International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 1191-1194

Received: 21-01-2018
Accepted: 23-02-2018

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Seed bacterialization induced proline content in *Sorghum bicolor* crop under severe drought condition

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Abstract
The sorghum seed bacterialization with four moisture stress tolerance (MST) rhizobacterial isolates viz., *Serratia marcescens* L1SC8, *Pseudomonas putida* L3SC1, *Enterobacter cloacae* L1CcC1 and *Serratia marcescens* L2FmA4 were found beneficial to mitigate drought stress effect in sorghum (*Sorghum biocolor* L). This was attributed to the increase in osmolytes i.e. proline accumulation. The proline in leaves of sorghum plants raised from bacterized seed increased significantly over control at severe drought condition where soil moisture was in the range of 8 to 20%. At soil moisture percent of 20.37%, 17.21% and 8.83%, the proline increase was 0.80 – 0.192 µmoles g^{-1}, 0.234 – 0.365 µmoles g^{-1} and 0.138 – 0.373 µmoles g^{-1} respectively over the control.

Keywords: Proline, osmolytes, sorghum, *Serratia marcescens*, *Pseudomonas putida*, *Enterobacter cloacae*, moisture stress condition

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. Now a days microbes associated with plant has been used for confer the resistance against stress and enhanced the crop productivity (Mayak *et al*., 2004; Glick *et al*., 2007; Marulanda *et al*., 2009; Yang *et al*., 2009) [20, 7, 19, 32]. With the help of various metabolic activities, it has been found that PGPR associated with rhizosphere help many cereals and vegetables plants to confer drought tolerance (Timmusk and Wagner, 1999; Mayak *et al*., 2004; Sandhya *et al*., 2009; Kasim *et al*., 2013) [29, 20, 22, 14]. Microbes may change the metabolic pathways and biochemical molecules in the plant system which leads to sustain the drought condition. Osmotic adjustment is the active accumulation compatible solutes in plants experiencing drought stress which is one of the key adaptations that helps plants tolerate drought-induced damage which protects enzymes, proteins, cellular organelles and membranes against oxidative damage (Kiani *et al*., 2007; Hoekstra and Buitink, 2001; Farooq *et al*., 2009; Huang *et al*., 2014) [15, 12, 6, 13]. Proline is one of the most important osmolytes which maintain cellular turgor and help plants lower water potential without decreasing actual water content (Yoshiba *et al*., 1997; Serraj and Sinclair, 2002) [33, 26] and also contributes to stabilizing sub-cellular structures (e.g. proteins and membranes), scavenging free radicals and buffering cellular redox potential (Ashraf and Foolad, 2007; Hayat *et al*., 2012) [3, 11]. Many studies indicated that plants with increased levels of proline would be better able to tolerate drought stress (Sankar *et al*., 2007; Alexieva *et al*., 2001; Lum *et al*., 2014; Silvente *et al*., 2012; Mafakheri *et al*., 2010) [25, 1, 17, 28, 18]. Treatment of plants with PGPR has been shown to lead to an increase in proline levels which helps plant to tolerate drought stress condition (Vardharajula *et al*., 2011; Naseem and Bano, 2014; Grover *et al*., 2014; Gururani *et al*., 2013; Sarma and Saikia, 2014) [30, 21, 8, 9, 23]. 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.

Material and Methods

Sampling, isolation and screening

A total 81 bacterial cultures 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 cultures was done on nutrient agar medium by pour plate technique. Out of 81 isolates, four effective bacterial inoculants (L1SC8, L3SC1, L1CcC1 and L2FmA4) were selected on the basis of their performance on plant growth parameter of sorghum *in vitro* condition. The selected four bacterial isolates L1SC8, L3SC1, L1CcC1 and L2FmA4 were identified as *Serratia marcescens, Pseudomonas putida, Enterobacter cloacae* and *Serratia marcescens*.

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 5 g of jaggery in 100 ml of water). 5 ml 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 upto 30 min.

Field Experiment

Assay of moisture stress tolerant bacterial inoculant was performed on the *var. Phule vasudha*. 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 (100 g) was taken at a uniform depth of 15 cm 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., 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

Proline Content

For determination of proline content, 1g leaves of the freshly harvested samples at flowering stage were homogenised with 10ml (3%) sulphosalicylic acid and centrifuged at 5000g at 28°C for 10min. The supernatant was used for proline estimation (Bates *et al.* 1973) [4]. The proline concentration was determined from the standard curve and estimated at micromoles per gram fresh weight.

Results and Discussion

Proline Content

Proline content was estimated from sorghum crop when the crop was at drought condition i.e. at the soil moisture level of 20.37%, 17.21 and 8.83%. The results (Table 1) indicate that four bacterial isolate viz., *Serratia marcescens* L1SC8, *Pseudomonas putida* L3SC1, *Serratia marcescens* L2FmA4 and *Enterobacter cloacae* L1CcC1 were statistically significant in production of proline over the untreated control. At different drought soil moisture levels of 20.37%, 17.21% and 8.83%, the proline increase was range of 0.80 – 0.192 µmoles g^{-1} fw, 0.234 – 0.365 µmoles g^{-1} fw and 0.138 – 0.373 µmoles g^{-1} fw respectively over the untreated control. Moisture stress tolerant (MST) bacterial inoculants showed significant increase in proline content as compared to untreated control under drought stress condition and the maximum proline production in sorghum leaves was observed at soil moisture level of 17.21%. *Serratia marcescens* L1SC8 seed bacterization showed highest increase in proline content over untreated control at 20.37% and 17.21% soil moisture condition followed by *Pseudomonas putida* L3SC1. The seed bacterization with *Pseudomonas putida* L3SC1 showed highest increase in proline content of leaves over untreated control at 8.83% soil moisture condition followed by *Serratia marcescens* L1SC8.

The seed treatment of sorghum seed with moisture stress tolerant bacteria isolated from semi-arid region of Ahmednagar district of western Maharashtra were observed to increase sorghum plant growth parameter under drought condition and confers the plant drought resistance by altering biochemical ways. Microbes have different activities responsible for changes in plant growth under moisture stress condition.

The proline content in sorghum plants raised with sorghum seed bacterization increased significantly over the untreated seed plant. It has been suggested that the increase in leaf proline contribute to the observed drought tolerance by protecting the plants from dehydration stress. Furthermore, proline accumulation helps to maintain the osmotic adjustment in plant cells thereby maintaining leaf turgor by generating more negative leaf water potential and thus it helps to maintain water movement into the leaf, and protects the organization of macromolecules and cell membranes during water deficit conditions (Thapa *et al.* 2011). It is reported that lettuce plants co-inoculated with *Pseudomonas mendocina* and *Glomus intraradices* show high accumulation of proline which enhanced the drought stress alleviation in lettuce (Kohler *et al.* 2008). Rice varieties bacterialized with PGPRs showed higher proline content in leaves at all the level of drought stress (Gusain *et al.* 2015) [10]. Proline production in response to the water stress and its increase in maize seedlings was observed when inoculated with PGPR. Thus seed bacterilization improve drought stress response in plant and alleviate drought stress effects (Sandhya *et. al.* 2011) [24]. Highest amount of proline content was observed in maize plant under drought stress conditions due to inoculation of *Pseudomonas fluorescens* strains (Ansary *et. al.* 2012) [2]. Wang *et al.* (2012) [31] reported that treatment of cucumber (*Cucumis sativa* L.) plants with a mixture of three PGPR strains (*Bacillus cereus* AR156, *Bacillus subtilis* SM21, and *Serratia* sp. XY21) increased leaf proline contents 3–4 fold relative to untreated controls. Grover *et al.* (2014) [8] found that isolates of *Bacillus* spp (KB122, KB129, KB133, and KB142) mitigate the impact of soil drought by significant increase in the leaf proline content in PGPR treated plants.

Effect of moisture stress tolerant bacterial inoculants on plant leaves

The results (Table 1) indicate that the total number of leaves was significantly more in MST bacterial inoculant treated seeds as compared to untreated seeds. The maximum increase in total leaves number was observed with bacterial inoculant L3SC1 *(Pseudomonas putida)* followed by, L1SC8 (*Serratia marcescens*), L2FmA4 (*Enterobacter cloacae*) and L1CcC1 *(Serratia marcescens)*. The results were statistically significant over the control.

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 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) [5] 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.

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

The results (Table 1) indicate that the grain yield obtained from the MST bacterial inoculated plant was numerically more than the untreated plants. However the bacterial inoculant 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 MST bacterial isolates was upto 17.01 percent.

The grain yield obtained from the MST bacterial inoculated plant was numerically more than the untreated plants. Arshad *et al.* (2008) 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) [27] found that PGPR 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. Glick *et al.* (2007) [7] and Zahir *et al.* (2008) also reported that PGPR produced higher crop yields and provide stress relief especially under drought conditions.

Table 1: Effect of selected moisture stress tolerant bacterial inoculant on proline content

Bacterial inoculant	Proline content (µmoles g^{-1} fw)						Plant growth parameter		
	Soil moisture level						No. of functional leaves under drought stage	No. of non-functional leaves under drought stage	Yield q ha^{-1}
	20.37%		17.21%		8.83%				
	Proline µmoles g^{-1}	Increase over control	Proline µmoles g^{-1}	Increase over control	Proline µmoles g^{-1}	Increase over control			
Serratia marcescens L1SC8	0.709[a]	0.192	0.776[a]	0.365	0.626[a]	0.364	7.04	5.36	26.03
Pseudomonas putida L3SC1	0.681[b]	0.164	0.682[b]	0.271	0.635[a]	0.373	7.36	5.13	25.33
Enterobacter cloacae L1CcC1	0.521[d]	0.004	0.645[b]	0.234	0.400[b]	0.138	6.21	5.58	24.34
Serratia marcescens L2FmA4	0.597[c]	0.080	0.667[b]	0.256	0.451[b]	0.189	6.45	5.43	25.91
Untreated control	0.517[d]		0.411[c]		0.262[c]		5.46	6.09	22.25
SE±	0.00308		0.0203		0.0319		0.098	0.0432	37.74
CD at 5%	0.0093		0.0613		0.096		0.297	0.130	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.

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