

Table 2: Effect of ACC deaminase containing MST bacterial isolates on plant growth parameter of sorghum

MST bacterial inoculant		Plant growth parameter under drought stress condition			
		No. of functional leaves	No. of non- functional leaves	Height of plant (cm)	Yield kg ha^{-1}
ACC deaminase positive	L1SC8	5.76	4.97	249.34	2550.30
	L3SC1	4.47	6.13	261.11	2526.34
	L1CcC1	4.56	4.12	238.11	2449.31
	L2FmA4	5.43	4.84	251.99	2392.78
ACC deaminase negative	L3SC4	4.23	6.37	245.59	2269.09
	L5SC1	3.89	6.71	225.43	2271.88
	L1CcC2	4.34	5.66	247.20	2248.52
	L1CcC3	4.56	5.37	250.89	1998.88
	L2FmA2	4.67	5.53	246.77	2088.85
	L2FmA6	5.64	4.16	236.40	2211.00
	SE(+/-)	0.771	0.0822	3.88	35.469
	CD@5%	0.229	0.2441	11.53	105.38

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