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Plant Growth Parameter in *Sorghum bicolor* as Influenced by Moisture Stress Tolerant Rhizobacteria during Mitigation of Drought

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A B S T R A C T

In recent years due to drought yield of crop is adversely affected. In this context to increase the crop yield by utilising drought prone region, influences of moisture stress tolerant rhizobacteria on growth parameter of sorghum plant were examined under moisture stress conditions. Four autochthonous moisture stress tolerant bacterial strain isolated from semiarid region identified as *Serratia marcescens* strain L1SC8, *Pseudomonas putida* strain L3SC1, *Enterobacter cloacae* strain L1CcC1 and *Serratia marcescens* strain L2FmA4. *Sorghum bicolor* crops inoculated these bacterial isolates were subjected to moisture stress conditions. These isolates showed enhanced 1-aminocyclopropane-1-carboxylic acid deaminase and IAA production. The enhanced ACC deaminase activity can helps plants to lower the deleterious effect of excess ethylene. Seed priming of these bacterial isolates enhanced germination%, functional leaves, height and yield of *Sorghum bicolor* significantly over control under drought conditions. Hence it can be conclude that these bacterial cultures can be potentially use as PGPR as well as drought stress mitigating cultures to mitigate deleterious effect of drought stress in *Sorghum bicolor* crops in arid and semi-arid areas.

Introduction

Crop plants have limitations to protect themselves against abrupt climate change occurring in nature including droughts as these crop plants are not adapted to such abrupt climate change. At a given space and time, therefore plants develop a wide range of strategies to cope with stress situations. Under conditions of water deficiency, drought escape and drought tolerance are two important strategies to ensure plant growth. There is limited reported information on the role of microbes on the sustenance of drought tolerance. Currently microbial communities associated with plant have been used for enhancing crop productivity and providing stress resistance (Mayak *et al.*, 2004; Glick *et al.*, 2007; Marulanda *et al.*, 2009; Yang *et al.*, 2009). Plant growth promoting rhizobacteria associated with rhizosphere help plants tolerate stress by various metabolic ways

include their ability to confer drought tolerance to many cereals and vegetables plants (Timmusk and Wagner, 1999; Mayak *et al.,* 2004; Sandhya *et al.,* 2009; Kasim *et al.,* 2013) and their ability to confer more than one type of biotic and/or abiotic stress tolerance (Timmusk *et al.,* 1999; Mayak *et al.,* 2004; Coleman-Derr and Tringe, 2014). Inoculation of plants with beneficial micro-organisms promotes plant growth and increases drought tolerance in arid or semiarid areas (Marulanda and others 2007). 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). 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). In addition to this some PGPR synthesize phytohormones that help plant to sustain against abiotic stress (Glick and Pasternak, 2003). Indole acetic acid (IAA) is most active auxin which stimulates plant growth and development is IAA. PGPR producing IAA when primed with plant resulted in enhanced root growth and formation root hairs (Dimpka *et al.,* 2009) ultimately increases water and nutrient uptake in plants (Mantelin and Touraine, 2004), helping plants to confer water stress condition (Egamberdieva and Kucharova, 2009). Drought or a distressed situation caused by lack of rainfall is a deadly natural environmental hazard. It is directly related to one of the basic requirements of any form of life (i.e. water, air and food) that is water and is indirectly related to food because crops and other plants and animals exclusively depend on water. The prominent *rabi*sorghum growing districts are Solapur, Pune and (Nagaraj *et al.,* 2013). The major limitations to sorghum survival and productivity are the occurrence of various abiotic stresses (drought and temperature etc.) at different crop growth stages. Early and mid-season droughts are common in *kharif*, while terminal drought occur during *rabi* season. Drought adversely affects some of the important physiological, biophysical and biochemical processes of the plant. The application of associated microbes to crop plants under drought conditions provides new insights into novel protocols to improve plant defense response to drought, which can be an important component of agricultural production systems affected by a changing climate. Therefore present investigation has been made to examine influence of moisture stress tolerant rhizobacteria on growth parameter of sorghum crop under moisture stress conditions.

Materials and Methods

Sampling, isolation and screening

Total 81 bacterial cultures were isolated from root samples of sorghum and allied weed plants *viz., Cassia cerassia, Fimbristylis miliacea, Argemone mexicana, Chrozophororattleri, 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 cultures was done on nutrient agar medium by pour plate technique. Out of 81 isolates, four effective bacterial

isolates (L1SC8, L3SC1, L1CcC1 and L2FmA4) were selected on the basis of their performance on plant growth parameter of sorghum in *in vitro* condition.

Biochemical characterization

Biochemical tests *viz*., starch hydrolysis, H_2S production, gelatinase test, citrate utilization, catalyse activity, oxidase activity, nitrate reduction, Urease Test and Gram's reaction were carried out as per standard procedures given by Aneja (2003) as well as Cappuccino and Sherman (1987) for biochemical confirmation of isolates.

Bacterial growth and seed treatment

Seed of sorghum were surface sterilized with 70% ethanol and then washed thrice with sterilized distilled water. A suspension of 24h young bacterial culture was prepared in sterile water. The optical density of bacterial culture was adjusted to 0.1 OD (to have 10^7cfu/ml) at 620nm.

A jaggery suspension was prepared (by boiling 5g of jaggery in 100 ml of water). 5ml of bacterial suspension was added to 20 ml of jaggery suspension to prepare the bacterial inoculant. The sorghum seed were treated with this bacterial inoculant and dried in the shade for 30 min before sowing.

Field experiment

The efficacy of moisture stress tolerant bacterial inoculant was performed on the *var. Phulevasudha.* Seeds were treated as described earlier and sown in plot size 2.7m x 1.65m with spacing 45cm x 15 cm at *vapasa* condition. Experiments were conducted in split plot arrangement in the form of randomized block design (RBD) with four replications

Monitoring soil moisture

At the time of each observations moisture content of soil was determined. Soil sample (100g) was taken at a uniform depth of 15cm from the surface of soil. Fresh weight (FW) of the samples was recorded and dry weight (DW) was determined after drying the soil in oven for 24h at 110°C till constant weight. Soil moisture was calculated by the formula

Soil moisture (%) = (FW – DW) / DW X 100

Monitoring plant growth parameter

The biometric observation *viz.*, germination %, numbers of functional leaves and stem height were recorded at 30 days interval.

Yield parameter

Yield per hectare was estimated on the basis of net plot yield multiplied by the number of plots present in hectare area and then expressed as yield q/ha.

Screening of drought stress tolerant bacterial isolates for ACC deaminase activity

All the four drought stress tolerant bacterial isolates were inoculated and grown in 5 ml of Trypticase 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), 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 (Ali *et al.,* 2013).

Screening of bacterial isolates for Indole-3-acetic acid (IAA)

Luria-Bertani broth amended with tryptophan (5mM) was inoculated with overnight raised bacterial cultures (0.5 OD at 600 nm) and incubated at 28°C for 72 h. Two ml of bacterial culture was centrifuged at 10000g for 20 min and supernatant was separated. The supernatant used for IAA estimation (Gordon and Weber, 1951). The amount of IAA produced by bacterial cultures was estimated by using standard curve for IAA.

Statistical analysis

The statistical analysis of the data was carried out for randomised block design (RBD) for field experimentation. Means and standard errors of the means were calculated. Results were evaluated by analysis of variance (ANOVA). The differences between the means of inoculated and control treatments were tested using the least significant differences test ($p < 0.05$) (Panse and Sukhatme, 1985).

Results and Discussion

On the basis of morphological and biochemical characterisation, these moisture stress tolerant bacteria were identified as *Serratia marcescens* strain L1SC8, *Pseudomonas putida* strain L3SC1, *Enterobacter cloacae* strain L1CcC1 and *Serratia marcescens* strain L2FmA4 (Table 1).

The soil moisture % of field was 48.90% at the time of sowing. Seed priming of each of these bacterial strains increased germination percentage compared to untreated control (Table 2). The increase in germination % was in the range of 16.77 (*Enterobacter cloacae* strain L1CcC1) to 22.98% (*Serratia marcescens* strain L1SC8).Similar observation i.e. increase in seed germination were also reported by Saravan kumar and others (2011). He reported there was an increase in germination of green gram seeds treated with different bacterial strains (*Pseudomonas fluorescens* Pf1, *Bacillus subtilis* strains EPB5, EPB 22, EPB 31) over untreated seeds under drought stress condition. Cowpea seeds bacterized with *Bacillus* sp. RM-2 showed significant increase in % germination in comparison to uninoculated control (Minaxi *et al.,* 2012). Sarma and Saikia (2014) found 90% germination rate when mung bean seeds were treated with *Pseudomonas aeruginosa* GGRJ21 while in control this was only 75%. The greater increase of root as well as shoot length was recorded in treated plants as compared to the control plants. Timmusk *et al.,* (2014) found an increase in seedling germination due to bacterial priming of wheat under drought stress condition.

Generally the sorghum crop shows water stress symptoms or drought symptoms at the soil moisture level of less than 30%. The symptoms of drought stress shows yellowing of the green functional leaves. Thus under drought stress condition the number of the green functional leaves decreases thereby decreasing the rate of photosynthesis and activities of plant. The numbers of functional leaves were significantly more in plants with MST bacterial inoculant treated seeds as compared to untreated seeds. The bacterial inoculation increased number of functional green leaves compared to the untreated controls (Table 3). *P. putida* showed more number of functional green leaves compared to others. The results of seed inoculation on

plant leaves were concordant with those reported by several authors. The rhizobacterial isolates containing ACC deaminase activity significantly increased the number of leaves of pea compared to uninoculated controls at different moisture levels (Zahir *et al.*, 2008). Bresson *et al.*, (2013) 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.

The positive effect of seed priming of bacterial strains was also observed in plant height (Table 4). The increase in plant height was in range of 26.71 to 47.89 cm depending upon bacterial inoculant and days of plant growth. The bacterial isolate *P. putida* strain L3SC1, *E. cloacae* strain L2FmA4 and *S. marcescens* strain 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. Similar observations reported by Figueiredo *et al.*, (2008), he reported increase in height of *Phaseolus vulgaris* L. plants treated with PGPR than non-inoculated controls under drought. Saravan kumar *et al.*, (2011) reported the significant improvement in plant growth characters of green gram over untreated seeds. Among the different bacterial strains used, *Pseudomonas fluorescens* Pf1 was found to increase the vigour index of the green gram seedlings. The increase shoot length (19.0 cm) was greater in *P. fluorescens* Pf1 treated seedlings compared to untreated control. Kang *et al.*, (2014) found that the treatment of culture filtrates of *Pseudomonas putida* H-2-3 to soybean seed had a significant increase in length of shoot (9.6%) over the control

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 (Table 5). In untreated control the yield was 22.25 q ha^1 whereas in *S. marcescens* strain L1SC8 treated plant the yield was 26.03 q ha^1 and followed by bacterial inoculant *S. marcescens* strain L2FmA4, *P. putida* strain L3SC1 and *E. cloacae* strain 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. Arshad*et al.*, (2008) also reported better grain yield in *Pisum sativum* inoculated with *Pseudomonas* spp. containing ACC-Deaminase i.e. up to 62% and 40% higher than the respective uninoculated as well as nonstressed control. Shakir *et al.*, (2012) 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

Seed priming with PGPR showed enhanced tolerance to drought stress

Treatment of plant seeds with ACC deaminase-containing bacteria has been reported to facilitate plant growth by reducing ACC and ethylene levels about 2-4 fold that is synthesized as a consequence of stressful conditions such as drought and (Glick *et al.*, 1999 and Mayak *et al.*, 2004). Therefore, all the moisture stress tolerant bacterial isolates were screened for ACC utilization by spotting on ACC (3mM) supplemented DF salts minimal medium plate. The result (Table 6) shows that the 4 moisture stress tolerant (drought tolerant) bacterial isolates were possessing ACC deaminase activity by

showing growth on DF salts minimal media. The increase in yield and sustain in drought is due to PGPR activities of moisture stress tolerant bacteria. MST bacterial isolates have ACC deaminase activities which lowers the deleterious ethylene level in plants.

Table.1 Morphological and biochemical characters of moisture stress tolerant bacteria

Sr. No.	Test	L1SC8	L3SC1	L1CcC1	L2FmA4
1	Colony morphology	Pinkish red	Cream	Mucoid pink	Red
2	Gram reaction	-ve	-ve	-ve	-ve
3	Shape	Short rod	Rod	Rod	Short rod
4	Catalase	-	+	-	-
5	Oxidase	-	+	-	-
6	Oxidative/fermentative	F	O	F	F
7	Casein hydrolysis	+	+	-	+
8	Starch hydrolysis	-	-	-	-
9	Litmus agar		Alkaline		
10	Lysine	+	+	-	+
11	Ornithine	+	+	+	-
12	Urease	-	-	-	-
13	Nitrate	+	-	+	+
14	H_2S	-	-	-	-
15	Citrate utilisation	+	+	+	+
16	VogesProskauer	+	-	+	+
17	Methyl red	+	-	-	-
18	Indole	-	-	-	-
19	Malonate	-	+	+	-
20	Esculine hydrolysis	+	+	+	+
21	Arabinose	-	-	+	-
22	Xylose	+	-	+	+
23	Adonitol	-	-	+	-
24	Rhamnose	-	-	-	-
25	Cellobiose	-	-	-	+
26	Melibiose	+	-	+	+
27	Saccharose	+	-	+	-
28	Raffinose	-	-	-/+	-
29	Trehalose	+	-	-/+	+
30	Glucose	+	-	+	+
31	Lactose	-	-	+	-
Probable genus species		***Serratia marcescens***	***Pseudomonas putida***	***Enterobacter cloacae***	***Serratia marcescens***

Table.2 Effect of MST bacterial inoculant on germination % of *rabi* sorghum *var. Phulevasudha* at moisture level 48.90%

Bacterial Inoculants	Germination%	% Increase over control
***Serratiamarcescens* L1SC8**	99.00a	22.98
***Pseudomonasputida*L3SC1**	98.50a	22.36
***Enterobactercloacae* L1CcC1**	94.00b	16.77
***Serratiamarcescens* L2FmA4**	98.00a	21.74
Untreated	80.50c	
SE (±)	**0.893**	
CD at 5%	**2.691**	

The means followed by the similar letter in column for each treatments are not different significantly ($p<0.05$). Data are average of four replicates

Table.3 Effect of MST bacterial inoculant on number of functional leaves in *rabi* sorghum *var. Phule vasudha*

Bacterial Inoculants	Number of functional plant leaves			
	30 DAS	60 DAS	90 DAS	120 DAS
	at soil moisture level			
	40.56%	32.16%	20.89%	8.97%
***Serratiamarcescens* L1SC8**	7.24ab	12.09ab	7.04b	3.25a
***Pseudomonasputida*L3SC1**	7.30a	12.28a	7.36a	3.34a
***Enterobactercloacae* L1CcC1**	7.01b	11.26c	6.21c	2.67b
***Serratiamarcescens* L2FmA4**	7.19ab	11.83b	6.45c	3.04a
Untreated control	6.97b	11.03c	5.46d	2.10c
SE (±)	**7.14**	**11.70**	**6.50**	**2.88**
CD at 5%	**0.08**	**0.092**	**0.098**	**0.101**

The means followed by the similar letter in column for each treatments are not different significantly ($p<0.05$). Data are average of four replicates.DAS : Days after sowing

Table4 Effect of MST bacterial inoculant on height of plant of *rabi* sorghum *var. Phule vasudha*

Bacterial Inoculants	Height of plant (cm)			
	30 DAS	60 DAS	90 DAS	120 DAS
	at soil moisture level			
	40.56	32.16	20.89	8.97
***Serratiamarcescens* L1SC8**	64.46a	187.32a	237.74b	250.59b
***Pseudomonasputida*L3SC1**	64.42a	187.11a	247.90a	262.11a
***Enterobactercloacae* L1CcC1**	62.69a	184.72a	222.88c	240.93c
***Serratiamarcescens* L2FmA4**	61.70a	176.27b	234.94b	254.37b
Untreated control	60.79a	177.68b	212.19d	214.22d
SE (±)	**1.32**	**1.53**	**1.79**	**1.49**
CD at 5%	**3.9798**	**4.60**	**5.41**	**4.49**

The means followed by the similar letter in column for each treatments are not different significantly ($p<0.05$). Data are average of four replicates

Table.5 Effect of MST bacterial inoculants on grain yield of *rabi* sorghum (*var. Phule vasudha*)

Bacterial inoculant	Yield (q ha^{-1})	% Increase in yield over control
***Serratiamarcescens* L1SC8**	26.03a	17.01
***Pseudomonasputida*L3SC1**	25.33ab	13.86
***Enterobactercloacae* L1CcC1**	24.34b	9.40
***Serratiamarcescens* L2FmA4**	25.91a	16.45
Untreated control	22.25c	
SE (±)	**37.74**	
CD at 5%	**113.75**	

The means followed by the similar letter in column for each treatments are not different significantly ($p<0.05$). Data are average of four replicates

Table.6 Plant growth promotion activities of bacterial isolates isolated from sorghum field

Bacterial inoculant	ACC deaminase activity	IAA production (µg ml^{-1})
***Serratia marcescens* L1SC8**	+	161.80
***Pseudomonas putida* L3SC1**	+	150.00
***Enterobactercloacae* L1CcC1**	+	143.00
***Serratia marcescens* L2FmA4**	+	197.60

The result (Table 6) indicates that the moisture stress tolerant (drought tolerant) bacterial isolates were producing Indole 3-Acetic acid in the range of 143.00 (*E. cloacae* strain L1CcC1) to 197.60 µg ml^{-1} (*S. marcescens* strain L2FmA4). Highest IAA produced by *S. marcescens* L2FmA4 (197.60 µg/ml) followed by another strains of *S. marcescens* L1SC8 (161.80 µg/ml). On the other hand *E. cloacae* L1CcC1 produced lower amount of IAA compared to others. Similarly Marulanda *et al.*, (2009) found that *Pseudomonas putida*, *Pseudomonas* sp., and *Bacillus megaterium* were able to promote shoot and root biomass and improved plant drought tolerance was associated with a higher production of indole-3-acetic acid (IAA) by bacterial strains, which stimulated plant root growth and thus the ability to take up water.

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