

**EFFECT OF PLANT GROWTH PROMOTING RHIZOBACTERIA
ON GROWTH, OSMOLYTES AND ANTIOXIDANT STATUS OF
PEARL MILLET UNDER DROUGHT CONDITION**

Thesis

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By

Megha Pandey

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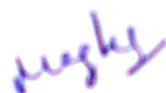
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(Megha Pandey)
Authoress

Dr. Anita Sharma
Associate Professor



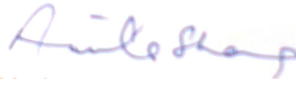
Department of Microbiology
College of Basic Sciences & Humanities
G.B. Pant Univ. of Agric. & Tech.,
Pantnagar - 263145,
Distt.: U.S. Nagar, (Uttarakhand), India

CERTIFICATE

This is to certify that the thesis entitled “**Effect of Plant growth promoting rhizobacteria on growth, osmolytes and antioxidant status of pearl millet under drought condition**” submitted in partial fulfilment of the requirements for the degree of **Doctor of Philosophy** with major in **Microbiology** and minor in **Molecular Biology and Biotechnology** of the College of Post-Graduate Studies, G.B. Pant University of Agriculture and Technology, Pantnagar, is a record of *bona-fide* research carried out by **Ms. Megha Pandey, Id. No. 35332**, under my supervision and no part of the thesis has been submitted for any other degree or diploma.

The assistance and help received during the course of this investigation have been duly acknowledged.

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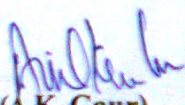
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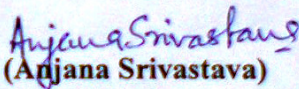
We, the undersigned, members of the Advisory Committee of **Ms. Megha Pandey, Id. No. 35332**, a candidate for the degree of **Doctor of Philosophy** with major in **Microbiology** and minor in **Molecular Biology and Biotechnology**, agree that the thesis entitled **“Effect of Plant growth promoting rhizobacteria on growth, osmolytes and antioxidant status of pearl millet under drought condition”** may be submitted in partial fulfilment of the requirements for the degree.

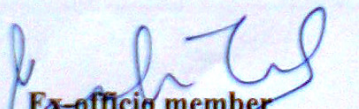

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Introduction



World population is increasing day by day at an alarming rate. It is estimated that by 2025, the world population will reach 8.0 billion (**United Nations, 2009**). Therefore, in order to ensure food and nutritional security for this seemingly ever-increasing population, agricultural productivity must increase proportionately. Earlier the green revolution has sufficiently increased food production through agricultural intensification, playing a significant role in reducing poverty. However, now the scenario is rapidly changing with population explosion. It is also interesting to note that only 10% of 13 billion hectare land of the whole world is under farming, although one third of the total land area is considered as potentially suitable for agriculture, to varying degree of adaptability. Given the current scenario of world agriculture, wherein the productivity of most of the food crops has reached a plateau, ensuring food and nutritional security seems to be a daunting task. Moreover agricultural land is further dwindling by rapid urbanization due to increasing population.

Agriculture is essentially a man made adjunct to natural ecosystem which is one of the most susceptible areas for climate change. Climatic factors constitute some of the main constraint on crops and live stock productions which are majorly exogenous and unchanging. Ironically, rapidly changing climatic conditions negatively affect plant productivity, thereby further posing a threat towards food security. These uncertain changes in environmental conditions lead to imbalanced homeostasis in the plant metabolism, is termed as stresses, which severely affects plant productivity. These stresses may originate from human activities or natural causes such as drought, salinity, extreme temperature, light intensities, air pollution, chemical toxicity and oxidative stress. Since plants have limited mechanisms of stress avoidance, they require flexible means of adaptation to cope up with these changing environmental vagaries. Every year a major portion of crop is lost due to these abiotic stresses as over 80% of global agriculture is rain-fed (**Cominelli and Tonelli, 2010**). Such disturbing trends indicate negative implications for food security and poverty alleviation programs, especially when viewed in conjunction with the increasing frequency of extreme weather conditions. Thus a major challenge to the world

farming community is keeping food production in sync with the increasing population. Meeting this challenge will require efforts in multiple areas, one of which will be the development of crops with increased environmental stress tolerance.

Under stress conditions, plant experiences either limited availability of essential nutrient or an excess of toxic material, both contribute equally to hamper the normal metabolism of plant and lead to a series of physiological, morphological, biochemical and molecular changes which adversely affect the plant health. Among several abiotic factors, water is the most crucial factor for the plant survival; therefore, success of any agricultural production system depends crucially on water availability. In broader sense, water stress includes both drought and salinity stress. These two aspects are becoming major factors for limiting plant productivity in many regions. It is proposed that it may further increase salinity in more than 50% agricultural land by the year 2050 (**Rodríguez *et al.*, 2005**).

According to Intergovernmental Panel on Climate Change (IPCC), these environmental stresses will further increase due to global climate change in the coming time (**Hirayama *et al.*, 2010**). According to *Food and Agriculture Organization* (FAO), the planet has approximately 1400 million km³ of water in totality; while, after subtracting the salty water of the oceans and the freshwater locked up in ice caps, only around 9000–14,000 km³ of water is potentially available for human consumption (**Shiklomanov, 1993**). Thus, water scarcity is a serious problem for agriculture which is further exacerbated by global climate change. In India, around 68% of the agricultural land is prone to drought in varying degrees. Thirty five % of the entire area, receiving rainfall between 750 mm and 1,125 mm, is considered drought prone (**NIDM, 2009**). Major states in India affected by drought are Rajasthan, Gujarat, Madhya Pradesh, Orissa and Andhra Pradesh.

Water deficit stress can be defined as a situation in which plant water potential and turgor are reduced enough to interface with normal functions (**Hsiao, 1973**). Plant reactions exist to circumvent the potentially harmful effects caused by a wide range of both abiotic and biotic stresses, including regulation of stomatal closure for improving water use efficiency, development of deep and secondary root system, accumulation of various osmoprotectants such as proline, glycine-betaine and aquaporins. Proline accumulation under stress is a phenomenon which is widely observed in various

organisms under drought and salinity stress. Apart from this drought stress causes the production of reactive oxygen radicals or species (ROS) or active oxygen species (AOS). These include H_2O_2 (hydrogen peroxide), $\text{O}_2^{\cdot-}$ (superoxide), O_2 (singlet oxygen) and OH (hydroxyl) radicals whose flux is known to increase under hostile conditions, enhancing leakage of electrons to molecular oxygen. These cytotoxic AOS can destroy normal metabolism through degradation of chlorophyll, oxidative damage to lipids, proteins and nucleic acids (**Fridovich, 1986**). Extended drought condition leads to interrupted reproductive development, premature leaf senescence, wilting and desiccation which cause plant death (**Schulze, 1986**). Plant cells are normally protected against such effects by complex defense mechanisms such as non-enzymic and enzymic antioxidants. The central role of antioxidant defense in biological systems is to quench or reduce the flux of oxygen intermediates, such as $\text{O}_2^{\cdot-}$ and H_2O_2 , to prevent the production of $\text{HO}^{\cdot}$, which is a most damaging reactive oxygen intermediate. ROS scavenging enzymes include enzymes such as ascorbate peroxidase (APX), superoxide dismutase (SOD), Guaiacol peroxidase (GPOX), catalase (CAT), and low molecular-weight antioxidants including ascorbic acid (ascorbate), glutathione and phenolic compounds (**Noctor and Foyer, 1998**).

Many methods such as traditional breeding technique, tissue culture, rDNA technology for crop improvement have been utilized till date and proved to be expensive, time consuming and still have very little success story. So, there was an urgent need for developing some biological tools, which could prove to be more ecofriendly and cost effective. Microorganisms are one such alternative which may play a powerful role as most of the land plants have a symbiotic relationship with a variety of soil microorganisms. In recent years, application of non-pathogenic bacteria in agriculture has shown positive results in relation to plant health, growth and productivity. Plant growth promoting rhizobacteria (PGPR) can be used as biofertilizer and can help in phytoremediation. PGPRs have a high potential for application in agriculture because they can improve plant growth through phytohormones (Indole acetic acid, Gibberallic acid) production, solubilization of mineral phosphate, antagonistic to plant pathogens, etc (**Glick, 1995**) under limited or stressed conditions. IAA acts as a suitable marker for bacterial effectiveness particularly under osmotic stress (**Boiero et al., 2007**). Underlying the mechanism,

these PGPRs induce some physico-chemical changes in plants against the pathogens and give tolerance to plants referred as ‘induced systemic tolerance’. Recent studies showed that rhizospheric bacteria can also help the plants in tolerating abiotic stresses. These microbes have various mechanisms to withstand the harsh conditions and can grow happily in such adverse conditions (**Marulanda *et al.*, 2009**). Many microorganisms are found in rhizosphere, the part of the soil attached to the root surface of a plant and affected by root exudates as well as microorganisms (**Bent, 2006; Lugtenberg and Kamilova, 2009**). Among different types of microorganisms, bacteria are found abundantly in the soil and it is well known from the literature that these rhizospheric organisms have very specific mechanism of interaction with the plants (**Galleguillos *et al.*, 2000**). They enter into root system of a host plant and impart their beneficial effect by having an endophytic life style (**Stone *et al.*, 2000**). Altered environmental conditions mainly affect the physiology of a plant and root exudation (**Compant *et al.*, 2010**). Increased CO₂ level in many cases results into increased C allocation and leads to altered composition of root exudates having difference in C/N ratio, change in concentration of signaling molecule. Thus, climate change, in particular, can show effect on microbial communities and their functioning. Rhizospheric bacteria have the potential to get accustomed to unfavorable conditions and may compensate for such harsh conditions (**Ruíz-Lozano and Azco, 2000; Marulanda *et al.*, 2009**). Various bacterial species differ significantly in their tolerance towards abiotic stress, which has been a vital focal point to provide a biological understanding of the adaptation and survival of living microorganisms under extreme environments. Indigenous microorganisms isolated from stressed sites are reported to exhibit high adaptability and tolerance to stress as compared to those isolated from non-stressed sites (**Marulanda *et al.*, 2006**). It is reported that under drought stress bacterial cells accumulate small compatible solutes called osmolytes that include amino acids like glutamate, glutamine, proline, alanine etc, quarternary amines like glycine betaine and sugars like sucrose, trehalose and polyglucosyl granules. These molecules improve cell growth under adverse osmotic conditions serving as osmoprotectants (**Csonka, 1989; Crowe and Crowe, 1992; Potts, 1994**) which maintain the cell turgidity, thus preventing degenerative processes. Recent studies indicated that osmolytes can also function as free-radical scavengers or chemical chaperones by directly stabilizing membranes and proteins (**Diamant *et al.*, 2001**).

However, an extensive mechanism behind the alteration in plant microbe relationship, plant physiology and finally ecosystem functioning is still to be worked out.

Soil-borne *Pseudomonads* have been precisely reckoned for their catabolic versatility and the main attraction has been paid for their excellent root-colonizing ability and capacity to produce a wide range of enzymes and metabolites that help the plant withstand varied biotic and abiotic stress conditions (**Vessey, 2003**). Due to the production of exopolysaccharide (EPS), bacteria like *Pseudomonas fluorescence* are able to survive under stressful conditions. EPS protects microorganisms from water stress by enhancing water retention and by regulating the diffusion of organic carbon sources (**Wilkinson, 1958; Hepper, 1975; Roberson and Firestone, 1992; Chenu, 1993; Chenu and Roberson, 1996**). Due to EPS, high-molecular-weight polymers that are secreted by microorganisms into the surrounding environment, microorganisms also attach irreversibly and colonize the roots due to involvement of a network of fibrillar material which permanently connects the bacteria to the root surface (**Bashan and Holguin, 1997**). Microbial polysaccharides can bind soil particles to form microaggregates (<250 µm diameter) and macroaggregates (>250 µm diameter) (**Edwards and Bremner, 1967; Tisdall and Oades, 1982; Oades, 1993**). Plant roots and fungal hyphae stabilize macroaggregates as they fit in the pores between micro-aggregates.

In areas of the world where drought is a common occurrence, the growth of many types of crops is either partially prevented or completely ceased. Subsequently, efforts are being made to develop drought-resistant crops that can survive in a prolonged stretch of water deficit. Millets are capable of surviving some of the most inhospitable ecosystems and provide food and fodder to millions where other quality cereals fail to grow. Among all the world's cereals, pearl millet (*Pennisetum glaucum*) is the sixth most important cereal crop (**Grains, 1996**). Today, pearl millet is planted on some 14 million hectares in Africa and 14 million hectares in Asia, thus reflecting its importance in today's agriculture. Despite this, pearl millet may be considered a "lost" crop because its untapped potential is still vast. It is poorly supported by both science and politics, as evident by the fact that few people outside India and parts of Africa have ever heard of this crop. As a result, it lags behind sorghum and far behind the other major grains in its genetic development. Pearl millet is usually grown as a dry

land dual purpose grain and fodder crop, although it is sometime irrigated in India, particularly the summer crop grown mainly as a forage crop (**Basavaraj *et al.*, 2010**). Being a hardy crop, it is relatively easy to grow under stressful regimes without hampering their net productivity. Specific varieties of pearl millet grow well under water deficit conditions. However, inter-varietal differences do exist among various varieties of pearl millet. Pearl millet varieties with high yield, tolerance to environmental stresses, disease and pest resistance are an integral part of developing the commercially potent and improved variety of pearl millet. This remains a goal of both national and international agencies.

Intrinsic properties of Pearl millet may help and to easily adapt to dry and arid soils of subtropical regions. Further to enhance the adaptability of crop and yield under harsh conditions like drought, understanding the interaction of pearl millet and PGPR might help to develop the strategy for its better cultivation. Moreover these studies may aid to better understand the inter-reliant role of various components of the antioxidative defense system in plants under the presence and absence of PGPRs. Therefore, the present work was carried out with the following objectives:

- Characterization of bacterial isolates on the basis of biochemical and plant growth promoting properties
- To determine the effect of bacterial isolates on the growth of different varieties of Pearl millet, Sorghum, Moth and Guar
- In vitro study of growth and stress tolerant mechanism of PGPR
- Screening of pearl millet varieties with potent PGPR, their drought tolerance potential and to compare the alterations in anti-oxidative system of drought sensitive and tolerant genotype in the presence of PGPR

*Review
of
Literature*

Agriculture is the largest private enterprise in India and will continue to be the lifeline of Indian economy in future. It is estimated that overall demand for food will increase as global population continues to rise from the calculated 6 billion people today to an anticipated population 9 billion by 2050 (**The world at six billion, 2009**). Moreover the inability of agrochemicals to increase crop yield beyond a threshold level has resulted in new challenges to make use of areas affected with various environmental limitations for agricultural purposes. Most of the fertile soils in favorable environments are being diverted towards alternate uses due to increasing urbanization which create pressure to expand agriculture. In general, the actual yield of most of crop plants is only 20% of that of attainable yield. External environment seems to be a dominant factor, responsible for limited crop production, such as excess or deficient water, high or low temperature, and soil salinity (**Boyer, 1982**). Therefore, improvement of plant defense against external environmental constraints might increase actual yield of various crops. Plants being sessile by nature are often exposed to various kinds of external limiting factors; therefore they have evolved several adaptive mechanisms against these limiting conditions. Recent progress in molecular biology has opened the door to uncover the adaptive mechanisms at the molecular level in plants (**Yamaguchi-Shinozaki and Shinozaki, 2005, 2006**).

According to IPCC, there had been an unprecedented warming trend during the 20th century. The average temperature of the earth's surface has risen by 0.74⁰C since the late 1800 (**IPCC, 2007**). A further increase of 1.5-6⁰C is projected up to 2100 (**IPCC, 2001, 2007**). Even with a temperature rise of 1-6⁰C, the IPCC predicts serious effects resulting into reduced crop yields leading to increased risk of hunger and spread of climate sensitive diseases. **Lobell et al. (2008)** have also predicted that increasing temperature and declining precipitation over semiarid regions are likely to reduce yield of major crops like corn, wheat, rice and other primary crops in the next two decades. These changes could have a substantial impact on global food security in the upcoming era (**Lobell et al., 2008**).

Adequate water supply is one of the most important factors responsible for crop production. Amount of irrigation water needed for crops is also climate

dependent, which remains inconsistent for most of the time. Under these circumstances, demand for irrigation water increases due to severe decrease in summer rainfall, increasing temperature and radiations. These variables are major factors involved in controlling vital physiological attributes (like onset of the reproductive cycle, length of the vegetative phase, timing of fruit setting or induction of whole plant senescence, flowering intensity etc). Assessments are needed to provide decision-makers with the information in order to develop appropriate plans to reduce the expected climate change or to adopt them. In such scenario, environmental stresses constitute a major barrier for agricultural production world over and especially in the Indian sub-continent.

2.1 What is Stress?

In general, stress is a force applied to an object per unit area. In biology, stress in plants could be defined as a change in growth condition(s) that disrupts metabolic homeostasis and requires an adjustment of metabolic pathways in a process that is usually referred to as acclimation or stress. It may be termed to that adverse condition in which an organism or plant cannot lead a normal life and has to switch over to adopt some specific physiological pathways. Change in external environmental condition is sensed by a plant which activates a cascade of signaling pathways. Three different types of compounds are involved in signaling pathways (1) compounds involved in the acclimation process such as antioxidants or osmoprotectants; (2) by-products of stress that appear in cells because of the disruption of normal homeostasis by the alteration(s) in growth conditions; and (3) signal transduction molecules involved in mediating the acclimation responses. Signaling molecules may be by-products of membrane degradation, different reactive oxygen species (ROS) or various oxidized compounds such as phenolic compounds or even some antioxidants (Mittler *et al.*, 2004)

2.1.1 Drought Stress

Plants are sessile and have evolved various mechanisms which enable them to adapt ever-changing environmental conditions. Drought is one of the most important manifestations of abiotic stress in plants. It is a major yield limiting factor of crop plants and continuously determines the natural distribution of plant species. Drought

exacerbates the effect of the other stresses to which plants are submitted (like salt and cold), leading to similar drought like effects. Plants have developed two strategies to resist drought: drought avoidance and dehydration tolerance (**Blum, 2005**). Drought avoidance refers to a plant's ability to maintain high water status even when water is scarce, for example by growing long roots to reach deep soil moisture, or reducing water loss by restricting the aperture of the stomata on leaf surfaces. Plants have to cope-up with drought stress at least at some point in their life cycle. They have evolved several mechanisms that allow them to adapt and survive transient periods of water deficit, if not at the whole plant level, at some level. According to the type of strategy adopted, plants are said to escape, avoid or tolerate drought stress, although these are not mutually exclusive. The plant drought response depends on the species inherent "strategy" but also on the duration and severity of the drought period. If drought stays for a long time it may cause oxidative damage due to over production of reactive oxygen species. As reactive oxygen species are common denominator in the detrimental effects of several environmental stresses, therefore enhancing the effect of antioxidant genes involved in the detoxification of reactive oxygen species could be a potential source of stress tolerance in crop plants.

2.2 Generation of ROS

Reactive oxygen species (ROS) are produced continuously as by product by various metabolic pathways, localized in different cellular compartments i.e., chloroplast, mitochondria and peroxisomes (**Navrot *et al.*, 2007**). Germinating seeds also produce ROS in the apoplast, where they are involved in cell wall loosening, regulation of growth and development, and pathogen defense. For example, apoplastic hydroxyl radicals ($\cdot\text{OH}$) in cress radicals and endosperm caps break dormancy by in vivo scission of cell wall polysaccharides at specific sites, acting via ABA or protein carbonylation (**Muller *et al.*, 2009 a, b**). Aerobic and photosynthetic organisms produced molecular oxygen ($\text{O}_2^{\bullet}$) along with Reactive Oxygen Species (ROS) in the earth environment about 2.7 billion years ago (**Halliwell, 2006**). Oxygen generated in the chloroplasts during photosynthesis accepts electrons passing through the photo systems, thus forming $\text{O}_2^{\bullet}$ which has two unpaired electrons with same spin quantum number which restrict their spin, thus it accept one electron at a time, leading to generation of ROS, which can damage the cells. Various biotic and abiotic stresses

such as salinity, UV radiation, drought, heavy metals, temperature extremes, nutrient deficiency, air pollution, herbicides and pathogen attack disturb the equilibrium between the production and the scavenging of ROS and is a major cause of loss of crop productivity worldwide (Shi *et al.*, 2001; Saxena *et al.*, 2011). These disturbances lead to sudden increase in intracellular level of ROS which significantly damages cellular structures. Further, $O_2^{\bullet -}$ leads to the formation of H_2O_2 , $OH^{\bullet}$ and other ROS. The ROS comprising $O_2^{\bullet -}$, H_2O_2 , $^1O_2$, $HO_2^{\bullet}$, $OH^{\bullet}$, $ROOH$, $ROO^{\bullet}$ and $RO^{\bullet}$ are highly reactive and toxic and cause damage to many cellular functions by damaging nucleic acids, oxidizing proteins, causing lipid peroxidation (LPO) which ultimately results in cell death (Gill and Tuteja, 2010; Fotopoulos *et al.*, 2010). ROS can damage cells as well as evoke the expression of a number of genes and signal transduction pathways. The subcellular location for generation of any ROS is very important, because it diffuses only very short distance before reacting with a cellular molecule. Various antioxidative defence mechanisms scavenge these ROS molecules under steady state conditions (Foyer and Noctor, 2005). Stress-induced ROS accumulation is counteracted by enzymatic antioxidant systems including SOD, APX, GPX, GST, and CAT and non-enzymatic low molecular metabolites i.e., ASH, GSH, α -tocopherol, carotenoids and flavonoids (Gratao *et al.*, 2005) including proline (Chen and Dickman, 2005). These antioxidants are found in almost all cellular compartments, demonstrating the importance of ROS detoxification for cellular survival.

2.2.1 Generation and perception of Reactive Oxygen Species (ROS) under drought stress

Root system is the first organ to detect a limitation in the water supply in a plant which sends signals to the leaves through xylem sap. Phytohormone such as abscisic acid is considered to a major root to shoot stress signal (Zhang *et al.*, 2002; Jiang and Hartung, 2008). When the stress signal reaches the leaves, it triggers stomatal closure and the plant shifts to a water saving strategy. Stomata play a major role in plant adaptation to stress by adjusting their opening patterns. Plants are able to control water loss by reducing transpiration flux, but they are concomitantly limiting the entrance of carbon dioxide (CO_2) (Carvalho, 2008). This will have direct and indirect implication on the reduction of net photosynthesis and on the overall production of ROS by plants under drought stress (Mittler, 2002). In fact, under drought stress, ROS production is enhanced through multiple ways. For instance, the

limitation on CO₂ fixation reduces NADP⁺ regeneration through Calvin cycle, hence provoking an over reduction of the photosynthetic electron transport chain. During photosynthesis and under drought stress there is maximum leakage of electrons to O₂ by the Mehler reaction (Smirnoff, 1993).

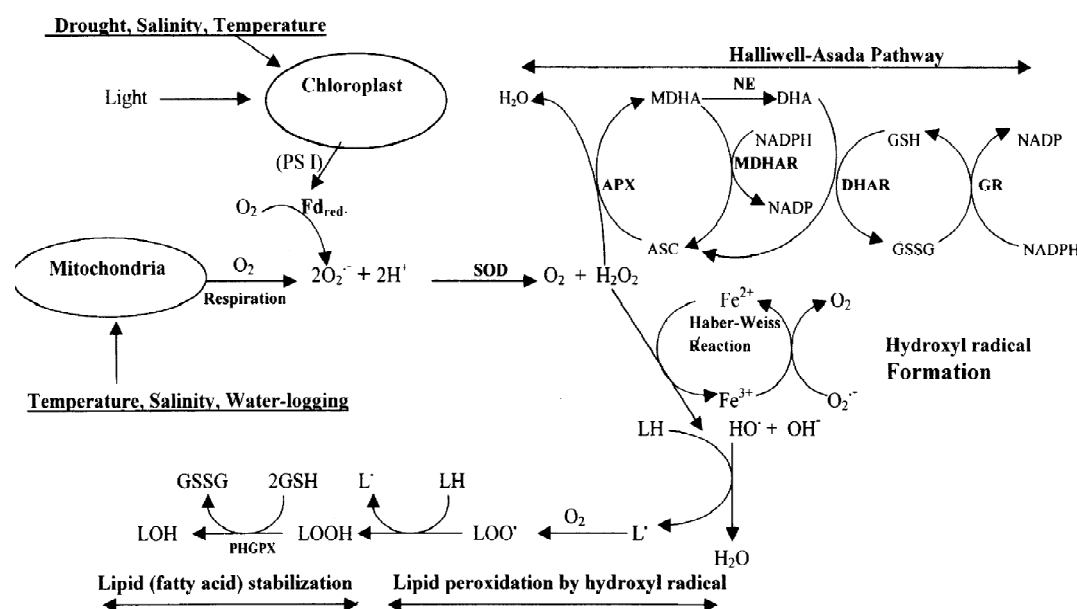


Fig. 1: Generation and scavenging of superoxide radical and hydrogen peroxide, and hydroxyl radical-induced lipid peroxidation and glutathione peroxidase-mediated lipid (fatty acid) stabilization (Halliwall, 2000)

Biehler and Fock (1996), estimated that in wheat under drought stress, leakage of photosynthetic electrons to the Mahler reaction was increased by approximately 50% as compared to unstressed wheat. In fact, under drought stress, the rate of photo respiration is also enhanced, especially when RuBP oxygenation is maximal due to limitation on CO₂ fixation (Noctor *et al.*, 2002). During evolution, the biological activities of ROS seem to have undergone several modifications. The continuous release of highly reactive ROS has necessitated the development of ROS scavengers to minimize the cytotoxic effects of ROS and its intermediates which are produced sequentially in the cell (Apel and Hirt, 2004).

2.2.2 Singlet oxygen (¹O₂)

The first excited state of oxygen (O₂) is the singlet oxygen (¹O₂) which is an unusual component of reactive oxygen species as it is not related to electron transfer

to O_2 . Insufficient energy dissipation during photosynthesis leads to formation of chlorophyll triplet state. This triplet state reacts with O_2 to give extremely reactive singlet oxygen, which during photosynthesis shows a powerful damaging effect on PSI and PSII as well as on the whole photosynthetic machinery.

2.2.3 Superoxide radicals ($O_2^{\bullet-}$)

The production of ROS is an usual consequence of aerobic respiration. When the terminal cytochrome c oxidase and the alternative oxidase react with O_2 , four electrons are transferred and H_2O is released. However, occasionally $O_2^{\bullet-}$ is produced upon reduction of O_2 during electron transport along with the non cyclic pathway in the ETC of chloroplasts at the level of PS I and other compartments of the plant cell. Usually $O_2^{\bullet-}$ is the first ROS to be generated which may trigger the formation of other ROS like $OH^{\bullet}$, and more possibly $^1O_2$ (Halliwell, 2006), which may cause peroxidation of membrane lipids and cellular weakening.

2.2.4 Hydrogen peroxide (H_2O_2)

The univalent reduction of $O_2^{\bullet-}$ produces H_2O_2 (Bhattacharjee, 2005). It is now well established that excess of H_2O_2 in the plant cells leads to the occurrence of oxidative stress by inactivating enzymes through oxidizing their thiol groups. H_2O_2 plays dual role in plants, at low concentrations, it acts as a signal molecule involved in signaling tolerance towards various biotic and abiotic stresses whereas, at high concentrations, it leads to Programmed cell death (Quan *et al.*, 2008). H_2O_2 has also been shown to act as a key regulator in a broad range of physiological processes, such as senescence (Peng *et al.*, 2005), photorespiration and photosynthesis (Noctor and Foyer, 1998a), stomatal movement (Bright *et al.*, 2006), cell cycle (Mittler *et al.*, 2004) and growth and development (Foreman *et al.*, 2003). H_2O_2 has also been accepted as a second messenger for the signals generated by means of ROS because of its long life and high permeability across membranes (Quan *et al.*, 2008). It is reported that H_2O_2 increases the activities of antioxidant enzymes along with the induction of their related-isoform(s) under non-NaCl-stress conditions (Tanoua *et al.*, 2009).

2.2.5 Hydroxyl radical ($OH^{\bullet}$)

Hydroxyl radicals are among the most reactive ROS known. In the presence of suitable transitional metals, especially Fe, $OH^{\bullet}$ can also be produced from $O_2^{\bullet-}$ and

H₂O₂ at neutral pH and ambient temperatures by the iron-catalyzed, O₂^{•-} driven Fenton reaction. These OH[•] are thought to be responsible for mediating oxygen toxicity *in vivo*. It potentially react with all the biological molecules i.e., DNA, proteins, lipids, and almost are the constituents of the cells. Due to the absence of any enzymatic mechanism for the elimination of this highly reactive ROS, excess production of OH[•] ultimately leads to cell death (Vranova *et al.*, 2002).

2.3 Consequences of ROS Generation in Plants

2.3.1 Lipid peroxidation (LPO)

Lipid peroxidation, a radical-mediated chain reaction initiated by abstraction of a hydrogen atom from a polyunsaturated lipid is terminated by chain-breaking antioxidants. The peroxidation of lipids is considered as the most damaging process known to occur in any living organism. Excessive peroxidation of membrane lipids disrupts the bilayer arrangement, decreases membrane fluidity, increases membrane permeability and modifies membrane-bound proteins. It is shown that during LPO, products are formed from polyunsaturated precursors that include small hydrocarbon fragments such as ketones, MDA, etc (Garg and Manchanda, 2009). Some of these compounds react with thiobarbituric acid (TBA) to form coloured products called thiobarbituric acid reactive substances (TBARS). LPO, in both cellular and organelle membranes, takes place when ROS levels reach to a threshold, thus it not only affects normal cellular functioning, but also aggravates oxidative stress through production of lipid-derived radicals (Montillet *et al.*, 2005). The overall process of LPO involves three distinct stages: initiation, progression and termination. Initiation step involves transition metal complexes, especially those of Fe and Cu. In aerobic environment, oxygen will react to the fatty acid at the carbon-centered lipid radical to give rise to ROO[•]. Once initiated, ROO[•] can further propagate the peroxidation chain reaction by abstracting a hydrogen atom from adjacent PUFA side chains. The resulting lipid hydroperoxide can easily decompose into several reactive species including: lipid alkoxyl radicals, aldehydes (malonyldialdehyde), alkanes, lipid epoxides, and alcohols (Fam and Morrow, 2003). The overall effects of LPO are to decrease membrane fluidity; make it easier for phospholipids to exchange between the two halves of the bilayer; increase the leakiness of the membrane to substances that do not normally

cross it other than through specific channels and damage membrane proteins, inactivating receptors, enzymes, and ion channels.

2.3.2 Protein oxidation

Protein oxidation is defined as irreversible covalent modification of protein that leads to cell death by necrosis, which is characterized by disruption of the cell membrane and cellular organelles. Protein oxidation is induced by ROS or byproducts of oxidative stress (**Ghezzi and Bonetto, 2003**). The oxidation of various amino acids viz., arginine, histidine, lysine, proline, threonine and tryptophan gives free carbonyl groups which may inhibit or alter their activities and increase susceptibility towards proteolytic attack (**Moller *et al.*, 2007**). Protein carbonylation may occur due to direct oxidation of amino acid side chains (**Shringarpure and Davies, 2002**). ROS target proteins contain sulfur-containing amino acids and thiol groups, irrespective of their location of synthesis and action.

2.3.3 DNA damage

DNA might get damaged due to exposure to the biotic and abiotic stress factors, in spite of its high stability and thereby exerts genotoxic stress (**Tuteja *et al.*, 2009**). Endogenously generated damage to DNA is known as “spontaneous DNA damage” which is produced by involvement of reactive metabolites ($\text{OH}^\bullet$, $\text{O}_2^\bullet$ and $\text{NO}^\bullet$). High level of ROS can cause damage to cell structures, nucleic acids, lipids and proteins. DNA damage includes base deletion, pyrimidine dimers, cross-links, strand breaks and base modification, such as alkylation and oxidation (**Gill and Tuteja, 2010**). It has been reported that $\text{OH}^\bullet$ is the most reactive and cause damage to all components of the DNA molecule, damaging both the purine and pyrimidine bases and also the deoxyribose backbone (**Halliwell and Gutteridge, 1999**). DNA damage results in various physiological effects, such as reduced protein synthesis, cell membrane destruction and damage to photosynthetic proteins, which affects growth and development of the whole plant (**Britt, 1999**).

2.3.4 ROS scavenging under drought

Plants have evolved various mechanisms for ROS scavenging. Major mechanisms involved are antioxidant systems for scavenging reactive oxygen species,

produced during normal or stressed physiological conditions. The antioxidant system can be divided into three groups: (1) antioxidant enzymes, (2) lipid soluble, membrane associated antioxidants (α -tocopherol, β -carotene, and ubiquinone) and (3) water soluble antioxidants (e.g., glutathione and ascorbate). Among these systems, antioxidant enzymes are most reactive and efficient protective mechanism against oxidative stress (**Halliwell and Gutteridge, 1999**).

Symptoms of oxidative damage viz. lipid peroxidation have been used to assess the increase in ROS production under drought stress. However, lack of symptoms does not imply that ROS formation is not occurring. Instead, the lack of symptoms is likely to result on the concomitant increase in cellular antioxidant defenses. The measure of specific antioxidants and antioxidant enzyme activities and/or expression analysis during water stress treatments has been generally accepted as an approach to assess the involvement of the scavenging system during drought stress (**Loggini et al., 1999; Pastori and Foyer, 2001**).

2.4 Antioxidants

The term antioxidant is used to describe any compound that is capable of quenching ROS without itself undergoing conversion to a destructive radical. Plants have developed an efficient defense system that includes antioxidant compounds and antioxidant enzymes to control and regulate ROS level in the cells. Ascorbic acid, α -tocopherol, flavonoids, glutathione and carotenoids are the non-enzymatic antioxidants that detoxify singlet oxygen and hydroxyl radical. The antioxidant enzymes are catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR).

2.4.1 Nonenzymatic antioxidants

2.4.1.1 Proline

Besides an osmolyte, proline is now considered as a potent antioxidant and potential inhibitor of Programmed cell death (PCD), thus it is now regarded as nonenzymatic antioxidants that microbes, animals, and plants require to mitigate the adverse effect of ROS (**Chen and Dickman, 2005**). Following salt, drought and metal

stress in plants there is a dramatic accumulation of Proline. Free Proline has been proposed to act as an osmoprotectant, a protein stabilizer, metal chelator, an inhibitor of LPO, $\text{OH}^\bullet$ and $^1\text{O}_2$ scavenger (Ashraf and Foolad, 2007; Trovato *et al.*, 2008). Therefore, proline is not only an important molecule in redox signaling, but also an effective quencher of ROS formed under salt, metal and dehydration stress conditions in all plants (Alia and Saradhi, 1991). It is suggested that the ability of proline to scavenge ROS and to inhibit ROS-mediated apoptosis can be an important function in response to cellular stress. Enhanced synthesis of Proline under drought or salt stress has been implicated as a mechanism to alleviate cytoplasmic acidosis and maintain NADP^+ -NADPH at values compatible with metabolism (Hare and Cress, 1997).

2.4.1.2 Carotenoids

In all the photosynthetic organisms, carotenoids like β -carotene, zeaxanthin and tocopherols serve as an important photo protective agent, either by dissipating excess excitation energy as heat or by scavenging ROS and suppressing LPO. Carotenoids are pigments that are found in plants and microorganisms. Carotenoids, a lipid soluble antioxidant play a multitude of functions in plant metabolism including oxidative stress tolerance. Carotenoids carry out three major functions in plants; first, they absorb light at wavelength between 400 and 550 nm and transfer it to the Chlorophyll (Sieferman-Harms, 1987). Second, they protect the photosynthetic apparatus by quenching a triplet sensitizer (Chl^3), $^1\text{O}_2$ and other harmful free radicals which are naturally formed during photosynthesis (Collins, 2001). Third, they are important for PSI assembly and the stability of light harvesting complex proteins as well as thylakoid membrane stabilization (Niyogi *et al.*, 2001).

2.4.2 Enzymatic antioxidants

2.4.2.1 Superoxide dismutase (SOD)

Superoxide dismutases are metalloenzymes which catalyze dismutation of $\text{O}_2^{\bullet-}$ to H_2O_2 and O_2 at a rate 104 times as fast as spontaneous dismutation at neutral pH (Halliwell and Gutteridge, 1999). SOD has been proposed to be important in plant stress tolerance and provide the first line of defense against the toxic effects of elevated levels of ROS. Three different kinds of SODs have been characterized in eukaryotes; a copper- and zinc-containing form (CuZnSOD) localized in the cytosol, a

manganese-containing form (MnSOD) in the mitochondria and a copper- and zinc-containing form in the extracellular matrix (ECSOD) (Marklund, 1982). SOD has been shown to play a central role in protecting cells and tissues against oxidant stress (Fridovich and Freeman, 1986; Tsan, 1997). Metalloenzyme SOD is the most effective intracellular enzymatic antioxidant which is ubiquitous in all aerobic organisms and in all subcellular compartments prone to ROS mediated oxidative stress. It is well established that various environmental stresses often lead to increased generation of ROS, where, The SODs remove $O_2^{\bullet-}$ by catalyzing its dismutation, one $O_2^{\bullet-}$ being reduced to H_2O_2 and another oxidized to O_2 .

The subcellular distribution of SOD isozymes is also distinctive. All forms of SOD are nuclear-encoded and targeted to their respective subcellular compartments by an amino terminal targeting sequence (Scandalias, 1990). The upregulation of SOD is implicated in combating oxidative stress caused due to biotic and abiotic stress and have a critical role in the survival of plants under stressed environment. Increase in SOD activity following drought stress was noted in three cultivars of *P. vulgaris* (Zlatev *et al.*, 2006), *A. philoxeroides* (Wang *et al.*, 2008) and *O. sativa* (Sharma and Dubey, 2005). Wang and Li (2008) studied the effect of water stress on SOD activities of total leaf and chloroplast in *Trifolium repens* L. and reported significant higher increase in SOD activity under water stress.

2.4.2.2 Catalases (CAT)

Catalase is a tetrameric heme-containing porphyrin enzyme and helps in degrading H_2O_2 via a catalytic reaction. It can also utilize H_2O_2 for the peroxidatic oxidation of various organic substrates (Durner and Klessig, 1995). It is mainly located in the peroxisome. In maize, it was present in mitochondria (Willekens *et al.*, 1995). The compartmentalization of catalase in peroxisomes does not preclude catalase from operating as a general sink of cellular H_2O_2 (Willekens *et al.*, 1997). It has lower substrate affinity compared to APX, since the reaction requires simultaneous access of two H_2O_2 molecules to the active site. Catalase is photosensitive and undergoes photoinactivation with subsequent degradation (Feierabend *et al.*, 1992). The tolerant dried bean seeds displayed high CAT and GR activities and low APX and SOD activities while the opposite trend was observed in immature desiccation intolerant seeds, suggesting that antioxidant enzymes may play a role in the acquisition of drying tolerance during seed development (Bailly *et al.*, 2001).

2.4.2.3 Guaiacol peroxidase (GPX)

Peroxidases which oxidize guaiacol (o-methoxyphenol), as a commonly used reducing substrate in vitro, are referred to as guaiacol peroxidases (GP). Guaiacol peroxidases (GPX) are haem-containing enzymes participating in many physiological processes in plants. They are located in cytosol, vacuole, cell wall, apoplast and extracellular medium, but not in organelles and are assumed to be involved in a range of processes related to plant growth and development. The expression pattern of these enzymes is organ-specific (**Ghamsari *et al.*, 2007**). GPX decomposes indole-3-acetic acid (IAA) and has a role in the biosynthesis of lignin and defense against biotic stress by consuming H₂O₂. GPX prefers aromatic electron donors such as guaiacol and pyragallol usually oxidizing ascorbate at the rate of around 1% that of guaiacol (**Asada, 1999**). Activity of GPX varies considerably depending upon plant species and stress conditions. A concomitant increase in GPX activity in both the leaf and root tissues of *Vigna radiata* has also been reported under salinity stress (**Panda, 2001**).

2.4.2.4 Ascorbate peroxidase (APX)

Ascorbate peroxidases (APXs), is one of the most important groups of antioxidant enzymes of plants. So far at least five APX isoforms have been identified in plants: cytosolic isoforms, mitochondrial isoforms, peroxisomal/glyoxysomal isoforms and chloroplastic isoforms. All the forms of APX are thought to function as scavengers of the H₂O₂ that is generated continuously in cells (**Miyake and Asada, 1996**). APX is a class I hemeperoxidase which is found in *Euglena* (**Shigeoka *et al.*, 1980**), *Cyanobacteria* (**Tel-Or *et al.*, 1986**), *Chlamydomonas* (**Miyake *et al.*, 1991**), and in a red alga *Galdieria partita* (**Kitajima *et al.*, 2002**). APX has also been found in the protozoan *Trypanosoma cruzi* (**Boveris *et al.*, 1980**) and insects (**Mathews *et al.*, 1997**). It has never been reported from animals, except for one case from bovine eye (**Wada *et al.*, 1998**). Nitric oxide (NO) can bind to the heme group of APX, thereby inhibiting its activity (**Clark *et al.*, 2000**). Expression of APX genes can be activated by many factors such as pathogen attack, mechanical pressure, injury, UV radiation (**Rao *et al.*, 1996**), salt stress (**Lopez *et al.*, 1996**; **Mittova *et al.*, 2004a, b**; **Gomez *et al.*, 2004**), low or high temperature and water deficiency (**Mittler and Zilinskas, 1994**).

2.5 Abiotic Stress Signals and Signaling Pathways in Plants

Cell signaling is initiated when an extracellular object, interacts with plasma membrane protein or receptor. Several adverse environmental conditions such as chilling, frost, high temperature, salinity, and abundance of pesticides, heavy metals, production of reactive oxygen species, UV radiation and nutrient deprivation serve as elicitors of abiotic stress for plant cell (**Mahajan and Tuteja, 2005**). Perception of signal occurs by the receptor present on outer membrane of a plant cell. Signal transduction occurs in a downstream manner and production of secondary messengers, such as calcium, inositol, phosphate and reactive oxygen species, is initiated. Similarly in animal cells various protein kinases are involved in biotic /abiotic stress responses (**Peck *et al.*, 2003**). In different environmental stresses two type of protein kinases get activated in plants namely mitogen-activated protein kinases (MAPKs) and calcium-dependent protein kinases (CDPKs) (**Ludwig *et al.*, 2005**).

Calcium is known as an important secondary messenger in plants which gives response for diverse stimuli. Due to external or internal signal, cytosolic concentration of calcium increases rapidly and transiently in the cell through different calcium transport channels. After sensing these calcium signals, sensory proteins alter their confirmation. The sensory proteins are divided into two groups: the sensory relays including calmodulin and calcineurin B-like protein and sensor protein kinases such as calcium dependent protein kinases as well as calcium and calmodulin dependent protein kinases. These sensory kinases interact with other associated proteins and initiate a phosphorylation cascade and trigger the responsive genes and related transcription factors. Ultimately the product of these genes results into plant adaptation and help them to survive in harsh environmental conditions.

Mitogen-activated protein kinases (MAPKs) cascade is a major signaling pathway under abiotic stress conditions. These MAP kinases are found in eukaryotic organisms as a conserved signaling module. Three major kinases viz. MAP kinase kinase kinase, MAP kinase kinase and MAP kinase are involved in this cascade. As soon as some adverse environmental conditions arrive, plasma membrane gets stimulated, as a result MAP kinase kinase kinase get activated and in-turn phosphorylate the serine threonine residue of MAP kinase kinase, since MAP3Ks is a serine/threonine kinase. MAP2Ks phosphorylate MAP kinase on threonine and

tyrosine residues. MAP kinases are able to phosphorylate diverse range of kinases and transcription factors.

2.6 Plant Growth Promoting Rhizobacteria (PGPR)

Various bacterial species, residing in rhizospheric zone of a plant, are known to exert beneficial effect on plant. These rhizospheric bacteria are known as plant growth promoting rhizobacteria (PGPR). The rhizosphere is the soil around the root zone and under the influence of the root exudates. It is a site with complex interactions between the root and associated microorganisms (Sylvia *et al.*, 1998). According to Whipps (2001), there are three basic categories of interactions (neutral, negative and positive) generally exist between the rhizobacteria and the growing plants. Most rhizobacteria associated with plants are commensals in which the bacteria establish an innocuous interaction with the host plants exhibiting no visible effect on the growth and overall physiology of the host (Beattie, 2006). In negative interactions, the phytopathogenic rhizobacteria produce phytotoxic substances such as hydrogen cyanide or ethylene, thus, negatively influence on the growth and physiology of the plants. Counter to these deleterious bacteria, there are some PGPRs that can exert a positive effect on plant growth by direct mechanisms such as solubilization of nutrients, nitrogen fixation, production of growth regulators, etc., or by indirect mechanisms such as stimulation of mycorrhizae development, competitive exclusion of pathogens or removal of phytotoxic substances (Bashan and de-Bashan, 2010). However, in accordance with their degree of association with the plant root cells, PGPRs can be classified into extracellular plant growth promoting rhizobacteria (ePGPR) and intracellular plant growth promoting rhizobacteria (iPGPR) (Martínez-Viveros *et al.*, 2010). iPGPRs can enter plant cells and produce specialized structures, the so-called nodules. ePGPRs, on the other hand, are found in the rhizosphere, or within the apoplast of the root cortex, but not inside the cells. According to their proximity to the plant root, ePGPRs can be divided into three classes: those living near, but not in contact with the roots; those colonizing the root surface; and those living in the spaces between cells of the root cortex (Gray and Smith, 2005). The bacterial genera such as *Agrobacterium*, *Arthrobacter*, *Azotobacter*, *Azospirillum*, *Bacillus*, *Burkholderia*, *Caulobacter*, *Chromobacterium*, *Erwinia*, *Flavobacterium*, *Micrococcous*, *Pseudomonas* and *Serratia* belong to ePGPR (Gray and Smith, 2005).

The iPGPR includes the endophytes and *Frankia* species both of which can symbiotically fix atmospheric N₂ with the higher plants (Verma *et al.*, 2010). As the effect of PGPR on plants was demonstrated, the concept of PGPR began to gain importance and a large number of bacterial strains have been isolated, screened (Cattelan *et al.*, 1999; Bertand *et al.*, 2001) and evaluated for plant growth promotion (Glick *et al.*, 1997; De Salamone, 2000; Bent *et al.*, 2001). Potential role of PGPRs in conferring resistance to water stress in tomatoes and peppers has been investigated (Mayak *et al.*, 2004). Fluorescent pseudomonads and species of *Bacillus* were reported with very high efficiency in host root colonization and production of growth metabolites resulting in improved strategic crop yield (Khalid *et al.*, 2004). Various mode of action of a *Bacillus subtilis* strain, FZB24 against phytopathogens are examined by Kilian *et al.* (2000) suggesting the role of the bacterium in plant vitality. According to Cakmakci *et al.* (2006), soil rhizobacterial populations are capable of exerting beneficial effect on many plants like wheat, potato, maize, grasses, pea and cucumber by colonizing rhizosphere. Application of PGPR increased the nodulation and nitrogen fixation of soya bean (*Glycine max* (L.) Merr.) over a wide range of root zone temperatures (RZTs) (Zhang *et al.*, 1996). Thus, it has been established that the inoculation of PGPRs can increase nodulation, nitrogen uptake, growth and yield response of crop plants. In addition to this, employing microorganisms as co-culture in biotization is also another important area of research (Sekar and Kandavel, 2010).

Plant growth-promoting rhizobacteria enhance plant growth either by direct or indirect mechanisms (Glick, 1995). Direct mechanisms of plant growth promotion may involve the synthesis of substances by the bacterium or facilitation of the uptake of nutrients from the environment (Glick *et al.*, 1999). Indirect promotion of plant growth occurs when PGPR lessen or prevent the deleterious effects of plant pathogens on plants by production of inhibitory substances or by increasing the natural resistance of the host (Nehl *et al.*, 1996; Cartieaux *et al.*, 2003).

Direct growth promoting mechanisms are as follows i) nitrogen fixation ii) solubilization of phosphorus iii) sequestering of iron by production of siderophores iv) production of phytohormones such as auxins, cytokinins, gibberellins and v) lowering of ethylene concentration (Kloepper and Schroth, 1978; Glick, 1995; Glick *et al.*, 1999).

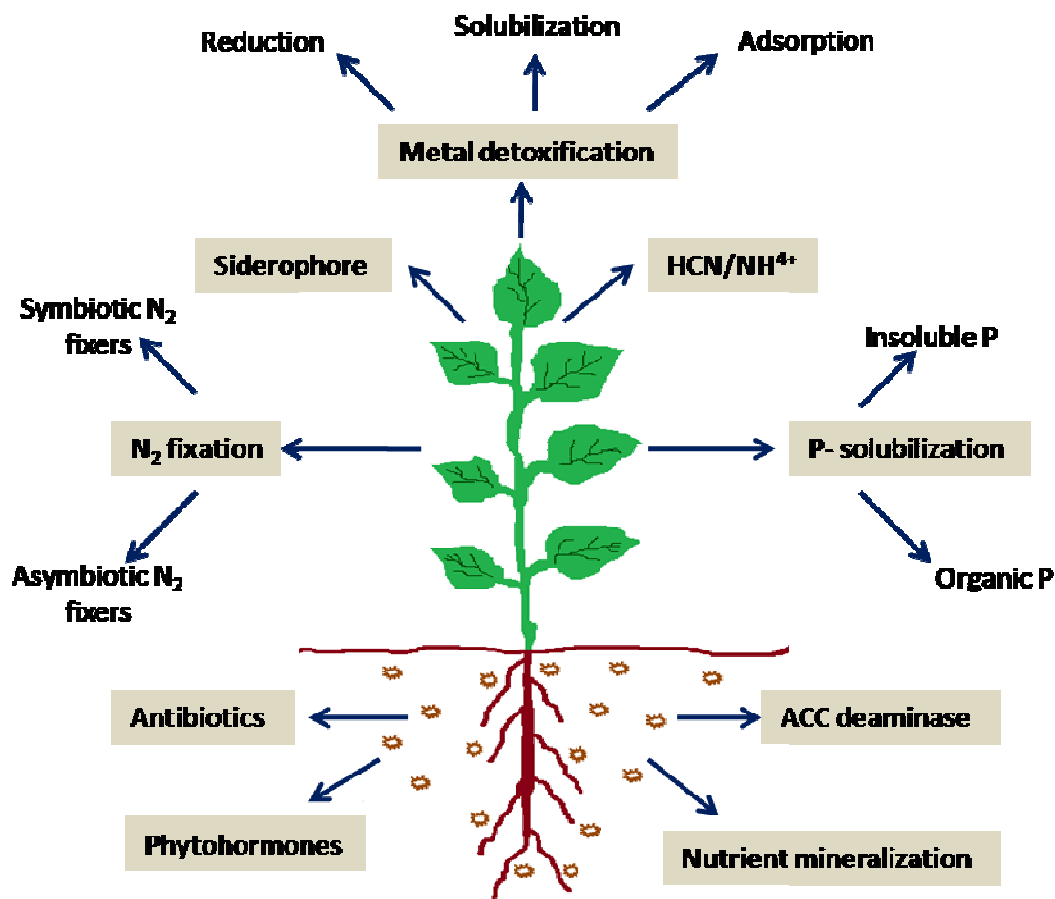


Fig. 2: Schematic diagram showing mechanisms of growth promotion by PGPR

In addition to *Rhizobium* spp., a number of free-living bacteria, for example *Azospirillum*, *Azotobacter* spp., are also able to fix nitrogen and provide it to plants (Bashan *et al.*, 1999). However, it is generally believed that free-living bacteria provide only a small amount of nitrogen in comparison to symbiotic bacteria that the bacterially-associated host plant requires (James and Olivares, 1998).

Phosphorus is one of the most essential nutrient requirements in plants. Ironically, soils may have large reservoir of total phosphorus (P) but the amounts available to the plants are usually a tiny proportion of the total phosphorus. Low availability of phosphorous to the plants is because of the vast majority of soil P is found in insoluble forms, while the plants can absorb only the soluble forms of phosphorus viz. monobasic (H_2PO_4^-) and the diabasic (HPO_4^{2-}) ions (Glass, 1989). Several phosphate solubilizing microorganisms (PSMs) are now recorded to convert the insoluble form of phosphorus to soluble form through acidification, chelation and

exchange reactions (**Hameeda et al., 2008**) and secretion of organic acids or protons (**Richardson et al., 2009**). Bacterial genera like *Azospirillum*, *Azotobacter*, *Bacillus*, *Beijerinckia*, *Burkholderia*, *Enterobacter*, *Erwinia*, *Flavobacterium*, *Microbacterium*, *Pseudomonas*, *Rhizobium* and *Serratia* are reported as the most significant phosphate solubilizing bacteria (**Sturz and Nowak, 2000; Sudhakar et al., 2000; Mehnaz and Lazarovits, 2006**).

Despite the fact that iron is the fourth most abundant element on earth, in aerobic soils, it is not readily assimilated by either bacteria or plants because ferric ion (Fe^{+3}), a predominant form of iron in nature is only sparingly soluble so that the amount of iron available for assimilation by living organisms is extremely low (**Ma, 2005**). Microorganisms and plants require a high level of iron, and obtaining sufficient iron is even more problematic in the rhizosphere where plant, bacteria and fungi compete for iron (**Loper and Buyer, 1991; Guerinot and Ying, 1994**). To survive with such a limited supply of iron, bacteria synthesize low-molecular mass siderophores (400–1500 Da), with an exceptionally high affinity for Fe^{+3} (K_a ranging from 1023 to 1052) and membrane receptors which bind with the Fe-siderophore complex and thereby facilitating iron uptake by microorganisms (**Nielands, 1981; Hider and Kong, 2010**). Direct benefits of bacterial siderophores on the growth of plants have been demonstrated in several experiments. For example, (i) several studies using radiolabeled ferric-siderophores as a sole source of iron showed that plants are able to take up the labeled iron (**Crowley et al., 1988; Robin et al., 2008**); (ii) mung bean plants, inoculated with the siderophore producing *Pseudomonas* strain GRP3 and grown under iron limiting conditions, showed reduced chlorotic symptoms and an enhanced chlorophyll level compared to uninoculated plants (**Sharma et al., 2003**); (iii) the Fe-pyoverdine complex synthesized by *Pseudomonas fluorescens* C7 was taken up by *Arabidopsis thaliana* plants, leading to an increased level of iron inside plant tissues and improved plant growth (**Vansuyt et al., 2007**).

PGPR can alter root architecture and promote plant development with the production of different phytohormones like IAA, gibberellic acid and cytokinins (**Kloepper et al., 2007**). **Swain et al. (2007)** reported a positive effect of IAA producing strains of *Bacillus subtilis* on *Dioscorea rotundata* L. They applied a suspension of *B. subtilis* on the surface of the plant, which resulted in an increase in the root: stem ratio as well as number of sprouts as compared un-inoculated plants.

Although ethylene is essential for the normal growth and development in plants but at high concentration it can be harmful as it induces defoliation and other cellular processes that may lead to reduced crop performance. It is reported that using 1-amino cyclopropane-1-carboxylic acid (ACC) deaminase activity, PGPR can divert ACC from the ethylene biosynthesis pathway in the root system of *Arabidopsis thaliana* (Desbrosses *et al.*, 2009). Thus, rhizobacteria assist in diminishing the accumulation of ethylene levels and re-establish a healthy root system needed to cope up with environmental stresses.

Indirect mechanisms of plant growth promotion by PGPR include i) antibiotic production ii) depletion of iron from the rhizosphere iii) synthesis of antifungal metabolites iv) production of fungal cell wall lysing enzymes v) competition for sites on roots and vi) induced systemic resistance (Dunne *et al.*, 1997; Liu *et al.*, 1995; Glick *et al.*, 1999).

2.7 Plant- Microbe Interaction: Molecular Aspect

The intimacy between the plants and the environment in rhizosphere is essential for better acquisition of water and nutrients by plants as well beneficial interactions of plants with soil-borne microorganisms (Ryan *et al.*, 2009). Plant interacts with different types of soil microorganisms such as plant growth promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi in its natural ecosystem, that confer resistance against biotic and abiotic stresses. They both have symbiotic relationship with each other (Saikkonen *et al.*, 1998) and can change the ecology of the host by providing tolerance to different stresses, increasing the uptake of water and nutrient from the soil and protecting the plants from pathogenic microorganisms which otherwise can confer severe diseases (Smith and Read, 1998). Plants colonized by these beneficial microorganisms show better growth than non-inoculated plants under nutrient deficient conditions (Wu and Xia, 2006). Bacteria enhance plant growth by altering root morphology due to production of plant hormone and subsequent increase in ion uptake (Persello-Cartieaux *et al.*, 2003). Soil and rhizospheric microbes are well known for production of plant hormones like cytokinin, gibberellin like substances, ethylene, jasmonate and auxins (Strzelczyk and Pokojaska-Burdziej, 1984; Kucey, 1988; Patten and Glick, 1996). For a stable interaction between plant and microbes, organism should have capacity to colonize

the roots. Bacteria should have some characteristics for efficient colonization such as motility (**Lugtenberg *et al.*, 1996**), prototrophy for amino acid (**Simons *et al.*, 1997**), high growth rate (**Simons *et al.*, 1996**) and antibiotic production (**Mazzola *et al.*, 1992**). Motility is an important factor for colonization so that microbes can attach to the plant root and after reaching the root epidermis, motile form is switched to non-motile form due to metabolic shift caused by flagellin synthesis (**Alexandre *et al.*, 1996**). According to **Moens and Vanderleyden (1996)** adhesive properties are also associated with bacterial flagellin but attachment is a crucial step for colonization. Another step towards colonization is the ability of that organism to grow on roots. For successful colonization microorganism has to compete for nutrient availability in external environment (**Simons *et al.*, 1996, 1997**). Molecular signals responsible for establishment of an organism are reported to be involved in plant and microbe interaction. Most of the functional properties of the microbes, essential for colonization are induced by root exudates. A bacterium recognizes root exudates as nutrient and signaling molecule which attracts bacteria towards the root and stimulates its growth. Bacterial sensor-regulator (two component system and type 3 secretion systems) are also found to be activated in rhizosphere and reported to have some role in plant and bacteria interaction (**Preston *et al.*, 1998**).

2.8 Abiotic Stresses: Adaptation of Microorganisms at Cellular Level

Different stress factors have significant influences on the performance of microorganisms in their natural environment. Selection and deployment of microorganisms in stressed ecosystems therefore require concerted research and technology development. Extensive research has been carried out on occurrence and functional diversity of agriculturally important microbes in stressed environments as reviewed by several authors (**Grahm, 1992; Zahran, 1999; Venkateswarlu *et al.*, 2008**). Besides, importance of PGPRs in maintaining root health and nutrient uptake, their tolerance to environmental stress is also well recognized (**Malhotra and Srivastava, 2009**) although, specific traits of promoting plant growth and development are limited at a given environment of plant–microbe interactions. The occurrence of *Rhizobium*, *Bradyrhizobium*, *Azotobacter*, *Azospirillum*, *Pseudomonas* and *Bacillus* has been reported from desert ecosystems, acid soils, saline and alkaline areas and highly eroded hill slopes of India (**Tilak *et al.*, 2005; Selvakumar *et al.*,**

2009; Upadhyay *et al.*, 2009). PGPR are adapted to adverse conditions and may compensate for such detrimental conditions (Marulanda *et al.*, 2007, 2009). The term induced systemic tolerance (IST) has also been proposed for PGPR-induced physical and chemical changes that result in enhanced tolerance of plants to abiotic stress (Yang *et al.*, 2009).

Under stress, most of the rhizobacteria produce osmoprotectants (K^+ , glutamate, trehalose, proline, glycine-betaine, proline, betaine and ectoine etc.) to modulate their cytoplasmic osmolarity (Blanco and Bernard, 1994). The periplasmic space of bacteria contains large quantity of highly anionic polysaccharides known as membrane derived oligosaccharides, which are too large to diffuse through the porin proteins and probably function to maintain the turgor pressure of the periplasm of these organisms. Many microorganisms produce exo-polysaccharides (EPS) as a strategy for growing, adhering to solid surfaces, and surviving adverse conditions. The physiological role of EPS depends on the ecological niches and natural environment from where microorganisms have been isolated. Indeed, EPS production is a process that requires a noticeable amount of energy (70% of total energy reserve) representing a significant carbon investment for microorganisms. However, the benefits related to EPSs production are significantly higher than the cost, considering the increased growth and survival of the microorganisms in their presence (Wolfaardt *et al.*, 1999). EPS possess a protective nature and form a layer surrounding the cells, providing an effective protection against high or low temperature and salinity or against possible predators. The inoculated seedlings with EPS producing microbes showed improved soil aggregation and root adhering soil and higher relative water content in the leaves (Sandhya *et al.*, 2009). Plants treated with exopolysaccharide (EPS) producing bacteria also display increased resistance to water stress (Bensalim *et al.*, 1998). An EPS producing strain *Pseudomonas putida* strain GAP-P45 could form biofilm on the root surface of sunflower seedlings and impart tolerance to plants against drought stress. Exopolysaccharides possess unique water holding and cementing properties, thus play a vital role in the formation and stabilization of soil aggregates and regulation of nutrients and water flow across plant roots through biofilm formation (Tisdall and Oadea, 1982; Roberson and Firestone, 1992).

Plants adaptation to environmental stresses is associated with metabolic adjustments that lead to the accumulation of several compatible organic solutes and water stress proteins like dehydrins ranging in size from 9 to 200 kDa (**Close, 2006**). Protein variation is an essential part of plant response to environmental stress as well as for adaptation to environmental conditions. Inoculation of lettuce (*Lactuca stiva* L.) with PGPR *Pseudomonas mendocina* augmented an antioxidant catalase under severe drought conditions, suggesting that they can be used as inoculants to alleviate the oxidative damage elicited by drought (**Kohler et al., 2008**).

2.8.1 Alleviation of abiotic stress in plants by rhizosphere and endophytic bacteria

Besides developing mechanisms for stress tolerance, microorganisms can also impart some degree of tolerance to plants towards abiotic stresses like drought, chilling injury, salinity, metal toxicity and high temperature. Rhizospheric and endorhizospheric microorganisms help plants tolerate abiotic stresses by various mechanisms including modification of plant response at genetic level (**Bashan and Holguin, 1998; Srivastava et al., 2008**). Under highly adverse conditions, plants exhibit a reduced leaf growth rate due to decreased water uptake, which restricts photosynthetic capacity. Plant involves a number of metabolic and physiological changes in response to salt stress and water deficiency (drought) (**Hu, 2005**). Inoculation of salt-stressed plants with PGPR strains alleviates the salinity stress in plants. An increase in salinity in the soil causes a physiological response or disorder in lettuce plants (**Han and Lee, 2005**). The long-term goal of improving plant–microbe interactions for salinity affected fields and crop productivity can be met with an understanding of the mechanism of osmoadaptation in *Azospirillum* sp. **Tripathi et al. (1998)** reported that in *Azospirillum* sp. there is an accumulation of compatible solutes such as glutamate, proline, glycine-betaine and trehalose in response to salinity/osmolarity; proline plays a major role in osmoadaptation through increase in osmotic stress that shifts the dominant osmolyte from glutamate to proline in *A. brasilense*. *Azospirillum*-inoculated sorghum plants had more water content, higher water potential and lower canopy temperature in their foliage. Hence, they were less drought-stressed than noninoculated plants. **Saleena et al. (2002)** have studied the diversity of indigenous *Azospirillum* sp. associated with rice cultivated along the coastline of Tamil Nadu.

When a plant is exposed to adverse climatic conditions, phytohormones play a key role in maintaining plant homeostasis (**Potters *et al.*, 2007**). Auxins, especially indole acetic acid (IAA) is produced in the shoot and transported to plant roots, where it enhances root growth, promotes the growth of lateral root and increases its efficiency of absorbing more nutrients from the soil. However higher concentration of auxin can be harmful to the root growth and is directly associated with the synthesis of ethylene (**Eliasson *et al.*, 1989; Jackson, 1991**). Likewise, in the rhizosphere, bacteria also show same type of beneficial effect by producing indole acetic acid (IAA) which enhances plant growth and improve plant health. Some PGPR strains are also reported to produce cytokinin and antioxidant, which degrade reactive oxygen species and accumulate the abscisic acid and impart tolerance towards oxidative stress (**Stajner *et al.*, 1997**). Recently, **Kohler *et al.* (2008)** demonstrated the greater activity of antioxidant catalase in lettuce plants under severe drought conditions when inoculated with PGPR, *Pseudomonas mendocina* and arbuscular mycorrhizal fungi (*Glomus intraradices* or *G. mosseae*). The same authors have suggested their role as inoculant to alleviate the oxidative damage elicited by drought. It is interesting to note in the current study that PGPB strain *P. fluorescens* Pf1 increased the accumulation of catalase and peroxidase when green gram plants were exposed to water stress. **Saravanakumar *et al.* (2011)** have reported that greater accumulation of antioxidant enzymes in green gram plants treated with *P. fluorescens* Pf1 could also be involved in reduction of oxidative stress generated during water stress. This in turn indicated an enhanced drought resistance in green gram plants bacterized with *P. fluorescens* Pf1 followed by *B. subtilis* EPB 22. Similarly, the concept of PGPR elicited 'induced systemic tolerance' to salt and drought was proposed by **Yang *et al.* (2009)**. Under water deficiency, maize seedlings inoculated with *Azospirillum brasilense*, however, displayed improved relative and absolute water contents in comparison to non inoculated treatments. Bacterial inoculation has also been shown to prevent a significant drop in water potential, in parallel with a concomitant increase in root growth, total aerial biomass and foliar area, as well as proline accumulation in leaves and roots. These effects were more significant at 75% reduction in water supply, compared to 50% (**Casanovas *et al.*, 2002**). **Timmusk and Wagner (1999)** were the first to show that inoculation of *Paenibacillus polymyxa* confers drought

tolerance in *Arabidopsis thaliana* through the induction of drought responsive gene, ERD15 (Early response to dehydration 15). Inoculation of *Azospirillum brasilense* Sp245 in wheat (*Triticum aestivum*) under drought stress resulted in a better water status and an additional “elastic adjustment” resulting in better grain yield and mineral quality (Mg, K and Ca) (Creus *et al.*, 2004). PGPR can have positive effects on vigour and productivity, especially under stress conditions. Seed inoculated with PGPR in asparagus (*Asparagus officinalis* L) resulted in a positive response and enhanced plant growth under drought (Liddycoat *et al.*, 2009). The phosphate-solubilising microorganisms can interact positively in promoting plant growth as well as P uptake of maize plants, leading to improved plant tolerance under water deficit conditions (Ehteshami *et al.*, 2007). On the basis of mutational studies of *Azospirillum*, Kadouri *et al.* (2003) proved the role of PHB synthesis and accumulation in enduring various stresses, viz. UV irradiation, heat, osmotic pressure, osmotic shock and desiccation. *Azospirillum*-inoculated wheat (*T. aestivum*) seedlings subjected to osmotic stress developed significant higher coleoptiles, with higher fresh weight and better water status than non inoculated seedlings (Alvarez *et al.*, 1996; Cereus *et al.*, 2005).

Another mechanism used by the PGPR is the production of ACC deaminase which hydrolyzes ACC (1-aminocyclopropane-1-carboxylic acid), the precursor molecule of ethylene and confer induced systemic tolerance (IST) against drought and salt. Many aspects of plant life are regulated by ethylene level. Biosynthesis of ethylene is subjected to tight regulation, involving transcriptional and post-transcriptional factors regulated by environmental cues, including biotic and abiotic stresses (Hardoim *et al.*, 2008). In the biosynthetic pathway of ethylene, S-adenosylmethionine (S-AdoMet) is converted by 1-aminocyclopropane-1-carboxylate synthase (ACS) to 1-aminocyclopropane-1-carboxylate (ACC), the immediate precursor of ethylene. Under stress conditions, plant hormone (ethylene) endogenously regulates plant homeostasis and results in reduced root and shoot growth. In the presence of ACC deaminase producing bacteria, plant ACC is sequestered and degraded by bacterial cells to supply nitrogen and energy. Furthermore, by removing ACC, the bacteria reduce the deleterious effect of ethylene, ameliorating plant stress and promoting plant growth (Glick, 2007).

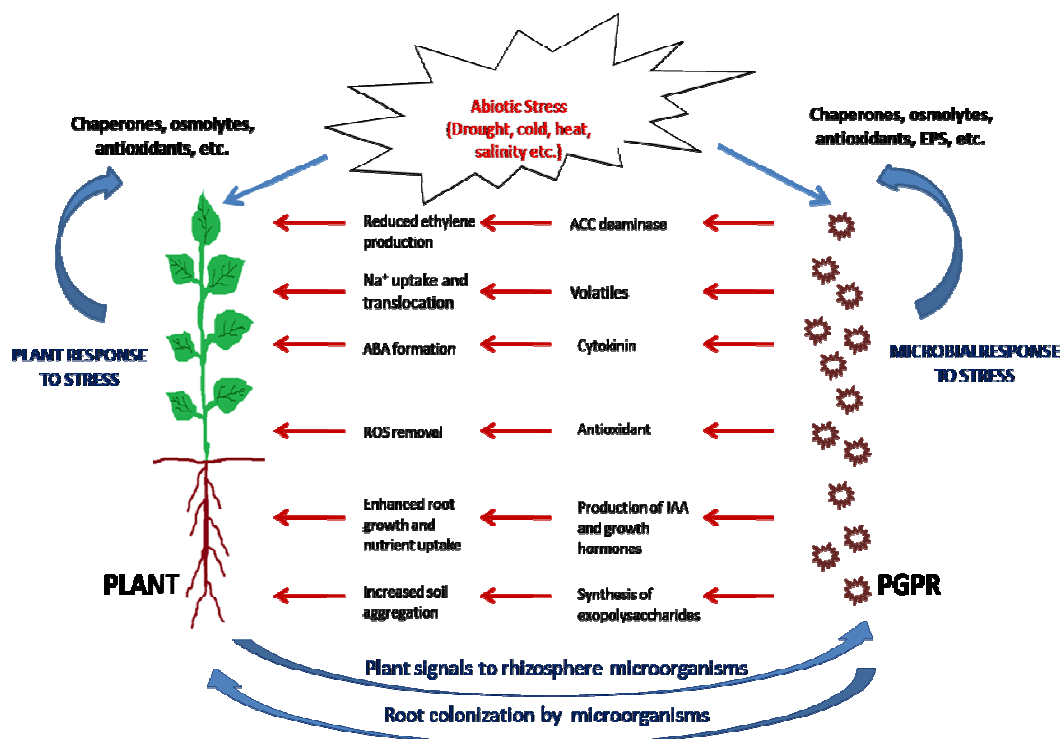


Fig. 3: Plant microbe interactions: helping plants to alleviate abiotic stress

Saleem *et al.* (2007) have reviewed the role of PGPR containing ACC deaminase in stress agriculture. Inoculation with ACC deaminase containing bacteria induce longer roots which might be helpful in the uptake of relatively more water from deep soil under drought stress, thus increasing water use efficiency of the plants under drought conditions (Zahir *et al.*, 2008). Chen *et al.* (2007) correlated proline accumulation with drought and salt tolerance in plants. Introduction of proBA genes derived from *Bacillus subtilis* into *A. thaliana* resulted in production of higher level of free proline resulting in increased tolerance to osmotic stress in the transgenic plants. Increased production of proline along with decreased electrolyte leakage, maintenance of relative water content of leaves and selective uptake of K^+ ions resulted in salt and drought tolerance in *Zea mays* coinoculated with *Rhizobium* and *Pseudomonas* (Bano and Fatima, 2009). Proline protects membranes and proteins against the adverse effects of high concentration of inorganic ions and temperature extremes. It also functions as a protein-compatible hydrotope, and as a hydroxyl radical scavenger (Smirnoff and Cumbes, 1989). Accumulation of proline buffers cellular redox potential under environmental stresses (Jain *et al.*, 2001; Wahid and Close, 2007). Trehalose metabolism in rhizobia is a key for signaling plant growth, yield and

adaptation to abiotic stress and its manipulation had a major agronomical impact on leguminous plants (Suarez *et al.*, 2008). Figueiredo *et al.* (2008) reported increased plant growth, N content and nodulation of *Phaseolus vulgaris* L. under drought stress due to coinoculation of *Rhizobium tropici* and *P. polymyxa*. *Phaseolus vulgaris* plants inoculated with *Rhizobium etli* over expressing trehalose-6-phosphate synthase gene had more nodules with increased nitrogenase activity and high biomass compared with plants inoculated with wild type *Rhizobium etli*. Three week old plants inoculated with *R. etli* (mutant cells) subjected to drought stress fully recovered whereas plants inoculated with wild type *Rhizobium etli* died. Microarray analysis of 7,200 expressed sequence tags from nodules of plants inoculated with over expressing trehalose-6-phosphate synthase gene (mutant strain) revealed up regulation of genes involved in stress tolerance, suggesting a signaling mechanism for trehalose (Suarez *et al.*, 2008).

Rhizobacteria inhabiting the sites exposed to frequent stress conditions, are likely to be more adaptive or tolerant and may serve as better plant growth promoters under stressful conditions (Lifshitz *et al.*, 1986). It has been reported that *P. polymyxa* inoculation is more effective in relatively harsh and poor quality conditions (Chanway and Holl, 1994). Drought-exposed barley plants when inoculated with *P. polymyxa* could tolerate stress, 2 weeks longer than uninoculated control plants (Timmusk, 2003). Isolation of indigenous microorganisms from the stress affected soils and screening on the basis of their stress tolerance and PGP traits may be useful in the rapid selection of efficient strains that could be used as bioinoculant for stressed crops.

2.8.2 Symbiotic fungi for alleviation of abiotic stress in plants

Arbuscular mycorrhizal (AM) symbiosis is often alleged to improve plant resistance to water deficit and drought stress through the alteration of plant physiology and the expression of plant genes (Subramanian and Charest, 1998; Ruiz- Lozano and Azcon, 2000). There are reports of AM-induced increases in drought tolerance, involving both increased dehydration and dehydration tolerance (Allen and Boosalis, 1983). AM fungi inoculation could decrease malondialdehyde and soluble protein content of the leaves and enhance activities of superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT) resulting in improved osmotic adjustment and drought tolerance plants (Wu and Xia, 2005). Inoculation of *Glomus versiforme* in citrus plants improved the osmotic adjustment of the plant

under drought stress through enhanced levels of non-structural carbohydrates, $K^{(+)}$, $Ca^{(+)}$ and $Mg^{(2+)}$ (Wu and Xia, 2006).

2.9 Pearl millet (*Pennisetum glaucum*)

Pearl millet (*Pennisetum glaucum* L.) R. Br. was domesticated from wild grasses of the southern Sahara approximately 4000 years ago. Since then it has become widely distributed across the semiarid tropics of Africa and Asia. It is also extensively grown as a summer annual grazing crop in the southern United States, tropical and subtropical regions of the world (Goldman *et al.*, 2003). In 2003, pearl millet was the world's sixth largest cereal crop with 29 million metric tons produced on 36 million hectare of cultivated land. Approximately half of the world's pearl millet is grown in Africa. Pearl millet, a C4 plant belonging to the family Poaceae, has a very high photosynthetic efficiency and dry matter production capacity. It is grown under the most adverse agroclimatic conditions where other crops fail to produce economic yields. Pearl millet is usually cultivated in regions with characteristically low and erratic rainfall, high mean temperature, high potential evaporation and infertile shallow soils with poor water holding capacity. In spite of this, pearl millet has a remarkable ability to respond to favorable environments because of its short developmental stages and capacity for high growth rate, thus making it an excellent crop for short growing season and under improved crop management. Pearl millet is highly nutritive and versatile food stuff, and is mainly used as whole seed, cracked seed, ground flour, dough or as grain like rice. It is a crop of vital importance to millions of people and is one of the world's most resilient crops. It is supremely adapted to harsh conditions such as drought, heat, aridity and production is likely to increase as the world gets hotter and drier. Of all the major cereals, it is able to tolerate extremes of hot and drought. It yields reliably in regions too hot and too dry to consistently support good yields of maize or even sorghum. It is easy to grow and suffer less from disease and insects pests than sorghum, maize or other crops (National research council, 1996).

2.9.1 Availability and utilization of pearl millet in India

Pearl millet production in India was characterized by subsistence cultivation during 1970s with a small marketable surplus. At individual level, India has the highest area (9.3mha) and production (9.5 m tons) of pearl millet. The major pearl millet

growing Indian states are Rajasthan, Maharashtra, Gujarat, Uttar Pradesh, and Haryana. These states also have the highest concentration of pearl millet consumers since bulk of the consumption for food takes place in the growing areas. Haryana used to be an important pearl millet growing state, but from 1980s, rice and wheat have replaced pearl millet, and now it accounts for only 9% of pearl millet production in the country. Pearl millet is primarily grown for food for human consumption and its dry fodder forms the basis of livestock ration during the dry period of year (November-January) in India (Kelley *et al.*, 1996). But in recent years, it is being geared to a more market oriented crop owing to the change in utilization from mainly food use to many other alternative uses such as animal feed, potable alcohol, processed food, etc.

Pearl millet production is confronted with relatively fewer biotic stresses compared to other crops. Among the diseases, downy mildew (*Sclerospora graminicola* (Sacc.) Schroet.) is the most important constraint in India and Africa, especially on hybrids in India. Other diseases of relatively minor importance include smut (*Moesziomyces penicillariae*), rust (*Puccinia substriata* var. *penicillariae*), blast (*Pyricularia grisea*), and ergot (*Daviceps foeniculis*). Insect-pests and parasitic weed *Striga* are significant challenges in Africa but not in India. Because of the cultivation of pearl millet largely in rainfed production systems of semiarid and arid regions of world, crop growth is constrained by several abiotic stresses. Drought is the primary abiotic constraint and is caused by low and erratic distribution of rainfall. The mean annual rainfall in pearl millet-growing areas in India ranges from 150 to 750mm and most of it is received during June -September (Harinarayana *et al.*, 1999). Pearl millet is very adaptable and thrives at higher elevations than most other tropical cereals, cultivated on soils ranging from rich loams to poor shallow upland soils. A great deal of work has been done on understanding the response of pearl millet to moisture stress at various growth stages with a view to understanding its adaptation to drought stress conditions. It has been conclusively established that effects of water stress depends on the developmental stages of the plant during which the crop is subjected to stress (Yadav *et al.*, 2012). Consequently, pearl millet research has concentrated on exploring the effects of drought at specific growth stages. Therefore, pearl millet may be utilized as a hardy and a model crop in conjunction with use of PGPR to monitor the plants capability in alleviating stress and surviving better in adverse environmental conditions.

*Materials
and
Methods*

3.1 Revival of Rhizospheric Bacteria

Eight species of *Bacillus* and one species of *Pseudomonas* were provided by rhizospheric biology lab, Department of Biological Sciences. All the bacterial isolates used in this study were checked for purity and maintained throughout the investigation. They were partially sequenced from Department of Biochemistry, University of Delhi, South Campus and identified as:

Designation	Bacterial strains
1B	<i>Bacillus cereus</i> 5507
4	<i>Bacillus subtilis</i>
14B	<i>Bacillus cereus</i> BSB 38
9F	<i>Bacillus cereus</i> JDM2-1
8B	<i>Bacillus cereus</i>
13B	<i>Bacillus cereus</i> RSR
P3	<i>Pseudomonas fluorescence</i>
8	<i>Bacillus cereus</i>
92	<i>Bacillus cereus</i>

3.2 Morphological and Culture Characterization

Isolated bacterial colonies were studied for their margin, shape, elevation, surface, pigmentation, size, cell shape and arrangement.

3.2.1 Gram's staining

A loopful of actively growing bacterial culture was heat fixed on a clean glass slide and stained as per the method of **Bergey's Manual of Determinative Bacteriology (1994)**. The slide was examined under oil immersion at 100X of a student microscope.

3.3 Biochemical Characterization

All the bacterial isolates were subjected to biochemical tests according to **Bergey's Manual of Systematic Bacteriology (1994)**.

3.3.1 Catalase test

Catalase activity was checked by growing the organism in tryptone broth. After 24 h of incubation at 30°C, two drops of H₂O₂ (3% v/v) were added on a drop of culture. Release of gas bubbles indicated a positive test.

3.3.2 Lipase production

Nutrient agar plates supplemented with 1% Tween 80 were spot inoculated with test bacteria and incubated at 30°C for 24 h. Clear zone around the colonies indicates the hydrolysis of Tween 80 and presence of lipase enzyme.

3.3.3 Starch hydrolysis

Starch agar plates were spot inoculated with the test organisms and incubated for 2-3 days at 30°C. After incubation, plates were flooded with iodine solution. Yellow zones against blue background around the colony indicated production of amylase or starch hydrolysis.

3.4 PGPR Properties

3.4.1 Siderophore production

Overnight grown bacterial cultures were spot inoculated on CAS (Chrome Azurol S) plates according to **Schwyn and Neilands (1987)**. Plates were incubated at 30°C for 48 h. Appearance of orange halo around the bacterial colonies against a dark blue background indicates siderophore production.

3.4.2 Phosphate solubilization

Pikovskaya's agar plates were spot inoculated with overnight grown bacterial cultures and incubated at 30°C for 48 h. Formation of clear zone around the colonies indicates a positive test of phosphate solubilization (**Pikovskaya, 1944**).

3.4.3 Indole acetic acid (IAA) production (Gorden and Webber, 1951)

Tubes of succinate broth with 100 µg/ml tryptophan and without tryptophan were inoculated with actively growing bacterial cultures individually and incubated

under shaking conditions at $28\pm 2^{\circ}\text{C}$ for 48 h. After incubation, the cultures were centrifuged at 10,000 rpm for 10 min. Two ml of Salkovsky reagent was added to 1.0 ml of culture supernatant and the resulting mixture was incubated at 30°C for 25 min. Development of pink colour indicates IAA production by the test bacteria. Absorbance was recorded at 530 nm using UV/VIS spectrophotometer (Ray-Leigh UV 2601).

3.4.3.1 Quantification of IAA

Reagents

1. Stock solution of indole acetic acid ($100\text{ }\mu\text{g/ml}$)
2. Salkovsky reagent: 1.0 ml FeCl_3 (0.5M) mixed with 50ml HClO_4 (35%).

3.4.3.2 Standard curve

Different volumes (50-500 μl) from the stock solution of IAA were taken in the tubes in duplicate. Final volume was made to 1.0 ml with distilled water (DW), to which 2 ml Salkovsky reagent was added and the mixture was incubated at 30°C for 25 min. Distilled water was taken as control. Absorbance was recorded at 530 nm. Graph was plotted using IAA concentrations verses absorbance.

3.4.4 NH_3 production

Bacterial cultures were grown in peptone water for three days at 28°C , in an incubator shaker at 120 rpm. After 3 days, 1.0 ml Nessler's reagent was added to 1.0 ml culture. Development of yellow to brown color indicated NH_3 production (**Dey *et al.*, 2004**).

3.5 Growth Curve

A single colony of all the bacterial strains was inoculated in 5 ml nutrient broth separately. Inoculated medium was incubated at 28°C at 120 rpm for 22-24 h, for the development of primary culture. Hundred μl of each primary culture was inoculated in a flask containing 100 ml nutrient broth. A sum of 27 flasks were inoculated and incubated at 28°C at 120 rpm and absorbance of bacterial culture was recorded at an interval of 1h interval upto 36 h. Graph was plotted between the absorbance and time.

3.6 pH Pattern of Nutrient Medium Inoculated with Bacterial Species

To determine the effect of bacterial growth on the pH of the medium, fresh bacterial culture of individual organism was inoculated separately in nutrient broth. Inoculated broth was incubated at 30°C and samples were withdrawn at an interval of four hr. After filtration through Whatmann no.1, samples were processed to determine their pH.

3.7 *In vitro* Study on Effect of Plant Growth Promoting Rhizobacteria on Seed Germination

Effect of PGPR on seed germination of pearl millet (*Pennisetum glaucum*) (var. CZIC 923) was observed under laboratory conditions.

3.7.1 Seed sterilization

For surface sterilization, seeds were washed thoroughly with sterilized DW and then treated with 0.2 % sodium hypochlorite for 5- 10 min and then washed thoroughly with sterilized DW. Seeds were then dipped in 75% ethanol for 1-2 min and again washed with sterilized DW under aseptic conditions. Sterilized seeds were dried over sterilize Whatmann no.1 filter paper in laminar bench.

3.7.2 Preparation of bacterial inoculum

Each bacterial strain was grown in 150 ml Erlenmeyer flask containing 50 ml of nutrient broth at 30°C for 24h. Bacterial cultures were grown up to a period to obtain an optical density of 0.6 (10^5 to 10^6 CFU ml⁻¹) at 600 nm wavelength using a UV/VIS spectrophotometer (Ray-Leigh UV 2601)

3.7.3 Seed bacterization

Bacterial cultures were applied by seed coating. Surface sterilized seeds were dried and treated with fresh bacterial culture. For adherence of bacterial cultures on to the seeds, 1% Carboxy methyl cellulose (CMC) was also added. Control obtained only broth.

Treated seeds were transferred on sterilized moist filter paper kept in Petri plates. Covered Petri plates containing bacterized seeds were incubated at 28°C in a growth chamber. Ten seeds were kept in a Petri plate. Data was recorded at 80% seed germination.

3.8 Screening of PGPRs on Four Crops in a Pot Experiment

3.8.1 Experimental details

Experiment was laid out in a completely randomized design with three replications for all the crops. The layout plan of the experiment is given below:

3.8.2 Soil collection for pot experiment

About 6 quintal soil was collected from the upper 0-15 cm soil layer from Ujhani (Distt. Badaun) during the month of March, 2010 and analyzed for the following physico-chemical properties.

Table 3.1 Physico-chemical analysis of soil samples

S. No	Properties	Method used
1.	Soil texture	Manual observation
2.	Soil pH (1:2.5) Soil water suspension	Beckman Glass electrode pH meter (Jackson, 1973)
3.	Organic carbon (%)	Modified Walkley and Black method (Jackson, 1973)
4.	Available mineralizable nitrogen (kg ha ⁻¹)	Alkaline KMnO ₄ method (Subbaiah and Asija, 1956)
5.	Available P ₂ O ₅ (kg ha ⁻¹)	Olsen's method (Olsen <i>et al.</i>, 1954)

3.8.3 Preparation of experimental pots

Soil was air dried and sieved with <5 mm mesh. Soil was sterilized by autoclaving on three consecutive days at 121°C for 60 min each. After sterilization, soil was filled into the pots of 500 gm capacity. The pots were irrigated with tap water and left for 1-2 days for equilibration.

3.8.4 Selection of seeds

Two varieties of pearl millet (*Pennisetum glaucum*) (CZP 2k9 and CZIC 923), two varieties of moth (*Vigna aconitifolia*) (CZM1 and CZM3), one variety of guar (*Cyamopsis tetragonoloba*) (RGC-936) and sorghum (*Sorghum bicolor*) (var. PC-23)

were selected for the pot experiment. All the varieties were supplied by Dr. Anurag Sadena, PS, CAZRI, Jodhpur, Rajasthan.

3.8.5 Seed sterilization

Sterilization of seeds was done as described earlier.

3.8.6 Treatments

All the bacterial cultures mentioned above along with a control (without bacteria) were used as a treatment.

3.8.7 Preparation of bacterial inoculation

Preparation of bacterial inoculum was done as described earlier.

3.8.8 Seed treatment

One ml of bacterial culture was put in a hole and the seed was placed. Control obtained only broth.

3.8.9 Sowing of seeds

Four seeds were sown in one pot. After emergence, two seedlings were maintained per pot. Pots were watered as and when required.

3.8.10 Growth conditions

Experiment was carried out in a poly house under following growth conditions: Temperature: $27 \pm 2^{\circ}\text{C}$, Photo period: 16/8 h day/night cycle, Light intensity: $400 \text{ Em}^{-2}\text{s}^{-1}$, (400-700 nm), Relative humidity: 60%.

3.8.11 Sample collection and analysis

Plants were harvested after 45 days of sowing and washed thoroughly with tap water followed by DW. After initial air drying, root/shoot fresh weight, height and root volume were recorded. Now, plant samples (root and shoot) were kept in paper bags, dried at 60°C in an electric oven for 48 h and weighed to determine the dry matter yield.

3.8.12 Plant height

Plant height was measured from the soil base to the tip of fully expanded leaf and values were recorded in cm.

3.8.13 Plant fresh weight

Shoot/root of the plant was weighed separately immediately after harvesting

3.8.14 Plant dry weight

Plant samples were dried in an oven at 60°C for two days to get a constant weight. Dry matter of root/shoot of the plants was recorded and values were expressed in grams.

3.8.15 Root volume

Root volume was also recorded by dipping the roots in water filled in measuring cylinder. The amount of water, which was displaced by roots, was recorded as root volume and expressed in ml.

3.8.16 Chemical analysis of plant samples

3.8.16.1 Determination of total nitrogen

Total nitrogen of the plant samples was determined by the Kjeldahl method (Iswaran, 1981) using two steps

- i) Sample preparation and digestion
- ii) Distillation

3.8.16.1.1 Sample preparation

Dried leaf sample (0.2 g) was taken and mixed with 5 ml conc. H_2SO_4 . Hydrogen peroxide (30%) was also added prior to digestion to minimize foaming. One g of salt/ catalyst mixture was added to each digestion tube. Mixture was consisted of 10 g of K_2SO_4 and 1 g of catalyst mixture (20 parts of CuSO_4 + 1 part of selenium powder). The samples were digested on a heating plate at 200°C. After digestion, samples were cooled to room temperature and diluted to make up the volume 50 ml with DW.

3.8.16.1.2 Distillation

Ten ml of digested sample was transferred to vacuum jacket of fully automated Kjeldahl distillation apparatus. Boric acid solution (4%) containing bromocresol green and methyl red indicator and 40% NaOH solution was filled in the containers attached with the apparatus. Ten ml of 4% boric acid solution and 10 ml of

NaOH was automatically transferred to the tube containing digest. After distillation, the boric acid was titrated against N/200 H₂SO₄. Blank was also titrated. Nitrogen content in the plant sample was calculated as follows:

$$N(\%) = \frac{TV \times \text{normality of } \frac{N}{200} \text{ H}_2\text{SO}_4 \times \text{volume of digestion} \times 100}{\text{Weight of sample} \times \text{aliquot taken}}$$

Titration value (TV) = Sample titration value - Blank titration value

3.8.16.2 Determination of phosphorus

3.8.16.2.1 Diacid digestion of sample

Diacid digestion of the sample was carried out using 9:4 mixture of HNO₃: HClO₄. Dried plant sample (0.2 g) was mixed with 5 ml of acid mixture and digested in a heating plate. Samples were heated at high temperature until the red NO₂ fumes ceased. It was further heated until liquid became colorless. After digestion, it was cooled and diluted to make up the volume to 50 ml with DW.

3.8.16.2.2 Phosphorus estimation in acid digests

Reagents

Vandomolybdate solution

Ammonium molybdate (22.5 g) was dissolved in 400 ml DW. Ammonium vandate (1.25 g) was dissolved in 300 ml boiling DW. Vandate solution was added to the molybdate solution. After cooling the solution, 250 ml of con.HNO₃ was added and diluted to 1 L.

3.8.16.2.3 Preparation of standard curve

Stock solution of KH₂PO₄

KH₂PO₄ (0.2195 g) was dissolved in 1 L DW to the final concentration of 50 µg P/ml (50 ppm). Final concentration of 0, 1, 2, 3, 4 and 5 ppm from stock solution were prepared. Ten ml of vandomolybdate reagent was added to each flask. Distilled water was added to make up the final volume. It was incubated for 30 min and absorbance was recorded at 420 nm with spectrophotometer (Ray-Leigh UV 2601). Absorbance versus concentration curve was plotted.

Estimation of P

Plant digest was taken in place of stock solution and the same procedure was followed as described for standard curve. P concentration is calculated using the standard curve.

$$\text{P in \%} = \text{Sample conc. (ppm)} \times \frac{1}{\text{Wt of sample (g)}} \times \frac{100}{\text{Aliquot (ml)}} \times \frac{\text{Final volume (ml)}}{10000}$$

3.8.16.3 Selection of crop and PGPR

Among all the crops, pearl millet and two most efficient PGPR (P3 and 14B) were selected for further studies.

3.9 Testing of Endophytic Nature of Selected PGPR

A pot experiment was set up for testing of endophytic nature of selected PGPR in a glass house as described earlier. Seeds of Pearl millet var. CZIC923 were surface sterilized as described previously. Both the bacterial cultures (P3 and 14B) were used for seed inoculation as described earlier. Plants were harvested after 30 days of germination and roots were collected.

3.9.1 Surface sterilization of pearl millet roots

Pearl millet roots were washed with tap water to remove attached soil. Subsequently, the roots were immersed in 70% ethanol for 3 min, washed with fresh sodium hypochlorite solution (0.2%) for 5 min, rinsed with 70% ethanol for 30 s, and finally washed five times with sterile distilled water. To confirm that the sterilization process was successful, the aliquots of the sterile distilled water used in the final rinse were inoculated on nutrient agar (NA) plates. The plates were examined for bacterial growth after incubation at 28 °C for 3 days. Root samples that did not show bacterial growth, were used for further analysis.

3.9.2 Isolation of endophytic bacteria from pearl millet root samples

Roots were dissected into pieces of 1 cm and aseptically transferred to Petri dishes containing 3% water-agar. Plates were incubated at room temperature for 3-4 days. The confirmation of isolates was done by streaking the same isolates on the plate containing 3% agar and sterile root extract as the only source of nutrients. The morphological identification of isolate was carried out by performing gram staining.

3.9.3 Isolation of genomic DNA from endophytic bacterial isolates and our standard organisms (P3 and 14B): CTAB Method by Jaufeerally-Fakim and Dookun (2000).

Procedure

1. Each bacterial isolate was inoculated in a 5 ml Nutrient Broth. All the cultures were incubated at 28°C.
2. Actively grown culture (1.5 ml) was centrifuged at 4000 rpm for 2 min. Supernatant was discarded.
3. Pellet was resuspended in 567 µl TE buffer by repeated pipetting.
4. 30 µl of 10% SDS and 3 µl of 20 mg/ml Proteinase K were added to the pellet, suspended in TE buffer to give a final concentration of 100 µg/ml Proteinase K in 0.5% SDS. Tubes were mixed thoroughly and incubated for 1h at 37°C.
5. 100 µl of 5 M NaCl was added to the tubes and mixed thoroughly.
6. 80 µl of 10% CTAB was added to the tubes, mixed thoroughly and incubated for 10 min at 65°C.
7. 800 µl of Chloroform/Isoamyl-alcohol (24:1) was added to the tubes, mixed and centrifuged at 11,000 rpm for 5 min.
8. Aqueous phase was collected to a fresh microcentrifuge tube, leaving the interface behind and an equal volume of chloroform/isoamyl alcohol (1:1) was added to the tube and centrifuged at 11,000 rpm for 5 min.
9. Supernatant was transferred to a fresh tube and 2 µl of RNase (10mg/ml) was added, mixed gently and incubated at 37°C for 1h.
10. Equal volume of Isopropanol was added to the tube, mixed gently and incubated at -20°C for overnight.
11. Tubes were centrifuged at 13,000 rpm for 15 min. Supernatant was discarded and pellet was air dried.
12. Pellet was dissolved in 50 µl of nuclease free water.

3.9.4 PCR amplification

DNA (extracted from all the samples) was subjected to polymerase chain reaction (PCR) for amplification of 16S rDNA genes were amplified by PCR. Universal primers GM3f as a forward primer and GM4r as a reverse primer were used according to **Muyzer *et al.* (1995).**

3.9.4.1 Primer details

Primer Name	Sequence (5'-3')	Product Size	T _m (°C)
GM3f	AGAGTTTGATCMTGG	~ 1500 bp	42.4
GM4r	TACCTTGTTACGACTT		43.3

PCR reaction mixture consisted of:

PCR mixture	Volume (μl)
Buffer - 10X	5 μl
dNTPs (10 mM)	2 μl
Primers (10 μM) GM3f	1.25 μl
GM4r	1.25 μl
<i>Taq</i> DNA polymerase (3 U/μl)	1 μl
Genomic DNA	1 μl
Sterile water	38.5 μl
Final volume	50 μl

The amplification programme was as follows;

1. Preheating at 95°C for 5 min
 2. Denaturation at 95°C for 1 min
 3. Annealing at 50°C for 1 min
 4. Extension at 72°C for 2 min
 5. Final extension at 72°C for 10 min
 6. Hold at 4 °C forever
- } 33 cycles

Five μl aliquot of PCR products were examined by horizontal electrophoresis in 1% agarose gel. PCR reactions were performed using a Peltier thermal cycler (Bio-Rad DNA Engine, ALD 1244).

3.9.5 Amplified ribosomal DNA restriction analysis (ARDRA)

16S rDNA amplicons were digested with two restriction enzymes in 20 µl reaction volume individually. The reaction mixture was incubated at 37°C with *MspI*, for 2 h while at 65°C for 4 h with *TaqI*.

Table 3.2 Restriction sites of restriction enzymes used in this study

<i>S.No.</i>	<i>Restriction Enzymes</i>	<i>Restriction Sites</i>
1	<i>MspI</i>	5'-C [↓] CGG-3' 3'-GGC [↓] C-5'
2	<i>TaqI</i>	5'-T [↓] CGA-3' 3'-AGC [↓] T-5'

The restriction digestion was set in 20 µl reaction mixture containing following ingredients:

Table 3.3 Reaction mixture

Reagent/Enzyme	Stock	Working concentration	Volume (µl)
Digestion buffer	10X	1X	2
Enzyme	10 U/µl	1U	0.1
Amplicon	-	-	15
TDW	-	-	2.9

3.9.4.1 Analysis of restriction digestion pattern on gel

Enzymes were inactivated by mixing the loading dye and restriction products were run on 2.5% agarose gel using TAE buffer (1x) at 60 V for 4h. After run, the gel was visualized under UV Gel Doc Imaging system (Bio-Rad). The size of restriction digestion band was determined by comparing with standard molecular weight marker. Gel was photographed by Gel Doc Imaging system (Bio-Rad). The profile of our standard organisms (P3 and 14B) was matched with DNA restriction profile of isolated endophytic bacteria.

3.10 Screening of Six Varieties of Pearl Millet Inoculated with PGPR

3.10.1 Particulars of the pot experiment

Same soil and growth conditions were maintained as mentioned earlier.

3.10.2 Varieties

Six pearl millet varieties (CZP 2k9, CZIC 923, CZP 2k3, CZP 2007, CZP9603 and CZP 9802) were used in the pot experiment for screening the most responsive and less responsive variety on the basis of growth promotion by PGPR.

3.10.3 Treatments

Control, *Pseudomonas fluorescence* (P3) and *Bacillus cereus* BSB 38 (14B) were used.

3.10.4 Seed sterilization and inoculum preparation

It was done as described earlier.

3.10.5 Seed treatment

Total four seeds were sown in a pot. One ml of bacterial culture was put in a hole and the seed was placed. Control obtained only broth.

3.10.6 Sowing of seeds

Four seeds per pot were sown. After seed emergence, two seedlings were maintained per pot. Pots were watered as and when required.

3.10.7 Harvesting and analysis

Plants were harvested after 45 days of sowing.

3.10.7.1 Physiological parameter

Root-shoot length, fresh and dry weight and root volume were recorded as described previously.

3.11 Effect of Three Different Soil Types on Two Varieties of Pearl Millet

3.11.1 Soil used

Three different soil types from Ujhani (UP), Mukteshwar (Uttarakhand- high altitude) and Pantnagar (Uttarakhand Tarai region) were used in the experiment. All

the three soil types were analyzed for the following physico-chemical properties (soil texture, pH, EC, organic carbon and macro nutrient analysis).

3.11.2 Variety

CZIC 923 and CZP 9603

3.11.3 Treatments

Soil type	Bacterial cultures
Mukteshwar (Type 1)	Control
	P3 (<i>Pseudomonas fluorescence</i>)
	14B (<i>Bacillus cereus</i> BSB 38)
Pantnagar (Type2)	Control
	P3 (<i>Pseudomonas fluorescence</i>)
	14B (<i>Bacillus cereus</i> BSB 38)
Ujhani (Type3)	Control
	P3 (<i>Pseudomonas fluorescence</i>)
	14B (<i>Bacillus cereus</i> BSB 38)

3.11.4 Seed sterilization and inoculum preparation

It was done as described earlier.

3.11.5 Seed treatment

One ml of bacterial culture was put in a hole and the seed was placed. Control obtained only broth.

3.11.6 Sowing of seeds

Four seeds were sown per pot. After emergence, two seedlings were maintained per pot. Pots were watered as and when required.

3.11.7 Harvesting and analysis

Plants were harvested after 45 days of sowing.

3.11.7.1 Physiological parameter

Root-shoot length, fresh and dry weight and root volume were recorded as described previously.

3.12 Growth of PGPR in Nutrient Medium Supplemented with Polyethylene Glycol (PEG 8000)

Four sets of nutrient broth with different water potential (-0.5, -0.75, -1.0, -1.5 MPa) were prepared by adding appropriate amount of polyethylene glycol according to Michel (1983).

Osmotic potential of the solution is measured following the van't Hoff's equation:

$$\Pi = i \times C \times R \times T$$

Where,

Π = osmotic potential,

i = van't Hoff factor,

C = molarity of the solution,

R = gas constant (0.0831 bar L K⁻¹ mol⁻¹),

Polyethylene glycol (PEG 8000) was used to increase water potential of the medium. Different concentrations viz. 20, 25, 30 and 35% PEG were used to increase the water potential up to -0.5, -0.75, -1.0, -1.5 MPa, respectively. Flasks containing 100 ml of media (NB+ PEG) were inoculated with 1% overnight raised culture of two bacterial isolates (P3, 14B). Control for each concentration was also used. Experiment was planned with three replicates. All the flasks were incubated at 28°C at 120 rpm for 24 h. Growth was estimated by measuring the optical density at 600 nm using a spectrophotometer. Growth of both the isolates at various stress level was recorded.

3.12.1 Plant growth promoting properties of both the strains under different water potential

Both the bacterial cultures (P3 and 14B), grown in nutrient broth supplemented with PEG-8000 for 24 h, were tested for plant growth promoting properties such as siderophore production, phosphate solubilization, NH₃ production, IAA production as described earlier.

3.12.2 Extraction and purification of exopolysaccharide (EPS) from PGPR with and without stress (Fett *et al.*, 1989, Sandhya *et al.*, 2009)

Bacterial cultures able to grow at maximum stress level were analyzed for their ability to produce EPS under zero stress and all the stress level described above. Flasks containing 100 ml media were inoculated with 1% overnight raised culture of two bacterial isolates (P3, 14B). Control for each concentration also used. Three replicates of each isolate and each concentration were also taken. All the flasks were incubated at 28°C at 120 rpm for 36 h. Exopolysaccharide production was checked in 12, 24 and 36 h.

10 ml of culture was collected in a centrifuge tube after 12, 24 and 36 h of incubation



Culture was centrifuged at 20,000xg for 20 min; highly viscous culture was diluted with 0.85% KCl before centrifugation



Supernatant was collected and filtered through 0.45 µm nitrocellulose membrane



Three volume of ice cold ethanol was mixed with supernatant and stored at 4°C for overnight



Precipitated EPS, obtained by centrifugation (10,000xg for 15 min) was dissolved in 2 ml distilled water



EPS was further purified by repeated dialysis in tap water and then distilled water



Volume of sugar extract was made 20 ml with distilled water



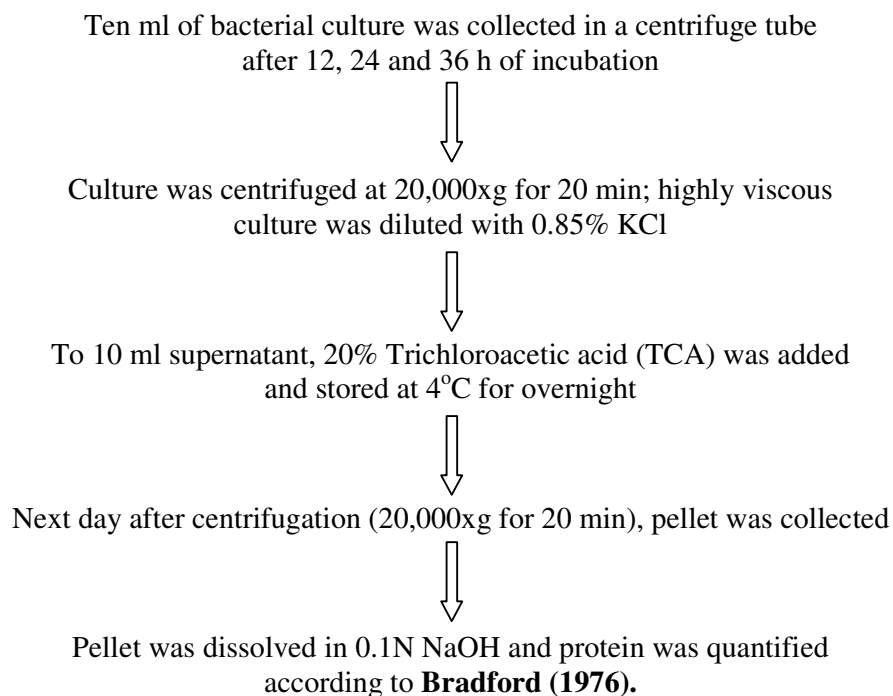
Total carbohydrate of EPS was determined according to **Dubois *et al.* (1956)**



In 1ml sugar solution, 5 % phenol was added. Then 5 ml (95% conc. H₂SO₄) was added and incubated for 10 min. Absorbance was taken at 490 nm using spectrophotometer (Ray-Leigh UV 2601)

3.12.3 Quantification of protein of PGPR under stress (Rajalingam *et al.*, 2008)

Bacterial cultures were also tested for their protein level under normal and stressed condition. Same experiment was set up as done for exopolysaccharide production.



3.12.3.1 Protein estimation by Bradford method

Bradford dye

Dye solution:

Commassie brilliant blue G-250	:	50 mg
Absolute alcohol	:	25 ml
Orthophosphoric acid 85 %	:	50 ml

The volume was made up to 500 ml with distilled water. After dissolving the salt, resulting solution was filtered through Whatmann filter paper No. 2, in dark. The dye was stored in a dark colored bottle at 4°C.

BSA (Bovine serum albumin) standard

Stock solution	:	10.0 mg/10 ml
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Glass test tubes having 5, 10, 20, 40, 60, 100 µl of BSA in duplicate were prepared. Extraction buffer was added to make up the final volume to 300 µl.

1. Three ml of dye was added in each tube.
2. The reaction mixture was incubated at room temperature for 15 min, in dark.
3. Absorbance was recorded at 595 nm against a reagent blank for all the samples.
4. Graph was plotted between the Absorbance Vs Concentration of protein (µg).
5. Amount of protein was determined by extrapolation from the standard curve of BSA.

3.12.4 Total soluble sugar content

To estimate sugar content in bacterial cells grown under stress conditions. Same experiment was set up as done for exopolysaccharide production. Three volume of ice cold ethanol was mixed with supernatant and stored at 4°C for overnight. The supernatant was used for quantitative estimation of sugar according to **Dubois *et al.* (1956)**.

3.12.5 Estimation of proline content

To determine the proline content in bacterial cells grown under stress conditions. Same experiment was set up as done for exopolysaccharide production. Cell pellet was boiled with 70-80% ethanol at 60°C in water bath for 45 min and centrifuged at 10,000xg for 15 min (**Sadasivam and Manickam, 1992**). Free proline accumulation was determined in the supernatant using the method of **Bates *et al.* (1973)**. Two ml of supernatant was mixed with 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent. The reaction mixture was incubated for one hour at 100°C in a water bath. After incubation, the reaction was terminated in an ice bath. Four ml of toluene was added to each reaction mixture tube. Chromophore was extracted by vigorous stirring on a vortex mixer. The absorbance of chromophore containing toluene layer was measured at 520 nm. Concentration of proline in the sample was computed from a standard curve of L-proline. Results were expressed in µg of free proline g⁻¹ fresh weight.

3.13 Screening of Tolerant/Susceptible Pearl Millet Varieties Inoculated With PGPR during Drought

3.13.1 Varieties

Three pearl millet varieties (CZP 2k9, CZIC923, CZP9603) were used in a pot experiment to screen the tolerant and susceptible varieties and to see the effect of PGPR in response to drought stress.

3.13.2 Treatments

Treatment included *Bacillus cereus* BSB 38 (14B), *Pseudomonas fluorescence* (P3) and one control (no bacterial treatment).

3.13.3 Seed sterilization and inoculation with PGPR

Same practice was followed as described earlier.

3.13.4 Soil used

Ujhani soil was used in the experiment. Soil was air dried, sieved, sterilized by autoclaving. After sterilization soil was filled into the pots of 500 g capacity.

3.13.5 Plant growth under drought stress

Pots were maintained in a poly house. Growth conditions maintained were: temperature: $27 \pm 2^\circ\text{C}$, photo period: 16/8 h day/night cycle, light intensity: $400 \text{ Em}^{-2}\text{s}^{-1}$, (400-700 nm), relative humidity: 60%. Soil water content was determined by drying the water saturated soil in the oven. Water holding capacity (WHC) of soil was determined by following method:

Whatman no. 1 filter paper was placed in the bottom of a porous cup. It was weighed and mass was recorded. Oven dried soil was filled in the cup gently and weight was recorded. Cup was placed into a shallow pan of water for 24 h allowing only the bottom of the cup to become wet. Soil was allowed to become saturated from the bottom of the cup to the surface. Cup was removed from the pan of water and placed in a humid enclosure for 24 h until drainage was completed and weight was recorded.

$$\% \text{ water holding capacity of the soil} = \frac{\text{Mass of the water contained in the saturated soil}}{\text{Mass of the saturated soil}} \times 100$$

Where,

Mass of the water contained in the saturated soil = mass of saturated soil - mass of dry soil

Mass of saturated soil = mass of cup, filter paper, and saturated soil - mass of cup and filter paper

Soil moisture was adjusted at 85 % of water holding capacity (WHC). Pots were weighed daily Soil moisture was maintained constant during the experiment by sprinkling with water.

After 45 days of sowing, drought stress was imposed by withholding the irrigation for 8 days. Harvesting was done for three times. First harvesting was done at 85% soil moisture content (control plants) after 45 days of sowing. Second and third harvesting was done at 60% and 35% moisture content at 4th and 8th day of drought, respectively.

3.13.6 Physiological parameter

Root-shoot height, fresh weight and root volume was recorded as described earlier.

3.13.7 Biochemical parameters

For biochemical analysis, fresh leaves were taken and crushed immediately in their respective buffers.

3.13.7.1 Estimation of proline content

Free proline was determined by the method of **Bates *et al.* (1973)**. Leaf material (0.1 g) was homogenized in 4 ml of sulfosalicylic acid (3% w/v in DW) and the homogenate was centrifuged at 10000xg for 30 min at room temperature. Two ml supernatant was mixed with 2 ml of glacial acetic acid and 2 ml of acid ninhydrin reagent. The reaction mixture was incubated for one hour at 100⁰C in a water bath. After incubation the reaction was terminated in an ice bath. To each reaction mixture tube, 4 ml of toluene was added and the chromophore was extracted by vigorous stirring on a vortex mixer. The absorbance of chromophore containing toluene layer was measured at 520 nm. Concentration of proline in the sample was computed from a standard curve of L-proline. Results were expressed in µg of free proline g⁻¹ fresh weight.

3.13.7.2 Malondialdehyde content

Procedure of **Heath and Packer (1968)** was followed to measure the MDA content. Leaf material (0.1 g) was homogenized in 3 ml of 0.1 % trichloroacetic acid (TCA). Homogenate was centrifuged at 10,000xg for 10 min. Crude extract (0.3 ml)

was mixed with 1.2 ml of 0.5% (w/v) 2-thiobarbituric acid (TBA) prepared in 20% (w/v) trichloroacetic acid (TCA). The mixture was incubated at 95°C for 30 min. and then centrifuged at 10000xg for 10 min. Obtained supernatant was collected and allowed to cool at room temperature. Absorbance of the supernatant was measured at 532 nm and 600 nm. Absorbance at 600 nm is subtracted from the absorbance at 532 nm for non-specific absorbance. The concentration of MDA was calculated by using an extinction coefficient of 155 mM⁻¹ cm⁻¹.

3.13.7.3 Hydrogen peroxide content

Hydrogen peroxide was measured spectrophotometrically after reaction with potassium iodide (KI) according to **Alexieva *et al.* (2001)**. Leaf material (0.1 g) was crushed in 1.0 ml of 0.1% (w/v) trichloroacetic acid (TCA) and centrifuged at 10000 rpm for 30 min at 4°C. Reaction mixture was consisted of 0.5 ml of supernatant, 0.5 ml of 0.1M potassium phosphate buffer and 2 ml of 1M KI reagent. The blank had 0.1% TCA in place of leaf extract. Reaction was allowed to develop for 1 h in dark and absorbance was measured at 390 nm. The amount of H₂O₂ was calculated using standard curve prepared with different dilutions of a working standard of 100 µM of H₂O₂.

3.13.7.4 Chlorophyll content

Concentration of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and caretenoid was analysed following the method of **Arnon, (1949)**. Leaf material (0.1 g) was collected and placed in a test tube, added with 10 ml of 80% acetone, the test tube were sealed with parafilm to prevent evaporation and then stored in dark for 24 h. The Chl *a*, Chl *b* and caretenoid concentrations were measured using a UV-Visible spectrophotometer at wavelengths 663 nm and 645 nm.

Solution of 80% acetone was used as a blank. Concentration of Chl *a*, Chl *b* and caretenoid (mg g⁻¹ FW) in the leaf tissues were calculated according to the following equations:

$$\text{Chl } a = \frac{(12.7 \times A_{663}) - (2.63 \times A_{645})}{\text{Weight (g)} \times 1000}$$

$$\text{Chl } b = \frac{(22.9 \times A_{645}) - (4.48 \times A_{663})}{\text{Weight (g)} \times 1000}$$

$$\text{Caretenoid content} = \frac{(1000 \times A_{470}) - \{1.9(\text{chl } a - \text{chl } b)\}}{214}$$

3.13.7.5 Anti-oxidative enzyme analysis

3.13.7.5.1 Preparation of extracts

For protein and enzyme extraction, 0.1 g of leaf samples were homogenized with 50 mM sodium phosphate buffer (PH 7.0) containing 1 mM ethylenediaminetetraacetic acid (EDTA) and 0.25% (w/v) polyvinylpyrrolidone (PVP). Whole extraction procedure was carried out at 4°C. The homogenate was centrifuged at 10,000xg for 15 min at 4°C and the supernatant collected was used to assay enzyme activity. Protein concentration in the enzyme extract was determined by the method of **Bradford (1976)** using bovine serum albumin as a standard.

3.13.7.5.2 Superoxide dismutase (SOD) activity

Superoxide dismutase (SOD, EC 1.15.1.1) activity was assayed spectrophotometrically as the inhibition of photochemical reduction of nitro-blue tetrazolium (NBT) at 560 nm (**Beauchamp and Fridovich, 1971**). The reaction mixture (3 ml) consisted of 50 mM Na-phosphate buffer (pH 7.8), 13 mM L-methionine, 75 μ M NBT, 10 μ M EDTA.Na₂, 2.0 μ M riboflavin and 0.3 ml enzyme extract. Test tubes, containing reaction mixture were incubated for 10 min in 4,000 lux at 35°C. One unit SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the rate of NBT reduction measured at 560 nm.

3.13.7.5.3 Ascorbate peroxidase (APX) Activity

Ascorbate peroxidase (APX, EC 1.11.1.11) activity was determined according to the method of **Nakano and Asada (1981)**. The reaction mixture contained 50 mM potassium phosphate (pH 7.0), 0.2 mM EDTA, 0.5 mM ascorbic acid, 2% H₂O₂ and 0.1 ml enzyme extract in a final volume of 3 ml. Decrease in absorbance at 290 nm for 1 min was recorded and the amount of ascorbate oxidized was calculated using extinction coefficient (2.8 mM⁻¹ cm⁻¹).

Difference in absorbance (ΔA_{290}) was divided by the ascorbate molar extinction coefficient (2.8 mM⁻¹ cm⁻¹) and the enzyme activity expressed as μ mol of H₂O₂ min⁻¹.mg⁻¹ protein, taking into consideration that 1.0 mol of ascorbate is required for the reduction of 1.0 mol of H₂O₂ (**McKersie and Leshem, 1994**).

3.13.7.5.4 Guaiacol Peroxidase (GPX) Activity

Guaiacol peroxidase (GPX, EC 1.11.1.7) activity was determined as described by **Urbanek *et al* (1991)**. Leaf material (0.1 g) was homogenized in 2.0 ml of extraction buffer (100 mM Phosphate buffer (pH 7) containing 0.1 mM EDTA in a pre-chilled mortar and pestle. The homogenate was centrifuged at 16,000xg for 20 min at 4°C. The supernatant fraction was used as a crude enzyme extract for determination of enzyme activity. Total reaction mixture volume was 2 ml, prepared in quartz cuvette. In 1850 µl of assay buffer containing, 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, 50 µl of enzyme extract was added. Reaction was started by adding 50 µl of guaiacol (5 mM) and 50 µl of H₂O₂ (15 mM) and increase in absorbance was recorded at 470 nm for 3 min. The formation of tetraguaiacol was quantified using its molar extinction coefficient (26.6 mM⁻¹cm⁻¹). The enzyme activity was expressed as µmol min⁻¹mg⁻¹protein.

3.13.7.5.5 Catalase (CAT) Activity

Leaves were homogenized in common extraction buffer. Specific Catalase (EC 1.11.1.6) activity was measured according to **Beers and Sizer (1952)** with minor modifications. The assay mixture consisted of 50 µL of the enzyme extract, 100 mM phosphate buffer (pH 7.0), 0.1 µM EDTA, and 20 mM H₂O₂ in a total volume of 1.5 ml. Decrease of H₂O₂ was monitored by reading the absorbance at 240 nm at the moment of H₂O₂ addition and 1 min later. The difference in absorbance (ΔA_{240}) was divided by the H₂O₂ molar extinction coefficient (36 M⁻¹cm⁻¹). Enzyme activity was expressed as µmol of H₂O₂ min⁻¹.mg⁻¹ protein.

3.13.7.5.6 Protein estimation by Bradford method

The method based on **Bradford (1976)** was used for protein estimation as described elsewhere.

3.14 *In vitro* Study of Drought Tolerance in Plants Inoculated with PGPR

After screening with PGPR, two pearl millet varieties (one tolerant and one susceptible) were selected for in vitro study of drought tolerance of plants inoculated with PGPR.

3.14.1 Varieties used

CZIC 923 and CZP 9603 variety of pearl millet were used for the further studies.

3.14.2 Soil used

Vermiculite was used in place of soil. It was sterilized by autoclaving on three consecutive days at 121°C for 60 min each. After sterilization, it was filled into the pots of 500 g capacity.

3.14.3 Treatments in pot experiment

Stress level	Culture
Control (No PEG)	Control
	P3 (<i>Pseudomonas fluorescence</i>)
	14B (<i>Bacillus cereus</i> BSB 38)
0.75 MPa (25% PEG)	Control
	P3 (<i>Pseudomonas fluorescence</i>)
	14B (<i>Bacillus cereus</i> BSB 38)
1.0 MPa (30% PEG)	Control
	P3 (<i>Pseudomonas fluorescence</i>)
	14B (<i>Bacillus cereus</i> BSB 38)

3.14.4 Seed sterilization and inoculation with PGPR

It was done as described previously.

3.14.5 Preparation of bacterial inoculation

Culture preparation was done as described earlier.

3.14.6 Seed treatment

Total four seeds were sown in a pot. One ml of bacterial culture was put in a hole and the seed was placed. Control obtained only broth.

3.14.7 Sowing of seeds

Four seeds were sown per pot. After emergence, two seedlings were maintained per pot. Pots were watered as and when required.

3.14.8 Growth conditions

Pots were maintained in a poly house. Following growth conditions were maintained: Temperature: $27\pm 2^{\circ}\text{C}$, Photo period: 16/8 h day/night cycle, Light intensity: $400\text{ Em}^{-2}\text{s}^{-1}$, (400-700 nm), Relative humidity: 60%.

3.14.9 Imposition of drought stress using polyethylene glycol (molecular wt. 8000)

The screened varieties of pearl millet were grown in the poly house under controlled conditions. Irrigation was done at proper interval to maintain a constant moisture level. Hogland's solution was given to the plants twice in a week. Twenty five day old seedlings were harvested and treated with different concentrations of PEG i.e. -0.75 and -1.0 MPa in Hogland's solution. Control plants were given simple Hogland's solution without PEG. After 12 h, the comparative studies were made between control and stressed plants by observing various biochemical and physiological changes in stress plants depending upon the intensity of the applied stress.

3.14.10 Physiological parameter

Root-shoot fresh and dry weight and root volume was recorded as described previously.

3.14.11 Biochemical parameter

Level of proline, H_2O_2 , MDA, chlorophyll and caretenoid content were measured as described earlier.

3.14.11.1 Measurement of electrolyte leakage

Electrolyte leakage (EL) was estimated according to **Dionisio-Sese and Tobita (1998)**. Fresh leaf samples were washed with triple DW and cut into small pieces (~1cm segments) and suspended in test tubes containing 10 ml of de-ionized water. Tubes were incubated in a water bath at 32°C for 2h. After incubation, electrical conductivity (EC1) of the bathing solution was recorded with an electrical conductivity meter (Eutech Instruments, Con 510). These samples were then

autoclaved at 121°C for 20 min to completely kill the tissues and release the electrolytes. Samples were then cooled to 25°C, and final electrical conductivity (EC2) was measured. The percent leakage of electrolytes was calculated using the formula $(EC1/EC2) \times 100$.

3.14.11.2 Relative water content of leaves

Relative water content was estimated according to **Teulat *et al*, (2003)**. Fresh weight of leaves (FW) was recorded immediately after harvesting. The leaves were left in distilled water for 24 h at 4°C in darkness and the turgid weight was recorded (TW). The dry weight (DW) was then measured after 24 h at 60°C. The RWC was calculated according to **Barrs and Wetherley (1968)** as

$$RWC (\%) = [(FW-DW) / (TW-DW)] \times 100.$$

3.14.11.3 Leaf water loss (LWL) assay

Fresh weight (W1) of leaves was recorded. After cutting from seedlings, the leaves were left to evaporate under room condition for 2 h and reweighed (W2). LWL was calculated by $(W1-W2)/ W1 \times 100\%$.

3.14.11.4 Extraction of exopolysaccharide (EPS) from leaf tissue

3.14.11.4.1 Preparation of leaf extracts (Naidu, 1998)

Fresh leaf tissue (100–200 mg) was placed in a 10 ml plastic centrifuge tube to which 5 ml of ice cold methanol: chloroform: water (MCW; 60:25:15) was added to the plant material. The centrifuge tube was then maintained in a beaker containing ice and the contents were completely homogenized with mortar and pestle. Homogenization continued until fibrous plant material liberated most of the chlorophyll. At this point, cells would have broken down with the release of their contents including osmoprotectants. Cold MCW was added and the centrifuge tube was maintained in ice to reduce heat generation and possible break-down of solutes. The grinding head was washed with 5 ml DW and this was added to the MCW-plant homogenate in the centrifuge tube. Addition of water also helped to break the MCW emulsion. The contents of the tube were shaken on a test tube mixer for 10 s and the resulting homogenate was centrifuged at 100xg for 10 min at room temperature. The clear upper methanol-water (MW) phase was removed; its volume was measured and stored below 4°C for the estimation of exopolysaccharide.

Total carbohydrate content in the leaf extract was determined according to **Dubois *et al.* (1956)** as described earlier.

3.14.11.5 Anti-oxidative enzymes:

Activity of superoxide dismutase, ascorbate peroxidase, guaiacol peroxidase and catalase, was measured as described earlier.

Statistical analysis

Analysis of variance was performed in a one way randomized design for screening of PGPR on four crop experiment using two way randomized designs were used for varietal difference of pearl millet and bacterial drought tolerance studies. Analysis of variance in three way randomized designs was performed in rest of the studies. The means were compared at ($p < 0.05$) to detect significant differences among treatments.

*Results
and
Discussion*

4.1. Revival of Bacterial Strains and Their Morphological Characterization

Nine bacterial isolates used in this study were collected from Rhizospheric Biology Lab, Department of Biological Sciences. They were maintained in plates/ slants throughout the investigation. Pure cultures were preserved in slants at 4⁰C. Bacterial cultures were observed for their morphological characters. Gram reaction was done for observing cell morphology of bacteria (**Plate1**). Observations are presented in table no.4.1

Table 4.1 Morphological characteristics of selected bacterial strains

Characters	Bacterial strains								
	14B	8	8B	1B	13B	P3	9F	92	4
Colony morphology									
Colony size	M	M	L	L	S	M	S	L	M
Margin	U	E	U	U	E	E	E	E	U
Surface	Wr	Sm	Wr	Wr	Sm	Sm	Sm	Sm	Sm
Chromogenesis	Cr	W	Cr	Cr	Cr	F	W	W	W
Form	I	Cir	I	I	Cir	Cir	Cir	Cir	I
Cell Morphology									
Gram reaction	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve
Shape	Rod	Rod	Long Rod	Rod	Rod	Rod	Rod	Rod	Rod
Arrangement	Sc	Sc	chain	Sc	Sc	Sc	Sc	Sc	Chain

M– Medium, S– Small, L– Large, E – Entire, U– Undulated, Sm– Smooth, F-Florescent, Wr– Wrinkled, Cir– Circular, I– Irregular, W-white, Sc– Scattered, Cr-cream, F-Florescent

4.2 Growth Pattern of Bacterial Isolates in Nutrient Broth

Bacterial isolates showed different growth pattern in nutrient medium. All the bacteria showed their maximum growth within 24 h. Bacteria 8, 1B, 92 and P3 were fast grower showing their log phase between 11 to 13 h while bacteria 9f, 8B, 14B, 4 and 13B were slow grower as compared to rest of the bacteria showing their log phase between 13 to 16 h (**Fig. 4.1 a, b and c**)

4.3 pH Pattern of Nutrient Medium Inoculated with Bacterial Species

During the lag phase (4-5 h) of the bacterial growth, pH of the medium dropped from about 7.1 to 6.4. The pH of the medium gradually increased from 6.4 to 8.15 between 4 h and 16 h, but at 20 h the pH went up to 8.6 in all the isolates (**Fig. 4.2, a, b and c**).

4.4 Characterization of Rhizospheric Bacteria for Their Biochemical and Plant Growth Promoting Properties

4.4.1 Catalase test

Out of nine tests bacterial isolates, 14B and P3 showed maximum catalase production while bacterial isolate 92, 9f and 4 were found positive. Rest of the isolates did not show catalase production.

4.4.2 Lipase production

Only 14B and P3 showed positive test for lipase production, as zone of clearance were observed in the nutrient agar media supplemented with 1% tween 80. Rest of the isolate did not give positive result for lipase production (**Plate2A**).

4.4.3 Starch hydrolysis

All the isolates showed positive starch hydrolysis test except 8B. Bacterial isolate 14B and 4 showed maximum amylase production (**Plate2B**).

4.4.4 Siderophore production

All the isolates with orange colored zone around their colonies were found positive for siderophore production on Chrome azurol 'S' agar plates except 92 (*Bacillus cereus*). P3 and 14B showed maximum siderophore production followed by 1B and 4 (**Plate2C**).

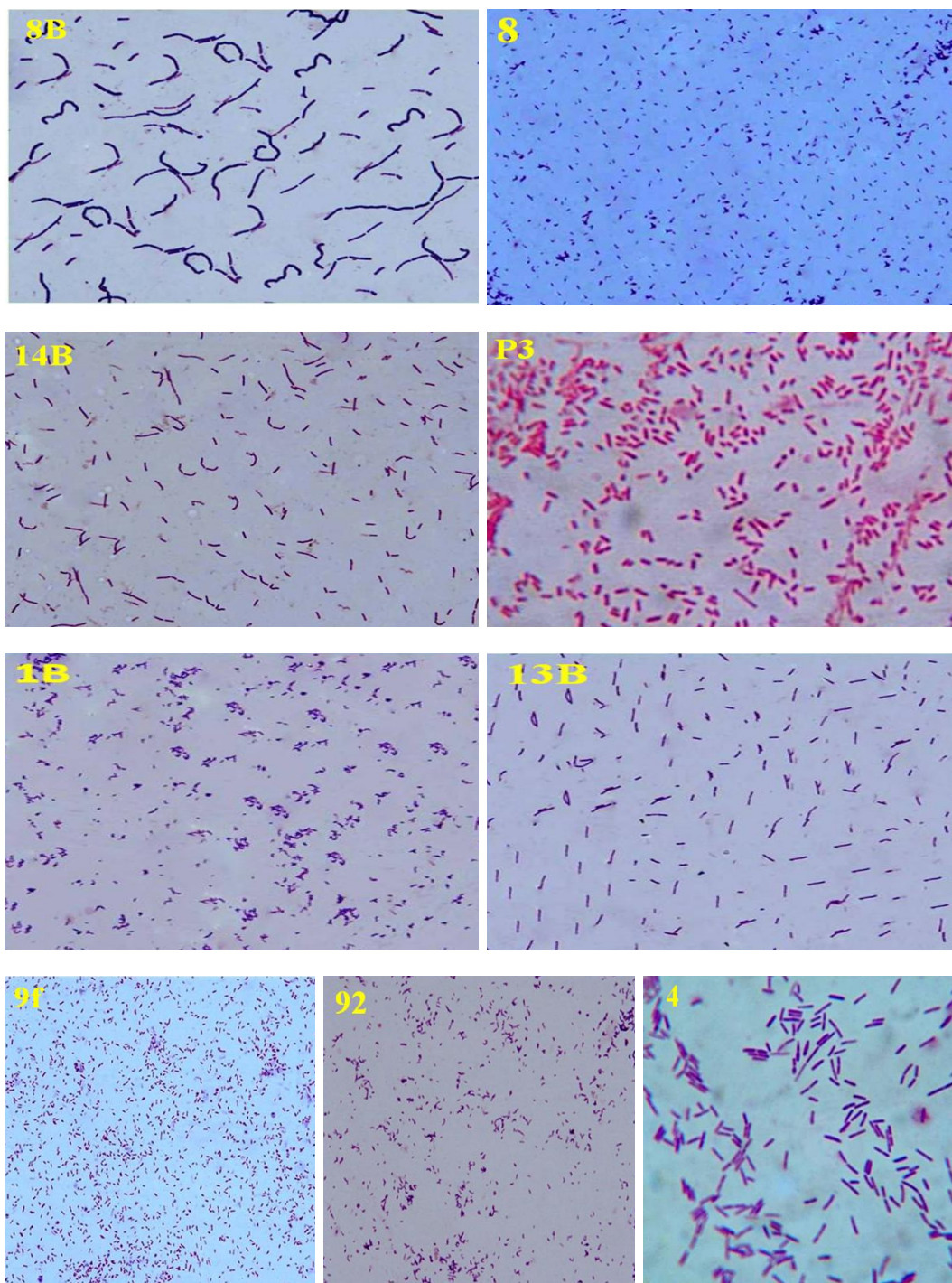


Plate 1: Cell morphology of bacterial species

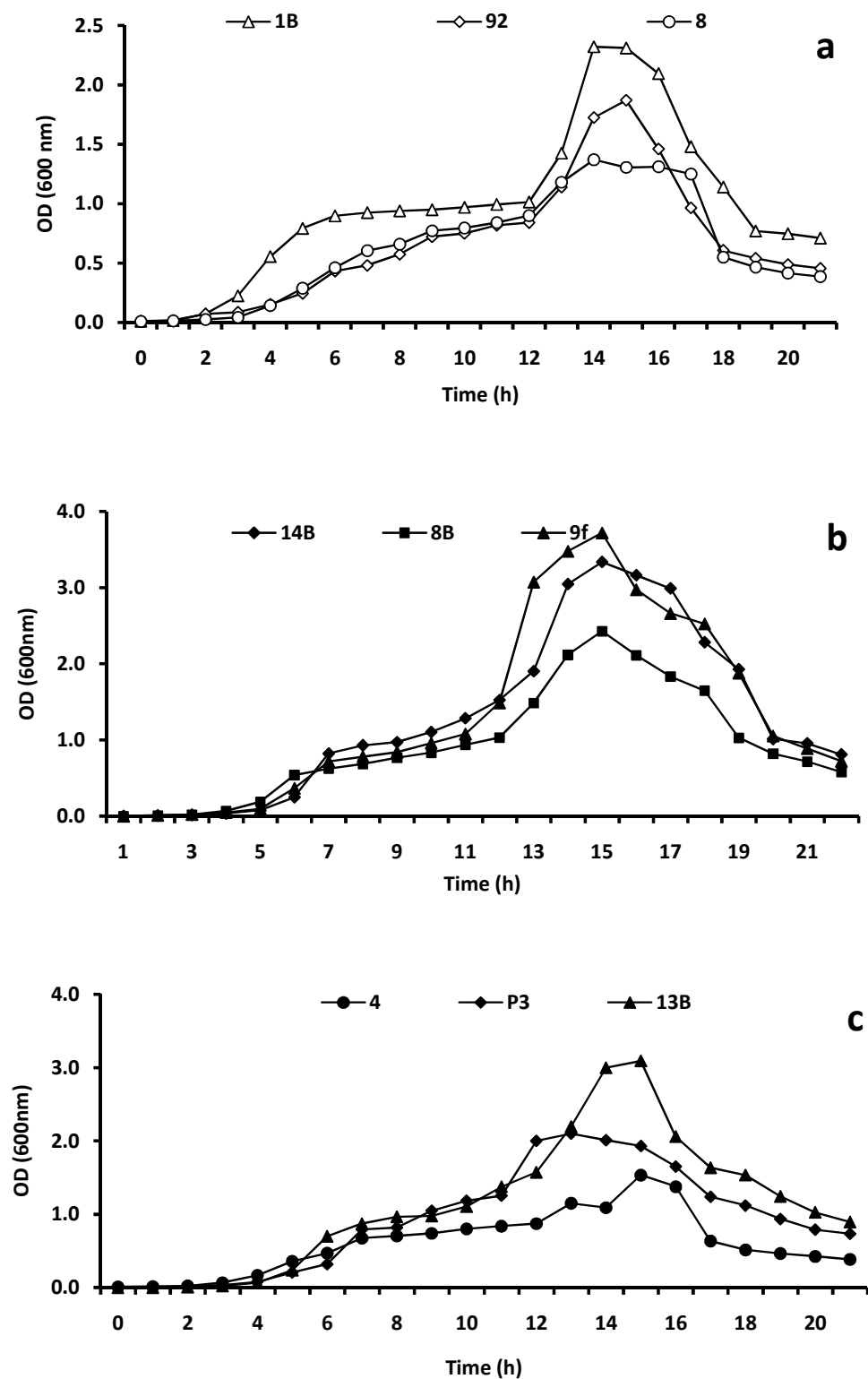


Fig. 4.1: a, b and c Growth of bacterial isolates in nutrient broth

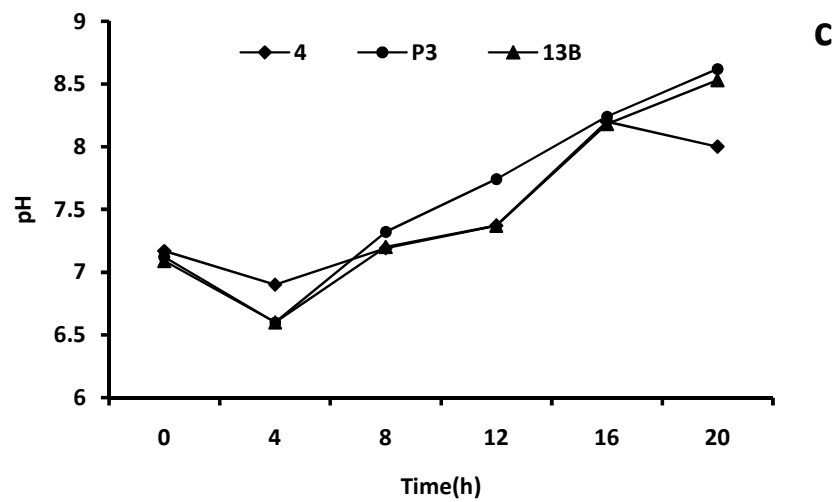
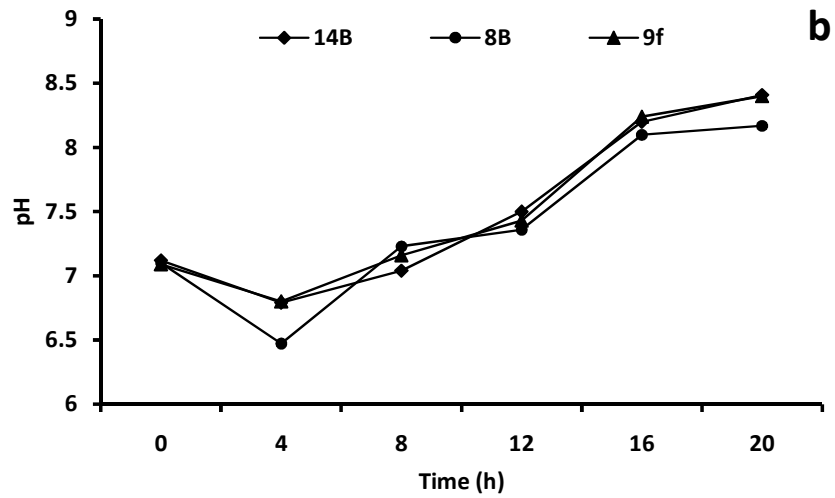
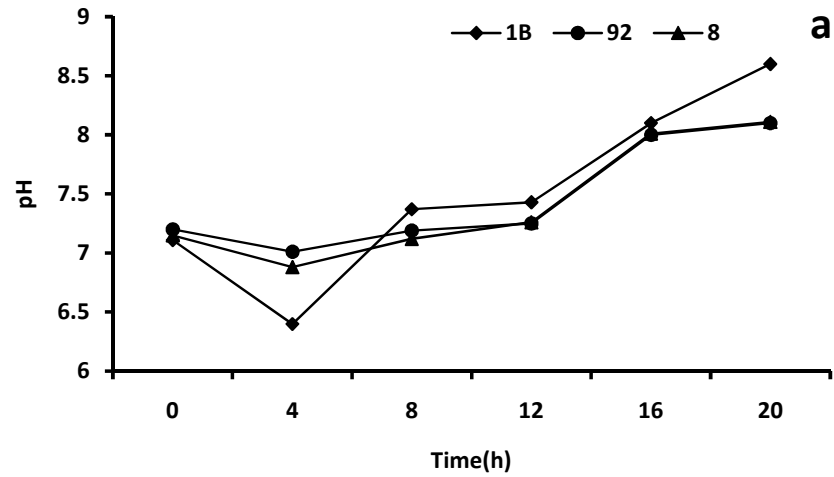


Fig. 4.2 a, b and c pH pattern of growing bacterial isolates in nutrient broth

4.4.5 Phosphate solubilization

Out of nine bacterial isolates, four (1B, 13B, 14B and P3) with clear zones around their colonies against milky background of Pikovaskya agar gave positive test for phosphate solubilization. P3 and 14B solubilized phosphate maximally (based on zone size) (**Plate2D**).

4.4.6 NH₃ production

P3 and 14B showed maximum production of NH₃. Bacterial isolate 4 and 1B produced a little amount of NH₃. Rest of the bacterial isolates did not show NH₃ production.

4.4.7 Indole acetic acid (IAA) production

All the bacterial isolates produced indole acetic acid in the presence of 100 µg/ml of tryptophan. The range of IAA production in all the test bacterial isolates was 0.516-3.36 µg/ml after 48 h of incubation in succinate broth. Maximum IAA production was recorded in P3 (3.37 µg/ml) and minimum in 13B (0.52 µg/ml) (**Fig. 4.3 and Plate2E**).

Table 4.2: Characterization of rhizospheric bacteria for biochemical and Plant growth promoting properties

Properties	Bacterial strains								
	14B	13B	1B	P3	92	9F	8B	8	4
Siderophore production	+++	+	++	+++	-	+	+	+	++
P-Solubilization	++	+	+	+++	-	-	-	-	-
Rhamnolipid production	-	-	-	+	-	-	-	-	-
Lipase production	+	-	-	++	-	-	-	-	-
Starch hydrolysis	++	+	+	+	+	+	-	+	++
Catalase test	++	-	-	++	+	+	-	-	+
IAA production	+++	+	+	+++	++	++	++	+	++
NH ₃ production	++	-	+	+++	-	-	-	-	+
+++ Good growth, ++ Moderate, + Survival, - No growth									

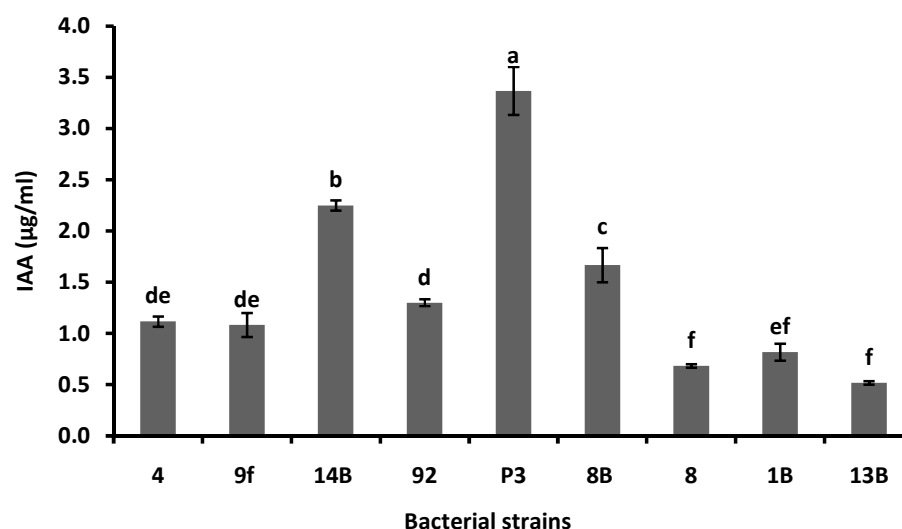


Fig. 4.3: IAA production by plant growth promoting rhizobacteria. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

Bacteria contributing towards sustainable plant growth promotion (directly or indirectly) are usually referred as plant growth promoting rhizobacteria (PGPR) or plant health promoting rhizobacteria (PHPR). By synthesizing hormones, siderophores, enzymes etc they facilitate the uptake of certain nutrients (Cakmakci *et al.*, 2006). Synthesis of antibiotics and antifungal compounds by PGPR help the plants indirectly preventing them from diseases (Jetiyanon and Kloepper, 2002; Glick, 2003; Guo *et al.*, 2004 and Saravanakumar *et al.*, 2009). Most of the test bacteria solubilized insoluble phosphorus and produced IAA, a growth hormone. P3 (*Pseudomonas fluorescens*) and 14B (*Bacillus cereus*) showed maximum plant growth promoting traits viz. siderophore, NH_3 , IAA production and phosphate solubilization. Several authors have isolated bacteria and characterized their biochemical and plant growth promoting properties (Vardharajula *et al.*, 2011; Saravanakumar *et al.*, 2011; Mohamed and Gomaa, 2012).

4.5. Effect of PGPR on Seed Germination

Pearl millet seeds treated with different bacterial strains revealed the significant improvement in germination rate over untreated seeds. In comparison to non treated seeds, 14B and P3 significantly enhanced (56.67 and 53.33% respectively) seed germination while minimum germination (16.67%) was recorded in treatment 8 and 13B (Fig. 4.4). Effect of plant growth promoting bacteria on seed germination in many crops is well documented by various authors (Liddycoat *et al.*, 2009; Vrbničanin *et al.*, 2011; Jha and Saraf, 2011).

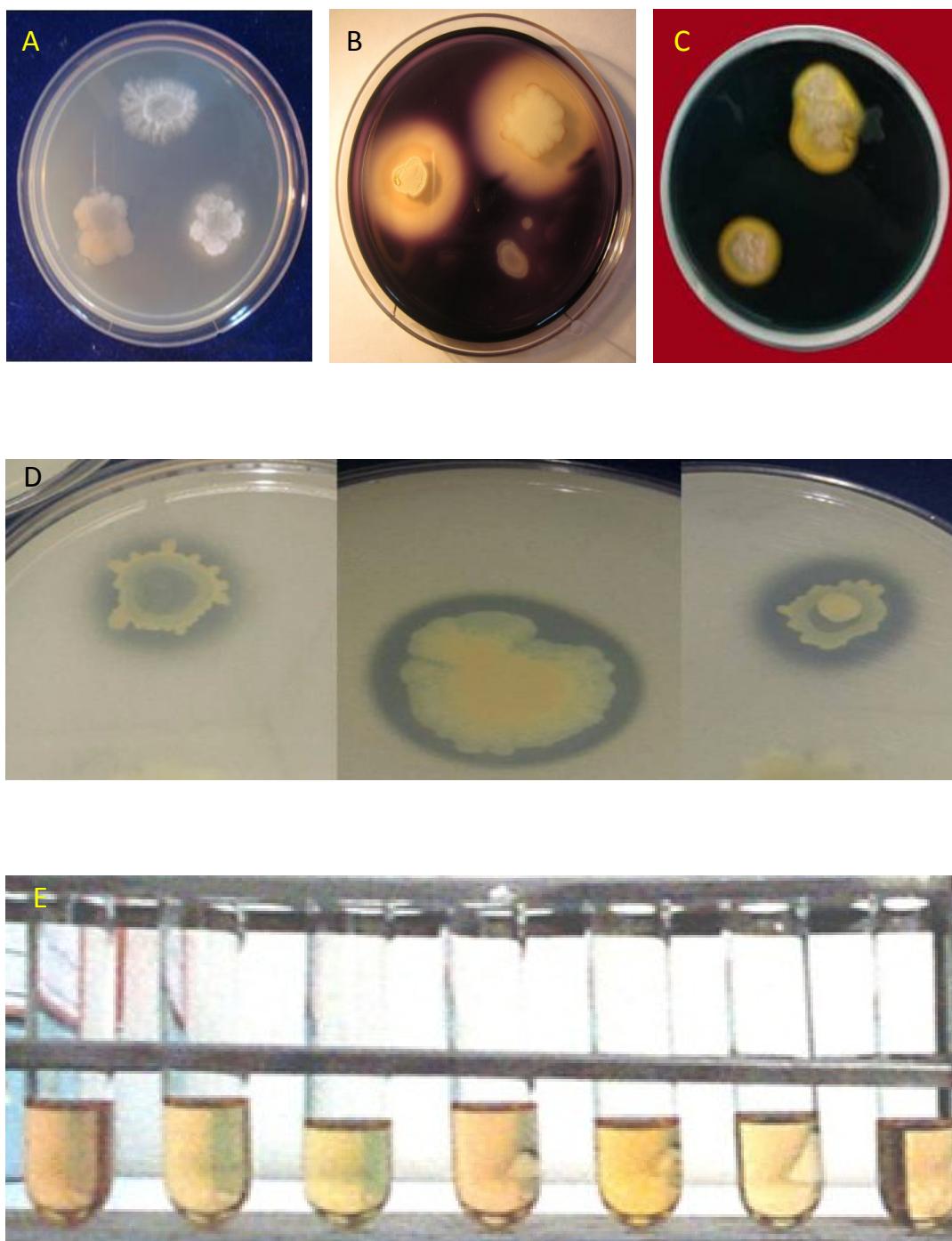


Plate 2: Biochemical tests, A- Lipase production, B- Starch hydrolysis, C- Siderophore production, D- Phosphate solubilization, E-IAA production

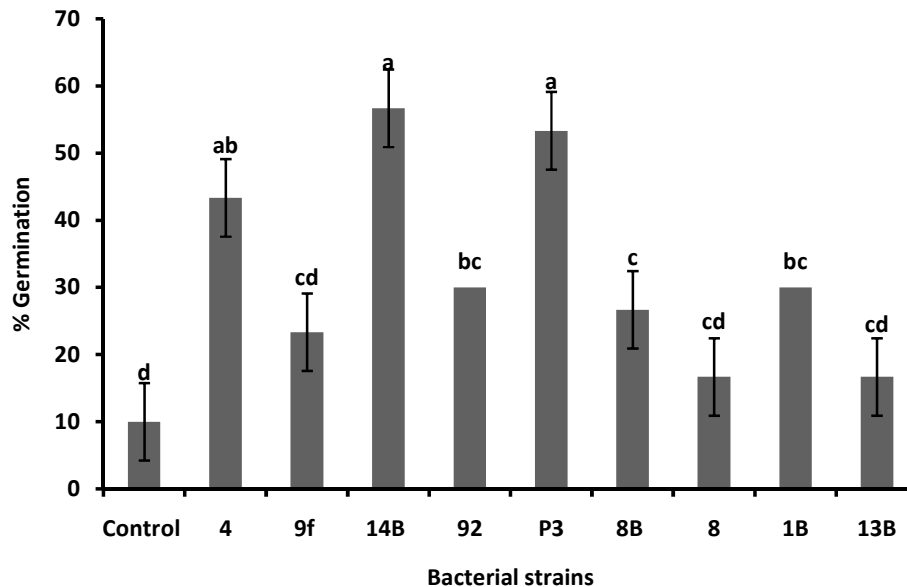


Fig. 4.4: Effect of bacterial cultures on seed germination of Pearl millet in 3 days after germination. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.6. Screening of PGPR on Four Crops in a Pot Experiment

The plant rhizosphere is a versatile and dynamic ecological environment of intense microbe–plant interactions for harnessing essential micro- and macro-nutrients from a limited nutrient pool (Jeffries *et al.*, 2003). A pot experiment was conducted to screen the most suitable crop among pearl millet, sorghum, guar and moth and the most responsive bacteria among nine test bacterial isolates. Effect of nine isolates on the growth of different varieties of four crops was observed. Each crop had shown different response, when grown in the presence of rhizospheric bacteria.

Table 4.3: Physicochemical properties of soil used in the pot experiment

Source	Soil texture	pH	EC	Moisture content (%)	OC content (%)	N (kg/ha)	P (kg/ha)	K (Kg/ha)
Ujhani, Distt. Badaun	Sandy	6.2	0.193	0.31	0.144	150.52	14.17	75.79

4.6.1 Effect of bacterial treatments on the plant growth promotion of pearl millet

Two varieties of pearl millet viz. CZIC923 and CZP2k9 were selected, for the pot experiment (**Plate3A and B**).

4.6.1.1 Plant height

4.6.1.1.1 Root length

In var. CZIC923, a maximum increase of 55.81% in treatment 14B in the root length was observed in pearl millet while 9f showed minimum root length (11.62%) which was found to be non-significant over control, 4, 92 and P3 treatment (**Fig. 4.5a**). Treatment P3 showed 185.71% increase in root length of var. CZP2k9 while treatment 4 showed minimum root length (44.2%) which was found to be non-significant over the control, 4 and 13B (**Fig. 4.6a**).

4.6.1.1.2 Shoot length

In case of var. CZIC923, maximum increase of 91.79% in shoot length was recorded in 14B treated pearl millet while minimum shoot length (31.7%) was observed in treatment 1B which was significant over the control but non-significant to 9f treatment (**Fig. 4.5b**). In var. CZP2k9, treatment of 14B increased shoot length by 74.04% as compared to control while minimum shoot length(14.88%) was recorded in 13B which was found to be non-significant over the control, 92 and 8 (**Fig. 4.6b**).

4.6.1.2 Plant fresh weight

4.6.1.2.1 Root fresh weight

In var. CZIC923 a significant increase of 238.46% was recorded in 14B treated plants while minimum root fresh was recorded in treatment 8 which was found to be non-significant over the control and treatment 4 (**Fig. 4.5c**). In var. CZP2k9, P3 increased root fresh weight by 220.40% followed by 9f (149.71%) and 14B (145.34%) as compared to control while rest treatment were non-significant over the control (**Fig. 4.6c**).

4.6.1.2.2 Shoot fresh weight

In case of variety CZIC923, a significant increase of 297.60% was recorded in 14B treated pearl millet as compared to control which was non-significant as compared to 13B while minimum shoot fresh weight was observed in 9f (61.87%)



A- Pearl millet (var. CZIC-923)

B- Pearl millet (var. CZP-2K9)



C- Sorghum (var. PC23)

D- Moth (var. CZM1)

E- Moth (var. CZM3)

F- Guar (var. RGC936)

Plate 3: Effect of PGPR on plant growth

which was non-significant over 1B treatment (**Fig. 4.5d**). In var. CZP2k9, a significant increase of 139.60% was observed in treatment P3 followed by 14B (131.11%) and 9f (112.46%) as compared to control while minimum shoot fresh weight (23.88%) was recorded in 13B which was found to be non-significant over the control, 92 and 4 (**Fig. 4.6d**).

4.6.1.3 Plant dry weight

4.6.1.3.1 Root dry weight

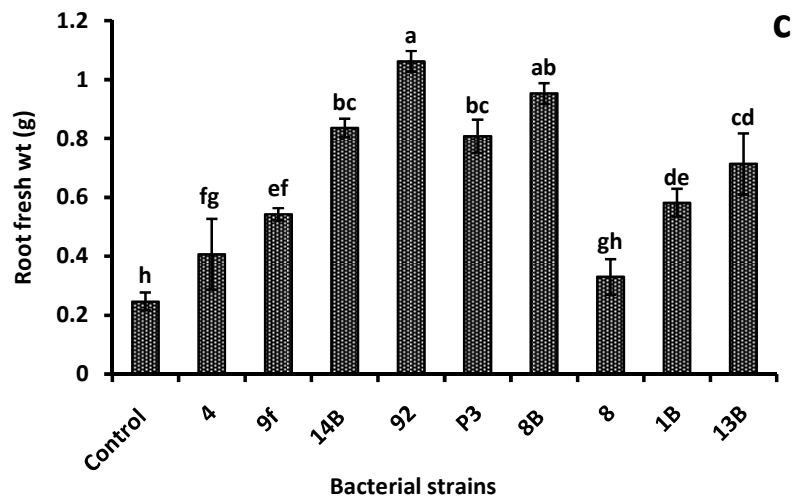
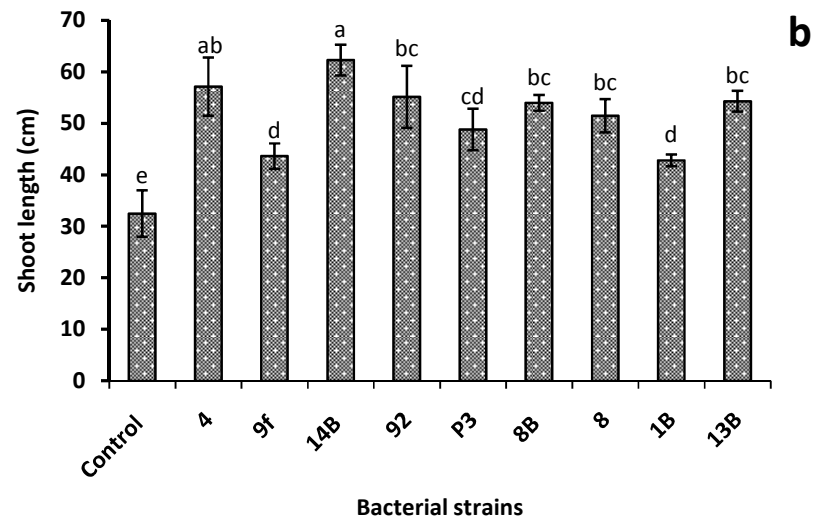
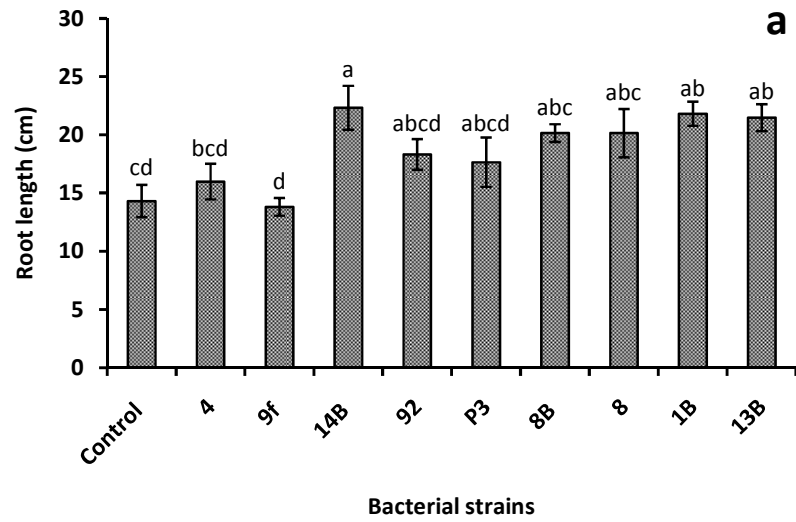
In pearl millet var. CZIC923, a significant increase of 280.55% in treatment 14B followed by treatment 92 (265.45%) was recorded as compared to control while it was found to be non-significant to treatment P3, 8B, 8 and 1B. Minimum root length was observed in 13B (118.18%) which was found to be significant as compared to control but non-significant with rest of the treatment except 14B and 92 (**Fig. 4.5e**). In var. CZP2k9, a maximum increase of 531.51% was observed in treatment P3 as compared to control but found to be non-significant over 1B treatment (500%). Minimum root dry weight (92.3%) was recorded in treatment 13B which was found to be non-significant over the control and treatment4 (**Fig. 4.6e**).

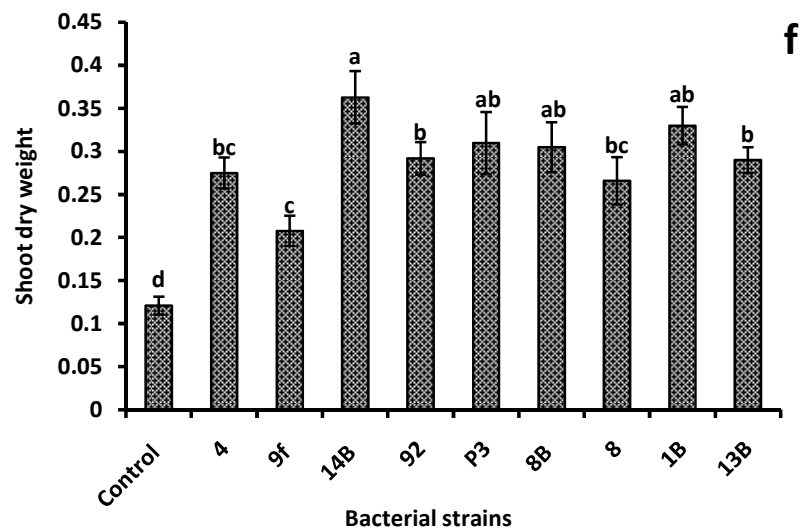
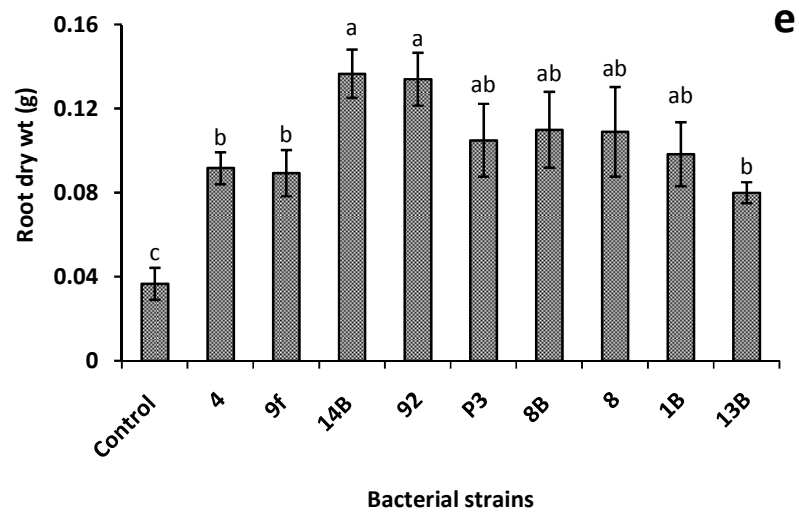
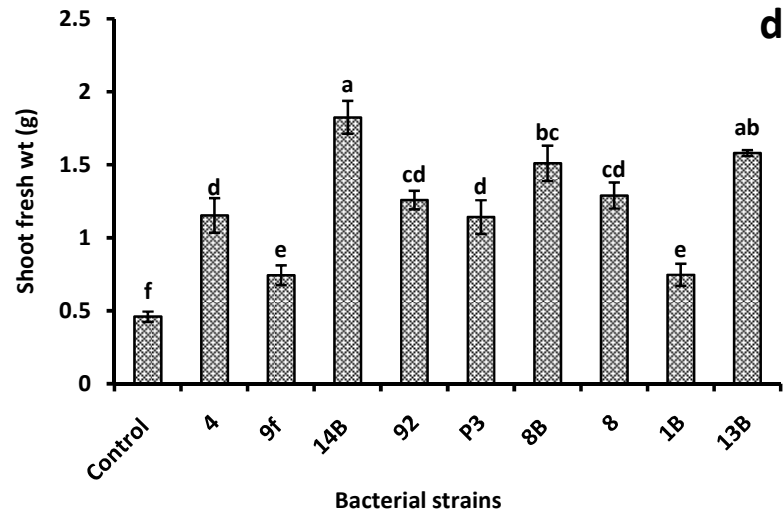
4.6.1.3.2 Shoot dry weight

In pearl millet var. CZIC923, a significant increase of 156.19% was recorded in 14B treatment as compared to control but it was found to be non-significant over the treatment 8B, P3 and 1B. Minimum shoot dry weight (68.7%) was recorded in 9f which was significant as compared to control but found to be non-significant over the treatment 4 and 8 (**Fig. 4.5f**). In var. CZP2k9, 146.15% increase was observed in treatment P3 as compared to control while it was non-significant over the treatment 8B and 8. A non-significant decrease of 21.25 and 18.88% in shoot dry weight was recorded in treatment 92 and 13B as compared to control (**Fig. 4.6f**).

4.6.1.4 Root volume

A significant increase of 379.61% in root volume of var. CZIC923 in treatment 14B was recorded as compared to control but non-significant over the treatment 1B. Minimum root volume was recorded in P3 (120%) which was found to be non-significant over the control and other treatments except 14B and 4 (**Fig. 4.5g**). In var. CZP2k9, a significant increase of 257.14% in treatment P3 was observed as





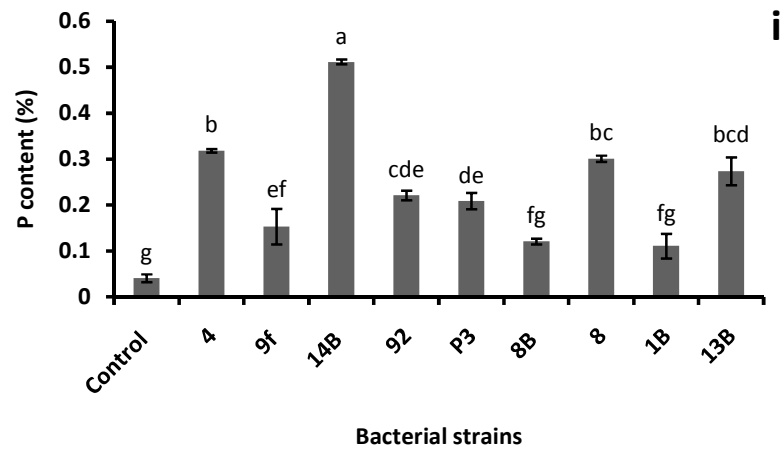
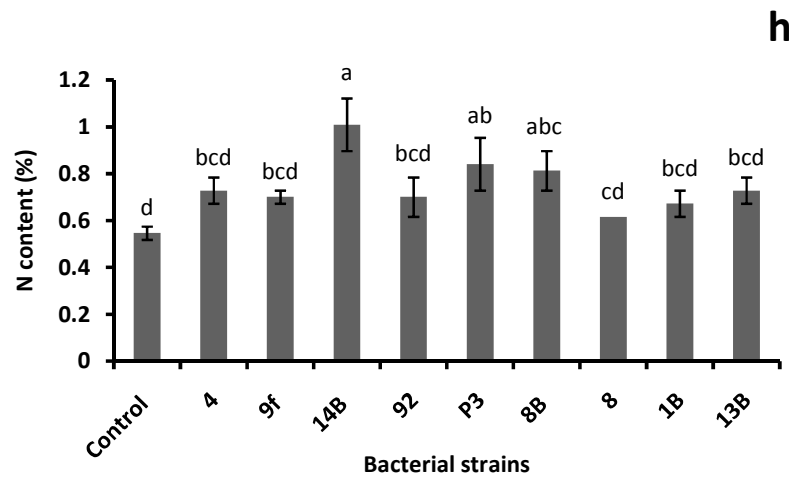
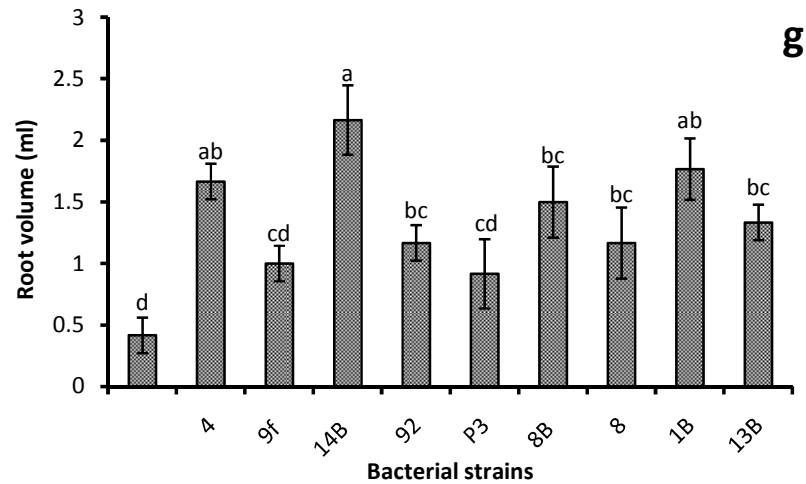
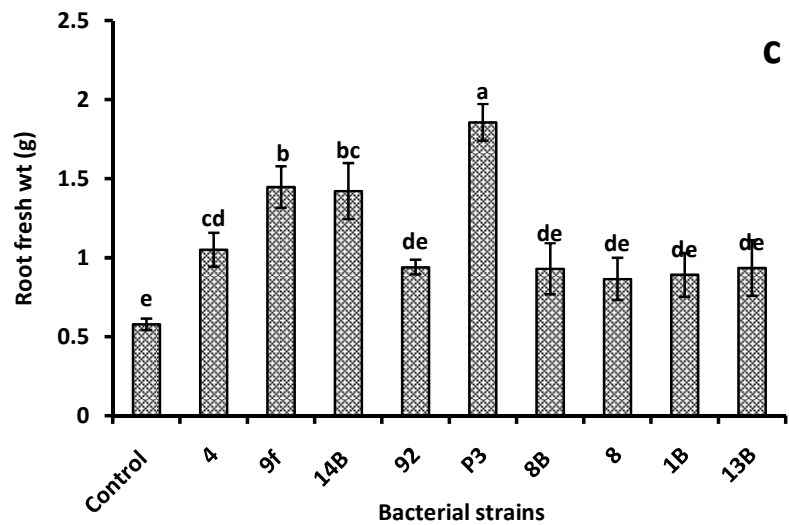
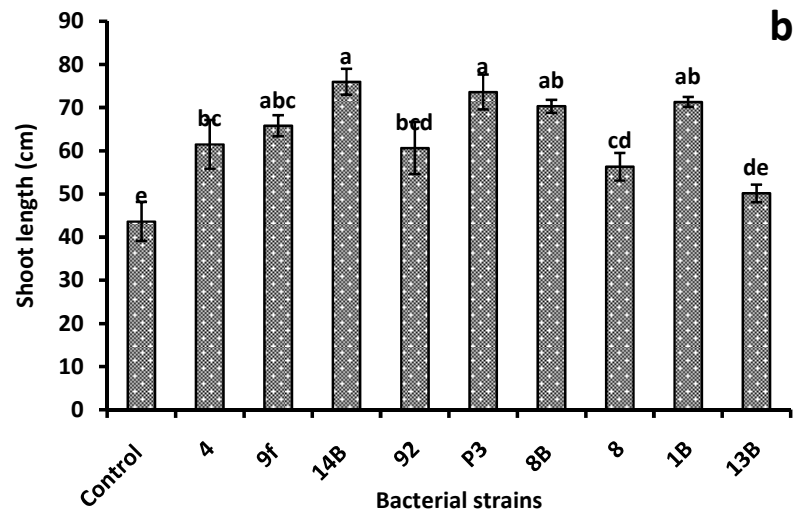
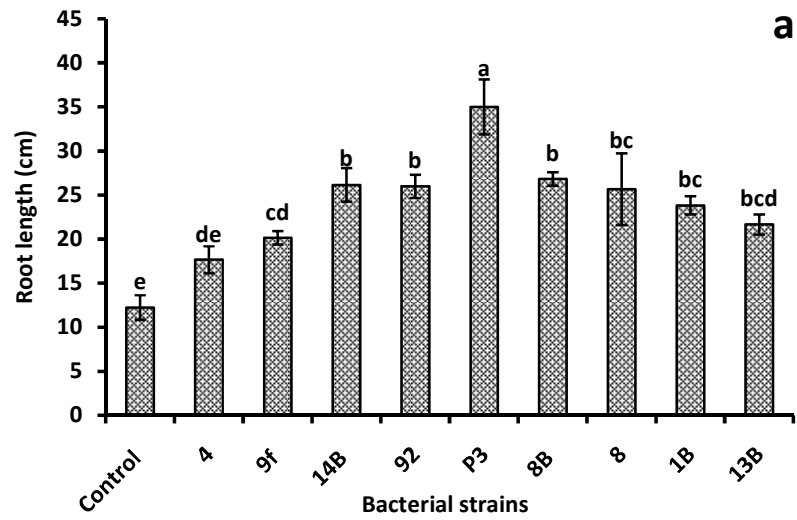
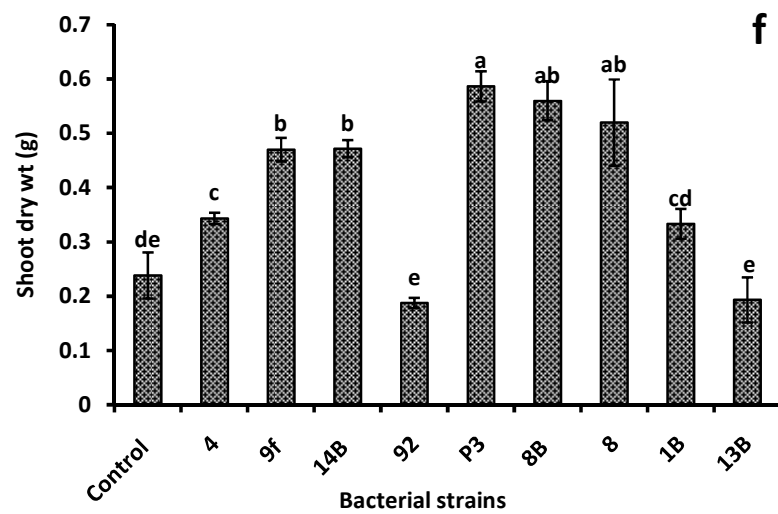
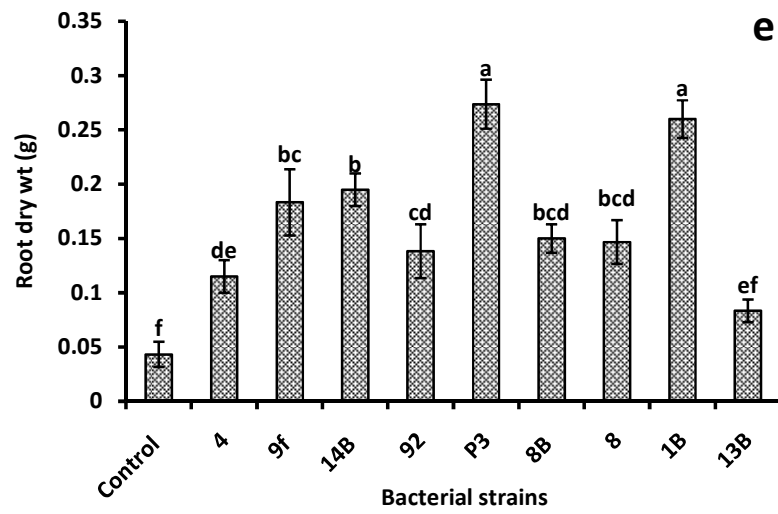
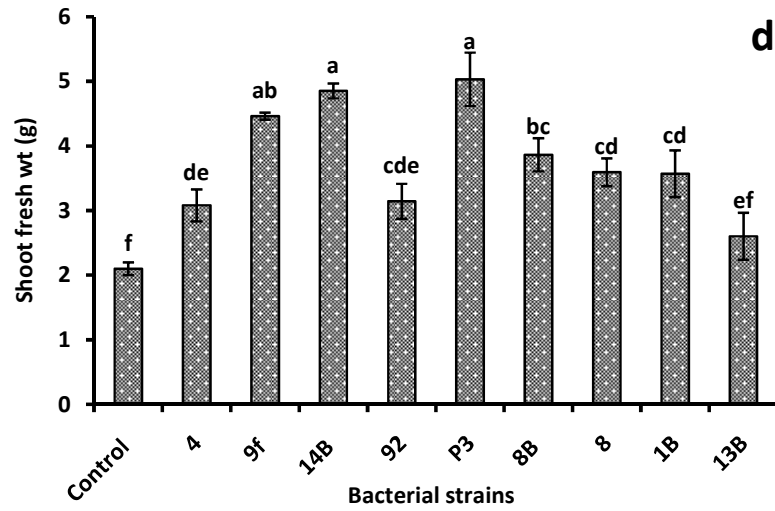


Fig. 4.5: Growth promotion of Pearl millet var.CZIC923 inoculated with PGPR. (a) Root length; (b) Shoot length; (c) Root fresh wt; (d) Shoot fresh wt; (e) Root dry wt.; (f) Shoot dry wt.; (g) Root volume; (h) N content (%); (i) P content (%) Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$





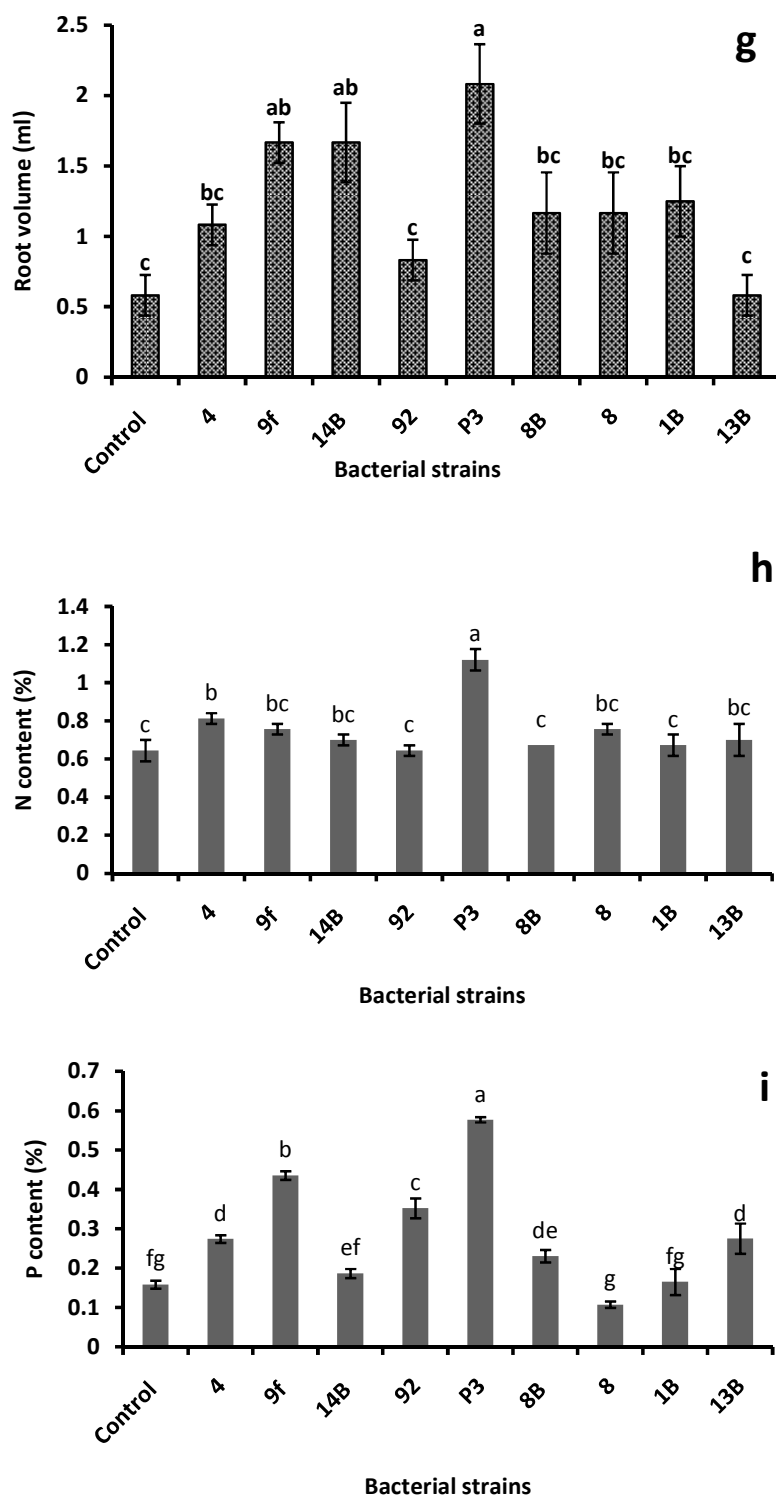


Fig. 4.6: Growth promotion of Pearl millet var.CZP2k9 inoculated with PGPR. (a) Root length; (b) Shoot length; (c) Root fresh wt; (d) Shoot fresh wt; (e) Root dry wt.; (f) Shoot dry wt.; (g) Root volume; (h) N content (%); (i) P content (%) Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

compared to control but non-significant over the treatment 9f and 14B. Minimum root volume was observed in 13B which was non-significant over the control and other treatment except P3, 14B and 9f (**Fig. 4.6g**).

4.6.1.5 Nitrogen (N) content in shoot (%)

An increase of 84.64% in N content was observed in treatment 14B while lowest N content (11.36%) was recorded in treatment 8 as compared to control in pearl millet var. CZIC923. In var. CZP2k9, P3 has shown 73.91% (maximum) increase, however treatment 92 showed lowest N content as compared to other treatments (**Fig. 4.5 h & 4.6h**).

4.6.1.6 Phosphorus (P) content in shoot (%)

An increase of 1151.28% in P content of pearl millet (var. CZIC923), was reported in treatment 14B. A minimum (170.51%) increase in P content was recorded in treatment 1B in the same variety. In case of var. CZP2k9, 14B significantly increased P content by 264.56%. Treatment 8 could not support enhancement of P content when compared to control (**Fig. 4.5i & 4.6i**).

4.6.2 Effect of bacterial treatments on the plant growth promotion of sorghum

A single var. PC23 of sorghum was used for screening the suitable crop and potent PGPR (**Plate3C**).

4.6.2.1 Plant height

4.6.2.1.1 Root length

A significant increase of 127.65% in treatment 4 was observed as compared to control but found to be non-significant over the treatment 92. Minimum root length (43.08%) was recorded in treatment 8B which was found to be non-significant over the rest of the treatments except 4 and 92 (**Fig. 4.7a**).

4.6.2.1.2 Shoot length

Treatment 4 significantly affected shoot length of sorghum as increase of 83.73% was recorded as compared to control while it was found to be non-significant with rest of the treatments. All the treatments had similar effect on shoot length (**Fig. 4.7b**).

4.6.2.2 Plant fresh weight

4.6.2.2.1 Root fresh weight

A significant increase of 349.69% was recorded in 13B treated sorghum plants as compared to control while it was non-significant over the treatment 8. Minimum root fresh weight (175.15%) was recorded in treatment 1B which was significant over the control while non-significant over the rest of the treatments except 14B, 8 and 13B (**Fig. 4.7c**).

4.6.2.2.2 Shoot fresh weight

A significant increase of 634.69% was recorded in treatment 4 as compared to control. A similar effect of all the treatments except treatment 4 on shoot fresh weight was found which was significant over the control (**Fig. 4.7d**).

4.6.2.3 Plant dry weight

4.6.2.3.1 Root dry weight

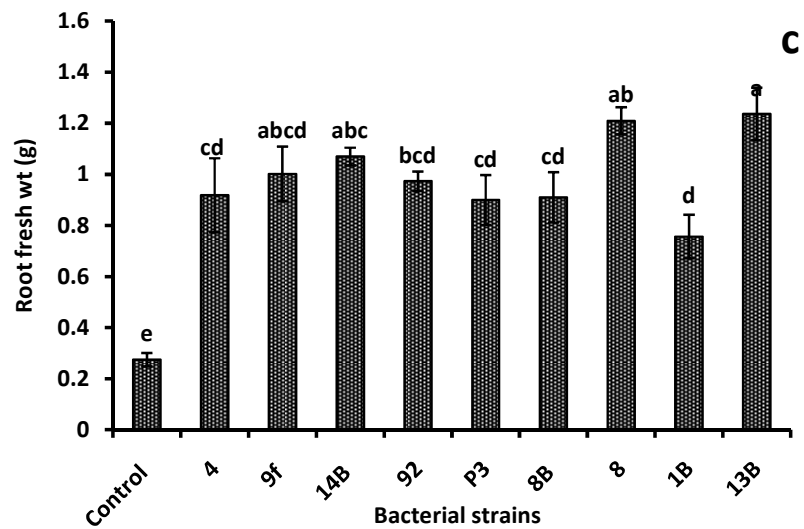
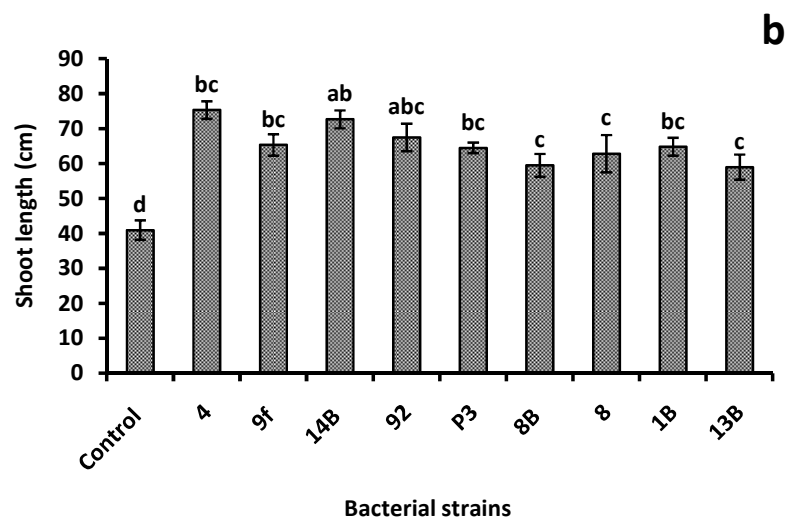
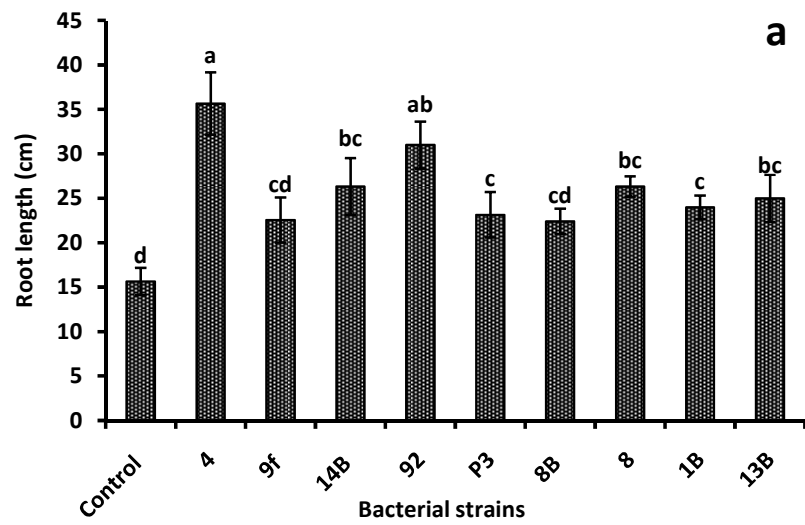
A significant increase of 352.58% was observed in treatment 8 as compared to control which was found to be non-significant over the treatment 1B. Minimum root length (48.7%) was observed in treatment 9f which was significant as compared to control but found to be non-significant over the rest of the treatments except 8, 1B and 13B (**Fig. 4.7e**).

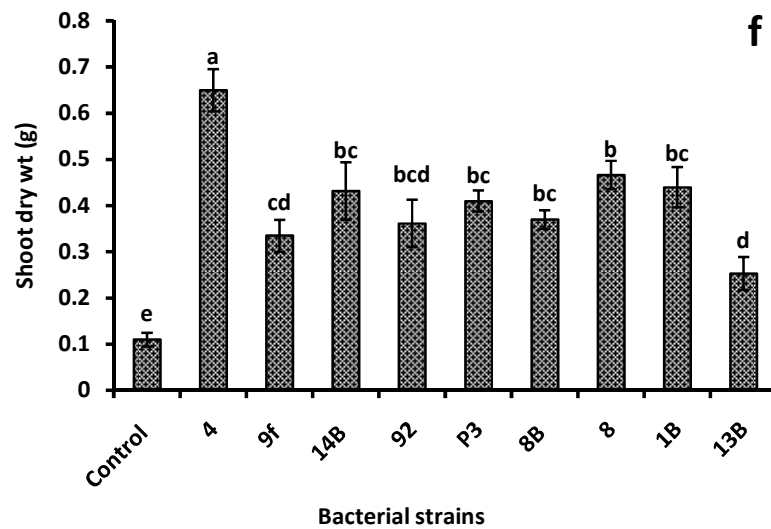
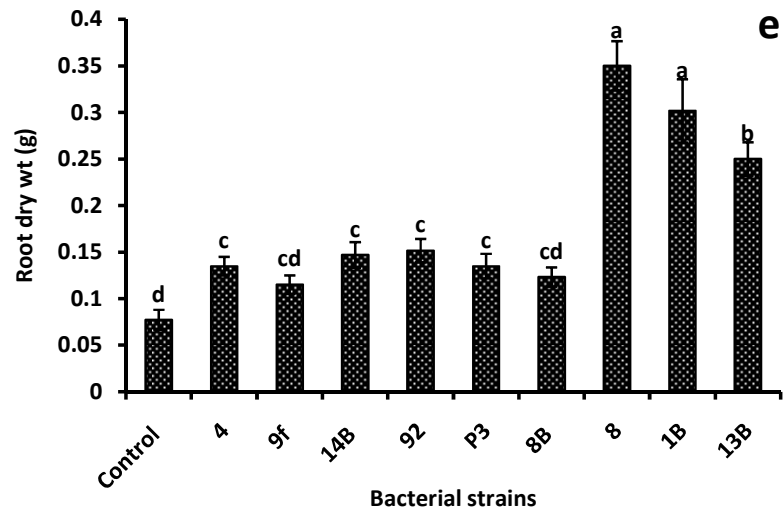
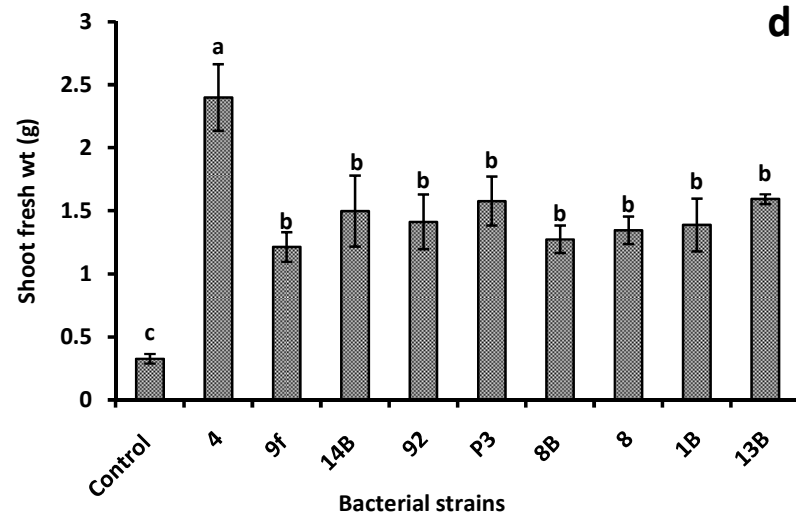
4.6.2.3.2 Shoot dry weight

Treatment 4 in sorghum showed 490.90% increase as compared to control. Minimum shoot dry weight (130.3%) was observed in treatment 13B which was found to be significant over the control but non-significant when compared with treatment 9f and 92 (**Fig. 4.7f**).

4.6.2.4 Root volume

A significant increase of 207.69% in treatment 4 was observed as compared to control while it was found to be non-significant over the other treatments. All the treatments had similar effect on root volume (**Fig. 4.7g**).





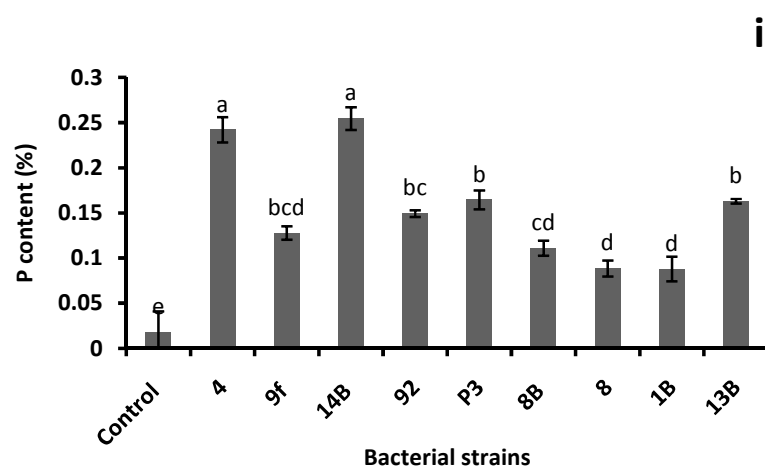
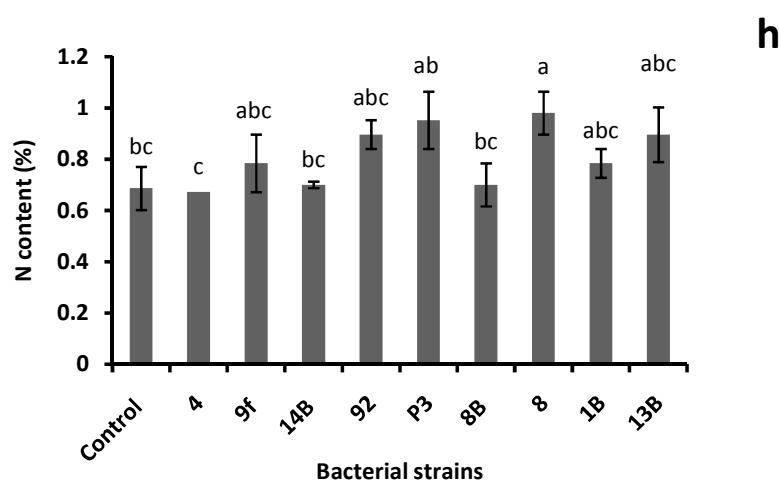
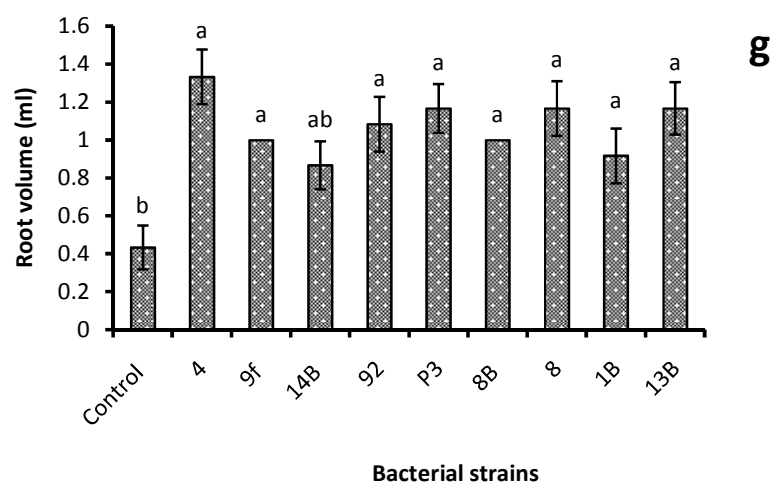


Fig. 4.7: Growth promotion of Sorghum var. PC23 inoculated with PGPR. (a) Root length; (b) Shoot length; (c) Root fresh wt; (d) Shoot fresh wt; (e) Root dry wt.; (f) Shoot dry wt.; (g) Root volume; (h) N content (%); (i) P content (%) Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.6.2.5 N content in shoot (%)

In sorghum (var.PC23), a significant increase of 42.85% in N content in treatment 8 was observed while a non-significant decrease in N content was observed in treatment 4 as compared to control and rest of the treatments except 8 and P3 (**Fig. 4.7h**).

4.6.2.6 P content in shoot (%)

A maximum increase of 1353.95% was observed in sorghum P content in treatment 14B which was significant as compared to control while non-significant to treatment 4. A minimum increase of 402.60% in was recorded in treatment 1B as compared to control but non-significant to treatment 8 (**Fig. 4.7i**).

4.6.3 Effect of bacterial treatments on the plant growth promotion of moth

Two varieties of moth (CZM1 and CZM3) were selected to observe the effect of PGPR on plant vigor in the pot experiment (**Plate3D and E**).

4.6.3.1 Plant height

4.6.3.1.1 Root length

In moth var. CZM1, a significant increase of 45.37% in treatment 8B was observed while it was found to be non-significant to rest of the treatments except control, 4 and 8 (**Fig. 4.8a**). In var. CZM3, there was no effect of bacterial treatments as compared to control while a significant decrease of 38.98 and 42.37% was recorded in treatment 9f and 13B respectively as compared to control (**Fig. 4.9a**).

4.6.3.1.2 Shoot length

In moth var. CZM1, a significant increase of 56.90% was recorded in P3 treatment as compared to control while it was found to be non-significant to rest of the treatments except 14B, 9f and 8 (**Fig. 4.8b**). In var. CZM3, there was no visual effect of bacterial treatment on shoot length when compared with control except in treatment 1B where a significant decrease of 24.26% was observed (**Fig. 4.9b**).

4.6.3.2 Plant fresh weight

4.6.3.2.1 Root fresh weight

In moth var. CZM1, a significant increase of 228.57% was recorded in 1B as compared to control while non-significant to treatment 4. There was no effect of

treatment 9f and 92 on root fresh weight as both were found to be non-significant over the control (**Fig. 4.8c**). In var. CZM3, P3 treatment has increased root fresh weight by 99.9% as compared to control while rest of the treatments were found to be non-significant as compared to control (**Fig. 4.9c**).

4.6.3.2.2 Shoot fresh weight

In variety CZM1, 85.50% increase was recorded in 8B treatment which was found to be non-significant to rest of the treatments except 9f, 14B, and 13B. Treatment 9f and 14B were ineffective as they were non-significant to control (**Fig. 4.8d**). In var. CZM3, only 22.61% increase was observed in treatment 8 as compared to control while non-significant to the treatment P3. A significant decrease of 12.53 and 23.4% was recorded in treatment 4 and 1B respectively as compared to control. Rest of the treatments was non-significant to control (**Fig. 4.9d**).

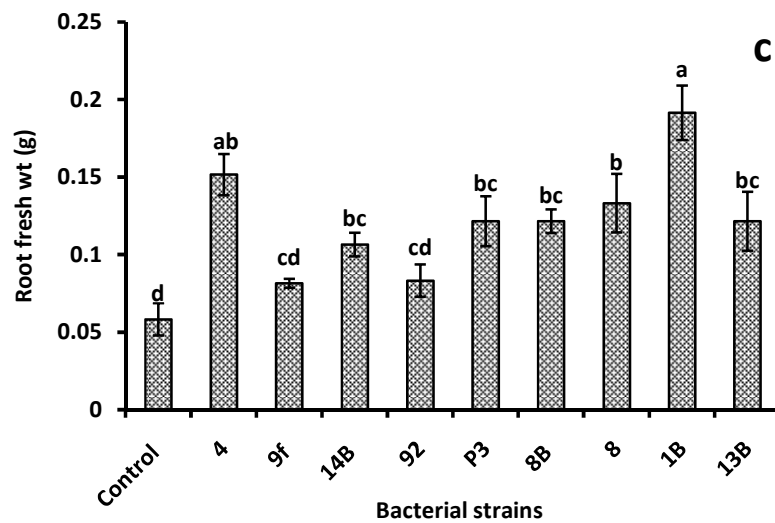
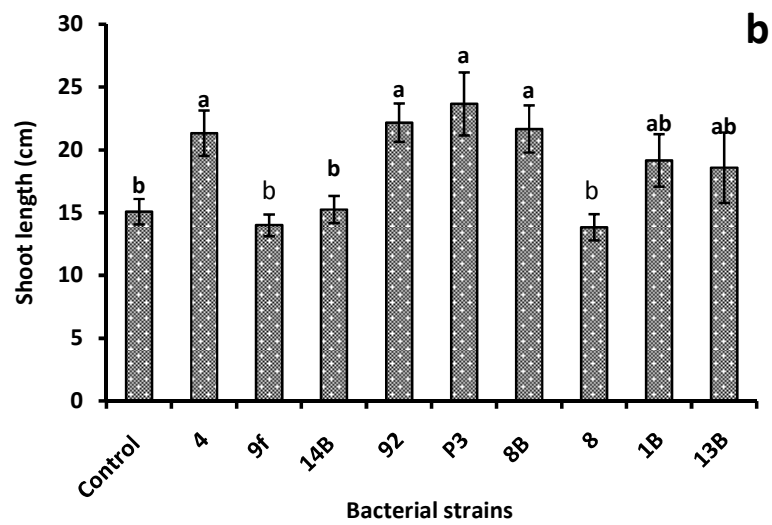
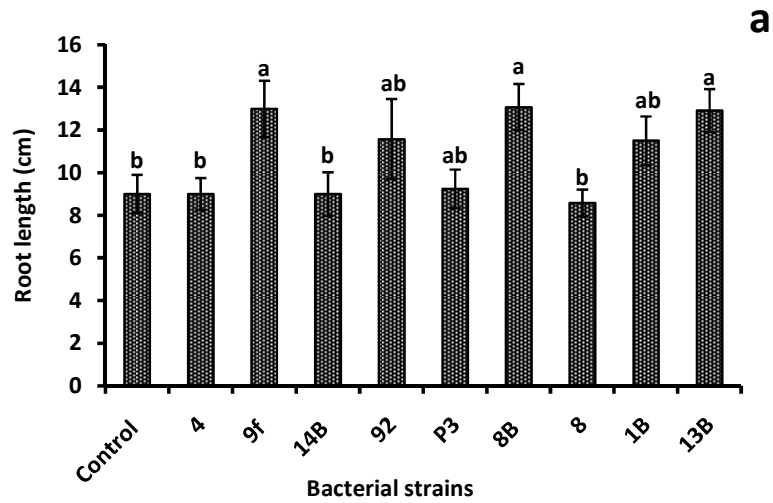
4.6.3.3 Plant dry weight

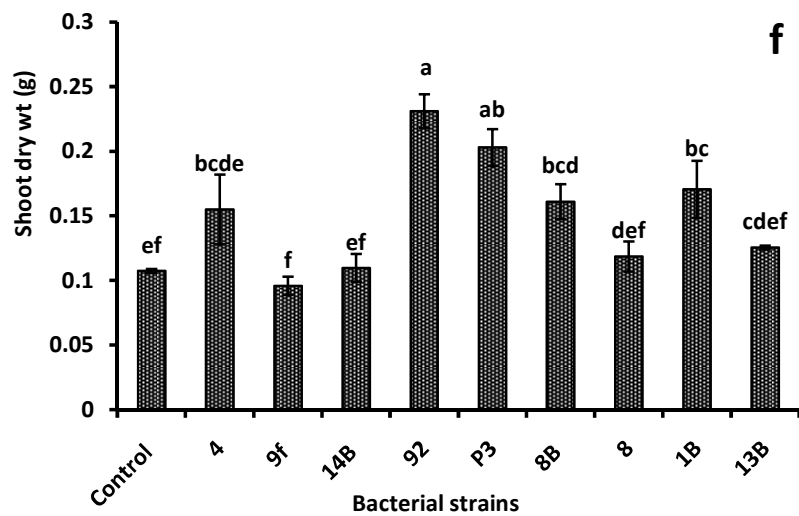
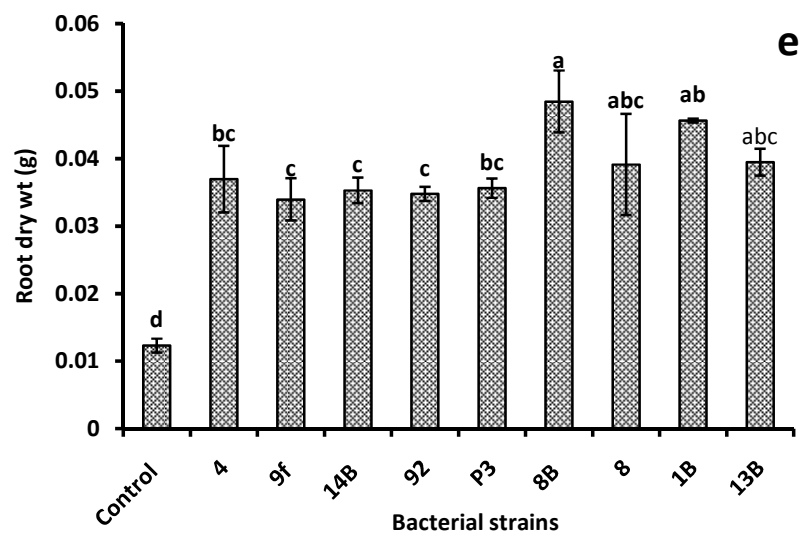
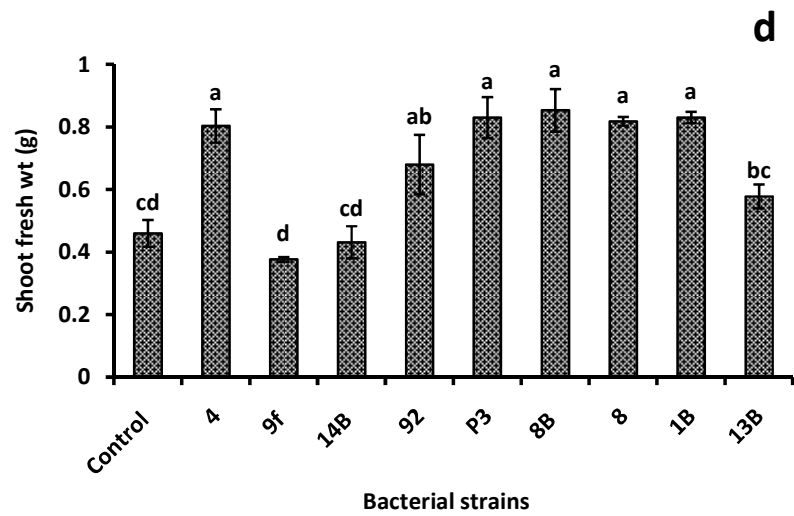
4.6.3.3.1 Root dry weight

In var. CZM1, a significant increase of 293.24% in root dry weight was reported in treatment 8B as compared to control while it was found to be non-significant to the treatments 8, 1B and 13B. Rest of the treatments was significant to control while non-significant to each other (**Fig. 4.8e**). In var. CZM3 treatment 8B and P3 showed a significant increase of 56.56% in root dry weight of over control but non-significant to treatment 4, 8 and 1B while rest of the treatments was non-significant over the control (**Fig. 4.9e**).

4.6.3.3.2 Shoot dry weight

In CZM1 var. of moth, treatment 92 showed a significant increase of 115.21% was recorded as compared to control while it was found to be non-significant over P3. Treatment 4, 9f, 14B, 8 and 13B were found to be non-significant as compared to control (**Fig. 4.8f**). In var. CZM3, treatment P3 and 92 showed enhancement of shoot dry weight as only 28.87% increase was observed in both the treatments as compared to control. Rest of the treatments was found to be non-significant as compared to control except treatment 13B (**Fig. 4.9f**).





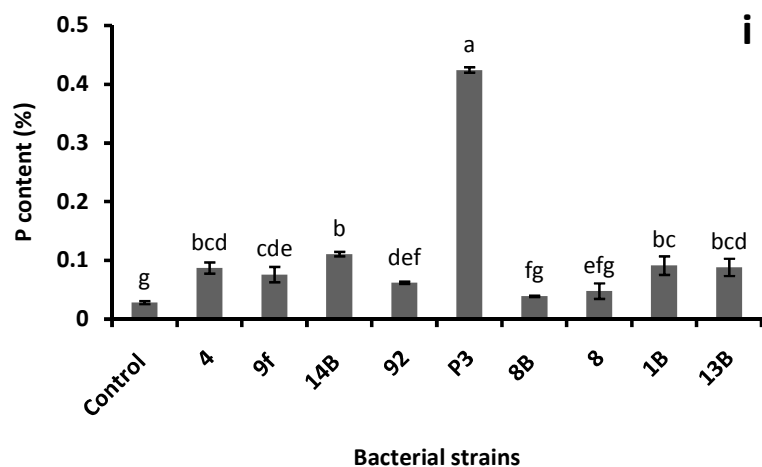
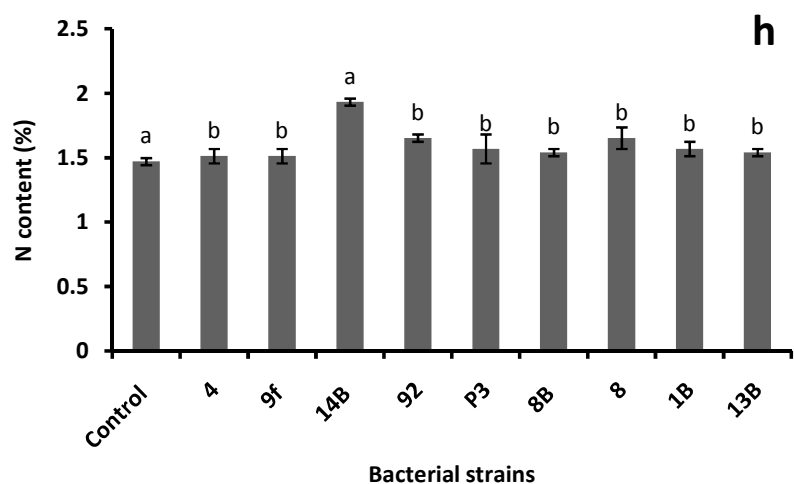
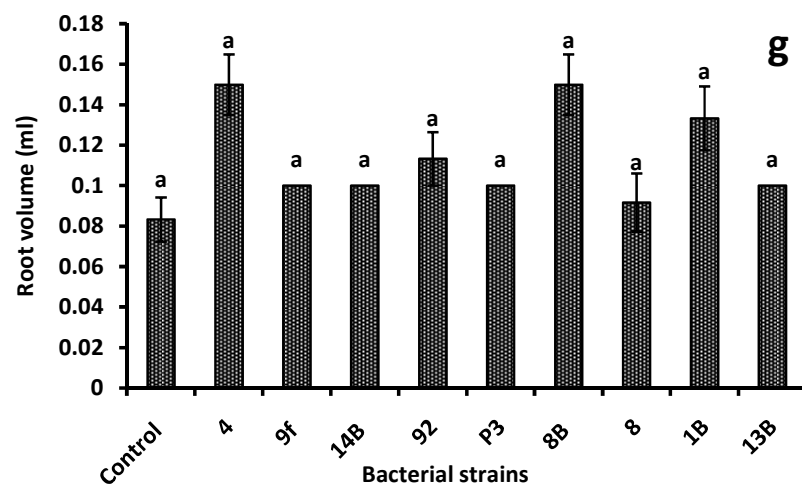
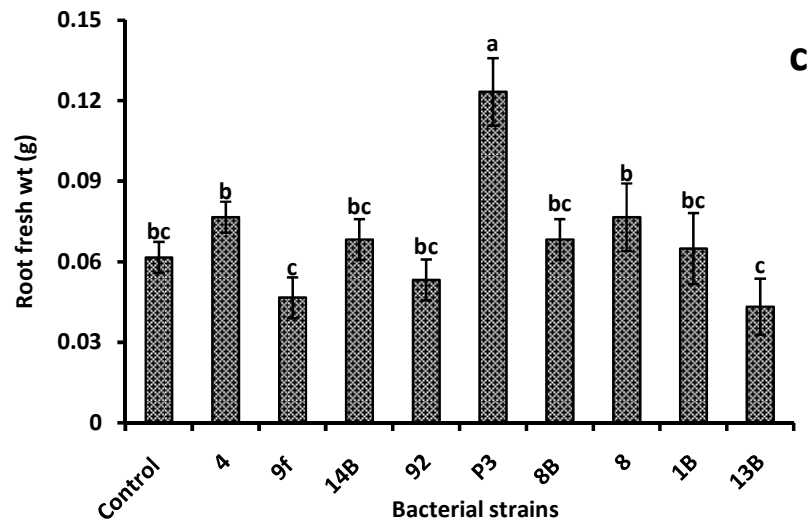
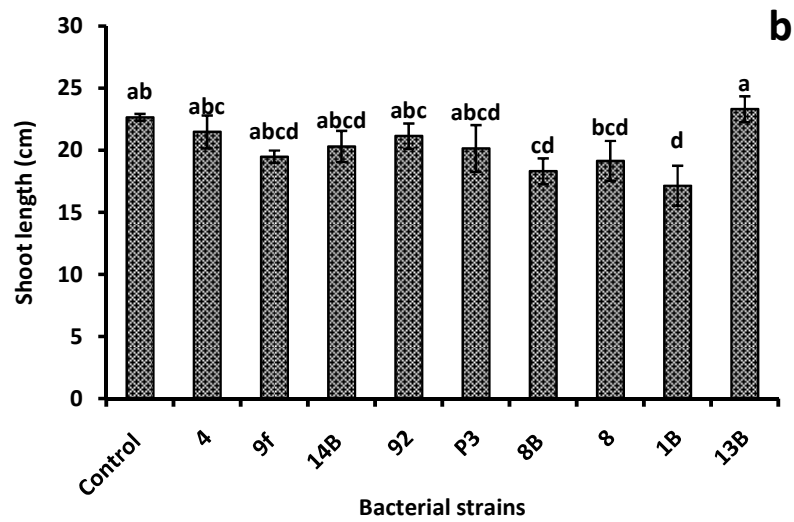
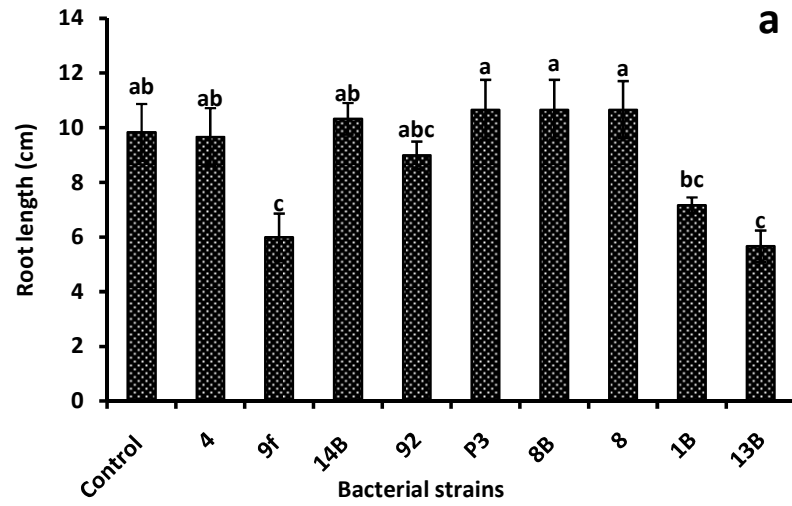
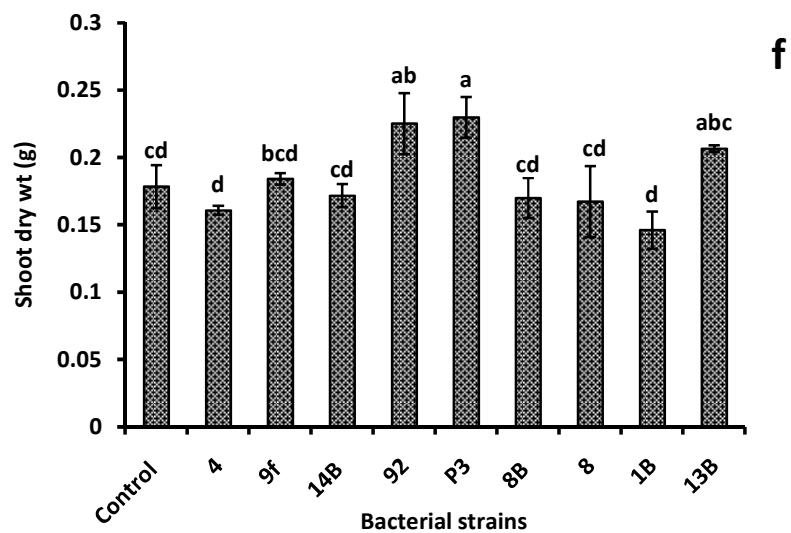
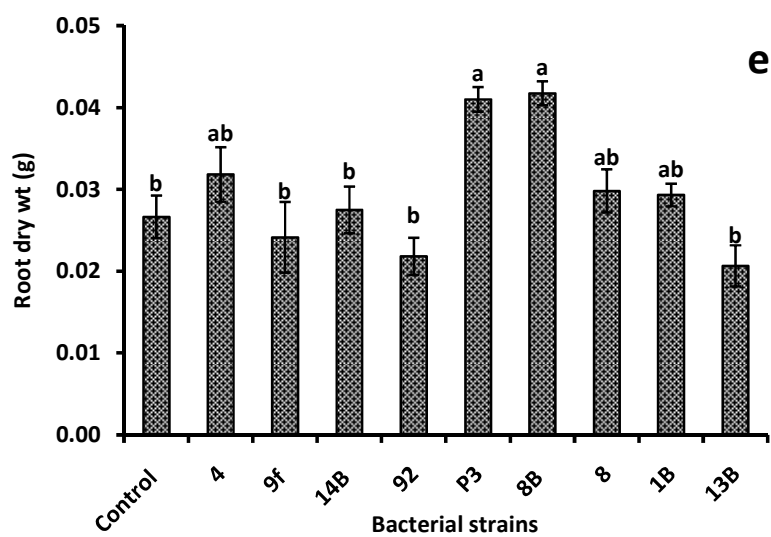
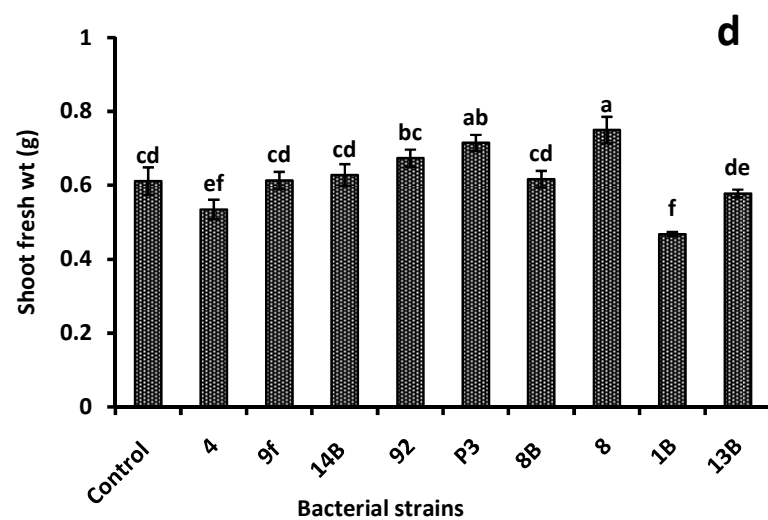


Fig. 4.8: Growth promotion of Moth var. CZM1 inoculated with PGPR. (a) Root length; (b) Shoot length; (c) Root fresh wt; (d) Shoot fresh wt; (e) Root dry wt.; (f) Shoot dry wt.; (g) Root volume; (h) N content(%); (i) N content (%) Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$





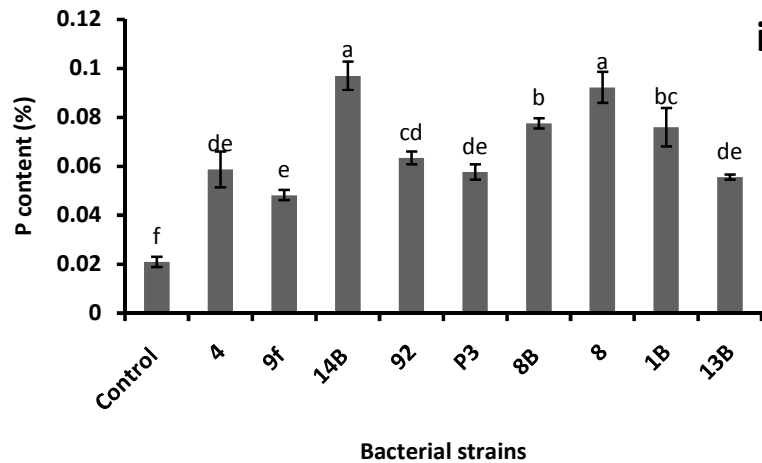
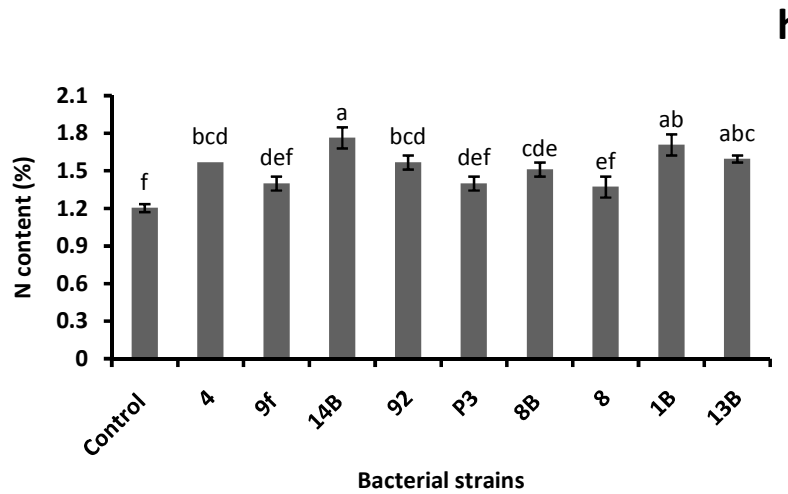
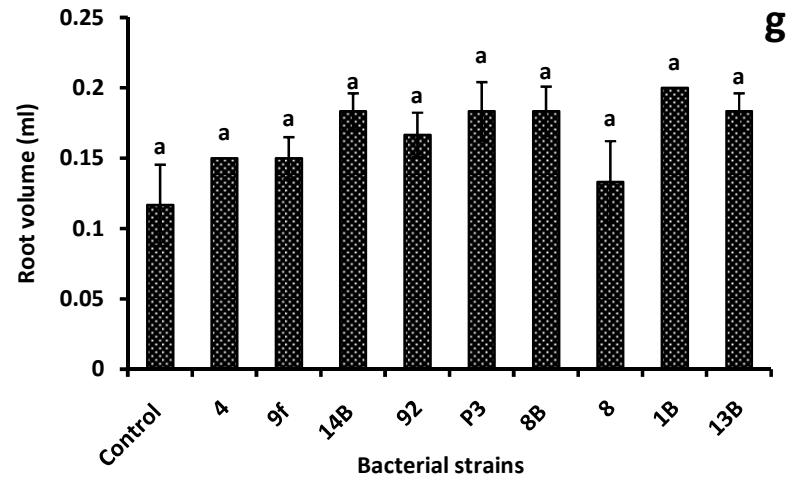


Fig. 4.9: Growth promotion of Moth var. CZM 3 inoculated with PGPR. (a) Root length; (b) Shoot length; (c) Root fresh wt.; (d) Shoot fresh wt.; (e) Root dry wt.; (f) Shoot dry wt.; (g) Root volume; (h) N content (%); (i) P content (%) Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.6.3.4 Root volume

In var. CZM1, all the treatments were non-significant over the control (**Fig. 4.8g**). In var. CZM3, all the treatments had similar effect on root volume as the control as all the treatments were found to be non-significant over the control (**Fig. 4.9g**).

4.6.3.5 N content in shoot (%)

In case of variety CZM1, a maximum increase of 31.42% in 14B treatment while a minimum increase of 2.85% in treatment 9f and 4 was recorded (**Fig. 4.8h**). In case of var. CZM3, 46.51% maximum increase was observed in treatment 14B and minimum increase of 8.95% was observed in treatment 8 as compared to control (**Fig. 4.9h**).

4.6.3.6 P content in shoot (%)

Treatment P3 gave maximum increase (1412.15%) in P content in CZM1. Minimum increase of 38.31% was recorded in treatment 8B as compared to control in same variety. In var. CZM3, 14B treatment was best as it showed 362.49% increase. Treatment 9f showed minimum increase of 130% in P content as compared to control in the same variety of moth (**Fig. 4.8i & 4.9i**).

4.6.4 Effect of bacterial treatments on the plant growth promotion of guar

A single var. of guar (RGC936) was used to observe the effect of test bacterial isolates on plant vigour in the pot experiment (**Plate 3F**).

4.6.4.1 Plant height

4.6.4.1.1 Root length

A significant increase of 63.26% in treatment 1B was observed as compared to control while a significant decrease in root length was observed in all the treatments except 92, 8B, and 1B. Treatment 14B and 13B were found to be non-significant over the control (**Fig. 4.10a**).

4.6.4.1.2 Shoot length

There was no significant increase in shoot length in any treatment as compared to control as all the treatments were non-significant over the control (**Fig. 4.10b**).

4.6.4.2 Plant fresh weight

4.6.4.2.1 Root fresh weight

A significant increase of 274.70% was recorded in treatment 92 as compared to control. Treatment 4, 8B, and 13B had no impact on root fresh weight as they were found to be non-significant over the control (**Fig. 4.10c**).

4.6.4.2.2 Shoot fresh weight

In guar (var. RGC936), a significant increase of 38.93% was recorded in treatment P3 as compared to control while rest of the treatments had no impact on shoot fresh weight as they were found to be non-significant over the control (**Fig. 4.10d**).

4.6.4.3 Plant dry weight

4.6.4.3.1 Root dry weight

A significant increase of 433.20% in root dry weight was observed in treatment 14B as compared to control while rest of the treatments was found to be non-significant over the control (**Fig. 4.10e**).

4.6.4.3.2 Shoot dry weight

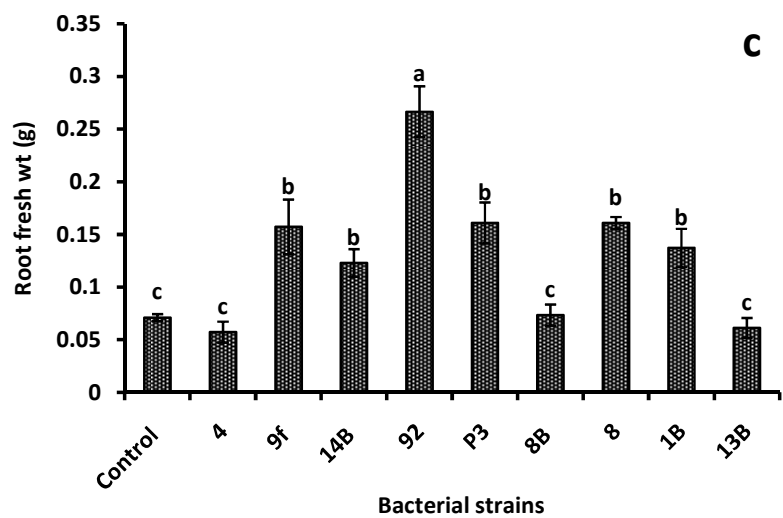
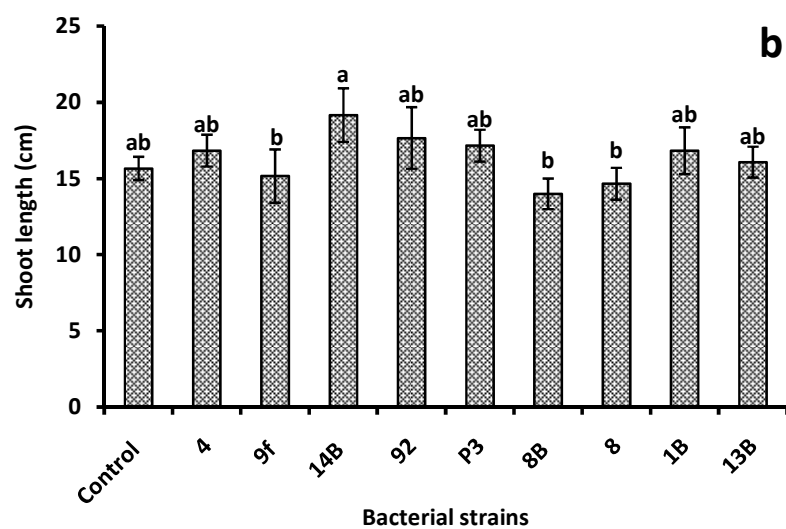
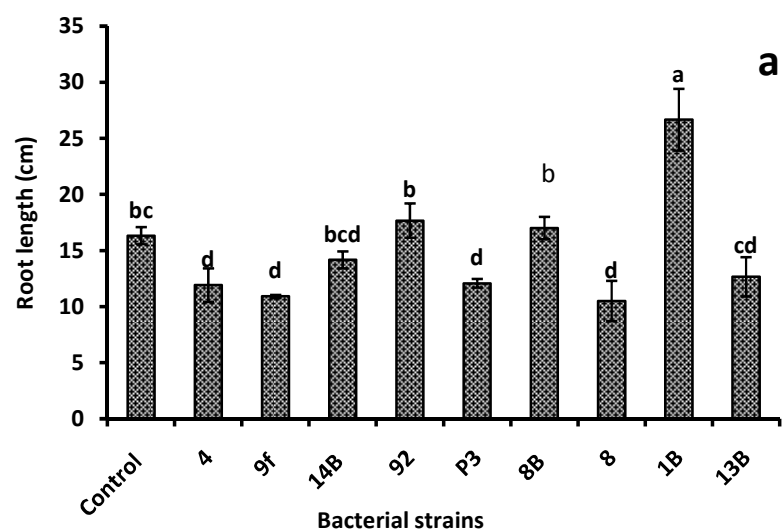
A significant increase of 49.05% was recorded in treatment P3 as compared to control while rest of the treatments had no impact on shoot dry weight as compared to control (**Fig. 4.10f**).

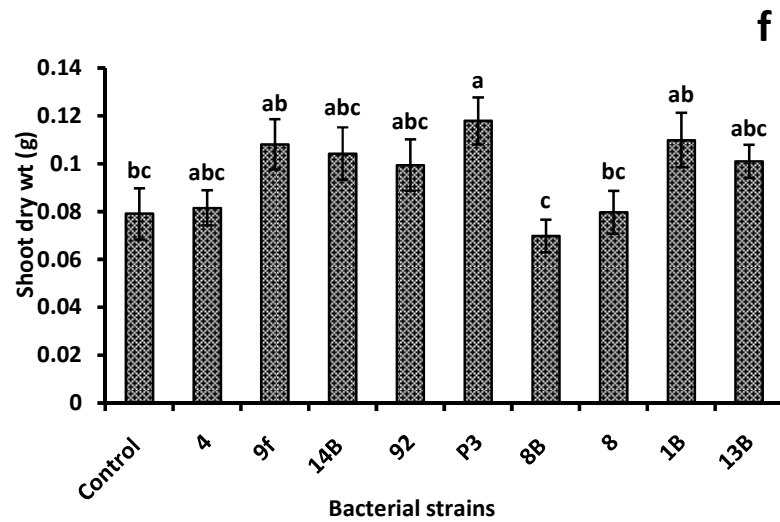
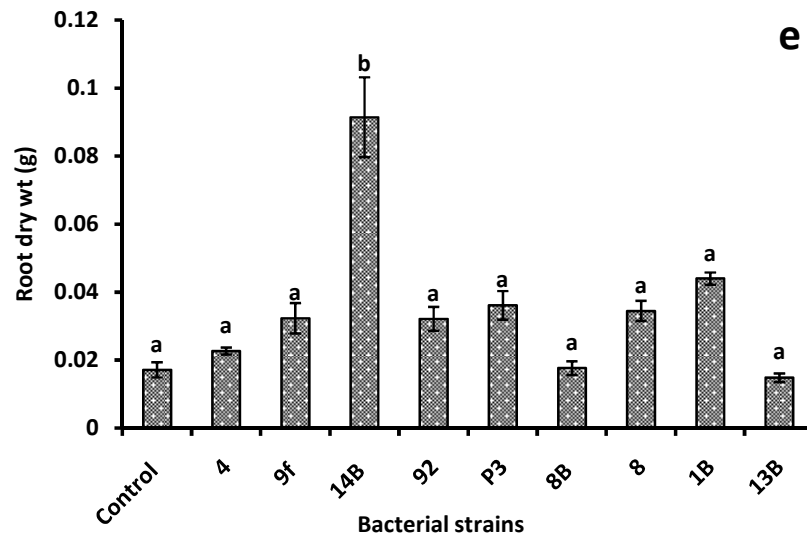
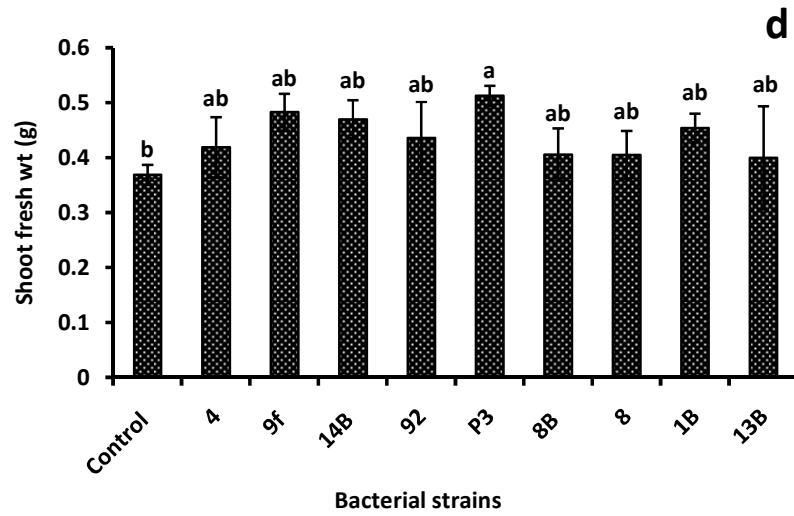
4.6.4.4 Root volume

Increase of 207.69% in root volume in treatment 4 was observed but found to be non-significant as compared to control. Rest all the treatments was also showed non-significant effect on root volume (**Fig. 4.10g**).

4.6.4.5 N content in shoot (%)

In case of guar var.RGC936, a maximum increase of 23.06% in P3 treatment was recorded as compared to control which was found to be non-significant over the treatment 1B and 13B while rest treatments were non-significant over the control in respect to increase in N content in shoot (**Fig. 4.10h**).





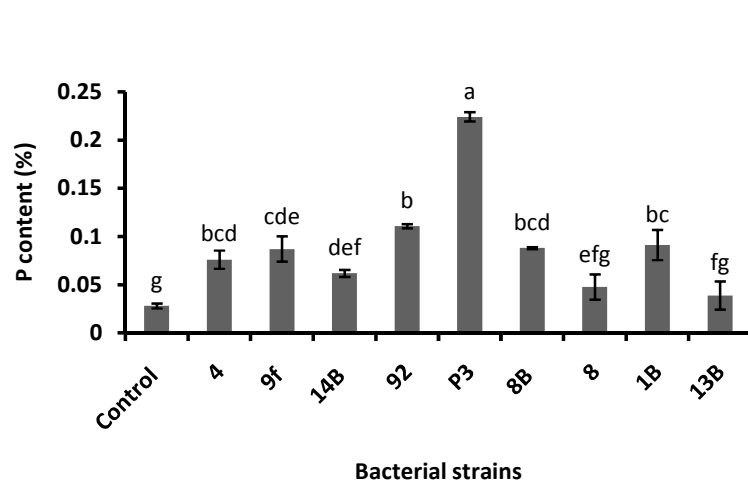
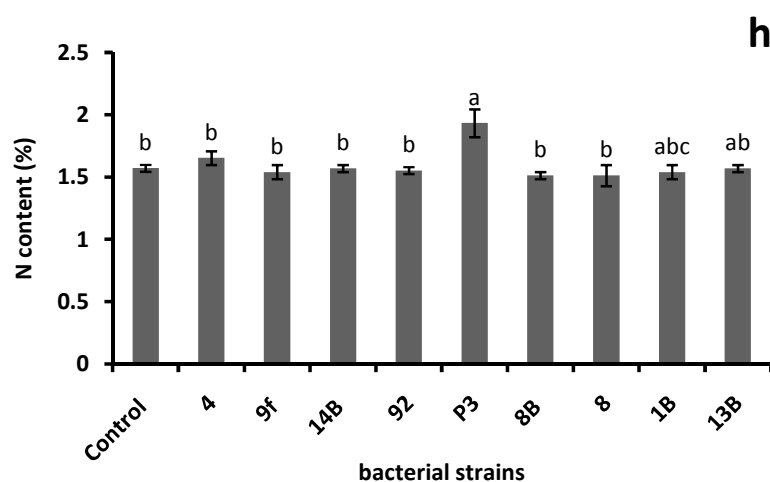
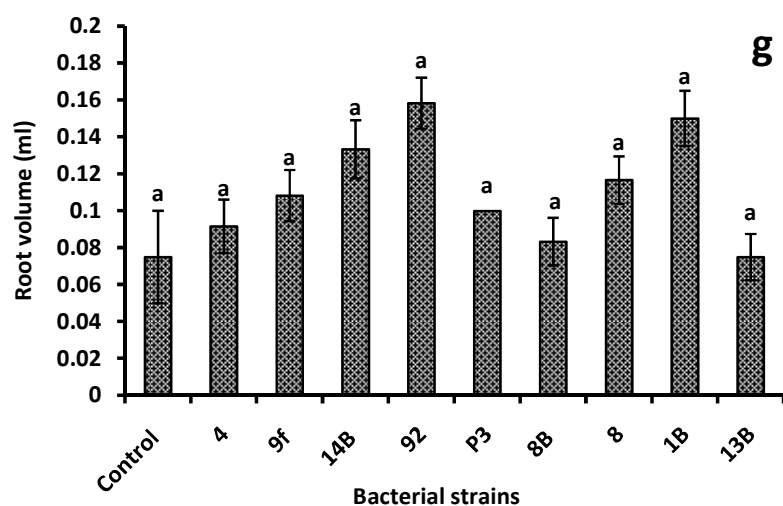


Fig. 4.10: Growth promotion of Guar RGC936 inoculated with PGPR. (a) Root length; (b) Shoot length; (c) Root fresh wt.; (d) Shoot fresh wt.; (e) Root dry wt.; (f) Shoot dry wt.; (g) Root volume; (h) N content (%); (i) P content (%) Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.6.4.6 P content in shoot (%)

Treatment P3 showed an increase of 699.10% in P content as compared to control while a minimum increase of 41.20% was recorded in treatment 8 as compared to control. Treatment 8 and 13B had no impact on P content as both were non-significant over the control (**Fig. 4.10i**).

In the present investigation, nine PGPR isolates were evaluated for their effect on plant growth and nutrient uptake and to ascertain the involvement of possible PGPR trait(s) in enhancing plant growth. Two rhizospheric bacteria (14B and P3) were found to be the most potent bacteria for plant growth promotion of var. CZIC923 and CZP2k9 of pearl millet respectively. They gave consistent results for all the parameters in both the varieties. Bacterial treatment 4 was most responsive in Sorghum but maximum enhancement in fresh weight and dry weight of root was recorded in treatment 13B and 8 respectively. Moth and Guar were not responsive towards growth promotory action of bacteria. Hence, among four test crops only pearl millet was selected for further study. Two bacteria (P3 and 14B) were selected as they gave consistent results in both the varieties of pearl millet. Both the bacteria showed maximum plant growth promoting traits viz. siderophore production, phosphate solubilization, NH_3 and Indole acetic acid (IAA) production. Thus, the presence of PGPR traits in an individual organism makes an organism a successful inoculant strain (**Dey *et al.*, 2004**). The ability of PGPR in growth promotion in various crops is well documented (**Burdman *et al.*, 2000**; **Akhtar *et al.*, 2012**). The results reported here corroborate earlier studies and indicate a future possibility that PGPR can be used to promote growth and health of crop plants (**Raj *et al.*, 2003**). **Mohamed and Gomma (2012)** reported plant growth promotion in radish plants mediated by *Bacillus subtilis* and *Pseudomonas fluorescens*. Seed bacterization of these isolates increased the root length, plant biomass, plant height and nutrient uptake in pots significantly over control. Similar study of plant growth promotion in pearl millet mediated by *Bacillus* spp. and *Pseudomonas* was reported by **Hameeda *et al.* (2006)**. Chelation of iron by microbial siderophores and phosphate solubilization have also been reported earlier as a part of PGPR activities involved in enhanced plant growth (**Kloepper *et al.*, 1988**; **Glick, 1995**). Pearl millet therefore was selected as a candidate crop for further evaluation of six different varieties inoculated with selected PGPR.

4.7 Testing of Endophytic Nature of Selected PGPR Using ARDRA

To test the endophytic nature of both the isolates (P3 and 14B), a pot experiment was conducted. Pearl millet seeds were inoculated with both the bacterial isolates. Plants were harvested after 30 days of germination and roots were taken for the isolation of endophytic bacteria. After 3-4 days of incubation the growth of organism was observed below the root samples. The microbial growth surrounding the root on water agar indicated that the organism gained their nutrient from the root only. Portion of this growth was then streaked on nutrient agar plates, The bacterium isolated from root sample of 14B inoculated plants was gram positive and showed similarity with 14B bacteria, while bacteria isolated from root sample of P3 inoculated plants was gram negative but colony morphology was not matched with P3 bacteria.

4.7.1 DNA isolation and amplification of 16S rDNA genes

Total genomic DNA from our bacterial isolates (P3 and 14B) and from endophytes of pearl millet roots was isolated and amplified using universal primers and a distinct band of 1500 bp was obtained in all the samples (**Plate 4A**).

4.7.2 ARDRA

Amplified 16S rDNA of all the samples were subjected to restriction digestion with *TaqI* and *MspI*. According to ARDRA pattern, 14B bacteria and endophyte isolated from 14B treated pearl millet roots had identical banding pattern while in case of P3, restriction pattern did not match (**Plate 4B**). From the above results we can conclude that 14B might be an endophyte in nature while P3 was not found to be an endophyte. Bacterial endophytes have been intensively studied in the context of agricultural practices rather than ecology or biodiversity (**Hallmann *et al.*, 1997; Sturz *et al.*, 2000**). Endophytic bacteria have been considered as possible inoculants for increasing plant productivity (**Hallmann *et al.*, 1997**). They have been isolated from roots of many crops using ARDRA previously (**Sturz *et al.*, 1997; Sun *et al.*, 2008; Panchal and Ingal, 2011**). 14B bacteria showed the endophytic nature as the banding pattern was same as that of endophyte which was isolated from roots of 14B treated plants. **Hallmann *et al.* (1997)** suggested that bacteria, which are isolated from surface-sterilized plant tissues and which do no apparent harm to the plant, could be considered endophytes.

4.8 Screening of different Varieties of Pearl Millet with Selected PGPR

To screen the genotype specific behavior in different varieties of pearl millet towards plant growth promotion in the presence of two selected PGPR (14B and P3), a pot experiment was conducted. By observing different growth parameters, effect of bacterial inoculation on plant growth was checked. Six different varieties viz. CZP2k9, CZIC923, CZP 2k3, CZP 2007, CZP9603 and CZP 9802 were used in the pot experiment (**Plate 5A, B, C, D and E**).

4.8.1 Plant height

4.8.1.1 Root length

In all the varieties both the bacteria showed significant increase as compared to control except var. CZP9603 in which both the isolates reduced the root length which was significant over the control plants (**Fig. 4.11a**). Treatment P3 recorded a maximum increase of 57.14% in root length of var. CZIC923 while in plants treated with 14B a maximum increase of 42.85% was observed over the var. CZP9603 in var. CZIC923 as well as CZP2k9. Among six varieties, irrespective of bacterial inoculation var. CZIC923 showed a significant increase of 50.14% in root length as compared to var. CZP9603 which recorded minimum root length (**Fig. 4.11b**).

4.8.1.2 Shoot length

Both the bacteria showed significant increase in length as compared to control in all the varieties of pearl millet except CZP9603 and CZP2003 in which there was no significant effect of bacterial inoculation on shoot length. Treatment P3 recorded a maximum increase of 20.76% in var. CZIC923 while a maximum increase of 24.59% in shoot length was observed in treatment 14B in var. CZIC923 as compared to var. CZP9603 which recorded minimum root length (**Fig. 4.12a**). Irrespective of bacterial inoculation a maximum increase of 39.68% in shoot length was recorded in var. CZIC923 which was significant from other varieties while significant reduction in shoot length was recorded in var. CZP 2007 which was found to be minimum (**Fig. 4.12b**).

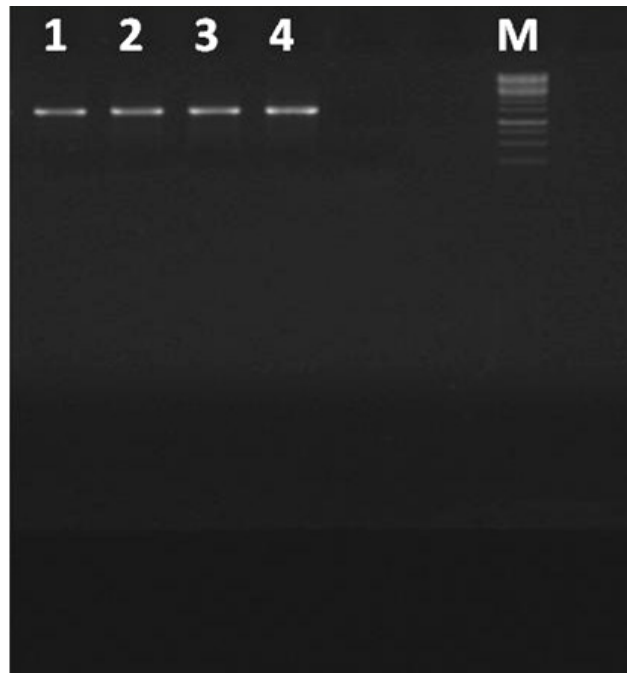


Plate 4A: 16S rDNA amplification of bacterial DNA

M: 1kb ladder; 1: 14B; 2: P3; 3 & 4: Endophyte isolated from 14B and P3 treated root of pearl millet respectively

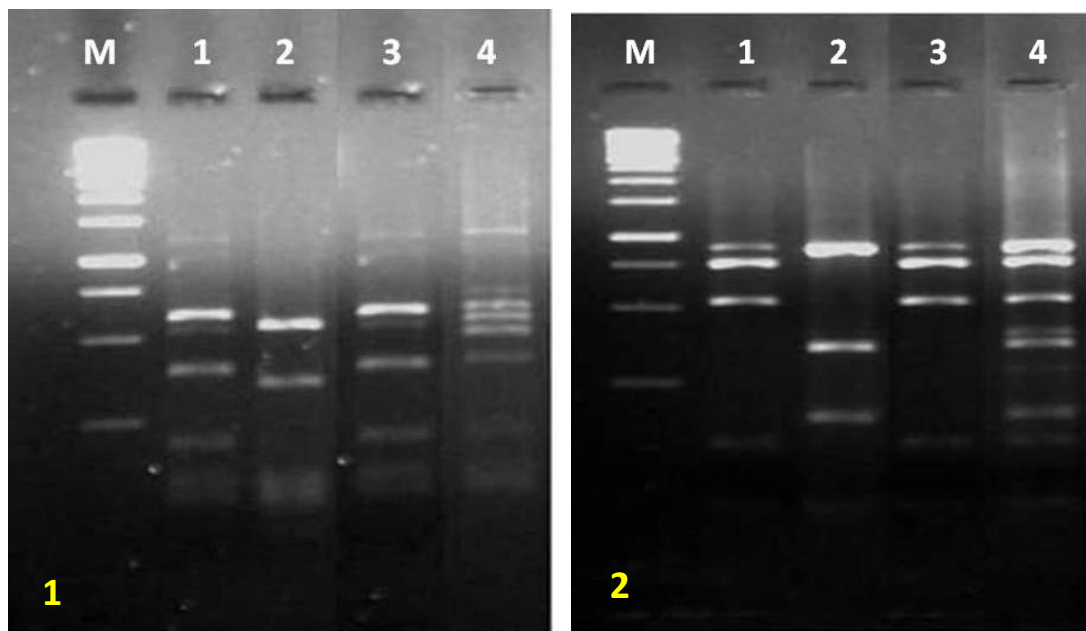


Plate 4B: Restriction pattern of 16S rDNA region of PGPR with (1): *MspI* and (2): *TaqI*

M: 100kb ladder; 1: 14B; 2: P3; 3 & 4: Endophyte isolated from 14B and P3 treated root of pearl millet respectively

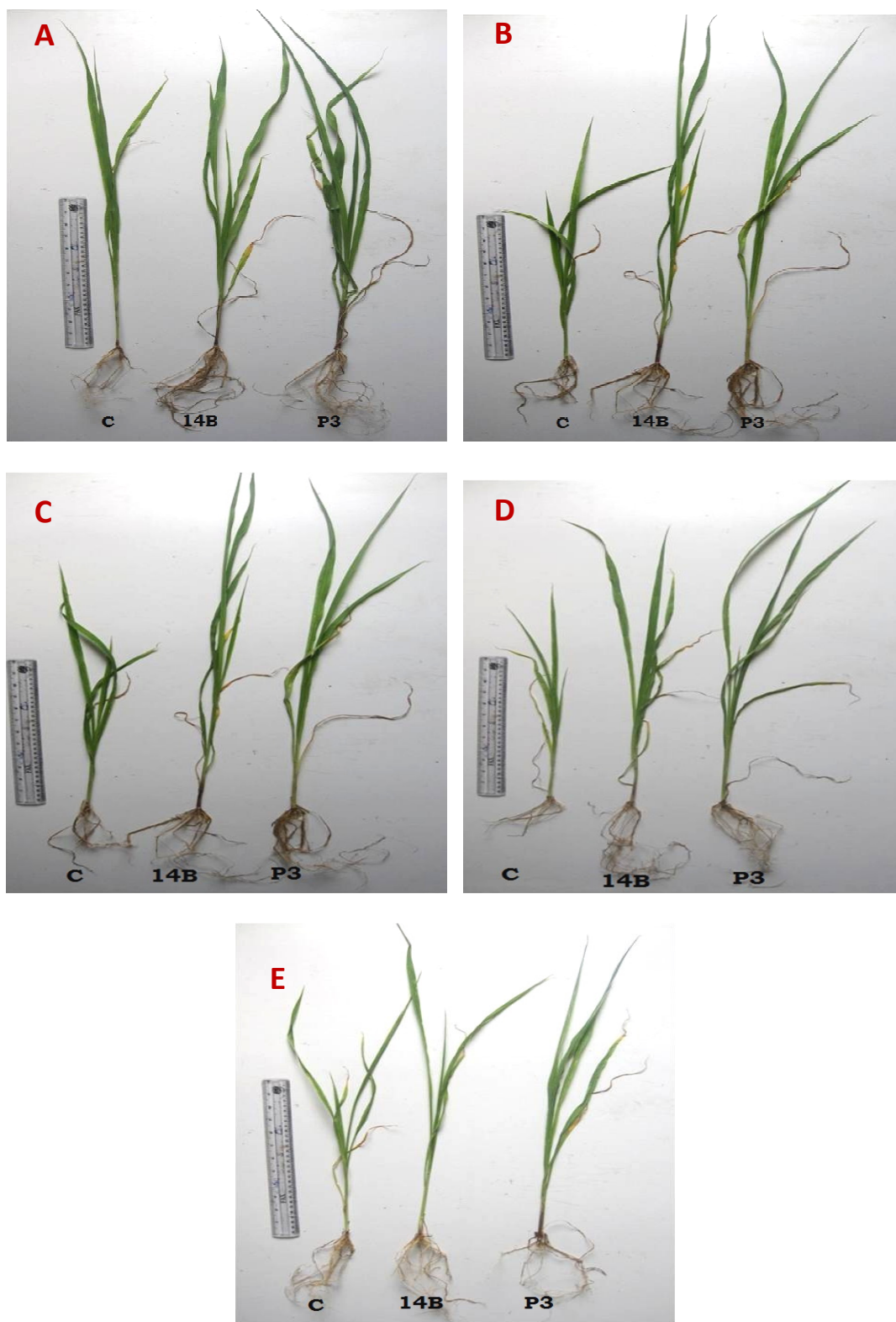


Plate 5: Effect of PGPR on different varieties of pearl millet: (A)- CZP 2003, (B)- CZP 9802, (C)- CZP 2k9, (D)- CZIC 923, (E)- CZP 9603

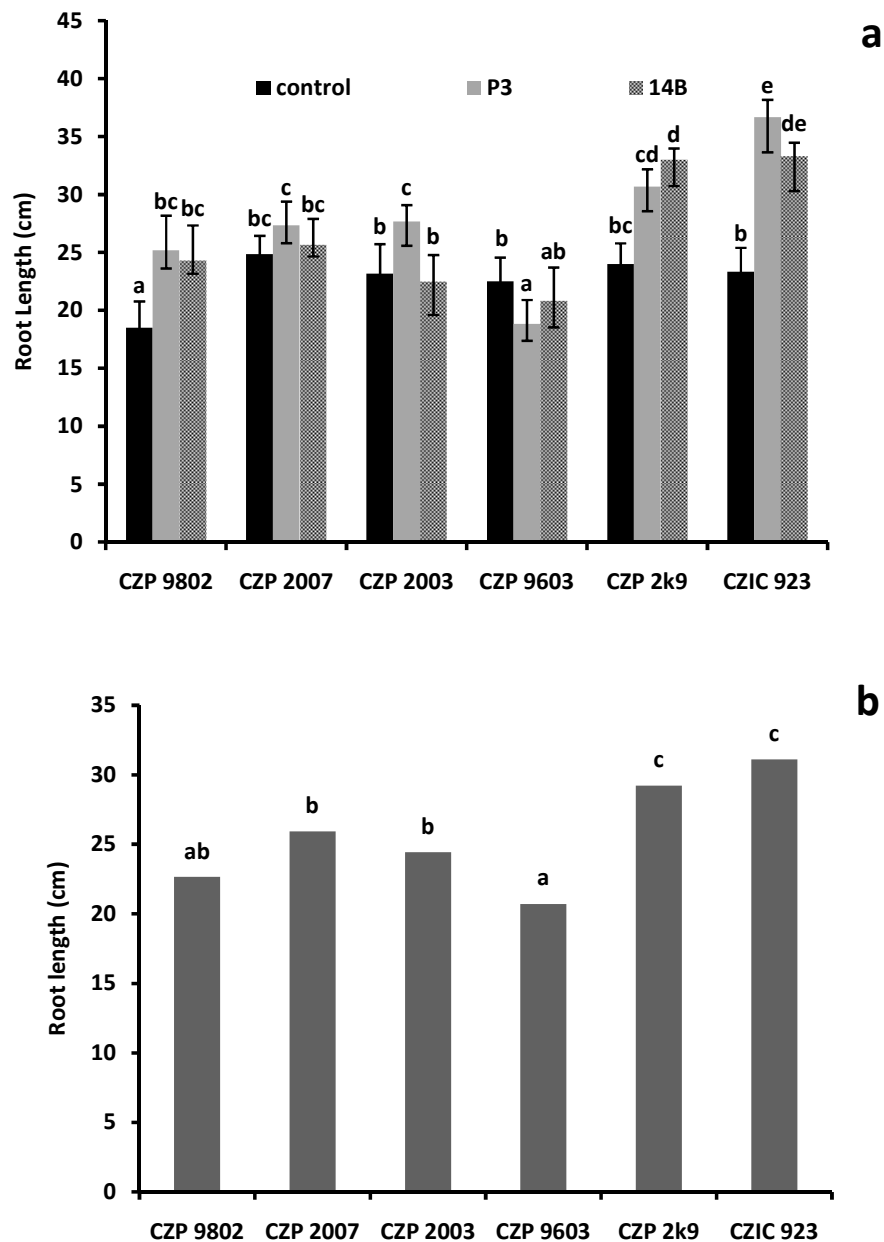


Fig. 4.11: Root length of different varieties of pearl millet (a) Effect of PGPR; (b) Irrespective of bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

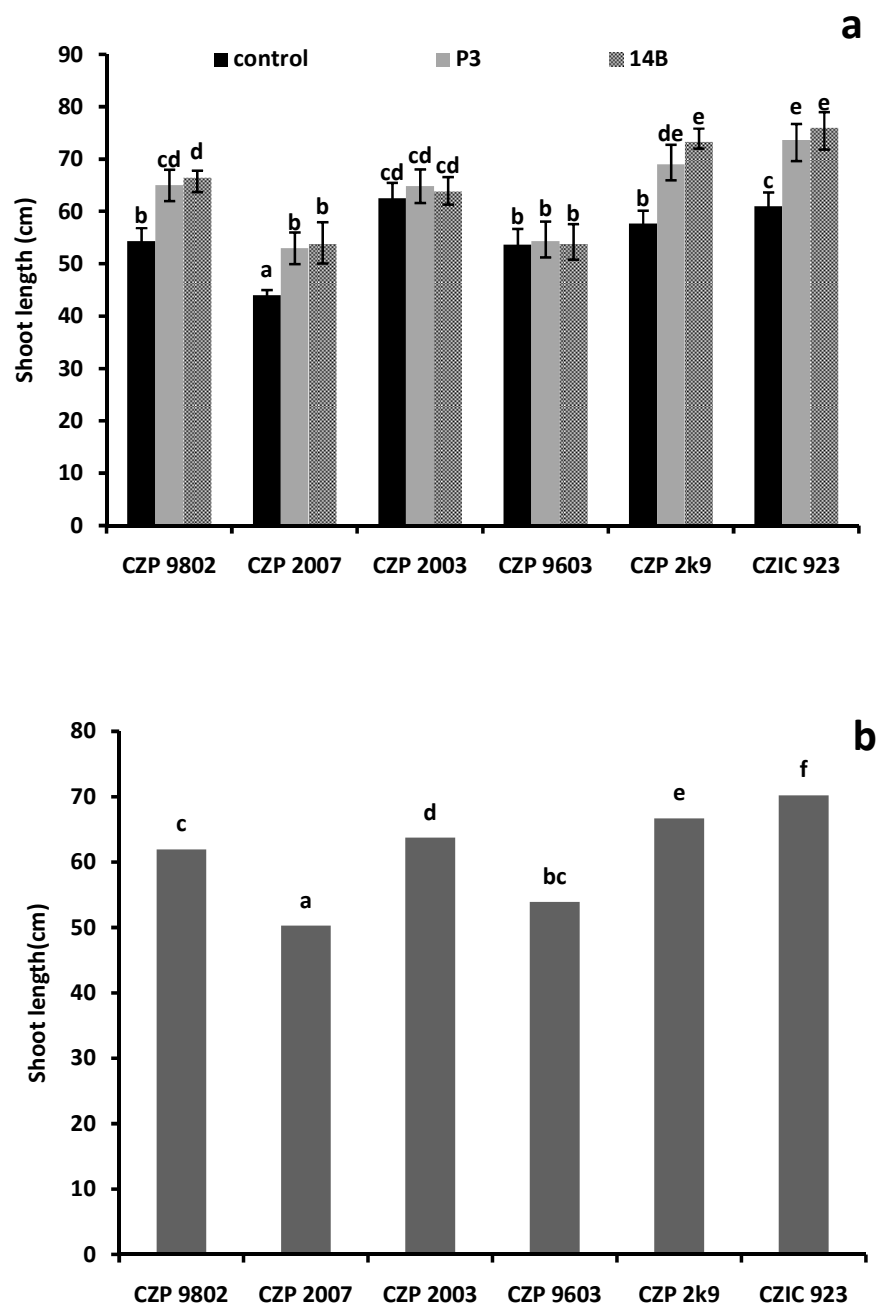


Fig. 4.12: Shoot length of different varieties of pearl millet (a) Effect of PGPR; (b) Irrespective of bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.8.2 Plant fresh weight

4.8.2.1 Root fresh weight

There was non-significant effect of bacterial inoculation in root fresh weight in var. CZP2003 and CZP9603 as compared to other varieties while in var. CZIC923 maximum root fresh weight was reported. In var. CZIC923, P3 has significantly increased root fresh weight (220.4%) followed by 14B (145.34%) over the var. CZP2003 (**Fig.4.13a**). Irrespective of bacterial inoculation, var. CZIC923 showed a significant increase of 382.02% in root fresh weight as compared to other varieties while minimum root fresh weight was observed in CZP2003 which was at par with CZP9603 (**Fig.4.13b**).

4.8.2.2 Shoot fresh weight

In var. CZIC923, 14B recorded a significant increase of 96.03% in shoot fresh weight as compared to control while it was found to be at par in treatment P3. In var. CZP9603, minimum shoot fresh weight was recorded as both the bacteria reduced the weight up to 18.11% significantly from var. CZP9603 (**Fig.4.14a**). Among six varieties, var. CZIC923 recorded a maximum increase of 69.38% in shoot weight which was significantly different from other while minimum weight was observed in var. CZP9603 (**Fig.4.14b**).

4.8.3 Plant dry weight

4.8.3.1 Root dry weight

In all the varieties except var. CZIC923 and CZP2k9, there was non-significant impact of bacterial inoculation on root dry weight as compare to control. In var. CZIC923, treatment 14B recorded a maximum increase of 90.93% while in treatment P3 a significant increase of 82.32% in root dry weight was observed when compared with var. CZP9603 (**Fig. 4.15a**). Irrespective of bacterial inoculation, among six varieties, var. CZIC923 increased root dry weight significantly up to 64.96% over the var. CZP9603 which recorded minimum root dry weight among all the varieties (**Fig. 4.15b**).

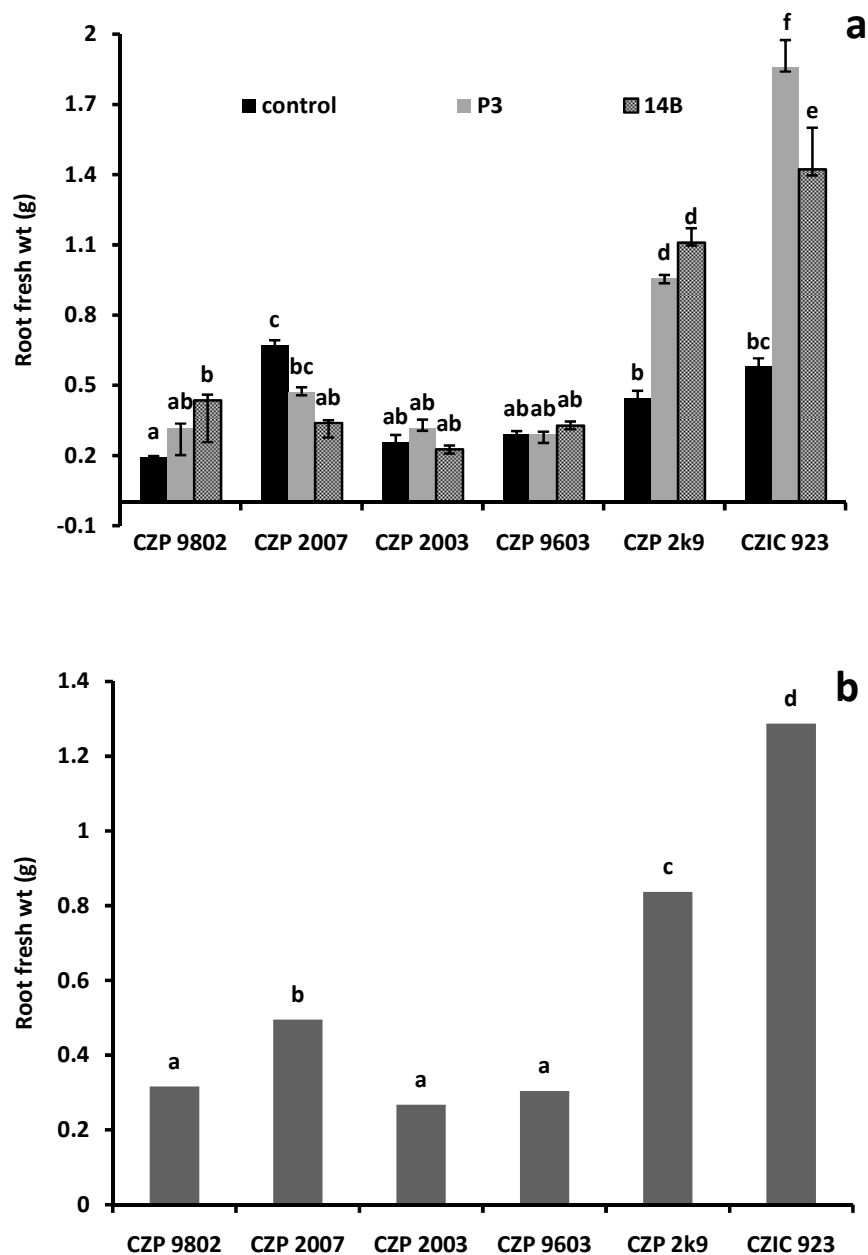


Fig. 4.13: Root fresh wt. of different variety of pearl millet (a) Effect of PGPR; (b) Irrespective of bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

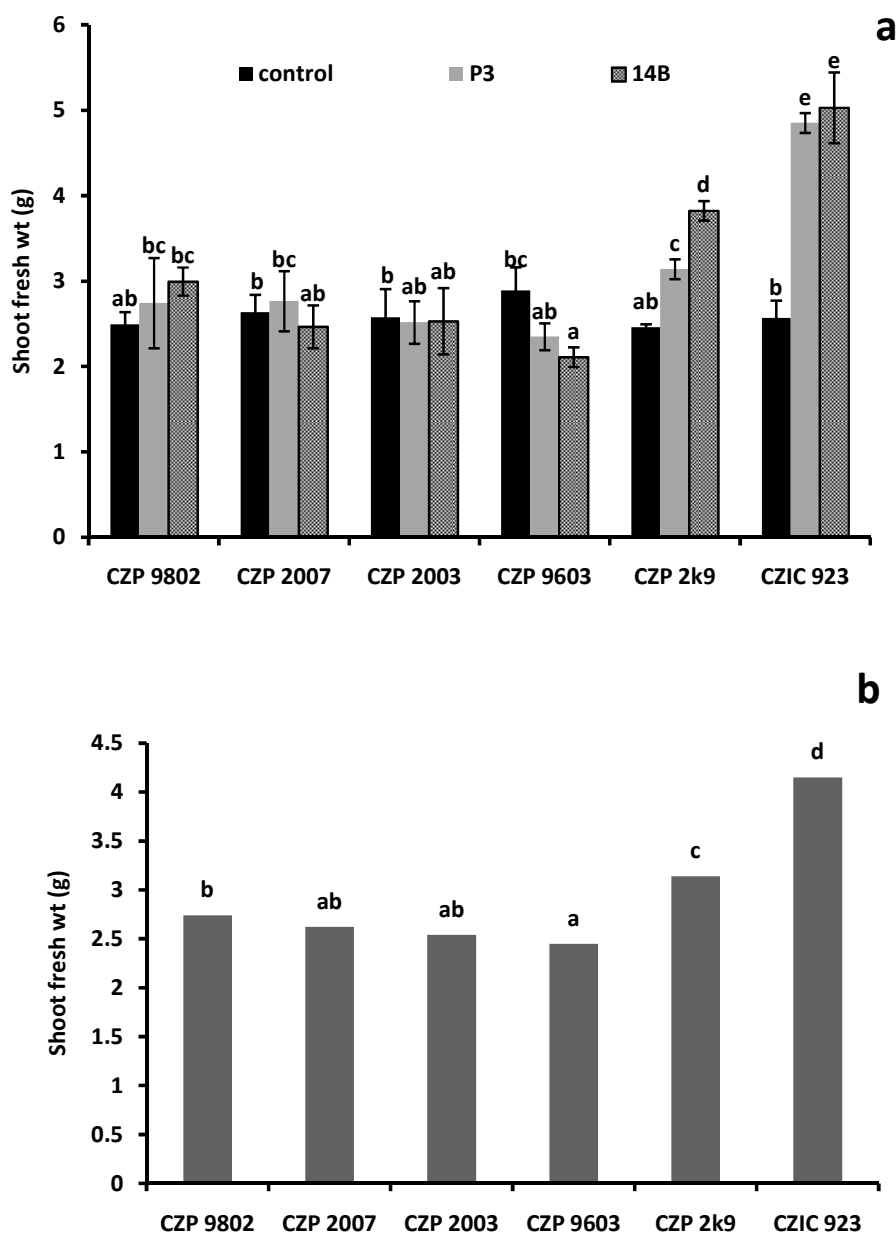


Fig. 4.14: Shoot fresh wt. of different varieties of pearl millet (a) Effect of PGPR; (b) Irrespective of bacterial inoculation. Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

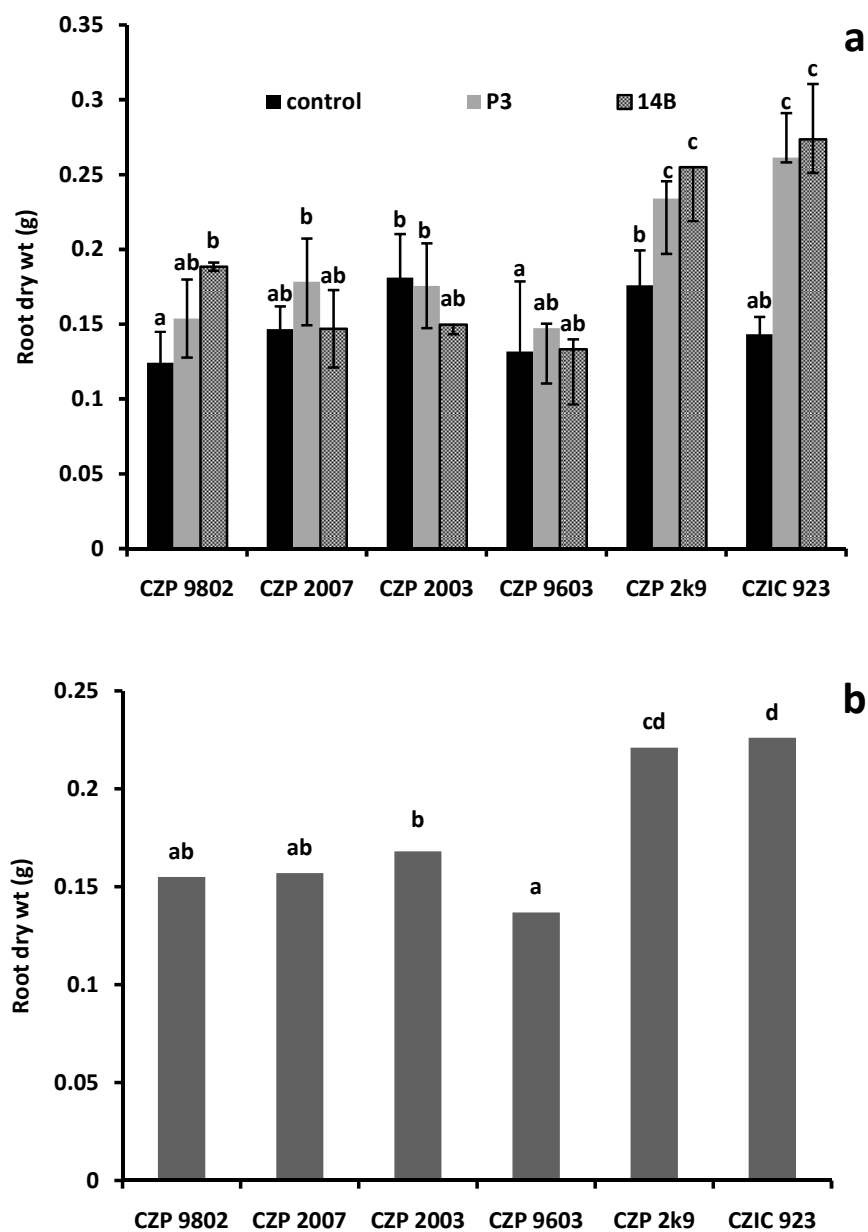


Fig. 4.15: Root dry wt. of different varieties of pearl millet (a) Effect of PGPR; (b) Irrespective of bacterial inoculation. Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.8.3.2 Shoot dry weight

Maximum increase in shoot dry weight was observed in var. CZIC923 as treatment 14B recorded a maximum increase of 42.99% while plants treated with P3 showed an increase of 26.53% as compared to control. A significant decrease was observed in both the treatments (P3 and 14B) in var. CZP9603 as compared to control. P3 treatment reduced the shoot dry weight up to 36.9% while in 14B treated plant, a significant reduction of 28.06% was recorded as compared to var. CZP9603 (**Fig. 4.16a**). Significantly higher shoot weight (40.7%) was recorded in var. CZIC923 over the var. CZP9603 which recorded minimum shoot dry weight irrespective of bacterial treatments (**Fig. 4.16b**).

4.8.4 Root volume

There was no significant effect of bacterial treatments on root volume in all the varieties except CZIC923 and CZP2k9 as compared to control. Treatments 14B and P3 recorded a maximum increase of 52.94 and 47.05% respectively in var. CZIC923 as compared to var. CZP2003 (**Fig. 4.17a**). Significantly higher root volume (40.7%) was recorded in CZIC923 among six varieties when compared irrespective of bacterial inoculation while it was at par with CZP2k9. Minimum root volume was observed in CZP2003 while it was at par with CZP9603 (**Fig. 4.17b**).

An experiment was conducted to observe the effect of bacterial inoculation in different varieties of pearl millet. According to **Bouton *et al.* (1979)** differences do exist among pearl millet genotypes in order to respond to bacterial inoculum. Var. CZIC923 was observed as the most responsive variety followed by CZP2k9 while var. CZP9603 was reported as the least responsive to growth promotory action of bacteria. Both the bacterial isolates (P3 and 14B) showed significant effect on plant growth promotion in pearl millet var. CZIC923 and CZP2k9 while same bacterial isolates showed non-significant effect on var. CZP9603 of the same crop. Our findings could be understood by the fact that plant roots offer a niche for the proliferation of soil bacteria that thrive on root exudates and lysates. While these bacteria utilize the nutrients that are released from the host for their growth, they also secrete metabolites into the rhizosphere. Several of these metabolites can act as signaling compounds in some varieties of a crop referred as host plant or some can act as inhibitory in different varieties of the same crop referred as non host plant. These signals are

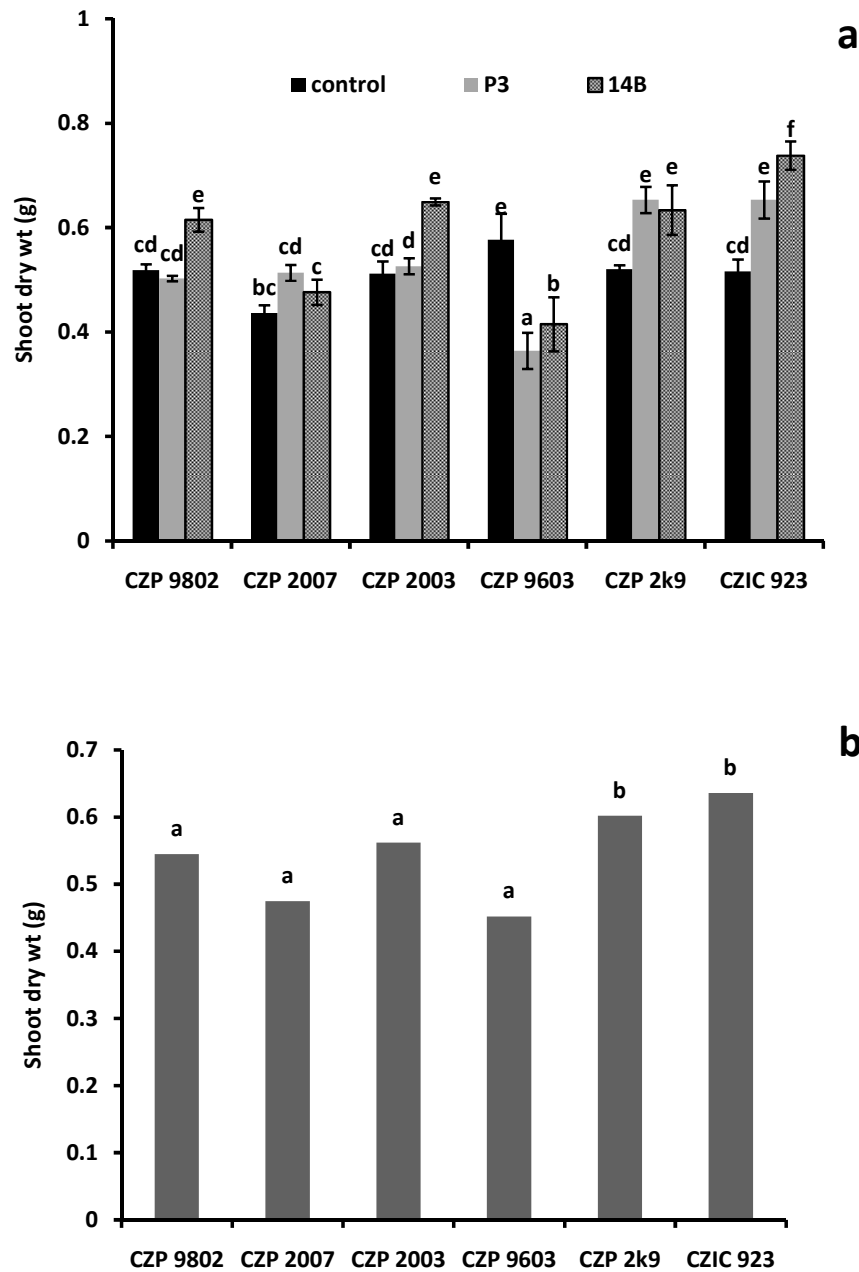


Fig. 4.16: Shoot dry wt. of different varieties of pearl millet (a) Effect of PGPR; (b) Irrespective of bacterial inoculation. Mean value ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

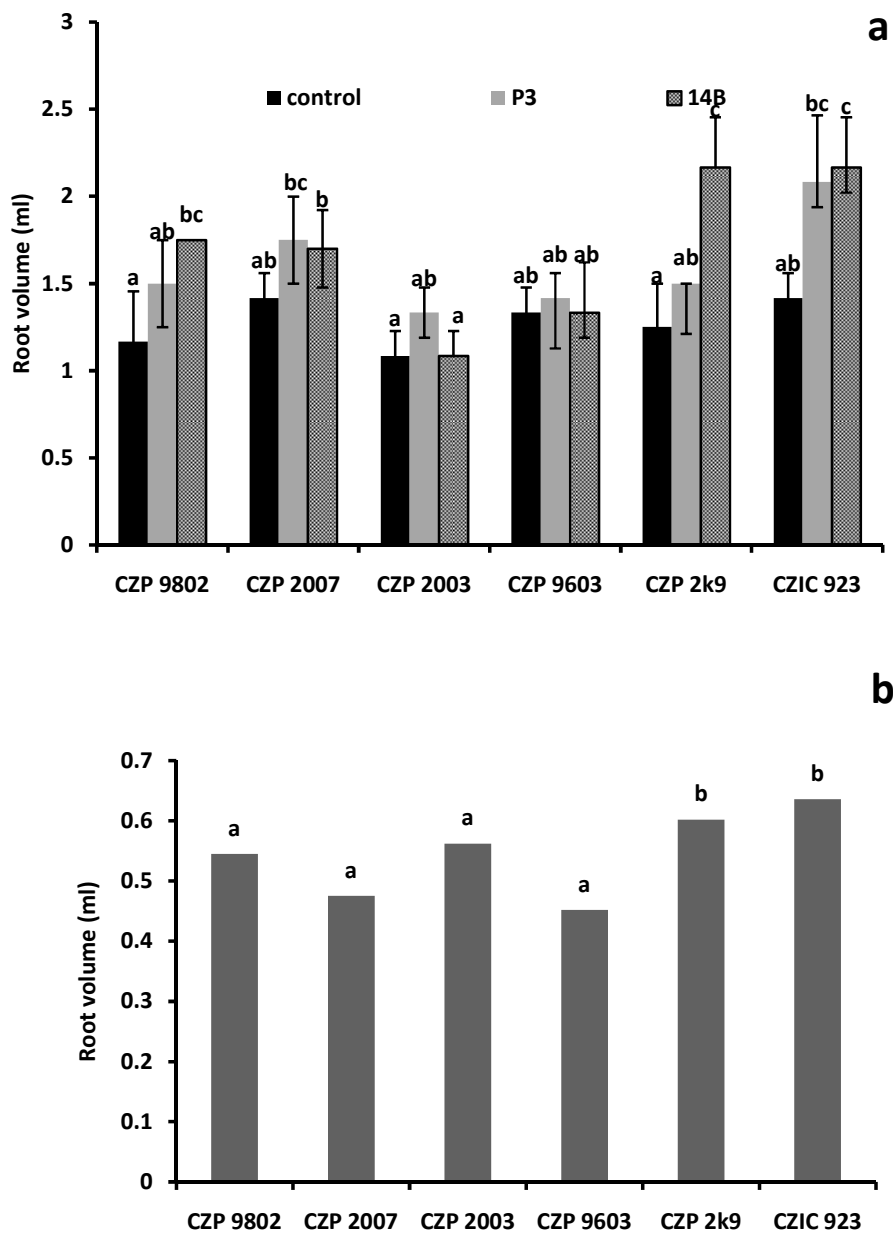


Fig.4.17: Root volume of different varieties of pearl millet (a) Effect of PGPR; (b) Irrespective of bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

perceived by neighboring cells within the same micro-colony, by cells of other bacteria that are present in the rhizosphere, or by root cells of the host plant (Van Loon and Bakker, 2003; Bais *et al.*, 2004; Gray and Smith, 2005; Kiely *et al.*, 2006). Growth promotion by soil microorganisms can be considered part of a continuum in which interactions between plants and microorganisms range from deleterious (pathogens) to beneficial (PGPR) (Glick *et al.*, 1999; Ryu *et al.*, 2005). The net effect of bacteria-plant interactions, including modulation and biosynthesis of plant hormones is influenced by environmental and host factors, such as temperature, water availability, host genotype and plant health (Preston, 2004). Cultivar-specific variation in disease suppression, plant growth promotion, antibiotic production and colonization has been described for several PGPR– plant interactions, but few studies have addressed the genetic and mechanistic basis of this variation (Smith and Goodman, 1999). However, advancement made for recalcitrant crop like pearl millet is still in its juvenile stage.

4.9 Effect of Three Different Soil Types on Two Varieties of Pearl Millet

The physicochemical properties of the soil are the main factors influencing the survival and activity of an inoculated strain as well as the growth of plants. This study examined the effect of different soil types viz. sandy, red clay and silty clay loam (Table 4.4) on the two varieties of pearl millet (CZIC923, CZP9603) inoculated with two strains P3 (*Pseudomonas fluorescens*) and 14B (*Bacillus cereus*), identified as most responsive strains as per our prior experiment (Plate6 A, B and C).

Table 4.4 Physico-chemical properties of three soil used in the experiment

Source	Soil texture	pH	EC	Moisture content (%)	OC content (%)	N (kg/ha)	P (kg/ha)	K (Kg/ha)
Ujhani, UP	Sandy	6.4	0.193	0.31	0.144	150.52	14.17	75.79
Mukteshwar, Uttarakhand	Red clay	5.98	0.312	6.00	1.56	324.17	8.81	270.29
Pantnagar, Uttarakhand	Silty clay loam	8.03	0.848	11.78	1.15	186.07	15.24	145.60



Plate 6A: Effect of Mukteshwar soil on two varieties 1- CZIC923 and 2- CZP9603 of pearl millet inoculated with PGPR



Plate 6B: Effect of Pantnagar soil on two varieties 1- CZIC923 and 2- CZP9603 of pearl millet inoculated with PGPR

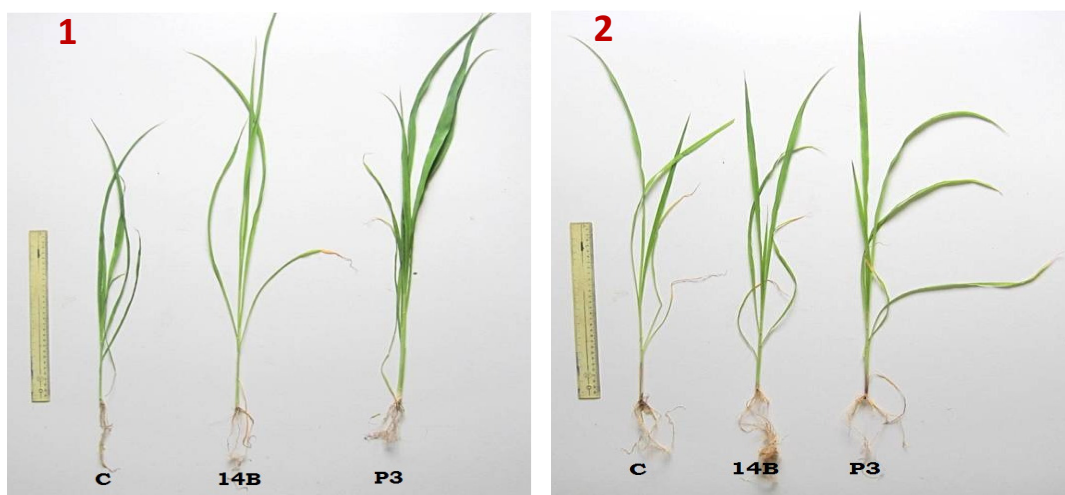


Plate 6C: Effect of Ujhani soil on two varieties 1- CZIC923 and 2- CZP9603 of pearl millet inoculated with PGPR

4.9.1 Plant height

4.9.1.1 Root length

In Mukteshwar soil, there was non-significant effect of bacterial inoculation on shoot length in both the varieties. Effects of bacterial inoculation were found to be significant in Pantnagar and Ujhani soil over the control. In Pantnagar soil, using var. CZIC923 treated with P3 increased the root length up to 29.43% while treatment 14B recorded a significant increase of 25.02% as compared to control. In var. CZP9603, treatment P3 recorded a significant decrease of 20.9% over the control while in treatment 14B a significant decrease of 17.9% was observed as compared to control. In Ujhani soil, there was significant increase in both the bacterial treatments as compared to control in var. CZIC923 whereas in case of var. CZP9603 there was no significant increase in both the treatments over the control. In var. CZIC923, in plants inoculated with P3 increased the root length up to 57.47% while treatment 14B recorded an increase of 70.24% as compared to control (**Fig. 4.18a**).

Significantly higher root length (71.18%) was recorded in Pantnagar soil as compared to other soil irrespective of bacterial inoculation and varieties of pearl millet (**Fig. 4.18b**).

4.9.1.2 Shoot length

In Mukteshwar soil, in var. CZIC923, both the bacterial treatment significantly increased the shoot length as compared to control. P3 treatment has recorded a significant increase of 49.19% while in 14B treatment a significant increase of 52.38% in shoot length was observed as compared to control. In var. CZP9603 14B treatment increased the shoot length significantly up to 22.56% while P3 treatment was found to be non-significant over the control. In Pantnagar soil, in var. CZIC923, P3 treatment recorded a significant increase of 11.82% in shoot length as compared to control while 14B was found to be non-significant over the control. In var. CZP9603 both the bacterial treatment was found non-significant over the control. In Ujhani soil, using var. CZIC923 treated with P3 increased shoot length significantly up to 35.33% while 14B treated plants recorded a significant increase of 25.15% in shoot length as compared to control. In var. CZP9603, 14B increased the shoot length up to 12.27% whereas P3 treatment was non-significant over the control (**Fig. 4.19a**).

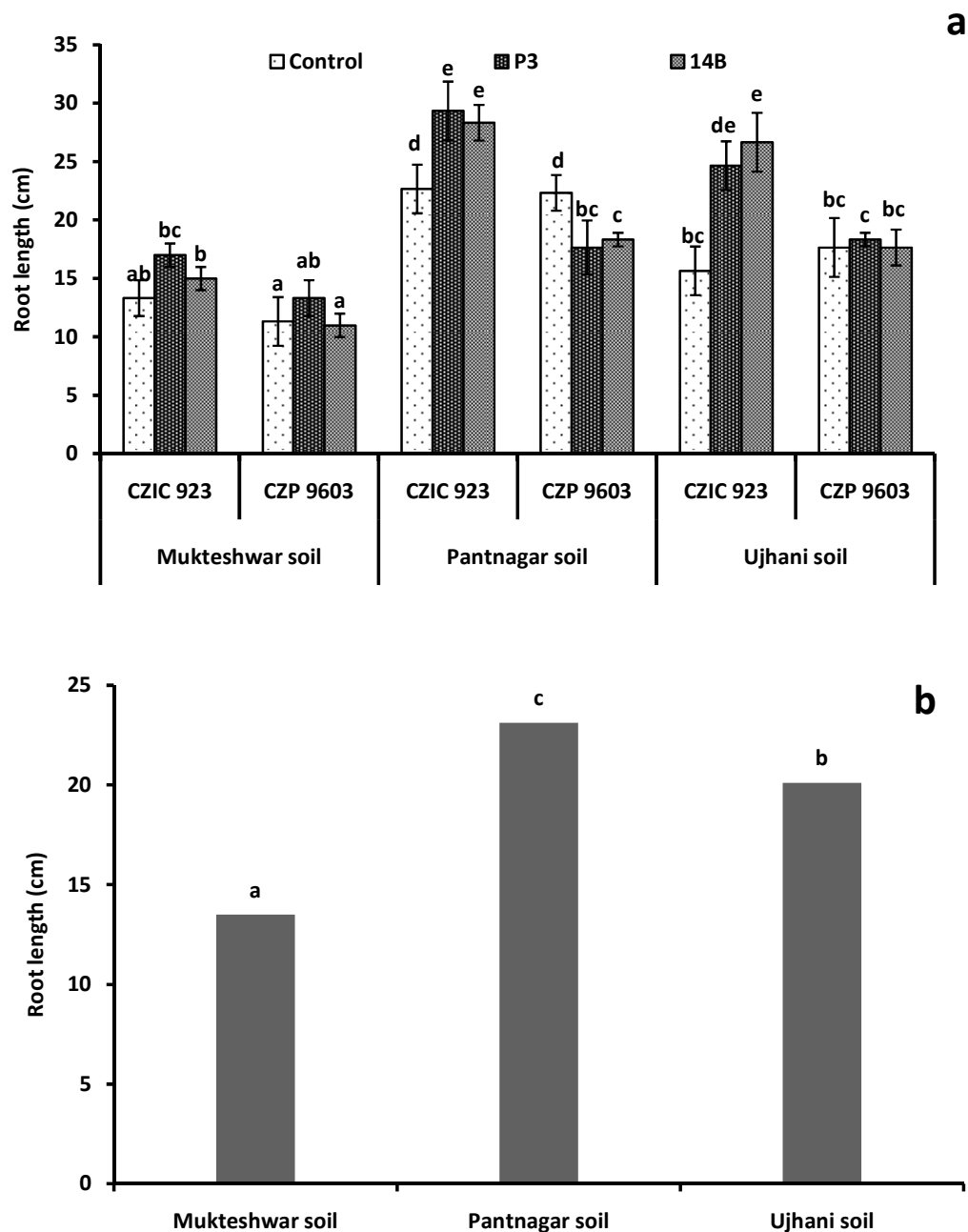


Fig. 4.18: Root length of two varieties of pearl millet in three soil types (a) Effect of PGPR; (b) Irrespective of varieties and bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

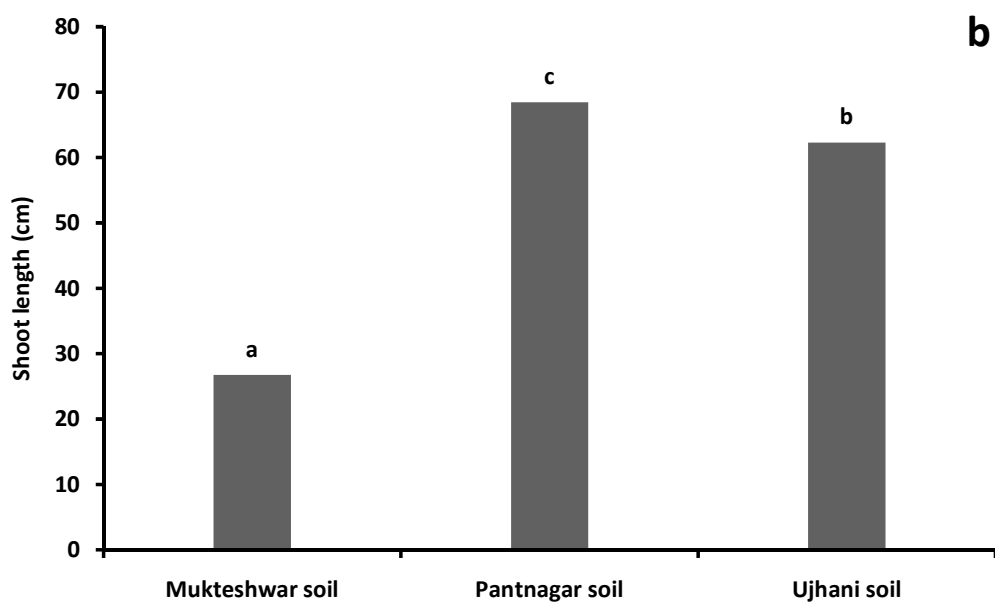
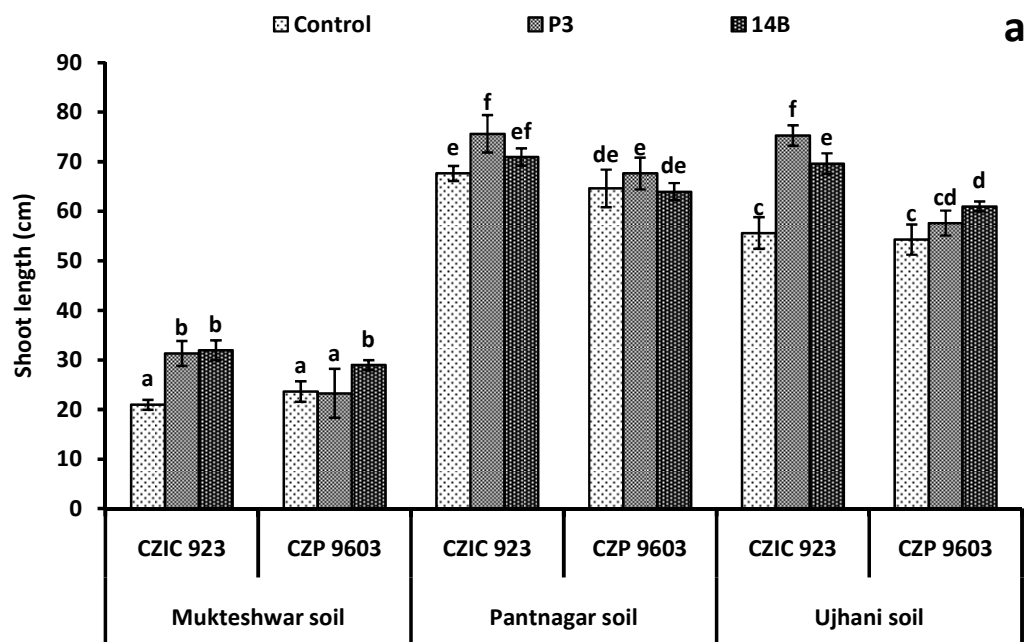


Fig. 4.19: Shoot length of two varieties of pearl millet in three soil types (a) Effect of PGPR; (b) Irrespective of varieties and bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

Impact of soil type on shoot length was similar as observed in root length. Irrespective of bacterial inoculation and variety, Pantnagar soil significantly increased root length (156.13%) as compared to Mukteshwar soil (**Fig. 4.19b**).

4.9.2 Plant fresh weight

4.9.2.1 Root fresh weight

In Mukteshwar soil, both the bacterial treatments were non-significant over the control in both the varieties. In Pantnagar soil, var. CZIC923 recorded a significant increase of 29.33% in both the bacterial treatments over the control. In var. CZP9603, both the bacterial treatments recorded a significant reduction in root fresh weight as compared to control. P3 treatment recorded a significant decrease of 11.11% whereas 14B reduced the root fresh weight up to 28.65% as compared to control. In Ujhani soil, P3 recorded a significant increase of 88.81% while 14B increased the root fresh weight up to 191.3% in var. CZIC923 as compared to control. In var. CZP9603, a significant increase of 26.7% was observed in P3 treated plants as compared to control whereas 14B treatment was found to be non-significant over the control (**Fig. 4.20a**).

A significant increase of 1263.33% in root fresh weight was observed in Pantnagar soil irrespective of other factors over Mukteshwar soil which has minimized the root fresh weight significantly as compared to control (**Fig. 4.20b**).

4.9.2.2 Shoot fresh weight

In Mukteshwar soil, in var. CZIC923, a significant increase in shoot fresh weight was found in both the isolates as compared to control. P3 treatment recorded a significant increase of 52.46% while in 14B treatment a significant increase of 29.01% in shoot fresh weight was observed as compared to control. In var. CZP9603, there was non-significant effect of bacterial inoculation over the control. In Pantnagar soil, in var. CZIC923, 14B significantly increased the shoot fresh weight up to 149.74% while in P3 treatment 85.08% increase was observed over the control while bacterial inoculation was ineffective in var. CZP9603. In Ujhani soil, using var. CZIC923 treated with P3 increased the shoot fresh weight up to 150.98% while in 14B a significant increase of 103.7% was recorded when compared with control (**Fig. 4.21a**). Irrespective of other factors, a maximum increase of 770.07% in shoot fresh weight was recorded in Pantnagar soil as compared to Mukteshwar soil (**Fig. 4.21b**).

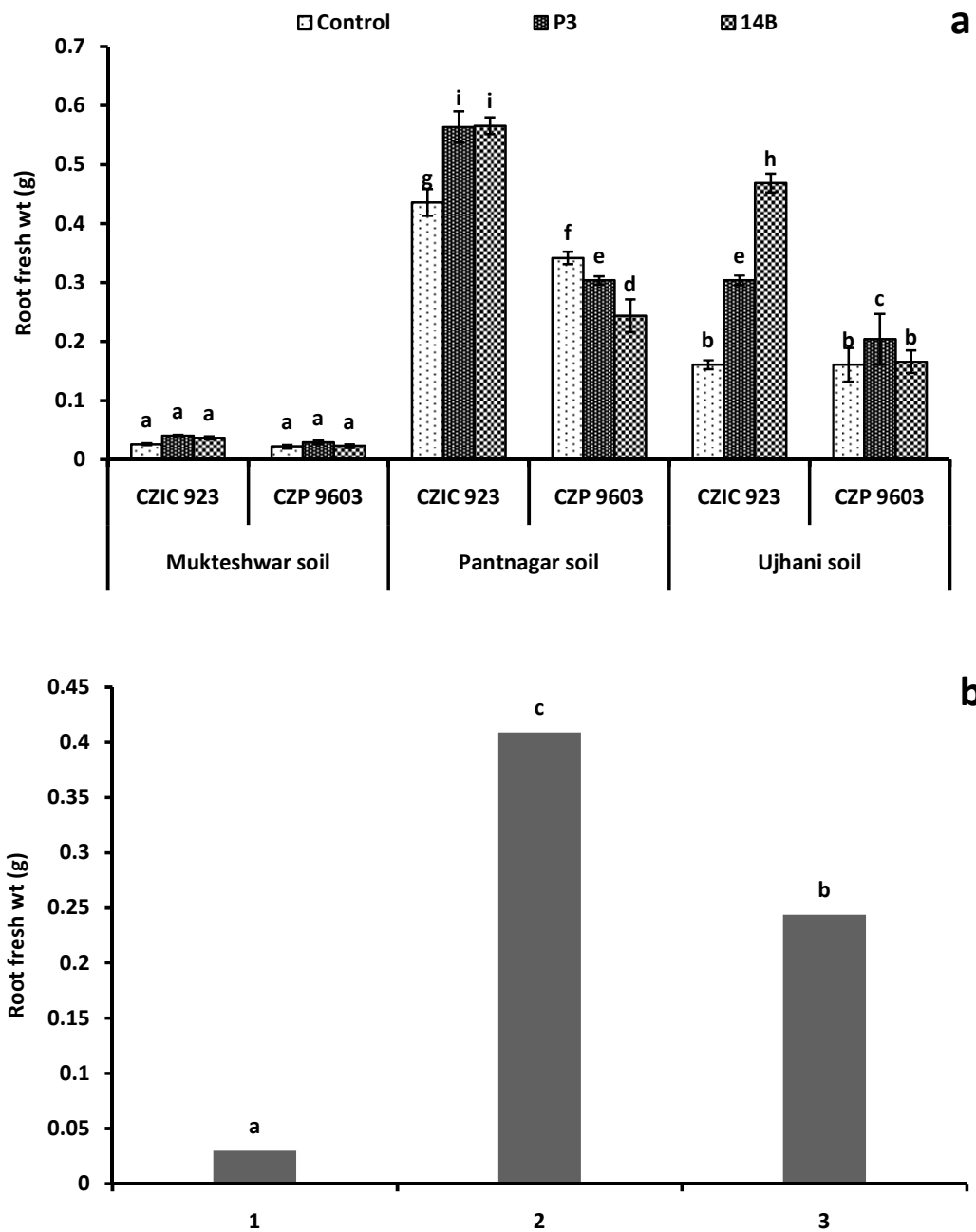


Fig. 4.20: Root fresh weight of two varieties of pearl millet in three soil types (a) Effect of PGPR; (b) Irrespective of varieties and bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

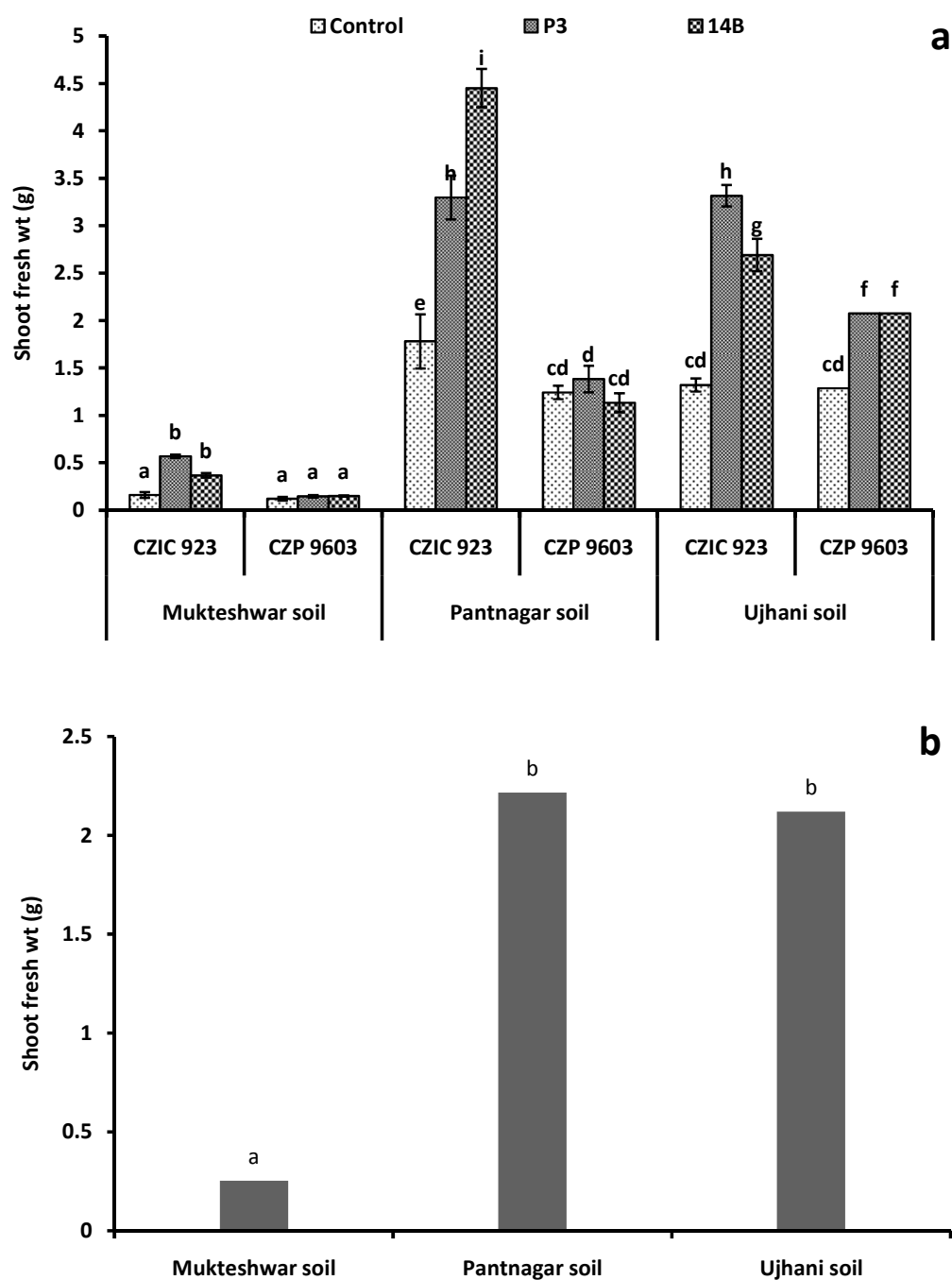


Fig. 4.21: Shoot fresh weight of two varieties of pearl millet in three soil types (a) Effect of PGPR; (b) Irrespective of varieties and bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.9.3 Plant dry weight

4.9.3.1 Root dry weight

In Mukteshwar soil, there was no effect of bacterial inoculation on root dry weight in both the varieties of pearl millet. In Pantnagar soil, in var. CZIC923, 41.71% significant increase was observed in P3 treated plants whereas 14B treatment reduced the root dry weight significantly up to 30.28% as compared to control. In var. CZP9603, a significant decrease of 36.87% in root dry weight was observed in 14B inoculated plants as compared to control whereas P3 treatment was non-significant over the control. In Ujhani soil, in var. CZIC923, P3 treatment recorded a significant increase of 57.53% while in 14B treatment, a significant increase of 102.73% was observed when compared with control. In pearl millet var. CZP9603, there was non-significant effect of treatments over the control (**Fig. 4.22a**). Irrespective of other factors, soil types followed similar trend as in shoot dry weight. In Pantnagar soil, maximum root dry weight (858.82%) was observed over the Mukteshwar soil and significantly minimum root dry weight was observed in Mukteshwar soil (**Fig. 4.22b**).

4.9.3.2 Shoot dry weight

In Mukteshwar soil, in var. CZIC923, P3 treatment recorded a significant increase of 144.11% in shoot dry weight as compared to control while 14B had non-significant effect over the control. In Pantnagar soil in var. CZIC923, P3 and 14B recorded a maximum increase of 55.48 and 61.72% respectively. In var. CZP9603, bacterial treatment had no effect on shoot dry weight over the control. In Ujhani soil, both the PGPR (P3 and 14B) increased the shoot dry weight up to 120.42% in var. CZIC923. In var. CZP2k9, a significant increase of 23.55 and 37.98% was observed in P3 and 14B treatment respectively (**Fig. 4.23a**). Irrespective of other factors, soil types followed the same trend in shoot dry weight as other parameters. Significantly maximum (980.95%) shoot dry weight was observed in Pantnagar soil as compared to Mukteshwar soil (**Fig. 4.23b**).

4.9.4 Root volume

In Mukteshwar soil, bacterial inoculation was ineffective in case of root volume in both the varieties of pearl millet. In Pantnagar soil, in var. CZIC923, a significant increase of 71.42% was observed in P3 treated plants whereas 14B

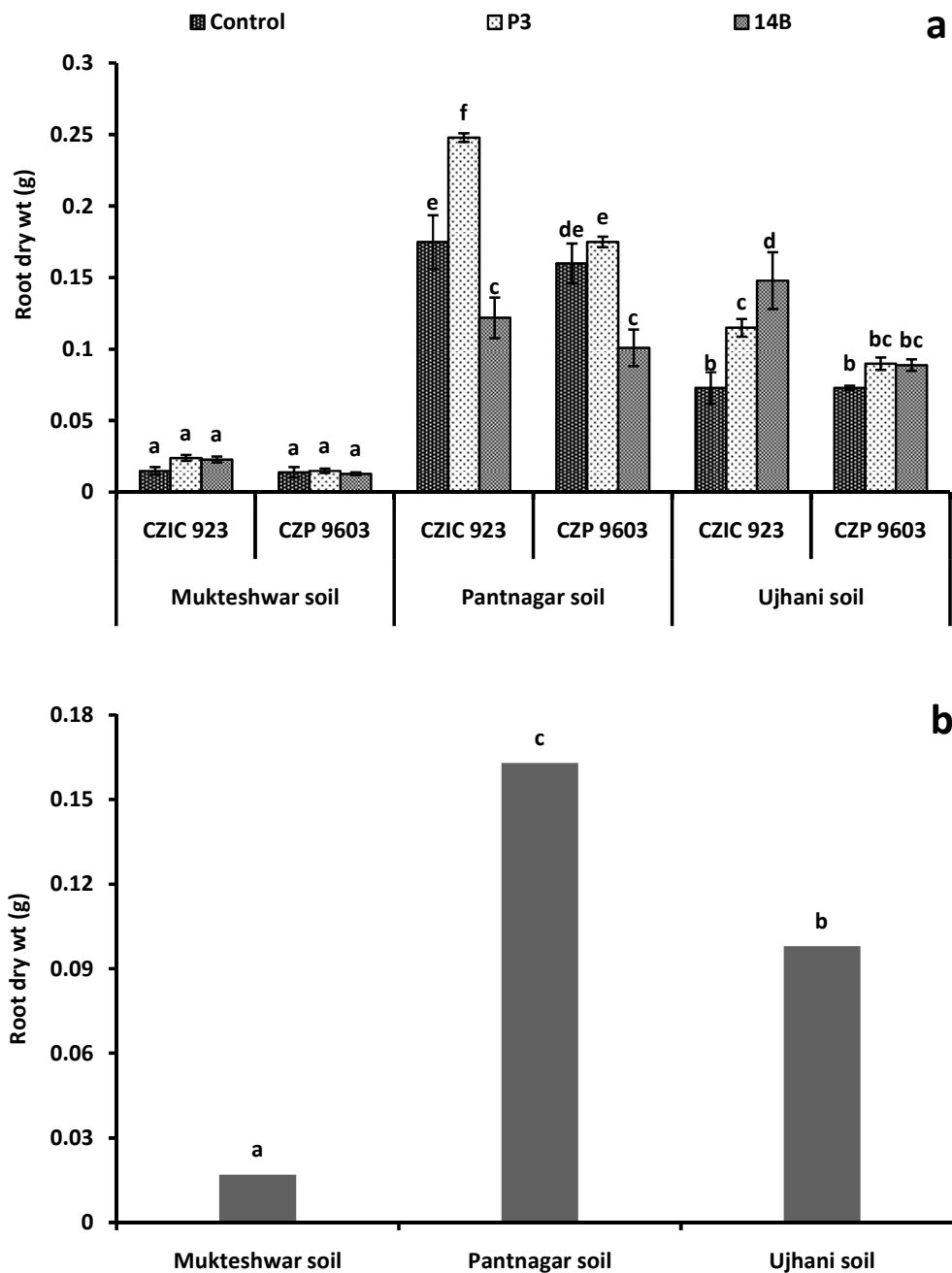


Fig. 4.22: Root dry weight of two varieties of pearl millet in three soil types (a) Effect of PGPR; (b) Irrespective of varieties and bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

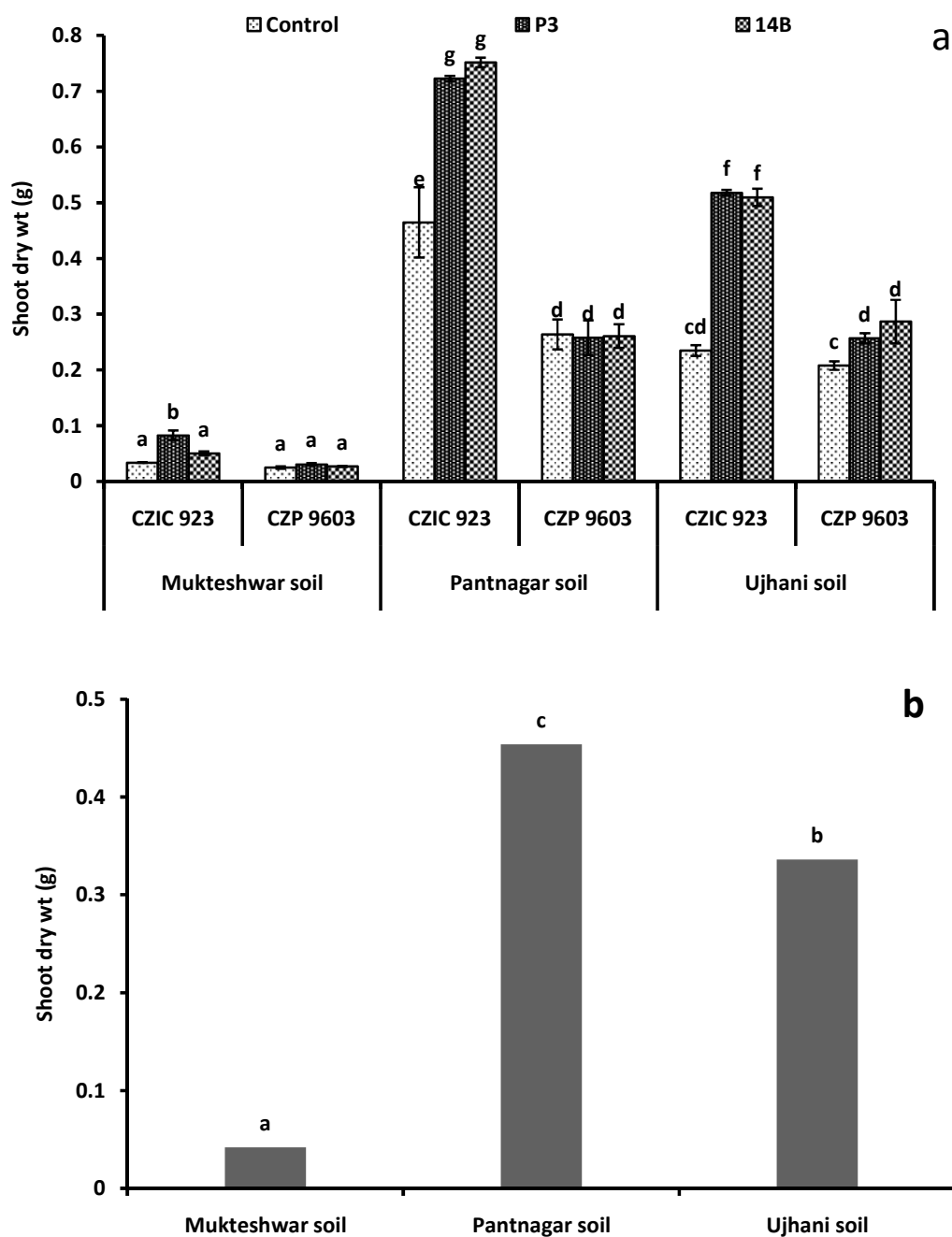


Fig. 4.23: Shoot dry weight of two varieties of pearl millet in three soil types (a) Effect of PGPR; (b) Irrespective of varieties and bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

recorded a significant increase of 89.28% in root volume as compared to control. Var. CZP9603 followed the same trend as in Mukteshwar soil. In Ujhani soil, in pearl millet var. CZIC923, P3 treatment recorded a significant increase of 43.33% in root volume. In 14B treatment, a significant increase of 55% was observed as compared to control. In var. CZP9603, both the bacterial treatment significantly reduced the root volume. P3 treatment recorded a significant reduction of 30.26% in root volume as compared to control while 14B was non-significant over the control (**Fig. 4.24a**). Irrespective of bacterial inoculation and variety, increase in root volume (850.0%) was similar in both the soil (Pantnagar and Ujhani) while minimum root volume was recorded in Mukteshwar soil (**Fig. 4.24b**).

An understanding of ecological conditions affecting bacterial inoculants is important when introducing microbes for increasing plant growth and productivity. In this study the influence of three different soil types on the growth promotory action of bacterial isolates for two varieties (CZIC923 and CZP9603) of pearl millet was investigated. In Pantnagar soil though both the varieties showed increased plant growth while in Ujhani soil, bacterial treatment has better stimulatory effect on plant growth. Plant growth was suppressed in Mukteshwar soil, where high pH might have made nutrient less available to plants. Similar work was reported by **Egamberdiyeva (2007)** in maize suggesting that the plant growth stimulating efficiency of bacterial inoculants was affected by soil nutritional condition. The bacterial inoculation had a much better growth promotory effect on pearl millet in nutrient deficient soil (Ujhani) than in nutrient rich soil (Pantnagar). A study on the effect of soil types on *Cassia* species by **Sharma et al. (1980)** also suggested the role of soil type on the plant growth as black soil was more effective in inducing plant growth.

The results obtained from this study suggest that soil texture, pH and nutritional level have great impact on plant growth stimulating efficiency of PGPR. The bacterial inoculation showed better stimulating effect on the growth of pearl millet in nutrient deficient soil while the stimulatory efficiency reduces in relatively rich soil such as clay loam soil.

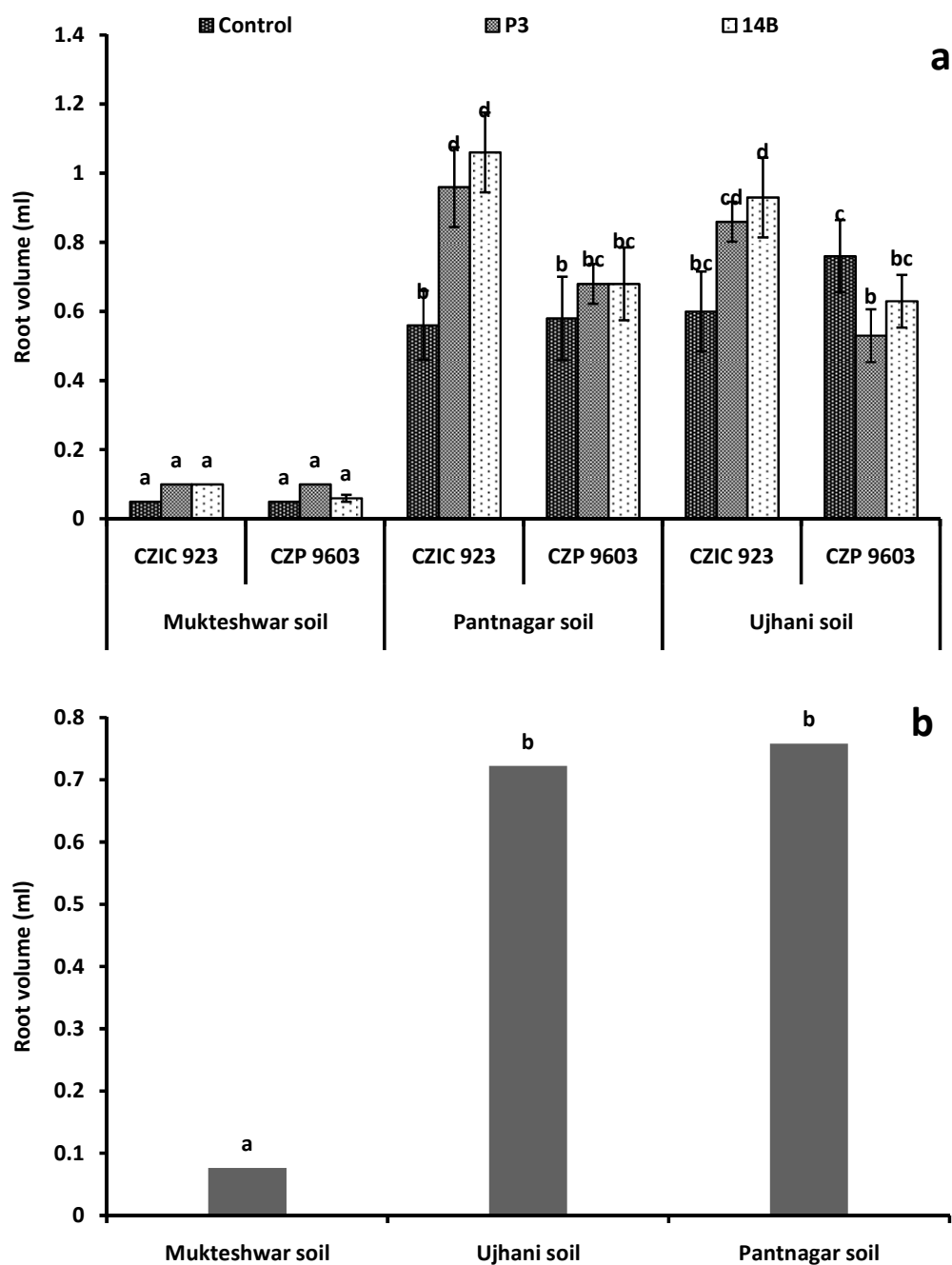


Fig. 4.24 Root volume of two varieties of pearl millet in three soil types (a) Effect of PGPR; (b) Irrespective of varieties and bacterial inoculation. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.10 Growth of PGPR in Nutrient Medium Supplemented with Polyethylene Glycol (PEG 8000)

Both the bacterial isolates (P3 and 14B) were screened for drought tolerance. Growth was recorded after 16 h in stress and non stress condition. Both the isolates showed maximum growth in control, whereas growth was significantly decreased with increased concentration of polyethylene glycol (PEG). Stress level of -1.5MPa seemed to be inhibitory for both the bacterial isolates as growth was reduced sharply. 14B showed higher growth as compared to P3 in all the concentrations of PEG.

4.10.1 Bacterial response to drought stress

4.10.1.1 Exopolysaccharide production

A non-significant increase in exopolysaccharide production was found with time in non stressed conditions in P3 (*Pseudomonas fluorescens*). In all the stress level a gradual increase in EPS content was observed with time except in -1.5MPa, in which decrease (9.63%) in EPS content was observed after 24 h. Maximum EPS content was recorded under stress level of -1.5 MPa at 24 h (**Fig. 4.25a**). Irrespective of PEG concentrations, a significant increase of 23.84% in level of exopolysaccharide was recorded at 24 h while a non significant increase was observed in 36 h (**Fig. 4.25b**).

In 14B (*Bacillus cereus*), in all the treatments a significant increase in EPS content was observed up to 24 h but a significant decrease was recorded at 36 h. Maximum EPS content was recorded under water potential of -1.0 MPa at 24 h having 190.34% increase in EPS content while further a significant reduction of 43.94% was observed in 36h. EPS content was significantly lowered down in -1.5 MPa water potential showing as compared to rest of the concentrations having a significant decrease of 40.72% in EPS content was recorded in 36h (**Fig. 4.26a**). Irrespective of PEG concentration a significant increase of 189.16% was observed at 24 h as compared to 12 h while EPS production lowered down significantly (30%) at 36 h (**Fig. 4.26b**).

4.10.1.2 Proline content

In P3 (*Pseudomonas fluorescens*), similar trend was found in case of proline as in EPS production. In control, a significant increase of 12.92% was recorded at 24 h while 10.23% increase in proline content was observed at 36 h. Similar trend was

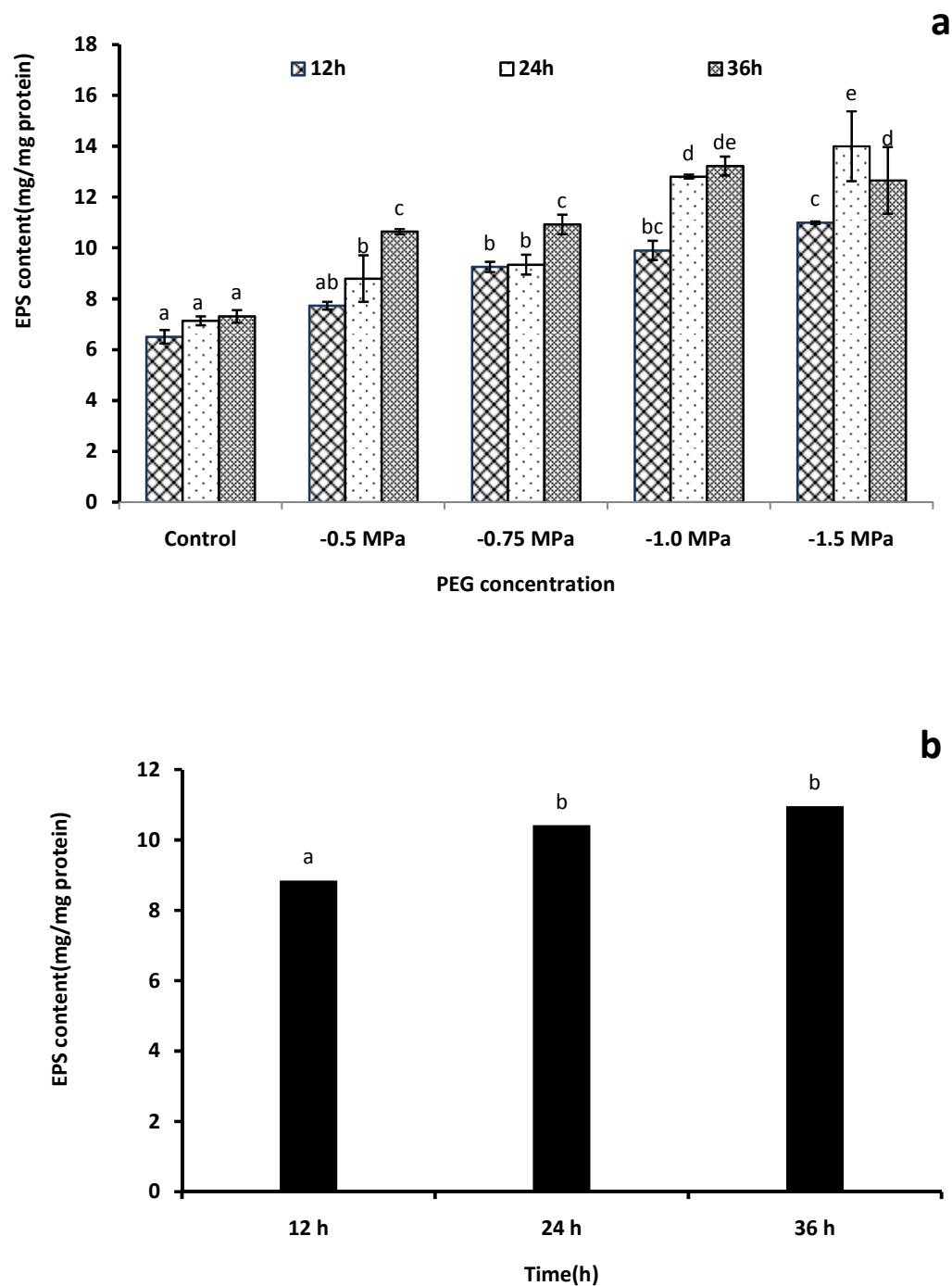


Fig. 4.25: EPS content in P3 (*Pseudomonas fluorescens*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

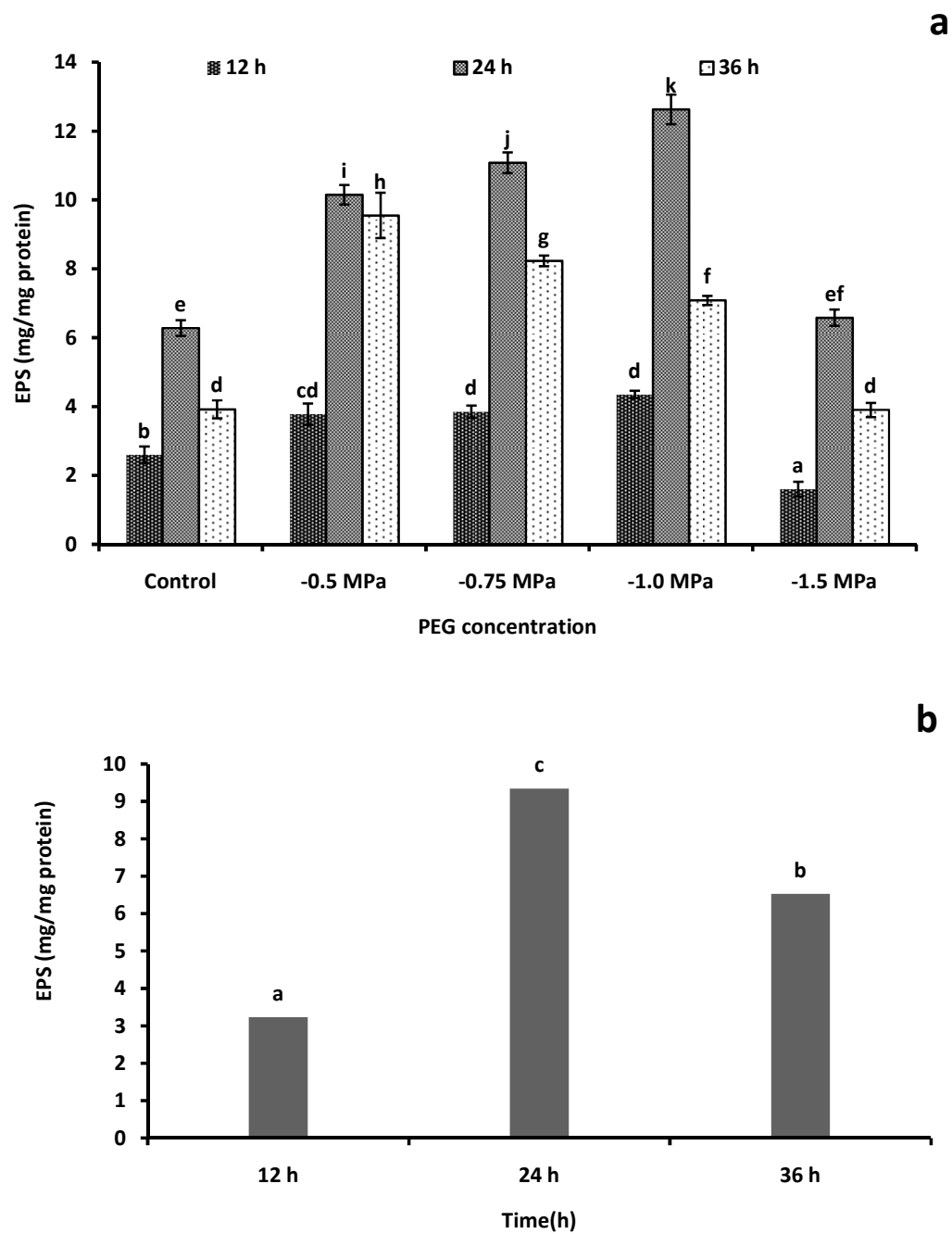


Fig. 4.26: EPS content in 14B (*Bacillus cereus*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

observed in water potential of -0.5 and -0.75 MPa. Bacteria could tolerate the stress level of -1.0 MPa having highest concentration of proline. Under -1.0 MPa, a significant increase of 15.8 and 38% was recorded at 24 h and 36 h respectively as compared to 12 h of growth. In water potential of -1.5 MPa, there was non-significant effect on proline content at 24 h and 36 h as compared to 12 h (**Fig. 4.27a**). Irrespective of PEG concentration the level of proline production significantly increased with time. A significant increase of 13% was recorded in 24 and 36 h respectively (**Fig. 4.27b**).

In case of 14B (*Bacillus cereus*) in control and -0.5 MPa osmotic potential, a gradual increase in proline content was observed with time while under water potential of -1.0 and -1.5 MPa, a significant decrease in proline content was observed after 24 h. Maximum proline concentration was observed under water potential of -0.75 MPa at 24 h, showing a significant increase of 33.17% and 54.5% at 24 h and 36 h respectively. Under water potential of -1.0 MPa, proline content was found significantly higher (15.89%) at 24 h whereas a significant decrease of 8.2% was recorded in 36 h. Under water potential of -1.5 MPa, no significant increase was observed in 24 h while a significant reduction of 32.38% was recorded at 36 h (**Fig. 4.28a**). Irrespective of PEG concentration, a significant increase in proline content was observed with time. In 24 h a significant increase of 16.75% and in 36 h 23.46% significant increase in proline content was recorded as compared to 12 h (**Fig. 4.28b**).

4.10.1.3 Total soluble sugar content

In P3 (*Pseudomonas fluorescens*), under all stress level a gradual increase in sugar content was observed with time except in -1.5MPa, in which decrease (11.8%) in EPS content was observed after 24 h. Under water potential of -0.75 and -1.0 MPa, significant increase of 54.7 and 63% was observed upto 24 h respectively while a non-significant increase was observed at 36 h. Maximum sugar content was found under -1.0MPa water potential at 36 h (**Fig. 4.29a**). In P3, irrespective of PEG concentration, a significant increase of 44.28% was recorded in total soluble sugar level in 24 h as compared to 12 h while a non significant increase was observed in 36 h as compared to 24 h (**Fig. 4.29b**).

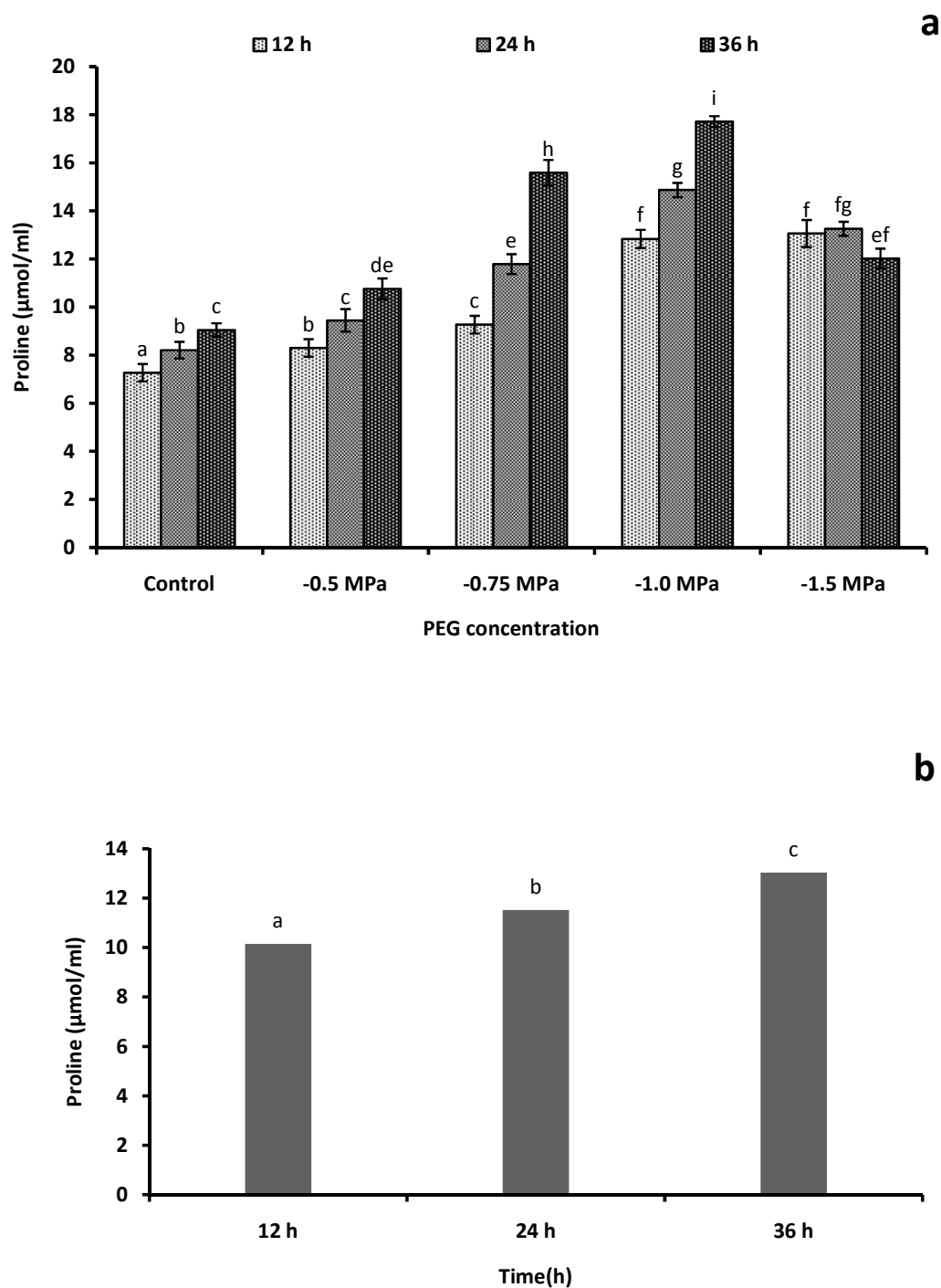


Fig. 4.27: Proline content in P3 (*Pseudomonas fluorescense*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

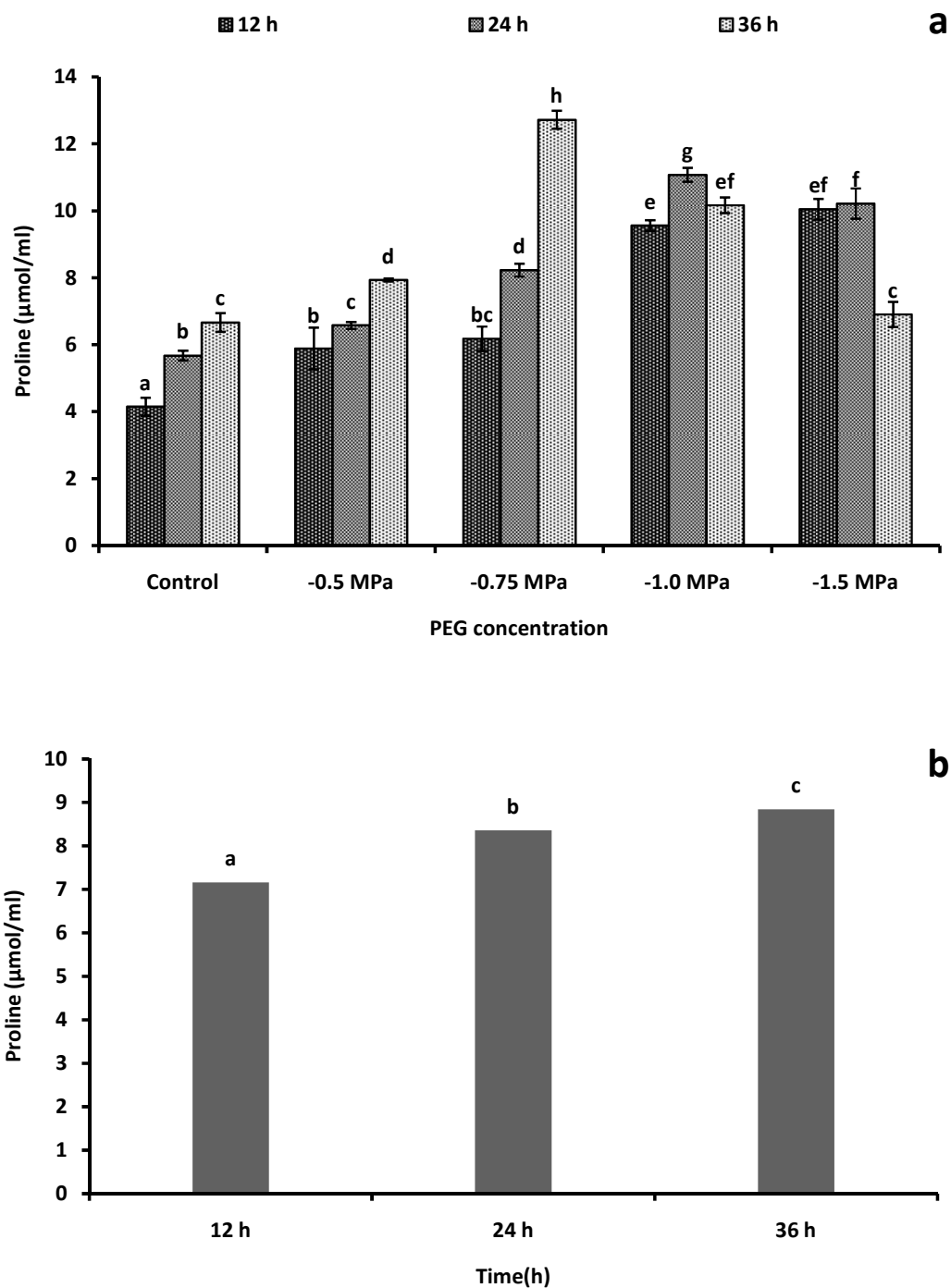


Fig. 4.28: Proline content in 14B (*Bacillus cereus*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

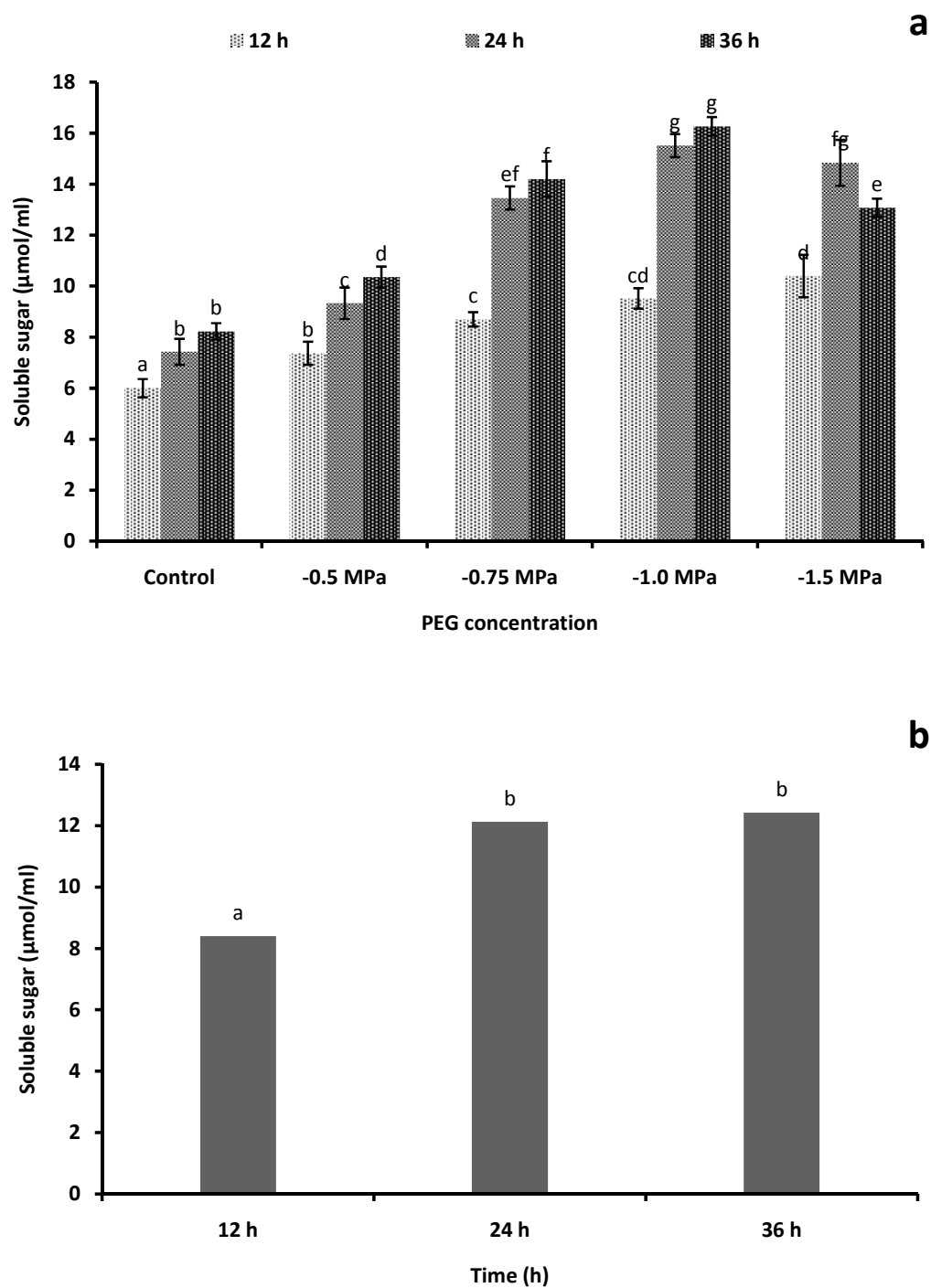


Fig. 4.29: Soluble sugar content in P3 (*Pseudomonas fluorescens*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

In 14B (*Bacillus cereus*), under non stressed conditions increase in sugar content was observed with time. In all the concentrations of PEG, sugar level was significantly increased in 24 h while a significant decrease was reported in sugar content in 36 h under all the stress levels. Under -1.5 MPa water potential, maximum concentration of sugar was observed as compared to other PEG concentration showing a significant increase of 46.64% was observed in 24 h as compared to 12 h while there was a decrease of 27.9% was reported in 36 h (**Fig. 4.30a**). A significant increase of 42.17% in sugar content irrespective of PEG level was reported in 24 h which was at par with 36 h (**Fig. 4.30b**).

4.10.1.4 Protein content

As the level of stress increased in the bacterial cell, production of EPS was increased while level of protein lowered down. Protein level increased significantly with time in control and -1.5 MPa while protein level was significantly decreased with time under other stress conditions. Maximum protein content was observed in control at 36 h showing a 96% increase as compared to 24 h (**Fig. 4.31a**). Irrespective of PEG concentration, a significant increase of 8.56% in 24 h and 39.24% in 36 h was recorded in protein level (**Fig. 4.31b**).

14B followed similar trend of protein content as in case of P3 for all the treatments. Maximum protein content was observed in control at 36 h showing a 39.3% increase as compared to 24 h Minimum level of protein was found in stress level of -1.0 MPa (**Fig.4.32a**). Irrespective of PEG concentration, a significant increase of 41.86% in 24 h was observed while in 36 h 18.5% increase in protein level was observed (**Fig.4.32 b**).

Table 4.5 Effect of drought stress (-1.0 MPa) on PGPR properties

Bacterial strain	Properties			
	Siderophore production	P-Solubilization	IAA production (µg/ml)	NH ₃ production
P3	++	++	1.66	+
14B	+	+	1.09	+

+++Good growth, ++Moderate, +Survival, -No growth

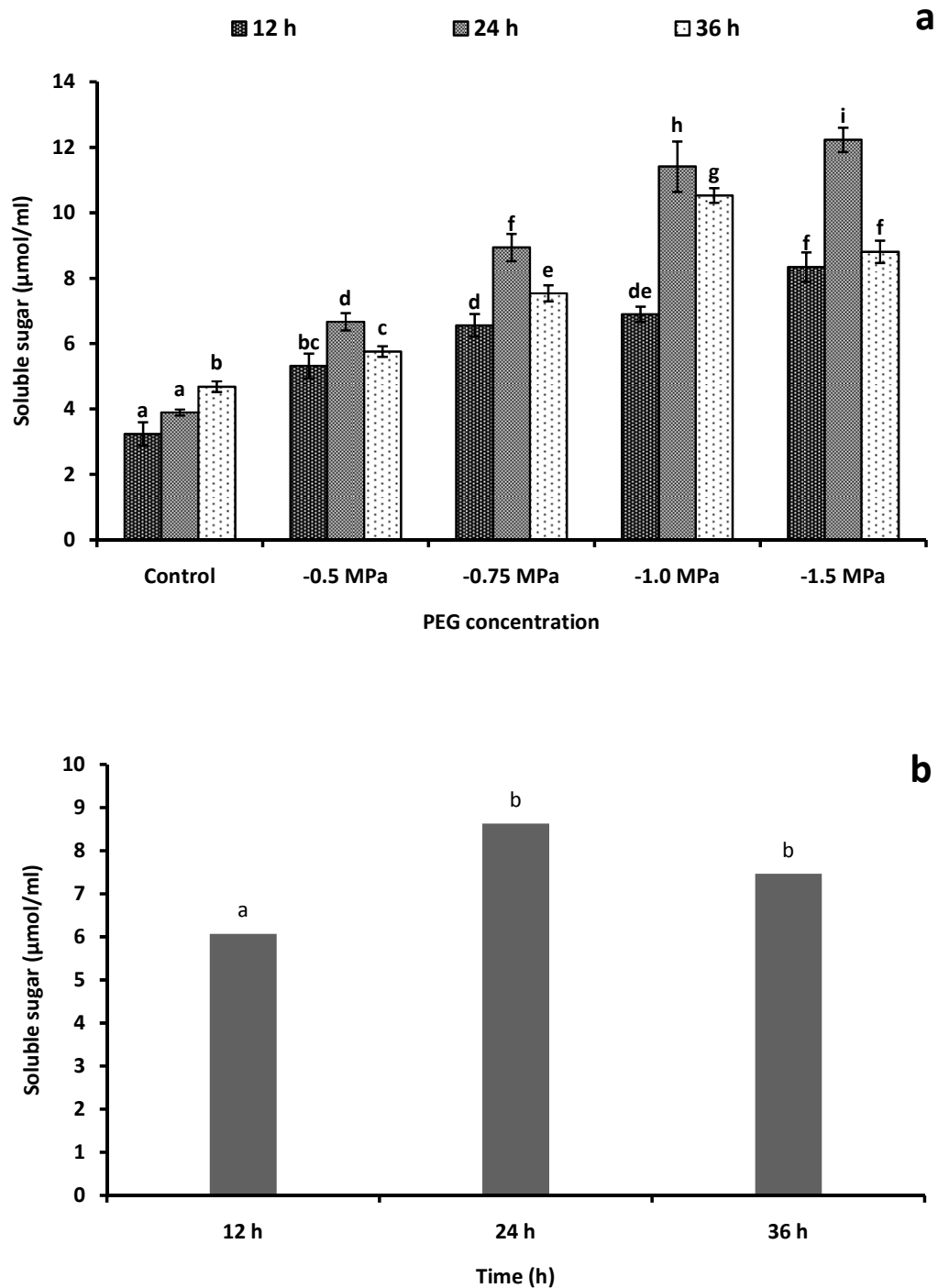


Fig. 4.30: Soluble sugar content in 14B (*Bacillus cereus*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

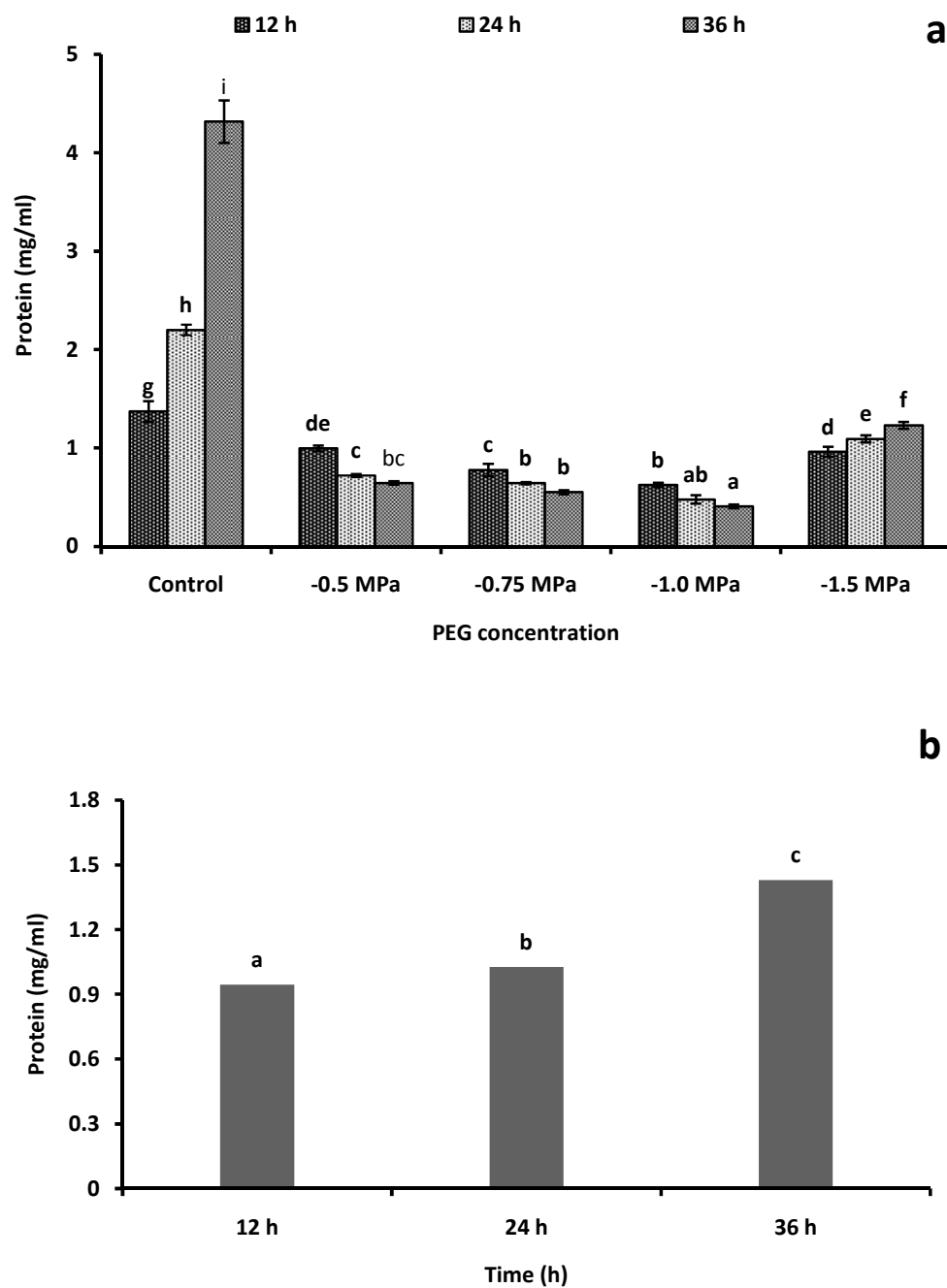


Fig. 4.31: Protein content in P3 (*Pseudomonas fluorescence*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

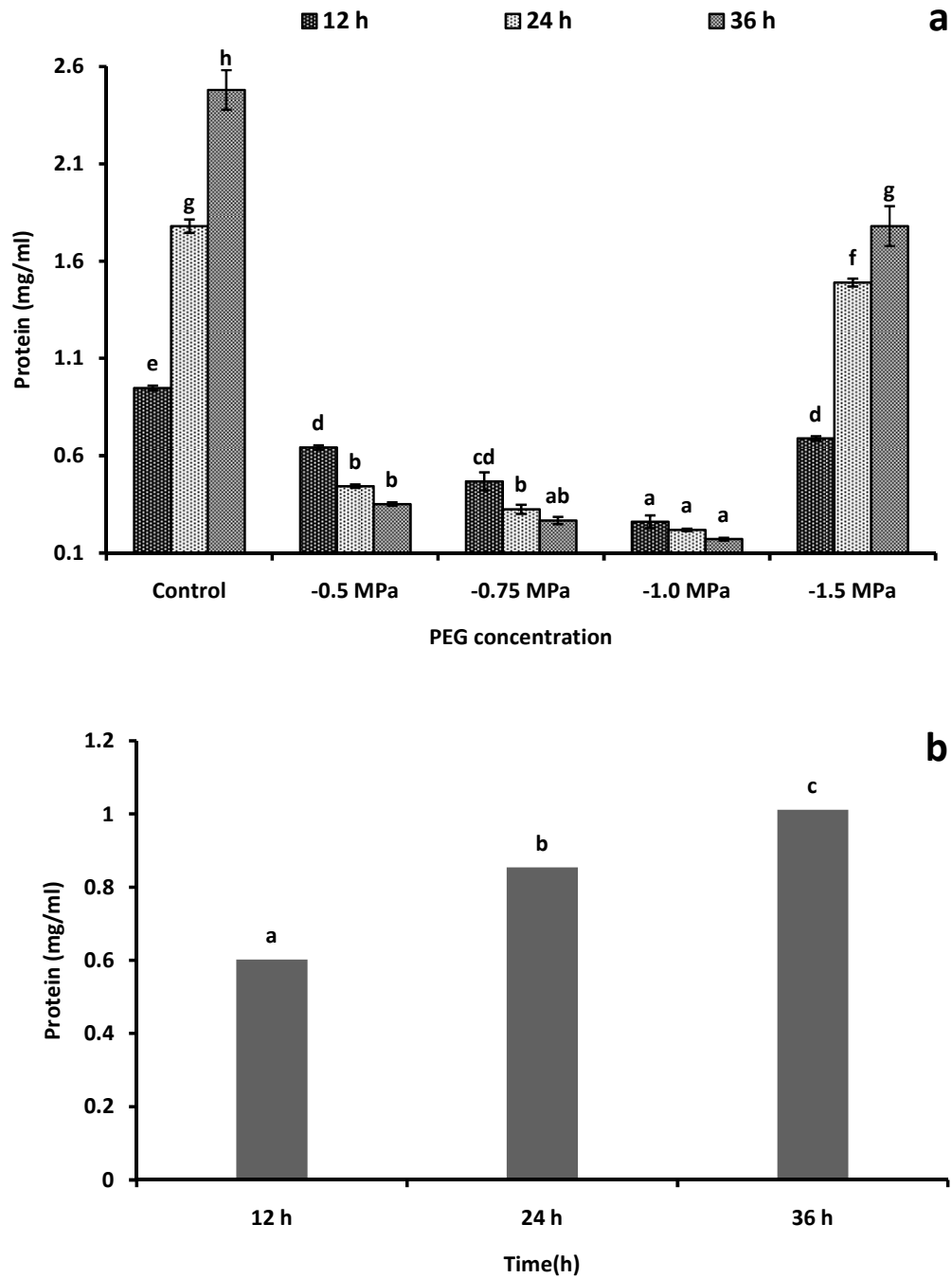


Fig. 4.32: Protein content in 14B (*Bacillus cereus*) in different time interval (a) Under different water potential; (b) Irrespective of water potential. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

Both the isolates also exhibited plant growth promoting properties (**Table 4.5**) like production of ammonia, siderophore, IAA, and P solubilization under drought stress conditions; significant reduction in plant growth promoting traits was observed under drought stress.

In this experiment, both the isolates were grown in nutrient broth medium with different concentration (-0.5, -0.75, -1.0 and -1.5 MPa) of PEG. Both could grow upto water potential of -1.0 MPa and possessed PGP properties (IAA, P-solubilization, production of siderophores and ammonia) both under osmotic potential of -1.0MPa. Water potential of -1.5 MPa seems to be inhibitory for both the bacterial isolates as the growth, EPS and osmolytes production lowered down significantly with time. In a similar study by **Sandhya et al. (2009)**, it was reported that *Pseudomonas* sp. strain GAP-P45 could tolerate maximum level of drought stress (-0.73 Mpa). The EPS production of these isolates was higher under stressed than under no stress conditions, and it increased by increasing stress level, indicating that EPS production in bacteria occurs as a response to the stress (**Roberson and Firestone, 1992**). Under stress conditions energy flow of the cells is directed toward protection mechanisms to synthesize osmolytes to protect themselves against fluctuations in osmotic conditions (**Timmusk, 2003**) and accumulate to higher levels to alleviate stress effects (**Raˆsaˆnen et al., 2004**). The accumulated osmolytes enhance the stability of proteins and membrane under water-limiting environments (**Kogut and Russell, 1987**). Both the isolate showed the accumulation of more proline, sugar and EPS under stress conditions as compared to non-stressed conditions, indicating the role of these metabolites in stress tolerance. However, the concentration of protein was reduced significantly under stress indicating the degeneracy under stress conditions. Under stress, proteins are used for polysaccharide production (**Roberson and Firestone, 1992**). Our findings were also supported by the study done by **Vardharajula et al. (2011)** in which *Bacillus* spp. strains HYD-B17, HYTAPB18, HYDGRFB19, BKB30, and RMPB44 secrete conspicuous amounts of EPS, proline and soluble sugar under stress conditions. EPS forms an organo-mineral sheath around the cells, which leads to an increase in microaggregates as an indirect additional effect, which improves the plant growth under drought stress by increasing aggregate stability of soil (**Alami et al., 2000**).

If we compare the level of EPS and osmolytes between *Pseudomonas fluorescens* (P3) and *Bacillus cereus* (14B), a significant increase was observed in case of P3. This could be understood by the fact that a non-specific physical stress such as increased osmotic pressure might cause the accumulation of EPS in *Bacillus* sp, thereby stimulating the kinase activity of KinD (a type of kinase) which in turn enhance the level of Spo0A gene, responsible for sporulation. This rise repressed the gene for SinI (a repressor of SpoA) and instead turned on genes for entry into sporulation (Rubinstein *et al.*, 2012). Most of the metabolic energy shifted towards spore formation as sporulation is highly energy demanding process hence the level of EPS and osmolytes lowered down in 14B as compared to P3.

Under water stress of -1.0 MPa, both the isolate showed the plant growth promoting properties which discussed earlier. PGP substances under drought stress conditions can be helpful in maintaining better nutritional status thus influencing plant microbe interactions hence can be used as criteria for the selection of efficient PGP strains for plant studies under harsh conditions (Vardharajula *et al.*, 2011). In addition to general plant growth, IAA stimulates stress tolerance because of physical and chemical changes in plant caused by these PGPR (Marulanda *et al.*, 2010). Inoculation with cadmium resistant strains *Pseudomonas tolaasi* and *Pseudomonas fluorescens* enabled *Brassica napus* to grow under cadmium stress because of the production of IAA and siderophores (Dell'Amico *et al.*, 2008). Similarly cytokinins are believed to be signals involved in mediating of environmental stresses (Jackson, 2002) and their balance is influenced by environmental cues (Timmusk, 2003). From above study we conclude that P3 and 14B were an efficient EPS producers under drought stress (-1.0 MPa) and possessed plant growth properties. Therefore, they were selected for inoculation of pearl millet plants under drought stress conditions.

4.11 Screening of Tolerant/Susceptible Pearl Millet Varieties Inoculated with PGPR during Drought

Drought stress is one of the most adverse factors for plant growth and productivity (Guoth *et al.*, 2008). In the present study, in order to determine whether the two bacterial isolates P3 (*Pseudomonas fluorescens*) and 14B (*Bacillus cereus*) could elicit antioxidant activities in different varieties of pearl millet under drought condition; a pot experiment was carried out. Three varieties of pearl millet (CZIC923, CZP2k9 and CZP9603) were used and inoculated with two PGPR (P3 and 14B). These different varieties were selected on the basis of their performance under glass

house conditions, inoculated with PGPR. Drought stress was imposed in 45 days old plants by withholding the irrigation. At different soil moisture level three times harvesting was done. Screening for identification of a potential drought tolerant and a susceptible variety, from among three pearl millet varieties was done through stress marker analysis and using various biochemical enzyme assays. A differential response among the three varieties provided a clear illustration of the fact that tolerant variety respond better for growth, antioxidative enzymes and proline accumulation (**Plate 7**).

4.11.1 Plant growth under drought

Drought stress drastically affected the growth of pearl millet plant as reflected by stunted growth, less vigor, and drying of leaves. After 45 days of germination, soil moisture in pots subjected to drought stress was 60 and 35% of WHC as compared to 85% of WHC in pots without stress.

4.11.1.2 Plant height

4.11.1.2.1 Root length

Root length was consistently decreased with increase in stress. In plants grown in normal condition, significant increase in root length of the plant treated with PGPR was observed when compared with control in all the test varieties of the pearl millet. In 4th day of the stress, bacterial treatment was effective in all the varieties over the control. In 8th day of stress, in pearl millet varieties CZP2k9 and CZP9603, a non significant effect of bacterial treatment was observed in root length. In var. CZIC923, a significant increase of 1.5 fold in root length in plants treated with P3 was observed while in treatment 14B, a 1.1 fold increase was recorded (**Fig. 4.33a**). Irrespective of bacterial inoculation and level of stress, maximum root length (11.20%) was observed in var.CZIC923 over the two varieties of pearl millet used (**Fig. 4.33b**).

4.11.1.2.2 Shoot length

A decrease in shoot length of all the varieties of pearl millet was observed with increase in stress. In var. CZP2k9, both the bacterial treatments were significantly effective over the control in stressed as well as non stress conditions while no significant increase in root length of the plants treated with bacteria was observed in var. CZIC923 and CZP9603 (**Fig. 4.34a**). A significant increase of 14% in shoot length was observed in var.CZIC923 as compared to other varieties irrespective of other factors (**Fig. 4.34b**).

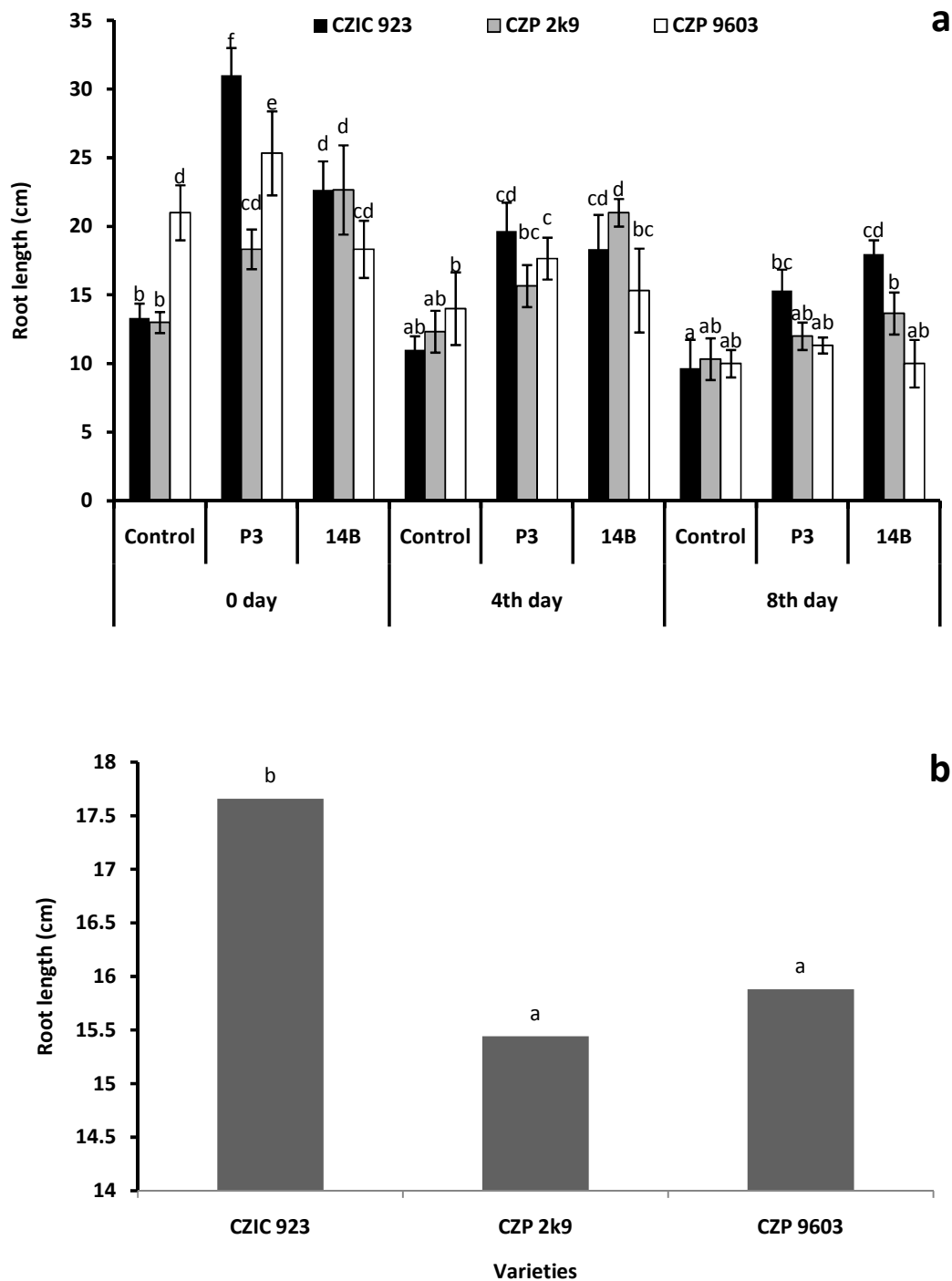


Fig. 4.33: Effect of drought on root length of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

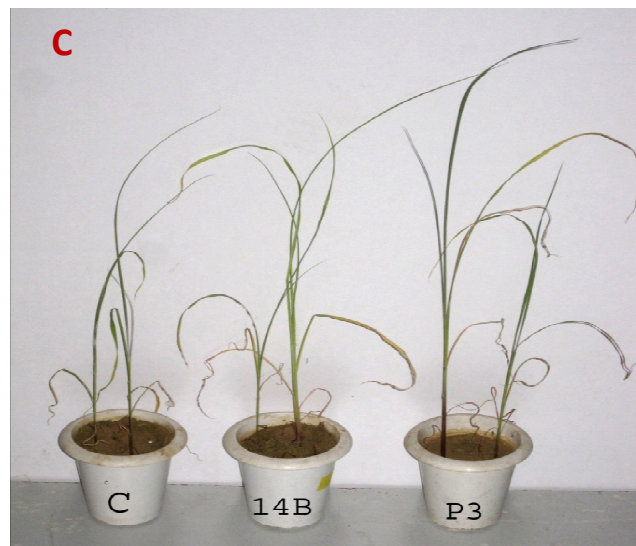
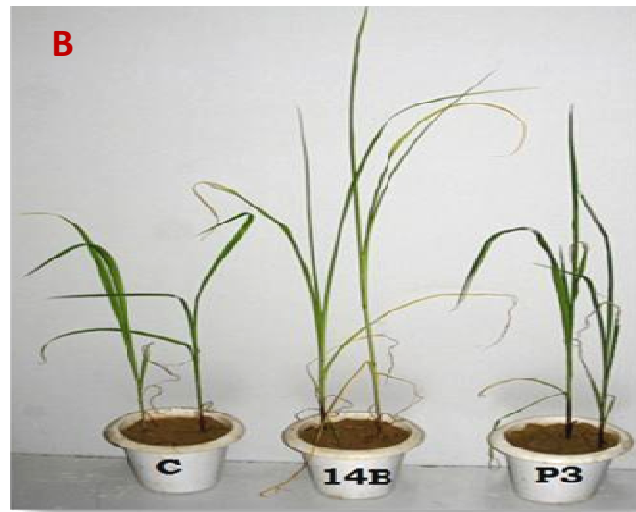
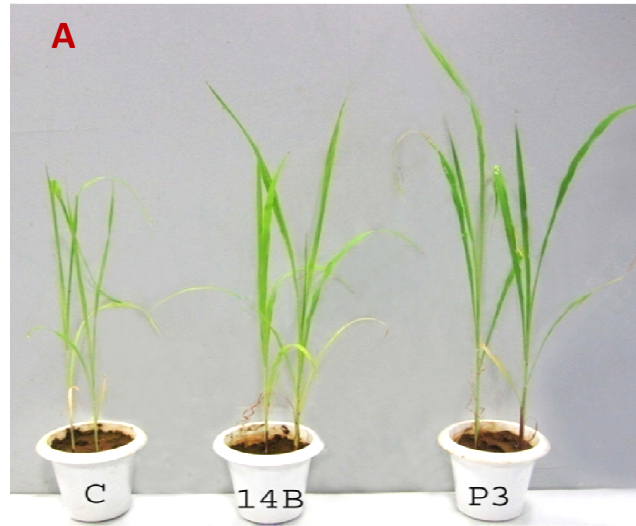


Plate 7: Effect of PGPR on growth of Perl millet: A- normal condition; B- 4 days after drought condition; c-8 days after drought

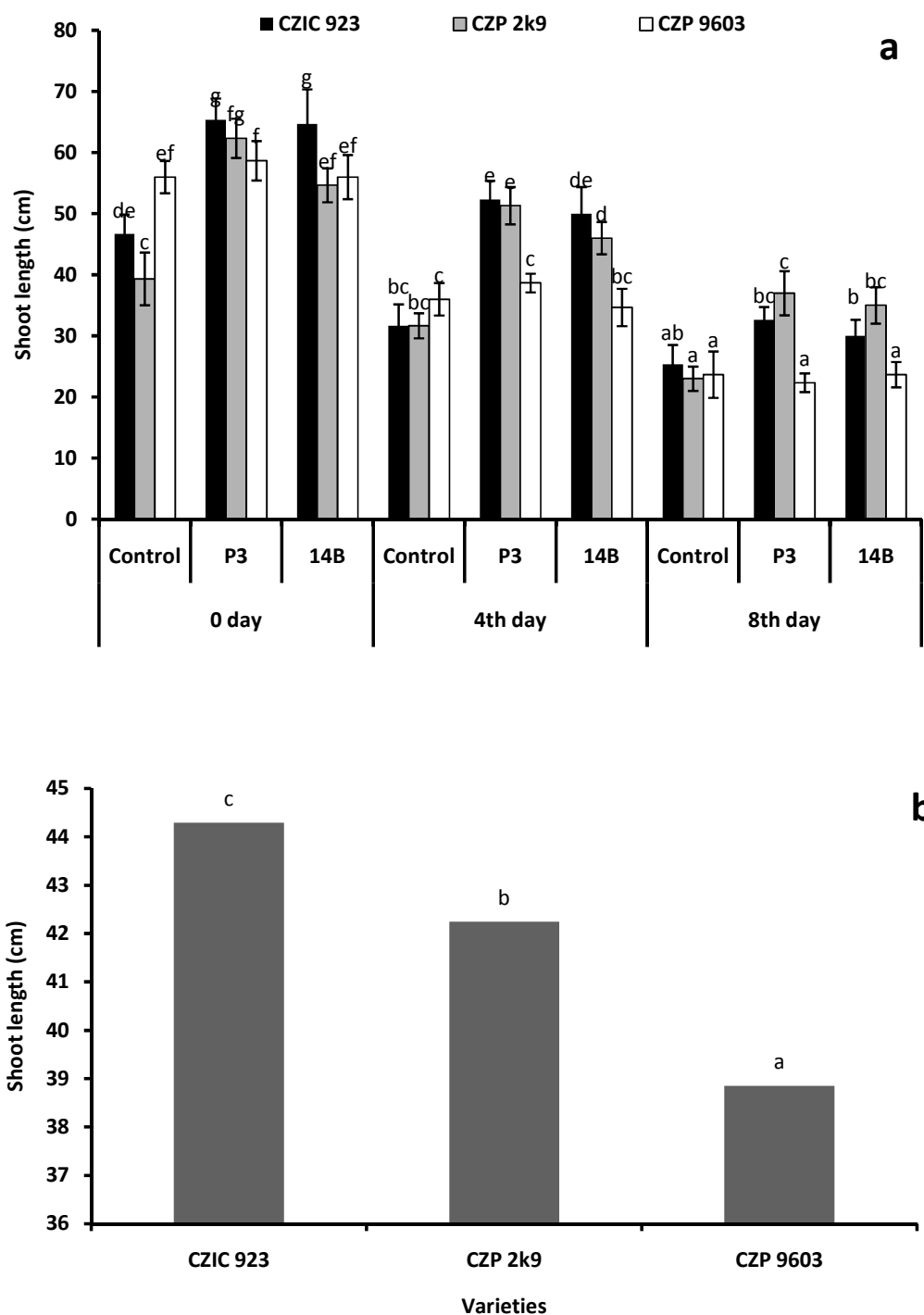


Fig. 4.34: Effect of drought on shoot length of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.11.1.2 Plant fresh weight

4.11.1.2.1 Root fresh weight

A continuous decrease in root fresh weight was observed with increasing stress in all the varieties tested. In var. CZP9603, bacterial treatment was found non-significant both in stressed as well as non stressed condition over that of the control. In 8th day of stress in var. CZIC923, a significant increase of 3.9 fold in root fresh weight was found in P3 treated plants while a significant increase of 2.5 fold was observed in 14B treated plants as compared to control. In var. CZP2k9, a significant increase of 3.1 and 2.8 fold was observed in P3 and 14B treated plants respectively (**Fig. 4.35a**). Irrespective of other factors, var. CZIC923 showed the maximum (121.73%) root fresh weight as compared to other varieties (**Fig. 4.35b**).

4.11.1.2.2 Shoot fresh weight

A gradual decrease in shoot length was observed in all the test varieties of pearl millet with stress. Var. CZP9603 followed almost similar trend as in case of root fresh weight. In var. CZIC923, both the bacterial isolates showed a significant increase of 2.1 fold in shoot fresh weight. In var. CZP2k9, 5.3 and 4.3 fold increase in shoot fresh weight was recorded in P3 and 14B respectively (**Fig. 4.36a**). Irrespective of bacterial inoculation and stress level var. CZIC923 showed maximum increase (58.33%) as compared to rest of two varieties of pearl millet (**Fig. 4.36b**).

Plants treated with PGPR showed enhancement in plant growth as compared to uninoculated plants under stress. Among the three varieties of pearl millet var. CZIC923 showed maximum plant growth as compared to other two varieties followed by var. CZP2k9 while minimum plant growth was recorded in var. CZP9603. Thus a higher plant growth in var. CZIC923, under stress, can be correlated with better stress tolerance characteristics of this variety. Similar correlation of plant with higher root biomass showing increased stress tolerance due to higher microbial activity was highlighted by **Haynes and Francis (1993)**. The increased microbial biomass thus leads to production of EPS under stressful environment, which improved soil permeability by increasing soil aggregation and maintaining higher water potential around the roots; in this way, there was an increase in the uptake of nutrients by plant, with an increase in plant growth; in addition, the bacteria protected the

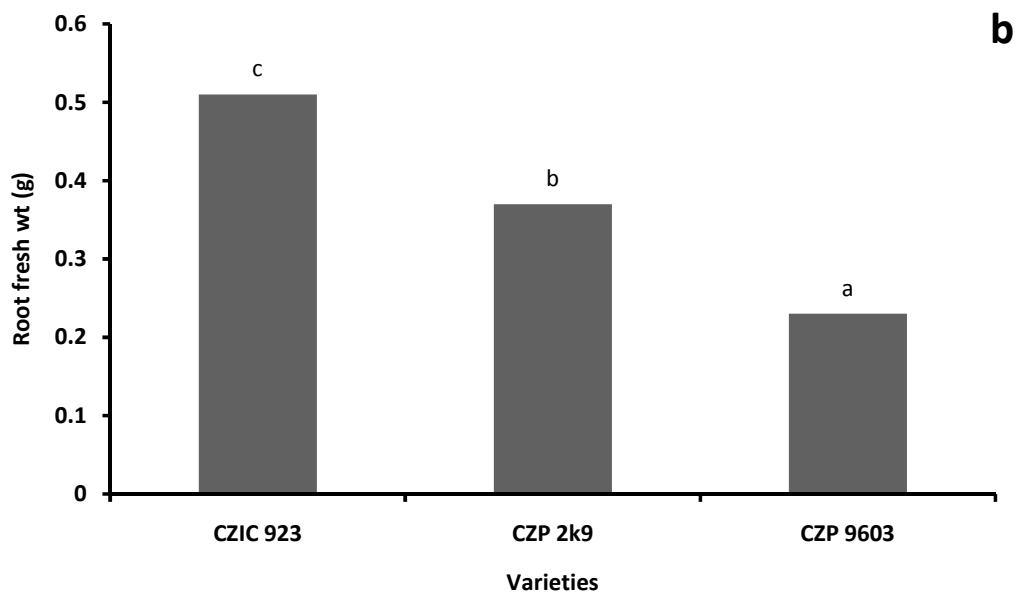
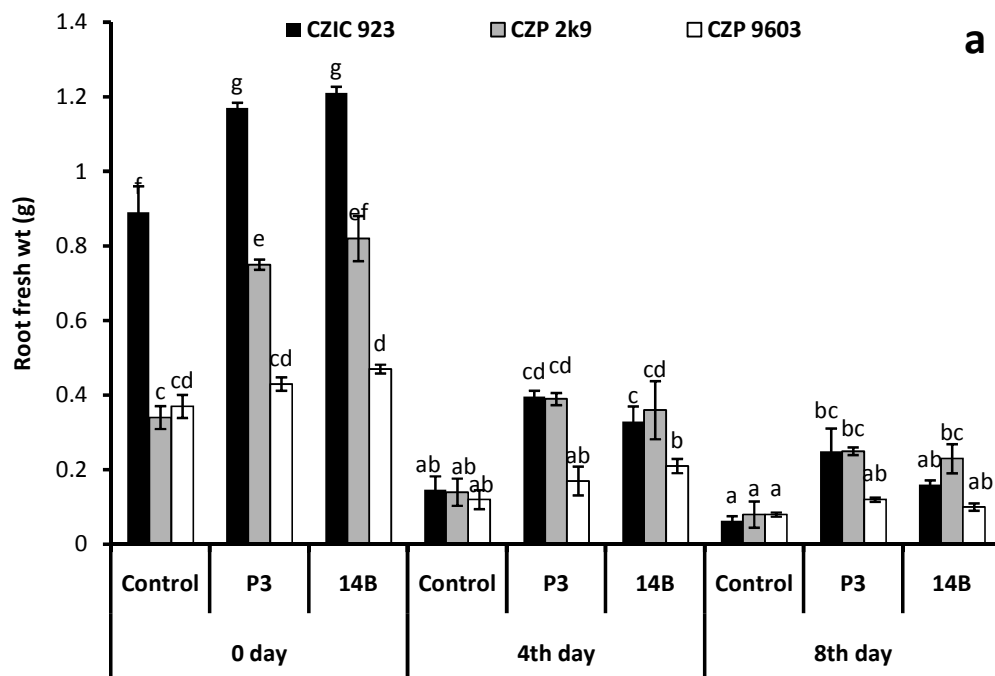


Fig. 4.35: Effect of drought on root fresh weight of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

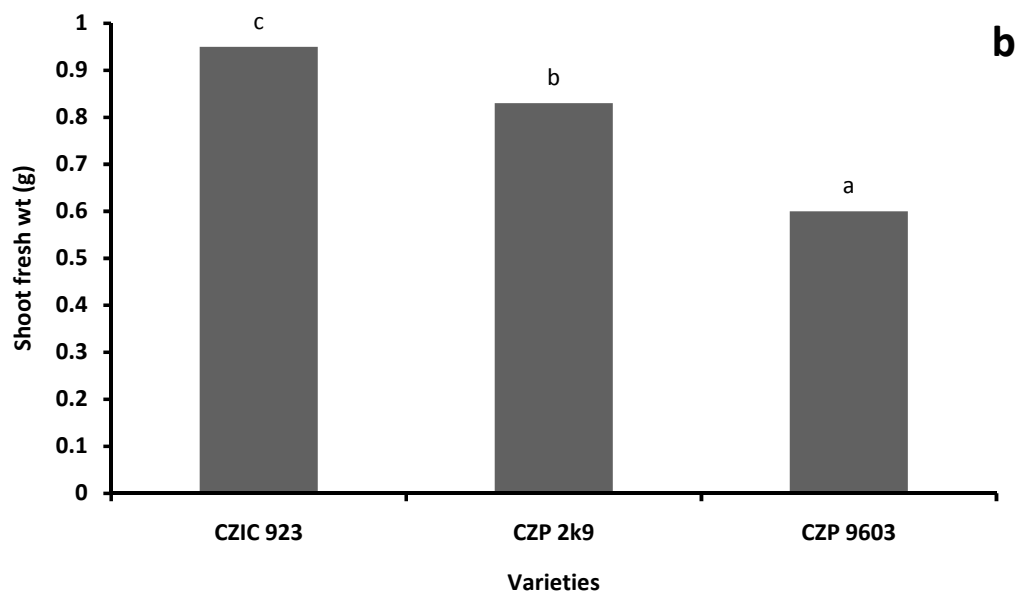
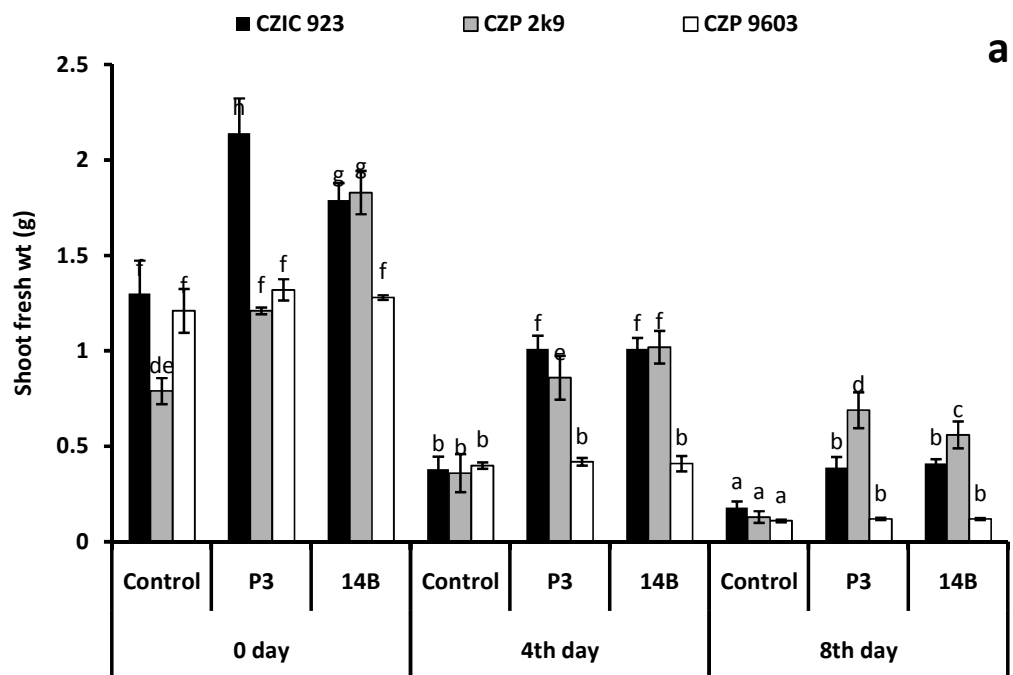


Fig. 4.36: Effect of drought on shoot fresh weight of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

seedlings from drought stress (Alami *et al.*, 2000; Miller and Wood, 1996). Higher EPS content could help the plants to take up a higher volume of water and nutrients from rhizosphere soil (Miller and Wood, 1996), resulting in a better growth of plants, and also, this was useful to counteract the negative effects of drought stress (Munns, 2002). In uninoculated seedlings, due to the absence of EPS producing bacterial populations, most of roots was devoid of EPS and thus was more susceptible to stress effect. Moreover, a higher population of EPS-producing bacteria on roots of inoculated plants may have stimulated root exudation (Tisdall, 1994; Miller and Wood, 1996; Wittenmayer and Merbach, 2005), with stimulation of growth of inoculated bacteria with higher EPS production in the rhizosphere (Fischer *et al.*, 2003). Similar work was reported in *P. putida* GAP-P45 by Sandhya *et al.* (2009).

4.11.2 Antioxidant marker analysis

4.11.2.1 Proline accumulation

Osmotic adjustment through the accumulation of cellular solutes, such as proline, has been suggested as one of the possible means for overcoming osmotic stress caused by the loss of water (Caballero *et al.*, 2005). It also acts as a storage compound for protein synthesis. Whenever a plant experiences a stress, inhibition of starch biosynthesis takes place and proline accumulation is used as a source of carbon and nitrogen for the survival of plant (Schellenbaum *et al.*, 1998).

A significant increase in proline content was recorded in the leaves of stressed plants in all the treatments. In plants with no stress, bacterial inoculation was non significant over the control while in stressed plants both the bacteria resulted in a significant increase in the proline content in all varieties of pearl millet. In 8th day of stress, a maximum increase of 1.85 fold in proline content was found in P3 treatment followed by 14B which recorded increase of 1.08 fold over the uninoculated plant in var. CZIC923. In var. CZP9603, a maximum increase of 1.2 fold was found in both the bacterial treatment over the control while var. CZP2k9 recorded an increase of 1.4 fold in both the bacterial treatment as compared to control (Fig. 4.37a). In pearl millet var. CZIC923 significant increase of 44.92% in proline level was found irrespective of bacterial inoculation and level of stress as compared to var.CZP9603 (Fig. 4.37 b). The differential increase in proline content provides a clear illustration of the fact that tolerant variety respond differently for proline accumulation, suggesting its vital role

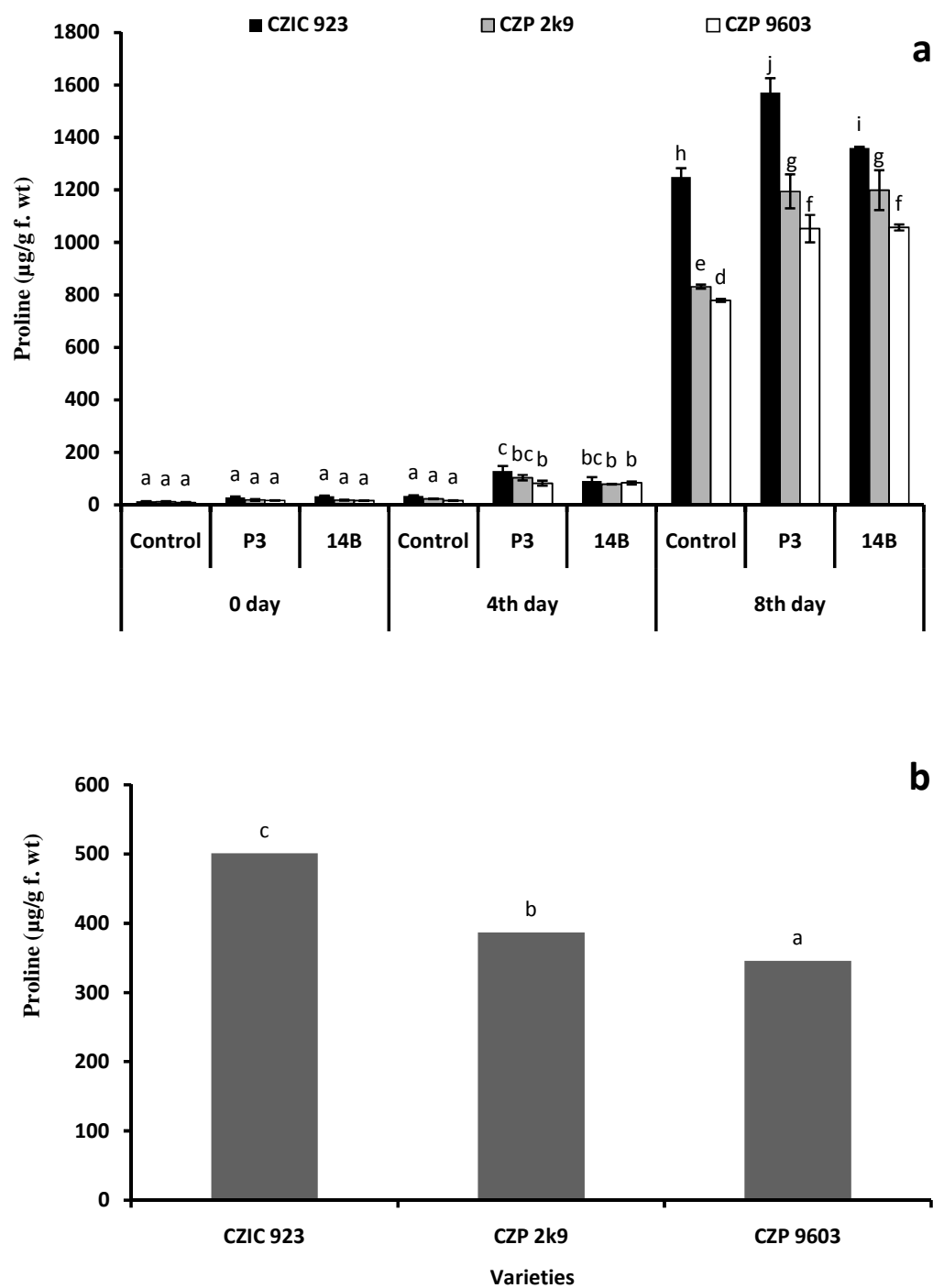


Fig. 4.37: Effect of drought on proline content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

as an osmoregulatory solute in plants (Kumar *et al.*, 2011). Similar varietal differences in proline accumulation were also observed in seedlings of other plants subjected to various abiotic stresses (Yadav *et al.*, 2004; Liu *et al.*, 2010; Cao *et al.*, 2011). Kohler *et al.* (2009) reported the increased accumulation of proline in the plants inoculated with *P. mendocina*, alone or in combination with either of the selected Arbuscular Mycorrhizal fungi under severe drought conditions. Thus, it is hypothesized in the current study that accumulation of proline in bacterial treated pearl millet plants might attribute to the bacterization of *P. fluorescens* (P3) followed by *B. cereus* (14B), thus suggesting their vital role in alleviating drought stress.

4.11.2.2 Malondialdehyde content

Malondialdehyde is one of many low molecular weight end products of lipid hydroperoxide decomposition and is most often measured as an index of lipid peroxidation. Lipid peroxidation has been established as major mechanisms to trace the cellular injury in many biological systems of plant and animal origin. In the present study it was observed that the MDA content gradually increased in all the treatments with increasing stress. In 0 day of stress there was non-significant effect of the treatments over the control while in 4th and 8th day of stress both the bacterial treatment significantly reduced the MDA content when compared with control in all the varieties of pearl millet. In 8th day of stress, both the bacteria (P3 and 14B) significantly recorded a 1.85 fold decrease in MDA content over the control in var. CZIC923. However, var.CZP2k9 recorded a 1.6 fold decrease in MDA content in plants treated with both the bacterial treatments. In var. CZP9603, significantly less (1.08 fold) MDA was observed in plants treated with P3 while reduction in MDA content was non-significant in 14B treated plants when compared to control (**Fig. 4.38a**). Among the varieties of pearl millet var. CZIC923 performed better as minimum MDA content (53.27%) was found in this var. when compared with var. CZP9603 of pearl millet (**Fig. 4.38b**). The observed changes in the MDA content were consistent with previous work done where an enhanced level of lipid peroxidation of grasses and maize was recorded under drought stress indicating the oxidative damage to plants. Thus signifying lipid peroxidation to be a direct consequence of increased ROS generation (Fu and Huang, 2001; Bai *et al.*, 2006). Similar results have been obtained by El-Tayeb (2006) where MDA content was

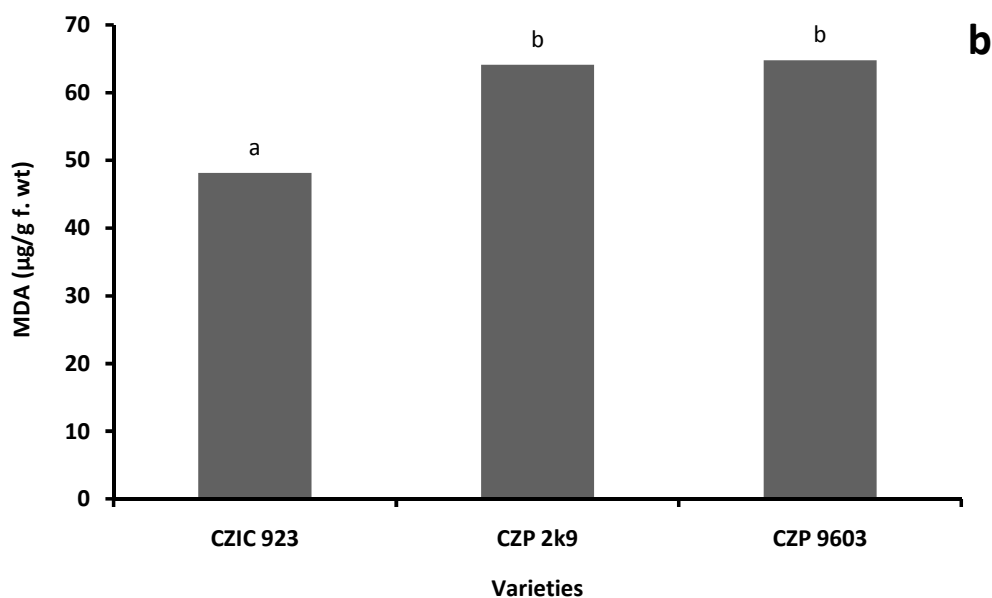
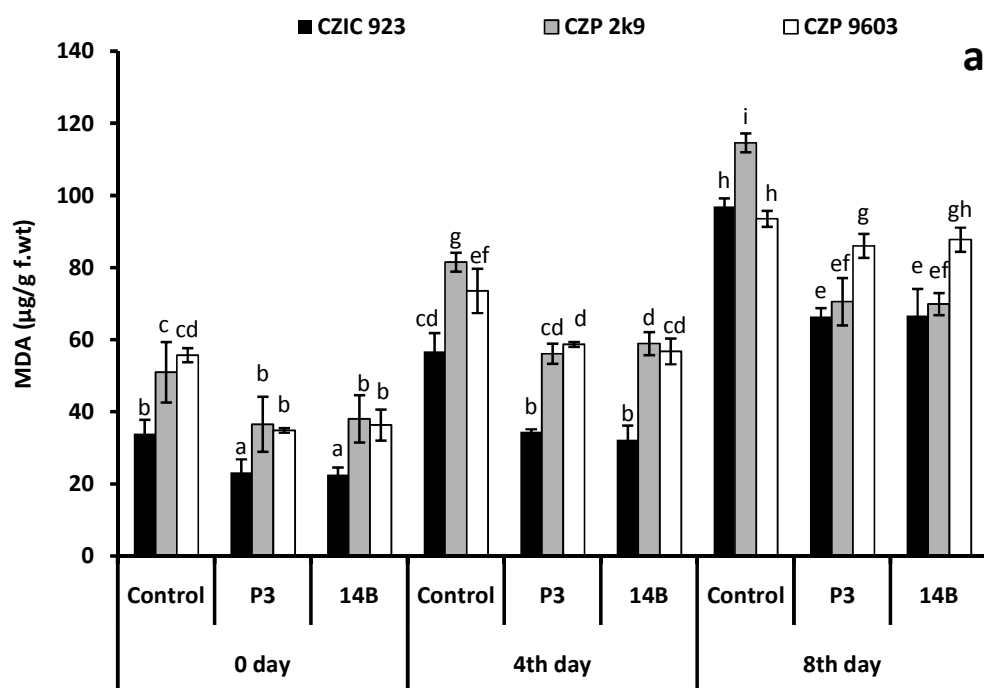


Fig. 4.38: Effect of drought on malondialdehyde (MDA) content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

lower in the leaves of drought tolerant *Phaseolus acutifolius* than in drought-sensitive ones. Similarly in the present study, MDA content significantly lowered down in bacterial treated plants. Our results support the view of **Tartoura (2010)** that MDA level was significantly lowered down in compost treated wheat plants as compared to uninoculated plants.

4.11.2.3 Chlorophyll content

A high chlorophyll and caretenoid value means that the applied stress had only a limited effect on the chlorophyll content of plants and on the overall metabolic status of the cell. This leads to increased photosynthetic rate, more dry matter production and higher productivity (**Mohan *et al.*, 2000**). Chlorophyll stability index (CSI) is an important parameter that reflects the ability of the affected plant to sustain photosynthesis under stress (**Sayed, 1999**). A gradual decrease in chlorophyll and caretenoid content was found with stress in all the varieties used.

In 8th day of stress, 1.75 fold increase in chl *a* was found in P3 treated plants and 1.31 fold increase was found in 14B treated plants when compared with control in var. CZIC923 Bacterial treatment was ineffective in var. CZP9603 and var.CZP2k9 of pearl millet in 8th day of stress (**Fig. 4.39a**). Irrespective of bacterial inoculation and level of stress var. CZIC923 showed significantly maximum increase (51.80%) in chl *a* over the other varieties (**Fig. 4.39 b**).

In 8th day of stress bacterial treatment had no effect on chl *b* content over the control in var. CZP9603. In var. CZIC923, P3 has significantly increased (1.51 fold) chl *b* content while an increase of 2 fold was observed in 14B treated plants over the control. In var. CZP2k9, a 1.8 fold increase was reported in P3 treatment however, increase of 1.4 fold in chl *b* content was observed in 14B treated plants as compared to control (**Fig. 4.40a**). Irrespective of bacterial inoculation and level of stress var. CZIC923 showed significantly maximum increase (54.05%) in chl *b* over the other varieties (**Fig. 4.40b**).

In case of caretenoid content, in all the three varieties of pearl millet bacterial treatment showed significant increase under normal condition while in 8th day of stress increase in caretenoid content was non-significant in bacteria treated plants over the control (**Fig. 4.41a**). Irrespective of bacterial inoculation and level of stress a

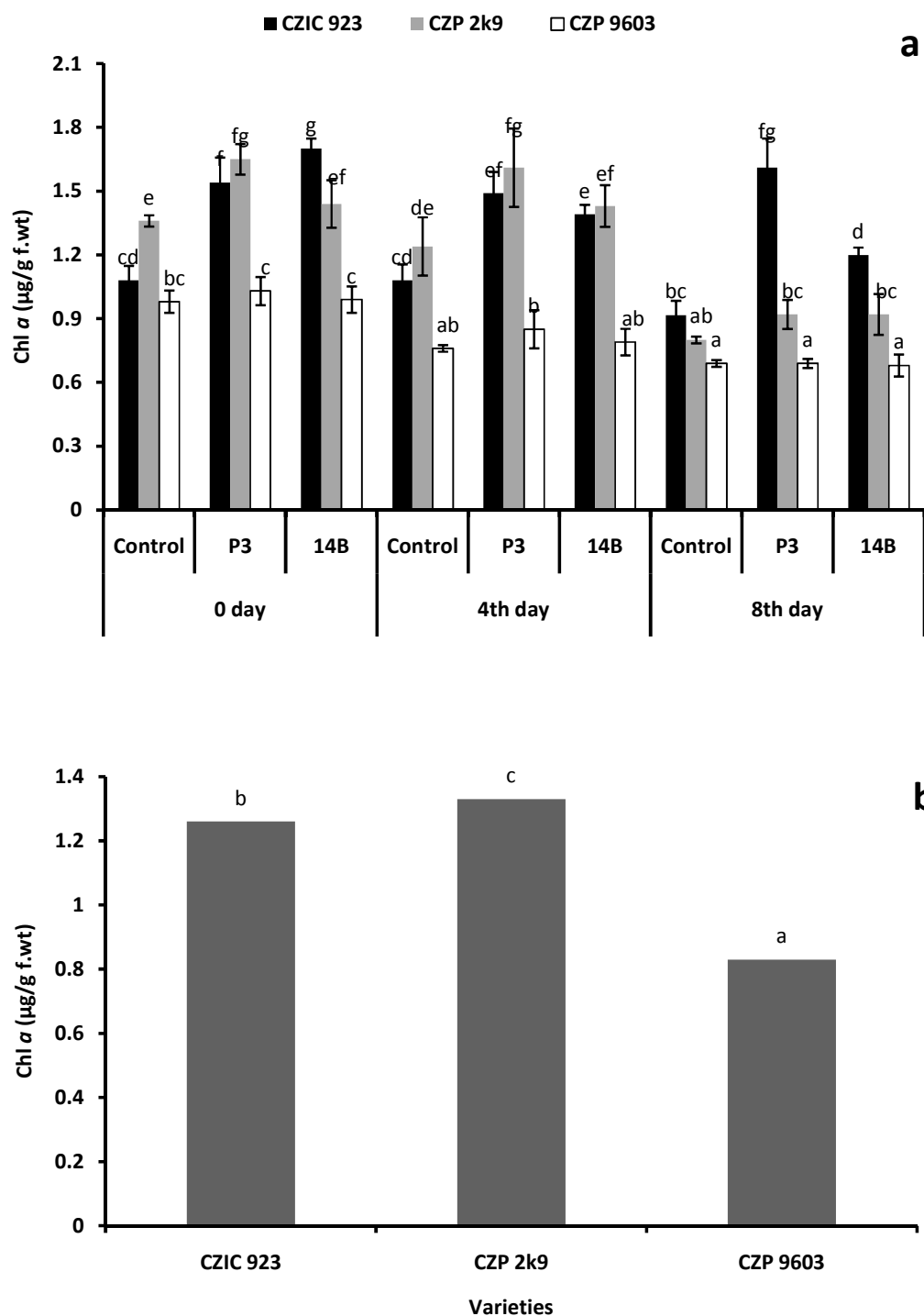


Fig. 4.39: Effect of drought on chlorophyll *a* (chl *a*) content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

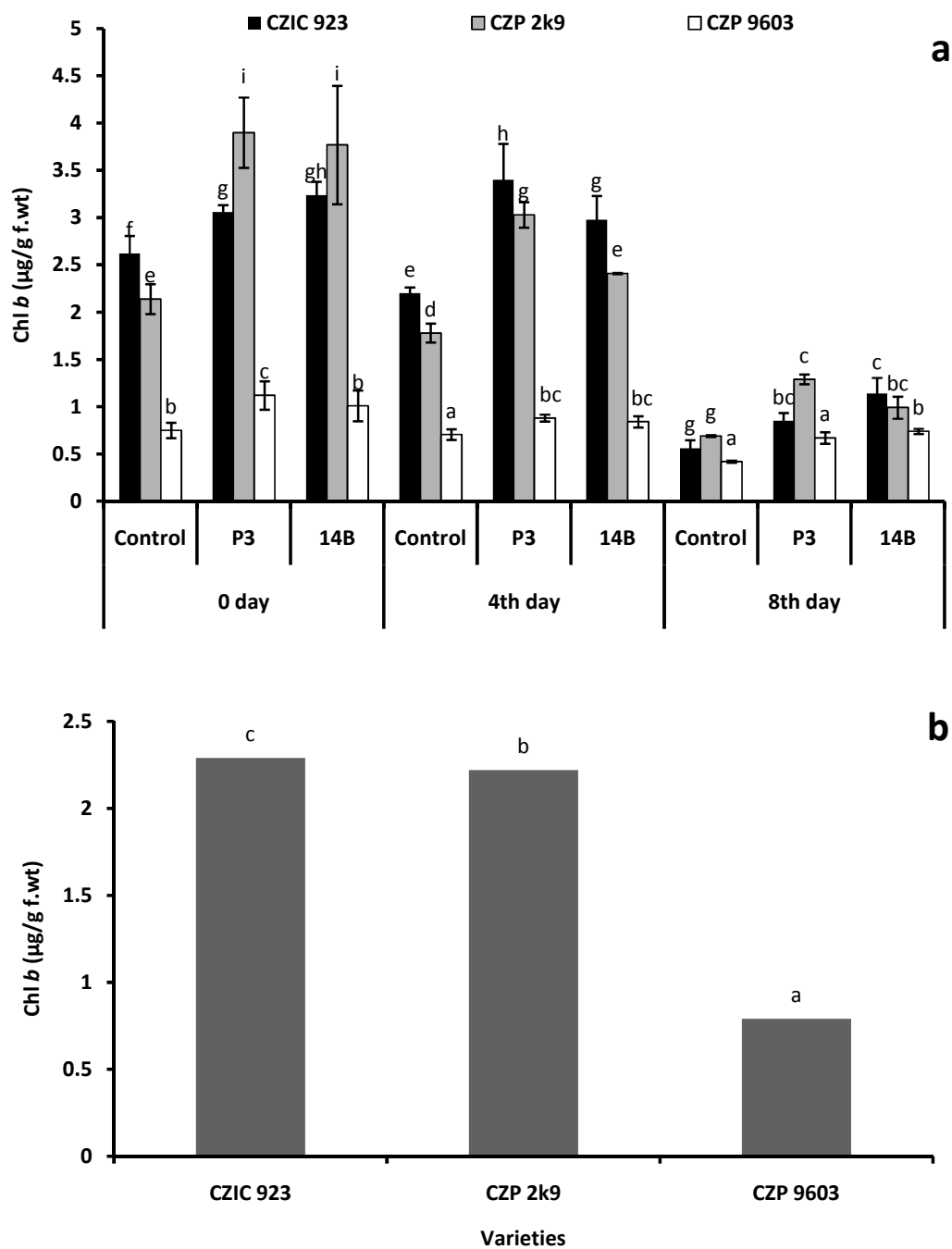


Fig. 4.40: Effect of drought on chlorophyll *b* (chl *b*) content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

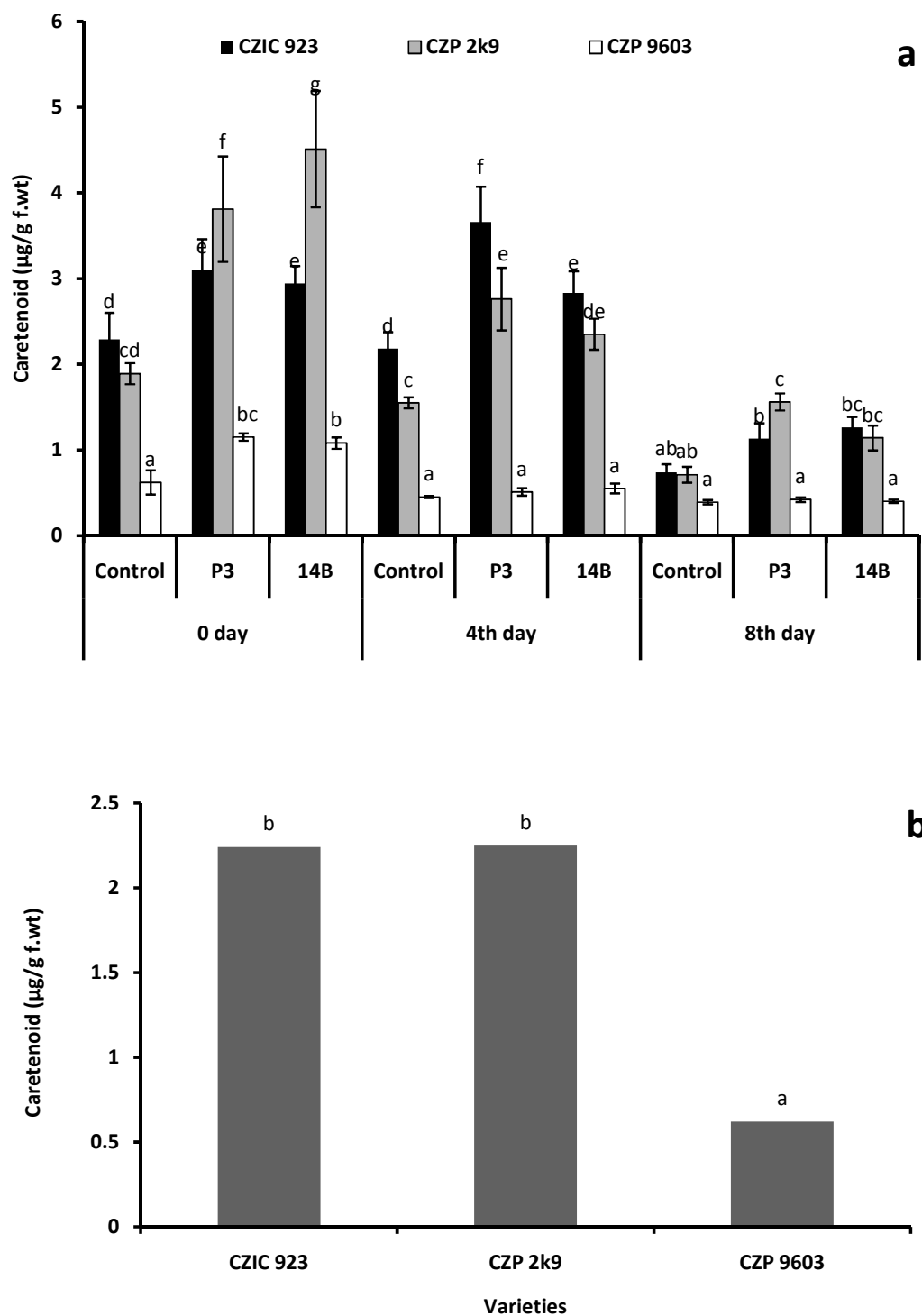


Fig. 4.41: Effect of drought on caretenoid content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

significantly maximum increase (261.21%) in caretenoid content was found in both the varieties (CZIC923 and CZP2k9) with minimum in var.CZP9603 (**Fig. 4.41b**). In a similar fashion **Wanichananan *et al.* (2003)** also correlated high chlorophyll, caretenoid and high proline content in salt tolerant rice variety. Lowest chlorophyll and caretenoid content was recorded for the var. CZP9603, which indicates a susceptible character to drought. Significant increase in chlorophyll and caretenoid content in plants inoculated with PGPR is supported by the study of **Gururani *et al.* (2012)** where they found improved photosynthetic performance in *Solanum tuberosum* when inoculated with *Bacillus* sp. under abiotic stress.

4.11.2.3 Hydrogen peroxide (H₂O₂) content

Hydrogen peroxide is a toxic compound that is produced as a result of dismutation of superoxide radicals in response to various abiotic stresses including drought stress. H₂O₂ is an important signaling molecule that plays a dual role in plants. Its levels are kept under check by a battery of H₂O₂ metabolizing enzymes (**Kaushal *et al.*, 2011**). At low concentrations, it acts as a signal molecule involved in signaling, triggering tolerance to various biotic and abiotic stresses (**Gill and Tuteja, 2010**). H₂O₂ has also been shown to act as a key regulator in a broad range of physiological processes such as senescence, photorespiration, photosynthesis and stomatal movement (**Quan *et al.*, 2008**). Any excessive increase in H₂O₂ has severe consequences for the affected cell. In our experiment, in all the test varieties of pearl millet a significant increase in H₂O₂ content was observed with stress. In plants grown in normal conditions, a non significant effect of bacterial treatment was observed over the control in all the three varieties used while in stress conditions both the bacterial treatments were significant over the control in all the varieties of pearl millet. In 8th day of stress, in var. CZIC923, P3 treatment has significantly reduced the H₂O₂ content upto 1.36 fold whereas, in 14B treatment a 1.07 fold reduction was reported. In var. CZP2k9, 1.33 and 1.43 fold decrease was found in H₂O₂ content in P3 and 14B treatment respectively. In var. CZP9603, P3 treatment recorded a significant decrease in H₂O₂ content of 1.2 fold and a 1.1 fold decrease was found in 14B treated plants (**Fig. 4.42a**). Irrespective of rest of the factors var.CZIC923 of pearl millet showed significant reduction in H₂O₂ content (24.85%) as compared to other varieties (**Fig. 4.42b**). These results are in agreement with data presented by **Selote and Chopra, (2010)** that the

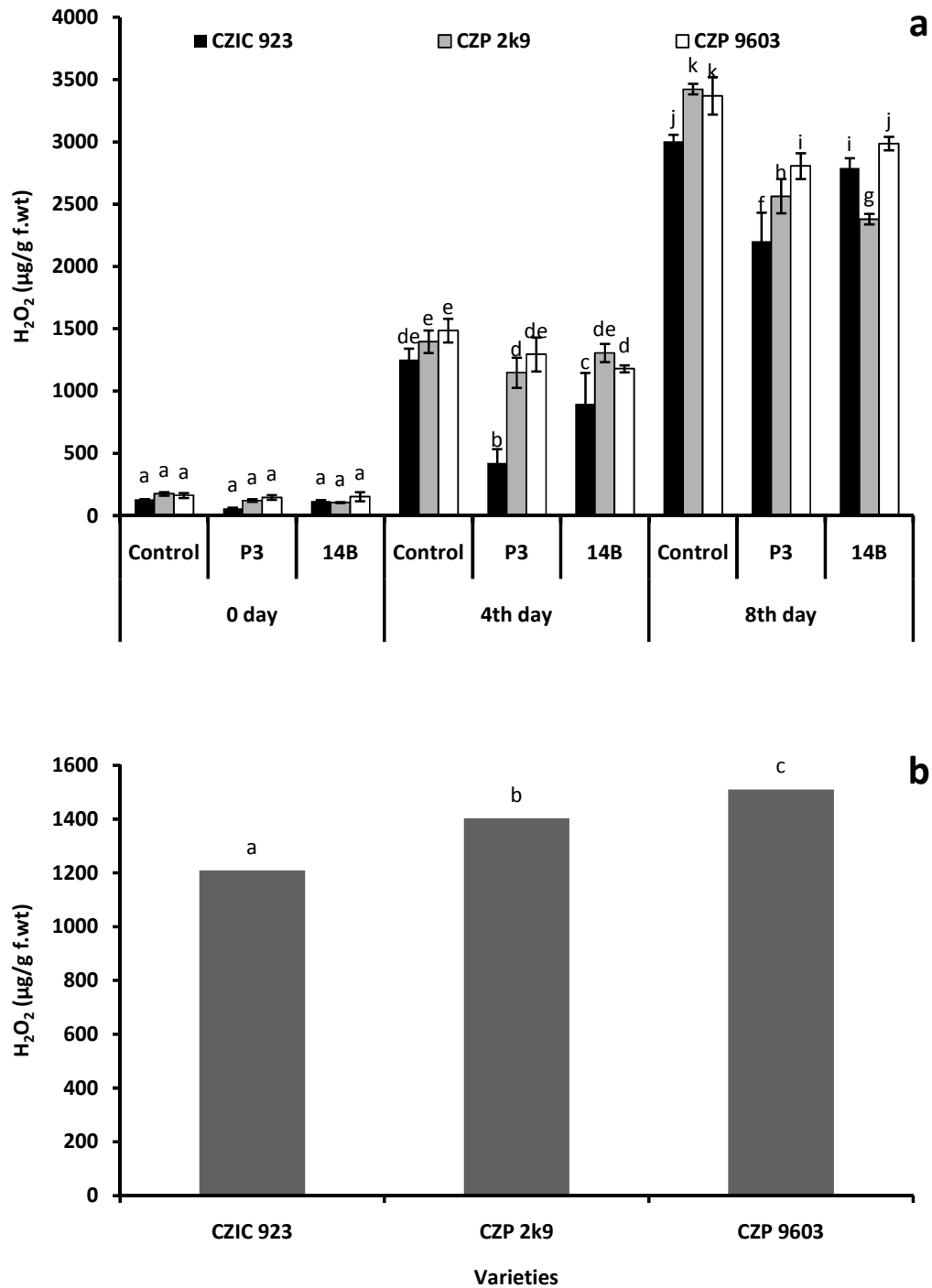


Fig. 4.42: Effect of drought on H₂O₂ content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

systematic increase in H₂O₂ levels is due to lack of increase in H₂O₂ scavenging antioxidant enzyme. The increase in H₂O₂ content of the plants on exposure to drought stress is a well documented phenomenon which may be due to the formation of O²⁻ (superoxide ion) by electron transport chains which dismutates to form H₂O₂ in chloroplast and mitochondria (**Elstner, 1991**). Stress induced H₂O₂ can also react with superoxide radicals to form highly reactive hydroxyl radicals (**Prousek, 2007**) in metal catalyzed fenton reaction. These hydroxyl radicals initiate self- propagating reactions leading to peroxidation of membrane lipids and destruction of proteins, finally leading to cell death (**Jaw and Ching, 1998**). From these results it may be concluded that the lower accumulation of H₂O₂ in PGPR treated plants could be due to the appropriate activities of antioxidant enzymes. Our results are supported by the study of **Moslemi et al. (2011)** where they stated that PGPR help in reduction of H₂O₂ during drought stress.

4.11.3 Antioxidative enzyme analysis

4.11.3.1 Superoxide dismutase (SOD) activity

The enzyme Superoxide dismutase (SOD) plays a prime role in protecting cells against oxidative stress since they dismutate O²⁻ to H₂O₂ and O₂. In the present study, an increase in SOD activity in pearl millet plants was observed in both the treatments including control after the imposition of stress and continued increasing till the last day (8th) of the study; this could help to mitigate the affect of superoxide radicals produced under oxidative stress. In 8th day of stress, in var. CZIC923 both the bacterial treatments (P3 and 14B) had significantly increased the SOD activity by 1.16 fold over the control. In var. CZP2k9, 1.18 fold increase in SOD activity was recorded in both the bacterial treatments while in case of var. CZP9603, an increase of 1.01 fold was observed in both the treatments over the control (**Fig. 4.43a**). Irrespective of bacterial inoculation maximum (29.10%) increase in SOD activity was reported in pearl millet var. CZIC923 as compared to var. CZP9603 (**Fig. 4.43b**).

A previous study on green gram plants inoculated with PGPR under water stress supports our results that that PGPB strain *P. fluorescens* Pf1 increased the accumulation of antioxidant enzymes when green gram plants were exposed to water stress (**Saravanakumar et al., 2011**). Recently, **Kohler et al. (2009)** demonstrated the greater activity of antioxidant catalase in lettuce plants under severe drought

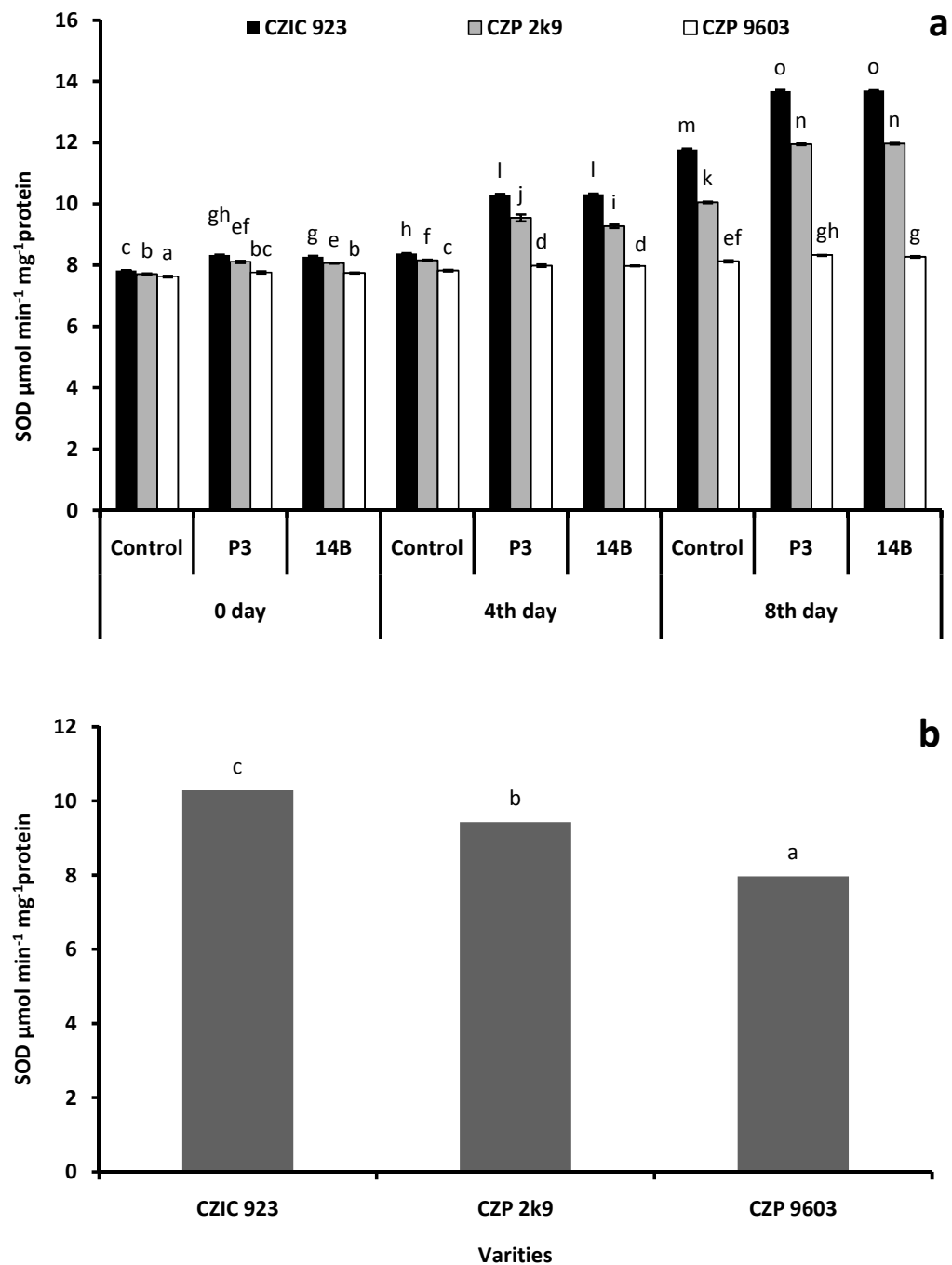


Fig. 4.43: Effect of drought on superoxide dismutase content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

conditions when inoculated with PGPR, *Pseudomonas mendocina* and arbuscular mycorrhizal fungi (*Glomus intraradices* or *G. mosseae*). The same authors have suggested that they can be used in inoculants to alleviate the oxidative damage elicited by drought. Similarly, the concept of PGPR elicited ‘induced systemic tolerance’ to salt and drought was proposed recently by **Yang *et al.* (2009)**.

4.11.3.2 Ascorbate peroxidase (APX) activity

Ascorbate peroxidase (APX) is one of the most important enzyme of Halliwell-Asada pathway, and catalyses the reduction of H₂O₂ to water. It has high specificity and affinity for ascorbate as reductant. Maximum activity of Ascorbate peroxidase was found in 8th day of stress. In control (0 day of stress) plants, effect of bacterial strains was non-significant over the control in two varieties (CZP2k9 & CZP9603) of pearl millet while in var. CZIC923, P3 has significantly increased the APX activity over the control. At 4th and 8th day of stress bacteria significantly increased the APX activity in all the varieties of pearl millet. In 8th day of stress in var. CZIC923, a significant increase of 1.8 fold in APX activity in P3 treated plants followed by 1.5 fold increase in 14B treated plants was observed. In var.CZP2k9, a significant increase of 2 fold in APX activity was observed in P3 treatment while 1.78 fold increases was found in 14B treatment as compared to control. In var.CZP9603, both the bacteria had significantly increased the APX activity upto 1.4 fold as compared to control (**Fig. 4.44a**). Irrespective of bacterial inoculation maximum APX activity (91.73%) was recorded in pearl millet var.CZIC923 as compared to var.CZP9603 (**Fig. 4.44b**). Increase in specific activity of APX under drought stress is in agreement with the results obtained by **Sairam and Srivastava, (2000)**, who have reported a positive correlation between increased H₂O₂ metabolizing enzyme activities and decreased lipid peroxidation in drought tolerant plant genotypes. Other authors have demonstrated that the high activity of the enzyme conferred tolerance to water deficit in several species of plants such as moss, *Poa pratensis* and maize (**Dhindsa and Matowe, 1981; Egert and Tevini, 2002; Wang and Huang, 2004**). Our results are supported by the results of **Dai *et al.* (2009)**, which showed that the level of superoxide dismutase, catalase and peroxidase were increased in *Brassica napus* L. under stress.

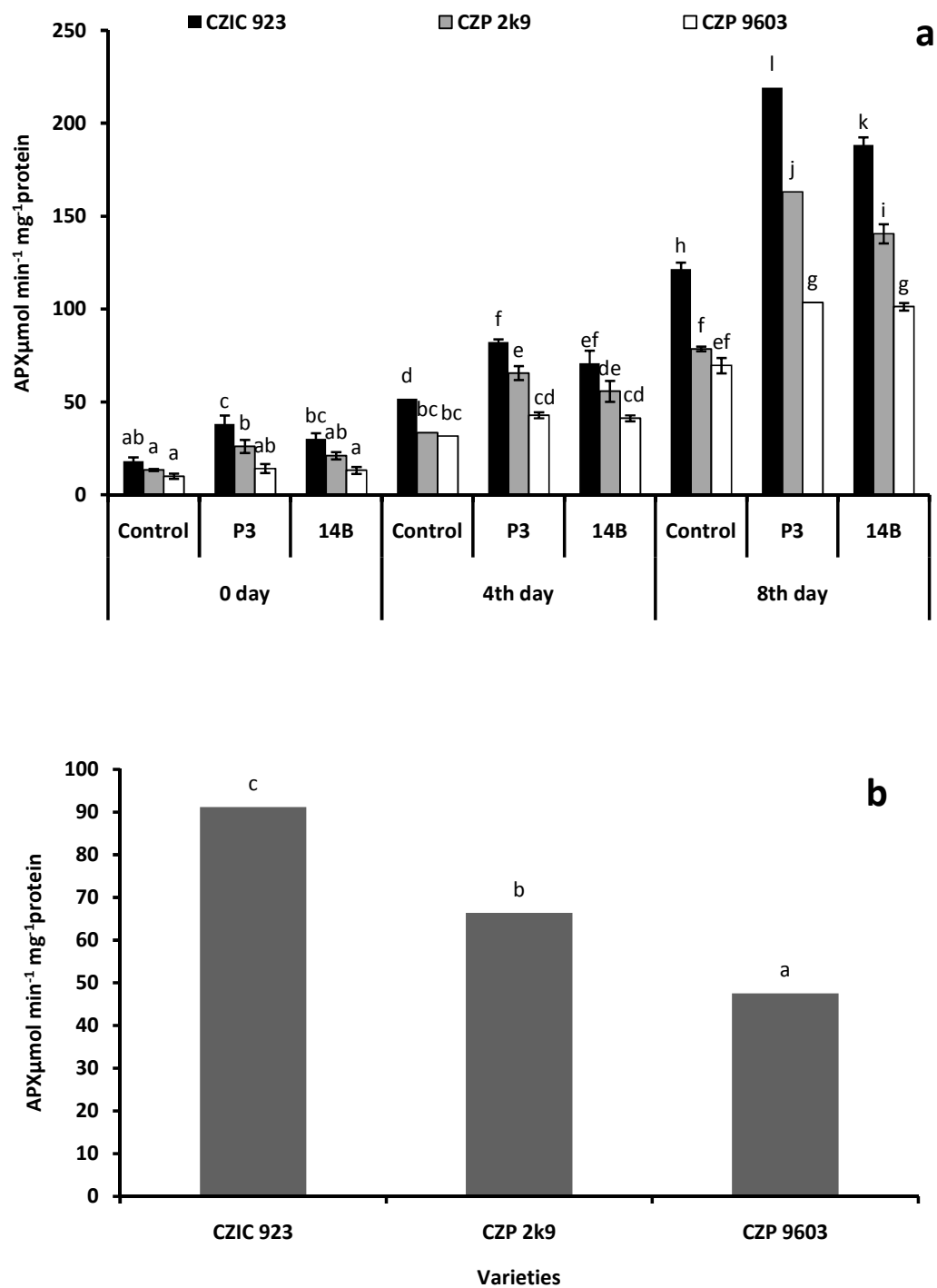


Fig. 4.44: Effect of drought on ascorbate peroxidase (APX) content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.11.3.3 Guaiacol peroxidase (GPX) activity

A gradual increase in GPX activity was found with stress in all the test varieties of pearl millet. Increased activity of GPX indicates that plants have responded positively to increased accumulation of H_2O_2 , by increasing the activities of H_2O_2 scavenging enzymes; this can be attributed to increased tolerance to salinity stress. A significant effect of bacterial treatment was observed in all the varieties under normal and stressed conditions. In 8th day of stress, in var. CZIC923 an increase of 2 fold in GPX activity was observed in both the bacterial treatments as compared to control. In var. CZP2k9, an increase of 1.9 fold was reported in P3 treatment while in 14B treated plants, an increase of 1.7 fold was found as compared to control. In var. CZP9603, both the treatments had increased the GPX activity upto 1.6 fold (**Fig. 4.45a**). Irrespective of drought stress and bacterial treatment var. CZIC923 showed maximum increase (118.11%) in GPX activity as compared to control (**Fig. 4.45b**). Similarly **Panda and Upadhyay, (2003)** also reported an increased activity of Guaiacol peroxidase in salt stressed roots of *Lemna minor*. **Khosravinejad et al. (2008)** observed a linear increase in GPX activity, along with ascorbate peroxidase and catalase activity under salinity stress in barley shoots and roots. Our results were in accordance with the study of **Gururani et al. (2012)** which shows that the inoculations with rhizobacteria strains 4 and 6 protected *S. tuberosum* against abiotic stress factors like water deficit, salinity, and heavy-metal toxicity. Plant tolerance to these stresses correlated with the increased expression levels of ROS-scavenging enzymes APX, SOD, CAT, DHAR, and GR, suggesting that inoculation with these strains triggered abiotic stress-related defense pathways under stressed conditions.

4.11.3.4 Catalase (CAT) activity

Catalase activity followed similar trend as in case of ascorbate peroxidase activity. In 8th day of stress in var. CZIC923, an increase of 2.3 fold and 1.8 fold in catalase activity was observed in P3 and 14B treated plants respectively. In var. CZP2k9, an increase of 2.8 and 2.3 fold was reported in P3 and 14B treated plants respectively while in CZP9603, both the treatments significantly enhanced the catalase activity upto 1.9 fold (**Fig. 4.46a**). A significant increase (112.21%) in catalase activity was found in pearl millet var. CZIC923 as compared to CZP9603 (**Fig. 4.46b**).

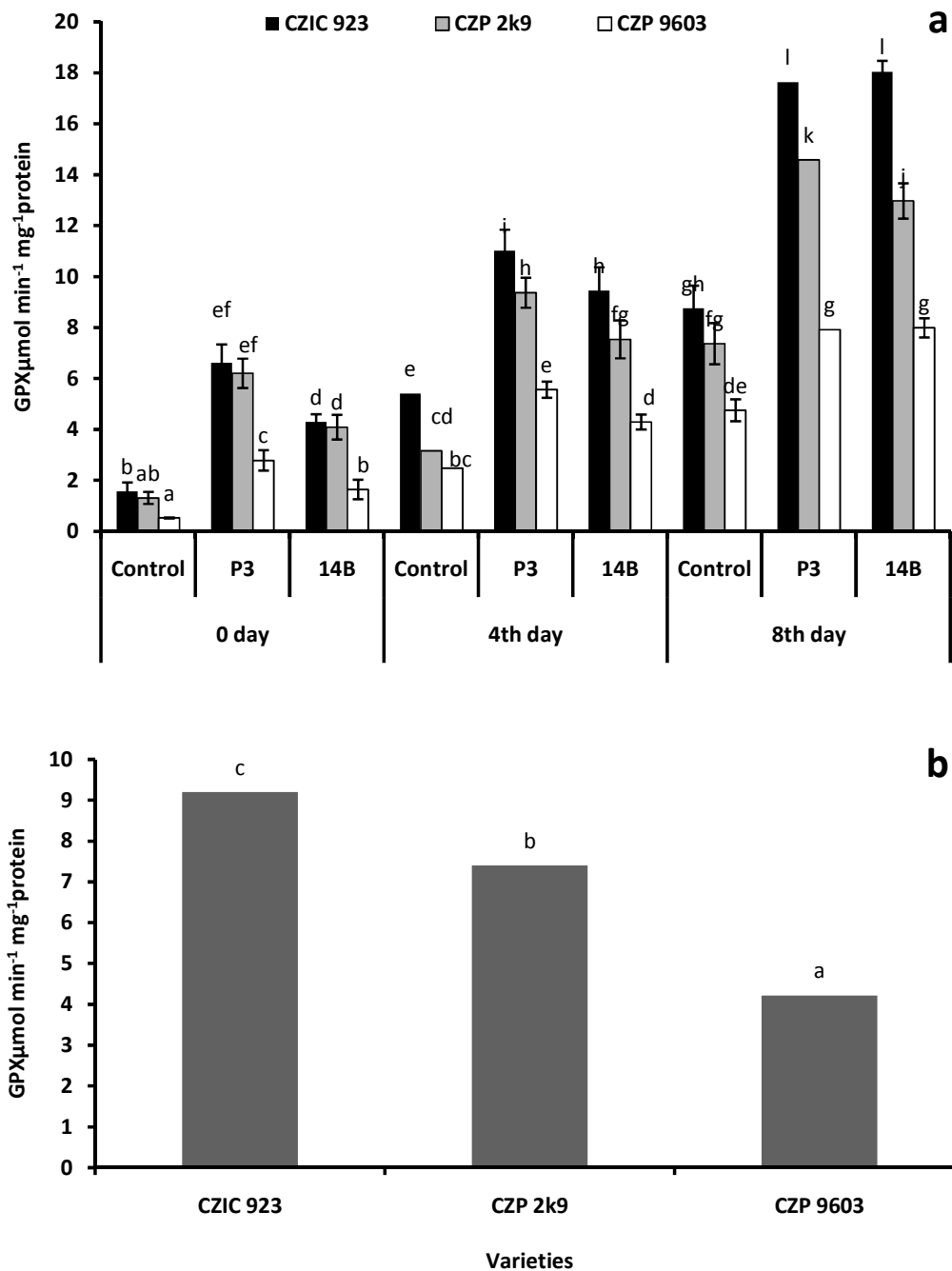


Fig. 4.45: Effect of drought on guaiacol peroxidase (GPX) content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

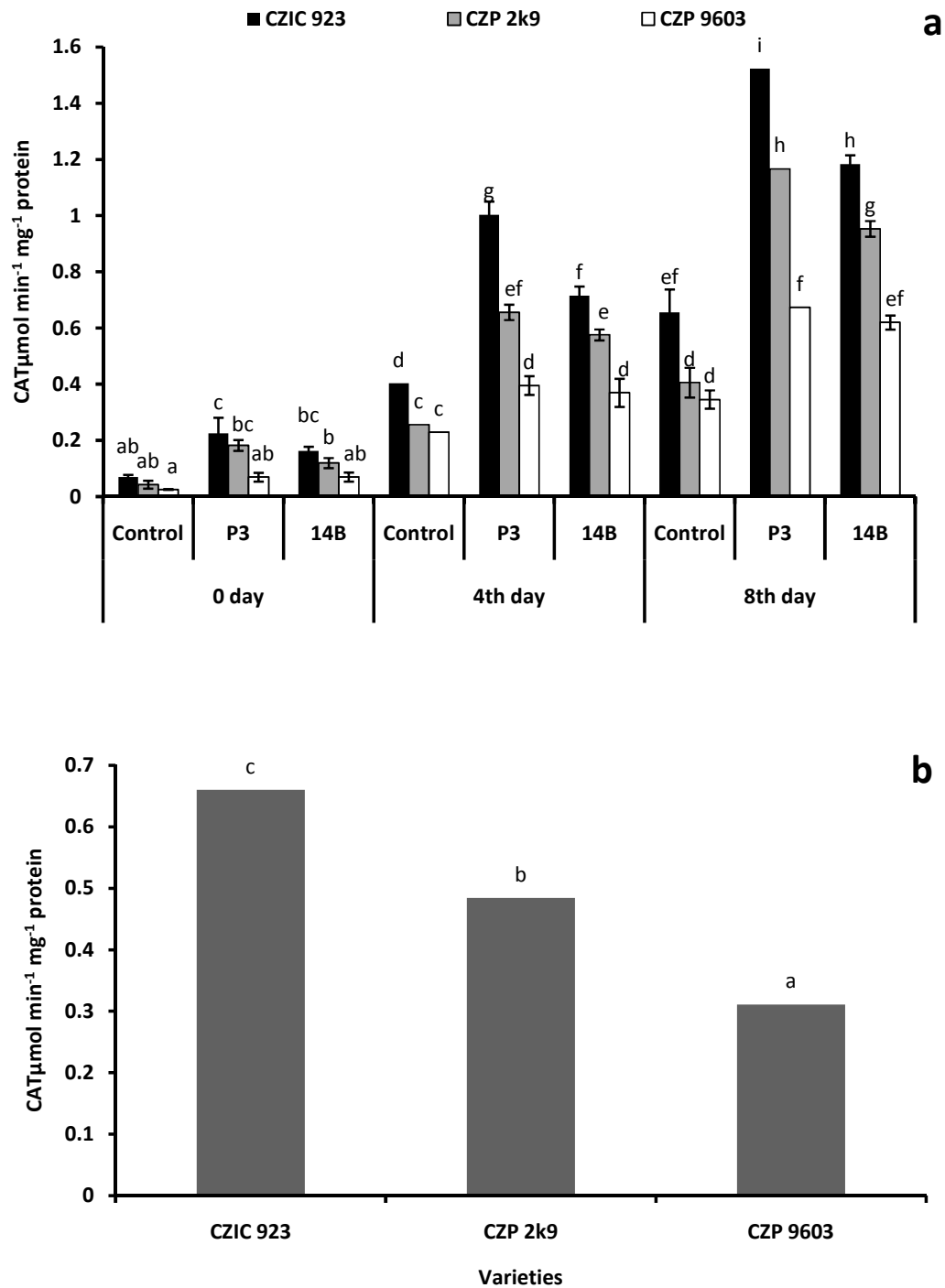


Fig. 4.46: Effect of drought on catalase (CAT) content of three varieties of pearl millet (days after drought) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

Our results are in agreement with the findings of **Kohler *et al.* (2006)** in lettuce plant, which is reported that the greater activity of antioxidant CAT and accumulation of under severe drought conditions were recorded when inoculated with PGPR, *Pseudomonas mendocina* and arbuscular mycorrhizal fungi. **Weisany *et al.* (2012)** reported the increased activity of catalase in soybean (*Glycine max* L.) under salinity stress.

In conclusion, it seems inoculation of P3 and 14B influenced biochemical and physiological parameters of pearl millet and helped plants tolerate drought stress to a higher level as compared to uninoculated seedlings. Inoculation with PGPR strains under drought stress resulted in increased level of proline, chlorophyll a, b and caretenoid and antioxidant enzymes (SOD, APX, CAT and GPX) activities in var. CZIC923 of pearl millet as compared to var. CZP9603. These features help PGPR to detoxify the compounds such as MDA and H₂O₂ accumulated in plant system during adverse conditions besides benefitting the survival of microbes. Further, from the results we can conclude that among three varieties of pearl millet var. CZIC923 was most drought tolerant and var. CZP9603 was the most susceptible one. So for comparative studies further we have chosen these two varieties.

4.12 *In vitro* Study of Drought Tolerance in Plants Inoculated with PGPR

Understanding abiotic stress responses in plants is an important and challenging topic in plant research (**Hirayama and Shinozaki, 2010**). It is known that plants have a well-organized defense system against ROS that works under control as well as stress conditions. Stress-induced variation of antioxidant activity is dependent on the severity and duration of stress. A large number of studies on various species indicated that stress alters the amount and the activities of the enzymes involved in scavenging of ROS and their corresponding steady-state levels of mRNA (**Gueta *et al.*, 1997; Herna'ndez *et al.*, 2009; Gill and Tuteja, 2010**).

Water deficit induced by Polyethylene glycol (PEG) affect physiological and biochemical changes in pearl millet. The plants were subjected to two progressive stresses: moderate (-0.75 MPa) and severe (-1.0 MPa) respectively. Extensive studies have been conducted for understanding the plant tolerance and oxidative

stress in response to water deficit. Osmotic solution such as PEG has been used to impose water stress by exposing the root system of plants can resolve the problem. PEG is used successfully to decrease the water potential of plants as it doesn't enter into the root (Zgallai *et al.*, 2005). This neutral polymer is being widely used to impose water stress in plants (Kumar *et al.*, 2011). PEG solution mimic dry soil more closely than solutions of low molecular osmotic pressure, which infiltrate the cell wall with solutes (Veslues *et al.*, 1998). In this experiment two varieties of pearl millet (CZIC923 and CZP9603) inoculated with P3 and 14B were exposed to different concentrations of Polyethylene glycol (PEG) after 25 days of germination. After 14 h of exposure, plants were tested for physiological and biochemical parameters (Plate 8A and B).

4.12.1 Plant growth under drought

Drought stress affected the growth of pearl millet seedlings as reflected by stunted growth, less vigor, and drying of leaves. Effect of drought stress was more visual in var.CZP9603 than var.CZIC923.

4.12.1.1 Plant fresh weight

4.12.1.1.1 Root fresh weight

A gradual decrease in root fresh weight was observed in both the varieties with increasing stress level. In var. CZIC923, under -1 MPa water potential, as compared to control an increase of 1.1 fold was observed in P3 and 14B treated plants was observed. In var. CZP9603, both the treatments had non-significant effect on root fresh weight (Fig. 4.47a). Irrespective of bacterial treatments and stress level, var. CZIC923 significantly increased (45.25%) root fresh weight as compared to var. CZP9603 (Fig. 4.47b).

4.12.1.1.2 Shoot fresh weight

In var. CZIC923, under -1 MPa water potential, an increase of 1.5 fold in shoot fresh weight was observed in P3 treated plants whereas a 1.7 fold increase in 14B treated plants was observed. In var. CZP9603, both the treatments had non-significant effect on root fresh weight (Fig. 4.48a). Irrespective of bacterial treatments and stress level in variety CZIC923, a significant increase of 48.67% was recorded as compared to var. CZP9603 (Fig. 4.48b).

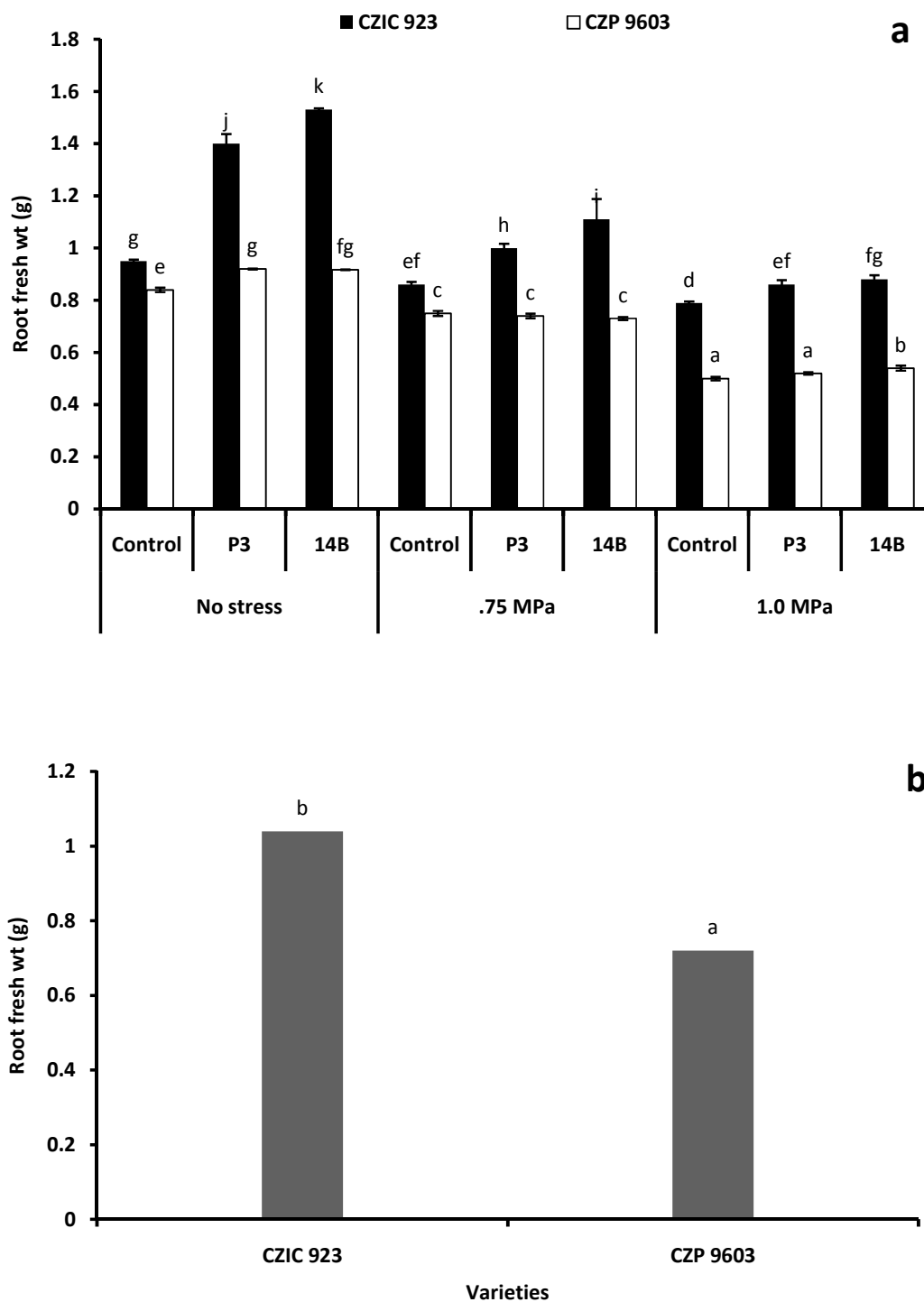


Fig. 4.47: Root fresh weight of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

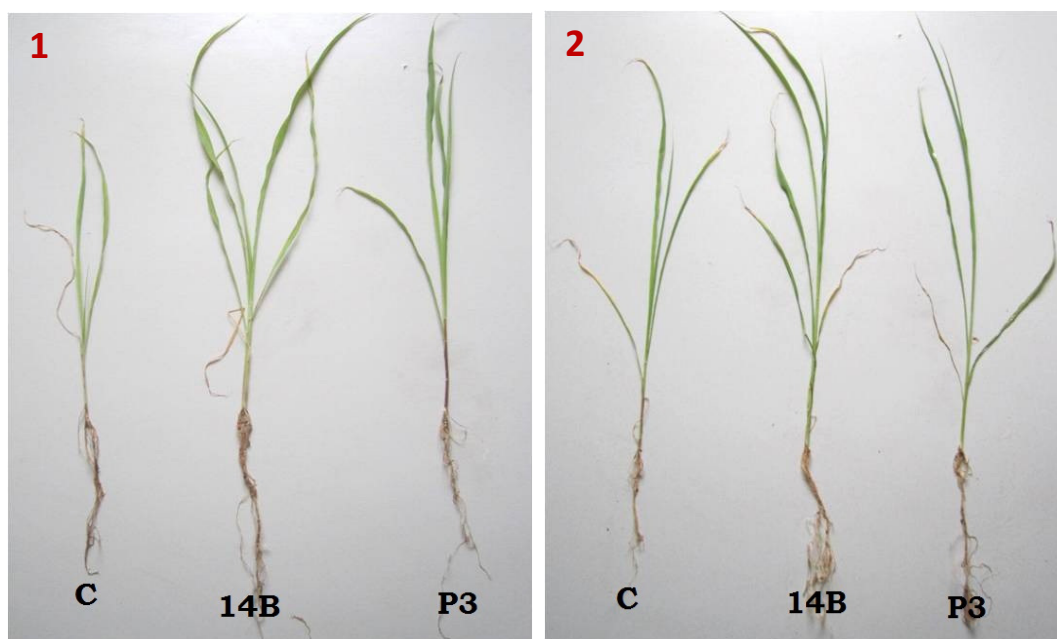


Plate 8A: Growth of Pearl millet var. CZIC 923 (drought tolerant) after 12 h (1) in hogland solution; (2) in -1.0 MPa (30%) PEG solution

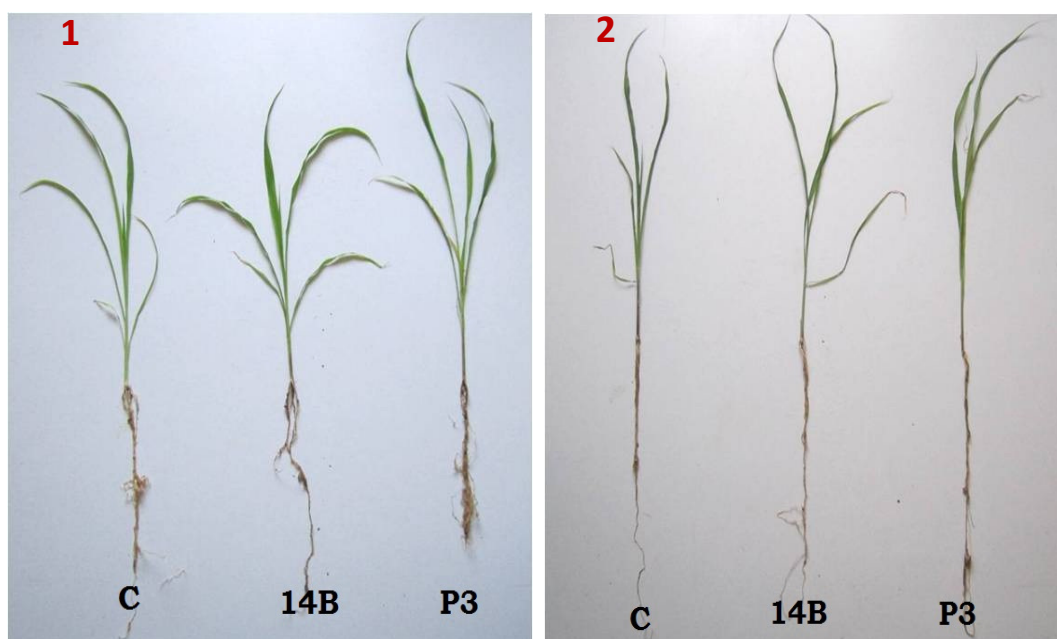


Plate 8B: Growth of Pearl millet var. CZP 9603 (drought susceptible) after 12 h (1) in hogland solution; (2) in -1.0 MPa (30%) PEG solution

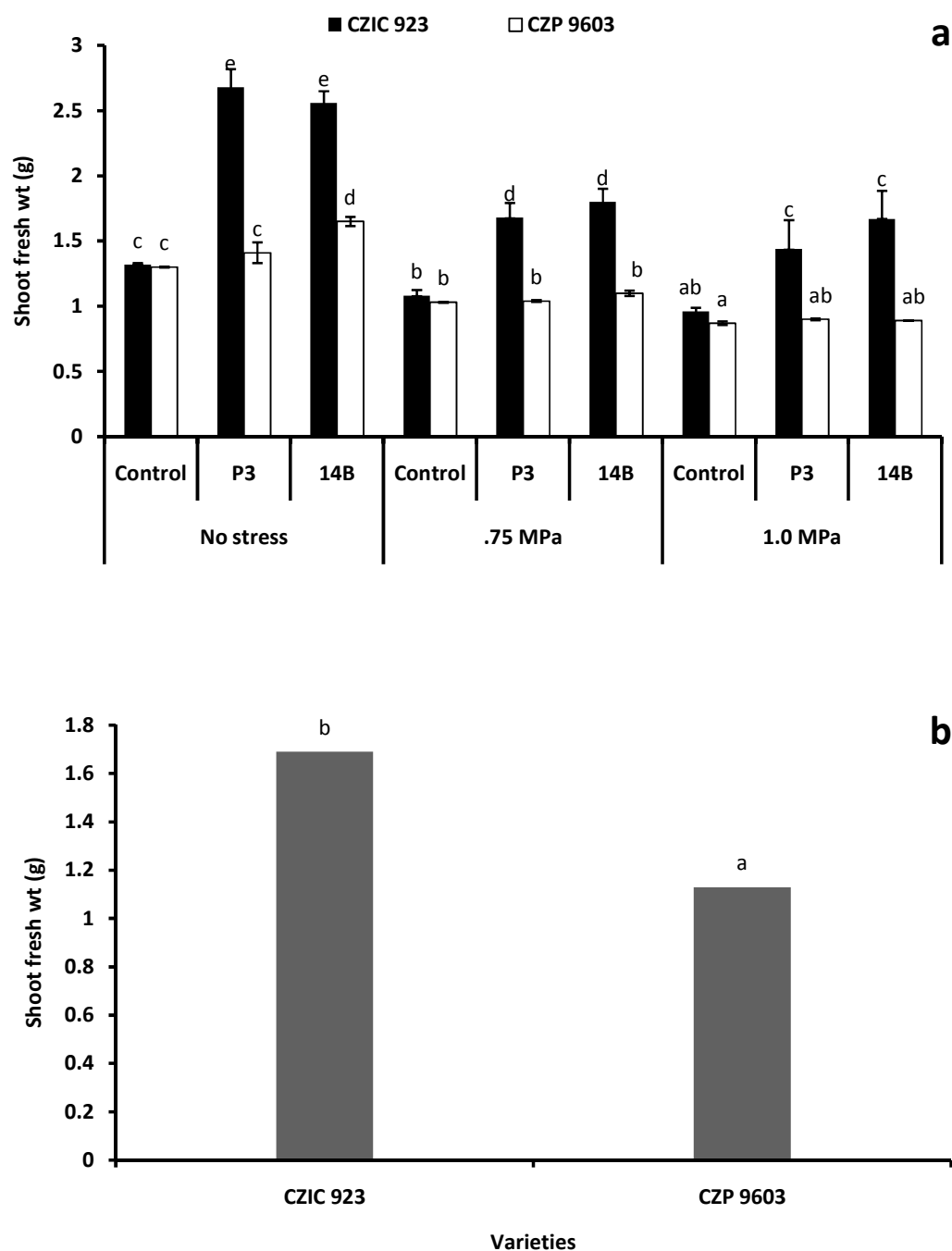


Fig. 4.48: Shoot fresh weight of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.12.1.2 Plant dry weight

4.12.1.2.1 Root dry weight

Similar trend was observed as in case of root fresh weight. In var. CZIC923 under -1.0MPa stress level, an increase of 2.1 fold in root dry weight in both the bacterial treatment was found (**Fig. 4.49a**). Irrespective of bacterial treatment and level of stress a significant increase of 48.57% was observed in case of var. CZIC923 as compared to var. CZP9603 (**Fig. 4.49b**).

4.12.1.2.2 Shoot dry weight

In var. CZP9603, bacterial treatment was non-significant over the control under -1 MPa of water potential. In var. CZIC923, P3 had significantly lower down the shoot dry weight upto 1.46 fold whereas a significant increase of 2.5 fold was observed in 14B treatment (**Fig. 4.50a**). In case of variety CZIC923, 44.44% increase was recorded as compared to var. CZP9603 irrespective of bacterial inoculation and level of stress (**Fig. 4.50b**).

4.12.1.3 Root volume

In plants grown in normal conditions, there was significant increase in both the bacterial treatments as compared to control in both the varieties. In var. CZIC923 non significant decrease was observed in both the concentration of PEG as compared to control while it was significant in case of var. CZP9603 (**Fig. 4.51a**). Irrespective of bacterial treatment and stress level, in case of var. CZP9603, non significant decrease was observed as compared to var. CZIC923 (**Fig. 4.51b**).

4.12.2 Antioxidant marker analysis

4.12.2.1 Proline accumulation

In normal condition, both the varieties showed non significant increase in proline content in inoculated plants as compared to uninoculated plants. In both the varieties, proline content significantly increased in inoculated plants as compared to control with increasing stress. In CZIC923, significantly maximum proline (1.85 fold) was observed in 14B as compared to control while a significant increase of 1.8 fold in P3 treatment in both the concentration of PEG. In case of var. CZP9603 both the bacterial treatments increased proline content up to 1.2 fold (**Fig. 4.52a**). Irrespective

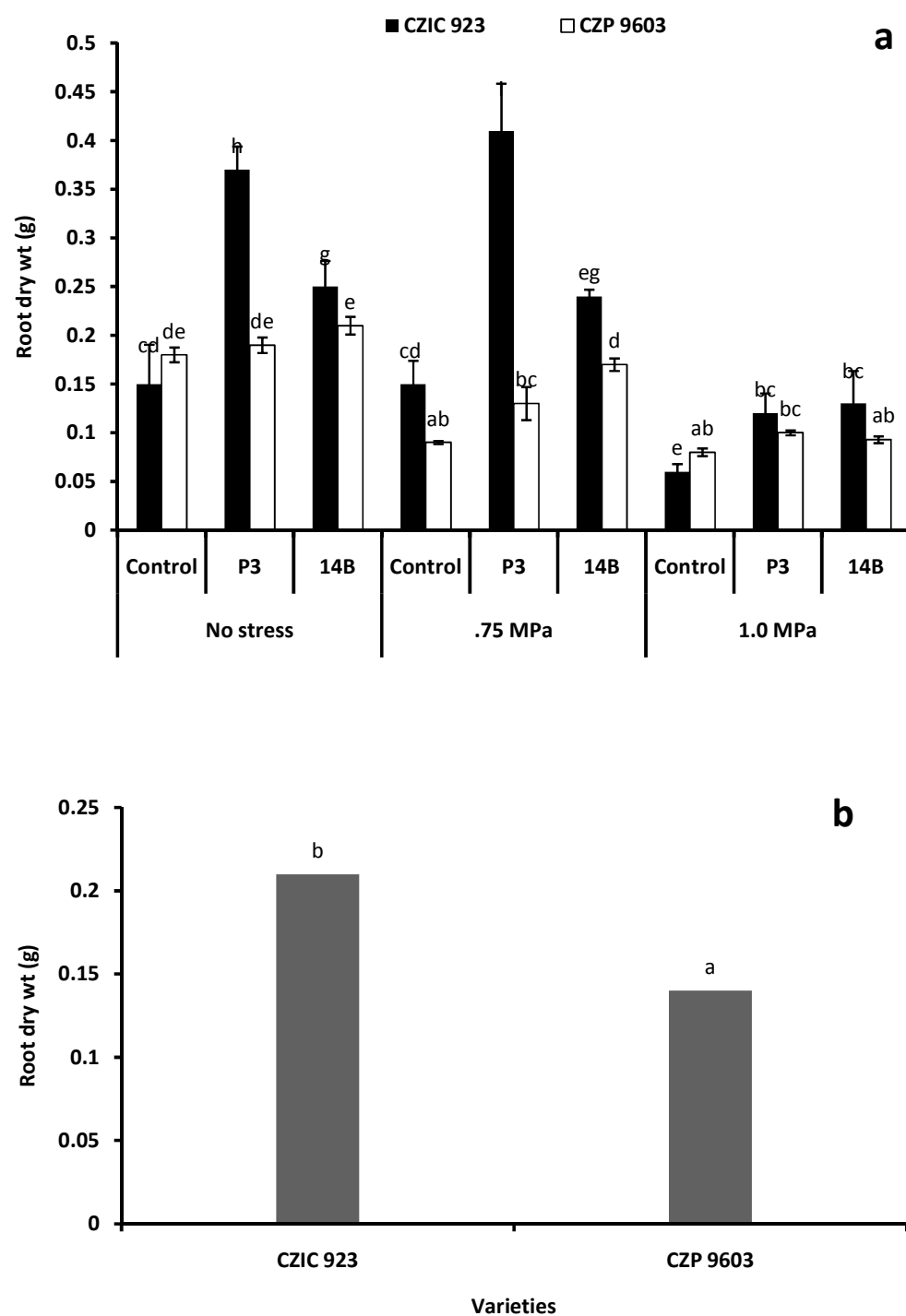


Fig. 4.49: Root dry weight of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

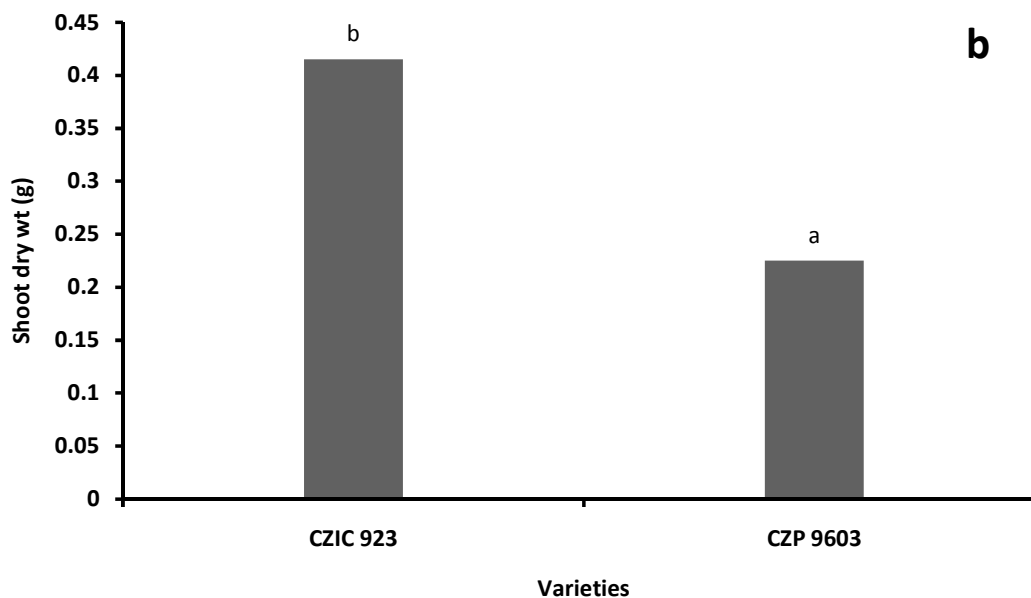
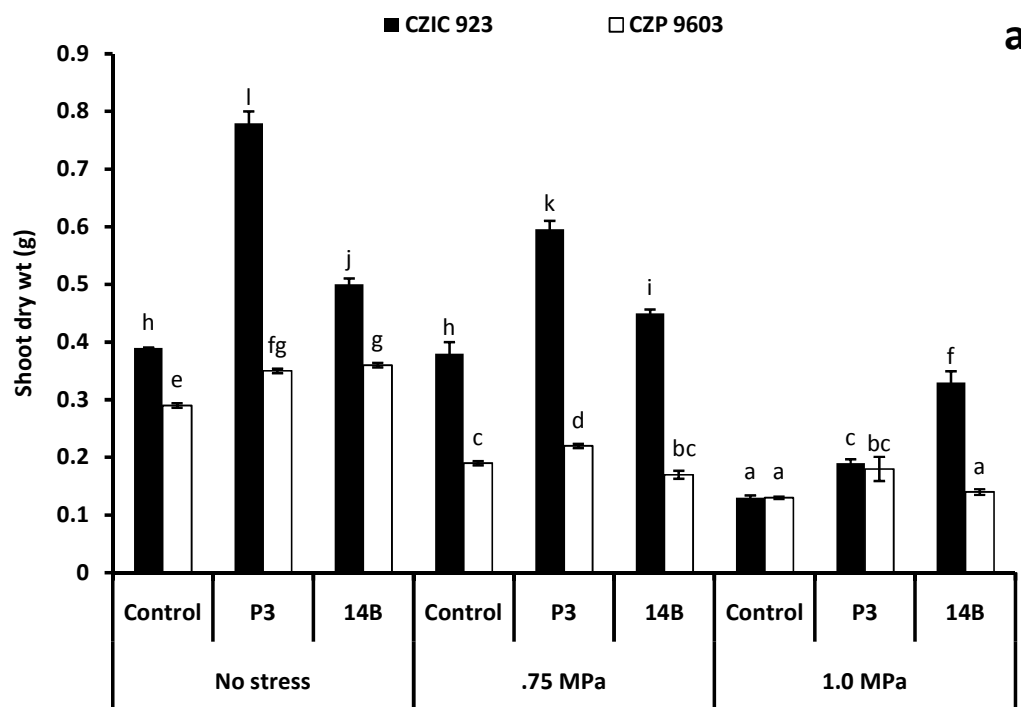


Fig. 4.50: Shoot dry weight of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

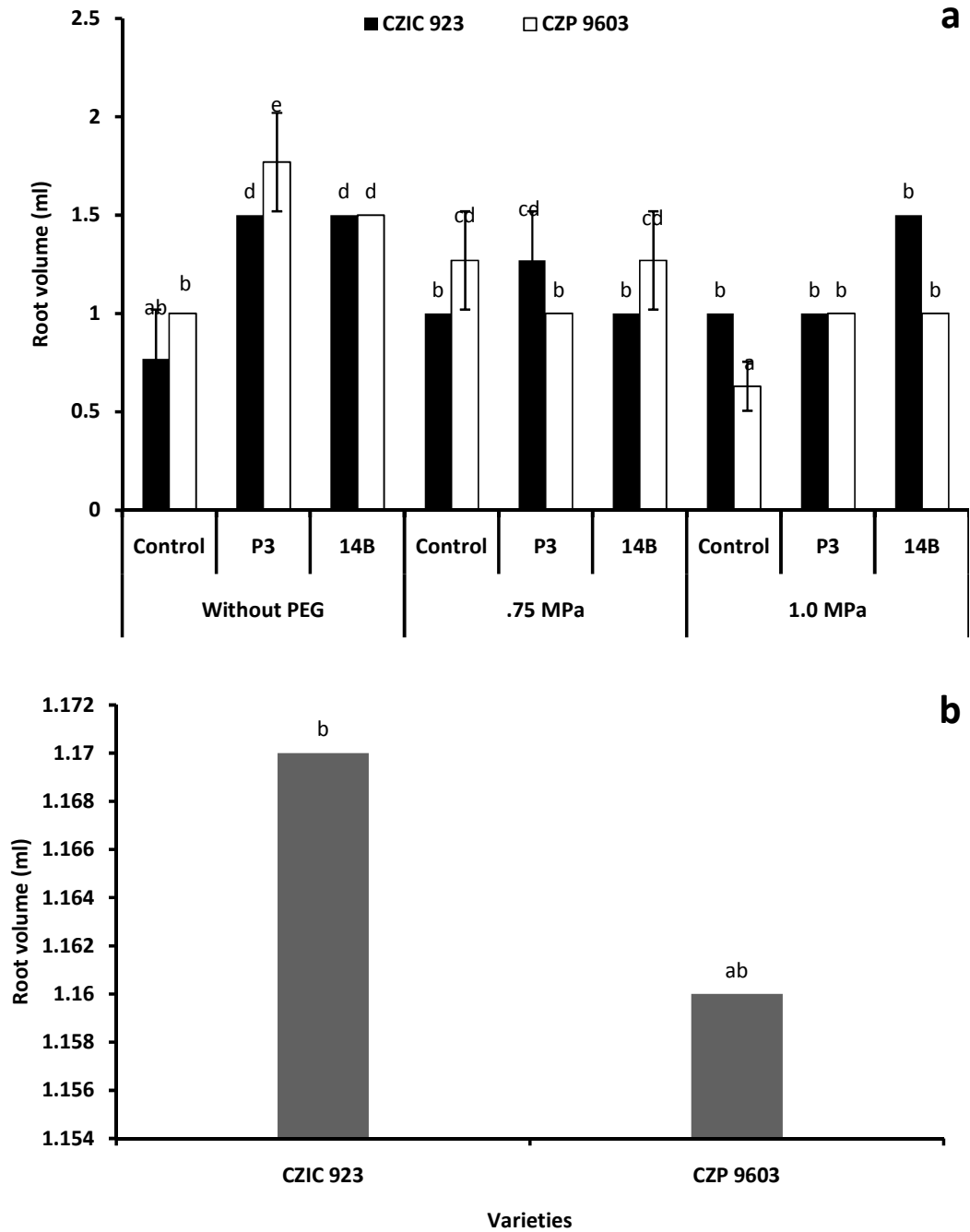


Fig. 4.51: Root volume of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

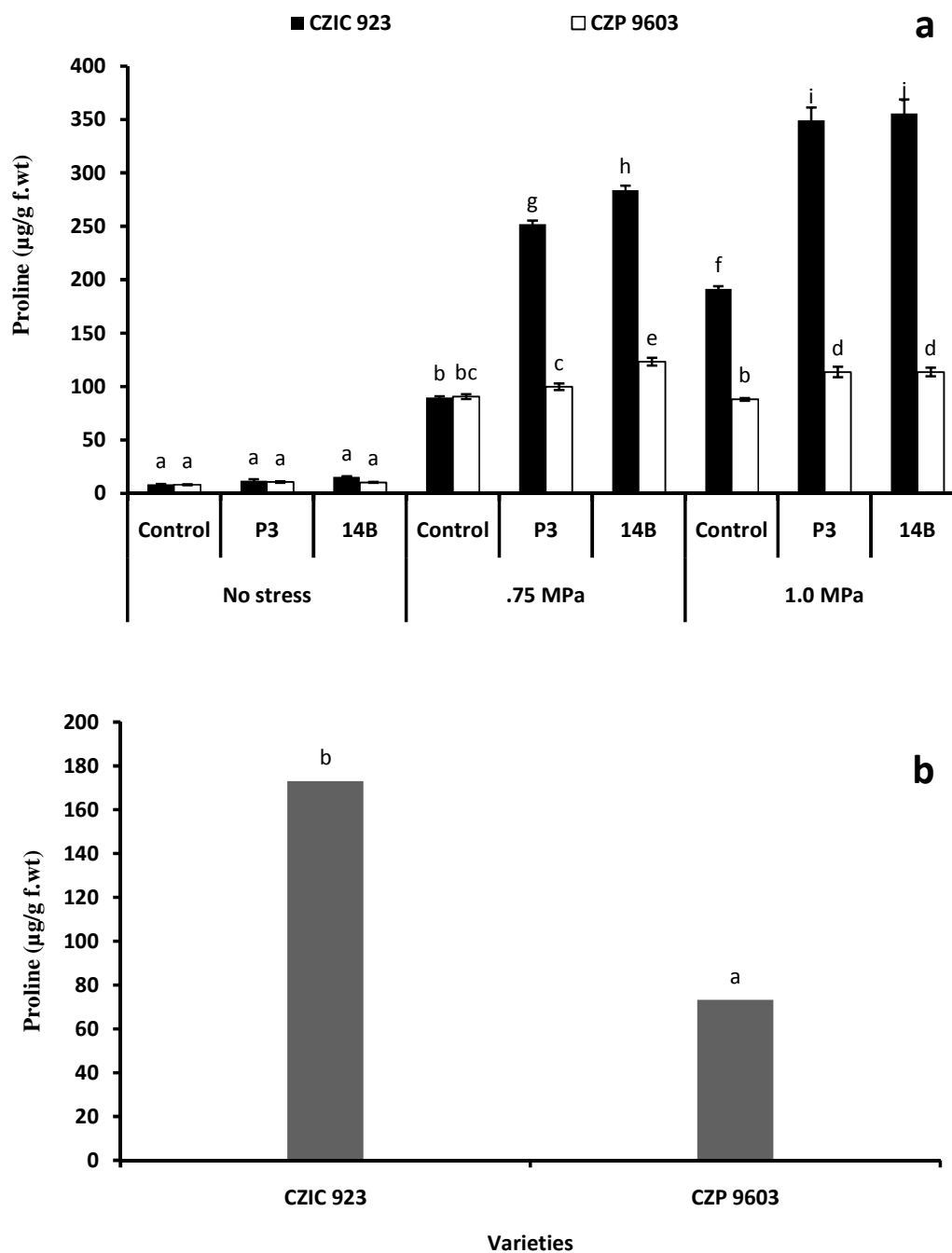


Fig. 4.52: Proline content of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

of bacterial inoculation and level of stress, the variety CZIC923 recorded significant increase of 136.26% in proline level under stress, over that in var.CZP9603 (**Fig. 4.52b**).

4.12.2.2 Malondialdehyde content

Membrane stability index under stress, as reflected by MDA (malondialdehyde) accumulation is proportional to the extent of free radical induced damage to membranes in plants. A gradual increase was observed in plants of both the varieties with stress. Under -1.0 MPa, in var. CZIC923, plants treated with P3 showed a significant increase of 1.3 fold in MDA content while an increase of 1.6 fold in 14B treated plants was observed. In var.CZP9603, an increase of 1.4 and 1.3 fold was observed in P3 and 14B treatments respectively (**Fig. 4.53a**). A significant decrease of 119.80% was observed irrespective of both the factors in var.CZIC923 as compared to var.CZP9603 (**Fig. 4.53b**).

4.12.2.3 Chlorophyll content

Chlorophyll content was analysed in two varieties of pearl millet, under increasing osmotic potential of PEG. A significant decrease was found in chlorophyll and caretenoid level in stressed plants as compared to control plants. In case of var. CZIC923, under stress of -.75MPa, *chl a* level was increased significantly (1.1 fold) in P3 as compared to control whereas in -1.0 MPa a non-significant increase was observed in both the treatments. In var.CZP9603 under -1 MPa, P3 has increased the level of *chl a* significantly (1.2 fold) while in case of 14B a significant decrease (1.1 fold) was observed as compared to control plants (**Fig. 4.54a**). In var. CZIC923, *chl b* content was significantly increased (1.4 fold) in both the bacterial treatments whereas in CZP9603 there was non-significant effect of bacterial treatment over the control plants (**Fig. 4.55a**). In case of caretenoid content under -1 MPa, in var. CZIC923 there was a non significant effect of bacterial treatment over the control. In var. CZP9603, P3 had significantly increased the caretenoid content up to 1.2 fold whereas 14B was found non significant over the control (**Fig. 4.56a**). Irrespective of PEG level or bacterial treatments, 7.79, 18.49 and 21.70% increase was found in *chl a*, *b* and caretenoid content respectively in var. CZIC923 as compared to var. CZP9603 (**Fig. 4.54b; 4.55b and 4.56b**).

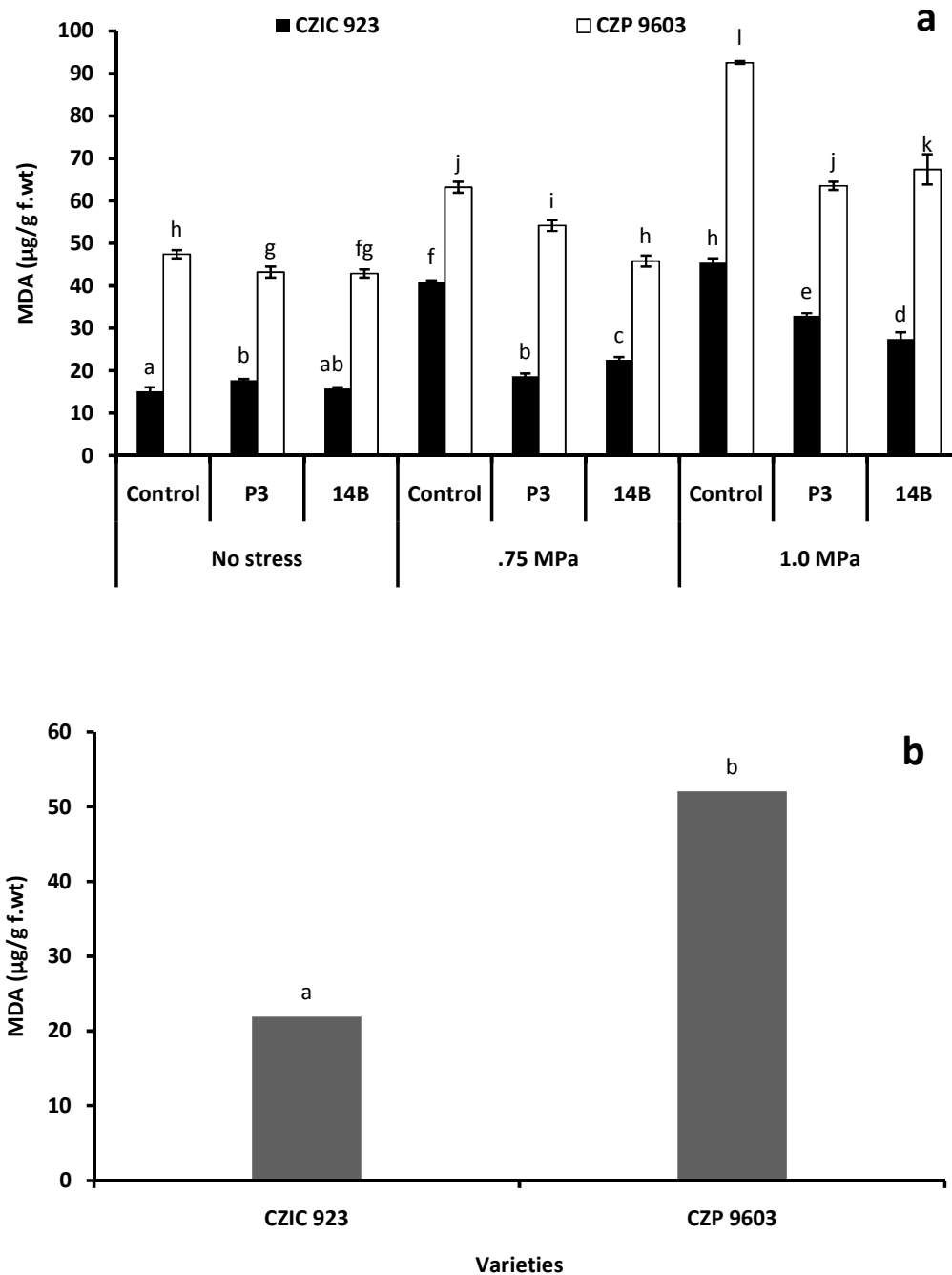


Fig. 4.53: Malondialdehyde (MDA) content of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

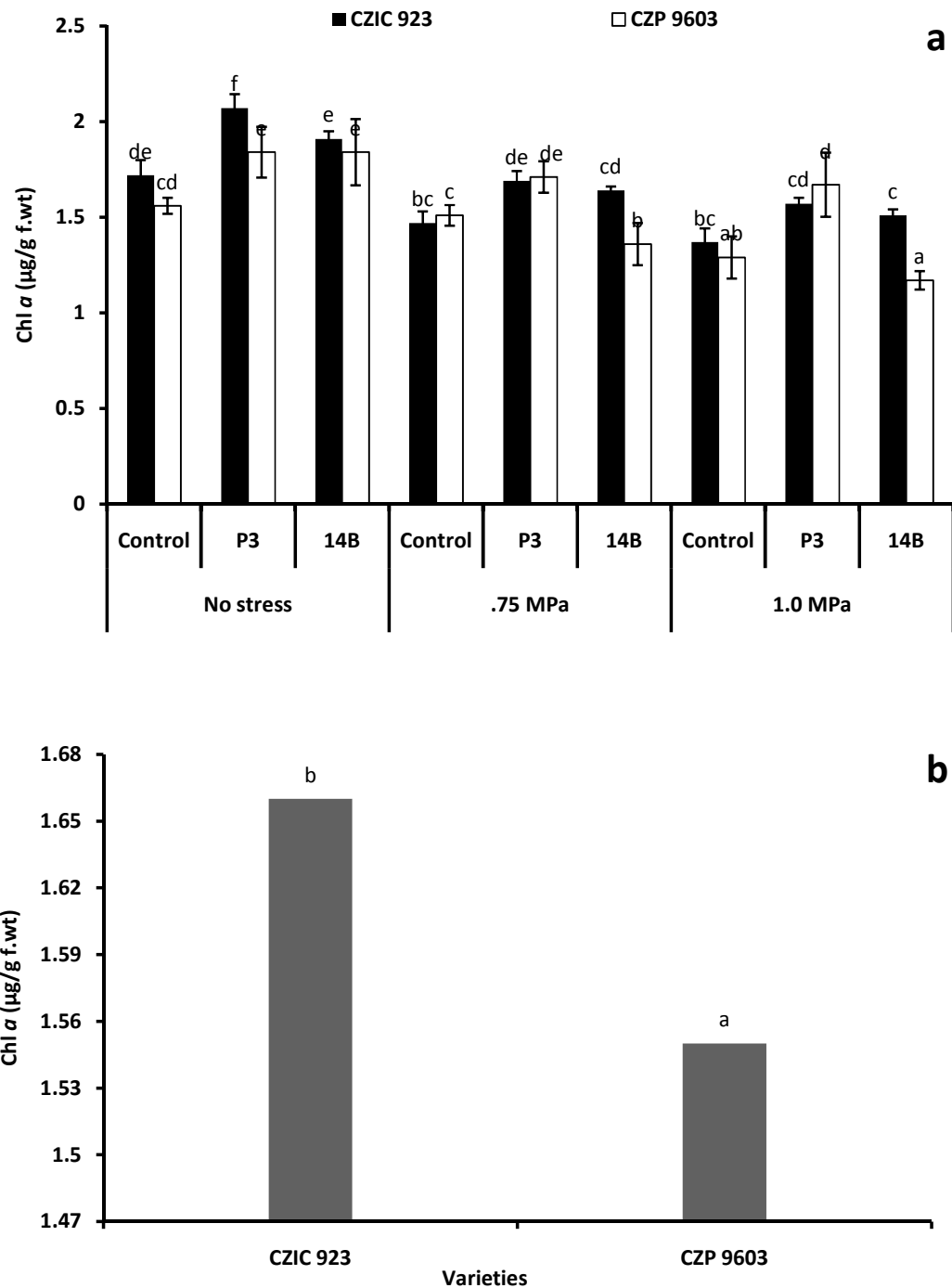


Fig. 4.54: Chlorophyll *a* (chl *a*) content of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

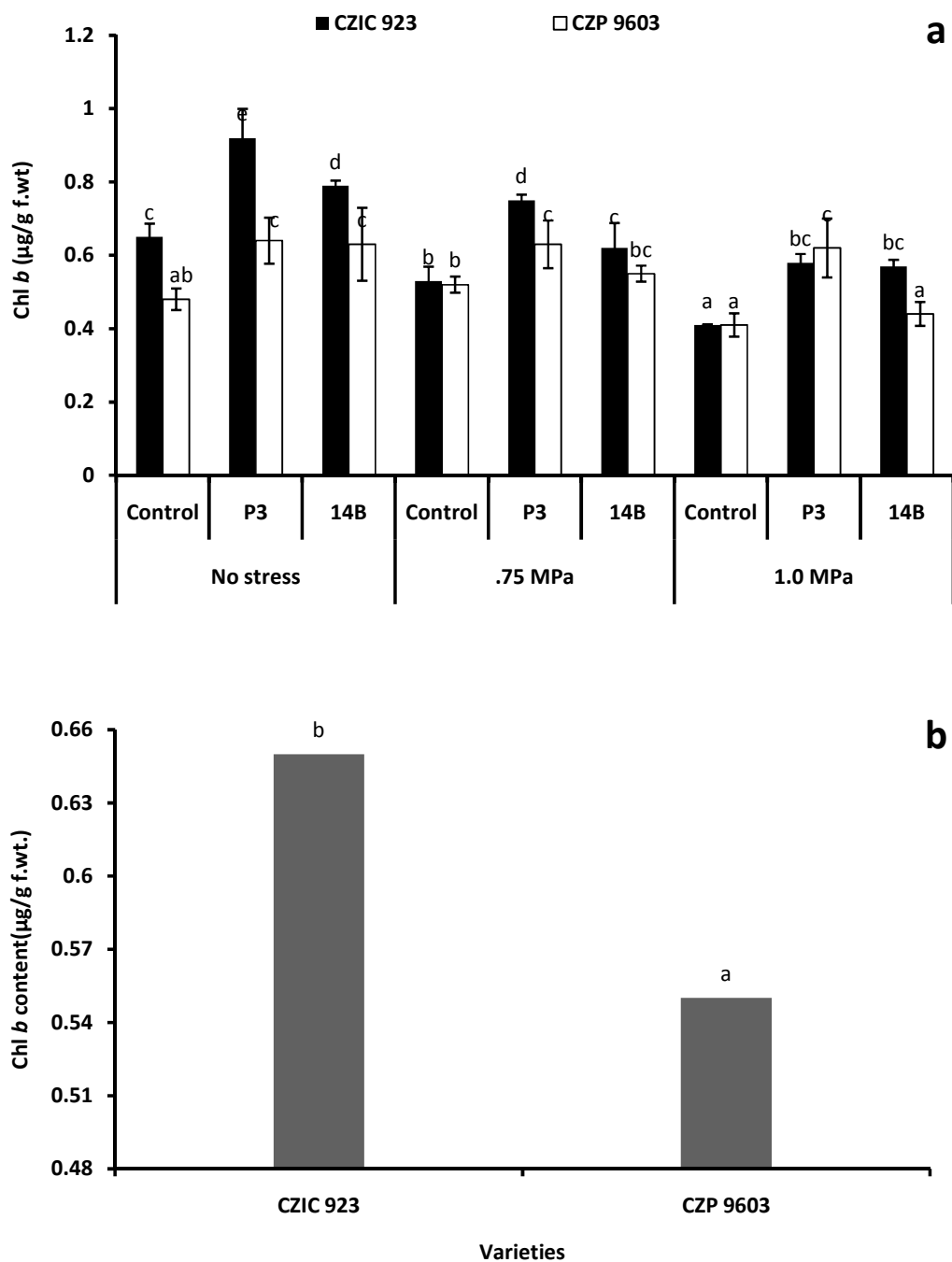


Fig. 4.55: Chlorophyll *b* (chl *b*) content of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

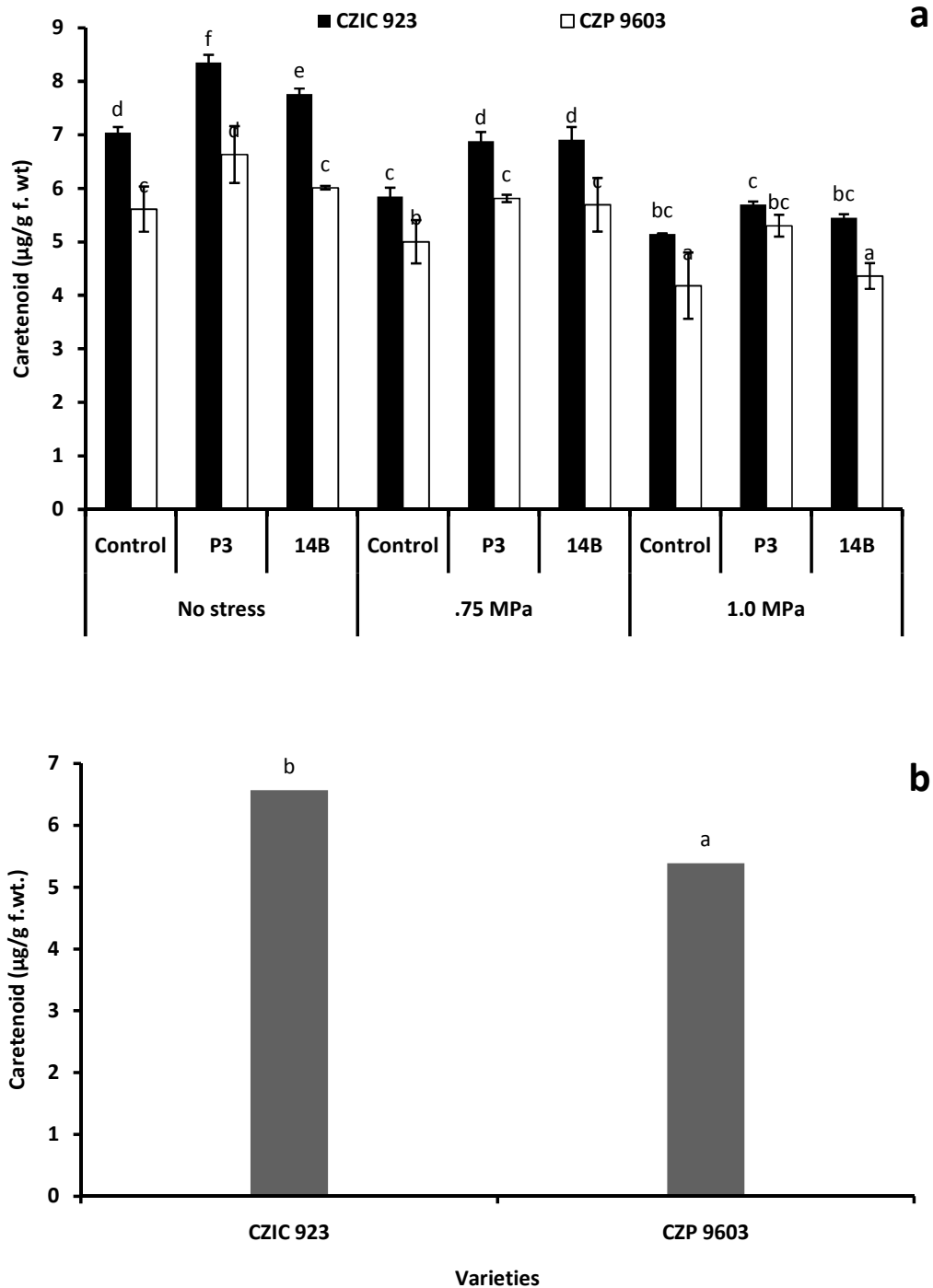


Fig. 4.56: Caretenoid content of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.12.2.4 Hydrogen peroxide (H₂O₂) content

Hydrogen peroxide is one of the most mobile reactive oxygen species, produced under stress. Increase in H₂O₂ level has severe consequences for the affected cell. A significant decrease was observed in bacterial treated plants in both the varieties while a gradual increase was observed in increasing stress. In var. CZIC923, a significant decrease of 1.28 fold in P3 treated plants was observed under -1 MPa while a 1.18 fold decrease was observed in 14B treatment as compared to control. In var. CZP9603, a decrease of 1.1 fold was observed in treatment P3 over the control whereas a 1.2 fold decrease was reported in 14B treated plants (**Fig. 4.57a**). 13.29% decrease was found in var. CZIC923 as compared to var. CZP9603 irrespective of rest of the factors (**Fig. 4.57b**).

4.12.2.5 Measurement of electrolyte leakage

Ion leakage is an indicator of membrane damage. In stress membrane damage is associated with generation of reactive oxygen species (ROS) which leads to membrane leakage. In the present experiment, the percent electrolyte leakage was increased with increase in the severity of applied drought stress. In the plants grown without stress non significant effect of bacterial treatments was found over the control in both the varieties of pearl millet. In -1 MPa osmotic potential, in var. CZIC923, a decrease of 1.15 fold was observed in both the bacterial treatments over the control while in var. CZP9603, a decrease of 1.1 fold in electrolyte leakage was found in both the bacterial treatments as compared to control (**Fig. 4.58a**). Irrespective of stress level and bacterial treatments a significant decrease of 125.37% was observed in CZIC923 as compared to var. CZP9603. Similar trend was observed as in case of H₂O₂ (**Fig. 4.58b**).

4.12.2.6 Relative water content of leaves

The relative water content of the leaves decreased with the onset of stress conditions in both the varieties. Almost similar trend was found as in H₂O₂ except in var. CZP9603, where effect of bacterial inoculation became non-significant in -1.0 MPa level of stress. In var. CZIC923 both the treatment significantly increased the relative water content of the leaves by 1.3 fold over the control (**Fig. 4.59a**). Irrespective of both of the factors in var. CZIC923, an increase of 68.32% was observed when compared with var. CZP9603 (**Fig. 4.59b**).

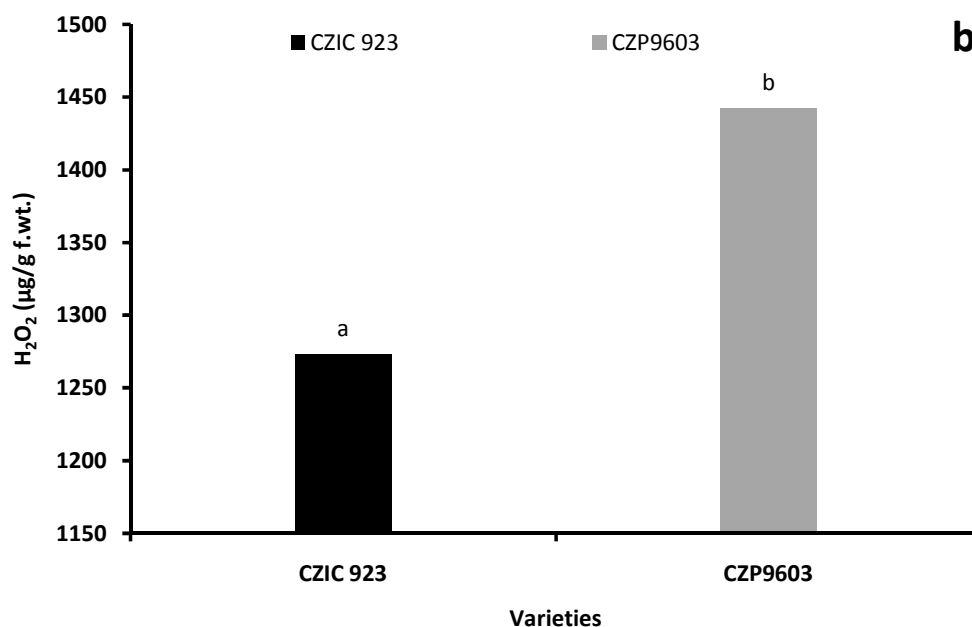
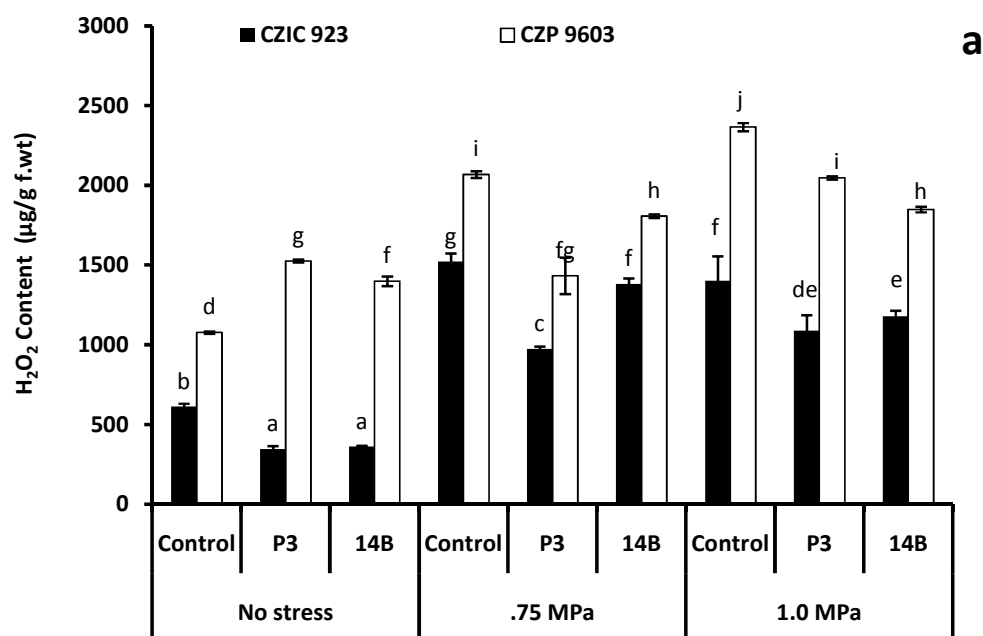


Fig. 4.57: H₂O₂ content of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

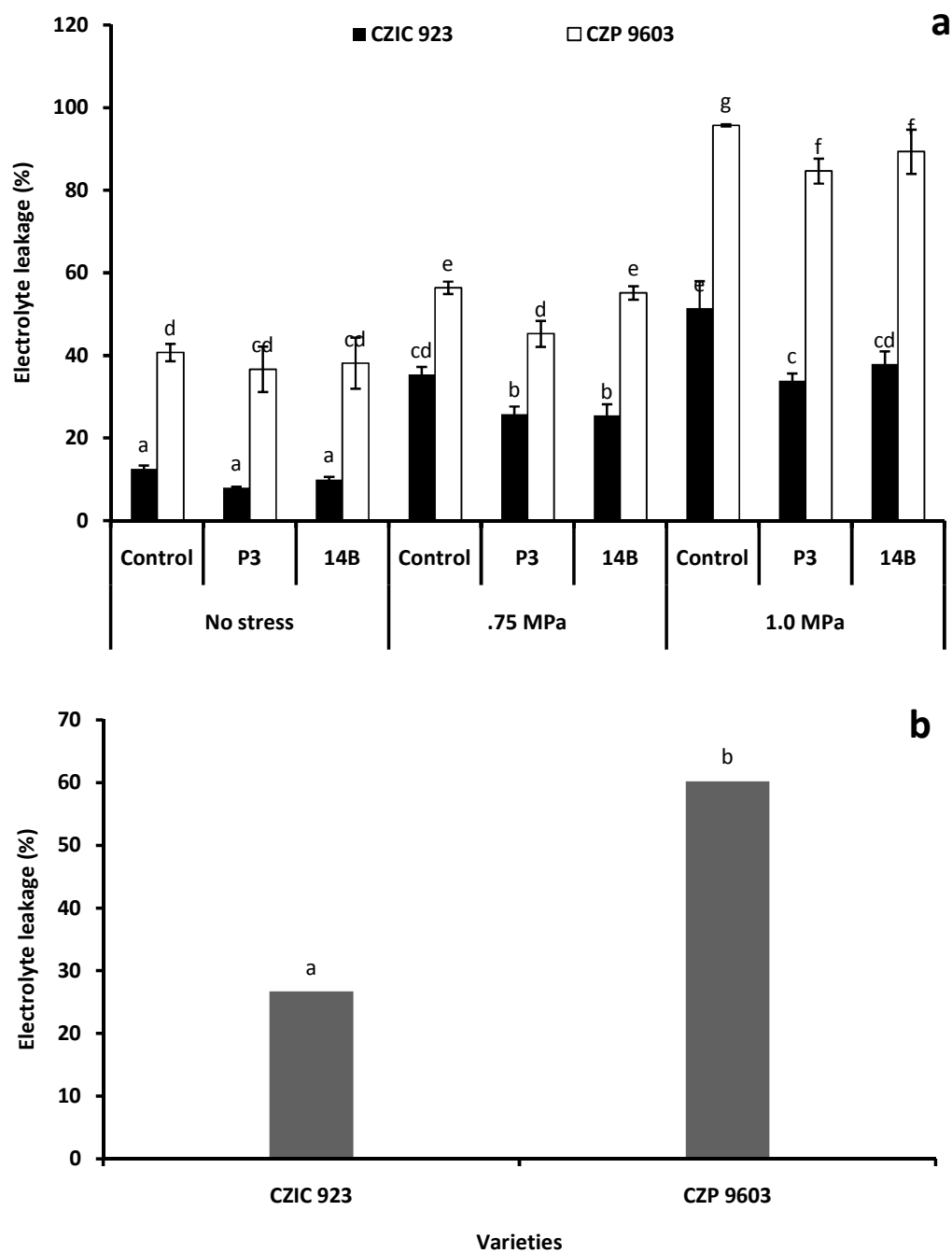


Fig. 4.58: Electrolyte leakage of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

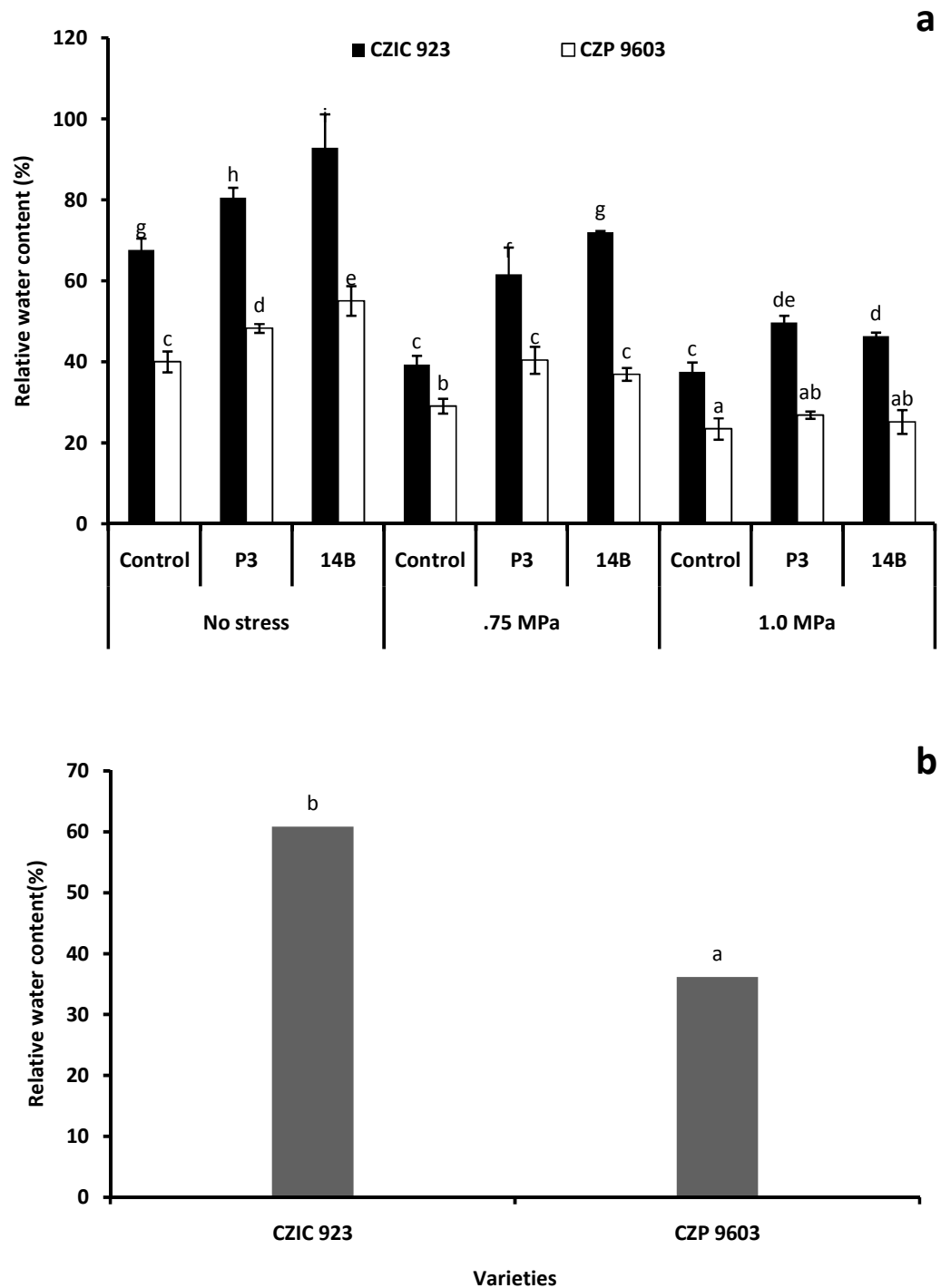


Fig. 4.59: Relative water content of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.12.2.7 Leaf water loss (LWL)

The percent leaf water loss was increased with increase in the severity of applied drought stress. In both the varieties bacterial treatment has significantly reduced the leaf water loss under stress and non stressed conditions. Under -1 MPa water potential, in var.CZIC923 P3 treatment lowered down the leaf water loss up to 1.3 fold while a decrease of 1.1 fold was observed in 14B treated plants. In case of var. CZP9603, both the bacterial isolates significantly reduced the leaf water loss by 1.2 fold (**Fig. 4.60a**). Irrespective of stress level and bacterial treatment, significant decrease of 21.96% was found in var. CZIC923 as compared to var. CZP9603 (**Fig. 4.60b**).

4.12.2.8 Leaf exopolysaccharide (EPS) content

In response to stress plant accumulate various types of osmoprotectants such as sugars, sugar alcohols and proline etc. In this experiment, increased level of leaf exopolysaccharide was found with the onset of stress. In var. CZIC923, there was significant increase in EPS content in bacterial treated leaves in all the stress level. P3 treated plants of var. CZIC923 showed the increase of 1.25 fold over the control while in 14B treatment an increase of 1.17 fold was observed as compared to control. In var. CZP9603, increase was non-significant in the treatments as compared to control (**Fig. 4.61a**). Irrespective of bacterial inoculation and level of stress, a significant increase of 415.60% was found in var.CZIC923 as compared to var.CZP9603 (**Fig. 4.61b**).

4.12.3 Antioxidant enzyme analysis

4.12.3.1 Superoxide dismutase (SOD) activity

A significant increase in superoxide dismutase specific activity was recorded under stress, in both the varieties as compared to control. In var. CZIC923, significant increase was observed in both the bacterial treatment as compared to control under stress as well as controlled condition. Under water potential of -1 MPa, both the bacteria treated plants of var. CZIC923 significantly increased the SOD activity up to 1.1 fold over the control. In var. CZP9603, 1.0 fold increase was found in both the treatments as compared to control (**Fig. 4.62a**). Irrespective of bacterial inoculation and level of stress, 57.47% increase was recorded in var. CZIC923 as compared to CZP9603 (**Fig. 4.62b**).

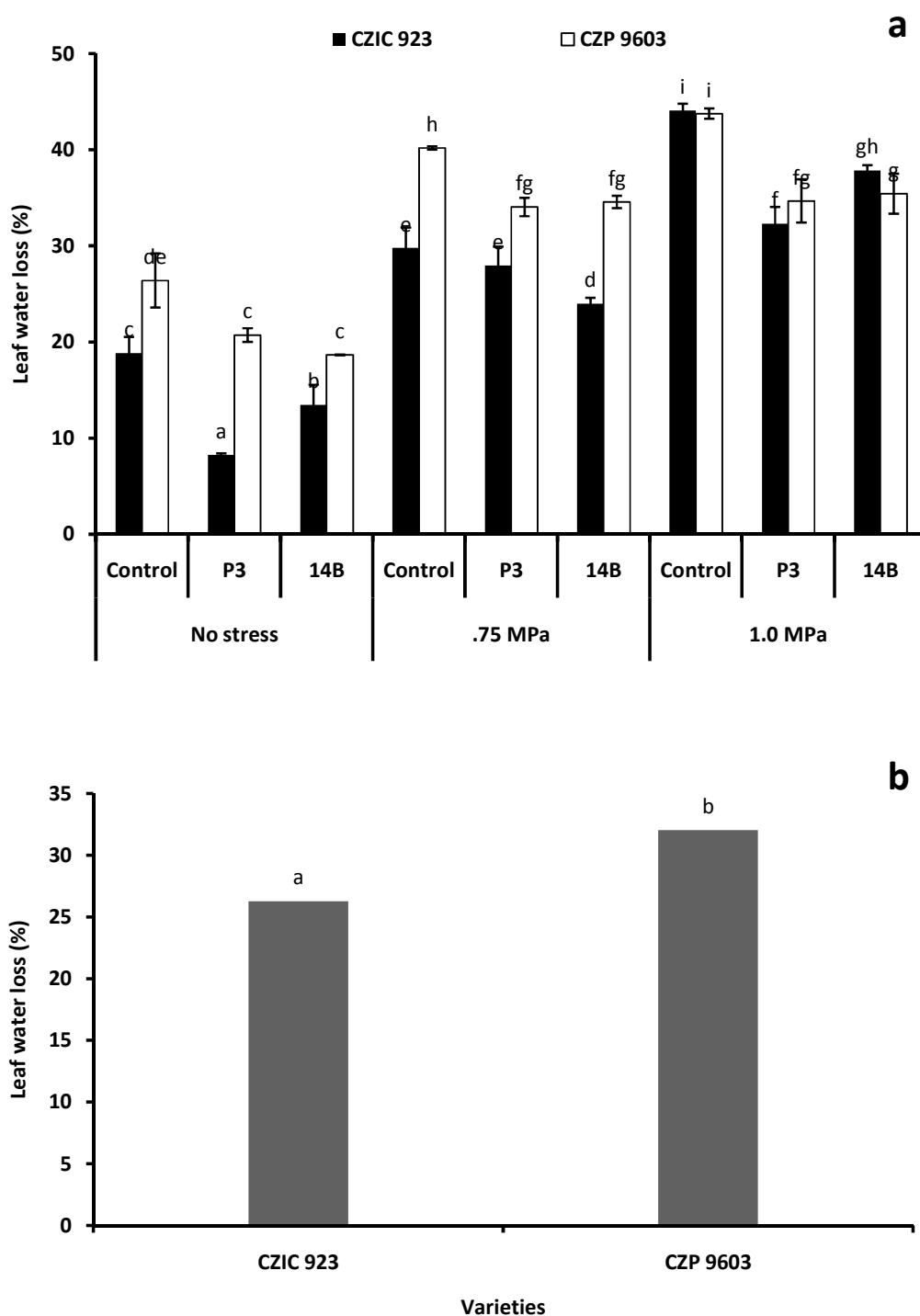


Fig. 4.60: Leaf water loss of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

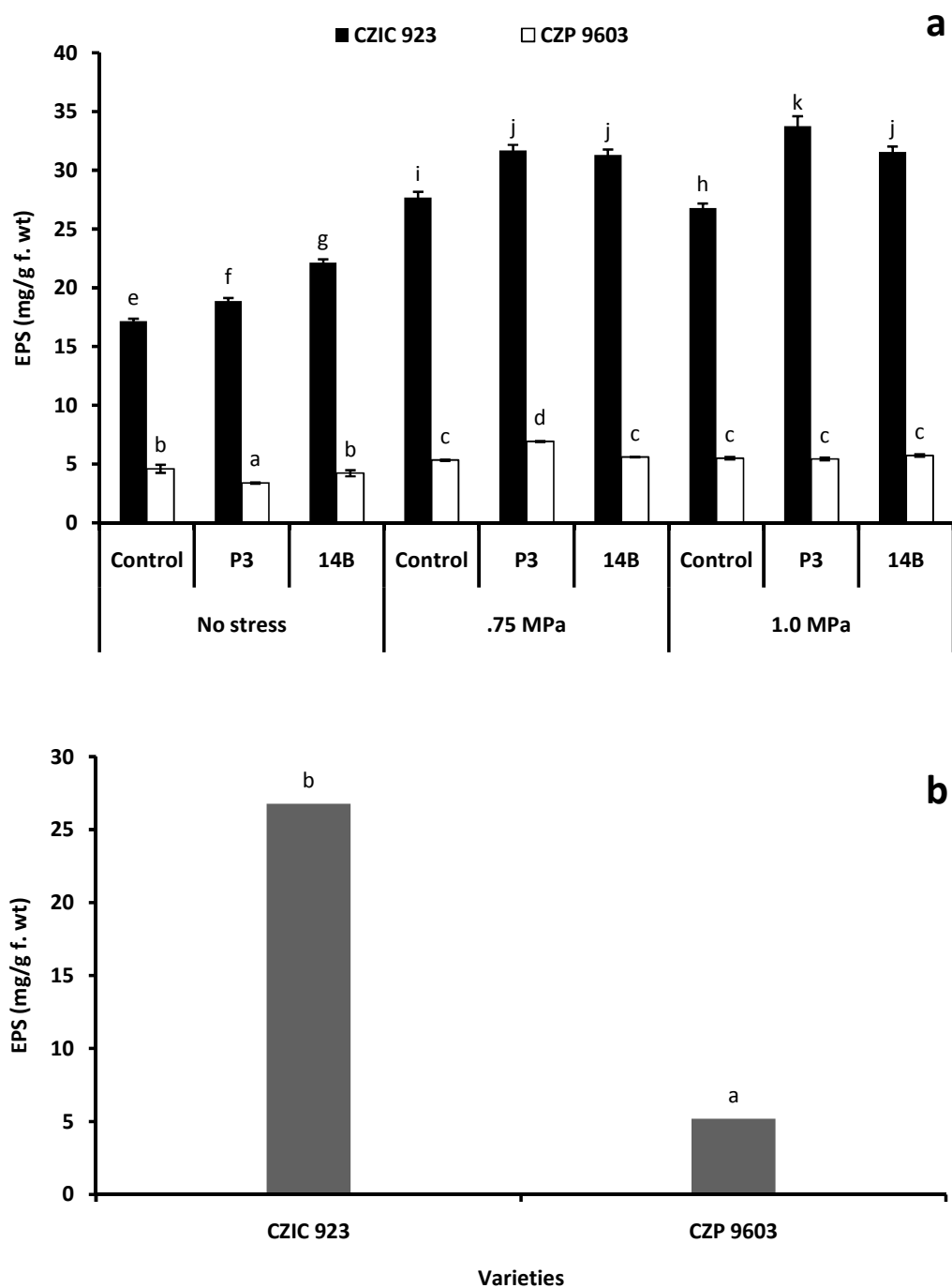


Fig. 4.61: Leaf exopolysaccharide (EPS) of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

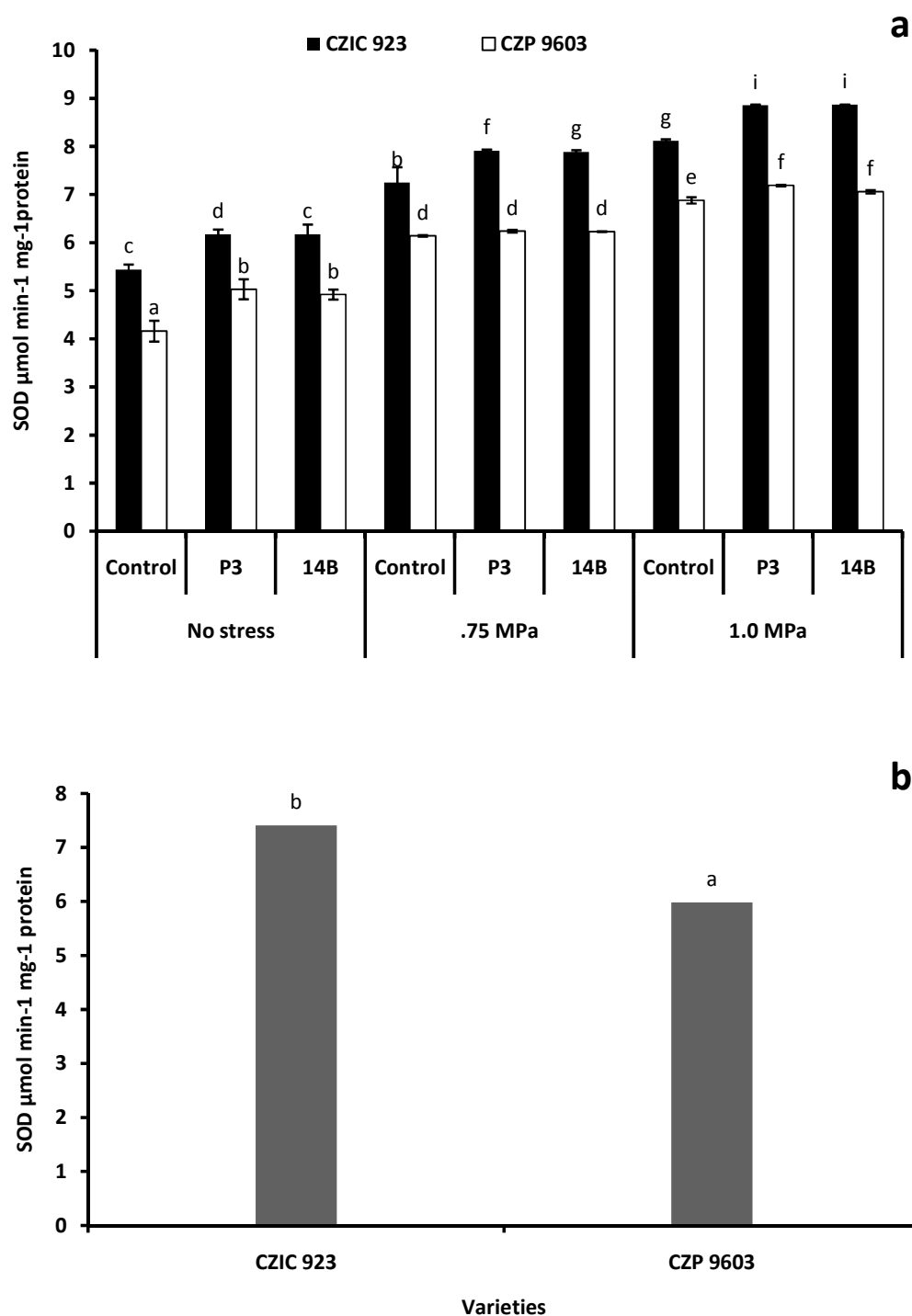


Fig. 4.62: Superoxide dismutase (SOD) of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

4.12.3.2 Ascorbate peroxidase (APX) activity

APX activity increased with increasing the severity of stress. In both the varieties, there was non-significant effect of bacterial treatment in non stress and stress level of -0.75MPa while in case of -1.0MPa , a significant increase was observed in bacterial treated plants as compared to control in both the varieties. In var. CZIC923, a significant increase of 1.5 and 1.3 fold was observed in P3 and 14B treated plants respectively over the control. In var. CZP9603, an increase of 1.3 and 1.1 fold in APX activity was found in P3 and 14B treatment respectively (**Fig. 4.63a**). A significant increase of 48.53% was observed in var.CZIC923 as compared to var.CZP9603 irrespective of rest of the factors (**Fig. 4.63b**).

4.12.3.3 Guaiacol peroxidase (GPX) activity

In both the varieties, control plants (without stress) showed non-significant increase in GPX activity treatments as compared to control. In -1.0MPa stress level, bacterial treatment showed significant increase in both the varieties, while bacterial treatment was ineffective in var. CZP9603 under -0.75MPa stress level. In case of var. CZIC923, a significant increase of 1.7 fold in plants treated with P3 treatment was found while a 1.37 fold increase was found in 14B treatment over the control. In var. CZP9603, an increase of 1.4 and 1.2 was observed over the control in P3 and 14B treated plants respectively (**Fig. 4.64a**). A significant increase of 57.47% in GPX activity was found irrespective of other factors in var.CZIC923 as compared to var.CZP9603 (**Fig. 4.64b**).

4.12.3.4 Catalase (CAT) activity

Similar trend was found in catalase activity as in case of GPX. An increase of 2 fold in catalase activity was observed in P3 treated plants of var.CZIC923 whereas in 14B treatment catalase activity was enhanced by 1.7 fold as compared to control. In var. CZP9603, an increase of 1.3 and 1.1 fold in catalase activity was found in P3 and 14B treated plants respectively (**Fig. 4.65a**). A significant increase (73.83%) was recorded in var. CZIC923 as compared to CZP9603 irrespective of all the factors (**Fig. 4.65b**).

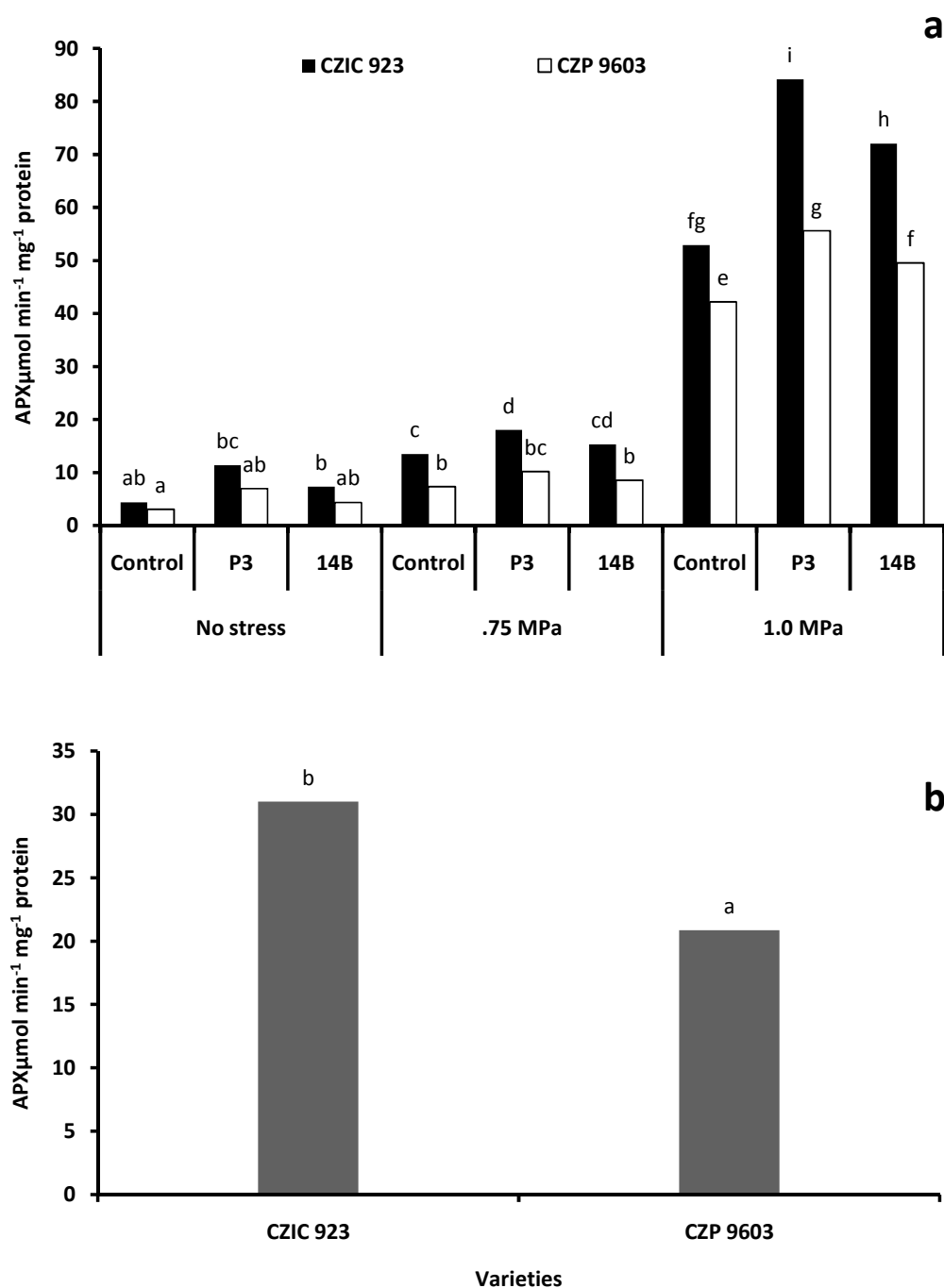


Fig. 4.63: Ascorbate peroxidase (APX) of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

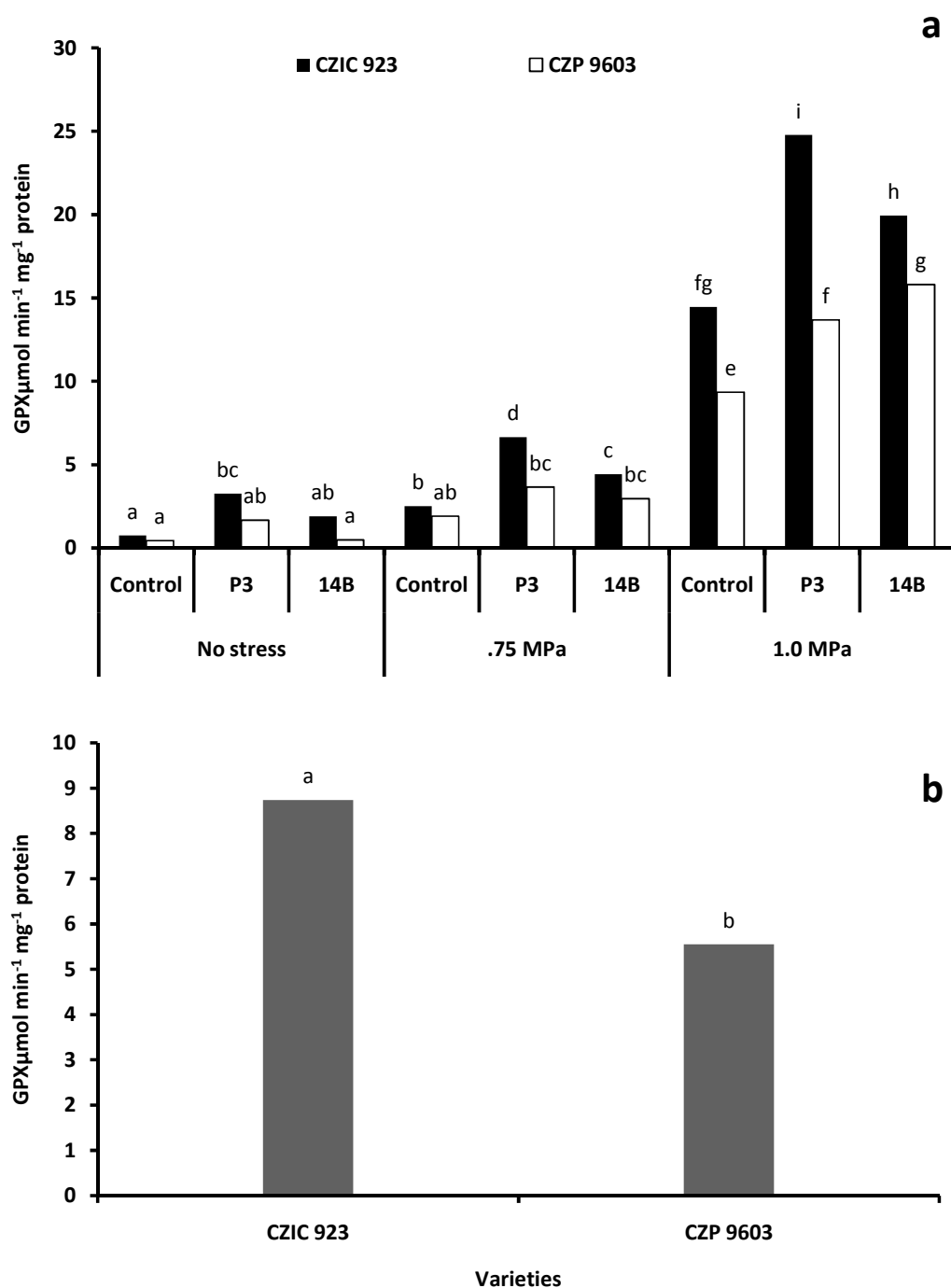


Fig. 4.64: Guaiacol peroxidase (GPX) of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

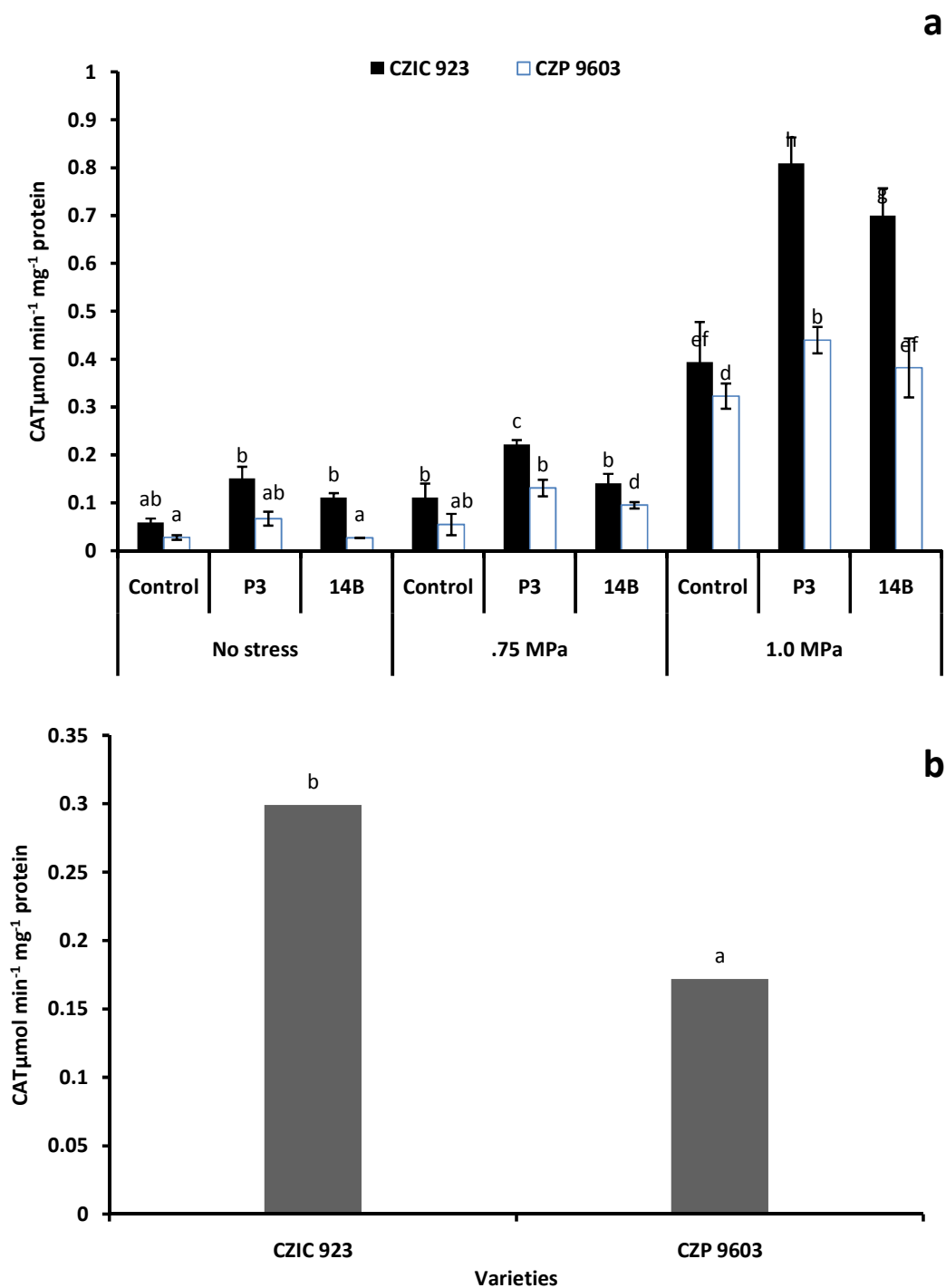
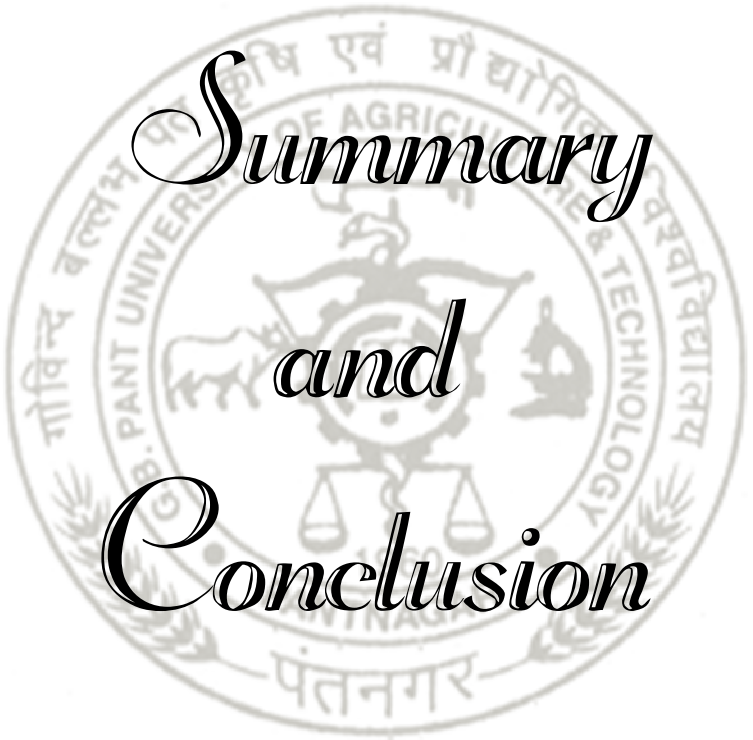


Fig. 4.65: Catalase (CAT) of two varieties of pearl millet grown under different concentrations of PEG (Polyethylene glycol) (a) Inoculated with PGPR; (b) Irrespective of PGPR and stress level. Mean values ($\pm$ S.D.) with different letters differed significantly at $p < 0.05$

In this experiment, inoculation of two varieties of pearl millet (CZIC923 and CZP2k9) with two rhizobacterial strains (P3 and 14B) isolated and identified in our laboratory induced plant tolerance when exposed to drought imposed by different concentration of PEG. Variety CZIC923 was highly drought tolerant as compared to var. CZP9603. Both the bacterial strains (*Bacillus cereus* and *Pseudomonas fluorescens*) also showed enhanced activity of reactive oxygen species (ROS) scavenging enzymes in var. CZIC923 as compared to var. CZP9603. Inoculation with these bacteria not only induced significant changes in the specific activity of different ROS-scavenging enzymes but also enhanced the proline production, photosynthetic efficiency and in turn improves the relative water content in leaf, leaf exopolysaccharide and reducing the leaf water loss and electrolyte leakage of inoculated plants, which proved to be a reason for the tolerance of abiotic stress. PGPR isolates belonging to *Pseudomonas* and *Bacillus* genera were reported to enhance resistance to water stress in green gram plants (Sarvanakumar *et al.*, 2011). Besides other beneficial properties, *Bacillus sp.* and *Pseudomonas* are known to reveal SOD activities and mitigate the oxidative stress in living systems (Gururani *et al.*, 2012). The bacterial SODs play an important role in their survival in the rhizosphere by facilitating the removal of free radicals (Wang *et al.*, 2007). In general, the survival of the bacterial strains is attributed to their contribution to the alleviation of abiotic stress and plant growth promotion (Dimkpa *et al.*, 2009b). Abiotic stress conditions cause an increase in ROS formation at the cellular level. ROS such as superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide, and hydroxyl radicals ($\cdot OH$) cause lipid peroxidation of membranes (Sgherri *et al.*, 2000; Hemavathi *et al.*, 2010). The antioxidant enzymes of the cell have the ability to remove the free radicals produced during abiotic stress conditions. These enzymes also protect the membranes and DNA from damage. Our results supported by a study done by Gururani *et al.* (2012), where they reported that the PGPR-inoculated and non-inoculated potato plants were subjected to different stresses induced by PEG, NaCl, and $ZnCl_2$ caused a putative changes in the expression of genes encoding the ROS-scavenging enzymes. The mRNA expression of SOD and APX in bacteria-inoculated plants growing under stress conditions increased considerably when compared with that in the non-inoculated stressed plants. Similarly, the mRNA expression levels of genes encoding for other antioxidative enzymes such as CAT, DHAR, and GR also increased in the

bacteria-treated plants. Bacterial inoculation has also been reported to positively influence root growth, relative water content, leaf exopolysaccharide, proline accumulation while reduction in H₂O₂ and MDA content in the leaves. Higher proline accumulation in potato plants inoculated with bacteria indicated higher plant tolerance to water stress. In a similar study done by **Creus *et al.* (2004)**, where they observed the increase in relative water content, water potential, and apoplastic water fraction when exposed the *Azospirillum* inoculated wheat plants to drought conditions and hypothesized the “elastic adjustment” as a crucial factor for increased drought tolerance in the wheat plants. Photosynthesis is the most important process on earth, directly or indirectly influencing the growth and survival of every organism. Therefore, it cannot be examined simply as an isolated phenomenon but must be studied within the context of whole-plant regulation. we measured the photosynthetic efficiency of pearl millet plants subjected to drought through Chlorophyll *a* and *b* content and caretenoid content. Inoculated plants showed enhanced chlorophyll and carotenoid level as compared to non-inoculated pearl millet plants. Earlier, several reports were published where these parameters were employed to examine the photosynthetic efficiency of different crop plants subjected to various stresses like drought, salinity, and heavy metal (**de Ronde *et al.*, 2004; Yusuf *et al.*, 2010; Mathur *et al.*, 2011**). In conclusion, the results showed here proves that the inoculations with rhizobacteria strains P3 and 14B protected pearl millet against drought as we discussed earlier. Plant tolerance to these stresses correlated with the increased levels of ROS-scavenging enzymes APX, SOD, CAT, and GPX, suggesting that inoculation with these strains might triggered abiotic stress-related defense pathways under stressed conditions.



Summary and Conclusion



Present study was planned to screen the potent plant growth promoting rhizobacteria, and to access their potential for drought tolerance in pearl millet. The findings of the study are summarized as follows-

- A sum of nine rhizospheric bacteria (eight *Bacillus* spp. and one *Pseudomonas* spp.) was characterized for their biochemical and plant growth promoting properties. P3 (*Pseudomonas fluorescens*) and 14B (*Bacillus cereus*) showed maximum plant growth promoting traits viz. siderophore, NH_3 , IAA production and phosphate solubilization.
- Effect of PGPRs on seed germination of pearl millet was tested. In comparison to untreated seeds, 14B and P3 significantly enhanced seed germination by 56.67 and 53.33%, respectively while minimum enhancement of 16.67% in seed germination was recorded in treatment 8 (*Bacillus cereus*) and 13B (*Bacillus cereus* RSR).
- A pot experiment was conducted under glass house condition to screen the most responsive crop among pearl millet, sorghum, moth and guar towards nine bacterial isolates. Maximum plant growth was achieved in pearl millet followed by sorghum, moth and guar. Maximum growth was recorded in variety CZIC923 and CZP2k9 by 14 B and P3, respectively. They gave consistent results for all the parameters in both the varieties. Hence, pearl millet was selected for further study and among nine bacteria, two bacteria (P3 and 14B) was selected.
- To test the endophytic nature of bacteria P3 and 14B, a pot experiment was conducted. Endophytes isolated from the roots were subjected to ARDRA along with our bacteria. 14B bacteria showed the endophytic nature as the banding pattern was same as that of endophyte which was isolated from roots of 14B treated plants while in case of P3, restriction pattern did not match.
- To compare the genotypic specificity in different varieties of pearl millet towards bacterial treatments, a pot experiment using six varieties of pearl millet inoculated with 14B and P3 was conducted. Var. CZIC923 was

observed as the most responsive variety followed by CZP2k9 while var. CZP9603 was reported as the least responsive to growth promotory action of bacteria. Both the bacterial isolates (P3 and 14B) showed significant effect on plant growth promotion in pearl millet var. CZIC923 and CZP2k9 while same bacterial isolates showed non-significant effect on var. CZP9603 of the same crop.

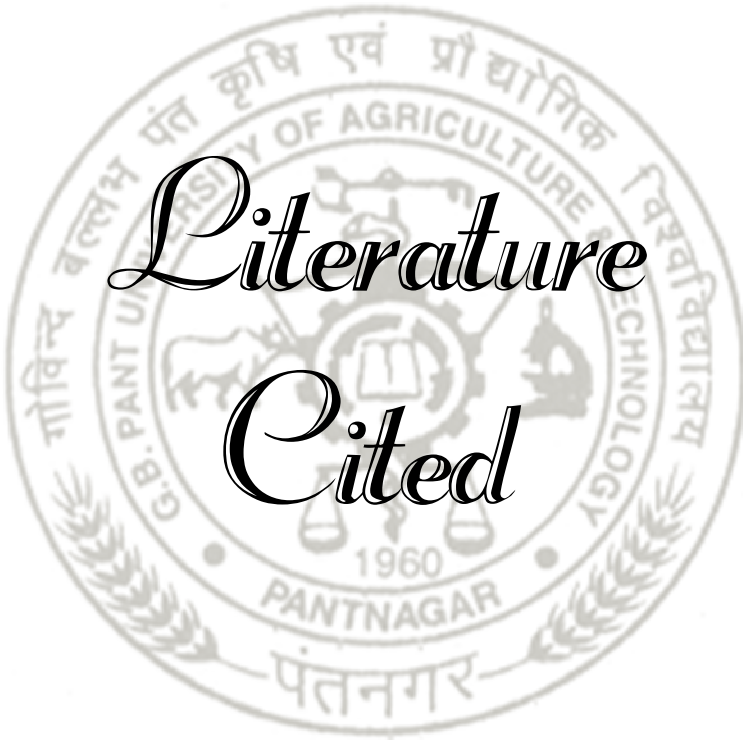
- To understand the effect of different soil types viz. sandy, clay loam and silty clay loam on the performance of two varieties of pearl millet (CZIC923, CZP9603) inoculated with P3 and 14B a glass house study was conducted. Results showed maximum plant growth in Pantnagar soil followed by soil of Ujhani and Mukteshwar. Bacterial response was significantly higher in Ujhani soil. The bacterial inoculation showed better growth promoting effect on the growth of pearl millet in nutrient deficient soil while the stimulatory efficiency reduces in relatively rich soil such as clay loam soil.
- Both the bacteria (P3 and 14B) were tested for their drought tolerance by growing them in nutrient broth medium with different concentration (-0.5, -0.75, -1.0 and -1.5 MPa) of polyethylene glycol (PEG). Both the isolates could grow up to water potential of -1.0 MPa and showed the presence of PGP properties (IAA, P-solubilization, production of siderophores and ammonia). Water potential of -1.5 MPa was inhibitory for both the bacterial isolates. P3 produced significantly higher level of EPS (13.23 mg ml⁻¹), soluble sugar (16.27 μmol ml⁻¹) and proline (17.72 μmol ml⁻¹) as compared to 14B in which a significantly lower amount of EPS (7.08 mg ml⁻¹), soluble sugar (10.53 μmol ml⁻¹) and proline (10.17 μmol ml⁻¹) was observed under -1.0 MPa of water potential in 36h.
- Screening was done to select a potential drought tolerant and a susceptible variety of pearl millet among three varieties through plant vigour, analysis of stress marker viz. Proline, H₂O₂, chlorophyll and malondialdehyde (MDA) and using various antioxidant enzyme assays. In inoculated plants of variety CZIC923, significantly higher amount of proline, chlorophyll *a*, chlorophyll *b*, caretenoid and specific activity of antioxidant enzyme was recorded followed by var. CZP2k9 and var. CZP9603. Among three varieties of pearl

millet tested, var. CZIC923 was most stress tolerant and var. CZP9603 was the most susceptible one.

- Two pearl millet varieties (CZIC923, CZP9603) selected on the basis of drought tolerance were subjected to two progressive drought stresses 1) moderate (-0.75 MPa) and 2) severe (-1.0 MPa) by using polyethylene glycol (PEG). Variety CZIC923 was highly drought tolerant as compared to var. CZP9603 as the response was further elevated by inoculating these bacteria which induced significant changes in the specific activity of different reactive oxygen species (ROS) scavenging enzymes (SOD, APX, CAT and GPX) and enhanced proline production, chlorophyll *a*, *b* and carotenoid level, relative water content of leaf, leaf EPS and reducing leaf water loss and electrolyte leakage of inoculated plants

Conclusion

The overall study shows the importance of rhizospheric bacteria in combating drought stress. The results proved that the inoculation with two plant growth promoting rhizobacteria P3 and 14B supported the growth of pearl millet against drought. Plants inoculated with rhizobacteria could tolerate the osmotic potential of -1MPa (10bars). Plant tolerance to drought correlated with the increased levels of ROS-scavenging enzymes (APX, SOD, CAT, and GPX), suggesting that inoculation with these bacteria triggered abiotic stress-related defense pathways under stressed conditions. Furthermore, this study raise the question whether the activity of antioxidant enzyme would increase or decrease in plants in response to bacterial inoculation because the literature is available in the context of both the facts. Our understanding of the plant-bacterial stress response mechanism is very limited. It would be a follow-up study for the up-coming researchers and to reach in a definite conclusion we must have to elucidate the whole stress-response of plant bacterial system at genetic level.



Literature Cited



LITERATURE CITED

- Akhtar, A., Hisamuddin, M. I., Robab, A. and Sharf, R. 2012.** Plant growth promoting Rhizobacteria: An overview. *J. Nat. Prod. Plant. Resour.* 2(1): 19-31.
- Alami, Y., Achouak, W., Marol, C. and Heulin, T. 2000.** Rhizosphere soil aggregation and plant growth promotion of sunflowers by an exopolysaccharide producing *rhizobium* sp. strain isolated from sunflower roots. *Appl. Environ. Microbiol.* 66(8): 3393-3398.
- Alexandre, G., Jacoud, C., Faure, D. and Bally, R. 1996.** Population dynamics of a motile and a non-motile *Azospirillum lipoferum* strain during rice root colonisation and motility variation in the rhizosphere. *FEMS Microbiol. Ecol.* 19: 271-278.
- Alexieva, V., Sergiev, I., Mapelli, S. and Karanov, E. 2001.** The effect of drought and ultraviolet radiation on growth and stress markers in pea and wheat. *Plant Cell Environ.* 24: 1337-1344.
- Alia, P. and Saradhi, P. 1991.** Proline accumulation under heavy metal stress. *J. Plant Physiol.* 138: 554-558.
- Allen, M. F. and Boosalis, M. G. 1983.** Effects of two species of VA mycorrhizal fungi on drought tolerance of winter wheat. *New Phytol.* 93: 67-76.
- Alvarez, M. I., Sueldo, R. J. and Barassi, C. A. 1996.** Effect of *Azospirillum* on coleoptiles growth in wheat seedlings under water stress. *Cereal Res. Communica.* 24(1): 101-107.
- Apel, K. and Hirt, H. 2004.** Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu.Rev. Plant Biol.* 55: 373-399.
- Arnon , D. I. 1949.** Copper enzymes in isolated chloroplast polyphenol oxidase in *Beta vulgaris*. *Plant physiol.* 24: 1-15.
- Asada, K. 1999.** The water-water cycle in chloroplasts: scavenging of active oxygens and dissipation of excess photons. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 50: 601-639.
- Ashraf, M. and Foolad, M. R. 2007.** Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Env. Exp. Bot.* 59: 206-216.

- Bai, L., Sui, F., Ge, T., Sun, Z., Lu, Y. and Zhou, G. 2006.** Effect of soil drought stress on leaf water status, membrane permeability and enzymatic antioxidant system of maize. *Pedosphere*. 16: 326-332.
- Bailly, C., Audigier, C., Ladonne, F., Wagner, M. H., Coste, F., Corbineau, F. and Côme, D. 2001.** Changes in oligosaccharide content and antioxidant enzyme activities in developing bean seeds as related to acquisition of drying tolerance and seed quality. *J. Exp. Bot.* 52: 701-708.
- Bais, H. P., Park, S., Weir, T. L., Callaway, R. M. and Vivanco, J. M. 2004.** How plants communicate using the underground information superhighway. *Trends Plant Sci.* 9(1): 26–32.
- Bano, A. and Fatima, M. 2009.** Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. *Biol. Fert. Soils*. 45: 405–413.
- Barrs, H. D. and Weatherley, P. E. 1968.** A re-examination of the relative turgidity technique for estimating water deficits in leaves. *Aus. J. Biol. Sci.* 15(3): 413-428.
- Basavaraj, G., Rao, P. P., Bhagavatula, S. and Ahmed, W. 2010.** Availability and utilization of pearl millet in India. *ICRISAT*. 8: 1-6.
- Bashan, Y. and de-Bashan, L. E. 2010.** Chapter two—How the plant growth-promoting bacterium *Azospirillum* promotes plant growth—a critical assessment. *Adv. Agron.* 108: 77–136.
- Bashan, Y. and Holguin, G. 1997.** *Azospirillum*-plant relationships: environmental and physiological advances (1990–1996). *Can. J. Microbiol.* 43: 03-121.
- Bashan, Y. and Holguin, G. 1998.** Proposal for the division of plant growth-promoting rhizobacteria into two classifications: biocontrol-PGPB (plant growth-promoting bacteria) and PGPB. *Soil Biol. Biochem.* 30: 1225–1228.
- Bashan, Y., Rojas, A. and Punte, M. E. 1999.** Improved establishment and development of three cacti species inoculated with *Azospirillum brasilense* transported into disturbed urban desert soil. *Can. J. Microbiol.* 45: 441-451.
- Bates, L. S., Waldren, R. P. and Teare, I. D. 1973.** Rapid determination of free proline for water–stress studies. *Plant Soil*. 39: 205– 207.
- Beattie, G. A. 2006.** Plant-associated bacteria: survey, molecular phylogeny, genomics and recent advances. In: Gnanamanickam SS (ed) *Plant-associated bacteria*. Springer, the Netherlands. 1–56. doi: 10.1007/978-1-4020-4538-7_1.

- Beauchamp, C. and Fridovich, I. 1971.** Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. *Anal. biochem.* 44(1): 276.
- Beers, R. F. and Sizer, I. W. 1952.** A spectrophotometric method for measuring the breakdown of hydrogen peroxide by catalase. *J. Biol. Chem.* 195(1): 133-140.
- Bensalim, S., Nowak, J. and Asiedu, S. 1998.** A plant growth promoting rhizobacterium and temperature effects on performance of 18 clones of potato. *Potato J.* 75: 145–152.
- Bent, E. 2006.** Induced systemic resistance mediated by plant growth-promoting rhizobacteria (PGPR) and fungi (PGPF). Multigenic and Induced Systemic Resistance in Plants (Tuzun S & Bent E, eds). 225–258. Springer, Berlin.
- Bent, E., Tuzun, S., Chanway, C. P., and Enebak, S. 2001.** Alterations in plant growth and in root hormone levels of lodgepole pines inoculated with rhizobacteria. *Canadian journal of microbiology.* 47(9): 793-800.
- Bergey's manual of systematic Bacteriology 1994.**
- Bertrand, H., Nalin, R., Bally, R., and Cleyet-Marel, J. C. 2001.** Isolation and identification of the most efficient plant growth-promoting bacteria associated with canola (*Brassica napus*). *Biology and Fertility of Soils.* 33(2): 152-156.
- Bhattacharjee, S. 2005.** Reactive oxygen species and oxidative burst: roles in stress, senescence and signal transduction in plant. *Curr. Sci.* 89: 1113-1121.
- Biehler, K. and Fock, H. 1996.** Evidence for the contribution of the Mehler-Peroxidase reaction in dissipating excess electrons in drought-stressed wheat. *Plant Physiol.* 112: 265-72.
- Blanco and Bernard, T. 1994.** Osmoadaptation in rhizobia: ectoine-induced salt tolerance. *J. Bacteriol.* 176: 5210–5217.
- Blum, A. 2005.** Drought resistance, water-use efficiency, and yield potential – are they compatible, dissonant, or mutually exclusive? *Aust. J. Agric. Res.* 56: 1159–1168.
- Boiero, L., Perrig, D., Masciarelli, O., Pena, C., Cassán F. and Luna, V. 2007.** Phytohormone production by strains of *Bradyrhizobium japonicum* and possible physiological and technological implications. *Appl. Microbiol. Biotechnol.* 74: 874–880.
- Bouton, J. H., Smith, R. L., Schank, S. C., Burton, G. W., Tyler, M. E., Littell, R. C., Gallaher, R. N. and Quesenberry, K. H. 1979.** Response of Pearl Millet Inbreds and Hybrids to Inoculation with *Azospirillum brasilense*. 19(1): 12-16.

- Boveris, A. H., Sies, H., Martino, E. E., Decampo, R., Turreus, J. F. and Stoppani, A. O. M. 1980.** Deficient metabolic utilization of hydrogen peroxide in *Trypanosoma cruzi*. *Biochemi. J.* 188: 643–648.
- Boyer, J. S. 1982.** Plant productivity and environment. *Sci.* 218: 443–448.
- Bradford, M. M. 1976.** A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 246–254.
- Bright, J. Desikan, R., Hancock, J. T., Weir, I. S. and Neill, S. J. 2006.** ABA-induced NO generation and stomatal closure in *Arabidopsis* are dependent on H₂O₂ synthesis. *The Plant J.* 45: 13:122.
- Britt, A. B. 1999.** Molecular genetics of DNA repair in higher plants. *Trends Plant Sci.* 4: 20-25.
- Burdman, S., Okon, Y. and Jurkevitch, E. 2000.** Surface characteristics of *Azospirillum brasilense* in relation to cell aggregation and attachment to plant roots. *Cri. Rev. microbiol.* 26(2): 91-110.
- Caballero, J. I., Verduzco, C. V., Galan, J. and Jimenz, E. S. D. 2005.** Proline accumulation as a symptom of drought stress in maize: A tissue differentiation requirement. *J. Exp. Bot.* 39: 889-897.
- Cakmakci, R., Donmez, F., Aydin, A. and Sahin, F. 2006.** Growth promotion of plants by plant growth-promoting rhizobacteria under greenhouse and two different field soil conditions. *Soil Biol. Biochem.* 38(6): 1482–1487.
- Carvalho, M. H. C. 2008.** Drought stress and reactive oxygen species, Production, scavenging and signaling. *Plant Signal. Behav.* 3(3): 156-165.
- Casanovas, E. M., Barassi, C. A. and Sueldo, R. J. 2002.** *Azospirillum* inoculation mitigates water stress effects in maize seedlings. *Cereal Res. Commun.* 30: 343-350.
- Cattelan, A. J., Hartel, P. G. and Fuhrmann, J. J. 1999.** Screening for plant growth-promoting rhizobacteria to promote early soybean growth. *Soil Sci. Soc. Am. J.* 63:1670–1680.
- Chanway, C. P. and Holl, F. B. 1994.** Growth of outplanted lodepole pine seedlings one year after inoculation with plant growth promoting rhizobacteria. *Forest Sci.* 40: 238–246.

- Chen, C. and Dickman, M. B. 2005.** Proline suppresses apoptosis in the fungal pathogen *Colletotrichum trifolii*. *Proceed. Nat. Acad. Sci.* 102:3459-464.
- Chen, M., Wei, H., Cao, J., Liu, R., Wang, Y. and Zheng, C. 2007.** Expression of *Bacillus subtilis* proAB genes and reduction of feedback inhibition of proline synthesis increases proline production and confers osmotolerance in transgenic *Arabidopsis*. *J. Biochem. Mol. Biol.* 40(3): 396–403.
- Chenu, C. 1993.** Clay or sand polysaccharide associations as models for the interface between microorganisms and soil: water-related properties and microstructure. *Geoderma*. 56: 143–156.
- Chenu, C. and Roberson, E. B. 1996.** Diffusion of glucose in microbial extracellular polysaccharide as affected by water potential. *Soil Biol. Biochem.* 28: 877–884.
- Clark, D., Durner, J., Navarre, D. A. and Klessig, D. F. 2000.** Nitric oxide inhibition of tobacco catalase and ascorbate peroxidase. *Mol. Plant Microbe Interac.* 13: 1380–1384.
- Close, T. J. 2006.** Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins. *Physiologia Plantarum*. 97(4): 795-803.
- Collins, A. 2001.** Carotenoids and genomic stability. *Mutat. Res.* 475:1-28.
- Cominelli, E. and Tonelli, C. 2010.** Transgenic crops coping with water scarcity. *New Biotechnol.* 27: 473-476.
- Compant, S. Marcel, G. A. van der Heijden, Angel, A. and Sessitsc, H. 2010.** Climate change effects on beneficial plant-microorganism interactions. *FEMS Microbiol. Ecol.* 73: 197–214.
- Creus, C. M., Sueldo, R. J. and Barassi, C. A. 2004.** Water relations and yield in *Azospirillum*-inoculated wheat exposed to drought in the field. *Can. J. Bot.* 82(2): 273–281.
- Creus, C., Graziano, M., Casanovas, E., Pereyra, M., Simontacchi, M., Puntarulo, S., Barassi, C. and Lamattina, L. 2005.** Nitric oxide is involved in the *Azospirillum brasilense* induced lateral root formation in tomato. *Planta*. 221(2): 297-303.
- Crowe, J. H. and Crowe, L. M. 1992.** Membrane integrity in anhydrobiotic organisms: toward a mechanism for stabilizing dry cells. In: Somero GN, Osmond CB, Bolis CL (eds) *Water and life*, 1st edn. Springer, Berlin, pp 87–103.

- Crowley, D. E., Reid, C. P. P. and Szaniszlo, P. J. 1988.** “Utilization of microbial siderophores in iron acquisition by oat,” *Plant Physiol.* 87: 685-688.
- Csonka, L. N. 1989.** Physiological and genetic responses of bacteria to osmotic stress. *Microbiol. Mol. Biol. Rev.* 53: 121–147.
- Dai, F., Huang, Y., Zhou, M. and Zhang, G. 2009.** The influence of cold acclimation on antioxidative enzymes and antioxidants in sensitive and tolerant barley cultivars. *Biologia Planta.* 53(2): 257-262.
- de Ronde, J. A., Cress, W. A., Kruger, G. H. J., Strasser, R. J. and van Staden, J. 2004.** Photosynthetic response of transgenic soybean plants, containing an Arabidopsis P5CR gene, during heat and drought stress. *J. Plant Physiol.* 161: 1211–1224.
- De Salamone, I. E. G. 2000.** *Direct beneficial effects of cytokinin-producing rhizobacteria on plant growth* (Doctoral dissertation).
- Dell’Amico, E., Cavalca, L. and Andreoni, V. 2008.** Improvement of *Brassica napus* growth under cadmium stress by cadmium-resistant rhizobacteria. *Soil Biol. Biochem.* 40(1): 74–84.
- Desbrosses, G., Contesto, C., Varoquaux, F., Galland, M. and Touraine, B. 2009.** PGPR-Arabidopsis interactions is a useful system to study signalling pathways involved in plant developmental control. *Plant Signal Behav.* 4: 321–323.
- Dey, R., Pal, K. K., Bhatt, D. M. and Chauhan, S. M. 2004.** Growth promotion and yield enhancement of peanut (*Arachis hypogaea* L.) by application of plant growth-promoting rhizobacteria. *Microbiol. Res.* 159(4): 371-394.
- Dhindsa, R. S. and Matowe, W. 1981.** Drought tolerance in two mosses: Correlated with enzymatic defense against lipid peroxidation. *J. Exp. Bot.* 32: 79–91.
- Diamant, S., Eliahu, N., Rosenthal, D. and Goloubinoff, P. 2001.** Chemical chaperones regulate molecular chaperones in vitro and in cells under combined salt and heat stresses. *J. Biol. Chem.* 276: 39586–39591.
- Dimkpa, C., Weinand, T. and Asch, F. 2009b.** Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant Cell Environ.* 32: 1682–1694.
- Dionisio-Sese, M. L. and Tobita, S. 1998.** Antioxidant responses of rice seedlings to salinity stress. *Plant Sci.* 135(1): 1-9.
- Dubois, M., Gilles, K. A., Hamilton, J. K., Rebers, P. A. and Smith, F. 1956.** Colorimetric method for determination of sugars and related substances. *Anal. chem.* 28(3): 350-356.

- Dunne, C., Crowley, J. J., Loccoz, Y. M., Dowling, D. N., and O`Gara, F. 1997.** Biological control of *Pythium ultimum* by *Stenotrophomonas maltophilia* W81 is mediated by an extracellular proteolytic activity. *Microbiol.* 143: 3921-3931.
- Durner, J. and Klessig, D. F. 1995.** Inhibition of ascorbate peroxidase by salicylic acid and 2, 6- dichloroisonicotinic acid, two inducers of plant defense responses. *Proceed. Nat. Acad. Sci.* **92**: 11312–11316.
- Edwards, A. P. and Bremner, J. M. 1967.** Dispersion of soil particles by sonic vibrations. *J. Soil Sci.* 18: 47–63.
- Egamberdiyeva, D. 2007.** The effect of plant growth promoting bacteria on growth and nutrient uptake of maize in two different soils. *Appl. Soil Ecol.* 36(2–3): 184–189.
- Egert, M. and Tevini, M. 2002.** Influence of drought on some physiological parameters symptomatic for oxidative stress in leaves of chives (*Allium schoenoprasum*). *Environ. Exp. Bot.* 48: 43–49.
- Ehteshami, S. M. R., Aghaalkhani, M., Khavazi, K. and Chaichi, M. R. 2007.** Effect of Phosphate Solubilizing Microorganisms on quantitative and qualitative characteristics of Maize (*Zea mays* L) under water deficit stress. *Pak. J. Biol. Sci.* 10(20): 3585-3591.
- Eliasson, L., Bertell, G. and Bolander, E. 1989.** Inhibitory action of auxin and root elongation not mediated by ethylene. *Plant Physiol.* 91: 310–314.
- Elstner, E. F. 1991.** Mechanism of oxygen activation in different compartments. in: E.J. Pell, K.L. Steffen (Eds.). Active Oxygen/Oxidative Stress and Plant Metabolism. *American Society of Plant Physiologists.* pp. 13-25.
- El-Tayeb, M. A. 2006.** Differential responses of pigments, lipid peroxidation, organic solutes, catalase and peroxidase activity in the leaves of two *Vicia faba* L. cultivars to drought. *Intern. J. Agri. Biol.* 8(1): 116–122.
- Fam, S. S. and Morrow, J. D. 2003.** The isoprostanes: unique products of arachidonic acid oxidation – a review. *Curr. Med. Chem.* 10: 1723–1740.
- Feierabend, J., Schaan, C. and Hertwig, B. 1992.** Photoinactivation of catalase occurs under both high- and low-temperature stress conditions and accompanies photoinhibition of photosystem II. *Plant Physiol.* 100: 1554-1561.
- Fett, W. F., Osman, S. F. and Dunn, M. F. 1989.** Characterization of exopolysaccharides produced by plant-associated fluorescent pseudomonads. *Appl. Environ. Microbial.* 55(3): 579-583.

- Figueiredo, M. V. B., Burity, H. A., Martinez, C. R. and Chanway, C. P. 2008.** Alleviation of drought stress in common bean (*Phaseolus vulgaris* L.) by co-inoculation with *Paenibacillus polymyxa* and *Rhizobium tropici*. *Appl. Soil Ecol.* 40: 182–188.
- Fischer, S. E., Miguel, M. J. and Mori, G. B. 2003.** Effect of root exudates on the exopolysaccharide composition and the lipopolysaccharide profile of *Azospirillum brasilense* cd under saline stress. *FEMS Microbiol. Lett.* 219: 53–62.
- Foreman, J., Demidchik, V., Bothwell, J. H., Mylona, P., Miedema, H., Torres, M. A., Linstead, P., Costa, S., Brownlee, C., Jones, J. D., Davies, J. M. and Dolan, L. 2003.** Reactive oxygen species produced by NADPH oxidase regulate plant cell growth, *Nature* 422: 442-446.
- Fotopoulos, V., Ziogas, V., Tanou, G. and Molassiotis, A. 2010.** Anjum N.A. (eds) et al. Involvement of AsA/DHA and GSH/GSSG Ratios in Gene and Protein Expression and in the Activation of Defence Mechanisms Under Abiotic Stress Conditions. Ascorbate-Glutathione Pathway and Stress Tolerance In Plants. DOI: 10.1007/978-90-481-9404-9_10 Springer Science+ Business Media B. V.
- Foyer, C. H. and Noctor, G. 2005.** Redox homeostis and antioxidant signaling: a metabolic interface between stress perception and physiological responses. *Plant Cell.* 17: 1866-1875.
- Fridovich, I. 1986.** Biological effects of the superoxide. *Rad., Arch. Biochem. Biophys.* 247: 1-11.
- Fridovich, I. and Freeman, B. 1986.** Antioxidant defenses in the lung. *Annu. Rev. Physiol.* 48: 693-702.
- Fu, J. and Huang, B. 2001.** Envolvement of antioxidants and lipid peroxidation in the adaptation of two cool-season grasses to localized drought stress. *Environ. Exp. Bot.* 45(2): 105–114.
- Galleguillos, C., Aguirre, C., Barea, J. M. and Azcon, R. 2000.** Growthpromoting effect of two *Sinorhizobium meliloti* strains (a wild type and its genetically modified derivative) on a non-legume plant species in specific interaction with two arbuscular mycorrhizal fungi. *Plant Sci.* 159: 57–63.
- Garg, N. and Manchanda, G. 2009.** ROS generation in plants: boon or bane? *Plant Biosys.* 143: 8-96.
- Ghamsari, L., Keyhani, E. and Golkhoo, S. 2007.** Kinetics Properties of Guaiacol Peroxidase Activity in *Crocus sativus* L. Corm during Rooting, *Iranian Biom. J.* 11(3): 137-146.

- Ghezzi, P. and Bonetto, V. 2003.** Redox proteomics: identification of oxidatively modified proteins. *Proteom.* 3: 1145-1153.
- Gill, S. S. and Tuteja, N. 2010.** Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48: 909-993.
- Glass, A. D. M. 1989.** Plant nutrition: an introduction to current concepts. Jones and Bartlett Publishers, Boston, p 234.
- Glick, B. R. 1995.** The enhancement of plant growth by free-living bacteria. *Can. J. Microbiol.* 41(2): 109-117.
- Glick, B. R. 2003.** Phytoremediation: synergistic use of plants and bacteria to clean up the environment. *Biotechnol. Adv.* 21(5): 383-393.
- Glick, B. R. 2007.** Promotion of plant growth by bacterial ACC deaminase. *Crit. Rev. Plant Sci.* 26: 227-242.
- Glick, B. R., Karaturovic, D. M. and Newell, P. C. 1995.** A novel procedure for rapid isolation of plant growth promoting *Pseudomonas*. *Can. J. Microbiol.* 41: 533-536.
- Glick, B. R., Liu, C., Ghosh, S. and Dumbrof, E. B. 1997.** Early development of canola seedlings in the presence of plant growth promoting rhizobacterium *Pseudomonas putida* GR 12-2. *Soil Biol. Biochem.* 29: 1233-1239.
- Glick, B. R., Patten, C. L., Holguin G. and Penrose, D. M. 1999.** Biochemical and genetic mechanisms used by plant growth promoting bacteria. Imperial College Press, London.
- Goldman, J. J., Hanna, W. W., Fleming, G. and Ozias-Akins, P. 2003.** Fertile transgenic pearl millet [*Pennisetum glaucum* (L.) R. Br.] plants recovered through microprojectile bombardment and phosphinothricin selection of apical meristem-, inflorescence-, and immature embryo- derived embryogenic tissues. *Plant Cell Reports.* 21: 999-1009.
- Gomez, J. M., Jimenez A., Olmos, E. and Sevilla, F. 2004.** Location and effects of long-term NaCl stress on superoxide dismutase and ascorbate peroxidase isoenzymes of pea (*Pisum sativum* cv. Puget) chloroplasts. *J. Exp. Bot.* 55: 119-130.
- Gordon, S. A. and Weber, R. P. 1951.** Colorimetric estimation of indoleacetic acid. *Plant Physiol.* 26(1): 192-195

- Graham, P. H. 1992.** Stress tolerance in *Rhizobium*, *Bradyrhizobium* and nodulation under adverse soil conditions. *Can. J. Microbial.* 38: 475–484.
- Grains, 1996.** Lost Crops of Africa: Board on Science and Technology for International \Development, Office of International Affairs-National Research Council. *The National Academies Press.* 1.
- Gratao, P. L., Polle, A., Lea, P. J. and Azevedo, R. A. 2005.** Making the life of heavy metal stressed plants a little easier. *Funct. Plant Biol.* 32: 481-494.
- Gray, E. J. and Smith, D. L. 2005.** Intracellular and extracellular PGPR: commonalities and distinctions in the plant–bacterium signaling processes. *Soil Biol. Biochem.* 37(3): 395–412.
- Guerinot, M. L. and Ying, Y. 1994.** “Iron: nutritious, noxious, and not readily available,” *Plant Physiol.* 104 (3): 815-820.
- Gueta-Dahan, Y., Yaniv, Z., Zilinskas, B. A. and Ben-Hayyim, G. 1997.** Salt and oxidative stress: similar and specific responses and their relation to salt tolerance in Citrus. *Planta.* 203: 460–469.
- Guo, J., Ying Qi, H., Guo, Y., Ge, H., Gong, L., Zhang, L. and Sun, P. 2004.** Biocontrol of tomato wilt by plant growth promoting rhizobacteria. *Biol. Contr.* 29: 66–72.
- Guóth, A., Tari, I., Gallé, Á., Csiszár, J., Cseuz, L. and Erdei, L. 2008.** Changes in photosynthetic performance and ABA levels under osmotic stress in drought tolerant and sensitive wheat genotypes. *Acta Biol. Szeged.* 52(1): 91-92.
- Gururani, M. A., Upadhyaya, C. P., Strasser, R. J., Woong, Y. J. and Park, S. W. 2012.** Physiological and biochemical responses of transgenic potato plants with altered expression of PSII manganese stabilizing protein. *Plant Physiol. Biochem.* 58: 182–194.
- Gururani, M. A., Upadhyaya, C. P., Venkatesh, J., Baskar, V. and Park, S. W. 2012.** Plant growth promoting rhizobacteria enhance abiotic stress tolerance in *Solanum tuberosum* through inducing changes in the expression of ROS scavenging enzymes and improved photosynthetic performance. *J. Plant Growth Regul.* In press.
- Halliwell, B. 2006.** Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. *Plant Physiol.* 141: 312–322.
- Halliwell, B. and Gutteridge, J. M. C. 1999.** Free radicals in biology and medicine, 3rd JKmsb ed. Oxford University Press, Oxford.

- Hallmann, J., Quadt-Hallmann, A., Mahafee, W.F. and Kloepper, J.W. 1997.** Bacterial endophytes in agricultural crops. *Can. J. Microbiol.* 43:895–914.
- Hameeda, B., Harini, G., Rupela, O. P., Wani, S. P. and Reddy, G. 2008.** Growth promotion of maize by phosphate-solubilizing bacteria isolated from composts and macrofauna. *Microbiol Res.* 163: 234–242.
- Hameeda, B., Rupela, O. P., Reddy, G. and Satyavani, K. 2006.** *Application of plant growth-promoting bacteria associated with composts and macrofauna for growth promotion of Pearl millet (Pennisetum glaucum L.). Biol. Fert. Soils.* 43(2): 221-227.
- Han, H. S. and Lee, K. D. 2005.** Plant Growth Promoting Rhizobacteria effect on antioxidant status, photosynthesis, mineral uptake and growth of Lettuce under soil salinity. *Research J. Agri. Biol. Sci.* 1(3): 210-215.
- Hardoim, P. R., van Overbeek, S. V. and van Elsas, J. D. 2008.** Properties of bacterial endophytes and their proposed role in plant growth. *Trends Microbiol.* 16: 463–471.
- Hare, P. D. and Cress, W. A. 1997.** Metabolic implications of stress-induced proline accumulation in plants. *Plant Growth Regul.* 21: 79-102.
- Harinarayana, G., Anand Kumar, K. and Andrews, D. J. 1999.** Pearl millet in global agriculture, in *Pearl Millet Breeding* (eds I.S. Khairwal, KN. Rai, D.J.Andrews, and G. Harinarayana), Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, India, pp. 479-506.
- Haynes, R. J. and Francis, G. S. 1993.** Changes in microbial biomass C, soil carbohydrate composition and aggregate stability induced by growth of selected crop and forage species under field conditions. *J. Soil Sci.* 44(4): 665–675.
- Heath, R. L. and Packer, L. 1968.** Photoperoxidation in isolated chloroplasts: I. Kinetics and stoichiometry of fatty acid peroxidation. *Arch. Biochem. Biophysic.* 125(1): 189-198.
- Hemavathi, Upadhyaya, C. P., Nookaraju, A., Young, K. E., Chun, S. C., Kim, D. H. and Park, S. W. 2010.** Enhanced ascorbic acid accumulation in transgenic potato confers tolerance to various abiotic stresses. *Biotechnol. Lett.* 32: 321–330.
- Hepper, C. M. 1975.** Extracellular polysaccharides of soil bacteria. In: Walker N (ed) *Soil microbiology, a critical review.* Wiley, New York, 93–111.

- Hernandez, I., Chacón, O., Rodriguez, R., Portieles, R., López, Y., Pujol, M. and Borrás-Hidalgo, O. 2009.** Black shank resistant tobacco by silencing of glutathione S-transferase. *Biochem. Biophys. Res. Commun.* 387: 300-304.
- Hider R. C. and Kong, X. 2010.** “Chemistry and biology of siderophores,” *Natural Product Rep.* 27(5): 637–657.
- Hirayama, T. and Shinozaki, K. 2010.** Research on plant abiotic stress responses in the post-genome era: past, present and future. *The Plant J.* 61(6): 1041–1052.
- Hsiao, T. C. 1973.** Plant response to water stress. *Aun. Rev. Plant Physiol.* 24: 524–570.
- Hu, C. 2005.** Induction of growth promotion and stress tolerance in *Arabidopsis* and tomato by plant growth promoting rhizobacteria. Dissertation. <http://hdl.handle.net/10415/769>.
- IPCC. 2001.** Climate Change Synthesis Report: Third Assessment Report of the Intergovernmental Panel on Climate Change .Cambridge: Cambridge University Press.
- IPCC. 2007.** Climate Change.Adaptation and Vulnerability, Summary for Policymakers, Intergovernmental Panel on Climate Change, Geneva, Switzerland.
- Iswaran, V. 1981.** A rapid titrimetric method of estimation of nitrogen in agricultural materials. *Zentralblatt für Mikrobiol.* 136(2): 176–180.
- Jackson, M. B. 1991.** Ethylene in root growth and development. In *The Plant Hormone Ethylene* (eds A.K. Mattoo & J. C. Suttle), 159–181. CRC Press, Boca Raton, FL, USA.
- Jackson, M. B. 2002.** Long-distance signalling from roots to shoots assessed: the flooding story. *J. Exp. Bot.* 53(367): 175-181.
- Jackson, M. C. 1973.** Soil Chemist Analysis. Prentice Hall Pvt. Ltd. India.
- Jain, M., Mathur, G., Koul, S. and Sarin, N. B. 2001.** Ameliorative effects of proline on salt stress induced lipid peroxidation in cell lines of groundnuts (*Arachis hypogea* L.). *Plant Cell Rep.* 20: 463–468.
- James, E. K. and Olivares, F. L. 1998.** “Infection and colonization of sugar cane and other graminaceous plants by endophytic diazotrophs, *Cri. Rev. Plant Sci.* 17(1): 77–119.

- Jaufeerally-Fakim, Y. and Dookun, A. 2000.** Extraction of high quality DNA from polysaccharides-secreting xanthomonads. *Sci. Technol. Res. J. Univer. Mauritius*. 6: 33-40.
- Jaw, N. L. and Ching, H. K. 1998.** Effect of oxidative stress caused by Hydrogen peroxide on senescence of rice leaves. *Bot. Bull Acad. Sin.* 39: 161–165.
- Jeffries, P., Gianinazzi, S., Perotto, S., Turnau, K. and Barea, J. 2003.** The contribution of arbuscular mycorrhizal fungi in sustainable maintenance of plant health and soil fertility. *Biol. Fert. Soils*. 37(1): 1-16.
- Jetiyanon, K. and Kloepper, J. W. 2002.** Mixtures of plant growth-promoting rhizobacteria for induction of systemic resistance against multiple plant diseases. *Biologic. Cont.* 24(3): 285–291.
- Jha, K. S. and Saraf, M. 2011.** Effect of Plant Growth Promoting Rhizobacteria on Seed Germination Behaviour and Seedling Vigor of *Jatropha curcas*. *Int. J. Biotech. Biosci.* 1(1): 1-10.
- Jiang, F. and Hartung, W. 2008.** Long-distance signalling of abscisic acid (ABA): The factors regulating the intensity of the ABA signal. *J. Exp. Bot.* 59(1): 37-43.
- Kadouri, D., Jurkevitch, E. and Okon, Y. 2003.** Involvement of reserve material poly- α -hydroxy butyrate (PHB) in *Azospirillum brasilense* in stress endurance and colonization. *Appl. Environ. Microbiol.* 69: 3244–3250.
- Kaushal, N., Gupta, K., Bhandhari, K., Kumar, S., Thakur, P. and Nayyar, H. 2011.** Proline induces heat tolerance in chickpea (*Cicer arietinum* L.) plants by protecting vital enzymes of carbon and antioxidative metabolism. *Physiol. Mol. Bio. Plants*. 17(3): 203-213.
- Kelley, T. G., Parthasarathy Rao, P., Weltzien, E. and Purohit, M. L. 1996.** Adoption of improved cultivars of pearl millet in an arid environment: straw yield and quality considerations in western Rajasthan. *Exp. Agric.* 32: 161-171.
- Khalid, A., Arshad, M. and Zahir, Z. A. 2004.** Screening plant growth promoting rhizobacteria for improving growth and yield of wheat. *J. Appl. Microbiol.* 96(3): 473–480.
- Khosravinejad, F., Heydari, R. and Farboodnia, T. 2008.** Antioxidant response of two barley varieties to saline stress. *Res. J. Biol. Sci.* 3(5): 486-490.
- Kiely, P. D., Haynes, J. M., Higgins, C. H., Franks, A., Mark, G. L., Morrissey, J. P. and O’Gara, F. 2006.** Exploiting new systems-based strategies to elucidate plant-bacterial interactions in the rhizosphere. *Microbial Ecol.* 51: 257–266.

- Kilian, M., Steiner, U., Krebs, B., Junge, H., Schmiedeknecht, G. and Hain, R. 2000.** FZB24 *Bacillus subtilis*-mode of action of a microbial agent enhancing plant vitality. *Pflanzenschutz Nachr Bayer*. 1: 72–93.
- Kitajima, S., Ueda, M., Sano, S., Miyake, C., Kohchi, T., Tomizawa, K., Shigeoka, S. and Yokota, A. 2002.** Stable form of ascorbate peroxidase from the red alga *Galdieria partita* similar to both chloroplastic and cytosolic isoforms of higher plants. *Biosci. Biotechnol. Biochem*. 66: 2367-2375.
- Kloepper, J. W. and Beauchamp, C. J. 1992.** A review of issues related to measuring colonization of plant roots by bacteria. *Can. J. Microbiol*. 38(12): 1219-1232.
- Kloepper, J. W. and Schroth, M. N. 1978.** Plant growth-promoting rhizobacteria on radishes. Proceedings of the 4th International Conference on Plant Pathogenic Bacteria, Vol. II, Station de Pathologie Végétale et Phyto-Bactériologie ed), pp. 879–882. Gilbert-Clarey, Tours, France.
- Kloepper, J. W., Gutierrez-Estrada, A. and McInroy, J. A. 2007.** Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. *Can. J. Microbiol*. 53(2): 159–167.
- Kloepper, J. W., Lifshitz, K. and Schroth, M. N. 1988.** *Pseudomonas* inoculants to benefit plant production. *ISI Atlas Sci. Anim. Plant Sci*. 1: 60-64.
- Kogut, M. and Russell, N. J. 1987.** Life at the limits. Considerations on how bacteria can grow at extremes of temperature and pressure, or with high concentrations of ions and solutes. *Sci. Prog*. 71: 381-399.
- Kohler, J., Hernández J. A., Caravaca, F., Roldán, A. 2008.** Plant growth promoting rhizobacteria and arbuscular mycorrhizal fungi modify alleviation biochemical mechanisms in water stressed plants. *Funct. Plant Biol*. 35: 141–151.
- Kohler, J., Hernández, J. A., Caravaca, F., and Roldán, A. 2009.** Induction of antioxidant enzymes is involved in the greater effectiveness of a PGPR versus AM fungi with respect to increasing the tolerance of lettuce to severe salt stress. *Environmental and Experimental Botany*. 65(2): 245-252.
- Kohler, J., Caravaca, F., Carrasco, L. and Roldán, A. 2006.** Contribution of *Pseudomonas mendocina* and *Glomus intraradices* to aggregate stabilization and promotion of biological fertility in rhizosphere soil of lettuce plants under field conditions. *Soil Use Manage*. 22(3): 298–304.

- Kozdroj, J., Trevors, J. T. and Van Elsas, J. D. 2004.** Influence of introduced potential biocontrol agents on maize seedling growth and bacterial community structure in the rhizosphere. *Soil Biol. Biochem.* 36: 1775–1784.
- Kucey, R. M. N. 1988.** Plant growth-altering effects of *Azospirillum brasilense* and *Bacillus* C-11–25 on two wheat cultivars. *J. Appl. Bacteriol.* 64:187–196.
- Kumar, R. R., Karajol, K. and Naik, G. R. 2011.** Effect of polyethylene glycol induced water stress on physiological and biochemical responses in pigeonpea (*Cajanus cajan* L. Millsp.). *Recent Res. Sci. Tech.* 3: 148-152.
- Liddycoat, S. M., Greenberg, B. M. and Wolyn, D. J. 2009.** The effect of plant growth-promoting rhizobacteria on asparagus seedlings and germinating seeds subjected to water stress under greenhouse conditions. *Can. J. Microbiol.* 55(4): 388–394.
- Lifshitz, R., Kloepper, J. W., Scher, F. M., Tipping, E. M. and Laliberte, M. 1986.** Nitrogen-fixing pseudomonads isolated from roots of plants grown in the Canadian high arctic. *Appl. Environ. Microbiol.* 51: 251–255.
- Liu, L., Kloepper, J. W. and Tuzun, S. 1995.** Induction of systemic resistance in cucumber against Fusarium wilt by plant growth promoting rhizobacteria. *Phytopathol.* 85: 695–698.
- Liu, Z. J., Guo, Y. K. and Bai, J. G. 2010.** Exogenous hydrogen peroxide changes antioxidant enzyme activity and protects ultrastructure in leaves of two cucumber ecotypes under osmotic stress. *J. Plant growth Regul.* 29: 171–183.
- Lobell, D., Burke, M., Tebaldi, C., Mastrandera, M., Falcon, W. and Naylor, R. 2008.** Prioritizing climate change adaptation needs for food security in 2030. *Sci.* 319: 607–610.
- Loggini, B., Scartazza, A., Brugnoli, E. and Navari-Izzo, F. 1999.** Antioxidant defense system, pigment composition and photosynthetic efficiency in two wheat cultivars subjected to drought. *Plant Physiol.* 119: 1091–1099.
- Loper, J. E. and Buyer, J. S. 1991.** “Siderophores in microbial interactions on plant surfaces,” *Mol. Plant-Microbe Interact.* 4: 5–13.
- Lopez, F., Vansuyt, G., Casse-delbart F. and Fourcroy, P. 1996.** Ascorbate peroxidase activity, not the mRNA level, is enhanced in salt-stressed *Raphanus sativus* plants. *Plant Physiol.* 97: 13–20.
- Ludwig, A. A., Saitoh, H., Felix, G., Freymark, G., Miersch, O., Wasternack, C., Boller, T., Jones, J. D. G. and Romeis, T. 2005.** Ethylene-mediated cross-talk between calcium-dependent protein kinase and MAPK signaling controls stress responses in plants. *Proceed. Nat. Acad. Sci.* 102: 10736–10741.

- Lugtenberg, B. and Kamilova, F. 2009.** Plant-growth-promoting rhizobacteria. *Annu. Rev. Microbiol.* 63: 541–556.
- Lugtenberg, B. J. J., van der Bij, A., Bloemberg, G., Woeng, T. C. A., Dekkers, L., Kravachenko, L., Mulders, I., Phoelich, C., Simons, M., Spaink, H., Tikhonovich, I., de Wager, I. and Wijffelman, C. 1996.** Molecular basis of rhizosphere colonisation by *Pseudomonas* bacteria. In *Biology of Plant–Microbe Interactions*. International Society for Molecular Plant–Microbe Interactions, St. Paul. 433–440.
- Ma, J. F. 2005.** Plant root responses to three abundant soil minerals: silicon, aluminum and iron. *Cri. Rev. Plant Sci.* 24(4): 267–281.
- Mahajan, S. and Tuteja, N. 2005.** Cold, salinity and drought stresses: An overview. *Arch. Biochem. Biophys.* 444: 139–158.
- Malhotra, M. and Srivastava, S. 2009.** Stress-responsive indole-3-acetic acid biosynthesis by *Azospirillum brasilense* SM and its ability to modulate plant growth. *Eur. J. Soil Biol.* 45: 73–80.
- Marklund, S. L. 1982.** Human copper-containing superoxide dismutase of high molecular weight. *Proc. Natl. Acad. Sci. USA* 79: 7634–7638.
- Martinez-Viveros, O., Jorquera, M. A., Crowley, D. E., Gajardo, G. and Mora, M. L. 2010.** Mechanisms and practical considerations involved in plant growth promotion by rhizobacteria. *J. Soil Sci. Plant Nutr.* 10: 293–319.
- Marulanda, A., Barea, J. M. and Azcón, R. 2006.** An indigenous drought-tolerant strain of *Glomus intraradices* associated with a native bacterium improves water transport and root development in *Retama sphaerocarpa*. *Microbiol. Ecol.* 52: 670–678.
- Marulanda, A., Jose-Miguel Barea and Azcon, R. 2009.** Stimulation of Plant Growth and Drought Tolerance by Native Microorganisms (AM Fungi and Bacteria) from Dry Environments: Mechanisms Related to Bacterial Effectiveness. *J. Plant Growth Regul.* 28: 115–124.
- Marulanda, A., Porcel, R., Barea, J. M. and Azcon, R. 2007.** Drought tolerance and antioxidant activities in lavender plants colonized by native drought tolerant or drought sensitive *Glomus* species. *Microb. Ecol.* 54(3): 543–552.
- Marulanda, A., Azcón, R., Chaumont, F., Ruiz-Lozano, J. M. and Aroca, R. 2010.** Regulation of plasma membrane aquaporins by inoculation with a *Bacillus megaterium* strain in maize (*Zea mays* L.) plants under unstressed and salt-stressed conditions. *Planta.* 232(2): 533–543.

- Mathews, M. C., Summers, C. B. and Felton, G. W. 1997.** Ascorbate peroxidase: a novel antioxidant enzyme in insects. *Arch. Insect Biochem. Physiol.* 34: 57–68.
- Mathur, S., Jajoo, A., Mehta, P. and Bharti, S. 2011.** Analysis of elevated temperature-induced inhibition of photosystem II using chlorophyll a fluorescence induction kinetics in wheat leaves (*Triticum aestivum*). *Plant Biol.* 13: 1–6.
- Mayak, S., Tirosh, T. and Glick, B. R. 2004.** Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* 42(6): 565–572.
- Mazzola, M., Cook, R. J., Thomashow, L. S., Weller, D. M. and Pierson, L. S. 1992.** Contribution of phenazine antibiotic biosynthesis to the ecological competence of fluorescent pseudomonads in soil habitats. *Appl. Environ. Microbiol.* 58: 2616–2624.
- McKersie, B. D. and Leshem, Y. Y. 1994.** Stress and stress coping in cultivated plants Kluwer Academic Publishes, London.
- Mehnaz, S. and Lazarovits, G. 2006.** Inoculation effects of *Pseudomonas putida*, *Gluconacetobacter azotocaptans*, and *Azospirillum lipoferum* on corn plant growth under greenhouse conditions. *Microb. Ecol.* 51(3): 326–335.
- Michel, B. E. 1983.** Evaluation of the water potentials of solutions of polyethylene glycol 8000 both in the absence and presence of other solutes. *Plant Physiol.* 72(1): 66–70.
- Miller, K. J. and Wood, J. M. 1996.** Osmoadaptation by rhizosphere bacteria. *Ann. Rev. Microbiol.* 50: 101–136.
- Mittler, R. 2002.** Oxidative stress, antioxidants and stress tolerance. *Trends Plant Sci.* 7: 405–10.
- Mittler, R. and Zilinskas, B. A. 1994.** Regulation of pea cytosolic ascorbate peroxidase and other antioxidant enzymes during the progression of drought stress and following recovery from drought. *The Plant J.* 5: 397–405.
- Mittler, R., Vanderauwera, S., Gollery, M. and Van Breusegem, F. 2004.** Reactive oxygen gene network of plants. *Trends Plant Sci.* 9: 490–498.
- Mittova, V., Guy, M., Tal, M. and Volokita, M. 2004a.** Salinity upregulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. *J. Exp. Bot.* 55: 1105–1113.

- Mittova, V., Theodoulou, F. L., Kiddle, G., Volokita, M., Tal, M., Foyer, C. H. and Guy, M. 2004b.** Comparison of mitochondrial ascorbate peroxidase in the cultivated tomato, *Lycopersicon esculentum*, and its wild, salt-tolerant relative, *L. pennellii* – role for matrix isoforms in protection against oxidative damage. *Plant, Cell Environ.* 27: 237–250.
- Miyake, C. and Asada, K. 1996.** Inactivation mechanism of ascorbate peroxidase at low concentrations of ascorbate; hydrogen peroxidase decomposes compound I of ascorbate peroxidase. *Plant Cell Physiol.* 37: 423–430.
- Miyake, C., Michihata, F. and Asada, K. 1991.** Scavenging of hydrogen peroxide in prokaryotic and eukaryotic algae: acquisition of ascorbate peroxidase during the evolution of cyanobacteria. *Plant Cell Physiol.* 32: 33–43.
- Moens, S. and Vanderleyden, J. 1996.** Functions of bacterial flagella. *Cri. Rev. Microbiol.* 22: 67–100.
- Mohamed, H. I. and Gomaa, E. Z. 2012.** Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescens* on growth and pigment composition of radish plants (*Raphanus sativus*) under NaCl stress. *Photosynthetica.* 50(2): 263-272.
- Mohan, M. M., Narayanan, S. L. and Ibrahim, S. M. 2000.** Chlorophyll stability index (CSI): its impact on salt tolerance in rice. *IRRN.* 25(2): 38-39.
- Moller, I. M., Jensen, P. E. and Hansson, A. 2007.** Oxidative modifications to cellular components in plants. *Annu. Rev. Plant Biol.* 58: 459-481.
- Montillet, J. L., Chamnongpol, S., Rustérucchi, C., Dat, J., van de Cotte, B., Agnel, J. P., Battesti, C., Inzé, D., Van Breusegem, F. and Triantaphylides, C. 2005.** Fatty acid hydroperoxides and H₂O₂ in the execution of hypersensitive cell death in tobacco leaves. *Plant Physiol.* 138: 1516-1526.
- Moslemi, Z., habibi, D., Asgharezadeh, A., Ardakani, M. R., Mohammadi, A. and Mohammadi, M. 2011.** Response of phytohormones and biochemical markers of maize to super absorbent polymer and plant growth promoting rhizobacteria under drought stress. *American Eurasian J. Agric. Environ. Sci.* 10(5): 787-796.
- Muller, K., Carsten, A. C., Linkies, A., Torres, M. A. and Leubner-Metzger, G. 2009a.** The NADPH-oxidase AtrbohB plays a role in Arabidopsis seed after-ripening. *New Phytologist.* 184: 885–897.
- Muller, K., Linkies, A., Vreeburg, R. A. M., Fry, S. C., Krieger-Liszkay, A. and Leubner-Metzger, G. 2009b.** In vivo cell wall loosening by hydroxyl radicals during cress seed germination and elongation growth. *Plant Physiol.* 150: 1855–1865.

- Munns, R. 2002.** Comparative physiology of salt and water stress. *Plant Cell Environ.* 25(2): 239–250.
- Muyzer, G., Teske, A., Wirsén, C. O. and Jannasch, H. W. 1995.** Phylogenetic relationships of *Thiomicrospira* species and their identification in deep-sea hydrothermal vent samples by denaturing gradient gel electrophoresis of 16S rDNA fragments. *Arch. Microbiol.* 164(3): 165-172.
- Nakano, Y. and Asada, K. 1981.** Hydrogen peroxide is scavenged by ascorbate-specific peroxidases in spinach chloroplasts. *Plant Cell Physiol.* 22: 867-880.
- National Research council. 1996.** Lost crops of Africa. Volume:1. Grains. National Academy Press. Washington, D.C.
- Navrot, N., Rouhier, N., Gelhaye, E. and Jaquot, J. P. 2007.** Reactive oxygen species generation and antioxidant systems in plant mitochondria, *Physiol. Plant.* 129: 185-195.
- Nehl, D. B., Allen, S. J., and Brown, J. F. 1997.** Deleterious rhizosphere bacteria: an integrating perspective. *Applied Soil Ecology.* 5(1): 1-20.
- Neilands, J. B. 1981.** “Iron absorption and transport in microorganisms,” *Annu. Rev. Nutr.* 1: 27–46.
- NIDM. 2009. Manual for drought management,** Ministry for agriculture and cooperation-Ministry for agriculture- Government of India. 1- 202.
- Niyogi, K. K., Shih, C., Chow, W. S., Pogson, B. J., DellaPenna, D. and Bjorkman, O. 2001.** Photoprotection in a zeaxanthin-and lutein-deficient double mutant of *Arabidopsis*. *Photosynthetic Res.* 67: 139-145.
- Noctor, G. and Foyer, C. H. 1998.** Ascorbate and glutathione: keeping active oxygen under control. *Plant Mol. Biol.* 49: 249-279.
- Noctor, G. and Foyer, C. H. 1998a.** A re-evaluation of the ATP: NADPH budget during C₃ photosynthesis. A contribution from nitrate assimilation and its associated respiratory activity? *J. Exp. Bot.* 49: 1895-1908.
- Noctor, G., Veljovic-Jovanovic, S., Driscoll, S., Novitskaya, L. and Foyer, C. H. 2002.** Drought and Oxidative Load in the Leaves of C₃ Plants: a Predominant Role for Photorespiration. *Ann. Bot.* 89: 841-850.
- Oades, J. M. 1993.** The role of biology in the formation, stabilization and degradation of soil structure. *Geoderma.* 56: 182–186.

- Olsen, S. R., Cole, C. V., Watanabe, F. S. and Dean, L. A. 1954.** Estimation of available phosphorus in soil by extraction with NaHCO_3 , U.S.D.A. Ciraza (Quoted from, Method of soil Analysis, C.A. Black 2nd ed.) 1165 Am. Soc. Agron., Inc. Medison Wisconsin, USA. (1035).
- Panchal, H. and Ingle, S. 2011.** Isolation and Characterization of Endophytes From the Root of Medicinal Plant *Chlorophytum borivillianum* (Safed musli). *J. Adv. in Develop. Res.* 2 (2) 205-209.
- Panda, S. K. 2001.** Oxidative response of green gram seeds under salinity stress. *Indian journal of plant physiology.* 6(4): 43.
- Panda, S. K. and Upadhyay, R. K. 2003.** Salt stress injury induces oxidative alterations and antioxidative defence in the roots of. *Lemna minor.* *Biol. Plant.* 48: 249-253.
- Pastori, G. M. and Foyer, C. H. 2001.** Identifying Oxidative Stress Responsive Genes by Transposon Tagging. *J. Exp. Bot.*, 52 (Special Issue).
- Patten, C. L. and Glick, B. R. 1996.** Bacterial biosynthesis of indole3-acetic acid. *Can. J. Microbiol.* 42: 207–220.
- Peck, S. C. 2003.** Early phosphorylation events in biotic stress. *Curr. Opin. Plant Biol.* 6: 334–338.
- Peng, C. L., Ou, Z. Y., Liu, N. and Lin, G. Z. 2005.** Response to high temperature in flag leaves of super high-yielding rice Peiai 64S/E32 and Liangyoupeijiu. *Rice Sci.* 12: 179-186.
- Persello-cartieaux, F., Nussaume, L. and Robaglia, C. 2003.** Tales from the underground: molecular plant–rhizobacteria interactions. *Plant, Cell Environ.* 26:189–199.
- Pikovskaya, R. I. 1948.** Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiologiya.* 17: 362–370.
- Potters, G., Pasternak, T. P., Guisez, Y., Palme, K. J. and Jansen, M. A. K. 2007.** Stress-induced morphogenic responses: growing out of trouble? *Trends Plant Sci.* 12: 98–105.
- Potts, M. 1994.** Desiccation tolerance of prokaryotes. *Microbiol. Rev.* 58: 755–805.
- Preston, G. M. 2004.** Plant perceptions of plant growth-promoting *Pseudomonas*. *Phil. Trans. R. Soc. Lond.* 359(1446): 907-918.

- Preston, G. M., Haubold B. and Rainey, P. B. 1998.** Bacterial genomics and adaptation to life on plants: implications for the evolution of pathogenicity and symbiosis. *Cur. Opin. Microbiol.* 1: 589–597.
- Prousek, J. 2007.** Fenton chemistry in biology and medicine. *Pure Appl. Chemist.* 79(12): 2325-2338
- Quan, L. J., Zhang, B., Shi, W. W. and Li, H. Y. 2008.** Hydrogen peroxide in plants: a versatile molecule of the reactive oxygen species network. *J. Integ. Plant Biol.* 50: 2-18.
- Ra`sa`nen, L. A., Saijets, S., Jokinen, K. and Lind-stro`m, K. 2004.** Evaluation of the roles of two compatible solutes, glycine betaine and trehalose, for the *Acacia senegal*_ *Sinorhizobium* symbiosis exposed to drought stress. *Plant Soil.* 260:237-251.
- Raj, N. S., Sarosh, B. R., Shetty, N. P., Shetty, H. S. and Reddy, M. S. 2003.** Plausible biochemical and molecular mechanisms involved in plant growth promoting rhizobacteria mediated resistance induction against pearl millet downy mildew disease. In: Proceedings of the 6 th International workshop on Plant growth promoting rhizobacteria, Indian Institute of Species Research, Calicut, India, 520-529.
- Rajalingam, D., Loftis, C., Xu, J. J. and Kumar. T. K. S. 2009.** Trichloroacetic acid-induced protein precipitation involves the reversible association of a stable partially structured intermediate. *Prot. Sci.* 18: 980-993.
- Rao, M. V., Paliyath, G. and Ormrod, D. P. 1996.** Ultraviolet-B- and ozone induced biochemical changes in antioxidant enzymes of *Arabidopsis thaliana*. *Plant Physiol.* 110: 125–136.
- Richardson, A. E., Barea, J. M., McNeill, A. M. and Prigent-Combaret, C. 2009.** Acquisition of phosphorus and nitrogen in the rhizosphere and plant growth promotion by microorganisms. *Plant Soil.* 321: 305–339.
- Roberson E, B., and Firestone, M. K. 1992.** Relationship between Desiccation and Exopolysaccharide Production in a Soil *Pseudomonas* sp. *Appl. Environ. Microbiol.* 58(4): 1284-1289.
- Robin, A., Vansuyt, G., Hinsinger, P., Meyer, J. M., Briat, J. F. and Lemanceau, P. 2008.** “Iron dynamics in the rhizosphere. Consequences for plant health and nutrition,” *Adv. Agronom.* 99: 183-225.
- Rodríguez, M., Canales, E. and Orlando, B. H. 2005.** Molecular aspects of abiotic stress in plants. *Biotechnología Aplicada.* 22: 1-10.

- Rubinstein, S. M., Kolodkin-Gal, I., Mcloon, A., Chai, L., Kolter, R., Losick, R. and David, A. 2012.** Weitz1Osmotic pressure can regulate matrix gene expression in *Bacillus subtilis*. *Mol. Microbiol.* doi:10.1111/j.1365-2958.2012.08201.x.
- Rui'z-Lozano, J. M. and Azco'n, R. 2000.** Symbiotic efficiency and infectivity of an autochthonous arbuscular mycorrhizal *Glomus* sp. from saline soils and *Glomus deserticola* under salinity. *Mycorrhiza*. 10: 137–143.
- Ryan, P. R., Dessaux, Y., Thomashow, L. S. and Weller, D. M. 2009.** Rhizosphere engineering and management for sustainable agriculture. *Plant Soil*. 321: 363–383.
- Ryu, C., Hu, C., Locy, R. D. and Kloepper, J. W. 2005.** Study of mechanisms for plant growth promotion elicited by rhizobacteria in *Arabidopsis thaliana*. *Plant Soil*. 268(1): 285-292.
- Sadasivam, S., Manickam, A. 1992.** Biochemical Methods for Agricultural Sciences. Wiley Eastern Limited, New Delhi, India.
- Saikkonen, K., Faeth, S. H., Helander, M. and Sullivan, T. J. 1998.** Fungal endophytes: a continuum of interactions with host plants. *Annu. Rev. Ecol. Syst.* 29:319–343.
- Sairam, R. K. and Srivastava, G. C. 2000.** Induction of oxidative stress and antioxidant activity by hydrogen peroxide treatment in tolerant and susceptible genotype. *Biol. Plant*. 43(3): 381–386.
- Saleem, M., Arshad, M., Hussain, S. and Bhatti, A. S. 2007.** Perspective of plant growth promoting rhizobacteria (PGPR) containing ACC deaminase in stress agriculture. *J. Ind. Microbiol. Biotechnol.* 34: 635–648.
- Saleena, L. M., Rangarajan, S. and Nair, S. 2002.** Diversity of *Azospirillum* strains isolated from rice plants grown in saline and non saline coastal agricultural ecosystems. *Microbial Ecol.* 44(3): 271–277.
- Sandhya, V., Ali, S. K. Z., Grover, M., Reddy, G. and Venkateswarlu, B. 2009.** Alleviation of drought stress effects in sunflower seedlings by the exopolysaccharides producing *Pseudomonas putida* strain GAP-P45. *Biol. Fert. Soils*. 46(1): 17-26.
- Saravanakumar, D., Kavino, M., Raguchander, T., Subbian, P., and Samiyappan, R. 2011.** Plant growth promoting bacteria enhance water stress resistance in green gram plants. *Acta Physiologiae Plantarum*. 33(1): 203-209.

- Saravanakumar, D., Lavanya, N., Muthumeena, K., Raguchander, T. and Samiyappan. R. 2009.** Fluorescent pseudomonad mixtures mediate disease resistance in rice plants against sheath rot (*Sarocladium oryzae*) disease. *Bio. Control.* 54(2): 273-286.
- Saxena, S. C., Joshi, P. K., Grimm, B. and Arora, S. 2011.** Alleviation of ultraviolet-C-induced oxidative damage through overexpression of cytosolic ascorbate peroxidase. *Biologia.* 66(6): 1052-1059.
- Sayed, A. F. M. 1999.** Alternative dietary protein sources for farmed tilapia, *Oreochromis* spp. *Aquacul.* 179(1-4): 149-168.
- Scandalias, J. G. 1990.** Response of plant antioxidant defense genes to environmental stress. *Adv. Genet.* 28: 1-41.
- Schellenbaum, L., Müller, J., Boller, T., Wienken, A. and Schüepp, H. 1998.** Effects of drought on non-mycorrhizal and mycorrhizal maize: changes in the pools of non-structural carbohydrates, in the activities of invertase and trehalose, and in the pools of amino acids and imino acids. *New Phytol.* 138: 59-66.
- Schulze, E. D. 1986.** Carbon dioxide and water vapour exchange in response to drought in the atmosphere and in the soil. *Ann. Rev. Plant Physiol.* 37: 247-274.
- Schwyn, B. and Neilands, J. B. 1987.** Universal chemical assay for the detection and determination of siderophores. *Anal. biochem.* 160(1): 47-56.
- Sekar, S. and Kandavel, D. 2010.** Interaction of plant growth promoting rhizobacteria (PGPR) and endophytes with medicinal plants— new avenues for phytochemicals. *J. Phytol.* 2: 91-100.
- Selote, D. S. and Chopra R. K. 2010.** Antioxidant response of wheat roots to drought acclimation. *Protoplasma.* 245: 153-163.
- Selvakumar, G., Joshi, P., Mishra, P. K., Bisht, J. P. and Gupta, H. S. 2009.** Mountain aspects influence the genetic clustering of psychrotolerant phosphate solubilizing Pseudomonads in the Uttarkhand Himalayas. *Curr. Microbiol.* 59: 432-438.
- Sgherri, C. L. M., Maffei, M. and Navari-Izzo, F. 2000.** Antioxidative enzymes in wheat subjected to increasing water deficit and rewatering. *J. Plant Physiol.* 157: 273-279.
- Sharma, A., Johri, B. N., Sharma, A. K. and Glick, B. R. 2003.** “Plant growth-promoting bacterium *Pseudomonas* sp. strain GRP3 influences iron acquisition in mung bean (*Vigna radiata* L. Wilzeck),” *Soil Biol. Biochem.* 35(7): 887-894.

- Sharma, H. K., Daiya, K. S. and Chawan, D. D. 1980.** Effect of different soil types on plant growth, leaf pigments and sennoside content in *Cassia* species. *Pharmaceutisch Weekblad*. 2(1): 573-575.
- Sharma, P. and Dubey, R. S. 2005.** Modulation of nitrate reductase activity in rice seedlings under aluminium toxicity and water stress: role of osmolytes as enzyme protectant. *J. Plant Physiol.* 162: 854-864.
- Shi, W. M., Muramoto, Y., Ueda, A. and Takabe, T. 2001.** Cloning of peroxisomal ascorbate peroxidase gene from barley and enhanced thermotolerance by overexpressing in *Arabidopsis thaliana*. *Gene*. 273: 23-27.
- Shigeoka, S., Nakano, Y. and Kitaoka, S. 1980.** Metabolism of hydrogen peroxide in *Euglena gracilis* Z by l-ascorbic acid peroxidase. *Biochem. J.* 186: 377-380.
- Shiklomanov, I. A. 1993.** World freshwater resources. In P. H. Gleick (ed.) *Water in Crisis: A Guide to the World's Freshwater Resources*. New York, Oxford University Press Shiklomanov, I.A. (1999). Paris, UNESCO.
- Shringarpure, R. and Davies, K. J. 2002.** Protein turnover by the proteasome in aging and disease. *Free Radic. Biol. Med.* 32: 1084-1089.
- Sieferman-Harms, D. 1987.** The light harvesting function of carotenoids in photosynthetic membrane. *Plant Physiol.* 69: 561-568.
- Simons, M., Permentier, H. P., de Weger, L. A., Wijffelman, C. A. and Lugtenberg, B. J. J. 1997.** Amino acid synthesis is necessary for tomato root colonisation by *Pseudomonas fluorescens* strain WCS365. *Mol. Plant-Microbe Interact.* 10: 102-106.
- Simons, M., vander Bij, A. J., Brand, I., de Weger, L. A., Wijffelman, C. A. and Lugtenberg, B. J. J. 1996.** Gnotobiotic system for studying rhizosphere colonisation by plant growth-promoting *Pseudomonas* bacteria. *Mol. Plant-Microbe Interact.* 9: 600-607.
- Smirnoff, N. 1993.** The role of active oxygen in the response of plants to water deficit and desiccation. *New Phytol.* 125: 27-58.
- Smirnoff, N. and Cumbes, Q. J. 1989.** Hydroxyl radical scavenging activity of compatible solutes. *Phytochem.* 28: 1057-1060.
- Smith, K. P. and Goodman, R. M. 1999.** Host variation for interaction with beneficial plant-associated microbes. *Ann. Rev. Phytopathol.* 37: 473-491.
- Smith, S. E., and Read, D. J. 1998.** Mycorrhizal symbiosis. *Plant Growth Regul.* 25: 1-71.

- Srivastava, S., Yadav, A., Seem, K., Mishra, S., Chaudhary, V. and Srivastava, C. S. 2008.** Effect of high temperature on *Pseudomonas putida* NBRI0987 biofilm formation and expression of stress sigma factor RpoS. *Curr. Microbiol.* 56(4): 453–457.
- Stajner, D., Kevresan, S., Gasic, O., Mimica-Dukic, N. and Zongli, H. 1997.** Nitrogen and *Azotobacter chroococcum* enhance oxidative stress tolerance in sugar beet. *Biol. Plantarum.* 39(3): 441–445.
- Stone, J. K., Bacon, C. W. and White, J. F. 2000.** An overview of endophytic microbes: endophytism defined. *Microbial Endophytes* (Bacon CW & White JF, eds), 3–29. Marcel Dekker Inc., New York.
- Strzelczyk, E. and Pokozska-Burdziej, A. 1984.** Production of auxin and gibberellins-like substances by mycorrhizal fungi, bacteria and actinomycetes isolated from soil and mycorrhizosperme of pine (*Pinus silvestris* L.). *Plant Soil.* 81: 185-194.
- Sturz, A. V. and Nowak, J. 2000.** Endophytic communities of rhizobacteria and the strategies required to create yield enhancing associations with crops. *J. Appl. Soil Ecol.* 15: 183–190.
- Sturz, A. V., Christie, B. R., Matheson, B. G. and Nowak, J. 1997.** Biodiversity of endophytic bacteria which colonize red clover nodules, roots, stems and foliage and their influence on host growth. *Biol Fertil Soils* 25:13–19.
- Suárez, R., Wong, A., Ramírez, M., Barraza, A., del Carmen Orozco, M., Miguel, A. Cevallos, Lara, M., Hernández, G. and Iturriaga, G. 2008.** Improvement of drought tolerance and grain yield in common bean by overexpressing trehalose-6-phosphate Synthase in Rhizobia. *Mol. Plant Microbe Interact.* 21(7): 958-966.
- Subbiah, B. V. and Asija, G. L. 1956.** A rapid procedure for the estimation of available nitrogen in soils. *Curr. Sci.* 25(8): 259-260.
- Subramanian, K. S. and Charest, C. 1998.** Arbuscular mycorrhizae and nitrogen assimilation in maize after drought and recovery. *Physiol. Plant.* 102: 285–296.
- Sudhakar, P., Chattopadhyay, G. N., Gangwar, S. K. and Ghosh, J. K. 2000.** Effect of foliar application of *Azotobacter*, *Azospirillum* and *Beijerinckia* on leaf yield and quality of mulberry (*Morus alba*). *J. Agric. Sci.* 134: 227–234.
- Sun, L., Qiu, F., Zhang, X., Dai, X., Dong, X. and Song, W. 2008.** Endophytic Bacterial Diversity in Rice (*Oryza sativa* L.) Roots Estimated by 16S rDNA Sequence Analysis. *Microbial Ecol.* 55: 415–424.

- Swain, M. R., Naskar, S. K. and Ray, R. C. 2007.** Indole-3-acetic acid production and effect on sprouting of yam (*Dioscorea rotundata* L.) minisetts by *Bacillus subtilis* isolated from culturable cowdung microflora. *Pol. J. Microbiol.* 56: 103–110.
- Sylvia, D. M., Fuhrmann, J. J., Hartel, P. G. and Zuberer, D. A. 1998.** Principles and applications of soil microbiology. Prentice-Hall, Inc. Upper Saddle River, NJ. 550 pp.
- Tanoua, G., Molassiotis, A. and Diamantidis, G. 2009.** Hydrogen peroxide-and nitric oxide-induced systemic antioxidant prime-like activity under NaCl-stress and stress-free conditions in citrus plants. *J. Plant Physiol.* 166: 1904-1913.
- Tartoura, K. A. H. 2010.** Alleviation of oxidative-stress induced by drought through application of compost in wheat (*Triticum aestivum* L.) Plants. *American-Eurasian J. Agric. Environ. Sci.* 9(2): 208-216.
- Tel-Or, E., Huflejt, M. and Packer, L. 1986.** Hydroperoxide metabolism in cyanobacteria. *Arch. Biochem. Biophys.* 246: 396–402.
- Teulat, B., Zoumarou-Wallis, N., Rotter, B., Ben Salem, M., Bahri, H. and This, D. 2003.** QTL for relative water content in field-grown barley and their stability across Mediterranean environments. *Theoret. Appl. Genetic.* 108(1): 181-188.
- The world at six billion, 2009.**
- Tilak, K. V. B. R., Ranganayaki, N., Pal, K. K., De, R., Saxena, A. K., Nautiyal, C. S., Mittal, S., Tripathi, A. K. and Johri, B. N. 2005.** Diversity of plant growth and soil health supporting bacteria. *Curr. Sci.* 89(1): 135–150.
- Timmusk, S. 2003.** Mechanism of action of the plant growth promoting Bacterium *Paenibacillus polymyxa*. Comprehensive Summaries of Uppsala Dissertations from the Faculty of Science and Technology 908. Acta Univ Upsaliensis Uppsala, Sweden, pp 40.
- Timmusk, S. and Wagner, E. G. H. 1999.** The plant growth-promoting rhizobacterium *Paenibacillus polymyxa* induces changes in *Arabidopsis* thaliana gene expression: a possible connection between biotic and abiotic stress responses. *Mol. Plant-Microb Inter.* 12: 951–959.
- Tisdall, J. M. 1994.** Possible role of soil microorganisms in aggregation in soils. *Plant Soil.* 159(1): 115-121.
- Tisdall, J. M. and Oadea, J. M. 1982.** Organic matter and water stable aggregates in soils. *J. Soil Sci.* 33: 141–163.

- Tripathi, A. K., Mishra, B. M. and Tripathi, P. 1998.** Salinity stress responses in plant growth promoting rhizobacteria. *J. Biosci.* 23(4): 463–471.
- Trovato, M., Mattioli, R. and Costantino, P. 2008.** Multiple roles of proline in plant stress tolerance and development. *Rendiconti Lincei.* 19: 325-346.
- Tsan, M. F. 1997.** Superoxide dismutase and pulmonary oxygen toxicity. *Proc. Soc. Exp. Biol. Med.* 214: 107-113.
- Tuteja, N., Ahmad, P., Panda, B. B. and Tuteja, R. 2009.** Genotoxic stress in plants: Shedding light on DNA damage, repair and DNA repair helicases. *Mutat. Res.* 681: 134-149.
- United Nations, 2009.** World Population Prospects: The 2008 Revision, Highlights, working paper number ESA/P/WP.201.
- Upadhyay, S. K., Singh, D. P. and Saikia, R. 2009.** Genetic diversity of plant growth promoting rhizobacteria from rhizospheric soil of wheat under saline conditions. *Curr. Microbiol.* 59(5): 489–496.
- Urbanek, H., Kuzniak-Gebarowska, E. and Herka, K. 1991.** Elicitation of defence responses in bean leaves by *Botrytis cinerea* polygalacturonase. *Acta. Phys. Plant.* 13: 43-50.
- Van Loon, L. C. and Bakker, P. A. H. M. 2003.** Signalling in rhizobacteria-plant interactions. In H. De Kroon and E. J. W. Visser (Eds.), Root ecology, (Ecological studies 168:297–330). Berlin, Heidelberg: Springer-Verlag.
- Vansuyt, G., Robin, A., Briat, J. F., Curie, C. and Lemanceau, P. 2007.** “Iron acquisition from Fe-pyoverdine by *Arabidopsis thaliana*,” *Mol. Plant-Microbe Interact.* 20(4): 441-447.
- Vardharajula, S., Ali, S. Z., Grover, M., Reddy, G. and Bandi, V. 2011.** Drought-tolerant plant growth promoting *Bacillus* spp.: effect on growth, osmolytes, and antioxidant status of maize under drought stress. *J. Plant Interact.* 6(1): 1-14.
- Venkateswarlu, B., Desai, S. and Prasad, Y. G. 2008.** Agriculturally important microorganisms for stressed ecosystems: Challenges in technology development and application’. In: Khachatourians GG, Arora DK, Rajendran TP, Srivastava AK (eds) *Agriculturally important Microorganisms*, vol 1. Academic World, Bhopal, pp 225–246
- Verma, J. P., Yadav, J., Tiwari, K. N., Lavakush and Singh, V. 2010.** Impact of plant growth promoting rhizobacteria on crop production. *Int. J. Agric. Res.* 5: 954–983.

- Veslues, P. E., Ober, E. S. and Sharp, R. E. 1998.** Root growth and oxygen relations at low water potentials. Impact of oxygen availability in polyethylene glycol solutions. *Plant Physiol.* 116: 1403-1412.
- Vessey, J. K. 2003.** Plant growth promoting rhizobacteria as biofertilizers. *Plant Soil* 255:571–586.
- Vranova, E., Atichartpongkul, S., Villarroel, R., Montagu, M. V., Inze, D. and Camp, W. V. 2002.** Comprehensive analysis of gene expression in *Nicotiana tabacum* leaves acclimated to oxidative stress. *Proc. Natl. Acad. Sci. U.S.A.* 99: 870-875.
- Vrbničanin, S., Božić, D., Sarić, M., Pavlović, D. and Raičević, V. 2011.** Effect of Plant Growth Promoting Rhizobacteria on *Ambrosia artemisiifolia* L. Seed Germination. *Pestic. Phytomed.* 26(2): 141–146.
- Wada, N., Kinoshita, S., Matsuo, M., Amako, K., Miyake, C. and Asada, K. 1998.** Purification and molecular properties of ascorbate peroxidase from bovine eye. *Biochem. Biophysic. Res. Commun.* 242: 256–261.
- Wahid, A. and Close, T. J. 2007.** Expression of dehydrins under heat stress and their relationship with water relations of sugarcane leaves. *Biol. Plant.* 51: 104–109.
- Wang, C. Q. and Li, R. C. 2008.** Enhancement of superoxide dismutase activity in the leaves of white clover (*Trifolium repens* L.) in response to polyethylene glycol-induced water stress. *Acta. Physiol. Plant.* 30: 841-847.
- Wang, L., Zhou, Q., Ding, L. and Sun, Y. 2008.** Effect of cadmium toxicity on nitrogen metabolism in leaves of *Solanum nigrum* L. as a newly found cadmium hyperaccumulator. *J. Hazardous Metals.* 154: 818-825.
- Wang, Y. J., Wang, H. M., Yang, C. H., Wang, Q. and Mei, R. H. 2007.** Two distinct manganese-containing superoxide dismutase genes in *Bacillus cereus*: their physiological characterizations and roles in surviving in wheat rhizosphere. *FEMS Microbiol. Lett.* 272: 206–213.
- Wang, Z. and Haung, B. 2004.** Physiological recovery of Kentucky bluegrass from simultaneous drought and Heat Stress. *Crop sci.* 44: 1729-1736.
- Wanichananan, P., Kirdmanee, C. and Vutiyanano, C. 2003.** Effect of salinity on biochemical and physiological characteristics in correlation to selection of salttolerance in aromatic Rice (*Oryza sativa* L.). *Sci. Asia.* 29: 333-339.

- Weisany, W., Sohrabi, Y., Heidari, G., Siosemardeh, A., and Ghassemi-Golezani, K. 2012.** Changes in antioxidant enzymes activity and plant performance by salinity stress and zinc application in soybean (*Glycine max* L.). *Plant Omics*. 5(2): 60.
- Whipps, J. M. 2001.** Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* 52:4 87–511.
- Wilkinson, J. F. 1958.** The extracellular polysaccharides of bacteria. *Bacteriol. Rev.* 22: 46–73.
- Willekens, H., Chamnongpol, S., Davey, M., Schraudner, M., Langebartels, C., Van Montagu, M., Inzé, D. and Van Camp, W. 1997.** Catalase is a sink for H₂O₂ and is indispensable for stress defence in C₃ plants. *EMBO J.* 16: 4806–4816.
- Willekens, H., Inze, D., Van Montagou, M. and Van Camp, W. 1995..** Catalases in plants. *Mol. Breeding*. 1: 207–228.
- Wittenmayer, L. and Merbach, W. 2005.** Plant responses to drought and phosphorus deficiency: contribution of phytohormones in root-related processes. *J. Plant Nut. Soil Sci.* 168(4): 531–540.
- Wolfaardt, G. M., Lawrence, J. R. and Korbe, D. R. 1999.** “Function of EPS,” in *Microbial Extracellular Polymeric Substances: Characterization, Structure and Function*, J. Wingender, T. R. Neu, and H.-C. Flemming, Eds., pp. 171–200, Springer, New York, NY, USA.
- Wu, Q. S. and Xia, R. X. 2005.** Effects of AM fungi on drought tolerance of citrus grafting seedling trifoliolate orange/cara cara. *Ying Yong Shen Tai Xue Bao*. 16(5): 865–869.
- Wu, Q. S. and Xia, R. X. 2006.** Arbuscular mycorrhizal fungi influence growth, osmotic adjustment and photosynthesis of citrus under well-watered and water stress conditions. *J. Plant Physiol.* 163(4):417–425.
- Yadav, D. K., Islam, S. S., and Tuteja, N. 2012.** Rice heterotrimeric G-protein Gamma subunits (RGG1 and RGG2) are differentially regulated under abiotic stress. *Plant Signaling & Behavior*. 7(7): 733–740.
- Yadav, R. S., Sharma, R. K. and Yadav, R. M. 2004.** Effect of simulated drought on free proline accumulation in some wheat (*Triticum aestivum* L.) genotypes. *Ind. J. Agric. Biochem.* 17: 43–44.

- Yamaguchi-Shinozaki, K. and Shinozaki, K. 2005.** Organization of cis-acting regulatory elements in osmotic- and cold-stress-responsive promoters. *Trends Plant Sci.* 10: 88–94.
- Yamaguchi-Shinozaki, K. and Shinozaki, K. 2006.** Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu. Rev. Plant Biol.* 57: 781–803.
- Yang, J., Kloepper, J. W. and Ryu, C. M. 2009.** Rhizosphere bacteria help plants tolerate abiotic stress. *Trends Plant Sci.* 14: 1-4.
- Yusuf, M. A., Kumar, D., Rajwanshi, R., Strasser, R. J., Tsimilli-Michael, M., Govindjee Sarin, N. B. 2010.** Overexpression of c-tocopherol methyl transferase gene in transgenic Brassica juncea plants alleviates abiotic stress: physiological and chlorophyll a fluorescence measurements. *Biochim. Biophys. Acta.* 1797: 1428–1438.
- Zahir, Z. A., Munir, A., Asghar, H. N., Arshad, M. and Shaharoon, B. 2008.** Effectiveness of rhizobacteria containing ACC-deaminase for growth promotion of peas (*Pisum sativum*) under drought conditions. *J. Microbiol. Biotech.* 18(5): 958-963.
- Zahran, H. H. 1999.** Rhizobium-Legume symbiosis and nitrogen fixation under severe conditions and in an arid climate. *Microbiol. Mole. Biol. Rev.* 3(4): 968–989.
- Zgallai, H., Steppe, K. and Lemeur, R. 2005.** Photosynthetic, physiological and biochemical responses of tomato plants to polyethylene glycol- induced water deficit. *J. Integr. Plant Biol.* 47(12): 1470-1478.
- Zhang, F., Dasthi, N., Hynes, R. K. and Smith, D. L. 1996.** Plant growth promoting rhizobacteria and Soybean [*Glycine max* (L.) Merr.] nodulation and nitrogen fixation at suboptimal root zone temperatures. *Ann. Bot.* 77: 453–459.
- Zhang, J., Sauter, A. and Hose, E. 2002.** Abscissic acid in xylem: Where does it come from, where does it go? *J. Exp. Bot.* 53: 27-32.
- Zlatev, Z. S., Lidon, F. C., Ramalho, J. C. and Yordanov, I. T. 2006.** Comparison of resistance to drought of three bean cultivars. *Biol. Plant.* 50: 389-394.



Appendices

Appendix-I

Media Composition	gL ⁻¹
Nutrient agar	
Peptone	3.0
Beef extract	5.0
Agar	20
Sodium succinate medium	
Sodium Succinate	5.0
Sodium glutamate	1.0
K ₂ HPO ₄	0.5
MgSO ₄ .7H ₂ O	0.2
(NH ₄) ₂ SO ₄	0.4
Agar-agar	15.0
pH	7.0 ± 0.2
Chrome Azurol's agar medium	
a) Dye solution	
Chromeazurol's dye	60.50 mg
Distilled water	50.0 ml
b) 1mM FeCl ₃ .6H ₂ O in 10mM HCl	10.0 ml
c) Hexadecyl trimethyl ammonium bromide	72.90 mg
Distilled water	40.0 ml
Solution (a) was added to solution (b) then solution (c) added in resultant. Whole solution was made upto 100 ml. Resultant dark solution was autoclaved and added to King's B agar medium before pouring.	
pH	6.8 ± 0.2

Pikovaskaya's Agar

Yeast extract	0.5
Dextrose	10
CaPO ₄	5.0
(NH ₄) ₂ SO ₄	0.5
KCl	0.2
MgSO ₄ .7H ₂ O	0.1
MnSO ₄	0.0001
FeSO ₄	0.001
Agar	20

Reagents**TRIS –EDTA (TE) Buffer**

Tris HCl	10 mM
EDTA	1 mM
pH	8

Sodium Dodecyl Sulphate 10 %(w/v)

Proteinase K 20 mg ml⁻¹ (w/v)

CTAB 10 % (w/v)

Phenol: Chloroform 1:1(v/v)

Chloroform: Isoamyl alcohol 24:1(v/v)

RNAase 10 mg ml¹

Chilled ethanol (absolute) 70%

TRIS Acetate EDTA (TAE)

TRIS Acetate 40mM

Na₂ EDTA 1mM

Loading dye (6X)

Sucrose	40% (w/v)
Bromophenol blue	0.25% (w/v)
Xylene Cyanol FF	0.25% (w/v)

Ethyedim Bromide

Stock	10.00mg ml ⁻¹ (w/v)
Working Solution	0.5µg ml ⁻¹

10% Trichloroacetic Acid (TCA)

Dissolve 10g TCA in 80 ml of distilled water and make up the final volume to 100 ml. Store in dark colored bottle.

0.25% Thiobabituric acid (TBA)

0.25g of TBA is dissolved in 80 ml of TCA and volume is made to 100ml.

Potassium phosphate buffer (0.1 M, pH 7.4-7.6)

Add 40 ml of 0.2M KOH to 50 ml of 0.2 M KH₂PO₄ and adjust the pH to 7.4-7.6 by adding 1 M KOH, (if necessary). Make up the final volume to 100 ml with distilled water.

EDTA solution (0.1 M)

Add 3.724g EDTA (disodium salt) in 60 ml of distilled water and adjust the pH to 8 (EDTA will not dissolve until the pH is adjusted to 8.0). Make the final volume 100 ml.

Potassium phosphate buffer (0.1 M, pH 7.0)

Add 30 ml. of 0.2 mol/L KOH to 50 ml of 0.2 M KH₂PO₄ and add distilled water (adjust the pH to 7.0 by adding 1 M KOH, if necessary). Make the final volume to 100 ml with distilled water.

85%, 50 ml orthophosphoric acid (OPA)

Dissolve 48.29 ml OPA (of available purity 88%) in 1.71 ml of distilled water to have a final volume of 50 ml.

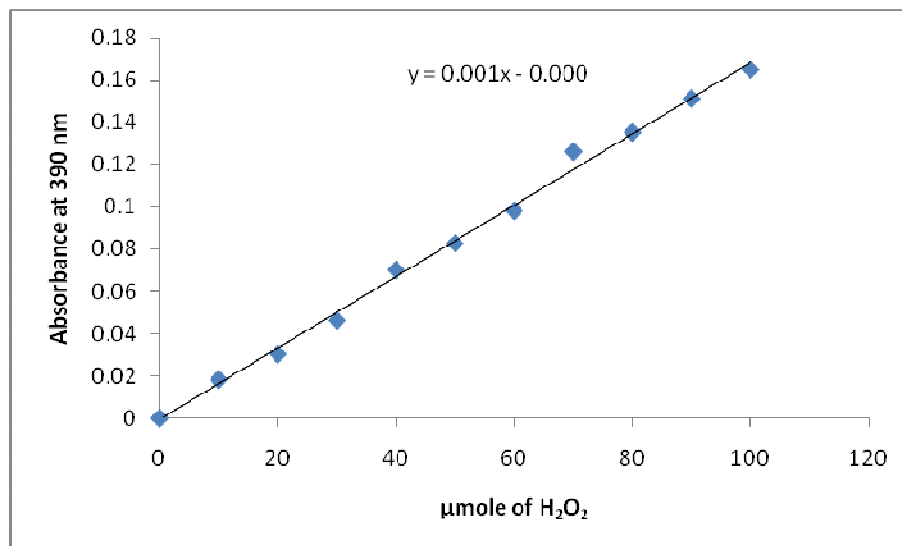
H₂O₂ stock For APX and GPX activity measurement (0.5 M)

515 µl of 9.7 M H₂O₂ (Available in lab) is diluted in 10 ml of distilled water.

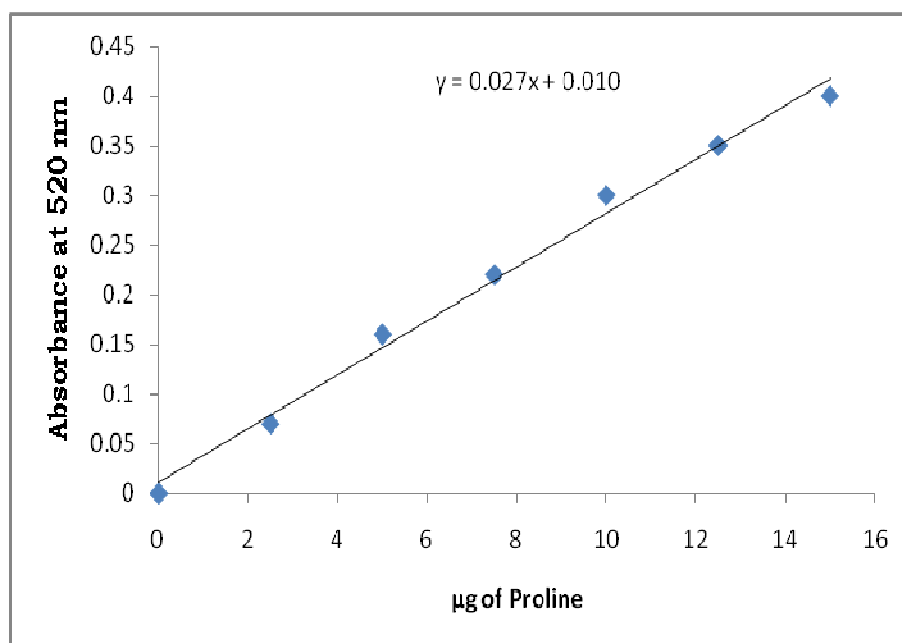
Nessler's Reagent

Mix 3ml of mercuric iodide, 3.5ml of Potassium Iodide, 12ml of sodium hydroxide in 81.5 ml of DW to have a final volume of 100 ml.

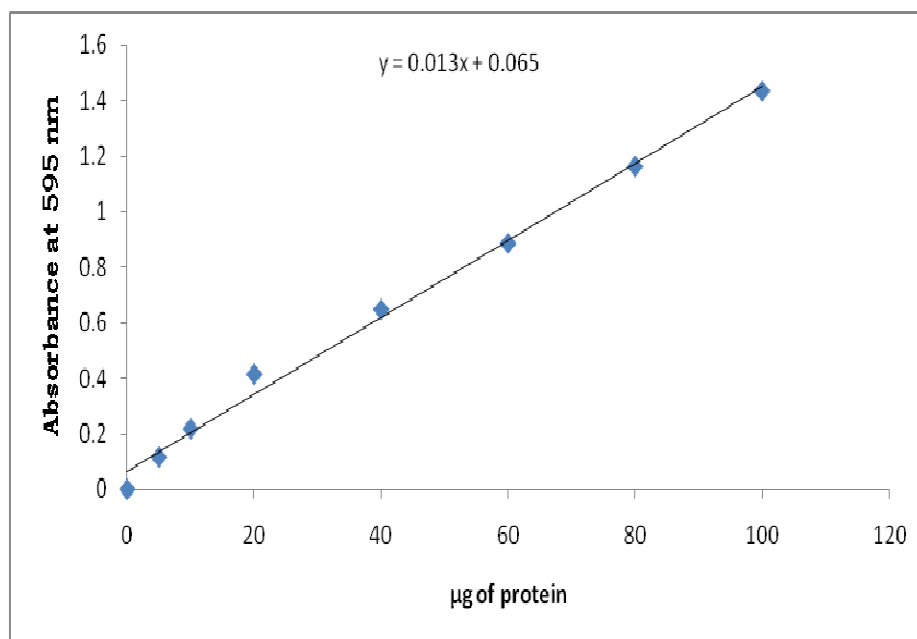
Appendix-II



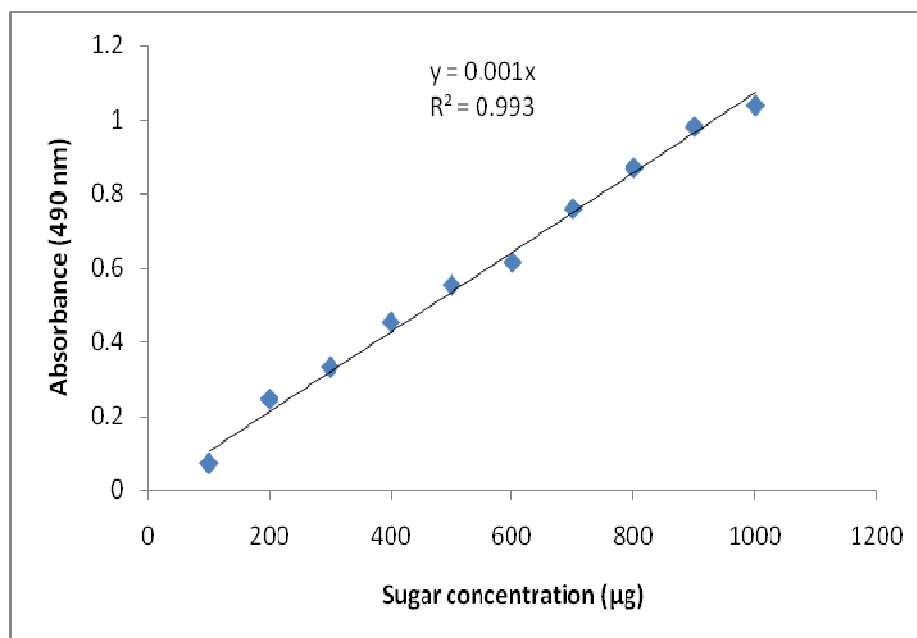
Standard curve for H₂O₂



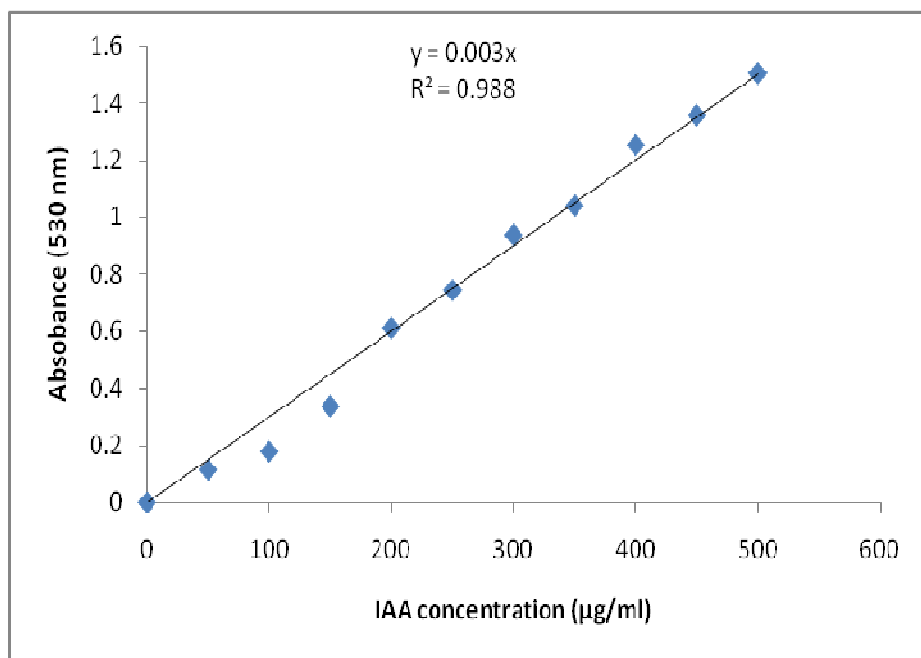
Standard curve for Proline



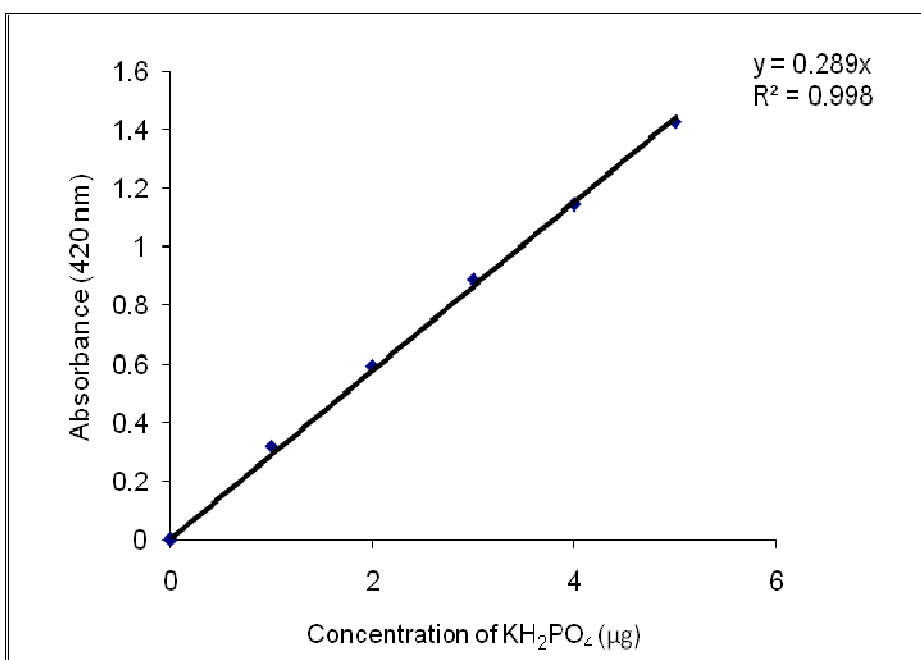
Standard curve for proteins (Bradford method)



Standard curve for sugar (Dubois method)



Standard curve of Indole Acetic acid (IAA)



Standard curve of KH_2PO_4 for estimation of phosphorus

Appendix-III

Table 1: Effect of PGPR on root length of six varieties of pearl millet

Variety	Control	P3	14B	Mean
CZP 9802	18.50 ^a	25.16 ^{bc}	24.33 ^{bc}	22.66 ^{ab}
CZP 2007	24.83 ^{bc}	27.33 ^c	25.66 ^{bc}	25.94 ^b
CZP 2003	23.16 ^b	27.66 ^c	22.50 ^b	24.44 ^b
CZP 9603	22.50 ^b	18.83 ^a	20.83 ^{ab}	20.72 ^a
CZP 2k9	24.00 ^{bc}	30.66 ^{cd}	33.00 ^d	29.22 ^c
CZIC 923	23.33 ^b	36.66 ^e	33.33 ^{de}	31.11 ^c
Mean	22.72 ^a	27.72 ^b	26.61 ^b	

Table 2: Effect of PGPR on shoot length of six varieties of pearl millet

Variety	Control	P3	14B	Mean
CZP 9802	54.33 ^b	65.00 ^{cd}	66.50 ^d	61.94 ^c
CZP 2007	44.00 ^a	53.00 ^b	53.83 ^b	50.27 ^a
CZP 2003	62.50 ^{cd}	64.83 ^{cd}	63.83 ^{cd}	63.72 ^d
CZP 9603	53.66 ^b	54.33 ^b	53.83 ^b	53.94 ^{bc}
CZP 2k9	57.66 ^b	69.00 ^{de}	73.33 ^e	66.66 ^e
CZIC 923	61.00 ^c	73.66 ^e	76.00 ^e	70.22 ^f
Mean	55.52 ^a	63.30 ^b	64.55 ^b	

Table 3: Effect of PGPR on root fresh wt. of six varieties of pearl millet

Variety	Control	P3	14B	Mean
CZP 9802	0.193 ^a	0.320 ^{ab}	0.436 ^b	0.316 ^a
CZP 2007	0.673 ^c	0.473 ^{bc}	0.340 ^{ab}	0.495 ^b
CZP 2003	0.258 ^{ab}	0.316 ^{ab}	0.227 ^{ab}	0.267 ^a
CZP 9603	0.290 ^{ab}	0.292 ^{ab}	0.329 ^{ab}	0.304 ^a
CZP 2k9	0.446 ^b	0.956 ^d	1.110 ^d	0.837 ^c
CZIC 923	0.580 ^{bc}	1.858 ^f	1.423 ^e	1.287 ^d
Mean	0.407 ^a	0.702 ^b	0.644 ^b	

Table 4: Effect of PGPR on shoot fresh wt. of six varieties of pearl millet

Variety	Control	P3	14B	Mean
CZP 9802	2.49 ^{ab}	2.74 ^{bc}	2.99 ^{bc}	2.74 ^b
CZP 2007	2.63 ^b	2.76 ^{bc}	2.46 ^{ab}	2.62 ^{ab}
CZP 2003	2.57 ^b	2.51 ^{ab}	2.53 ^{ab}	2.54 ^{ab}
CZP 9603	2.89 ^{bc}	2.35 ^{ab}	2.11 ^a	2.45 ^a
CZP 2k9	2.45 ^{ab}	3.14 ^c	3.82 ^d	3.14 ^c
CZIC 923	2.56 ^b	4.85 ^e	5.03 ^e	4.15 ^d
Mean	2.60 ^a	3.06 ^b	3.16 ^b	

Table 5: Effect of PGPR on root dry wt. of six varieties of pearl millet

Variety	Control	P3	14B	Mean
CZP 9802	0.124 ^a	0.153 ^{ab}	0.188 ^b	0.155 ^{ab}
CZP 2007	0.146 ^{ab}	0.178 ^b	0.147 ^{ab}	0.157 ^{ab}
CZP 2003	0.181 ^b	0.175 ^b	0.149 ^{ab}	0.168 ^b
CZP 9603	0.131 ^a	0.147 ^{ab}	0.133 ^{ab}	0.137 ^a
CZP 2k9	0.176 ^b	0.234 ^c	0.255 ^c	0.221 ^{cd}
CZIC 923	0.143 ^{ab}	0.261 ^c	0.273 ^c	0.226 ^d
Mean	0.150 ^a	0.191 ^b	0.191 ^b	

Table 6: Effect of PGPR on shoot dry wt. of six varieties of pearl millet

Variety	Control	P3	14B	Mean
CZP 9802	0.518 ^{cd}	0.502 ^{cd}	0.615 ^e	0.545 ^a
CZP 2007	0.436 ^{bc}	0.513 ^{cd}	0.477 ^c	0.475 ^a
CZP 2003	0.511 ^{cd}	0.526 ^d	0.649 ^e	0.562 ^a
CZP 9603	0.577 ^e	0.364 ^a	0.415 ^b	0.452 ^a
CZP 2k9	0.520 ^{cd}	0.653 ^e	0.633 ^e	0.602 ^b
CZIC 923	0.516 ^{cd}	0.653 ^e	0.738 ^f	0.636 ^b
Mean	0.513 ^a	0.535 ^b	0.588 ^c	

Table 7: Effect of PGPR on root volume of six varieties of pearl millet

Variety	Control	P3	14B	Mean
CZP 9802	1.16 ^a	1.50 ^{ab}	1.75 ^{bc}	1.47 ^b
CZP 2007	1.41 ^{ab}	1.75 ^{bc}	1.70 ^b	1.62 ^b
CZP 2003	1.08 ^a	1.33 ^{ab}	1.08 ^a	1.16 ^a
CZP 9603	1.33 ^{ab}	1.41 ^{ab}	1.33 ^{ab}	1.36 ^{ab}
CZP 2k9	1.25 ^a	1.50 ^{ab}	2.16 ^c	1.63 ^{bc}
CZIC 923	1.41 ^{ab}	2.08 ^{bc}	2.16 ^c	1.88 ^c
Mean	1.27 ^a	1.59 ^b	1.70 ^b	

Table 8: Effect of PGPR on root length of two varieties of pearl millet in three different soils

Variety	Mukteshwar soil			Mean	Pantnagar soil			Mean	Ujhani soil			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	13.33 ^{ab}	17.00 ^{bc}	15.00 ^b	15.11 ^b	22.66 ^d	29.33 ^c	28.33 ^c	26.77 ^f	15.66 ^{bc}	24.66 ^{de}	26.66 ^c	22.33 ^c	17.22 ^a	23.66 ^b	23.33 ^b	21.40 ^b
CZP 9603	11.33 ^a	13.33 ^{ab}	11.00 ^a	11.88 ^a	22.33 ^d	17.66 ^{bc}	18.33 ^c	19.44 ^d	17.66 ^{bc}	18.33 ^c	17.66 ^{bc}	17.88 ^{cd}	17.11 ^a	16.44 ^a	15.66 ^a	16.40 ^a
Mean	12.33 ^a	15.16 ^b	13.00 ^a	13.50 ^a	22.50 ^d	23.50 ^d	23.33 ^d	23.11 ^c	16.66 ^b	21.50 ^c	22.16 ^{cd}	20.11 ^b	17.16 ^a	20.05 ^b	19.50 ^b	

Table 9: Effect of PGPR on shoot length of two varieties of pearl millet in three different soils

Variety	Mukteshwar soil			Mean	Pantnagar soil			Mean	Ujhani soil			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	21.00 ^a	31.33 ^b	32.00 ^b	28.11 ^b	67.66 ^c	75.66 ⁱ	71.00 ^{ef}	71.44 ^e	55.66 ^c	75.33 ⁱ	69.66 ^c	66.88 ^d	48.11 ^a	60.77 ^d	57.55 ^c	55.48 ^b
CZP 9603	23.66 ^a	23.33 ^a	29.00 ^b	25.33 ^a	64.66 ^{de}	67.66 ^c	64.00 ^{de}	65.44 ^d	54.33 ^c	57.66 ^{cd}	61.00 ^d	57.66 ^c	47.55 ^a	49.55 ^{ab}	51.33 ^b	49.48 ^a
Mean	22.33 ^a	27.33 ^b	30.50 ^c	26.72 ^a	66.16 ^e	71.66 ⁱ	67.50 ^e	68.44 ^c	55.00 ^d	66.50 ^e	65.33 ^e	62.27 ^b	47.83 ^a	55.16 ^b	54.44 ^b	

Table 10: Effect of PGPR on root fresh wt. of two varieties of pearl millet in three different soils

Variety	Mukteshwar soil			Mean	Pantnagar soil			Mean	Ujhani soil			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.026 ^a	0.041 ^a	0.037 ^a	0.034 ^a	0.436 ^g	0.564 ⁱ	0.566 ⁱ	0.522 ^d	0.161 ^b	0.304 ^e	0.469 ^h	0.311 ^c	0.207 ^c	0.303 ^d	0.357 ^e	0.289 ^b
CZP 9603	0.022 ^a	0.029 ^a	0.023 ^a	0.025 ^a	0.342 ^f	0.304 ^e	0.244 ^d	0.297 ^c	0.161 ^b	0.204 ^c	0.166 ^b	0.177 ^b	0.175 ^b	0.179 ^b	0.144 ^a	0.166 ^a
Mean	0.024 ^a	0.035 ^a	0.030 ^a	0.03 ^a	0.389 ^e	0.434 ^g	0.405 ^f	0.409 ^c	0.161 ^b	0.254 ^c	0.317 ^d	0.244 ^b	0.191 ^a	0.241 ^b	0.251 ^b	

Table 11: Effect of PGPR on shoot fresh wt. of two varieties of pearl millet in three different soils

Variety	Mukteshwar soil			Mean	Pantnagar soil			Mean	Ujhani soil			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.162 ^a	0.571 ^b	0.371 ^b	0.368 ^b	1.783 ^e	3.300 ^h	4.453 ⁱ	3.178 ^f	1.322 ^{cd}	3.318 ^h	2.693 ^g	2.444 ^e	1.089 ^b	2.396 ^c	2.505 ^c	1.997 ^b
CZP 9603	0.123 ^a	0.148 ^a	0.152 ^a	0.141 ^a	1.244 ^{cd}	1.385 ^d	1.135 ^{cd}	1.255 ^c	1.288 ^{cd}	2.077 ^f	2.078 ^f	1.814 ^d	0.885 ^a	1.204 ^b	1.122 ^b	1.070 ^a
Mean	0.142 ^a	0.359 ^b	0.261 ^b	0.254 ^a	1.513 ^d	2.342 ^e	2.794 ^f	2.216 ^b	1.305 ^c	2.697 ^f	2.385 ^e	2.129 ^b	0.987 ^a	1.800 ^b	1.813 ^b	

Table 12: Effect of PGPR on root dry wt. of two varieties of pearl millet in three different soils

Variety	Mukteshwar soil			Mean	Pantnagar soil			Mean	Ujhani soil			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.015 ^a	0.024 ^a	0.023 ^a	0.020 ^a	0.175 ^c	0.248 ^f	0.122 ^c	0.182 ^c	0.073 ^b	0.115 ^c	0.148 ^d	0.112 ^c	0.088 ^b	0.129 ^c	0.097 ^b	0.105 ^b
CZP 9603	0.014 ^a	0.015 ^a	0.013 ^a	0.014 ^a	0.160 ^{de}	0.175 ^c	0.101 ^c	0.145 ^d	0.073 ^b	0.090 ^{bc}	0.089 ^{bc}	0.084 ^b	0.082 ^{ab}	0.093 ^b	0.067 ^a	0.081 ^a
Mean	0.014 ^a	0.020 ^a	0.018 ^a	0.017 ^a	0.168 ^c	0.212 ^f	0.111 ^{cd}	0.163 ^c	0.073 ^b	0.102 ^c	0.118 ^d	0.098 ^b	0.085 ^a	0.111 ^b	0.082 ^a	

Table 13: Effect of PGPR on shoot dry wt. of two varieties of pearl millet in three different soils

Variety	Mukteshwar soil			Mean	Pantnagar soil			Mean	Ujhani soil			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.034 ^a	0.083 ^b	0.505 ^f	0.056 ^b	0.465 ^e	0.723 ^g	0.752 ^g	0.647 ^e	0.235 ^{cd}	0.518 ^f	0.510 ^f	0.421 ^d	0.245 ^b	0.441 ^c	0.437 ^c	0.374 ^b
CZP 9603	0.025 ^a	0.031 ^a	0.027 ^a	0.027 ^a	0.264 ^d	0.258 ^d	0.261 ^d	0.261 ^c	0.208 ^c	0.257 ^d	0.287 ^d	0.251 ^c	0.166 ^a	0.182 ^a	0.191 ^a	0.180 ^a
Mean	0.030 ^a	0.057 ^b	0.038a ^b	0.042 ^a	0.365 ^d	0.491 ^f	0.506 ^f	0.454 ^c	0.221 ^c	0.388 ^{de}	0.398 ^e	0.336 ^b	0.205 ^a	0.312 ^b	0.314 ^b	

Table 14: Effect of PGPR on root volume of two varieties of pearl millet in three different soils

Variety	Mukteshwar soil			Mean	Pantnagar soil			Mean	Ujhani soil			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.05 ^a	0.10 ^a	0.10 ^a	0.83 ^c	0.60b ^c	0.86 ^{cd}	0.93 ^d	0.80 ^c	0.56 ^b	0.96 ^d	1.06 ^d	0.86 ^c	0.40 ^a	0.64 ^b	0.70 ^b	0.583 ^b
CZP 9603	0.05 ^a	0.10 ^a	0.06 ^a	0.07 ^a	0.76 ^c	0.53 ^b	0.63 ^{bc}	0.64 ^b	0.58 ^b	0.68 ^{bc}	0.68 ^{bc}	0.65 ^b	0.46 ^a	0.43 ^a	0.45 ^a	0.454 ^a
Mean	0.05 ^a	0.10 ^a	0.08 ^a	0.076 ^a	0.68 ^c	0.70 ^{cd}	0.78 ^d	0.72 ^b	0.57 ^b	0.82 ^d	0.87 ^d	0.758 ^b	0.436 ^a	0.541 ^b	0.579 ^b	

Table 15: Exopolysaccharide production in P3 in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	6.51 ^a	7.73 ^{ab}	9.26 ^b	9.91 ^{bc}	11.00 ^c	8.85 ^a
24 h	7.14 ^a	8.80 ^b	9.35 ^b	12.81 ^d	14.01 ^e	10.42 ^b
36 h	7.31 ^a	10.65 ^c	10.93 ^c	13.23 ^{de}	12.66 ^d	10.96 ^b
Mean	6.99 ^a	9.06 ^b	9.84 ^c	11.98 ^d	12.56 ^d	

Table 16: Exopolysaccharide production in 14B in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	2.60 ^b	3.78 ^{cd}	3.85 ^d	4.35 ^d	1.60 ^a	3.23 ^a
24 h	6.28 ^e	10.15 ⁱ	11.08 ^j	12.63 ^k	6.58 ^{ef}	9.34 ^c
36 h	3.92 ^d	9.55 ^h	8.23 ^g	7.08 ^f	3.90 ^d	6.53 ^b
Mean	4.27 ^a	7.82 ^b	7.72 ^b	8.02 ^b	4.02 ^a	

Table 17: Proline production in P3 in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	7.27 ^a	8.30 ^b	9.27 ^c	12.84 ^f	13.06 ^f	10.15 ^a
24 h	8.21 ^b	9.45 ^c	11.79 ^e	14.87 ^g	13.26 ^{fg}	11.52 ^b
36 h	9.05 ^c	10.76 ^{de}	15.59 ^h	17.72 ⁱ	12.02 ^{ef}	13.03 ^c
Mean	8.17 ^a	9.50 ^b	12.21 ^{cd}	15.15 ^e	12.78 ^d	

Table 18: Proline production in 14B in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	4.15 ^a	5.89 ^b	6.18 ^{bc}	9.56 ^c	10.05 ^{ef}	7.16 ^a
24 h	5.68 ^b	6.58 ^c	8.23 ^d	11.08 ^g	10.22 ^f	8.36 ^b
36 h	6.67 ^c	7.94 ^d	12.72 ^h	10.17 ^{ef}	6.91 ^c	8.84 ^c
Mean	5.50 ^a	6.80 ^b	9.04 ^c	10.27 ^d	9.06 ^c	

Table 19: Total soluble sugar production in P3 in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	6.00 ^a	7.37 ^b	8.70 ^c	9.52 ^{cd}	10.40 ^d	8.40 ^a
24 h	7.43 ^b	9.33 ^c	13.46 ^{ef}	15.52 ^g	14.84 ^{fg}	12.12 ^b
36 h	8.23 ^b	10.36 ^d	14.21 ^f	16.27 ^g	13.08 ^e	12.42 ^b
Mean	7.22 ^a	9.02 ^b	12.12 ^c	13.77 ^e	12.77 ^d	

Table 20: Total soluble sugar production in14B in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	3.24 ^a	5.32 ^{bc}	6.56 ^d	6.90 ^{de}	8.34 ^f	6.07 ^a
24 h	3.90 ^a	6.67 ^d	8.94 ^f	11.41 ^h	12.23 ⁱ	8.63 ^b
36 h	4.69 ^b	5.76 ^c	7.54 ^e	10.53 ^g	8.81 ^f	7.46 ^b
Mean	3.94 ^a	5.92 ^b	7.68 ^c	9.61 ^d	9.79 ^d	

Table 21: Protein content inP3 in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	1.37 ^g	0.996 ^{de}	0.776 ^c	0.625 ^b	0.961 ^d	0.946 ^a
24 h	2.20 ^h	0.720 ^c	0.642 ^b	0.478 ^{ab}	1.093 ^e	1.027 ^a
36 h	4.317 ⁱ	0.644 ^{bc}	0.551 ^b	0.407 ^a	1.23 ^f	1.43 ^a
Mean	2.629 ^b	0.787 ^a	0.656 ^a	0.503 ^a	1.096 ^a	

Table 22: Protein content in14B in stressed and non stressed conditions in different time interval

Time	Concentration of PEG					
	Control	-0.5 MPa	-0.75 MPa	-1.0 MPa	-1.5 MPa	Mean
12 h	0.948 ^e	0.643 ^d	0.469 ^{cd}	0.261 ^a	0.689 ^d	0.602 ^a
24 h	1.78 ^g	0.444 ^b	0.325 ^b	0.219 ^a	1.49 ^f	0.854 ^a
36 h	2.48 ^h	0.352 ^b	0.268 ^{ab}	0.172 ^a	1.78 ^g	1.012 ^a
Mean	1.740 ^b	0.480 ^a	0.354 ^a	0.217 ^a	1.32 ^b	

Table 23: Effect of PGPR on root length of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	13.33 ^b	31.00 ^f	22.66 ^d	22.33 ^d	11.00 ^{ab}	19.66 ^{cd}	18.33 ^{cd}	16.33 ^c	9.66 ^a	15.33 ^{bc}	18.00 ^{cd}	14.33 ^b	11.33 ^a	22.00 ^d	19.66 ^c	17.66 ^b
CZP 2k9	13.00 ^b	18.33 ^{cd}	22.66 ^d	18.00 ^c	12.33 ^{ab}	15.66 ^{bc}	21.00 ^d	16.33 ^c	10.33 ^{ab}	12.00 ^{ab}	13.66 ^b	12.00 ^a	11.88 ^a	15.33 ^b	19.11 ^c	15.44 ^a
CZP 9603	21.00 ^d	25.33 ^e	18.33 ^{cd}	21.55 ^d	14.00 ^b	17.66 ^c	15.33 ^{bc}	15.66 ^{bc}	10.00 ^{ab}	11.33 ^{ab}	10.00 ^{ab}	10.44 ^a	15.00 ^b	18.11 ^c	14.55 ^b	15.88 ^a
Mean	15.77 ^c	24.88 ^f	21.22 ^c	20.62 ^c	12.44 ^b	17.66 ^d	18.22 ^d	16.11 ^b	10.00 ^a	12.88 ^b	13.88 ^b	12.25 ^a	12.74 ^a	18.48 ^c	17.77 ^b	

Table 24: Effect of PGPR on shoot length of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	46.66 ^{de}	65.33 ^g	64.6 ^g	58.88 ^f	31.66 ^{bc}	52.33 ^e	50.00 ^{de}	44.66 ^d	25.33 ^{ab}	32.66 ^{bc}	30.00 ^b	29.33 ^b	34.55 ^b	50.11 ^e	48.22 ^c	44.29 ^c
CZP 2k9	39.33 ^c	62.33 ^{fg}	54.66 ^{ef}	52.11 ^e	31.66 ^{bc}	51.33 ^e	46.00 ^d	43.00 ^d	23.00 ^a	37.00 ^c	35.00 ^{bc}	31.66 ^b	31.33 ^a	50.22 ^c	45.22 ^d	42.25 ^b
CZP 9603	56.00 ^{ef}	58.66 ^f	56.00 ^{ef}	56.88 ^f	36.00 ^c	38.66 ^c	34.66 ^{bc}	36.44 ^c	23.66 ^a	22.33 ^a	23.66 ^a	23.22 ^a	38.55 ^c	39.88 ^c	38.11 ^c	38.85 ^a
Mean	47.33 ^c	62.11 ^g	58.44 ^f	55.96 ^c	33.11 ^c	47.44 ^e	43.55 ^d	41.37 ^b	24.00 ^a	30.66 ^{bc}	29.55 ^b	28.07 ^a	34.81 ^a	46.74 ^c	43.85 ^b	

Table 25: Effect of PGPR on root fresh weight of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.89 ^f	1.17 ^g	1.21 ^g	1.09 ^f	0.146 ^{ab}	0.396 ^{cd}	0.33 ^c	0.29 ^c	.063 ^a	0.25 ^{bc}	0.16 ^{ab}	0.16 ^{ab}	0.36 ^c	0.60 ^c	0.56 ^c	0.51 ^c
CZP 2k9	0.34 ^c	0.75 ^e	0.82 ^{ef}	0.64 ^c	0.14 ^{ab}	0.39 ^{cd}	0.36 ^{cd}	0.30 ^c	0.08 ^a	0.25 ^{bc}	0.23 ^{bc}	0.19 ^b	0.19 ^a	0.46 ^d	0.47 ^d	0.37 ^b
CZP 9603	0.37 ^{cd}	0.43 ^{cd}	0.47 ^d	0.42 ^d	0.12 ^{ab}	0.17 ^{ab}	0.21 ^b	0.16 ^{ab}	0.08 ^a	0.12 ^{ab}	0.10 ^{ab}	0.10 ^a	0.19 ^a	0.24 ^{ab}	0.26 ^b	0.23 ^a
Mean	0.53 ^d	0.78 ^e	0.83 ^e	0.72 ^c	0.14 ^{ab}	0.32 ^c	0.30 ^c	0.25 ^b	0.07 ^a	0.21 ^b	0.16 ^b	0.15 ^a	0.25 ^a	0.44 ^b	0.43 ^b	

Table 26: Effect of PGPR on shoot fresh weight of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	1.30 ^f	2.14 ^h	1.79 ^g	1.74 ^f	0.38 ^b	1.01 ^f	1.01 ^f	0.80 ^d	0.18 ^a	0.39 ^b	0.41 ^b	0.32 ^b	0.62 ^b	1.18 ^e	1.07 ^d	0.95 ^c
CZP 2k9	0.79 ^{de}	1.21 ^f	1.83 ^g	1.27 ^e	0.36 ^b	0.86 ^e	1.02 ^f	0.75 ^d	0.13 ^a	0.69 ^d	0.56 ^c	0.46 ^c	0.43 ^a	0.92 ^c	1.14 ^{de}	0.83 ^b
CZP 9603	1.21 ^f	1.32 ^f	1.28 ^f	1.27 ^e	0.40 ^b	0.42 ^b	0.41 ^b	0.41 ^c	0.11 ^a	0.12 ^a	0.12 ^a	0.12 ^a	0.57 ^b	0.62 ^b	0.60 ^b	0.60 ^a
Mean	1.10 ^d	1.55 ^e	1.63 ^f	1.43 ^c	0.38 ^b	0.76 ^c	0.81 ^c	0.65 ^b	0.14 ^a	0.40 ^b	0.36 ^b	0.30 ^a	0.54 ^a	0.90 ^b	0.93 ^b	

Table 27: Effect of PGPR on proline content of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	13.33 ^a	27.90 ^a	32.09 ^a	24.42 ^a	33.82 ^a	129.87 ^c	90.37 ^{bc}	84.69 ^b	1249.38 ^h	1572.09 ^j	1360.49 ⁱ	1393.99 ^e	432.18 ^c	576.62 ^e	494.32 ^d	501.04 ^c
CZP 2k9	12.09 ^a	18.52 ^a	17.78 ^a	16.13 ^a	23.20 ^a	103.5 ^{bc}	79.28 ^b	68.65 ^b	831.60 ^c	1194.56 ^g	1199.01 ^g	1075.06 ^d	288.96 ^a	438.84 ^c	432.02 ^c	386.61 ^b
CZP 9603	8.88 ^a	16.79 ^a	15.31 ^a	13.66 ^a	15.80 ^a	82.47 ^b	83.70 ^b	60.65 ^b	779.26 ^d	1052.59 ^f	1056.79 ^f	962.88 ^c	267.98 ^a	383.95 ^b	385.26 ^b	345.73 ^a
Mean	11.43 ^a	21.07 ^a	21.72 ^a	18.07 ^a	24.27 ^a	105.26 ^b	84.45 ^b	71.33 ^b	953.41 ^c	1273.08 ^e	1205.43 ^d	1143.97 ^c	329.71 ^a	466.47 ^c	437.20 ^b	

Table 28: Effect of PGPR on malondialdehyde content of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	33.97 ^b	23.22 ^a	22.58 ^a	26.59 ^a	56.77 ^{cd}	34.41 ^b	32.25 ^b	41.14 ^b	96.98 ^h	66.45 ^c	66.66 ^c	76.70 ^d	62.57 ^c	41.36 ^a	40.50 ^a	48.14 ^a
CZP 2k9	50.96 ^c	36.56 ^b	38.06 ^b	41.86 ^b	81.50 ^g	56.12 ^{cd}	58.92 ^d	65.51 ^c	114.62 ⁱ	70.53 ^{ef}	69.89 ^{ef}	85.01 ^e	82.36 ^c	54.40 ^b	55.62 ^b	64.13 ^b
CZP 9603	55.70 ^{cd}	34.83 ^b	36.34 ^b	42.29 ^b	73.54 ^{ef}	58.70 ^d	56.77 ^{cd}	63.00 ^c	93.55 ^h	86.02 ^g	87.74 ^{gh}	89.10 ^f	74.26 ^d	59.85 ^c	60.28 ^c	64.80 ^b
Mean	46.88 ^b	31.54 ^a	32.33 ^a	36.91 ^a	70.60 ^c	49.74 ^b	49.31 ^b	56.55 ^b	101.72 ^e	74.33 ^d	74.76 ^d	83.60 ^c	73.07 ^b	51.87 ^a	52.13 ^a	

Table 29: Effect of PGPR on hydrogen peroxide content of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	132.34 ^a	58.86 ^a	116.95 ^a	102.71 ^a	1252.84 ^{de}	422.71 ^b	898.76 ^c	858.10 ^b	3004.44 ^j	2203.45 ^f	2791.11 ⁱ	2666.33 ^d	1463.21 ^c	895.01 ^a	1268.94 ^b	1209.05 ^a
CZP 2k9	176.22 ^a	122.76 ^a	106.66 ^a	135.21 ^a	1397.53 ^e	1148.15 ^d	1306.17 ^{de}	1283.95 ^c	3423.21 ^k	2563.95 ^h	2380.25 ^g	2789.13 ^e	1665.65 ^d	1278.28 ^b	1264.36 ^b	1402.76 ^b
CZP 9603	163.55 ^a	147.16 ^a	153.48 ^a	154.73 ^a	1486.42 ^e	1294.8 ^{de}	1179.75 ^d	1320.32 ^c	3369.63 ^k	2805.92 ⁱ	2985.68 ^j	3053.74 ^f	1673.20 ^d	1415.96 ^c	1439.63 ^c	1509.60 ^c
Mean	157.37 ^a	109.59 ^a	125.70 ^a	130.89 ^a	1378.93 ^d	955.22 ^b	1128.23 ^c	1154.12 ^b	3265.76 ^g	2524.44 ^e	2719.01 ^f	2836.40 ^c	1600.68 ^c	1196.42 ^a	1324.31 ^b	

Table 30: Effect of PGPR on chlorophyll a content of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	1.08 ^{cd}	1.54 ^f	1.70 ^g	1.44 ^g	1.08 ^{cd}	1.49 ^{ef}	1.39 ^e	1.32 ^f	0.916 ^{bc}	1.61 ^{fg}	1.20 ^d	1.24 ^e	1.03 ^b	1.54 ^f	1.43 ^e	1.33 ^c
CZP 2k9	1.36 ^e	1.65 ^{fg}	1.44 ^{ef}	1.48 ^g	1.24 ^{de}	1.61 ^{fg}	1.43 ^{ef}	1.43 ^g	0.80 ^{ab}	0.92 ^{bc}	0.92 ^{bc}	0.88 ^c	1.13 ^c	1.39 ^e	1.26 ^d	1.26 ^b
CZP 9603	0.98 ^{bc}	1.03 ^c	0.99 ^c	1.00 ^d	0.76 ^{ab}	0.85 ^b	0.79 ^{ab}	0.80 ^b	0.69 ^a	0.69 ^a	0.68 ^a	0.69 ^a	0.81 ^a	0.86 ^a	0.82 ^a	0.83 ^c
Mean	1.36 ^e	1.65 ^{fg}	1.44 ^{ef}	1.48 ^g	1.24 ^{de}	1.61 ^{fg}	1.43 ^{ef}	1.43 ^g	0.80 ^{ab}	0.92 ^{bc}	0.92 ^{bc}	0.88 ^c	1.13 ^c	1.39 ^e	1.26 ^d	1.26 ^b

Table 31: Effect of PGPR on chlorophyll b content of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	2.62 ^f	3.06 ^g	3.24 ^{gh}	2.97 ^d	2.20 ^e	3.40 ^h	2.98 ^g	2.86 ^d	0.56 ^a	0.85 ^{bc}	1.14 ^c	0.85 ^b	1.79 ^d	2.43 ^e	2.45 ^e	2.29 ^c
CZP 2k9	2.14 ^e	3.90 ⁱ	3.77 ⁱ	3.27 ^e	1.78 ^d	3.03 ^g	2.41 ^e	2.41 ^c	0.69 ^a	1.29 ^c	0.99 ^{bc}	0.99 ^b	1.54 ^c	2.74 ^f	2.39 ^e	2.22 ^b
CZP 9603	0.75 ^b	1.12 ^c	1.01 ^b	0.96 ^b	0.706 ^a	0.88 ^{bc}	0.84 ^{bc}	0.80 ^b	0.42 ^a	0.67 ^a	0.74 ^b	0.61 ^a	0.62 ^a	0.89 ^b	0.86 ^b	0.79 ^a
Mean	1.84 ^d	2.69 ^g	2.67 ^g	2.40 ^c	1.56 ^c	2.43 ^f	2.08 ^c	2.02 ^b	0.55 ^a	0.94 ^b	0.95 ^b	0.81 ^a	1.32 ^a	2.02 ^c	1.90 ^b	

Table 32: Effect of PGPR on caretenoid content of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	2.29 ^d	3.10 ^e	2.94 ^e	2.78 ^d	2.18 ^d	3.66 ^f	2.83 ^e	2.89 ^d	0.74 ^{ab}	1.13 ^b	1.26 ^{bc}	1.04 ^b	1.74 ^c	2.63 ^e	2.34 ^d	2.24 ^b
CZP 2k9	1.89 ^{cd}	3.81 ^f	4.51 ^g	3.40 ^e	1.55 ^c	2.76 ^e	2.35 ^{de}	2.22 ^c	0.71 ^{ab}	1.56 ^c	1.14 ^{bc}	1.13 ^b	1.38 ^b	2.71 ^e	2.66 ^e	2.25 ^b
CZP 9603	0.62 ^a	1.15 ^{bc}	1.08 ^b	0.95 ^b	0.45 ^a	0.51 ^a	0.55 ^a	0.50 ^a	0.39 ^a	0.42 ^a	0.40 ^a	0.40 ^a	0.48 ^a	0.69 ^a	0.68 ^a	0.62 ^a
Mean	1.60 ^c	2.69 ^f	2.84 ^f	2.38 ^c	1.39 ^c	2.31 ^e	1.91 ^d	1.87 ^b	0.6 ^a	1.03 ^b	0.93 ^b	0.86 ^a	1.20 ^a	2.01 ^b	1.89 ^b	

Table 33: Effect of PGPR on Ascorbate Peroxidase (APX) activity of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	18.15 ^{ab}	38.16 ^c	30.3 ^{bc}	28.86 ^c	51.66 ^d	82.20 ^f	70.88 ^{ef}	68.24 ^f	121.5 ^h	219.22 ^l	188.30 ^k	176.34 ⁱ	63.77 ^c	113.2 ^g	96.48 ^f	91.15 ^c
CZP 2k9	13.45 ^a	26.10 ^b	21.1 ^{ab}	20.22 ^b	33.39 ^{bc}	65.63 ^e	55.83 ^{de}	51.62 ^e	78.59 ^f	163.12 ^j	140.60 ⁱ	127.43 ^h	41.81 ^a	84.95 ^e	72.51 ^d	66.42 ^b
CZP 9603	10.04 ^a	14.20 ^{ab}	13.24 ^a	91.50 ^g	31.72 ^{bc}	42.90 ^{cd}	41.26 ^{cd}	38.63 ^d	69.63 ^{ef}	103.57 ^g	101.31 ^g	12.49 ^a	37.13 ^a	53.56 ^b	51.93 ^b	47.54 ^a
Mean	13.88 ^a	26.15 ^b	21.53 ^b	20.52 ^a	38.92 ^c	63.58 ^e	55.99 ^d	52.83 ^b	89.91 ^f	161.97 ^h	143.40 ^g	131.76 ^c	47.57 ^a	83.90 ^c	73.64 ^b	

Table 34: Effect of PGPR on Guaiacol Peroxidase (GPX) activity of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	1.570 ^b	6.616 ^{ef}	4.30 ^d	4.16 ^b	5.41 ^e	11.02 ⁱ	9.44 ^h	8.62 ^d	8.759 ^{gh}	17.63 ^l	18.04 ^l	14.81 ^f	5.246 ^d	11.75 ^g	10.594 ^f	9.198 ^c
CZP 2k9	1.31 ^{ab}	6.203 ^{ef}	4.09 ^d	3.86 ^b	3.16 ^{cd}	9.37 ^h	7.53 ^{fg}	6.69 ^c	7.37 ^{fg}	14.6 ^k	12.97 ^j	11.64 ^e	3.948 ^b	10.05 ^f	8.201 ^e	7.40 ^b
CZP 9603	0.530 ^a	2.786 ^c	1.646 ^b	1.65 ^a	2.47 ^{bc}	5.56 ^e	4.29 ^d	4.11 ^b	4.756 ^{de}	7.916 ^g	7.99 ^g	6.89 ^c	2.586 ^a	5.422 ^d	4.644 ^c	4.217 ^a
Mean	1.136 ^a	5.202 ^c	3.345 ^b	3.228 ^a	3.683 ^b	8.648 ^e	7.092 ^d	6.474 ^b	6.962 ^d	13.376 ^f	13.002 ^f	11.113 ^c	3.927 ^a	9.075 ^c	7.813 ^b	

Table 35: Effect of PGPR on Catalase (CAT) activity of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.07 ^{ab}	0.226 ^c	0.16 ^{bc}	0.153 ^b	0.403 ^d	1.003 ^g	0.716 ^f	0.707 ^f	0.656 ^{ef}	1.523 ⁱ	1.183 ^h	1.121 ^h	0.376 ^b	0.917 ^c	0.687 ^d	0.660 ^c
CZP 2k9	0.043 ^{ab}	0.183 ^{bc}	0.12 ^b	0.115 ^b	0.256 ^c	0.656 ^{ef}	0.576 ^e	0.496 ^d	0.406 ^d	1.166 ^h	0.953 ^g	0.842 ^g	0.235 ^a	0.668 ^d	0.550 ^c	0.484 ^b
CZP 9603	0.026 ^a	0.07 ^{ab}	0.07 ^{ab}	0.055 ^a	0.230 ^c	0.396 ^d	0.370 ^d	0.332 ^c	0.346 ^d	0.673 ^f	0.620 ^{ef}	0.546 ^e	0.201 ^a	0.380 ^b	0.353 ^b	0.311 ^a
Mean	0.046 ^a	0.160 ^b	0.117 ^b	0.108 ^a	0.296 ^c	0.685 ^f	0.554 ^e	0.512 ^b	0.470 ^d	1.121 ^h	0.918 ^g	0.836 ^c	0.271 ^a	0.655 ^c	0.530 ^b	

Table 36: Effect of PGPR on Superoxide dismutase (SOD) activity of three varieties of pearl millet under drought conditions in different days interval

Variety	0 day			Mean	4 th day			Mean	8 th day			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	7.83 ^c	8.33 ^{gh}	8.28 ^g	8.15 ^d	8.38 ^h	10.29 ^l	10.32 ^l	9.66 ^g	11.78 ^m	13.68 ^o	13.70 ^o	13.05 ⁱ	9.33 ^e	10.76 ^h	10.77 ^h	10.29 ^c
CZP 2k9	7.71 ^b	8.11 ^{ef}	8.07 ^e	7.96 ^c	8.16 ^f	9.55 ^j	9.28 ⁱ	9.00 ^f	10.06 ^k	11.95 ⁿ	11.97 ⁿ	11.33 ^h	8.64 ^d	9.87 ^g	9.77 ^f	9.43 ^b
CZP 9603	7.64 ^a	7.77 ^{bc}	7.75 ^b	7.72 ^a	7.83 ^c	7.99 ^d	7.98 ^d	7.93 ^{bc}	8.13 ^{ef}	8.33 ^{gh}	8.28 ^g	8.25 ^e	7.87 ^a	8.03 ^c	8.00 ^{bc}	7.97 ^a
Mean	7.73 ^a	8.07 ^c	8.03 ^{bc}	7.94 ^a	8.12 ^d	9.28 ^f	9.19 ^e	8.86 ^b	9.99 ^g	11.32 ^h	11.31 ^h	10.87 ^c	8.61 ^a	9.55 ^c	9.51 ^b	

Table 37: Effect of PGPR on root length of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	21.0 ^{bc}	26.0 ^d	26.0 ^d	24.33 ^c	19.0 ^a	25.5 ^d	22.0 ^c	22.16 ^a	19.0 ^a	23.0 ^c	22.5 ^c	21.50 ^a	19.66 ^a	24.83 ^d	23.50 ^c	22.66 ^b
CZP 9603	21.0 ^{bc}	21.5 ^{bc}	22.0 ^c	21.5 ^a	18.5 ^a	21.5 ^{bc}	22.0 ^c	20.66 ^a	19.5 ^{ab}	22.0 ^c	20.5 ^{bc}	20.66 ^a	19.66 ^a	21.66 ^b	21.50 ^b	20.94 ^a
Mean	21.0 ^b	23.5 ^d	22.25 ^c	22.25 ^b	18.75 ^a	22.25 ^c	22.0 ^c	21.00 ^a	19.25 ^a	24.0 ^d	23.25 ^d	22.17 ^b	19.66 ^a	23.25 ^c	22.50 ^b	

Table 38: Effect of PGPR on shoot length of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	52.0 ^c	63.0 ^g	61.0 ^g	58.66 ^e	41.0 ^b	56.5 ^f	48.0 ^{cd}	48.50 ^c	41.0 ^b	50.5 ^{de}	46.0 ^c	45.83 ^b	44.66 ^a	56.66 ^c	51.66 ^d	51.0 ^b
CZP 9603	51.5 ^e	51.5 ^e	58.5 ^f	53.83 ^d	48.5 ^d	50.0 ^{de}	47.5 ^{cd}	48.66 ^c	41.5 ^b	38.5 ^a	40.0 ^{ab}	40.0 ^a	47.16 ^b	46.66 ^b	48.66 ^c	47.50 ^a
Mean	51.75 ^c	57.25 ^g	59.75 ^h	56.25 ^c	44.75 ^c	53.25 ^f	47.75 ^d	48.58 ^b	41.25 ^a	44.50 ^c	43.0 ^b	42.91 ^a	45.91 ^a	51.66 ^c	50.16 ^b	

Table 39: Effect of PGPR on root fresh wt. of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.95 ^g	1.40 ^j	1.53 ^k	1.29 ^f	0.86 ^{ef}	1.00 ^h	1.11 ⁱ	0.99 ^e	0.79 ^d	0.86 ^{ef}	0.88 ^{fg}	0.85 ^c	0.87 ^c	1.09 ^d	1.17 ^e	1.04 ^b
CZP 9603	0.84 ^e	0.92 ^g	0.917 ^{fg}	0.89 ^d	0.75 ^c	0.74 ^c	0.73 ^c	0.74 ^b	0.50 ^a	0.52 ^a	0.54 ^b	0.52 ^a	0.70 ^a	0.72 ^b	0.73 ^b	0.72 ^a
Mean	0.89 ^f	1.16 ^g	1.22 ^h	1.09 ^c	0.80 ^d	0.87 ^e	0.92 ^f	0.86 ^b	0.65 ^a	0.69 ^b	0.71 ^c	0.68 ^a	0.78 ^a	0.91 ^b	0.95 ^c	

Table 40: Effect of PGPR on shoot fresh weight of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	1.32 ^c	2.68 ^e	2.56 ^e	2.19 ^e	1.08 ^b	1.68 ^d	1.80 ^d	1.52 ^d	0.96 ^{ab}	1.44 ^c	1.67 ^c	1.36 ^c	1.12 ^{ab}	1.93 ^c	2.01 ^c	1.69 ^b
CZP 9603	1.30 ^c	1.41 ^c	1.65 ^d	1.45 ^d	1.03 ^b	1.04 ^b	1.10 ^b	1.06 ^b	0.87 ^a	0.90 ^{ab}	0.89 ^{ab}	0.89 ^a	1.06 ^a	1.12 ^{ab}	1.21 ^b	1.13 ^a
Mean	1.31 ^d	2.05 ^f	2.11 ^f	1.82 ^c	1.05 ^b	1.36 ^{de}	1.45 ^e	1.29 ^b	0.91 ^a	1.17 ^c	1.28 ^d	1.12 ^a	1.09 ^a	1.53 ^b	1.61 ^c	

Table 41: Effect of PGPR on root dry weight of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.15 ^{cd}	0.37 ^h	0.25 ^g	0.25 ^d	0.15 ^{cd}	0.41 ⁱ	0.24 ^{eg}	0.27 ^e	0.06 ^a	0.12 ^{bc}	0.13 ^{bc}	0.10 ^a	0.12 ^a	0.30 ^d	0.20 ^c	0.21 ^b
CZP 9603	0.18 ^{de}	0.19 ^{de}	0.21 ^e	0.19 ^c	0.09 ^{ab}	0.13 ^{bc}	0.17 ^d	0.13 ^b	0.08 ^{ab}	0.10 ^{bc}	.093 ^{ab}	0.092 ^a	0.12 ^a	0.14 ^{ab}	0.16 ^b	0.14 ^a
Mean	0.16 ^c	0.28 ^f	0.23 ^e	0.22 ^c	0.13 ^b	0.27 ^f	0.21 ^d	0.20 ^b	0.072 ^a	0.11 ^b	0.11 ^b	0.098 ^a	0.12 ^a	0.22 ^b	0.18 ^c	

Table 42: Effect of PGPR on shoot dry weight of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.39 ^h	0.78 ⁱ	0.50 ^j	0.56 ^f	0.38 ^h	0.596 ^k	0.45 ⁱ	0.47 ^e	0.13 ^a	0.19 ^c	0.33 ^f	0.22 ^c	0.30 ^d	0.52 ^f	0.43 ^c	0.415 ^b
CZP 9603	0.29 ^e	0.35 ^{fg}	0.36 ^g	0.33 ^d	0.19 ^c	0.22 ^d	0.17 ^{bc}	0.19 ^b	0.13 ^a	0.18 ^{bc}	0.14 ^a	0.15 ^a	0.21 ^a	0.25 ^c	0.22 ^b	0.225 ^a
Mean	0.34 ^f	0.56 ⁱ	0.43 ^h	0.44 ^c	0.29 ^d	0.41 ^g	0.31 ^e	0.33 ^b	0.13 ^a	0.185 ^b	0.23 ^c	0.18 ^a	0.25 ^a	0.39 ^c	0.32 ^b	

Table 43: Effect of PGPR on root volume of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.77 ^{ab}	1.50 ^d	1.50 ^d	1.26 ^c	1.00 ^b	1.27 ^{cd}	1.00 ^b	1.09 ^b	1.00 ^b	1.00 ^b	1.50 ^d	1.17 ^{bc}	0.92 ^a	1.26 ^b	1.33 ^b	1.17 ^b
CZP 9603	1.00 ^b	1.77 ^c	1.50 ^d	1.42 ^d	1.27 ^{cd}	1.00 ^b	1.27 ^{cd}	1.18 ^{bc}	0.63 ^a	1.00 ^b	1.00 ^b	0.88 ^a	0.97 ^a	1.26 ^b	1.26 ^b	1.16 ^{ab}
Mean	0.88 ^{ab}	1.63 ^d	1.50 ^d	1.34 ^c	1.13 ^b	1.13 ^b	1.13 ^b	1.13 ^b	0.82 ^a	1.00 ^b	1.25 ^c	1.02 ^a	0.94 ^a	1.26 ^b	1.29 ^b	

Table 44: Effect of PGPR on proline level of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	8.52 ^a	11.85 ^a	15.56 ^a	11.98 ^a	89.63 ^b	251.85 ^g	283.7 ^h	208.39 ^c	191.48 ^f	349.26 ⁱ	355.56 ^j	298.77 ^d	96.54 ^d	204.32 ^e	218.27 ^f	173.05 ^b
CZP 9603	8.15 ^a	10.74 ^a	10.37 ^a	9.75 ^a	90.74 ^{bc}	100 ^c	123.33 ^e	104.69 ^b	88.15 ^b	113.70 ^d	113.70 ^d	105.18 ^b	62.35 ^a	74.81 ^b	82.47 ^c	73.21 ^a
Mean	8.34 ^a	11.29 ^a	12.96 ^a	10.86 ^a	90.19 ^b	175.93 ^d	203.52 ^e	156.54 ^b	135.82 ^c	231.48 ^f	234.63 ^f	201.98 ^c	79.45 ^a	139.57 ^b	150.37 ^c	

Table 45: Effect of PGPR on hydrogen peroxide level of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	611.85b	345.19a	361.48a	439.51a	1521.48g	973.33c	1382.22f	1292.35c	2365.93j	2047.41i	1848.89h	2087.41e	1499.75d	1121.98a	1197.53b	1273.09 ^a
CZP 9603	1077.04d	1525.93g	1398.53f	1333.83c	2068.15i	1432.59fg	1807.41h	1769.38d	1402.96f	1088.89de	1179.26e	1223.71b	1516.05d	1349.14c	1461.73d	1442.31 ^b
Mean	844.44a	935.56b	880a	886.67 ^a	1794.82f	1202.96c	1594.82e	1530.87 ^b	1884.45g	1568.15e	1514.07d	1655.56 ^c	1507.90c	1235.56a	1329.63b	

Table 46: Effect of PGPR on malondialdehyde content of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	15.16 ^a	17.74 ^b	15.8 ^{ab}	16.24 ^a	40.97 ^f	18.71 ^b	22.58 ^c	27.42 ^b	45.48 ^h	32.90 ^e	27.42 ^d	35.27 ^c	33.87 ^b	23.12 ^a	21.94 ^a	26.31 ^a
CZP 9603	47.42 ^h	43.23 ^g	42.90 ^{fg}	44.52 ^d	63.23 ^j	54.19 ⁱ	45.81 ^h	54.41 ^e	92.58 ^l	63.55 ^j	67.42 ^k	74.52 ^f	67.74 ^e	53.66 ^d	52.04 ^c	57.81 ^b
Mean	31.29 ^b	30.49 ^a	29.36 ^a	30.38 ^a	52.10 ^f	36.45 ^d	34.19 ^c	40.92 ^b	69.03 ^g	48.23 ^e	47.42 ^e	54.89 ^c	50.81 ^c	38.39 ^b	36.99 ^a	

Table 47: Effect of PGPR on chlorophyll a content of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	1.72 ^{de}	2.07 ^f	1.91 ^c	1.90 ^c	1.47 ^{bc}	1.69 ^{de}	1.64 ^{cd}	1.60 ^c	1.37 ^{bc}	1.57 ^{cd}	1.51 ^c	1.48 ^b	1.52 ^a	1.78 ^b	1.68 ^b	1.66 ^b
CZP 9603	1.56 ^{cd}	1.84 ^e	1.84 ^e	1.75 ^d	1.51 ^c	1.71 ^{de}	1.36 ^b	1.53 ^{bc}	1.29 ^{ab}	1.67 ^d	1.17 ^a	1.38 ^a	1.45 ^a	1.74 ^b	1.46 ^a	1.55 ^a
Mean	1.64 ^c	1.96 ^e	1.87 ^d	1.82 ^c	1.49 ^b	1.70 ^c	1.50 ^b	1.56 ^b	1.33 ^a	1.62 ^c	1.34 ^a	1.43 ^a	1.49 ^a	1.76 ^c	1.57 ^b	

Table 48: Effect of PGPR on chlorophyll b content of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.65 ^c	0.92 ^e	0.79 ^d	0.79 ^d	0.53 ^b	0.75 ^d	0.62 ^c	0.64 ^c	0.41 ^a	0.58 ^{bc}	0.57 ^{bc}	0.52 ^a	0.53 ^b	0.75 ^d	0.66 ^c	0.65 ^b
CZP 9603	0.48 ^{ab}	0.64 ^c	0.63 ^c	0.58 ^b	0.52 ^b	0.63 ^c	0.55 ^{bc}	0.57 ^b	0.41 ^a	0.62 ^c	0.44 ^a	0.49 ^a	0.47 ^a	0.63 ^c	0.54 ^b	0.55 ^a
Mean	0.57 ^c	0.78 ^e	0.71 ^d	0.69 ^c	0.53 ^{bc}	0.69 ^d	0.59 ^c	0.60 ^b	0.41 ^a	0.59 ^c	0.50 ^b	0.50 ^a	0.50 ^a	0.70 ^c	0.60 ^b	

Table 49: Effect of PGPR on caretenoid content of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	7.04 ^d	8.35 ^f	7.76 ^e	7.72 ^e	5.85 ^c	6.88 ^d	6.91 ^d	6.55 ^d	5.15 ^{bc}	5.70 ^c	5.45 ^{bc}	5.43 ^b	6.02 ^c	6.98 ^d	6.71 ^d	6.57 ^b
CZP 9603	5.61 ^c	6.63 ^d	6.01 ^c	6.08 ^c	5.00 ^b	5.81 ^c	5.69 ^c	5.50 ^b	4.18 ^a	5.30 ^{bc}	4.36 ^a	4.61 ^a	4.93 ^a	5.91 ^c	5.35 ^b	5.39 ^a
Mean	6.33 ^c	7.49 ^e	6.89 ^d	6.90 ^c	5.43 ^b	6.35 ^c	6.3 ^c	6.02 ^b	4.67 ^a	5.50 ^b	4.90 ^a	5.02 ^a	5.47 ^b	6.45 ^c	6.03 ^a	

Table 50: Effect of PGPR on percent electrolyte leakage of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	12.55 ^a	7.96 ^a	9.95 ^a	10.15 ^a	35.40 ^{cd}	25.78 ^b	25.51 ^b	28.90 ^b	51.50 ^e	33.95 ^c	37.91 ^{cd}	41.12 ^c	33.15 ^b	22.56 ^a	24.46 ^a	26.72 ^a
CZP 9603	40.72 ^d	36.65 ^{cd}	38.15 ^{cd}	38.51 ^c	56.41 ^e	45.26 ^d	55.16 ^e	52.28 ^d	95.71 ^g	84.66 ^f	89.34 ^f	89.90 ^e	64.28 ^d	55.53 ^c	60.88 ^d	60.23 ^b
Mean	26.64 ^b	22.31 ^a	24.05 ^{ab}	24.33 ^a	45.91 ^e	35.52 ^c	40.34 ^d	40.59 ^b	73.61 ^h	59.31 ^f	63.62 ^g	65.51 ^c	48.72 ^c	39.04 ^b	42.67 ^b	

Table 51: Effect of PGPR on relative water content of leaves of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	67.65 ^g	80.53 ^h	92.88 ⁱ	80.35 ^e	39.36 ^c	61.65 ^f	72.02 ^g	57.68 ^d	37.50 ^c	49.76 ^{de}	46.38 ^d	44.55 ^c	48.17 ^c	63.97 ^d	70.43 ^e	60.86 ^b
CZP 9603	40.01 ^c	48.30 ^d	55.08 ^e	47.79 ^c	29.11 ^b	40.42 ^c	36.95 ^c	35.49 ^b	23.46 ^a	26.89 ^{ab}	25.17 ^{ab}	25.17 ^a	30.86 ^a	38.54 ^b	39.10 ^b	36.16 ^a
Mean	53.83 ^d	64.41 ^f	73.98 ^g	64.10 ^c	34.24 ^b	51.03 ^d	54.48 ^e	46.58 ^b	30.48 ^a	38.33 ^c	35.78 ^b	34.86 ^a	39.52 ^a	51.26 ^b	54.75 ^c	

Table 52: Effect of PGPR on percent leaf water loss of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	18.83 ^c	8.28 ^a	13.46 ^b	13.52 ^a	29.77 ^c	27.97 ^c	23.99 ^d	27.24 ^c	44.06 ⁱ	32.32 ^f	37.84 ^{gh}	38.07 ^e	30.89 ^c	22.85 ^a	25.09 ^b	26.28 ^a
CZP 9603	26.39 ^{de}	20.71 ^c	18.66 ^c	21.92 ^b	40.18 ^h	34.04 ^{fg}	34.57 ^{fg}	36.26 ^d	43.75 ⁱ	34.66 ^{fg}	35.42 ^g	37.94 ^e	36.77 ^d	29.80 ^c	29.55 ^c	32.04 ^b
Mean	22.61 ^c	14.49 ^a	16.06 ^b	17.72 ^a	34.97 ^g	31.01 ^e	29.28 ^d	31.75 ^b	43.91 ⁱ	33.49 ^f	36.63 ^h	38.01 ^c	33.83 ^c	26.33 ^a	27.32 ^b	

Table 53: Effect of PGPR on leaf exopolysaccharide (EPS) content of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	17.16 ^c	18.88 ^f	22.15 ^g	19.39 ^d	27.68 ⁱ	31.68 ^j	31.29 ^j	30.21 ^e	26.79 ^h	33.75 ^k	31.56 ^j	30.70 ^f	23.88 ^b	28.10 ^c	28.33 ^c	26.76 ^b
CZP 9603	4.59 ^b	3.39 ^a	4.22 ^b	4.06 ^a	5.33 ^c	6.92 ^d	5.59 ^c	5.95 ^c	5.50 ^c	5.44 ^c	5.73 ^c	5.5 ^b	5.14 ^a	5.25 ^a	5.18 ^a	5.19 ^a
Mean	10.87	11.13 ^a	13.19 ^b	11.73 ^a	16.50 ^c	19.29 ^f	18.44 ^e	18.08 ^b	16.15 ^c	19.59 ^f	18.64 ^e	18.13 ^b	14.51 ^a	16.68 ^b	16.76 ^b	

Table 54: Effect of PGPR on Ascorbate peroxidase (APX) activity of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	4.42 ^{ab}	11.37 ^{bc}	7.37 ^b	7.72 ^b	13.48 ^c	18.03 ^d	15.26 ^{cd}	15.59 ^c	52.89 ^{fg}	84.15 ⁱ	72.03 ^h	69.69 ^e	23.60 ^{bc}	37.85 ^e	31.55 ^d	31.00 ^b
CZP 9603	3.07 ^a	6.96 ^{ab}	4.34 ^{ab}	4.79 ^a	7.37 ^b	10.21 ^{bc}	8.51 ^b	8.70 ^b	42.23 ^e	55.60 ^g	49.56 ^f	49.13 ^d	17.55 ^a	24.26 ^c	20.80 ^b	20.87 ^a
Mean	3.74 ^a	9.16 ^b	5.85 ^a	6.25 ^a	10.43 ^{bc}	14.12 ^c	11.89 ^c	12.15 ^b	47.56 ^d	69.88 ^f	60.79 ^e	59.41 ^c	20.57 ^a	31.05 ^c	26.18 ^b	

Table 56: Effect of PGPR on Catalase (CAT) activity of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.059 ^{ab}	0.151 ^b	0.111 ^b	0.106 ^b	0.111 ^b	0.222 ^c	0.141 ^b	0.158 ^c	0.394 ^{ef}	0.809 ^h	0.70 ^g	0.634 ^e	0.188 ^b	0.394 ^d	0.317 ^c	0.299 ^b
CZP 9603	0.028 ^a	0.067 ^{ab}	0.027 ^a	0.040 ^a	0.055 ^{ab}	0.131 ^b	0.095 ^b	0.094 ^d	0.323 ^d	0.440 ^b	0.382 ^{ef}	0.382 ^d	0.135 ^a	0.213 ^b	0.168 ^{ab}	0.172 ^a
Mean	0.043 ^a	0.108 ^{bc}	0.069 ^{ab}	0.073 ^a	0.083 ^b	0.176 ^d	0.118 ^c	0.126 ^b	0.358 ^e	0.625 ^g	0.541 ^f	0.508 ^c	0.161 ^a	0.303 ^c	0.242 ^b	

Table 57: Effect of PGPR on Superoxide Dismutase (SOD) activity of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	5.44 ^c	6.17 ^d	6.17 ^c	5.93 ^b	7.25 ^f	7.91 ^{gh}	7.89 ^g	7.69 ^e	8.12 ^g	8.86 ⁱ	8.87 ⁱ	8.62 ^f	6.94 ^c	7.65 ^d	7.64 ^d	7.41 ^b
CZP 9603	4.16 ^a	5.03 ^b	4.92 ^b	4.70 ^a	6.14 ^d	6.24 ^d	6.23 ^d	6.21 ^c	6.88 ^e	7.19 ^f	7.06 ^{ef}	7.04 ^d	5.73 ^a	6.15 ^b	6.07 ^b	5.98 ^a
Mean	4.80 ^a	5.60 ^b	5.55 ^b	5.32 ^a	6.70 ^c	7.08 ^d	7.06 ^d	6.95 ^b	7.50 ^e	8.03 ^f	7.96 ^f	7.83 ^c	6.33 ^a	6.90 ^b	6.86 ^b	

Table 58: Effect of PGPR on Guaiacol Peroxidase (GPX) activity of two varieties of pearl millet under different stress level imposed by poly ethylene glycol

Variety	Without PEG			Mean	.75 MPa			Mean	1.0 MPa			Mean	Bacterial strains			Mean
	Control	P3	14B		Control	P3	14B		Control	P3	14B		Control	P3	14B	
CZIC 923	0.754 ^a	3.25 ^{bc}	1.91 ^{ab}	1.97 ^b	2.52 ^b	6.64 ^d	4.42 ^c	4.53 ^c	14.46 ^{fg}	24.79 ⁱ	19.94 ^h	19.73 ^e	5.91 ^b	11.56 ^d	8.76 ^c	8.74 ^a
CZP 9603	0.436 ^a	1.67 ^{ab}	0.496 ^a	0.87 ^a	1.91 ^{ab}	3.66 ^{bc}	2.95 ^{bc}	2.84 ^b	9.35 ^e	13.69 ^f	15.81 ^g	12.95 ^d	3.90 ^a	6.34 ^b	6.42 ^b	5.55 ^b
Mean	0.595 ^a	2.46 ^b	1.20 ^b	1.42 ^a	2.22 ^b	5.15 ^d	3.69 ^c	3.68 ^b	11.90 ^e	19.24 ^g	17.88 ^f	16.34 ^c	4.90 ^a	8.95 ^c	7.59 ^b	

VITA

Megha Pandey, authoress of this manuscript was born on 20th July 1986 at Champawat (Uttarakhand). She completed her High-school in 2001 from U. P. Board and Intermediate in 2003 from U. A. Board. She did her Graduation from V.G.P.G College, Lohaghat in 2007. She took admission for the degree of M.Sc. (Microbiology) in the college of Basic Science and Humanities, G. B. P. U. A. & T., Pantnagar in 2007. She received the Teaching Assistantship of the University during her Degree Programme. In the year 2009 she joined the Department of Microbiology in the same university for Doctoral degree. She has qualified Uttarakhand-State Eligibility Test in 2012. She has been selected for the post of grade IIB officer in Gujarat State Fertilizers & Chemicals, Limited, Vadodara through campus placement.

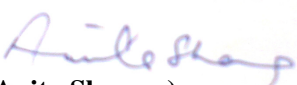
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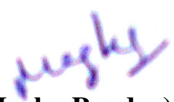
*Megha Pandey
Indira Gandhi Marg
Champawat,
Uttarakhand-262523
INDIA
E-mail: pandeymegha22@gmail.com*

Name	: Megha Pandey	Id. No.	: 35332
Sem. & year of admission	: 1 st Sem., 2009-10	Degree	: Ph.D.
Major	: Microbiology	Department	: Microbiology
Minor	: Molecular Biology and Biotechnology		
Thesis title	: Effect of Plant growth promoting rhizobacteria on growth, osmolytes and antioxidant status of pearl millet under drought condition		
Advisor	: Dr. Anita Sharma		

ABSTRACT

Drought is one of the most important manifestations of abiotic stress in plants. It is a major yield limiting factor of crop plants. In this regard, plant growth promoting rhizobacteria (PGPR) have been reported to play important role. Besides developing mechanisms for their own survival under stress, bacteria also impart some degree of tolerance to plants towards abiotic stresses. In this study, nine bacterial isolates (eight *Bacillus* sp and one *Pseudomonas*) were characterized for their plant growth promoting properties and tested for plant growth promotion in different varieties of four crops (pearl millet, sorghum, guar and moth). Out of 9 bacterial isolates, two isolates: P3 (*Pseudomonas fluorescens*) and 14B (*Bacillus cereus*) showed maximum plant growth promoting traits and were found to enhance plant growth of var. CZIC923 and CZP2k9 of pearl millet, respectively. 14B was found to be an endophyte through ARDRA. In an experiment for screening the genotypic specific behavior of pearl millet, six varieties of pearl millet inoculated with two PGPRs, var. CZIC923 was most responsive followed by CZP2k9 while var. CZP9603 was least responsive for plant growth promotory action of bacteria. The effect of three soil types on the performance of two PGPRs in two varieties (CZIC923 and CZP9603) of pearl millet was checked. Results showed the maximum plant growth in Pantnagar soil followed by soil of Ujhani and Mukteshwar. However, bacterial response was significantly higher in Ujhani soil. Bacterial growth and osmolyte production was checked in nutrient medium supplemented with different concentrations of polyethylene glycol (PEG). Both the isolates could grow up to water potential of -1.0 MPa and showed PGPR properties. In comparison to 14B, P3 produced increased level of exopolysaccharide (EPS), soluble sugar and proline. Three varieties of pearl millet viz. CZIC923, CZP2k9 and CZP9603 inoculated with P3 and 14B in a pot experiment under drought condition resulted in increased level of proline, antioxidant enzymes viz. Superoxide dismutase (SOD), Ascorbate peroxidase (APX), Catalase (CAT) and Guaiacol peroxidase (GPX) activities in plants. Among three varieties of pearl millet, var. CZIC923 was most drought tolerant while var. CZP9603 was susceptible. In vitro experiment using pearl millet variety CZIC923 and CZP9603 inoculated with P3 and 14B induced plant tolerance when exposed to drought (imposed by different concentration of PEG). Variety CZIC923 was highly drought tolerant as compared to var. CZP9603 as the response was further elevated by inoculating these bacteria which induced significant changes in the specific activity of different reactive oxygen species (ROS) scavenging enzymes (SOD, APX, CAT and GPX) and enhanced proline production, chlorophyll *a*, *b* and caretenoid level, relative water content of leaf, leaf EPS and reducing leaf water loss and electrolyte leakage of inoculated plants. The overall study showed the importance of rhizospheric bacteria in combating drought stress.

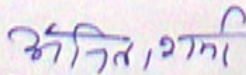

(Anita Sharma)
Advisor

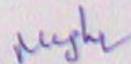

(Megha Pandey)
Authoress

विद्यार्थी का नाम : मेधा पांडे	परिचयांक संख्या : 35332
बठ्ठास एवं प्रवेश का वर्ष : प्रथम, 2009-10	उपाधि : पीएच0 डी0
मुख्य विषय : सूक्ष्म जीव विज्ञान	विभाग : सूक्ष्म जीव विज्ञान
सहायक विषय : आणविक जीव विज्ञान एवं जैव प्रौद्योगिकी	
शोध बन्ध शीर्षक : पादप वृद्धि कारक जीवाणुओं का बाजरा के वृद्धि, ओस्मोलाइट्स एवं एंटीऑक्सीडेंट स्थिति पर सूखा परिस्थितियों का प्रभाव।	
सलाहकार : डा0 अनीता शर्मा	

सारांश

सूखा, पौधों में अजीब तनाव में सबसे महत्वपूर्ण अभिव्यक्तियों में से एक है। यह फसली पौधों की सीमित उपज के लिए एक प्रमुख कारक है। इस संबंध में पौधों के विकास को बढ़ावा देने वाले जीवाणु महत्वपूर्ण भूमिका निभाते हैं। अजीब तनाव के तहत अपने स्वयं के अस्तित्व के लिए तंत्र विकसित करने के अलावा जीवाणु अजीब तनावों में पौधों के लिए सहिष्णुता प्रदान करते हैं। इस अध्ययन में नौ पृथक जीवाणुओं (आठ बैसिलस स्पीसीज और एक स्त्रैटोमोनास स्पीसीज) का उनके पादप वृद्धि कारक विशेषता एवं चार फसलों (बाजरा, ज्वार, ग्वार और मूँग) की विभिन्न किस्मों में पौधों की वृद्धि एवं विकास को बढ़ावा देने के लिए परीक्षण किया गया। नौ पृथक जीवाणुओं में से दो जीवाणुओं पी-3 (स्त्रैटोमोनास फ्लुओरिसन्स) तथा 14-बी (बैसिलस सिरस) ने पौधों में अधिकतम विकास गुणों को प्रदर्शित किया तथा बाजरा की दो किस्मों CZIC923 एवं CZP2k9 में पौधों की वृद्धि को बढ़ाया। आनुवंशिक विशिष्ट व्यवहार की छटनी के लिए बाजरा की छः किस्मों का दो पादप वृद्धि कारक जीवाणुओं के साथ परीक्षण किया गया। जीवाणुओं का पादप वृद्धि कारक प्रभाव CZIC923 में सबसे अधिक उसके बाद CZP2k9 में तथा सबसे कम CZP9603 में पाया गया। तीन मृदा प्रकार में इन जीवाणुओं के वृद्धि कारक प्रदर्शन को बाजरा की दो किस्मों (CZIC923 एवं CZP9603) के साथ परीक्षण किया गया। पतनगर मृदा में सबसे अधिक पौधों की वृद्धि दर्ज की गयी। उझानी मृदा में पौधों की वृद्धि में अच्छा जीवाणु उत्प्रेरक प्रभाव दिया, जबकि मुक्तेश्वर की मृदा में पौधों की वृद्धि में कोई वृद्धि कारक प्रभाव नहीं पाया गया। दोनों जीवाणुओं की वृद्धि तथा ओस्मोलाइट्स उत्पादकता को विभिन्न पोलिइथाईलीन ग्लाइकोल (PEG) सांद्रता से युक्त पुष्टिकार तरल माध्यम में देखा गया। दोनों जीवाणु-1 MPa के जलीय विभव में भी पादप वृद्धि कारक गुणों को प्रदर्शित कर सके। 14बी की तुलना में पी-3 ने अधिक एक्सोपोलीसेक्केराइड, घुलनशील शक्कर तथा प्रोलीन उत्पादित किया। तीन बाजरा की किस्मों (CZIC923, CZP2k9 तथा CZP9603) का पी-3 एवम 14बी के साथ सूखा परिस्थिति में गमलों में परीक्षण करने पर पौधों में प्रोलीन तथा एंटीऑक्सीडेंट जैसे सुपरऑक्साइड डिस्म्यूटेज, एस्कोर्बेट परॉक्सीडेज, कैटालेज तथा गॉयकॉल परॉक्सीडेज की सक्रियता अधिक पायी गयी। इन तीनों बाजरा की किस्मों में CZIC923 सूखे के प्रति अधिक सहनशील तथा CZP9603 कम सहनशील पाई गई। बाजरा की दो किस्मों CZIC923 एवं CZP9603 का जब पी-3 एवं 14बी के साथ सूखा परिस्थिति में (जो की विभिन्न पोलिइथाईलीन ग्लाइकोल (PEG) सांद्रता के साथ दिया गया) परीक्षण किया गया तो CZIC923, CZP9603 की तुलना में अधिक सहनशील पाई गयी। जीवाणु युक्त पौधों में प्रतिक्रियाशील ऑक्सीजन प्रजातियाँ (ROS) को निष्क्रिय करने वाले किण्वक (Enzyme) (सुपरऑक्साइड डिस्म्यूटेज, एस्कोर्बेट परॉक्सीडेज, कैटालेज तथा गॉयकॉल परॉक्सीडेज) की विशिष्ट सक्रियता प्रमापी पाई गयी साथ ही प्रोलीन, क्लोरोफिल ए, बी एवं केरेटीनोइड की मात्रा, पत्ती में सापेक्ष पानी की मात्रा, पत्तियों की एक्सोपोलीसेक्केराइड, पत्तियों में पानी की कम हानी तथा इलेक्ट्रोलाइट रिसाव अधिक पाई गयी। अंततः यह अध्ययन दर्शाता है कि मूल परिवेश में रहने वाले जीवाणु सूखा तनाव की परिस्थितियों में महत्वपूर्ण भूमिका निभाते हैं।


(अनीता शर्मा)
सलाहकार


(मेधा पांडे)
लेखिका