Biotic priming of *Abelmoschus esculentus* (L.) Moench seeds using various phosphate solubilizing bacteria against drought stress

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CERTIFICATE

This is to certify that the thesis entitled **"Biotic priming of *Abelmoschus esculentus* (L.) Moench seeds using various phosphate solubilizing bacteria against drought stress"** submitted to the University of Calicut by Smt. **Pravisya, P.,** in partial fulfillment of the requirements for the degree of **Doctor of Philosophy** in **Botany** of the **University of Calicut**, is a *bona fide* record of the research work undertaken by her in this department under my supervision and guidance and this work or any part of it has not been submitted elsewhere for the award of any other degree, diploma, associate ship, fellowship or other similar title or recognition.

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DECLARATION

I, **Pravisya, P.,** do hereby declare that the Ph. D thesis entitled **"Biotic priming of *Abelmoschus esculentus* (L.) Moench seeds using various phosphate solubilizing bacteria against drought stress"** is a research work accomplished by me under the supervision of **Dr. K. M. Jayaram, Professor (Rtd.), Plant Physiology and Biochemistry Division, Department of Botany, University of Calicut** in partial fulfillment of the requirements for the award of the degree of **Doctor of Philosophy** in **Botany, University of Calicut.** I also declare that any part of this work has not been submitted by me for the award of any other degree or diploma, and it represents original work done by me.

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ABBREVIATIONS

ABA	-	Abscisic acid
ABS/CSo	-	Absorption flux per cross section
AOS	-	Active oxygen species
APX	-	Ascorbate peroxidise
BC	-	*Burkholderia cepacia*
BS	-	*Bacillus subtilis*
BSA	-	Bovine serum albumin
CAT	-	Catalase
CFU	-	Colony forming unit
CSI	-	Chlorophyll stability index
DCMU	-	3 (3,4dichlorophenyl) -1, 1-dimethyl urea
DCPIP	-	2, 6, Dicholrophenolindophenol
DHAR	-	Dehydroascorbate reductase
DI/CSo	-	Dissipated energy flux per cross section
DIo/RC	-	Dissipated energy flux per reaction center
DTNB	-	5 , 5-dithio-bis(2-nitrobenzoic acid)
EDTA	-	Ethylenediamine tetra-acetic acid
ET/CSo	-	Electron transport flux per cross section
ETo/RC	-	electron transport flux per reaction center
Fm	-	Maximum Chl *a* fluorescence
Fv/Fo	-	The ratio of photochemical to non photochemical quantum efficiencies
GPOX	-	Guaiacol peroxidase
GR	-	Glutathione reductase
GS	-	Glutamine synthatase
H_2O_2	-	Hydrogen peroxide

HEPES	-	(N-(2-Hydroxyethyl) piperazine-N-(2- ethane sulphonic acid)
HSP	-	Heat shock protein
LEA	-	Late embryogenesis abundant proteins
MDA	-	Malondialdehyde
MDHA	-	Monodehydroascorbate
MDHAR	-	monodehydroascorbate reductase
mM	-	Millimolar
MV	-	Methyl viologen
NaCl	-	Sodium chloride
NADH	-	Nicotinamide adenine dinucleotide
NaN3	-	sodium azide
NBT	-	Nitroblue tetrazolium
NPQ	-	Non-photochemical quenching
$O_2^{\bullet-}$	-	Superoxide
^oC	-	Degree celsius
PBQ	-	Para-benzoqunone
PF	-	*Pseudomonas fluorescens*
PI(abs)	-	Performance index on absorption basis
PS I	-	Photosystem I
PSII	-	Photosystem II
RC/CSo	-	Density of active reaction centres per cross section
ROS	-	Reactive oxygen species
RPM	-	Rotation per minute
SEM	-	Scanning electron microscopy
SOD	-	Superoxide dismutase
TBA	-	Thiobarbituric acid
TCA	-	Trichloroacetic acid
TR/CSo	-	Trapping flux per cross section
TRo/RC	-	Trapping flux per reaction center

WS	-	Water stress
WW	-	Well watered
ΦEo	-	Electron transport quantum yield

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INTRODUCTION

Over the past decades, global climate change was faster due to increasing levels of atmospheric carbon dioxide (OECD, 2016). The climate change mainly disturbs the abiotic factors in the environment and results in various types of abiotic stresses such as drought, salinity and flood, where as abiotic stress induced by heavy metals are mainly due to anthropogenic activities. These changes are the major challenges in the agriculture sector. In fact, agriculture is closely interconnected with the environment and any changes in climatic condition directly affect the crop establishment and yield (Miguel and Nicholls, 2017). However, the effect of climate change seems to be varying in accordance with the nature of crop and stage of development at which the plants are exposed (Zhang *et al.,* 2018). Abiotic stress alters plant-pest interactions and enhances the susceptibility of host plants to pathogens and reduces competitive ability of host plants with weeds (Panday *et al*., 2017), so climate change indirectly causes induction of dual stress in plants.

Agriculture is a complex ecosystem which is closely related to climate, so any change in climate causes a reduction in productivity. As per the report of the Intergovernmental Panel on Climate Change (IPCC, 2014), many countries will experience an increase in average temperature, depletion of water resources, desertification and periods of heavy precipitation. Apart from these changes climate change can modify texture of soil, salinity and accumulation of other chemical compounds such as heavy metals, cause reduction in arable land. These changes make a drastic reduction in food production for the growing population of the world. So urgent steps are needed for increasing the production of major food grains like maize, rice and wheat, to around 70% of the existing quantity, to meet the global food requirement in 2050 (Hellin *et al*., 2012).

Climate change reduces the renewable surface water and ground water resources in most dry subtropical regions by an alternation in rain fall which results in water stress (IPCC, 2014). In agriculture, crop productivity is influenced by the water quality and quantity (Sarwat and Tuteja, 2017). The intensity of drought stress increases by about 50% to 200% on global agriculture productivity and reduced the agriculture land area during the 21st century (Zhao and Dai, 2017). Plants imposed to water stress at the reproductive stage shows a higher reduction in yield (Zhang *et al*., 2018). It is reported that water stress at flowering stage decline the rate of photosynthesis and results in decrease in the allocation of photosynthetic assimilates to floral organs, this in turn causes increased rate of flower abscission and eventually decreases the productivity.

Drought stress is the main factor that limits crop productivity. Various analyses have revealed that abiotic stress, due to drought, heat, cold and salinity are the major factors that prevents the crop from their full yield potential. Living organisms are subjected to water stress either due to water scarcity in the environment or due to non-availability of water because of the presence of excessive amount of salts in water. Water stress leads to substantial variation in morphology, anatomy, physiology and biochemistry of plants which ultimately reflected on the yield potentials (Kramer, 1969). It is one of the major environmental constraints on plant growth and combined with high temperature and soil physico-chemical deficiencies, restricts crop production all over the world (Ceccarelli, 1984; Agarwal *et al.,* 1999). Drought inhibits the photosynthesis of plants, causing changes in chlorophyll contents and damage to the photosynthetic apparatus and the photosynthetic electron transport through PSII was inhibited (Chakir and Jenson, 1999). Both short and prolonged period of water stress affect nutrient characteristics, photosynthesis and other metabolic activities of plants and ultimately the growth and productivity of such plants are adversely affected. Plants

encounters drought stress at the vegetative stage considerably reduces plant height (Singh and Singh, 2010).

Plants contain about 80-90% of water and drought stress induce water imbalance in plants which in turn cause growth retardation and increased wilting and nutrient deficiencies. To cope up with harmful effects plants shows various morphological, physiological, biochemical and genetic adaptations. To alleviate the harmful effect of ROS, plants accumulate low molecular weight substances as a first line defense, which cannot intercept the normal function of plants and are called osmolytes. Osmolytes reduces cell osmotic potential and improve water absorption and maintain cell turgor. Osmolytes are classified into two according to the nature of compound: 1. nitrogen-containing compounds (proline and other amino acids, quaternary ammonium compounds and polyamines) and 2. hydroxy compounds (sucrose, polyhydric alcohols and oligosaccharides) (McCue and Hanson, 1990).

Long term water stress disturbs the equilibrium between ROS production and their scavengers, the antioxidant enzymes, leading to the quick production of ROS in multiple sites of the plants including chloroplasts, mitochondria, peroxisomes, the endoplasmic reticulum and plasma membrane. Higher production of ROS was neutralized by increased activity of non enzymatic antioxidant enzymes (Zandalinas *et al*., 2018).

In chloroplasts, ROS generation occurs in different sites and mainly coupled with the electron transport chain (ETC) which acts as the primary source of singlet oxygen. Apart from this, singlet oxygen production may also take through membrane damage, catalyzed by lipoxygenase (Asada, 2006; Foyer and Noctor, 2003). Reduction in light absorption due to improper functioning of light harvesting complex leads to failure of electron acceptance by iron-sulfur centre. As a result Fe-S over reduction and induces the ROS production. These electrons reduces oxygen molecules in plant cell and which

trigger generation of superoxide radicals and hydrogen peroxide popularly known as Mehler reaction (Gill and Tuteja, 2010).Abiotic stress interrupt the balance between light harvesting and energy utilization extending the half-life of singlet chlorophyll (1Chl*). This can promote the production of triplet chlorophyll (3Chl*) which reacts with ground state triplet oxygen ($3O_2$) and results in the generation of singlet oxygen and damage photosystem II (Zolla and Rinalducci, 2002). According to Cruz de Carvalho (2008), cellular dehydration results in acceleration of the rate of Mehler reaction is up to 50% due to interruption in ETC. Any over reduction of the electron transport chain is expected to enhance the probability of singlet oxygen generation in PSII (Fischer *et al*., 2013).

Mitochondrial production of ROS is accompanied with normal respiration process, however various abiotic and biotic stress increase the rate of ROS production (Jezek and Hlavata, 2005; Navrot *et al*., 2007). During stressed condition insufficient NAD^+ supply and fully reduced ubiquinone in mitochondrial electron transport chain (complex I and II) favours the production of ROS (Torres, 2002). Drought induced enhancement in photorespiration leads to hydrogen peroxide production in peroxisomes (Sharma *et al.*, 2012). Peroxisomal H_2O_2 production in plants via the enhanced activity of glycolate oxidase plays an important role in photorespiration (Foyer *et al*., 2009). Drought stress accelerates stomatal closure and results in the reduction of intercellular carbon dioxide which facililitate increase in glycolate production and RuBP oxygenation (Cornic and Briantais, 1991), thus enhance the peroxisomal H_2O_2 generation. Cytosol act as a source of $O_2^{\cdot-}$ generation by the activity of enzymes located in them such as xanthine dehydrogenase and the aldehyde oxidase (Yesbergenova *et al*., 2005; Zarepour *et al*., 2010)

Plants exhibit a well known antioxidant system consisting of enzymatic antioxidants such as superoxide dismutase (SOD), peroxidases (POD), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR) and non-enzymatic antioxidants like ascorbate (AsA), glutathione (GSH), thioredoxin, carotenoids, tocopherols etc., for balancing the production of ROS (Gill and Tuteja 2010; Lata and Prasad, 2011). The efficiency of ROS scavenging enzymes reduces the consequence of oxidative stress in plants and the detoxification of ROS is a step by step process.

The superoxide radicals are highly reactive ROS, so plants quickly convert it into hydrogen peroxide and oxygen molecule. This reaction is catalyzed by the enzyme superoxide dismutase; with the help of metal ions Fe^{3+} and Cu^{2+}. This process is considered as a first step in ROS scavenging. Haber-Weiss process is another method to detoxify super oxide, in which superoxide radicals reduce metal ions and then react with hydrogen peroxide to form hydroxyl radicals through the second step, commonly known as Fenton reaction.

Hydrogen peroxide is detoxified by a number of enzymes; viz. peroxidases (POD) such as guaiacol peroxidase (GOPX), ascorbate peroxidase (APX) and catalase (CAT). Among this CAT is considered as the first line defender in H_2O_2 scavenging, which dissociates H_2O_2 into water and oxygen (Lata and Prasad, 2011). In the case of guaiacol peroxidases, guaiacol is used as the electron donor at the time of H_2O_2 neutralization (Asada, 2006).

Ascorbate peroxidase is another antioxidative enzyme mainly found in plants thylakoid membranes which effectively detoxify $\mathbf{H_2O_2}$ in the presence of ascorbate. Ascobate-glutathione cycle (AGC) commonly known as Halliwell-Asada pathway is a major pathway for scavenging H_2O_2 and regenerating ascorbic acid (AsA) and glutathione (GSH). Ascrobate-

glutathione cycle (AGC) is formed by following enzymes; ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate reductase (DHAR) and glutathione reductase (GR) and non enzymes like AsA and GSH (Gill and Tuteja, 2010).

The AGC cycle is initiated by oxidation of ascorbate by APX enzyme and resulting in the formation of monodehydroascorbate radical (MDHA). MDHA directly reduced in to AsA in the presence of NAD(P)H and MDHAR. Otherwise two molecules of MDHA dismutate to form AsA and DHA and the DHA is disproportionated into AsA by reduction with glutathione is catalyzed by DHAR located in the stroma. Regeneration of GSH was catalyzed by the enzyme GR with NADPH as the reductant (Heldth, 2005). Phenolics are a major non enzymatic antioxidant that serve as electron donors and stabilize unpaired electrons and terminaties the Fenton reaction (Jung *et al*., 2002). They have the ability to alter peroxidation kinetics through changing the lipid packing order and declining the membrane fluidity (Arora *et al*., 2000). These modifications prevent the diffusion of ROS and terminate the spreading of ROS chain (Jung *et al*., 2002).

Austin *et al.* (1969) reported that primed carrot seed showed faster growth in the field than untreated ones. According to the authors, this was due to the increased length of the embryo of the treated seeds, primarily due to the enhanced cell division during hardening treatment than due to cell expansion. Plants raised from primed seeds results earlier defense responses and exhibit an increased resistance to biotic or abiotic stresses compared with plants raised from unprimed or non primed seeds (Conrath, 2011; Balmer *et al*., 2015). Priming of seeds with various chemicals such as salicylic acid, 2,6-dichloroisonicotinic acid (Kauss *et al*., 1992), benzothiadiazole (Katz *et al*., 1998), β- aminobutyric acid (BABA) (Oostendorp *et al*., 2001) or even with water enable the plants to improve abiotic stress tolerance. Van Hulten *et al.*

(2006) opined that priming accelerated and increased the plant's ability to activate the defense mechanism to cope with certain stress conditions. In this perspective, priming represents an important ecological adaptation to resist environmental changes.

Microorganisms in soil play an important role in the germination of seeds, seedling growth and establishment. Plant growth promoting rhizobacteria (PGPR) is an important group of microbes that improve plant growth and development, under normal environmental conditions. These bacteria exist and proliferate in the rhizosphere and root exudates serve as a nutrient medium for microbial growth (Doornbos *et al*., 2012). Mainly rhizobacteria improve plant's stress tolerance in two ways (i.e. indirect and direct method of growth promotion); indirect growth promotion by preventing or decreasing destructive effects of plant pathogens (Nehl *et al*., 1997; Cartieaux *et al*., 2003), through the production of some metabolites which reduce pathogen population and increase the synthesis of siderophores which reduce the iron accessibility for certain pathogens (Kloepper, 1996; Arora *et al*., 2001; Bhattacharyya and Jha, 2012). PGPR can also enhance plant resistance by induced systemic resistance; resulting in the protection of plant from pathogen attack (Saravanakumar and Samiyappan, 2007). The direct growth promotion of the PGPR occurs in different ways; primary via facilitating and enrichment of nutrient in soil by fixing atmospheric nitrogen, solubilize minerals (so increase its availability to plants) and ultimately producing phytohormones (Patten and Glick, 2002). Apart from this beneficial role, these microorganisms reduce the harmful effect of different stresses in plants and increase plant's tolerance potential against various abiotic stresses like salinity, drought, flooding and heavy metal toxicity (Ma *et al*., 2011b).

Phosphorus (P) is a macro- nutrient element necessary for the early establishment and normal plant growth and development. It accelerates tillering, good pod formation and seed setting. But it's low mobility or insoluble form or even inadequate quantity in soil results poor uptake by plants, which consequently reduce growth and metabolism. Many soil micro-organisms are able to solubilize the unavailable forms of Calcium and Phosphorus through their metabolic activities. Obsessive and excessive use of chemical fertilizers coupled with irrigation has rendered the soils unfit for cultivation which can be replaced by environmental friendly liquid bio-fertilizers. Application of inoculums to the seeds of host plants are still a usual practice for improving plants tolerance potential (Graham-Weiss *et al.,* 1987).

Phosphorous deficiency occurs in both type of soil, including nutrient rich and nutrient deficient (Suriyagoda *et al*., 2011). Most of the Indian soils are deficient in available form of P and its requirement is met by the addition of phosphatic fertilizers but the use efficiency of applied Phosphorus rarely exceeds 30% due to its fixation as Fe and Al phosphates in acid soil and Ca and Mg phosphates in alkaline soils. In this context, phosphate solubilizing micro-organisms play an important role in the utilization of unavailable native phosphates as well as phosphates (Lagreid *et al.,* 1999).

Phosphorus solubilizing microorganisms (PSMs) play an important role in phosphorus enrichment in soil by mobilizing phosphorus through the release of organic and inorganic substances into soil (Sharma *et al*., 2013). This increases the availability of phosphorus in the soil for plants. Bacteria are observed as more effective than fungi in phosphorous solubilization (Sharma *et al*., 2013). Within soil bacterial flora, ectorhizospheric strains such as *Pseudomonas*, *Bacilli*, *Rhizobium*, *Enterobacter* and endo-symbiotic

rhizobia are recorded as successful strains of phosphate solubilizers (Khan *et al*., 2009). The phosphorus solubilizing bacteria (PSBs) such as *Bacillus megaterium, Bacillus circulans, Bacillus subtilis, Bacillus polymyxa, Bacillus ircalmous* and *Pseudomonas striata* are effective strains of phosphate solubilizers, which play an important role in improving the chemical and physical nature of the soil. It also increases the availability of minerals in the soil and accelerates the plant growth (Rodriguez and Fraga, 1999; Ravikumar *et al.,* 2010). Other than as a macroelement, phosphorus play an important role in drought stress alleviation in plants due to its significant role in root development and the ability to adjust physiological, morphological and biochemical processes of plant during stress exposure (Cortina *et al*., 2013; Liu *et al*., 2015).

Abelmoschus esculentus (L.) Moench (okra) is an economically important vegetable crop belongs to the family Malvaceae grew in tropical and sub-tropical regions of the world (Gemede *et al*., 2015). Although it's original home is Ethiopia, Sudan and North-Eastern African countries, India ranks number one in consumption. Okra is sensitive to frost, low temperature, water logging and drought conditions (Ariyo, 1993; Oyelade *et al.,* 2003). Fresh leaves, buds, flowers, pods, stems and seeds of okra are used for various purposes. The green tender fruits of okra are rich in vitamins, calcium, potassium and other mineral matters (Camciuc *et al.,* 1998) and consumed as vegetables for making salads, soups and stews, in fresh or dried, fried or boiled form. It is also well known that mucilage of okra has medicinal property; it binds to cholesterol and bile acid carrying toxins dumped into the liver. Okra seeds are rich in oil (20% to 40%) with linoleic acid as the important constituent which is essential for human nutrition (Andras *et al*., 2005). The fruit of okra has high quantity of soluble fiber as gums and pectin

which facilitate to lower serum cholesterol, reducing the risk of heart diseases. It is abundant in antioxidant enzymes which has important beneficial health effects on a number of human diseases like cardiovascular disease, type 2 diabetes, digestive diseases and some cancers (Kahlon *et al*., 2007; Atawodi *et al*., 2009; Sabitha *et al*., 2011). Jaleel *et al*. (2009) reported that drought stress in okra cause reduction in the plant length, number of leaves, leaf area photosynthetic pigments and yield. Okra plants tolerate drought stress up to a particular level but severe stress cause plant death.

Agriculture system in the state of Kerala is mainly depended on rain fall, but nowadays fluctuation in rainfall occurs which adversely affect the farming community and negatively affect agriculture productivity of the state. The potential of microorganisms to fight against drought is well studied. So the present investigation is focused to find out the suitable microorganism that improves okra plant growth and yield under drought conditions by subjecting the seeds to priming treatment and also the mechanism operating in ROS scavenging under stress.

Hypothesis

It is hypothesized that okra seeds treated with phosphate solubilizing bacteria show better resistance against water stress and the various metabolic pathways will be up/down regulated during the treatment. Keeping in mind the above hypothesis, the following objectives are put forth to prove the hypothesis.

Objectives

Major objectives of the present study are:

1) Inoculation of okra seeds with different species of Phosphate Solubilizing Bacteria (PSB) such as *Bacillus subtilis, Pseudomonas fluorescens* and *Burkholderia cepacia* to select the suitable bacteria strain that exhibiting better drought tolerance.

2) To find the effective concentration of PSBs for priming treatment of okra seeds.

3) To study the drought tolerance potential of okra (bacteria treated and untreated) by evaluating morphological, physiological and biochemical parameters.

4) To evaluate the effect of drought stress on different yield parameters in bacteria treated and untreated okra.

REVIEW OF LITERATURE

Plant growth and productivity are greatly influenced by various environmental factors like low and high light intensity, water availability, flooding, various salt and metal concentrations, low and high temperature, UV radiation and availability of the nutrients. Since plants are sessile, changes in these factors may impose stress condition in plants. As a consequence of these stresses, plants undergo various morphological, physiological, biochemical and molecular changes (Taiz and Zaiger, 2002). Extreme stress conditions enhance the production of various oxygen free radicals like super oxide, hydrogen peroxide, hydroxyl radicals and singlet oxygen and these active oxygen species react with proteins and lipids in the cell membrane and induce oxidative damages to cell membrane. Photosynthesis is greatly affected by reactive oxygen species (ROS) burst which mainly happens due to the denaturation of membrane protein and enzymes associated with various processes of photosynthesis. To alleviate the harmful effects of abiotic stresses plants exhibit the different mechanisms such as accumulation of osmolytes, enhanced activity of the antioxidant system, reduced leaf area, increased root length, increased cuticle thickening etc. These morphological and metabolic changes further results in the reduction in yield of many crop plants (Pandey *et al*., 2017).

Although about 70 % of the earth's surface is occupied by water (Küppers *et al*., 2014; Siddique and Bramley 2014), only about 2.5 % is freshwater (Gleick and Palaniappan, 2010) and all the living organisms depends upon this available water. Increased urbanization due to population explosion reduced the quality of water reflected as water deficiency stress in the agriculture sector. The declining trend of rainfall as a result of global warming also play a key role in water stress (Farahani *et al*., 2009; Mishra

and Cherkauer, 2010; Vadez *et al*., 2012). In India agricultural sector is mainly dependent on the rainfall. The changing climatic pattern contributes the severe reduction in availability of sufficient water supply, which in turn adversely affected the productivity and economy of the country.

Drought stress induces various morphological, physiological, anatomical, biochemical and molecular changes in plants to withstand the negative effect of stress and this changes ultimately leads to a reduction in the yield of plants. Alteration in the root, leaf area and growth of the plants are main morphological adaptations in response to water stress. Photosynthesis, respiration and stomatal conductance are the important physiological processes affected during water stress (Anjum *et al.*, 2011 a, b). Reduction in cellular water status induce the production of osmolytes like proline, sugar, glycine betaine, protein and active oxygen species (AOS) and also enhances the activity of the enzyme associated with scavenging AOS (Foyer and Shigeoka, 2010; Zlatev and Lidon, 2012). In order to cope up with water stress, plants over express some genes especially code for the stress resistant proteins, antioxidant enzymes etc. to induce the tolerance capacity of plants (Tuna *et al*., 2010). Drought stress results in anatomical variation in plants like distribution of sclerenchymatic cells in the cortex, variation in phloem and xylem diameter etc. in root which in turn facilitate conduction of water from dry soil (Canne-Hilliker and Kampny, 1991; Ristic and Cass, 1991; Makbul *et al*., 2008).

As a result of limited resource availability during water stress, plants reduce their growth rate which is observed as a reduction in shoot growth and leaf area (Schuppler *et al*., 1998; Parent *et al*., 2010). In crop plants, the first and foremost effect of drought is impaired germination and poor establishment (Harris *et al*., 2002). It is reported that cell elongation is reduced under water stress due to the interruption in water supply from the

xylem to cells (Nonami, 1998). Earlier findings suggest that water deficiency in cells inhibit cell division which results in growth retardation and reduction in leaf expansion (Rucker *et al*., 1995; Hussain *et al*., 2008). Optimization in leaf area due to water stress provides additional water saving under stress and increase water-use efficiency in plants (Condon *et al*., 2002). As a result of these, overall fresh and dry biomass production of plants will decrease (Zhao *et al*., 2006).

Enhancement in root growth was considered as an adaptive strategy towards drought stress tolerance and alternation in root morphology helps to improve water status under drought (Nguyen *et al*., 1997). Various studies conducted in *Catharanthus roseus*, maize and rice revealed that water stress results in the increase in root growth (Sacks *et al*., 1997; Kavar *et al*., 2008; Jaleel *et al*., 2008). Xyloglucan endotransglucosylase expression is known to be inducing in roots upon water stress which directly promotes the root growth and root surface area during water stress (Sengupta and Reddy, 2011). Drought imposes altered expression of different proteins (Dixit *et al*., 2014), for ensuring water availability under stress, which can improve photosynthesis and yield during the drought periods (Basu *et al*., 2016). Furthermore, it has been reported that water stress activate changes in root anatomy, such as the decrease in suberization and compact development of sclerenchyma layer, which improves the water retention during stress conditions (Huang and Fry 1998; Steudle, 2000; Henry *et al*., 2012)

Increased production of cytokinin in drought exposed plant's roots improves the growth and enhances the acquisition of nutrients and water in drought resistant plants (Dodd, 2003; Schachtman and Shin, 2007). Root-leaf signaling mediated through phytohormones such as abscisic acid (ABA), cytokinins, ethylene and photosynthetic enzyme like malate reduces water

loss through stomatal closure during drought (Guerrero and Mullet, 1986; Taiz and Zeigar, 2002).

Stomatal closure results in the reduction of carbon dioxide assimilation in plants, which in turn reduces photosynthesis and increase photorespiration (Athar and Ashraf, 2009; Flexas *et al*., 2004). Reduction in intercellular carbon dioxide distribution interrupts primary photochemistry of plants which further leads to the production of active oxygen species and thereby cause damages to photosynthetic pigments and apparatus (Lawlor and Cornic, 2002). Decrease in carbon influx during water stress damage RuBP, which decline Rubisco activitiy, ATP production and oxidation of nicotinamide adenine dinucleotide (NADPH) in the Calvin cycle (Farooq *et al*., 2012). According to Tausz *et al*. (2001), *Pinus canariensis* L. imposed to water stress decrease the rate of net photosynthesis by oxidative burst and reduced stomatal conductance. To reduce drought induced damages during photosynthesis, plants adopt various mechanisms like thermal energy dissipation, xanthophyll cycle and water-water cycle (Niyogi, 1999; Demmig-Adams and Adams, 1996; 2006).

Osmotic adjustment is a main adaptive mechanism exhibited by plants against drought stress to keep up normal cellular water content under stress conditions (Hsiao *et al*., 1976; Munns, 1988; Živčák *et al*., 2009). Plants achieve osmoregulation under stress by accumulating various low molecular weight substances called compatible solutes, like soluble sugars, sugar alcohols, proline, glycinebetaine (GB), organic acids, trehalose, etc., (Cechin *et al*., 2006; Kiani *et al*., 2007; Farooq *et al*., 2008, 2009a, b). These compatible solutes, reduce the harmful effect of drought stress mainly through two mechanisms: one is by accumulation of these solute in leaf, helps to maintain leaf turgor which regulates stomatal conductance and improve influx of carbon (Kiani *et al*., 2007), and second mechanism, enhancement of

osmolytes in root's increase the ability to intake water from the dry soil (Chimenti *et al*., 2006). Compatible solutes improve dehydration tolerance not only through osmotic adjustment but also *via* avoiding membrane damages by stabilizing proteins, lipids and enzymes (Le and McQueen-Mason, 2006; Ashraf and Foolad, 2007; Galvani, 2006).

Chloroplast, mitochondria and microbodies are important sources of ROS generation in plants (Dybing *et al*., 1976; Møller, 2001; Foyer and Noctor, 2003). Mehler-reaction occurring in chloroplast is the main source of ROS production during photosynthesis (Asada and Takahashi, 1987). Photorespiration in C3 plants is another way of production of ROS like hydrogen peroxide (Slesak *et al*., 2007). Moreover over reduction of electron transport chain in mitochondria and various detoxification mechanism operated by endoplasmic reticulum also act as ROS sources in plants under normal condition (Urban *et al*., 1997; Møller, 2001).

Plants possess dynamic antioxidant system in order to withstand the negative effect of reactive oxygen species and they keep equilibrium between the production of ROS and activity of the antioxidant system. But stress induced alteration in normal metabolic processes of plants cause the oxidative burst in them which enhances the activity of antioxidant system (Anjum *et al*., 2011a,b; Hasanuzzaman *et al*., 2013). ROS scavenging machinery is a combination of enzymatic and non enzymatic antioxidants. Where, superoxide dismutase (SOD), guaiacol peroxidases (GOPX), catalase (CAT), ascorbate peroxidase (APX), dehydroascorbate reductase (DHAR), monodehydroascorbate reductase (MDHAR), glutathione reductase (GR) comprises of enzymatic antioxidant system and ascorbate, glutathione, thioredoxin, carotenoids, tocopherols together form non-enzymatic antioxidant system (Gill and Tuteja 2010; Poiroux-Gonord *et al*., 2013; Das

and Roychoudhury, 2014). The performance of this antioxidant machinery during stress condition determines the stress tolerance potential of plants.

During stress, various metabolic changes help in increasing plant's stress tolerance potential, but simultaneously may reduce the yield in many crop plants. This loss in yield may be due to the increased energy need for operating these defense machineries. Drought induced reduction in crop yield not only depend upon the intensity of stress but also on the growth and age of plants which experience the stress. Studies conducted on this problem revealed that plants exposed to stress during flowering or reproductive stage of plants results in greater yield loss. Drought also causes a decrease in the quality of fruit or grain; and the reports indicate that drought stress exposed plants produced fruits with reduced starch, carbohydrate, lipid, protein and mineral content (Galieni *et al*., 2015**)**. Drought induced reduction in quality and quantity of food crops results in insecurity in food and nutrition for the growing population. So scientists are making efforts to introduce alternative methods to increase food production by producing plants having high stress tolerance and yield capacity.

Through conventional method like plant breeding (Atlin and Lafitte, 2002) successful abiotic stress tolerant varieties were produced especially against drought and salinity stress in major economically important crops like rice, maize, wheat, sugarcane, legumes, cotton etc. (Saranga *et al*., 2001; Valkoun, 2001; Witcombe *et al.*, 2008; Badu-Apraku and Yallou, 2009; Ashraf, 2010; Shahbaz and Ashraf, 2013). However the method of improving stresse tolerance in plants, production of tolerant variety through plant breeding is a time consuming process. Moreover, water stress tolerance in plants is a complex multigenic trait controlled by a large number of genes and loci on chromosomes collectively called as qualitative trait locus (QTL)

(Mohammadi *et al*., 2005). The interaction between QTLs and climate factors makes QTL mapping a risky task (Tuberosa and Salvi, 2006).

Transgenic methods are used to produce tolerant plants by transfer of desired genes (Ashraf, 2010). The method gives special emphasis to the genes which encode phytohormones, osmolytes and antioxidants playing important role in stress tolerance. The genes encoding glycine betaine expression improved drought stress tolerance in plants (Zhang *et al*., 2008a). Similarly, *Beta* gene in *Escherichia coli* incorporated in maize plant increase drought tolerance by improving glycine betaine production (Quan *et al*., 2004). Drought stress tolerance in soybean (*Glycine max* (L.) Merr.) and tobacco (*Nicotiana tabacum* L.) was improved by engineering genes responsible for proline synthesis (De Ronde *et al*., 2004; Gubiš *et al*., 2007). According to Kudo *et al*. (2017) over expression of *DREB1A and OsPIL1* genes improved drought tolerance in transgenic plants. Over expression of the gene encodes for superoxide dismutase has been engineered and found to improve drought tolerance in alfalfa, potato and rice (Perl *et al*., 1993; McKersie *et al*., 1996; Wang *et al*., 2005). GM tobacco was produced by the over expression of genes for APX and MDHAR synthesis (Eltayeb *et al*., 2007). Transferring of LEA protein genes enhance drought tolerance in wheat, sorghum, and rice (Xu *et al*., 1994; Cheng *et al*., 2002).

Even though GM crop production promises the stress tolerance to plants there are many problems associated with GM crops. Some problems associated with GM crops are toxicity, allergenicity, intolerance, poor nutritional quality and safety of the food for human consumption (Costa *et al*., 2011). Various reports suggests the negative impact of GM crops in environment and human health are due to (i) gene flow to weed plants through pollination increase its ecological fitness; (ii) loss of natural germplasm; (iii) increase the resistance of unwanted microorganism through

gene flow; (iv) its interaction may destroy potential non-target organisms (Dale *et al*., 2002; Velkov *et al*., 2005; Chandler and Dunwell, 2008; Warwick *et al*., 2009). Apart from this drawback, the technique is expensive and not easy to practice.

Worldwide efforts are taken by researchers to identify a technically feasible, cost-effective strategy suitable for farmers for practicing in field level. In the recent years the phenomenon of 'priming' of plants was noticed as one among the effective methods to manage drought stress without compromising much on the yield. This technique overcomes many of the drawbacks of breeding and transgenic method. In 'priming' seedling/seed attain a unique physiological state called 'primed' state, after a pre-exposure or pre soaking to a priming agent and which will enhance the ability of the plant to withstand a subsequent harsher environment (Goellner and Conrath, 2008). Primed plants exhibit a quick and rapid response to the stressed condition, i.e., in the primed state, amplification of innate/ inherent stress tolerance capacity of plants was enhanced (Conrath *et al*., 2006; Jakab *et al*., 2005).

Seed priming is defined as pre-soaking of seeds with a solution containing priming agent and after that, the hydrated seeds are dried, this initiate germination metabolisms within the seeds except for the radical appearance (McDonald, 1999). The technique of priming using living organisms is known as biological or biotic priming (McQuilken *et al*., 1998; Ashraf and Foolad, 2005). Reddy (2012) explained 'biopriming' more in biotic stress control aspect and reported that application of beneficial bacteria inoculums in the seeds and protect the plants from disease. Biopriming help to initiate various physiological processes related to germination in seeds but with inhibited the plumule and radicle emergence until the seeds sown in soil or germinating medium (Anitha *et al*., 2013). Seed priming with bacteria

results in bacteria multiplication in the spermosphere even before sowing and it ensures the earlier infection of endophytic bacteria in seeds (Taylor and Harman 1990). These events induce quick germination and growth of seedlings (Moeinzadeh *et al*., 2010). Priming with plant growth promoting rhizobacteria has been reported in various crops such as carrot, sweet corn (Callan *et al*., 1990, 1991) and tomato (Harman and Taylor, 1988; Legro and Satter, 1995; Warren and Bennett, 2000; Jensen *et al*., 2001). PGPR is a major group of root-colonizing bacteria that have received global attention and they exhibit capacity to produce different types of enzymes and metabolites which improve plants tolerance potential against biotic and abiotic stresses (Mayak *et al*., 2004; Glick *et al*., 2007; Kim *et al*., 2009; Pineda *et al*., 2013; Chauhan *et al*., 2015). PGPR play a key role in plant growth promotion by nutrient acquisition and disease control (Yang *et al*., 2009; Dimpka *et al*., 2009; Grover *et al*., 2010). The advantages of PGPR is that it can make association with both monocotyledonous and dicotyledonous plants and offer biotic and/or abiotic stress tolerance to wide range of plants (Timmusk and Wagner, 1999; Timmusk *et al*., 1999; Mayak *et al*., 2004; Sandhya *et al*., 2009; Kasim *et al*., 2013; Coleman-Derr and Tringe, 2014). PGPR improves plant's drought tolerant capacity by altering physiological, molecular and biochemical metabolisms such as water and nutrient relations, carbohydrate metabolism, protein metabolism, hormone metabolism as well as antioxidant defense responses (Huang *et al*., 2014).

Earlier studies suggest that plant-associated microorganisms can improve plant's responses to various environmental stresses, including water stress (Budak *et al*., 2013; Cooper *et al*., 2014). Investigations on plant-microbial association suggest that plant growth promoting bacteria not only improve plants stress tolerance but also increase the crop productivity (Mayak *et al*., 2004; Glick *et al*., 2007; Marulanda *et al*., 2009; Yang *et al*., 2009). Among the various plant microbal interactions, the association between

mycorrhizhal fungi and plants were well studied (Rodriguez and Redman, 2008; Bonfante and Anca, 2009; Singh *et al*., 2011; Aroca and Ruíz-Lozan, 2012; Azcon *et al*., 2013)

Root morphology is an important trait in drought stress tolerance and enhanced root system improves water absorption (Bacon *et al*., 2002; Yu *et al*., 2007; Huang *et al*., 2014). Root length and diameter, number of the secondary root, production of root hairs etc. determine the capacity of water absorption (de Dorlodot *et al*., 2007; Vacheron *et al*., 2013). Roots show morphological changes during stress condition depending upon soil nature (Bengough *et al*., 2006; Forde, 2009; Tuberosa, 2012). PGPR inoculation with various plants was reported to improve root growth (Kloepper, 1992; Kloepper *et al*., 2004; Ngumbi, 2011). Moreover, it is suggested that PGPR induces alterations in root morphology and improve root surface area, thereby leads to increase in water and nutrient intake which directly enhance plant growth (Somers *et al*., 2004; Timmusk *et al*., 2014).

Naseem and Bano (2014) reported that maize seeds inoculated with *Alcaligenes faecalis* (AF3) showed a 10% increase in root length as compared to untreated plants under water stress. They suggest that this enhancement in root improved water absorption under stress and increases the drought tolerance potential of maize. Naveed *et al*. (2014) found that maize treated with *Burkholderia phytofirmans* strain PsJN enhanced root biomass by 70 and 58% in Mazurka and Kaleo cultivars respectively. *Enterobactor* sp treatment in same variety improved root growth by 47 and 40% respectively over their control plants under dehydration stress. Similar to this report Yasmin *et al*. (2013) reported that maize treated with PGPR increased root length by 43.3% during water stress. Wheat inoculated with *Bacillus thuringiensis* showed two to three fold longer root hairs and denser lateral roots (Timmusk *et al*., 2014).

Furthermore, these studies prove that PGPR inoculation improves plant water absorption under drought stress by alternating the root morphology.

Plants inoculated with PGPR maintain normal shoot growth under stress and this resulted in enhanced yield of plants. Vardharajula *et al*. (2011) reported that inoculation of *Bacillus* sp in maize enhances shoot growth during water deficient condition as compared to un-inoculated plants. Similar observations were reported by Timmusk *et al*. (2014) in wheat plants inoculated with PGPR showed 78% increase in biomass. Studies conducted by Lim and Kim (2013) reported that pepper inoculated with *Bacillus licheniformis* K11 enhanced their biomass by 50% under drought. Similar observation was reported in different crops including sorghum, wheat, sunflower green gram, mung bean and maize (Sandhya *et al*., 2010; Arzanesh *et al*., 2011; Saravanakumar *et al*., 2011; Castillo *et al*., 2013; Kasim *et al*., 2013; Grover *et al*., 2014; Naseem and Bano, 2014; Naveed *et al*., 2014; Sarma and Saikia, 2014).

Photosynthesis is the most affected physiological process during water stress. Under water deficient condition, the chlorophyll content decreases due to degradation by production of ROS leading to reduction in photosynthesis. Pre-treatment of soyabean with *Pseudomonas putida* H2-3 improved photosynthesis during stress by increasing chlorophyll content which in turn resulted in increased plant growth (Kang *et al*., 2014a). Similar observations were reported in various plants like *Ocimum basilicum,* wheat, runner bean, coriander, *Arachis hypogaea* L. (Heidari and Golpayegani, 2012; Stefan *et al*., 2013; Mathivanan *et al*., 2017; Vishwakarma *et al*., 2017; Warwate *et al*., 2017). PGPR inoculation improves the maximum quantum yield of PSII thus alleviate the effect of drought stress during photosynthesis (Briantais *et al*., 1996). Fv/Fm (maximum quantum yield of PSII) values of PGPR inoculated potato plant were were observed in the range of 0.79 to 0.83 during drought

stress, whereas the PGPR untreated plants had aquantum yield of 0.45–0.71 (Gururani *et al.,* 2013). Performance index (PI) of cotton, was also improved in PGPR treated drought exposed plants. The PI represents overall performance of PSII i.e., the density of the reaction centers in the chlorophyll pool, trapped excitation energy and its conversion in the electron transport chain (Strasser *et al*., 1999; Tsimilli-Michael *et al*., 2000). Maize seeds treated with *Burkholderia* sp, exhibit high water use efficiency under drought stress and thereby increased the photosynthetic rate and a reduction in the rate of transpiration (Fan *et al*., 2015). It was also reported that, the treatment with *Burkholderia* improved the stomatal conductance without increasing water loss through transpiration. A similar observation was also reported in grapevines and soybean (Zhang *et al*., 1997; Rolli *et al*., 2015).

Water stress results in alternation in various physio-chemical properties of soil and this negatively affect the growth of soil microorganisms. To mitigate the harmful effects of water stress, bacteria like PGPR produce exopolysaccharides (EPS) (Roberson and Firestone, 1992). This exopolysaccharide improves soil water holding capacity and soil permeability by improving soil aggregation which maintains higher water potential around the roots (Miller and Wood, 1996; Alami *et al*., 2000; Selvakumar *et al*., 2012). Naseem and Bano, (2014) reported that maize seeds inoculated with *Proteus penneri* (Pp1), *Pseudomonas aeruginosa* (Pa2) and *Alcaligenes faecalis* (AF3) increased soil moisture content under drought stress condition.

Plant growth promoting bacteria improve nutrient availability during favorable and unfavorable situation through acidification of the soil by secreting low molecular weight organic acids such as gluconic acid that chelates the cations bound to phosphate (Richardson *et al*., 2009, Kundan *et al*., 2015). According to Kausar *et al*. (2018) PGPR inoculation in groundnut seeds improves nutrient availability by reducing soil pH. Delshadi *et al*.

(2017) observed that PGPR treated *Onobrychis sativa* exposed to drought stress showed a reduction in soil pH during stress condition which helps the plants to absorb nutrients from the soil and maintain normal growth.

Leaf relative water content (RWC) is taken as an important parameter to measure plant water status. Reduction in RWC is an indirect measurement of cell turgor loss, which decreases in cell expansion as a consequence of stress in plants (Ashraf, 2010; Lu *et al*., 2010; Castillo *et al*., 2013). It was reported that plants showed better RWC in drought condition as a part of adaptation strategy (Jarvis and Jarvis, 1963). RWC is used as a good parameter for evaluating tolerance potential of PGPR priming against drought stress. Grover *et al*. (2014) observed that sorghum seeds primed with PGPR, *Bacillu*s spstrain KB 129 increased RWC by 24 % under drought. Similar observation was reported in maize (Sandhya *et al*., 2010; Vardharajula *et al*., 2011; Bano *et al*., 2013; Naveed *et al*., 2014; Naseem and Bano, 2014) that higher RWC helps the plants to cope up with oxidative and osmotic stresses imposed by water stress; this would also improve the productivity of plant. According to the observation of Casanovas *et al*., (2002) in *Zea mays* seeds inoculated with *Azospirillum brasilense* BR11005spp RWC was increases due to the bacterial absicisic acid (ABA) that stimulate stomatal closure, thereby alleviating water loss under drought. The findings of Dodd *et al*. (2010) also supported that PGPR improve plant's RWC through alterations various physiological processes

Increase in osmolytes in PGPR treated plants was reported in various plants, such as maize (Sandhya *et al*., 2010; Vardharajula *et al*., 2011; Naseem and Bano, 2014), sorghum (Grover *et al*., 2014), potato plants (Gururani *et al*., 2013), mung bean (Sarma and Saikia, 2014), and *Arabidopsis thaliana* L. (Cohen *et al*., 2015). Wang *et al*. (2012) observed that cucumber inoculated with a mixture of three PGPR strains (*Bacillus cereus* AR156,

Bacillus subtilis SM21 and *Serratia* sp. XY21) increased leaf proline contents by 3-4 folds when compared to untreated plants and they suggested that the enhancement in leaf proline protect the cucumber from dehydration. According to Paul *et al*. (2008) PGPR secretes osmolytes in response to water stress and encourage plant growth. Inoculation of *Pseudomonas putida* GAP-P45 in maize helped to keep the plant biomass, RWC and OP during drought stress through higher production of proline (Sandhya *et al*., 2010). Similar result was observed in maize plant treated with *Pseudomonas fluorescens* (Ansary *et al*., 2012). *B. thuringiensis* (Bt) treatment enhanced proline production in *L. dentate* which improve drought alleviation capacity (Armada *et al*., 2014). Sandhya *et al*. (2010) suggested that the enhancement of proline production in PGPR treated plants is as a result of up regulation of genes participated in proline synthesis and accumulation of proline facilitate to retain cellular water status and protect membrane proteins and lipid from oxidative stress. Like this, PGPR mixture containing *Pseudomonas jessenii* R62, *Pseudomonas synxantha* R81 and *Arthrobacter nitroguajacolicus* strainYB3, strain YB5 enhanced plant growth in water stress tolerant (Sahbhagi) and water stress sensitive (IR-64) rice cultivars (Gusain *et al*., 2015). Proline accumulation under water stress is stress tolerant and suceptible varieties accumulated proline in different levels and this suggest that proline play an important role in osmoregulation under drought (Gusain *et al*., 2015).

Different reports suggest that maize treated with PGPR *Pseudomonas putida* GAP-P45 (Sandhya *et al*., 2010) and *Azospirillum lipoferum* (Bano *et al*., 2013) could mitigate the harmful effect of drought by accumulating free amino acids and soluble sugars during stress. Over expression of the gene trehalose-6-phosphate synthase responsible for the synthesis of trehalose (a sugar actively participated in osmoregulation) in *Phaseolus vulgaris* treated with *Rhizobium etli* improved the stability of membrane and enzymes under

stress condition (Suarez *et al*., 2008; Yang *et al*., 2010). It is well known that trehalose plays a significant role as a signaling molecule in plants under stress condition (Paul *et al*., 2008). Thus activation of sugar metabolism in PGPR treated plants in response to stress is an important strategy for improving plant growth and yield under stress condition (Rodriguez *et al*., 2008).

Various reports suggest that PGPR improves glycine betaine synthesis under stress condition by accumulating choline. *B. subtilis* GB03 inoculated *Arabidopsis* (Zhang *et al*., 2010) and *Klebsiella variicola* F2, *P. fluorescens* YX2 and *Raoultella planticola* YL2 treated in maize recorded higher production of choline which in turn results in the enhancement in glycine betaine (GB) metabolism. Enhancement in GB content stimulated leaf relative water content (LRWC) retention capacity and also improved the dry weight of plants (Glick *et al*., 2007; Zhang *et al*., 2010; Gou *et al*., 2015). According to Zhang *et al*. (2010) over expression of PEAMT gene under osmotic stress in *Arabidopsis* inoculated with *B. subtilis* GB03 resulted in the increase in choline and glycine betaine contents. Augmentation in GB production induced by PGPR, reduced water loss due to osmotic stress (Nadeem *et al*., 2010; Bashan *et al*., 2014). Accumulation of polyamines also effectively mitigated osmotic stress and cadaverine produced by bacteria is synergetic to polyamines that improved root growth in plants. Enhanced production of cadaverine produced by *A. brasilense* Az39 stimulated root growth and helped to mitigate osmotic stress in rice Cassan *et al*. (2009).

It is well known that plants have enzymatic and non-enzymatic scavenging enzymes to alleviate the harmful effects of stress (Helena and Carvalho, 2008; Simova-Stoilova *et al*., 2008). Evaluation of the specific activity of antioxidant enzyme upon water stress is an important tool to understand the tolerance capacity of plants against stress condition. Various investigations revealed that there is a positive correlation between enhanced

antioxidant system activity and the tolerance potential of plants (Contour-Ansel *et al*., 2006; Guo *et al*., 2006). Drought stress tolerant plants exhibit an effective active oxygen scavenging system (Apel and Hirt, 2004; Huang *et al*., 2014). Hence several studies conducted on drought stress tolerance potential through bacteria treatment consider the specific activity of antioxidant enzymes as an important parameter to measure the tolerance capacity of the plant during water stress.

Potato inoculated with *Bacillus pumilus* str. DH-11 and *Bacillus firmus* str. 40, recorded an up-regulation of the specific activity of antioxidant enzymes like ascorbate peroxidase and catalase (Gururani *et al*., 2013). They also noticed that the CAT activity of PGPR treated plants was two times higher than untreated plants under water stress and the increase in the activity of reactive oxygen scavenging system upon drought is the foremost reason for the enhancement of drought tolerant potential in potato. According to Saravanakumar *et al*. (2011) improvement in the activity of CAT in green gram inoculated with *Pseudomonas fluorescens* Pf1 and *Bacillus subtilis* EPB is positively correlated with drought tolerance. PGPR mediated increase in CAT activity and improvement in water stress tolerance was also observed in different crop plants like cucumber (Wang *et al*., 2012), maize (Sandhya *et al*., 2010; Sarma and Saikia, 2014; Vardharajula *et al*., 2011) and wheat (Kasim *et al*., 2013). The previous investigations clearly indicated that the significant role of antioxidant enzymes in PGPR-mediated drought tolerance. Gururani *et al*. (2013) observed that higher accumulation of enzymatic and non enzymatic antioxidants in *Solanum tuberosum* inoculated with PGPR.

Non enzymatic antioxidant system in plants mainly consists of ascorbate, glutathione, flavonoids, carotenoids and tocopherols (Ma *et al*., 2008). Among these compounds, glutathione and ascorbate are major non enzymatic antioxidants that scavenge reactive oxygen species associated with

AsA-GSH cycle (Noctor and Foyer, 1998). There are fewer reports about bacteria mediated augmentation in AsA and GSH content in stressed plants, Ruíz-Sánchez *et al*. (2011) reported that co-inoculation of *Azospirillum* and *Arbuscular* mycorrhiza in rice enhanced the AsA and GSH content during water deficient condition. A similar observation was reported by Armada *et al*. (2014) in maize plant co-inoculated with mycorrhizal fungi and *Bacillus thuringiensis*

The equilibrium between the phytohormones such as auxins, gibberellins (GAs), cytokinins (CKs), ethylene (ET), and abscisic acid (ABA) regulate the plant growth. Auxin, GAs and CKs stimulate plant growth but ethylene and abscisic acid inhibit growth (Taiz and Zeiger, 2010). Drought stress is known to facilitate the increase in the production of plant growth inhibiting hormones like ethylene and abscisic acid (Farooq *et al*., 2009b). According to Dodd *et al*. (2010) and Bresson *et al*. (2014) plant growth promoting bacteria stabilize growth by altering plant hormone system. PGPR promote the reduction in ethylene synthesis (Glick *et al*., 1998; Belimov *et al*., 2009), change in the equilibrium between cytokinins and abscisic acid (Figueiredo *et al*., 2008; Cohen *et al*., 2009) also modify auxin signaling (Contesto *et al*., 2010).

Among the phytohormones, auxin (IAA), play a key role in plant growth and development, it regulates the different type of cellular metabolisms such as vascular tissue differentiation, production of lateral roots and adventitious roots, cell division, stem and root elongation, and gravitropism (Glick, 1995). Inoculation of *Pseudomonas putida* and *Bacillus megaterium* to *Trifolium repens* L. enhanced shoot and root growth and water content during drought and these changes are positively correlated with increase in the auxin production by PGPR treatment (Marulanda *et al*., 2009). *Phyllobacterium brassicacearum* treated *Arabidopsis* plants recorded higher

modification in root topology and this was due to the higher production of IAA in these plants (Contesto *et al*., 2010; Bresson *et al*., 2014). Modification in root topology helps the plants to mitigate the negative effect of drought stress.

Higher production of ethylene is observed in response to various stresses signaling like water stress, extreme temperature, salinity, heavy metal toxicity etc. (Johnson and Ecker, 1998). 1-aminocyclopropane-1- carboxylate (ACC) is the intermediate product of ethylene in higher plants. Previous studies suggested that plant growth promoting bacteria produce an enzyme called ACC deaminase which hydrolyzes 1-Aminocyclopropane-1-carboxylate into ammonia and alpha-ketobutyrate thus reduce the production of ethylene during stress condition (Glick *et al*., 1998; Shaharoona *et al*., 2006). As a result of deactivation of ACC, plants can maintain normal growth under stressed condition (Siddikee *et al*., 2011). Arshad *et al*. (2008) reported that pea inoculated with *Pseudomonas* sp. reduced the production of ethylene during drought stress. *Solanum lycopersicum* and *Capsicum annuum* treated with *Achromobacter piechaudii* ARV8 recorded maximum reduction in ethylene synthesis during stress and ameliorate the deleterious effect of drought stress (Mayak *et al*., 2004). A similar observation was reported by Lim and Kim (2013) in *Bacillus licheniformis* K11 inoculated pepper plant, which could tolerate drought stress and had better survival compared to non-treated plants.

Abscisic acid is known as an antitranspirant and it regulates stomatal movement during drought (Porcel *et al*., 2014; Cohen *et al*., 2015). While stomatal closure during drought inhibits water loss through transpiration, ABA plays an important role in drought stress tolerance. Plant growth promoting bacteria induce the production of ABA during stress and thereby increase drought stress tolerance. Higher production of ABA in *Bacillus* inoculated lettuce under drought was observed by Arkhipova *et al*. (2007) and they suggested that this enhancement in ABA induce stress tolerance in

lettuce. According to Cohen *et al*. (2008) *Arabidopsis* treated with *Azospirillum brasilense* Sp245 produce a higher amount of ABA content and the enhancement was significantly correlated with drought alleviation potential of plants. Various studies revealed that increased drought tolerance as a result of enhancement in ABA production is due to the reduction in transpiration, root hydraulic conductivity and up regulation of aquaporin activity (Aroca *et al*., 2006; Zhou *et al*., 2012).

Overall activation of stress tolerance mechanisms cause a higher reduction in the yield of crop plants. Sivakalai and Krishnaveni (2017) reported that seed biopriming with *Azospirillum* and *Pseudomonas fluorescens* in pumpkin increase yield and yield components. Previous results suggest that *Pseudomonas fluorescens* inoculation in sugar beets can improve fruit length, girth, circumference and 100 seed weight (Suslow and Schroth, 1982). Aamir *et al.* (2013) report that the number of pods and yield were increased in mung bean treated with Rhizobium and PGPR. Priming with *Azospirillum, Azotobacter,* and *Pseudomonas,* enhanced the crop growth rate, yield and quality in various medicinal plants (Baser-Kouchebagh *et al.,* 2013). Sakthivel *et al.,* (2009) noticed that the co-inoculation of *Pseudomonas* + *Azotobacter* + *Azospirillum* in tomato variety PKM-1 increased fruit yield. According to Gravel *et al.* (2007) co-inoculation of *Pseudomonas putida* and *Trichoderma atroviride,* in hydroponic condition promoted the reproductive growth of tomato. Priming enriches grain mineral content in both stressed and unstressed conditions. Enhancement in water absorption under stressed and unstressed condition increased the nutrient content in barley (Tabassum *et al*., 2018). The increase in chlorophyll contents and water usage efficiency caused better protection against membrane damage during stress which might have increased the pollen viability and assimilate partitioning, thus resulted in the yield of better number of grains and grain weight (Arshad *et al*., 2017) under drought stress.

MATERIALS AND METHODS

3.1 Plant material

Seeds of *Abelmoschus esculentus* (L.) Moench (Okra) cv Arka anamika, procured from Regional Agricultural Research Station (Palakkad Disrict, Kerala State) were used to raise plants for the present study.

3.2 Bacteria strains

The bacteria strains such as, *Bacillus subtilis* 10224 (BS), *Pseudomonas fluorescens* 9768 (PF) and *Burkholderia cepacia*10618 (BC) for the experimental studies were collected from IMTECH, Chandigarh, India. *Bacillus subtilis* (BS) was maintained on nutrient agar medium and the inoculum was prepared by incubating two-day old cultures of BS at 28ºC on a rotary shaker (150 rpm) for 48 hrs in nutrient broth. The inoculum of *Pseudomonas fluorescens* (PF) was maintained in King's B medium prepared by incubating one day old culture of PF at 28ºC on a rotary shaker at 150 rpm for 24 hrs in nutrient broth. The bacteria strain of *Burkholderia cepacia*(BC) was maintained in tryptic soy agar and the inoculum was prepared by incubating two day old cultures of BC at 28ºC on a rotary shaker (150 rpm) for 48 hrs in nutrient broth. All the bacteria cultures when attained a concentration of approximately 10^{-7}cfu (Colony Forming Units) ml (0.5 OD at 600 nm) was used as inoculums for the priming treatment.

Fig. 1: Bacteria strains for inoculation a) *Bacillus subtilis*(BS), b) *Pseudomonas fluorescens* (PF) and c) *Burkholderia cepacia* (BC)

3.3 Priming treatment

Healthy seeds of okra were were selected and sterilized with 1% (w/v) sodium hypochlorite solution for 5 min, followed by several washing with double distilled water. Then inoculated with 25 ml each of the inoculums of *B. subtilis*, *P. fluorescens* and *B. cepacia* at a concentration of 10^{-7} CFUml. Pre-weighed healthy okra seeds were subjected to priming treatment by soaking the seeds in the bacteria cultures for 6 hrs and then air dried until the inoculated seeds attain a weight equal to the initial weight. These primed seeds were used for the field experiment.

3.4 Field experiment

The seeds primed (treated) with *B. subtilis*, *P. fluorescens* and *B. cepacia* and unprimed/untreated (control) were sown in polythene bags filled with 5 kg of sterilized soil, and after the establishment only one healthy plant was retained in each bag. Fifty plants were chosen from the control and treated sets of each bacteria strain. After 17 days of vegetative growth, the 50 plants from each set were equally divided into two sets each. One set each of all the bacteria treated and control plants, was regularly watered with 500 ml of water and the other set was kept without water for 7 days. The leaf samples were collected for physiological and biochemical analyses on the 0^{th}, 1^{st}, 3^{rd}, 5^{th} and 7^{th} day. After 7 days of drought stress, all the plants were watered uniformly and the leaf samples were collected on the 1^{st}, 2^{nd} and 3^{rd} days of re-watering, to determine the physiological and biochemical changes, if any, during this period. Physiological and biochemical parameters were analyzed for determining the drought tolerance potential and recovery mechanisms. The field experiments were conducted in a randomized block design in a green house, at a temperature of 28 ± 2 °C, the light intensity of 150 µmol m^2s^{-1} and relative humidity of 60 ±5 %. The third leaf from the base was used for the physiological and biochemical analyses.

3.5 Determination of effective concentration of different bacteria inoculums

For determining effective concentration of different bacteria inoculums, okra seeds were inoculated with different concentration of bacteria culture (10^{-5}, $10^{-6,}$ $10^{-7,}$ 10^{-8} and 10^{-9} cfu) by serial dilution. The concentration showed maximum enhancement in growth attributes and drought stress tolerance when compared to other concentration were selected via analyze leaf moisture and chlorophyll stability index.

3.5.1 Determination of leaf moisture content (LMC)

To determine the leaf moisture content (LMC), freshly collected samples of leaves were cut into small discs and measured the fresh weight (FW) using an electronic balance. Immediately after taking the fresh weight, the samples were kept in a hot air oven initially at 100^{o}C for one hour, then the temperature of the oven was adjusted to 60^{o}C and the samples were kept overnight. Dry weight (DW) of the samples was measured on the next day and the process of drying and weighing was repeated until the dry weight of the sample becomes constant.

Leaf Moisture Content % = [(Fresh weight-Dry weight) ÷ (Fresh weight)] x100

3.5.2 Determination of chlorophyll stability index (CSI)

Chlorophyll was extracted according to the method of Arnon (1949) described in 3.8.1 and CSI calculated by following formula,

CSI= Total chlorophyll of stressed plant / Total chlorophyll of control plant

3.6 Determination of bacteria association with okra root using scanning electron microscope (SEM)

To determine the association of bacteria with okra root using scanning electron microscope (SEM), roots were fixed in 2.5% glutaraldehyde,

prepared in 0.1 M phosphate buffer (pH 7.2) for 5 minutes. Fixed specimens were rinsed twice with phosphate buffer and dehydrated by passing through an ascending series of acetone (70, 80, 90 and 100%). Five minutes incubation time was provided in each acetone series. Dried specimens were mounted on to grooves cut on aluminium stubs using double side adhesive conducting carbon tapes to expose the sections. Then the specimens were gold-palladium coated and further photomicrographs were taken using the photographic attachment of the FESEM (Carl-Zeiss Gemini 300 Field Emission Scanning Electron Microscope).

3.7 Morphological parameters

3.7.1 Leaf area

Leaves collected from the treated and control plants of okra were brought to the laboratory, washed thoroughly and blotted. Leaf area of all the treated and control plants was determined by sketching the outer surface area on a graph paper and thereby calculated the leaf area which was expressed in cm^2.

3.7.2 Shoot length

To determine shoot length, the experimental plants were uprooted and brought to the laboratory, washed thoroughly using tap water and blotted to remove water content adhered on the surface. Length of the shoot was measured manually, using a graduated meter scale and expressed in centimeter.

3.7.3 Root length

The experimental plants of okra were carefully pulled out from the soil causing less damage to the root system. The roots were thoroughly washed in running tap water, blotted to remove excess water adhered on the roots and

length of the roots was measured manually, using a graduated meter scale and expressed in centimeter.

3.7.4 Number of lateral roots

The plants were carefully pulled out from the soil with utmost care and the roots were thoroughly washed in running tap water, blotted to remove excess water adhered on the roots and the number of lateral roots was counted manually.

3.8 Physiological parameters

3.8.1 Leaf pigment composition

Leaf pigment composition, such as total chlorophyll and carotenoid content, was quantified according to the method of Arnon (1949). Fresh samples of leaves were washed with water and blotted with filter paper. One hundred milligrams of fresh leaf sample was weighed using an electronic balance and crushed in 80% acetone using a clean mortar and pestle. The homogenate was centrifuged at 5000 rpm for 10 min and the supernatant was collected. The residue was re-extracted with 80% acetone and the supernatant was pooled. The process of extraction and centrifugation was repeated until the pellet became colorless. The final volume of the pooled supernatant was noted. The absorbance was read at 470 nm, 646 nm, 663 nm and 750 nm using a UV-VIS spectrophotometer against the solvent blank of 80% acetone.

$$Total\ chlorophyll\ = \frac{20.12(A_{646} - A_{750}) + 8.02(A_{663} - A_{750})}{Fresh\ weight\ of\ the\ sample} x\ Volume$$

$$Carotenoid\ content = \frac{1000(A_{470}) + 3.27(chla - chlb)}{Fresh\ weight\ of\ the\ sample x229} x\ Volume$$

3.8.2 Estimation of photosynthetic electron transport activities

The photosynthetic electron transport activities were analyzed polarographically using Oxygraph Plus oxygen electrode system (DW1/AD, Hansatech, Norfolk, UK) which consists of a highly sensitive S1 Clark Type polarographic oxygen electrode disc mounted within a DW1/AD electrode chamber and connected to the Oxygraph Plus electrode control unit (OXYG1, Hansatech). The DW1/AD electrode chamber provides a highly versatile solution to measurements of dissolved oxygen in liquid phase samples with clear cast acrylic construction providing excellent sample visibility and uniform illumination. Precise temperature control of the sample and electrode disc is achieved by connecting the water jacket of the DW1/AD to a thermo-regulated circulating water bath. Thylakoids from okra leaves were isolated at $4^{o}C$ and Photosystem I (PSI) (O_2 uptake) and Photosystem II (PSII) (O_2 evolution) activities were measured as described by Puthur (2000). The light dependent O_2 uptake/evolution was measured by irradiating the thylakoid suspension with white light (1800 µmol $m^{-2}s^{-1}$) continuously, provided by a 100W halogen lamp (LS2, Hansatech). The activities of PSI and PSII were expressed in terms of µmol of O_2 consumed (PSI)/evolved (PSII) $min^{-1}mg^{1}$ chlorophyll.

3.8.2.1 Preparation of thylakoid membranes

Thylakoid membranes were isolated from the leaves of experimental okra plants according to the method of Puthur (2000). The fresh leaves were cut into pieces and 100mg of fresh leaf tissue was gently homogenized with a chilled mortar and pestle using an ice-cold isolation buffer, containing 400 mM sucrose, 20 mM tricine (pH 7.8) and 10 mM NaCl. The homogenate was filtered through 6 layers of mira cloth to remove large debris and the filtrate was centrifuged at 5000 rpm for 6 min at $4^{0}C$. The supernatant was discarded, and the thylakoid pellets were suspended in 500 µl suspension buffer (pH 7.5)

containing 10 mM NaCl, 20 mM HEPES [N-(2-Hydroxyethyl) piperazine-N(2-Ethanesulphonic acid)], 100 mM sucrose and 2 mM $MgCl_2$ and it was transferred to a clean chilled tube and stored on ice to minimize the loss of activity.

3.8.2.2 Estimation of total chlorophyll concentration in the thylakoid suspension

To compare the results obtained using different chloroplast preparations, the total chlorophyll content of the thylakoid samples was estimated according to the method of Arnon (1949). Twenty microliter of the thylakoid suspension was added to the test tube containing 3 ml, 80% acetone. The tube was covered with parafilm and the contents of the tubes were mixed thoroughly using a vortex mixer to dissolve the chlorophyll, and the homogenate was centrifuged at 5000 rpm for 5 min to pellet any particulate material, and the supernatant was collected. The absorbance of the supernatant was measured at 645 nm, 663 nm and 750 nm using a UV-VIS Spectrophotometer against the solvent blank (80% acetone).

The total chlorophyll concentration in the thylakoid suspension was calculated using the following equation:

Total chlorophyll in the thylakoid suspension = [20.12 (A646 - A750) + 8.02 (A663 – A_{750})] dilution factor.

3.8.2.3 Assay of photosystem I (PSI) and photosystem II (PSII) activities

PSI and PSII activities were analyzed using Oxygraph Plus oxygen electrode system (DW1/AD, Hansatech, Norflok, UK) according to the protocol of Puthur (2000). PSI activity was measured in terms of oxygen consumption after PSII activity was blocked initially by adding DCMU to the medium. The reaction mixture consisted of suspension buffer, reduced 2, 6-

dichlorophenolindophenol (DCPIP) (0.1mM), ascorbate (600 µM), methyl violegen (MV) (500 µM), NaN_3 (1 mM) and DCMU (5 µM). Thylakoid suspension equivalent to 20µg chlorophyll was added and the volume was made upto 1ml with reaction buffer. Electron transport to PSI was maintained by artificial electron donors, ascorbate and DCPIP in the medium. Ascorbate acted as reductant by donating electrons to DCPIP and further the electrons supplied by reduced DCPIP to plastocyanin were transferred to PSI. Electrons from PSI are bypassed to an artificial electron acceptor, MV in the reaction mixture instead of being accepted by FeS centre. Finally MV reacts with oxygen molecules in the medium and produces H_2O_2. The dissociation of H_2O_2 into oxygen and H_2O by catalase in plant tissue is inhibited by NaN_3 in the reaction mixture. Thus the oxygen consumption by the activity of PSI alone is measured by oxygen electrode system.

PSII activity was measured in terms of oxygen evolution by using para-benzo quinone (pBQ) as an artificial electron acceptor and it will scavenge the electrons from plastoquinone. The transfer of the electron from plastoquinone to cytochrome is terminated and so the activity of PSII alone can be measured. Splitting of water for transferring the electrons to PSII results in the evolution of oxygen molecules in the medium and it was measured by Oxygraph Plus system. The reaction mixture (1ml) in DW1/AD electrode chamber consisted of the reaction buffer, pBQ (500 µM) and isolated thylakoid suspension equivalent to 20 µg chlorophyll.

3.8.3 Chlorophyll *a* fluorescence measurement

Chlorophyll *a* fluorescence parameters were analyzed by using Plant Efficiency Analyzer (Handy PEA; Hansatech Ltd., King's Lynn, Norflok, UK), which is a portable fluorometer having high resolutions (Strasser *et al*., 2004). All measurements were performed on the upper surfaces of the mature leaves after dark adapted for a period of 20 min using the leaf exclusion clips

and then they were illuminated with continuous red light of high intensity (3000 μmolm $^{-2}$ s $^{-1}$). All measurements were recorded up to one second with a data acquisition rate of 10μs for the first 2 min and at 1ms.

Thereafter, the various fluorescence parameters, maximal fluorescence (Fm), the activity of the water-splitting complex on the donor side of the PSII (Fv/Fo), PSII structure function index (SFI), performance index (PI), electron transport quantum yield (φ_{Eo}), and the primary photochemistry ($\Phi_o/(1- \Phi_o)$) were measured. The phenomenological energy fluxes were figured as energy pipeline leaf model [RC/CSo (concentration of the active reaction centers per cross section), ABS/CSo (the number of photons absorbed per cross section), TR/CSo (the maximal trapping rate of an exciton measured per cross section), ET/CSo (the electron transport flux per cross section) and DI/CSo (the dissipation rate per cross section)]. PSII energy fluxes per reaction center (RC) [flux of absorption per reaction center (ABS/RC), trapping flux per reaction center (TR /RC), electron transport flux per reaction center (ETo /RC) and dissipated energy flux per reaction center (DI /RC)] was figured as specific membrane model. Data were analyzed; radar plot, energy pipeline and specific membrane models were deduced using Biolyzer HP3 software.

3.9 Biochemical parameters

Estimation of various biochemical parameters like proline, total free amino acids, total soluble sugar content**,** total protein, malondialdehyde (MDA), reactive oxygen species (ROS) and antioxidants was done following standard procedures. The colour intensity of the resultant solution of all the biochemical estimations was measured using UV-VIS spectrophotometer (Systronics 2201).

3.9.1 Soil analysis

3.9.1.1 Determination of soil moisture content

The soil moisture content was determined according to the method of Robert *et al*. (1987). Soil samples from the experimental set up were collected after stopping the irrigation at the above mentioned intervals. For this, the soil was collected from the polythene bags after removing the experimental plants, and then thoroughly mixed for uniform distribution of moisture content. Immediately after mixing, 250 g of soil was weighed using a pre-weighed container made up of aluminium foil. The container along with the soil was then oven dried at 100°C initially for 4 hrs and then transferred to a temperature of 60°C. The dry mass of soil samples was recorded every day until the weight become constant. The soil moisture content was calculated as per the following equation:

Soil Moisture Content = [(Fresh weight-Dry weight) ÷ (Fresh weight)] x100

3.9.1.2 Determination of Soil pH

For this purpose, a soil suspension was prepared by thoroughly mixing one gram of soil with 25 ml of distilled water (1:25). This mixture kept undisturbed for some time and then filtered carefully. The pH of the filtrate was measured using a pH meter (Model-Eutech).

3.9.2 Plant analysis

3.9.2.1 Determination of leaf relative water content percentage (LRWC)

To determine the leaf relative water content (LRWC), freshly collected samples of leaves was cut into small discs and the fresh weight was measured (FW) using an electronic balance. Immediately after taking the fresh weight, the discs were floated on distilled water and kept under the dark condition at room temperature. After 6 hrs, the discs were blotted to remove water content

from the surface and weight of the discs was again determined and this was considered as the turgid weight (TW). Further, the samples were kept in a hot air oven initially at 100^{o}C for one hour, then the temperature of the oven was adjusted to 60^{o}C and there the samples were kept overnight. Dry weight (DW) of the samples was measured on the next day and the process of drying and weighing was repeated until the dry weight of the sample became constant. The relative water content percentage was calculated according to the method proposed by Weatherly (1950).

$$\text{Relative water content}\,\% = \frac{(\text{Fresh weight- Dry weight})}{(\text{Turgid weight} - \text{Dry weight})} \times 100$$

3.9.2.2 Determination of leaf osmotic potential

Leaf osmotic potential was measured according to the method proposed by Hura *et al*. (2007) by using a vapour pressure osmometer (Wescor 5520, USA). Calibration of the chamber was done using 100, 290 and 1000 mmol/kg standard solutions (Wescor). Cell sap from the leaves was collected by the freeze-thawing method. For this, five milligram of fresh leaf tissue was taken and pooled together in an Eppendorf tube. Leaf tissue were frozen in liquid nitrogen and then kept in a deep freezer (-80^{o}C) for 30 min. For the estimation, leaf samples were thawed at room temperature and the extruding sap was collected with a 10 µL pipette and quickly transferred to the disc chamber of the osmometer and readings were recorded.

3.9.2.3 Quantification of total protein content

The total protein content of the leaf tissue was quantified using Folin-Ciocalteau reagent according to the method of Lowry *et al*. (1951). For this, 500 mg of the leaf tissue was weighed and homogenized in 5 ml phosphate buffer (pH 7.0) using a pre-chilled glass mortar and pestle. An equal volume of 10% trichloroacetic acid (TCA) was added to the homogenate. This

mixture was kept in a refrigerator at 4ºC for 1 hr for flocculation. The protein precipitate was collected by centrifugation at 5000 rpm for 10 min at 4° C, the supernatant was discarded. The residue was washed twice with cold 2% TCA followed by washing with 30% perchloric acid to remove starch. Diethyl ether was used to extract lipids and 80% acetone, to remove the pigments. The pellet obtained after centrifugation was dried and later digested in 1ml 0.1 N NaOH by heating in a water bath for 10 min. After cooling, the suspension was cleared by centrifugation at 5000 rpm for 10 min at 4° C and the supernatant was collected. From this, known volume of aliquots were pipetted out and made up to 1 ml by adding distilled water. To the aliquots, 5 ml of alkaline copper reagent was added and shaken well. After 10 min, 0.5 ml of 1 N Folin- Ciocalteau reagent was added and shaken well immediately. The tubes were kept for 30 min for colour development. The OD of the resultant solution was read at 700 nm. Bovine serum albumin (BSA) fraction V powder was used as the standard.

3.9.2.4 Total free amino acids

Total free amino acids were analyzed according to the method of Moore and Stein (1948). Five hundred milligrams of fresh leaf samples were taken and homogenized using a clean mortar and pestle with 80% (v/v) ethanol. The extract was centrifuged at 10000 rpm for 15 min at 4°C and the supernatant was collected. The supernatant was made up to 10 ml with 80% ethanol. From this 0.1ml of the sample was pipetted out and mixed with 1ml of ninhydrin reagent in a test tube. Tubes were kept in a boiling water bath for 20 min and further 5 ml of diluent (prepared by mixing an equal volume of water and n-propanol) was added to it. This mixture was then cool to room temperature and the absorbance was read at 570 nm against a reagent blank. The standard curve was plotted by using leucine (0.05 to 0.2mM) in 0.1 M citrate buffer at pH 5.

Preparation of the reagent: The reagent solution was prepared by dissolving 20 g of ninhydrin and 3 g of hydrindantin in 750ml of methyl cellosolve. The solution was stirred carefully to avoid the formation of air bubbles in the solution and to this solution, 250 ml of sodium acetate buffer (pH 5.5) was added and the resulting reddish reagent solution was immediately transferred to a one litter dark glass bottle. Only freshly prepared reagent was used for this experiment.

3.9.2.5 Proline

Proline content was determined as described by the method of Bates *et al*. (1973). Five hundred milligram of fresh leaf tissue was weighed and homogenized in 5 ml of 3% (w/v) aqueous sulfosalicylic acid using a clean glass mortar and pestle. The homogenate was transferred to centrifuge tubes and centrifuged at 10000 rpm, for 10 min. at room temperature and the supernatant was collected for the estimation of proline. Two milliliter of the supernatant was taken in test tubes in triplicate and an equal volume of glacial acetic acid and acid ninhydrin (2.5%) (prepared by dissolving 1.25 g of ninhydrin in a mixture of 30 ml of glacial acetic acid and 20 ml of 6 M-ortho phosphoric acid) was added to it. The tubes were then heated in a boiling water bath for 1 hr and then the reaction was terminated by placing the tubes on an ice bath. Four milliliter of toluene was added to the reaction mixture and stirred well for 20-30sec. on a vortex mixer. The chromophore-toluene layer was separated carefully, and the colour intensity of the solution was measured at a wavelength of 520 nm. L-proline was used as the standard.

3.9. 2.6 Quantification of total soluble sugar content

Two hundred milligrams of fresh leaf tissue was weighed and homogenized in 80% ethyl alcohol using a clean glass mortar and pestle. The homogenate was collected and then centrifuged at 10000 rpm for 10 minutes

at 4ºC, the supernatant was collected and the pellet was re-extracted using 80% alcohol and the supernatant was pooled. The combined supernatant was used for the estimation of total soluble sugar content following the method of Dubois *et al*. (1956). From this supernatant, a known volume of the aliquot was taken in a clean test tube and made up to 1ml using distilled water and added 0.1ml of 5 % (w/v) phenol and mixed well. Then 5 ml of concentrated sulphuric acid was added to the tube quickly from a burette. After cooling, the optical density of the resultant solution was measured at 490 nm. D-glucose was used as the standard.

3.9.2.7 Quantification of Reactive Oxygen Species (ROS)

3.9.2.7.1 Superoxide anion

Quantification of superoxide anion was done as per the protocol of Doke (1983). Five hundred milligram of leaf tissue was cut into 1 mm x 1 mm fragments and immersed in 10 mM potassium-phosphate buffer (pH 7.8) containing 0.05% NBT (w/v) and 10mM NaN_3 and left for 1 hr at room temperature for incubation. After incubation, 2 ml of the reaction solution was taken and heated at 85^0C for 15 min and then cooled rapidly. After cooling the optical density of the resultant solution was measured at a wavelength of 580 nm. Sodium nitrate was used as the standard.

3.9.2.7.2 Hydrogen peroxide

Five hundred milligram of leaf tissue was weighed and homogenized with 0.1% (w/v) of 5 ml TCA in a pre-chilled glass mortar and pestle. The homogenate was centrifuged at 12000 rpm for 15 min at 4º C. The supernatant was collected; from this 0.5 ml supernatant was pipetted out and mixed with 0.5 ml of 10 mM potassium phosphate buffer (pH 7.0) and 1 ml of 1M potassium iodide. The absorbance of the resultant solution was recorded at 390 nm. Hydrogen peroxide (Analytical) was used as the standard.

3.9.2.8 Quantification of Malondialdehyde (MDA) content

Two hundred milligrams of leaf tissue was weighed in duplicate and homogenized in 5ml of 5% TCA. The homogenate was centrifuged at 12000 rpm for 15 min at room temperature. The supernatant was collected and used for the estimation of MDA. The MDA content determination was done according to the method of Heath and Packer (1968). Two milliliter of the supernatant was mixed with an equal volume of 0.5% of thio-barbituric acid (TBA) in 20% TCA. This solution was heated at 95^{o} C for 30 min, cooled and then centrifuged at 3000 rpm for 2 min at room temperature. The supernatant was collected and the absorbance of the supernatant was measured at 532 nm and 600 nm. The absorbance value at 532 nm was corrected for non-specific turbidity by subtracting from the absorbance value at 600 nm; and then the MDA content was calculated using its extinction coefficient of 155 mM^{-1} cm^{-1}.

3.9.2.9 Assay of Antioxidant system

3.9.2.9.1 Enzymatic antioxidant

3.9.2.9.1.1 Superoxide dismutase (SOD) (EC 1.15.1.1)

From the fresh leaf tissue, 500 mg was weighed and ground to fine powder using a pre-chilled mortar and pestle with liquid nitrogen. The ground tissue was homogenized using 50mM phosphate buffer of pH 7.8. A pinch of polyvinylpolypyrrolidone (PVPP) was added to the above as phenolic binder. The homogenate was centrifuged at 14000 rpm for 20 min in a refrigerated centrifuge (Thermo scientific X1R) at 4ºC. The supernatant was collected and used for the enzyme assay. Estimation of SOD activity was done as per the modified protocol of Giannopolitis and Ries (1977) by monitoring the ability of SOD to inhibit the photochemical reduction of nitroblue tetrazolium (NBT). The reaction mixture consisted of 0.1 ml of 1.5 M sodium carbonate,

0.3 ml of 0.13 M methionine, 0.3 ml of 10 µM EDTA, 0.3 ml of 13 µM riboflavin, 0.3 ml of 0.63 mM nitrobluetetrazolium (NBT) and 100 µl of enzyme extract. The reaction mixture was made up to 3ml using phosphate buffer (50mM; pH 7.8). Different assay systems were set, viz. dark-control, light-control and test samples. Test tubes containing only assay mixture without enzyme extract were served as controls. One set of the control was illuminated under a fluorescent lamp for 30 min (light-control) and the other set was kept in dark for 30 min (dark-control). One set of test sample (tubes containing assay mixtures with enzyme extract) was also illuminated and the other set was kept in dark. The dark control served as blank and illuminated control as an absolute light control. The formazan accumulation in different tubes was quantified by recording the absorbance of the developed blue colour at 560 nm against the blank. Results were expressed as units SOD mg protein^{-1}. One unit of SOD was defined as the enzyme activity that inhibited the photo reduction of NBT to blue formazan by 50%.

3.9.2.9.1.2 Catalase (CAT) (EC 1.11.1.6)

The catalase (CAT) activity of the fresh leaf tissue was determined following the method of Kar and Mishra (1976). Five hundred milligram leaf tissue was weighed and homogenized using a pre-chilled glass mortar and pestle in a medium consisting of 50 mM phosphate buffer (pH 7.0) and 100 mg of polypyrrolidone as phenolic binder. The homogenate was filtered through two layered muslin cloth. The filtrate was centrifuged at 14000 rpm for 20 min at 4ºC in a refrigerated centrifuge. The supernatant was collected and used for the enzyme assay. The activity of CAT was determined as a decrease in absorbance at 240 nm for 1 min following the decomposition of one unit of the enzyme was defined as µmoles H_2O_2 decomposed per min per mg protein. Assay system consisted of 1 ml of 50 mM phosphate buffer (pH 7.0), 2 ml enzyme extract and 1 ml of 30 mM H_2O_2. The phosphate buffer and

enzyme extract were pipetted out and thoroughly mixed in a test tube and then added H_2O_2 to initiate enzyme activity. Immediately after the addition of H_2O_2, enzyme activity was measured at 240 nm for 90 sec at 15 sec interval. The CAT activity was measured in terms of µmol H_2O_2 oxidized $min^{-1}gram^{-1}$ protein content.

3.9.2.9.1.3 Ascorbate peroxidase (APX) (EC 1.11.1.11)

The ascorbate peroxidase (APX) activity was assayed by using the method of Nakano and Asada (1981) by following the decrease in absorbance at 290 nm due to AsA oxidation with slight modifications. For this 500 mg fresh leaves were homogenized with 5 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 0.2 mM EDTA and 0.1mg PVPP in a pre-chilled mortar and pestle. The homogenate was centrifuged at 4ºC for 20 min.14,000 rpm and the supernatant was used for measuring the APX activity. To measure the APX activity 3ml assay system was prepared by mixing 0.5 mM AsA 0.015 ml(from 100 mM stock), and 0.1 mM EDTA in 50 mM sodium phosphate buffer 2.58 ml (pH 7.0). Hundred micro liter of cytosolic enzyme extract was added to the buffer mix and the enzyme reaction was initiated by adding 0.296 ml of 100 mM H_2O_2. H_2O_2 dependent oxidation was followed by monitoring the decrease in absorbance at 290 nm. One unit of APX activity was defined as the amount of enzyme that oxidized one µmol of AsA per min at room temperature under the above conditions.

3.9.2.9.1.4 Guaiacol peroxidase (GOPX) (EC 1.11.1.7)

Five hundred milligram of leaf tissue was weighed and ground to fine powder in a pre-chilled mortar and pestle using liquid nitrogen. The powder was homogenized and prepared 10% homogenate using 50 mM Tris-HCl buffer (pH 7.5) and 50 mg of PVPP as phenolic binder. The extract was filtered through two layered muslin cloth. The filtrate was centrifuged at 4°C

for 20 min at 15,000 rpm in a refrigerated centrifuge. The supernatant was transferred to a test tube and stored in an ice bath and used for the enzyme assay. GOPX activity was measured according to the method of Gasper *et al*. (1975), following the H_2O_2 dependent oxidation of guaiacol (extinction coefficient 26.6 mM^{-1} cm^{1}) at 420 nm. The 3ml assay mixture consisted of 2.87 ml 100 mM phosphate buffer (pH 7.8), 30 µl of 1% guaiacol and 100 µl enzyme extract. The blank was prepared by adding 50mM Tris-HCl buffer (pH 7.5) to the reaction mixture, instead of enzyme extract. All the components were mixed thoroughly. Twelve micro liters of H_2O_2 was added to the assay mixture to initiate the enzyme activity. Immediately after the addition of H_2O_2, increase in the absorbance due to oxidation of guaiacol was measured at 420 nm for 3 min at intervals of 30 sec. One unit of GOPX activity was defined as the amount of enzyme that caused the formation of 1µM of tetraguaiacol per minute.

3.9.2.9.1.5 Glutathione reductase (GR) (EC 1.6.4.2)

Glutathione reductase (GR) activity was assayed by following the method of Carlberg and Mannervik (1975). From the fresh leaf samples, 500 mg tissue was weighed and homogenized with 5 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 0.2 mM EDTA and 0.1mg PVPP using a pre-chilled mortar and pestle. The homogenate was centrifuged at 4°C for 20 min at 14,000 rpm and the supernatant was collected. GR activity was determined by measuring the oxidation of NADPH at 340 nm. For this 3 ml reaction mixture was prepared by mixing 3 mM EDTA 0.3 ml, 0.1 mM NADPH 0.3 ml, and 1mM oxidized glutathione (GSSG) 0.3 ml in 1.8 ml phosphate buffer (pH 7.6) and finally add 0.3ml enzyme extract to the buffer system. The activity of GR was measured by measuring the decrease in the absorbance per minute. One unit of the enzyme activity is defined as the amount of enzyme required to oxidize 1µmol of NADPH per min.

3.9.2.9.1.6 Monodehydroascorbate reductase (MDHAR) (EC 1.1.5.4)

Monodehydroascorbate reductase (MDHAR) activity was measured following the method of Hossain *et al*. (1984). Five hundred milligram fresh leaf tissue was weighed and homogenized with 5 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 0.2 mM EDTA and 0.1mg PVPP using a pre-chilled mortar and pestle. Centrifugation of the homogenate was done at 4ºC for 20 min at 14,000 rpm and the supernatant was collected. For measuring the MDHAR activity a reaction mixture was prepared containing 0.1 mM EDTA, 0.25% of triton X100, 3 mM NADH, 30 mM ascorbate and 0.25 units ascorbate oxidase in 1.8ml of phosphate buffer (150 mM). Add 300 µl of enzyme extract to this and the MDHAR activity was determined by measuring the oxidation of NADH at 340 nm. One unit of the enzyme activity is defined as the amount of enzyme required to oxidize one µmol of NADH.

3.9.2.9.1.7 Dehydroascorbate reductase (DHAR) (EC 1.8.5.1)

Dehydroascorbate reductase (DHAR) activity was measured as per the protocol given by Dalton *et al*. (1993). Fresh leaves (500 mg) were homogenized with 5 ml of 50 mM sodium phosphate buffer (pH 7.0) containing 0.2 mM EDTA and 0.1 mg PVPP using a pre-chilled mortar and pestle. The homogenate was centrifuged at 4ºC for 20 min at 14,000 rpm. The supernatant was collected and used for measuring the DHAR activity. The assay mixture consisted of 1 mM EDTA, 15 mM reduced glutathione and 2 mM dehydroascorbate in 1.5 ml phosphate buffer (pH 7.0). To this mixture add 300 µl of the enzyme extract and the increase in absorbance was recorded at 265 nm. One unit of the enzyme activity is defined as the amount of enzyme catalyzing the formation of 1 µmol of ascorbate per min.

The enzyme activity was calculated using the following equation:

$$Enzyme\ activity\ (Units/ml) = \frac{Vx(change\ in\ absorbance/\ \min)}{\square.d.v.}$$

where,

V = total volume (ml),

ε = extinction coefficient of substrate (that disappears) or the product (which appears),

d = path distance of cuvette in cm (1 cm), and

v = aliquot volume (ml)

The value calculated above represent the activity of 1 ml enzyme extract in 'units' as the total number of moles of product formed or substrate disappeared in one min. Since, one enzyme unit represents one micromole of product formed or substrate disappeared in 1min, one ml of enzyme solution would contain 'units' equivalent to that calculated from 1 ml extract.

Specific activity was calculated after determining soluble protein concentration according to Bradford (1976).

$$Specific\ activity = \frac{Activity\ in\ Units}{mg\ protein/ml\ enzyme\ extract}$$

3.9.2.9.2 Non-enzymatic antioxidant system

3.9.2.9.2.1 Total phenolics

The total phenolic content was determined using Folin-Denis reagent according to the method of Folin and Denis, (1915). For the quantification of total phenolics, 100 mg of chopped fresh leaf tissue was weighed using an electronic balance and homogenized in 80% ethanol (v/v) using a clean mortar and pestle. The homogenate was then centrifuged at 10000 rpm for 20 min at 4ºC and the supernatant was collected in a china dish. The residue was re-extracted with 80% ethanol and was again centrifuged. The known volume

of supernatant was pipetted in to a clean test tube and made up to 2 ml using distilled water and 0.5 ml of 1 N Folin-Denis reagent was added to it. The contents were thoroughly mixed, and after 3 min, 2 ml of 1N sodium carbonate was added. After thorough mixing, the mixture was kept for 1hr for colour development and OD of the resultant solution was measured at 700 nm. Tannic acid was used as the standard.

3.9.2.9.2.2 Ascorbate (AsA) content

Measurement of ascorbate (AsA) content was done according to the method of Chen and Wang (2002). Two hundred milligram leaf tissue was weighed using an electronic balance and homogenized with 5 ml of 5% TCA (w/v) by centrifugation at 12000 rpm for 15 min at 4°C. The supernatant was collected and used for the quantification of AsA content. An aliquot of 1 ml of the supernatant was mixed well with 0.3 ml of 200 mM NaH_2PO_4. To this, 0.5 ml of 10 % (v/v) TCA, 0.4 ml of 42 % (v/v) H_3PO_4, 0.4 ml of 4 % (w/v) bipyridyl (dissolved in 70% alcohol) and 0.2 ml of 3 % $FeCl_3$ (w/v) were successfully added. The mixture was then incubated at 42°C for 15 min. The absorbance of the resultant solution was measured immediately after incubation at 524 nm. The ascorbate content was calculated from a standard curve prepared by using different concentrations of AsA.

3.9.2.9.2.3 Glutathione (GSH) content

The glutathione (GSH) content was estimated according to the method of Chen and Wang (2002). For this 200 mg of leaf tissue was weighed using an electronic balance and homogenized with 5 ml of 5% (w/v) TCA. The homogenate was centrifuged at 12000 rpm for 15 min at 4° C, and the supernatant was collected and used for the estimation of reduced glutathione (GSH) content. To an aliquot of 0.5 ml, 2.6 ml of 150 mM NaH_2PO_4 buffer (pH 6.8) and 0.18 ml of 3 mM 5 dithio-bis-2-nitrobenzoic acid (DTNB) dissolved in 100 mM phosphate buffer (pH 6.8) were added. After 5 min, the absorbance of the resultant solution was read at 412 nm and the GSH content

was calculated from a standard curve using varying concentrations of reduced glutathione.

3.10 Characterization of yield

Yield, from all the treated and control plants, was done on 9th day of fruiting, at which stage the fruits were able to consume. Yield components like fruit length, fresh weight and fruit per plant were determined. Fruit length was measured manually using a graduated meter scale and expressed in centimeters. Fresh weight of fruit was determined using an electronic weighing balance. Fruits per plant were determined manually by counting the fruits of randomly selected three plants.

3.10.1 Nutritional value of fruits

Nutritional values, of 9day old tender fruits of okra such as total protein, total soluble sugar content and total amino acid content were quantified as per the standard methods described previously (3.9.1.2.3; 3.9.1.2.4 and 3.9.1.2.6).

3.10.1.1 Quantification of carbohydrate content

Total carbohydrate content in 9day old okra fruits was estimated according to the method of Hedge and Hofreiter (1962). One hundred milligram of fresh fruit tissue was weighed and hydrolyzed with 2.5 N HCl in a water bath for 3 hrs and cooled to room temperature. It was neutralized with solid sodium carbonate until the effervescence ceased. The volume was made up to 100 ml and centrifuged at 6000 rpm for 10 min at room temperature and collected the supernatant. From the supernatant, known volume was taken and made up to 1 ml with distilled water. 4 ml of anthrone reagent was added and heated for 8 min in a boiling water bath. It was then cooled rapidly and optical density of the resultant solution was measured at 630 nm. D-Glucose was used as the standard.

3.10.1.2 Quantification of total lipid

Total lipid content of tender, 9day old fruits was quantified as per the modified protocol of Folch method (Folch *et al*., 1957; Christie, 1993). One gram of fresh fruit tissue was weighed and homogenized thoroughly in chilled diethyl ether using a clean glass mortar and pestle. The homogenate was centrifuged at rpm for 10 min and the supernatant was collected in a pre-weighed China dish. The sediment was homogenized again extracted using diethyl ether and the supernatant was added to the bulk in the China dish. The China dish containing the combined supernatant was kept in a hot air oven at 60°C for 24 hrs. The China dish along with the content left after evaporation was weighed and the difference between the final weight and initial weight was noted.

3.10.1.3 Quantification of elements

Determination of elements such as Mn, Zn, Ca, Mg and Fe were done according to the method of Allan (1969) and were analyzed using an atomic absorption spectrophotometer (Model: GBC932 Plus-3000). For this 9 days old fruits of different treatments and control were sampled and dried at 60ºC in a hot air oven. Known weights of the dried samples were digested by refluxing in a mixture of nitric acid and perchloric acid in the ratio of 10:4 until the solution became colourless using Kjeldahl's flask heated on a heating mantle. Then the digest was filtered and further transferred to a standard flask and the volume was made up to 50 ml and stored in screw-capped containers. This filtrate was used for the estimation of Mn, Zn, Ca, Mg and Fe.

3.10.1.4 Quantification of Phosphorus

Available phosphorus in the fruit was quantified by following Brays method No. 1 (Bray and Kurtz, 1945). To determine fruit phosphorus 5 g of air-dried soil was mixed with 50 ml of Bray and Kurtz extracting solution (0.03 N NH_4F in 0.025 N HCl). An aliquot (5 ml) of the extract was taken; the

volume was made up to 20 ml with distilled water. To this 4 ml Murphy-Riley colour developing solution (solution containing 250 ml of 2.5M H_2SO_4, 75 ml ammonium molybdate solution, 50 ml ascorbic acid solution, 25 ml of antimony potassium tartrate solution and 100 ml of distilled water) was added. After 15 min the intensity of blue colour was measured using a UV-VIS Spectrophotometer at 730 nm.

3.11 Data analysis

Data obtained from all the above studies were statistically analysed and Analysis of variance (ANOVA) was performed using SPSS software 19.0. Means were compared using the Duncan test at 5% probability level. All the data are an average of recordings from three independent experiments each with three replicates (*i.e.* n=9). The data represent mean ± standard error (SE).

I. Preliminary screening

I.a Leaf moisture content

Leaf moisture content of okra treated with different concentration of various bacteria cultures exhibited significant differences (Fig.1. A, B, C). When comparing the leaf moisture content of different concentration of bacteria culture (i.e., 10^{-5},10^{-6},10^{-7}, 10^{-8} and 10^{-9} cfu/ml) during irrigated condition no significant change was observed in lower concentration (i.e., 10^{-5} and 10^{-6} cfu/ml) of bacteria treated okra compared to control plants, whereras in the case of higher concentration of bacteria inoculated (10^{-7}, 10^{-8} and 10^{-9} cfu/ml) plants, significant variation was observed, but no significant variation in leaf moisture content was recorded in higher concentration of bacteria inoculated (10^{-7}, 10^{-8} and 10^{-9} cfu/ml) okra (Fig.1. A, B, C). Similar results were recorded in drought exposed period. Higher reduction in leaf moisture content was observed in all plants exposed to drought stress after seven days of stress (Fig.1. A, B, C). Hence, for the further evaluation of drought tolerance potential of okra the treatment cultuers of 10^{-7} cfu/ml were selected.

I.b Chlorophyll stability index (CSI)

Chlorophyll stability index of *A. esculantes* plants treated with different concentration of various bacteria culture exhibited significant differences (Fig.2.A, B, C). During comparing the CSI of different concentration of bacteria culture (i.e., 10^{-5},10^{-6},10^{-7}, 10^{-8} and 10^{-9} cfu/ml), there is no significant change in CSI was observed in lower concentration (i.e., 10^{-5} and 10^{-6} cfu/ml) of bacteria treated okra compared to control plants, during irrigated and non- irrigated condition (Fig.1,A,B,C). The higher concentration of bacteria inoculation (10^{-7}, 10^{-8} and 10^{-9} cfu/ml) recorded more or less similar effect in CSI during water stressed and unstressed

condition. CSI was decreased with increasing stress exposed days and severe reduction was observed beyond the seven days of stress (Fig.1. A, B, C).

I.c Localisation of bacteria using SEM

Different bacteria colonization in root of *A. esculentus* was vishualized using scanning electron microscope (Fig.A)

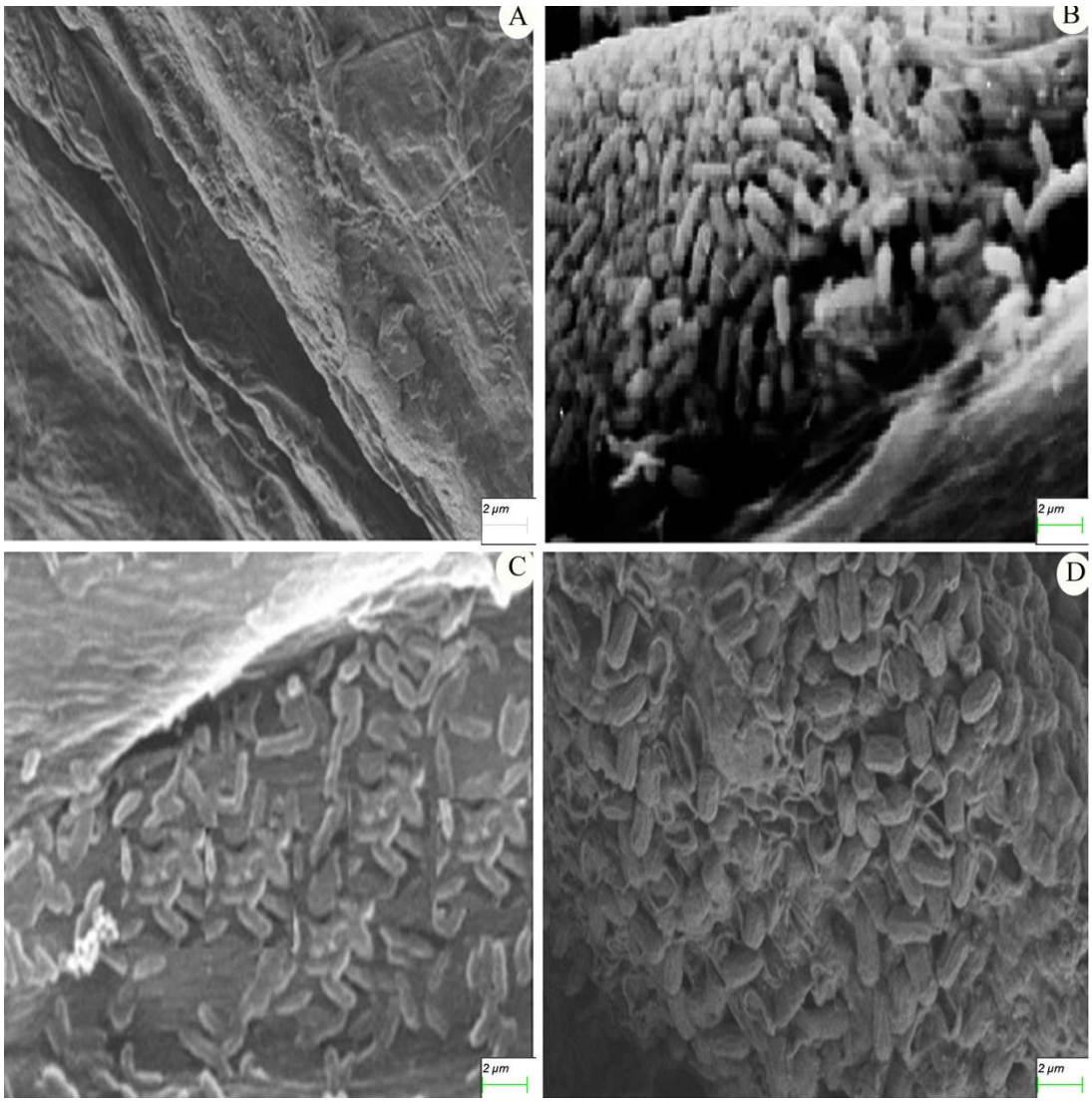

Fig A: Root epidermal colonization of different bacteria in okra A) Control B) *Pseudomonas fluorescens* C) *Burkholderia cepacia* and D) *Bacillus subtilis*

4.1 CHAPTER 1

4.1.1 Plant growth analysis

Increase in various growth parameters like leaf area, shoot length and root length were observed in *A. esculantes* plants treated with phosphate solublising bacteria [*Pseudomonas fluorescens* (*PF*)*, Bacillus subtilis* (BS) and *Bruckholderia cepacia* (BC)] when compared to untreated control plants (WW) during irrigated condition. Water stress for a period of seven days significantly reduced the growth parameters and the reduction was noticed in the order; untreated okra (WS)>*Bruckholderia cepacia* treated plants (WS+BC)>*Bacillus subtilis* treated plants (WS+BS)>*Pseudomonas fluorescens* (WS+PF) treated plants.

4.1.1.1 Leaf area

Leaf area of the *A. esculantes* plants treated with bacteria culture exhibited significant differences when compared to untreated plants in control experiments. Upon comparing the leaf area, increase in leaf area related to the bacteria treatments was in the order; PF treated plants >BS treated plants >BC treated plants (63.25>50.5> 46.5) (Fig.1.1). The leaf area of bacteria untreated plants was highly reduced (26.75 cm^2) compared to bacteria treated plants. Among the bacteria treated plants, the highest leaf area was observed in PF treated plant. However, no significant changes were observed in the leaf area during drought stress exposure period i.e., 1d., 3d., 5d., 7d. and recovery period i.e., 1R., 2R and 3R (Fig.1.1 and Fig. 1A).

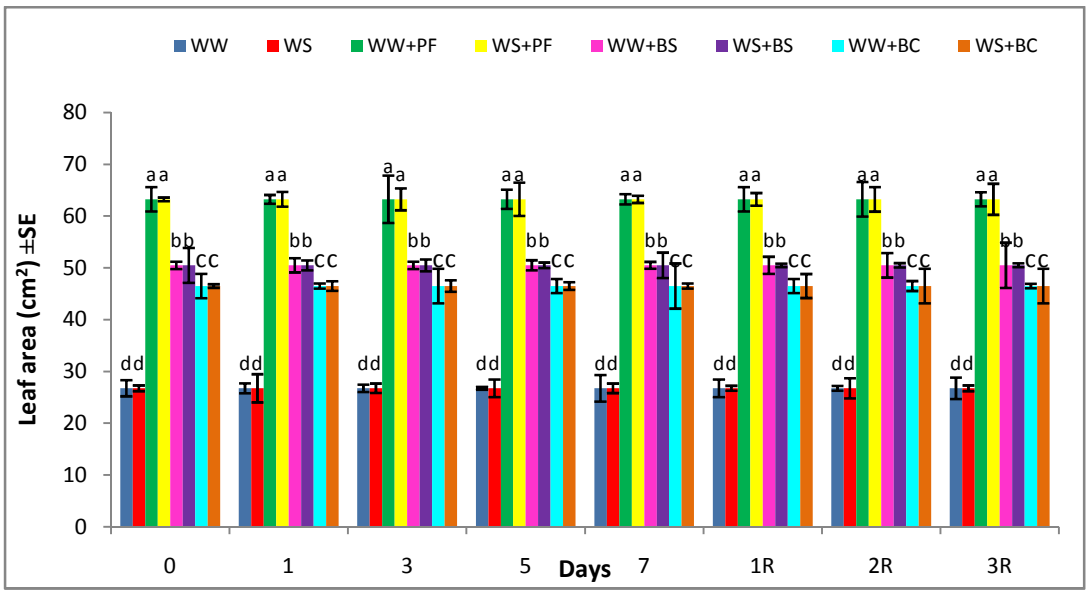

Fig.1.1. Variation in leaf area during drought stress and recovery in okra inoculated with different bacteria. (WW- well irrigated, WS-water stressed, WW+PF-well watered okra inoculated with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,* WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,* WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.1.1.2 Shoot length

The shoot length was significantly different between bacteria treated and untreated okra plants during well watered condition, maximum shoot length was observed in PF treated plants, the increase in shoot length was in the order WW+PF>WW+BS >WW+BC>WW during well-watered condition (Fig.1.2 and Fig. 1B). Drought stress significantly reduced shoot growth, the higher reduction was observed in untreated plants and the lower reduction was recorded in PF treated plants during stress (i.e., 1d, 3d, 5d and 7d.) and the order of reduction in shoot length was observed as WS>WS+BC>WS+BS>WS+PF (Fig.1.2). On the first day of drought exposure, a significant reduction in shoot length was recorded in untreated plants, but the reduction was least in bacteria treated plants. The shoot growth retardation in the bacteria treated plants were observed in the order

WS+BC>WS+BS >WS+PF (Fig.1.2). On the third day of drought exposure, the shoot length in bacteria untreated plants was significantly reduced but bacteria treatment alleviated drought induced reduction in shoot length in okra. Reduction in the shoot growth of bacteria treated okra exposed to drought were observed in the order WS+BC>WS+BS >WS+PF (Fig.1.2). Shoot growth retardation in okra during fifth day of drought was recorded to be WS>WS+BC>WS+BS>WS+PF (Fig.1.2). On the seventh day of drought exposure a significant reduction in shoot growth in okra was observed, the highest reduction was observed in untreated plants. The order of reduction in shoot growth on the seventh day in stressed plants was recorded as WS>WW+BC>WW+BS>WW+PF (Fig.1.2 and Fig. 1B).

Re-watering regained the shoot growth in all plants. During the first day of re-watering, a significant increment in shoot growth was observed, the highest increase in shoot growth was recorded in PF treated plants and the lowest was noticed in the untreated drought exposed plants and the order of increase in shoot growth was observed as WS+PF>WS+BS>WS+BC>WS (Fig.1.2 and Fig. 1B). During the second day of re-watering, all the bacteria treated drought exposed plants recovered shoot growth similar to control plants and shoot length was recorded as WS+PF>WS+BS>WS+BC (Fig.1.2 and Fig. 1B). The third day of rehydration resulted in the recovery of stress imposed reduction of shoot growth in all plants and the shoot length was in the order WS+PF>WS+BS>WS+BC>WS (Fig.1.2 and Fig. 1B).

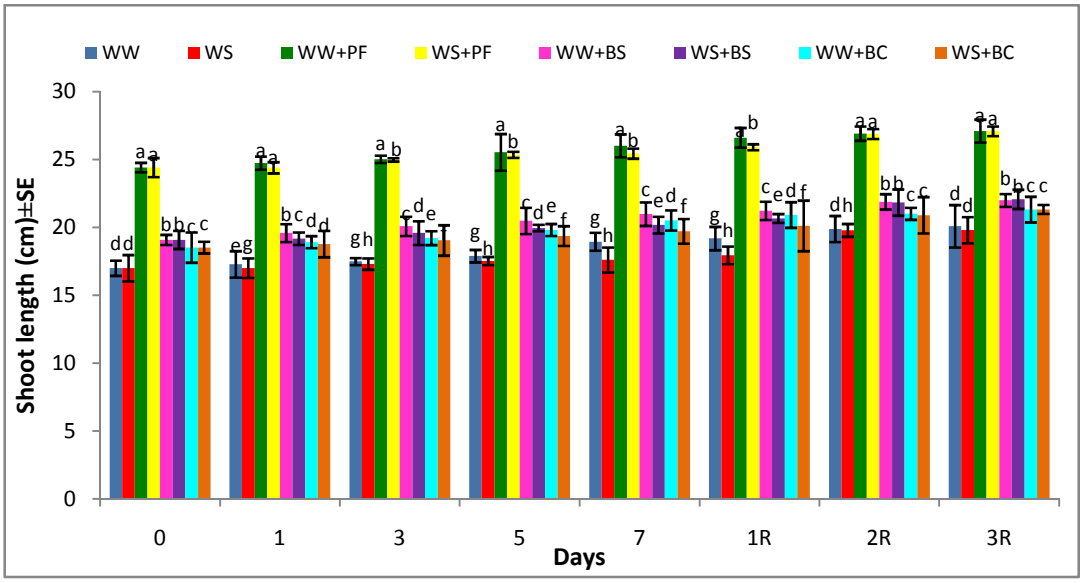

Fig.1.2. Variation in shoot length during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculated with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,* WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,* WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.1.1.3 Root length

Significant differences were observed in the bacteria treated and untreated okra plants during well watered condition. Here, the maximum root length was observed in PF treated plants and the increment in root length due to bacteria treatment was observed in the order WW+PF>WW+BS> WW+BC>WW in irrigated plants (Fig.1.3 and Fig. 1C). Drought significantly increased root growth in stress exposed plant, the highest increment in root length was recorded in PF inoculated plants and lowest was observed in the untreated plants, under stress exposure i.e., 1d, 3d, 5d and 7d. The order of promotion in root length was observed as WW+PF>WW+BS>WW+BC>WW (Fig.1.3). During the first day of drought exposure, a significant increase in the root length in PF treated okra plants,

was observed whereas, the stress induced increment in root growth was lesser in untreated stress exposed plants. The increase root growth of bacteria treated plants were observed in the order WS+PF>WS+BS>WS+BC (Fig.1.3). It was noticed that, the third day of water stress significantly improved the root length in bacteria treated plants especially in PF inoculated okra, but in the bacteria untreated plants root length was much reduced. The increase in root growth was on the third day of stress was observed in the order WS+PF>WS+BS>WS+BC>WS (Fig.1.3). On the fifth day of drought, highest increment in root growth was observed in PF treatment, whereas the lowest was observed in bacteria untreated water stressed plants. The increasing trend of root growth on the fifth day of stress exposure was recorded in the order WS+PF>WS+ BS >WS+ BC>WS (Fig.1.3). On the seventh day of drought exposure, a significant increase in root growth was observed in PF treated okra, and a lesser increase in root length was recorded in bacteria untreated plants. The order of increase in root growth on the seventh day in stress imposed plants was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.1.3 and Fig. 1C).

Rehydration of water stressed plants resulted in the restoration of normal root length i.e., root growth as similar to control plants. Faster regain of normal root growth was observed in PF treated okra plants within three days of re-watering and it was observed as the time limit for complete recovery of all the plants exposed to drought. The first day of re-watering resulted in significant reduction in the promotion of root growth. Enhanced down regulation of root growth during re-watering of drought exposed okra was observed in PF treated plants, but in bacteria untreated plants, the actual root length was regained slowly. The order of increase in down regulation of root growth was observed as WS+PF>WS+BS >WS+BC>WS (Fig.1.3). On the second day of re-watering, bacteria treated plants showed normal root growth, while in the case of bacteria untreated plants, actual root growth was

not regained. WS+PF>WS+BS>WS+BC>WS is the order of regain of normal root growth (Fig.1.3). The third day of re-watering resulted in complete recovery of root length in bacteria treated plants and untreated plants in the order WS+PF>WS+BS >WS+BC>WS (Fig.1.3 and Fig. 1C).

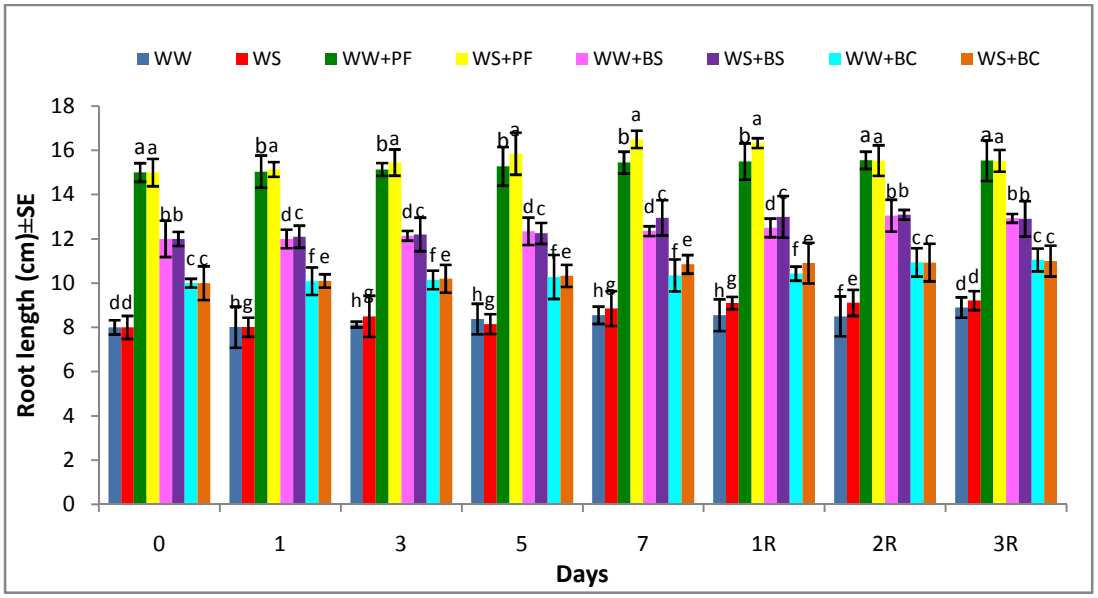

Fig.1.3. Variation in root length during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed, WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens* ,WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,*WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.1.1.4. Number of lateral root

A significant difference in the number of lateral roots was observed in the bacteria treated plants and the untreated okra during irrigated condition. An increased number of lateral root was recorded in PF treated plants and the increase in the number of lateral roots in okra was recorded as WW+PF>WW+BS>WW+BC>WW in irrigated plants (Fig.1.4 and Fig. 1C). Water stress induced more number of lateral roots, increased number of lateral root was noted in PF treated plants and lower number was observed in

untreated plants, under stress i.e., 1d, 3d, 5d and 7d, the order of increase in lateral root number was WW+PF>WW+BS>WW+BC>WW (Fig.1.4). During the first day of water stress, a significant increase in the number of lateral roots was observed in PF treated okra plants but in the untreated stress exposed plants, the increase in number of lateral roots was lesser. Stress induced lateral root production in bacteria treated and untreated plants on the first day of drought exposure was noted in the order WS+PF>WS+BS >WS+BC (Fig.1.4). During the third day of drought, a significant increment in lateral root production was observed in PF inoculated okra while in the un-inoculated plants, the lateral root production was reduced. Lateral root production on the third day of stress was observed in the order, WS+PF>WS+BS >WS+BC>WS (Fig.1.4). On the fifth day of the drought exposure, highest lateral root production was observed in PF treated plants, and lowest was recorded in untreated plants compared to control plants. Increased lateral root production on the fifth day of stress exposure was recorded in the order WS+PF>WS+BS>WS+ BC>WS (Fig.1.4). A significant increase in the number of lateral roots was observed in okra plants on the seventh day of water stress. PF treated okra recorded a significant increase in lateral root number and lesser production of lateral roots was observed in the untreated plants. The increased production of lateral roots between the treatments was observed in the order WS+PF>WS+BS>WS+ BC>WS (Fig.1.4 and Fig. 1C).

Re-watering of stressed plants resulted in the down regulation of lateral root production. Bacteria treated okra plants recovered normal lateral root number on the second day of treatment but in the case of untreated plants, three days of re-watering was required for the complete recovery. The first day of re-watering resulted in a significant reduction in the production of lateral roots. Enhanced down regulation of root growth during re-watering of drought exposed okra was observed in PF treated plants, but the untreated

plants the regeneration of lateral roots was lesser. The order of decrease in root growth was observed as WS+PF>WS+BS >WS+BC>WS (Fig.1.4). On the second day of re-watering, bacteria treated plants showed normal lateral root production, while in the case of bacteria untreated plants the actual lateral root number is the order WS+PF>WS+BS >WS+BC>WS (Fig.1.4). The third day of re-watering resulted in complete recovery of lateral root production in all stress exposed plants similar to the control plants. Increased lateral root production in bacteria treated and untreated plants were observed as WS+PF>WS+BS >WS+BC>WS (Fig.1.4 and Fig. 1C).

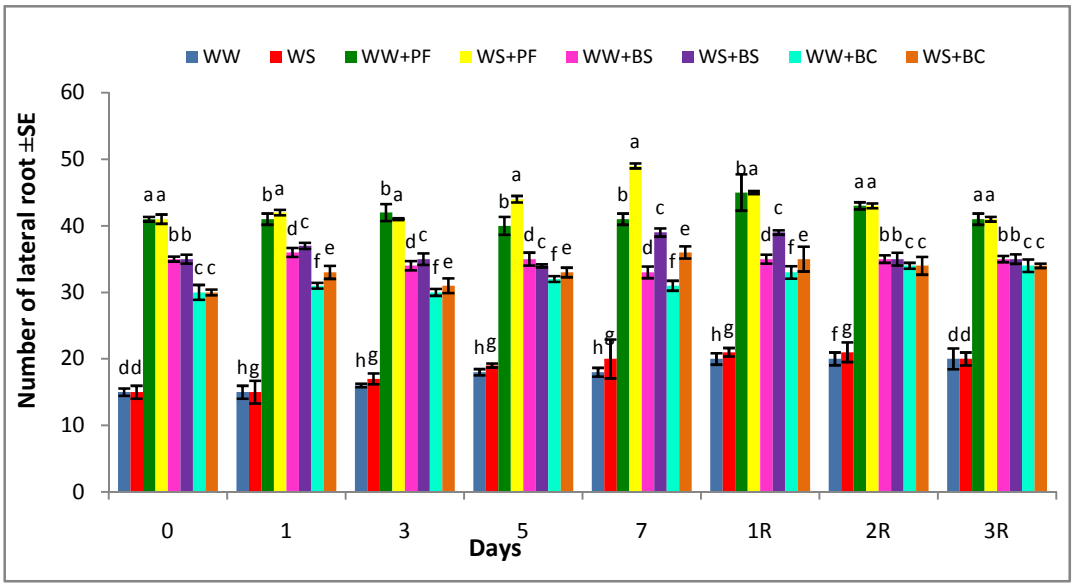

Fig.1.4. Variation in total number of lateral roots during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,* WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,* WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.1.2 Discussion

Water stress was considered as an important abiotic stress imparted by climate change which leads to worldwide reduction in plant growth and yield. (Chaves *et al*., 2003). Various morphological adaptations have been developed by plants to alleviate the harmful effect of drought. Plants cope up with drought driven imbalance in water status mainly through two mechanisms; by the reduction of water loss and increased water uptake (Basu *et al.,* 2015). Plants achieve optimum water availability during dry condition by increased root surface area, hydraulic conductance, etc. and thus, maintain normal growth and yield penalty in crop plants under drought as similar to normal conditions. Plants prevent excessive water loss upon drought through the closing of stomata, change in leaf angle and reduction in leaf area. The present study has monitored the effect of different bacteria i.e., *P. fluorescens* (*PF*)*, B. subtilis* (BS) and *B.cepacia* (*BC*) on plant growth under well watered, stressed (1d, 3d, 5d, 7d) and recovery condition (1R,2R and 3R)

4.1.2.1 Leaf area

The morphological parameters are the first visible changes as a result of phosphate solublizing bacteria treatment in okra. Leaf was considered as the primary organ of vascular plants which receive sunlight for photosynthesis i.e., it acts as a source for growth and yield. Increase in leaf area directly increases the number of photoreceptors like chlorophyll and it results in the enhancement in photosynthetic efficiency of okra, leads to increased stress tolerance potential of plants. While comparing the results, it was observed as bacteria treatment increased the leaf area in okra plants during stress and recovery period, moreover, PF treated plants showed the highest leaf area. These results are in agreement with the findings of Al-Ahmadi *et al.* (2017) that increased leaf area in *P. fluorescence* treated *Crocus sativus* L. and suggested that *Pseudomonas* sp. treatment improved leaf area through altered phytohormones system increased the accumulation of minerals like phosphorous and nitrogen and also through the production some enzymes in

modifying ethylene level in plants. Similar results were also observed in *Burkholderia* sp. treated pineapple (Baldotto *et al.*, 2010). According to Baset *et al*. (2010) inoculation of *Bacillus* and *Azospirillum* in Musa improved the leaf area. Co-inoculation of *Rhizobium* + *Pseudomonas* + *Bacillus* in groundnut improved leaf surface area (Mathivanan *et al*., 2014). *Pantoea agglomerans* RK-92 treated cabbage (*Brassica oleracea*) also effectively increased leaf area of the seedlings (Turan *et al.,* 2014).

4.1.2.2 Shoot length

The primary function of the stem was to support leaves, conduction of water and nutrients, photochemical reaction and also to supply the byproducts of photosynthesis through specific vascular conducting tissues. Reduction in water availability minimize shoot and leaf growth in order to decrease the water loss and expand root system to increase water availability (Parent *et al.,* 2010). Significant reduction in shoot length was observed in bacteria untreated water stressed plants but, phosphate solublizing bacteria (PSB) increased stem growth in treated plants which positively correlated with expanded root system of bacteria treated plants under well watered and stressed condition which confirms the sufficient water availability to plants even under stress condition. Even though, drought cause a significant reduction in stem elongation of the untreated okra, phosphate solublizing bacteria effectively alleviate the negative effects of drought by maintaining the nutrient and water absorption under stress condition. Among the studied PSB's *P. florescence* (PF) showed higher solubilization capacity of minerals from the soil, which resulted in increased shoot growth in *P. fluorescence* treated okra plants and also showed increased root growth, there by help the plants to absorb nutrients and maintain normal growth under stress condition. This reduction of stress effect in bacteria treated plants result in faster recovery in rehydration time. The *B. subtilis* and *B.cepacia* treated plants effectively alleviated the harmful effect of drought in okra, but not as much as the effect of *P. florescence* treatment. As per the suggestion of Agami *et al.*

(2016), a higher reduction in shoot length of drought exposed PGPR untreated basil plant was due to the changes in the metabolic process of the plant including chlorophyll destruction and the cell division. According to Shao *et al.* (2009) drought results in reduction of tissue water content and turgor pressure of the cell, thereby inhibiting cell enlargement and division causing a reduction in plant growth. Dual inoculation of AM + *Azospirillum* in rice plant promotes plant growth under well-watered conditions due to the alleviation of the harmful effect of drought on photosynthetic perfomance of plants (Ruíz-Sánchez *et al.,* 2011). Similar trend was also observed in *Azospirillum* treated maize plants by Bano *et al.* (2013). *Pseudomonas nitroreducens* improves *A. thaliana* and *Lactuca sativa* growth via the up-regulation of *CycB1pro:GUS* and *CyclinB* genes activity which stimulates the cell growth and enhanced uptake of nitrate (Trinh *et al.,* 2018). Previous studies of Kang *et al.* (2014b) supported the present result that, inoculation of *Burkholdera cepacia* SE4, *Promicromonospora* sp. SE188 and *A. calcoaceticus* SE370 in cucumber increase drought tolerence.

4.1.2.3 Root

Plant roots connect the soil to other vegetative parts of the plant, absorb water and minerals from the soil and play a leading role in the plant growth, so root biomass is directly related to plant growth. Root biomass plays an important role in soil water retention, several studies revealed that, roots could alter the soil structures, by (i) volumetric occupancy of roots in soil pore space (Scholl *et al.,* 2014) (ii) water retention in roots (Taleisnik *et al.,* 1999) and (iii) the release of root exudates (Traoré *et al.,* 2000). Compared to the control plants, increased root growth and lateral root production was observed in bacteria treated plants. Among bacteria treated plants, *P. florescence* treatment was more effective in okra compared to *B. subtilis* and *B. cepacia* treated okra. This root growth promotion improves drought tolerance potential, enhancement in root growth and lateral root production in drought exposed okra plants. On the other hand, PF treated

plants showed increased root growth and the number of lateral roots on drought exposure followed by *B. subtilis* and *B. cepacia* treated plants when compared to untreated plants. Increase in root length and number of lateral root production enhance drought tolerance in plants by improving soil water retention, which reflected an increased relative water content in stressed plants. A similar observation was also reported by Gao *et al.* (2018) that increased lateral root production by plants improves water retention capacity within the soil. Root induced alternation in soil water retention cause change in soil organic matter and soil bulk density from each other (Wang *et al.,* 2017), which maintains the plant growth during dry condition. Plant growth promoting rizobacteria like *Pseudomonas*, *Azospirillum* and *Bacillus* maximize the auxin synthesis in plants which in turn results loosening of root cell wall, thus increasing the of number root exudates (Chen *et al.,* 2013; Glick 2012; Notz *et al.,* 2001; Prigent-Combaret *et al.,* 2008). Root exudates contain sugars, organic acids, amino acids, phenolic compounds, and some secondary metabolites (Bais *et al.,* 2006) that facilitate in the PGPR growth and enhance colonization around the root. *Bacillus* Notz LZR216 inoculation improves root growth and altered root architecture in cytokinin receptor mutants *Arabidopsis* (Wang *et al.,* 2018a). Similarly, *Bacillus megaterium* promotes changes in root system morphology by altering cytokinin-signaling pathway (Ortíz-Castro *et al.,* 2008). *Pseudomonas* treatment modifies cytokinin signaling in wild variety of *Arabidopsis* (Hann *et al.,* 2014). Furthermore, Spaepen *et al.* (2007) reported that, an increase in root growth in bacteria treated plants due to the enhanced ability of plants for phosphorous acquisition. According to Kudoyarova *et al.* (2017) *P. extremaustralis* inoculation in wheat increase auxin level resulted in the production of cytokinin which in turn lead to increased the root mass.

4.2. CHAPTER 2

4.2.1 Physiological parameters

4.2.1.1 Photosynthetic pigment analysis

4.2.1.1.1 Total Chlorophyll content

Bacteria treatment significantly improved chlorophyll content in irrigated plants, the increasing order of chlorophyll content was observed as WW+PF>WW+BS >WW+BC>WW. Water stress resulted in the reduction of chlorophyll content throughout the stress period (1-7th day), later period of drought stress i.e., fifth and seventh day of drought recorded a significant reduction in chlorophyll content in all the treatments. PF treatment effectively alleviated the drought induced decrease in chlorophyll compared to control plants and other bacteria treated and untreated plants. After PF treated plants, a decrease in the degradation of chlorophyll due to drought stress was observed in BS treated plants throughout the stress period (i.e., 1-7th) compared to control plants, BC treated plants and bacteria untreated plants. Among bacteria treatment, BC treatment in okra was noted as least effective to prevent degradation of chlorophyll during drought exposure however, BC treated plants recorded a significant increase in chlorophyll content when exposed to stress compared to untreated stressed plants (Fig.2.1). Higher reduction in chlorophyll content was observed in water stressed bacteria and untreated okra plants.

Recovery study revealed that, all the treatments regained its actual chlorophyll content during re-watering (Fig.2.1). On the first day of re-watering, all plants were recorded a significant increase in chlorophyll content and the order of chlorophyll content among different treatments was observed as WS+PF>WS+BS>WS+BC>WS. On the second day of re-watering, the drought exposed bacteria treated plants recovered normal chlorophyll content

similar to control plants and the chlorophyll content in bacteria treated plants were in the order WS+PF>WS+BS>WS+BC. Chlorophyll content increased in untreated plants, but not similar to the control plants. On the third day of re-watering, all the drought stressed plants regained chlorophyll content, in which higher chlorophyll content was observed in PF treated plants and lower in bacteria untreated plants and the order of increase in chlorophyll content in okra after recovery was observed as WW+PF>WW+BS >WW+BC>WW.

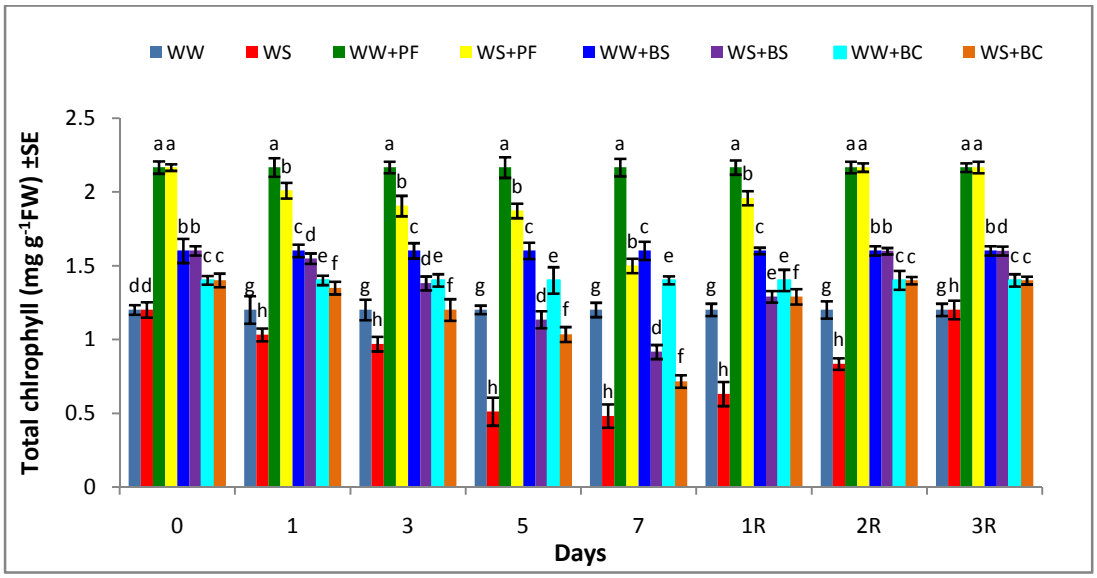

Fig.2.1. Variation in total chloophyll content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,*WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,* WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.2.1.1.2 Carotenoid content

Carotenoid content showed an increasing trend in the drought exposed plants, higher carotenoid was observed in untreated plants on the 7th day of drought exposure when compared to control and bacteria treated plants. There

is no significant increase in the carotenoid content on the 1st day of stress exposure in both treated and untreated plants (Fig.2.2). However, from the third day onwards a significant rise in carotenoid content was recorded in all plants under drought and higher accumulation of carotenoid was registered in bacteria untreated plants compared to control and other bacteria treated stress exposed plants. A lesser quantity of carotenoid was recorded in PF treated plants compared to other bacteria treated plants and well watered plants, the increasing order of carotenoid content on the third day of water stress was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.2). On the fifth day of stress, a steep increase in carotenoid content was observed in the untreated plants compared to control okra plant, whereas, PF treated okra plants showed a significant reduction in carotenoid content. The accumulation pattern of carotenoid content in okra on the fifth day of drought in different treatments were recorded as WS>WS+BC>WS+BS>WS+PF (Fig.2.2). Bacteria treated and untreated okra plants subjected to seven days of drought stress exhibited significant increment in the carotenoid content, of which the prominent increase in carotenoid content was noticed in untreated plants. Although the rise in carotenoid content in bacteria treated plants was lesser than untreated plants, among the bacteria treated okra, PF treated plants showed a significant reduction in carotenoid content and the order of increase in water stressed plants were observed in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.2).

Carotenoid content decreased in plants during rehydration and a sudden decrease in carotenoid content was observed in the bacteria treatment. During the first day of re-watering, the carotenoid content decreased in all the plants and the reduction in carotenoid content was observed in the order WS+PF>WS+BS >WS+BC>WS (Fig.2.2). On the second day of re-watering, bacteria treated plants recovered carotenoid content and the carotenoid content in the rehydration treatment were in the order WS+PF>WS+BS >WS+BC>WS (Fig.2.2). The third day of watering resulted in the recovery of

carotenoid content in all stress imposed plants and the range of carotenoid content between the treatments were observed as WS+PF>WS+BS >WS+BC>WS (Fig.2.2).

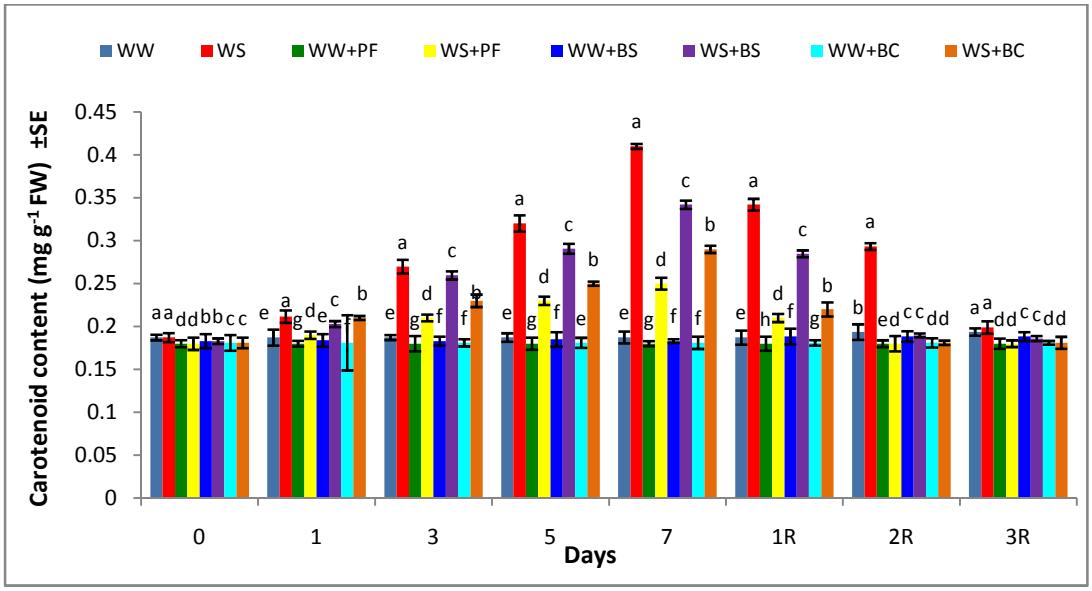

Fig.2.2. Variation in carotenoid content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,* WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,*WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.2.1.1.3 Photosystem II activity

Drought resulted significant damages in the reaction center of photosystem II resulted in the reduction of photosynthesis. It was observed that photosystem II was more susceptible to drought in both bacteria treated and untreated plants. However, bacteria treated plants showed a significant increase in PSII activity during irrigation and the order of activity between the bacteria treated and untreated okra was recorded as WW+PF>WW+BS >WW+BC>WW. On the first day of drought, no significant changes were observed in the PSII activity in bacteria treated and the untreated plants

(Fig.2.3). But from the third day onwards, the reduction of PSII activity was remarkable in all the treatments. Higher reduction in PSII activity was observed in the untreated plants and a lower reduction was observed in PF treated plants compared to control and other bacteria treated stress exposed plants (Fig.2.3). The fifth day of drought significantly reduced PSII activity in the untreated plants, whereas the decline in the PSII activity of bacteria treated plants were significantly lower compared to stress untreated plants. Among the bacteria treatments, PF treated plants record lesser reduction in PSII activity with respect to their control and other bacteria treated stressed plants i.e., (BS and BC treated okra), the order of reduction in PSII activity of okra plants under stress condition was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.3). The seventh day of water stress resulted in a higher reduction in the reaction centers which in turn reflected a reduction in the PSII activity of okra, severe reduction in PSII activity was recorded in bacteria untreated plants and the lower reduction was observed in PF treated plants over respective control plants and other bacteria treated plants. The order of reduction in bacteria treated and untreated plants were recorded as WS>WS+BC>WS+BS>WS+PF (Fig.2.3).

All the plants regain PSII activity during rehydration, on the first day of re-watering resulted in a significant increase in PSII activity in bacteria treated and untreated plants, though, a complete restoration of PSII activity was not observed. PSII activity restoration on the first day of re-watering was observed as WS+PF>WS+BS>WS+BC>WS (Fig.2.3). The second day of re-watering resulted in complete restoration of PSII activity (i.e., PSII activity similar to their control plants) in bacteria treated plants, whereas in the case of untreated plants the PSII activity was not completely recovered and the increase in PSII activity between the treatments was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.3). The third day of watering resulted

in the recovery of PSII activity in all stress imposed plants and the order of PSII activity was observed as WS+PF>WS+BS >WS+BC>WS (Fig.2.3).

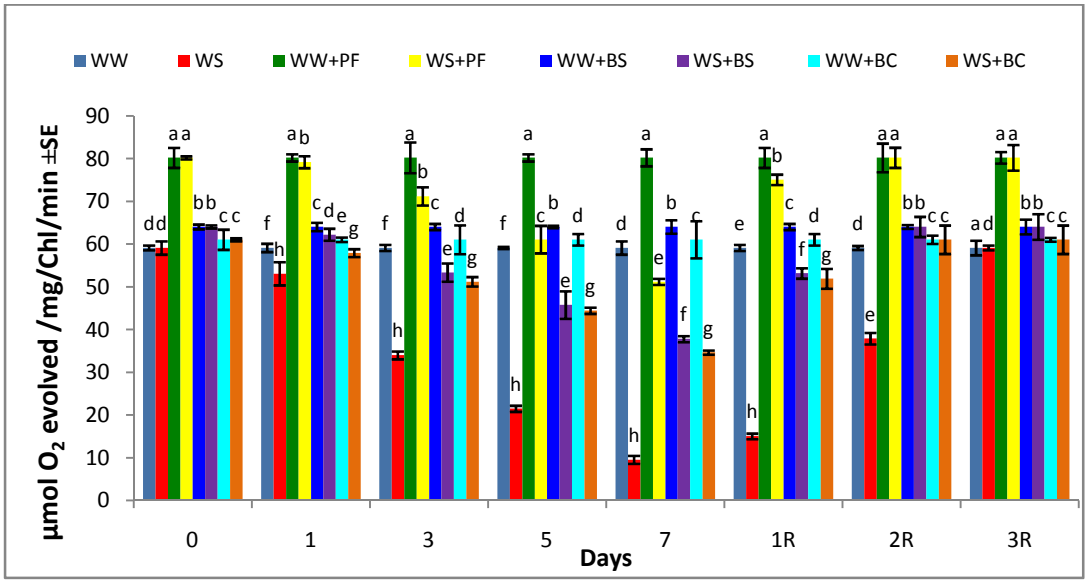

Fig.2.3. Variation in phoosystem II activity during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,*WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,* WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.2.1.1.4 Photosystem I activity

The photosystem I activity, in okra during regularly irrigated condition significantly improved in bacteria treated plants and the order of increase in PSI activity was recorded in the order WW+PF>WW+BS>WW+BC>WW. In drought susceptible plants, higher reduction in photosystem I activity was recorded in the untreated plants and a lower reduction was observed in PF treated plants. During the first day of water stress, no significant change in PSI activity was observed in bacteria treated and untreated plants (Fig.2.4). During the third day of drought, a significant decrease in the activity of the

PSI was observed in all plants subjected to drought. Higher reduction in PSI activity was observed in untreated plants and a lower reduction was observed in PF treated plants when compared to control and other bacteria treated stress exposed plants (Fig.2.4). On the fifth day of stress, a significant decline in PSI activity was observed in untreated plants, however, the decrease in PSI activity of bacteria treated plants was significantly lesser than the drought induced reduction in the untreated plants. Among bacteria treated plants, PF treated plants recorded the lower reduction in PSI activity compared to their control plants and other stressed bacteria treated plants i.e., (BS and BC treated okra), the order of reduction in PSI activity, on the fifth day of water stress was recorded as WS>WS+BC>WS+BS>WS+PF (Fig.2.4). The seventh day of drought, PSI activity of okra significantly decreased and higher reduction in PSI activity was observed in the untreated plants and a lower reduction was observed in the PF treated plants with respect to control plants and other bacteria treated plants. The order of reduction in PSI activity of bacteria treated and untreated plants were recorded as WS>WS+BC> WS+BS>WS+PF (Fig. 2.4).

All the plants recovered the PSI activity at the time of rehydration, but bacteria treated plants showed faster recovery when compared to the untreated plants. The first day of re-watering significantly improved the PSI activity in bacteria treated plants while, the increase in the PSI activity in untreated plants was recorded to be lesser when compared to bacteria treated plants. Recovery status of PSI activity on the first day of re-watering was recorded as WS+PF>WS+BS>WS+BC>WS (Fig. 2.4). The second day of rehydration lead to a complete recovery of PSI activity (i.e., PSI activity as similar to control plants) in bacteria treated plants and partial increase in the PSI activity was observed in untreated plants however a complete reimbursement was not observed. The increase in PSI activity between the treatments were recorded as WS+PF>WS+BS>WS+BC>WS (Fig. 2.4). The third day of watering

resulted in a complete recovery of PSI activity in all stress imposed plants and the order of PSI activity between the treatments was observed as WS+PF>WS+BS >WS+BC>WS (Fig. 2.4).

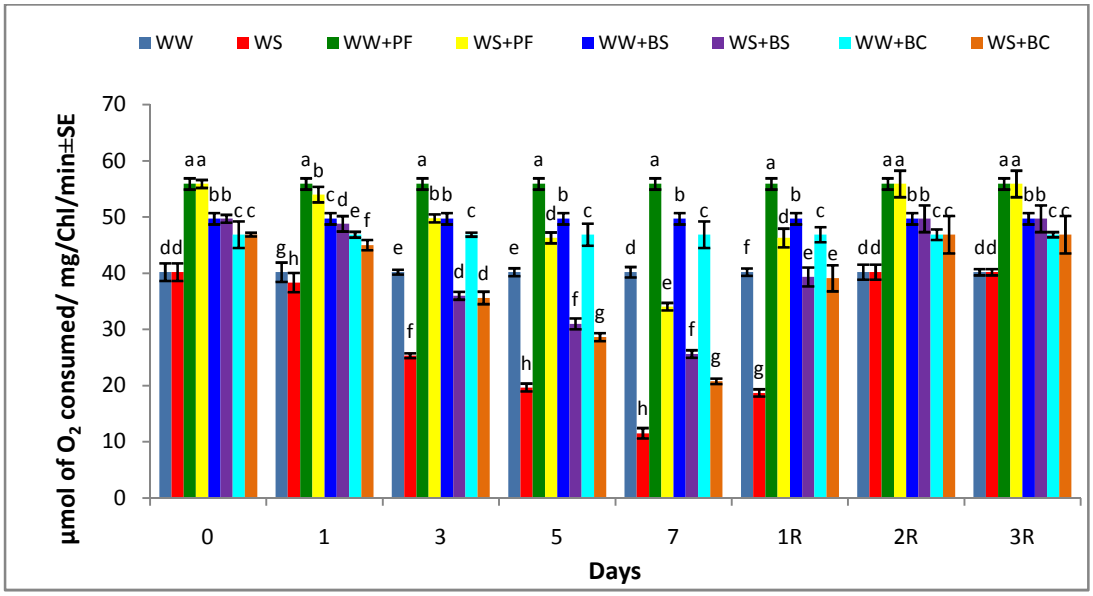

Fig.2.4. Variation in phoosystem I activity during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,*WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,* WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.2.1.1.5 Chlorophyll *a* fluorescence parameters

4.2.1.1.5.1 Electron donation to PSII

Among the analyzed chlorophyll *a* fluorescence parameters the ratio of electron donation to PSII (F_V/F_o) has increased significantly in bacteria treated plants when compared to the untreated plants under irrigated condition. The increase in electron donation due to the bacteria treatment was observed in the order WW+PF>WW+BS>WW+BC>WW (Fig.2.5 A). On the first day of drought, a significant change in the rate of electron donation to PSII was

recorded by bacteria treated and untreated plants with respect to their control plants (Fig.2.5 B). During the third day of drought, a significant decline in the rate of electron donation to PSII was observed in all the treatments. Enhancement in the reduction of electron donation to PSII was observed in untreated plants and a lesser decrease was recorded in PF treated plants over their respective control and other bacteria treated drought stressed okra. The rate of reduction in electron donation to PSII on the third day of stress in various treatments was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.5 C). Fifth day of water stress significantly reduced the rate of electron donation in the untreated plants, but the bacteria treatment significantly improved the rate of electron donation to PSII even under drought condition, and the order of decline in the rate of electron donation to PSII in the various treatment was observed in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.5 D). A significant decrease in the rate of electron donation to PSII was observed in all okra plants exposed to seven days of drought. The untreated plants showed a higher decrease in the rate of electron donation to PSII compared to bacteria treated plants and among the treatments, PF treated okra showed a lesser reduction in the electron donation to PSII with respect to their control and other bacteria treated plants. The decreasing order of electron donation to PSII was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.5 E).

Okra recovered a normal rate of electron donation to PSII upon re-watering. Bacteria treatment resulted in a quick recovery in the rate of electron donation to PSII in okra compared with untreated plants. The first day of re-watering significantly improved the rate of electron donation to PSII in bacteria treated okra, however, in untreated plants the restoration of electron donation to PSII was slower with respect to bacteria treated plants. After the first day of re-watering, the rate of increase in electron donation to PSII in okra with various treatments was recorded as WS+PF>WS+BS> WS+BC>WS (Fig.2.5 F). The second day of rehydration resulted in complete

recovery in the rate of electron donation to PSII (i.e., a rate of electron donation similar as their control plants) in the bacteria treated plants, while in untreated okra the recovery rate of electron donation to PSII was partial. The increment in the rate of electron donation to PSII between the treatment was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.5 G). The third day of rehydration resulted in complete recovery of electron donation to PSII in all stress imposed plants and the order was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.5 H).

4.2.1.1.5.2 Performance index (PI_{ABS})

The performance index (PI_{ABS}) of PSII was improved by bacteria treatment in the control condition compared to untreated plants (WW+PF>WW+BS>WW+BC>WW) (Fig.2.5 A). The decrease in PI_{ABS} was observed in stress exposed okra, but the reduction was less in bacteria treated plants. There was no significant change in $PI_{ABS,}$ when exposed to the first day of drought stress. But from the third day of water stress significantly reduced PI $_{(ABS)}$ in untreated plants, whereas the decline in PI_{ABS} was much reduced in bacteria treated plants. Lesser reduction in PI_{ABS} has observed in PF treated plants with respect to untreated plants and other bacteria treated plants (Fig.2.5 B). On the third day of drought, a decrease in the PI_{ABS} of okra treated with different bacteria was observed as WS>WS+BC>WS+BS> WS+PF (Fig.2.5 C). On the fifth day of drought, okra without bacteria treatment recorded a significant decrease in PI_{ABS} and the lower reduction was observed in PF treated okra. The decrease in PI_{ABS} in drought exposed plants was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.5 D). On the seventh day of drought, a drastic decrease in PI_{ABS} was observed in the untreated plants with respect to control plants, while the reduction in bacteria treated plants was lesser than the untreated plants. Among the bacteria treated plants, PF treatment recorded a lesser reduction in PI_{ABS} with respect to the control

plants and other stress exposed plants. The decrease in PI_{ABS} during stress was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.5 E).

Re-watering of okra resulted in an increment in PI_{ABS} in all the stress exposed plants. The First day of re-watering resulted in a significant increase in PI_{ABS} in both bacteria treated and untreated okra, even though faster recovery was recorded in bacteria treated plants. Among the bacteria treated plants, PF treatment resulted in a significant increment in $PI_{ABS,}$ on the first day of watering and the restoration of PI_{ABS} with different treatment was observed as WS+PF>WS+BS>WS+BC>WS (Fig.2.5 F). The second day of watering resulted in complete restoration of PI_{ABS} in bacteria treated okra. Increase in PI_{ABS} was observed in the untreated plants but fails to recover PI_{ABS} activity completely. The order of increase in PI_{ABS} between the treatment was in the order WS+PF>WS+BS>WS+BC>WS (Fig.2.5 G). The third day of re-watering resulted in complete recovery of PI_{ABS} in all the stress imposed plants and the order of recovery was WS+PF>WS+BS >WS+BC>WS (Fig.2.5 H).

4.2.1.1.5.3 Maximal fluorescence (Fm)

There is a significant difference between maximal fluorescence (Fm) of PSII in bacteria treated plants and untreated plants of control experiments, the order of increase in Fm of okra treated with different bacteria was observed as WW+PF>WW+BS>WW+BC>WW (Fig.2.5 A). Fm of PSII decreased under drought stress, higher reduction in Fm was recorded in the untreated drought susceptible okra compared to control plants and the bacteria treated drought exposed plants. The change in Fm value of okra plants exposed to the first day of water stress was insignificant with respect to control plants (Fig.2.5 B). The decline in Fm value of okra exposed to the third day stress was higher in the untreated plants and lesser in PF treated plants with respect to control plants. The decreasing order of Fm value of okra

on the third day of water stress was recorded as WS>WS+BC>WS+BS> WS+PF (Fig.2.5 C). Fm value in okra exposed to the fifth day of drought resulted in a significant decrease, higher decline in Fm value was observed in untreated plants and a lower reduction was recorded in PF treated plants compared to control and other bacteria treated plants. The order of reduction in Fm value between the treatments induced by the fifth day of drought WS>WS+BC>WS+BS>WS+PF (Fig.2.5 D). A drastic decline in Fm value was observed in the untreated okra subjected to the seventh day of drought however, the Fm value decreases appreciably in bacteria treated plants. Reduction in Fm value observed in PF treated plants with respect to their control plants and other bacteria treated plants. The decrease in Fm value on the seventh day of water stress was in the order WS>WS+BC>WS+BS> WS+PF (Fig.2.5 E).

Okra regains the normal Fm value during rehydration and faster recovery of Fm value was recorded in bacteria treated plants. The first day of watering significantly increased the Fm value of okra, however, in bacteria treated plants the Fm value regained faster than the untreated plants. The increase in Fm value as a result of re-watering in okra with various treatments was in the order WS+PF>WS+BS>WS+BC>WS (Fig.2.5 F). A complete revival of Fm value in bacteria treated plants was observed on the second day of rehydration, Fm value found to be increased in the untreated okra even though, the recovery was partial. Increase in Fm value in stressed plants with different treatments was observed as WS+PF>WS+BS >WS+BC>WS (Fig.2.5 G). The third day of re-watering resulted in a complete revival of Fm in each stress imposed plants and the Fm value was recorded in the order WS+PF>WS+BS >WS+BC>WS (Fig.2.5 H).

4.2.1.1.5.4 Structure function index (SFI)

Structure function index (SFI) of PSII provides information about the structural and functional changes in PSII reaction center exposed to stress. A slight variation in SFI was observed in bacteria treated plants and untreated plants in normal irrigated condition, the difference in SFI of okra inoculated with different bacteria was in the order WS+PF>WS+BS >WS+BC>WS (Fig.2.5 A). Reduction in SFI of PSII was observed in drought affected okra, but the SFI was highly reduced in bacteria treated okra compared to untreated plants. The reduction in SFI of okra subjected to the first day of water stress was insignificant in both bacteria treated and untreated plants (Fig.2.5 B). However, a minor reduction in SFI was observed in untreated plants on the third day of stress. Reduction in SFI was negligible in bacteria treated plants and among different bacteria treatments, PF treated okra recorded lesser reduction in SFI with respect to untreated plants and other bacteria treated plants. Within the different treatments, decrease in the SFI of PSII in okra on the third day of stress was in the order, WS>WS+BC>WS+BS>WS+PF (Fig.2.5 C). Prolonged water stress (i.e., 5d and 7d) negatively influenced the SFI of PSII in the untreated plants and resulted in a higher reduction in SFI. Okra treated with bacteria significantly improved SFI of PSII, in bacteria treated okra PF treated plants was recorded lesser reduction in SFI. The SFI in drought exposed plants were recorded in the decreasing order as WS>WS+BC>WS+BS>WS+PF (Fig.2.5 D). Severe reduction in SFI of PSII was observed in okra when exposed to the seventh day of drought, at the same time lower reduction in SFI was observed in PF treated plants when compared to their control plants and other stress exposed plants. A higher reduction was noted in the untreated plants, the order of decrease in SFI during the drought was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.5 E).

Rehydration resulted in the revival of SFI in okra, bacteria treated plants recorded quick recovery of SFI in okra compared to untreated plants. The first day of re-watering increased SFI in all plants, still, bacteria treated plants recorded faster regain of SFI. The increasing rate of SFI in okra under various treatments was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.5 F). During the second day of re-watering, the bacteria treated plants regain the SFI of PSII (i.e., SFI as similar as their control plants), in the untreated okra, SFI was increased but not completely recovered. Increase in the SFI of PSII between the treatments were recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.5 G). The third day of rehydration resulted in the restoration of okra PSII's SFI and the order of restoration was noted to be WS+PF>WS+BS >WS+BC>WS (Fig.2.5 H).

4.2.1.1.5.5 Electron transport quantum yield (φ_{Eo})

Significant improvement in electron transport quantum yield (φ_{Eo}) was observed in bacteria treated irrigated plants, higher φ_{Eo} was recorded in PF treated plants and lower φ_{Eo} was noted in the untreated plants. The order of φ_{Eo} of okra during the control condition was observed as WW+PF>WW+BS> WW+BC>WW (Fig.2.5A). A significant reduction in φ_{Eo} was observed in okra during stress, but bacteria treatment significantly improved φ_{Eo} even under drought. The reduction in φ_{Eo} due to the first day of stress exposure was insignificant (Fig.2.5B), whereas the plants showed significant difference in φ_{Eo} from the third day of water stress, the untreated plants recorded increased reduction in φ_{Eo} and lower reduction was observed in PF treated okra compared to control and other bacteria treated plants. The decreasing order of electron transport quantum yield (φ_{Eo}) between the treatments was noted as WS>WS+BC>WS+BS>WS+PF (Fig.2.5C). The fifth day of drought significantly reduced the φ_{Eo} in all plants, the higher reduction in φ_{Eo} was recorded in untreated okra compared with control and bacteria treated plants.

However, the stress imposed reduction was less in bacteria treated plants, a lower reduction was recorded in PF treated plants over the respective control plants. The reduction in φ_{Eo} on the fifth day of drought was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.5D). Significant decrease in φ_{Eo} was observed in okra exposed to the seventh day of drought, while the increasd φ_{Eo} reduction was observed in the untreated plants and PF treated plants showed lesser decrease in φ_{Eo} compared to the control plants and the range of reduction in φ_{Eo} during stress exposure was in the order WS>WS+BC>WS+ BS>WS+PF (Fig.2.5E).

At the time of re-watering okra recovers normal φ_{Eo}, yet bacteria treated plants recorded quick recovery. First day of rehydration improved the rate φ_{Eo} in all plants; however, the bacteria treated plants recorded increased φ_{Eo} than bacteria untreated plants. The order of φ_{Eo} between the treatments after the first day of re-watering was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.5F). The second day of re-watering resulted in a complete recovery in φ_{Eo} of bacteria treated plants, but the untreated okra plants fail to recover completely. Increasing order of φ_{Eo} between the treatment was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.5G). The third day of rehydration resulted in a complete recovery of φ_{Eo} in untreated plants and the order was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.5H).

4.2.1.1.5.6 Primary photochemistry ($\Phi_o/(1- \Phi_o)$)

Bacteria treated plants were registered with a significant improvement in the primary the photochemistry of okra compared to untreated plants. The increase in primary photochemistry due to bacteria treatment in okra plants was observed as WW+PF>WW+BS>WW+BC>WW (Fig.2.5 A). A drastic reduction was observed in the primary photochemistry in all plants when subjected to drought stress, but the rate of reduction in primary

photochemistry was reduced in bacteria treated plants. The decrease in the primary photochemistry of okra due to the drought stress on the first day was insignificant (Fig.2.5 B), but the third day of water stress recorded a significant drop in primary photochemistry. However, the decline was more prominent in bacteria untreated plants compared to bacteria treated plants. Bacteria treatment significantly reduced the drought induced decrement in primary photochemistry. Among them, PF treated plants recorded a lesser reduction in the primary photochemistry. Drought induced decrease in primary photochemistry of okra with different treatment on the third day of stress was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.5 C). Plants exposed to fifth day of drought without bacteria treatment recorded a significant decrease in primary photochemistry and the lower decline was observed in PF treated okra. The decreasing rate of primary photochemistry in drought exposed plants was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.5 D). The seventh day of drought recorded a drastic drop in primary photochemistry, in bacteria untreated plants recorded higher reduction with respect to control plants, while the reduction in bacteria treated plants was lesser than the untreated plants. Among the bacteria treated plants, PF treated okra recorded lesser reduction in primary photochemistry with respect to the control plants and other stress exposed plants. The decline in primary photochemistry due to the water stress exposure was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.5 E).

All plants regain normal primary photochemical activity during re-watering, bacteria treatment resulted in a sudden recovery compared with untreated plants. First day of rehydration significantly improved the rate of primary photochemistry in all plants however; regain of primary photochemistry in bacteria treated plants was faster than the untreated plants. After the first day of re-watering, the rate of primary photochemistry in okra with various treatments was recorded as WS+PF>WS+BS>WS+BC>WS

(Fig.2.5 F). The second day of rehydration resulted in a complete recovery of the rate of primary photochemistry in bacteria treated plants and in the untreated plants, the recovery rate of primary photochemistry was slower. Increase in primary photochemistry between the treatment was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.5 G). The third day of rehydration resulted in a full recovery of primary photochemistry in all stress imposed plants and the order was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.5 H).

4.2.1.1.5.7 Energy flux model of PSII

Various specific energy flux model of PSII was studied in bacteria treated and untreated plants in control, stressed and recovery state. Using specific energy flux model of PSII per cross section, the parameters investigated were absorption per cross section (ABS/CSo), trapping energy per cross section (TR/CSo), rate of electron transport per cross section (ET/CSo) and dissipation energy per cross section (DI/CSo). Specific energy flux model of PSII per reaction center was used to analyze absorption (ABS/RC), trapping energy (TR_O/RC) rate of electron transport (ET_O/RC) and dissipation energy (DI_O/RC) per active reaction center of PSII was investigated.

4.2.1.1.5.7.1 Reaction center density per cross section

Drought cause reduction in reaction center density per cross section (RC/CS_O). There is no significant change in RC/CSo between untreated plants and bacteria treated plants during regularly watered period (Fig.2.6.A, Fig.2.7.A, Fig.2.8.A and Fig. 2.9.A). Drought impaired reduction in RC/CSo was insignificant on the first day of drought stress (Fig.2.6.B, Fig.2.7.B, Fig.2.8.B and Fig. 2.9.B). The third day of water stress showed a significant reduction in RC/CSo in the untreated plants, however, the decline in RC/CSo

in bacteria treated plants was lower, within bacteria treated plants a lesser reduction in RC/CSo was observed in PF treated plants with respect to untreated plants and also in other bacteria treated plants. The reduction in RC/CSo on the third day of drought in okra was observed as in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.6. C, Fig.2.7.C, Fig.2.8.C and Fig. 2.9.C). Plants exposed to the fifth day of drought showed a significant reduction in RC/CSo, a higher decline in RC/CSo was observed in okra without bacteria treatment and a lower reduction was observed in PF treated okra with respected to control plants and other bacteria treated plants. The decreasing rate of RC/CSo in drought exposed plants was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.6.D, Fig.2.7.D, Fig.2.8.D and Fig. 2.9.D). The seventh day of drought resulted in a higher reduction in RC/CSo in the untreated plants with respect to control plants whereas the reduction observed in bacteria treated plants was lesser than the untreated plants. Among the bacteria treatments, the PF treated okra recorded a lesser reduction in RC/CSo compared to control plants and other stress exposed plants. The decrease in RC/CSo upon water stress was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.6.E, Fig.2.7.E, Fig.2.8.E and Fig. 2.9. E).

All plants recovered normal RC/CSo during re-watering, bacteria treatment resulted in a faster recovery of RC/CSo in okra compared to untreated plants. The first day of re-watering resulted in a significant improvement in RC/CSo, but the plants cannot recover the actual RC/CSo, although the bacteria treated plants showed a steep hike in regaining RC/CSo compared to untreated plants. After the first day of re-watering, the rate of increase in RC/CSo in okra with various treatments was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.F, Fig.2.7.F, Fig.2.8.F and Fig. 2.9. F). The second day of rehydration resulted in a complete recovery of RC/CSo in bacteria treated plants, but in the untreated plants, a complete recovery was

not observed. Enhanced RC/CSo by re-watering between the treatment was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.G, Fig.2.7.G, Fig.2.8.G and Fig. 2.9. G). The third day of rehydration resulted in a complete recovery of RC/CSo in all the stress exposed plants and the order was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.H, Fig.2.7.H, Fig.2.8.H and Fig. 2.9. H).

4.2.1.1.5.7.2 Absorption per cross section (ABS/CSo) and (ABS/RC) reaction center

Increase in absorption per cross section (ABS/CSo) and absorption per active reaction center (ABS/RC) was observed in bacteria treated plants in irrigated condition compared to untreated plants. The enhancement in ABS/CSo and ABS/RC between the treatments was observed as WW+PF>WW+BS>WW+BC>WW (Fig.2.6.A, Fig.2.7.A, Fig.2.8.A, Fig. 2.9.A, Fig.2.10.A, Fig.2.11.A, Fig.2.12.A and Fig. 2.13.A). Water stress resulted in a decrease in ABS/CSo and ABS/RC however, the bacteria treatment significantly improved the drought induced reduction in ABS/CSo and ABS/RC compared to untreated plants. The rate of ABS/CSo and ABS/RC in the treatments were recorded as WW+PF>WW+BS> WW+BC>WW. The decrease in ABS/CSo and ABS/RC was observed in okra subjected to the first day of drought, but the drop was less significant (Fig.2.6.B, Fig.2.7.B, Fig.2.8.B, Fig. 2.9.B, Fig.2.10.B, Fig.2.11.B, Fig.2.12.B and Fig. 2.13.B). On the third day of stress, a significant decrease was observed in ABS/CSo and ABS/RC in untreated plants but in the case of bacteria treated plants the reduction was lesser. PF treated plants recorded a lower reduction in ABS/CSo and ABS/RC during the third day of stress with respect to untreated plants and the other bacteria treated plants. The order of drought induced decline in ABS/CSo and ABS/RC on the third day of stress among the different treatments were WS>WS+BC>WS+BS>WS+PF

(Fig.2.6.C, Fig.2.7.C, Fig.2.8.C, Fig. 2.9.C, Fig.2.10.C, Fig.2.11.C, Fig.2.12.C and Fig. 2.13.C). When plants exposed to the fifth day of water stress without bacteria treatment, a significant decrease in ABS/CSo and ABS/RC and a lower reduction was observed in PF treated okra compared to the control plants and other bacteria treated plants. The drought imposed decrease in ABS/CSo and ABS/RC among the treatment was observed as WS>WS+BC> WS+BS>WS+PF (Fig.2.6.D, Fig.2.7.D, Fig.2.8.D, Fig. 2.9.D, Fig.2.10.D, Fig.2.11.D, Fig.2.12.D and Fig. 2.13.D). The seventh day of drought resulted in a drastic decline in ABS/CSo and ABS/RC in untreated plants with respect to control plants, but the decline in ABS/CSo and ABS/RC in bacteria treated plants was recorded lesser than the untreated plants. In bacteria treated plants a lower reduction in ABS/CSo and ABS/RC was observed in PF treated plants compared to control and bacteria treated plants. The decrease in ABS/CSo and ABS/RC during stress exposure was recorded in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.6.E, Fig.2.7.E, Fig.2.8.E, Fig. 2.9.E, Fig.2.10.E, Fig.2.11.E, Fig.2.12.E and Fig. 2.13.E).

All the plants recovered a normal ABS/CSo and ABS/RC upon re-watering, bacteria treated plants acquired normal ABS/CSo and ABS/RC faster than the untreated plants. The first day of re-watering resulted in a significant increase in ABS/CSo and ABS/RC in all the treatments, but complete recovery was not observed. Bacteria treated plants recovered the ABS/CSo and ABS/RC faster than the untreated plants. First day of re-watering resulted an increase in ABS/CSo and ABS/RC in okra in following order WS+PF>WS+BS >WS+BC>WS (Fig.2.6.F, Fig.2.7.F, Fig.2.8.F, Fig. 2.9.F, Fig.2.10.F, Fig.2.11.F, Fig.2.12.F and Fig. 2.13.F). The second day of rehydration resulted in complete recovery of ABS/CSo and ABS/RC in the bacteria treated plants, an increase in ABS/CSo and ABS/RC was recorded in untreated plants but the recovery was incomplete. Increase in ABS/CSo and ABS/RC between the treatment was recorded as WS+PF>WS+BS>WS+BC>

WS (Fig.2.6.G, Fig.2.7.G, Fig.2.8.G, Fig. 2.9.G, Fig.2.10.G, Fig.2.11.G, Fig.2.12.G and Fig. 2.13.G). The third day of rehydration resulted in complete recovery of ABS/CSo and ABS/RC in all the stress imposed plants and the order of recovery among the different treatments was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.H, Fig.2.7.H, Fig.2.8.H, Fig. 2.9.H, Fig.2.10.H, Fig.2.11.H, Fig.2.112.H and Fig. 2.13.H).

4.2.1.1.5.7.3 Trapping energy per cross section (TR_O/CSo) and reaction center (TR_O/RC)

A slight increase in trapping energy per cross section was observed in bacteria treated plants under irrigated condition (Fig.2.6.A, Fig.2.7.A, Fig.2.8.A, Fig. 2.9.A, Fig.2.10.A, Fig.2.11.A, Fig.2.12.A and Fig. 2.13.A). Water stress significantly reduced trapping energy per cross section (TR_O/CSo) and trapping energy per reaction center (TR_O/RC) in both bacteria treated and untreated okra plants during the stress exposure. The first day of stress did not cause any significant change in trapping energy per cross section (TR_O/CSo) and trapping energy per reaction center (TR_O/RC) (Fig.2.6.B, Fig.2.7.B, Fig.2.8.B, Fig. 2.9.B, Fig.2.10.B, Fig.2.11.B, Fig.2.12.B and Fig. 2.13.B). Whereas, from the third day onwards the stress exposed plants showed a significant difference in trapping energy per cross section (TR_O/CSo) and trapping energy per reaction center (TR_O/RC). Higher reduction in TR_O/CSo and TR_O/RC was observed in the untreated plants however, the decrease in TR_O/CSo and TR_O/RC was much lesser in bacteria treated plants. The order of decrease in TR_O/CSo and TR_O/RC in the treatments was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.6.C, Fig.2.7.C, Fig.2.8.C, Fig. 2.9.C, Fig.2.10.C, Fig.2.11.C, Fig.2.12.C and Fig. 2.13.C). The fifth day of drought stress showed a higher reduction in TR_O/CSo and TR_O/RC in the untreated plants but a lesser reduction was observed in PF treated plants. The order of reduction in TR_O/CSo and

TR_O/RC activity in stress exposed plants was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.6.D, Fig.2.7.D, Fig.2.8.D, Fig. 2.9.D, Fig.2.10.D, Fig.2.11.D, Fig.2.12.D and Fig. 2.13.D). The seventh day of stress, significantly reduced the TR_O/CSo and TR_O/RC in all the stress exposed plants. A higher reduction was observed in the untreated plants but the reduction was lesser in bacteria treated plants compared to untreated water stressed plants. Among the bacteria treated plants, PF treated plants showed a lesser reduction in TR_O/CSo and TR_O/RC exposed to drought and the order of reduction in stress exposed plants were recorded as WS>WS+BC>WS+BS> WS+PF (Fig.2.6.E, Fig.2.7.E, Fig.2.8.E, Fig. 2.9.E, Fig.2.10.E, Fig.2.11.E, Fig.2.12.E and Fig. 2.13.E).

The first day of re-watering significantly increased trapping by PSII in bacteria treated okra, however, in untreated plants the regain was slower compared to bacteria treated plants. After the first day of re-watering, the rate of increase in trapping energy to PSII in okra with various treatments was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.F, Fig.2.7.F, Fig.2.8.F, Fig. 2.9.F, Fig.2.10.F, Fig.2.11.F, Fig.2.12.F and Fig. 2.13.F). The second day of rehydration led to a full recovery of PSII trapping energy (i.e., trapping energy per reaction center and cross section of PSII was similar as their control plants) in bacteria treated plants, while in the untreated plants complete recovery was not observed. Increase in the rate of trapping of light energy to PSII among the treatment was recorded as WS+PF>WS+BS> WS+BC>WS (Fig.2.6.G, Fig.2.7.G, Fig.2.8.G, Fig. 2.9.G, Fig.2.10.G, Fig.2.11.G, Fig.2.12.G and Fig. 2.13.G). The third day of rehydration lead to full recovery of trapping energy to PSII in all stress imposed plants and the increasing order of trapping energy per reaction center and cross section of PSII was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.6.H, Fig.2.7.H, Fig.2.8.H, Fig. 2.9.H, Fig.2.10.H, Fig.2.11.H, Fig.2.12.H and Fig. 2.13.H).

4.2.1.1.5.7.4 Electron transport per cross section and reaction center

A significant increase in electron transport per cross section and reaction center was observed in bacteria treated plants and untreated plants under irrigated condition. The rate of electron transport per cross section and reaction center (ET/CSo and ET/RC) in the irrigated condition among various treatments were in the order WW+PF>WW+BS>WW+BC>WW (Fig.2.6.A, Fig.2.7.A, Fig.2.8.A, Fig. 2.9.A, Fig.2.10.A, Fig.2.11.A, Fig.2.12.A and Fig. 2.13.A). Drought stress causes significant reduction in ET/CSo and ET/RC in both bacteria treated and untreated okra however, first day of drought exposure did not show any significant variation between well watered and water stressed plants (Fig.2.6.B, Fig.2.7.B, Fig.2.8.B, Fig. 2.9.B, Fig.2.10.B, Fig.2.11.B, Fig.2.12.B and Fig. 2.13.B). From the third day of stress, the reduction in ET/CSo and ET/RC between treatments was in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.6.C, Fig.2.7.C, Fig.2.8.C, Fig. 2.9.C, Fig.2.10.C, Fig.2.11.C, Fig.2.12.C and Fig. 2.13.C). The fifth day of drought stress caused a significant decrease in ET/CSo and ET/RC in untreated okra, a lower reduction was observed in PF treated okra. The decrease in ET/CSo and ET/RC in drought exposed plants was in the order WS>WS+BC>WS+BS> WS+PF (Fig.2.6.D, Fig.2.7.D, Fig.2.8.D, Fig. 2.9.D, Fig.2.10.D, Fig.2.11.D, Fig.2.12.D and Fig. 2.13.D). The seventh day of drought resulted in a drastic decrease in ET/CSo and ET/RC in the untreated plants with respect to control plants, while the reduction observed in bacteria treated plants was lesser than the untreated plants. Among the different bacteria treated plants, PF treated okra recorded a lesser reduction in ET/CSo and ET/RC with respect to the control plants and other stress exposed plants, the decrease in ET/CSo and ET/RC during stress exposure was in the order WS>WS+BC>WS+BS> WS+PF (Fig.2.6.E, Fig.2.7.E, Fig.2.8.E, Fig. 2.9.E, Fig.2.10.E, Fig.2.11.E, Fig.2.12.E and Fig. 2.13.E). Okra recovered a normal rate of electron donation to PSII upon re-watering, bacteria treatment resulted in a quick recovery of ET/CSo and ET/RC of PSII in okra compared to untreated plants.

The first day of re-watering significantly improved the ET/CSo and ET/RC of PSII in bacteria treated okra. However, in untreated plants the restoration in the rate of electron donation to PSII was slower compared to bacteria treated plants. After the first day of re-watering, the rate of increase in ET/CSo and ET/RC of PSII in okra with various treatments was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.F, Fig.2.7.F, Fig.2.8.F, Fig. 2.9.F, Fig.2.10.F, Fig.2.11.F, Fig.2.12.F and Fig. 2.13.F). The second day of rehydration resulted in a complete recovery of ET/CSo and ET/RC (i.e., PSII activity as similar as their control plants) of bacteria treated plants, while the untreated okra did not recover completely the rate of electron donation to PSII. Increment of electron donation to PSII between the treatment was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.G, Fig.2.7.G, Fig.2.8.G, Fig. 2.9.G, Fig.2.10.G, Fig.2.11.G, Fig.2.12.G and Fig. 2.13.G). The third day of rehydration resulted in a complete restoration of ET/CSo and ET/RC in all stressed plants and the difference between the electron transport was observed as WS+PF>WS+BS >WS+BC>WS (Fig.2.6.H, Fig.2.7.H, Fig.2.8.H, Fig. 2.9.H, Fig.2.8.10, Fig.2.11.H, Fig.2.12.H and Fig. 2.13.H).

4.2.1.1.5.7.5 Dissipation per cross section DI_O/CSo

Drought stress resulted in an increase in dissipation per cross section DI_O/CSo and dissipation per active reaction center DI_O/RC in okra. There was no significant difference in dissipation between bacteria treated and untreated plants under irrigated condition (Fig.2.6.A, Fig.2.7.A, Fig.2.8.A, Fig. 2.9.A, Fig.2.10.A, Fig.2.11.A, Fig.2.12.A and Fig. 2.13.A) and on the first day of stress (Fig.2.6.B, Fig.2.7.B, Fig.2.8.B, Fig. 2.9.B, Fig.2.10.B, Fig.2.11.B, Fig.2.12.B and Fig. 2.13.B). On the third day of stress, DI/CSo and DI/RC showed a significant increase and the order of increase in dissipation among the treatments were observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.6.C, Fig.2.7.C, Fig.2.8.C, Fig. 2.9.C, Fig.2.10.C, Fig.2.11.C, Fig.2.12.C and Fig. 2.13.C). On the Fifth day of drought, okra without bacteria treatment recorded a significant increase in DI/CSo and DI/RC and a lesser reduction was

observed in PF treated okra. The decreasing rate of DI/CSo and DI/RC in drought exposed plants was observed as WS>WS+BC>WS+BS>WS+PF (Fig.2.6.D, Fig.2.7.D, Fig.2.8.D, Fig. 2.9.D, Fig.2.10.D, Fig.2.11.D, Fig.2.12.D and Fig. 2.13.D). The seventh day of drought resulted in a drastic increase in DI/CSo and DI/RC untreated plants compared to control plants, while the increase observed in bacteria treated plants was lesser than the untreated plants. Among the bacteria treated plants, PF treatment recorded a lesser increase in DI/CSo and DI/RC with respect to their control plants and other stress exposed plants, the increase in DI/CSo and DI/RC during stress exposure was noted in the order WS>WS+BC>WS+BS>WS+PF (Fig.2.6.E, Fig.2.7.E, Fig.2.8.E, Fig. 2.9.E, Fig.2.10.E, Fig.2.11.E, Fig.2.12.E and Fig. 2.13.E). Okra recovered the normal rate of DI/CSo and DI/RC upon re-watering, bacteria treatment resulted in quick recovery of DI/CSo and DI/RC in the PSII of okra compared with the untreated plants. The first day of re-watering significantly improved the rate of electron donation to PSII in bacteria treated okra. However, in untreated plants, the restoration of DI/CSo and DI/RC PSII was slower with respect to bacteria treated plants.

After first day of re-watering, the rate of decrease in DI/CSo and DI/RC in PSII of okra with various treatments was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.F, Fig.2.7.F, Fig.2.8.F, Fig. 2.9.F, Fig.2.10.F, Fig.2.11.F, Fig.2.12.F and Fig. 2.13.F). The second day of rehydration resulted in a complete recovery in the rate of DI/CSo and DI/RC of PSII (i.e., PSII activity as similar as their control plants) of bacteria treated plants, while the untreated okra did not completely recover the dissipation by PSII. Decrease in DI/CSo and DI/RC of PSII between the treatment was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.2.6.G, Fig.2.7.G, Fig.2.8.G, Fig. 2.9.G, Fig.2.10.G, Fig.2.11.G, Fig.2.12.G and Fig. 2.13.G). The third day of rehydration resulted in a complete recovery of PSII DI/CSo and DI/RC in all stress imposed plants and the order was recorded as WS+PF>WS+BS >WS+BC>WS (Fig.2.6.H, Fig.2.7.H, Fig.2.8.H, Fig. 2.9.H, Fig.2.10.H, Fig.2.11.H, Fig.2.12.H and Fig. 2.13.H).

4.2.2 Discussion

4.2.2.1 Effect of bacteriapriming on photosynthesis

4.2.2.1.1 Photosynthetic pigments

Photosynthesis is an important biological process of transforming the energy from sunlight into photochemical energy by photosynthetic organisms (Antal *et al.,* 2018). Chlorophyll molecules act as major photoreceptors in photosynthesis and play an important role in energy absorption from visible spectrum of red (620-750 nm) and blue lights (450-495 nm). Other pigments like carotenoid and xanthophylls act as accessory pigments and absorb solar energy in other ranges of the spectrum and conduct the energy to the reaction center of major photosynthetic pigments (chlorophyll) by resonance transfer through rings and double bonds in pigment structure (Murata and Nishiyama, 2018).

Reduction in chlorophyll and carotenoid content has a major impact on drought stress (Guo *et al*., 2016), that the enhanced activity of proteolytic enzymes like chlorophyllase and enhanced production of ROS, cause membrane disintegration and damage to chlorophyll (Hattori *et al*., 2008). In bacteria treated plants, higher chlorophyll content was observed as a result of increased availability of nutrient content due to modification in root morphology by bacteria was a possible mechanism which improves the synthesis of photosynthetic pigments. Increased chlorophyll content was observed in PF treated plants, and also recorded a lower reduction in chlorophyll content when exposed to drought. Minimum reduction in chlorophyll content was also recorded in BS and BC treated plants compared to untreated plants. Saikia *et al*. (2018) observed that inoculation of plant growth promoting bacterium in *Vigna mungo* and *Pisum sativum* significantly improved chlorophyll content by increasing the availability of nutrients and this increase in chlorophyll content improved photosynthesis even under

stress condition. *Bacillus subtilis* inoculation in lettuce plant alters the stress imposed changes in phytohormone i.e., which increased the production of cytokinins, and also resulted in an increase in chlorophyll synthesis assisting the plants to reduce the chlorophyll degradation (Arkhipova *et al.,* 2005). Similar results were also observed in sunflower inoculated with a mixture of *Azotobacter chroococcum* and *Bacillus polymyxa* (Singh *et al.,* 2015).

Bacteria treatment facilitates a minimum reduction in chlorophyll content in stress imposed plants which cause a faster recovery in these plants. ROS induced damages in chlorophyll were alleviated by the bacteria treatment, through enhanced the activity of ROS scavengers. Delayed recovery in bacteria untreated plants directly correlated with the lower enhancement in enzyme activity during stress. The bacteria treated plants also showed higher metabolite content and lower osmotic potential, which help to maintain a higher RWC and it prevents the chlorophyll degradation under drought stress.

Carotenoids play an important role in photoprotection (Munne-Bosch and Penuelas, 2003), the increase in carotenoid content due to water stress indicate a higher need for photoprotection to primary photosynthetic pigments (Elsheery and Cao, 2008). In stressed conditions, thylakoids act as a source of reactive oxygen species due to the alteration in metabolism (Reddy *et al.,* 2004). Carotenoids protect photosynthetic machinery in four ways, (a) reacting with intermediate products of lipid peroxidation and stop the chain reactions, (b) active scavenging $^1O_2$ and produce heat as a by-product, (c) preventing the production of singlet oxygen by reacting with triplet chlorophyll and excited chlorophyll and (d) dissipating the excess excitation energy, via the xanthophyll cycle (Das and Roychoudhury, 2014).

Carotenogenesis in chromoplasts was influenced by developmental and environmental signals and the major carotenoids, violaxanthin and β-carotene

increased progressively during stress treatment (Nisar *et al*., 2015). The chlorophyll, carotenoid ratio was a good indicator of stress in plants (Hendry and Price, 1993). Similar to above findings an increase in carotenoid content was observed in all okra plants (both bacteria treated and untreated) exposed to drought stress. However, in bacteria treated drought exposed plants the increase in carotenoid content was lesser than untreated plants during stress exposure. This indicates that, higher photodamage due to drought stress occurs in bacteria untreated drought exposed plants. In contradictory with our results, Hernandez *et al.* (2014) reported that in lettuce the PGPR (*Azospirillum brasilense* + *Pantoea dispersa*) treatment had a positive effect on plant growth and the contents of carotenids. Similarly, PGPR and P_2O_5 alone and in combination inoculated in soybean (*Glycine max* L.) showed a significant increase in the carotenoid content (Dwivedi and Gopal, 2013). The application of different strains of PGPR treatments showed that the enhancement in carotenoid content during co-inoculation with *Azospirillum* and *Pseudomonas* in normal and stress conditions (Ahmadi *et al.,* 2013). The combined treatment of *Rhizobium leguminosarum*, *Bacillus megaterium* and *Bacillus mucilaginous* enhanced the carotenoid content when compared to all other treatment of black gram and *Arachis* (Rajasekaran, 2009; Mathivanan *et al.,* 2017).

4.2.2.1.2 Activity of PSI and PSII

In drought stressed okra plants, photosystem II (PSII) activity decreased vertically, this result was significantly correlated with various other parameters like reduction in RWC, leaf osmotic potential, ROS production and activity of ROS scavenging enzymes. Whereas, bacteria treated plants exhibited a lesser reduction in PSII activity and the order of reduction in PSII activity among bacteria treated stressed plants was observed as BC+ WS>BS+WS>PF+WS. The reduction in PSII photodamage in bacteria treated

plants positively correlated with osmolytes production, RWC level and their active antioxidant system. It was already reported that, the performance of PSII was determined by various environmental factors and prolonged drought stress dramatically decreased the activity of PSII (Woo *et al.,* 2008; Marcińska *et al.,* 2017). PSII is a multi-subunit pigment-protein complex composed of D1, D2 found in plants thylakoid membranes, the main component of PSII are reaction centers (RCs), the chlorophyll (Chl) *a*/*b* light-harvesting complex (LHCII) and the oxygen-evolving complex (OEC) (Allahverdiyeva *et al.,* 2013). Earlier studies revealed that, drought stress cause destruction of OEC of PSII and PSII RCs which affects PSII photochemistry (Sperdouli and Moustakas, 2012) and phosphorylation of PSII proteins (Chen *et al.,* 2016). Water stress mediated degradation of PSII proteins D1, D2 and LHCII also resulted in decrease in PSII activity (Yuan *et al.,* 2005, Duan *et al.,* 2006). Enhanced ROS production during drought also leads to the degradation in pigment content due to the destruction of protein, DNA and other cellular functions (Chen *et al.,* 2016; Montillet *et al.,* 2005). According to Nishiyama *et al.* (2011), ROS have a role in inhibiting the synthesis of D1 protein, which was essential for the repair of photodamaged PSII complexes. Accumulation of soluble metabolites in chloroplasts helps the plants to mitigate the harmful effect of water stress PSII (Ashraf and Foolad, 2007; Reddy *et al.,* 2004). Enhanced activity of antioxidant system and augmentation in osmolytes may also facilitate faster recovery in PSII activity in bacteria treated plants.

With increasing duration of water stress, PSI activity was noted to be reduced in okra. The higher reduction in PSI activity was observed in bacteria untreated plants, whereas the reduction in bacteria treated plants was lesser than untreated plants. Among the different bacteria used, PF treated plants exhibited a lesser reduction in PSI activity and the order of decline in PSI activity in bacteria treated plants was observed as BC+ WS>BS+WS>

PF+WS. According to the findings of Huang *et al.* (2013), water stress resulted in the closure of stomata, reduces the availability of carbon dioxide, which induce over reduction of the iron-sulfur centers (PSI acceptor side) and finally results in photoinhibition of PS I. Lower production of ROS in bacteria treated plants may result in an improvement of PSI activity of okra under water stress. The findings of Sonoike (2006) are in agreement with these results, that enhanced production of ROS during water stress reduces iron-sulfur centers and hydroxyl peroxide, impaired the PSI activity. In plants the harmful effect of PS I photoinhibition was alleviated by blocking linear electron flow (LEF) and promoting cyclic electron flow (CEF) that CEF was considered as a major alternation in electron sink for the dissipation of excess excitation energy in CO_2 restricted condition (Huang *et al.,* 2013; Driever and Baker, 2011). CEF also promote higher NPQ level to protect PSII from photoinhibition (Huang *et al*., 2012). So lower reduction in PSI activity of bacteria treated plants also protects PSII from the deleterious effect of drought stress. Faster recovery of PSI in bacteria treated plants significantly correlated with their active ROS defense mechanism.

4.2.2.1.3 Chl *a* fluorescence Analysis of PSII

Chlorophyll *a* fluorescence is a tool to analyze the physiological state of photosystem II (PSII) components, electron transport chain components and the cooperation of light-dependent and light-independent biochemical mechanism (Strasser *et al*., 2004; Suggett *et al*., 2010; Kalaji *et al*., 2011). There are few reports about the monitoring of bacteria mediated drought stress using chlorophyll *a* fluorescence. The efficiency of water splitting complex OEC (Fv/Fo) was reduced in plants under water stressed condition. This led to the donor side photoinhibition of PSII and the result was significantly correlated with PSII activity under the stressed condition. Severe reduction in Fv/Fo was observed in the untreated plants resulting in the higher

reduction of PSII activity in plants. The efficiency of OEC complex was affected differently in drought stressed okra treated with various bacteria. PF treatment improved the efficiency of Fv/Fo in okra plants under the stressed condition. This in turn, affected a lesser reduction in PSII activity of PF treated plants under water stress. In bacteria treated plants, BC treatment showed a higher reduction in Fv/Fo efficiency, suggesting that BC treatment was not effective in okra plants to improve drought tolerance compared to other bacteria treatment. Similar observations were made by Samaniego-Gámez *et al*. (2016). According to the authors, PGPR treatment improves PSII activity under drought through protecting reduction of OEC complex.

Performance index (PI_{ABS}) is a quantitative representation of plant vitality, calculated from the density of the RC per chlorophyll, relative expression of primary photochemistry and total electron transport (Strasser *et al*., 2004). In irrigated condition, PI was higher in bacteria treated okra compared to untreated plants, amongst the bacteria treatment in PF treated okra recorded higher PI. Drought stress resulted in the reduction in PI of all plants, however higher reduction in PI was observed in the untreated stress exposed plants. Performance index was used to analyze the drought factor index (DFI) in plants, DFI was defined as relative drought induced reduction in PI during a stressed period (Kalaji *et al*., 2017). When compared the PI of different bacteria treated drought exposed plants, PF treated plants showed a lesser reduction upon water stress and BC treated plants showed a higher reduction in PI of okra. The reduction in PI during drought exposure was due to the changes in antenna size, reduction in trapping efficiency and interaction in electron transport from QA (Strasser *et al*., 2004). Our results were in agreement with this report that, drought exposed plants lower TRo/CS, TRo/RC and ETo/CS, ETo/RC in the untreated plants and among bacteria treated plants, it was higher in PF treated plants and lower in BC treated plants. The lesser reduction in TRo/CS, TRo/RC and ETo/CS, ETo/RC may

accelerate the faster recovery of PI in bacteria treated plants during re-watering.

Maximal fluorescence (Fm) value was the output of fully reduced QA, Here the Fm value exhibited a reduction in drought stressed plants and maximum reduction in Fm value was recorded in the untreated plants whereas, minimum Fm reduction was noticed in PF treated plants. The BC treated plants showed a higher reduction in Fm value compared with other bacteria treated plants. Yamane *et al.* (2008) suggested that, higher reduction in Fm value in stressed plants is due to the degradation of chlorophyll protein, in agreement with this report the present study suggested that chlorophyll content in the untreated drought exposed plants reduced dramatically. Reduction in Fm value indicates that, failure of energy transfer from LHC II linear electron transport pathway, the trapped energy is used for photochemical reaction emitted as heat and fluorescence (Zhang, 2017). Drought stress results in a decline in Fm value and increase in fluorescence of *Lycium ruthenicum* Murr. (Guo *et al.,* 2016). The minimal reduction in Fm value of bacteria treated okra indicates the successful transfer of trapped energy to Z pathway. These results improved the photosynthetic rate of okra under stress and also facilitate quick recovery of plants.

Structural and functional index of PSII (SFI) provides information about the structural and functional changes in stressed plants. In okra the SFI value was reduced under drought stress exposure. A severe reduction was observed in the untreated plants and a lower reduction was observed in bacteria treated plants. Reduction in SFI activity in bacteria treated okra plants vary with different bacteria treatments, the lower reduction in SFI value was reported in PF treated plants followed by BS treated plants, the higher reduction was observed in BC treated plants. Drought stress enhances the production of ROS in plants that damage the protein components of

photosystem II and lead to photoinhibition (Das and Roychoudhury, 2014). Water stress also affects the repair and synthesis of D1 protein, this in turn, results in structural modification and loss of PSII function (Giardi *et al.,* 1996; Derks *et al.,* 2015). ROS production and SFI value in this study suggests that bacteria treated plants showed lower accumulation of ROS. This reduction in ROS production may also facilitate the sudden recovery during re-watering.

Electron transport quantum yield of PSII (φEo) evaluate the efficiency of PSII photochemistry and transfer of energy absorbed by photosynthetic pigments to the photochemical reaction. φEo value declined in the untreated water stressed plants, but the decline was lesser in bacteria treated plants. PF treated plants showed a lower reduction in φEo value compared to other bacteria treated plants. BC showed a higher reduction in φEo value among bacteria treated drought exposed plants. Drought induced stomatal closure reduced carbon dioxide assimilation and resulted in the reduction in φEo in potato plants (Mankowska *et al.,* 2018). Drought stress causes degradation of chlorophyll resulting in the decline in solar light absorption that interrupts the excitation of PSII and energy transfer. The higher rate of restoration in chlorophyll pigments was observed in bacteria treated plants resulting in faster recovery of φEo value.

Drought stress damage of PSII associated protein resulted in a decline in primary photochemical yield of plants. In drought exposed okra, primary photochemical activity was decreased. The maximum reduction in primary photochemistry was observed in the untreated plants in which Fv/Fo, SFI and PSII activity was also reduced, suggesting that, drought incited higher damage in OEC complex and PSII protein complex, causing a reduction in primary photochemistry. A similar result was observed in potato exposed to drought stress and recovery (Mankowska *et al.,* 2018). Lesser reduction in Fv/Fo, SFI and PSII activity was observed in bacteria treated plants that alleviate the

harmful effect of drought stress in the primary photochemistry of okra and thus lead to faster recovery upon re-watering in photochemical activity.

Drought stress mediated fluctuation in PSII energy fluxes per CSo was analyzed by phenomenological leaf models of okra leaves. A similar observation was made by Marcińska *et al.* (2017) in drought exposed oat plants. ABS/CSo, TRo/CSo and ET/CSo were reduced in drought exposed untreated plants, but in bacteria treated okra plants the reduction in ABS/CS, TRo/CS and ET/CS was lesser. BC treated plants are more susceptible to drought stress and PF treated plants showed a lesser reduction in PSII energy fluxes per CSo bacteria treated plants. ABS/RC, TRo/RC and ET/RC was decreased in drought stressed okra plants, however, the reduction was less in the case of PF treated plants, lower reduction in ABS/CSo, TRo/CSo and ET/CSo was linearly correlated with lesser reduction of ABS/RC, TRo/RC and ET/RC of PF treated plants. Various findings suggested that, the reduction in ABS/CSo, TRo/CSo and ET/CSo was directly proportional to activity of ABS/RC, TRo/RC and ET/RC due to antenna size variation of reaction center which directly influence ABS/CSo, TRo/CSo and ET/CSo. This revealed that bacteria treatments significantly modify the size of antennae in okra which facilitates an increase in photosynthetic rate and faster recovery under the stressed condition.

Dissipation per cross section and dissipation per reaction center DI/CSo and DI/RC significantly increased in water stressed bacteria treated plants, dissipation is the indirect measurement of photo-inhibition in plants. These results indicate that, bacteria treatment significantly reduces photo-inhibition in plants and PF treatment was more effective in okra for alleviating drought related photosynthetic reduction.

4.3 CHAPTER 3

4.3.1 Biochemical parameters

4.3.1.1 Soil analysis

4.3.1.1.1 Soil moisture content

Soil moisture content was significantly reduced in drought exposed plants, during well watered condition, higher soil moisture content was detected in bacteria treated plants growing in soil and the order of soil moisture content was observed as WW+PF>WW+BS>WW+BC>WW (Fig.3.1). The first day of drought stress, induced a slight reduction in soil moisture content between the treatments and the reduction in soil moisture content between the treatments was observed in the order WS>WS+BC>WS+BS>WS+PF (Fig.3.1). A gradual reduction in soil moisture content was observed on the third day of drought exposure, higher reduction in soil moisture content was noticed in untreated soil compared to the control plants. Whereas, in bacteria treated soil recorded lesser reduction, among these soil with PF treatment plants recorded higher soil moisture content compared to other bacteria treated plants and the order of reduction in soil moisture content was observed as WS>WS+BC>WS+BS >WS+PF (Fig.3.1). The fifth day of the drought stress resulted in considerable decrease in soil moisture content compared to the first and third day of drought exposure (Fig.3.1). However, the bacteria treated soil recorded remarkable soil moisture retention and higher soil moisture content was observed in the soil of PF treated plants. The order of reduction in soil moisture content in different treatments of *A.esculentus* was recorded as WS>WS+BC>WS+BS >WS+PF. The maximum reduction in soil moisture content was observed on the seventh day of drought in bacteria untreated drought imposed plants, however, drought driven reduction in soil moisture content was much reduced in PF treated plants (Fig.3.1). The decline in soil moisture content was recorded in the order WS>WS+BC>WS+BS>WS+PF. Apart from other analyzed parameters all plants recovered actual soil moisture on the first day

of re-watering (Fig.3.1) and the soil moisture content of drought exposed plants after the first day of watering was observed the same as regularly irrigated plants i.e., WS+PF>WS+BS >WS+BC>WS

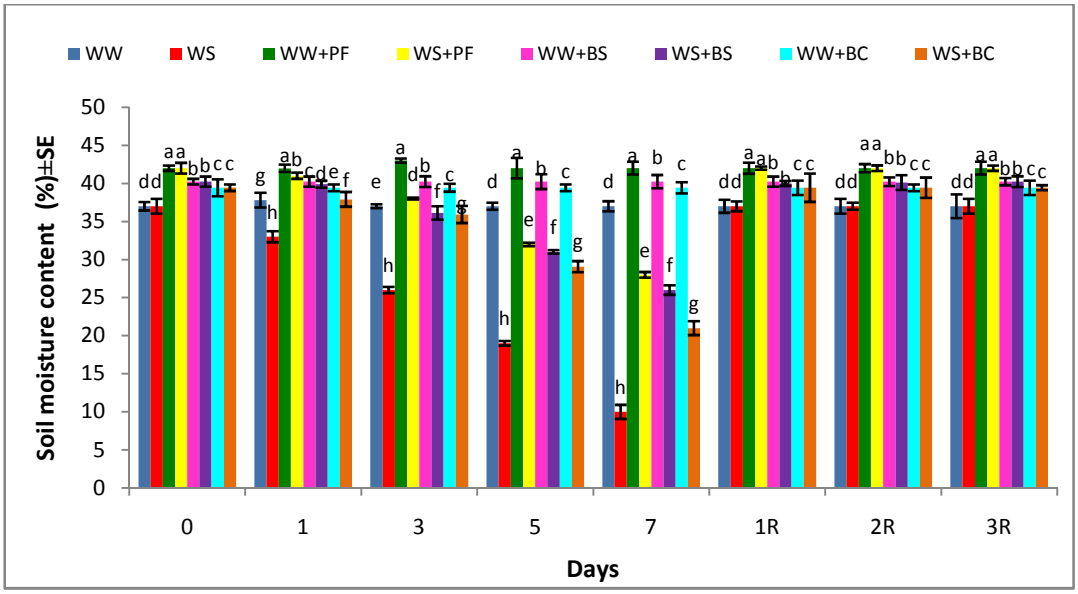

Fig 3.1. Variation in soil moisture content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,* WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS-water stressed okra inoculated with *B. subtilis*, WW+BC-well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.1.1.2. Soil pH

There is negligible difference in soil pH between soil harboring bacteria treated and untreated plants in irrigated condition. Drought stress didn't affect any significant difference in soil pH of untreated plants, similar observation was noticed in soil from bacteria treated plants on the first and third day of water stress compared to the irrigated plants and the order of pH level of the soil on the first and third day of drought stress was observed as WS>WS+BC>WS+BS>WS+PF (Fig.3.2). Although soil having bacteria treated plants showed a gradual reduction in soil pH on the fifth and seventh day of drought stress. On the fifth day of drought, higher reduction in soil pH

was observed in soil having PF treated plants and the order of soil pH was recorded in the order WS>WS+BC>WS+BS>WS+PF (Fig.3.2). During the observation period, a higher reduction in soil pH was recorded on the seventh day of drought stress, the maximum reduction in pH was noticed in soil having PF treated plants compared to soil having other bacteria treatments (i.e., BS and BC treated). The order of soil pH on the seventh day of stress was accounted as WS>WS+BC>WS+BS>WS+PF (Fig.3.2). Regain in soil pH was observed during rehydration period. On the first day of re-watering, faster recovery in soil pH was observed in PF treated plants compared to other bacteria treated plants and the order of soil pH was observed as WS>WS+BC>WS+BS>WS+PF (Fig.3.2). The second day of re-watering resulted in the recovery of soil pH in all bacteria treated plants and the order was recorded WS>WS+BC>WS+BS>WS+PF (Fig. 3.2). The third day of re-watering recorded the soil pH in the order WS>WS+BC>WS+BS>WS+PF (Fig. 3.2).

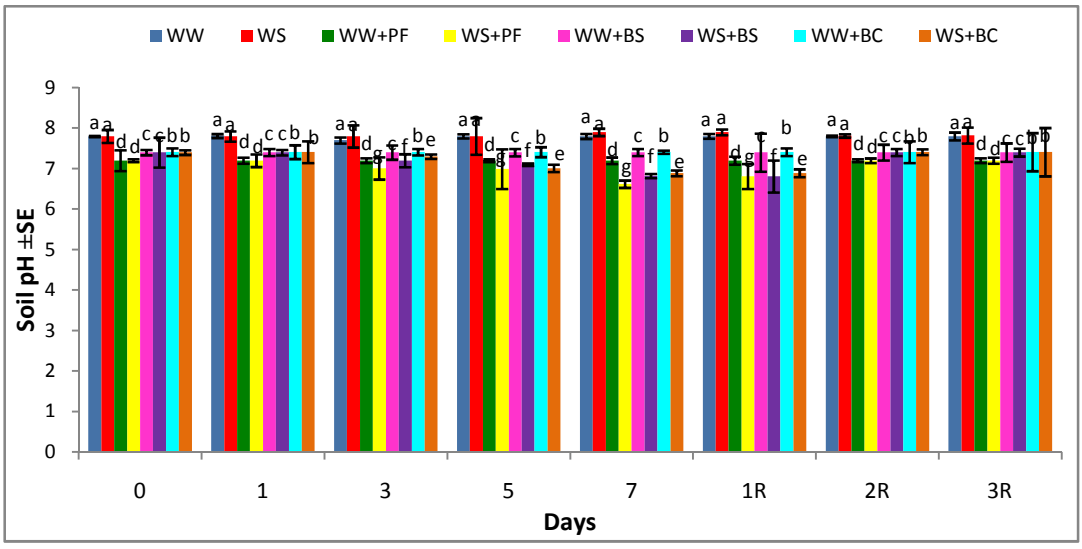

Fig 3.2. Variation in soil pH during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.1.2 Plant analysis

4.3.1.2.1 Leaf relative water content (LRWC)

Leaf relative water content showed significant variation in regularly watered plants and the difference in LRWC was observed in the order WW+PF>WW+BS >WW+BC>WW. During water stressed period (1-7th day of stress exposure) a gradual reduction in LRWC was observed in bacteria treated and untreated plants. The first day drought stress cause a minimal reduction in LRWC, however within drought exposed plants untreated plants recorded higher reduction in LRWC and lesser decrement was noticed in PF treated plants. The order of LRWC in drought exposed plants was observed as WS+PF>WS+BS>WS+BC>WS. Higher reduction LRWC was observed in untreated plants during the third day of drought stress but the reduction was lesser in bacteria treated plants. LRWC on the third day of drought stress in okra treated with different bacteria and without treatment were observed in the order WS+PF>WS+BS>WS+BC>WS (Fig. 3.3). The fifth day of drought stress resulted in a maximum reduction in LRWC in untreated plants and the lesser decline was accounted by PF treated plants. The variation in LRWC was observed on the fifth day of drought stress in the order WS+PF>WS+BS >WS+BC>WS (Fig. 3.3). A steep reduction in LRWC was noticed in all plants during the seventh day of drought stress. Untreated plants recorded higher reduction in LRWC and bacteria treated plants recorded lesser reduction in LRWC when exposed to drought stress. The order of LRWC during the seventh day of drought was observed as WS+PF>WS+ BS>WS+BC>WS (Fig.3.3).

A gradual increase in LRWC was recorded in all stress exposed plants during re-watering, on the first day of re-watering all stressed plants recorded increase in LRWC, but didn't regain LRWC level similar to control plants (regularly irrigated plants). Improvement in LRWC between the treatments on

the first day of re-watering was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.3.3). Second day of re-watering resulted in the revival of LRWC in all bacteria treated plants whereas, in the untreated plants the regain of LRWC was incomplete. The increment in LRWC on the second day of watering was in the order WS+PF>WS+BS>WS+BC>WS. Untreated plants recovered LRWC on the third day of rehydration and after the recovery LRWC status of the plants was observed in the order WS+PF>WS+BS>WS+BC>WS (Fig.3.3).

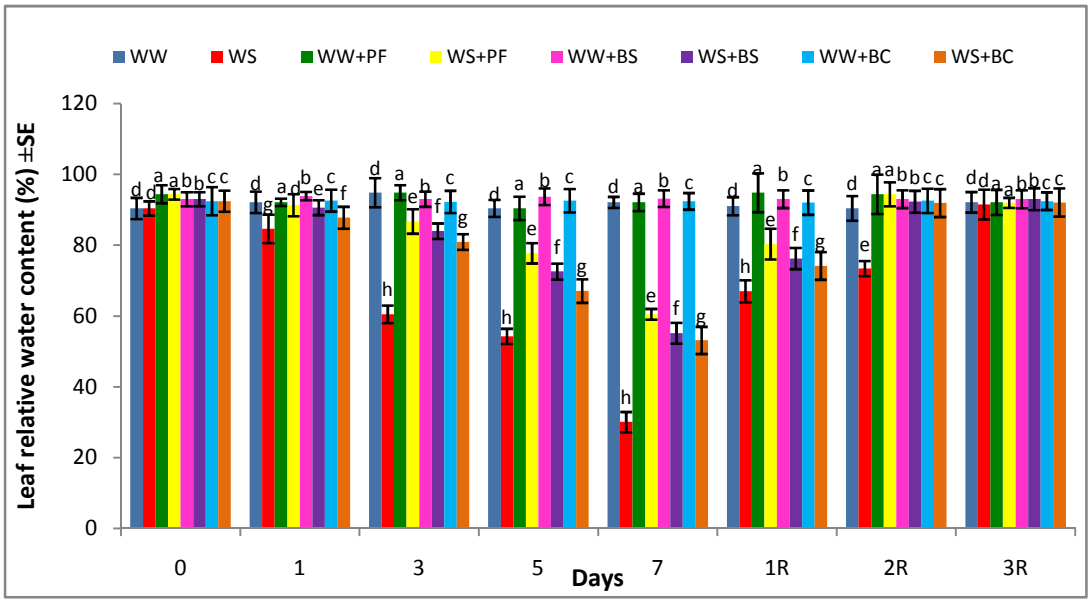

Fig 3.3. Variation in leaf relative water content during drought stress and recovery in okra inoculated with different bacteria. (WW-well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P.fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B.cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.1.2.2 Leaf osmotic potential (LOP)

Leaf osmotic potential becomes more negative (reduce) during drought stress in all plants, a slight variation in LOP was observed in all okra plants

susceptible to the first day of drought stress. The order of reduction in LOP was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.4). On the third day of drought stress remarkable variation in leaf osmotic potential was noticed, higher reduction in LOP was recorded in PF treated plants and the minimal decrease was accounted in untreated plants. The decreasing order of LOP was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.4) on the third day of stress, LOP was further declined during the fifth day of drought stress, PF treated plants showed more reduction in LOP during this period and lesser reduction was noticed in untreated plants. The fifth day of water stress resulted in a decrease in LOP of okra in the order WS+PF>WS+BS> WS+BC>WS (Fig. 3.4). Among the observed period, a significant decrease in LOP was observed on the seventh day of drought stress, upon this period PF treated plants registered more reduction in LOP and lower was observed in untreated plants. The increase in LOP was accounted in the order WS+PF>WS+BS>WS+BC>WS (Fig. 3.4).

Drought driven reduction in leaf osmotic potential was recovered during the re-watering period. On the first day of re-watering leaf osmotic potential was increased, PF treated plants recovered LOP faster than other bacteria treated and untreated plants. The order of recovery in LOP was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.4). All bacteria treated plants revived LOP on the second day of watering but a partial revival in LOP was recorded by untreated plants and the order of recovery was observed as WS+PF>WS+BS>WS+BC>WS, but bacteria untreated plants did not regain actual LOP (Fig. 3.4). The third day of re-watering resulted in the regain of normal osmotic potential in all plants and the order of leaf osmotic potential between the treatment was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.4).

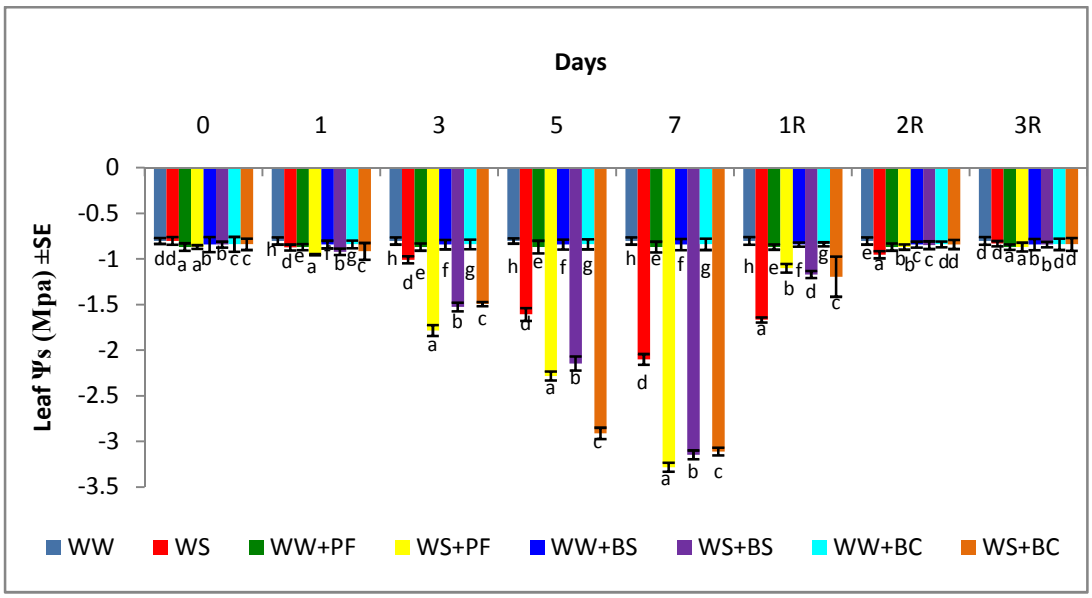

Fig. 3.4. Variation in leaf osmotic potential during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS-water stressed okra inoculated with *B. subtilis*, WW+BC-well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.1.2.3 Total Protein

A significant difference was recorded in total protein content between bacteria treated and untreated plants (WW+PF>WW+BS>WW+BC>WW) (Fig. 3.5) in the irrigated condition. A gradual increase was observed in total protein content in bacteria treated and untreated plants when exposed to drought stress up to the 3rd day of drought exposure (Fig. 3.5). PF treated plants registered higher protein content during the first day of drought stress and untreated plants accounted lesser increment in protein content. The order of protein content was recorded as WS+PF>WS+BS>WS+BC>WS upon the first day of drought exposure (Fig. 3.5). Beyond that a steep hike in protein content was observed on the third day of water stress in all stress exposed plants. Increase in total protein content was recorded in PF treatment

compared to control plants and lesser increment was recorded in untreated plants. The order of increase in protein content was recorded as WS+PF>WS+BS>WS+BC>WS (Fig. 3.5). Thereafter the protein production was significantly reduced in all drought susceptible plants. On the fifth day of drought stress, protein content was significantly reduced in untreated plants however, the reduction was much lesser in bacteria treated plants especially in PF treated plants. The rate of reduction in total protein content in okra exposed to the fifth day of drought stress was in the order WS>WS+BC>WS+BS>WW+PF (Fig.3.5). Among the observed period, a significant reduction in protein content was recorded on the seventh day of water stress. Untreated plants recorded a higher reduction in protein content and PF treated plants recorded lesser reduction in protein content. Drought induced reduction in total protein content during the seventh day was observed in the order WS>WS+BC>WS+BS>WW+PF (Fig. 3.5).

Okra regain normal protein level (similar to control plants) under re-watering, bacteria treated plants showed faster regain of protein content upon rehydration (Fig. 3.5). The first day of re-watering resulted in a remarkable increase in the protein content in bacteria treated okra plants compared to untreated plants, increase in protein content during first day was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig. 3.5). The second day of re-watering resulted in complete regain of protein content in bacteria treated plants, while in bacteria untreated okra partially regains the protein content. Increase in the protein content on the second day of rehydration was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.5). The third day of rehydration recorded complete recovery of protein content in all plants and the order of protein content was observed as WS+PF>WS+BS>WS+BC>WS (Fig.3.5).

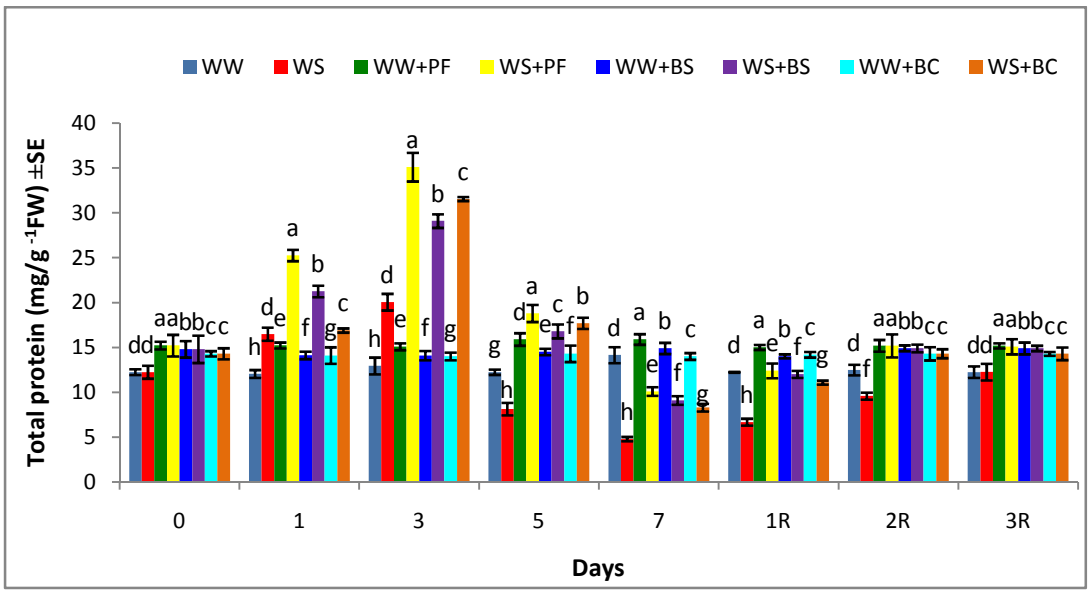

Fig. 3.5. Variation in leaf osmotic potential during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS-water stressed okra inoculated with *B. subtilis*, WW+BC-well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.1.2.4 Amino acids

During irrigated condition, no significant difference was observed in total amino acid content between the bacteria treated and untreated plants. But a remarkable increase in total amino acid content was observed in drought stressed plants (Fig. 3.6). During the first day of the drought exposure, all the treatments showed a considerable enhancement in total amino acid content, in which the PF treated plants exhibited a significant increase in total amino acid content compared to control plants. Minor increase in amino acid content was recorded in untreated plants and the order of increase in amino acid in drought exposed plants was observed as WS+PF>WS+BS>WS+BC>WS (Fig.3.6). The third day of drought stress considerably increased the amino acid content in okra, higher amino acid accumulation was noticed in PF treated plants and

minimal augmentation was recorded in untreated plants compared to control plants and the increase in amino acid in drought exposed plants was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.6). More increase in amino acid content was observed on the fifth day of drought stress, the same pattern of increase in amino acid content was observed during this period i.e., the incrementing order was WS+PF>WS+BS>WS+BC>WS (Fig 3.6). Within the observed period, seventh day of drought stress drastically increased the amino acid content in all the plants, but a sharp increase in aminoacid content was observed in PF treated plants and lesser increment was noted in untreated plants. The augmentation of amino acid during the seventh day of stress was recorded as WS+PF>WS+BS>WS+BC>WS (Fig. 3.6).

Water stress mediated increase in total amino acid content was reduced under rehydration. The first day of watering caused are remarkable reduction in total amino acid content in all drought stressed plants, bacteria treated plants recorded significant reduction in total amino acid content compared to untreated plants. The total amino acid content in all the treatments was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.6). Recovery of total amino acid content was recorded on the second day of watering in bacteria treated plants however, untreated plants partially revive the total amino acid content during the second day of re-watering. The total amino acid content upon the second day of re-watering was recorded as WS>WS+PF>WS+BS >WS+BC (Fig. 3.6). The third day of re-watering resulted in the normal total amino acid regain in all drought exposed plants and order of total amino acid content between the treatments were observed as WS+PF>WS+BS >WS+BC>WS (Fig. 3.6).

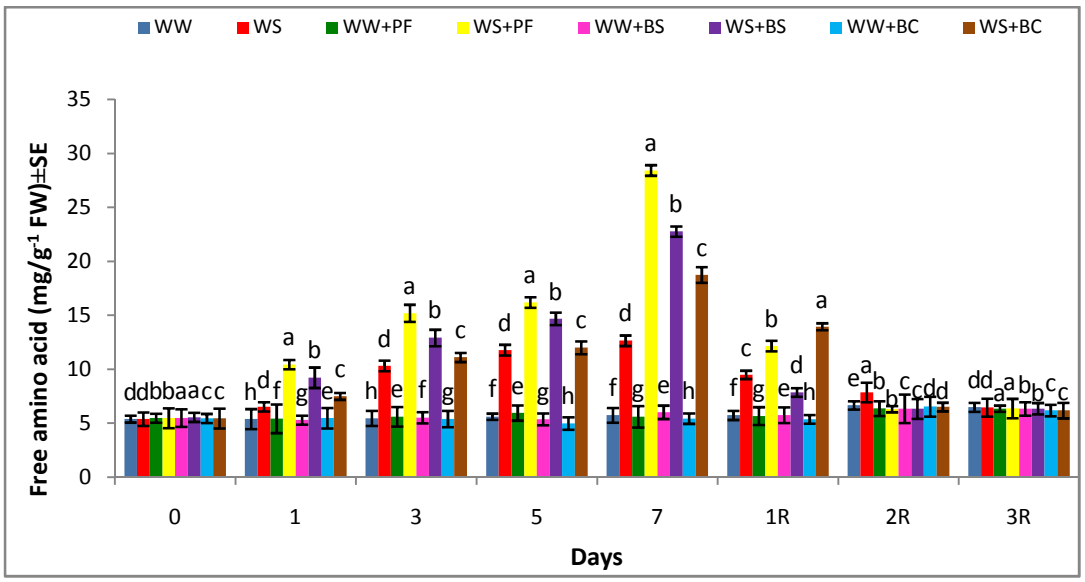

Fig. 3.6. Variation in free aminoacid content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS-water stressed okra inoculated with *B. subtilis*, WW+BC-well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.1.2.5 Proline

A gradual increase in proline content was observed during the drought period (1-7th day of water stress) in bacteria treated and untreated plants (Fig. 3.7). The first day of drought stress significantly increased the proline production in all plants, PF treated plants recorded a remarkable increase in proline content and untreated plants recorded minor increase compared to control plants. The order of enhancement in proline content on the first day of drought stress was observed as WS+PF>WS+BS>WS+BC>WS (Fig.3.7). The third day of drought stress registered further improvement in proline content, like the first day of stress increased quantity of proline was observed in PF treated plants and lesser production was recorded in untreated plants when compared against the control plants. The increase in proline content in

different treatments was observed in the order WS+PF>WS+BS>WS+BC>WS (Fig. 3.7). Similar to earlier days observation higher proline level was recorded in PF treated plants and lower increment was noticed in untreated plants exposed to five days of drought stress. The enhancement in proline content in drought susceptible plants on the fifth day of drought stress was observed as the order WS+PF>WS+BS>WS+BC>WS (Fig. 3.7). Throughout the observed period of drought stress condition (1^{st}- 7^{th} day of drought stress) a steep increase in proline content was observed on the seventh day of drought stress. Similar to other stressed periods, remarkable increase in proline production was recorded by PF treated plants and minimal proline accumulation was observed in untreated plants compared to control plants. The order of increment in proline level was recorded as WS>WS+BC>WS+BS>WS+PF (Fig. 3.7).

Water stress induced the up-regulation of proline production was decreased during re-watering. During the first day of rehydration, proline content was significantly reduced in all plants, bacteria treated plants showed more reduction in prol'ine level compared to untreated plants. The order of increase in proline content on the first day of rehydration was observed as WS>WS+BS>WS+BC>WS+PF (Fig. 3.7). All bacteria treated plants recorded regain in proline content on the second day of watering and the order of recovery was observed as WS>WS+PF>WS+BS>WS+BC. Although bacteria untreated plants showed significant decrease in proline content, only partial revival is occurred (Fig. 3.7). The third day of re-watering resulted in regain of normal proline level in all plants and order of proline content in re-watered plants was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.7).

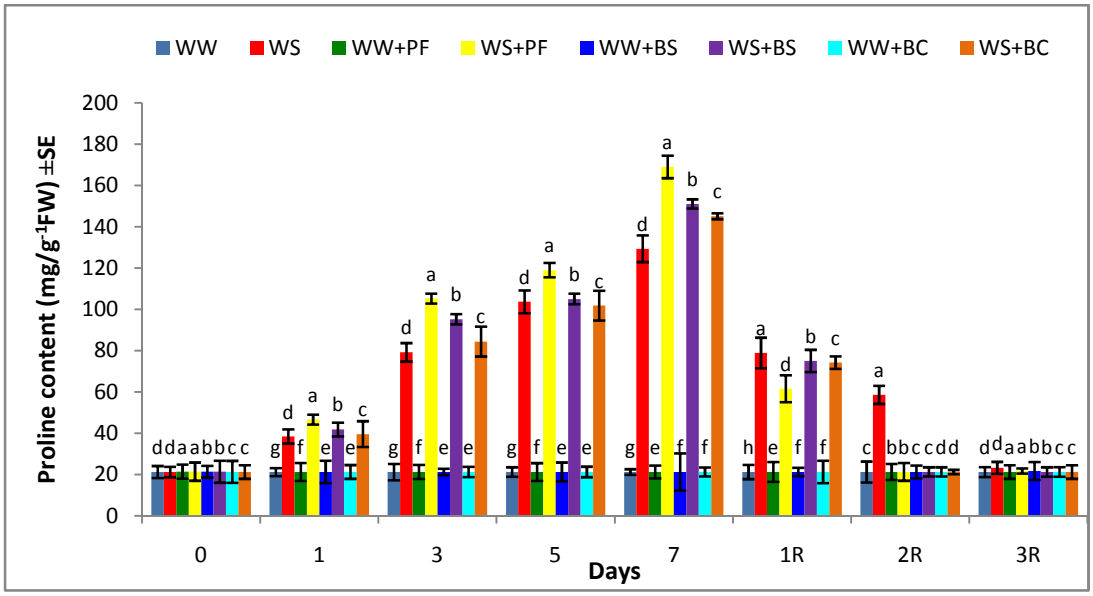

Fig. 3.7. Variation in proline content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS-water stressed okra inoculated with *Bacillus subtilis*, WW+BC-well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.1.2.6 Soluble sugar

Considerable variation in soluble sugar content was observed in bacteria treated and untreated plants upon the irrigated period. PF treated plants recorded higher increment in soluble sugar content during irrigated condition and the order of increase in soluble sugar level was recorded as WS+PF>WS+BS>WS+BC>WS (Fig. 3.8). Progressive enhancement in soluble sugar content was observed in drought exposed plants during stress period. On the first day of drought stress, a slight variation in soluble sugar content was observed. Higher soluble sugar level was observed in PF treated plants and the lower level was observed in untreated plants compared to control plants. The hike in soluble sugar production during the first day of

stress exposure was recorded in the order WS+PF>WS+BS >WS+BC>WS (Fig. 3.8). A significant increase in soluble sugar content was observed during the third day of water stress in all plants. Although a sharp increase in soluble sugar in stress exposed plants were observed in PF treated plants and lesser enhancement was observed in untreated plants when compared against the control plants. The order of increase in soluble sugar production was observed as WS+PF>WS+BS >WS+BC>WS (Fig. 3.8). The fifth day of drought stress resulted in a remarkable increase in soluble sugar content, in drought imposed plants. PF treated plants showed higher soluble sugar content compared to control plants however, the soluble sugar content recorded in untreated plants was much reduced compared with control plants. The order of accumulation of soluble sugar content on the fifth day of drought stress was observed as WS+PF>WS+BS >WS+BC>WS (Fig. 3.8). During the observed days,the seventh day of water stress resulted in sharp increment in soluble sugar accumulation, PF treated plants recorded higher soluble sugar content compared to other bacteria treated plants and untreated plants. The augmentation of soluble sugar content during drought stress exposed plants was recorded as WS+PF>WS+BS >WS+BC>WS (Fig. 3.8).

Drought induced increase in soluble sugar content revert during re-watering. Upon the first day of rehydration soluble sugar content was significantly reduced in drought exposed plants, bacteria treated plants showed a significant reduction in soluble sugar content compared to untreated plants. The order of recovery of soluble sugar level on the first day of rehydration was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.8). All bacteria treated drought exposed plants recorded regain of soluble sugar level on the second day of watering and the order of soluble sugar content was observed as WS>WS+BC>WS+BS>WS+PF, while in untreated plants a remarkable reduction in soluble sugar level was observed but, the regain in soluble sugar content was partial (Fig. 3.8). The third day of re-watering

resulted in retaining normal soluble sugar level in drought exposed plants and the order of soluble sugar content between the treatment was observed as WS+PF>WS+BS>WS+BC>WS (Fig. 3.8).

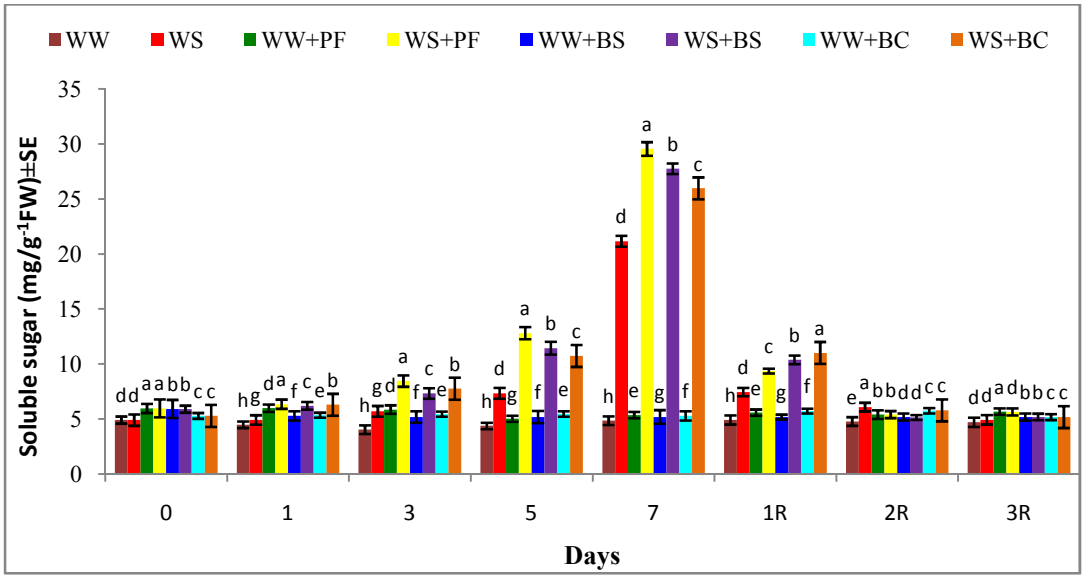

Fig. 3.8. Variation in soluble sugar content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WW+BS-water stressed okra inoculated with *B. subtilis*, WW+BC-well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.3.2 Discussion

4.3.2.1 Soil moisture content (SMC)

Soil moisture content was an important parameter used to measure the intensity of drought stress, water stress severely affect soil water status and soil-water-plant continuum. In the present study SMC declined vertically under seven days of water stress, but the bacteria treated soil recorded better water status under stress condition. Present findings demonstrate that PF treatment was more effective to ameliorate the drought stress imparted

reduction in SMC, however the other two (BS and BC) bacteria kept a higher SMC when compared with bacteria untreated plants growing soil. Soil micro-organisms were directly related to the soil environment affecting changes in soil particles directly affect their life, during drought stress, soil water content was much reduced leading to dehydration of microbial cell and finally leading death of the organism (Schimel *et al.,* 2007). To overcome the crucial situation they produce extracellular polymeric substances (Zhang *et al.,* 2013), which acts as a coating to the cell wall and creates favorable condition for their growth which prevents the dehydration of cell (Rossi *et al.,* 2012). Recent findings suggest that this EPS play an important role in water retention under drought stress. Kroener *et al.* (2018) suggested that the mucilaginous substances produced by micro-organisms occupy in soil pores will increase the water retention capacity of the soil under water deficient condition. A decline in soil hydraulic conductivity was observed by Volk *et al.* (2016) in *Pesudomonas* treated soils which facilitate the water absorption by plants. Studies showed that PGPR modify the soil characters by increasing the production of EPS, they also suggest that, EPS improve soil water status mainly due to the three reasons (i) high water retention capacity; (ii) change soil constitution pore space and (iii) alternation in soil surface moisture (Zheng *et al.*, 2018). These findings support the present investigations results i.e., bacteria treated soil recorded higher soil moisture content during stress exposure. This may also lead to the normal growth of okra under stress. The improved water retention capacity of bacteria treated soil also cause faster recovery of soil water status during re-watering.

4.3.2.2 Soil pH

Soil pH was considered as the key factor which determines the availability of nutrients in the soil like phosphorous, nitrogen, iron etc. decrease in soil pH increase solubilization of mineral nutrients (Zahid *et al.,*

2015). Higher reduction in soil pH was recorded in okra treated with PF, BS and BC growing soil, it was already proved that PGPR promotes plant growth by altering the soil physical and chemical characters (Abd-Alla, 1994). By increasing the drought stressed days more reduction in soil pH was observed in PF treated plants growing soil. According to Chen *et al.* (2006) phosphate solubilizing bacteria application decrease soil pH and increase availability of phosphorous in soil through the production of various microbial metabolites. Xiao *et al.* (2017) suggested that plant growth was dependent on soil microbe's diversity, pH and nitrogen and potassium content in the soil. Increase in nitrogen availability in soil by PGPR application was reported by Cakmakci *et al.* (2007). Our results were agreement with the findings of Das and Singh (2014), that PGPR application with organic manure in mung bean decrease the soil pH and also report that PGPR application improves the nutrient status of the soil by reducing soil pH.

Reduction in soil pH by plant microorganisms was an important strategy to enhance the plant growth and this may accomplish either by the production of siderophores or acidic organic compounds (Van Loon, 2007). Various reports suggested that PSB decrease in soil pH by producing the gluconic acid and mineral acids (Chen *et al*., 2006; Deubel *et al*., 2000), alkaline phosphatases (Rodroguez and Fraga, 1999), phytohormones and H^+ protonation (Xiao *et al*., 2017), anion exchange, chelation and siderophores production (Sugihara *et al*., 2010). In agreement with our results Adnan *et al*. (2017) reported that PSB reduce pH of alkaline soil and improved plants growth.

4.3.2.3 Plant water status, metabolits and osmoregulation

4.3.2.3.1 Leaf relative water content

Long term water stress results in soil drying which interrupts the water absorption and leads to a decline in leaf relative water content. Leaf relative

water content was considered as the best tool to analyze the growth and metabolic status of plants under stress condition (Alizadeh, 2002). In okra treated with different bacteria i.e., *P. fluorescens*, *B. subtilis* and *B. cepacia* showed better water status throughout stress exposed period. Within bacteria treated okra plants, PF treatment was more efficient to keep the relative water content under severe drought condition i.e., 7^{th} day of drought exposure. The higher LRWC content in bacteria treated plants directly related with alternation in root morphology of plants and also the increased osmolyte content in these plants. Many PGPB interfere the equilibrium between auxin and cytokinin, they play an important role in organogenesis which results in an increase in root length, lateral root number and root hairs (Vacheron *et al.,* 2013), these modifications facilitate increase in water absorption by plants. Maize treated with different bacteria such as *Proteus penneri*, *Pseudomonas aeruginosa* and *Alcaligenes faecalis* improve cellular water status via enhanced production of exopolysaccharide (Naseem and Bano, 2014). Similar to these results, Kanwal *et al*. (2017) reported that PGPR application improves the drought stress tolerance in wheat through preventing higher reduction in relative water content. According to the suggestion of Agami *et al.* (2016) increases in the LRWC in PGPR treated basil due to the augmentation in proline production and this feature was considered as an adaptive response to reduce osmotic stress induced by drought stress. Increased LRWC in *Bacillus* treated drought stressed sorghum plants were considered as counteract against osmotic stress under drought stress (Grover *et al.,* 2014). Higher LRWC in okra bacteria treated plants under drought stressed period accelerate faster regain of cellular water status during re-watering. Whereas in untreated plants drought induced reduction in LRWC was more than bacteria treated plants this slow down the regain of water status during re-watering.

4.3.2.3.2 Leaf osmotic potential

The reduction in cellular water status results in higher osmotic stress in plants, to cope up with this state plants increase the productions of organic solutes this event is generally known as the osmotic adjustment (Taize and Zeiger, 2010). Enhanced synthesis of osmolyte in stressed plants increases the osmotic potential of plant tissues, which confirm the accessibility of water from the dry soil. Hence the decrease in osmotic potential of water stressed bacteria treated plants improves the water absorption. During drought higher reduction in the osmotic potential of PF treated okra plants corroborate the PF treatment was more effective to improve the drought stress tolerance in okra plants when compared with other bacteria treatments (i.e., BS and BC) and untreated plants. Karamanos and Papatheohari (1999) suggested that leaf water potential was an indicator of plant water balance under the stressed condition. *Azospirillum lipoferum* inoculation in maize, modified different metabolic pathways during stress which results in change in the production of sugars, glucose, hormones and secondary metabolites, leading to the alternation in leaf water potential (Rozier *et al*., 2017). Similar observations have been reported in maize treated with *Bacillus* by Vardharajula *et al*. (2011). The enhanced accumulation of osmolytes resulted in the lowering of osmotic potential which helps the okra plants to absorb water from dry soil at a faster rate and causes fast recovery of bacteria treated plants during re-watering, these results were in agreement with the observations made in wheat (Wang *et al*., 2018b). Increase in the number of compatible solutes under stress facilitate ROS detoxification, protein stabilization and cell membrane protection and also leading to the reduction in osmotic potential of cells, which in turn improve water diffusion into the root cell, thereby maintaining a higher turgor potential (Abid *et al*., 2018).

Rehydration of okra after seven days of drought stress resulted in the reduction in LOP. This result was significantly related to the reduction in osmolytes like protein, proline, amino acids and sugar content. Faster reduction in osmolytes content was recorded by bacteria treated plants results in faster regain of LOP in okra during re-watering. However, untreated plants showed a slower revival in osmolytes content which in turn resulted in the slower recovery of leaf osmotic potential.

4.3.2.3.3 Total protein content

The increase in protein content was observed in bacteria (PF, BS and BC) treated and untreated plants during initial days of drought exposure was may be due to the enhanced production of stress related protein like late embryogenesis abundant (LEA) proteins, heat shock proteins, water channel proteins like aquaporins and ROS scavenging enzymes. Several studies revealed that drought stress promotes the synthesis of some proteins (dehydrin, HSPs, LEA etc.) which actively involved in osmotic balance and protect other proteins and also degradation of some other proteins such as the protein involved in various metabolisms like photosynthesis (Chutia and Borah, 2012). According to Wang *et al.* (2013) the higher expression of aquaporin increased in *T. salsuginea* in response to drought stress enhanced the drought tolerance capacity in plants. Enhancement in the synthesis of Late Embryogenesis Abundant (LEA) proteins was a common mechanism under stress as it improves the tolerance potential in drought stressed plants (Dang *et al.,* 2014). Drought stress induced the production heat shock proteins, which are the important shielding proteins having a key role in many cellular processes like protein synthesis and targeting (Coca *et al*., 1994). An increment in synthesis of different HSP'S like HSP70, HSP60, HSP90, and HSP100 are induced by drought stress (Wang *et al*., 2004).

However, after 3rd day of drought stress, total protein content was decreased in both bacteria treated and untreated plants. Similar observations were made by Varadarajula *et al*. (2011) in maize. Progressive water stress resulted in decrease in total protein content of plants and suggested that the decline in protein was due to the hydrolysis of protein to amino acids. In agreement with this report the present observation there is a corresponding increase in total amino acid content during 5th and 7th day of water stress in bacteria treatment, especially in PF treated plants. This increment in amino acid content facilitates an increase in drought tolerance in bacteria treated plants. However, in untreated plants, there is no significant relation between protein degradation and increase in amino acid content during drought stress resulting in a decrease in tolerance potential of these plants. Faster restoration of protein content in bacteria treated plants may be due to the enhanced activity of ROS scavengers which actively scavenge stress induced ROS production and facilitate quick recovery of protein content in bacteria treated plants than untreated plants.

4.3.2.3.4 Total amino acid content

Apart from a precursor of protein synthesis, amino acids play an important role in the osmotic adjustment in drought stressed plants. Higher accumulation of total amino acids in PSB treated plants than untreated plants during drought stress enhanced the potential of osmoregulation. In support to above observation, Shahjee *et al*. (2002) reported that microbial inoculation ensures drought stress tolerance in plants by increased amino acid synthesis. Earlier studies reported that, enhanced production of amino acids like histidine, glycine, alanine, tyrosine, cysteine, valine, methionine, tryptophan, phenylalanine, lysine, hydoxyproline and sarcosine in PGPR treated cauliflower, maize and wheat ensure water stress tolerance potential (Harrigan *et al*., 2007; Bowne *et al*., 2012; Witt *et al*., 2012; Ekinci *et al*., 2014).

Although amino acids are functioning as osmolytes each of them have a specific role in stress alleviation, proline, glycine and alanine inhibited stomatal opening, while histidine and methionine induce stomatal opening of *Vicia faba* (Kumar and Sharma, 1989). Moreover, Rai and Rana (1996) reported that histidine and proline promoted mineral absorption in *Phaseolus* seedlings. According to Harrigan *et al*., (2007) histidine plays an important role in plant growth and development and abiotic stress mitigation in plants. Some amino acids like tyrosine and phenylalanine act as precursors for secondary metabolites, which are actively participated in active oxygen species scavenging (Less and Galili, 2008; Gill and Tuteja, 2010). Drought and salinity stress induce glycine betaine synthesis in plants, which act as osmolyte and keep up cell water content, stabilize the proteins, maintain membrane structures, shield to photosynthetic apparatus and protect cytoplasm and chloroplasts from the adverse effects of high osmotic stress (Munns, 2002; Ashraf and Harris, 2004; Su *et al*., 2006; Raza *et al*., 2007). Similar to this results higher amino acid content was recorded in bacteria treated okra and this may be the reason for the increment in amino acid content in these plants. Various studies revealed that drought induce the activity of different enzymes such as choline monooxygenase (CMO) in beet (*Beta vulgaris* L.), betaine aldehyde dehydrogenase (BADH) in spinach (*Spinacia oleracea* L.), which result in the accumulation of amino acids (Yang *et al*., 2008; Zhang *et al*., 2011).

Enhanced production of aromatic amino acid was observed in Timothy treated with *B. subtilis* B26 under drought-stress conditions (Gagné-Bourque *et al*., 2016) this change attributes to improve tolerance potential of plants. Previous reports suggest that the augmentation in amino acid content in bacteria treated plants not only due to the improvement in the amino acid synthesis in plants, but also bacteria directly contribute their amino acid to the plants (Lyons *et al*., 1990). According to Taylor *et al*. (2004), amino acids

serve as energy resource during stress condition which increases the tolerance potential of plants. Higher synthesis of amino acid in bacteria treated plants during stress efficiently alleviate the stress induced damages in okra this assist faster recovery of amino acid content. However, the amino acid productions in the untreated plants were not sufficient to protect the plants from osmotic stress as this slow down the recovery process of these plants.

4.3.2.3.5 Proline

Proline, an essential amino acid which plays an important role in the stress tolerance of plants (Valentovic *et al*., 2006; Chen *et al*., 2007). Proline reduces membrane damage due to drought by acting as osmolytes, balance of intracellular redox homeostasis, ROS scavenger, cellular signaling, chemical chaprones (Hanson and Burnet, 1994; Maneesuwannarat *et al*., 2013; Liang *et al*., 2013). During drought stress, they act as acompatible solute and regulate cell osmoticum and protect cell components from dehydration (Farooq *et al*., 2009b). Osmoregulation during water stress assist to maintain cell turgor, leading to normal cell enlargement and plant growth under drought stress apart from this modification proline regulate stomatal movement during water stress and kept stomata partially open helping to maintain carbon assimilation (Alves and Setter, 2004). Earlier reports suggest that there was a positive correlation between proline biosynthesis and GSH production, because γ-glutamyl phosphate precursor of GSH was derived from the proline biosynthetic pathway (Hoque *et al*., 2008; Liang *et al*., 2013).

In the present study, proline content was significantly improved with increasing drought stress. Remarkable increase in proline content in bacteria treated plants suggested that bacteria treated plants improve stress tolerance of okra and in agreement with previous reports that, these plants also recorded higher LRWC, LOP and GSH content during stress condition which improves stress tolerance potential of bacteria inoculated okra. Amongst the treatments,

PF treated plants showed higher proline content, directly related to improving stress tolerance of these plants via improved cell water content and ROS scavenging. Lower proline accumulation was recorded in untreated plants and these plants are more susceptible to drought stress with a lower LRWC, LOP, and GSH content. While proline serves as an osmolyte which helps to maintain plant water status under dehydration bacteria treated plants showed faster recovery potential during re-watering. During re-watering period untreated plants recorded higher proline content compared to bacteria treated plants and this facilitate higher absorption of water and cope up with water loss during dehydration stress. But in the case of bacteria treated plants, improved water status during stress condition by accumulating different osmolytes like proline alleviate drought induced damages in okra and also maintain plant water status which assists in faster recovery of proline content in these plants.

According to Chen *et al*. (2007) induction of proBA genes derived from *B. subtilis* into *A. thaliana* lead to enhanced proline production, which was correlated with the increase in osmotic tolerance in transgenic plants. Similar observations were made in *Burkholderia* (Porcel and Ruiz-Lozano, 2004; Barka *et al*. 2006), Arthrobacter (Sziderics *et al*., 2007) and *Bacillus* (Gururani *et al*., 2013). Authers suggested that PGPR play an important role in plants for mitigating the harmful effect of drought stress trough higher accumulation of proline.

4.3.2.3.6 Total soluble sugar

Enhancement in total soluble sugar content in okra plants treated with bacteria, directly related with their osmotic adjustment. Several studies revealed that the increase in soluble sugar content increases the drought stress tolerance in plants. According to Osakabe *et al*. (2013) soluble sugars regulates the stomatal closure and increase the drought tolerant efficiency in

plants. While in stressed condition sugar not only acts as osmolyte but also as ROS scavenger (Krasensky and Jonak, 2012). A similar observation was made by Arabzadeh (2012), that the accumulation of sugar in response to water stress inhibits the oxidation of cell membrane. Sugars accumulated in plants under dehydration maintain leaf turgdity and prevent the dehydration of membranes and proteins (Crowe *et al*., 2001; Sawhney and Singh, 2002). Apart from this function, sugars play an important role as a signaling molecule during seed germination, photosynthesis and senescence. Soluble sugar accumulation facilitates the production of proline under osmotic stress which considers as an important osmolyte (Hellmann *et al*., 2000).

Sugars increase drought tolerance potential of plants through an important mechanism in which substitution of the hydroxyl groups of sugar for water helps to maintains hydrophilic interactions within the membranes and during water stress sugars interact with proteins through hydrogen bond thus improve the membrane protein stability (Leopold, 1994). Various reports proved that sugars can act as biological glass in the cytoplasm of dehydrated cells which increases the drought stress resistant and helps to survive in dry condition (Leopold, 1994; Buitink *et al*., 1998). So, soluble sugar content was selected as a bio-marker for selecting drought tolerance wheat varieties (*Triticum durum* Desf.) (Al Hakimi *et al*., 1995). Earlier reports suggest that drought stress alter the expression of gene encode sugar synthesis in plants, up regulate the genes encoding enzymes participated in gluconeogenesis such as fructose-biphosphate aldolase (Cramer *et al*., 2007), in phosphorylation of sugars like hexokinase (Whittaker *et al*., 2001) and synthesis of galactinol synthase (Taji *et al*., 2002). Apart from above stated function sugars act as a carbon source during stressful condition which reduces the harmful effect of the stress (Wingler *et al*., 1998; Rizhsky *et al*., 2004; Cramer *et al*., 2007).

In conjunction to above statements, it was revealed that higher accumulation of sugar content in bacteria treated plants positively regulate drought stress tolerance via compatible solute and active oxygen scavenger. It was also well documented among bacteria treated plants that, PF treatment was more effective than other bacteria treatment. Higher accumulation of sugar in bacteria treated plants during drought stress improves water status and membrane stability which in turn increase the recovery of plants during re-watering. High sugar content in bacteria treated plants in control condition was related with the corresponding improvement in the photosynthetic efficacy of these plants. Lesser reduction in photosynthetic parameters help to synthesis more sugar for osmotic adjustment during drought stress. Lower sugar accumulation in untreated plants during stress may also be related to higher photosynthetic damage in these plants. In agreement with this result Bano and Fatima (2009) suggested that higher accumulation of soluble sugar improved drought stress tolerance. In addition Yang *et al*. (2013) augmentation in sugar content during water stress helps rapid recovery in blue grass under re-watering.

Similar to the above stated results, various reports opinioned that PGPR increases drought tolerance. In maize seedlings treated with *Bacillus* strains recorded higher sugar content due to starch degradation, which improve the drought stress tolerance potential of plants (Nayer and Reza, 2008). Enhanced trehalose accumulation was reported in *Phaseolus vulgaris* treated with *Rhizobium etli,* which confer drought stress tolerance and co-inoculation of *Rhizobium tropici* and *Paenibacillus polymyxa* improve sugar accumulation by over expressing a trehalose-6-phosphate synthase gene (Figueiredo *et al*., 2008).

4.4 CHAPTER 4

4.4.1 Reactive oxygen species production

4.4.1.1 Superoxide

A significant difference in superoxide generation was observed in bacteria treated and untreated plants that are well watered. Superoxide content during irrigation condition was recorded as WW>WW+BC>WW +BS>WW+PF (Fig.4.1). The first day of the drought stress in okra showed a significant improvement in the superoxide generation and the higher superoxide content was recorded in untreated plants and the lesser amount was observed in PF treated plants. The increase in the superoxide content during the first day of drought stress was observed as WS>WS+BC>WS+ BS> WS+PF (Fig.4.1). The third day of the drought stress resulted in further increased superoxide production in bacteria treated and untreated plants, higher superoxide production was observed in untreated plants and a lower quantity was recorded in PF treated plants similar to the first day of drought stress. The hike in superoxide generation on the third day of drought stress was observed as WS>WS+BC>WS+BS>WS+PF (Fig.4.1). The fifth day of drought stress keep up the similar trend in superoxide production like previous days of stress. However, the superoxide content was significantly increased on the fifth day of drought stress and the enhancement was observed as in the order WS>WS+BC>WS+BS>WS+PF (Fig.4.1). Higher quantity of superoxide production was recorded on the seventh day of water stress compared to other observed days in all stress exposed plants, whereas the augmentation in superoxide content followed the same pattern in all treatments i.e., WS>WS+BC>WS+BS>WS+PF (Fig.4.1).

The decline in superoxide content was observed in drought stressed plants during rehydration. The first day of the re-watering resulted a significant reduction in superoxide generation and the production of

superoxide in each treatment was observed in the order WS>WS+PF>WS+BS>WS+BC (Fig.4.1). The second day of re-watering resulted in complete recovery from drought induced superoxide production (i.e., superoxide content was alike their control plants) in bacteria treated plants, but in untreated plants, the recovery of superoxide production was incomplete and the superoxide content in re-watered plants was recorded as WS>WS+PF>WS+BS>WS+BC (Fig.4.1). The third day of watering resulted in a reduction in superoxide content and all plants in untreated plants recorded actual superoxide content like their control plants. The increasing order of superoxide content in okra was recorded as WS>WS+PF>WS+BS>WS+BC during the third day of re-watering (Fig.4.1).

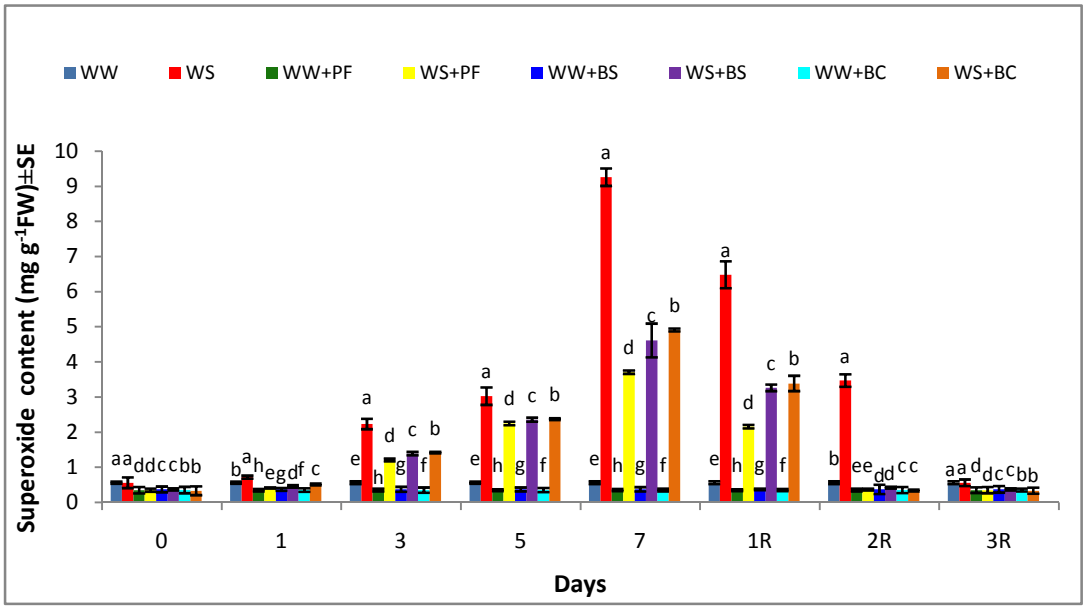

Fig.4.1. Variation in leaf area during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS-well watered okra inoculated with *B. subtilis*, WW+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.1.2 Hydrogen peroxide

Considerable difference in hydrogen peroxide production was recorded in bacteria treated and untreated plants during irrigated condition. Hydrogen peroxide content during regularly watered condition was recorded as in the order WS>WS+BC>WS+BS>WS+PF (Fig.4.2). The first day of water stress recorded a remarkable increase in the hydrogen peroxide content, higher hydrogen peroxide was recorded in untreated plants and minimal was recorded in PF treated plants. The increasing order of hydrogen peroxide content in drought stressed plants was recorded as WS>WS+BC>WS+BS> WS+PF (Fig.4.2). The third day of water stress resulted in increased hydrogen peroxide content in the whole stressed plants (i.e., bacteria treated and untreated plants) even though, a steep increase in the hydrogen peroxide production was recorded in untreated plants and lower increment was recorded in PF treated plants compared to control plants. The enhancement in hydrogen peroxide content on the third day of drought stress was recorded as WS>WS+BC>WS+BS>WS+PF (Fig.4.2). Synonymous to the trend of previous days hydrogen peroxide generation was higher in untreated plants on the fifth day of drought stress and lesser hydrogen peroxide content was recorded in PF treated plants with respect to control plants. The enhancement in the hydrogen peroxide content in drought stressed plants was observed in the order WS>WS+BC>WS+BS>WS+PF (Fig.4.2). Hydrogen peroxide content significantly elevated on the seventh day of water stress and the augmentation was recorded in a similar range as in the previous days observation i.e., WS>WS+BC>WS+BS>WS+PF (Fig.4.2).

Re-watering resulted in the reduction in hydrogen peroxide production, the first day of rehydration resulted in a remarkable reduction in hydrogen peroxide content and it was recorded as in the order WS>WS+BC>WS+BS> WS+PF (Fig.4.2). The second day of re-watering resulted in a complete

recovery from stress induced hydrogen peroxide production in all bacteria treated plants, although a significant reduction in hydrogen peroxide production was observed in untreated plants, the recovery was incomplete and the hydrogen peroxide content in re-watered plants was recorded as in the order WS>WS+BC>WS+BS>WS+PF (Fig.4.2). The third day of watering resulted in a complete recovery of all the drought stressed plants, i.e., the hydrogen peroxide content was recorded same as regularly watered plants and the order of hydrogen peroxide content in okra was recorded as WS>WS+BC>WS+BS>WS+PF (Fig.4.2).

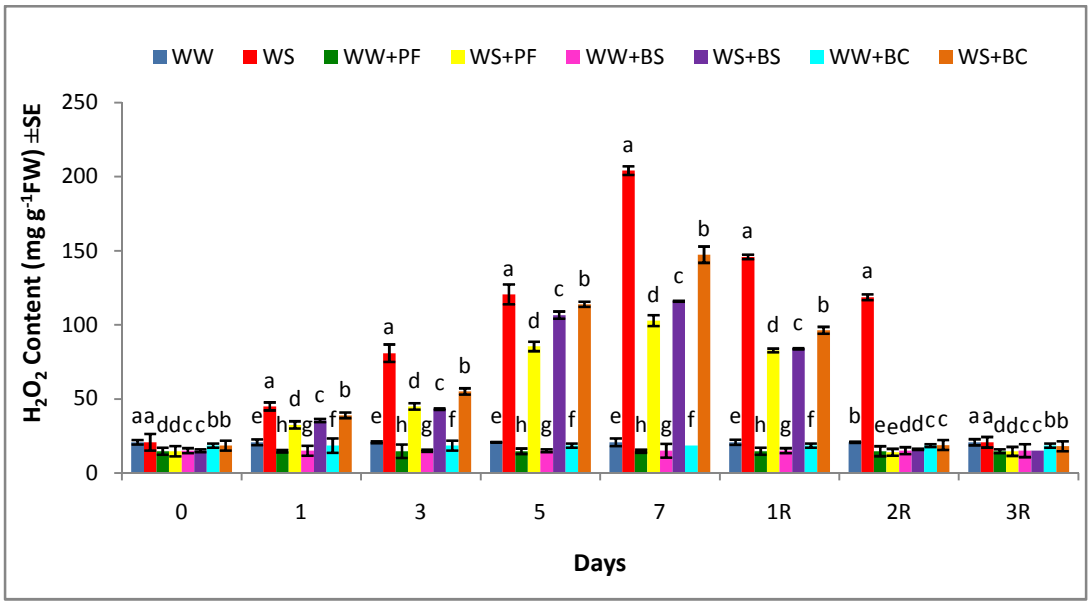

Fig.4.2. Variation in hydrogen peroxide content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed, WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia*. 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.2 Membrane damage

A significant difference in the MDA content was observed between the bacteria treated and untreated okra plants in okra during regularly irrigated condition as in the order WW>WW+BC>WW+BS>WW+PF (Fig.4.3). MDA content progressively increased in all plants exposed to drought stress. The first day of the drought stress recorded a significant increase in the MDA content in bacteria treated and untreated plants compared with control plants. The order of increase in MDA content was observed as WS>WS+BC>WS+ BS>WS+PF (Fig.4.3). On the third day of drought stress, a considerable increase in MDA content was observed in untreated plants and minimal increment was observed in PF treated plants compared to BS and BC treated plants and the increase in MDA content were recorded in the order WS>WS+BC>WS+BS>WS+PF (Fig.4.3). The fifth day of the drought significantly increased MDA content in untreated plants, but the enhancement in MDA content was found lesser in PF treated plants compared with other bacteria treatments and untreated drought stressed plants. The order of augmentation in MDA content in drought stressed plants was observed as WS>WS+BC>WS+BS>WS+PF (Fig.4.3). The seventh day of the water stress recorded higher quantity of lipid peroxidation in untreated plants, which resulted in significant enhancement of MDA content and a lesser amount of MDA was recorded in PF treated drought stressed plants with respect to other bacteria treated and untreated plants. The quantity of MDA was recorded in the order WS>WS+BC>WS+BS>WS+PF upon seventh day of water stress (Fig.4.3).

Rehydration resulted in a decrease in MDA content, during the first day of re-watering, the MDA content in stressed plants significantly reduced and recorded in the order WS>WS+BC>WS+BS>WS+PF (Fig.4.3). All the bacteria treated plants recovered from membrane damage on the second day

of re-watering and the MDA content was observed in the order WS>WS+BC>WS+BS>WS+PF (Fig.4.3). The third day of watering resulted in a complete recovery from drought induced lipid peroxidation in okra and the MDA content in the treatments are recorded as WS>WS+BC> WS+BS>WS+PF (Fig.4.3).

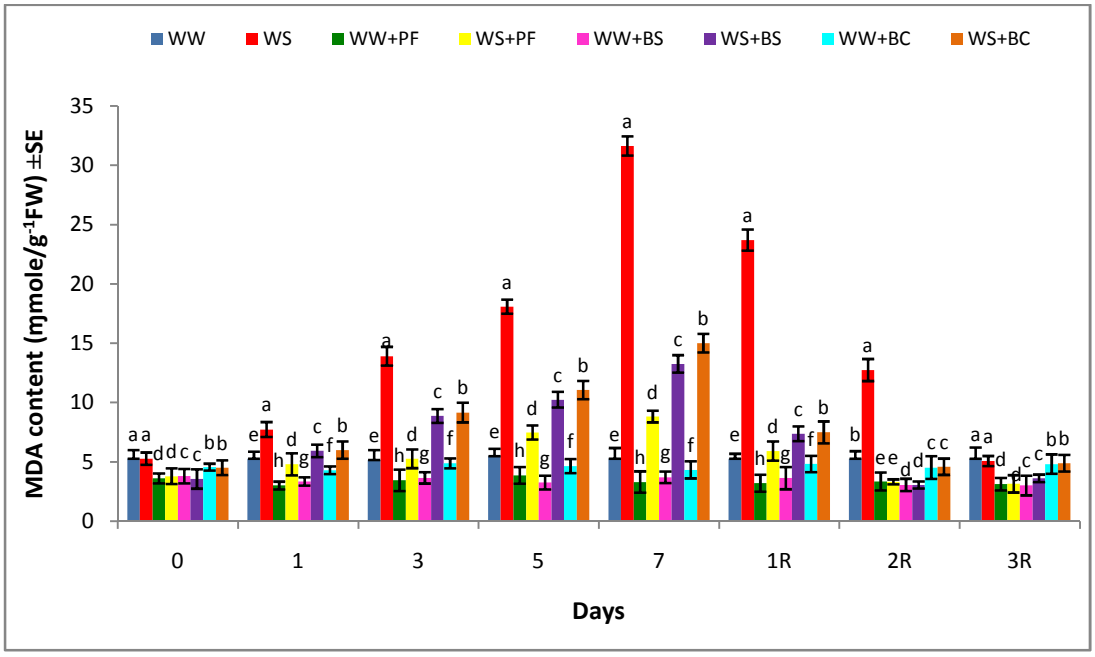

Fig.4.3. Variation in MDA content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3 ROS scavenging system

4.4.3.1 Enzymatic antioxidants

4.4.3.1.1 Superoxide dismutase (SOD)

Drought stress induced increase in SOD specific activity was significantly higher in bacteria treated plants compared to untreated plants

throughout the stress period. On the first day of drought stress, SOD specific activity in BS treated plants was vertically increased whereas, in untreated plants slight increase in SOD specific activity was recorded. The order of SOD specific activity upon the first day of the drought stress was observed in the order WS+BS>WS+PF>WS+BC>WS (Fig.4). The third day of the drought stress results in further increase in SOD specific activity in all plants, however, a steep increase in specific activity was observed in PF treated plants and lesser increment was recorded in untreated plants compared to the control plants. The up-regulation of SOD specific activity in water stressed plants were recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4). Similar to earlier days observation, the fifth day of water stress recorded higher SOD specific activity in bacteria treated plants and it was accounted in the order WS+PF>WS+BS>WS+BC>WS (Fig.4). Within the observed period, a significant increase in SOD specific activity was observed on the seventh day of water stress in PF treated plants and a minimal increase was recorded in untreated plants. On the seventh day of the water stress drought stress imparted up-regulation of SOD specific activity was observed in the order WS+PF>WS+BS>WS+BC>WS (Fig.4).

Down regulation of SOD specific activity was observed during rehydration; the first day of re-watering resulted in a significant decrease in SOD specific activity in bacteria treated plants and it was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4). The second day of watering resulted in full recovery from stress induced up regulation of SOD specific activity in bacteria treated plants. Although untreated plants showed a significant reduction in SOD specific activity they are not completely recovered and the SOD specific activity of plants was recorded in the order WS>WS+PF>WS+BS>WS+BC (Fig.4). The third day of watering resulted in recovery of SOD specific activity of all the stress exposed plants and SOD specific activity was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4).

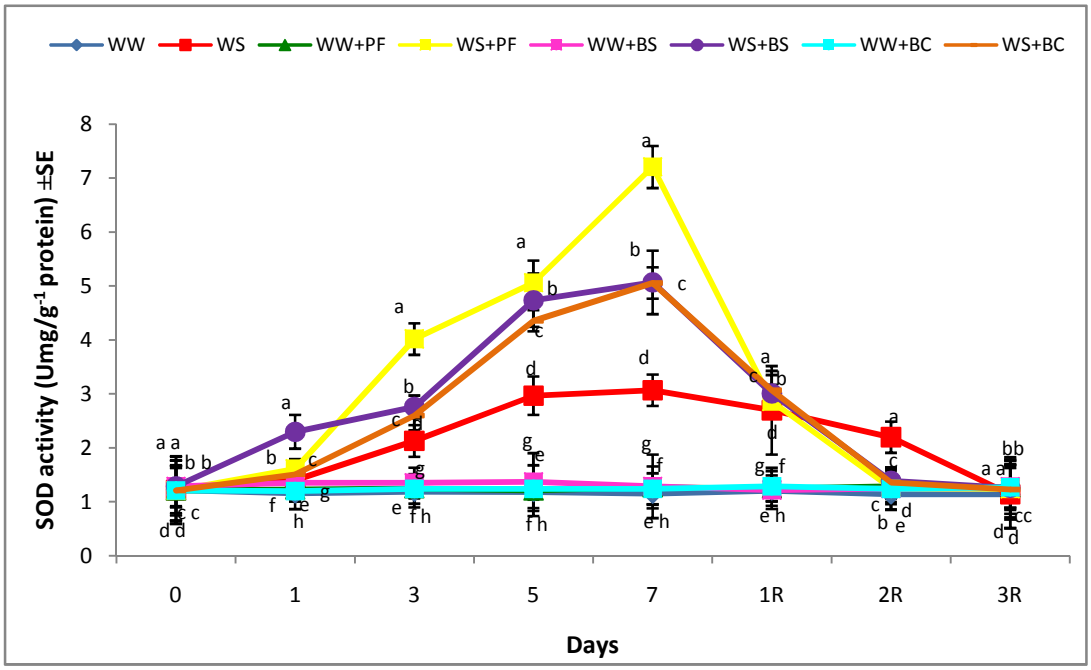

Fig.4.4. Variation in SOD specific activity during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B.cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.1.2 Catalase (CAT)

Catalase specific activity was significantly improved in bacteria treated plants compared to untreated plants during the water stressed condition. The first day of the drought stress enhanced CAT specific activity in BS treated plants compared to other bacteria treated plants (i.e., PF and BC treated plants) and untreated plants. The order of increase in specific activity of CAT upon the first day of the water stress was recorded as WS+BS>WS+PF> WS+BC>WS (Fig.4.5). The third day of drought stress resulted in more increase in CAT specific activity in all the plants, although a sharp increment

in CAT specific activity was accounted in PF treated plants but the increment recorded in untreated plants was lesser compared to the control plants. The up-regulation in CAT specific activity was increased during the third day of stress and the increment was recorded in the order WS+PF>WS+BS>WS+BC >WS (Fig.4.5). Similar to the observation of earlier days, the fifth day of water stress recorded higher CAT specific activity in bacteria treated plants (especially in PF treated plants) and it was in the decreasing order WS+PF>WS+BS>WS+BC>WS (Fig.4.5). Within the observed days, a significant increment in CAT specific activity was observed on the seventh day of the stress in PF treated plants and a minimal increase was recorded in untreated plants compared to control plants. Drought stress imparted up regulation of CAT specific activity in the order WS+PF>WS+BS>WS+BC >WS (Fig.4.5).

A decrease in CAT specific activity was observed during re-watering, the first day of rehydration resulted in significant decrease in CAT specific activity in bacteria treated plants and the CAT specific activity was recorded in as the order WS>WS+PF>WS+BC>WS+BS (Fig.4.5). The second day of rehydration resulted in complete recovery from drought imparted enhancement of CAT specific activity in all bacteria treated plants. Untreated plants recorded remarkable deduction in CAT specific activity even though, they are not completely recovered and the CAT specific activity of the plants was recorded as WS>WS+PF >WS+BS>WS+BC (Fig.4.5). The third day of watering, a complete recovery of all plants and CAT specific activity accounted as in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.5).

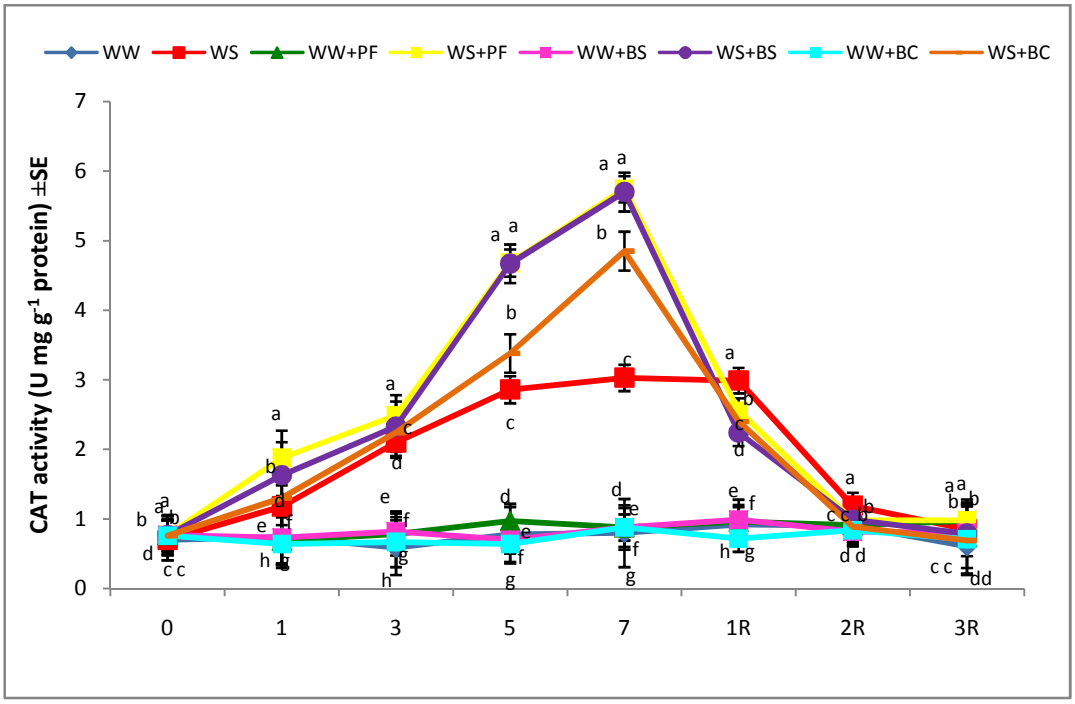

Fig.4.5. Variation in CAT specific activity during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens,* WW+BS- well watered okra inoculated with *B. subtilis,* WS+BS- water stressed okra inoculated with *B. subtilis,* WW+BC- well watered okra inoculated with *B. cepacia,* WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days) . Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.1.3 Ascorbate peroxides (APX)

Specific activity of ascorbate peroxidase gradually enhanced in drought stressed plants compared to untreated plants during the drought stress period. On the first day of drought stress, APX specific activity was increased in BS treated plants followed by PF and BC treated plants recorded higher APX specific activity and a minimal increase was observed in untreated plants. The order of increase in APX specific activity was noticed in the order WS+BS>WS+PF>WS+BC>WS (Fig.4.6). The APX specific activity showed a steep increase on the third day of drought stress in PF treated plants, however, the drought stress imparted increment in APX specific activity was

lesser when compared to the control plants. When the plants exposed to the third day of drought the augmentation in APX specific activity was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.6). Same as the previous days observation fifth day of water stress accounted the up-regulation of APX specific activity in bacteria treated plants and it was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.6). During the stress period, a remarkable increase in APX specific activity was recorded on the seventh day of stress and the up-regulation was observed as WS+PF>WS+BS>WS+ BC>WS (Fig.4.6).

Reduction in APX specific activity was reported upon re-watering, the first day of rehydration significantly reduced the APX specific activity in bacteria treated plants and the APX specific activity after the first day of re-watering was observed in the order WS+BS>WS+BC>WS+PF>WS (Fig.4.6). The second day of watering resulted in full recovery of APX specific activity in all the bacteria treated plants but, in the untreated plants a reduction in CAT specific activity was failed to attain complete recovery. The APX activity in re-watered plants on the second day of rehydration observed in the order WS>WS+PF>WS+BS>WS+BC (Fig.4.6). During the third day of watering resulted complete recovery of all the plants was noticed and APX specific activity was expressed in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.6).

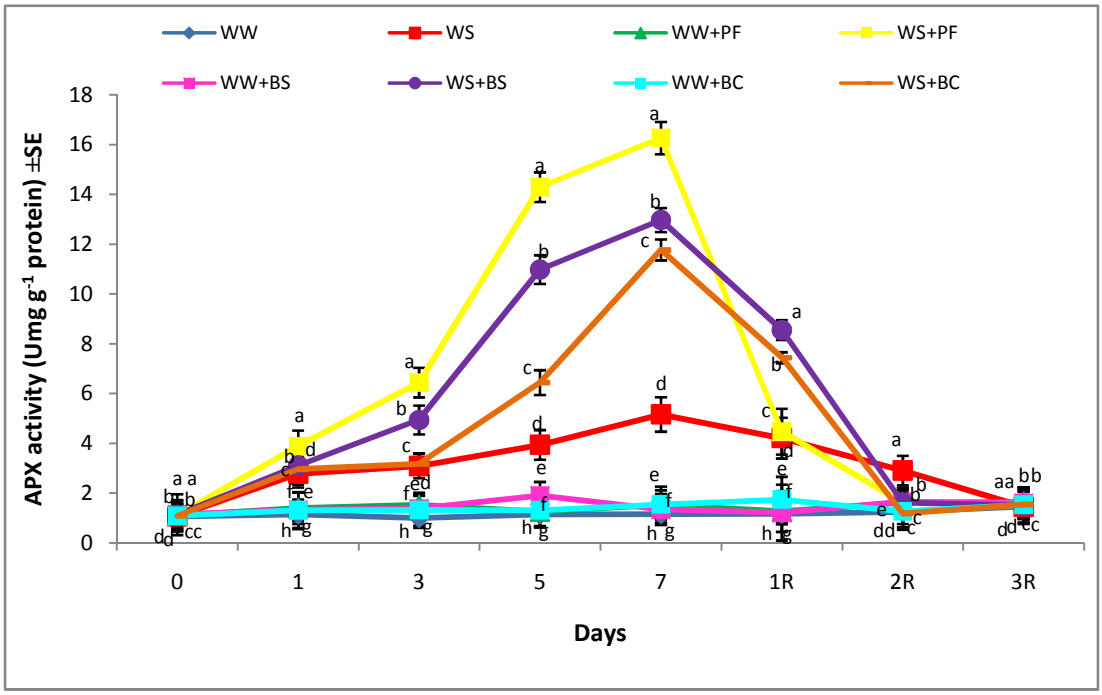

Fig.4.6. Variation in APX specific activity during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia*. 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.1.4 Guaicol peroxidase (GOPX)

In general guaicol peroxidase specific activity was steadily increased in bacteria treated and untreated plants during the stressed period. On the first day of drought stress, GOPX specific activity was up-regulated and higher activity recorded in PF treated plants followed by BS and BC treated plants and lower improvement was recorded in untreated plants. The order of increase in the specific activity of GOPX on the first day of water stress was reported in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.7). A significant increase in GOPX activity was observed on the third day of drought stress,

more GOPX specific activity was accounted for PF treated plants and the lesser increase was recorded in the untreated plants. The increase in GOPX specific activity during the third day of drought stress was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.7). Same as the previous days observations, fifth day of water stress recorded as enhancement in GOPX specific activity in PF treated plants and the enhancement recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.7). During the experimental period, a remarkable enhancement in GOPX specific activity was recorded on the seventh day of water stress in PF treated plants and lower increment was observed in untreated plants. Water stress induced enhancement in GOPX specific activity was observed as WS+PF>WS+BS>WS+BC>WS (Fig.4.7) on the seventh day of water stress.

Reduction in GOPX specific activity was recorded during watering, the first day of the re-watering significantly down-regulated the GOPX specific activity in bacteria treated plants and observed as in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.7). The second day of watering helps the bacteria treated plants to recover the GOPX specific activity completely. A remarkable reduction in GOPX specific activity was observed in untreated plants yet, a partial recovery was recorded and the GOPX specific activity of plants was in the order WS>WS+PF>WS+BS>WS+BC (Fig.4.7). The third day of watering resulted in the recovery of all the stress exposed plants and GOPX specific activity accounted as WS+PF>WS+BS>WS+BC>WS (Fig.4.7).

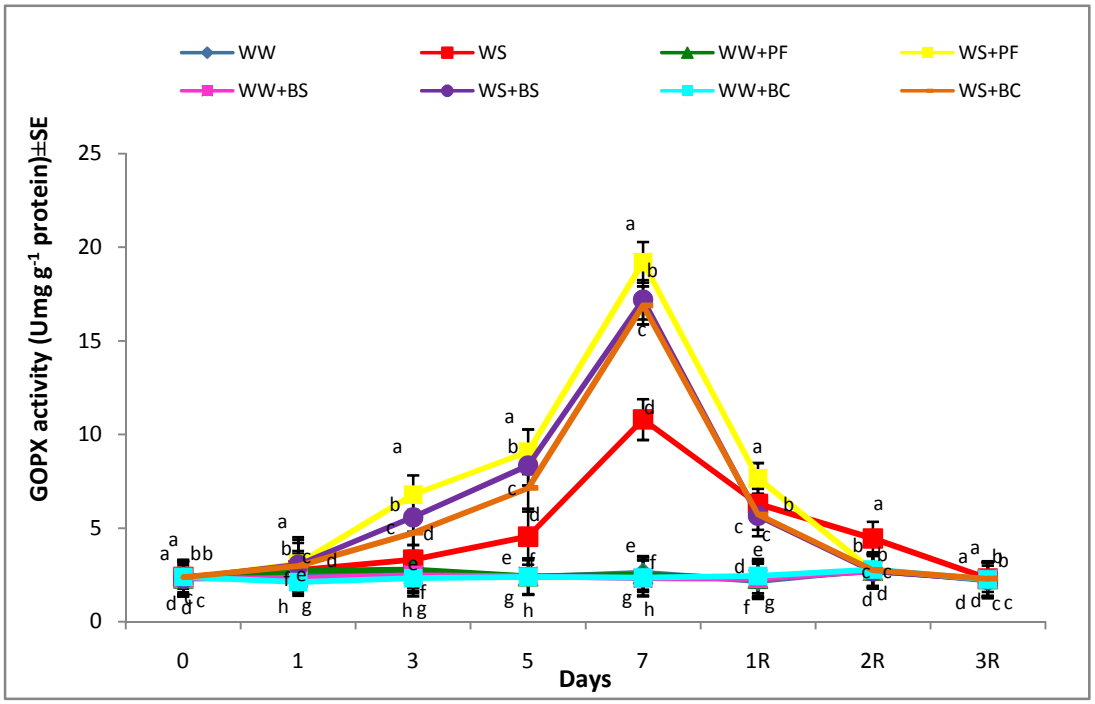

Fig.4.7. Variation in GOPX specific activity during drought stress and recovery in okra inoculated with different bacteria. (WW-well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens,* WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS-well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.1.5 Glutathione reductase (GR)

A progressive enhancement in the specific activity of glutathione reductase was recorded when exposed to drought stress, higher GR specific activity was recorded in bacteria treated plants compared to untreated plants. The first day of the water stress recorded a slight increase in GR specific activity in bacteria treated and untreated plants. The order of specific activity during the first day of drought stress was in the order WS+PF>WS+BS>WS+ BC>WS (Fig.4.8). On the third day of the drought stress, the GR specific activity was further improved in all the plants, whereas a sharp increase in

specific activity of GR was observed in PF treated plants and a slighter increase was accounted in untreated plants against the control plants. The increase in GR specific activity was sustained during third day of the drought stress and the enhancement in GR specific activity was observed as WS+PF>WS+BS>WS+BC>WS (Fig.4.8). The fifth day of water stress also recorded higher GR specific activity in bacteria treated plants and it was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.8). Among the study period, a significant increase in GR specific activity was recorded on the seventh day of water stress in PF treated plants and a lesser hike was observed in untreated plants. Water stress induced enhancement in GR specific activity was observed as in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.8) on the seventh day of water stress.

A decrease in the activity of GR was observed during rehydration, the first day of re-watering significantly reduced the GR specific activity of bacteria treated plants and GR activity was recorded in the order WS>WS+PF>WS+BS>WS+BC (Fig.4.8). The second day of re-watering resulted in complete recovery of GR activity in all the bacteria treated plants from drought stress induced up-regulation. Significant reduction in GR specific activity was observed in untreated plants but they partially recovered the GR activity and the specific activity of GR in plants was recorded as WS>WS+PF>WS+BS>WS+BC (Fig.4.8). The third day of watering resulted in stress recovery of all the plants and GR specific activity was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.8).

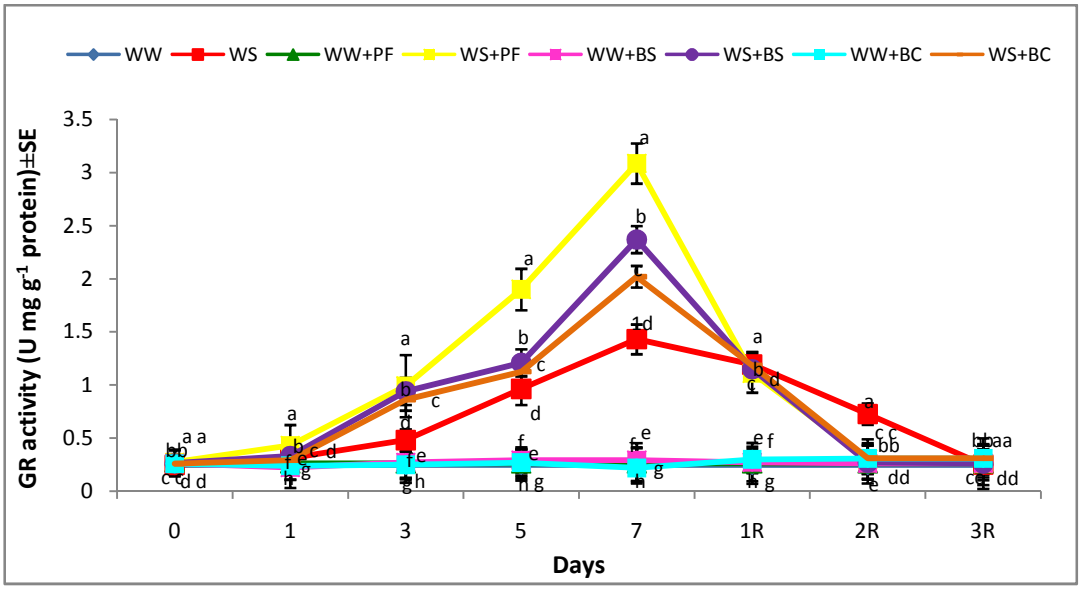

Fig.4.8. Variation in leaf area during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed, WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis,*WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B.cepacia.* 1,3,5 and 7 represent stressed days; 1R, 2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.1.6 Monodehydroascorbate reductase (MDHAR)

Monodehydroascorbate reductase (MDHAR) specific activity significantly increased with increasing days of drought stress, bacteria treated plants showed higher MDHAR activity than untreated plants throughout the stress period (Fig.4.9). During the first day of drought stress, little increase in MDHAR specific activity was recorded in bacteria treated and untreated plants. The order of specific activity during the first day of drought stress was recorded in the order WS+BS>WS+PF>WS+BC>WS (Fig.4.9). A hike in MDHAR specific activity was observed on the third day of drought stress in all the stressed plants. Whereas, the PF treated plants showed more increase in MDHAR specific activity compared to other bacteria treated and untreated plants and the order of up-regulation in MDHAR specific activity was noticed

in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.9). Further increase in MDHAR specific activity was observed on the fifth day of drought stress, similar to the other day's observation in PF treated plants that showed a higher MDHAR specific activity with respect to the other bacteria treated and untreated plants. The order of increase in MDHAR specific activity in drought stressed plants were recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.9). Moreover, a significant increase in MDHAR specific activity was recorded on the seventh day of stress in PF treated plants and the lesser rise was recorded in untreated plants. Water stress induced up-regulation of MDHAR specific activity was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.9) on the seventh day of stress.

Rehydration resulted in a decrease in MDHAR specific activity, on the first day of re-watering MDHAR specific activity remarkably reduced in the bacteria treated plants and the order of MDHAR specific activity in re-watered plants was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.9). The second day of the hydration resulted in complete reversal of MDHAR specific activity in all the bacteria treated plants. However, in untreated plants, specific activity of MDHAR was significantly reduced and the recovery was incomplete. The MDHAR specific activity of plants on the second day of re-watering was recorded in the order WS>WS+PF>WS+BS>WS+BC (Fig.4.9). The third day of watering resulted in recovery of all plants and MDHAR specific activity was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.9).

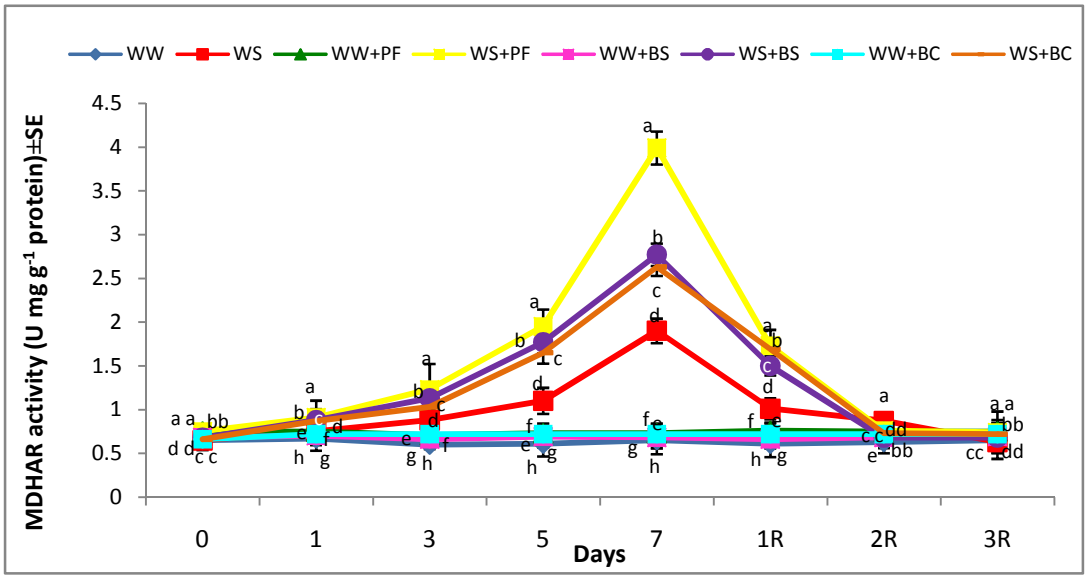

Fig.4.9. Variation in MDHAR specific activity during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B.cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.1.7 Dehydroascorbate reductase (DHAR)

Water stress increased DHAR specific activity remarkably in bacteria treated plants were compared to the untreated plants during the stress period (Fig.4.10). On the first day of the water stress, specific activity of DHAR significantly increased in PF treated plants with respect to other bacteria treated and untreated plants. The order of DHAR specific activity during the first day of drought stress was recorded in the order WS+PF>WS+BS> WS+BC>WS (Fig.4.10). Further increase in DHAR specific activity was observed on the third day of drought stress in all stress exposed plants, however, a sharp increase in DHAR specific activity was observed in PF

treated plants and a minimal enhancement was recorded in untreated plants compared to the control plants. The increase in DHAR specific activity was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.10). Fifth day of the water stress recorded a higher DHAR specific activity in the bacteria treated plants (i.e., PF treated plants) and DHAR specific activity in stress exposed plants were recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.10). Among the observed period, DHAR specific activity was up-regulated significantly in PF treated plants and the lower increase was noticed in untreated plants. Drought stress induced up regulation of DHAR specific activity was recorded as in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.10).

However, re-watering decreased the DHAR specific activity, on the first day of rehydration with a significant decrease in DHAR specific activity was observed in bacteria treated plants compared to the untreated plants and the order of DHAR specific activity was recorded as WS+PF>WS+BS> WS+BC>WS (Fig.4.10). The second day of rehydration resulted in a complete recovery of DHAR specific activity in all the bacteria treated plants. In untreated plants, a significant reduction in DHAR specific activity was observed however, not completely recovered. DHAR specific activity of plants on the second day of rehydration was recorded in the order WS>WS+PF >WS+BS>WS+BC (Fig.4.10). The third day of rehydration caused recovery of all the plants and DHAR specific activity was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.10).

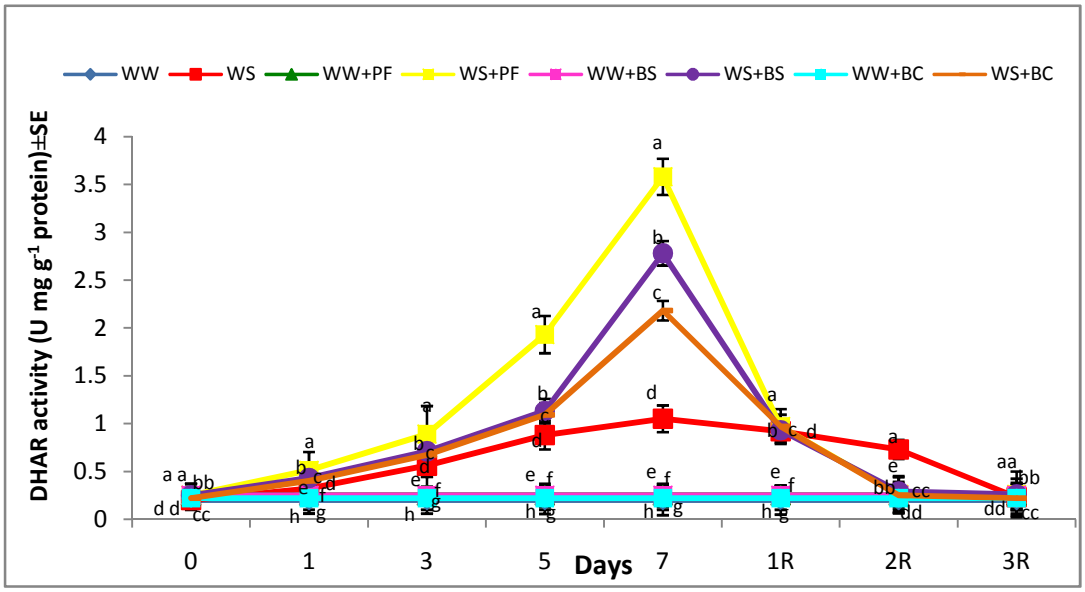

Fig.4.10. Variation in DHAR specific activity during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia*. 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.2 Non enzymatic antioxidants

4.4.3.2.1 Phenolics

The phenolic content was gradually increased with drought exposure. Higher accumulation of phenolic content was recorded in PF treated plants during regularly watered period followed by BS and BC treated plants and a lower level of phenolic content was recorded in untreated plants (Fig.4.11). On the first day of drought stress, okra treated with PF recorded a remarkable enhancement in phenolic content and lower enhancement was recorded by untreated plants when compared to control plants. The accumulation of phenolic content in drought stressed plants was recorded as

WS+PF>WS+BS>WS+BC>WS (Fig.4.11). The third day of the drought stress resulted in a progressive increase in phenolic content and the order of accumulation was observed as WS+PF>WS+BS>WS+BC>WS (Fig.4.11). Similar to the trend of first and second days, a higher phenolic content was recorded in PF treated plants on the fifth day of drought stress and lesser phenolic accumulation was recorded in untreated plants. The augmentation in the phenolic content in drought stressed plants was observed in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.11). During the experimental period, a higher quantity of phenolic content was recorded on the seventh day of water stress in PF treated drought stressed plants and untreated plants were recorded a lesser enhancement in phenolic content compared to control plants. The accumulation of phenolic in drought exposed plants were recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.11).

Rehydration resulted in a reduction in phenolic synthesis, the first day of the re-watering resulted in a remarkable decrease in phenolic content and it was recorded in each treatment as in the order WS>WS+PF>WS+BS> WS+BC (Fig.4.11). On the second day of re-watering, the complete regain of phenolics content was observed in all bacteria treated plants. A significant reduction in phenolic content was observed in untreated water stressed plants however, only partially recovered. The increasing order of phenolic content on the second day of re-watering was observed as WS>WS+PF >WS+BS>WS+BC (Fig.4.11). The third day of watering resulted in the recovery of phenolic content same as in the case of control plants and the order of phenolic content in okra was recorded as WS+PF>WS+BS> WS+BC>WS (Fig.4.11).

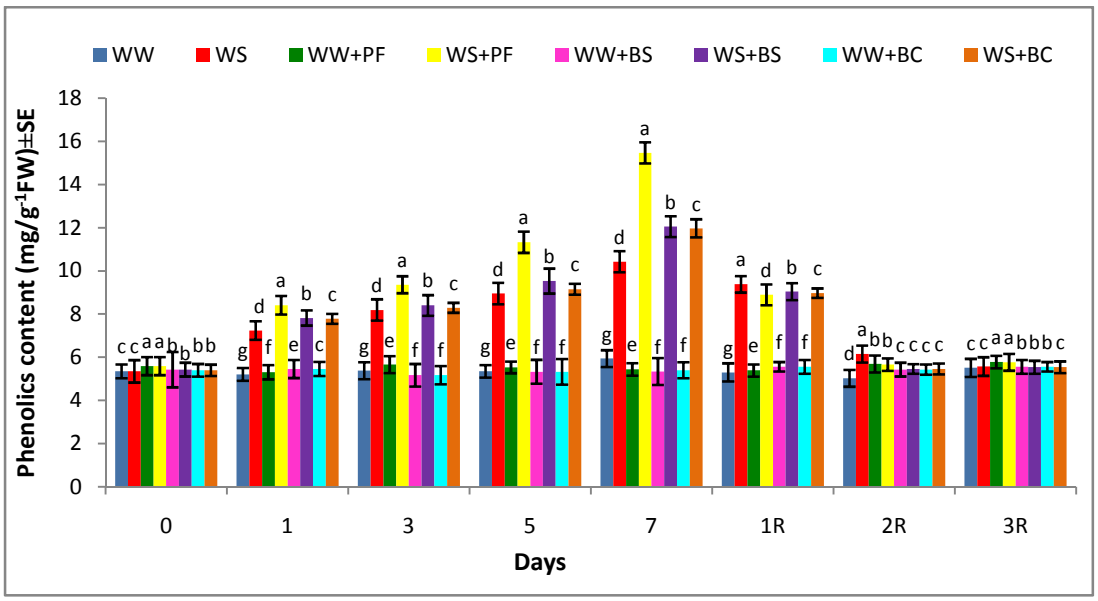

Fig.4.11. Variation in phenolics content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS-well watered okra inoculated with *B. subtilis*, WS+BS- water stressed okra inoculated with *B. subtilis,*WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.2.2 Ascorbate (AsA)

Bacteria treated okra showed a significant difference in ascorbate content during regularly watered condition against untreated plants. Among the bacteria treated plants, PF treated plants recorded a higher quantity of ascorbate followed by BS treated and BC treated plants (Fig.4.12). Drought stress resulted in progressive enhancement in ascorbate content, increased ascorbate content during water stress period was recorded by the PF treated plants followed by BS and BC treated plants. A lower level of ascorbate content was recorded in untreated water stressed plants. The first day of drought stress resulted in a significant increase in the ascorbate level in PF treated okra and lesser augmentation was recorded in untreated plants. The

increment of ascorbate in drought stressed plants was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.12). Plants exposed to the third day of drought stress showed an enhancement in ascorbate content and the order of increase in ascorbate content was in the order WS+PF>WS+BS>WS+ BC>WS (Fig.4.12). Similar to initial days of observation, higher ascorbate content was recorded in bacteria treated plants on the fifth day of drought stress and lesser ascorbate accumulation was recorded in untreated plants. The enhancement in the ascorbate content in drought stressed plants was observed in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.12). Within the stressed days, a significant increase in ascorbate content was observed on the seventh day of water stress in PF treated plants and the untreated plants recorded lesser improvement in ascorbate content compared to control plants. The enhancement pattern of ascorbate content on the seventh day of drought exposure were recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.12).

Rehydration resulted in the decrease in ascorbate content in the stressed plants, during first day of the re-watering a significant reduction in ascorbate content was recorded in drought stressed plants. Ascorbate content in re-watered plants was recorded in the order WS+PF>WS+BS>WS+BC >WS (Fig.4.12). The second day of rehydration resulted in complete recovery of ascorbate content in all the bacteria treated plants, a remarkable reduction in ascorbate content was observed in untreated water stressed plants but not entirely recovered. The elevation in the level of ascorbate content was observed as in the order WS>WS+PF>WS+BS>WS+BC (Fig.4.12). The third day of re-watering resulted in the recovery of ascorbate content in all the plants and the order of ascorbate content in okra was recorded as WS+PF>WS+BS>WS+BC>WS (Fig.4.12).

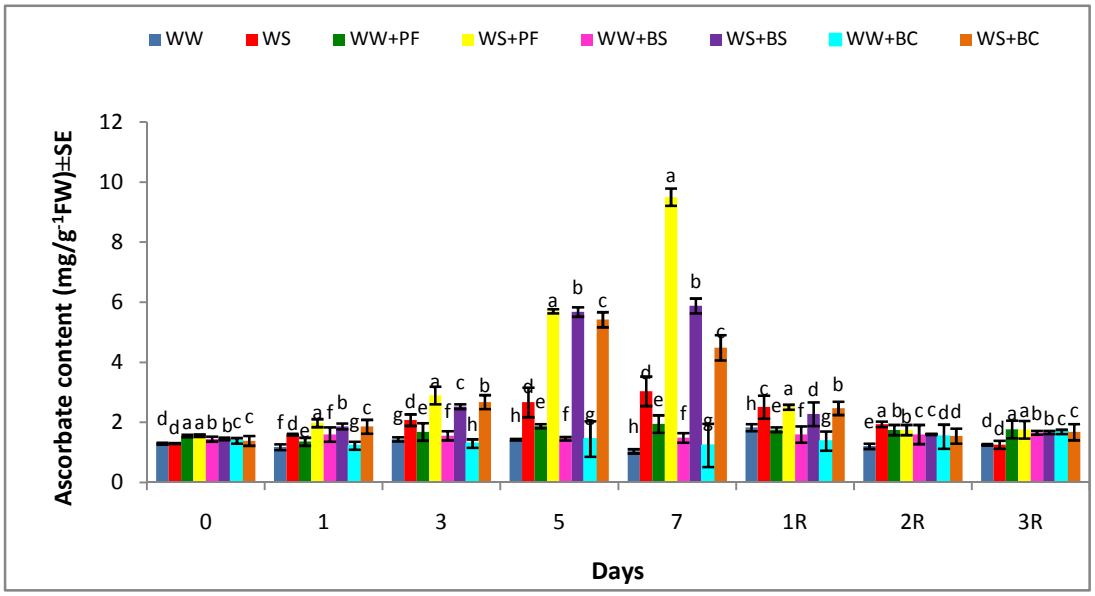

Fig4.12. Variation in ascorbate content during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed ,WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS-water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.3.2.3 Glutathione (GSH)

Like other non-enzymatic antioxidants, the glutathione content also increased during drought stress. There is a significant variation in glutathione content between the bacteria treated and untreated plants under regularly irrigated condition (Fig.4.13**)**. Higher glutathione content was observed in BS treated plants during watered condition preceding to PF and BC treated okra recorded a slight increase in glutathione content when compared to untreated plants (Fig.4.13**)**. A considerable increase in glutathione content was observed between the bacteria treated and untreated plants during the first day of drought stress. Maximum augmentation in the level of glutathione was

recorded by PF treated plants and the minimum was noticed in untreated plants when compared against control plants and the increasing order of glutathione content in stressed plants was recorded as WS+PF>WS+BS> WS+BC>WS (Fig.4.13**)**. The third day of drought stress resulted in a gradual increase in the glutathione content in all the stressed plants, whereas a sheer increment in the glutathione content was recorded in PF treated plants and untreated plants. The rise in glutathione content during the third day of stress was recorded in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.13). Compared to earlier water stressed days, a higher glutathione content was recorded in PF treated plants on the fifth day of drought stress and the minimal increase was observed in untreated plants. Drought induced increment in glutathione content was recorded as in the order WS+PF>WS+BS>WS+BC>WS (Fig.4.13**)** on the fifth day of water stress. During the observed intervals, a significant increase in glutathione content was observed on the seventh day of drought stress, higher elevation in glutathione was recorded in PF treated plants and the lower increase was registered in untreated plants compared to the control plants. The order of increase in glutathione was observed as WS+PF>WS+BS>WS+BC>WS (Fig.4.13).

Re-watering resulted in a decline in glutathione synthesis, first day of rehydration resulted in a significant reduction in glutathione content and the difference in glutathione production between the treatment was in the order WS+BS>WS+BC>WS>WS+PF (Fig.4.13). Second day of the rehydration resulted in the complete regain of stress driven enhancement of glutathione synthesis in bacteria treated plants, and a significant reduction in glutathione production in untreated plants, however, they are not completely recovered and the glutathione content in re-watered plants was recorded as WS>WS+PF>WS+BS>WS+BC (Fig.4.13). Third day of rehydration resulted in a complete recovery of all plants i.e., normal glutathione content and the

order of hydrogen peroxide content in okra was recorded as WS>WS+BS >WS+PF>WS+BC (Fig.4.13).

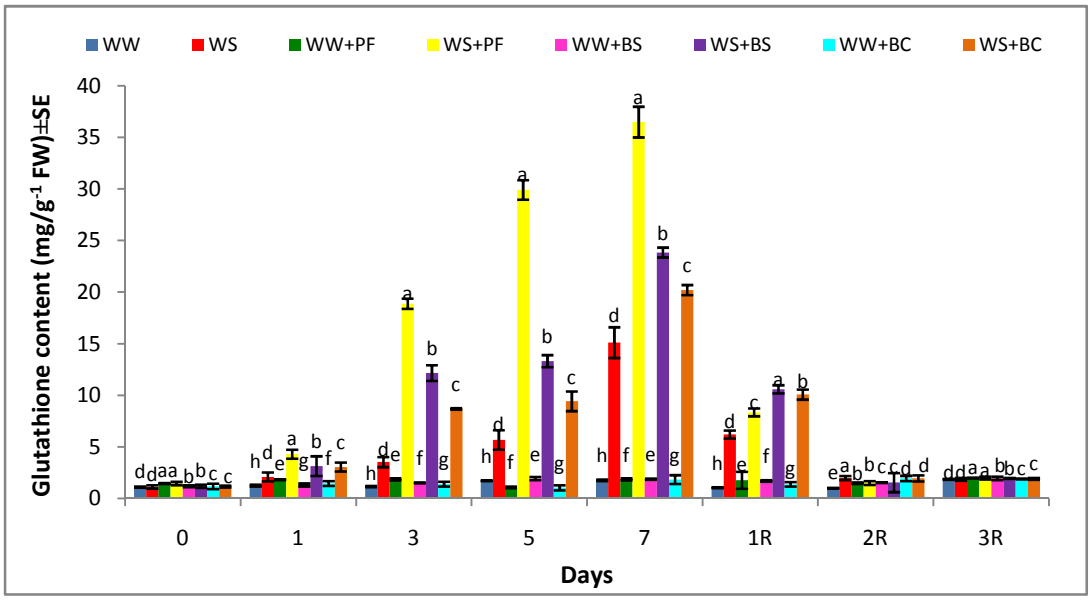

Fig.13. Variation in leaf area during drought stress and recovery in okra inoculated with different bacteria. (WW- well watered, WS-water stressed, WW+PF-well watered okra inoculate with *P. fluorescens*, WS+PF-water stressed okra inoculated with *P. fluorescens*, WW+BS- well watered okra inoculated with *B. subtilis*, WS+BS-water stressed okra inoculated with *B. subtilis*, WW+BC- well watered okra inoculated with *B. cepacia*, WS+BC- water stressed okra inoculated with *B. cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

4.4.4 Discussion

4.4.4.1 Production of reactive oxygen species (ROS) and lipid peroxidation

Generally, plants keep equilibrium between ROS production and scavenging during unstressed condition, abiotic stresses disturb this equilibrium and results in oxidative burst. During abiotic stress, ROS production was increased mainly through two ways 1. As a result of disruptions in metabolic pathways held in various organelles such as

chloroplast, peroxisome and mitochondria 2. ROS produced for signaling purpose (Gilroy *et al.,* 2014; 2016; Choudhury *et al*., 2017). Abiotic stress induces the reduction of molecular oxygen to chem2ically active reactive oxygen species (ROS), such as singlet oxygen ($1O_2$), superoxide radical ($O_2^{\cdot-}$), hyroxyl radical ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2) (Sharma and Dietz, 2009). According to Sofo *et al*. (2005) the ROS production accelerates under dehydration due to the interruption of electron transport system and oxidizing metabolic activities occurring in chloroplast, mitochondria and microbodies.

Higher oxidative stress was recorded in untreated okra plants, which directly correlated with the increased quantity of $O_2^{\cdot-}$ and H_2O_2 production under drought stress. In contrast, PSB treatment down regulated the production of $O_2^{\cdot-}$ and H_2O_2 levels during drought stress in okra. Enhancement in the production of osmolytes and antioxidants in drought stressed bacteria treated okra limiting the ROS production. PF treated plants recorded a higher antioxidant activity during stress condition that resulted in the lower production of super oxide and hydrogen peroxide. Maximum ROS generation was recorded in untreated plants, linearly correlated with lesser production of antioxidant enzymes. Enhanced accumulation of osmolytes and activity of ROS scavenging enzymes during stressed condition protect okra from oxidative stress and results in faster regain during re-watering. This view is further supported by Moslemi *et al*., (2011). According to the authors PGPR inoculation in maize significantly reduced the production of ROS by inducing the production of antioxidant enzymes and osmolytes. Zhou *et al*., (2016) observed that, minimal ROS production in *Arabidopsis* inoculated with *Bacillus megaterium* BOFC15 and suggested that, secretion of spermidine and polyamine (PA) by the bacterium under drought stress plays an important role in diminishing oxidative damage.

Lipids, proteins and nucleic acids are the major macromolecules damaged by ROS. Under drought stress, ROS damage cell membrane by the oxidation of hydrogen from methylene group of polyunsaturated fatty acids of membrane lipids, eventually leading to the production of aldehydes including malonyldialdehyde (MDA), lipid alkoxy radicals, alkanes and lipid epoxides (Apel and Hirt, 2004). So the quantity of MDA in plant tissue during stress condition can be considered as a physiological marker of membrane damage (Gill and Tuteja, 2010).

In the present study, drought stress cause an increase in membrane damage quantified as the increased level of MDA content. Inoculation of PSB's decreased MDA content suggested that, PSB's can maintain the membrane integrity against oxidative damage imposed by water stress. Among PSB treated plants, PF treatment effectively alleviates drought induced lipid peroxidation. Moreover, proper scavenging of ROS in PSB treated plants facilitated faster recovery from stress induced membrane damage. Jha and Subramanian (2014) reported that, inoculation of PGPR reduces the osmotic stress thus the plants record decline in MDA content. Koc (2015), also suggest that application of plant growth promoting bacteria and arbuscular mycorrhizal fungi act as an anti-stress agent in strawberry under stress condition. PGPR inoculation reduces drought induced cellular damages, leading to the reduction in MDA content and membrane permeability (Sahin *et al*., 2015). According to Gusain *et al*. (2015) PGPR treated rice seedlings did not show drought stress symptom similar to untreated plants. PGPR reduced the harmful effect of the drought stress by lowering lipid peroxidation in wheat compared to un-inoculated plants during drought and subsequent recovery (Mishra *et al*., 2016). Our results are supported by the earlier reports by Sandhya *et al*. (2010), the authors observed that *Bacillus* sp. treated maize plants were recorded with lower MDA under stress condition when compared to control. In agreement with our results Gusain *et al*. (2015)

and Shukla *et al*. (2012) suggested that increased accumulation of osmolyte and enhanced activity of ROS scavenging system increase membrane stability under the stress condition.

4.4.4.2 Antioxidant system

Antioxidant system actively involves in the detoxification of ROS molecule and maintains equilibrium between the activities of antioxidant enzymes and produces ROS under normal environmental condition. Any changes in the environmental condition disturb this equilibrium and it leads to the concomitant increase in ROS. Based on the activity of antioxidant molecules, they can be classified as enzymatic and non enzymatic antioxidants. Enzymatic antioxidants remove free radicals by breaking them and convert them to non-toxic molecule, this conversion is a multi step process; in the first step most dangerous free radical, the superoxide and hydroxyl radicals are converted to hydrogen peroxide (H_2O_2) and then to water, assisted by cofactors such as copper, zinc, manganese and iron. Non-enzymatic antioxidants act by interrupting free radical chain reactions (Shahidi and Zhong, 2010; Nimse and Pal, 2015). Both non-enzymatic and enzymatic antioxidant activity has found to be improved during the stress condition.

4.4.4.2.1 Enzymatic anti oxidant system

To minimize the oxidative stress induced by drought, plants increase the activity of ROS detoxification system (Ahamed *et al.,* 2014). The enzymes involved in the reduction of ROS were localized in different subcellular compartments like mitochondria, chloroplast, peroxisome and cytsol because they are the important sites of ROS production under stress. Superoxide Dismutase (SOD), Catalase (CAT), Ascorbate Peroxidase (APX), Monodehydroascorbate reductase (MDHAR), Dehydroascorbate reductase

(DHAR), Glutathione Reductase (GR) and Guaiacol Peroxidase (GOPX) are the major components of enzymatic antioxidant system.

Superoxide dismutase (SOD) was a metalloenzyme that initiates the first line defense against ROS accumulation by rapid conversion of superoxides ($O_2^{\cdot}-$) into H_2O_2 and O_2. By the detoxification of $O_2^{\cdot}-$, SODs reduce the hazard on hydroxyl radical ($OH^{\cdot}$) formation through the metal catalyzed Haber-Weiss-type reaction (Gill and Tuteja, 2010). Apart from this, SOD has a key role in nuclear transcription, as a RNA binding protein, a synthetic lethal interaction and a signal modulator in glucose metabolism (Chung, 2017). PSB inoculation improves the SOD activity in stressed okra plants, which is reflected as the reduction in superoxide content during stress. Higher SOD activity was observed in PF treated plants and the reduction in both membrane damage and superoxide production was observed in this plants. Lower reduction in photosystem activity especially PSII activity and damage was also observed in PF treated plants was correlated with higher SOD activity. The accumulation of super oxide also decreased in BS and BC treated plants compared to control plants, but not as much as PF treated plants. Severe reduction in SOD activity was recorded in untreated plants which result in higher superoxide content and membrane damage under water stress. Quick recovery in SOD activity was also observed in PSB treated plants might be due to the steep reduction in generation of superoxide during recovery period i.e., PSB treatment effectively managed the ROS production during drought results in faster recovery. Slow regeneration of superoxide may result slower regain in SOD activity in untreated plants during rehydration. Saravanakumar *et al*. (2011) reported that green gram inoculated with *Pseudomonas* strains significantly improves SOD activity under water stress and this plays an important role in drought stress tolerance. A significant increment in specific activity of SOD in PGPR treated sunflower

was recorded under drought stress compared to un-inoculated plants (Singh *et al.,* 2015).

Hydrogen peroxide (H_2O_2) was generated in plants as a result of photorespiration, ß-oxidation of fatty acids and dismutation of superoxides (Sagi and Fluhr, 2006; Andre *et al.,* 2013) and it can diffuse through aquaporins of intra and intercellular membranes of cells (Bienert *et al.,* 2007). H_2O_2 plays a dual role as ROS and as a signaling molecule, accumulated hydrogen peroxide act as a signal for cell apoptosis under the stressed condition. A Higher quantity of hydrogen peroxide produced during stress efficiently detoxified by various enzymes like catalase (CAT), ascorbate peroxide (APX), guaicol peroxide (GOPX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR).

Catalase, a heme-containing redox enzyme found in all aerobic organisms, actively involves in the conversion of H_2O_2. The conversion may be a direct one to H_2O and O_2 or indirectly by oxidizing substrates like methanol, ethanol, formaldehyde, formate, or nitrite by using hydrogen peroxide (Sharma and Ahamed, 2014). Catalase plays a key role in early growth and establishment of seedlings by detoxifying H_2O_2 under water stress (Bailly *et al*., 2008; Jiang and Zhang, 2002). Significant improvement in CAT activity was recorded in PSB treated plants, among PSB treatments PF treated plants showed higher increment in CAT activity. This in turn results in lesser membrane damage and hydrogen peroxide content in plants. BS and BC treatment effectively alleviate drought induced hydrogen peroxide production and membrane damage. However, it was observed that, concomitant increase in CAT activity during water stress in untreated plants was not sufficient to control the generation of hydrogen peroxide which resulted in the augmentation in MDA content and hydrogen peroxide. The lower membrane

damage in PSB treated plants accelerates the recovery of plants. These observations were supported by the findings of Ünyayar *et al*. (2005) that CAT activity was increased in tolerant *Lycopersicon peruvianum* and decreased in sensitive *L. esculentum* under drought stress. Over expression of *Escherichia coli* catalase (*KatE*) with a chloroplast-targeting signal sequence protects the thiol-modulated enzymes in the Calvin cycle in tobacco plants, thereby mitigating the inhibition of photosynthesis under photooxidative stress (Foyer *et al*., 1994).

Guaiacol peroxidases, the heme-containing enzymatic ROS scavenger that oxidizes aromatic electron donors such as guaiacol and pyragallol in the presence of H_2O_2 (Sharma *et al*., 2012). Guaiacol peroxidase was a part of many important biosynthetic mechanisms involved in the defense against abiotic and biotic stresses (Gill and Tuteja, 2010). In drought stressed okra plants, PSB inoculated plants showed higher GOPX activity which improved oxidative stress tolerance potential. In bacteria treated plants PF treatment was more significant to alleviate the harmful effect of the drought stress, these plants showed a lower increase in H_2O_2 under the stressed condition, it significantly increased the membrane integrity, chlorophyll content and PSI and PSII activity under water stress. BS and BC treated plants have recorded with lesser drought tolerant capacity compared to PF treated plants. Lower GOPX activity in untreated plants assist increased membrane damage and decrease in the metabolic process including photosynthesis. Increased GOPX activity during the stressed condition in PSB treated plants actively scavenge hydrogen peroxides and reduce membrane damage due to ROS leading to quick regain in GOPX activity than untreated plants. A similar observation was made by Heidari and Gopayegani (2012) in basil treated with PGPR, that significantly improved water stress tolerance with increased GOPX activity. Besides this, Žuna-Pfeiffer *et al.* (2010) suggested that, guaiacol peroxidase regulate the production of phenolics, in our observation also a concomitant

increase in phenolics content was observed with increasing drought exposed days.

Ascorbate peroxidase (APX), glutathione reductase (GR), monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) are the potent scavengers of hydrogen peroxide and they together operate ascorbate-glutathione cycle in plants to detoxify hydrogen peroxide to water and molecular oxygen (Shan *et al.,* 2015). Enhanced hydrogen peroxide generation was observed in chloroplasts, peroxisomes and mitochondria due to stress induced alteration in metabolic pathways. Ascorbate peroxide oxidizes hydrogen peroxide using ascorbate as an electron donor and produces monodehydroascorbate (MDHA) radical. MDHA was less active than other free radicals and dissociate to dehydroascorbate (DHA) and ascorbate in the presence of monodehydro ascorbate reductase (MDHAR). Ascorbate was regenerated from dehydro ascorbate by the action of the enzyme dehydroascorbate reductase, glutathione reductase regenerate GSH in the AsA-GSH cycle using NADPH as a cofactor (Bartoli *et al.,* 2017).

Ascorbate peroxidase was observed in plastid-containing organisms (Teixeira *et al.,* 2004; Passardi *et al*., 2007; Nedelcu *et al.,* 2008). Different isoforms of APX was observed in the chloroplast stroma, thylakoids (Groden and Beck 1979; Miyake and Asada 1992), cytosol, mitochondria and peroxisomes (Jiménez *et al*., 1998). Approximately 70–80% of the hydrogen peroxide produced during stress was scavenged by APX enzymes present in the chloroplasts (Bartoli *et al*., 2017). APX is observed as the most significant peroxidase participated in H_2O_2 scavenging using ascorbate as a substrate and it is the first step of AsA-GSH cycle (Michalak, 2006). Ascorbate plays an important role in reducing the ROS production in chloroplast by actively scavenging the products of water-water cycle and also helps to dissipate excess electron from photosynthetic electron transport (Asada, 2006).

Hence, the enhanced production of APX during drought stress increases the tolerance potential of plants. The increment in APX specific activity in PSB treated plants is directly correlated with the improvement in photosystem activity under stress. Higher activity of APX was observed in PF treated plants with a lower oxidative damage and a lesser reduction in photosystem activity. This result revealed that, drought induced generation of H_2O_2 in chloroplast effectively detoxified by APX. BS and BC treated plants also showed an increase in APX activity this also cause a lower reduction in photosystem activity and membrane stability under water stress. However, the increase in APX activity in response to drought stress in untreated plants fails to scavenge the ROS induced by drought stress which have resulted in an increase in membrane damage and a decrease in photosystem activity. PBS inoculation in okra further enhanced APX activity, preventing the H_2O_2 mediated cell damage, and there by thus helps to faster recovery during rehydration.

Monodehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) are mainly associated with restoration of ascorbate in AsA-GSH cycle. MDHAR, a soluble monomeric flavin enzyme that catalyzes the reduction to AsA from the MDHA radical using NADH as an electron donor (Hossain and Asada, 1985). MDHAR reduce monodehydroascorbate by active conversion to DHA and AsA (Drazkiewicz *et al.,* 2003). DHAR catalyze the reduction of DHA to AsA. In plants, isoforms of MDHAR and DHAR has been located in the chloroplast, mitochondria, peroxisome and cytosol. Hyperactivity of these enzymes during various abiotic stresses ensure plant's tolerance potential. (Dalton *et al*., 1993; Bérczi and Møller, 1998; Jiménez *et al*., 1998; Mittova *et al*., 2000; Shimaoka *et al*., 2000).

Increased DHAR and MDHAR activity was observed in bacteria treated plants under drought stress and this enhancement improved the

drought tolerance potential in okra by active detoxification of hydrogen peroxide and potent recycling of AsA. Moreover, it was observed that the activity of DHAR and MDHAR directly related to a reduction in hydrogen peroxide generation in okra and also the increase in AsA. The bacteria treated plants generated higher DHAR and MDHAR activity and lower hydrogen peroxide content during drought stress. Among the bacteria treated plants, PF treatment was more effective because these plants record higher DHAR and MDHAR activity during the severe stress conditions, compared to other bacteria treated plants. Untreated plants recorded lesser DHAR and MDHAR activity leading to higher oxidative destruction in these plants. According to Fazeli *et al*. (2007) DHAR and MDHAR play a crucial role in improvement of drought stress tolerance potential of plant through reducing superoxide and hydrogen peroxide radical and which was in agreement with our results, the earlier reports suggest that the up regulation of DHAR increase the plant's drought tolerance (Osipova *et al*., 2011; Kang *et al*., 2013). It was noticed that, the increased expression of DHAR has been shown to increase drought tolerance in different crops like potato (Eltayed *et al*., 2011), rice (Kim *et al*., 2013) and tobacco (Eltayed *et al*., 2007). These results support the present observation that enhancement in MDHAR and DHAR activity promote plant's drought stress tolerance. Earlier reports suggest that over expression of MDHAR and DHAR genes improve oxidative stress tolerance and increase the AsA content in plants (Guo *et al*., 2006; Plomion *et al*., 2006; Zhou *et al*., 2015). Similarly, Ma (2007) and Zhang *et al*. (2008b) reported that an increase in DHAR activity under drought stress have been reported in a range of plant species. The increased activities of these enzymes accelerate hydrogen peroxide detoxification under drought stress in kentucky bluegrass (Bian and Jiang, 2009).

The activity of MDHAR and DHAR was down-regulated in okra during rehydration phase, faster restoration of enzyme activity was recorded

in bacteria treated plants compared to untreated plants and this might be due to the low stress severity of bacteria treated plants. A Similar observation was made in *Prunus* (Sofo *et al*., 2005) and kentucky bluegrass (Bian and Jiang, 2009) that the quick reduction in enzyme activity during re-watering directly related to enhancement in drought tolerance potential of the plants.

Glutathione reductase (GR) a flavin-containing enzyme, besides acting as an antioxidant, it regulates plant growth and development not only under stress but also under optimum conditions (Swanson and Gilroy 2010). Drought stress induced increase in GR activity improved the drought alleviation potential of plants (Pastori and Trippi, 1992). The present observation revealed that, higher GR activity was observed in PSB treated plants, especially in PF treated okra plants. Enhanced GR activity triggers reduction in H_2O_2 in PF, BS and BC treated plants during drought stress and also improved the production of GSH as a part of AsA-GSH cycle. However, the up- regulation of GR activity was lesser in untreated plants resulting in an increase in the severity of drought stress and reduction in recovery during re-watering. This finding was further supported by the observation of Ma *et al*. (2011a). Similarly, drought-induced abundance of GR in cowpea and *P. vulgaris* increased water stress tolerance potential (Contour-Asel *et al*., 2006; Torres-Frankin *et al*., 2008). Pang and Wang, (2010) opined that, there is a positive correlation between drought tolerance potential of plants and GR activity.

4.4.4.2.2 Non-enzymatic antioxidant

Non enzymatic antioxidants are directly reacting with ROS and effectively detoxify them, among the non-enzymatic antioxidants AsA is initiate the ROS scavenging (Foyer and Halliwell, 1976). AsA plays an import ant role in maintaining the equilibrium between the antioxidant system and ROS production during abiotic stresses (Reddy *et al*., 2004). The

augmentation of AsA is positively correlated with drought stress tolerance (Kefeli, 1981). Ascorbate operates two hydrogen peroxide scavenging systems in plants, water-water cycle in chloroplast (Asada, 1999) and the AsA–GSH cycle (Foyer and Halliwell, 1976; Foyer and Noctor, 2011).

In addition, AsA regulates various physiological processes, including plant defense against oxidization, activation of co-factor of enzymes, plant cell division, cell expansion, senescence, plant growth and development (Horemans *et al*., 2000). Therefore, enhanced accumulation of AsA under water stress help to maintain the normal metabolisms during extreme stress condition. Ascorbate has a major role in photosynthesis, in the Mehler reaction with APX to control the redox state of photosynthetic electron carriers and as a cofactor for violaxanthin (Smirnoff and Wheeler, 2000). Apart from this, AsA also acts as an electron donor in photosystem II during photoinhibition (Smirnoff, 2011). This might be the reason for the lesser reduction in PSII activity in bacteria treated plants under severe stress conditions.

In our study, the increase in AsA content in response to drought stress was higher in bacteria treated plants. Within bacteria treated plants, PF treatments plants recorded higher AsA than BS and BC treated plants. This enhancement was directly proportionate to the activity of MDHAR and DHAR. Whereas, drought induced increase in AsA content was lesser in untreated plants. This increases the oxidative damage due to ROS in these plants, which in turn delayed the recovery of the plants during re-watering.

Glutathione (GSH), a low-molecular-weight thiol tripeptide, plays a major role in plant stress tolerance through detoxification of ROS, redox homeostasis and modifies phytohormone signaling pathways via suitable alteration of the thiol group cysteine residues (Das and Roychoudhury, 2014). GSH was commonly observed in cell compartments such as cytosol,

endoplasmic reticulum, vacuoles, and mitochondria. However, it was predominantly observed in chloroplast (Millar *et al*., 2003). It detoxifies cytotoxic H_2O_2 and also reacts with singlet oxygen, superoxide radicals, and hydroxyl radicals. While GSH actively involved in ROS scavenging and in the regeneration of ascorbate in Ascorbate-Glutathione cycle, it helps to increase the tolerance against oxidative stress (Murmu *et al*., 2017). According to Zechmann *et al*. (2011) GSH regulate auxin metabolism and Diaz-Vivancos *et al*. (2010 a,b) reported that, GSH initiate cell division by activating the expression of genes involved in cell cycle, so in the present study higher accumulation of GSH in bacteria treated plants during drought facilitate plant growth stress through regulating auxin metabolism and transport. Apart from this, GSH act as an AsA regenerator assisting to improve the efficiency of AsA- GSH cycle and lead to effective ROS detoxification in bacteria treated plants compared to untreated plants. Although, an increase in GSH content was observed in untreated plants, fails to detoxify the drought induced ROS production under water stress in okra and this has resulted in lagging of GSH restoration during re-watering. Enhanced activity of GR in PSB was facilitating in the increased production of GSH in bacteria treated plants. The earlier findings report that glutathione helped to overcome oxidative stress in tobacco (Foyer and Noctor, 2005; Del-Rio *et al*., 2006).

Phenolic substances a group of secondary metabolites including flavonoids, tannins, hydroxycinnamate esters and lignin, exhibits antioxidant properties and mitigate oxidate stress during unfavourable condition (Oszmanski, 1995; Gumul *et al*., 2007). Accumulation of phenolic substances was one of the general tolerance responses to abiotic stresses in plants (Winkel-Shirley, 2001). During stressed condition phenolic modify the peroxidation kinetics of membrane through altering the lipid bilayers and to

decrease the fluidity of the membrane, in addition, it serves as terminators of free radicals (Schroeter *et al*., 2002).

Increase in phenolic content in bacteria treated plants enhances the tolerance potential of okra against drought induced oxidative burst. PF treated plants showed higher phenolic content than that of BS, BC and untreated plants. It revealed that PF treatment was more effective in drought stress tolerance in okra plants. The higher quantity of phenolics effectively minimizes the lipid peroxidation by ROS and recorded a significant reduction in ROS content and MDA content in these plants. Effective stress alleviation upon stress exposure resulted in a faster recovery in bacteria treated plants. In agreement with our result Erdogan *et al*. (2016) reported that PGPR inoculated plants recorded higher phenolic content during stressed condition. And they suggested that enhancement in phenolic content was directly correlated with ROS detoxifying capacity and ROS scavenging enzyme activity.

4.5 CHAPTER 5

4.5.1 Yield parameters

4.5.1.1 Number of fruits per plant

There is a considerable difference in the number of fruits per plant between bacteria treated plants and untreated plants. The maximum number of fruits was recorded in regularly watered plants and it was observed in the order WW+PF>WW+BS>WW+BC>WW. Drought stress resulted in significant reduction in the number of fruits per plant, lesser reduction in fruit number was observed in bacteria treated plants and the decreasing order of fruit number in drought exposed plants were recorded as WS>WS+BC> WS+BS>WS+PF (Table . 1).

4.5.1.2 Fruit length

Significant variation in fruit length was observed between well watered bacteria treated and untreated plants. Higher fruit length was recorded in bacteria inoculated plants. The order of increment in fruit length in okra was recorded as WW+PF>WW+BS>WW+BC>WW. Drought stress resulted in a remarkable reduction in fruit length, lower reduction in fruit length was observed in PF and higher reduction was recorded in untreated plants. The order of reduction in fruit length was observed as WS>WS+BC>WS+BS> WS+PF (Table .1, Fig. 1).

4.5.1.3 Fruit fresh weight

Bacteria treatment significantly increases the fruit fresh weight in well watered plants. PF treated plants have recorded with higher fruit length and a lesser fruit length was observed in untreated plants. The order of increase in fruit fresh weight was observed in the order WW+PF>WW+BS> WW+BC>WW. However, drought stress caused a reduction of fruit fresh

weight. Significant reduction in fruit fresh weight was observed in untreated plants compared to the control plants. Drought induced reduction in fruit fresh weight was lesser in PF treated plants, the increasing order of fruit fresh weight in drought exposed plants was observed as WS+PF>WS+BS> WS+BC>WS (Table .1).

4.5.2 Nutritional value

4.5.2.1 Protein

Fruit protein content was significantly improved by bacteria treatment and the increasing order of protein content was recorded as WW+PF>WW+BS>WW+BC>WW in regularly watered plants. While protein content was significantly reduced in drought exposed untreated plants, but the reduction was negligible in bacteria treated plants. The order of reduction in protein content in drought encountered plants was recorded in the order WS>WS+BC>WS+BS>WS+PF (Table .1).

4.5.2.2 Total amino acid

The total amino acid content was significantly improved in bacteria treated plants bearing fruits and the increasing order of amino acid content was recorded in the order WW+PF>WW+BS>WW+BC>WW. In water stressed plants, higher reduction in fruit amino acid content was observed, untreated plants have recorded with higher reduction in amino acid content and lesser reduction was noticed in PF treated plants compared with the regularly watered plants. The decrease in amino acid content due to drought was recorded in the order WS>WS+BC>WS+BS>WS+PF (Table .1).

4.5.2.3 Carbohydrate

Carbohydrate content in the fruit was significantly increased in fruits bacteria treatment in well watered and stressed plants. Highest carbohydrate

content in regularly irrigated plants was recorded by PF treated plants and the lowest by drought stressed untreated plants. Increasing order of fruit carbohydrate content in regularly irrigated plants was recorded as WW+PF>WW+BS>WW+BC>WW. Whereas, drought exposure remarkably reduce the fruit carbohydrate content in untreated plants, but bacteria treated plants recorded lesser reduction in fruit carbohydrate content. Among bacteria treated plants, PF recorded better carbohydrate content. Reduction in fruit carbohydrate content in drought exposed plants was observed in the order WS>WS+BC>WS+BS>WS+PF (Table .1).

4.5.2.4 Sugar

A significant increase in fruit sugar content was recorded in bacteria treated plants compared to untreated plants. Within bacteria treated plants, highest fruit sugar content was in recorded in PF treated plants and the order of increase in sugar content was recorded as WW+PF>WW+BS> WW+BC>WW. All drought exposed plants recorded a remarkable reduction in fruit sugar content PF treated plants recorded a lesser reduction in sugar content and a higher reduction was observed in untreated plants. The order of increase in sugar content was noticed as WS>WS+BC>WS+BS>WS+PF (Table .1).

4.5.2.5 Lipid

Bacteria treatment significantly improved lipid content in irrigated plants and the increasing order of lipid content was observed as WW+PF>WW+BS>WW+BC>WW. Increased reduction in fruit lipid content was noticed in untreated drought exposed plants compared to control plants. The reduction in fruit lipid content due to water stress was observed in bacteria treated plants and PF treated plants were recorded with lesser

reduction in lipid content and the increasing order of lipid content in drought exposed plants was observed as WS+PF>WS+BS >WS+BC>WS (Table . 1).

4.5.3 Mineral composition

4.5.3.1 Phosphorous (P)

Phosphorous content was noted to be significantly increased, during well watered condition, higher phosphorous content was detected in bacteria treated plants bearing fruit and the increasing order of phosphorous content in fruit was observed as WW+PF>WW+BS> WW+BC>WW. Compared to control plants, significant decrease in phosphorus content was recorded in drought exposed plants bearing fruit. However, the reduction in phosphorous content in fruit was much lesser in bacteria treated drought susceptible plants, among which PF treated plants showed better phosphorous content in fruit, followed by *B. subtilis* and *B. cepacia* treated plants. Drought stress resulted in a drastic reduction in P content in untreated plants bearing fruit (Table .1).

4.5.3.2 Potassium (K)

Potassium content in fruit was showed significant variation in regularly watered plants and the variation in potassium content was observed in the order WW+PF>WW+BS >WW+BC>WW. Drought significantly affects the potassium content in fruit, whereas PF treated plants bearing fruit showed a lesser reduction in potassium content with respect to their control plants. Higher reduction in potassium content was observed in untreated plants. The variation in potassium content of drought exposed okra bearing fruit was recorded in the order WS+PF>WS+BS >WS+BC>WS (Table .1).

4.5.3.3 Zinc

Considerable variation in zinc content was observed in regularly irrigated bacteria treated and untreated plants bearing fruit. PF treated plants bearing fruit recorded higher zinc content and lesser zinc content was observed in untreated plants bearing fruit. The order of increase in zinc content was recorded as WW+PF>WW+BS>WW+BC>WW. Drought significantly reduces zinc accumulation in fruit. The decrease in fruit zinc content was observed higher in untreated plants bearing fruit with respect to control plants. Stress induced reduction in zinc content was lower in PF treated plants producing fruit. The decreasing order of zinc content in fruits of drought exposed plants are recorded as WS>WW+BC>WW+BS>WW+PF. (Table . 1).

4.5.3.4 Calcium (Ca)

A significant difference in calcium content was observed in regularly watered bacteria treated and untreated plants producing fruit. Increase in fruit calcium content in unstressed bacteria treated and untreated plants were recorded as WW>WW+BC>WW+BS>WW+PF. Bacteria treatment significantly reduced the calcium content in drought exposed plants producing fruit. Higher reduction in fruit calcium content was recorded in untreated plants bearing fruit susceptible to drought stress. Whereas, the fruits produced by PF treated plants showed minimum decrease in calcium content compared to regularly watered plants producing fruit. The increasing order of fruit calcium content in drought stressed plants are recorded as WS+PF>WS+BS> WS+BC>WS (Table. 1).

4.5.3.5 Magnesium (Mg)

Magnesium content in fruit was recorded with a significant variation in regularly watered bacteria treated and untreated plants and the difference in

magnesium content was observed in the order WW+PF>WW+BS >WW+BC>WW. Drought induced reduction in magnesium content in fruit was lesser in bacteria treated plants, PF treated plants recorded lower reduction in fruit magnesium content compared to control plants. Among drought imposed plants, higher decrease in the assimilation of magnesium was recorded in untreated plants. The fruit magnesium content of drought exposed plants was recorded in the order WS+PF>WS+BS>WS+BC>WS (Table. 1).

4.5.3.6 Iron (Fe)

A remarkable difference in fruit iron content was observed in unstressed bacteria treated and untreated plants. Higher iron content was noticed in PF treated plants and lower iron content was recorded by untreated plants. The variation in fruit iron content was recorded in the order WW+PF>WW+BS >WW+BC>WW. Iron intake was significantly reduced in all water stressed plants, untreated plants were recorded a significant reduction in iron content of fruits. PF treated plants showed a lesser decrease in fruit iron content compared to control plants. The order of iron assimilation in drought exposed plants was recorded as WS+PF>WS+BS>WS+BC>WS (Table. 1).

Table.1: Yeild and nutritional parameters of fruits subjected to drought stress and irrigated plants treated with different bacteria (WW- well watered, WS-water stressed,WW+PF-well watered okra inoculate with *Pseudomonas fluorescens*, WS+PF-water stressed okra inoculated with *Pseudomonas fluorescens*, WW+BS- well watered okra inoculated with *Bacillus subtilis*, WS+BS- water stressed okra inoculated with *Bacillus subtilis*, WW+BC- well watered okra inoculated with *Burkholderia cepacia*, WS+BC- water stressed okra inoculated with *Burkholderia cepacia.* 1,3,5 and 7 represent stressed days; 1R,2R and 3R represent recovery days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

		WW (±SE)	WS (±SE)	WW+PF(±SE)	WS+PF(±SE)	WW+BS(±SE)	WS+BS(±SE)	WW+BC(±SE)	WS+BC(±SE)
Fruit length (cm)		15.01±0.45^{f}	6.5±0.53^{h}	21.50±0.45^{a}	17.01±0.32^{d}	18.00±0.54^{b}	15.30±0.71^{e}	17.23±0.63^{c}	14.29±0.67^{g}
Fresh weight (g)		12.70±0.73^{e}	7.83±0.84^{h}	15.21±0.35^{a}	13.77±0.47^{d}	14.23±0.78^{b}	11.12±0.58^{f}	13.99±0.79^{c}	10.32±0.12^{g}
Fruit per plant		9.00±0.23^{g}	5.00±0.25^{h}	17.00±0.32^{a}	15.00±0.30^{b}	14.00±0.34^{d}	11.00±0.27^{e}	13.00±0.24^{c}	10.00±0.23^{f}
Carbohydrate (mg/g^{-1})		3.40±0.72^{g}	2.96±0.69^{b}	6.45±0.33^{a}	6.03±0.64^{b}	4.93±0.68^{c}	4.52±0.35^{d}	4.42±0.43^{e}	4.12±0.29^{f}
Lipid (mg/g^{-1})		0.30±0.01^{f}	0.20±0.06^{g}	0.60±0.05^{a}	0.55±0.01^{b}	0.50±0.05^{c}	0.43±0.03^{d}	0.50±0.01^{c}	0.37±0.22^{e}
Sugar (mg/g^{-1})		15.15±0.62^{g}	13.59±0.75^{h}	24.37±0.42^{a}	23.19±0.34^{b}	19.22±0.55^{c}	18.68±0.39^{e}	19.07±0.59^{d}	17.89±0.46^{f}
Amino acid (mg/g^{-1})		24.58±0.83^{g}	20.58±0.63^{h}	46.43±0.58^{a}	43.99±0.19^{b}	29.02±0.22^{c}	27.71±0.40^{e}	28.35±0.62^{d}	26.23±0.54^{f}
Protein (mg/g^{-1})		122.87±2.12^{g}	64.05±1.03^{h}	203.92±1.83^{a}	203.13±2.37^{b}	156.86±0.26^{c}	155±3.01^{d}	154.68±2.19^{e}	153.74±2.03^{f}
Minerals (mg/g^{-1})	Zn	0.55±0.001^{f}	0.45±0.002^{h}	0.67±0.03^{a}	0.62±0.01^{c}	0.65±0.08^{b}	0.58±0.07^{e}	0.61±0.07^{d}	0.053±0.05^{g}
	Ca	48.20±.0.89^{g}	35.6±0.37^{h}	79.80±0.62^{a}	74.60±0.38^{b}	65.70±0.034^{c}	59±0.75^{e}	64.8±0.12^{d}	53.60±0.27^{f}
	Fe	0.62±0.04^{g}	0.56±0.03^{h}	0.82±0.01^{a}	0.79±0.02^{b}	0.77±0.02^{c}	0.73±0.09^{e}	0.75±0.03^{d}	0.65±0.04^{f}
	Mg	27.64±0.92^{g}	20.85±0.11^{h}	39.10±0.19^{a}	35.40±0.27^{d}	36.40±0.37^{b}	31.99±0.22^{e}	35.64±0.43^{c}	29.45±0.59^{f}
	P	54.80±0.18^{f}	45.18±0.65^{h}	69±0.34^{a}	65.22±0.17^{b}	61.22±0.29^{c}	54.89±0.54^{e}	58.35±0.61^{d}	52.01±0.57^{g}
	K	218.03±2.19^{g}	202.01±1.09^{h}	299.17±2.10^{a}	295.11±1.40^{b}	264.96±3.01^{c}	258.17±2.15^{e}	262.89±2.93^{d}	251.67±3.64^{vvvvvv}

4.5.4 Discussion

Global climate change negatively affects plant yield due to the alteration in different abiotic and biotic factors. Abiotic factors are widespread all over the world and among them drought is an important factor which affects plant growth, productivity and quality of the crop (Ferrara *et al*., 2011; Rebey *et al*., 2012). Water stress activates different signaling pathways in plants which resulte significant changes in plant metabolic gene expression (Jahangir *et al*., 2009; Zhao, 2012; Clauw *et al*., 2015; Wang *et al*., 2015). As a consequence, there will be significant variation in the morphological and biochemical characters of storage organs (*i.e*., fruit or tubers), which may decrease the nutritional and health values of food crops.

4.5.4.1 Yield parameters

Drought stress during reproductive stage significantly reduces yield parameters like number of fruits per plant, fresh weight of fruit and fruit length in okra. But the active response of drought amelioration mechanisms in bacteria treated plants resulted in lesser reduction in the yield of okra. Among the bacteria strains under study, PF treated plants respond earlier and faster against drought induced damages which results in higher yield even in drought exposed plants. Former reports suggest that, PGPR improves yield in plants by improving mineral acquisition, hormone alternation and improved photosynthetic efficiency of various crop plants such as broccoli (Yildirim *et al*., 2011), cabbage (Tanwar *et al*., 2013), cucumber (Egamberdieva *et al*., 2011). However, there are lesser reports about the improvement of yield parameters by PGPR under stress condition. Similar to our report, Arvin *et al*. (2012) observed that brassica inoculated with *P. fluorescens* and *P. putida* showed better yield under drought stress. Our results were also further supported by the findings of Arkhipova *et al*. (2007) and Ullah *et al*. (2016).

The authors suggested that, PGPR mediated alteration in plant morphology and physiological metabolisms helps to keep the yield increased in plants.

4.5.4.2 Nutritional value

4.5.4.2.1 Protein

Protein is a major component of crops, around 10–30% of the total dry matter consists of protein and it was suggested that, both genetic and environmental factors influence the protein content of fruit (Wang and Frei, 2011). Various abiotic stresses like drought, heat and elevated ozone reduces photosynthesis and this in turn results in the reduction of grain quality (Black *et al*., 2000; Dupont and Altenbach, 2003; Barnabas *et al*., 2008; Booker *et al*., 2009). In addition, accelerated leaf senescence during stress for remobilization of various metabolites and minerals for stress alleviation has also resulted in the reduction in grain protein content (Triboi and Triboi-Blondel, 2002). In drought stressed untreated plants, a significant reduction was observed and this was due to the increased severity of stress. However, the bacteria treated plants keep up the protein content under water stress this might be due to the proper induction of mitigation methods in these plants. In addition, *P. fluorescens* treated plants were recorded higher osmolytes accumulation and antioxidant activity during drought stressed period, which increase photosynthesis under stress condition and improve the protein content of fruits. Previously, it was reported that, water stress induced later reproductive stage decrease the fruit protein content (Rotundo and Westgate, 2009; Fois *et al*., 2011). Likewise, Kirnak *et al*. (2010) reported that, water stress in soybeans reduces the protein content in seeds.

4.5.4.2.2 Total amino acids

Okra fruits were rich in essential amino acids like aspartic acid, proline, glutamic acid, arginine, leucine, alanine, lysine, serine, and phenylalanine, methionine, isoleucine, histidine, cysteine, and tyrosine (Sami *et al*., 2013; Farinde *et al*., 2007; Gemede *et al*., 2015). Drought stress significantly reduced the amino acid content in fruit, a remarkable reduction in total amino acid content was observed in untreated plants, whereas the reduction was negligible in bacteria treated plants. The higher reduction in amino acid content in untreated water stressed okra bearing fruits was may be due to the re-allocation of amino acids from storage organ to vegetative parts of okra, to cope up with stress induced damage and also various metabolic alternation that cease the activation of enzymes participated in amino acid synthesis. There are earlier reports that, PGPR improved the stress tolerance in plants by accelerating amino acid synthesis and also contribute their own amino acid to maintain the osmo-regulation and ROS detoxification in stressed plants to fulfill the stress induced requirement of amino-acid in okra and reduce higher deduction in amino acid content in fruits (Lyons *et al*., 1990).

4.5.4.2.3 Carbohydrates

Water stress during the reproductive stage directly affects various enzymes involved in carbohydrate synthesis, this results in lower accumulation of carbohydrate in drought stressed plants (Saeedipour and Moradi, 2011). Bacteria treatment significantly improved the carbohydrate content in okra, whereas, drought exposure resulted in a reduction in fruit carbohydrate content more in untreated okra compared to bacteria treated plant. This revealed that, bacteria treatment effectively managed the drought induced enzyme changes in fruits. PF treatment was found more effective in okra when compared to other bacteria treated. In agreement with our results,

similar results were observed in barley (Thitisaksakul *et al*., 2012) and corn (Ali *et al*., 2013). Earlier reports suggested that, drought stress interrupt ADP-glucose pyrophosphorylase (AGPase) which facilitate the reduction of starch synthesis in tuber crops like cassava and potato (Ballmer *et al*., 2012; Jiang *et al*., 2012; Vandegeer *et al*., 2013). Drought induced reduction in photosynthesis results in the reduction of starch synthesis and lead to a decline in total carbohydrate content (Yamagata *et al*., 1987; Gebbing and Schnyder, 1999).

4.5.4.2.4 Total soluble sugars

Sugar is an important component of fruits, different reports suggest that water stress resulted in a reduction in total soluble sugar content in fruits like tomato (Patanè *et al*., 2011), onion (Kumar *et al*., 2007) and beet root (Stagnari *et al*., 2014). The reduction in sugar content in storage organs of plants was due to the translocation of sugar to vegetative parts of the plants to cope up with stress induced damages. Similar to the above observations, in okra reduction in total soluble sugar was observed in stressed plants. However, the bacteria treated plants exhibited only a minor reduction in sugar content especially the PF treated plants and this suggest that, bacteria induced stress alleviation mechanisms helps to maintain the sugar level in the fruits.

4.5.4.2.5 Lipid

In okra with increasing drought susceptibility, the lipid content was found to be content decreased, a higher reduction in total lipid content was observed in bacteria untreated plants. The significant reduction in poly saturated fatty acids content in drought exposed sunflower (Flagella *et al*., 2010), groundnut (Dwivedi *et al*., 1996) and sage (Bettaieb *et al*., 2009) was reported. Different studies suggest that, the reduction in fatty acid composition was due to the decline in enzyme activity involved in lipid

synthesis and conversion (Bouchereau *et al*., 1996; Flagella *et al*., 2010; Laribi *et al*., 2009; Bettaieb *et al*., 2010). Interruption in transfer of fatty acids from the plastids to the cytosol was also affected by environmental stresses (Flagella *et al*., 2010). Apart from these observations, Ahmad *et al*. (2014) opinion that, drought imposed production of reactive oxygen species (ROS) cause oxidation of lipids which play a major role of reduction in lipid quantity. So the lesser reduction in lipid content in bacteria treated plants, particularly in PF treated plants was may be due to the higher antioxidant enzyme activity during drought exposure.

4.5.4.2.6 Mineral elements

Mineral content in crops are related to genotypes, environmental conditions, soil properties and maturity of crops (Martínez-Ballesta *et al*., 2011). Water plays a major role in mineral assimilation in plants hence, water shortage directly interrupt mineral uptake in plants through the decrease in transpiration rates and weakens the active transport and membrane permeability (Misle *et al*., 2014). The increase in mineral (Zn, Ca, Fe, Mg, P and K) accumulation in bacteria treated plants even under the stress condition was due to the alternation in root morphology and the activity of bacteria which solubilize the minerals in the soil. According to Martínez-Ballesta *et al*. (2011) soil K^+ absorption was severely affected by water stress and resulting in a reduction in crop quality. Previous studies in watermelon exposed to water stress suggested that, K^+ and Mg^{2+} contents decline dramatically (Rouphael *et al*., 2008). Moreover, Oktem (2008), opined that, severe drought significantly reduced the intake of Fe, Zn and Cu from the soil, resulting in a reduction in the quantity of these minerals in corn grains (Oktem, 2008).

SUMMARY AND CONCLUSION

The present study is an attempt to enhance the drought tolerance potential of okra using three phosphate solublizing bacteria (*P. fluorescens*, *B. subtilis* and *B. cepacia*). For experimentation Okra seeds were pre-treated with various concentrations (cfu) of bacteria and standardized the concentration as 10^{-7}cfu/ml based on chlorophyll stability index and leaf moisture content analysis. The plants showed severe wilting symptoms on the seventh day of drought exposure and beyond 7 days of drought exposure, untreated plants cannot recover. After the stress exposure, the plants were re-watered and various morphological, physiological, biochemical and yield parameters were analyzed during this period (i.e. stressed and recovery). The major conclusions are summarized below.

a) The results pertaining to soil physical characters revealed that the bacteria inoculation modified the soil physical properties like soil moisture content and soil pH which facilitate to improve plants drought tolerance potential. Here higher water retention capacity and low pH were recorded in bacteria inoculated okra growing soil during drought stress. In the present study PF treated plants showed higher SMC and lower soil pH followed by BS and BC treated plants. Lower SMC and higher soil pH were noticed in untreated plants growing soil. Higher SMC during drought ensures the water availability during water stress whereas the reduction in soil pH confirms the nutrient accessibility to plants.

b) The inoculation of bacteria in okra seeds also significantly improved various growth parameters like leaf area, shoot length, root length and lateral root number. Among the bacteria treated plants, PF treated okra

recorded better improvement in morphological parameters. Followed by this, plants of BS and BC treatment also improved the morphological parameters. The drought induced reduction in plant growth parameters was alleviated by modifying metabolic processes, and the additional energy required for this processes might be resulted in growth retardation. However bacteria mediated acceleration in plant defense system against drought stress corroborate stress tolerance without much alteration in plant growth.

c) The imbalance in chlorophyll and carotenoid content i.e., stress induced increase in caroteinoid content revealed the higher photodamage in plants. In our observation higher caroteinoid content was noticed in untreated plants which untreated plants was more susceptible to drought stress compare to bacteria treated plants. The reduction in photosynthetic yield was higher in untreated plants might be due to the higher reduction in photosynthetic pigments (i.e., chlorophyll) and increased damage to photosynthetic proteins. Drought interrupts the electron transport in chloroplast and resulted in higher ROS production and damage to reaction centers consecutively recorded as reduction in PSI and II activity. However, bacteria treated plants lower reduction in plant water status (i.e., RWC) and higher antioxidant system helps to reduce drought induced damage in the reaction center. PF performed better than other two bacteria.

d) The chlorophyll *a* fluorescence parameters like Fv/Fo, PI_{ABS}, Fm, $\varphi_{Eo,}$ primary photochemistry and energy flux model of PSII were analysed. Untreated plants subjected to water stress recorded higher reduction as compared to bacteria treated plants. The higher reduction in Fv/Fo in drought exposed okra indicated damage of the water-splitting complex on the donor side of PSII due to drought induced reduction in cellular

water status and ROS generation. Increase in Fm value resulted in the lower efficiency photosystems to convert light energy into chemical energy. Bacteria inoculation in okra plants alleviates the donor side and/or acceptor side inhibition of PS II. Water stress initiated ROS production and resulted in damage to photosystem proteins which lead to reduction in structural functional index. As a consequence of reduction in SFI, primary photochemistry and quantum electron yield also decrease. Reduction in Fv/Fo, Fm, φ_{Eo} and primary photochemistry of PSII parameters resulted plant vitality decrease (PI_{ABS}). While the stress induced decrease in Fv/Fo, PI_{ABS}, Fm, φ_{Eo} and primary photochemistry of PSII was lesser in bacteria treated plants as compared to untreated plants.

e) The energy flux leaf model of Chl *a* fluorescence related parameters suggested that drought stress cause inactivation of RCs in okra leaves. In bacteria treated drought encountered plants, the stress-induced reduction in energy fluxes of absorption and trapping per leaf cross-section [(ABS/CS) and (TRo/CS)], were comparatively lesser and it was higher in the case of untreated plants. Among bacteria treated plants, PF treated plants recorded lesser reduction in ABS/CS and TRo/CS than BS and BC treated plants, this point out that PF treatment is more efficient to tolerate drought. It was also observed that bacteria treated plants reduced the drought assisted reduction of electron transport ETo/CS, this might be due to the minimal stress effect on PSII activity. Lesser reduction in ETo/CS in bacteria treated plants also leads to decrease in dissipation energy by these plants. Dissipation energy of PSII per cross section was increased in untreated plants and it is positively correlated with a reduction in trapping energy and chlorophyll degradation in these plants under drought stress.

f) In okra, the absorption energy flux and trapping energy flux per reaction center (ABS/RC and TRo/RC) decreased during severe stress conditions in untreated plants (i.e., 5^{th} and 7^{th} day of stress) and it might be due to the variation in antenna size. The dissipation per reaction centre (DIo/RC) was increased in untreated okra subjected to drought stress and was due to the incapability of RCs in trapping the light energy during stress. This also led to a reduction in electron flow per RC (ETo/RC). However, the reduction was lesser in bacteria treated plants compared to untreated plants, PF treated okra recorded better performance among bacteria treated plants. This revealed the fact that bacteria treatment significantly alleviated drought induced damage in reaction center.

g) Higher plant water status during drought stress improved plant stress tolerance. Here bacteria treated plants recorded in increased RWC during drought stress. This is due to the lowering in osmotic potential by increased accumulation of osmolytes like soluble sugar, free amino acids and proline. This facilitates the absorption of water from the dry soil. Higher osmolytes content, RWC and lower leaf osimotic potential was recorded by PF treated plants, followed by BS and BC treated plants. From this, it is revealed that, the bacteria treated plants have the ability to keep cellular water content under drought stressed. Higher reduction in protein content in untreated plants directly correlated with higher ROS production. However in bacteria treated plants increased activity of ROS system protect the protein from oxidative stress.

h) Drought imparted reduction in cellular water content enhanced ROS production and membrane damage was noticed in untreated plants during the drought exposure period which positively correlated with higher reduction in plant water status, increased photo-inhibition and

lower enhancement in enzymatic and non-enzymatic antioxidant system in these plants. Early up-regulation of different enzymatic (SOD, CAT, APX, GOPX, MDHAR, DHAR and GR) and non enzymatic antioxidants (AsA, GSH and phenolics) activities in bacteria treated plants triggered reduction in ROS production.

i) Rapid response of plant defense systems in all bacteria treated plants accelerated recovery in these plants. Among bacteria treated plants, PF treated drought stress encountered plants recorded faster and efficient defense response. So PF treatment was considered as a better method for enhancing the drought stress tolerance in okra. The faster recovery during re-watered period was also monitored in PF treated plants. Moreover, better yield in okra was also recorded in PF treated plants with improved nutritional quality when compared to BS and BC treated plants and untreated plants. The present venture constituts an eco-friendly approach in enhancing drought stress tolereance of okra plants and it is an easily practiciable biopriming method in the field.

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LIST OF PUBLICATIONS AND PRESENTATIONS

Research papers in journals

I. **Pravisya, P.** and Jayaram, K. M. (2015). Priming of *Abelmoschus esculentus* (L.) Moench (okra) seeds with liquid phosphobacterium: An approach to mitigate drought stress. *Tropical Plant Research,* 2: 276-281.

II. **Pravisya, P.**, Jayaram, K. M. and Yusuf A. (2018). Biotic Priming with *Pseudomonas fluorescens* induce drought stress tolerance in *Abelmoschus esculentus* (L.) Moench (Okra). *Physiology and Molecular Biology of Plants,* (IF: 1.15) (Doi.org/10.1007/s12298-018-0621-5).

III. **Pravisya, P.**, Jayaram, K. M. and Yusuf A. (2018). Pre treatment with *Bacillus subtilis* mitigates drought induced photosynthesis reduction in *Abelmoschus esculentus* (L.) Moench (okra) by modulating antioxidant system and photochemical activity. *Scientia Horticulturae,* (under review).

Papers in seminars/conference proceedings

I. **Pravisya P**., Shintu, P.V., Vidya, P. and K.M. Jayaram. (2014). Response of phosphate solubilizing bacteria on drought in okra (*Abelmoschus esculentus* (L.) Moench) plants. **Proceedings of 26th Kerala Science Congress,** Pookode, Wayanad: 28-31 January.

II. **Pravisya, P.,** Jayaram, K. M. and Yusuf A. (2016). Response of *Bacillus subtilis* treatment in okra (*Abelmoschus esculentus* (L.)

Monech) under water stress. **Proceedings of 28th Kerala Science Congress**, University of Calicut, Malappuram: 28-30 January.

III. **Pravisya, P**., Jayaram, K. M. and Yusuf A. (2017). Impact of *Bacillus subtilis* (BS) treatment on the photochemical and antioxidant mechanism in okra exposed to drought stress and recovery. **8*th* International Conference on Photosynthesis and Hydrogen Energy Research for sustainability** held at Hyderabad University, India, October 30-November 03.

IV. **Pravisya, P.,** Jayaram, K. M. and Yusuf A. (2017). *Pseudomonas fluorescens* (PF) treatment induces drought tolerance potential of *Abelmoschus esculentus* (L.) Moench (okra). **National Seminar on Modern Trends in Conservation, Utilization and Improvement of Plant Genetic Resources,** 23- 24 November, organized by Gregor Mendel Foundation University of Calicut with Department of Botany, University of Kerala.

V. **Pravisya, P.,** Jayaram, K. M. and Yusuf A. (2018). Biopriming alleviate drought stress induced reduction in photochemistry of *Abelmoschus esculentus* (L.) Moench (okra). **International conference on recent scenario in plant science research-climate change and its associated variations**, held at Annamalai University, India, 23-25 March.

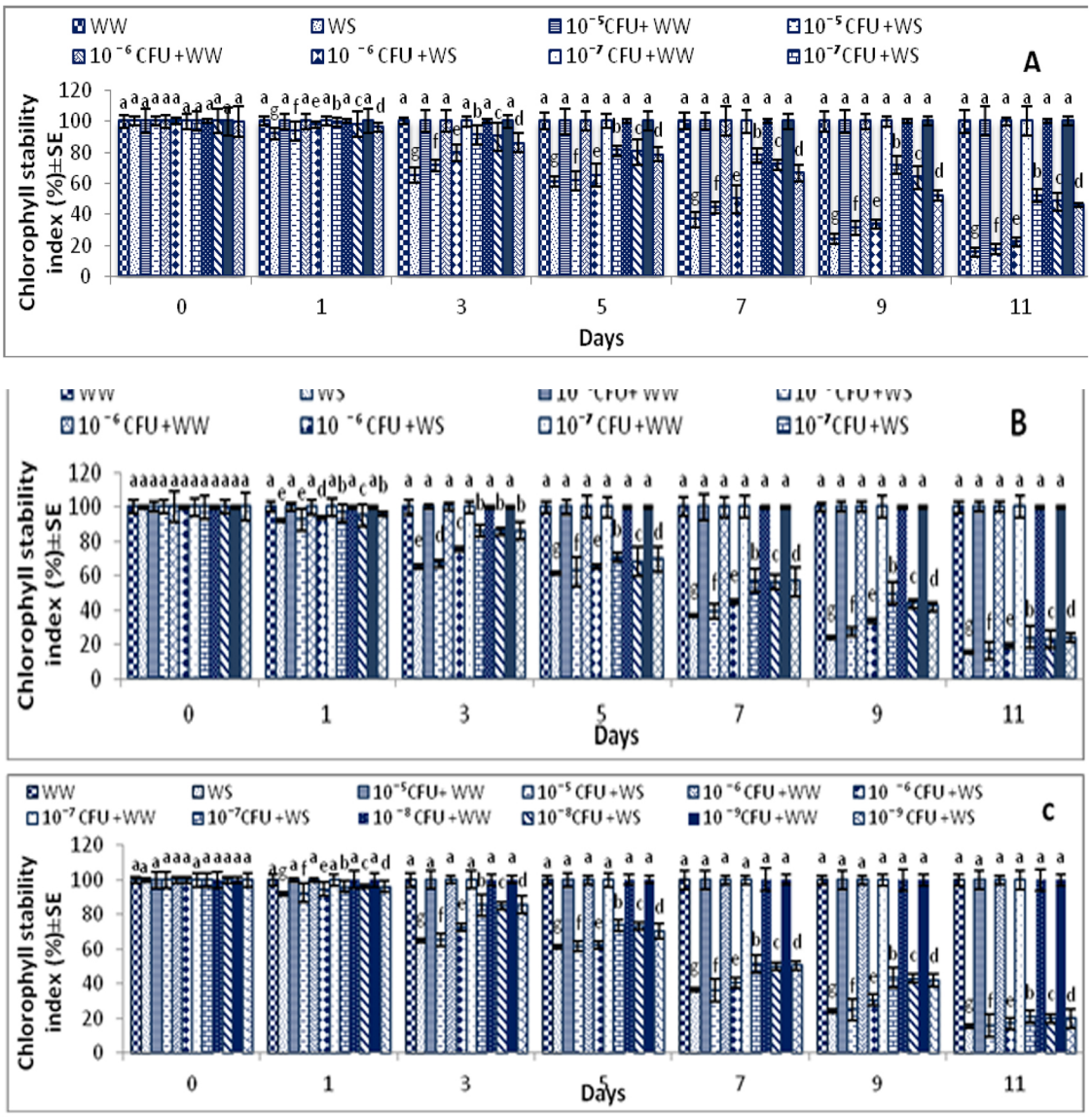

Fig. 2. Effect of different bacteria [*P. fluorescens* (A) *B. subtilis* (B), *B. cepacia* (C)] inoculation concentration (10^{-5},10^{-6},10^{-7}, 10^{-8} and 10^{-9} cfu/ml) in chlorophyll stability index of okra. (WW- well irrigated, WS-water stressed, 1,3,5,7,9 and 11 represent stressed days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

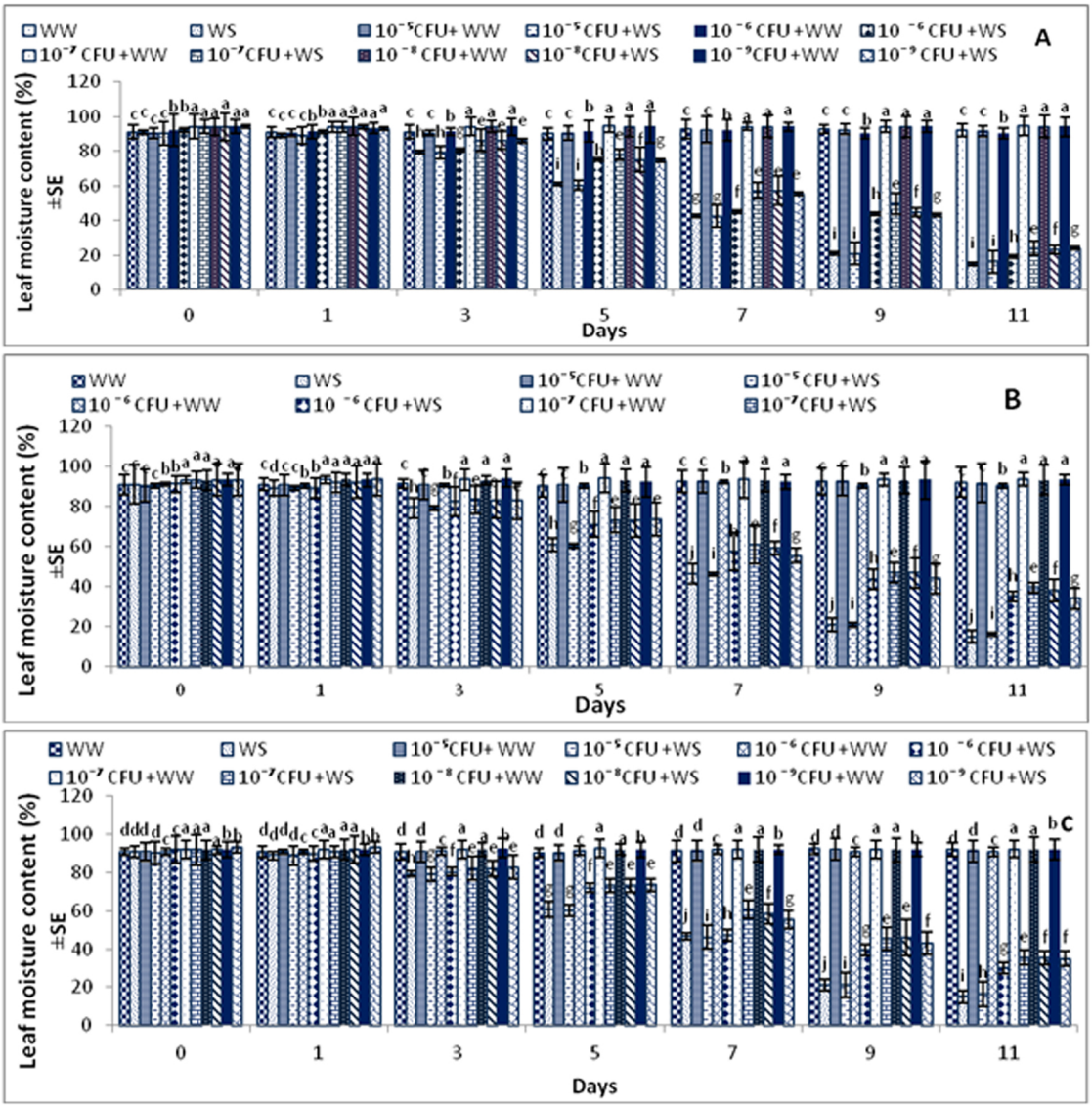

Fig.1. Effect of different bacteria [*Pseudomonas fluorescens* (A) *Bacillus subtilis* (B), *Burkholderia cepacia* (C)] inoculation concentration (10^{-5},10^{-6},10^{-7}, 10^{-8} and 10^{-9} cfu/ml) in leaf moisture content of okra. (WW- well irrigated, WS-water stressed, 1,3,5,7,9 and 11 represent stressed days). Each letter indicate statistical difference $p \leq 0.05$ value followed by the same letter in a bar indicate no significant difference between the treatment following one way ANOVA.

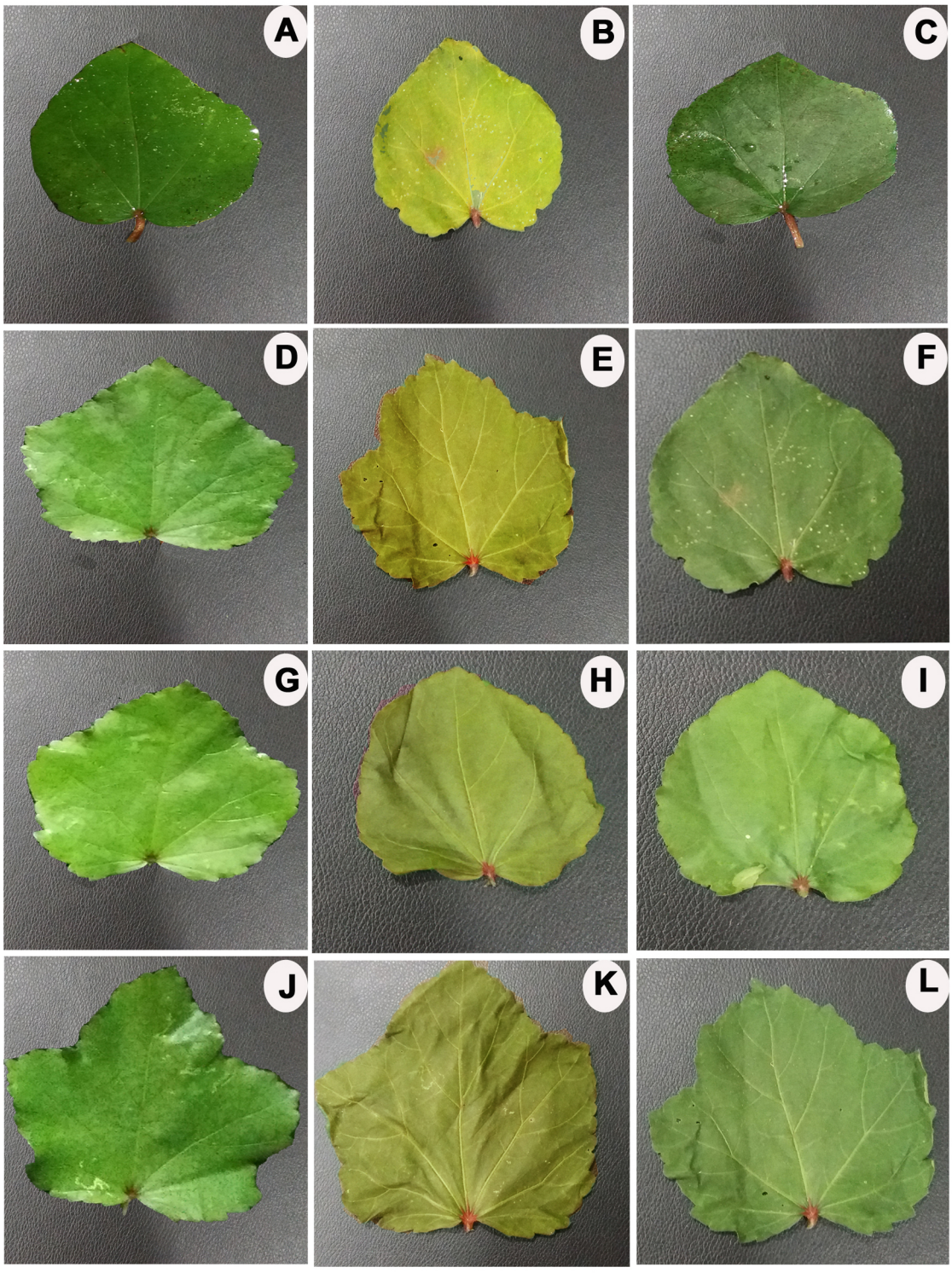

Fig 1.A: Variations in leaf area of *A. esculentus* during watered (A, D, G, J) drought (B, E, H, K) and recovery (C, F, I, L) period A) untreated well watered B) untreated water stressed C) untreated rcovered D) well watered *B. subtilis* treated E) water stressed *B. subtilis* treated F) *B. subtilis* treated recovered G) well watered *B. cepacia* treated H) water stressed *B. cepacia* treated I) *B. cepacia* treated recovered J) well watered *P. fluorescens* treated K) water stressed *P. fluorescens* treated L) *P. fluorescens* treated recovered.

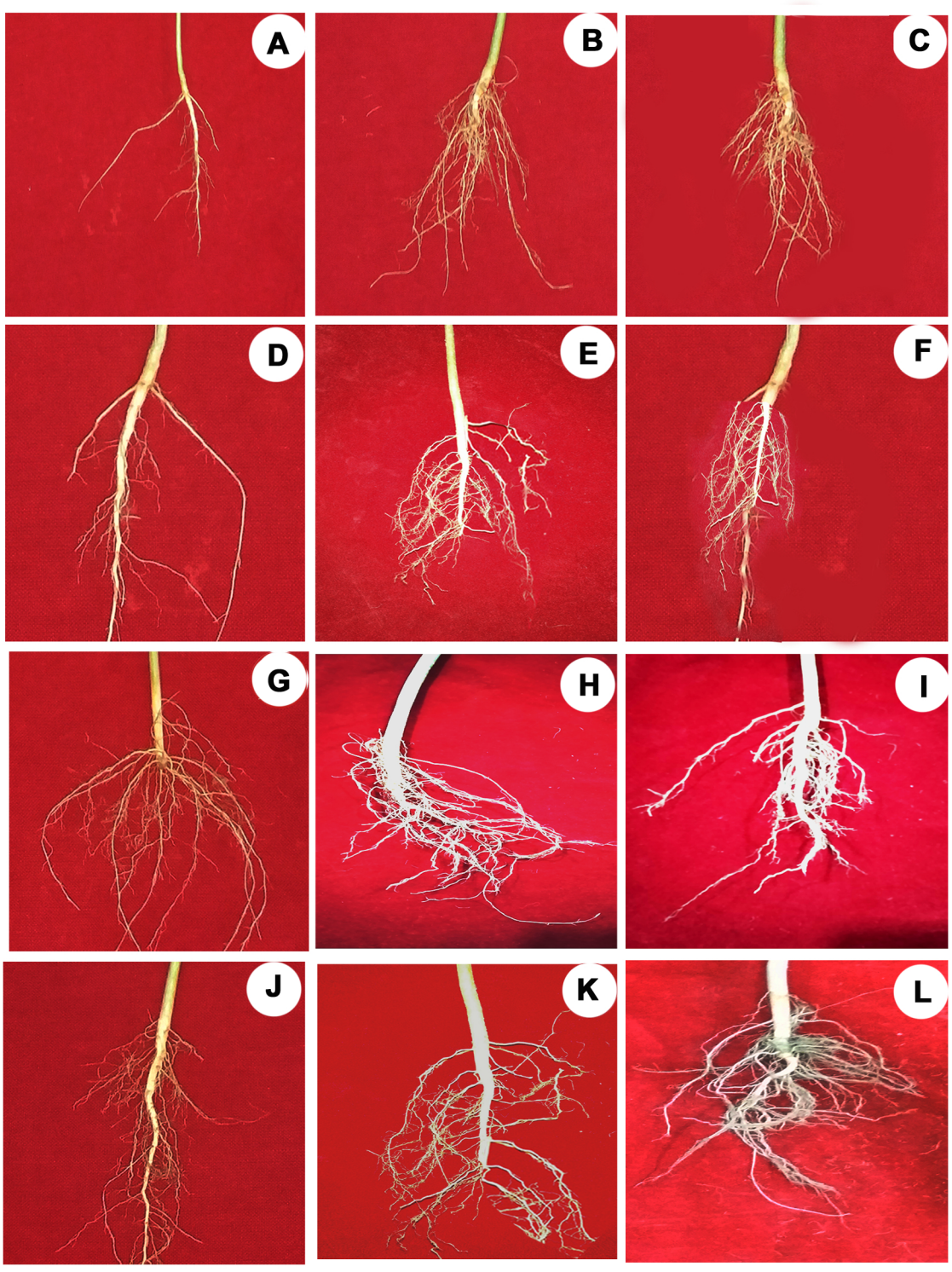

Fig 1.C: Variations in root morphology of *A. esculentus* during watered (A,D,G,J) drought (B, E,H,K) and recovery (C,F,I,L) period A) untreated well watered B) untreated water stressed C) untreated recovered D) well watered *B. cepacia* treated E) water stressed *B. cepacia* treated F) *B.cepacia* treated recovered G) well watered *B. subtilis* treated H) water stressed *B. subtilis* treated I) *B. subtilis* treated recovered J) well watered *P. fluorescens* treated K) water stressed *P. fluorescens* treated L) *P. fluorescens* treated recovered.

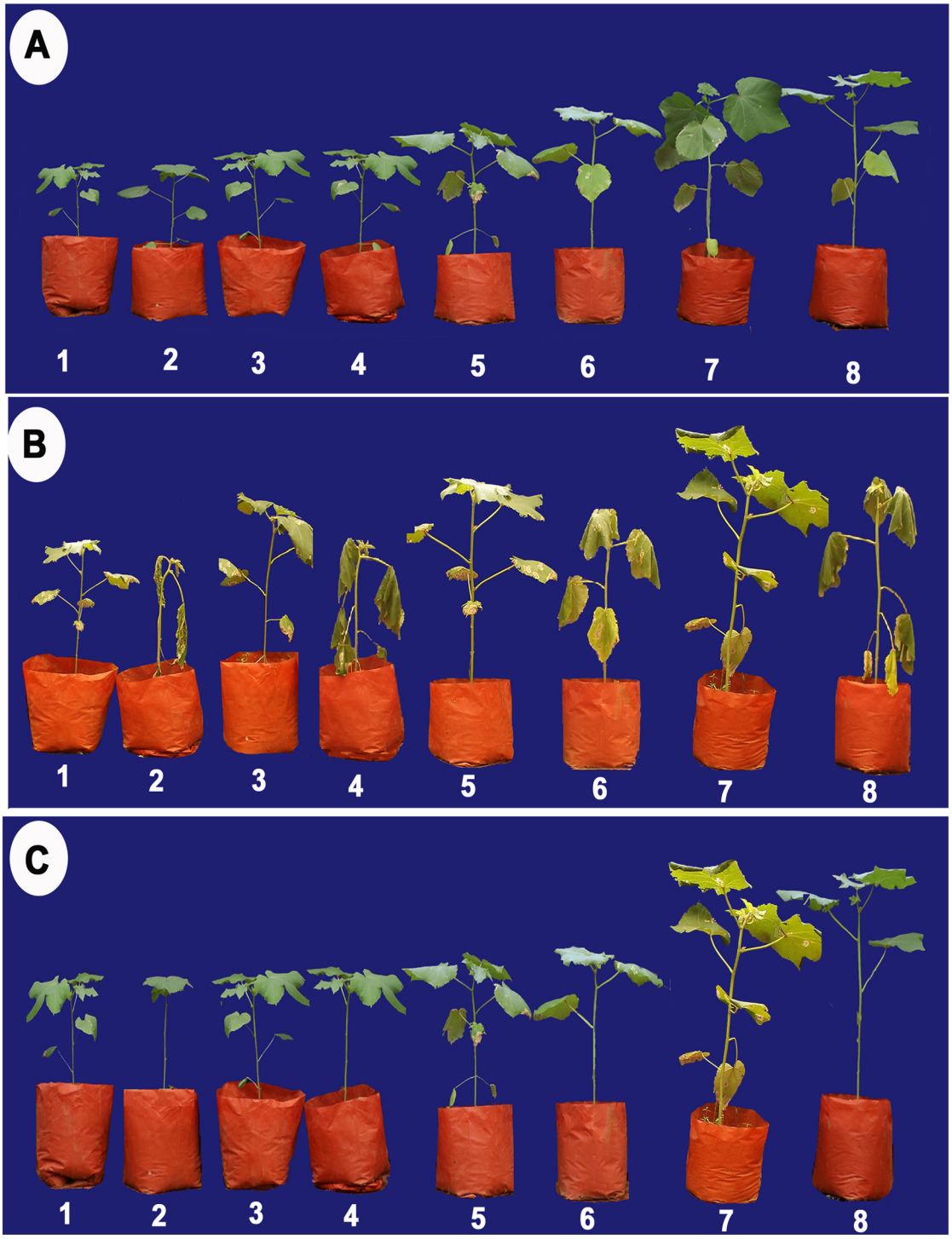

Fig 1.B: Growth of *A. esculentus* treated with different bacteria exposed to drought and recovery period A) regularly watered B) drought stressed C) recoverd 1) untreated well watered 2) untreated water stressed 3) well watered *B. subtilis* treated 4) water stressed *B. subtilis* treated 5) well watered *B. cepacia* treated 6) water stressed *B. cepacia* treated 7) well watered *P. fluorescens* treated 8) water stressed *P. fluorescens* treated.

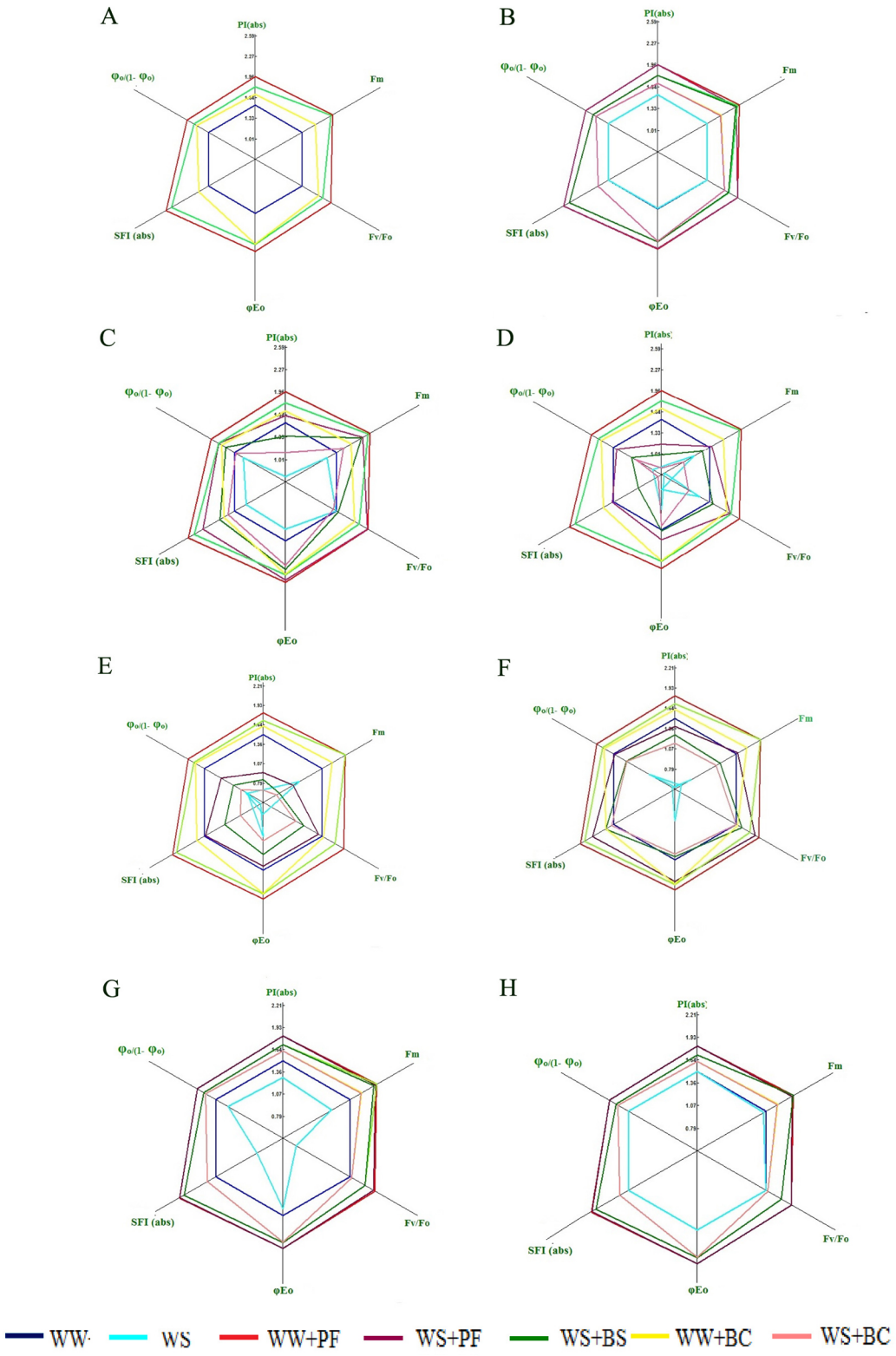

Fig. 2.5: Radar plot of selected Chl *a* fluoresence parameters recorded in *A. esculentus* without bacteria treatment and with different bacteria treatment [*B. cepacia* (BC) *B. subtilis* (BS) and *P. fluorescens* (PF)] after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd of water stress), D (5th of water stress), E (7th of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering).

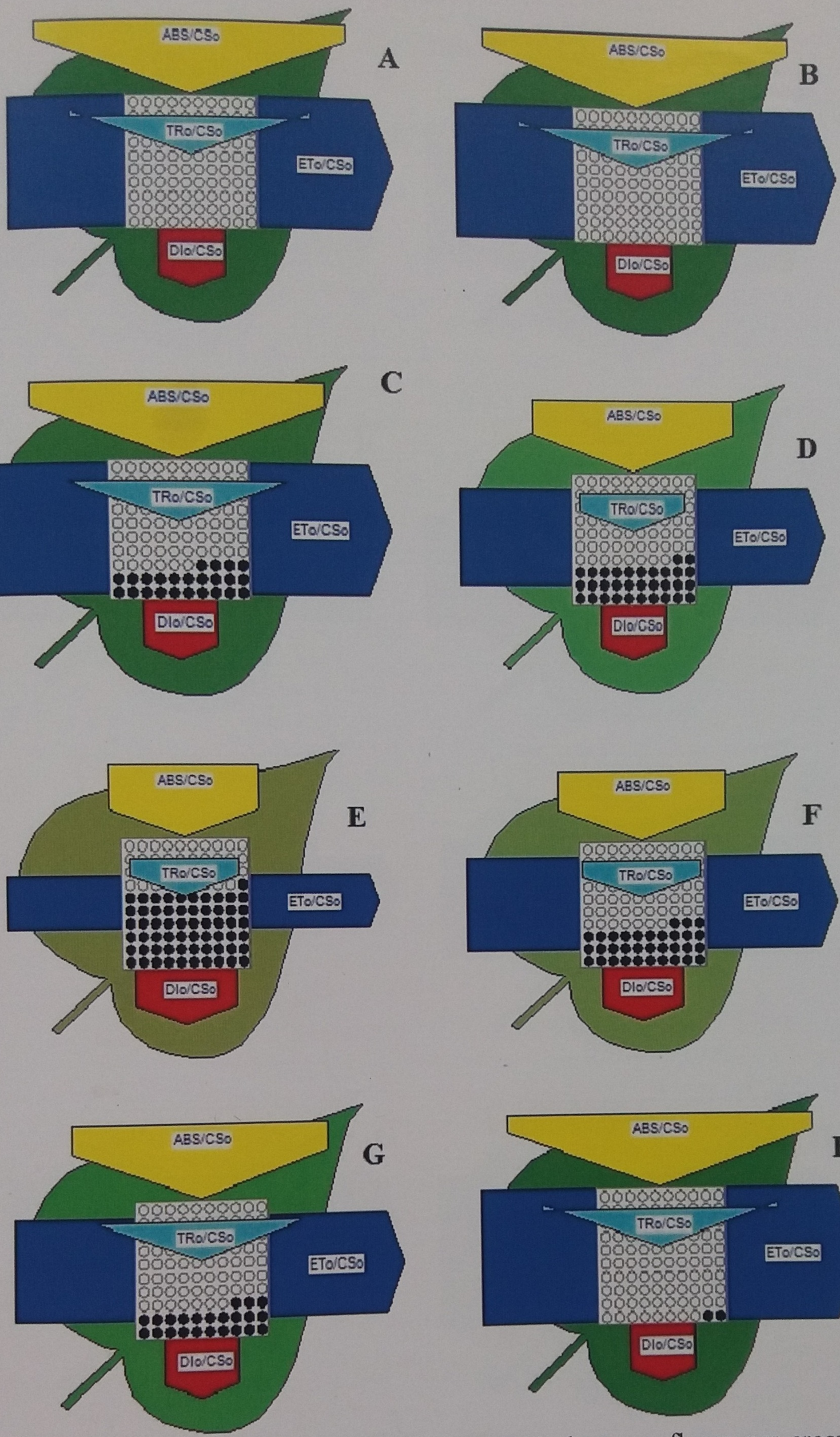

Fig. 2.6: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CSo) in untreated *A. esculentus* after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd day of water stress), D (5th day of water stress), E (7th day of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering). Different parameters represent in different colours ABS/CSo (Yellow), TRo/CSo (Light blue), ETo/CSo (Blue) and DI/CSo (Red). The value of each parameters can be seen in relative changes in width of each arrow. White circle represent active reaction centers and dark circle represent inactive reaction centers.

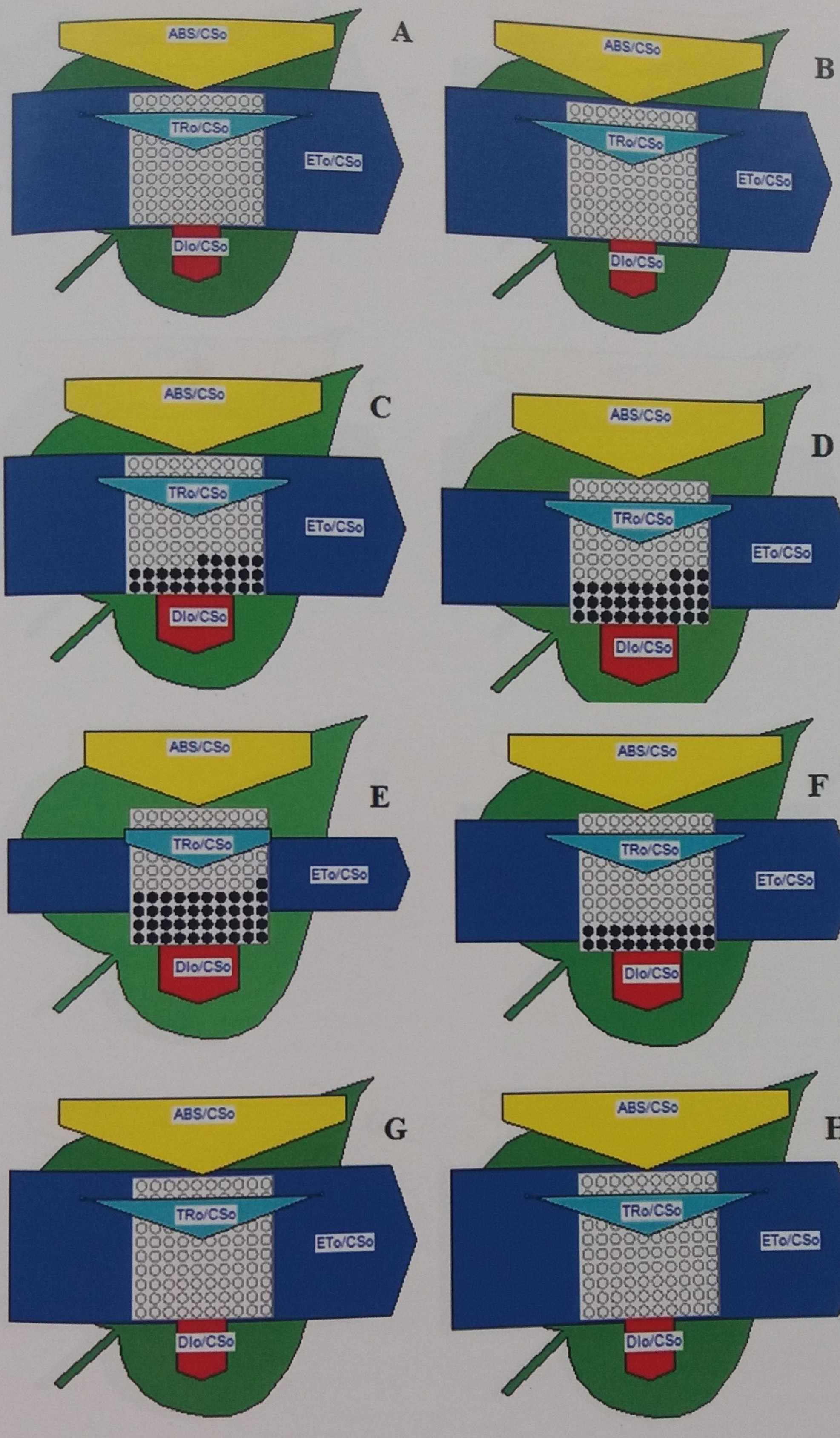

Fig. 2.7: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CSo) in *A. esculentus* treated with *B. cepacia* treatment after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd of water stress), D (5th of water stress), E (7th of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering). Different parameters represent in different colours ABS/CSo (Yellow), TRo/CSo (Light blue), ETo/CSo (Blue) and DI/CSo (Red). The value of each parameters can be seen in relative changes in width of each arrow. White circle represent active reaction centers and dark circle represent inactive reaction centers.

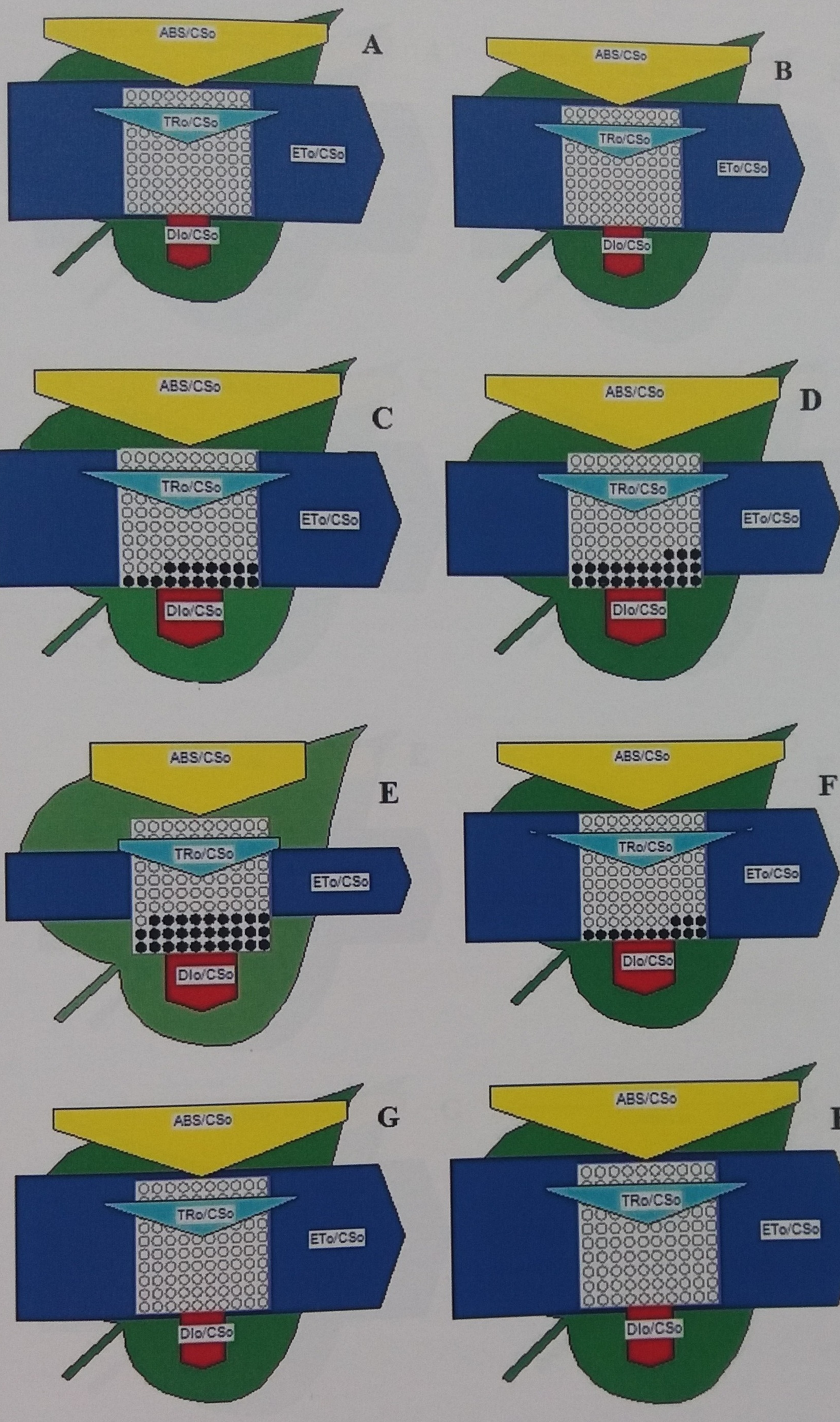

Fig. 2.8: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CSo) in *A. esculentus* treated with *B. subtilis* treatment after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd day of water stress), D (5th day of water stress), E (7th day of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering). Different parameters represent in different colours ABS/CSo (Yellow), TRo/CSo (Light blue), ETo/CSo (Blue) and DI/CSo (Red). The value of each parameters can be seen in relative changes in width of each arrow. White circle represent active reaction centers and dark circle represent inactive reaction centers.

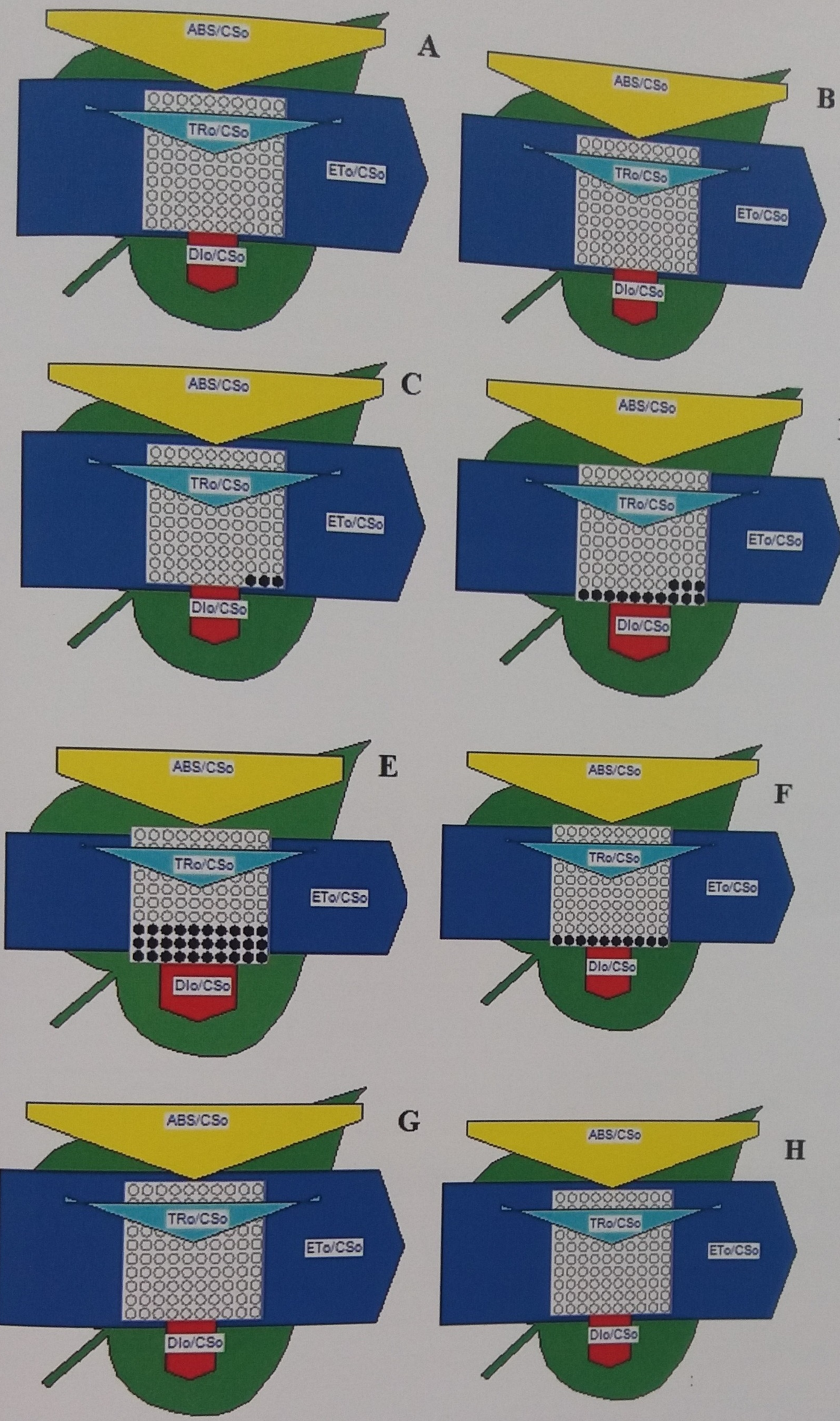

Fig. 2.9: Energy pipeline leaf model of phenomenological energy fluxes per cross section (CSo) in *A. esculentus* treated with *P. fluorescens* treatment after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd day of water stress), D (5th day of water stress), E (7th day of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering). Different parameters represent in different colours ABS/CSo (Yellow), TRo/CSo (Light blue), ETo/CSo (Blue) and DI/CSo (Red). The value of each parameters can be seen in relative changes in width of each arrow. White circle represent active reaction centers and dark circle represent inactive reaction centers.

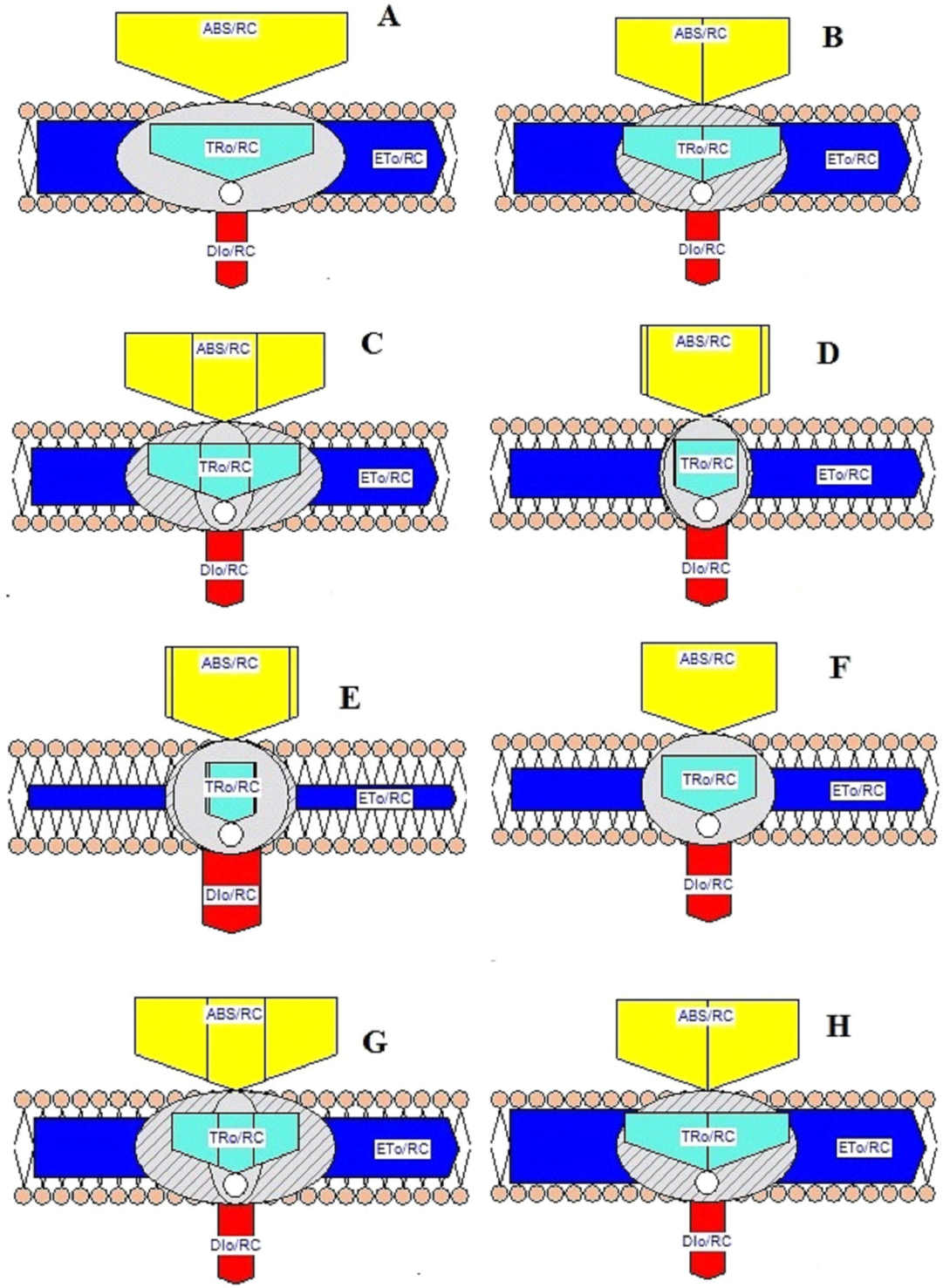

Fig. 2.10: Specific membrane model of energy fluxes per reaction center (RC) untreated *A. esculentus* after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd day of water stress), D (5th day of water stress), E (7th day of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering). Different parameters represent in different colours ABS/CSo (Yellow), TRo/CSo (Light blue), ETo/CSo (Blue) and DI/CSo (Red). The value of each parameters can be seen in relative changes in width of each arrow.

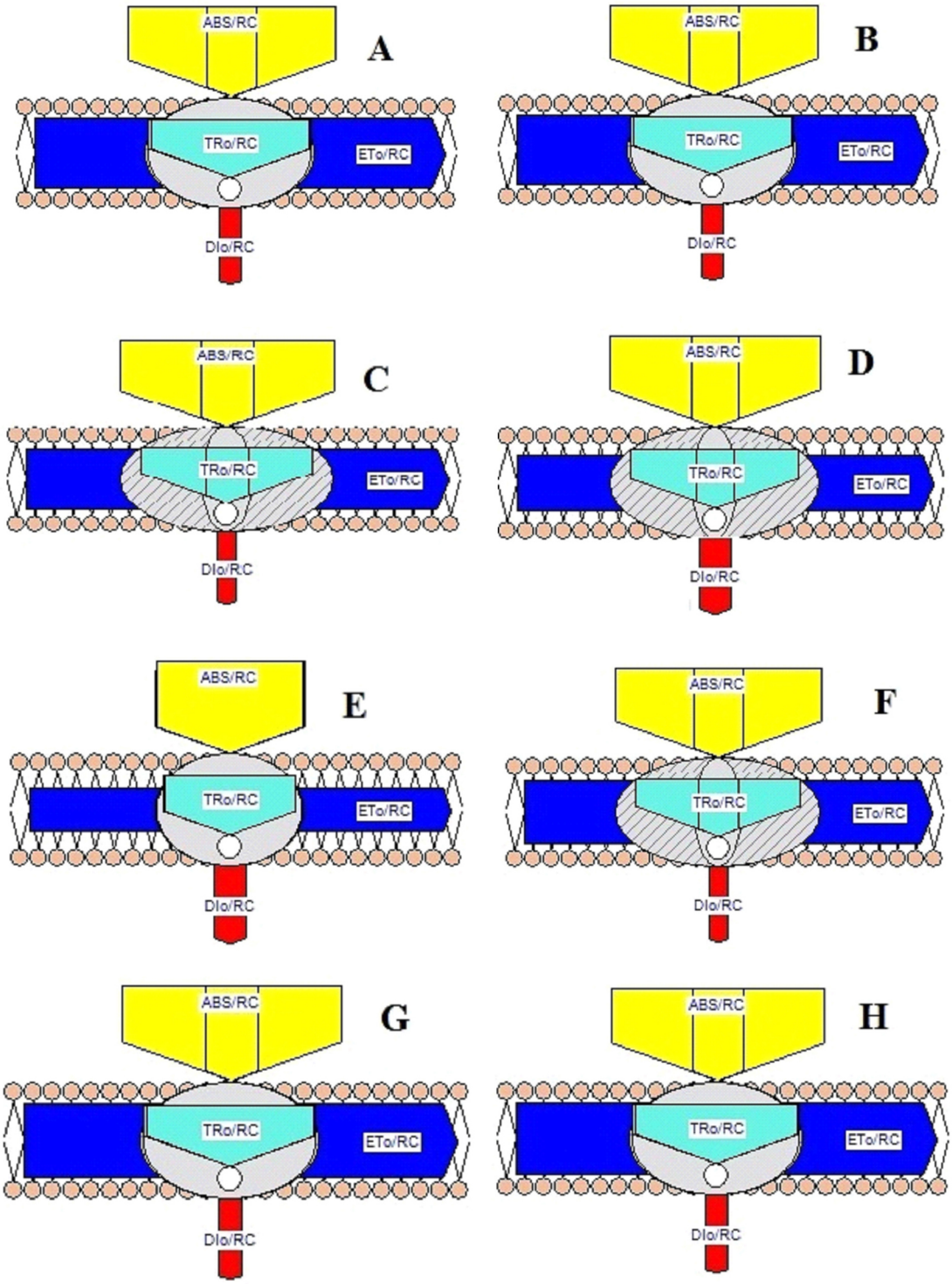

Fig. 2.11: Specific membrane model of energy fluxes per reaction center (RC) in *A. esculentus* treated with *B. cepacia* treatment after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd day of water stress), D (5th day of water stress), E (7th day of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering). Different parameters represent in different colours ABS/CSo (Yellow), TRo/CSo (Light blue), ETo/CSo (Blue) and DI/CSo (Red). The value of each parameters can be seen in relative changes in width of each arrow.

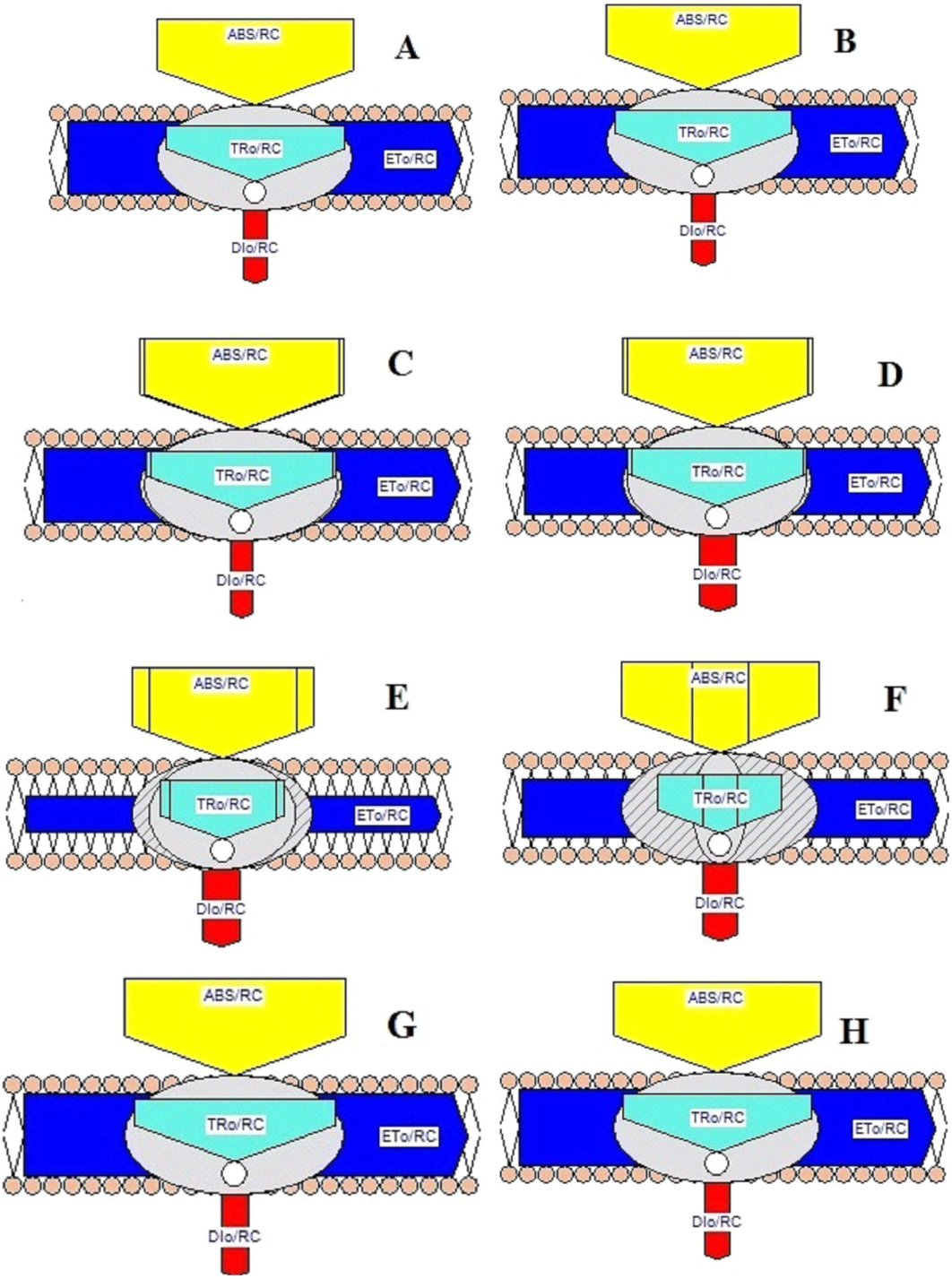

Fig. 2.12: Specific membrane model of energy fluxes per reaction center (RC) in *A. esculentus* treated with *B. subtilis* treatment after exposure to drought stress and recovery for different time intervals [A (well watered condition), B (1st day of water stress), C (3rd day of water stress), D (5th day of water stress), E (7th day of water stress), F (1st day of re-watering), G (2nd day of re-watering) and H (3rd day of re-watering). Different parameters represent in different colours ABS/CSo (Yellow), TRo/CSo (Light blue), ETo/CSo (Blue) and DI/CSo (Red). The value of each parameters can be seen in relative changes in width of each arrow.