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ORIGINAL ARTICLE

Retention of Water and Stomatal Conductance in Sorghum Plant as Influenced by Bacterial Species Under Drought Condition

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ABSTRACT
The relative water content (RWC) and stomatal conductance (SC) in sorghum (Sorghum bicolor, L.) leaves were assessed under water stress condition by sorghum seed priming with Serratia marcescens strain L1SC8, Pseudomonas putida strain L3SC1, Enterobacter cloacae strain L1CcC1and Serratia marcescens strain L2FmA4.RWC was higher in leaves of seed priming plants. Water stress resulted in decreased RWC of plant leaves. Seed priming withSerratia marcescens strain L1SC8 showed higher RWC over untreated control followed by Enterobacter cloacae strain L1CcC1. However SC decreased in seed primed stressed plants leaves compared to non-primedstressed plant leaves. Pseudomonas putida strain L3SC1 showed lowest SC as compared to other treatments and control. Thus seed priming with moisture stress tolerant bacterial cultures increase the RWC while decrease the SC which was the major attributing factors for drought tolerance in sorghum.
Keywords : *relative water content, stomatal conductance, sorghum, Serratia marcescens, Pseudomonas putida, Enterobacter cloacae , moisture stress, drought*

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INTRODUCTION

Drought is one of the major abiotic stresses that adversely affects majority of the world's crop growth and productivity. Drought stress is common in many parts of the world and more than 50% of the globe is arid or semi-arid or is subjected to some kind of drought stress. Plant response to drought stress, at cellular and molecular level, limits plant growth and yield [12]. The adaptation mechanism of plant drought tolerance may involve promotion of root extension, allowing an efficient water uptake [9, 13]. Leaf relative water content (RWC) is an important indicator of water status in plants; it reflects the balance between water supply to the leaf tissue and transpiration rate [8]. Appropriate relative water content (RWC) in plant is probably the most important component to maintain the physiological and cellular activities in plant in normal as well as drought condition. Similarly stomatal conductance is also important which determines the rate of passage of carbon dioxide (CO_2) entering, or water vapor exiting through the stomata of a leaf. Less stomatal conductance indicates increasing water use efficiency as consequence of the relative improvement in intracellular CO_2 and partial stomatal closure.Numerous microorganisms are associated with rhizosphere of plants. Some of these have positive effects on plants. Certain bacteria survive under water stress conditions which help plants to sustain in water stress conditions by inducing some physiological changes in plant system.

In the present study, we evaluated the effect of the moisture stress tolerant bacteria *Serratia marcescens* strain *L1SC8, Pseudomonas putida* strain *L3SC1*, *Enterobacter cloacae* strain *L1CcC1*and *Serratia marcescens* strain *L2FmA4* on the relative water content and stomatal conductance of sorghum leaves under drought stress condition where the parameter RWC and SC render the plants more tolerant to drought stress.

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 of field from where samples were taken 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. The selected four isolates L1SC8, L3SC1, L1CcC1 and L2FmA4 were identified as *Serratia marcescens, Pseudomonas putida, Enterobacter cloacae* and *Serratia marcescens* [7].

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 upto 30 min.

Field experiment

Efficacy of moisture stress tolerant bacterial inoculant was assessed on sorghum*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 (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 over for 24h at 110°C till constant weight. Soil moisture was calculated by the formula

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

Relative water content assay

The method described by Henderson and Davies-Jr. [6] was followed for determination of relative water content. Relative water content of sorghum leaves was determined by taking weight of fresh flag leaves of plants raised from seed priming with bacterial inoculant and untreated control seed plants. Initially midribs from the leaflets were removed after excision and fresh weight (FW) was taken. The leaflets were floated on the distilled water for three hours, blotted with tissue paper and again leaflet weight was taken to obtain fully turgid weight (TW). The leaves were dried in oven at 95°C to obtain dry weight (DW). Relative water was calculated by following formula: RWC (%) = (FW - DW) / (TW – DW) x 100

Measurement of stomatal conductance

The observations on stomatal conductance were recorded with the help of Portable Infrared Gas Analyzer (IRGA). Measurement of stomatal conductance was recorded on the adxial surface of third fully expanded leaf from the top by using IRGA. An intact leaf of plant was clamped into the chamber and observations were taken. During the measurements stomatal conductance was logged, computed and stored in the memory. This measurement was made between 10.00 am to 12.00 noon. Stomatal conductance expressed as mol $H_2Om^{-2}S^{-1}$.

RESULTS AND DISCUSSION

Relative water content

The result (Table 1) indicate that the moisture stress tolerant bacterial inoculant increase the relative water content of leaves to sustain the drought. The increase of RWC was in the range of 8.10 to 11.53 percent. In general RWC below 70 percent in plant leaves shows the wilting and drought stress symptoms. All the bacterial inoculant increases the RWC. The increase in RWC was statistically significant over control. Thus the moisture stress tolerant bacterial inoculants sustain the drought condition by increasing the RWC in plant.

The drought stress resulted in decreased relative water content (RWC) in plant leaves reflects a loss of turgor results in reduced plant growth. By measuring the relative water content, one can get the water status of plant because it is involved in the metabolic activity in tissues [1,3, 8]. An increase in RWC should be considered an important drought tolerance enhancement strategy and could be used as a major tool in screening PGPR bacterial isolates for drought stress-alleviating potential. The moisture stress tolerant bacterial inoculant increase the relative water content of leaves to sustain the drought. In our study all the bacterial inoculant increases the RWC. The increase in RWC was statistically significant over control. Thus the MST bacterial inoculants sustain the drought condition by increasing the RWC in plant. Sandhya *et al.* [14] reported that seed bacterization of maize with strains of *Pseudomonas* spp. had increased relative water content in inoculated maize leaves as compared to uninoculated drought stressed maize leaves. Yasmin *et al.* [16] reported that PGPR treated *Zea mays* showed increase in relative water content of leaves by 21.7-28.4% under drought stressed conditions. The microbial inoculation increased the relative water content of leaves under drought stress. Naseem and Bano [10] reported that seed bacterization of maize with EPS-producing bacterial strains in combination with their respective EPS, showed maximum increase in relative water content of plant leaves. Plants having higher yield under drought stress need to maintain higher RWC. Castillo *et al.* [3] reported that *Achromobacter xylosoxidans* (SF2) and *Bacillus pumilus* (SF3 and SF4) treated sunflower (*Helianthus annuus* L.) showed highest increase in relative water content (80%) for co-inoculated seedlings following by singly inoculated seedlings (72%) and uninoculated seedlings (70.4%). It has been found that mung bean treated with *Pseudomonas aeruginosa* GGRJ21 showed higher leaf relative water content (23%) compared to the uninoculated normal plants under drought conditions [15]. Grover *et al.* [5] reported that treatment of sorghum (*Sorghum bicolor* L.) with PGPR resulted in increased relative water content in leaves under stress conditions. Naveed *et al.* [11] reported that bacterial inoculation improved the physiological traits and growth of both maize cultivars and enhanced their capacity to tolerate drought via increase in relative water content. They found that *Burkholderia phytofirmans* PsJN inoculation resulted in 30 % maximum increase in relative leaf water content compared to control under drought stress.

Stomatal conductance

The result (Table 2) indicates that the bacterial treatments decreased the stomatal conductance compared to untreated control. The most efficient treatment in decreasing this value was bacterial inoculant L3SC1 followed by L2FmA4, L1CcC1 and L1SC8.

Stomatal conductance estimates the rate of gas exchange (i.e. carbon dioxide uptake) and transpiration (i.e. water loss) through the leaf stomata. Less Stomatal conductance under drought conditions indicates a better water use efficiency of these plants. All the four MST bacterial inoculants showed decrease in stomatal conductance compared to untreated control. Benabdellah *et al.* [2] reported decline in stomatal conductance in inoculated *Trifolium repens* plants treated with AMF or B2 inoculants compared to non-inoculated control under drought condition. Yasmin *et al.* [16] reported that maize seedlings primed with the PGPR isolates showed decrease in stomatal conductance under drought stress and results in enhanced water use efficiency and ultimately growth of plant. Cohen *et al.* [4] found that inoculation of *Arabidopsis thaliana* with *Azospirillum brasilense* decreased stomatal conductance compared to uninoculated control.

Table 1. Effect of MSTB inoculants on Relative water content of sorghum leaves

Relative water content (%) at drought stress condition		
Treatment(s)	**RWC**	**Increase over control**
Serratiamarcescens L1SC8	73.84 [a]	11.53
Pseudomonasputida L3SC1	70.41[b]	8.10
Enterobactercloacae L1CcC1	71.34[b]	9.01
Serratiamarcescens L2FmA4	71.28[b]	8.97
Untreated	62.31	
SE(+)	**0.579**	
CD at 5%	**1.747**	

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 2. Effect of MST bacterial inoculants on stomatal conductance

Stomatal conductance mol $H_2Om^{-2}S^{-1}$during drought stress condition	
Treatment(s)	**Stomatal conductance**
Serratiamarcescens L1SC8	0.153^{b}
Pseudomonasputida L3SC1	0.140^{a}
Enterobactercloacae L1CcC1	0.143^{a}
Serratiamarcescens L2FmA4	0.142^{a}
Untreated control	0.191^{c}
SE (±)	0.00169
CD at 5%	0.00508

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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