बाजरा में पादप वृद्धि उत्प्रेरक राइजोबैक्टीरिया द्वारा सूखा के प्रति दैहिक सहनशीलता

PGPR-Elicited Systemic Tolerance to Drought in Pearl Millet (*Pennisetum glaucum* L.)

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PGPR-Elicited Systemic Tolerance to Drought in Pearl Millet (*Pennisetum glaucum* L.)

A Thesis

By

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This is to certify that the thesis entitled **"PGPR-ELICITED SYSTEMIC TOLERANCE TO DROUGHT IN PEARL MILLET (*PENNISETUM GLAUCUM* L.)"** submitted to the Post-Graduate School, Indian Agricultural Research Institute, New Delhi, in partial fulfillment of the requirements for the degree of **DOCTOR OF PHILOSOPHY in MICROBIOLOGY** by **Mr. MANJUNATHA B S** embodies the results of bona fide research work carried out by him under my guidance and supervision. No part of the thesis has so far been submitted anywhere for the publication or for any other degree or diploma.

All the assistance and help received during the course of the investigation has been duly acknowledged.

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Place: ICAR-IARI,

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Dedicated To
My Parents…
&
The Farmers
of India…

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Drought is one of the most common environmental stresses that affect growth and development of plants and continues to be an important challenge to agricultural researchers. Since the dawn of agriculture, mild to severe drought stress has been one of the major production limiting factors. The recent trends in global climate change and increasing erratic weather patterns are likely to aggravate these further. Prolonged drought is one of the most important abiotic stresses limiting global crop production and is likely to further increase in future due to climate change.

If the stress is prolonged, plant growth and productivity are severely diminished. The average yields from the major crop plants may reduce by more than 50% due to drought stress.Climate models have predicted increased severity and frequency of drought under the ongoing global climate change scenarios (IPCC 2007). Drought severely affects plant growth and development with substantial reductions in seed germination, crop growth rate, biomass accumulation and crop productivity. It impairs normal growth, disturbs water relations and reduces water use efficiency in plants. Plant's vegetative as well as reproductive stages are intensely influenced by drought stress. Crop improvement for evolving better varieties can help to tolerate abiotic stresses to some extent. However, such strategies are long drawn and cost intensive. There is a need to develop simple and low cost biological methods for management of abiotic stresses. These are cost-effective and thus, have become an integral part of modern agriculture. Such approaches must enhance and sustain agricultural productivity and at the same time be safe from environmental and health perspectives.

Pearl millet is a major cereal and fodder crop in India. Pearl millet is the staple food of majority of the poor and small land holders, as well as feed and fodder for livestock in the rainfed regions of N-W India and grows primarily in the arid and semi-arid regions. It is usually grown under the most adverse agro-climatic conditions where other crops like sorghum and maize fail to produce economic yields. Pearl millet is one of the most drought resistant field crops. It is able to grow in poor fertility soils and in areas which are frequently exposed to water stress during either the vegetative or reproductive phases. Pearl millet in near future may extend into regions that are too dry due to its exceptional ability to tolerate drought. It is comparatively a hardy crop but

even this crop does not realize its full yield potential under water scarcity conditions. Water deficit stress has significant consequences on the development and growth of pearl millet. Critical stages of pearl millet which are severely affected by water deficit stress are flowering and grain formation phases and these results in drastic reduction in yield.

In India, pearl millet is the third most widely cultivated food crop after rice and wheat. It was grown in 7.128 million ha during 2015-16 (Directorate of Millet Development, 2017). The major pearl millet growing states are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana which account for more than 90% of pearl millet acreage in the country. As an average of the latest data of four years (2012-13 to 2015-16), Rajasthan, Uttar Pradesh, Maharashtra, Haryana and Gujarat accounted for 94.82% of total area under pearl millet and contributed to 87.70% of total production. It is usually grown under rain fed conditions and in regions intermittently exposed to water stress. It is frequently exposed to low moisture conditions during its growth phase and this may lead to40-46% reduction in yield (Radhouane, 2013).

Bacteria play an important role in influencing higher plants' response to abiotic stresses. Beneficial bacteria can improve plant growth and development under stressed conditions and, subsequently, enhance yield (Creus *et al.*, 1998). Many of the drought tolerant microorganisms have showed to be effective in alleviating effects of drought stress in inoculated plants (Marulanda *et al.,* 2007). Osmotolerant exopolysaccharide producing *Pseudomonas putida* strain GAP-P45 helped to mitigate drought stress in sunflower seedlings and maize plants (Sandhya *et al*., 2009; 2010). Enhancement of drought tolerance in plant by plant growth promoting rhizobacteria (PGPR) was for the first time reported in *Arabidopsis thaliana* inoculated with *Paenibacillus polymyxa* B2 (Timmusk and Wagner, 1999). The inoculated plants could survive drought stress remarkably longer compared to the untreated control plant. Drought stress mitigation by PGPRs has also been reported in tomato, pepper, canola, bean, lettuce and sunflower (Mayak *et al.,* 2004; Forchetti *et al*., 2010). *Burkholderia phytofirmans* PsJN has been reported to promote growth under drought stress in a number of plant species, including vegetables, potato and grapevine (Sessitsch *et al*., 2005).The endophytes bacteria improves the germination, seedling emergence, promote plant growth and establishment under adverse environmental conditions and consequently enhance plant growth and yield (Long *et al.,* 2008).

PGPRs have been observed to induce elicitation of a range of abiotic and biotic elicitors for conferring tolerance to drought stress in plants. These induce a number of physical and chemical changes in plants, resulting in enhanced tolerance to abiotic stresses in plants termed as induced systemic tolerance (IST) (Yang *et al*., 2008). *Achromobacter piechaudii* ARV8 isolated from the arid and salty environments of Arava region of southern Israel conferred resistance to drought stress in tomatoes seedlings by reducing the production of stress hormone ethylene (Mayak *et al*., 2004). Inoculation of wheat (*Triticum aestivum*) with *Azospirillum brasilense* Sp245 under drought stress resulted in a better water status and an additional ‘‘elastic adjustment’’ leading to better grain yield and mineral quality (Mg, K and Ca) (Creus *et al*., 2004). Bacterial endophytes actively altered the physiology of the host plant exposed to drought stress (Hardoim *et al*., 2012). Introduction of different rhizobial species, *A. caulinodans* ORS 571, *Sinorhizobium meliloti* 1021 and *Mesorhizobium huakui* 93 enhanced rice growth by stimulating photosynthetic activity and enhancing resistance to drought (Chi *et al*., 2005). Many of the microorganisms augmented levels of antioxidative enzymes in inoculated plants under drought conditions (Kohler *et al*., 2008). Decreased generation of ROS in colonized plants was observed to confer abiotic stress tolerance (Rodriguez *et al*., 2009).

PGPRs are also known to prime the plant against drought stress by inducing the differential expression of drought stress-responsive genes. Inoculation with *Paenibacillus polymyxa* conferred drought tolerance in *Arabidopsis thaliana* through the induction of drought responsive genes, ERD15 (EARLY RESPONSE TO DEHYDRATION 15) and of an ABA-responsive gene, RAB18 (Timmusk *et al.,* 1999).*P. indica*, an endophytic fungus, conferred drought tolerance to *Arabidopsis* by priming the aerial parts of the plant for an early and high expression of (drought) stress-responsive genes (Sherameti *et al*., 2008). Genes of the ethylene signaling pathway were expressed differentially in the presence of beneficial diazotrophic endophytes in sugarcane (Cavalcante *et al*., 2007). In view of this, the present study was proposed with the following objectives.

1. To evaluate the water-stress mitigation potential of osmotolerant bacteria in pearl millet.

2. To evaluate the influence of osmotic stress on plant growth promoting activities, during plant-endophyte association.
3. To decipher the bacteria-mediated mechanism of stress tolerance under different levels of water stress.

Drought is a prolonged period of abnormally low rainfall, leading to a shortage of water, soil moisture deficit and hydrological imbalance. It is commonly occurs in all climatic regions and lasts regularly a season or more; leading to shortage of water. It causes adverse effect on crops and natural vegetation. It starts slowly, has a long duration and covers a vast area. In India, it upsets the country's food security by seriously affecting our agricultural economy, which is heavily dependent on monsoon and Government of India has declared 216 districts of the country as drought pronein2016(Ministry of Agriculture and farmers Welfare). The most challenging task ahead of researchers from India and worldwide is to mitigate the hazard of climate change and provide food for ever growing population in the world. The climate change and global warming, results changes in precipitation pattern and consequently drought have negative effects on crop production and productivity (St Clair and Lynch, 2010).

Global warming, its associated effects are expected to cause abiotic stresses, such as flooding, drought and extreme temperature that are bound to have adverse effects on crop production. Since 1900, more than 11 million people died as a result of droughts and one-third of world population has been affected from drought. Drought is one of the most important factor that cause of ill-health and death because they deny access to sufficient water and often cause malnutrition and famine. Drought's prolongation and its intensity have increased due to global warming. Whereas regional droughts have occurred in the past, the widespread and spatial extent of current droughts is broadly static, with expected change in the hydrological cycle, under global warming. Current IPCC projections of increasing temperature and mean sea levels; and heightened the intensity of droughts and rainstorms suggest that substantial population migrations will be taking place within the next 30-50 yrs particularly in coastal areas.

2.1 Different types of drought

2.1.1 Meteorological drought

It is defined as degree of dryness and the duration of the dry period in comparison to some "normal" or "average". The meteorological drought must be considered as region specific since the atmospheric conditions that result in deficiencies of precipitation are highly variable from region to region.

2.1.2 Agricultural drought

It is defined **as** circumstances when soil moisture is insufficient and results in the lack of crop growth and production. It primarily concerns itself with short-term drought situation. It links various features of meteorological drought to agricultural impacts and concentrating on precipitation shortages, inadequate soil water, reduced ground water and reservoir levels needed for irrigation. Plant water demand depends on prevailing weather conditions, biological characteristics of the specific plant, its stage of growth, and the physical and biological properties of the soil.

2.1.3 Hydrological drought

It is associated with the effects of periods of precipitation followed by shortfall on surface or surface water supply, resulting in considerable impact on society. Hydrological drought is usually out of phase with the occurrence of meteorological and agricultural droughts. It takes longer for precipitation deficiencies to show up in the components of the hydrological system such as soil moisture, stream flow ground water and reservoir levels. As results, these impacts are out of phase with impacts in other economic sectors.

2.2 Distribution of drought

At present 38% land area worldwide is affected by drought and the rest has minimum level of drought exposure (Dilley *et al*., 2005). Drought impacted around 56% of the land mass and 300 million people are endangered across the 18 states (IPCC, 2007). At present total 16% of the country area is drought prone and 68% of sown area is threatened to drought. In India there are different pearl millet growing states which are drought affected such as **Andra Pradesh** (Chittoor, Kadapa, Kurnool, Nellore, Anantapur, Prakasam, and Srikakulam), **Gujarat** (Bhavanagar, Banaskanta, Kheda, Bharuch, Jamnagar, Kutch, Meshana, Rajkot, , Ahmedabad and Surendranagar), **Haryana** (Gurugram, Bhiwani, Rohtak, Mahendragarh), **Karnataka** (Bangalore Rural, Ramanagara, Kolar, Chickballapur, Tumakuru, Chitradurga, Davanagere, Chamarajanagar, Mysuru, Mandya, Ballari, Koppal, Raichur, Kalaburgi, Yadgir, Bidar, Belagavi, Bagalkote, Vijapura, Gadag, Haveri, Dharwad, Shivamogga, Hassan, Kodagu, Uttara Kannada, Chikkamagalur, Ballari, Koppal, Raichuru, Kalaburagi, Yadagir, Bidar, Belagavi, Bagalkote, Vijayapura, Gadag, Haveri, Dharwad.), **Madhya Pradesh** (Katni, Shahdol, Umaria, Anuppur, Tikamgarh, Rewa, Jabalpur, Sidhi, Sagar, Damoh, Seoni, Sigroli, Sheopur, Chhatarpur, Bhind, Panna, Satna, Dindori, Shivpuri,

Mandsaur, Morena, Jhabua, Bhopal, Ujjain, Neemuch, Vidishia, Raisen, Rajgarh, Khandwa, Ratlam, Narsinghpur, Guna, Betul, Burhanpur, Agar Malwa, Sehore, Indore, Dhar, Shajapur, Harda, Chhindwara, Dewas,Ashoknagar,Khargone,Hoshangabad, Badwani), **Maharashtra** (Nashik, Dhule, Pune, Satara, Sangli, Aurangabad, Jalna, Beed, Latur, Osmanabad, Nanded, Parbhani, Hingoli, Buldhana, Akola, Yavatmal, Nagpur, Gadchiroli), **Rajasthan** (Ajmer, Banswara, Baran, Barmer, Bhilwara, Chittorgarh, Churu, Dungarpur, Hanumangarh, Jaipur, Jaisalmer, Jalor, Jhunjhunu, Jodhpur, Nagaur, Pali, Rajsamand, Udaipur, Pratapgarh),**Uttar Pradesh** (Sant Ravidas Nagar, Sonbhadra, Sultanpur, Mirzapur, Ballia, Sidharthnagar, Shahjahanpur, Banda, Pratapgarh, Chandauli, Etawah, Basti, Baghpat, Jaunpur, Faizabad, Gonda, Kannauj, Barabanki, Sant Kabir Nagar, Jhansi, Jalaun, Gorakhpur, Hathras, Etah, Allahabad, Chitrakoot, Kanpur Nagar, Lucknow, Deoria, Mainpuri, Maharajganj, Agra, Auraiya, Pilibhit, Amethi, Mahoba, Rae Bareily, Kushinagar, Kanpur Dehat, Kaushambi, Fatehpur, Ambedkar Nagar and Balrampur).

2.2.1 Factors contributing to drought

Drought is the resultant of a number of factors. The most important one related to the amount of water vapour present in the atmosphere, it produces precipitation. More rains, hail, sleet and snow can occur where there are low air pressure and moist. In presence of high air pressure, less moisture is available for precipitation. This results in a deficit of water for the areas above which they move. When winds shift air masses and warm, dry, continental air drift above an area in contrast to cooler, moist and oceanic air masses. El Nino, affects the temperature of ocean's water, which has also an impact on precipitation levels because in years when the temperature cycle is present and it can shift the air masses over the ocean surfaces, makes wet places dry and dry places wet. Finally, deforestation for building construction and agriculture combined results erosion which can also cause drought.

2.3 Effect of drought stress on plant growth

Different environmental factors affect plant growth and development such as drought, salinity, nutrient imbalance and extreme temperature are the most important environmental constraints to crop production and productivity worldwide**.** These stresses significantly affect and reduce agricultural productivity. Climate change, prolonged drought with erratic rainfall cause multiplicative effects of abiotic stresses on crop and seriously threatens the sustainable agricultural production. Water

availability is one of the major component which limits plant growth and productivity (Boyer, 1982). Agricultural regions are affected by drought stress may experience yield losses of more than 50% for major crops in the world. Drought stress majorly affects plant at morphological, biochemical, physiological and molecular levels resulting in stunting of plant growth and development, leading to reduction in crop productivity. Numerous stresses caused by water stress lead to extensive crop loss worldwide (Mittler, 2006). These drought related stresses might increase in the near future because of unpredictable global climate change. When compared to other stresses, drought hinder the plant growth and development, it is one of the most devastating, economically damaging and affect virtually every aspect of physiology and metabolism plant.

2.3.1 Effect drought on pearl millet growth

Drought is the one of the most important constraint for pearl millet production in the semiarid and arid regions of south Asia and Africa. It poses the important environmental stress to plant survival, distribution and crop productivity, causing drastic reduction in economic yield. Pearl millet has three growth phases: The first developmental stage or vegetative phase, starts at planting and ends at panicle initiation and in addition to this the emergence of leaves, roots, and tillers develop throughout this phase (Maiti and Bidinger, 1981). Drought during this phase can cause slow seed germination and induce seedling death. As a result, the overall millet yield can be reduced (Yadav *et al.,* 2012). The second developmental stage or panicle initiation phase, goes from panicle initiation to flowering (Maiti and Bidinger, 1981). The leaf, stem and panicle are elongated during panicle this phase; tillering is going to complete and stigmas start emerging on the panicle. Water stress during this stage has neglected effect on yield because its lost will be compensated by the secondary tillers (van Oosterom *et al.,* 2001). Finally, the third phase or grain filling and maturity stage start at flowering and ends at physiological maturity. Dark layer at the bottom of the seed indicates the end of this phase (Maiti and Bidinger, 1981). When drought occurs during grain filling, it reduces yield by reducing the number of panicle and grain weight (Bidinger *et al.,* 1987b). The grain yield compensation strategies under drought depends on the plant growth stage Mahalakshmi and Bidinger (1985b) and compared yield compensation strategies when drought occurs at panicle initiation and flowering **(Table 1).** They analysed, that compensation of yield loss was complete under drought at

panicle initiation, but incomplete at flowering stage and the duration of water deficit after flowering also influences its effect on grain yield. As short duration water stress after flowering can have a slight yield reducing effect where as long duration water stress after flowering can harshly reduce yield (Mahalakshmi *et al.,* 1987). Grain yield reduction as a result of drought stress after flowering can be explained by a reduction in grain production for both main shoot and tillers. As a result, 75% of pearl millet yield loss occurs under drought stress. For instance, Mahalakshmi *et al.* (1988) found that one additional day of water stress caused a yield loss of 0.9% of the total yield.

Table 1. Drought susceptible crops

Crop	**Growth stage**	**Yield reduction**	**References**
Rice	Reproductive	53–92%	Lafitte *et al*. (2007)
Barley	Seed filling	49–57%	Samarah (2005)
Maize	Grain filling	79–81%	Monneveux *et al*. (2006)
Pigeonpea	Reproductive	40–55%	Nam *et al*. (2001)
Common beans	Reproductive	58–87%	Martínez *et al*. (2007)
Cowpea	Reproductive	60–11%	Ogbonnaya *et al*. (2003)
Pearl millet	Reproductive	40-46%	Leila *et al.* (2013)

2.4 Effect of water deficit stress on plant growth and development

Effect of drought is dependent on plant's developmental stage, duration and intensity of water restriction. On other hand, plant's adaptive strategies determine the tolerance level, and consequently survival by the plant under these conditions of inadequate water supply (Kramer and Boyer, 1995).Water deficit affects different aspects related to plant development, such as seed germination, root and shoot development, photosynthesis, flowering and grain formation, reduction in leaf dry matter etc. (Costa, 2011). There is a stunting of plant growth due to water deficiency, and consequent repercussion on production parameters, such as number of grains and pods per plant. It was reported that drought stress and salt stress are closely related and their mechanisms also overlap. In particular, water deficiency results in disruption in photosynthesis, increased photorespiration, decrease in photosynthesis rate, reduction in leaf area, stomatal closing, nutrient acquisition and altered homeostasis of cells,

leading to increased production of ROS (Reactive oxygen species) such as superoxide, hydrogen peroxide, and hydroxyl radicals (Fontana, 1992; Miller *et al*., 2010; Santos and Carlesso, 1998). Even though reactive oxygen species also act as signals for the activation of stress response and defence pathways at low concentration (Pitzschke *et al*., 2006), its enhanced production during stress can pose a serious threat to the cells.

Drought is a situation that lowers plant water potential and turgor to the extent that plants face difficulties in executing normal physiological and biochemical functions. Water stress changes plant physiology and biochemistry (Abdullah *et al.,* 2011). Fundamental changes that occurred as a result of dehydration include changes in physiological and biochemical processes (Sangtarash, 2010), membrane structure and ultrastructure of subcellular organelles (Yordanov *et al.,* 2003) and water relations (Gorai *et al.,* 2010). Plant growth under drought stress is influenced by loss of turgor, stomatal closure, inhibition in cell growth and enlargement, altered photosynthesis, changes in plant metabolites (Bartels and Sunkar, 2005), respiration, carbohydrates, growth promoters, ion uptake, nutrient metabolism (Farooq *et al.,* 2008) and nutrient uptake (Akinci and Losel, 2010). However, its impact depends on the duration and intensity of stress (Chaves *et al.,* 2009), genetically determined plant sensitivity and capacity (Valladares *et al.,* 2007), developmental stage and species of plant (Jaleel *et al.,* 2008), soil type and climate (DaMatta and Ramalho, 2006).

Summarily, under the condition of water stress the plant could adapt through changes in molecular and physiological mechanisms but have to pay the price in the form of reduced biomass yields (Boutraa and Sanders, 2001). Severe deficit of water may result in the arrest of photosynthesis, reduction in turgor, water potential, solutes concentrations in the cytosol and increase of extra-cellular matrices and also lead to the inhibition in cell enlargement (Bhatt and Srinivasa Rao, 2005). Subsequently, continuous accumulation of abscisic acid (ABA) and compatible osmolytes, overproduction of reactive oxygen species result in wilting and finally plant death (Jaleel *et al.,* 2008).

2.5 Causes of growth reduction by drought stress

Shoot growth cessation, decreases in photosynthesis and root growth, stomatal closure; moderate increases in ROS are the main causes of growth reduction due to drought stress (Chaves, 1991). Drought stress, at mild intensity inhibits stomatal

conductance and leaf photosynthesis in green plants (Medrano *et al.,* 2002). There is reduction in photosynthesis due to stomatal limitation and cell growth is also affected, resulting in stunting of plants (Chaves, 1991; Galmes *et al*., 2007; Bousba *et al*., 2009). Chloroplast and other photosynthetic pigments are damaged; PSI and PSII systems are affected resulting in an overall decrease in photosynthetic efficiency of the plant; plant WUE decreases (Liu *et al*., 2006; Zlatev, 2009; Damayanthi *et al.,* 2010; Anjum *et al*., 2011). This results in decreased availability of photosynthates for plant growth. There is stunting of root growth; absorptive area of root is decreased. As a result, there is decrease in uptake of water and nutrients by the plant and ultimately it leads to nutrient deficiency also.

2.6 Strategies for combating drought

A variety of strategies used to improve the drought tolerance of crops, including traditional selection methods, molecular breeding programs and transgenic approaches are useful but are time consuming and resource intensive technologies (Flexas *et al*., 2013). However, recent reports have indicated the potential of microorganisms in improving plant's tolerance to abiotic stresses. Inoculation of *Paenibacillus polymyxa* confers drought tolerance in *Arabidopsis thaliana* was for the first time reported by Timmusk *et al*. (1999). Whereas in case of wheat (*Triticum aestivum*) the inoculation of *Azospirillum brasilense* Sp245 under drought stress resulted in a better water status and an additional 'elastic adjustment' leading to better grain yield and mineral quality (Creus *et al.,* 2004). Several PGPRs are reported to induce drought stress tolerance in some plants such as maize, sunflower and green gram (Sandhya *et al.,* 2009; Kasim *et al.,* 2013).

Microbes can play a significant role in drought stress alleviation in crops. Besides influencing the physico-chemical properties of rhizospheric soil through production of exopolysaccharides and formation of biofilm, microorganisms can also impact higher plants' response to abiotic stresses like drought, chilling injury, metal toxicity, salinity and high temperature, through different mechanisms like induction of antioxidative enzymes and osmo-protectants etc. in plant cells. Use of these microorganisms per-se can alleviate stresses in crop plants; a new and emerging application in agriculture. Bacterial inoculation has also been shown to prevent a significant drop in water potential, in parallel with a simultaneous increase in root growth, plant biomass and leaf area. There is increased accumulation of compatible

osmolytes such as proline as well, in roots and leaves. The effects are more significant at higher water stress levels as compared to lower stress (Casanovas *et al.,* 2002). Inoculation with *Azospirillum* resulted in higher content of micronutrients Mg, K and Ca in grains and reduced grain yield losses in wheat exposed to water deficit. There was increase in plant water content, RWC, leaf water potential and apoplastic water fraction (Creus *et al.,* 2004)

Drought stress greatly affects and reduces agricultural productivity. Abiotic stresses are one of the primary causes of crop loss worldwide, causing average yield losses of more than 50% for major crops. Prolonged drought can cause different effects on crops which seriously impact agricultural production, a wide range of adaptations and mitigation strategies are required to cope with the effects of drought on crop production. Efficient resource management and low cost technologies need to be developed for improved crop productivity and alleviation of drought stress in crop plants.

PGPRs are adapted to adverse conditions and may protect plants from the deleterious effects of drought stress, thus increasing crop productivity in arid or semi-arid areas (Kavamura *et al*., 2013). Therefore, identification and development of eco-friendly strategies that can ameliorate plant growth in response to water stresses are an immediate need in agricultural systems that have to cope with the jeopardies of climate change. Endophytic bacteria may in future be even more important than rhizospheric bacteria, because it can escape competition from rhizospheric microorganisms and have more intimate contact with plant tissues. Endophytes are microbes which live inside plants without causing any disease to the plants and some of these may even confer benefits to their plant host such as abiotic stress reduction, increased vegetative and root growth (Hardoim *et al.,* 2008).

2.6.1 Endophytic bacteria

Plants are naturally associated with mutualistic microorganisms that include endophytes. Endophytic bacteria have been found in virtually every plant studied and they colonize the internal tissues of their host plant. Till date not even a single plant species have been reported which was devoid of endophytes (Sturz, 2000). Both monocotyledonous and dicotyledonous plants ranging from woody tree species, such as oak and pear, to herbaceous crop plants such as sugar beet and maize crops can

harbour endophytic bacteria. These are ubiquitous in most plant species, residing latently or actively colonizing plant tissues and can form different relationships including symbiotic, mutualistic, commensalistic and trophobiotic. In general, endophytic microbes originate from the epiphytic microbial communities of the rhizosphere and phylloplane, as well as these may be transmitted through endophyte-infested seeds or planting materials.

Endophytes are diverse microbes which include fungi and bacteria. These endophytes spend the entire or part of their life cycle living inside the plant causing no apparent symptoms of disease and in fact establish a mutualistic association with plants (Long *et al.*, 2008). Endophytic bacteria have been isolated from a large diversity of plants. Endophytic populations, like rhizospheric populations, are conditioned by biotic and abiotic factors (Seghers *et al*., 2004), but endophytic bacteria could be better protected from biotic and abiotic stresses than rhizospheric bacteria (Hallmann *et al*., 1997). Endophytic bacteria in a single plant host are not restricted to a single species but comprise several genera and species.

2.6.2 Endophytic bacteria colonization within the plant

Prior to colonizing the inside of a plant, endophytic bacteria first colonize the rhizosphere (Nowak, 2000) or the phyllosphere (Pillay and Nowak, 1997). Microorganisms can gain entry into plants by chance as well; however, those microbes that enter a plant's interior accidentally, generally do not survive for long periods of time. True endophytic colonization is confirmed with proper spread among different parts of the plant and the maintenance of endophytic state for bacterial generations within the plant environment (Van Over Beek *et al*., 2006). Endophytic bacteria rely on the nutritional supply offered by the plant; any parameter affecting the nutritional status of the plant could consequently affect the endophytic community. Endophytes employ different mechanisms to gain entry into plants. They may enter the plant through different points; these entry points include tissue wounds (Agarwal and Shende, 1987), lenticels (Scot *et al*., 1996), stomata (Roos and Hattingh, 1983), root cracks (Sorensen and Sessitsch, 2006) and germinating radicles (Gagn *et al*., 1987). Entry through root cracks is recognized as the main portal of entry for bacterial colonization. Besides gaining entrance to plants through natural openings or wounds, other mechanisms may

include through the production of cell wall degradative enzymes like cellulase and pectinase (Quadt-Hallmann, 1997; Thekkiniath *et al*., 2010).

Upon reaching the inside of the plant, an endophyte may localize itself at the point of entry or may be spread throughout the plant (Hallmann *et al*., 1997). Plant xylem has been observed to be a selective environment for nitrogen-fixing endophytic bacteria such as *Acetobacter diazotrophicus* (Dong *et al*., 1994), *Gluconacetobacter diazotrophicus* (Cocking, 2003), *Herbaspirillum seropedicae* (James *et al*., 2002),and *Serratia marcescens* (Gyaneshwar *et al*., 2001).

Some endophytes colonize nutrient-rich intercellular spaces of plant hosts using them to spread inside host plants (Dong *et al*., 1994). Some systemic bacterial colonizers can also use the lumen of xylem vessels to spread throughout the plant (Compant *et al*., 2005b, 2008a). However, only few endophytes are able to colonize aerial vegetative plant parts (Hallmann, 2001). *Herbaspirillum seropedicae* entered the rootsthrough cracks at the point of lateral root emergence and colonized the rootintercellular spaces, aerenchyma and cortical cells, with a fewpenetrating the stele to enter the vascular tissue (James *et al*., 2002).

Bacterial flagella and the plant transpiration stream support their movements inside plants (James *et al*., 2002; Compant *et al*., 2005b). *Azoarcus* sp. type IV pili are involved in adherence to the plant surfaces, an essential step towards endophytic colonization (Dorr *et al*., 1998). *Azoarcus* mutants affected in pili were incapable of systemic spread into rice shoots (Dörr *et al*., 1998). Non motile mutants of *Salmonella enterica* were incapable of colonizing or had only a reduced invasion capacity in *Arabidopsisthaliana* (Cooley *et al*., 2003).

2.6.3 Interactions between plants and endophytic bacteria

Quorum-sensing (QS) is one of the most important bacterial traits to coordinate population behaviour (von Bodman *et al*., 2003). Bacterial communication by autoinducer molecules like *N*-acyl homoserine lactone plays an essential role in endophytic colonization. QS mutant strains of *Burkholderia kururiensis* M130, impaired to produce; and respond to one type of (AHL), showed decreased root and aerial rice tissue colonization when compared to the wild-type strain (Suarez-Moreno *et al*., 2010). By using the quorum quenching approach, they showed that a mutant of the rice endophyte *Azospirillum lipoferum* B518, that constitutively expressed AttM lactonase (an enzyme that hydrolyzes the lactone ring of AHLs), increased the synthesis

of proteins linked to transport and chemotaxis (Boyer *et al*., 2008). This suggested that QS in this strain was dedicated to regulate functions involved in root colonization. Even the beneficial effects of endophytic colonization (i.e. increases in root length and branching) were reduced in QS mutant strains.

Bacterial signal molecules such as lumichrome and lipochito-oligosaccharides were potentially involved in host growth stimulation (Mehboob *et al*., 2009). Rice endophyte metagenome survey indicated high abundance of genes encoding proteins for autoinducer synthesis and detection (Hardoim *et al*., 2012). Three different autoinducer systems identified were autoinducer-2 system (AI-2), the AHL system, and the diffusible signal factor system (DSF), indicating a need for concerted gene regulation for colonization by endophytic bacteria. Many bacterial pathogens and symbionts might secrete or inject proteins (called effectors) to interact with plant cells. The function of effectors, secreted by symbionts, is still unknown, but they often differ from those from pathogens (Deakin and Broughton, 2009).

Colonization of wheat by *Azorhizobium caulinodans* and *Azospirillum brasilense* was stimulated by flavonoids (Webster *et al*., 1998), as was colonization by *Azorhizobium caulinodans* of two *Brassica napus* (oilseed rape) varieties (O'Callaghan *et al*., 2000). Flavonoids are better known for their role in inducing the expression of *nod* genes that code for enzymes producing Nod factors. Neither Nod genes nor Nod factors are required for the endophytic colonization of *Arabidopsis thaliana* or wheat (Gough *et al*., 1997; Webster *et al*., 1998). Therefore, the role of flavonoids in stimulating colonization may be related to regulating other bacterial genes, such as those for phytoalexin resistance; type III secretion (Perret *et al*., 1999; Viprey *et al*., 1998); or genes for the synthesis of lipopolysaccharides (Reuhs *et al*., 2005), participating in the interaction with the plant.

2.7 Plant growth promotion by endophytic bacteria

The interaction between endophytic bacteria and host plants has not been fully understood. It is well established that some of these interactions are beneficial to the plant (Long *et al*., 2008). The fact that endophytes have close association with internal tissues of host plant has increasingly gained them scientific and commercial interest due to their potential to improve plant quality and growth (Schulz *et al*., 1999). Endophytic bacteria have been discovered to have several beneficial effects on host plants. These can promote plant growth and yield and can act as biocontrol agents. They

improve plant nutrition through nitrogen fixation and phosphate solubilization (Long *et al*., 2008). Bacterial endophytes were reported to be transmitted via seeds in cactus plant, and these were subsequently found to assist the cactus seedlings to establish and grow on barren rock (Puente *et al*., 2009a).Moreover, endophytes have also been reported to trigger induced systemic resistance (ISR)-based plant growth promotion (Ait Barka *et al*., 2002).

The beneficial effects of bacterial endophytes on their host plant appear to occur through similar mechanisms described for plant growth-promoting rhizobacteria (PGPR) (Kloepper *et al*., 1991a; Höflich *et al*., 1994). Endophytes have been reported to possess a diversity of plant growth promoting activities. These have been reported to improve plant nutrient status by nitrogen fixation (Baldani and Baldani, 2005), IAA production (Taghavi *et al.,* 2009), phosphorus solubilization (Dias *et al*., 2009), ACC deaminase activity (Manjunatha *et al*., 2015) and Gibberellic acid production (Sgroy *et al*., 2009). Most of the endophytic bacteria are reported to possess multiple plant growth promoting activities (Rashid *et al*., 2011).

2.7.1 Nitrogen fixation

A naturally occurring *Gluconacetobacter diazotrophicus* endophyte in sugarcane, colonizes xylem and fixes nitrogen (Cocking, 2003). Brazilian sugarcane cultivars can derive a substantial part of their nitrogen requirement from biological nitrogen fixation by endophytic diazotrophic bacteria. Nitrogen-fixing bacteria were isolated from the stems of wild and cultivated rice on a modified Rennie's medium (Elbeltagy *et al*., 2001). Application of endophytic bacterial inoculants supplying N requirement have increased plant growth and yield in sustainable manner, efficiently, in various crops. Some of the promising endophytic biofertilizers include the members of *Achromobacter, Azoarcus*, *Gluconoacetobacter, Burkholderia*, *Herbaspirillum* and *Serratia* (Rothballer *et al.,* 2008; Franche *et al.,* 2009). Efficient N supply by endophytic diazotrophic bacteria in kallar grass and sugarcane suggests the possible avenues of biological nitrogen fixation in interior niches of plants. It is evident from the reports that the *Gluconoacetobacter diazotrophicus* is the main contributor of endophytic biological nitrogen fixation in sugarcane, and it has the ability to fix approximately150 Kg N ha $^{-1}$ year $^{-1}$ (Muthukumarasamy *et al.,* 2005). Inoculation of *Herbaspirillum* sp. into rice seedlings, maintained in N-free Hoagland solution containing ^{15}N-labelled N, showed ^{15}N dilution amounting upto 40% increase in total

N of plant (Baldani *et al.*,2000). These investigations suggest that endophytic diazotrophs have a considerable potential to increase the productivity of non-legumes including important cash crop plants.

Effect of *Azospirillum* inoculation; and N-fertilization effect on grain yield and on the phyllosphere endophytic diversity of nitrogen-fixing bacteria in a rice rainfed crop was assessed and it improved N-content of grain and production of rainfed rice crop (Pedraza *et al*., 2008). Nitrogen fixation is a prime requisite for plant growth particularly in crops like cereals and pulses. N_2 fixers, also called‘ diazotrophs’ play a critical role in the plant ecosystem by reducing dinitrogen (N_2) to ammonia (NH_3) (Dilworth, 1974). Previous reports have indicated that diazotrophs showed ameliorating effects on nutrient uptake, stress tolerance and overall plant growth promotion (Sachdev *et al.,* 2009; Bhattacharyya and Jha, 2012; Gururani *et al*., 2012). Diazotrophic endophytes also trigger the plant growth and soil fertility (Singh *et al.,* 2017).

2.7.2 Phosphate solubilization

Phosphorus is found in soil in an insoluble state that cannot be used directly by plants. Many endophytic bacteria are known to possess various mechanisms to convert insoluble phosphate to soluble forms. Phosphate solubilization has been reported to be a common trait in endophytic bacteria and the majority of endophytic population from soybean, strawberry, sunflower and cactus (59-100%) were able to solubilize mineral phosphate (Palaniappan *et al*., 2010). It was also suggested that these microbes participated in phosphate assimilation (Kuklinsky-Sobral *et al.,* 2004). Many endophytic bacteria isolated from sunflower grown in drought regime were revealed to possess phosphate solubilizing ability (Forchetti *et al*., 2007).

Majority of endophytes were capable of solubilizing Fe/Ca -phosphates and pulverizing rock. These bacteria were also present in cactus seeds. Many of the phosphate solubilizing isolates were also diazotrophic, thus providing the host plant with N next to P (Puente *et al*., 2009a). The endophytes were tested in pot experiments, where endophyte-free cacti growing on mineral phosphate rock were amended with endophytes or nutrients, or were grown under sterile conditions. The bacterized plants grew well without nutrient addition and were comparable to fertilized plants, whereas the endophyte-free cacti failed to develop. This indicated that the endophytes were able to provide the developing plantlets with phosphate as well as nitrogen (Puente *et al*.,

2009b).Bacterial endophytes have been reported to promote plant growth by a mechanism of phosphate solubilization activity (Verma *et al.,* 2001).

2.7.3 IAA production

IAA producing endophytic bacteria have also been isolated from multiple plants including poplar, soybean, potato, strawberry etc. IAA production by endophytic bacteria has been associated with the promotion of plant root growth, enhanced production of lateral roots and increase in root volume and biomass (Taghavi *et al.,* 2009). Endophytic bacteria isolated from organically grown rice were observed to possess high IAA production ability (Duangpaeng *et al*., 2011). Inoculation with these isolates in rice increased root and shoot length, number of shoots per plant and shoot height. The endophytic *Bacillus* and *Paenibacillus* strains from medicinal plant *Lonicera japonica* were screened for use as potential plant growth promoters (Zhao *et al*., 2015). *Bacillus* strain 170 had the highest indoleacetic acid (IAA) production. Regression analysis showed a significant positive correlation between IAA production and increase in root length of the endophyte inoculated wheat seedlings.

2.7.4Gibberellic acid production

Gibberellins are tetracyclic diterpenoid acids that are involved in a number of developmental and physiological processes in plants (Crozier *et al*., 2000). This hormone helps in seed germination, seedling emergence, stem and leaf growth, floral induction and flower and fruit growth (King and Evans, 2003; Sponsel, 2003). Gibberellins are also implicated in promotion of root growth, root hair abundance, inhibition of floral bud differentiation in woody angiosperms, regulation of vegetative and reproductive buddormancy and delay of senescence in many organs of a range of plant species (Reinoso *et al.,*2002).It may be unsurprising then that phytohormones are key components of plant-microbe interactions. Certain bacteria have the ability to produce multiple phytohormones including gibberellins (GA) (Tsavkelova *et al*., 2006).

Indigenous endophytic *Bacillus* sp, *Micrococcus* sp, *Pseudomonas* sp, *Flavobacterium* sp and *Serratia* sp., isolated from tropical legume crops *viz*. redgram, blackgram, greengram, cowpea and chickpea were observed to produce a number of phytohormones including gibberellic acid (UmaMaheswari *et al.,* 2013). The production of GA by these bacteria ranged from 0.75 to 2.83 g ml^{-1}. Endophytic bacteria from halophyte *Prosopis strombulifera* such as *Lysinibacillus fusiformis,*

Achromobacter xylosoxidans, Brevibacterium halotolerans, and *Bacillus licheniformis* produced significantly high amounts of GA (Sgroy *et al*., 2009). Gibberellic acid production was also demonstrated in *Bacillus siamensis* strain BE 76isolated from stem of banana (*Musa* sp.)in medium supplemented with and without L-tryptophan (Ambawad *et al.,* 2018). They suggested that these GA producing bacteria could be useful for production biofertilizers to enhance growth and productivity of banana as well as to decrease harmful chemical fertilizers usage.

2.7.5 Microbe mediated drought stress alleviation

Crop production encounters various abiotic stresses particularly in the arid and semi-arid regions. Abiotic stressors, such as soil salinity, freezing, extremely high temperature, drought, flooding, often inhibit plant growth either directly by interfering with normal plant functioning or indirectly by the synthesis of excess stress-related ethylene (ET) and subsequent growth inhibition. Water stress injuries include retarded growth, leaf lesions, wilting and loss of cell membrane properties ensuing from changes in membrane fluidity. Plant-associated bacteria play a key role in adaptation of the host plant to a changing environment (Hallmann *et al*., 1997).

Plant growth promoting endophytic bacteria enhance plant growth by an array of mechanisms like phosphate solubilization, biological nitrogen fixation, rhizosphere engineering, phytohormone production, antifungal activity, siderophore production, production of volatile organic compounds (VOCs), production of 1-Aminocyclopropane-1-carboxylate deaminase (ACC), induction of systemic tolerance to water stress, promoting beneficial plant-microbe symbioses etc. (Bhattacharya and Jha, 2011). Endophytic bacteria actively respond to various abiotic stress factors that hamper the overall agricultural scenario. After successful colonization, rhizosphere as well as endophytic bacteria may alleviate temperature or drought stress in plants (Aroca and Ruiz-Lozano, 2009) by inducing a systemic response (Yang *et al.,* 2009). This demonstrates the potential role of certain bacterial strains for use in agriculture.

Plant-growth-promoting rhizobacteria (PGPR) can modulate plant's physiological status with response to water deprivation, thereby improving plant survival under such stressful conditions (Marasco *et al.,* 2012). *Phyllobacterium brassicacearum* strain STM196 inoculated *Arabidopsis thaliana* showed changes in transpiration rate and there was also reproductive delay which improved plant's

resistance to drought (Bresson *et al.,* 2013). Cucumber plants inoculated with a consortium of three PGPR strains belonging to *Bacillus cereus*, *Serratia* sp. and *Bacillus subtilis* showed enhanced tolerance to drought through a decrease in lipid peroxidation (Wang *et al*., 2012). An increase in superoxide dismutase activity and leaf proline content was also noted in inoculated plants as compared to control under drought stress. An increase in photosynthetic rate was observed on inoculation with *Azospirillum* and *Pseudomonas fluorescens* in *Pinus halepensis* and rice, respectively (Rincon *et al*., 2008; Ruiz-Sanchez *et al.,* 2011). Increased production of osmolytes by PGPR, under drought stress conditions, is also postulated to improve survival of plants (Vanderlinde *et al.,* 2010).

Inoculation of the alpine plant species *Chorispora bungeana* with endophytic *Clavibacter* sp. Enf12 isolated from the same plant growing under snow enhanced plant growth both at 20 and 0ºC. It also significantly attenuated the production of ROS, oxidative damage and electrolyte leakage. Inoculation also led to elevated levels of antioxidant enzymes and proline, indicating improved control of oxidative damage and increased hardiness (Ding *et al*., 2011). Similarly, a cold-tolerant *Serratia marcescens* SRM isolate from summer squash significantly enhanced biomass and nutrient uptake in wheat seedlings under cold conditions. *S. marcescens* had several PGP traits, including IAA production and phosphate solubilization, and these activities were retained at 4ºC (Selvakumar *et al*., 2008).The important factor in growth stimulation of the osmotolerant bacteria is their ability to produce IAA. Improvement in root proliferation in inoculated drought-stressed rice plants is likely to be induced by IAA hormone, apparently for enhanced water uptake (Yuwono *et al.* 2005; Dimkpa *et al*., 2009).

Ethylene is a plant hormone that plays a vital role in plant developmental processes as well as in stress signalling (Glick, 2004). Under normal conditions, ethylene helps in seed germination, root hair development, root elongation, leaf and petal abscission, fruit ripening and organ senescence (Abeles *et al*., 1992; Siddikee *et al*., 2011). However, during the stress response, plants produce high levels of ethylene that acts antagonistically for normal function and is deleterious to plant growth. ACC is the immediate precursor of ethylene in all higher plants. ACC 7 deaminase is a multimeric enzyme that cleaves ACC to α-ketobutyrate and ammonia and thereby decreases ethylene levels in host plants (Glick, 2005; Sun *et al*., 2009).

Ethylene levels in the plant may be regulated by cleaving ACC or inhibiting its production; in either case, bacterial efficiency increases in close proximity to the plant cells in which ethylene biosynthesis occurs (Hardoim *et al.,* 2008). Bacteria with ACC deaminase activity frequently provide a range of other benefits and have been postulated to be major forerunners in the transition from chemicals to bacterial plant growth promotion in agricultural systems (Glick, 2014).

Inoculation with ACC deaminase producing *B. licheniformis* K11 strain enhanced drought tolerance in pepper (Hui and Kim, 2013). ACC deaminase producing *Pseudomonas* spp. increased root length in *Pisum*, leading to enhanced uptake of water under drought stress conditions (Zahir *et al.,* 2008). Reduced activity of the antioxidant enzymes ascorbate peroxidaseand glutathione peroxidasein *Bacillus* spp. inoculated maize plants indicated increased tolerance to drought stress (Vardharajula *et al*., 2011). *A. brasilense* producing trehalose increased drought tolerance and plant growth in maize (Rodriguez *et al.,* 2009). Inoculation with *B. licheniformis* K11 strain increased expression of genes Cadhn, VA, sHSP and CaPR-10 in pepper under drought stress (Hui and Kim, 2013).Numerous other studies have also correlated the beneficial effects of inoculation with ACC deaminase producing endophytic bacteria with increased stress tolerance and growth in suboptimal conditions. Inoculation of maize, wheat, cotton, canola, groundnut and tomato with ACC-deaminase producing bacteria *Achromobacter piechaudii* AVR8, *Serratia proteamaculans* M35, *Klebsiella oxytoca* Rs-5,*Pseudomonas* sp. and *Enterobacter cloacae* CAL2 increased host biomass production, lowered Na^+ and enhanced K^+ content compared to uninoculated plants (Nadeem *et al*., 2010). Plant growth-promoting bacterium *A. piechaudii* strain ARV8 also enhanced resistance to water stress in tomato and pepper plants (Mayak *et al*., 2004).

In addition to ACC deaminase and ET levels, other endobacterial factors are likely to play roles in plant stress tolerance and growth. A study also revealed the positive effect of five ACC deaminase producing endophytes on the adaptation to abiotic stress by pepper (*Capsicum annuum*) (Sziderics *et al*., 2007). Under moderate stress, four of the five isolates increased plant biomass. *Microbacterium* sp. EZB22, the only studied strain devoid of ability to produce IAA, failed to promote growth, despite its ACC deaminase activity, indicating that growth enhancement was likely due to several bacterial plant growth promoting activities. *Arthrobacter* sp. EZB4 and*Bacillus*

sp. EZB8 were able to attenuate the induction of several stress-related genes in pepper, indicating reduced stress (Sziderics *et al*., 2007).

Many plant-associated bacteria are able to degrade the ET precursor ACC by (bacterially-encoded) ACC deaminase and utilize the end products as carbon and nitrogen sources. Thus, endophytic bacteria may promote plant growth as a consequence of the action of bacteria expressing the enzyme 1-aminocyclopropane-1-carboxylate (ACC) deaminase. Hence, this forms an efficient sink for ACC. Concomitantly, these bacteria lower the ET levels in colonized plant tissue and restore plant growth under stressful conditions (Glick *et al*., 2007). Production of ACC deaminase and associated plant growth promotion, by root elongation and increase of plant biomass, has been reported for numerous endophytic species, including many *B. phytofirmans* and *B. cepacia* isolates, *Methylobacterium fujisawaense,* as well as for *Pseudomonas, Arthrobacter* and *Bacillus* spp. (Nadeem *et al*., 2010).

PGPR inoculants have been used for plant growth promotion and mitigation of drought stress (Ruiz *et al.,* 2011). Some reports showed that *Pseudomonas chlororaphis* induced drought tolerance in *A. thaliana* by synthesizing volatile compounds (Cho *et al*., 2008). Exopolysaccharides producing microbes enhanced plant growth under drought stress (Naseem and Bano, 2014), probably by improving soil aggregate stability (Alami *et al*., 2000). Rhizobacteria were isolated from maize grown under semi-arid and arid conditions. *B. cereus* and *Bacillus pumilus* strains isolated from semi-arid conditions proved to be more effective in enhancing drought tolerance in plants compared to *Pseudomonas* strains isolated from arid conditions.

2.8 Different mechanisms for development of systemic tolerance against abiotic stresses

2.8.1 Induced systemic tolerance

Plant growth promoting bacteria (PGPB) help the associated plants to counter abiotic stresses such as drought, nutrient deficiency, high temperature, low temperature, salt, presence of toxic metals etc. PGPB induced physical and chemical changes in plants which results in improving the immune response of plants to abiotic stresses are termed as 'induced systemic tolerance' (Yang *et al*.,2009).The bacterial products that elicit induction of induced systemic tolerance are of diverse mechanisms and show their induction in plants which possibly possess receptors for the respective ligands. These

inducers may be 1-aminocyclopropane-1-carboxylate deaminase activity, antioxidants, cytokinin, volatile organic compounds and quorum-sensing signals (van Loon, 2007). Role of volatile organic compounds such as 2, 3-butanediol produced by *Bacillus* sp. in induced systemic tolerance has been reported by Ryu *et al*. (2004).

In response to the abiotic stresses, plant produces ethylene to regulate plant homeostasis. Beyond a threshold level, the production of ethylene is inhibitory as it reduces root and shoot development and hence described as "stress ethylene". Some of the endophytic bacteria have property to produce 1-aminocyclopropane-1-carboxylate-deaminase, which can degrade the immediate precursor of ethylene from root exudates and convert it to α-ketobutyrate and ammonia and thus, can promote growth of the plant in the vicinity (Glick *et al*., 2007). In addition to this, there are various other mechanisms through which induced systemic tolerance is generated in response to stresses. It includes volatile organic compounds mediated salt tolerance (Zhang *et al.,* 2008); affecting abscisic acid signalling of plants during stress through production of cytokinin (Figueiredo *et al.,* 2008); and through production of antioxidant catalase (Kohler *et al.,* 2008). The role of phytohormones produced by associative bacteria during drought stress in the promotion of plant growth has been well described (Egamberdieva, 2009). Indole-3-acetic acid producing bacteria in drought condition can stimulate formation of well-developed roots for providing sufficient water from soil (Marulanda *et al.,* 2009).

In *A. thaliana,* jasmonate or ethylene mediated induced systemic resistance by *Bradyrhizobium* sp. strain ORS278 has been reported by Cartieaux *et al*. (2008). While transcriptome analysis indicated SA mediated induced systemic resistanceby endophytic bacteria *Paenibacillus alvei* in *A. thaliana* (Tjamos *et al*., 2005). *B. cereus* strain AR156 was reported to trigger induced systemic tolerance against abiotic stress in *A. thaliana* through salicylic acid and jasmonic acid/ethylene signaling pathways in an NPR1-dependent manner (Niu *et al.,* 2011).

B. phytofirmans strain PsJN has a wide host spectrum, including wheat, maize and grapevine, and has been implicated in a range of beneficial abiotic stress tolerance. Photosynthetic rate, water-use efficiency and chlorophyll content of wheat inoculated with *B. phytofirmans* PsJN were improved with respect to control under field conditions, ultimately resulting in increased grain yield (Naveed *et al.,* 2014a). In maize, shoot and root biomass, leaf area and photosynthetic efficiency was higher in

drought exposed plants inoculated with both *B. phytofirmans* and *Enterobacter* sp. strain FD17 with respect to uninoculated control. *B. phytofirmans* offered more efficient protection against drought, indicating that physiological responses to endophyte inoculation are specific to the plant and microbial genotypes (Naveed *et al.,* 2014b). *B. phytofirmans* strain *PsJN* induces resistance to grey mould and increases tolerance to low non-freezing temperatures in grapevines. Following growth at 4°C, more rapid and greater up-regulation of the plant stress related gene transcripts and metabolites was observed in the plant in presence of the bacteria, indicating a priming effect of the endophyte (Theocharis *et al.,* 2012).

2.8.2 Phytohormone Production

Phytohormones such as auxins, cytokinins, gibberellins, ethylene and abscisic acid (ABA) play key roles in the regulation of plant growth and development. These are involved in fundamental and complex developmental pathways and also help in dynamic responses to the environment (Salisbury and Ross, 1992; Durbak *et al.,* 2012). When plants encounter suboptimal environmental conditions, the levels of endogenous phytohormones are often insufficient and lead to stunting of plant growth (Tsakelova *et al*., 2006). It is maybe unsurprising then that phytohormones are key components of plant-microbe interactions. Certain bacteria have the ability to produce phytohormones including auxins, gibberellins and cytokinins (Bottini *et al.,* 2004; Tsavkelova *et al.,* 2006) and many rhizosphere microorganisms produce or modulate phytohormones under *in vitro* conditions (De Salamone *et al*., 2005). These phytohormones could be used as signalling molecules between bacteria and plants and also the existing crosstalk between IAA and ethylene biosynthesis has been exploited as a means of communication (Spaepen *et al.,* 2007; Yuan *et al.,* 2008). Furthermore, bacteria can also influence and regulate phytohormone production by the plant. Consequently, many PGPB with the ability to produce phytohormones can affect the plant's hormonal balance. In fact, one of the mechanisms employed by PGPR strains ensuring plant survival under drought stress is modification in content of bacterial phytohormones, such as auxins, gibberellins, cytokinins, ethylene and abscisic acid (ABA) (Manero *et al*., 2001).

One of the most important factors in the growth stimulation of plants by rhizobacteria is their ability to produce IAA. IAA has been reported to be effective in imparting osmotic stress tolerance to bacteria (Boiero *et al.,* 2006). Plants inoculated

with *Pseudomonas putida* were able to survive drought stress due to the production of IAA (Marulanda *et al*., 2009). Pereyra *et al.* (2012) reported that wheat seedlings inoculated with *Azospirillum* were able to cope with osmotic stress due to morphological modifications in coleoptile xylem architecture. This was attributed to upregulation of the indole-3-pyruvate decarboxylase gene and enhanced IAA synthesis in *Azospirillum*. Improvement in root proliferation in inoculated drought-stressed rice plants was likely induced by this hormone (Yuwono *et al.,* 2005), apparently for enhanced water uptake. IAA production by PGPR causes modifications in root system architecture by increasing the number of root tips and the root surface area, thus increasing water and nutrient acquisition (Mantelin and Touraine, 2004), which helps plants to cope with water deficit (Egamberdieva and Kucharova, 2009). It has also been reported that bacterial VOCs from *B. subtilis* strain GB03 caused growth promotion in *Arabidopsis* by upregulating transcripts involved in auxin homeostasis (Zhang *et al*., 2007).

Inoculation of *Herbaspirillum frisingense*strain GSF30T with miscanthus seedlings, a temperate grass endophyte, promoted root and shoot growth. The transcriptome analysis identified that the jasmonate response and ethylene signalling was altered by the presence of *H. Frisingense* (Straub *et al.,* 2013b). The endophytic bacterial strains were isolated from sweet potato (Khan and Doty, 2009). Sweet potato cuttings inoculated with those endophytic bacterial strains that produced IAA and auxin produced roots first and grew more rapidly than uninoculated cuttings. *H. Frisingense* strain GSF30T was demonstrated to produce IAA in culture (Rothballer *et al*., 2008) and auxin was concluded to be the likely mechanism behind increase in seedling growth of the wheat plants inoculated with *B. subtilis* (Egorshina *et al*., 2012). *Azospirillum* spp. are considered to increase plant growth primarily via root stimulation by auxin, with nitrogen fixation and production of other phytohormones playing lesser roles (Steenhoudt and Vanderleyden, 2000). These effects may well be applicable in field situations, for example *Azospirillum* sp. strain B510, isolated from surface-sterilized stems of rice, significantly increased tiller number and yields of field grown rice plants following re-inoculation of seedlings (Isawa *et al.,* 2010); while three *Pseudomonas* strains enhanced growth and spike length in wheat in both laboratory and field conditions (Iqbal and Hasnain, 2013). These effects were attributed to phytohormone production rather than nitrogen fixation in both the cases.

Production of other phytohormones by rhizobacteria was also reported to enhance drought tolerance in the inoculated plants. Physiological modifications in soybean plants inoculated by the gibberellins secreting rhizobacterium *Pseudomonas putida*strain H-2–3 improved plant growth under drought conditions (Sang-Mo *et al.,* 2014). Production of gibberellins and ABA by *Azospirillum lipoferum* alleviated drought stress in maize plants (Cohen *et al.,* 2009). Cellular dehydration induces biosynthesis of ABA, which is commonly known as a stress hormone because of its prodigious accumulation during water stress conditions. ABA is involved in water loss regulation by control of stomatal closure and stress signal transduction pathways (Yamaguchi *et al*., 1994).

Arabidopsis plants were inoculated with *A. Brasilense*strain Sp245 producing ABA under drought stress conditions (Cohen *et al*., 2008). The inoculated plants had elevated levels of ABA compared to non-inoculated ones. *A. brasilense* strain Sp245 enhanced plant biomass, altered root system architecture by increasing lateral root number, stimulating photosynthesis and decreasing water loss. Changes in all these parameters could be correlated with incremented ABA levels. There was improvement in plant survival and seed yield. Proline levels and relative water content were improved in leaves; and there was decrease in stomatal conductance and malondialdehyde. PGPR *Phyllobacterium brassicacearum* strain STM196, isolated from the rhizosphere of *Brassica napus*, improved osmotic stress tolerance in inoculated *Arabidopsis* plants by elevating ABA content, leading to decreased leaf transpiration (Bresson *et al*., 2013). Inoculation of *Platycladus orientalis* container seedlings with cytokinin producing *B. Subtilis* has been reported to interfere with suppression of shoot growth, thus conferring drought stress resistance (Liu *et al*., 2013).

2.8.3 ACC deaminase activity

Plant synthesizes the gaseous hormone ethylene (C_2H_4) in plant tissues from the precursor 1-aminocyclopropane-1-carboxylic acid (ACC). It is involved in various developmental and physiological processes of plants, such as seedling emergence, root hair, tissue differentiation, development and elongation, leaf and flower senescence, lateral bud development, fruit degreening, anthocyanin synthesis, and ripening, production of volatile compounds responsible for aroma in fruits etc. (Abeles *et al*., 1992; Frankenberger and Arshad, 1995; Spaink, 1997; Bleecker and Kende, 2000). Ethylene regulates plant responses to biotic and abiotic stresses (Penninckx *et al.,*

1998). Under normal conditions, plants synthesize ethylene in required amounts, conferring beneficial effects on plant growth and development; however, in response to abiotic and biotic stresses there is often increase in ethylene production that has an adverse effect on plant growth. Ethylene is thought to be responsible for senescence in all plants (Ali *et al.,* 2012). Ethylene biosynthesis is increased during drought stress that results in reduced root and shoot growth.

Many investigations have indicated that some PGPR strains possess ACC deaminase enzyme activity (Glick, 2007), which can cleave the plant ethylene precursor ACC in to ammonia and α-ketobutyrate, thereby lowering the ethylene level (Shaharoona *et al*., 2006). Many studies have demonstrated the presence of ACC deaminase activity in a wide range of soil microorganisms including the fungus *Penicillium citrinum* (Honma, 1993) and various bacteria (Blaha *et al*., 2006; Madhaiyan *et al.,* 2007; Kuffner *et al.,* 2008; Chinnadurai *et al*., 2009). Bacterial ACC deaminase activity is relatively common. In one study, 12% of isolated *Rhizobium* spp. from various sites in southern and central Saskatchewan possessed this enzyme (Duan *et al*., 2009). In another study, ACC deaminase activity/genes were found in a wide range of bacterial isolates including *Agrobacterium, Azospirillum, Achromobacter, Burkholderia, Rhizobium, Ralstonia, Enterobacter*, and *Pseudomonas* (Blaha *et al*., 2006). In a model described by Glick *et al*. (1998), the main visible effect of seed inoculation with ACC deaminase-producing bacteria, under gnotobiotic conditions, was the enhancement of root elongation (Glick *et al*., 1995; Hall *et al.,* 1996; Shah *et al*., 1997).

The plants inoculated with PGPB containing ACC deaminase enzyme activity can have longer roots (Glick *et al.,* 1999) and can be better able to resist the inhibitory effects of ethylene stress imposed by heavy metals, drought, salinity and flooding (Burd *et al*., 2000; Zahir *et al*., 2008; Mayak *et al.,* 2004; Grichko and Glick, 2001). Besides, treatment of plant seeds or roots with bacteria, containing ACC deaminase, typically reduces ACC and ethylene levels about 2-4 fold (Penrose and Glick, 2001). The role of ACC deaminase enzyme activity in plant growth promotion has been clearly investigated in the symbiosis of *B. phytofirmans* strain PsJN and canola; and a constructed knock-out mutant of *B. phytofirmans* strain PsJN lacking ACC deaminase activity. The PsJN mutant was no longer able to promote the elongation of roots of canola seedlings. Concisely, PGPB containing ACC deaminase could be used as

successful inoculant because of having an effective strategy for improving growth and yield of crops via adjusting ethylene level in plants.

Bacteria occurring on root surfaces containing ACC deaminase have been shown to modify the sensitivity of root and leaf growth to soil drying, apparently by influencing ethylene signalling (Bashan and Holguin, 1998). Thus, the deleterious effect of ethylene is abated by the removal of ACC, thereby ameliorating plant stress and promoting plant growth (Glick, 2007). Inoculation of *Pisum* with *Pseudomonas* spp. induced longer roots, which led to an increased uptake of water from soil, under drought stress conditions (Zahir*et al*., 2008). The ACC deaminase activity of *Achromobacter piechaudi* was shown to confer tolerance to water deficit stress in tomato and pepper, resulting in significant increases in fresh and dry weights (Mayak *et al.,* 2004). Ethylene production was reduced in inoculated plants compared to non-inoculated controls, with improved recovery from water deficiency, although inoculation did not influence relative water contents. Studies on drought stressed pea inoculated with ACC deaminase activity-containing *Variovorax paradoxus* strain, as against ACC deaminase mutant strain, showed hormone signalling-mediated plant growth improvement, yield and water-use efficiency (Belimov *et al.,* 2009). Inoculating pepper with *B. Licheniformis*strain K11 increased ACC deaminase production, thus imparting tolerance to cope with drought stress (Hui and Kim, 2013).

2.8.4 Osmolyte production and accumulation

Plants under water stress conditions have a greater necessity to adjust osmotically, in order to alleviate cell turgidity losses. Plants adaptation to environmental stresses is associated with metabolic adjustments that lead to the accumulation of several compatible organic solutes like glycine betaines, sugars, polyamines, proline, quaternary ammonium compounds, polyhydric alcohols and other amino acids (Yancey *et al*., 1982). As a response to water stress, plants increase the synthesis of osmolytes, thus increasing osmotic potential within cells (Farooq *et al.,* 2009). It is well documented that enhanced biosynthesis of glycine betaine-like quaternary compounds, increases plants adaptability to various types of abiotic stresses (Sakamoto and Murata, 2002; Chen and Murata, 2008).

The accumulation of osmolytes, such as glycine betaine, proline and trehalose, is the most frequent acclimatization response observed in plants and bacteria under water stress conditions (Chen *et al*., 2008; Gruszka *et al*., 2007; Rodriguez *et al*., 2009).

PGPRs exudate osmolytes in response to drought stress, which probably acts synergistically with plant produced osmolytes and stimulate plant growth (Paul and Nair, 2008). Consistent with this, the beneficial effects of osmolyte producing rhizobacteria on rice were more significant when the stress conditions were more severe: differences in shoot dry weight, root dry weight and number of tillers between inoculated rice plants and non-inoculated controls were more prominent under severe drought (Yuwono *et al.,* 2005).

Proline synthesis results in osmotic adjustment, free radical scavenging and stabilization of subcellular structures in plant cells to overcome the detrimental effects of drought (Hare *et al*., 1998). Increased proline content in plants inoculated with *Bacillus* strains under drought stress was attributed to the upregulation of gene for *P5CS* involved in biosynthesis of proline and inhibition of expression of the gene for *ProDH*, which acts during metabolism of proline (Yoshiba *et al*., 1997). Proline synthesis has been observed to be increased in osmotically stressed plants in the presence of *Burkholderia* (Barka *et al.,* 2006). Introduction of *proBA* genes derived from *B. subtilis* into *A. thaliana* led to enhanced proline production, which was correlated to the acquisition of osmotic tolerance in transgenic plants (Chen *et al*., 2008). Elevation of leaf proline levels in maize plants was triggered during drought stress, which was further enhanced on inoculation with *P. fluorescens* (Ansary *et al*., 2012).

Inoculation of *Arabidopsis* with *B. Subtilis* strain GB03 induced elevated glycine betaine content and its precursor choline levels in plants, imparting them with drought tolerance. However, GB03-induced drought tolerance was lost in the xipotl mutant of *Arabidopsis* with reduced choline production (Zhang *et al*., 2010). Rapid accumulation of glycine betaine in *Oryza* inoculated with *Pseudomonas pseudoalcaligenes* has been reported to confer stress tolerance (Jha *et al.,* 2011). Probably glycine betaine produced by the osmotolerant bacteria acted synergistically with the plant-produced glycine betaine in response to stress, and in this way, increased drought tolerance.

Accumulation of soluble sugars as osmolytes is another adaptive mechanism that helps towards osmotic adjustment under drought stress. It is well documented that starch hydrolysis leads to higher sugar levels (Enebak *et al*., 1997). There is also accumulation of amino acids due to hydrolysis of proteins, which occurs in response to changes and this helps in osmotic adjustments (Iqbal *et al*., 2011; Krasensky and Jonak,

2012). An increase in soluble sugar content in drought-stressed plants is also observed (Dekankova *et al*., 2004). Starch depletion and higher sugar content were simultaneously observed in grapevine leaves during drought stress (Patakas and Noitsakis, 2001). PGPRs have been reported to improve drought stress tolerance by increasing the accumulation of amino acids and soluble sugars in inoculated stressed plants. *Azospirillum lipoferum* has been reported to increase maize growth, while accumulating free amino acids and soluble sugars during drought stress (Qudsaia *et al*., 2013). Maize seedlings inoculated with *Bacillus* strains displayed higher sugar content due to starch degradation, thus imparting resistance to plants during drought stress (Mohammadkhani and Heidari, 2008).

Maize plants inoculated with *Bacillus* sp. displayed higher levels of proline, sugars and free amino acids, thus increasing plant biomass, relative water content, leaf water potential and root adhering soil/root tissue ratio (Vardharajula *et al*., 2011). Some of the reports also demonstrated that adverse effects of drought stress on plant growth under uninoculated condition might be attributed to declining sugar levels and their enhanced accumulation probably led to increased tolerance to drought (Sandhya *et al.,* 2010). Maize seedlings inoculated with *Pseudomonas* sp. increased soluble sugar content compared to uninoculated control (Bano and Fatima, 2009). The increase in sugar content was attributed to hydrolysis of starch for providing sugar for osmotic adjustment to negate the effect of drought stress.

2.8.5 Nutrient acquisition

Abiotic stresses disrupt root–microbe associations that play a major role in plant nutrient acquisition, which result in retarded growth and reduced yield. A number of endophytic bacteria have the ability to form associative-symbioses with plants and to fix bioavailable nitrogen, within unspecialized tissues of the host plant; as compared to nodule formation during legume-rhizobia interaction, which is responsible for biological nitrogen fixation. PGPRs help in nutrient acquisition by the plant, through various mechanisms and thus help in reducing the negative impacts of drought stress. *Pseudomonas monteilii, Bacillus sp.* and *Cronobacter dublinensis* inoculation in *Ocimum basilicum* L. increased the nutrient uptake and reduced the antagonistic effects of abiotic stress (Rakshapal *et al*., 2013). Several diazotrophic bacterial species have been repeatedly identified as being associated as bacterial endophytes of sugarcane in Brazil. These species include *Gluconacetobacter diazotrophicus, Azospirillum*

amazonense, and Herbaspirillum seropedicae (Kirchhof *et al.,* 1998; Monteiro *et al*., 2012a). *Gluconacetobacter diazotrophicus* can be endophytic in sugarcane and has been identified in electron microscopic studies using immune-gold labelling techniques (James *et al.,* 1994). Both *G. diazotrophicus* and the mild plant pathogen *Herbaspirillum* sp. have been recorded in high numbers in sugarcane roots, stems and leaves (James and Olivares, 1998; Olivares *et al.,* 1996). *H. rubrisubalbicans* has the ability to colonize sugarcane endophytically (James *et al.,* 1997). These species of diazotrophic bacteria are likely to be key contributors to the significant biological nitrogen fixation that has been observed in field experiments using nitrogen balance and nitrogen isotope dilution techniques in Brazilian sugarcane (Baldani and Baldani, 2005; Boddey *et al.,* 1991; Dobereiner *et al.,* 1993; James, 2000).

In rice and maize, BNF contribution is similarly derived from a number of different species including members of *Azoarcus, Azospirillum, Herbaspirillum, Bacillus* and *Klebsiella* (Monteiro *et al.,* 2012a). In field experiments using wild rice, grain yields increased to the equivalent of using an additional nitrogen fertilizer application of 40 kg N/ha following inoculation with *H. seropedicea* (Baldani *et al*., 2000; Pereira and Baldani, 1995). Sixteen percent of plant nitrogen in field-grown Miscanthus plants was estimated to be derived from BNF, despite non-limiting soil nitrogen (Keymer and Kent, 2013). However, *A. diazotrophicus* colonization of sugarcane is inhibited by high N-fertilization (Fuentes-Ramırez *et al.,* 1999), and exogenous nitrogen fertilizer has been demonstrated to reduce the number of diazotrophic endophytes cultured from sugarcane (Pariona-Llanos *et al.,* 2010).

Uptake of phosphorus an important plant nutrient is affected in plants exposed to drought stress. PGPR mediated enhanced phosphorus uptake has been associated with the ability to solubilize P and increase its uptake (Gyaneshwar *et al.,* 2002). PGPR inoculated wheat plants given 75% recommended dose of fertilizer (RDF) gave yields equivalent to full RDF indicating supplementation of the fertilizer requirement of the crop by PGPR (Shaharoona *et al.,* 2008). PGPR promote root development and alter root architecture by the production of phytohormones such as indole acetic acid (IAA) resulting in increased root surface area and numbers of root tips, thus providing new sites for nutrient uptake (Kloepper *et al.*, 2007). Similar observations were recorded in tomato plants also (Hernandez and Chailloux, 2004), suggesting that PGPR increased plant uptake of mineral.

2.8.6 Induction of antioxidative enzymes and improved antioxidant status

Much of the injury caused on plants under abiotic stress is due to oxidative damage at the cellular level; and which is the result of imbalance between the formation of reactive oxygen species (ROS) and their detoxification. The most common aspect of adverse environmental conditions is the increased production of reactive oxygen species (ROS) within several subcellular compartments of the plant cell (Breusegem *et al*., 2001). Reactive oxygen species can occur as a by-product of regular cellular metabolism such as in photosynthesis. However, under stress, their formation is usually acerbated. Drought stress leads to the disruption of the photosynthetic apparatus and also increase in photorespiration, thus altering the normal homeostasis of cells, subsequently resulting in higher production of reactive oxygen species. Photosystem II is affected most by drought stress, particularly, within the oxygen-evolving complex and the reaction centres (Toivonen and Vidaver, 1988; He *et al*., 1995).

In general, these reactive oxygen species particularly superoxide and hydroxyl radicals are damaging to necessary cellular components such as DNA, proteins and lipids. Lipid peroxidation disrupts the membrane integrity of the plant cell. As a result, essential solutes leak out of organelles and from the cell, causing disruption in membrane function and metabolic imbalances. DNA is the blueprint for both future form and function. Any damage to its integrity could mean that proteins that would have been essential for optimal function of the plant will not be synthesized. However, these reactive oxygen species also act as a signal for the activation of stress-response and defence pathways (Pitzschke *et al*., 2006).Therefore, it is imperative to regulate the reactive oxygen species levels through the coordination of ROS production and reactive oxygen species scavenging systems to manage oxidative damage and simultaneously modulate signalling events.

Different plants have evolved different complex protective mechanisms to prevent the damage initiated by free radicals. Plants are armed with antioxidant defence systems constituting both enzymatic and non-enzymatic components that work in concert to alleviate the oxidative damage occurring during drought by the scavenging of reactive oxygen species (Miller *et al*., 2010). Enzymatic components include superoxide dismutase (SOD), ascorbate peroxidase (APX), catalase (CAT) and glutathione reductase (GR). Non-enzymatic components contain ascorbic acid, cysteine and glutathione (Price *et al*., 1994). High activities of antioxidant enzymes are linked

with oxidative stress tolerance in plants (Stajner *et al*., 1997). Superoxide dismutase regulates the cellular concentration of $O_2$and H_2O_2. The latter is broken down by catalases and peroxidases. Under moderate stress conditions, the radicals are efficiently scavenged by this antioxidant defence system. However, in periods of more severe stress in desiccation-sensitive plants, the scavenging system becomes saturated by the increased rate of radical production, and damage is inevitable. In *Xerophyta viscosa*, activities of superoxide dismutase, ascorbate peroxidase and glutathione reductase werefound to increase during dehydration (Sherwin and Farrant, 1998).Increased activity of different antioxidative enzymes and greater accumulation of antioxidants has been reported in response to drought stress in PGPR inoculated plants, thereby reducing adverse effect of drought stress (Han and Lee, 2005).

Inoculation of lettuce (*Lactuca stiva L*.) with *Pseudomonas mendocina* augmented antioxidative enzyme CAT under severe drought conditions, suggesting that they can be used as inoculants to alleviate oxidative damage elicited by drought (Kohler, 2008). Inoculation with PGPR strains under drought stress has also resulted in decreased antioxidant enzymes' activities. Maize plants inoculated with five drought tolerant plant growth promoting strains namely *Pseudomonas stutzeri, Pseudomonas entomophila, Pseudomonas putida, Pseudomonas montelli* and *Pseudomonas syringae* were subjected to drought stress and inoculated plants showed significantly lower activity of antioxidant enzymes in inoculated plants as compared to uninoculated plants, indicating a lowering of stress in the plants(Sandhya *et al*., 2010). Inoculation of basil (*Ocimum basilicum* L.) with three bacterial species such as *A. brasilense, Bacillus lentus and Pseudomonas* sp.,revealed that application of rhizobacteria under water stress improved antioxidant status and photosynthetic pigments content in basil plant.

2.8.7 Priming of drought stress responsive genes

In addition to physiological, biochemical and cellular changes, there are also changes in many genes and gene products in response to drought stress that occur at the transcriptional, post transcriptional and translational levels (Lata *et al.,* 2015). Some responses to water stress are related to changes in ion flux, stomatal closing, production of osmoprotectant metabolites, hormonal regulation and alterations in plant growth patterns (Shinozaki *et al.,* 2000). ABA dependent and independent pathways are known to mediate changes in gene expression in plants during water deficit stress. ABA-dependent pathways are thought to mediate changes in gene expression through an

ABRE-element and b-ZIP transcription factors (Busk and Pages, 1998), while the other pathway is through MYC and MYB elements and transcription factors (Yamaguchi-Shinozaki and Shinozaki, 1993).

Plants regulate their hydric state via stomatal closing, a process that is influenced by the hormone abscisic acid (ABA), which is synthesized mainly in the leaves. ABA synthesis is stimulated by dehydration conditions; and ABA plays an important role in response to the drought stress (Xiong *et al.,* 2002). Hydric deficit also decreases photosynthesis, leading to stomatal closing and to decreases in the intercellular CO_2 concentration (Taiz *et al.,* 2012). Although, not many reports are available on priming of the drought stress responsive plant genes by microbes, inoculation of PGPRs has been reported to modify plant response at the gene level under stress conditions, since these are capable of eliciting drastic physiological changes that modulate growth and development of the plant. PGPR-mediated modulation of the expression of drought stress responsive gene was for the first time reported by Timmusk and Wagner (1999). They reported induction of the drought stress responsive gene *ERD 15* in *A. thaliana* due to inoculation with *Paenibacillis polymyxa*. Genes of the ethylene (a plant stress hormone) signalling pathway were differentially expressed in the presence of beneficial endophytes in sugarcane (Vinagre *et al*., 2006).

Inoculation of *Piriformospora indica* conferred drought-stress tolerance to *Arabidopsis*, and this was associated with the priming of the expression of a quite diverse set of stress-related genes in the leaves, resulting in enhanced tolerance to stress (Sherameti *et al*., 2008). Inoculation of *Azospirillum* sp. and *Herbaspirillum* sp.inmaize provided tolerance under drought stress (Cura *et al*., 2017).They also studied the expression of*ZmVP14* gene which is involved in the biosynthesis of abscisic acid and observed that there was lower expression of *ZmVP14* gene in the inoculated plants. Inoculation with *Pseudomonas simiae* strain AU, confirmed the involvement of transcription factors (*DREB*/*EREB*), osmoprotectants (*P5CS, GOLS*), and water transporters(*PIP* and *TIP*) of these genes was upregulated in soybean plants (*Glycine max* L.) leading to drought tolerance (Vaishnav and Choudhary, 2018).

2.8.8 Exopolysaccharide production

The water potential of agricultural soil is a key parameter that determines the availability of water, oxygen and nutrients to plants and microorganisms. Availability

and retention of water by soil is determined to a very large extent by physico-chemical and structural properties of the soil. The complex and dynamic interactions among microorganisms, roots, soil and water in the rhizosphere induce changes in the physico-chemical and structural properties of rhizospheric soil (Haynes and Swift, 1990; Tisdall and Oades, 1980). These properties are disturbed under drought stress, making soil unsuitable for soil microbial activity and growth of plants. Water availability controls the production and consumption of protein and polysaccharides by bacteria (Roberson and Firestone, 1992) and thus, indirectly influences soil structure. Bacteria produce exopolysaccharides which play an important role in influencing soil structure. Production of these exopolysaccharides is reportedly enhanced under water stress. Microbial polysaccharides can bind soil particles to form micro-aggregates (<250 μm diameter) and macro-aggregates (>250 μm diameter; Oades, 1991).

Bacteria like *Pseudomonas* can survive under stress conditions due to the production of exopolysaccharide (EPS), which protects microorganisms from water stress by enhancing water retention and by regulating the diffusion of organic carbon sources (Chenu and Roberson, 1996). EPS also help the microorganisms to irreversibly attach and colonize the roots, due to involvement of a network of fibrillar material that permanently connects the bacteria to the root surface. Some investigations showed that the polysaccharides produced by *Azospirillum* played a crucial role in soil aggregation (Bashan *et al*., 2004). The EPS released into soil as capsular and slime materials by soil microbes can be absorbed by clay surfaces due to cation bridges, Van der Waals forces, hydrogen bonding and anion adsorption mechanisms, thus forming a protective capsule around soil aggregates (Tisdall and Oades 1982; Degens *et al*., 1994). The modification in physical properties of soil due to aggregation has been demonstrated to contribute to water and nutrient uptake by plant roots.

Plants treated with EPS-producing bacteria display increased resistance to water stress (Bensalim *et al.,* 1998). In some experiments they observed a significant increase in root adhering soil per root tissue (RAS/RT) ratio in sunflower rhizosphere inoculated with the EPS-producing rhizobial strain YAS34 under drought conditions (Alami *et al*., 1998). Similar results were obtained with wheat seedlings inoculated with *P. polymyxa* (Gouzou *et al*., 1993) and *P. agglomerans* under salt stress (Amellal *et al*., 1998). The higher level of EPS producing *P. putida* strain GAP-P45, isolated from sunflower rhizosphere, was used as seed treatment to alleviate drought stress effects in sunflower

seedlings (Sandhya *et al*., 2009). Inoculation of *Pseudomonas* sp. strain GAP-P45 increased the survival rate, plant biomass and root adhering soil/root tissue ratio of sunflower seedlings subjected to drought stress. Inoculated bacteria efficiently colonized the root adhering soil and rhizoplane and increased the percentage of stable soil aggregates. Scanning electron microscope studies showed the formation of biofilm of inoculated bacteria on the root surface.

2.9 Osmotolerant bacteria

Osmotolerant bacteria are those well adapted to environments with high osmotic pressures at high sugar concentrations. Osmophiles are similar to halophilic organisms, both having low water activity. They are found in all three domains of life: Bacteria, Archaea and Eukarya and with great diversity of organisms.

2.9.1 Adaptive mechanisms in bacteria for osmotic stress

Microorganisms respond to increase in osmolarity by accumulating osmolytes in the cytosol of their cell, which protects them from cytoplasmic dehydration (Yancey *et al*., 1982). These compatible solutes confer protection against the deleterious effects of low water activity, maintain the appropriate cell volume, and protect intracellular macromolecules and help in osmotic adjustment (Csonka and Epstein, 1996). The spectrum of compatible solutes used by microorganisms comprises only a limited number of compounds: trehalose, glycerol, glucosyl glycerol, proline, glutamate and some of the derivatives such asproline, betaine, ectoine, quaternary amines and their sulfonium analogues such as glycine betaine, carnitine and dimethylsulfoniopropionate. Many sulfate esters such as choline-*O*-sulfateand *N*-acetylated diamino acids and small peptides such as *N*- acetylornithine and *N*-acetyl glutaminyl glutamine amide. Under osmotic stress, the accumulation of these osmoprotectants is achieved by either endogenous *de novo* synthesis or uptake mediated by specific transporters (Kempf and Bremer, 1998).

Halophilic microbes usually adopt either of the two strategies of survival in saline environments: salt-in strategy and compatible solute strategy (Ventosa *et al*., 1998). Compatible solute strategy is employed by the majority of moderately halophilic and halotolerant bacteria, some yeasts, algae and fungi. In this strategy, cells maintain low concentrations of salt in their cytoplasm by balancing osmotic potential through the synthesis or uptake of organic compatible solutes. Hence, these microbes are able

to adapt to a wide range of salt concentrations. The compatible solutes include polyols such as sugars, glycerol, and their derivatives, amino acids and their derivatives, and quaternary amines such as ectoines and glycine betaine. The salt-in strategy is employed by true halophiles, including halophilic archaea and extremely halophilic bacteria. These microorganisms are adapted to high salt concentrations and cannot survive when the salinity of the medium is lowered. They generally do not synthesize organic solutes to maintain the osmotic equilibrium. This adaptation involves the selective influx of K^+ ions into the cytoplasm. All enzymes and structural cell components must be adapted to high salt concentrations for proper cell function.

Under osmotic stress, the accumulation of these osmoprotectants is achieved by either endogenous *de novo* synthesis or uptake mediated by specific transporters (Kempf and Bremer, 1998). Certain species of halophilic *Pseudomonas halosaccharolytica*and *Pseudomonashalophila*had the ability to synthesize and accumulate ectoine as compatible solute (Severin *et al*., 1992). Glycine betaine was a potent osmoprotectant for *Rhizobium meliloti* (Sauvage *et al*., 1983). Some reports showed that glycine betaine uptake was strongly stimulated when a variety of *R. melilotii* strains were subjected to an osmotic upshock (Le Rudulier and Bernard, 1986). Glycine betaine was a potent osmoprotectant for species of *Azospirillum* and *Pseudomonas* (Hartmann, 1988; Pocard *et al*., 1994). Proline betaine (stachydrine) was potent osmoprotectant for *R. melilotii* (Bernard *et al*., 1986). Proline betaine uptake was strongly stimulated when cells were transferred to a medium of elevated osmolality (Gloux and Le Rudulier, 1989).

Salt tolerant *Rhizobium* spp. was reported to accumulate glutamate when cells were grown at elevated osmolality (Hua *et al*., 1982). *Rhizobium* spp. accumulated K^+ when exposed to osmotic shock (Yap and Lim, 1983). *P. pseudoalcaligenes* an efficient salt tolerant biocontrol strain *de-novo* synthesized certain amino acids for salt tolerance (Paul *et al*., 2005). Glutamate also accumulated within *Pseudomonas* spp. and *Azospirillum* spp. during growth at elevated osmolatily (Pocard *et al*., 1994; Madkour *et al*., 1990). Glutamate and proline were the predominant amino acids when *A. brasilense* was grown in the presence of moderately high NaCl concentration (e.g. 0.3-0.5M NaCl). In some reports they have shown that trehalose accumulation by *R. meliloti* occurred primarily when cells were subjected to severe osmotic stress. Trehalose was the major compatible solute in a variety of pseudomonad species

(D'Souza *et al*., 1993; Smith *et al.*, 1994). Hartmann *et al.* (1991) reported that trehalose was a compatible solute in *A. brasilense* strain SP7and *Azospirillum halopraeferens* strain AU4. Pocard *et al.* (1994) showed that glucosylglycerol was a predominant compatible solute in *Pseudomonas mendocina* and *P. pseudoalcaligens.*

The adaptation of diazotrophs to osmotic stress is of great significance, because soil salinity inhibits many of the vital bacterial plant growth-promoting activities, such as nitrogen fixation and phytohormone production (Miller and Woods, 1996). There were deleterious effects of salt stress on growth and nitrogenase activity of *Azotobacter chroococcum* (Madkour *et al*., 1990). Nitrogenase activity in *Azospirillum* and *Klebsiella pneumoniae* were affected by salt stress (Rao and Venkateshwarulu, 1985). Biocontrol activities of *P. pseudoalcaligens* and *P. fluorescens* were not affected by high salinity, since these microbes accumulated compatible osmolytes as a mechanism for salt tolerance (Paul *et al*., 2005; Paul and Nair 2008).*A. brasilense* accumulated glycine betaine, to restore growth and acetylene reduction activity, under salt stress (Chowdhury *et al*., 2006). Production of phytohormone indole acetic acid was also affected at 100mM NaCl concentration (Tripathi and Mishra, 1998b).

Bacterial EPS can protect bacteria from various stresses. Production of EPS is used as criteria for the isolation of stress tolerant microorganisms. A *Pseudomonas* sp. strain increased its EPS production during desiccation (Roberson and Firestone, 1992). The production of EPS possibly enhances water retention in the microbial environment and seems to regulate the diffusion of carbon sources such as glucose. Bacteria like *Pseudomonas* can survive under stress conditions due to the production of EPS, which protects microorganisms from water stress by enhancing water retention and by regulating the diffusion of organic carbon sources (Chenu and Roberson, 1996). Cellular defences against desiccation-induced damage include accumulation of compatible solutes, exopolysaccharide production and enzyme synthesis to combat oxidative stress (Leblanc *et al*., 2008). Concentration and composition of microbial EPS dramatically changed under stress conditions. Capsular material of *A. Brasilense* strain Sp245 was found to contain high molecular weight carbohydrate complexes (lipopolysaccharide–protein (LP) complex and polysaccharide–lipid (PL) complex that could be responsible for protection under extreme conditions like desiccation. Addition of these complexes to a suspension of de capsulated cells of *A. Brasilense* strain Sp245 significantly enhanced survival under drought stress.

3.1 Bacterial cultures used

Thirty one osmotolerant endophytic bacteria isolated from pearl millet, mustard and cluster bean available in the germplasm of Division of Microbiology, IARI, New Delhi-110012, were used in the present study.

3.2 Growth and maintenance of endophytic bacteria

Endophytic bacteria were grown on nutrient agar or nutrient broth (Atlas, 2004)(Appendix I). The stock cultures were maintained on slants of nutrient agar medium and were refrigerated at 4ºC. Sub culturing was done as and when required.

3.3 Sterilization of media and glassware

All media were autoclaved at 15 psi (1.06 kg/cm^2) pressure for 20 min. Glasswares used in the experiment were sterilized in a hot air oven at 180ºC for 2 hr. Thermolabile chemicals were filter sterilized using 0.22 µm sterile disposable syringe filters.

3.4 Test crop

Seeds of drought susceptible pearl millet variety (composite-443), which is recommended under irrigated conditions, were collected from the Division of Agronomy, Indian Agricultural Research Institute, New Delhi-110012.

3. 5 Screening for water deficit stress alleviation in pearl millet crop

The thirty one osmotolerant endophytic bacterial isolates were used for screening for their effect on plant growth under water deficit stress conditions under Phytotron conditions. Six inch plastic pots containing 1 kg sterile soil were used for the experiment. Pearl millet seeds were inoculated with 48 hr old broth cultures of osmotolerant endophyticbacteria (approx. 1x10^7 cfu/ml) before sowing. Four seeds per pot were sown and 15 days after sowing, 30 days stress was given. Soil was maintained at 50% field capacity (FC). In absolute control treatment uninoculated plants were maintained at FC and in uninoculated control treatment, plants were maintained at 50% FC. Three replications per treatment were maintained. Data on shoot, root fresh and dry weight was recorded 45 days after sowing (DAS).

Five culturesshowing best performance for most of the parameters were selected for further studies.

3.5.1 Effect on seed germination and seedling vigour

Seeds of pearl millet cultivar composite-443 were surface sterilized with 0.1% $HgCl_2$ solution for 3 min followed by 70% ethanol for 30 seconds. These were then thoroughly washed with sterile water to remove traces of $HgCl_2$and ethanol. The surface sterilized seeds were soaked in 48 hr old broth cultures of the selected isolates for 1 hr and then excess broth was drained. The seeds were then kept for germination on petriplates containing 0.8% sterile agar supplemented with 0%, 20% and 40% PEG 6000. Appropriate uninoculated controls were maintained. Ten seeds per petriplate and three replications of each treatment were maintained. Petriplates were incubated at 30±2°C for 3 days. After 3 days, data on percent seed germination, fresh weight of seedling and radicle and plumule length were recorded.

Two most promising isolates were selected on the basis of seed germination, fresh weight of seedlings, radicle and plumule length and these were used for further studies.

3.5.2 Effect of osmotic stress on growth of the selected isolates

Effect of osmotic stress on the growth of the selected isolates was studied by two methods, determining optical density using a spectrophotometer at regular intervals as well as determining growth kinetics by using Bioscreen C.

3.5.2.1 Spectrophotometer method

Nutrient broth supplemented with different concentrations of PEG 6000 (0%, 20%, 30%, 40%and 50%) were used to study the effect of osmotic stress on the selected osmotolerant isolates. Appropriate uninoculated controls were maintained. The tubes were inoculated with two days old 2% inoculum of the selected isolates and incubated at 28±2ºC for 48 hr on an orbital shaker. Growth of the isolates on different concentrations of PEG 6000 was determined by taking O.D. at 600 nm using a spectrophotometer.

3.5.2.2 Bioscreen C method

Nutrient broth supplemented with different concentrations of PEG 6000 (0, 10%, 20%, 30% and 40%) was prepared and after autoclaving these were used to study

the effect of osmotic stress on the selected osmotolerant isolates. Cell growth of the bacterial strains was measured using a Bioscreen C lab system (Oy Growth Curves Ab Ltd, Helsinki, Finland). Nutrient broth @294µL was transferred to Honeycomb wells and to this 2% (6µL) inoculum of the desired culture broth was added. Three replicates per treatment and one blank were maintained. The filled plates were immediately placed in the Bioscreen for analysis. The optical density at 600 nm (OD600) was measured every twenty minutes at 30 °C for a period of 24 hr under static condition. Microsoft Excel was used for drawing growth curves.

3.6 Effect of osmotic stress on plant growth promoting activities of the selected osmotolerant isolates

3.6.1 Phosphate solubilization ability

Two isolates were selected for studying the effect of osmotic stress on phosphate solubilization ability. Pikovyskaya's broth supplemented with 20% and 30% PEG 6000 was prepared and dispensed in tubes. Pikovyskaya's broth without PEG 6000 was used as control treatment. To each of the tubes containing 5 ml of the broth, 25 mg of tri calcium phosphate was added individually. The tubes were then inoculated with 2% inoculum of the desired culture broth. For each treatment, three replications were maintained. These were incubated at 28±2ºC for 7 days on an orbital shaker. The broth cultures were then transferred to the centrifuge tubes and centrifuged at 10,000 rpm for 8 min. Phosphate solubilized was determined in the supernatant by the method of King (1932), improved by Jackson (1967). In each case, one ml of the supernatant was transferred to fifty ml of the volumetric flasks. Ten ml of chloro molybidic acid (see Appendix I) was added and the volume was made to around 42-45 ml. To this five drops of stannous chloride were added. The volume was made up to 50 ml and immediately observations were taken at 600 nm using Perkin Elmer spectrophotometer (Model Lambda EZ 201). The quantity of phosphate solubilized was expressed as (µg P solubilized/mg protein).

3.6.1.1 Protein estimation

One ml of the sample was taken and cells were pelleted by centrifuging at 10,000 rpm for 8 min using a SPINWIN centrifuge. The pellets were then suspended in0.5 ml of 2N NaOH and then kept in boiling water bath for 10 min. These were allowed to cool, then 0.5 ml 2N HCI was added for neutralization. The samples were

then transferred to glass tubes and 5 ml of solution C (see Appendix I) was added to it. These were mixed thoroughly and allowed to stand at room temperature for 10 min. To this, 0.5 ml of 1:1 diluted Folin's reagent was added with rapid and immediate mixing. Tubes were then incubated for 30 min at room temperature for colour development. Intensity of blue colour was measured at absorbance maxima of 660 nm using a Perkin Elmer Spectrophotometer (Model Lambda EZ 201).

3.6.2 Acetylene reduction ability

The selected cultures were inoculated in semi-solid modified Rennie's combined carbon medium supplemented with 20% and 30% PEG 6000. Semi-solid modified Rennie's combined carbon medium without PEG 6000 was used as control treatment. Broth cultures of the isolates were inoculated in test tubes containing Rennie's combined carbon medium and these were incubated at 28±2ºC for 3 days in a B.O.D. incubator. After incubation, ARA activity was determined using a Gas chromatograph by the method of Hardy (Hardy *et al*., 1971). The cotton plugs were removed and the tubes were plugged with sterile suba seals. Ten per cent air space (v/v) of the tubes was replaced with acetylene and the tubes were further incubated for 24 hr. Appropriate uninoculated controls were maintained. Three replications per treatment were maintained. After incubation, 1 ml air sample was removed from the tubes and injected into GC for analysis. The ethylene produced by reduction of acetylene was assayed using a Gas chromatograph (Nucon 5765 model) with a FID detector having Porapak N column. The carrier gas was nitrogen. The operating conditions were: Injector temperature: 110ºC, Column temperature: 75ºC and Detector temperature: 110ºC. Standard ethylene (100 vpm) was used to calculate the amount of ethylene produced. The growth in test tubes was carefully collected and protein content was estimated as described in section 3.6.1.1. ARA was expressed in terms of nmoles of ethylene produced per mg protein per hr. The equation used to calculate ethylene produced was as follows:

$$\text{Nano moles of } C_2H_4\text{/mg protein/hour} = \frac{C \times Ps \times As \times V}{P_{STD} \times A_{STD} \times T \times P}$$

Where,

C = Concentration of ethylene in standard in nano moles

P_S= Peak area of the sample

A_S= Attenuation used for sample

V= Volume of air space in the test tube in ml
P_{STD}= Peak area of standard ethylene
A_{STD} = Attenuation used for the standard ethylene
T= Incubation time in hours
P= Protein content of bacterial growth in test tube in mg

3.6.3 IAA production

To study the effect of osmotic stress on IAA production ability of the selected isolates, Luria broth (see Appendix I) supplemented with 20% and 30% PEG 6000 was prepared. After autoclaving, filter sterilized tryptophan solution @ 100 µg/ml broth was added individually to each tube. Luria broth without PEG 6000 was used as control treatment. The tubes were then inoculated with 2% inoculum of the desired culture broth and the tubes were incubated at 28±2ºC on an orbital shaker for 5 days. Three replications for each treatment were maintained.

After incubation, IAA production by the isolates was determined by the method of Hartmann (Hartmann *et al.*, 1983). One ml of the culture was transferred aseptically to eppendorf tube and centrifuged at 10,000 rpm for 5 min. The supernatant was used for the detection of IAA, whereas the pellet was used for protein estimation by Lowry's method as described in the section (3.6.1.1). For the detection of IAA, to 1 ml of the supernatant 4 ml of the reagent (see Appendix I) was added and mixed thoroughly. The tubes were incubated for 30 min to allow the colour to develop before taking reading at 530 nm in a Perkin Elmer spectrophotometer (model Lambda EZ 201). IAA production by the cultures was expressed as µg IAA produced/mg protein.

3.6.4 ACC deaminase activity

To study the effect of osmotic stress on ACC deaminase activity of the selected isolates, these were grown in 5 ml of nutrient broth supplemented with 20% and 30% PEG 6000 at 30°C for two days until they reached stationary phase. Then cells were collected by centrifugation, washed twice with 0.1 M Tris-HCl (pH 7.5), suspended in 2 ml of DF salts broth(see Appendix I)supplemented with 5 mM final concentration ACC, incubated at 30°C with shaking for another 48 hr. The induced bacterial cells were harvested by centrifugation for 10 min at 10,000 g, washed twice with 0.1 M Tris-HCl (pH 7.5), and resuspended in 200 µl of 0.1 M Tris-HCl (pH 8.5).The cells were labilized by adding 5% toluene (v/v) and then vortexed at the highest speed for 30 sec. Fifty µl of labilized cell suspension was incubated with 5 µl of 0.5 M ACC in an

eppendorf tube at 30°C for 30 min. The negative control for this assay included 50 µl of labilized cell suspension without ACC. The blank included 50 µl of 0.1 M Tris-HCl (pH 8.5) with 5 µl 0.5 M ACC. The samples were then mixed thoroughly with 500 µl of 0.56 N HCl by vortexing. The cell debris was removed by centrifugation at 20,000 X g (Sigma centrifuge 3K30) for 5 min. A 500 µl aliquot of the supernatant was transferred to a 13 X 100 mm glass test tube and mixed with 400 µl of 0.56N HCl and 150 µl of DNF solution (0.1 g 2, 4-dinitrophenylhydrazine in 100 ml of 2N HCl) and the mixture was incubated at 30°C for 30 min. One ml of 2N NaOH was added to the sample before the absorbance was measured at 540 nm.

3.6.5 Gibberellic acid production

To study the effect of osmotic stress on gibberellic acid production by the selected isolates, King's B broth (see Appendix I)supplemented with 20% and 30% PEG 6000 was prepared, inoculated and incubated at 28°C, for 7 days. King's B broth without PEG 6000 served as control. After incubation the samples were centrifuged at 8000 rpm for 10 min. Supernatant was used for Gibberellic acid estimation whereas pellet was used for protein estimation by Lowry's method as described earlier in the section (3.6.1.1). For Gibberellic acid, 1 ml of the supernatant was pipetted out separately into the test tubes and 0.133 ml of potassium ferro cynide solution was added to 1 ml of 30% HCl and the mixture was incubated at 27^{o}C for 75 min. The blank was prepared with 55%HCl. Absorbance was measured at 254 nm in a UV-spectrophotometer.

3.6.6Exopolysaccharide production

To study the effect of osmotic stress on exopolysaccharide production by the selected isolates, nutrient broth (NB) supplemented with 20% and 30% PEG 6000 was prepared. Nutrient broth without PEG 6000 was used as control treatment. The tubes were inoculated and incubated at 30°C under shaking conditions (120 rpm) for 7 days and after incubation,10 ml aliquots were removed from the growth medium and transferred to plastic centrifuge tube. The cell biomass was separated from the culture supernatant using centrifugation (10,000g for 15 min). The supernatant liquid was then transferred to a new centrifuge tube. Three volumes of chilled absolute ethanol were added to the supernatant liquid to precipitate exopolysaccharides. The tube was inverted several times for thorough mixing. Centrifugation (10,000rpm for 5 min) was used to separate the supernatant from the precipitate. The pelleted exopolysaccharide was dried

at 50°C for 24 hr to remove any ethanol residues. The weight of the dried exopolysaccharides was recorded and expressed as mg exopolysaccharide produced/mg protein.

3.6.7 Effect of osmotic stress on plant growth promoting activities during plant-microbe association

Two levels of osmotic stress were maintained using 20% and 30% PEG 6000 concentrations for studying the effect of osmotic stress on plant growth promoting activities during plant-microbe association. Pearl millet seeds were surface sterilized with 0.1% mercuric chloride for 3 min followed by thorough washings with sterile water. These seeds were kept for germination on soft agar (0.8%). After germination, the seedlings were transferred to flasks containing sterile distilled water. Log phase MCL-1 and MKS-1 cultures were used to inoculate these flasks. Ten seedlings per flask and appropriate controls were maintained. The flasks were kept under controlled conditions of the National Phytotron facility, IARI, India for 15 days. The flasks were maintained at optimal temperature for growth of pearl millet plants, 30±2ºC at day and 25±2ºC at night. After 15 days, the flasks were removed, the water in the flasks was collected and centrifuged to remove debris. IAA, GA and exopolysaccharide present in the supernatant were quantified as described earlier. For ethylene determination after 15 days, the flasks were plugged with subaseals to make them airtight and were further incubated for another 24 hr. At the end of incubation period, ethylene present in the head space was quantified by using gas chromatography (Mayak *et al*., 2004).

3.7 Molecular characterization

3.7.1Genomic DNA extraction

The two selected osmotolerant endophytic bacterial cultures were grown by inoculating a single colony from a freshly streaked nutrient agar medium plate in 5 ml of Luria broth and incubating it at 28±2ºC for 24 hr. Log phase cultures were used for isolation of total genomic DNA using ZR Genomic DNA II isolation kit (prolab). In an eppendorf1.5 ml of culture was taken and centrifuged at 10,000 rpm for 2 min and the supernatant was discarded. The pellet was resuspended in 350µl of genomic lysis buffer. It was allowed to stand at room temperature for 5-10 min. This was then transferred to a Zymo spin column in a collection tube and centrifuged at 10,000 rpm for 2 min. Zymo spin column was then transferred to a new collection tube, 200 µl of DNA pre wash buffer was added to the spin column centrifuge and again centrifuged

at 10,000 rpm for 2 min. Then 400 µl of g-DNA wash buffer was added to the spin column and again centrifuged at 10,000 rpm for 2 min. The spin column was then transferred to a new clean micro centrifuge tube and 50 µl of DNA elution buffer was added to the spin column. It was incubated for 5 min at room temperature and then centrifuged at 10,000 rpm for 2 min to elute the DNA.

3.7.2Amplification of 16S rDNA

The 16S rRNA gene from the bacterial genomic DNA was amplified using forward primer 5' AGAGTTTGATCCTGGCTC3' and reverse primer 5'TACGGTACCTTGTTACGACTT3' (Lane, 1991). In a reaction mixture 50-100 ng of template DNA, primer 10 pmol each, dNTP (200µM each), 2.5UTaq DNA polymerase (MbI Fermentas) were used. Final volume of reaction mixture was adjusted to 25 µl. Amplification was carried out under standard conditions (initial denaturation at 94ºC for 5 min, 30 cycles of denaturation at 95ºC for 30 sec, 30 cycles of annealing at 55ºC for 40 sec, extension at 72ºC for 1 min and final extension at 72ºC for 8 min). PCR product was resolved in 0.8% of agarose gel in 1xTAE buffer incorporated with (10 mg/ml) ethidium bromide, run at 60 V for 1.30 hr and visualized on a gel documentation system (Alpha Imager). Amplified product was purified by using Nucleo spin gel (Macherey-Nagel) PCR cleanup kit according to the instructions given in the manual.

3.7.3 Identification of the selected isolates

Amplified 16S rDNA of the selected isolates was sequenced. Sequencing of the purified DNA was done using automated fluorescent sequencer. The sequence was compared from BLAST search with known and identified cultures of NCBI database and then submitted to the Genbank.

3.8 Pot experiment

Earthen pots (14 inch size) were used for the experiment. Each pot contained 10 kg soil. Pearl milletcompositevariety-443seeds were inoculated with 48 hr old broth cultures (approx. $1x10^7$ cfu/ml) of the selected promising osmotolerant endophytic bacterial strains MCL-1 and MKS-1. The seeds were sown in the pots and single plant per pot was maintained. Plants were maintained at three levels of water regimes namely no stress (Field capacity), mild stress (-0.5 MPa) and severe stress (-1MPa). For

maintaining desired water stress, moisture content of soil was regularly monitored and water was replenished, in the pots, as and when required and stress was subjected 15 days after sowing. Twelve replications per treatment were maintained. After 50 days of growth, sampling was done to determine the effect of water stress on various plant physiological and biochemical parameters. Three replications were used for sampling and the remaining replications per treatment were used for determination of plant nitrogen, phosphorus, potassium, yield and dry weight after harvesting the crop. Carbohydrates content of the soil was determined at the time of crop harvest.

3.8.1 Measurement of net photosynthetic rate, stomatal conductance and transpiration velocity

Net photosynthetic rate (Pn), transpiration velocity (E) and stomatal conductance (gs) were measured on the 4thyoungest fully expanded leaf from the top at 60 DAS between 9:00–11:00 a.m. All the 9 plants from each treatment group were used for the measurement. The photosynthetic active radiation (PAR), temperature and CO_2 concentration during the measurements were 300 l mol m^{-2} s^{-1}, 30 C and 400 l mol mol^{-1} respectively. A portable LI-6400XT photosynthesis measurement system was used for measuring these parameters.

3.8.2 Plant physiological and biochemical parameters

Three replications per treatment were used for determination of various plant physiological and biochemical parameters. The plant physiological and biochemical parameters determined were membrane stability index (MSI), relative water content (RWC), chlorophyll, proline, glycine betaine, amino acid, total sugar, starch and total phenolics content.

3.8.2.1Membrane stability index

Membrane stability was determined by the modified method of Ibrahim and Quick (2001). One gram sample consisting of 10 leaf segment, 7 cm long, were rinsed in distilled water and placed in 16x150mm test tube with 10 ml of 0.4 M mannitol. The tubes were held overnight at room temperature. Conductance was measured with an electrical conductivity meter after calibration with a calibration solution. Test tubes were then autoclaved for 10 min at 120ºC and conductance was measured again. Membrane stability was expressed as:

$$MSA = (1 - T1/T2) \times 100$$

Where,
T1= was the conductivity reading before autoclaving
T2=was the conductivity reading after autoclaving.

3.8.2.2 Relative water content

Relative water content was determined by the method of Krishna *et al*. (2005). One gram of fully expanded leaves was collected and 8 mm discs were made and fresh weight of these were estimated and then these were floated over distilled water in petriplates for 6 hr. At the end of this period, these discs were surface dried and saturated weight was recorded. Thereafter the samples were dried in an oven (70ºC) for 24 hr and dry weight was recorded. Relative water content was then calculated as:

$$RWC = (Fresh\ wt - Dry\ wt) / (Saturated\ wt - Dry\ wt)$$

3.8.2.3 Proline content

Proline content was determined by the method of Bates *et al.* (1973). Leaf sample (1g) was grinded with 5 ml of 3% 5-sulfosalicylic acid (w/v in water). Samples were centrifuged at 10,000 rpm for 30 min. One ml of supernatant was mixed with 2 ml of ninhydrin reagent (see Appendix I). The mixture was boiled for 1 hr and then colour was extracted with 2 ml toluene. O.D was measured at 520 nm using a spectrophotometer and proline content in the samples was quantified using a standard curve.

3.8.2.4 Glycine betaine content

Glycine betaine content was determined by the method of Grieve and Grattan (1983). Leaf sample (0.5g) was homogenized with 5ml 0.2M phosphate buffer (pH-7.4). Filtrate was centrifuged at 10,000rpm for 10min at 4ºC temperature. Then 0.5M, 2ml perchloricacid was added to the pellet and this was incubated for 10 min in cold. After that 3M KOH was added to the mixture until the pH was raised to 4.5 and this was then centrifuged at 10,000rpm for 15min. Out of this, 2ml supernatant was incubated in cold overnight with 0.2ml potassium tri-iodide solution. After incubation, this mixture was centrifuged for 10min at 10,000rpm and 1ml supernatant was transferred to a test tube and 20 drops of concentrated $H_2SO_4$was added in cold

condition. After 2 hr of incubation at 0ºC, precipitate was collected by centrifugation at 10,000rpm for 10min. Now precipitate was dissolved in 10ml dichloroethane and incubated for 2 hr. After completion of incubation period, absorbance was measured at 365nm using a spectrophotometer.

3.8.2.5 Amino acid content

Amino acid content was determined by the method of Chen *et al.* (2006). One gm of freeze dried leaf sample was grinded in 5 ml methanol-chloroform-water (60:25:15 v/v) and mixture was incubated at 60ºC for 2 hr. The samples were centrifuged at 10,000 rpm for 30 min. The total amino acid content in the samples was determined by heating 1 ml of the supernatant with 1 ml of 0.1M acetate buffer (see Appendix I) and 1 ml of 5% ninhydrin (in ethanol) at 95ºC for 15 min. Then samples were cooled to room temperature and absorbance was measured at 570 nm using a spectrophotometer. Standard was prepared by using glycine (10-100 µg/ml).

3.9.2.6 Total sugar content

Total sugar content was determined by the method of Dubois *et al*. (1956). The samples were prepared as described for amino acids and 0.2 ml supernatant was taken and volume was made up to 1 ml with distilled water. To this 1 ml of 5% phenol (in water) and 5 ml of 96% sulphuric acid was added. The mixture was then shaken well, kept for 10 min and again shaken. It was then incubated at 25-30ºC for 20 min. O.D was taken at 490 nm. Glucose was used to prepare the standard curve.

3.9.2.7 Total phenolics content

Total phenolics content was determined by the method of Singleton *et al.* (1965). Fresh leaf tissue (500 mg) was grinded in 5 ml ethanol (80% v/v) using mortar and pestle. Sample was centrifuged at 10,000 rpm for 30 min. The pellet was resuspended in 2.5 ml ethanol and vortexed. This suspension was recentrifuged at 10,000 rpm for 30 min. An aliquot of 0.375 ml of this phenolics extract was mixed with 2.5 ml 1/10 diluted Folin-Ciocalteu reagent and 0.75 ml of 7.5% (w/v) Na_2CO_3 and was vortexed for 10 sec. The mixture was incubated at 45ºC in a shaking water bath for 15 min. Phenolics were measured at 750 nm using catechol (100-1000 ng/ml) as the standard.

3.8.2.8 Starch content

Starch content was determined by the method of Ait Barka *et al*. (2006). Fresh leaf tissue (500 mg) of freeze-dried leaf samples were homogenized individually at 4ºC in mortar containing 0.1M phosphate buffer, pH 7.5 (see Appendix I). The homogenates were centrifuged at 10,000g for 30 min and the pellets were resuspended in dimethyl sulfoxide-8M HCl (4:1 v/v). Starch was dissolved over 30 min at 60ºC with agitation (60 rpm). After centrifugation for 30 min at 10,000g, 0.2 ml supernatant samples were mixed with 0.2 ml iodine-HCl solution (0.06% KI and 0.003% I_2 in 0.05M HCl) and 2 ml of distilled water. The absorbance was read at 600 nm after 15 min of incubation at room temperature. Glucose was used to prepare the standard curve.

3.8.2.9 Chlorophyll estimation

Chlorophyll content was determined by the method of Arnon (1949). Fresh leaf sample (50mg) was cut into uniform size discs of 2 to 3 mm and these were then transferred into test tube. To this5 ml of DMSO was added and these tubes were incubated at 37 ºC for 4hr. After that absorbance was taken at 645 and 663 nm against DMSO as blank using a spectrophotometer. Total chlorophyll was estimated by the formula:

$$\text{Total Chl} = 20.2(A_{645}) + 8.02(A_{663}) \times V/(1000 \times W)$$

Where, A= absorbance at specific wavelength
V= final volume of chlorophyll extract in 100% DMSO
W= fresh weight of tissue extract

3.9 Root architecture determination

Root system architecture was determined by the method of Ansari *et al*. (1995). Three replications per treatment were used for root architecture determination. Plants were up-rooted carefully at 60 DAS and soil was removed by gentle shaking without damaging root; and root adhering soil was removed carefully by gently washing with tap water. The thoroughly washed fresh roots were carried to lab for determination of Root volume, Root surface area, Root length and Root diameter by using root scanner (Epson Expression 11000XL Graphic Arts Model).

3.10 Phyto hormone production

3.10.1 IAA estimation

Leaf samples (5g) were homogenized with liquid N_2 into powder and 2.5ml per gram fresh weight of 100% methanol was added to it. This was left overnight at 4°C in dark. Then it was centrifuged at 16,000g for 10 min at 4°C and the supernatant was transferred into a new fresh tube and 2ml of methanol was added to it and again it was left in dark at 4°C for 1-2 hr. Then it was centrifuged at 16,000g for 10 min at 4°C and the supernatant was transferred to a new tube and concentrated using a speed vacuum and volume was reduced to $1/10^{th}$ of the initial volume. To this 1ml of HPLC grade water was added and pH was adjusted to >9.0 with 1M KOH. Then 1ml of ethyl acetate was added and this was centrifuged at 16,000g for 10 min at 4°C. The lower aqueous phase was transferred to a new tube and pH was adjusted to < 3 with concentrated acetic acid and equal volume of ethyl acetate was added to it. It was again centrifuged at 16,000g for 5 min and then the upper organic phase was taken and dried completely in speed vacuum and dissolved in 150µl of methanol. This was filtered using syringe filter (0.22 µm) and 50 µl was injected into HPLC. In mobile phase two solvents (Solvent-A 90% methanol: 0.3% acetic acid: 10% HPLC water and solvent-B 10% methanol: 0.3% acetic acid: 90% HPLC water) were used. Fluorescence detector (Retention emission 280nm: Excitation 320nm) was used in IAA estimation.

3.10.2 GA estimation

Leaf sample (2g) were homogenized in 10 ml sodium phosphate buffer (50mM, pH7.5), containing 0.02% sodium diethyl dithio-carbonate extract. These were kept overnight at 4°C on a shaker at 150 rpm and centrifuged the next day at 10,000g at 4°C for 10 min. Supernatant was taken and volume made up to 5ml with sodium phosphate buffer (50mM, pH7.5). The supernatant was partitioned with 2.5ml/ 5ml diethyl ether in a separating funnel and aqueous phase was collected. The pH of the aqueous phase was adjusted to pH2.5 using 1N HCl and again partitioned twice with 5 ml petroleum ether. Aqueous phase was recollected after partitioning and repartitioned twice with diethyl ether. After collecting the aqueous phase the extract was again partitioned twice with 2.5 ml ethyl acetate and ether phase was collected. This ether phase was again partitioned twice with 5ml 0.2M K_2HPO_4. Aqueous phase was collected and its pH was adjusted to 2.5 by using concentrated H_3PO_4. The aqueous phase was later partitioned twice with 5 ml ethyl acetate and ether phase was collected which was dried after

filtering through a funnel containing sodium sulphate salt on the filter paper. The ethyl acetate extract was dissolved in 2ml methanol and used for estimation of GA. Sample was filtered through 0.22µm Millipore syringe filter before 20 µl sample was injected into HPLC. Mobile phase used was 65% methanol: 35% HPLC water. Variable length detector was used for GA estimation.

3.10. 3 ABA estimation

Frozen leaf sample (1 g) were homogenized three times with 10 ml of 80% v/v acetone (80 ml acetone, 1 ml glacial acetic acid and 100mg of 2,6 di-tart-butyl 4-methyl phenol in a total volume of 100 ml) and collected in a 100 ml volumetric flask. The tissue residue was then homogenized with pestle and mortar with acetone (80% v/v). The homogenate was filtered through WhatmanNo.1 filter paper. The filtrate was transferred to the boiling flask of rotary flask vacuum evaporator for removing acetone. As the acetone was evaporated, the lipid soluble material was deposited on the walls of the boiling flask. This was dissolved in 15 acetic acid solution and the amber colored aqueous solution was transferred into small vials. Before injecting the sample into HPLC, the samples were filtered with 0.45mm Millipore syringe filter using 2.5 ml plastic syringe and 20 µl sample was injected into HPLC. Mobile phase used was 1% acetic acid in 95% methanol and volume was made up by using HPLC grade water. Variable wavelength detector (265nm) was used in ABA estimation.

3.11.1 Plant dry weight

Three replications per treatment were used for root and shoot dry weight determination. The plants were harvested and after oven drying of the samples dry weight of roots and shoots were recorded.

3.11.2 Plant nutrient analysis

Oven dried grain and shoot samples were grinded and were analysed for nitrogen, phosphorus and potassium content.

3.11.2.1 Nitrogen content

Five hundred milligram of finely powdered sample was taken in 100 ml volumetric flask and 15 ml of $H_2SO_4$was added to it. This was allowed to stand for 30 min. To this 5g sodium thiosulphate was added and again it was allowed to stand for 30 min. The volumetric flask was heated slowly till frothing continued and after that it was heated briskly. Digestion was continued for half an hour after the colour of the

digest became transparent, then it was cooled and water was added to make up the volume to 100 ml. To this a few glass beads and 100ml of 40% NaOH solution was added along the sides of the kjeldahl flask. It was immediately connected to distillation bulb in the distillation unit. Ammonia evolved was collected in 25 ml boric acid solution and distillation was continued till 150 ml distillate was collected. Ammonia evolved was titrated against standard sulphuric acid (0.1N) using mixed indicator (0.5g Bromo cresol green and 0.1g Methyl red indicator in 100 ml ethanol).A blank containing all the reagents except the digested sample was also run.

3.11.2.2 Phosphorous content

Five hundred milligram of finely ground and powdered sample was mixed with 15 ml diacid (nitric acid: perchloric acid, 9:4)in volumetric flask and flask were placed on a hot plate in an acid proof digestion chamber having fume exhaust system. This was heated at 100^{o}C for 1 hr and then the temperature was raised to 200^{o}C. Digestion was continued until the contents became colourless and only dense fumes appeared and contents were reduced to 2-3 ml. Flask was removed from hot plate, cooled and water was added to make up the volume to 100 ml. It was then filtered through Whatman filter paper No.42. From this 5 ml of filtrate sample was taken into 50 ml volumetric flask and 5 N HNO_3 and 5 ml 0.25% ammonium metavanadate solution was added to it. This was mixed thoroughly and then 5 ml of 5% ammonium molybdate solution was added. Volume was made up to 50 ml with distilled water in a volumetric flask. It was thoroughly mixed and absorbance was recorded at 470 nm and reagent blank was used for setting zero.

3.11.2.3 Potassium content

Samples were processed as described earlier in section3.11.2.2 for Phosphorus estimation**.** Potassium content was determined in the digested samples using a flame-photometer.

3.12 Measurement of antioxidative enzymes

Leaf samples for the antioxidative enzyme assays were collected at 60 DAS.

3.12.1 Enzyme extraction

One gram leaf sample were homogenized with 10 ml 0.1M phosphate buffer, pH 7.0, containing 0.5mM EDTA in case of SOD, CAT and 0.5 mM EDTA and 1mM

ascorbic acid in case of APOX. The homogenate was centrifuged at 15,000g for 30 min at 4°C. The supernatant was collected and used for enzyme assays.

3.12.2 Superoxide dismutase (SOD)

Superoxide dismutase activity (SOD) was estimated by recording the decrease in optical density of formazone made by superoxide radical and nitro-blue tetrazolium (NBT) dye by the enzyme superoxide dismutase (Dhindsa *et al.*, 1981). The 3 ml reaction mixture contained 13.3mM methionine, 50 mM potassium phosphate buffer (pH 7.8), 75 mM NBT, 2 µM riboflavin, 0.1 mM EDTA, 50 mM sodium carbonate, 0.1 ml enzyme extractand0.9 to 0.95 ml of water. Reaction was started by adding 2 µM riboflavin (0.1 ml). Glass test tubes containing the reaction mixture were illuminated for 15 min at a light intensity of 3600 lux. Switching off the light stopped the reaction and the tubes were immediately covered with a black cloth. A non-irradiated reaction mixture containing enzyme extract, which does not develop color, was used as blank. Control was lacking enzyme in the reaction mixture and developed maximum color. The absorbance at 560 nm was recorded using a spectrophotometer. One unit of SOD was defined as the amount of enzyme required for causing 50% inhibition of the reduction of NBT. The enzyme activity was expressed as U/g of fr. wt.

3.12.3 Catalase (CAT)

Catalase activity was measured at 25°C according to the method given by Aebi (1984). Three ml of reaction mixture contained, 1.5 ml of 100 mM phosphate buffer (pH 7.0), 0.5 ml of 75 mMH_2O_2, 0.1 ml enzyme extract and 950 µl of distilled water. Control contained enzyme extract and phosphate buffer devoid of H_2O_2.Catalase activity was estimated by the decrease in absorbance of H_2O_2 (ε = 39.4 mM^{-1} cm^{1}) at 240 nm. The enzyme activity was expressed as H_2O_2 reduced mM/ min/mg of fr. wt.

3.12.4 Ascorbate peroxidase (APX)

Extraction method followed was same as in case of SOD except the extraction buffer contained 1 mM ascorbic acid (0.0176 g in 100 ml buffer) in addition to other ingredients. Ascorbate peroxidase was assayed by the method of Nakano and Asada (1981). The 3 ml reaction mixture contained, 0.5 mM ascorbic acid (0.5 ml of 3.0 mM), 50 mM potassium phosphate buffer (pH 7.0) (1.5 ml of 100 mM), 0.1 mM EDTA (0.1 ml of 3.0mM), 0.1 ml enzyme, 0.1 mM H_2O_2 (0.1 ml of 3.0 mM), 0.7 ml water to make a final volume of 3.0 ml. The reaction was started with the addition of 0.2 ml of

hydrogen peroxide. Decrease in absorbance for a period of 10 sec was measured at 290 nm in an UV- visible spectrophotometer. The initial and final contents of ascorbic acid were calculated by comparing with a standard curve drawn with known concentration of ascorbic acid. The enzyme activity was expressed as ascorbic acid oxidized (initial reading – final reading = quantity of ascorbic acid oxidized) per min/g fr. wt.

3.12.5 Ascorbic acid

Leaf sample (0.5 g) was homogenized with 10 ml of tricholoacetic acid. Homogenate was centrifuged at 5000rpm at 4^{o}C temperature. Supernatant was used for estimation of ascorbic acid. From this supernatant, 4 ml of the extract was mixed with 2 ml of 2% dinitrophenylhydrazine followed by the addition of 1 drop of 10% thiourea (in 70% ethanol). Then mixture was heated for 15 min in a boiling water bath at 100^{o}C. After cooling to room temperature, 5 ml of 80% (v/v) $H_2SO_4$was added to the mixture at 0^{o}C (in an ice bath). The absorbance was recorded at 530 nm.

3.12.6 Glutathione

Glutathione content was determined by the method of Smith *et al.* (1985).Leaf sample (1g) was homogenized with 10ml of cold 5% metaphosphoric acid. The homogenate was centrifuged at 10,000g for 15 min at 4^{o}C and the supernatant was used for analysis of reduced glutathione (GSH) and oxidized glutathione (GSSG). For total glutathione (GSH + GSSG) 1ml aliquot of supernatant was neutralized with 1.5 ml of 0.5 M phosphate buffer (pH 7.5), followed by addition of 50 µl of water. For GSSG assay, another 1 ml of the aliquot was neutralized with 1.5 ml of 0.5 M phosphate buffer (pH 7.5), followed by addition of 50 µl of 2-vinylpyridine, to mask the GSH, and the contents of the tube were vortexed until an emulsion formed. The tube was then incubated for 60 min at room temperature.

The 3 ml reaction contained 0.2 mM phosphate buffer (pH 7.5), 5 mM EDTA, 0.6 mM DTNB, 3 units of GR and water. Reaction was started by adding 0.1 ml of extract sample as described above. The reaction rate was monitored by measuring the change in absorbance at 412 nm for 1 min. The concentration of glutathione was quantified by referring to a standard curve based on GSH in the range of 0 to 50 µm ml^{-1}.

3.13 Lipid peroxidation

Leaf tissue (0.5 g) was homogenized in 10 ml of 0.1 % (w/v) TCA. The homogenate was centrifuged for 20 min (10,000 rpm). Supernatant was collected and 1 ml of supernatant was mixed with 4 ml 0.5% TBA diluted in 20 % TCA. This was incubated in water bath at 95°C for 30 min and cooled in an ice bath. In case the solution was not clear, it was centrifuged for a further 10 min (10,000 rpm). The absorbance was measured at 532 and 600 nm. O.D. 600 nm values were subtracted from the MDA-TBA complex values at 532 nm and MDA concentration was calculated using the Lambert-Beer law with an extinction coefficient ε= 155 $mM^{-1}cm^{-1}$. Results were presented as μmols MDA g^{-1}fr.wt.

Formula= (WL2 (523)-WL1 (600))/155*20

WL1= absorbance measured at 600 nm
WL2= absorbance measured at 523 nm

3.14 Induction of drought stress responsive genes

Leaf samples were harvested from pearl millet plants at field capacity and severe stress treatments, for RNA isolation at 60 DAS. Samples were immediately frozen in liquid nitrogen and stored at -80°C for isolating RNA.

3.14.1 Designing and synthesis of drought responsive genes /house-keeping gene primers forRT-PCR

PCR primers were designed using Primer-3 Software based on the sequence information available in databases (NCBI). Primers of *Elongation factor* **(PgEF-1a)** were designed from cDNA sequences of pearl millet. All primers used were submitted to NCBI database for BLAST search and confirmed to specifically anneal only with their corresponding genes. During designing primers all the parameters for RT primers were considered and followed systematically. All the designed primers were synthesized by ABA Biotech Company **(Table2).**

3.14.2 RNA isolation for RT-PCR

Pearl millet total RNA was extracted from the harvested leaf samples using GENETIX RNA Sure plant kit according to the manufacturer's instructions. Prior to use all glass wares, mortar and pestle were treated with chloroform, double autoclaved

and dried in an oven. Working bench, pipettes and hands were cleaned using RNase out solution.

Table 2. List of primers used for gene expression analysis in plant under water deficit stress conditions

Gene name	Primer sequence	
PgEF-1a	Forward	5'GTTACAACCCAGACAAGATTGC3'
PgEF-1a	Reverse	5'TGGACCTCTCAATCGTGTTG3'
PgAP2	Forward	5'GCAGAAGAGATTGCTGATGA3'
PgAP2	Reverse	5'GAGGGCTTTGAAGAAGAGAG3'
PgCSD	Forward	5'CTGTGGGATCATTGGACTTCAG3'
PgCSD	Reverse	5'CACATGTCCAGGGATGTTTAGAC3'
CaP5CS	Forward	5'TGGGTGTTGAAGGTCTCTTG3'
CaP5CS	Reverse	5'GGAAGGTTCTTGTGGGTGTAG3'
PgDREB2A	Forward	5'GGTTCACATAGGTCTGAGATGG3'
PgDREB2A	Reverse	5'TAGGTTCAAGTGGCTCGAATAC3'
SbSNAC1	Forward	5'TGGATCATGCACGAGTACAG3'
SbSNAC1	Reverse	5'TCTCCCACTCGTTCTTCTTATTG3'
PgAPX	Forward	5'CTCCTTCAGCTCCCAAGTGAC3'
PgAPX	Reverse	5'CAGTTCAGAGAGCCTGAGGTG3'
PgLEA	Forward	5'CATCGTCGGCAACTTCACCATC3'
PgLEA	Reverse	5'ACATTGGTCGATCAGATCACAGAG3'
SbYUC	Forward	5'CTGTACCGCGTGGGATTCTC3'
SbYUC	Reverse	5'GAGGAGACTATCTTGGCTCCA3C3'
SbGA20ox	Forward	5'GGTACAAGAGCTGCCTGCAC3'
SbGA20ox	Reverse	5'CTGCGTGAAGCGCATGAGGTC3'
SbNCED	Forward	5'GTACGTGCTCACCTTCGTCCAC3'
SbNCED	Reverse	5'GTGATGAAGGTGCCGTGGAAGC3'

3.14.3 Steps followed were as under

1. In a precooled mortar and pestel 100mg plant material was taken and was homogenized well using liquid nitrogen.
2. To the homogenized 100mg of tissues from step1, 350µl buffer RLB1 and 3.5µl β-mercaptoethanol was added and vortexed well.
3. The RNA Sure shredder was placed in collection tube (2ml) and the mixture was applied to itand this was centrifuged at 11.000xg for 1min. The filtrate was then transferred to a new microfuge tube of 1.5 ml capacity.
4. To the above solution 350µl of ethanol (70%) was added and mixed by pulse vortexing (10-15sec).
5. In a precooled mortar and pestel 100mg plant material was taken and was homogenized well using liquid nitrogen.
6. To the homogenized 100mg of tissues from step1, 350µl buffer RLB1 and 3.5µl β-mercaptoethanol was added and vortexed well.
7. The RNASure shredder was placed in collection tube (2ml) and the mixture was applied to it and this was centrifuged at 11.000xg for 1min. The filtrate was then transferred to a new microfuge tube of 1.5 ml capacity.
8. To the above solution 350µl of ethanol (70%) was added and mixed by pulse vortexing (10-15sec).
9. A new RNA Sure Plant column was taken and placed in collection tube and the lysate was loaded. This was centrifuged at 11,000xg for 30 sec. The columns were then placed in new collection tubes (2ml).
10. RDB solution 350µl was added and this was centrifuged at 11,000xg for 1 min to dry the membrane.
11. For on column DNase digestion,10µl of DNase was added to 90 µl of DNase reaction buffer in a 1.5 ml microcentrifuge tube. This was mixed properly and 95µl was applied directly onto the centre of silica membrane of the RNA Sure Column. This was incubated at room temperature for 15 min.
12. To the RNA Sure column 600µl of buffer RWB3 was added. This was centrifuged for 30 sec at 11,000xg. The filtrate was discarded. The column was transferred into a new collection tube.

13. To the column 200µl of buffer RWB3 was added and this was centrifuged for 2-3 min at 11000xg.The column was placed in a new nuclease free 1.5 ml collection tube.

3.14.4 Checking concentration, purity and integrity of RNA

Amount and purity ratio of RNA was quantified spectrophotometrically by using Nano Drop 1,000 (Nano Drop Technologies, Inc., DE, USA) at wavelengths of 260 nm and 280 nm. The RNA samples were also checked on 1.5 % agarose gel, which was prepared by dissolving 1.5 g of agarose in 100 ml of 1 X TBE followed by boiling until all the agarose dissolved. The gel was allowed to cool and 1 µl of ethidium bromide (10 mg/ml) was added to it, poured into a casting gel tray, and was allowed to solidify.

3.14.5 Preparation of RNA sample for loading

The 2.5 µl of RNA sample was taken in a 0.2 ml tube to which 2 µl of 2X RNA loading dye and 6 µl RNase free water was added and incubated at 60°C for 5 min followed by cooling on ice for 5 min. After giving a flush spin, the sample was loaded onto the wells of the gel. The gel was run at 70 V for 20 min and then visualized under UV light and picture was taken in the Gel documentation system (Alpha lmager).

3.14.6 Agarose gel electrophoresis

Material and Reagents

1. Agarose
2. 5X Tris-Borate-EDTA(TBE) Buffer (pH 8.0)
   - Tris base 54 g
   - EDTA 0.5 M (pH 8.0) 20 ml
   - Boric acid 27.5 g

   The buffer was autoclaved and stored at room temperature.
   - Final volume 1000 ml with distilled water
3. Gel loading dye (2X)
   - 95% formamide
   - 0.025% SDS

- 0.025% bromophenol blue
- 0.025% xylene cyanol FF
- 0.025% ethidium bromide
- 0.5 mM EDTA.

4. Mini gel apparatus and power supply
5. Gel documentation system: Alpha lmager

3.14.6.1 Procedure

The mini gel apparatus was setup as described by (Sambrook and Russell, 2001). The appropriate size of comb was selected and both ends of the slab were sealed by a tape to avoid leakage during casting of the gel.

- An agarose gel (1%) was prepared by heat dissolution of 1g of agarose in 100 ml 1X

  TBE buffer. Ethidium bromide from stock solution (10mg/ml) was added to a final concentration of 0.5 µg ml^{-1}.
- The gel was allowed to cool to almost 45-50°C before pouring into the gel plate.
- Once the gel was solidified, it was placed in the electrophoresis tank after removing the comb and tape.
- The tank was filled with 1 X TBE buffer till the gel was completely submerged.
- The total RNA samples were mixed with appropriate volume of 2X loading dye before loading.
- The samples were loaded and run at 5 v/cm.
- After 30 min of electrophoresis, the agarose gel was viewed using gel documentation system.

3.14.7qRT-PCR analysis

Two-step RT-PCR analysis was carried out to study gene expression.

3.14.7.1 First strand cDNA synthesis from total RNA

First-strand cDNA was synthesized from 1.5 µg of DNase-treated total RNA by iscript reverse transcriptase in 20µl reaction volume using 5x iscript reaction mix by

following the manufactures instructions. Various steps involved in cDNA synthesis are mentioned below.

In sterile PCR tube, the following components were added in the indicated order and mixed gently.

3.15.7.2 cDNA Reaction Mixture:

Component	**Volume/20µl reaction**
5x iscript Reaction mix	4µl
Iscript R- Transcriptase	1µl
RNA Template	Variable
Nuclease free water	Variable
Total volume	**20 µl**

When using larger amount of input RNA (>1µg) the reaction was scaled up to ensure optimum synthesis efficiency.

3.14.7.3 Thermal conditions for cDNA synthesis:

The complete reaction mix was incubated in a thermal cycler using the following protocol.

Component	**Thermal conditions**
Priming	5 min at 25°C
Reverse transcription	20 min at 46°C
RT-inactivation	1 min at 95°C
Optional step	Hold at 4°C

Once cDNA synthesis was completed the cDNA was stored at -20°C. Q-PCR was performed for all the primers listed in **(Table 2)** using cDNAs diluted to a working concentration.

1. 50X Tris-Acetic-EDTA(TAE) Buffer (pH 8.0)

- Tris base 242 g
- Glacial acetic acid 57.1 ml
- EDTA 0.5 M (pH 8.0) 200 ml

  Final volume 1000 ml with distilled water.

The buffer was autoclaved and stored at room temperature.

2. Gel loading dye (6X)

- 10mM Tris-HCI (pH 7.6)
- 0.03% bromophenol blue
- 0.03% xylene cyanol FF
- 60% glycerol
- 60mM EDTA

The PCR products (5µL) were analysed by agarose (1%) gel electrophoresis, as described in the earlier section.

3.14.7.4 Gene-specific primers for qPCR

Gene specific primers for qRT-PCR were designed manually and the primer characteristics were analyzed using Oligo analyzer (http://eu.idtdna.com/calc/analyzer). The sequence of primers used for qRT-PCR analysis is given in the **Table 2**.

3.14.7.5 Quantitative RT-PCR - reaction mix

Component	Volume/10µl reaction
PCR grade water	3.4µl
2x KAPA master mix	5 µl
10 µM primer-F	0.2 µl
10 µM primer-R	0.2 µl
Template cDNA	1 µl
50x ROX high	0.2 µl

The contents of the qPCR reaction were mixed and the qRT-PCR was carried out in Step One real time PCR machine (Applied Biosystems).

3.14.7.6 q-PCR conditions

Step	Hold	Cycle (40cycles)	
		Denature	Anneal/Extend
Temperature	95.0°C	95.0°C	60.0°C
Time	3 min	15 sec	30 sec

Expression data were normalized using endogenous control gene **(*PgEF-1a*).** Relative fold change was calculated using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). Each data point represents average of three technical replicates. The expression was represented in the form of relative fold change which is the relative change in expression of genes under stress conditions as compared with its expression under control conditions.

3.15 Soil Character determination

Soil samples were collected at the time of harvest for determination of soil carbohydrate content, Root adhering soil/ Root tissue ratio and water stability of soil aggregates.

3.15.1 Soil carbohydrate content

Soil carbohydrate content was determined by the method of Safarick and Santruckova (1992). Root adhering soil sample (10mg) were weighed and transferred into glass test tube and 2 ml water was added. One ml of 5% phenol solution was added and immediately after mixing, 5ml concentrated sulphuric acid was added rapidly and contents were mixed well by vortexing for 10sec. Then these were allowed to stand at room temperature for 1 hr. A soil blank was run with water instead of phenol solution. The test tubes were centrifuged for 10 min at 4600rpm. Absorbance of the sample was measured at 485nm (maximum absorbance caused by carbohydrates) and 575 nm (absorbance corresponding to blanks) against the phenol blank (no soil was added into water) and the soil blank were measured against sulphuric acid at 485nm.

3.15.2 Root adhering soil/ root tissue ratio

Root adhering soil was determined by the method of Sandhya *et al.* (2009). Plants were uprooted and given two gentle shakings to remove the loosely adhering bulk soil. The tightly adhered soil to root surface were removed and collected in plastic bags and air dried at room temperature. Roots were washed with tap water and dried in an oven for 48hr at 150^{o}C. Both soil and root dry weight were recorded and RAS ratio were calculated.

3.15.3 Water stability of soil aggregates

Soil samples (500g) were collected immediately after harvesting of crop and air dried at room temperature. The air dried soil samples were sieved with 3mm sieve and

soil aggregates were collected and 50 g was weighed for each replication. These soil aggregates were soaked for 10 min in 6 different sieves (4mm, 2mm, 1mm, 0.5mm, 0.2mm, 0.1mm)and different soil aggregates were retained in sieves according to the size which were collected and filtered by using filter paper. The filter paper were dried under room temperature and weight of the material retained on filter paper was recorded separately for different sieves.

3.16. Statistical analysis

Data generated were statistically analyzed using OPSTAT statistical software.

4.1 Screening for waterstress alleviation in pearl millet crop by the selected endophytic bacterial isolates

Out of the 31 isolates screened, inoculation with only 6 endophytic bacteria was observed to significantly improve shoot fresh weight under waterdeficit stressconditions (**Table 3**). Highest shoot fresh weight was observeddue to inoculation with isolateMKS-1**.**Other isolates which improved shoot fresh weight were KPSR-2, MCL-1, MMS-3, MMS-5 and MAS-2.Only three isolates namely MMS-3, MAL-2 and MAL-3 improved root fresh weight. Only three isolates significantly improved root dry weight namely CPSR-2, MMS-3 and MAL-2 while shoot dry weight was significantly enhanced by isolates MMS-3 and MKS-1. Five isolates showing best performance for most of the parameters were selected for further studies.

4.2 Effect of endophytic bacterial isolates on seed germinationand seedling vigour under osmotic stress

No effect of osmotic stress on seed germination was observed. Under both no stress and osmotic stress conditions 83.33% seed germination was observed in uninoculated control treatments (**Table 4**). Although, inoculation with isolate MKS-1 did improve seed germination under no stress condition, it was statistically at par with the control treatment. Under osmotic stress condition no positive effect of inoculation on seed germination was observed. However, under both these conditions inoculation with some of the cultures had a negative effect on seed germination viz. MMS-3 and MMS-5 under no stress condition and KPSR-2 and MMS-5 under osmotic stress condition.

Seedling vigour was improved due to inoculation with most of the isolates under no stress conditions as indicated byincreased radicle and plumule length ofseedlings (**Plate 1 and Table 4**). Under osmotic stress conditions, inoculation with isolate MKS-1 significantly improved plumule length while both the isolates MKS-1 and MCL-1 significantly improved radicle length and seedling fresh weight.

Table 3. Effect of the selected osmotolerant endophytic bacteria on plant growth under water deficit stress conditions

Treatments*	Shoot FW (g)	Root FW(g)	Shoot DW(g)	Root DW(g)
100% FC**	33.73	2.93	6.83	0.73
50% FC	23.42	1.17	3.85	0.4
NAD-3	12.47	0.40	2.12	0.22
NAD-7	15.40	0.75	2.48	0.36
NAD-9	12.53	0.53	2.13	0.24
NAD-17	20.77	0.93	3.32	0.38
MRD-4	20.93	0.85	2.79	0.44
MRD-8	19.00	0.68	3.34	0.31
MRD-9	20.67	0.88	3.51	0.44
MRD-17	20.23	1.16	2.85	0.45
RPRL-14	22.57	1.05	3.56	0.39
KPSR-2	26.33	1.39	4.22	0.47
KPSR-17	23.50	1.13	3.13	0.38
KPRL-6	24.43	1.11	3.16	0.33
KPRL-10	15.86	0.70	2.82	0.32
KPRR-22	11.27	0.84	2.06	0.32
KPSS-13	22.10	0.86	2.12	0.39
KPSR-5	23.33	1.00	3.89	0.41
CPSR-2	18.79	1.06	2.92	0.50
RPSL-9	17.56	0.60	2.87	0.27
RPSL-2	15.43	0.96	2.57	0.31
RPSR-5	24.00	0.82	3.78	0.40
MCL-1	27.37	1.30	4.16	0.58
MMS-3	29.30	1.43	4.99	0.49
MMS-5	30.67	1.36	3.98	0.43
MMR-1	20.96	0.97	4.52	0.43
MKS-1	32.33	1.20	5.03	0.36
MKL-4	15.30	1.06	2.62	0.41
MKR-2	20.53	1.08	2.80	0.30
MAS-1	17.27	1.38	2.68	0.40
MAS-2	26.67	1.28	3.74	0.47
MAL-2	23.20	1.40	3.56	0.59
MAL-3	25.87	1.52	4.11	0.47
SEm±	1.04	0.08	0.30	0.03
CD at 5%	2.94	0.23	0.86	0.09

* Except for absolute control treatment in all the other treatments soil was maintained at 50% field capacity

** Uninoculated control treatment with soil maintained at field capacity

Values are means of three replications

Table 4. Effect of the selected osmotolerant isolates on percent germination and seedling vigour of Pearl millet under osmotic stress

Isolates	Seed germination (%)		Plumule length (cm)		Radicle length (cm)		Fresh weight (mg)	
	Control	20 %*	Control	20 %	Control	20%	Control	20%
Uninoculated	83.33	83.33	1.29	0.61	1.84	1.33	288.33	175.67
KPSR2	80.00	51.67	1.48	0.40	0.47	1.25	256.00	192.33
MCL-1	86.67	83.33	1.65	0.62	2.89	1.45	300.67	205.33
MMS-3	56.67	70.00	1.23	0.38	2.44	1.11	268.33	177.33
MMS-5	61.67	63.33	1.40	0.34	1.51	1.33	267.67	168.00
MKS-1	90.00	83.33	1.56	0.70	2.95	1.57	350.33	213.67
SEm+	5.81	3.66	0.03	0.02	0.14	0.02	9.22	3.70
CD at 5%	18.11	11.42	0.10	0.08	0.44	0.06	28.72	11.53

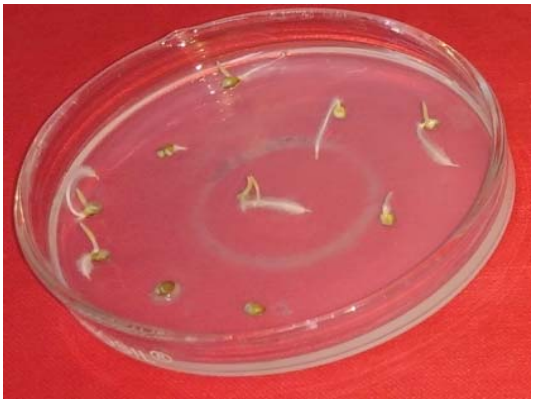
Values are means of three replications

*20% PEG 6000 concentration

The isolates MCL-1 and MKS-1 performed best for most of the plant parameters studied and hence were selected for further studies.

4.3 Screening of endophytic bacterial isolates for osmotolerance

Both the isolates MCL-1 and MKS-1 which improved shoot and root growth as well as improved seedling fresh weight were selected and effect of osmotic stress on their growth was studied. It was observed that in presence of 20% PEG 6000 there was less than 25% reduction in growth of these isolates (**Table 5**) indicating these isolates to be moderately tolerant to osmotic stress. However, in presence of 30% PEG 6000 there was more than 50% reduction in their growth. Although, the isolates were able to grow in the presence of 40% and 50% PEG 6000, growth was drastically reduced.



MCL-1 with 20% PEG

MKS-1 with 20% PEG

Uninoculated control

Plate 1. Effect of inoculation with the osmotolerant endophytic bacteria on pearl millet seed germination

4.3 Screening of endophytic bacterial isolates for osmotolerance

Both the isolates MCL-1 and MKS-1 which improved shoot and root growth as well as improved seedling fresh weight were selected and effect of osmotic stress on their growth was studied. It was observed that in presence of 20% PEG 6000 there was less than 25% reduction in growth of these isolates (**Table 5**) indicating these isolates to be moderately tolerant to osmotic stress. However, in presence of 30% PEG 6000 there was more than 50% reduction in their growth. Although, the isolates were able to grow in the presence of 40% and 50% PEG 6000, growth was drastically reduced.

4.3.1 Screening of endophytic bacterial isolates for osmotolerance by using bioscreen

Isolates MCL-1 and MKS-1 were selected for studying effect of osmotic stress on their growth. It was observed that in presence of 10% PEG 6000 there is clear cut log phase within short period in both isolates **(Fig 1 A and B)** indicating that these isolates have no stress at 10% PEG 6000. However, in presence of 20% PEG 6000 both the isolates taken slightly more time for showing clear cut log phase it indicates that slightly it have negative affect on growth and in 30% and 40% PEG 6000 both the isolates has taken more time for showing log phase and there is no stationery and decline phase it indicates that higher level of osmotic stress has negative affect on growth of isolates.

4.4 Effect of osmotic stress on plant growth promoting activities of the osmotolerantendophytic bacterial isolates

Effect of osmotic stress on various plant growth promoting activities of the selectedosmotolerantendophytic bacterial cultures were determined.

Table 5. Effect of osmotic stress on growth of the selected osmotolerant endophytic bacteria

Growth obtained (in O.D 600nm)		
Treatments		
	MCL-1	**MKS-1**
Control	0.69	0.70
20% PEG	0.55	0.58
30% PEG	0.31	0.21
40% PEG	0.13	0.12
50% PEG	0.04	0.07
SEm±	0.01	0.01
CD at 5%	0.03	0.02

Values are means of three replications

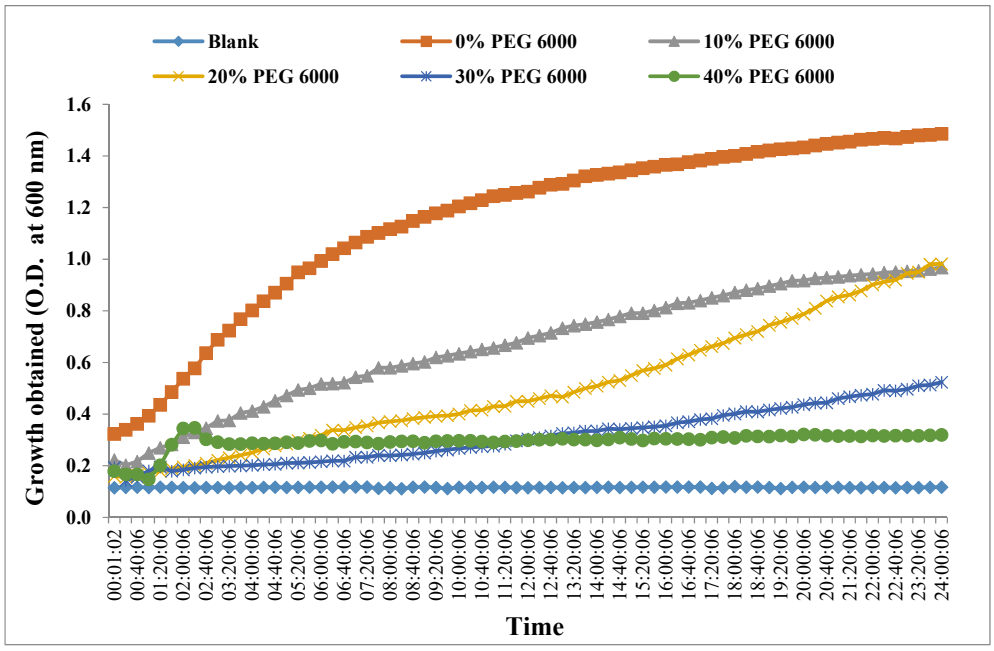
4.4.1 Phosphate solubilization ability

Both the selected isolates were phosphate solubilizers. There was a significant increase in phosphate solubilization by both the cultures in presence of osmotic stress as compared to control conditions (**Table 6**). However, significantly higher

Table 6. Effect of osmotic stress on P-solubization by the selected osmotolerant endophytic bacteria

Treatments	**P-solubilization (µg P solubilised/mg protein)**	
	MCL-1	**MKS-1**
Control	186.96	132.66
20%	438.45	744.24
30%	437.02	447.78
	SEm±	CD@ 5%
Factor A(Osmotic stress)	20.60	64.18
Factor B (Culture)	16.82	52.40
Factor(A) x (B)	29.13	90.76

A) MCL-1



B) MKS-1

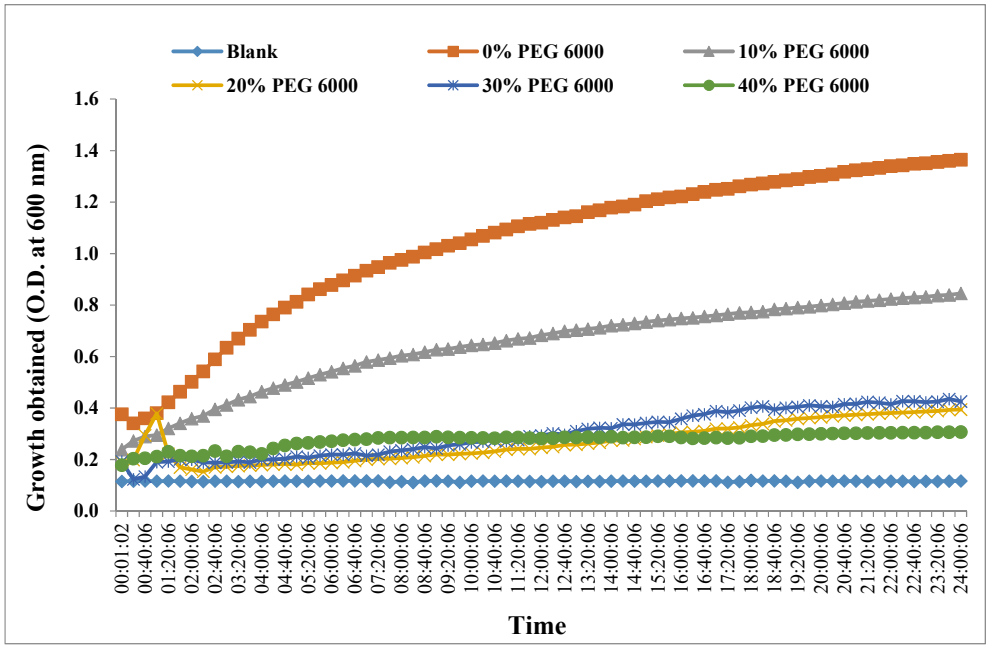


Fig 1. Effect of osmotic stress on growth of the selected osmotolerant endophytic bacteriausing Bioscreen C.

Phosphate solubilization by both MCL-1 and MKS-1 isolates was observed at 20% PEG concentration as compared to control treatments. Further increase in PEG 6000 concentration to 30% had an inhibitory effect on phosphate solubilization ability of isolate MKS-1, while no such effect was observed in case of isolate MCL-1. However, at all the osmotic stress conditions, isolate MKS-1 showed higherP-solubilization ability than isolate MCL-1.

4.4.2 IAA Production

Both the selected isolates were IAA producers. There was a significant increase in IAA production by both the cultures in presence of osmotic stress as compared to control conditions **(Table 7)**. Highest IAA production was observed in both the isolates MCL-1 and MKS-1 at 20%PEG 6000 concentration.Further increase in PEG 6000 concentration to 30% had an inhibitory effect on IAA production ability of isolate MCL-1. However, an increase in PEG 6000 concentration to 30% had a positive effect on IAA production ability of the isolate MKS-1.

4.4.3 Acetylene reduction ability

The selected isolates MCL-1 and MKS-1 did not show any nitrogen fixing ability as determined by acetylene reduction assay neither under control conditions nor on exposure to osmoticstress conditions.

4.4.4Gibberellic acid production

Both the selected isolates were gibberellic acid producers. There was a significant increase in gibberellic acid production by MCL-1in the presence of 20% PEG 6000 concentration as compared to control conditions (**Table 8**). Further increase in PEG 6000 concentration to 30% led to a significant increase in gibberellic acid production ability of the isolate as compared other treatments. However, in case of culture MKS-1, significantly higher gibberellic acid production was observed under control conditions as compared to the other two osmotic stress conditions. At 20% and 30% PEG 6000 concentrations the gibberellic acid production ability of the isolate significantly decreased.

Table 7. Effect of osmotic stress on indole acetic acid production by the selected osmotolerant endophytic bacteria

Treatments	IAA Produced (µg IAA produced/mg protein)	
	MCL-1	**MKS-1**
Control	14.72	53.25
20%	108.96	121.27
30%	85.21	273.59
	SEm±	CD@ 5%
Factor A(Osmotic stress)	6.99	21.76
Factor B (Culture)	5.70	17.77
Factor(A) x (B)	9.88	30.78

Table 8. Effect of osmotic stress on gibberellic acid production by the selectedosmotolerant endophytic bacteria

Treatments	Gibberellic acid Produced (µg GA produced/mg protein)	
	MCL-1	**MKS-1**
Control	544.84	770.24
20%	632.04	451.39
30%	700.06	599.77
	SEm±	CD@ 5%
Factor A(Osmotic stress)	6.54	20.38
Factor B (Culture)	5.34	16.64
Factor(A) x (B)	9.25	28.82

4.4.5 ACC deaminase activity

ACC deaminase activity of both the isolates was determined and both the isolates MCL-1 and MKS-1possessed ACC deaminase activity (**Table 9**).There was a significant increase in ACC deaminase activity by MCL-1in the presence of 20% PEG 6000 concentration as compared to control conditions.Further increase in PEG 6000 concentration to 30% had an inhibitory effect on itsACC deaminase activity.However, isolate MKS-1 showed highest ACC deaminase activity at 30% PEG 6000 concentration as compared to all other treatments and its ACC deaminase activity in presence of 20% PEG 6000 concentration was statistically at par with control treatment.

Table 9. Effect of osmotic stress on ACC deaminase activity by the selected osmotolerant endophytic bacteria

Treatments	**ACC deaminase activity (µM α-ketobutyrate produced/mg protein)**	
	MCL-1	**MKS-1**
Control	148.69	530.34
20%	554.32	596.62
30%	377.51	761.03
	SEm±	CD@ 5%
Factor A(Osmotic stress)	33.56	104.55
Factor B (Culture)	27.40	85.37
Factor(A) x (B)	47.46	147.86

4.4.6 Exopolysaccharide production

Both the selected isolates were exopolysaccharide producers. There was a significantincrease in exopolysaccharide production by both the cultures with an increase in osmotic stressas compared to control conditions (**Table 10**). Highest exopolysaccharide production by both the cultures was observed at 30% PEG 6000 concentration. Isolate MKS-1 showed higher exopolysaccharide production at both the concentrations of PEG 6000.

Table 10. Effect of osmotic stress on exopolysaccharide production by the selected osmotolerant endophytic bacteria

Treatments	Exopolysaccharide produced (mg/mg protein)	
	MCL-1	**MKS-1**
Control	0.12	0.19
20%	0.43	1.42
30%	0.73	1.83
	SEm±	CD@ 5%
FactorA(Osmotic stress)	0.014	0.044
FactorB (Culture)	0.01	0.04
Factor(A) x (B)	0.02	0.06

4.5 Identification of the selected osmotolerantendophyticbacteria through 16S rDNA sequencing

Identification of the isolates was done by partial sequencing of the 16S rDNA**(Plate 2**). The amplified 16S rDNA of isolate MCL-1 had following sequence of nucleotide.

TGAGCGCCCCCCCGAAGGTTAAGCTACCCACTTCTTTTGCAGCCCACTCCC
ATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGC
ATTCTGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAG
ACTCCAATCCGGACTACGACGAGCTTTGTGAGATTAGCTCCACCTCGCGG
CTTTGCAACCCTCTGTACTCGCCATTGTAGCACGTGTGTAGCCCTACTCGT
AAGGGCCATGATGACTTGACGTCGTCCCCACCTTCCTCCGGTTTATCACCG
GCAGTCTCCCTAGAGTTCCCACCATTACGTGCTGGCAAATAAGGATAGGG
GTTGCGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACG
ACAGCCATGCAGCACCTGTCTCACAGTTCCCGAAGGCACAAGTCCATCTC
TGGTCTCTTCTGTGGATGTCAAGAGTAGGTAAGGTTCTTCGCGTTGCATCG
AATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGA
GTTTTAACCTTGCGGCCGTACTCCCCAGGCGGTCTACTTAATGCGTTAGCT
TGAGAGCCCAGTGTTCAAGACACCAAACTCCGAGTAGACATCGTTTACGG
CGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGTGCAT

GAGCGTCAGTCTTTGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAG
ATCTCTACGCATTTCACCGCTACACCTGGAAATTCTACCCCCCTCTACAAG
ACTCTAGTTCGCCAGTTCGAAATGCTATTCCTAGGTTGAGCCCAGGGCTTT
CACATCTCGCTTAACAAACCGCCTGCGCACGCTTTACGCCCAGTAATTCCG
ATTAACGCTCGGACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGC
CGGTCCTTCTTCTGTAGGTAACGTCACAGATGAGCCGTATTAAGACTCACC
CTTTCCTCCCTACTGAAAGTGCTTTACAACCCGAAGGCCTTCTTCACACAC
GCGGCATGGCTGCATCAGGGTTTCCCCCATTGTGCAATATTCCCCACTGCT
GCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCTGATCATC
CTCTCAGAACAGCTAGGGATCGTCGCCTTGGTGAGCCATTACCTCACCAA
CTAGCTAATCCCACCTAGGTTCATCCAATCGCGAGAGGCCCGAAGGTCCC
CCTCTTTCCCCCGTAGGGCGTATGCGGTATTAGCAGTCGTTTCCAACTGTT
ATCCCCCTCGACTGGGCAGATCCCTAGGCATTACTCACCCGTCCGCCGCTC
GCCACCTCATAAGTAAACTCTACTTGTGCTGCCGCTCGACTGCATG

Based on nucleotide sequence matching from BLAST search it showed 99% homology with *Shewanellaputrefaciens*.

The amplified 16s rDNA of isolate MKS-1 had following sequence of nucleotide.

TGATCCACGATTACTAGCGATTCCGACTTCATGGAGTCGAGTTGCAGACTC
CAATCCGGACTACGACGCACTTTATGAGGTCCGCTTGCTCTCGCGAGGTCG
CTTCTCTTTGTATGCGCCATTGTAGCACGTGTGCAGCCCTGGTCGTAAGGG
CCATGATGACTTGAAGCCGTACCAAGAAACCTAAAGTTTATCACCGGCAG
TCTCCTTTGAGTTCCCACCATGACGTGCTGGCAACAAAGGATAAGGGTTG
CGCTCGTTGCGGGACTTAACCCAACATTTCACAACACGAGCTGACGACAG
CCATGCAGCACCTGTCTCAGAGTTCCCGAAGGCACTCCCGCATCTCTGCA
GGATTCTCTGGATGTCAAGACCAGGTAAGGTTCTTCGCGTTGCATCGAATT
AAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCATTTGAGTTT
TAACCTTGCGGCCGTACTCCCCAGGCGGTCGACTTAACGCGTTAGCTCCG
GAAGCCACGCCTCAAGGGCACAACCTCCAAGTCGACATCGTTTACGGCGT
GGACTACCAGGGTATCTAATCCTGTTTGCTCCCCACGCTTTCGCACCTGAG
CGTCAGTCTTCGTCCAGGGGGCCGCCTTCGCCACCGGTATTCCTCCAGATC
TCTACGCATTTCACCGCTACACCTGGAAATCTACCCCCCTCTACGAGACTC
AAGCTTGCCAGTTTCAAATGCAGTTCCCAGGTTGAGCCCGGGGATTTCAC
ATCTGACTTAACAAACCGCCTGCGTGCTATATACGGCCAGTAATTCCGATT

AACGCTTGCACCCTCCGTATTACCGCGGCTGCTGGCACGGAGTTAGCCGG
TGCTTCTTCTGCGAGTAACGTCAATGGCTAAGGTTATTAACCTCAACCCCT
TCCTCCTCGCTGAAAGTACTTTACAACCCGAAGGCCTTCTTCATACACGCG
GCATGGCTGCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCC
TCCCGTATGAGTCTGGACCGTGTCTCAGTTCCAGTGTGGCTGGTCATCCTC
TCAGACCAGCTAGGGATCGTCGCCTAGGTGAGCCTTTACCCCACCTACTA
GCTAATCCCATCTGGGCACATCTGATGGCATGAGGCCCGAAGGTCCCCCA
CTTTGGTCCGAAGACGTTATGCGGTATTAGCTACCGTTTCCAGTAGTTATC
CCCCTCCATCAGGCAGTTTCCCAGACATTACTCACCCGTCCGCCGCTCGTC
AGCAAATCAGCAAGCTGATTTCTGTACCGCTCGACTGCAT

Based on nucleotide sequence matching from BLAST search it showed 98% homology with *Cronobacterdublinensis.*

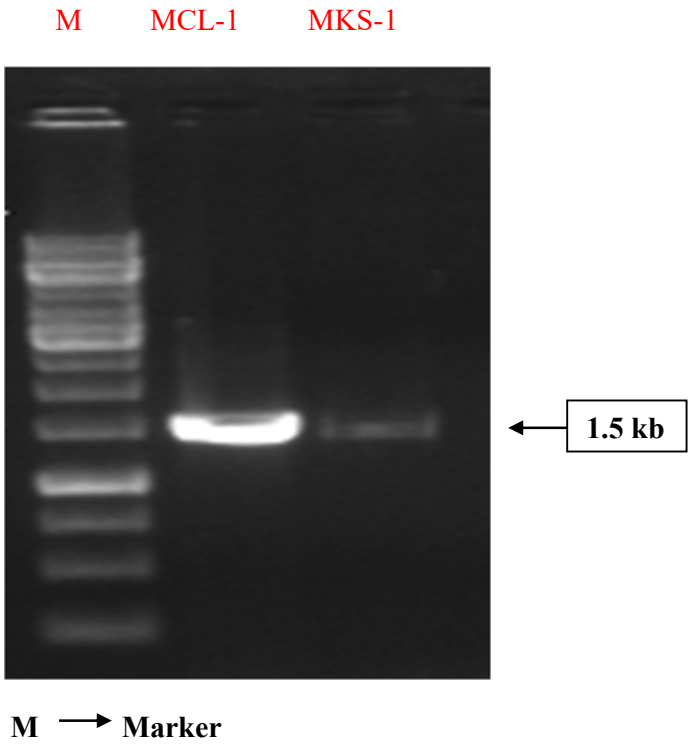


Plate 2. Amplified 16S rDNA of bacterial isolates

4.6 *In vivo* characterization of osmotolerantendophyticbacteria for plant growth promoting activities during plant-endophyteassociation

The selected strains *Shewanellaputrefaciens* strain MCL-1 and *Cronobacterdublinensis* strain MKS-1 were tested for various plant growth promoting

traits such as indoleacetic acid, gibberellic acid and exopolysaccharide production under *in vivo* conditions. These strains were also used for determining ethylene production by the plants under stress conditions.

4.6.1IAA Production

In the absence of osmotic stress conditions, there was very little IAA production during plant-endophyteassociation (**Table 11 and Plate 3**). However, on exposure to osmotic stress using 20 and 30% PEG 6000,significant increase in IAA production during plant-endophyte association was observed as compared to control conditions. Although, at 20% PEG concentration,IAAproduction in both the inoculated treatments was at par and statistically significantly more than control treatment, however at 30% PEG 6000 concentration, IAA production in *S.putrefaciens* strain MCL-1 inoculated treatment was significantly higher than *C.dublinensis* strain MKS-1 treatment.

Table 11. Effect of osmotic stress on indole acetic acid production during plant-endophyticassociation

Treatments	**Indole acetic acid produced (µg/ml)**		
	MCL-1	**MKS-1**	**Uninoculated**
Control	2.70	2.83	1.35
20%	3.42	3.49	2.85
30%	5.35	5.02	3.47
	SEm±	CD@ 5%	
Factor A (Osmotic stress)	0.03	0.08	
Factor B (Culture)	0.02	0.07	
Factor (A) x (B)	0.04	0.11	

Uninoculated control | MCL-1 with 20% PEG 6000 | MCL-1 with 30% PEG 6000

Uninoculated control | MKS-1 with 20% PEG 6000 | MKS-1 with 30% PEG 6000

Plate 3. Effect of osmotic stress on plant growth promoting activities of the osmotolerant endophytic bacteria during plant-endophytic association

4.6.2 Gibberellic acid production

Under all the three osmotic stress treatments, gibberellicacid production was observed during plant-endophyteassociation (**Table 12**). There was a significant increase in gibberellic acid production in presence of 20% PEG 6000 concentration as compared to control condition. Inoculated treatments showed higher gibberellic acid production at 20% PEG 6000 concentration as compared to control and 30% PEG 6000 treatments. Higher gibberellic acid production was observed in *C. dublinensis* strain MKS-1 inoculated treatment in presence of 20% PEG 6000 as compared to other treatments.

Table 12. Effect of osmotic stress on gibberellic acidproduction during plant-endophytic association

Treatments	**Gibberellic acid produced (µg /ml)**		
	MCL-1	**MKS-1**	**Uninoculated**
Control	75.31	112.80	96.71
20%	179.10	417.20	409.90
30%	125.35	370.35	378.57
	SEm±	CD@ 5%	
Factor A (Osmotic stress)	0.94	2.94	
Factor B (Culture)	0.77	2.40	
Factor (A) x (B)	1.34	4.16	

4.6.3 Exopolysaccharide production

Under all the three osmotic stress treatments, exopolysaccharide production during plant-endophyteassociation was observed (**Table 13**). There was a significant increase in exopolysaccharide production at 20% PEG 6000 concentration and further increase in osmotic stress to 30% had an inhibitory effect on exopolysaccharide production during plant-endophyte association.

Table 13. Effect of osmotic stress on exopolysaccharide production during plant-endophytic association

Treatments	Exopolysaccharide produced (mg /ml)		
	MCL-1	**MKS-1**	**Uninoculated**
Control	0.09	0.10	0.03
20%	0.13	0.13	0.09
30%	0.11	0.12	0.02
	SEm±	CD@ 5%	
Factor A(Osmotic stress)	0.004	0.001	
Factor B (Culture)	0.003	0.001	
Factor(A) x (B)	0.005	0.002	

4.6.4 Ethylene production

Ethylene production by the plants could not be detected under all the three osmotic stress conditions during plant-endophytic association.

4.7 Effect of inoculation with the osmotolerantendophytic bacteria on plant growth at 60 DAS

The effect of inoculation with the osmotolerantendophytic bacteria,*S.putrefaciens*strain MCL-1 and *C.dublinensis* strain MKS-1,on growth of pearl millet under water deficit stress conditions were studied (**Table 14 and Plate 4**). Shoot fresh weight was not significantly reduced under mild waterdeficitstress condition. However, exposure to severe waterdeficitstress significantly reduced shoot fresh weight of pearl millet. Inoculation with the osmotolerant*S. Putrefaciens*strain MCL-1 and *C. dublinensis* strain MKS-1 significantly increased shoot fresh weight under all water regimes as compared to uninoculatedcontrol conditions. Highest shoot fresh weight was obtained due to inoculation with *S. Putrefaciens*strain MCL-1 under mild waterdeficitstress conditions. Shoot fresh weight obtained in both the inoculated treatments under severe waterdeficitstress conditions was also statistically significantly

higher than uninoculated field capacity (no stress) control treatment. Again, higher shoot fresh weight was obtained due to inoculation with *S. Putrefaciens*strain MCL-1.

There was significant reduction in root fresh weight due to exposure to both mild and severe water deficitstress conditions. Inoculation had a positive effect on root fresh weight and significantly increased root fresh weight under all water regimesas compared to uninoculatedcontrol conditions. Highest root fresh weight was observed due to inoculation with *S. Putrefaciens*strain MCL-1 under field capacity. Under mild and severe water deficitstress conditions also significantly higher root fresh weight was observed in inoculated plants as compared to uninoculated plants. Under mild waterdeficitstress conditions, root fresh weight obtained due to inoculation with both the strains was at par. However, under severe waterdeficitstress conditions, better response was observed due to inoculation with *S. Putrefaciens*strain MCL-1.

There was significant reduction in shoot dry weight due to exposure to both mild and severe water deficitstress conditions. Inoculation with the osmotolerant*S. putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1 significantly increased shoot dry weight under all water regimes as compared to uninoculatedcontrol conditions. Highest shoot dry weight was obtained due to inoculation with *S. Putrefaciens*strain MCL-1 under field capacity. Under mild and severe water deficitstress conditions also significantly higher shoot dry weight was observed in the inoculated plants as compared to uninoculated plants. Under mild waterdeficitstress conditions, shoot dry weight obtained due to inoculation with both the strains was at par. However, under severe waterdeficitstress conditions, better response was observed due to inoculation with *S. Putrefaciens*strain MCL-1.

There was significant reduction in root dry weight due to exposure to both mild and severe water deficitstress conditions. Inoculation with the osmotolerant*S. Putrefaciens*strain MCL-1 and *C. dublinensis* strain MKS-1 significantly increased root dry weight under all water regimes as compared to the uninoculatedcontrol conditions. Highest root dry weight was obtained due to inoculation with *S. Putrefaciens*strain MCL-1 under field capacity. Under mild waterdeficitstress conditions, root dry weight obtained due to inoculation with both the strains was at par. However, performance of *S. Putrefaciens*strain MCL-1 was better under severe waterdeficitstress conditions as compared to mild waterdeficitstress conditions, while *C. dublinensis* strain MKS-1 performed better under mild waterdeficitstress conditions.

Table 14. Effect of inoculation with the osmotolerant endophytic bacteria on plant growth under water deficit stress conditions at 60 DAS

Treatments		Plant Biomass							
		Shootfr. wt.(g)		**Root fr.wt.(g)**		**Shoot dry. wt.(g)**		**Root dry.wt.(g)**	
Field capacity	Control	25.22		3.05		5.26		0.81	
	MCL-1	52.83		9.81		15.67		2.07	
	MKS-1	49.49		7.80		9.43		1.66	
Mild stress	Control	24.99		1.96		3.54		0.57	
	MCL-1	58.19		5.02		13.67		1.38	
	MKS-1	37.00		5.37		13.63		1.54	
Severe stress	Control	17.33		1.95		2.56		0.40	
	MCL-1	54.16		6.80		14.71		1.70	
	MKS-1	44.46		4.80		10.00		1.09	
		SEm ±	CD @ 5%	SEm ±	CD @ 5%	SEm ±	CD @ 5%	SEm ±	CD @ 5%
Factor A(Water stress)		0.61	1.83	0.14	0.41	0.27	0.82	0.04	0.13
Factor B (Culture)		0.61	1.83	0.14	0.41	0.27	0.82	0.04	0.13
Factor(A) x (B)		1.06	3.18	0.24	0.71	0.47	1.42	0.07	0.22

A) Field capacity

Field capacity control	Field capacity MCL-1	Field capacity MKS-1

B) Mild stress

Mild stress control	Mild stress MCL-1	Mild stress MKS-1

C) Severe stress

Severe stress control	Severe stress MCL-1	Severe stress MKS-1

Plate 4. Effect of inoculation with the osmotolerant endophytic bacteria on growth of pearl millet under water deficit stress conditions (60 DAS)

4.7.1 Effect of inoculation of the osmotolerant endophytic bacteria on plant yield at harvest

The effect of inoculation with the osmotolerant endophytic bacteria, *S. Putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1, on pearl millet cob yield, grain yield and plant biomass under water deficit stress conditions were studied (**Table 15**). No adverse effect of water deficit stress on pearl millet cob yield was observed. Inoculation with the osmotolerant *S. putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased cob yield under all water regimes as compared to uninoculated control conditions. Only exception was severe water deficit stress conditions where cob yield obtained due to inoculation with *S. Putrefaiens*strain MCL-1 was at par with uninoculated control treatment. Highest cob yield was obtained due to inoculation with *C. dublinensis* strain MKS-1 under field capacity. Under mild waterdeficitstress conditions, cob yield obtained due to inoculation with both the strains was at par. However, cob yield obtained due to inoculation with *C. dublinensis* strain MKS-1 under severe waterdeficitstress treatment was at par with that obtained under mild water deficit stress conditions.

No adverse effect of mild water deficit stress on pearl millet grain yield was observed, however, severe water deficit stress significantly reduced grain yield. Inoculation had a positive effect on grain yield and significantly increased grain yield under all water regimes as compared to uninoculated control conditions. Highest grain yield was observed due to inoculation with *C. dublinensis* strain MKS-1 under mild water deficit stress conditions which was also statistically at par with the yield obtained due to inoculation with this strain under field capacity conditions. Under severe water deficit stress conditions also performance of *C. dublinensis* strain MKS-1 was better than *S. putrefaiens*strain MCL-1. There was no adverse effect of mild water deficitstress on the plant biomass; however, severe water deficit stress significantly reduced the plant biomass (**Table 15**). Inoculation with the osmotolerant *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased the plant biomass at harvest under all water regimes as compared to uninoculated control conditions. Highest plant biomass at harvest was obtained due to inoculation with *S. Putrefaiens*strain MCL-1under field capacity. However, both the inoculated treatments were statistically at par. Under mild water deficit stress conditions performance of *C. dublinensis* strain MKS-1 was better while again under severe water deficit stress conditions the performance of both the strains was at par.

Table 15. Effect of inoculation with the osmotolerant endophytic bacteria on plant yield under water deficit stress conditions

Treatments		Yield				Plant Biomass (dry wt g)/plant	
		Ear yield (g)		Grain yield (g)/ear head			
Field capacity	Control	3.18		2.36		16.31	
	MCL-1	5.44		3.75		29.55	
	MKS-1	7.51		4.99		27.92	
Mild stress	Control	3.00		2.29		17.23	
	MCL-1	5.63		3.84		23.01	
	MKS-1	6.46		5.37		28.37	
Severe stress	Control	2.61		1.73		12.47	
	MCL-1	3.07		2.68		21.04	
	MKS-1	6.16		3.65		20.06	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.21	0.60	0.10	0.29	0.38	1.06
Factor B (Culture)		0.21	0.60	0.10	0.29	0.38	1.06
Factor(A) x (B)		0.37	1.04	0.17	0.51	0.65	1.84

4.7.2 Effect of inoculation with the osmotolerant endophytic bacteria on plant nutrient status under water deficit stress conditions

The effect of inoculation with the osmotolerant endophytic bacteria, *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1, on nitrogen, phosphorous and potassium content of both plant biomass and grains were determined under water deficit stress conditions (**Table 16a and b**). A decrease in nitrogen (%) in the plant was observed under water deficit stress conditions; however, inoculation significantly improved nitrogen (%) in all water regimes as compared to uninoculated control conditions. Highest nitrogen (%) was observed in the plant inoculated with *S. Putrefaciens* strain MCL-1 under mild water deficit stress conditions while under severe water deficit stress conditions both the cultures showed similar performance. A

decrease in nitrogen (%) in grain was observed only under mild water deficit stress conditions; however, inoculation significantly improved nitrogen (%) under these conditions. There was no impact of inoculation on nitrogen (%) of grain in severe water deficit stress conditions.

Table 16a. Effect of inoculation with the osmotolerant endophytic bacteria on plant nutrient status under water deficit stress conditions

Water deficit stress condition adversely affected plant phosphorous (%) only under severe water deficit stress condition while no such adverse effect of water deficit

Treatments		**N in plant (%)**		**P in plant (%)**		**K in plant (%)**	
Field capacity	Control	0.42		0.18		4.21	
	MCL-1	0.43		0.29		4.80	
	MKS-1	0.49		0.32		4.88	
Mild stress	Control	0.34		0.16		4.16	
	MCL-1	0.58		0.26		5.04	
	MKS-1	0.42		0.23		4.92	
Severe stress	Control	0.32		0.08		4.18	
	MCL-1	0.52		0.19		5.24	
	MKS-1	0.53		0.17		5.54	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.01	N/A	0.00	0.01	0.06	0.18
Factor B (Culture)		0.01	0.02	0.00	0.01	0.06	0.18
Factor(A) x (B)		0.01	0.03	0.01	0.02	0.10	0.31

stress on grain phosphorous (%) was observed (**Table 16a and b**). Inoculation with the osmotolerant *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased phosphorous (%) in plant and grain under all water regimes as

compared to uninoculated control conditions. Highest phosphorous (%) was observed in plant due to inoculation with *C. dublinensis* strain MKS-1 under field capacity conditions; whereas in case of grain the highest phosphorous (%) was obtained due to inoculation with *S. Putrefaciens* strain MCL-1 under severe water deficit stress conditions.

Table 16b. Effect of inoculation with the osmotolerant endophytic bacteria on plant nutrient status under water deficit stress conditions

Treatments		**N in grain (%)**		**P in grain (%)**		**K in grain (%)**	
Field capacity	Control	0.47		0.13		3.11	
	MCL-1	0.89		0.21		6.04	
	MKS-1	0.88		0.21		7.04	
Mild stress	Control	0.78		0.13		3.61	
	MCL-1	0.94		0.18		4.77	
	MKS-1	0.88		0.25		5.08	
Severe stress	Control	1.05		0.12		3.39	
	MCL-1	1.05		0.27		3.70	
	MKS-1	1.06		0.21		4.51	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.02	0.07	0.00	0.01	0.07	0.22
Factor B (Culture)		0.02	0.07	0.00	0.01	0.07	0.22
Factor(A) x (B)		0.04	0.13	0.01	0.01	0.13	0.38

No adverse effect of water deficit stress on plant potassium (%) was observed (**Table 16a and b**).Inoculation with the osmotolerant *S. putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased potassium (%) in plant and grain under all water regimes as compared to uninoculated control conditions. Only exception was potassium (%) of grains of *S. Putrefaciens* strain MCL-1 inoculated plants under

severe water deficit stress conditions which was at par with that of uninoculated control plants. Highest potassium (%) was observed in plant due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions and in case of grain highest potassium (%) was obtained due to inoculation with *C. dublinensis* strain MKS-1 under field capacity.

4.7.3 Effect of inoculation with the osmotolerant endophytic bacteria on plant photosynthetic parameters under water deficit stress conditions

The effect of inoculation with the osmotolerant endophytic bacteria, *S. putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1, on different photosynthetic parameters were determined under water deficit stress conditions (**Table 17**). Photosynthetic rate was observed to significantly increase under mild water deficit stress conditions. Although severe water deficit stress significantly reduced photosynthetic rate as compared to mild water deficit stress, photosynthetic rate was higher than that observed under field capacity conditions. Inoculation with these osmotolerant endophytic bacteria significantly increased the photosynthetic rate under all water regimes as compared to uninoculated control conditions. Highest photosynthetic rate was obtained due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions. Under all other inoculated water deficit stress treatments, photosynthetic rate was observed to be at par while lower values were observed under field capacity treatment.

Stomatal conductance was observed to significantly increase under mild water deficit stress conditions, while no adverse effect of severe water deficit stress on stomatal conductance was observed. There was no effect of inoculation on stomatal conductance under field capacity conditions. However, under mild and severe water deficit stress conditions inoculation with the osmotolerant *S. putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased stomatal conductance as compared to uninoculated control conditions. Only exception was *C. dublinensis* strain MKS-1 treatment under mild water deficit stress conditions which was at par with uninoculated control plants. Highest stomatal conductance was observed due to inoculation with *S. Putrefaciens* strain MCL-1 under severe water deficit stress conditions.

Table 17. Effect of inoculation with the osmotolerant endophytic bacteria on plant photosynthetic parameters under water stress conditions

Treatments		Photosynthetic rate (µmol $m^{-2}s^{-1}$)		Conductance (mol/m^2/s)		Transpiration rate (mmol/m^2/s)		Chlorophyll content (mg/g)	
Field capacity	Control	12.48		0.05		1.70		1.04	
	MCL-1	13.42		0.06		2.28		2.46	
	MKS-1	15.12		0.06		2.52		3.83	
Mild stress	Control	17.61		0.08		3.18		1.76	
	MCL-1	23.72		0.11		3.81		3.93	
	MKS-1	23.47		0.10		3.75		2.07	
Severe stress	Control	14.37		0.06		2.37		0.84	
	MCL-1	23.55		0.12		4.33		1.65	
	MKS-1	25.09		0.10		3.52		1.71	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.17	0.49	0.00	0.01	0.06	0.16	0.06	0.18
Factor B (Culture)		0.17	0.49	0.00	0.01	0.06	0.16	0.06	0.18
Factor (A) x (B)		0.29	0.85	0.01	0.02	0.10	0.28	0.11	0.32

Transpiration rate was observed to significantly increase under mild water deficit stress conditions. Although, severe water deficit stress significantly reduced transpiration rate as compared to mild water deficit stress, transpiration rate was higher than that observed under field capacity conditions. Under all the water regimes, inoculation with these osmotolerant endophytic bacteria significantly increased the transpiration rate as compared to uninoculated control conditions. Highest transpiration rate was observed due to inoculation with *S. Putrefaciens*strain MCL-1under severe

water deficit stress conditions. Under mild water deficit stress conditions transpiration rates in both the inoculated treatments were observed to be at par.

Chlorophyll content was observed to significantly increase under mild water deficit stress conditions while no adverse effect of severe water deficit stress on the chlorophyll content was observed which was observed to be statistically at par with the field capacity treatment. Under all the water regimes, inoculation with these osmotolerant endophytic bacteria significantly increased the chlorophyll content as compared to uninoculated control conditions. Highest chlorophyll content was observed due to inoculation with *S. putrefaciens* strain MCL-1under mild water deficit stress conditions. However, effect of inoculation was prominently more under water deficit stress conditions. Performance of *S. putrefaciens* strain MCL-1was superior to *C. dublinensis* strain MKS-1. Under severe water deficit stress conditions chlorophyll content in both the inoculated treatments were observed to be at par.

4.7.4 Effect of inoculation with the osmotolerant endophytic bacteria on plant root system architecture under water deficit stress conditions

The effect of inoculation with the osmotolerant endophytic bacteria, *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1, on plant root system architecture under water deficit stress conditions were studied (**Table 18 and Plate 5**). A decrease in root volume was observed under water deficit stress conditions. Inoculation with the osmotolerant endophytic bacteria significantly increased the root volume under all the water regimes as compared to uninoculated control conditions. Highest root volume was observed due to inoculation with *C. dublinensis* strain MKS-1 under field capacity. This strain also performed well under mild and severe water deficit stress conditions. A decrease in root surface area and root length was observed under mild water deficit stress conditions. While under severe water deficit stress conditions a significant increase in these parameters was noted. Inoculation with the osmotolerant endophytic bacteria significantly increased the root surface area and root length under all the water regimes as compared to uninoculated control conditions. Highest root surface area and root length was observed due to inoculation with *C. dublinensis* strain MKS-1 under field capacity. This strain also performed well under mild and severe water deficit stress conditions. A decrease in the root diameter was observed under water deficit stress conditions. Inoculation with the osmotolerant endophytic bacteria did not have any effect on the root diameter under field capacity

and mild water deficit stress conditions, however, under severe water deficit stress conditions inoculation significantly increased the root diameter as compared to uninoculated control conditions.

Table 18. Effect of inoculation with the osmotolerant endophytic bacteria on plant root system architecture under water deficit stress conditions

Treatments		**Root volume (cm^3)**		**Root surface area (cm^2)**		**Root length (cm)**		**Root diameter (mm)**	
Field capacity	Control	2.97		147.98		660.89		0.72	
	MCL-1	5.35		279.65		1394.94		0.65	
	MKS-1	9.04		577.49		2481.13		0.64	
Mild stress	Control	1.57		106.51		560.84		0.55	
	MCL-1	4.29		313.48		2354.40		0.47	
	MKS-1	6.28		379.38		2164.71		0.59	
Severe stress	Control	1.54		223.08		1538.21		0.32	
	MCL-1	5.52		409.21		1948.78		0.60	
	MKS-1	6.94		474.75		2138.58		0.48	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.20	0.61	10.86	32.52	126.99	N/A	0.02	0.07
Factor B (Culture)		0.20	0.61	10.86	32.52	126.99	380.23	0.02	N/A
Factor(A) x (B)		0.35	1.06	18.81	56.33	219.95	658.58	0.04	0.12

A) Field capacity

Field capacity control | Field capacity MCL-1 | Field capacity MKS-1

B) Mild stress

Mild stress control | Mild stress MCL-1 | Mild stress MKS-1

C) Severe stress

Plate 5. Effect of inoculation with the osmotolerant endophytic bacteria on pearl millet root architecture under water deficit stress conditions (60 DAS)

4.7.5 Effect of inoculation with the osmotolerant endophytic bacteria on plant hormone status under water deficit stress conditions

The effect of inoculation with the osmotolerant endophytic bacteria, *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1, on the plant hormone status was studied under water deficit stress conditions (**Table 19**).A decrease in ABA production was observed under water deficit stress conditions. Under field capacity conditions there was no effect of inoculation with *S. Putrefaciens* strain MCL-1 on plant ABA content and inoculation with *C. dublinensis* strain MKS-1 led to a decrease in plant ABA content. However, inoculation with these osmotolerant endophytic bacteria significantly increased the ABA content under mild and severe water deficit stress conditions as compared to uninoculated control conditions. Highest ABA content was obtained due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions. This strain performed well under both mild and severe water deficit stress conditions while *S. Putrefaciens* strain MCL-1 increased plant ABA content only under severe water deficit stress conditions.

Plant GA content was observed to significantly increase under water deficit stress conditions. Inoculation with these osmotolerant endophytic bacteria significantly increased plant GA content under both field capacity and mild water deficit stress conditions. However, under severe water deficit stress conditions, inoculation with *C. dublinensis* strain MKS-1 significantly increased plant GA content as compared to uninoculated control plants. Highest GA production was observed due to inoculation with *C. dublinensis* strain MKS-1 under mild water deficit stress conditions.

Plant IAA content was very low and under uninoculated conditions it was below the detection limit. Inoculation with the osmotolerant endophytic bacteria increased plant IAA content under all the water regimes. Under severe water deficit stress conditions, plants inoculated with *S. putrefaciens* strain MCL-1 had higher IAA content while under mild water deficit stress conditions plants inoculated with *C. dublinensis* strain MKS-1 had higher plant IAA content.

Table 19. Effect of inoculation with the osmotolerant endophytic bacteria on plant hormone status under water deficit stress conditions

Treatments		ABA (µmol/g)		GA (ppm/g)		IAA (ng/g)	
Field capacity	Control	988.76		49.79		2.088	
	MCL-1	1047.32		75.70		2.349	
	MKS-1	670.37		131.99		2.119	
Mild stress	Control	563.56		195.69		2.097	
	MCL-1	980.29		232.58		1.893	
	MKS-1	1357.14		367.34		2.133	
Severe stress	Control	392.26		60.16		1.399	
	MCL-1	1354.88		60.75		2.286	
	MKS-1	1665.76		68.04		2.106	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		21.29	63.74	1.16	3.44	0.11	N/A
Factor B (Culture)		21.29	63.74	1.16	3.44	0.09	N/A
Factor(A) x (B)		36.87	110.41	2.00	5.95	0.16	N/A

4.7.6 Effect of inoculation with the osmotolerant endophytic bacteria on plant biochemical status under water deficit stress conditions

The effect of inoculation with the osmotolerant endophytic bacteria, *S. Putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1, on the biochemical status of the plant under water deficit stress conditions was studied. The various biochemical parameters studied were proline, glycine betaine, amino acid, total sugars, starch and

total phenolics (**Table 20a and b**).A significant decrease in proline content was observed under water deficit stress conditions. Inoculation with these osmotolerant endophytic bacteria significantly increased the proline content under all the water regimes as compared to uninoculated control conditions. Highest proline content was observed due to inoculation with *C. dublinensis* strain MKS-1 under mild water deficit stress and severe water deficit stress conditions. Proline content of *S. Putrefaciens*strain MCL-1 inoculated plants under severe water deficit stress conditions was also at par with that of *C. dublinensis* strain MKS-1.

Table 20a. Effect of inoculation with the osmotolerant endophytic bacteria on plant biochemical parameters under water deficit stress conditions

Treatments		**Proline (mmol/g fr. wt)**		**Glycine betaine (µ mol/g fr. wt)**		**Amino acid (mg/g fr. wt)**	
Field capacity	Control	0.46		16.47		0.31	
	MCL-1	0.48		52.93		0.32	
	MKS-1	0.48		46.67		0.32	
Mild stress	Control	0.29		13.81		0.31	
	MCL-1	0.51		35.88		0.32	
	MKS-1	0.54		40.97		0.32	
Severe stress	Control	0.37		11.76		0.31	
	MCL-1	0.53		30.00		0.32	
	MKS-1	0.54		28.82		0.32	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.00	0.01	0.39	1.16	0	N/A
Factor B (Culture)		0.00	0.01	0.39	1.16	0	0
Factor (A) x (B)		0.00	0.01	0.67	2.00	0	N/A

A significant decrease in glycine betaine content was observed under water deficit stress conditions. Inoculation with these osmotolerant endophytic bacteria significantly increased the glycine betaine content under all water regimes as compared to uninoculated control conditions. Highest glycine content was obtained due to inoculation with *S. Putrefaciens* strain MCL-1under field capacity. Under mild water deficit stress conditions higher glycine betaine content was observed in *C. dublinensis* strain MKS-1 inoculated plants while under severe water deficit stress conditions glycine betaine content of the plants inoculated with either of the two strains was statistically at par.

Table 20b. Effect of inoculation with the osmotolerant endophytic bacteria on plant biochemical parameters under water deficit stress conditions

Treatments		**Total sugar (mg/g fr. wt)**		**Starch (mg/g fr. wt)**		**Total phenolics (mg/g fr. wt)**	
Field capacity	Control	26.99		0.56		1.04	
	MCL-1	27.84		1.68		2.86	
	MKS-1	28.40		5.45		2.75	
Mild stress	Control	27.61		0.72		0.63	
	MCL-1	27.93		5.49		4.04	
	MKS-1	27.93		5.34		5.83	
Severe stress	Control	27.63		0.59		1.73	
	MCL-1	28.32		1.61		4.38	
	MKS-1	28.48		6.85		6.32	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.15	N/A	0.06	0.19	0.15	0.44
Factor B (Culture)		0.15	0.45	0.06	0.19	0.15	0.44
Factor (A) x (B)		0.26	N/A	0.11	0.33	0.25	0.75

There was no effect of water deficit stress or inoculation on amino acid and total sugar contents of the plants. There was no adverse effect of water deficit stress on starch content on the plants. However, inoculation significantly improved the starch content under all water regimes. Highest starch content was obtained due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions. Starch content of both the inoculated treatments under mild water deficit stress conditions was comparable.

There was no significant change in total phenolics content under mild and severe water deficit stress conditions as compared to field capacity conditions. However, inoculation with the selected osmotolerant endophytic bacteria *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly enhanced total phenolics content under all water regimes as compared to uninoculated control conditions. Highest phenolics content was obtained due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions. Under both the water stress conditions severe as well as mild water deficit stress, *C. dublinensis* strain MKS-1 inoculated plants had higher total phenolics content.

4.7.7 Effect of inoculation with the osmotolerant endophytic bacteria on plant physiological status under water deficit stress conditions

The effect of inoculation with the osmotolerant endophytic bacteria, *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1, on the physiological status of the plant under water deficit stress conditions was studied. The various physiological parameters studied were membrane stability index, relative water content and lipid peroxidation and these parameters were affected due to water deficit stress (**Table 21**).Membrane stability index (MSI) was comparable under mild and severe water deficit stress conditions, however, there was significant reduction in MSI under field capacity conditions of the uninoculated control plants. Inoculation with the osmotolerant*S. Putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1 significantly increased MSI under all the water deficit stress conditions as compared to uninoculated control conditions. Highest membrane stability index was observed due to inoculation with both the strains under field capacity conditions. There was no adverse effect of water deficit stress on relative water content (RWC) of the leaves. Inoculation with the osmotolerant *S. putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1 significantly increased RWC of leaves under all the water regimes as compared to uninoculated control conditions. Highest relative water content was

obtained due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions which was comparable to that of *S. Putrefaciens* strain MCL-1 under severe water deficitstress conditions. A significant increase in lipid peroxidation was observed under water deficit stress conditions. Inoculation under field capacity conditions had an adverse effect on lipid peroxidation. Under mild water deficit stress conditions inoculation with *S. Putrefaciens* strain MCL-1 did not have any adverse effect on lipid peroxidation, however, inoculation with *C. dublinensis* strain MKS-1 significantly increased lipid peroxidation. There was no adverse effect of inoculation on lipid peroxidation under severe water deficit stress conditions.

Table 21. Effect of inoculation with the osmotolerant endophytic bacteria on plant physiological parameters under water deficit stress conditions

Treatments		**MSI (%)**		**RWC (%)**		**Lipid peroxidation MDA (n moles of TBARS/g fr.wt)**	
Field capacity	Control	12.10		63.30		17.59	
	MCL-1	25.90		80.16		32.39	
	MKS-1	25.80		83.56		27.66	
Mild stress	Control	19.00		61.56		25.51	
	MCL-1	23.20		73.91		25.12	
	MKS-1	23.00		75.23		42.24	
Severe stress	Control	16.40		62.18		31.31	
	MCL-1	21.30		83.35		26.62	
	MKS-1	20.30		82.97		30.37	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.65	1.96	0.85	2.56	1.06	3.16
Factor B (Culture)		0.65	1.96	0.85	2.56	1.06	3.16
Factor(A) x (B)		1.13	3.394	1.48	4.43	1.83	5.48

4.7.8 Effect of inoculation with the osmotolerant endophytic bacteria on antioxidative enzymes activity in plant under water deficit stress conditions

The response of pearl millet to water deficit stress conditions in terms of activity of antioxidative enzymes superoxide dismutase, catalase and ascorbate peroxidase were studied (**Table 22**).There was an increase superoxide dismutase enzyme activity (SOD) under water deficit stress conditions. Inoculation with the osmotolerant *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased superoxide dismutase activity under field capacity conditions while under mild water deficit stress conditions SOD enzyme activity was increased only in case of *C. dublinensis* strain MKS-1 inoculated plants. Under severe water deficit stress conditions, a decrease in SOD enzyme activity was observed in the inoculated treatments. There was an increase in catalase enzyme activity only under severe water deficit stress conditions. Inoculation with these osmotolerant endophytic bacteria significantly increased the catalase enzyme activity under all water regimes as compared to uninoculated control conditions. Highest catalase enzyme activity was observed due to inoculation with *C. dublinensis* strain MKS-1 under mild water deficit stress conditions. However, catalase ezyme activity of *S. Putrefaciens*strain MCL-1 inoculated plants under mild and severe water deficit stress conditions was observed to be at par with that of *C. dublinensis* strain MKS-1 under mild water deficit stress conditions. Ascorbate peroxidise enzyme activity was not detected in any of the treatments.

Table 22. Effect of inoculation with the osmotolerant endophytic bacteria on antioxidative enzymes activity in plant under water deficit stress conditions

Treatments		SOD(u/g fr.wt)		Catalase(µM H_2O_2reduced/min/g fr.wt)	
Field capacity	Control	2.63		0.67	
	MCL-1	7.37		1.62	
	MKS-1	9.37		1.37	
Mild stress	Control	11.78		0.71	
	MCL-1	12.08		2.15	
	MKS-1	13.57		2.23	
Severe stress	Control	10.65		1.04	
	MCL-1	5.45		2.06	
	MKS-1	1.17		1.60	
		SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.09	0.27	0.06	0.19
Factor B (Culture)		0.09	N/A	0.06	0.19
Factor(A) x (B)		0.16	0.47	0.11	0.33

4.7.9 Effect of inoculation with the osmotolerant endophytic bacteria on antioxidant status in plant under water deficit stress conditions

The response of pearl millet to water deficit stress conditions in terms of antioxidant content was studied (**Table 23**).

There was a significant decrease in total ROS under mild water deficit stress conditions as compared to field capacity conditions. However, total ROS under severe water deficit stress conditions was significantly higher than mild water deficit stress conditions and comparable to that observed under field capacity conditions. Inoculation with the osmotolerant *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 did not affect total ROS under field capacity or severe water deficit stress conditions. However, under mild water deficit stress conditions total ROS was significantly increased.

Ascorbic acid content was significantly reduced under severe water deficit stress conditions. Inoculation with the osmotolerant *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased ascorbic acid under water deficit stress conditions as compared to uninoculated control conditions. Highest ascorbic acid content was observed due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions. Under all the three water regimes *C. dublinensis* strain MKS-1 inoculated plants had higher ascorbic acid content.

There was significant increase in reduced glutathione (GSH) content under mild and severe water deficit stress conditions, while oxidized glutathione (GSSG) content was significantly enhanced only under severe water deficit stress conditions. Inoculation with the osmotolerant *C. dublinensis* strain MKS-1 and *S. putrefaciens* strain MCL-1significantly increased reduced glutathione (GSH) content under field capacity and severe water deficit stress conditions, respectively, as compared to uninoculated control conditions. In the other inoculated treatments reduced glutathione (GSH) content was comparable to uninoculated control treatments. Inoculation with the osmotolerant *C. dublinensis* strain MKS-1 and *S. Putrefaciens*strain MCL-1significantly increased oxidised glutathione (GSSG) content under field capacity and mild water deficit stress conditions, respectively, as compared to uninoculated control conditions. In the other inoculated treatments, oxidized glutathione (GSSG) content was comparable to uninoculated control treatments.

Table 23. Effect of inoculation with the osmotolerant endophytic bacteria on antioxidant status in plant under water deficit stress conditions

Treatments		Ascorbic acid(µmol/g fr. wt)		Reduced glutathione (GSH) (µ mol/ml)		Oxidized glutathione (GSSH) (µ mol/ml)	
Field capacity	Control	3.67		30.67		33.33	
	MCL-1	4.55		35.00		36.67	
	MKS-1	4.87		44.43		44.43	
Mild stress	Control	3.60		41.10		35.53	
	MCL-1	4.29		46.67		43.30	
	MKS-1	4.76		45.57		38.90	
Severe stress	Control	2.83		40.00		44.47	
	MCL-1	4.56		48.90		47.80	
	MKS-1	4.90		43.30		44.43	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		0.05	0.16	0.89	2.65	0.93	2.78
Factor B (Culture)		0.05	0.16	0.89	2.65	0.93	2.78
Factor (A) x (B)		0.09	0.28	1.54	4.59	1.61	4.81

4.8 Effect of inoculation with the osmotolerant endophytic bacteria on soil structure under water deficit stress conditions

Effect of inoculation with the osmotolerant endophytic bacteria *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 on soil structure was determined.

4.8.1 Soil carbohydrate content

It was observed that under water deficit stress conditions there was increase in soil carbohydrate content (**Table 24**).Inoculation with the osmotolerant endophytic bacteria *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly enhanced soil carbohydrate content under water deficit stress conditions as compared to the uninoculated control conditions. Highest soil carbohydrate content was obtained due to inoculation with *S. Putrefaciens* strain MCL-1 under severe water deficit stress conditions. Under both mild water deficit stress condition as well as severe water deficit stress condition soil carbohydrate content in *S. Putrefaciens* strain MCL-1inoculated treatments was observed to be significantly more than *C. dublinensis* strain MKS-1 inoculated treatments or uninoculated controls.

4.8.2 Root adhering soil/root tissue ratio

Significant increase in root adhering soil/root tissue ratio was observed under water deficit stress conditions (**Table 24**). Inoculation with the osmotolerant endophytic bacteria *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly increased root adhering soil/root tissue ratio under water deficit stress conditions as compared to the uninoculated control conditions. Highest root adhering soil/root tissue ratio was obtained due to inoculation with *C. dublinensis* strain MKS-1 under severe water deficit stress conditions. The *C. dublinensis* strain MKS-1 was observed to perform better than *S. putrefaciens* strain MCL-1. However, performance of both the inoculated treatments under field capacity and mild water deficit stress conditions was at par.

4.8.3Soil aggregate stability

Soil aggregate stability was not affected by water deficit stress (**Table 24**).Inoculation with the osmotolerant endophytic bacteria *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1 significantly enhanced soil aggregate stability under water deficit stress conditions as compared to uninoculated control conditions. Highest soil aggregate stability was observed due to inoculation with *C. dublinensis* strain MKS-1 under field capacity conditions. Higher soil aggregate stability values were obtained in inoculated treatments under mild water deficit stress as compared to severe water deficit stress conditions. Soil aggregate stability values were higher in treatments inoculated with *C. dublinensis* strain MKS-1.

Table 24. Effect of inoculation with the osmotolerant endophytic bacteria on soil structure under water deficit stress conditions

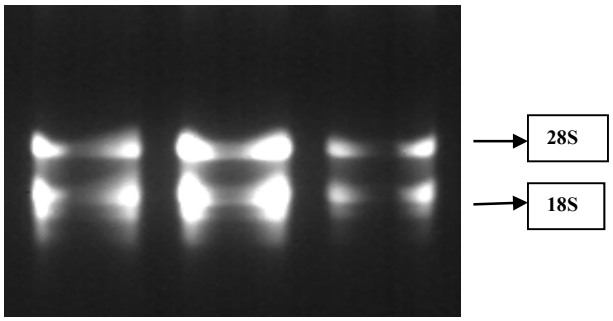
Treatments		Soil carbohydrate content (µg/g)		Root adhering soil/ root tissue ratio (g/g)		Soil aggregate stability (mm)	
Field capacity	Control	614.1		63.83		0.82	
	MCL-1	885.3		68.84		1.02	
	MKS-1	836.4		68.40		1.24	
ild stress	Control	653.1		77.47		0.84	
	MCL-1	887.0		82.24		0.93	
	MKS-1	798.2		80.49		1.02	
Severe stress	Control	740.2		77.09		0.87	
	MCL-1	958.1		79.02		0.89	
	MKS-1	841.8		85.25		0.96	
		SEm±	CD@ 5%	SEm±	CD@ 5%	SEm±	CD@ 5%
Factor A (Water stress)		3.70	11.08	0.55	1.64	0.01	0.03
Factor B (Culture)		3.70	11.08	0.55	1.64	0.01	0.03
Factor(A) x (B)		6.41	19.19	0.95	2.84	0.02	0.05

4.9 Effect of inoculation with the osmotolerant endophytic bacteria on plant drought stress responsive genes under water deficit stress conditions

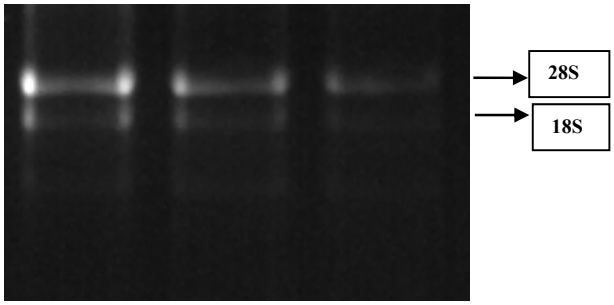
Total RNA isolated from pearl millet leaf tissues was analysed by TBE agarose gel electrophoresis. All the RNA samples showed intact 28S and 18S rRNA bands (**Plate 6A**) indicating that the quality of RNA was good. About 1.0 µg of total RNA was reverse transcribed with Super Script^{TM} III Reverse Transcriptase using oligo-dT

primer. The quality of cDNA was checked by using *PgEF-1a*gene primers. All the cDNA yielded good PCR amplification and expected amplicon size (**Plate 6B**). It showed that the cDNA prepared was of good quality. These cDNA were used to analyse the drought stress responsive and microbe-induced regulation of genes by quantitative RT-PCR analysis.

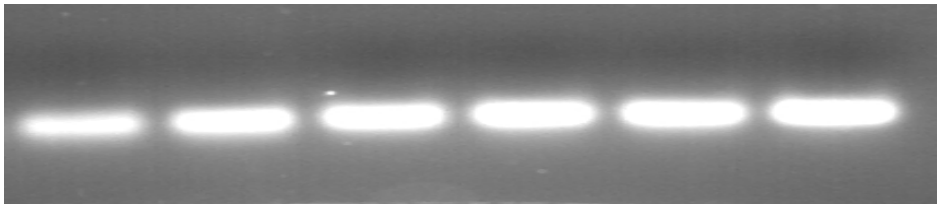
A) FC Cont FCMCL-1 FCMKS-1



SS Cont SSMCL-1 SSMKS-1



B) FC Cont FCMCL-1 FCMKS-1 SS Cont SSMCL-1 SSMKS-1



FC Cont –Field capacity controlSS Cont- Severe stress control
FCMCL-1-Field capacity culture-1SSMCL-1- Severe stress culture-1
FCMKS-1-Field capacity culture-2SSMKS-1-Severe stress culture-2

Plate 6. Total RNA isolation (A) and cDNA confirmation with *PgEF-1a*

The effect of inoculation on the expression of drought stress responsive genes was determined under control (Field capacity) and severe water deficit stress conditions in pearl millet and genes were selected based on physiological and biochemical parameters. The expression analysis of *PgAP2, PgCSD, PgDREB2A, CaP5CS, PgAPX, SbSNAC1, PgLEA, SbYUC, SbGA20oX* and *SbNCED* genes was studied with elongation factor *PgEF*-1a as housekeeping gene. Osmotolerant endophytic bacteria mediated change in the expression levels of different genes were analysed by comparing the expression levels of genes in tissue under water deficit stress with their respective controls by quantitative RT-PCR analysis.

4.9.1 Expression analysis of phyto hormonal gene regulation in pearl millet under water deficit stress conditions

Real time expression analysis was carried out to study the expression pattern of genes under control (Field capacity) and severe water deficit stress conditions. Apetala 2 (AP2) is a gene and member of large family of transcription factor AP2/EREBP (ethylene-responsive element-binding proteins). AP2/EREBPs belong to a superfamily of plant specific transcription factors characterized by presence of an AP2 DNA-binding domain of 60 amino acids. In *Arabidopsis thaliana* AP2 plays a role in the ABC model of flower development. EREBPS containing a single AP2 domain are involved in regulatory network of response to hormones and environmental signals involving DREBs (dehydration responsive element binding proteins) and ERFs (ethylene responsive factors). *AP2* with an important role in controls flowering, seed size, seed weight, and accumulation of seed oil and protein in *Arabidopsis* encodes a putative transcription factor distinguished by a novel DNA binding motif referred to as the *AP2* domain. There was no change in the expression of *PgAP2*gene in uninoculated control plants under control and severe water deficit stress conditions **(Figure 2).** Plants inoculated with *S. Putrefaciens* strain MCL-1 showed lowered expression of *PgAP2*gene under control conditions while the expression of this gene was enhanced 2 fold under severe deficit stress conditions. Plants inoculated with *C. dublinensis* strain MKS-1 showed nearly 2 fold increase in the expression of *PgAP2*gene under control conditions while the expression of this gene was enhanced 6 fold under severe deficit stress conditions.

Auxin is an essential plant hormone, plays crucial role in diverse aspects of growth and development involved in diverse processes, such as cell division,

expansion, differentiation, flowering, lateral root formation and senescence. Auxin function responses to environmental stresses such as drought, salinity and pathogen attack. The flavin monooxygenases (FMO) encoded by plant *YUCCA* gene catalyze a rate-limiting step in the tryptamine pathway for indole-3-acetic acidbiosynthesis. Overexpression of *YUC* genes in Arabidopsis leads to overproduction of auxin

Expression of *SbYUC* gene was nearly 2 fold up regulated under severe water deficit stress conditions as compared to control conditions in uninoculated plants (**Figure 2**). In *S. putrefaciens* strain MCL-1 inoculated plants, under control conditions, not much change in the expression of *SbYUC* gene as compared to uninoculated plants was observed while the expression of this gene was nearly 6 fold upregulated under severe water deficit stress conditions. In *C. dublinensis* strain MKS-1 inoculated plants, under control conditions, nearly 7.8 fold increase in the expression of *SbYUC* gene as compared to uninoculated plants was observed while the expression of this gene was 12.6 fold upregulated under severe water deficit stress conditions.

Biologically active gibberellin (GA) plays an essential role on plant growth and development. Gibberellins are involved in different plant developmental processes such as seed germination and development, stem elongation, leaf expansion, induction of flowering, flower development. GA 20-oxidase (GA 20-ox) and GA 3b-hydroxylase (GA 3b-hy) are enzymes that catalyze the late steps in the formation of active GA and are potential control points in the regulation of GA biosynthesis. There was no change in the expression of *SbGA20oX* gene under severe water deficit stress conditions as compared to control conditions in uninoculated plants (**Figure 2**). In *S. putrefaciens* strain MCL-1 inoculated plants, under control conditions, there was slight down regulation in the expression of *SbGA20oX* gene as compared to uninoculated plants was observed while the expression of this gene was nearly 3 fold upregulated under severe water deficit stress conditions. In *C. dublinensis* strain MKS-1 inoculated plants, under control conditions, nearly 2.6 fold increase in the expression of *SbGA20oX* gene as compared to uninoculated plants was observed while the expression of this gene was 5.9 fold upregulated under severe water deficit stress conditions.

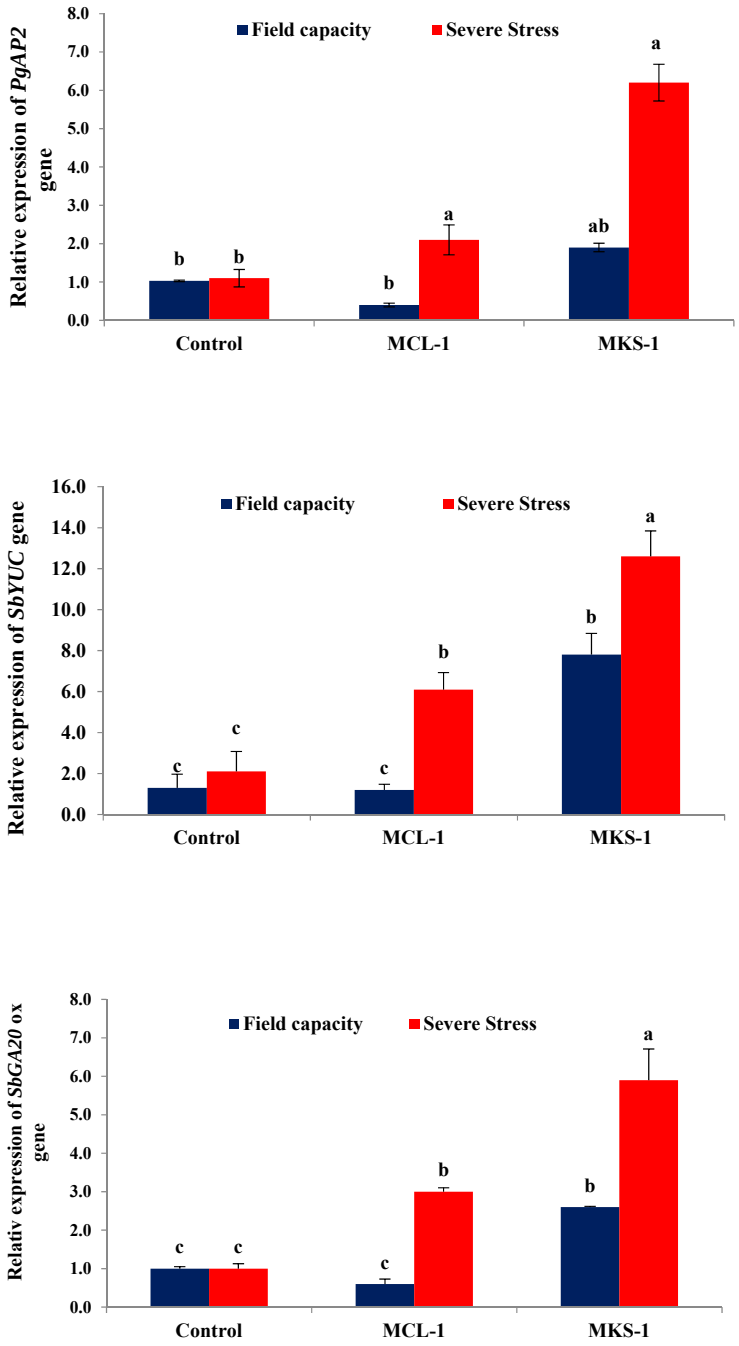


Fig 2. Expression analysis of phytohormonal gene regulation in pearl millet under water deficit stress conditions

4.9.2 Expression analysis of antioxidative enzyme gene regulation in pearl millet under water deficit stress conditions

When plants are exposed drought stress there is production of reactive oxygen species (ROS) and it can cause oxidative damages to the cells. Antioxidative defense, can detoxify the ROS present in the plants. Ascorbate peroxidase (APX) enzyme plays a key role for catalysing the conversion of H_2O_2 into H_20 by using ascorbate as electron donor. The APX gene expression is regulated in response to drought stress. There was slight down regulation of the expression of *PgAPX* gene under severe water deficit stress conditions as compared to control conditions in uninoculated plants (**Figure 3**). In *S. putrefaciens* strain MCL-1 inoculated plants, under control conditions, slight upregulation of *PgAPX* gene as compared to uninoculated plants was observed while the expression of this gene was further slightly upregulated under severe water deficit stress conditions. In *C. dublinensis* strain MKS-1 inoculated plants, under control conditions, nearly 1.5 fold increase in the expression of *PgAPX* gene as compared to uninoculated plants was observed while the expression of this gene was slightly down regulated under severe water deficit stress conditions.

Superoxide dismutase (SOD) is one of the most important antioxidant enzyme and constitute the first level of defence against oxidative stress in plants. SOD catalyzes the dismutation of $O2^-$ to H_2O_2 and O_2. There are different forms: iron SOD (FeSOD), manganese SOD (MnSOD) and copper/zinc SOD (CuZnSOD). They are localized in different parts of the cell, mitochondria for MnSOD and chloroplast for FeSOD. CuZnSOD isoforms (CSD) are CSD1 in cytosol, CSD2 in chloroplasts and CSD3 in peroxisomes. Increased in the expression of CSD gene enhances oxidative stress tolerance under under water stress conditions. There was decrease in the expression of *PgCSD* gene in uninoculated control plants under severe water deficit stress conditions as compared to control conditions (**Figure 3**). There was 1.6 fold increase in the expression of *PgCSD* gene in *S. Putrefaciens* strain MCL-1 inoculated plants under control conditions. Under severe water deficit stress conditions decrease in the expression of this gene as compared to control conditions was observed, however, the expression levels were higher than those of uninoculated plants. Plants inoculated with *C. dublinensis* strain MKS-1 showed lower expression levels of *PgCSD* gene as compared to uninoculated control plants under control conditions. Under severe water deficit stress conditions there was no change in the expression of *PgCSD*gene in uninoculated control plants and those inocultated with *C. dublinensis* strain MKS-1.

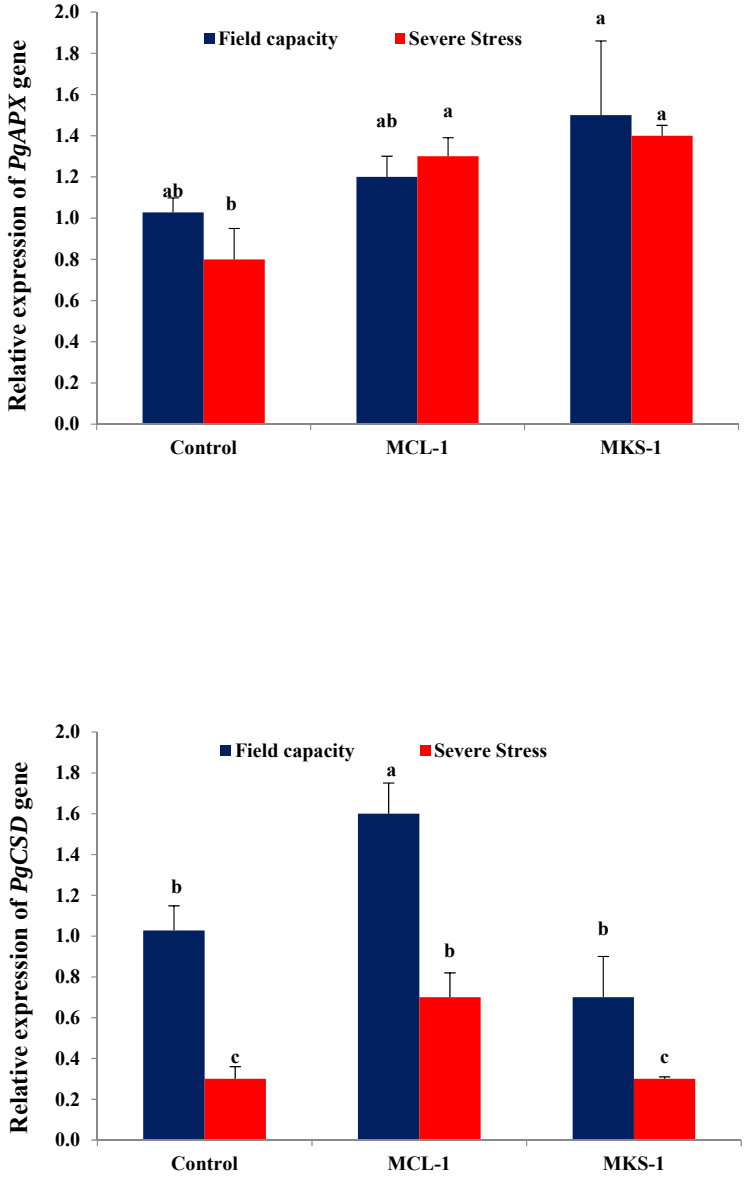


Fig 3. Expression analysis of antioxidative enzyme gene regulation in pearl millet under water deficit stress conditions

4.9.3 Expression analysis of osmolyte gene regulation in pearl millet under water deficit stress conditions

Δ-1-pyrroline-5-carboxylate synthetase enzymes, which catalyse the rate-limiting step of proline biosynthesis, are encoded by two closely related P5CS genes in Arabidopsis. Transcription of the P5CS genes is differentially regulated by drought, salinity and abscisic acid, suggesting that these genes play specific roles in the control of proline biosynthesis.

Proline is the one of the most common compatible osmolyte accumulated in higher plants under water stress conditions. The regulation of proline of Δ-1-pyrroline-5-carboxylate synthase (P5CS) controlling biosynthesis. The activity of P5CS represents a rate limiting step in proline biosynthesis, which is controlled at the level of P5CS transcription and through feedback inhibition of P5CS by proline. An increased level of proline was found to correlate with improved water stress tolerance plants. There was slight down regulation of the expression of *CaP5CS* gene under severe water deficit stress conditions as compared to control conditions in uninoculated plants (**Figure 4**). In *S. putrefaciens* strain MCL-1 inoculated plants, under control conditions, no change in the expression of *CaP5CS* gene as compared to uninoculated plants was observed while the expression of this gene was nearly 2 fold upregulated under severe water deficit stress conditions. In *C. dublinensis* strain MKS-1 inoculated plants, under control conditions, nearly 1.5 fold increase in the expression of *CaP5CS* gene as compared to uninoculated plants was observed while the expression of this gene was nearly 2 fold upregulated under severe water deficit stress conditions.

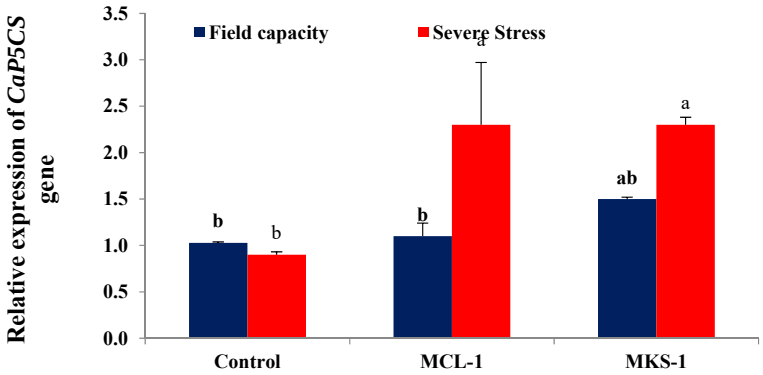


Fig 4. Expression analysis of osmolyte gene regulation in pearl millet under water deficit stress conditions

4.9.4 Expression analysis of *PgLEA* gene (protein) regulation in pearl millet under water deficit stress conditions

Late embryogenesis abundant (LEA) proteins are mainly involved in protecting higher plants from damage caused by environmental stresses. LEA protein is a large protein family that accumulates protein at late stages of seed development and vegetative tissues in response to drought stress. LEA protein expression are regulated by dehydration, signal transduction pathways and LEA genes. Expression of *PgLEA* gene was considerably down regulated in all other treatments as compared to uninoculated control treatment (**Figure 5**). There was considerable down regulation of expression of *PgLEA* gene under severe water deficit stress conditions as compared to control conditions in uninoculated plants. In *S. putrefaciens* strain MCL-1 inoculated plants, under control conditions, considerable down regulation of *PgLEA* gene as compared to uninoculated plants was observed while the expression of this gene was slightly upregulated under severe water deficit stress conditions as compared to inoculated control plants. In *C. dublinensis* strain MKS-1 inoculated plants, as compared to control conditions; there was slight upregulation of the expression of *PgLEA* gene under severe water deficit stress conditions.

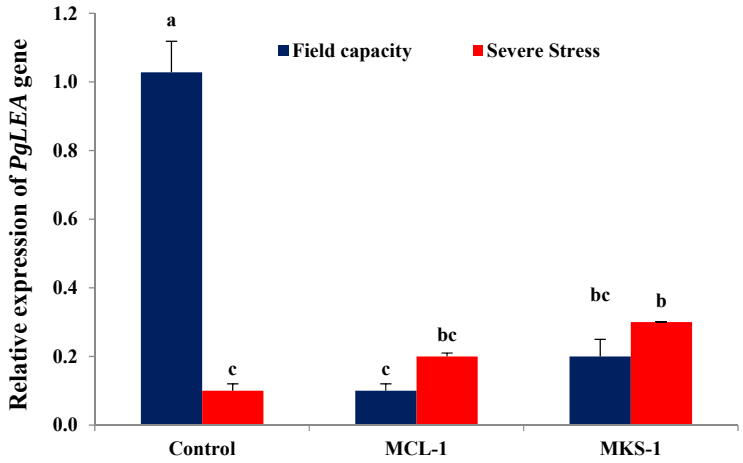


Fig 5. Expression analysis of *PgLEA* gene (protein) regulation in pearl millet under water deficit stress conditions

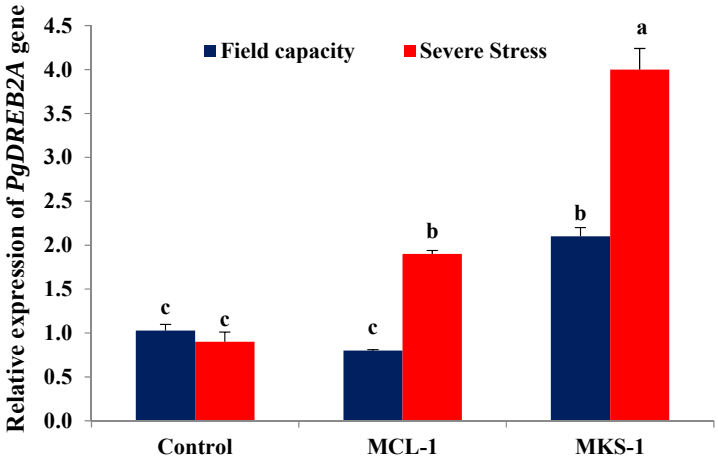
4.9.5 Expression analysis of transcription factorsregulation in pearl millet under water deficit stress conditions

DREB2A is a key transcription factor involved in the signal transduction network that controls the plant's response to dehydration and heat stress. DREB2A is an ethylene-responsive element binding factor/ APETALA2 (ERF/AP2) family transcription factor that governs the expression of different stress-inducible target genes such as specific cis-acting element, the dehydration responsive element/C-repeat (DRE/CRT). The constitutive expression of DREB2A leads to an overproduction of DREB2A and enhanced target gene induction during stress and increase tolerance to the drought stress in plants. There was a slight decrease in the expression of *PgDREB2A* gene under severe water deficit stress conditions as compared to control conditions in uninoculated plants (**Figure 6A**). In *S. Putrefaciens* strain MCL-1 inoculated plants, the expression of *PgDREB2A* gene was slightly down regulated under control conditions while the expression of this gene was 2 fold upregulated under severe water deficit stress conditions. In *C. dublinensis* strain MKS-1 inoculated plants the expression of *PgDREB2A* gene was 2 fold upregulated under control conditions while the expression of this gene was 4 fold upregulated under severe water deficit stress conditions.

SbSNAC1 is the member of plant-specific NAC transcription factor superfamily that plays most important role in the abiotic stress response in sorghum. The SbSNAC1 contain a typical NAC conserved domain at its N-terminus and a diverse C-terminal region. The expression of *SbSNAC1* was induced under drought stress. *SbSNAC1* expression relatively higher in roots with responds to abscisic acid. NAC genes regulate a variety of plant growth and developmental processes such as root development, floral morphogenesis, seed development, leaf senescence, stress inducible flowering induction, and fiber development. Overexpression of NAC transcription factor enhance tolerance to drought stress in plants. There was slight upregulation of expression of *SbSNAC1* gene under severe water deficit stress conditions as compared to control conditions in uninoculated plants (**Figure 6A**). In *S. Putrefaciens* strain MCL-1 inoculated plants, under control conditions, down regulation of *SbSNAC1* gene as compared to uninoculated plants was observed while the expression of this gene was 4 fold upregulated under severe water deficit stress conditions. In *C. dublinensis* strain

MKS-1 inoculated plants, under control conditions, nearly 2 fold increase in the expression of *SbSNAC1* gene as compared to uninoculated plants was observed while the expression of this gene was 6 fold upregulated under severe water deficit stress conditions.

Abscisic acid (ABA) is one of the most important plant hormone plays a crucial roles in many cellular processes such seed germination, vegetative growth, seed development, dormancy and environmental stress responses. A key step in ABA biosynthesis in plants is catalyzation of 9□*cis*epoxycarotenoid dioxygenase (NCED which cleaves 9□*cis* xanthophylls to xanthoxin, a precursor of ABA. The biosynthesis of ABA in *Arabidopsis* is controlled by a small family of *NCED* genes. Expression of *SbNCED* gene was nearly 2.5 fold up regulated under severe water deficit stress conditions as compared to control conditions in uninoculated plants (**Figure 6B**). In *S. putrefaciens* strain MCL-1 inoculated plants, under control conditions, there was slight down regulation in the expression of *SbNCED* gene as compared to uninoculated plants was observed while the expression of this gene was nearly 2.2 fold upregulated under severe water deficit stress conditions. In *C. dublinensis* strain MKS-1 inoculated plants, under control conditions, nearly 811.3 fold increase in the expression of *SbNCED* gene as compared to uninoculated plants was observed while the expression of this gene was 1779.4 fold upregulated under severe water deficit stress conditions.



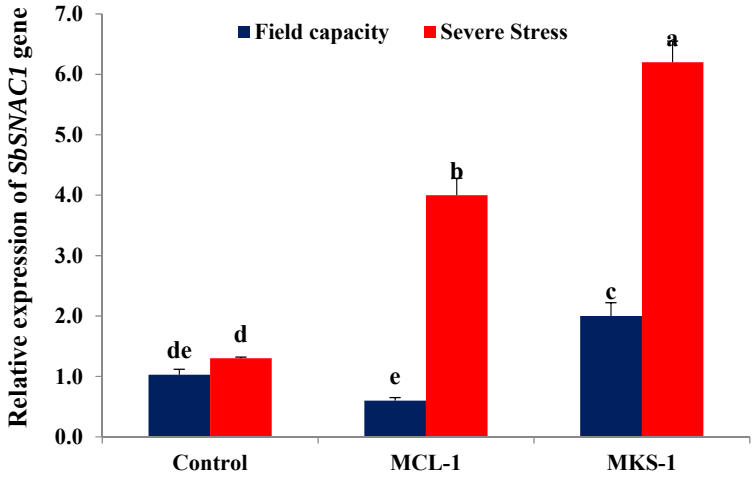


Fig 6.A. Expression analysis of transcription factors regulation in pearl millet under water deficit stress conditions

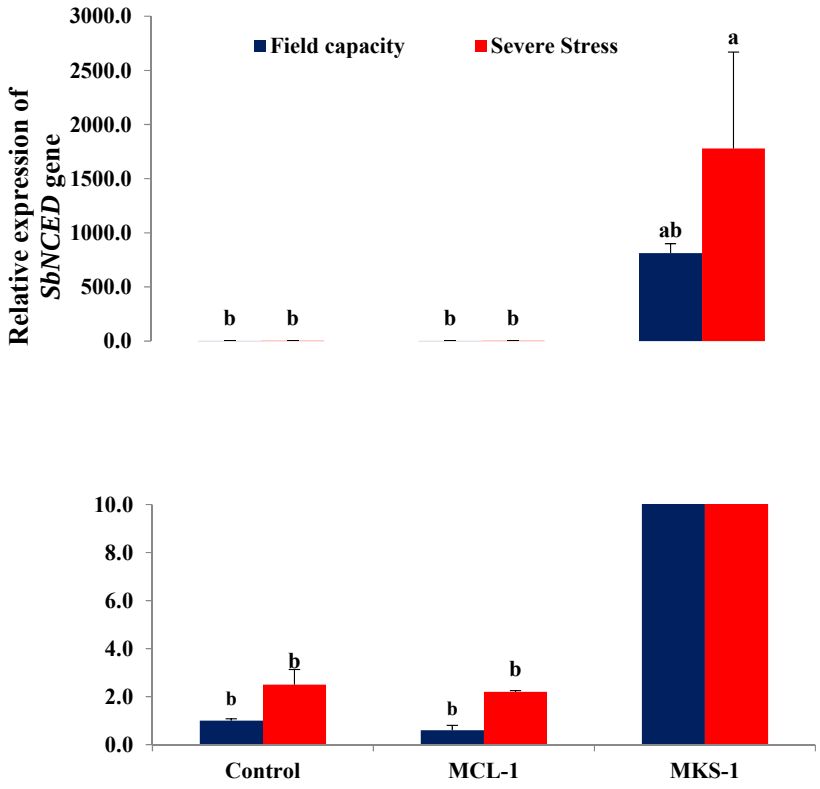


Fig 6.B. Expression analysis of phytohormonal gene regulation in pearl millet under water deficit stress conditions

Drought is one of the most common environmental stresses that affect growth and development of plants and continues to be an important challenge to agricultural researchers. Since the dawn of agriculture, mild to severe drought stress has been one of the major production limiting factors. The recent trends in global climate change and increasing erratic weather patterns are likely to aggravate these further. Prolonged drought is one of the most important abiotic stresses limiting global crop production and is likely to further increase in future due to climate change. If the stress is prolonged, plant growth and productivity are severely diminished. The average yields from the major crop plants may reduce by more than 50% due to drought stress.Climate models have predicted increased severity and frequency of drought under the ongoing global climate change scenarios (IPCC 2007). Drought severely affects plant growth and development with substantial reductions in seed germination, crop growth rate, biomass accumulation and crop productivity. It impairs normal growth, disturbs water relations and reduces water use efficiency in plants.Plant's vegetative as well as reproductive stages are intensively influenced by drought stress.

Crop improvement for evolving better varieties can help to tolerate abiotic stresses to some extent. However, such strategies are long drawn and cost intensive. There is a need to develop simple and low cost biological methods for management of abiotic stresses. Endophytic bacteria which are in intimate contact with the plants are known to have even stronger influence on their growth and development (Rosenblueth and Martinez-Romero, 2006; Gupta *et al*., 2013). Many of the endophytes increase plants tolerance to abiotic stresses. Hence, a deeper understanding of these endophytic bacterial functions is needed if we aim to use these endophytes for improving our crop productivity and for alleviation of water deficit stresses to which a plant is exposed. Beneficial endophytic bacteria having tolerance to osmotic stress have been reported to reduce the deleterious effects of drought to crops and improve plant growth and yield. These are cost-effective and thus, have become an integral part of modern agriculture. Such approaches must enhance and sustain agricultural productivity and at the same time be safe from environmental and health perspectives.

Pearl millet is a major cereal and fodder crop in India. Pearl millet is the staple food of majority of the poor and small land holders, as well as feed and fodder for

livestock in the rainfed regions of N-W India and grows primarily in the arid and semi-arid regions. It is usually grown under the most adverse agro-climatic conditions where other crops like sorghum and maize fail to produce economic yields. Pearl millet is one of the most drought resistant crops for commercial production. It is able to grow in poor fertility soils and in areas which are frequently exposed to water stress during either the vegetative or reproductive phases. Pearl millet in near future may extend into regions that are too dry due to its exceptional ability to tolerate drought. It is comparatively a hardy crop but even this crop does not realize its full yield potential under water scarcity conditions. Water stress has significant consequences on the development and growth of pearl millet. Critical stages of pearl millet which are affected severely by water deficit stress are flowering and grain formation phases and this result in drastic reduction in yield.

In India, pearl millet is the third most widely cultivated food crop after rice and wheat. It is grown on 7.128 million ha during 2015-16 (Directorate of Millet Development, 2017). The major pearl millet growing states are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh and Haryana which account for more than 90% of pearl millet acreage in the country. As an average of the latest data of four years (2012-13 to 2015-16), Rajasthan, Uttar Pradesh, Maharashtra, Haryana and Gujarat accounted for 94.82% of total area under pearl millet and contributed to 87.70% of total production. It is usually grown under rain fed conditions and in regions intermittently exposed to water deficit stress. It is frequently exposed to low moisture conditions during its growth phase and it leads to 40-46% reduction of yield (Radhouane, 2013). Water is very important for getting optimum yields in pearl millet. Both vegetative and reproductive stages of crop are susceptible to drought stress resulting in decreased germination, plant growth, flowering and cob formation.

During the present investigation, 31 osmotolerant endophytic bacterial isolates were screened for their ability to alleviate water deficit stress in pearl millet. Inoculation with only 6 endophytic bacteria was observed to significantly improve shoot fresh weight under water deficit stress conditions. Five isolates showed best performance for most the parameters studied. Inoculation with endophytic bacteria minimized the drought stress-imposed effects and significantly increased shoot biomass and root biomass in maize (Naveed *et al.,* 2014).Plant growth promoting rhizobacteria have been reported to improve growth of maize, pepper, canola, bean, lettuce and wheat under

drought stress (Glick *et al.,* 1997; Sandhya *et al.,* 2010; Chaoqiong *et al.,* 2017).Under diverseenvironmental conditions, bacterial endophytes are able to communicate and interact with the plant more efficiently than rhizosphericbacteria (Ali *et al.,* 2012; Coutinho *et al.,* 2015).Many of the drought tolerant microorganisms have proved to be effective in alleviating effects of drought stress in inoculated plants (Marulanda *et al.,* 2007).

In the present investigation, no effect of osmotic stress on seed germination was observed. Seed inoculation with both isolates did not have any significant effect on seed germination. Drought stress decreased the germination and also delayed the emergence of seeds in four vegetable species (Jamil *et al.,* 2006). Although, inoculation with isolate MKS-1 improved seed germination under no stress condition, it was statistically at par with the control treatment. Osmotolerant rhizobacteria improved seed germination and seedling vigour in mustard under osmotic stress conditions (Bandeppa *et al.,* 2015). In contrast, in the present investigation, under all the water levels studied, inoculations with most of the osmotolerant endophytic bacterial isolates did not have any positive effect on seed germination. However, seedling vigour was improved due to inoculation, as indicated by increased radicle and plumule length and fresh weight of seedling. Under water deficit stress conditions, especially, the effect of inoculation was very evident and there was considerable increase in seedling vigour. Under osmotic stress conditions, inoculation with isolate MKS-1 significantly improved plumule length while both the isolates MKS-1 and MCL-1 significantly improved radicle length and seedling fresh weight. In general, inoculation with PGPRs can enhance germination and seedling emergence (Zahir *et al.,* 2004). Maize was protected from inhibitory effects of drought stress by the bacterial endophytes (Naveed *et al.,* 2014). Best performance for most of the parameters was observed due to inoculation with MCL-1 and MKS-1 isolates.

The isolates MCL-1 and MKS-1 improved shoot and root growth as well as improved seedling fresh weight, and hence were selected for further studies. Effect of higher osmotic stress on their growth was studied using PEG 6000 to create osmotic stress in the medium. Higher concentration of PEG 6000 had an adverse effect on their growth. Both the isolates did not show much reduction in growth at 20% and 30% PEG 6000, however, higher concentration of PEG 6000 had a deleterious effect on their growth of. With an increase in PEG 6000 concentration 40% and 50% in the medium,

there was a concomitant decrease in growth of bacterial isolates. Recent reports have also indicated the ability of osmotolerant bacteria to grow in medium in presence of 40% and 50%PEG 6000 (Bandeppa *et al.,* 2015; Das *et al.,* 2017; Rathi *et al.,*2018). However, osmotic stress is known to affect growth of bacteria (Malakar *et al.,* 2014), which supported our observations. Growth of drought tolerant *Rhizobium* sp. NBRI2505 *sesbania* strain was drastically reduced on exposure to PEG 6000 (Rehman and Nautiyal, 2002). Endophytic bacteria isolated from different crops exposed to water deficit stress are generally adapted to survive under these conditions (Timmusk *et al.*, 2014).

Based on their 16S rDNA homology, the osmtolerant endophytic bacterial strains MCL-1 and MKS-1 were identified as *Shewanella putrefaciens* and *Cronobacter dublinensis* respectively. These bacteria had been isolated from leaf and stem tissues of mustard, respectively. Bacteria belonging to diverse genera have been isolated from drought affected area across the country. The endophytic bacterial genera observed in the plant tissues were *Macrococcus caseolyticus*; and *Pseudomonas aeruginosa* have earlier been reported as the dominant diazotrophic endophytic bacterial species colonizing pearl millet and remaining stabilized throughout the latter's various growth stages under field conditions (Gupta *et al*., 2013).Based on their 16S rDNA homology *Bacillus* genus was observed to be the most predominant group of osmotolerant endophytic bacteria isolated from drought susceptible and drought resistant varieties of pearl millet (Manjunatha *et al.,* 2018).

The two osmotolerant endophytic bacterial isolates *Shewanella putrefaciens*strain MCL-1 and *Cronobacter dublinensis* strain MKS-1 showing best performance for most of the plant parameters studied and these were selected for further studies. The selected endophytic bacteria *Shewanella putrefaciens* strain MCL-1 and *Cronobacter dublinensis* strain MKS-1 were screened for plant growth promoting activities under different osmotic stress conditions. The mechanism of plant growth promotion of endophytic bacteria is similar to that of plant growth promoting rhizobacteria (PGPRs), hence, these endophytes were also screened for their PGP activities under osmotic stress conditions. PGP activities include phosphorus solubilisation, nitrogen fixation, plant hormone synthesis and regulation of plant ethylene levels (Gamalero and Glick, 2011). Both the selected endophytic bacterial strains were observed to possess multiple PGP activities. Both the cultures showed

improvement in their P-solubilization, ACC deaminase, IAA, GA and EPS production abilities under osmotic stress conditions. None of the cultures possessed nitrogenase activity. Multiple PGP activities have been reported in osmotolerant bacteria belonging to diverse genera such as *Pseudomona*s spp. (Sandhya *et al*., 2010), *Bacillus safensis* and *Ochrobactrum pseudogregnonense* (Chakraborty *et al*., 2012), *Bradyrhizobium* spp. (Marinković *et al*., 2013), *Citricoccus zhacaiensis* (Selvakumar *et al*., 2015), *Bacillus* spp. (Manjunatha *et al.,* 2018). Phosphate solubilization has been reported to be a predominant trait present in bacteria isolated from plants exposed to drought (Maheshwari, 2012).There are recent reports on osmotolerant endophytic bacteria isolates from pearl millet possessing multiple plant growth promoting activities under osmotic stress conditions (Manjunatha *et al.,* 2015). Amongst these osmotolerant bacteria, there was high incidence of IAA production and P-solubilization ability. Some of these were also found to possess ACC deaminase activity, however, there was very low incidence of diazotrophs amongst the osmotolerant bacteria. These findings are very similar to our results.

During the present investigation, the plant growth promoting activities were significantly enhanced under lower osmotic stress concentrations obtained by exposure of the selected bacteria to 20% PEG 6000, however, further increase in osmotic stress concentration to 30% PEG 6000 led to decrease in some of these PGP activities. Abiotic stress responsive increase in IAA production has been observed in *Azospirillum brasilense* (Malhotra and Srivastava, 2008). Sandhya *et al*., (2010), in contrast, reported decrease in different PGP activities such as P-solubilization, production of phytohormones (IAA, gibberellic acid and cytokinins), under osmotic stress conditions as compared to non-stressed conditions. Osmotolerant endophytes isolated from pearl millet were also observed to possess multiple plant growth promoting activities under osmotic stress conditions (Manjunatha *et al.,* 2015).Osmotolerant *Bacillus cereus* and *Bacillus* sp. strains were reported to possess multiple PGP activities relevant to mitigation of drought stress in mustard plant (Bandeppa *et al.,* 2018).

During plant-microbe association, under *in vivo* conditions, effect of osmotic stress on plant growth promoting traits was observed. It was observed that under osmotic stress there was increase in most of the PGP traits. There was increase in IAA, GA and exopolysaccharide production, during association between the plant and the selected osmotolerant endophytic bacteria, on exposure to osmotic stress as compared

to control condition. Some reports have indicated that IAA production by endophytic bacteria has been associated with the promotion of plant root growth, enhanced production of lateral roots and increase in root volume and biomass (Taghavi *et al.,* 2009). At lower concentrations, auxins are known to stimulate root growth and thereby improve plant's capacity for nutrient and water acquisition (Overvoorde *et al.,* 2010). There was variable response to osmotic stress on the plant growth promoting activities during association between mustard and rhizobacterial strains *Bacillus cereus* and *Bacillus* sp. (Bandeppa *et al.,* 2018). There was an increase in IAA and exopolysacchardide production at lower level of osmotic stress, while higher level of osmotic stress had an inhibitory effect on these.

Gibberellic acid production was increased during plant-endophyte association under osmotic stress conditions. GA production ability has been reported in different PGPRs (Dimkpa *et al*., 2009).Gibberellins are known to regulate plant growth and development and improve seed germination(Magome *et al*., 2004).Exposure to 20% PEG 6000 hada beneficial effect on GA production during plant-endophyte association. However, at higher level of osmotic stress also was not detrimental to GA production as the values were comparable to control treatment. These observations are supported by earlier findings on association between mustard and rhizobacterial strains *Bacillus cereus* and *Bacillus* sp. where no detrimental effect of exposure to higher osmotic stress on GA production was observed (Bandeppa *et al.,* 2018).Under osmotic stress conditions exopolysaccharide production was enhanced during plant-endophyte association. There was a significant increase in exopolysaccharide production at 20% PEG 6000 concentration and further increase in osmotic stress to 30% had an inhibitory effect on exopolysaccharide production. Osmotic stress has been known to enhance exopolysaccharide production by bacteria (Vardharajula and Ali, 2014; Bandeppa *et al.,* 2018).Inoculation with EPS producing bacteria was reported increase plants resistance to water stress (Viscardi *et al.,* 2016).

In the present investigation, effect of the selected osmotolerant endophytic bacteria on pearl millet growth under water deficit stress conditions was studied. Water deficit in the soil hindered plant growth resulting in decreased production of plant biomass. Water deficit stress conditions predominate in rainfed areas and harm crop production, as it restricts physiological and biochemical processes by changing water availability that ultimately affects growth and yield (Debaeke and Abdellah, 2004).

Drought stress obstructs cell expansion by causing cellular dehydration, which evokes osmotic stress (Bartels and Sunkar, 2005). Osmotolerant endophytic bacterial cultures isolated from different crop plants grown in regions facing low soil moisture stress when used as bio-inoculants increase plant growth and tolerance to drought. Inoculation with osmotolerant *Shewanella putrefaciens* strain MCL-1 and *Cronobacter dublinensis* strain MKS-1 increased shoot and root fresh weight under all water deficit stress as compared to uninoculated control conditions, which may have led to better plant health and growth. PGPRs inoculants isolated from drought affected area can assist host plant to cope with drought stress (Sandhya *et al.,* 2010)**.** Inoculation of endophytic bacterial isolates such as *Burkholderia phytofirmans PsJN* and *Enterobacter sp. FD17*minimized the drought stress-imposed effects and increased shoot and root biomass in maize (Naveed *et al*., 2014).Inoculation with osmotolerant bacteria improved shoot, rootfresh and dry weight of the pearl millet plants under water deficit stress conditions (Das *et al.,* 2017).

Production and productivity of crops in terms of ear yield and total nutrient content were considerably affected under water deficit stress. PGPR inoculation has been reported to improve crop productivity under water deficit stress conditions. Reduction in vegetative biomass accumulation due to water deficit stress subsequently limits plant yield (Vile *et al*., 2012). Inoculation of plants with PGPRs can increase productivity of crops under a water deficit stress condition (Chanway and Holl, 1994). The PGPR inoculated plants displayed increased drought tolerance in arid and semi-arid areas as well as increase in growth and yield to remarkable levels (Marulanda *et al*., 2007). This supported our results, that inoculation of pearl millet with osmotolerant *S. putrefaciens* strain MCL-1 and *C. dublinensis* srain MKS-1 enhanced plant biomass at harvest under all water deficit stress conditions as compared to uninoculated control conditions.

Inoculationwith osmotolerant *S. putrefaciens* strain MCL-1 and *C. dublinensis* srain MKS-1 presumably led to decrease in stress levels of the plant under water deficit stress conditions. Ethylene is a stress hormone and is produced at higher concentration under water deficit stress condition (Khalid *et al*., 2006). Under water deficit stress conditions, the ethylene hormone endogenously controls plant homeostasis and results in reduced root and shoot growth. Due to production of ethylene there is stunting of plant growth, leading to reduction in biomass accumulation, which may eventually

affect crop yield. However, degradation of the ethylene precursor ACC by bacterial ACC deaminase releases plant stress and rescues normal plant growth (Mayak *et al*., 2004). PGPR bacteria which produces the enzyme, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, facilitate plant development and growth by reducing the ethylene levels, and inducing tolerance to various abiotic stresses (Nadeem *et al*., 2007; Zahir *et al*., 2008). Many workers have reported decrease in ethylene production by the inoculated plants, which have led to improvement in plant growth, under water deficit stress conditions. Mayak *et al.* (2004) also reported that inoculation with PGPR containing ACC deaminase confers resistance against drought stress in tomatoes and peppers. Both strains used in the present investigation possess ACC deaminase activity. It is likely that the stress induced accelerated synthesis of ethylene was reduced by inoculation of these strains with ACC deaminase activity, resulting in longer roots thereby helping the plant to take water from deeper layers of soil (Reid and Renquist,1997; Dodd *et al*., 2010; Naveed *et al*., 2014).Thus, these PGPRs provide significant protection from damage caused by various abiotic stress conditions (Jaleel, 2007).

Inoculation of *Bacillus licheniformis* K11 possessing ACC deaminase was able to increase growth of pepper plants under drought stress (Sziderics *et al*., 2007). Many researchers have reported better root growth in plants inoculated with bacteria containing ACC-deaminase (Glick *et al.,* 1995; Shaharoona *et al.,* 2006; Mayak *et al.,* 2004; Arshad *et al.,* 2008). Osmotolerant endophytic bacteria might also have indirectly promoted shoot growth through root growth which provides nutrients to the growing plant. The inoculated wheat seedlings showed greater tolerance to water stress as compared to uninoculated, at different water deficit stress levels (Nwaga *et al.,* 2010). Another PGPR strain, *Achromobacter piechaudii* ARV8 which produces ACC deaminase, also conferred resistance to drought stress in tomato (*Solanum lycopersicum L*.) and pepper (*Capsicum annuum* L.) plants (Mayak *et al*., 2004). These findings supported also our results that inoculation of pearl millet with osmotolerant *S. putrefaciens* strain MCL-1 and *C. dublinensis* srain MKS-1 enhanced plant and root growth under all water deficit stress conditions as compared to uninoculated control conditions.

Phytohormones such as IAA, abscisic acid, ethylene, cytokinins and gibberellins are produced by plants, which are important for their growth and

development (Barea and Brown, 1974; Frankenberger and Arshad, 1995; Teale *et al*., 2006; Egamberdieva, 2013). These hormones plays a crucial role in plants to help them escape abiotic stress or survive stressful conditions (Skirycz and Inze, 2010; Fahad *et al.,* 2015). PGPR are able to synthesize phytohormones that stimulate plant cell growth and division and help plant to become tolerant against environmental stresses (Glick and Pasternak, 2003).In abiotic stress conditions, phytohormone homeostasis is disturbed resulting in stunting of plant growth. Exogenous application of phytohormones such as auxins, gibberellins and zeatin significantly improved seed germination, seedling and root growth in plants exposed to salt stress (Egamberdieva, 2009). Similar effect was also noted when plants were inoculated with IAA producing *Pseudomonas aureantiaca* and *Pseudomonas extremorientalis* strains. There is thus, improvement in plant biomass due to acquisition of phytohormones of bacterial origin which helps in restoring the plant hormone homeostasis.

Physiologically most active auxin, IAA is known to influence many plant growth and developmental processes such as cell division, differentiation and extension; seed germination; root and xylem development; vegetative growth and help in development of resistance under water stress conditions (Teale *et al*., 2006; Navarro. 2008). IAA helps in lateral root formation and branching in roots. Osmotic stresses greatly impact auxin transport (Potters *et al*., 2007, 2009) and is one of the important reasons for stunting of growth on exposure to osmotic stress. Nearly 80% rhizospheric microorganisms from various crops have the ability to synthesize and release auxins (IAA) as secondary metabolites (Patten and Glick, 1996). Rhizobacteria secreted IAA will presumably add to the endogenous pool of plant IAA (Glick, 2012; Spaepen *et al*., 2007). Bacterial synthesis of IAA increases root length and surface area, thus providing the plant greater access to soil nutrients and water, thereby, mitigating the ill effects of water stress (Dimkpa *et al*., 2009). PGPR inoculation of *Phyllobacterium brassicacearum* strain STM196 increased the length of lateral root (Kechid *et al*., 2013) as well as density and length of root hairs, which led to a greater exchange of surface area with soil, and thus a higher water flux through the whole root system up to the leaves of the plant.

In the present study, increase in the production of IAA by the selected osmotolerant endophytic bacteria was noted, during plant-microbe association, especially under osmotic stress conditions. This presumably led to improved root

biomass, as was observed. Probably due to IAA production by the inoculated endophytic bacteria, root system architecture was also improved, as evidenced by increase in root volume, area and length. Changes in root system architecture and root biomass resulted in better mining of water from deeper layer of the soil, thereby mitigating water deficit stress in the inoculated plants. This is supported by our earlier observation, on improvement in water content of shoots of inoculated plants, during the initial screening of the 31 osmotolerant endophytic bacterial isolates.

Abscisic acid (ABA) plays an important role in the physiological processes of plants and it is crucial for the response to environmental stresses such as drought (Porcel *et al.,* 2014; Cohen *et al.,* 2015). Higher ABA content in plant organs under drought stress conditions results in physiological changes and modulation of plant growth (Farooq *et al.,* 2009).Cellular dehydration induces the biosynthesis of ABA, a stress hormone during water deficit condition (Kaushal and Wani, 2015). ABA hormone is involved in water loss regulation by controlling stomatal closure and stress signal transduction pathways (Yamaguchi-Shinozaki and Shinozaki, 1994). PGPR that helps increase the concentration of ABA can enhance plant's ability to tolerate drought stress. Some reports have shown that *Bacillus* spp. treated lettuce (*Lactuca sativa L.)* plants had increased amounts of ABA when compared to non-treated plants (Arkhipova *et al.,* 2007). Cohen *et al.* (2008) reported that *Arabidopsis* plants treated with PGPR *Azospirillum brasilense*Sp245 had increased ABA content than non-treated plants and enhanced the drought tolerance in plants.

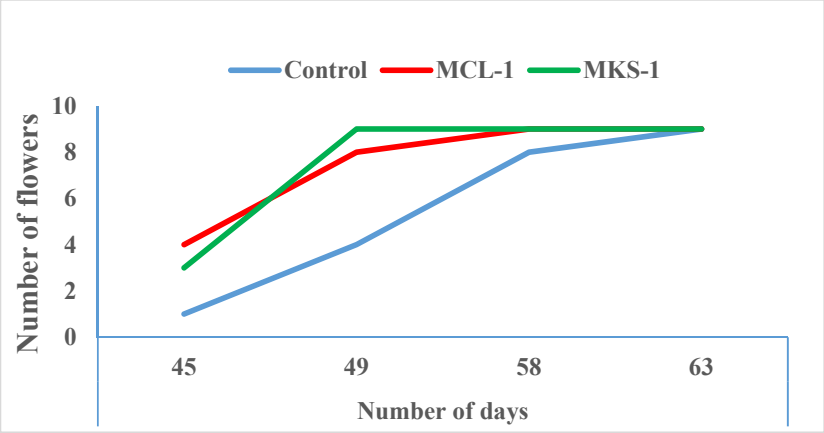
There are different mechanisms by which ABA is able to enhance drought tolerance. One of the mechanism by which ABA enhances drought tolerance is via regulation of leaf transpiration and root hydraulic conductivity (Aroca *et al.,* 2006). Another mechanism by which ABA enhances drought tolerance by upregulation of aquaporin's (Zhou *et al.,* 2012).PGPR *Phyllobacterium brassicacearum* strain STM196, isolated from rhizosphere of *Brassica napus* enhanced osmotic stress tolerance in inoculated Arabidopsis plants by elevating ABA content, leading to decreased leaf transpiration (Bresson *et al.,* 2013). Inoculation of *Platycladus orientalis* seedlings with *Bacillus subtilis* elevated the levels of ABA in shoots and increased stomatal conductance conferring drought stress resistance (Liu *et al.,* 2013).These reports are also supported by our observation, on elevated levels of ABA in inoculated plants and confirming the water deficit stress tolerance in pearl millet, in the present study. A decrease in ABA production was observed under water deficit stress

conditions. However, inoculation with these osmotolerant endophytic bacteria enhanced ABA content in plants under water deficit stress conditions as compared to uninoculated control conditions.

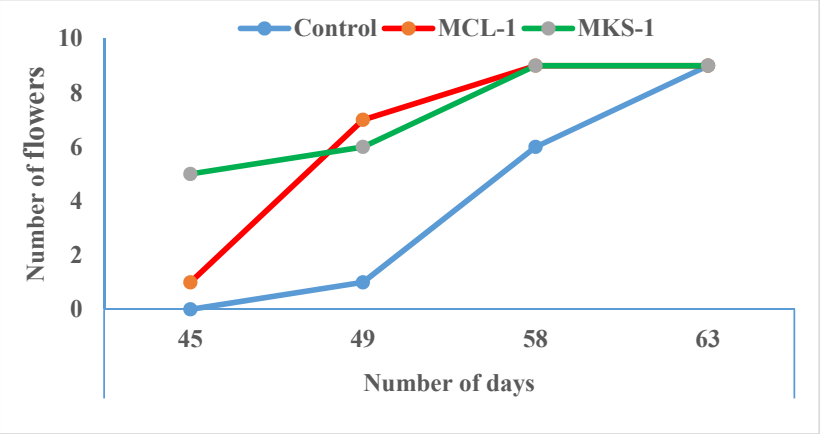
Gibberellins are tetracyclic diterpenoid acids that are involved in plant developmental and physiological processes (Crozier *et al.,* 2000; Davies 1995). Generally, gibberellins are involved in the processes of seed germination, seedling emergence, leaf expansion, stem elongation, floral induction and fruit growth (King and Evans 2003; Pharis and King 1985; Sponsel 2003; Magome *et al*., 2004). Gibberellins are also associated in promotion of root growth, root hair abundance, and inhibition of floral bud differentiation in woody angiosperms, regulation of vegetative and reproductive bud dormancy of plant species (Tanimoto 1987; Bottini and Luna 1993; Fulchieri *et al.,* 1993; Reinoso *et al.,* 2002;).There is decrease in GA levels during exposure of plants to water deficit stress which contributes to growth restriction. Colebrook *et al*. (2014) reported that the role of GA in controlling plant response to abiotic stress is becoming increasingly evident. Recently reports indicated that there is enhanced plant water use and reduced stomatal resistance in tomato at low salinity condition due to GA3 treatment (Maggio *et al*., 2010). GA3 application improved salinity tolerance in crops grown under saline condition maybe by restoring the hormonal homeostasis (Iqbal and Ashraf, 2013). Thus, GA producing bacterium presumably can enhance plant growth under water deficit stress by restoring the homeostasis of this hormone in plants.

In the present investigation, both the selected osmotolerant endophytic bacteria were observed to be GA producers during studies under *in vitro* conditions; and during *in vivo* plant-microbe interactions also, GA production during the plant-microbe association was observed. Bacterial origin GA may have added to the endogenous pool of plant GA and helped restore GA homeostasis under water deficit stress conditions leading to mitigation of water deficit stress in pearl millet. GA is known to induce flowering in plants; and reduced plant GA levels may lead to inhibition of flowering (Wilson *et al*., 1992). In the present investigation, flowering in the inoculated plants initiated early as compared to the uninoculated controls, which was presumably due to the contribution of bacterial GA to plant GA pool. This presumably resulted in restoration of plant GA homeostatsis leading to earlier initiation of flowering in inoculated plants, as compared to uninoculated control, under stress conditions **(Fig 7. A, B, C).**

A. Field capacity



B. Mild stress



C. Severe stress

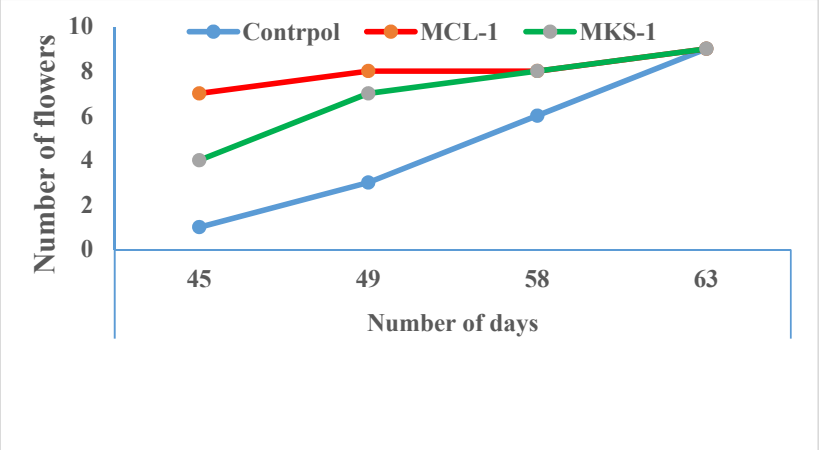


Fig 7. Effect of inoculation on flower initiation under water deficit stress conditions

Drought stress influences the availability and transport of soil nutrients, as nutrients are carried to the roots by water. The nutrients transport from soil to roots is highly dependent on the moisture content of soils (Silva *et al*., 2010). Drought stress therefore decreases nutrient diffusion and mass flow of water soluble nutrients such as nitrate, sulfate, Ca, Mg, and Si (Barber, 1995; Selvakumar *et al.,* 2012).The nutrient transport is thus, considerably affected under drought stress and leads to decrease in nutrient content in the plants. In the present investigation also, a decrease in nitrogen, phosphorus and potassium content in shoot and grain was observed under water deficit stress supporting the earlier findings. Concomitant with this, there was also decrease in root biomass and volume which may have led to a decrease in absorption of these nutrients from soil. Nutrients and water uptake by roots is highly influenced by root biomass and volume (Fageria, 2004) and hence, any decrease in these parameters will affect plant nutrient status which was observed in the present investigation.

Inoculated plants had higher nitrogen, phosphorus and potassium content in plants and higher phosphorus and potassium content in grains. These plants also showed better root biomass which thereby, increased the absorptive area of roots. As a result, presumably there was higher uptake of these nutrients from soil resulting in the observed higher contents of these nutrients in grains and shoot of the inoculated plants. Inoculation of the two superior Phosphorus solubilizing bacterial isolates (PSB1 and PSB8) enhanced seedling growth (shoot and root length, shoot and root dry weight) and nutrient contents (N, P and K)in plant tissues compared to control (Sarker *et al.,* 2014). PGPRs are known to play stimulatory role in improving nutrient uptake by the plant on exposure to abiotic stresses (Egamberdiyeva, 2007). In addition, the inoculated osmotolerant endophytic bacteria also possessed P-solubilizing ability which was also enhanced under osmotic stress. Phosphorus is one of the most important major nutrient which becomes deficient under drought stress (Allen, 2006) leading to stunting of plant growth. Phosphorus solubilizing bacteria are known to influence P-availability in soil (Richardson *et al.,*2011). These osmotolerant endophytic bacteria solubilize the unavailable forms of phosphorus in soil and transform it to readily available form, thereby increasing its availability and uptake by the plant.

Photosynthesis is one of the most important processes on earth to sustain life by capturing and converting light energy to chemical energy which is further utilized by the living organisms (Zargar *et al.,* 2017).Drought stress causes detrimental effect on

photosynthetic capacity of plants(Ashraf and Harris, 2013). There is considerable damage to photosynthetic pigments resulting in an overall reduction in plant chlorophyll content and photosynthetic capacity is thus, affected (Chutia and Borah, 2012). Reduction in photosynthesis is attributed to the decrease in turgor pressure, closure of stomata, limitation of gas exchange, reduction in CO_2 assimilation, impaired photosynthetic apparatus mainly PSI & PSII and enhanced metabolite fluxes (Chaves *et al.,* 2003; Jaleel *et al.,* 2009).Decreasing of chlorophyll content in bean, *Carthamus tinctorius* and *Paulownia imperialis* was observed under drought stress(Beinsan *et al.,* 2003; Siddiqi *et al.,* 2009; Astorga and Melendez, 2010).Water deficit stress reduces the water potential and there is turgor loss, resulting in stomatal closure and it leads to decline in the photosynthesis rate (Hoekstra *et al.,* 2001; Chen and Murata, 2008; Yang *et al.,* 2010;Alcazar *et al*., 2011). Under water deficit stress it is generally accepted that stomatal limitation is the main determinant to reduced photosynthesis (Cornic, 2000) and it results in decrease in both photosynthesis and internal $C0_2$ concentration, which leads to inhibition of total photosynthetic metabolism, thereby resulting in reduced net photosynthetic rates. The stomatal conductance and leaf water interact with each other and there is always a good correlation between them, even under drought stress (Ortuño. 2004).There is a drought-induced root- to-leaf signalling, which is promoted by soil drying, transpiration stream, resulting in stomatal closure.

Plant photosynthetic parameters such as transpiration rate, stomatal conductance and photosynthetic rates are thus considerably reduced under water deficit stress conditions. In the present investigation, the photosynthetic parameters were significantly impacted due to water deficit stress. However, plant photosynthetic parameters were significantly increased due to inoculation with the osmotolerant bacterial strains *S. putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1. Photosynthetic rate, chlorophyll content and water-use efficiency improved due to inoculation with *Burkholderia phytofirmans*PsJN in wheat as compared to control treatment under field conditions (Naveed *et al.,* 2014a).In maize, shoot and root biomass, leaf area and photosynthetic efficiency was higher under drought imposed plants due to inoculation with both *B. phytofirmans* and *Enterobacter sp.* FD17 as compared to control treatment (Naveed *et al.,* 2014b). *Bacillus subtilis* strain LK14isolated from medicinal plant *Moringa peregrine* inoculated with *Solanum lycopersicum* significantly increased shoot and root biomass and chlorophyll a and b contents as compared to control plants (Khan *et al.,* 2016).There was an increase in

chlorophyll content of the inoculated plants under water deficit stress which may have contributed to the observed increase in plant biomass. Inoculation with PGPRs significantly increased chlorophyll content under water deficit stress conditions (Heidari and Golpayegani, 2012). Endophytic bacterial strains 130, 135, and 170 isolated from the medicinal plant *Lonicera japonica* could produce wheat growth promoting molecules *in vitro* and increase wheat growth *i.e.*, stem length, root length, fresh weight, dry weight, and chlorophyll content *in vivo* (Zhao *et al.,* 2015), thereby supporting our findings. Inoculation with osmotolerant endophytic bacteria significantly improved all of the photosynthetic parameters studied. *Pinus halepensis* inoculated with *Pseudomonas fluorescens* (Rincon *et al*., 2008) and *Azospirillum* inoculated rice (Ruiz-Sanchez *et al*., 2011) displayed increased photosynthetic capacity. Inoculation with *Burkholderia phytofirmans* strain PsJN improved photosynthesis, chlorophyll content (22%) and efficiency of PSII (10%) of the maize cultivar Mazurka compared to the control treatment (Naveed *et al.,* 2014b). Another earlier study indicated a significant enhancement of transpiration velocity, stomatal conductance and net photosynthetic rates due to inoculation of rice with endophytic rhizobia (Chi *et al*., 2005). Plant-growth-promoting rhizobacteria (PGPR) possessed tremendous potential for modulating the physiological response to water deprivation, thus ensuring plant survival under such stressful conditions (Marasco *et al.,* 2012). Inoculation of *Arabidopsis thaliana* with *Phyllobacterium brassicacearum* strain STM196 enhanced resistance to water deficit through changes in transpiration rate and reproductive delay (Bresson *et al*., 2013).All these findings support our observations on increase in plant photosynthetic parameters due to inoculation with osmotolerant endophytic under water deficit stress conditions in pearl millet.

Drought stress affects physiological and biochemical properties of plants. There are different mechanisms are associated with plant's adaptation to environmental stresses. Plants adaptation to drought stress is associated with metabolic adjustments that lead to the accumulation of several compatible solutes such as proline, glycine betaine, sugars, aminoacids, polyamines and quaternary ammonium compounds(Yancey *et al.,* 1982;Close, 1996).These solutes maintain cellular turgor and help plants lower water potential without decreasing actual water content (Serraj and Sinclair, 2002).As the turgor pressure decreases, cell accumulates osmolytes for maintaining osmolarity to sustain life under drought stress (Zargar *et al.,* 2017).Drought stress is often accompanied by an increase in compatible solutes, specifically proline

(Farooq *et al.,* 2008). Proline is one of the most important osmolytes that accumulate in plants experiencing drought stress (Huang et al., 2014). In many plants, an increase in proline levels under drought stress has been correlated with drought tolerance in pea, chickpea, rice and soybean (Sankar *et al.,* 2007; Alexieva *et al.,* 2001; Mafakheri *et al.,* 2010; Lum *et al.,* 2014; Silvente *et al.,* 2012). In maize, proline level increased at 100 fold under low water potential (Voetberg and Sharp,1991).These studies indicate that plants with increased levels of proline would be able to tolerate drought stress. Inoculation led to an increase in starch and total phenolics content in plants presumably due to bacterial-mediated improvement in plant photosynthetic capacity. There was also bacterial induced increase in proline, glycine betaine, starch and phenolics content in the treated plants, thereby enhancing their tolerance to water deficit stress. Various workers have reported increase in proline contents in inoculated plants under drought stress (Sandhya *et al*., 2010). A consortium of three plant-growth-promoting rhizobacterial strains decreased monodehydroascorbate (MDA); enhanced leaf proline content and photosynthetic activity in cucumber leaves over control under drought stress (Wang *et al*., 2012). PGPR inoculation with plants has been shown to an increase in proline levels. This has been demonstrated in maize, sorghum, potato plants, mung bean, Arabidopsis and tomato (Naseem and Bano, 2014; Grover *et al.,* 2014; Gururani *et al.,* 2013; Sarma and Saikia, 2014; Cohen *et al.,* 2015; Shintu and Jayaram, 2015). High levels of proline probably help in maintaining cell water status, membrane integrity and prevent protein denaturation under stress (Yoshiba *et al*., 1997). Proline accumulation also helps in decreasing oxidative damage to the plants under drought stress (Nayer and Reza, 2008). Our results also supported the previous findings on increase in proline content in plants exposed to water deficit stress. We also observed an increase in starch content in the inoculated plants under water deficit stress conditions. This was presumably due to an increase in the chlorophyll content of the inoculated plants. There was also a concomitant increase in the other photosynthetic parameters such as photosynthetic rate, transpiration, stomatal conductance leading to higher rate of photosynthesis. This might have led to higher accumulation of starch in the inoculated plants under water deficit stress conditions.

Choline plays a significant role in the development of stress resistance in plants, mainly for enhancing glycine betaine biosynthesis and accumulation (Zeisel,2006; Zhang *et al.,* 2010). There are many reports on the role of microbes such as *B.subtilis* GB03 in *Arabidopsis* (Zhang *et al.,* 2010) and *Klebsiella varicola* F2, *P. fluorescens*

YX2 and *Raoultella planticola* YL2 in maize in inducing increase in biosynthesis and accumulation of choline as a precursor in glycine betaine metabolism, resulting in the accumulation of glycine betaine, thereby, improving leaf relative water content (RWC) and drymater weight (DMW) (Glick *et al.,* 2007; Zhang *et al.,* 2010; Gou*et al.,* 2015).Enhanced accumulation of solutes such as glycine betaine was induced by PGPR strains under water deficit stress conditions that regulated plant stress responses by preventing water losscaused by osmotic stress (Nadeem *et al.,* 2010; Bashan *et al.,* 2014); and osmotic stressed plants inoculated with PGPR strains such as *B. subtilis* GB03 and *Pseudomonas spp.* accumulated significantly higher glycine betaine than those in plants without inoculation (Sandhya *et al.,*2010). These studies indicate that plants with increased levels of glycine betaine would be able to tolerate drought stress. In our study also we observed an increase in accumulation of glycine betaine content in inoculated plants under water deficit stress conditions which probably enhanced their tolerance to water deficit stress. This was probably one of the factors responsible for the observed improvement in leaf relative water content and dry matter content of the inoculated plants under water deficit stress conditions.

Drought stress causes an accumulation of oxygen free radicals due to conformational changes in structure of protein, changes in electron transport chain and affects the membrane characteristics of living organisms through phospholipid fatty acid composition changes (Russell *et al.,* 1995; Vriezen *et al.,* 2007; Bérard *et al.,* 2015). Oxidative accumulation of free radicals induces protein denaturation and lipid peroxidation that ultimately leads to cell lysis (Potts, 1999). The various physiological parameters studied were membrane stability index, relative water content and lipid peroxidation and these parameters were affected due to water stress. Lipid peroxidation is also associated with water stress in plants (Pandey *et al*., 2010; Zhou *et al*., 2009; Lin and Kao, 2000). There is also an increase in generation of ROS under water stress conditions, which causes injury and thus, damages the cell membranes. There are positive correlation between drought stress sensitivity and membrane damage (Quan *et al*., 2004). Reduced water content has a detrimental effect on membrane integrity leading to increase in electrolyte leakage. High electrolyte leakage was observed in maize under drought stress as compared to control conditions. Drought stress is known to severely impact plant water status as was observed in the present investigation. The relative water content is a good indicator of water deficit stress (Fisher, 2000). Due to

inoculation with the osmotolerant endophytic bacteria, lipid peroxidation was significantly reduced in the plants along with a concomitant increase in membrane stability and relative water content under water deficit stress.

Relative water content in plant leaves is considered as one of the most important criteria for measuring plant water status because it is involved in plant metabolic activities. A decline in RWC reflects a loss of turgor pressure that results in limited cell expansion and consequentially, it will reduce growth in plants (Ashraf, 2010; Lu *et al.,* 2010; Castillo *et al.,* 2013). Therefore, an increase in relative water content should be considered an important drought tolerance improvement strategy. Relative water content is one of parameter used for screening PGPR potential for drought stress alleviation. There was an increase in membrane stability and water content of plants. Under drought stress relative water content in maize seedlings was considerably lowered while there was increase in electrolyte leakage and inoculation with plant growth promoting rhizobacterium *Pseudomonas* sp. considerably improved relative water content while lowering electrolyte leakage (Sandhya *et al*., 2010). Several investigations have reported that under drought stress, PGPR-treated plants maintained relatively higher relative water content compared to non-treated plants for example, Grover *et al.* (2014) reported that sorghum plants inoculated with PGPR, *Bacillus* spp. strain KB 129 under drought stress showed 24% increase in relative water content compared to control plants and similar results have also been reported in maize (Sandhya *et al.,* 2010; Vardharajula *et al.,* 2011; Bano *et al.,* 2013; Naveed *et al.,* 2014; Naseem and Bano, 2014). All these reports indicated that higher relative water content may help plants counteract the oxidative and osmotic stresses caused by drought stress and potentially contributing to greater productivity under drought stress. Casanovas *et al.* (2002) suggested that high relative water content in maize treated with *Azospirillum brasilense* BR11005was a result of bacterial abscisicacid that induced stomatal closure and mitigated drought stress. Increase in relative water content may have been a result of alterations of the sensitivity of physiological processes such as stomatal closure and also due to a reduction in the inhibitory effect of drought on the roots and the development of more effective root system in the inoculated plants (Dodd *et al.,* 2010; Naveed *et al.,* 2014b).

Exposure of plants to different abiotic stresses are known to induce oxidative stress that leads generation of reactive oxygen species (ROS) (Cheeseman, 2007). The

different kinds of ROS species in plants include superoxide anion radicals (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (OH^-) and singlet oxygen($^1O_2$)and alkoxy radicals. ROS reacts with proteins, lipids and deoxyribonucleic acid causing oxidative damage and impairing the normal functions of plant cells (Mittler, 2002; Miller *et al.,* 2010; Farooq *et al.,* 2009; Hasanuzzaman *et al.,* 2014). To overcome these effects, plants develop antioxidant defense systems comprising both enzymatic and non-enzymatic components that serve to prevent ROS accumulation and alleviate the oxidative damage occurring during drought stress. The enzymatic antioxidants superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), glutathione reductase (GR), and ascorbate peroxidase (APX) are the most important components in the scavenging system of ROS (Noctor and Foyer, 1998; Helena and Carvalho, 2008; Farooq *et al.,* 2009; Gill and Tuteja, 2010; Hasanuzzaman *et al.,* 2014). The response of pearl millet to water deficit stress conditions in terms of activities of antioxidative enzymes superoxide dismutase, catalase and ascorbate peroxidase was studied. There was an increase in SOD and catalase enzyme activity due to inoculation with the osmotolerant *S. putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1 in pearl millet over uninoculated control under water deficit stress conditions. These results are supported by earlier reported observations on enhancement in antioxidative enzyme activities in presence of PGPRs under water deficit stress conditions. Saravanakumar *et al.* (2011) reported an increase in the activity of CAT in green gram plants treated with *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPB. This increase was correlated with the observed drought tolerance. An association of CAT production and drought tolerance has also been observed in cucumber, maize and wheat (Wang *et al.,* 2012; Sandhya *et al.,* 2010; Sarma and Saikia, 2014; Vardharajula *et al.,* 2011; Kasim *et al.,* 2013). Inoculation of cucumber plants with *Bacillus cereus* AR156, *Bacillus subtilis* SM21 and *Serratia* sp. XY21, a consortium of three PGPR strains enhanced superoxide dismutase (SOD) activity, proline content and photosynthetic activity in leaves; and decreased leaf monodehydroascorbate (MDA) over control under drought stress (Wang *et al*., 2012). Similar to our findings an improvement in the antioxidants status was also observed by Heidari and Golpayegani (2012).

Plants possess a variety of antioxidant compounds such ascorbate and glutathione to keep ROS levels low and avoid the toxicity of these molecules Pandey *et al.,* 2017). Non-enzymatic antioxidant compounds, such as ascorbicacid, cysteine,

glutathione, α-tocopherol and carotenoids, also neutralize ROS (Sharma *et al.,* 2012; Kaushal and Wani, 2015). In the present investigation, the antioxidant compounds such as ascorbic acid and glutathione contents were increased under water deficit stress conditions due to inoculation with the osmotolerant *S. putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1 in pearl millet over the uninoculated control treatment. Inoculation of cowpea plants with *Bradyrhizobium* and *Actinomadura* showedincrease in the level of ascorbate and exhibited a greater oxidative and membrane protection by reacting directly or indirectly with ROS (Santos *et al.,* 2018). Glutathione occurs in reduced and oxidized forms and protects the cell membrane against ROS damage (Sytar *et al.,* 2013). PGPR inoculation of cowpea plants with *Bradyrhizobium* and *Actinomadura* showed decrease in reduced and increase in oxidized glutathione content in the nodules of cowpea plants under salt stress conditions. These PGP bacteria antioxidant system detoxifies reactive oxygen species generated in cowpea nodule under salt stress conditions and increased tolerance to salt stress (Santos *et al.,* 2018).

Drought stress impacts on physico-chemical and biological properties of soil and which makes soil unsuitable for microbial activities and crop yield. Soil water availability controls the production and consumption of protein and polysaccharides by bacteria (Robersonand Firestone, 1992) and thus indirectly influences soil structure. There is complex and dynamic interaction between soil microbes, roots, soil and water which greatly impacts physico-chemical and structural properties of soil (Haynes and Swift 1990; Tisdall and Oades, 1980). Microbial polysaccharides bind to soil particles and enhance the formation of stable aggregates (Sandhya *et al*., 2009). Exopolysaccharide production by microorganisms protects them from drought stress condition and facilitates their survival. Capsular material of *A. brasilense* Sp245 contains high molecular weight carbohydrate complexes responsible for protection under extreme conditions like desiccation. The suspension of decapsulated cells of *A. brasilense* Sp245 significantly enhanced survival under drought stress (Konnova *et al.,* 2001). Inoculation with exopolysaccharide producing bacteria increased plant's resistance to water stress (Bensalim *et al*., 1998). A significant correlation between exopolysaccharide production by cowpea *Bradyrhizobium* strains and their desiccation tolerance was observed (Hartel and Alexander, 1986). Exopolysaccharides possibly enhance water retention in the microbial environment and prevent ill effects of dessication and water potential fluctuations on the microbe (Hepper, 1975; Wilkinson,

1958; Amellal *et al*., 1998). Exopolysaccharide producing bacteria have been shown to improve permeability by increasing soil aggregation and maintaining higher water potential around the roots, thereby increasing the uptake of nutrients by the plant with an increase in plant growth and protection from drought stress (Miller and Wood, 1996; Alami *et al.,* 2000; Selvakumar *et al.,* 2012).

Stable soil aggregates influence water holding capacity of soil thereby influencing the amount of water available to the plant. Significant increase in exopolysaccharide production by the selected osmotolerant endophytic bacteria during plant-microbe association under osmotic stress conditions was observed under *in vitro* conditions. In the pot experiment also, in inoculated treatments, increase in soil carbohydrate content was observed, which was significantly enhanced under water deficit stress conditions. This presumably also helped in improving soil aggregate stability. Both these factors, soil carbohydrate content and soil aggregate stability are known to play an important role in enhancing availability of water to the plant. Presumably, these traits also played important role in alleviation of water deficit stress in pearl millet.

Formation of biofilm of inoculated bacteria on root surface and improved soil properties was reported as probable mechanism for improved drought tolerance of sunflower seedlings (Sandhya *et al*., 2009). Significant increase in root adhering soil per root tissue (RAS/RT) ratio was observed in sunflower rhizosphere inoculated with EPS producing bacterial strain YAS34 under drought conditions (Alami *et al.,* 2000). Inoculation with *Pseudomonas sp.* strain GAP-P45 increased the survival, plant biomass and RAS/RT of sunflower seedlings subjected to drought stress. The inoculated rhizobacteria could efficiently colonize the root adhering soil, rhizoplane and increase the percentage of stable soil aggregates. Better aggregation of RAS presumably led to increased uptake of water and nutrients from rhizospheric soil, thus ensuring plant growth and survival under drought stress (Sandhya *et al.,* 2009). In our study also we observed increase in soil carbohydrate content, soil aggregate stability and root adhering soil/root tissue ratio due to inoculation with osmotolerant endophytic bacteria which probably enhanced tolerance to water deficit stress in pearl millet.

The effect of inoculation on the expression of the drought stress responsive genes was studied under control and severe water deficit stress conditions in pearl millet. The genes were selected based on response of the crop to inoculation in terms

of microbe-mediated modulation of the physiological and biochemical parameters. Osmotolerant endophytic bacteria-mediated changes in the expression levels of different genes were analysed by comparing the expression levels of genes in inoculated plants under water deficit stress with their respective controls by using the quantitative RT-PCR analysis. Inoculation with the selected endophyte was observed to have induced changes in the gene expression of the plants under water deficit stress conditions leading to systemic tolerance to drought. Up-regulation of genes involved inauxin responses and down-regulation of genes involved in ethylene responses were identified in tomato plants due to inoculation with endophytic *Pseudomonasfluorescens*FPT9601-T5(Wang *et al.,*2005).PGPRs have been also reported to induce changes in the expression of drought stress responsive genes in the plants. Timmusk and Wagner (1999) were the first to report induction of drought stress responsive *ERD 15* gene due to inoculation with *Paenibacillus polymyxa*. Analysis of the expression of the drought stress responsive genes such as *PgAP2, PgCSD, CaP5CS, PgDREB2A, SbSNAC1, PgAPX, PgLEA, SbYUC, SbGA20ox and SbNCED* in pearl millet under control and severe water deficit stress conditions were carried out in the present study. It was observed that inoculation considerably influenced the expression of all these genes.AP2/EREBPs (ethylene-responsive element-binding proteins) belong to a superfamily of plant specific transcription factors characterized by the presence of an AP2 DNA-binding domain of 60 amino acids (Weigel, 1995; Okamuro *et al.,* 1997). Expression of *PgAP2* in inoculated plants was considerably enhanced under both the water regimes conditions, although higher expression was observed under severe water deficit stress conditions, indicating their expression was bacteria-induced. EREBPs containing a single AP2 domain are involved in the regulatory networks of response to hormones, pathogen attack, and environmental signals involving DREBs (dehydration responsive element binding proteins) and ERFs (ethylene responsive factors) (Xu *et al.,* 2011; Licausi *et al.,* 2013; Jisha *et al.,* 2015).

The expression of *SbYUC* gene was nearly 2 fold up regulated under severe water deficit stress conditions as compared to control conditions in uninoculated plants. Inoculation with *S. putrefaciens* strain MCL-1 under control conditions, not much change in the expression of *SbYUC* gene as compared to uninoculated plants was observed while the expression of this gene was nearly 6 fold upregulated under severe water deficit stress conditions. Where as in case of *C. dublinensis* strain MKS-1

inoculated plants, under control conditions, nearly 7.8 fold increase in the expression of *SbYUC* gene as compared to uninoculated plants was observed, while the expression of this gene was 12.6 fold upregulated under severe water deficit stress conditions. IAA is the major plant auxin that is synthesized by members of the *YUCCA* (YUC) family of flavin monooxygenases that catalyse a rate-limiting step in auxin biosynthesis. Overexpression of *AtYUC6* in potato established enhanced drought tolerance through regulated ROS homeostasis (Park *et al.,* 2013). Auxins are also known to be involved in improving root biomass, modulating root architecture by increasing root surface area, root length, root diameter etc. Our results indicated that inoculation increased the expression of *SbYUC* gene involved in auxin biosynthesis. Hence, higher expression of *SbYUC* gene may have led to higher accumulation of IAA, which was also supported by data on plant IAA status. This in turn, improved root biomass and root architecture, which is again, supported by our observations on these parameters under severe water deficit stress conditions. All these presumably also had a role to play improving the inoculated plant's tolerance to water deficit stress.

There was no change in the expression of *SbGA20oX* gene under severe water deficit stress conditions as compared to control conditions in uninoculated plants. There was increase in the expression of *SbGA20oX* gene due to inoculation with both *S. putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1 under severe water deficit conditions compared to uninoculated control under severe water deficit stress conditions. Plant hormone GA biosynthesis requires the expression of enzyme gene GA 20-oxidase gene (*OsGA20ox*1-4), GA 3-oxidase genes (*OsGA3ox1, 2*) in rice plant (Kitanaga *et al.,* 2014). Gibberellins stimulate cell elongation, germination and flowering in plants. The inoculated plants not only showed higher expression of *SbGA20oX* gene under severe water deficit stress condition but were observed to be taller than the uninoculated control plants exposed to similar stress conditions. Moreover, another observation was also noted that there was earlier induction of flowering in the inoculated plants under severe water deficit stress condition also. All these observations again indicated the advantage conferred by the selected bacteria in the inoculated plants by inducing higher expression of *SbGA20oX* gene under severe water deficit stress conditions.

Expression of *PgAPX* gene was down regulated under severe water deficit stress conditions as compared to control conditions in uninoculated plants. Inoculation with the osmotolerant endophytic *S. putrefaciens* strain MCL-1 and *C. dublinensis* strain

MKS-1 did not show any change in the expression of *PgAPX* gene under no stress conditions. However, there was upregulation of the expression of this gene in inoculated plants under severe water deficit stress conditions. Previous reports have also demonstrated that plant-associated microorganism's attenuate salt-induced lipid peroxidation as well has higher CAT and APX activities resulting in enhanced salt tolerance (Baltruschat *et al.,* 2008). PGPR have been known to mediate abiotic stress tolerance in plants through modulation of expression levels of ROS-scavenging enzymes (Gururani *et al.,* 2013). High levels of ABA induce antioxidant defence genes, such as SOD, GPX, APX, and CAT, that can scavenge ROS during salinity related damage from osmotic or ionic stress (Bharti *et al.,* 2016). PGPR, *Paenibacillus yonginensis* DCY84^T enhanced salt stress tolerance in *Panax ginseng* seedlings by activating defence-related systems like high transcription levels of ABA synthesis-related genes and antioxidant defence genes so as to help the plant to tolerate a long period of salinity stress (Sukweenadhi *et al.,* 2018). The transcript of genes for antioxidative enzymes (APX, CAT, POD, MnSOD, GPX and GR) increased in the PGPR-inoculated wheat plants (Bharti *et al.,* 2016). Plant tolerance to water deficit stress was correlated with the increased expression of *PgAPX* gene, suggesting that endophytic bacteria triggered the drought stress-related defence pathways in pearl millet.

There was decrease in the expression of *PgCSD* gene in the uninoculared control plants under severe water deficit stress conditions as compared to control conditions and the expression was increased under inoculated control conditions and the gene expression was decreased under inoculated severe water deficit stress conditions. In *Arabidopsis* miR398 regulates mRNAs encoding two copper superoxide dismutase (CSD) enzymes and a cytochrome c oxidase subunits are down-regulated in response to copper and stress resulting in decreased CSD1 and CSD2 mRNA and protein accumulation (Dugas *et al.,* 2008).Expression of *PgCSD* in inoculated plants was considerably enhanced under both the water regimes, indicating their expression was bacteria-induced. However, under water deficit stress conditions, uninoculated plants showed higher SOD enzyme activity presumably due to an increase in the activity of antioxidative enzyme MnSOD under water deficit stress condition. The transcript of genes for antioxidative enzyme MnSOD increased in the PGPR-inoculated wheat plants (Bharti *et al.,* 2016). Presumably antioxidative enzyme MnSOD is more

active under water deficit stress conditions as compared to no stress conditions (Rubio *et al.,* 2002).

The expression of *CaP5CS* gene was slightly down regulated under severe water deficit stress conditions as compared to control conditions in the uninoculated plants. Inoculation with the osmotolerant endophytic bacteria in the plants upregulated the expression of *CaP5CS* gene under severe water deficit stress conditions. Transcription of the *P5CS* genes is differentially regulated by drought and these genes play specific roles in the control of proline biosynthesis in *Arabidopsis* (Szekely *et al.,* 2008). Proline synthesis is regulated not only at the level of enzyme activity, but is also influenced by the level of *P5CS* gene expression (Kavi Kishor *et al.,* 2005; Wang *et al.,* 2017). In the present study, it was observed that the induction of higher expression of *CaP5CS* gene was also supported by higher accumulation of proline under severe water deficit stress. Similar findings were also reported in *Arabidopsis* and common bean on exposure to drought (Yoshiba *et al.,* 1995; Chen *et al.,* 2009). In recent studies, it was reported that *OsP5CS1* and *OsP5CS2* genes were co-expressed in tobacco plants, leading to increased proline accumulation and reduced oxidative damage to cells under abiotic stress conditions (Zhang *et al.,* 2014). The over expression of *P5CS2* gene was observed due to inoculation with the avirulent *Pseudomonas syringae* strains in *Arabidopsis* (Fabro *et al.,* 2004). The expression of *CaP5CS* gene in inoculated plants was higher under severe water deficit stress conditions as compared to uninoculated control conditions. Thus, under severe water deficit stress, there was bacterial priming of the expression of *CaP5CS* gene leading to enhanced accumulation of proline under severe water deficit stress condition. As a result, there was increase in the tolerance of the inoculated plant to severe water deficit stress condition.

Inoculation with *S. putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1, under control conditions, led to down regulation of *PgLEA* gene as compared to uninoculated plants., Under severe water deficit stress conditions, the expression of this gene was upregulated in case of *C. dublinensis* strain MKS-1 as compared to uninoculated plants under similar stress condition. *PgLEA* gene codes for low molecular weight proteins, which are mainly involved in protecting the plants from detrimental effects of abiotic stresses, especially drought stress (Hong-Bo *et al*., 2005). Higher expression of this gene under severe water deficit stress condition was induced in the inoculated treatments indicating that there was bacterial priming of this gene for

protecting the plants from deleterious effects of water shortage. In soybean, plants did not shown *LEA* gene expression under well-watered conditions and the higher gene expression was found in uninoculated plants subjected to drought stress. Only plants inoculated with *Bradyrhizobium japonicum* showed an enhanced level of *LEA* gene expression under well-watered conditions and a reduced level under drought stress conditions (Porcel *et al.,* 2005). Inoculation with *A. chroococcum* 76A enhanced the expression of *LEA* gene under salt stress, with higher expression levels observed in inoculated tomato plants (Oosten *et al.,* 2018). Our results indicate that inoculation increases expression of *PgLEA* gene involved in water deficit stress responses bacteria-induced and water deficit stress tolerance in pearl millet.

The expression of the *PgDREB2A* gene was down regulated under control conditions while the expression of this gene was 2 fold upregulated under severe water deficit stress conditiondue to inoculation with *S. putrefaciens* strain MCL-1; and 4 fold upregulated under severe water deficit stress condition due to inoculation with *C. dublinensis* strain MKS-1. Transcription factor *DREB2A* interacts with a cis-acting dehydration-responsive element (DRE) sequence and activates expression of the downstream genes involved in drought- and salt-stress response in *Arabidopsis thaliana*(Sakuma *et al.,* 2006).*DREBs* are transcriptional factors in signal transduction pathways involved in the regulation of abiotic stress responsive genes (Shinozaki and Yamaguchi-Shinozaki, 2007). *DREB*2 and *DREB* 1 are transcription factors for *LEA* which acts to initiate the transcription of genes (Shinozaki, *et al*., 1997). *DREB* transcription factors and *DRE* element serves as signal transduction under conditions of drought, salinity and coldstresses; and can control the expression of several target functional genes involved in plant's tolerance to these abiotic stresses (Qiang *et al*., 2000). Expression of *PgDREB2A* in the inoculated plants was considerably enhanced under both control and severe water deficit stress conditions, although higher expression was observed under water deficit stress conditions, indicating their expression was bacteria-induced. Thus, both these endophytic bacterial strains were observed to induce changes in the expression of transcription factor *DREB2A* which itself is involved in activation of expression of drought stress responsive genes, indicating that these bacteria were involved in eliciting systemic tolerance of the plant to drought.

There was no change in the expression of SbSNAC1 geneunder severe water deficit stress conditions as compared to control conditions in uninoculated plants. Inoculation with the *S. Putrefaciens*strain MCL-1 pearl millet plants, under control conditions, led to down regulation of expression of *SbSNAC1* gene as compared to uninoculated plants, while the expression of this gene was 4 fold upregulated under severe water deficit stress conditions. Whereas in case of *C. dublinensis* strain MKS-1 inoculated plants, under control conditions, nearly 2 fold increase in the expression of *SbSNAC1* gene as compared to uninoculated plants was observed while the expression of this gene was 6 fold upregulated under severe water deficit stress conditions.*SbSNAC1* is a member of the plant-specific NAC transcription factor superfamily that plays an important role in the abiotic stress response in sorghum (Lu *et al.,* 2013). The *SbSNAC1* protein consists of a typical NAC conserved domain at its N terminus and a diverse C-terminal region. The expression of *SbSNAC1*is induced by various abiotic stresses, such as drought and salinity. The expression of *SbSNAC1*, a sorghum NAC gene, conferred improved drought tolerance in transgenic *Arabidopsis* (Lu *et al.,* 2013).Transcription factor *SbSNAC1* confers drought tolerance to the plant *via* ABA-dependent pathway (Gahlaut*et al.,* 2016). NAC TFs are involved in the regulation of drought-related genes as transcriptional activators or repressors (Wang*et al.,* 2016).The overexpression of *ZmNAC111* in transgenic maize yielded enhanced water-use efficiency and increased expression of some drought responsive genes (Mao *et al.,* 2015).The maize NAC gene,*ZmSNAC1*, was cloned and characterized. It was observed to be induced by drought stress and its overexpression in transgenic *Arabidopsis* yielded enhanced dehydration tolerance (Lu *et al.,* 2012). In the present investigation the NAC gene, (*SbSNAC1*) was considerably up-regulated in pearl millet under severe water deficit conditions due to inoculation with the osmotolerant endophytic bacteria indicating their expression was bacteria-induced. These results suggest that the selected endophytic bacteria played an important role in the regulation of the response of the inoculated plant to severe water deficit stress through modulation of the expression of *SbSNAC1*, a transcription factor involved in conferring drought tolerance to plants through ABA-dependent pathway, which was also supported by our observations on enhanced accumulation of plant hormone ABA in the inoculated plants under severe water deficit stress conditions.

Expression of *SbNCED* gene was nearly 2.5 fold up regulated under severe water deficit stress conditions as compared to control conditions in uninoculated plants.

Inoculation with *S. putrefaciens* strain MCL-1 and *C. dublinensis* strain MKS-1 led to over expression of *SbNCED* gene under severe water deficit stress conditions. 9-cis-epoxycarotenoid dioxygenase (NCED) is a key enzyme involved in the biosynthesis of abscisic acid (ABA).The higher expression of *SbNCED* gene in inoculated plants under severe water deficit stress conditions was also supported by our data on the plant ABA status under severe water deficit conditions which also showed very high levels of ABA accumulation under these conditions in the inoculated plants. Overexpression of *NCED* gene resulted in ABA accumulation and increased drought tolerance in tomato, cowpea, tobacco, rice and Arabidopsis (Thompson et al., 2000; Iuchi *et al.,* 2001; Qin and Zeevaart, 2002; Pedrosa *et al.,* 2017; Sultana *et al.,* 2014; Tong *et al.,* 2017). An osmotic-inducible *VaNCED1* gene was isolated from a drought-resistant cultivar of *Vitis amurensis* and constitutively overexpressed in a drought-sensitive cultivar of *Vitis vinifera* (He *et al.,* 2018). Enhanced ABA accumulation may have contributed to improved tolerance of pearl millet to severe water deficit stress.

Thus, in the present study bacterial priming for drought stress tolerance in pearl millet under water deficit stress condition appeared to be primarily through ABA-dependent pathway.

The present study clearly demonstrates that inoculation with the osmotolerant endophytic bacteria can help alleviate water deficit stress in plants. There is improvement in plant health, crop productivity, nutrient status and photosynthetic efficiency. The inoculated osmotolerant PGPR ameliorate detrimental effects of water deficit stress on plants through production of phytohormones, EPS and ACC deaminase activity. They also enhance systemic tolerance of the inoculated plant to drought stress through modulation of osmolyte content, antioxidants and antioxidative enzyme status; and priming the plant for drought stress tolerance through induction of drought stress responsive genes.

Drought is one of the most common environmental stresses that affect growth and development of plants and impact agricultural productivity in arid and semi-arid regions. Pearl millet is a major cereal and fodder crop in India and is usually grown under rainfed conditions. Water deficit stress influences vegetative as well as reproductive stages of pearl millet. Yield reductions of up to 50% have been reported under water deficit stress conditions. Plant growth-promoting rhizobacteria (PGPR) can positively influence crop growth and productivity. Endophytic bacteria colonizing plants growing in water stressed environments are tolerant to water stresses. The beneficial osmotolerant endophytic bacteria can influence plant growth and development under similar environmental conditions. Thus, osmotolerant endophytic bacteria possessing plant growth promoting activities can be harnessed for alleviation of water deficit stress in plants.

In the present investigation, 31 osmotolerant endophytic bacteria isolated from different plants growing under different environmental conditions were characterized and evaluated for alleviation of water deficit stress in peal millet. These osmotolerant endophytic bacterial isolates were evaluated for their stress mitigation potential in pearl millet cultivar composite-443under *in vitro* conditions. Based on their alleviation of water deficitstress, two cultures MCL-1 and MKS-1 were selected for further studies. Effect of different concentrations of PEG6000 on their growth was determined. The isolates were also characterized for plant growth promoting activities like ARA, P solubilization, IAA production, GA production, exopolysaccharides production and ACC deaminase activity and the influence of osmotic stress on these activities under both *in vitro* and *in vivo* conditions was determined. Influence of these isolates on seed germination and seedling vigour under water deficit stress conditions was also assessed.These isolates were identified as *Shewanella putrefaciens* and *Cronobacter dublinensis*by 16S rDNA sequencing.

A pot culture experiment was carried out under water deficit stress conditions to evaluate the effect of these cultures on the growth of pearl millet. Effect of water deficit stress and inoculation on various plant physiological and biochemical parameters *viz.* amino acid, Glycine betaine, proline, sugars,starch and phenolics content, membrane stability index, lipid peroxidation, relative water content was

studied. Effect on photosynthetic efficiency parameters such as plant photosynthetic rate, stomatal conductance, transpiration rate, chlorophyll content was evaluated. Effect of inoculation on plant hormones such as ABA, GA and IAA was determined. Plant antioxidative enzyme activity and antioxidant status like superoxide dismutase, catalase, ascorbate peroxidase, ascorbic acid and glutathione were also determined. Plant growth parameters like yield and biomass, plant root system architecture were studied. Effect of inoculation on soil structure parameters such as soil carbohydrate content, root adhering soil/root tissue ratio and soil aggregate stability was determined. Plant nutrient status and effect of inoculation on plant drought stress responsive genes was also determined.

The results obtained from the investigation are briefed here under:

1. Thirty one osmotolerant endophytic bacteria isolated from pearl millet, mustard and cluster bean available in the germplasm of Division of Microbiology, IARI, New Delhi-110012, were used in the present investigation.
2. These osmotolerant isolates were screened for water deficit stress alleviation in pearl millet and 6 isolates significantly improved shoot fresh and dry weight under water deficit stress conditions.
3. Highest shoot fresh weight was observed due to inoculation with isolate MKS-1and shoot dry weight was significantly enhanced by isolates MMS-3 and MKS-1.
4. Only three isolates MMS-3, MAL-2, MAL-3 and CPSR-2, MMS-3, MAL-2 significantly improved root fresh weight and root dry weight respectively.
5. Seed inoculation with both isolates did not have any significant effect on seed germination.
6. Under osmotic stress conditions, isolate MKS-1 significantly improved plumule length while isolates MKS-1 and MCL-1 significantly improved radicle length and seedling fresh weight.
7. Based on nucleotide sequence matching from BLAST search, isolate MCL-1 was identified as *Shewanella putrefaciens*and isolate MKS-1was identified as *Cronobacter dublinensis.*
8. Both the strains showed clear cut log phase within short period in presence of 10% PEG 6000 it indicating that these strains have no stress at 10% PEG 6000.

9. Both the strains taken slightly more time for showing clear cut log phase in presence of 20% PEG 6000 it indicates that slightly it have negative affect on growth.
10. In presence of 30% and 40% PEG 6000 both the strains has taken more time for showing log phase and there is no stationery and decline phase it indicates that higher level of osmotic stress has negative affect on growth of isolates.
11. Both the selected bacterial strains were P-solubilizers, IAA, GA and exopolysaccharide producers.
12. There was significant increase in P-solubilization ability, IAA and GA production activity by both the cultures in presence of osmotic stress.
13. Highest phosphate solubilisation was observed at 20% and IAA production at 30% PEG6000 concentration by *C. dublinensis* strain MKS-1.
14. Both the cultures did not possess nitrogen fixation ability.
15. Highest exopolysaccharide production was observed by *C. dublinensis* strain MKS-1 at 30% PEG 6000 concentration. .
16. Both the cultures tested positive for ACC deaminase activity.
17. Both the cultures produced gibberellic acid and there was positive effect of higher osmotic stress on GA production ability of *S. putrefaciens* strain MCL-1, while higher osmotic stress had a negative effect on GA production ability of *C. dublinensis* strain MKS-1.
18. Highest GA production was observed at 30% PEG6000 concentration by culture *S. Putrefaciens* strain MCL-1.
19. Under *in vivo* conditions, osmotic stress significantly increased IAA, exopolysaccharide and GA production during plant-endophyte association.
20. Significant reduction in root length, shoot length, fresh weight as well as dry weight was observed when pearl millet plants were exposed to water deficit stress condition. Inoculation with osmotolerant *S. Putrefaciens* strain MCL-1and *C. dublinensis* strain MKS-1significantly improved all these parameters under water deficit stress conditions.
21. Plant physiological and biochemical parameters *viz*, proline, glycine betaine, starch, phenolics, and chlorophyll contents were significantly reduced under water deficit

stress conditions and due to inoculation with both these strains there was significant improvement all these parameters.

22. Inoculation with both the strains significantly improved plant hormone status such as ABA, IAA and GA under water deficit stress conditions.
23. There was significant decrease in membrane stability and relative water content (RWC) of plants under water deficit stress conditions. Both the parameters were significantly improved due to inoculation.
24. Lipid peroxidation was considerably increased due to exposure to water deficit stress conditions. However, inoculation with these strains did not have much effect on lipid peroxidation under water deficit stress conditions.
25. Plant antioxidative enzymes activity and antioxidant status like Superoxide dismutase, Catalase, Glutathione and Ascorbic acid were significantly improved due to inoculation under water deficit stress conditions.
26. Inoculation with both the strains significantly improved photosynthetic parameter such as photosynthetic rate, stomatal conductance, transpiration rate and chlorophyll content under water deficit stress conditions.
27. Under water deficit stress conditions there was drastic reduction in soil carbohydrate content, soil aggregate stability and root adhering soil ratio. Inoculation with the selected osmotolerant strains significantly enhanced all these soil parameters.
28. Inoculation with both the selected strains significantly improved yield, biomass and grain content under water deficit stress conditions.
29. Inoculation with both the strains significantly improved root system architecture such as root volume, surface area, diameter and length under water deficit stress conditions.
30. Inoculation with both the strains significantly improved N, P and K content in plant and P and K content in grains under water deficit stress conditions.
31. Inoculation with both the strains upregulated the expression of drought stress responsive genes *PgCSD, PgAP2, PgDREB2A, CaP5CS, SbSNAC1, SbYUC, SbGA20OX* and *SbNCED* under severe water deficit stress conditions as compared control conditions.

32. Inoculation with both the strains upregulated expression of drought stress responsive gene *PgAPX* under severe water deficit stress conditions as compared to uninoculated control under similar stress conditions.

33. Expression of gene *PgCSD* was higher under control conditions as compared to severe water deficit stress conditions for all the treatments.

34. There was differential expression of the drought stress responsive gene *SbNCED*. The expression of this gene was significantly upregulated in *C. dublinensis* strain MKS-1 inoculated plants under both control and severe water deficit stress conditions. While there was no effect of inoculation with *S. putrefaciens* strain MCL-1 on the expression of this gene under both control and severe water deficit stress conditions.

ABSTRACT

"PGPR-elicited Systemic Tolerance to Drought in Pearl Millet (*Pennisetum glaucum* L.)"

Drought is one of the most common environmental stresses that affect growth and development of plants and impact agricultural productivity in arid and semi-arid regions of the world. Pearl millet is one of the major cereal and fodder crop in India and is usually grown under rainfed conditions. It is usually grown as rainfed crop, however, water stress considerably hinders its growth and yield. Thus, osmotolerant endophytic bacteria possessing plant growth promoting activities can be harnessed for alleviation of water deficit stress in plants.

Thirty one osmotolerant endophytic bacteria isolated from pearl millet, mustard and cluster bean were used in the present investigation. These osmotolerant endophytic bacterial isolates were evaluated for their potential in alleviation of water deficit stress in pearl millet cultivar composite-443 under *in vitro* conditions. Only 6 endophytic bacteria significantly improved shoot and root fresh weight and shoot and root dry weight of plants under water deficit stress as compared to uninoculated control conditions. Five isolates KPSR-2, MCL-1, MMS-3, MMS-5 and MKS-1 showing better performance for most of the parameters studied were selected for evaluating their effect on seed germination and seedling vigour under water deficit stress conditions. Inoculation with two isolates MCL-1 and MKS-1 was observed to improve seedling fresh weight and radicle length under osmotic stress condition. Based on their potential for alleviation of water deficit stress, two promising osmotolerant endophytic bacterial isolates MCL-1 and MKS-1 were selected for further studies.

Effect of different concentrations of PEG6000 on their growth was determined by using spectrophometer and bioscreen techniques. It was observed that in presence of 20% and 30% PEG 6000, there was less reduction in their growth. However, at higher concentrations the isolates were able to grow but growth was drastically reduced. Effect of different concentration of PEG 6000 on their PGPR activities under *in vitro* condition and during plant endophyte-association were determined, under control, mild stress (20%) and severe stress (30%)conditions. Under in vitro conditions PGPR activities studied were P-solubilization, IAA production, GA production, exopolysaccharide production, ARA and ACC deaminase activity. During plant-endophyte association effect of the association under osmotic stress on production of IAA, GA and exopolysaccharide was determined. The isolates were identified as *Shewanella putrefaciens* strain MCL-1 and *Cronobacter dublinensis* strain MKS-1 by 16S rDNA sequencing.

A pot culture experiment was carried out under field capacity, mild water deficit stress (-0.5 MPa) and severe water deficit stress (-1 MPa) conditions to evaluate the effect of these cultures on pearl millet growth. Inoculation had a beneficial effect on plant biomass, yield and root system architecture under field capacity, mild water deficit stress and severe water deficit stress conditions. Photosynthetic parameters viz. stomatal conductance, transpiration rate and chlorophyll content were significantly enhanced in the inoculated plants under water deficit stress conditions. Various plant physiological parameters viz. glycine betaine, proline, starch and phenolics were significantly enhanced in the inoculated plants under water deficit stress conditions. Phytohormone production viz. ABA, IAA and GA were significantly enhanced in the inoculated plants under water deficit stress conditions. Inoculation with the osmotolerant bacteria significantly improved membrane stability index and relative water content in plants under water deficit stress conditions. Activity of antioxidative enzymes viz. catalase and superoxide dismutase: and antioxidative status viz. glutathione and ascorbic acid were significantly enhanced in the inoculated plants exposed to water deficit stress conditions. Plant and grain nutrient status such as nitrogen, phosphorus and potassium contents were considerably enhanced under water deficit stress conditions due to inoculation with selected strains. Inoculation with these osmotolerant strains significantly enhanced soil carbohydrate content, soil aggregate stability and root adhering soil ratio under both mild water deficit stress and severe water deficit stress conditions. The response of inoculation with *Shewanella putrefaciens* strain MCL-1 was markedly more under both mild and severe water deficit stress than that with *Cronobacter dublinensis* strain MKS-1. However, for most of the parameters studied in present investigation, the inoculated plants gave better response under severe water deficit stress as compared to mild water deficit stress and field capacity conditions. Expression of drought responsive genes *PgAP2, PgDREB2A, CaP5CS, SbSNAC1, SbYUC, SbGA20OX* and *SbNCED* was upregulated due to inoculation with both the strains under severe water deficit stress condition as compared to uninoculated control. Inoculation with both the strains upregulated *PgAPX, PgLEA* and *PgCSD* gene expression under severe water deficit stress conditions as compared to uninoculated control under similar stress condition.

As indicated in the present study, there was bacteria mediated elicitation of systemic tolerance to severe water deficit stress in pearl millet. Thus, osmotolerant endophytic bacteria possessing plant growth promoting activities can be used for drought stress alleviation in crops and to improve plant growth under water deficit stress.

Keywords: Drought stress tolerance, pearl millet, endophytes, osmotolerance

सारांश

बाजरा में पादप वृद्धि उत्प्रेरक राइजोबैक्टीरिया द्वारा सूखा के प्रति दैहिक सहनशीलता

अनावृष्टि एक मुख्य सामान्य वातावरणीय तनाव है जो पादप वृद्धि एवं विकास को प्रभावित करता है और शुष्क एवं अर्द्धशुष्क क्षेत्रों में उत्पादन पर प्रभाव डालता है। भारत में बाजरा एक मुख्य धान्य एवं चारा फसल है एवं आमतौर पर शुष्क दशाओं में उगाया जाता है। यह फसल साधारणतया असिंचित क्षेत्रों में उगाया जाता है यद्यपि जल प्रतिबल इसकी वृद्धि एवं उपज में बाधा डालता है। इस प्रकार पादप वृद्धि क्रियाशीलता को बढ़ाने वाले परासरण सहनशील अन्तः पादप जीवाणुओं का इस्तेमाल पौधों में पानी की कमी में होने वाले प्रतिबल के उपशमन के लिए किया जा सकता है।

इस अनुसंधान में बाजरा, सरसों एवं ग्वार से विलगित किये गये 31 परासरण सहनशील अन्तः पादप जीवाणुओं का प्रयोग किया गया। इन परासरण सहनशील अन्तः पादप जीवाणुओं का बाजरा की मिश्रित प्रजाति 443 में कृत्रिम परिवेश में जल प्रतिबल के उपशमन मूल्यांकन के लिए किया गया। इनमें से केवल 6 अन्तः पादप जीवाणुओं द्वारा जल प्रतिबल दशा में अनुपचारित के सापेक्ष प्ररोह एवं जड़ के ताजा एवं शुष्क भार में सार्थक सुधार देखा गया। पॉच आईसोलेट यथा के0पी0स0आर0–2, एम0सी0एल0–1, एम0एम0एस0–3, एम0एम0एस0–5 एवं एम0के0एस0–1 जिन्होने अध्ययन किये गये अधिकतर मापदंडो में अच्छा कार्य निष्पादन किया, उनका जल प्रतिबल दशा में बीज अंकुण एवं बीजांकुर औज के आधार पर मूल्यांकन किया गया। आईसोलेट एम0सी0एल0–1 एवं एम0के0एस0–1 के निवेशन द्वारा जल प्रतिबल दशा में बीजांकुर के ताजा भार एवं मूलांकुर की लम्बाई में सुधार देखा गया। उनके जल प्रतिबल उपशमन की क्षमता के आधार पर 2 परासरण सहनशील अन्तः पादप जीवाणुवीय आईसोलेट एम0सी0एल0–1 एवं एम0के0एस0–1 को आगे अध्ययन के लिए चयनित किया गया।

पैग 6000 के विभिन्न सांद्रण का उनकी वृद्धि पर प्रभाव का निर्धारण सपैक्ट्रोफोटोमीटर एवं बायोस्क्रीन तकनीकी द्वारा किया गया। 20 एवं 30 प्रतिशत सांद्रता पर उनकी वृद्धि में कम ह्रास देखा गया। यद्यपि उच्च सांद्रण दशा में उनकी वृद्धि तो हुई लेकिन वृद्धि में काफी कमी दर्ज की गई। विभिन्न पैग 6000 सांद्रता का उनकी पादप वृद्धि को बढ़ाने की क्रियाशीलता का कृत्रिम परिवेश एवं अन्तः पादप साहचर्य दशा में नियंत्रित, सौम्य (20:) एवं कठोर प्रतिबल (30:) दशा में प्रभाव देखा गया। कृत्रिम परिवेश दशा में पादप वृद्धि को बढ़ाने की क्रियाशीलता जैसे फॉस्फोरस घुलनशीलता, इण्डोल एसिटिक अम्ल उत्पादन, जिबरेलिक अम्ल उत्पादन, एक्जोपॉलीसैक्राईड उत्पादन, एसिटीलीन अपचयन क्रियाशीलता एवं ए0सी0सी0डी0 एमीनेज क्रियाशीलता थी। पादप साहचर्य की उपस्थिति में प्रतिबल दशा में इण्डोल एसिटिक अम्ल, जिबरेलिक अम्ल एवं एक्जोपॉलीसैक्राईड निर्धारित किए गए। 16 एस0आर0डी0एन0ए0 सिक्वैंसिंग द्वारा एम0सी0एल0–1 सैवनेला पट्रीफेसिंस प्रजाति एम0के0एस0–1 क्रोनेबैक्टर डबलिनैंसिस प्रजाति के रूप में पहचान की गई।

गमला में प्रक्षेत्र दशा, सौम्य जल कमी प्रतिबल दशा (–0.5 एम0पी0ए0) एवं कठोर जल कमी प्रतिबल दशा (–1 एम0पी0ए0) में इन संवर्धनों का बाजरे की वृद्धि पर प्रभाव का मूल्यांकन किया गया। निवेशन का पादप जैव भार, उपज एवं जड़ संरचना पर प्रक्षेत्र दशा, सौम्य जल न्यूनता प्रतिबल दशा एवं कठोर जल न्यूनताप्रतिबल दशा में लाभदायक पाया गया। निवेशित पौधों में जल न्यूनता प्रतिबल की दशा में प्रकाश संश्लेषणीय मापदंड जैसे रंध्रीय चालकता, वाष्पोत्सर्जन दर एवं पर्ण हरित की मात्रा में सार्थक वृद्धि दर्ज की गई। विभिन्न पादप कार्यिकी मापदंडो जैसे ग्लाइसीन बीटेन, प्रोटीन,मंड एवं फिनोलिक्स में भी निवेशित पौधों में सार्थक वृद्धि पायी

गई। निवेशित पौधों में जल न्यूनता प्रतिबल की दशा में पादप हॉर्मोन जैसे ए0बी0ए0, आई0ए0ए0 एवं जी0ए0 में भी महत्वपूर्ण वृद्धि दर्ज की गईं। जल न्यूनता प्रतिबल की दशा में निवेशित पौधों में परासरण सहनशील जीवाणुओं के निवेशन द्वारा मैम्ब्रेन स्थायित्व इण्डैक्स,रिलेटिव वाटर की मात्रा में भी उल्लेखनीय सुधार हुआ। एंटी ऑक्सीडेटिव एनजाईम जैसे कैटालेज, सुपर ऑक्साईड डिस्म्यूटेज एवं एंटी ऑक्सीडेटिव स्टेटस जैसे ग्लूटाथियोन और एस्कार्बिक अम्ल की मात्रा में जल न्यूनता प्रतिबल की दशा में निवेशित पौधों में उल्लेखनीय बढ़ौतरी पायी गई। पौधें एवं अनाज में पौषक तत्वों का स्तर जैसे नत्रजन, फॉस्फोरस और पौटाश की मात्रा चयनित स्ट्रेन द्वारा निवेशित पौधों में जल न्यूनता प्रतिबल की दशा में काफी वृद्धि पायी गई। सौम्य एवं कठोर जल न्यूनता प्रतिबल दोनो दशाओं में इन परासरण सहनशील जीवाणुओं द्वारा निवेशित करने से भूमि कार्बोहाइड्रेट की मात्रा, भूमि संघठन स्थायित्व एवं जड़ पर मिट्टी के चिपकने का अनुपात में सार्थक बढ़ोतरी पायी गई। क्रोनेबैक्टर डबलिनैंसिस प्रजाति एम0के0एस0–1 के सापेक्ष 1 सैवनेला पट्रीफेसिंस प्रजाति एम0सी0एल0–1 द्वारा निवेशित करने से, सौम्य एवं कठोर जल न्यूनता प्रतिबल दोनो दशाओं में प्रतिक्रिया अधिक रही। यद्यपि, इस अध्ययन में अधिकतर मापदंडो में निवेशित पौधों में कठोर जल न्यूनता प्रतिबल की दशा में सौम्य एवं प्रक्षेत्र दशा के सापेक्ष, अनुपचारित के मुकाबले प्रतिक्रिया अच्छी रही। शुष्क प्रभाविता जीन पी0जी0ए0पी0–2, पी0जी0डी0आर0ई0बी02ऐ0, सी0ऐ0पी05सी0एस0, एस0बी0एस0एन0सी01, एस0बी0वाई0यू0सी0, एस0बी0जी0ए020ओ0एक्स0 एवं एस0बी0एन0सी0ई0डी0 का इन दोनो प्रजातियों के निवेशन से कठोर जल न्यूनता प्रतिबल की दशा में अनुपचारित के सापेक्ष अपरेगूलेशन हुआ। कठोर जल न्यूनता प्रतिबल की दशा में इन दोनों प्रजातियों के निवेशन से अनुपचारित के सापेक्ष पी0जी0ए0पी0एक्स0, पी0जी0एल0ई0ऐ0 एवं पी0जी0सी0एस0डी0जीन की प्रभाविता समान दशाओं में अपरेगूलेटिड हुई।

वर्तमान अध्ययन से यह इंगित हुआ कि बाजरा में कठोर जल न्यूनता प्रतिबल जीवाणु दैहिक निष्कर्षण सहनशीलता है। इस प्रकार परासरण सहनशील अन्तः पादप जीवाणु जिनमें पादप वृद्धि बढ़ाने की क्रियाशीलता है उनको शुष्क प्रतिबल उपशमन तथा जल न्यूनता प्रतिबल की दशाओं में पादप वृद्धि में सुधार के लिए प्रयोग किया जा सकता है।

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APPENDICES-I

MEDIA COMPOSITION

❖ **Composition of growth media used in study**

1. Nutrient agar (gm/lit)

Beef extract	3
Peptone	5
NaCl	5
Agar	18
Distilled Water	1 Lit
pH	7

2. Nutrient broth (gm/lit)

Beef extract	3
Peptone	5
NaCl	5
Distilled Water	1 Lit
pH	7

3. Luria Broth (gm/lit)

Tryptone	10
Yeast extract	5
NaCl	5
Distilled Water	1 Lit
pH	7

4. **King's B medium(gm/lit)**

Proteose peptone	20
Glycerol	10
K_2HPO_4	1.5
$MgSO_4.7H_20$	1.5
Agar	16-18
pH	7.2

5. **DF-salts minimal medium (Dwonkin and Foster, 1958)(gm/lit)**

KH_2PO_4	4
$NaHPO_4$	6
$MgSO_4.7H_2O$	0.2
Glucose	2
Na gluconate	2
Citric acid	2
$(NH4)_2$ SO4	2
Trace element solution	0.1 ml
$FeS0_4.7H_20$ solution	0.1ml
Agarose	18
H_3Bo_3	10mg
$MnSo4.H_20$	11.1mg
$ZnSo4.2H_20$	124.6mg
$CuSo4.\%H_20$	28.22mg
$Mo0_3$	10.0mg
Distilled Water	0.1 Lit

6. Modified rennies combined carbon medium(gm/lit)

Solution-A

K_2HPO_4	0.8
KH_2PO_4	0.2
Nacl	1
$Na_2FeEDTA$	28mg
Yeast extract	100mg
Mannitol	5
Na-lactate	0.5ml
Distilled Water	0. 9 Lit
Agar	15
Sucrose	5

Solution -B

MgSo4	0.2
$CaCl_2.2H_20$	0.06
Distilled Water	100 ml
Biotin	5µl
PABA	10µl
pH	7

Both were autoclaved separately cooled and mixed filter sterilized

7. Pikovyskya's medium(gm/lit)

Glucose	10
Tricalcium phosphate	5
Ammonium sulphate	0.5
Sodium chloride	0.2
Magnesium sulphate.$7H_20$	0.1
Potassium chloride	0.2
Yeast extract	0.5
Manganesr sulphate	Trace
Ferrous sulphate	Trace
Agar	15
Distilled Water	1 Lit
pH	7

APPENDICES-II

REAGENTS /BUFFER/ SOLUTION

1. **Alkaline Solution**

   50 ml of Solution A (2% Na2Co3 in 0.1N NaOH) mixed with 1 ml solution B (0.5 ml 1% sodium potassium tartarate solution having 0.5 ml 0.5% CuSo4).

2. **Chloromolybdic acid**

Ammonium molybdate	15g
12N HCL	342 ml
Distilled Water	400 ml

   Dissolve 15g of ammonium molybdate in 400 ml of warm distilled water. Add 342 ml of 12 N HCL and cool.

3. **Reagents for protein estimation**

Solution-A

2 gm NaOH +10 gm of Na_2CO_3 dissolved in 500 ml of distilled water

Solution B

1% Na-K-tartarate + 0.5 % of $CuSO_4$

Solution-C

50 ml of solution A +1 ml of solution B

Folin-Cicaltaeu reagent

Distilled water and folin-Cicaltaeu reagent (In 1:1 Proportion)

Alkaline picrate reagent (for detection of HCN)

0.5 % Picric acid prepared in 2% Na_2CO_3

4. **Indole acetic acid (IAA) reagent**

$FeCl_3$ (0.5M)	15 ml
H_2So_4 conc. Sp.gr (1.84)	300 ml
Distilled water	500 ml

5. **Ninhydrin reagent for proline estimation**

Acetic acid	80 ml
Phosphoric acid con 85%	8 ml
Ninhydrin	1.25g

Make up volume to 100 ml

6. **Acetate buffer**

A) To make 0.2 M acetic acid solution and dilute 11.55 ml of acetic acid in 1000 ml with distilled water

B) To make 0.2 M sodium acetate solution take 22.22g sodium acetate in distilled water

Mix 6 ml solution A with 4 ml of solution B

7. **0.1 M phosphate buffer (pH-7.5)**

Solution-A

To prepare 0.1 M potassium dihydrogen phosphate dissolve 13.61g KH_2PO_4 in 1000 ml distilled water

Solution-B

To prepare 0.1 M disodium hydrogen phosphate dissolve 18.8g $Na_2HPO_4.2H_20$ in 1000 ml distilled water

To make 100 ml 0.1 M phosphate buffer with pH 7.5 mix 15 ml solution-A and 85 ml solution-B