International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Isolation and Testing of Bacterial Endophytes for Seed Quality Parameters Under Induced Moisture Stress in Rabi Sorghum (Sorghum Bicolor L.)

R M Channappagoudar[1], N K Biradarpatil [2], Ashok S Sajjan[3], P U Krishnaraj[4], M S L Rao[5]

[1]Ph.D (Agri.) Student, E-mail: rajashri6196@gmail.com
[2]Professor of (SS&T), Former Dean (PGS), University of Agricultural Sciences, Dharwad-580 005, Karnataka (India).
[3]Professor of (SS&T), University of Agricultural Sciences, Dharwad, Karnataka, (India).
[4]Professor of Microbiology, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad, Karnataka, (India).
[5]Professor, Dept. of Plant Pathology, College of Agriculture, Dharwad, University of Agricultural Sciences, Dharwad, Karnataka, (India).

Abstract

Rabi sorghum is the most important post rainy season cereal crop in peninsular India, grown predominantly under rainfed conditions. There is decline in the productivity of *rabi* sorghum under rainfed areas. Many endophytic bacteria live in association with their host and play crucial role in health and development of plant. The aims of this work were to isolate and testing of the bacterial endophytes for seed quality parameters of *rabi* sorghum under induced moisture stress (0%, 5%, 10%, 15%, 20% and 25% PEG 8000). The isolation was done from root, stem and seeds of sorghum plant. Among the five isolates tested for plant growth promoting traits AUST 3 recorded higher amount of IAA (15.25 µg/ml broth) and inorganic phosphate solubilization (14.73 ppm) and all the five isolates along with reference isolate were positive for ACC deaminase activity. Data indicated that increase in osmotic stress caused a significant decrease in seed quality parameters in all treatments under study. The isolate AUST 3 recorded higher seed germination (46.67%), root length (7.77 cm), shoot length (6.63 cm) and seedling vigour index (673) at highest PEG concentration (25% PEG 8000). From the overall results it was concluded that the promising isolate AUST showed comparatively better performance than other endophytes under higher concentration of PEG induced water stress.

Key words: *Sorghum bicolor*, isolation, polyethylene glycol and endophytes

---

***** *Part of the Ph.D (Agri.) Thesis submitted by the first author to the Department of Seed Science and Technology, College of Agriculture, Dharwad, UAS, Dharwad – 580 005, India.*

Introduction

Sorghum [*Sorghum bicolor* (L.)] is grown primarily in arid and semi-arid regions of India (Maharashtra, Andhra Pradesh, Karnataka and Tamil Nadu). India contributes about 16 per cent to the global sorghum production. The *rabi* sorghum accounts 2735.62 hectare of area with 2825.73 tonnes production and 1033 kg ha^{-1} productivity (Anon., 2021) in India.

In arid and semi-arid regions, drought is regarded as the primary abiotic stress. Moisture stress has the greatest impact on plant growth and production than any other abiotic stress (Shao *et al.*, 2009). Water stress causes irregular seed germination, poor and non-uniform establishment of seedlings and reduction of stem diameter, stem length, leaf area (Anjum *et al.*, 2011). The development of techniques for fast and homogeneous growth of seeds could be a sustainable approach for better agricultural productivity (Osburn and Schroth, 1989). In this context, improving seed quality and germination, as well as establishing a good plant stand, through 'seed priming' is a sustainable approach for increasing crop yield and performance (McDonald, 2000). The use of plant growth promoting endophytes to enrich the micronutrients in plants is one of the revolutionary ways to improve crop productivity as well as seed quality. These are the organisms residing inside the plant tissue without causing any harm to the plants.

The inoculation of beneficial bacteria through seed bio-priming results in increased plant growth and development. Because of their greater colonization adaptability under biotic and abiotic stress conditions, endophytic microbial strains appear to be more beneficial and stable than rhizospheric microbial strains for seed priming. Breeding methods are time-consuming, labor-intensive and expensive. As a result, in recent years, the plant-biotic approach, which incorporates the use of microbes such as endophytes, has emerged as an alternative cultural method for sustainable agriculture.

Methodology

Experimental site

The research studies were carried out in the Department of Seed Science and Technology and Microbial Genetics Laboratory and the pot experiment was conducted in the Department of Agronomy, College of Agriculture, University of Agricultural Sciences, Dharwad.

Seed Source

The seeds of sorghum variety SPV 2217 were procured from the Seed Unit, University of Agricultural Sciences, Dharwad, Karnataka for conducting the experiment

Isolation of bacterial endophytes

The plant samples were washed under running tap water for 10-15 min. to remove adhering soil particles and air-dried. The separated plant roots, stems and seeds were weighed up to one gram on a weighing balance. The samples were then surface-sterilized by dipping in 70% ethanol for 1 min, 3% sodium hypochlorite for 3 min. followed by 70% ethanol wash for 1 min (Kharwar *et al.*, 2008). They were then rinsed four times with sterile water and air dried in laminar flow.

These surface sterilized samples were then macerated in one ml of distilled water in pestle and mortar. The dilutions were prepared upto 10^{-5} dilutions. One hundred micro liters from each dilution of the respective sample was then poured in their respective petri plates so labeled from 10^{-1} to 10^{-5} containing nutrient agar medium (NA) and then spread with spreader for the isolation of the bacterial endophytes. The plating was done in triplicate for each dilution. The plates were incubated at 37 $^{\circ}$C for 72 - 96 hrs

(Long *et al*., 2003). Sterility check was performed by imprinting the surface sterilized plant samples of roots, stems and seeds in the media.

Seed priming with endophytes under PEG 8000 induced moisture stress

The experiment was conducted by selecting the five potent endophyte strains which showed significant tolerance for drought and salinity. The seeds were primed with potent endophytes strains along with reference strains in nutrient broth for 12 hr and further air dried. The blotter paper soaked with different concentrations of PEG 8000 used for germination.

Indole acetic acid (IAA) production

IAA production in different isolates of endophytes were detected according to Gordon and Weber (1951) by inoculating bacterial suspension in 10 ml nutrient broth containing L- tryptophan 5mM (L-Trp) separately and incubated at 28±2 °C @ 180 rpm for 3 days. The culture was centrifuged at 5000 rpm for 5 min. Appearance of pink colour confirmed the production of IAA. The amount of IAA (µg ml^{-1}) was determined quantitatively by adding 1 ml of Salkowski's reagent (1 ml of 0.5ml $FeCl_3$ in 50 ml of 35% perchloric acid) to 1 ml of culture supernatant along with uninoculated broth with Salkowski's reagent as a reference. The mixture was incubated in dark for 30 min @ room temperature. Absorbance of pink colour was measured spectroscopically (Biospectrophotometer Basic, Eppendof) at 535 nm after 20 min and quantified IAA concentration by standard curve.

Estimation of inorganic phosphate solubilized by endophytic isolates

Phosphate solubilization, of the cultures were tested for estimation of inorganic phosphate solubulized by endophytic bacteria in liquid medium which contains $Ca_3 (PO_4)_2$, at the concentration of 5 g l^{-1} as phosphorous source. The bacterial endophytes were inoculated to 100 ml of Pikovskaya broth (Pikovskaya, 1948). The flasks were incubated at 28 °C for 15 days. After 5 days, the Pikovskaya broth cultures were centrifuged at 9000 rpm for 20 mins. The supernatant was used for the estimation of inorganic phosphorus released by cultures by molybdenum-blue method (Murphy and Riley, 1962).

One millilitre of the supernatant of each bacterial isolate was taken in a 50 ml volumetric flask. To this, add 10 ml of chloromolybdic acid and mix thoroughly and the volume was made to three-fourth with double distilled water. Add 0.25 ml of Chlorostannous acid and volume was made upto 50 ml with double distilled water. The blue colour was developed after 15 minutes and spectrophotometer readings were taken at 610 nm. Blank readings were also taken. Standard graph was plotted using known concentrations of standard potassium dihydrogen phosphate solution (KH_2P0_4). The amount of inorganic phosphorus released into broth was calculated from the standard graph.

ACC deaminase activity

The qualitative estimation of ACC deaminase in the endophytic bacterium was done by method of Govindasamy *et al*. (2008) by streaking on Dworkin and Foster (DF) medium plates (Dworkin and Foster, 1958) minimal medium containing ACC as only nitrogen supply. Incubated was done at 28±2 °C for 3-4 days and observed for growth of different bacterial isolates.

Molecular identification of bacterial endophytes

Efficient bacterial isolates known for its beneficial traits were characterized by 16S rRNA gene (rDNA)

sequence analysis. The genomic DNA was isolated according to Chachaty and Saulnier, 2000. The DNA fragment was amplified from 16S rRNA gene. After amplification the fragment got sequenced after purification from Eurofins, Bangalore. The sequences were aligned with NCBI database using BLAST programme.

Primer	Sequence	Reference
27F	5'AGAGTTTGATCCTGGCTCAG 3'	Lane, (1991)
1492R	5'ACGGCTACCTTGTTACGACTT 3'	

Treatment details

Factor I: Endophyte strains (best 5 isolates, E_1-E_5)
Reference strain E6 (*Pseudomonas fluorescens*)
E_7: Control

Factor II: Drought conditions
D_1: 0% PEG 8000
D_2: 5 % PEG 8000
D_3: 10 % PEG 8000
D_4: 15 % PEG 8000
D_5: 20 % PEG 8000
D_6: 25 % PEG 8000

Observations recorded
Seed germination (%)
Hundred seeds in four replications were drawn at random from each treatment and the germination test was conducted by using Paper towel method as per the International Seed Testing Association procedure (Anon., 2011) in a cabinet maintained at 25±1 $^{\circ}$C constant temperature and 95 per cent relative humidity. The number of normal seedlings was counted manually at the end of tenth day to assess the total germination.

Root length (cm)
Ten normal seedlings in each sample were randomly chosen for the measurement of root length on the day of final count (10^{th} day) and average was calculated. The length of the root was measured from collar region to the tip of root and expressed in centimeter (cm).

Shoot length (cm)
Ten normal seedlings in each sample were randomly chosen for the measurement of shoot length on the day of final count (10^{th} day) and average was calculated. The shoot length was measured from the collar region to the point of attachment of cotyledon tip and expressed in centimeter (cm).

Seedling vigour index
The seeding vigour index was calculated by adopting the method suggested by Abdul - Baki and Anderson (1973) and expressed in number by using below formula.

Seedling vigour index I = Germination (%) × [Root length (cm) + Shoot length (cm)]

Results and Discussion

Molecular identification of the potent bacterial endophytes

Sequencing of the 16S rRNA gene has served as an important tool for molecular identification of bacteria over a number of years. The isolates AUST 3 and AUST 20 were identified as *Staphylococcus edaphicus* and *Myroides odoratimimus* respectively with 99 percent similarity (Table 1). In the present study, the efficient endophyte isolated from sorghum root belonged to group *Staphylococccus edaphicus* which showed plant growth promoting traits IAA production, phosphate solubilization, GA production and having antifungal activity. The similar results were reported by Araujo *et al*., 2020, where the isolate *S. edaphicus* promotes the maize plant growth under hydric stress and having the PGP traits. The other isolate AUST 20 (*M. odoratimimus*) has shown to improve the plant growth under moisture and salt stress condition in the present study is reported to be low human pathogen (Maraki *et al*., 2012). The *Myroides* potassium-solubilizing bacteria (KSB) improve the plant dry weight and uptake of both K and nitrogen (N) by tobacco seedlings and helps in maintaining the availability of soil nutrients (Zhang and Kong, 2014). Due to their human pathogenic nature care should be taken while using them as bioinoculant as it may lead to spread of disease

Plant growth promoting traits of the bacterial endophytes

The results of the plant growth promoting traits of the bacterial endophytes are presented in the Table 2.

Indole acetic acid (IAA) production is one of the plant growth promoting properties of endophytic bacteria that stimulate and facilitate plant growth. Among the isolates tested, AUST 3 (*S. edaphicus*) produced maximum amount of IAA (15.25 µg ml^{-1} broth), followed by the reference isolate, *P. fluorescens* (15.20 µg ml^{-1} broth) and AUST 20 - *M. odoratimimus* (13.60 µg ml^{-1} broth). IAA is a phytohormone that regulates many important physiological processes in plants, including tissue differentiation, root initiation and elongation, cell enlargement and division (Leveau and Lindow, 2005). Microorganisms that produce IAA promote the overall development and enlargement of plant roots, resulting in a larger root surface area and the plant's ability to obtain more nutrients from the soil (Boiero *et al.*, 2007).

The highest phosphate solubilization potential was shown by the reference isolate *P. fluorescens* (14.73 ppm) followed by AUST 3 - *S. edaphicus* (14.50 ppm) and the least was recorded by AUST 55 (7.33 ppm). As potential phosphate solubilizers, bacteria capable of producing a halo/clear zone due to inorganic phosphate solubilization in the surrounding medium were chosen. Phosphate solubilizing bacteria (PSB) either directly synthesis organic acids such as gluconic and citric acid or indirectly solubilize P by lowering soil pH, converting insoluble phosphate to soluble form that plants can absorb (Bashan *et al.*, 2013). PSB inoculants with beneficial traits could be used as a biofertilizer in sustainable agriculture (Panhwar *et al.*, 2012).

The role of ACC-deaminase in reducing stress ethylene levels via enzymatic hydrolysis of ACC into -ketobutyrate and ammonia has been proposed as one of the critical mechanisms of PGPB in promoting plant growth under extreme environmental conditions. Microbial species that exhibit optimum growth and ACC-deaminase activity under extreme environmental conditions are likely to be useful in extreme environmental locations. All five selected bacterial endophytes have shown positive result for ACC deaminase activity.

Effect of seed priming with bacterial endophytes on seed quality parameters under PEG 8000 induced moisture stress

Among the interaction effects, the treatment E_4 (AUST 44) had significantly highest germination (93.67%) at D_1 (0% PEG-8000) osmotic stress level followed by E_6 (*P. fluorescens)* has recorded on par seed germination (93.00%) at D_1 (0% PEG-8000) and significantly least seed germination (35.67%) was recorded in the E_7 (control) at D_6 (25% PEG-8000) higher osmotic stress level) (Table 3). It was observed that germination percentage with decreasing water potential of the environment probably was caused by the low hydraulic conductivity of the environment, where PEG 6000 makes water unavailable to seeds, affecting the imbibitions process of the seed which is fundamental for germination (Lobato *et al.*, 2008).

Among the interaction effects, the treatment E_2 (AUST 20) had significantly highest shoot length (18.23 cm) at D_1 (0% PEG-8000) osmotic stress level followed by E_1 (AUST 3) has recorded shoot length (17.57 cm) at D_1 (0% PEG-8000) and significantly least shoot length (4.13 cm) was recorded in the E_7 (control) at D_6 (25% PEG-8000) osmotic stress level (Table 4). Reduced seedling growth may also be due to the inhibition of processes such as cell division, enlargement and differentiation caused by water deficit condition (Schmidhalter and Oertli, 1991). Moisture stress greatly affected the hypocotyl length rather than growth of radical which indicates that the length of hypocotyl was more sensitive to drought stress (Kumar *et al*., 2011).

Among the interaction effects, the treatment E_2 (AUST 20) had significantly highest root length (15.17cm) at D_1 (0% PEG-8000) osmotic stress level followed by E_1 (AUST 3) has recorded on par root length (15.00 cm) at D_1 (0% PEG-8000) and significantly least root length (4.67 cm) was recorded in the E_7 (control) at D_6 (25% PEG-8000) higher osmotic stress level (Table 5). The effect of PEG induced water stress on reduced growth (root-shoot length) is due to a decrease in moisture absorption by the endosperm and embryonic axis and a delay in carbohydrate translocation to the embryonic axis.

Among the interaction effects, the treatment E_2 (AUST 20) had significantly highest seedling vigour index (3083) at D_1 (0% PEG-8000) osmotic stress level followed by E_1 (AUST 3) has recorded seedling vigour index (2975) at D_1 (0% PEG-8000) and significantly least seedling vigour index (313) was recorded in the E_7 (control) at D_6 (25% PEG-8000) higher osmotic stress level (Table 6).

Conclusion

The present research work was conducted to evaluate germination per cent and early seedling growth stage of endophytic bacteria in *rabi* sorghum through artificially created water stress by PEG of molecular weight 8000 in laboratory conditions. Breeding methods are time-consuming, labor-intensive and expensive. As a result, in recent years, the plant-biotic approach, which incorporates the use of microbes such as endophytes, has emerged as an alternative cultural method for sustainable agriculture. So is suggested that the findings may be helpful and fruitful for selection of moisture stress in sorghum under the discussed traits.

References

1. Abdul-Baki A S and Anderson J D, 1973, Vigour determination in soybean by multiple criteria. *Crop Science,* 13: 630-633.

2. Anjum S A, Farooq M, Wang L C, Xue L L, Wang S G, Wang L and Chen M, 2011, Gas exchange and chlorophyll synthesis of maize cultivars are enhanced by exogenously-applied glycinebetaine under drought conditions. *Plant, Soil and Environment*, 57(7): 326-331.
3. Anonymous, 2011, ISTA-International Rules for Seed Testing. Annual meeting of International Seed Testing Association, 31: 1-152.
4. Anonymous, 2021, Area, production and productivity of *rabi* sorghum. (*www.indiastat.com*).
5. Araújo V L, Junior M A, de Souza Júnior V S, de Araújo Filho J C, Fracetto F J, Andreote F D, *et al*., 2020, Bacteria from tropical semiarid temporary ponds promote maize growth under hydric stress. *Microbiological Research*, 1: 240.
6. Bashan Y, Kamnev A A, de Bashan L E, 2013, A proposal for isolating and testing phosphate-solubilizing bacteria that enhance plant growth. *Biology and Fertility of Soils*, 49: 1-2
7. Boiero M L, Perrig D, Masciarelli O A, Penna C, Ruiz O A, Cassán F D *et al*., 2007, Plant-growth-promoting compounds produced by two agronomically important strains of *Azospirillum brasilense* and implications for inoculant formulation. *Applied Microbiology and Biotechnology*, 75(5): 1143-1150.
8. Chachaty E and Saulnier P, 2000, Isolating chromosomal DNA from bacteria. In: Rapley, R.(Ed.), The nucleic acid protocols handbook, *Humana Press Inc, Totowa*,1(12): 29-32.
9. Dworkin M and Foster J, 1958, Experiments with some microorganisms which utilize ethane and hydrogen. *Journal of Bacteriology*, 75(5): 592-603.
10. Gordon S A and Weber R P, 1951, Colorimetric estimation of indoleacetic acid. *Plant Physiology*, 26(1): 192.
11. Govindasamy V, Senthilkumar M, Gaikwad K and Annapurna K, 2008, Isolation and characterization of ACC deaminase gene from two plant growth-promoting rhizobacteria. *Current Microbiology*, 57(4): 312-317.
12. Kharwar R, Verma V, Strobel G, Ezra D, 2008, The endophytic fungal complex of *Catharanthus roseus* (L.) G. Don. *Current Science*, 95: 228-232.
13. Kumar R R, Karajol K, Naik G R, 2011, Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeonpea (*Cajanus cajan* L. Millsp). *Recent Research in Science and Technology*, 3(1): 148-152.
14. Lane D J, 1991, 16S/23S rRNA sequencing. Nucleic acid techniques in bacterial systematic, New York, p. 115-218.
15. Leveau J H and Lindow S E, 2005, Utilization of the plant hormone indole-3-acetic acid for growth by *Pseudomonas putida* strain 1290. *Applied and Environmental Microbiology*, 71(5): 2365-2371.
16. Lobato A K S, Neto, O, Costa R C L, Santos Filho, B G, Silva F K S, Cruz, F J R, *et al*., 2008, Germination of sorghum under the influences of water restriction and temperature. *The Journal of Agricultural Science*, 3: 220-224.
17. Long H H, Furuya N, Kurose D, Takeshita M and Takanami Y, 2003, Isolation of endophytic bacteria from *Solanum* sp. and their antibacterial activity against plant pathogenic bacteria. *Journal of Faculty of Agriculture, Kyushu University*, 48: 21-28.
18. Maraki S, Sarchianaki E and Barbagadakis S, 2012, *Myroides odoratimimus* soft tissue infection in an immunocompetent child following a pig bite: case report and literature review. *The Brazilian Journal of Infectious Diseases*, 16(4): 390-392.

19. McDonald M B, 2000, Seed priming. In: Black M, Bewley J D, Seed technology and its biological basis. Sheffield Academic Press, Sheffield, p 287-325.
20. Murphy J A and Riley J P, 1962, A modified single solution method for the determination of phosphate in natural waters. *Analytica Chimica Acta*, 1(27): 31-6.
21. Osburn R M and Schroth M N, 1989, Effect of osmopriming sugar beet seed on exudation and subsequent damping-off caused by *Pythium ultimum. Phytopathology*, 78: 1246-1250.
22. Panhwar Q A, Othman R, Rahman Z A, Meon S and Ismail M R, 2012, Isolation and characterization of phosphate-solubilizing bacteria from aerobic rice. *African Journal of Biotechnology*, 11(11): 2711-2719.
23. Pikovskaya R I, 1948, Mobilization of phosphorus in soil in connection with vital activity of some microbial species. *Mikrobiologiya*, 17: 362-370.
24. Schmidhalter U and Oertli J J, 1991, Germination and seedling growth of carrots under salinity and moisture stress. *Plant and Soil*, 132(2): 243-251.
25. Shao H B, Chu L Y, Jaleel C A, Manivannan P, Panneerselvam R and Shao M A, 2009, Understanding water deficit stress-induced changes in the basic metabolism of higher plants biotechnologically and sustainably improving agriculture and the eco-environment in arid regions of the globe. *Critical Reviews in Biotechnology*, 29(2): 131-151.
26. Zhang C and Kong F, 2014, Isolation and identification of potassium-solubilizing bacteria from tobacco rhizospheric soil and their effect on tobacco plants. *Applied Soil Ecology*, 82: 18-25.

Table 1: Percent identity of potent bacterial endophytes through 16S rRNA gene sequencing.

Sl No.	Isolates	Organism Name	Accession Number	Percent identity
1	AUST 3	*Staphylococcus edaphicus*	MRZN01000038.1	99%
2	AUST 20	*Myroides odoratimimus*	CP037427.1	99%

Table 2: Plant growth promoting traits of the bacterial endophytes

Isolates	**IAA (µg/ml broth)**	**P solubilization (ppm)**	**ACC deaminase activity**
AUST 3	15.25	14.50	+
AUST 20	13.60	11.32	+
AUST 29	8.05	8.44	+
AUST 44	11.05	9.56	+
AUST 55	7.23	7.33	+
Pseudomonas fluorescens	15.20	14.73	+

Note: IAA- Indole acetic acid, ACC deaminase - Alpha Amino cyclopropane carboxylic acid (ACC) deaminase

Table 3: Effect of seed priming with bacterial endophytes on seed germination under PEG 8000 induced moisture stress under *in vitro* condition in *rabi* sorghum

Seed germination (%)							
E x D	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**	**Mean (E)**
E_1 : AUST 3	91.33	90.67	86.67	75.67	57.00	46.67	74.67
E_2 : AUST 20	92.33	90.00	85.33	72.33	55.00	45.00	73.33

E_3 : AUST 29	89.33	86.67	76.67	68.33	52.67	40.67	69.06
E_4 : AUST 44	93.67	88.67	82.33	73.00	55.67	44.67	73.00
E_5 : AUST 55	89.00	86.33	74.00	68.67	52.33	39.33	68.28
E_6 : *P. fluorescens*	93.00	88.00	80.33	71.67	54.67	43.67	71.89
E_7 : Control	87.33	83.67	70.33	65.33	48.00	35.67	65.06
Mean (D)	90.86	87.71	79.38	70.71	53.67	42.24	
		S.Em ±			**CD at 1 %**		
E		0.44			1.64		
D		0.41			1.52		
E x D		1.08			4.02		

Note: E = Endophytes, D = Moisture stress

Moisture stress levels: D_1 = PEG 8000 0%, D_2 = PEG 8000 5%, D_3 = PEG 8000 10%, D_4 = PEG 8000 15%, D_5 = PEG 8000 20%, D_6 = PEG 8000 25%

Table 4: Effect of seed priming of bacterial endophytes on shoot length under PEG 8000 induced moisture stress under *in vitro* condition in *rabi* sorghum

Shoot length (cm)							
E x D	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**	**Mean (E)**
E_1 : AUST 3	17.57	17.37	13.00	11.73	9.80	6.63	12.68
E_2 : AUST 20	18.23	17.30	12.50	11.00	9.27	6.53	12.47
E_3 : AUST 29	16.57	15.53	11.53	9.50	8.47	4.77	11.06
E_4 : AUST 44	17.17	16.80	14.60	11.57	9.60	5.43	12.53
E_5 : AUST 55	16.53	15.43	11.67	9.20	7.60	4.57	10.83
E_6 : *P. fluorescens*	17.00	16.63	13.23	11.23	8.77	5.50	12.06
E_7 : Control	15.20	13.43	10.67	7.87	6.43	4.13	9.62
Mean (D)	16.90	16.07	12.46	10.30	8.56	5.37	
		S.Em ±			**CD at 1 %**		
E		0.07			0.26		
D		0.06			0.24		
E x D		0.17			0.63		

Note: E = Endophytes, D = Moisture stress

Moisture stress levels: D_1 = PEG 8000 0%, D_2 = PEG 8000 5%, D_3 = PEG 8000 10%, D_4 = PEG 8000 15%, D_5 = PEG 8000 20%, D_6 = PEG 8000 25%

Table 5: Effect of seed priming of bacterial endophytes on root length under PEG 8000 induced moisture stress under *in vitro* condition in *rabi* sorghum

Root length (cm)							
E x D	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**	**Mean (E)**
E_1 : AUST 3	15.00	14.47	13.83	11.23	10.33	7.77	12.11
E_2 : AUST 20	15.17	13.50	12.27	10.03	9.00	7.23	11.20

E_3 : AUST 29	14.20	12.57	10.63	9.37	7.63	5.53	9.99
E_4 : AUST 44	14.73	13.07	12.50	10.33	8.73	6.80	11.03
E_5 : AUST 55	13.23	12.63	10.20	8.93	6.87	5.33	9.53
E_6 : *P. fluorescens*	14.80	13.73	12.67	9.53	9.20	6.53	11.08
E_7 : Control	12.20	11.40	9.53	7.57	6.00	4.67	8.39
Mean (D)	14.19	13.05	11.66	9.57	8.25	6.27	
		S.Em ±			**CD at 1 %**		
E		0.06			0.24		
D		0.06			0.22		
E x D		0.16			0.58		

Note: E = Endophytes, D = Moisture stress

Moisture stress levels: D_1 = PEG 8000 0%, D_2 = PEG 8000 5%, D_3 = PEG 8000 10%, D_4 = PEG 8000 15%, D_5 = PEG 8000 20%, D_6 = PEG 8000 25%

Table 6: Effect of seed priming of bacterial endophytes on seedling vigour index under PEG 8000 induced moisture stress under *in vitro* condition in *rabi* sorghum

Seedling vigour index							
E x D	**D_1**	**D_2**	**D_3**	**D_4**	**D_5**	**D_6**	**Mean (E)**
E_1 : AUST 3	2975	2887	2326	1738	1148	673	1958
E_2 : AUST 20	3083	2772	2113	1521	1005	620	1852
E_3 : AUST 29	2748	2435	1699	1289	849	419	1573
E_4 : AUST 44	2987	2649	2231	1599	1021	548	1839
E_5 : AUST 55	2649	2423	1618	1246	758	390	1514
E_6 : *P. fluorescens*	2957	2673	2081	1487	982	525	1784
E_7 : Control	2393	2077	1351	1009	597	313	1290
Mean (D)	2828	2559	1917	1413	909	498	
		S.Em ±			**CD at 1 %**		
E		9.60			35.78		
D		8.89			33.13		
E x D		23.52			87.65		

Note: E = Endophytes, D = Moisture stress

Moisture stress levels: D_1 = PEG 8000 0%, D_2 = PEG 8000 5%, D_3 = PEG 8000 10%, D_4 = PEG 8000 15%, D_5 = PEG 8000 20%, D_6 = PEG 8000 25%