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MICROBIAL DIVERSITY OF MOISTURE STRESS TOLERANT RHIZOBACTERIA ASSOCIATED WITH SORGHUM AND ALLIED WEEDS DURING SORGHUM CROP PRODUCTION UNDER DROUGHT CONDITION

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ABSTRACT
In the present investigation the microbial diversity particularly bacteria in the moisture stress (drought) soil environment depends on the types of host plant root on which these bacterial type colonize have been studied. Total 81 bacterial colonies isolated from sorghum and allied weed plants *viz, Cassia cerassia, Fimbristylis miliacea, Argemone mexicana, Chrozophoro rottleri, Fumaria parviflora* and *Euphorbia esula* using NA media. Out of which 42 were obtained as root endophytic isolates whereas 39 isolates were obtained as root ectophytic isolates. All these endophytic and ectophytic bacterial culture were able to grow in *in vitro* condition at 13% soil moisture level indicating that they sustain the severe drought condition in soil. From this work it can be concluded that these moisture stress tolerant bacteria would be present and sustain under drought stress condition.

INTRODUCTION

A little is known about the soil microbial diversity and the potential contribution of this microbial diversity to global biogeochemical cycling. Water deficit is the most common stress affecting plant growth in arid and semiarid regions. In such areas the native soil microflora plays any role in sustenance of the plant to drought/water stress condition is little known. Plants growing under extreme moisture stress conditions in arid and semiarid region experienced water stress and nutrient deficiencies but adopted these detrimental conditions by several mechanisms. Microbes associated with this plant experiencing drought also adopted these adverse conditions. (Ruý´z-Lozano *et al.,* 1996; Marulanda *et al.,* 2008). Soil microorganisms including beneficial microbes found to be associated in extreme environmental conditions. *Achromobacter piechaudii* ARV8 has been isolated from a rhizosphere soil sample from a *Lycium shawii* plant growing from the region having the annual rainfall below 50mm. (Mayak *et al.*, 2004). Bacteria belonging to the genera *Brevibacillus, Paenibacillus*, and *Bacillus* have been found to survive in extreme thermophilic environment (Verma *et al.*, 2014). Total twenty six bacteria isolated and identified as *Pseudomonas* and *Bacillus* species from rhizospheric soil samples of maize growing under semi-arid region (Harran Plain) of Turkey (Cevheri, 2012). Sustainable systems require the understanding of interactions between plants and microorganisms, especially those having a direct abiotic stress tolerance and the adaptation and survival of these microorganisms in extreme environments. Whether the microbial diversity depends on moisture stress condition and the plant type surviving under these condition is not yet known. Ahmednagar district situated in Western region of Maharashtra rainfall have annual rainfall below 500 mm and categorised under semiarid region. Sorghum is grown in this area in post-rainy season for food and fodder purpose. Therefore present investigation has been made to identify and assess the microbial diversity of moisture stress tolerant rhizobacteria associated with sorghum and allied weeds in drought soil ecosystem. The future aspect of our work is to screen the ability of these rhizobacteria helps to sustain and increase the yield of sorghum under the drought condition.

MATERIALS AND METHODS

Sample site and collection

Root samples of sorghum and allied weed plants *viz, Cassia cerassia, Fimbristylis miliacea, Argemone mexicana, Chrozophoro rottleri, Fumaria parviflora* and *Euphorbia esula* growing in sorghum field under moisture stress conditions during *rabi* seasons were collected from five different locations of semi-arid region (rainfall was less than 500mm) of Ahmednagar. The *rabi* sorghum crop at the time of root sample collection was either in milk stage/dough stage/grain filling stage whereas the allied plants were either in flowering stage/

full growth stage depending on the location under drought condition in the month of January. A total of fourteen root samples were obtained from the plants.

Soil sampling and moisture analysis

Soil samples were collected at the time of collection of root samples to determine the moisture stress status of the soil from the same fields having vertisols soil texture. The soil samples were drawn from the root zone of the sorghum and allied weed plants with the help of poger (from a soil depth of 15-20cm), collected in plastic bag and brought to laboratory. The soil samples were weight immediately and after drying in an oven at 120°C temperature for 24 h so as to estimate the soil moisture during soil moisture stress condition. Soil moisture was calculated by following formula

$$\text{Soil moisture \%} = \frac{(\text{Initial wt. of soil} - \text{Final wt. of soil})}{\text{Final wt. of soil}}$$

Isolation of ectophytic and endophytic moisture stress tolerant bacteria

Isolation of ectophytic and endophytic bacterial flora from root samples of sorghum and allied weed plants were done on nutrient agar medium. For isolation of ectophytic bacterial flora 10gm root from each sample was suspended in 100ml sterilized water and stirred by magnetic stirrer. For isolation of endophytic bacteria, the roots were washed thoroughly with tap water followed by distilled water to remove the ectophytic bacterial flora and then crushed in mortar pestle with 10ml sterile water. The crushed material was allowed to settle for 10min and one ml clear supernatant aliquot from each was plated on sterilised nutrient agar (NA) medium. Plates were incubated at 28 ± 2°C for 48h. All the plates were observed for the appearance of different bacterial colonies. Bacterial colonies with different growth characteristics (shape, size, colour and growth) obtained in this isolation were selected and were purified by further streaking on freshly prepared NA plates. The pure cultures of these bacterial isolates (81) were designated and used for further experimentation.

Survival of bacterial isolates at different soil moisture level

Survival of bacterial isolates at different soil moisture level was studied as per the method used by Sharma and Singh (2014) with some modifications. The pots were also disinfected by methylated alcohol. These pots were filled with known quantity of sterile soil and then saturated with sterile distilled water. 5ml of bacterial suspension of each isolates was added in previously labelled respective pots after 24 h. A pot without bacterial inoculant was maintained as control. These pots were kept at ambient temperature under laboratory condition. The moisture percentages in these pots were estimated by weighing the soil filled pots at regular interval. When the moisture percentage reached at desirable level, the presence of bacteria in pot soil was checked. For this purpose 1g of soil samples from respective pot at particular soil moisture level was used for estimation for bacterial presence by serial dilution method and streaking on nutrient agar plates. Presence of bacteria was checked at 50%, 30%, 25%, 20%, 15% and 13% soil moisture level.

RESULTS AND DISCUSSION

Assessment of drought stress in sorghum field

The soil moisture content of soil samples in rabi sorghum field of sorghum crop at milk stage/ dough stage/grain filling stage from five drought prone fields at different locations in semi-arid region of Ahmednagar district, where drought condition prevailed, was estimated by following standard method. Results revealed that the soil moisture content was variable from 11.79 percent to 13.46 percent with an average soil moisture content of 13.04% which indicate the severe drought condition during

Table 1: Isolation of bacteria from root samples of sorghum and weeds in sorghum field surviving in drought stress condition in Ahmednagar district

Field Location of sorghum crop	Field moisture status	Root sample of	No. of bacterial types associated with plant root	No. of isolates of ectophytic habitat	No. of isolates of endophytic habitat
Location 1	13.38	Sorghum at dough stage	8	4	4
		Sorghum at milk stage	6	3	3
		*Cassia cerassia (*Weed*)*	4	3	1
			18	**10**	**8**
Location 2	11.79	Sorghum at Dough stage	3	1	2
		*Fimbristylis miliacea (*Weed)	6	1	5
		*Argemone mexicana (*Weed)	4	2	2
		*Chrozophora rottleri (*Weed)	4	3	1
		*Fumaria parvifolra (*Weed)	8	4	4
		Withania somnifera (Weed)	5	3	2
		Euphorbia esula (Weed)	5	3	2
			35	**17**	**18**
Location 3	13.46	Sorghum at grain filling stage	8	4	4
		Fimbristylis miliacea(Weed)	8	2	6
			16	**6**	**10**
Location 4	13.37	Sorghum at dough Stage	5	3	2
			5	3	2
Location 5	13.20	Sorghum at grain filling stage	5	3	4
			5	3	4
Total no. of isolates			**81**	**39**	**42**

Table 2: Diversity for cultural and morphological characteristics of ectophytic bacteria present on root samples of sorghum and allied weeds in moisture stress soil

Bacterial isolate no.	Locations	Host details and growth stage	Appearance	Bacterial colony Form	Growth	Elevation	Gram reaction	Bacterial Cell Shape
1	Location 1	Sorghum	Dough stage	Red	Circular	Abundant	Umbonate	-ve Rod
2				Offwhite	Irregular	Moderate	Flat	-ve Rod
3				Dull Yellow	Circular	Moderate	Raised	-ve Short rod
4				Yellow	Circular	Slight	Raised	-ve Short rod
5			Milk stage	White	Irregular	Abundant	Flat	+ve Rod
6				Offwhite	Circular	Moderate	Flat	-ve Cocci
7				Yellow	Circular	Slight	Raised	-ve Rod
8		*Cassia cerassia*	Flowering stage	Mucoid pink	Circular	Abundant	Convex	-ve Rod
9				White	Circular	Abundant	Raised	-ve Rod
10				Dull yellow	Irregular	Moderate	Flat	-ve Rod
11	Location 2	Sorghum	Dough stage	Dull yellow	Irregular	Abundant	Flat	-ve Rod
12		*Fimbristylis miliacea*	Flowering stage	Dull yellow	Circular	Abundant	Flat	-ve Short rod
13		*Argemone mexicana*	Flowering stage	White	Circular	Abundant	Raised	-ve Rod
14				Dull yellow	Irregular	Moderate	Flat	-ve Rod
15		*Chrozophora rottleri*	Flowering stage	Offwhite	Irregular	Abundant	Flat	-ve Rod
16				Light brown	Circular	Slight	Raised	-ve Rod
17				Offwhite	Circular	Slight	Raised	-ve Rod
18		*Fumaria parviflora*	Flowering stage	White	Circular	Abundant	Flat	-ve Rod
19				Brown	Circular	Slight	Flat	-ve Cocci
20				Pale yellow	Irregular	Slight	Raised	-ve Rod
21				Light pink	Circular	Slight	Raised	-ve Rod
22		*Withania somnifera*	Flowering stage	Mucoid yellow	Circular	Abundant	Raised	-ve Rod
23				Dull yellow	Irregular	Abundant	Flat	-ve Rod
24				White	Circular	Moderate	Convex	-ve Rod
25		*Euphorbia esula*	Flowering stage	Offwhite	Rhizoid	Abundant	Flat	+ve Rod
26				Dull yellow	Irregular	Moderate	Raised	-ve Rod
27				Yellow	Circular	Slight	Raised	-ve Rod
28	Location 3	Sorghum	Grain filling stage	Pale yellow	Circular	Abundant	Convex	-ve Rod
29				Dull yellow	Irregular	Abundant	Flat	-ve Rod
30				Yellow	Circular	Slight	Raised	-ve Short rod
31				Offwhite	Circular	Moderate	Raised	-ve Short rod
32		*Fimbristylis miliacea*	Flowering stage	White	Irregular	Abundant	Flat	-ve Rod
33				Dull yellow	Irregular	Moderate	Flat	-ve Rod
34	Location 4	Sorghum	Dough stage	White	Circular	Abundant	Convex	-ve Rod
35				Offwhite	Circular	Moderate	Raised	-ve Rod
36				Yellow	Circular	Moderate	Convex	-ve Rod
37	Location 5	Sorghum	Grain filling stage	Offwhite	Circular	Abundant	Raised	-ve Rod
38				White	Irregular	Moderate	Flat	-ve Rod
39				Dull yellow	Circular	Slight	Raised	-ve Rod

the milk stage/dough stage/grain filling stage of sorghum crop.

Association and diversity of bacteria

The presence of ectophytic and endophytic bacterial types on/in root systems of sorghum and allied weeds *viz., Cassica cerassia, Fimbristylis miliacea, Aregemone mexicana, Chrozophora rottleri, Fumaria parviflora, Withania somnifera* and *Euphorbia esula* surviving under severe drought condition having 11.79 to 13.46 percent soil moisture were detected during isolation. In all 81 bacterial isolate of different colony types were obtained during the isolation from sorghum roots and weed roots. The number of bacterial types associated with sorghum root and weed roots were variable. The results (Table 1) indicate that the diversity and association of bacterial types on sorghum root and on weeds root varied with the location. Maximum numbers of bacterial types were found on the roots of weed *Fumaria parviflora* followed by other weeds. Maximum eight types of bacterial colonies were obtained from the roots of sorghum and *Fumaria parviflora* and *Fimbristylis miliacea*. Maximum bacterial isolates i.e. 42 were obtained as root endophytic isolates whereas 39 isolates were obtained as root ectophytic isolates. On the individual root habitat these bacterial isolates varied in their colony characters (colony colour, colony form, type of growth, elevation. Gram reaction and cell shape). The number of endophytic or ectophytic bacterial isolate types varied with plant type. Maximum number of ectophytic and endophytic bacterial types were obtained from location 2 where soil moisture content was less than other locations.

The results (Table 2) showed that the 39 ectophytic bacterial isolates obtained from the roots of sorghum and weeds differ in their cultural and morphological characters. At location 1, the roots of sorghum crop (at dough stage) harbour four ectophytic bacterial isolates which produce red, offwhite, dull yellow and yellow colonies and were differed from each other. Similarly the sorghum plant in the same location at milk stage harbours the white, offwhite and yellow bacterial colonies. At location 2, the sorghum crop (in dough stage) harboured only one ectophytic bacteria which produce dull yellow colonies. At location 4 the sorghum crop (in dough stage) harbour three types of bacteria which produced white, offwhite and yellow colonies. At location 3 the sorghum crop (at mature stage) harbour four types of bacteria which produced white, dull yellow, yellow, cream and offwhite colonies. At location 5, the sorghum crop harbour three ectophytic bacterial colonies

Table 3: Cultural and morphological characteristics of isolated endophytic bacteria from root samples of sorghum and allied weeds in moisture stress plant root environment

Bacterial isolate no.	Locations	Host details and growth stage		Bacterial colony		Gram reaction		Bacterial Cell Shape
			Appearance	Form	Growth	Elevation		
1	Location 1	Sorghum	Dough stage	Red	Circular	Abundant	Convex	-ve Rod
2				Yellow	Circular	Slight	Raised	-ve Rod
3				Offwhite	Irregular	Abundant	Flat	-ve Rod
4				Pinkish Red	Circular	Abundant	Convex	-ve Short rod
5		Sorghum	Milk stage	White	Irregular	Abundant	Flat	+ve Rod
6				Dull yellow	Irregular	Abundant	Flat	-ve Short rod
7				Yellow	Circular	Moderate	Convex	-ve Rod
8		*Cassia cerassia*	Flowering stage	Transparent	Irregular	Abundant	Flat	+ve Cocci
9	Location 2	Sorghum	Dough stage	Dull yellow	Irregular	Abundant	Flat	-ve Rod
10				White	Irregular	Abundant	Flat	+ve Cocci
11		*Fimbristylis miliacea*	Flowering stage	Red	Irregular	Abundant	Umbonate	-ve Short rod
12				White	Circular	Moderate	Raised	+ve Rod
13				Red	Circular	Moderate	Umbonate	-ve Rod
14				Yellow	Circular	Slight	Convex	-ve Rod
15				Pink	Circular	Slight	Convex	-ve Rod
16		*Argemone mexicana*	Flowering stage	Yellow	Circular	Slight	Raised	-ve Rod
17				Dull yellow	Irregular	Abundant	Flat	-ve Rod
18		*Chrozophora rottleri*	Flowering stage	Yellow	Circular	Slight	Raised	-ve Rod
19		*Fumaria parvifolra*	Flowering stage	White	Circular	Abundant	Raised	-ve Rod
20				Offwhite	Irregular	Abundant	Raised	-ve Rod
21				Yellow	Circular	Slight	Raised	-ve Rod
22				Red	Circular	Moderate	Convex	-ve Rod
23		*Withania somnifera*	Flowering stage	White	Rhizoid	Abundant	Flat	+ve Rod
24				Yellow	Circular	Slight	Raised	-ve Rod
25		*Euphorbia esula*	Flowering stage	Dull yellow	Irregular	Abundant	Raised	-ve Rod
26				Yellow	Circular	Slight	Raised	-ve Rod
27	Location 3	Sorghum	Grain filling stage	Pale Yellow	Circular	Slight	Raised	-ve Short rod
28				White	Irregular	Moderate	Flat	+ve Rod
29				Offwhite	Irregular	Moderate	Flat	-ve Rod
30				Dull yellow	Irregular	Moderate	Raised	-ve Rod
31		*Fimbristylis miliacea*	Flowering stage	White	Irregular	Abundant	Flat	+ve Rod
32				Dull yellow	Irregular	Abundant	Raised	-ve Rod
33				Offwhite	Irregular	Moderate	Flat	+ve Rod
34				Brownish	Circular	Slight	Convex	+ve Rod
35				Lemon yellow	Circular	Slight	Convex	+ve Cocci
36				Orange	Circular	Abundant	Convex	-ve Rod
37	Location 4	Sorghum	Dough stage	Dull yellow	Irregular	Abundant	Flat	-ve Rod
38				Yellow	Circular	Moderate	Raised	-ve Rod
39	Location 5	Sorghum	Grain filling stage	White	Irregular	Abundant	Flat	+ve Rod
40				Pink	Circular	Moderate	Convex	-ve Rod
41				Offwhite	Irregular	Moderate	Flat	-ve Short rod
42				Yellow	Circular	Slight	Raised	-ve Short rod

which produce offwhite, white and yellow growth. The various weeds which were present in the sorghum field harbour mucoid pink, white, dull yellow, offwhite, light brown, brown, pale yellow and light pink bacterial colonies. Thus it is apparent from the results that at the same location the bacterial types under water stress condition varied depending upon the plant roots on/in which they survive for their habitat. Most of the bacteria were rod shaped or short rod except one isolate of cocci from sorghum root and one isolate of cocci from *Fumaria parviflora* weed root. All the isolates were Gram –ve in their reaction except isolate no. 5 and 35 which were Gram +ve in reaction and were from sorghum root and *Euphorbia esula* weed root.

These bacterial isolates form either circular or irregular or rhizoid type of bacterial colonies with flat, raised or convex and umbonate elevation which was dependent on bacterial isolates.

The 42 endophytic bacterial isolates (Table 3) isolated from the roots of sorghum and allied weed in moisture stress rhizosphere environment of sorghum field produced either red, yellow, offwhite, pinkish red, white, dull yellow, pink, transparent, brownish, lemon yellow and orange colonies and were specific to the root sample subjected for isolation. These bacterial isolates were rod, short rod or cocci shape. The colonies were circular, irregular, rhizoid in their form with convex, raised, flat and umbonate type of elevation. These isolates were either Gram –ve and Gram +ve in their reaction. Thus there was diversity in bacterial isolates under moisture stress (drought) condition in sorghum field and the diversity was related with the plant type surviving under the drought stress condition. Similar pattern was reported by (Cevheri, 2012) who isolated twenty six bacteria from rhizospheric soil samples of maize growing under semi-arid region (Harran Plain) of Turkey and characterized them by morphological and biochemical tests. Gram-negative bacteria were *Pseudomonas* sp. and Gram-positive were *Bacillus* species. Several bacterial isolates found and survived in arid or semi-arid region has been reported by Mayak *et al.*, 2004; Marulanda *et al.*,2009; Minaxi, *et al.*, 2012; Sharma *et al.*, 2013 and Yasmin *et al.*, 2013. In the present study, 42 endophytic bacteria isolated from root samples of sorghum and allied weeds. Similarly drought tolerant endophytic bacteria isolated from the grasses of Kutch, India (Akbari *et al.*, 2016).

Assessment of moisture stress tolerant (MST) bacterial isolates to survive under different soil moisture regime.

Since the bacterial isolates were isolated from moisture stressed drought condition field, their survival under moisture stress condition (of different moisture regime) was studied. The different soil moisture regime representing moderate drought (50%, 40%, 30%, 25% soil moisture) and severe drought (20%, 15% and 13% soil moisture) were obtained in pot soil under *in vitro* condition as described. The eighty one bacterial isolates were inoculated in the individual pots of particular soil moisture content and incubated to see their survival under that soil moisture condition. The results indicate that all the bacterial culture were able to grow in *in vitro* condition at 13% soil moisture level indicating that they sustain the severe drought condition in soil. Similarly total seventeen flurescent *Pseudomonas* sp isolated from arid and semiarid condition could grow at minimum water potential in *in vitro* condition (Ali *et al.,* 2013).

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