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VARIABILITY IN STRESS TOLERANCE AND POTENTIAL OF ENDOPHYTIC BACTERIA TO IMPROVE CHICKPEA GROWTH UNDER WATER LIMITED CONDITIONS

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Plant growth promoting bacteria have been employed as a biotechnological tool for ameliorating the impacts of water deficit stress on plants. However, endophytic bacteria colonizing within plant tissues could be of great benefit to the crops. In the present study, forty strains of endophytic bacteria were isolated and tested for their survivability under water deficit conditions. Twelve bacterial isolates showing the highest potential to survive under water deficit conditions were evaluated for inducing stress tolerance in chickpea seedlings at different levels of water deficit stress (i.e. -0.04, -0.70 and -1.24 MPa) under gnotobiotic conditions. Bacterial inoculation with isolates Cs8, Cs10 and Cp7 prominently increased the root length of chickpea over un-inoculated control. Significantly higher root/shoot dry biomass was observed in response to inoculation with bacterial isolates Cs8, Cs10, Cp3 and Cp7 as compared to un-inoculated control. Moreover, all the endophytic bacterial isolates were capable to produce auxins, catalase, oxidase and exopolysaccharides. It is concluded that the endophytic bacteria colonizing internal tissues of pods and shoot of chickpea have better potential to improved growth and biomass of chickpea seedling under reduced water conditions.

Keywords: Endophytic bacteria, drought, chickpea, PEG 6000.

Abbreviations: PGPR, Plant Growth Promoting Rhizobacteria; DMSTI, Dry Matter Stress Tolerance Index; LB, Lauria-bertani; TSB, Tryptic soy broth; PEG, Polyethylene glycol

INTRODUCTION

The production of agricultural crops is reducing due to the prevalence of drought like situations in the world (Fahad *et al.*, 2017). Inoculation with plant growth promoting bacteria has been devised as a beneficial strategy for improving the fitness of crop plants especially under harsh environmental conditions. Bacteria in the rhizosphere face various conditions to ensure their survival through competition with indigenous microflora for nutrition and harsh environmental conditions before playing their role as plant growth promoter (Martinez *et al*., 2010). Predation by protozoans aggravates the problem of inoculums failure in field conditions by limiting survival of plant growth promoting bacteria (PGPB) in the rhizosphere. Ultimately, the role of rhizosphere bacteria as plant growth promoter is verily dependent on their capability to survive and flourish under hostile environments (Rivera *et al*., 2008).

While in distressed conditions some bacteria become advantageous due to their capability to enter, survive and express their plant beneficial traits inside the plant tissues. They live for the entire or part of their life cycle inside the plant without being pathogenic and are termed as endophytes (Hardoim *et al*., 2008). Bacteria as endophytes escape/avoid the competition with indigenous microflora for space and resources and adverse soil conditions. They get incubation in plant tissue at optimal temperature, space and nutrition (Beattie, 2007; Rosenblueth and Martinez-Romero, 2006). Endophytic bacteria are almost similar in their plant growth promoting attributes to rhizobacteria. They increase plant vigor and induce tolerance/resistance against biotic/abiotic stresses like drought (Hallmann *et al*., 1997; Naveed *et al*., 2014) and modulate the plant stress response (Dudeja and Giri, 2014). The plant growth promoting mechanisms by endophytic bacteria include the production of phytohormones, nitrogen fixation, siderophores production and production of antimicrobial compounds against plant pathogens (Hallmann *et al*.,1997; Rosenblueth and Martinez-Romero, 2006; Mitter *et al*., 2013). It has been observed that endophytic bacteria improve plant growth, biomass, root growth and protect the plants from drought by supplying growth regulators, osmolytes and nutrients (Kavamura *et al*., 2013; Egamberdieva, 2017).

Using endophytes on behalf of their advantages to avoid harsh soil environments is a new trend in sustainable agriculture. Though, researchers have tried different endophytes to improve growth, physiology, yield and abiotic stress tolerance in cereals. However, endophytes in legumes other than

rhizobium have not been studied yet. Therefore, in present investigation, we have isolated and identified the potential plant growth promoting endophytic bacteria from the roots, stems, leaves and pods tissues of chickpea for ameliorating impacts of water deficit on chickpea.

MATERIALS AND METHODS

***Isolation and preservation of endophytic bacteria*:** Healthy and disease free plant tissues (roots, stems, leaves, pods) of chickpea (grown in arid to semi-arid regions of Punjab-Pakistan) were sampled, washed, blotted and stored in refrigerator before the initiation of isolation procedure. Isolation of the endophytic bacteria was carried out in Soil Microbiology and Biochemistry Lab, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. The plant tissues were surface sterilized by dipping in alcohol (70%) for 1 min followed by 3-5 min dip in sodium hypochlorite (3.5%) and 4 times washing with sterilized distilled water (Long *et al*., 2008). Sterilized plant tissues were crushed in sterilized saline (0.85%) water. Dilution plate technique was followed to isolate the endophytic bacteria by using tryptic soy agar (TSA) as a growth medium (Evers *et al*., 2015). The plates were incubated at 28±1°C for 48 h to observe bacterial growth. Prolific bacterial colonies from each tissue were separated and purified by repeated streaking and preserved in glycerol (40%) at -40°C for future use. Ten isolates from each tissue were selected for further experimentation and coded accordingly (Table 1).

Table 1. Coding of endophytic bacteria isolated from roots, stems, leaves and pods tissues of chickpea.

Roots	Stems	Leaves	Pods
[1]Cr1	[2]Cs1	[3]Cl1	[4]Cp1
Cr2	Cs2	Cl2	Cp2
Cr3	Cs3	Cl3	Cp3
Cr4	Cs4	Cl4	Cp4
Cr5	Cs5	Cl5	Cp5
Cr6	Cs6	Cl6	Cp6
Cr7	Cs7	Cl7	Cp7
Cr8	Cs8	Cl8	Cp8
Cr9	Cs9	Cl9	Cp9
Cr10	Cs10	Cl10	Cp10

[1]Chickpea roots (Cr), [2]Chickpea stems (Cs), [3]Chickpea leaves (Cl), [4]Chickpea pods (Cp)

***Water deficit stress abiding ability of endophytic bacteria*:** Bacterial isolates from respective tissues were subjected to various levels of osmotic stress employed by using polyethylene glycol (PEG-6000) such as -0.05, -0.65, -1.57, -2.17 and -2.23 MPa. Osmotic stress treatments were established in sterilized 15 mL test tubes containing 7 mL Lauria-bertani (LB) broth by the addition of Polyethylene glycol 6000 (PEG-6000) (Busse and Bottomley, 1989) Osmotic potential of broth media was measured by osmometer (OSMOMAT-030-D, Gonotec, Germany) at respective PEG-6000 levels. Freshly prepared culture of each isolate (0.5 OD ≈ 10^7 cells mL^{-1}) was inoculated into the sterilized conical flask and incubated for two days in orbital shaking incubator at 28 ± 1°C and 100 rpm. Three sets of each treatment and a control without bacteria were maintained. Bacterial growth was determined by measuring optical density at λ 600 nm using spectrophotometer (Nicolet Evolution 300, Thermo Electron Corporation) (Asghar *et al*., 2015) and bacterial cell counts were determined by dilution plate technique. Bacteria showing higher OD and cell count under water deficit stress were considered as water deficit stress tolerant.

***Plant growth promoting potential of endophytic bacteria*:** Water deficit stress tolerant endophytic bacterial isolates, three from each part i.e. root (Cr1, Cr4, Cr10), stem (Cs6, Cs8, Cs10), leaf (Cl3, Cl6, Cl7) and pod (Cp3, Cp7, Cp10) were selected for plant growth promoting experiment in pouches using chickpea (*Cicer arietinum* L.) cv. Punjab 2008 as test crop under controlled conditions in growth room of Soil Microbiology and Biochemistry Lab, Institute of Soil and Environmental Sciences, University of Agriculture Faisalabad. Freshly prepared inocula of each bacterial isolate in LB broth (0.5 OD = 10^7 cells mL^{-1}) were used for seed inoculations. Surface sterilized pre-germinated seeds were dipped in inoculum for 20 min for inoculation. Control was maintained by dipping seeds in sterilized inoculum. Pouches were arranged on the shelf of growth room in completely randomized design with three replications. Day length was adjusted as 14 h dark and 10 h light and suitable temperature 22-25°C was maintained. The plants were irrigated with ½ strength Hoagland's solution. After three days of sowing, the water deficit stress treatments were applied by using PEG-6000 in ½ strength Hoagland's solution to maintain water potentials of -0.04, -0.70 and -1.24 MPa (Michel, 1983). Plants were harvested after 20 days and root/shoot fresh/dry biomass and length parameters were recorded.

***Dry matter stress tolerance index (DMSTI)*:** Dry matter stress tolerance index was calculated by dividing the dry weight of inoculated seedlings under stress (DWI or St) with the dry weight of un-inoculated and unstressed seedlings (DWUI and USt) (Ashraf *et al*., 2006).

$$\text{DMSTI} = \frac{(\text{DWI and St})}{(\text{DWUI and USt})} \times 100$$

Where DWI is the dry weight of inoculated seeding; DWUI is the dry weight of un-inoculated seedling; St = stressed seedling; USt = un-stressed seedling.

***Bacterial characterization*:** Auxin production in the presence and absence of L-tryptophan was determined spectrophotometrically (Nicolet Evolution 300, Thermo Electron Corporation) following the procedure described by Sarwar *et al*. (1992). Exopolysaccharides production was

assessed using RCV-glucose media as described by Ashraf *et al*. (2004). Catalase and oxidase activities were observed using the procedures by MacFaddin (1980) and Steel (1961), respectively. Microbial aggregation ability was determined using modified procedure of Madi and Henis (1989) and calculated using the formula

$$\% \text{ Aggregation} = \frac{OD2 - OD1}{OD2} \times 100$$

Where, OD1 (absorbance of aliquot after 20 min settling of fresh inocula) and OD2 (after 1 min vortex of the inocula) were measured at 540 nm on spectrophotometer.

***Statistical analysis*:** Data were analyzed by two-way analysis of variance (ANOVA) (Steel *et al*., 1997) and means were compared using LSD test. Software used for the analysis was Statistix 8.1 (Analytical Software, USA).

RESULTS

***Water deficit stress tolerance capability of endophytic bacteria*:** Following the OD and cell count of bacterial strains isolated from chickpea roots (Table 2) at various water potentials (-0.65 MPa, -1.57 MPa, -2.17 MPa, -2.23 MPa). A significant decrease in cell count and OD was recorded with gradual decrease in water potentials. However, bacterial isolates Cr3, Cr5 and Cr10 remained prominent among all bacterial isolates with higher OD values at -0.65 MPa. Whereas at -1.57 MPa, the OD values of Cr3, Cr7 and Cr10 were higher as compared to other bacterial isolates. At -2.17 and -2.23 MPa, the OD values trend was quite different from each other showing prominent group of three isolates as (Cr4, Cr7, Cr10) and (Cr1, Cr3, Cr5), respectively. As for as, bacterial cell counts at various water potential levels were concerned, bacterial isolate Cr3 was most prominent with significantly high cell counts at -0.65, -1.57, -2.17 and -2.23 MPa water potentials followed by Cr2 with prominence at -1.57, -2.17 and -2.23 MPa. In addition to Cr2 and Cr3, other isolates also showed significantly high cell counts at -0.65 MPa (Cr8, Cr9), -1.57 MPa (Cr5), -2.17 MPa (Cr10) and -2.23 MPa (Cr7). From overall observation for OD values and cell counts, bacterial isolates Cr2, Cr3 and Cr10 were

Table 2. Drought tolerance assay of endophytic bacteria isolated from chickpea roots at different PEG induced osmotic stress levels.

Isolates	Optical Density (OD) (n = 3)						Cell Count (CFU $mL^{-1} \times 10^{-3}$) (n = 3)					
	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean
Cr1	4.8ijk	4.7jk	4.2lmn	4.0n-q	3.8pqr	4.3EF	38.8b-f	35.8fg	20.8mno	20.4m-p	11.6stu	25.5F
Cr2	5.8f	5.2gh	4.9ij	3.1s	2.7t	4.3E	41.1abc	40.1a-d	26.2hij	25.3hi	15.9qr	29.7B
Cr3	7.1a	6.9a	6.2d	4.2lmn	3.7r	5.6A	41.9ab	41.2abc	34.2g	29.0ijk	18.1opq	32.9A
Cr4	4.6k	4.7jk	4.8jk	4.9ij	4.8ijk	4.8D	42.6a	41.1abc	23.6i-m	21.8h	14.6rst	28.7BC
Cr5	6.3cd	6.0de	5.1hi	4.0m-p	3.9o-r	5.1C	38.5c-f	37.1d-g	26.1hij	21.8lmn	11.4tu	27.0DE
Cr6	5.7f	5.4g	3.8pqr	2.7t	1.8v	3.9G	36.6efg	36.2fg	20.9mno	22.4lmn	14.8rs	26.2EF
Cr7	6.6b	5.8f	5.4g	4.7jk	3.0s	5.1C	40.4a-d	38.4c-f	23.1j-m	24.4klm	17.4u	28.7BC
Cr8	5.2gh	4.8ijk	4.1mno	2.4u	1.9v	3.7H	42.5a	41.5abc	24.2i-l	20.8i-l	11.9pqr	28.2CD
Cr9	5.1hi	4.9ij	4.4l	3.8qr	3.0s	4.2F	41.2abc	42.3a	21.7lmn	19.1mno	9.1stu	26.7EF
Cr10	7.0a	6.5bc	5.9ef	4.3lm	2.5tu	5.2B	42.0ab	39.8a-e	22.3k-n	26.8n-q	10.3u	28.2CD
Mean	5.8A	5.5B	4.9C	3.8D	3.1E		40.6A	39.4B	24.3C	23.2D	13.5E	
LSD	Drought (0.08), Isolate (0.11), Drought* Isolate (0.25)						Drought (1.04), Isolate (1.57), Drought* Isolate (3.28)					

Table 3. Drought tolerance assay of endophytic bacteria isolated from chickpea stem at different PEG induced osmotic stress levels.

Isolates	Optical Density (OD) (n = 3)						Cell Count (CFU $mL^{-1} \times 10^{-3}$) (n = 3)					
	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean
Cs1	4.1l	3.9m	2.5t	1.5w	1.4w	2.7I	28.8cde	22.5g-o	18.1m-u	13.8t-x	9.3xy	18.5CD
Cs2	5.4f	5.4f	4.4jk	2.4t	1.4w	3.8F	21.6i-p	23.4f-m	16.2q-v	15.3s-v	9.9wxy	17.3D
Cs3	4.3k	3.6n	2.8r	1.4w	1.7v	2.8H	34.9ab	27.9c-g	20.9j-r	17.6n-v	1.1z	20.5BC
Cs4	4.4jk	4.4jk	3.9m	3.9m	2.8qr	3.9E	21.9h-o	24.4d-k	20.3j-s	15.1s-w	1.6z	16.7D
Cs5	5.8e	4.4jk	2.6s	1.5w	1.5w	3.2G	31.6bc	27.1c-h	22.7g-o	16.5p-v	7.0y	21.0AB
Cs6	5.9de	6.0d	6.5ab	6.6a	6.3c	6.2A	27.3c-h	29.5bcd	23.6e-l	21.4i-q	15.1s-w	23.4A
Cs7	4.4jk	5.9de	6.4bc	3.2o	3.0p	4.6C	38.7a	28.4c-f	19.8j-s	15.0s-w	9.4xy	22.3AB
Cs8	5.2g	5.2fg	5.3fg	4.8h	4.6i	5.0B	22.5g-o	21.5i-q	24.5d-k	17.4o-v	15.6r-v	20.3BC
Cs9	4.2l	4.5ij	4.3k	2.0u	1.1x	3.2G	24.5d-j	22.3h-o	18.3l-u	13.1u-x	9.1xy	17.5D
Cs10	6.4bc	4.4jk	3.9m	3.6n	2.9pq	4.2D	31.0bc	26.5c-i	22.9g-n	19.1k-t	12.3v-y	22.4AB
Mean	5.0A	4.8B	4.3C	3.1D	2.7E		28.3A	25.4B	20.7C	16.4D	9.0E	
LSD	Drought (0.04), Isolate (0.06), Drought* Isolate (0.13)						Drought (1.71), Isolate (2.41), Drought* Isolate (5.40)					

identified as most water deficit stress tolerant among the tested bacterial isolates.

The bacterial isolates from the stem part of chickpea showed significant decrease in the OD value and cell count with increase in the intensity of PEG induced drought i.e. -0.05 to -2.23 MPa (Table 3). The isolates Cs6 and Cs8 showed significantly higher OD values among all bacterial isolates at all water deficit stress levels (-0.65, -1.57, -2.17 and -2.23 MPa) followed by Cs7 which also remained prominent for its OD at -0.65, -1.57, and -2.23 MPa. At -2.17 MPa, bacterial isolate Cs4 was also a prominent fellow for its higher OD value along with Cs6 and Cs8. For cell count, Cs6 was most prominent with higher number of cells at -0.65, -1.57, -2.17 and -2.23 MPa as compared to other bacterial isolates. Bacterial isolate Cs10 was following Cs6 with its prominence in cell count at -1.57, -2.17 and -2.23 MPa. At -1.57 and -2.23 MPa and Cs8 also showed prominent cell count along with Cs6 and Cs10. Whereas Cs3 joined Cs6 and Cs7 for prominent cell count at -0.65 MPa and Cs6 and Cs10 at -2.17 MPa. Isolates Cs6, Cs8 and Cs10 were selected as most efficient to survive and flourish under water deficit conditions on behalf of their OD values and cell counts.

Significant decrease in OD values and cell counts of endophytic bacterial isolates from leaf were observed with decrease in water potential from -0.05 to -2.23 MPa (Table 4). The water potential -0.05 MPa was considered as control or normal water potential of media for bacterial growth. At -0.65 MPa, bacterial isolates Cl2, Cl3 and Cl6 were prominent with higher OD values among other bacterial isolates. At -1.57 MPa, bacterial isolates Cl3, Cl6 and Cl8 shows higher OD values but at -2.17 MPa Cl8, Cl9 and Cl10 showed higher OD values among other bacterial isolates. The OD values of bacteria isolates Cl3, Cl7 and Cl9 was prominently higher at -2.23 MPa. For cell count the strains (Cl3, Cl6, Cl7), (Cl3, Cl6, Cl10), (Cl2, Cl3, Cl6), and (Cl1, Cl3, Cl7) were prominent among other tested bacterial isolates at -0.65, -1.57, -2.17 and -2.23 MPa, respectively. On behalf of OD values and cell counts data collectively, the bacterial isolates Cl3, Cl6 and Cl7 were designated as most water deficit stress tolerant.

Table 4. Drought tolerance assay of endophytic bacteria isolated from chickpea leaves at different PEG induced osmotic stress levels.

Isolates	Optical Density (OD) (n = 3)						Cell Count (CFU $mL^{-1} \times 10^{-3}$) (n = 3)					
	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean
Cl1	4.2gh	4.0hi	2.5no	1.6uv	1.4v	2.7D	32.2bcd	25.8d-j	21.4h-q	15.1q-t	6.0vwx	20.1BC
Cl2	5.0e	4.4f	3.0l	1.4v	0.8wx	2.9C	28.3c-g	24.8e-l	19.5j-s	16.3o-t	4.2wx	18.6CDE
Cl3	6.7a	4.8e	3.8i	1.5uv	1.8st	3.7A	37.2ab	33.2bc	24.5e-m	18.3l-s	8.4uvw	24.3A
Cl4	3.5j	3.3jk	3.1kl	1.9rs	1.0w	2.5E	25.2e-k	22.1g-o	18.9k-s	13.8stu	2.0wx	16.4DE
Cl5	3.9i	3.5j	2.7mn	1.5uv	0.9wx	2.5E	28.3c-g	24.8e-l	21.7h-p	15.8o-t	5.0vwx	19.1CD
Cl6	5.3d	4.5f	3.4j	2.5no	1.4v	3.4B	30.6cde	27.2c-i	23.9f-n	17.8n-s	5.1vwx	20.9BC
Cl7	5.9c	4.0hi	2.5no	2.3pq	2.1qr	3.3B	42.0a	28.8c-f	18.1m-s	15.4p-t	8.0uvw	22.5AB
Cl8	6.4b	4.3fg	3.5j	2.9lm	1.6tu	3.7A	25.9d-j	20.8i-r	21.2i-q	14.4r-u	1.9wx	16.8DE
Cl9	4.4fg	2.5op	2.5op	3.1kl	2.3pq	2.9C	27.9c-h	18.9k-s	18.3l-s	10.7tuv	4.4vwx	16.1E
Cl10	3.3j	2.7mn	2.2q	3.8i	0.7x	2.5E	31.0b-e	25.1e-k	22.9f-n	4.1wx	0.7x	16.8DE
Mean	4.8 A	3.8 B	2.9 C	2.2 D	1.4 E		30.8 A	25.2 B	21.1 C	14.2 D	4.6 E	
LSD	Drought (0.07), Isolate (0.10), Drought* Isolate (0.22)						Drought (2.07), Isolate (2.92), Drought* Isolate (6.53)					

Table 5. Drought tolerance assay of endophytic bacteria isolated from chickpea pods at different PEG induced osmotic stress levels.

Isolates	Optical Density (OD) (n = 3)						Cell Count (CFU $mL^{-1} \times 10^{-3}$) (n = 3)					
	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean	-0.05 (MPa)	-0.65 (MPa)	-1.57 (MPa)	-2.17 (MPa)	-2.23 (MPa)	Mean
Cp1	4.1i	3.9j	3.4lm	2.5q	1.3u	3.1F	31.2cd	24.5f-i	16.8jk	5.4n-r	2.6pqr	16.1DE
Cp2	5.0f	4.5h	3.5kl	3.5klm	0.9w	3.5E	38.4ab	30.4cde	16.2jk	2.3pqr	1.2r	17.7CDE
Cp3	6.7a	6.7a	4.8g	3.5kl	1.8t	4.7A	41.9a	35.5bc	25.2e-h	15.6jkl	9.4mno	25.5A
Cp4	3.5kl	3.4lm	2.9o	2.0s	0.9w	2.6G	35.9bc	27.1def	16.9jk	5.1n-r	0.6r	17.1DE
Cp5	3.8j	3.5lm	2.7p	1.4u	1.1v	2.5G	35.2bc	26.5def	16.1jk	1.8qr	0.7r	16.0DE
Cp6	5.4d	5.6d	4.5h	2.7p	1.3u	3.9C	28.3def	19.5ij	17.6jk	7.4nop	4.8n-r	15.5E
Cp7	5.8c	6.0c	3.4lm	2.2r	2.1rs	3.9C	37.0ab	24.4f-i	19.8hij	10.0lmn	7.4n-q	19.7BC
Cp8	6.5b	5.3e	4.6h	2.9o	1.7t	4.2B	35.9bc	20.5g-j	17.2jk	8.4mno	1.6r	16.7DE
Cp9	4.4h	4.6h	4.4h	3.1n	2.2r	3.8D	37.9ab	25.6d-g	18.3jk	9.1mno	0.7r	18.3BCD
Cp10	3.4lm	3.3m	3.9j	3.6k	3.4lm	3.5E	39.3ab	27.1def	18.6jk	13.5klm	4.0o-r	20.5B
Mean	4.9A	4.7B	3.8C	2.8D	1.7E		36.1A	26.1B	18.3C	7.9D	3.3E	
LSD	Drought (0.05), Isolate (0.07), Drought* Isolate (0.16)						Drought (1.77), Isolate (2.51), Drought* Isolate (5.60)					

Table 6. Performance of endophytic bacteria to promote shoot/root length (cm) and dry mass under water deficit axenic conditions.

	Root length (cm) (n = 3)				Shoot length (cm) (n = 3)			
Isolates	**No PEG**	**15%PEG**	**30%PEG**	**Mean**	**No PEG**	**15%PEG**	**30%PEG**	**Mean**
Control	14.6 g-k	12.5 m-p	9.2 rs	12.1 G	15.2 g-m	13.0 opq	11.2 rst	13.2 F
Cr2	19.7 a	15.6 e-h	10.5 qr	15.2 CDE	19.0 a	16.2 e-i	11.0 rst	15.4 CDE
Cr3	19.0 ab	14.1 h-l	11.7 pq	14.9 DEF	18.7 ab	14.1 l-o	12.3 pqr	15.1 DE
Cr10	14.7 g-j	13.0 k-p	15.2 e-h	14.3 F	15.1 h-n	14.0 mno	14.7 j-n	14.6 E
Cs6	13.5 i-o	12.0 n-q	8.5 s	11.3 G	14.1 l-o	11.4 rs	10.3 st	11.9 G
Cs8	18.1 abc	15.5 e-h	14.0 h-m	15.9 ABC	18.5 ab	16.5 c-g	15.3 g-m	16.8 A
Cs10	16.5 def	17.6 bcd	15.0 f-i	16.3 AB	16.8 c-f	17.9 abc	13.0 opq	15.9 BC
Cl3	13.7 i-m	11.8 pq	8.9 s	11.6 G	13.7 nop	12.2 qr	9.9 tu	11.9 G
Cl6	18.1 abc	13.0 k-p	12.6 l-p	14.6 EF	17.8 a-d	14.0 mno	12.9 opq	14.9 DE
Cl7	13.6 i-n	11.9 opq	8.7 s	11.4 G	14.3 k-o	12.3 pqr	8.7 u	11.7 G
Cp3	15.0 f-i	16.0 efg	15.8 efg	15.6 BCD	17.3 b-e	16.4 d-h	16.0 e-j	16.6 AB
Cp7	18.1 abc	14.2 h-k	17.6 bcd	16.6 A	18.6 ab	14.9 i-n	16.3 e-i	16.6 AB
Cp10	14.8 ghi	13.2 j-p	16.7 cde	14.9 DEF	15.5 f-l	15.9 f-j	15.6 f-k	15.7 CD
Mean	16.1 A	13.9 B	12.6 C		16.5 A	14.5 B	12.9 C	
LSD	Drought (0.44), Isolate (0.91), Drought* Isolate (1.57)				Drought (0.41), Isolate (0.82), Drought* Isolate (1.42)			

	Root dry weight (g) (n = 3)				Shoot dry weight (g) (n = 3)			
Isolates	**No PEG**	**15%PEG**	**30%PEG**	**Mean**	**No PEG**	**15%PEG**	**30%PEG**	**Mean**
Control	1.04 c-g	0.53 mno	0.22 p	0.60 EF	0.24 e-m	0.23 g-n	0.18 l-o	0.22 DE
Cr2	1.23 a-e	0.97 f-i	0.21 p	0.81 CD	0.28 d-j	0.30 c-g	0.12 opq	0.23 CDE
Cr3	1.28 abc	0.79 h-k	0.36 op	0.81 CD	0.31 b-e	0.22 h-n	0.21 j-n	0.25 CD
Cr10	1.13 c-g	0.73 j-m	0.78 i-l	0.88 BC	0.25 e-l	0.24 e-m	0.25 e-m	0.25 CD
Cs6	0.55 l-o	0.21 p	0.21 p	0.32 G	0.23 f-n	0.18 l-o	0.06 q	0.16 F
Cs8	1.27 a-d	1.15 b-g	0.97 f-i	1.13 A	0.36 abc	0.31 b-e	0.24 f-m	0.30 AB
Cs10	1.20 b-f	1.46 a	0.38 nop	1.01 AB	0.31 b-f	0.30 c-g	0.22 i-n	0.27 ABC
Cl3	1.11 c-g	0.37 op	0.19 p	0.56 F	0.25 e-l	0.17 mno	0.16 nop	0.20 EF
Cl6	1.01 e-i	0.62 k-n	0.48 no	0.70 DE	0.35 a-d	0.25 e-m	0.21 j-n	0.27 ABC
Cl7	0.55 l-o	0.21 p	0.17 p	0.31 G	0.22 h-n	0.19 k-o	0.09 pq	0.17 F
Cp3	1.13 c-g	1.06 c-g	1.03 d-h	1.07 A	0.41 a	0.29 c-i	0.24 f-m	0.31 A
Cp7	1.37 ab	0.94 g-j	1.04 c-g	1.12 A	0.38 ab	0.25 e-l	0.18 l-o	0.27 ABC
Cp10	1.21 b-f	1.06 c-g	1.04 c-g	1.10 A	0.31 b-f	0.26 e-k	0.24 f-m	0.27 BC
Mean	1.08 A	0.78 B	0.54 C		0.30 A	0.25 B	0.18 C	
LSD	Drought (0.07), Isolate (0.14), Drought* Isolate (0.24)				Drought (0.02), Isolate (0.04), Drought* Isolate (0.07)			

*PEG: Polyethylene glycol

The cell count and OD values of the bacterial isolates from pods (Table 5) showed significant decrease with increase in PEG induced water deficit conditions (i.e. -0.05, -0.65, -1.57, -2.17 and -2.23 MPa). At -0.65 MPa, Cp3, Cp6 and Cp7 were most prominent for OD while Cp2, Cp3, Cp4 and Cp10 showed significantly higher cell count. Bacterial isolates Cp3, Cp6 and Cp8 showed higher OD at -1.57 MPa. At -2.17 and -2.23 MPa, bacterial isolates (Cp2, Cp3, Cp10) and (Cp7, Cp9, Cp10), respectively, remained prominent for OD among other bacterial isolates. Bacterial isolates Cp3, Cp7 and Cp10 showed significantly higher cell count at -1.57 and -2.17 MPa. At -2.23 MPa, significantly higher cell counts were recorded for Cp3, Cp6 and Cp7. Overall analysis of the OD values and cell count data, the bacterial isolates Cp3, Cp7 and Cp10 were selected as most efficient water deficit stress abiding bacterial strains among other bacterial isolates.

***Plant growth promoting activity of endophytic bacteria under water deficit stress*:** Water deficit stress tolerant bacterial isolates (Cr2, Cr3, Cr10, Cs6, Cs8, Cs10, Cl3, Cl6, Cl7, Cp3, Cp7 and Cp10) selected from OD values and cell counts at various PEG-6000 induced water potential levels were tested for their potential as plant growth promoting bacteria. Chickpea seedling growth was observed with the inoculation of endophytic isolates (Cr2, Cr3, Cr10, Cs6, Cs8, Cs10, Cl3, Cl6, Cl7, Cp3, Cp7 and Cp10) under normal (No PEG) and water deficit stress (PEG 15 and 30%) situations. Water deficit stress caused significant decrease in the length (21%) and dry biomass of root (50%) and shoot (40%) (Table 6).

Under normal conditions, inoculation with Cr2, Cr3, Cs8, Cs10, Cl6, and Cp7 showed a significant increase in root length in comparison to un-inoculated control (Table 6). At

15% PEG, Cr2, Cr3, Cs8, Cs10, Cp3, and Cp7 significantly increased the root length as compared to respective un-inoculated control. At 30% PEG, Cr3, Cr10, Cs8, Cs10, Cl6, Cp3, Cp7 and Cp10 inoculation significantly increased root length over un-inoculated control. However, maximum root length was recorded by inoculation with Cr2, Cs10, Cp7 at no PEG, 15 and 30% PEG, respectively. Inoculation of Cs8, Cs10 and Cp7 appeared to be the most efficient for increasing root length (51%, 63%, and 90%, respectively) as a whole.

Shoot length (Table 6) was increased significantly by the inoculation with (Cr2, Cr3, Cs8, Cs10, Cl3, Cl6, Cp3 and Cp7, Cr2, Cs8, Cs10, Cp3, Cp7 and Cp10), and (Cr10, Cs8, Cs10, Cl6, Cp3, Cp7 and Cp10) over respective un-inoculated controls at no PEG, 15 and 30% PEG, respectively. Maximum shoot length at no PEG, 15 and 30% PEG was observed due to the inoculation of Cr2, Cs10 and Cp7, respectively. Whereas overall efficiency of Cs8, Cp7 and Cp10 inoculation was prominent among other bacterial isolates for improving shoot growth.

Root dry weight (Table 6) was significantly increased only by the inoculation of Cp7 over un-inoculated control at normal conditions except Cs6 and Cl7 which significantly decreased it. At 15% PEG, Cs10 inoculation showed maximum root dry biomass but all other bacterial isolates except Cr3, Cl3 and Cl6 remained statistically different from un-inoculated control. The bacterial isolates from pods (Cp3, Cp7, Cp10) showed maximum and significant increase in the root dry biomass over un-inoculated control at 30% PEG followed by Cs8, Cr10 and Cl6, respectively. Looking into the main effects of endophytic bacteria inoculation, bacterial isolates from roots (Cr2, Cr3, Cr10), pods (Cp3, Cp7, Cp10) and stem (Cs8, Cs10) showed significant increase in root dry biomass as compared to un-inoculated control whereas bacterial isolates from leaf (Cl3, Cl6) remained statistically at par with control and Cs6 and Cl7 reduced the root dry biomass.

Shoot dry biomass (Table 6) was significantly increased over un-inoculated control by Cs8, Cl6, Cp3 and Cp7 under normal conditions. Only Cs8 inoculated seedlings showed a significant increase over un-inoculated control at 15% PEG whereas other all inoculations remained statistically similar to control. At 30% PEG, inoculation with endophytic bacteria remained similar to un-inoculated control for shoot dry biomass. However, Cs6 and Cl7 inoculation reduced the shoot dry biomass at 30% PEG as compared to un-inoculated control. The main effect of inoculation showed no effect of isolates from roots but a significant improvement with isolates from pods.

As a whole, bacterial isolates Cs8 and Cp7 inoculation showed significant improvements in root/shoot length and root/shoot dry biomass as compared to un-inoculated control followed by Cs10 (increased root length, root/shoot dry biomass) and Cp3 (increased shoot length, root/shoot dry biomass) and remained prominent among the inoculated isolates.

Dry matter stress tolerance index (Figure 1) was significantly improved by the inoculation of Cr2, Cs8, Cs10, Cl3, Cp3 and Cp10 under 15% PEG induced water deficit stress in comparison to un-inoculated control. At 30% PEG induced water deficit stress, Cr10, Cs8, Cp3 and Cp10 significantly improved the dry matter stress tolerance index as compared to un-inoculated control. Bacterial isolates Cs8, Cp3 and Cp10 were most efficient among other inoculated bacterial isolates for improving dry matter stress tolerance index.

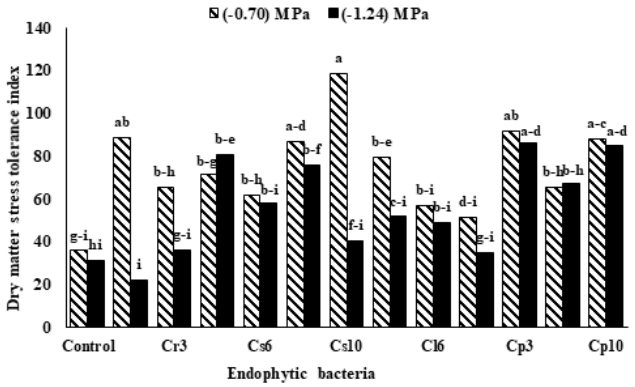

Figure 1. Performance of endophytic bacteria to promote dry matter stress tolerance index under water deficit axenic conditions. (n = 3).

***Characterization of selected endophytic bacteria*:** The bacterial isolates from stem Cs8, Cs10 belonged to *Ochrobactrum* genius whereas the bacterial isolates from pods (Cp3, Cp7) were *Stenotrophomonas* (Table 7). All the bacterial strains were capable to produce catalase, oxidase and exopolysaccharides. Endophytic bacterial isolates from stem had higher aggregation capability (Cs8, Cs10; 4.40%, 4.27%,

Table 7. Characterization of endophytic bacteria.

Characters of different endophytic isolate	Cs8	Cs10	Cp3	Cp7
Aggregation assay	4.40%	4.27%	3.45%	3.12%
Auxin production (Without L-TRP)	3.23 mg mL^{-1}	2.98 mg mL^{-1}	3.60 mg mL^{-1}	3.78 mg mL^{-1}
(With L-TRP)	19.89 mg mL^{-1}	21.29 mg mL^{-1}	25.67 mg mL^{-1}	29.53 mg mL^{-1}
Catalase	+++	++	++	+
Oxidase	+	+	+	+
Exopolysaccharides	+++	++	++	++

respectively) than the bacterial isolates from pods (Cp3, Cp7; 3.45%, 3.12%, respectively). Auxin production in the absence of L-tryptophan was higher by Cp7 (3.78 mg mL^{-1}) followed by Cp3 (3.60 mg mL^{-1}), Cs8 (3.23 mg mL^{-1}) and Cs10 (2.98 mg mL^{-1}), respectively. In the presence of L-tryptophan, production of auxin was increased in all the isolates as Cs8, Cs10, Cp3, and Cp7, 19.89, 21.29, 25.67 and 29.53 mg mL^{-1}, respectively.

DISCUSSION

Water deficit stress is main constraint to the survival of plant growth promoting bacteria in the rhizosphere (Vurukonda *et al*., 2016). Whereas the plant have lower osmotic potential as compared to rhizosphere in order to uptake water and nutrients for their growth and development (Hsiao, 1979; Nobel, 1999). Endophytic bacteria identified in this study are capable to survive and grow at substantially low osmotic potential (-2.23 MPa) isolated from different tissues (root, stem, leaves, pods) of chickpea. Bacteria show varying capacity to adapt osmotic stress conditions (Naz *et al*., 2009; Sgroy *et al*., 2009) depending on their genetic diversity and makeup (Trabelsi *et al*., 2009). Survivability of bacteria might be enhanced by different mechanisms like osmoregulation with the production and accumulation of compatible osmolytes (trehalose, ectoine, glycine betaine) and extracellular proteases and change in cell morphology (Busse and Bottomley, 1989; Smith and Smith, 1989; Das *et al*., 2015). Accumulation of glutamate and ionic potassium inside the bacterial cell maintains the water relation and safeguard it from severe osmotic stress (Botsford and Lewis, 1990). Bacterial ability to produce exopolysaccharides, oxidase and catalase helps them to sustain under drought through the development of multicellular layer (biofilm) and scavenging reactive oxygen species to avoid cell/ membrane/ nucleic acid rupture (Goyal *et al*., 1986; Boumahdi *et al*., 1999; Vanderlinde *et al*., 2010). Therefore, amino acid, proline, soluble sugars and exopolysaccharides producing ability of bacteria are regarded as indicators of drought tolerance (Vardharajula *et al*., 2011). Possibility for water deficit stress adaptability by endophytic bacteria is certain due to their niches inside the different tissues of plants where osmotic potential likely to be at the lowest in comparison to the rhizosphere. Therefore, these water deficit stress tolerant endophytes could be a potential resource for improving plant growth under drought.

Top three strains capable to survive under PEG induced water deficit stress were selected from each niche (root, stem, leaves, pods) and tested for improving the growth of chickpea seedlings under drought in gnotobiotic conditions. Though, growth of seedlings was reduced significantly due to water deficit stress as compared to control. However, inoculation with selected bacteria significantly improved the root/shoot length and biomass over un-inoculated plants. Drought disrupts the normal functioning of plant varying their physiological and biochemical responses, whereas, plant growth promoting bacteria induce certain plant functioning to ameliorate/reduce the stress impact (Yang *et al*., 2009; Vardharajula *et al*., 2011). Improvement in growth and biomass of chickpea seedlings under water limited conditions in this study might be due to the ability of endophytic strains to produce auxins (for increased root growth to increase exploring area), exopolysaccharides (to increase water and nutrient holding capacity of rhizosphere). Moreover, the production of siderophores, nutrient solubilization, and plant growth hormones like gibberellin and cytokinins would have participated in improving water deficit stress tolerance and improved growth of chickpea seedlings (Hallmann *et al*., 1997; Rosenblueth and Martinez-Romero, 2006; Mitter *et al*., 2013). Bacteria having the ability to colonize roots at a higher rate can improve drought tolerance of plants through increasing P nutrition and vigor of the plant under stress (Arachevaleta *et al*., 1989; Hallmann *et al*., 1997; Verma *et al*., 2001; Azevedo and Araujo, 2003; Wakelin *et al*., 2004).

***Conclusions*:** Endophytic bacterial isolates from stem and pods of chickpea seemed to be the prominent among the inoculants. These isolates improve the growth of chickpea seedlings normal as well as in water deficit conditions. Role of these endophytic bacteria for promoting rhizobia-legume symbiosis may also be explored in future.

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