

**PHYSIO-CHEMICAL STUDIES ON EFFECT OF ENDOPHYTIC
BACTERIA IN COMBATING INDUCED SALINITY STRESS IN
SUGARCANE.**

by

Mr. PANKAJ SURESH CHOUGULE
(Reg. No. 2015/61)

**A Thesis submitted to the
MAHATMA PHULE KRISHI VIDYAPEETH,
RAHURI - 413 722, DIST.AHMEDNAGAR,
MAHARASHTRA, INDIA.**

In partial fulfilment of the requirements for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

BIOCHEMISTRY



**DEPARTMENT OF BIOCHEMISTRY
POST GRADUATE INSTITUTE
MAHATMA PHULE KRISHI VIDYAPEETH,
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2023**

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2023

CANDIDATE'S DECLARATION

I hereby declare that this thesis or part
there of has not been submitted
by me or other person to any
other University or Institute
for a Degree or
Diploma

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Date: / /2023

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This is to certify that the thesis entitled, “**PHYSIO-CHEMICAL STUDIES ON EFFECT OF ENDOPHYTIC BACTERIA IN COMBATING INDUCED SALINITY STRESS IN SUGARCANE**”, submitted to the Faculty of Agriculture, Mahatma Phule Krishi Vidyapeeth, Rahuri, Dist. Ahmednagar (Maharashtra) in partial fulfilment of the requirement for the award of the degree of **DOCTOR OF PHILOSOPHY (AGRICULTURE)** in **BIOCHEMISTRY**, embodies the results of a piece of bonafide research work carried out by **MR. CHOUGULE PANKAJ SURESH** under my guidance and supervision and that no part of the thesis has been submitted for any other degree or diploma.

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

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Place: M.P.K.V., Rahuri

Date: / /2023

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations	Description
%	Per cent
cm	Centimeter
mM	Millimeter
M	Meter
dSm ⁻¹	deci Siemens per meter
DTPA	Diethylene triamine penta acetic acid
AB-DTPA	Ammonium bicarbonate- Diethylene triamine penta acetic acid
mg kg ⁻¹	milligram per kilogram
kg ha ⁻¹	Kilogram per hectare
mg m ⁻³	mega gram per cubic meter
meL ⁻¹	Milli equivalent per litre
<i>et al.</i>	And other (et alli)
etc.	Et cetera
i.e.	That is
Kg	Kilogram
kg ha ⁻¹	kilogram per hectare
g kg ⁻¹	Gram per kilogram
Ha	Hectare
Viz	Videlicet (namely)
ppm	Parts per million
pH	Puissance de hydrogen
EC	Electrical conductivity
OC	Organic carbon
CaCO ₃	Calcium carbonate
N	Nitrogen
NaCl	Sodium Chloride
SAR	Sodium Adsorption Ratio
ESP	Exchangeable Sodium Percentage
P	Phosphorous
K	Potassium
Ca	Calcium
Mg	Magnesium
Fe	Iron
CAT	Catalase
APX	Ascorbate peroxidase
SOD	Superoxide dismutase
RLWC	Relative leaf water content
S.D	Standard Deviation
Min	Minimum

Max	Maximum
M	Molar
N	Normal
HgCl ₂	Mercuric chloride
Vol.	Volume
Eq.	Equivalent
EBT	Endophytic bacteria treatment
RS	Reducing sugar
NRS	Non reducing sugar
TS	Total sugar
O _c	degree celsius
DAP	Days after planting
EB	Endophytic bacteria
SS	Salt stress
CEB	Consortia endophytic bacteria
GA	<i>Gluconacetobacter</i>
HERB	<i>Herbaspirillum</i>
AAR	<i>Azoarcus</i>
NA	Nutrient agar
FW	Fresh weight
V/V	Volume/Volume
G	Gram
TBARS	Thiobarbituric acid reactive substance
Hr	Hour
APX	ascorbate peroxidase
NFM medium	Nitrogen free media
MDA	Malondialdehyde
ACC	1-aminocyclopropane-1-carboxylate
IAA	Indole-3-acetic acid
BNF	Biological Nitrogen Fixation
ROS	Reactive Oxygen Species
GB	Glycine betaine

ABSTRACT

Physio-chemical studies on effect of endophytic bacteria in combating induced salinity stress in sugarcane.

by

MR. PANKAJ SURESH CHOUGULE

A candidate for the degree

of

DOCTOR OF PHILOSOPHY (AGRICULTURE)

in

BIOCHEMISTRY

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Research Guide: Dr. A.A. Kale

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A pot culture experiment was carried on “Physio-chemical studies on effect of endophytic bacteria in combating induced salinity stress in sugarcane” to study the effect of endophytic bacteria on biochemical parameters in sugarcane grown under imposed salinity stress conditions. Two contrasting varieties viz., CoM-0265 (salt tolerant) and CoC-671 (salt susceptible) were obtained from Central Sugarcane Research Station, Padegaon and planted in pot. Salt stress was imposed by irrigating NaCl solutions of 0, 2, 4, 8 dSm⁻¹ and grown in the pot containing sodic soil grown under glasshouse conditions using completely Randomized Block Design.

The endophytic bacteria were isolated from the sugarcane nodes grown on saline soil using selective media viz., LGIP media for *Gluconacetobacter*, Rennies media for *Azospirillum*, nitrogen free malate for *Herbaspirillum* and *Azoarcus* media for *Azoarcus*. These isolated endophytes were confirmed by colour of colony grown on specific media and by characterizing biochemical tests viz., oxidase, gelatin liquefaction, urease, H₂S production and catalase. It was revealed that all tests were positive except gelatin liquefaction. All the endophytes isolated were screened for salt tolerance by growing on respective selective media containing different levels viz., 2, 4, 8 dSm⁻¹ salt concentrations. It was observed that all the endophytes tolerated upto 8 dSm⁻¹ salt stress conditions. The individual endophytic bacterial culture and consortia was prepared having CFU 1 X 10⁸ and was applied to the sugarcane setts at the time of planting and to plants at 15, 30, 45 and 60 DAP. The salt stress was imposed after 2 month after germination by applying salt solutions with interval of 8 days. The leaf samples were collected for physio-biochemical analysis after visual symptoms were observed on plants and it was approximately 3.5 months (105

days) after germination i.e. tillering stage. The physiological parameters recorded were leaf area, shoot length, root length and RLWC and biochemical parameters viz., total chlorophyll content, chlorophyll stability index, lipid peroxidation, membrane injury index, proline, glycine betaine, RS, NRS and TS were estimated. The enzymes viz., catalase, ascorbate peroxidase and superoxide dismutase were also assayed.

The current outcome focus on role of isolated endophytic bacteria and their consortia were investigated for their potential to ameliorate plant salinity stress. Analyses of various morpho-physiological parameters, osmolytes and antioxidative enzymes conducted to investigate endophytic bacteria mediated the salt stress tolerance.

The morpho-physiological parameters viz., leaf area, shoot length, root length, relative leaf water content, total chlorophyll content and chlorophyll stability index were decreased with increase in imposed salt stress levels in both the varieties CoM-0265 and CoC-671 in both endophyte treated and untreated plants. The mean values indicated that the more decrease in morpho-physiological parameters were observed in CoC-671 variety than CoM-0265 under control condition (without endophytes treated). By the application of different endophytic bacteria along with consortia it was noticed that morpho-physiological parameters were increased over the control (with endophytes treated). Among the various salt stress levels the plant grown under sodic soil has more morpho-physiological parameters than imposed salt stress levels in both the varieties treated with endophytic bacteria. Further, least decrease in morpho-physiological parameters with imposed salt stress were observed in endophytes bacteria consortia (CBC) treatment followed by *Gluconacetobacter* in salt tolerant CoM-0265 and CoC-671 variety respectively.

The biochemical parameters viz., lipid peroxidation and membrane injury index it were noticed that with increase in imposed salt stress levels in both the varieties CoM-0265 and CoC-671 in both endophyte treated and untreated plants. The higher mean values were observed in salt susceptible CoC-671 variety than salt tolerant CoM-0265 variety in endophyte untreated plant under stress condition. It was observed that endophytes and their consortia ameliorate negative effect of salt stress by decreasing lipid peroxidation and membrane injury index over the control without application of bacterial endophytes in each of salt stress treatment in both the varieties. However, more decrease was observed in control plants (without salt stress) than the salt stress plants. Among the various salt stress levels the plant grown under sodic soil showed better performance of lipid peroxidation and

membrane injury index in imposed salt stress in both the varieties under the increased salt stress levels respectively.

In case of osmolytes viz., proline, glycine betaine, reducing sugar, non-reducing sugar and total sugar the increase in salt stress levels resulted in increased in osmolytes in both the endophyte treated and untreated plants. The higher mean values were observed in salt susceptible variety CoC-671 than salt tolerant variety CoM-0265 under imposed salt stress levels. By the application of different endophytes along with their consortia it was noticed that osmolytes were further increased over the control (without application endophytes) in each salt stress treatment in both the varieties. Among the various salt stress levels the plant grown at 8 dSm^{-1} have more mean value of osmolytes than other imposed salt stress levels in both the varieties by the application of endophytic bacteria. The significant increase in osmolytes under imposed salt stress levels were observed in consortia of endophytic bacteria (CEB) treatment followed by *Gluconacetobacter* treatment in salt tolerant CoM-0265 and CoC-671 variety respectively.

Similarly, antioxidative enzymes viz., catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) the increase in salt stress levels resulted in increased in antioxidative enzymes in both the endophyte treated and untreated plants. The higher mean values were observed in salt susceptible variety CoC-671 than salt tolerant variety CoM-0265 under imposed salt stress levels. By the application of different endophytes along with their consortia it was noticed that antioxidative enzymes were further increased over the control (without application endophytes) in each salt stress treatment in both the varieties. Among the various salt stress levels the plant grown at 8 dSm^{-1} have more mean value of antioxidative enzymes than other imposed salt stress levels in both the varieties by the application of endophytic bacteria. The significant increase in antioxidative enzymes under imposed salt stress levels were observed in consortia of endophytic bacteria (CEB) treatment followed by *Gluconacetobacter* treatment in salt tolerant CoM-0265 and CoC-671 variety respectively.

Thus in conclusion it was emphasized that by the application of endophytes, the osmolytes and antioxidant activity were increased under the imposed salt stress levels. Further chlorophyll stability index was increased however lipid peroxidation was decreased in both varieties of sugarcane by the application of endophytes. It was revealed that by the application of endophytes the membrane injury index was overcome and

Abstract contd....**Mr: Pankaj S. Chougule**

RLWC, root length, shoot length, and leaf area was increased. Therefore the endophytes alleviate the salt stress in sugarcane by modulating the biomolecules and biochemical pathway.

1. INTRODUCTION

Sugarcane (*Saccharum officinarum* L., Family: Poaceae) is considered to be an important industrial, tropical and subtropical region perennial cash crop cultivated globally in more than 100 countries (Mohanraj et al., 2021) for sugar, ethanol, bagasse, or lignocellulose and has been subjected to extensive multidimensional research (Gupta et al., 2022).

At harvest of the total above ground dry biomass, sugarcane stalks account for about 75 % and the leaves and tops around 25 % respectively. Biochemical composition of sugarcane crop is 69-75 % water, 8-16% sugars, 8-16 % fiber, 0.5 – 1.0 % nitrogen, 0.3 – 0.8 % ash, 0.5-2.0 % reducing sugars, 0.2 – 0.6 % inorganic compounds (phosphates, chlorides, sulphates etc.), and 0.5 – 1.0 % (organic compounds proteins, organic acids, pigments etc). The composition of sugarcane juice at maturity have around 70-80 % water, 18-22 % sucrose, 2 -4 % glucose, 2-4 % fructose, 1.5 –4.5 % salts of inorganic acids, 1.0 – 3.0 % salts of organic acid, 0.1 - 0.5 % carboxylic acids, 0.5- 2 % of amino acids 0.5- 0.6 % proteins, 0.01-0.05 % starch (Kaavya et al., 2019).

Sugarcane is highlighted for its role as a feedstock for both sugar and ethanol production. Thus, it is estimated that 80% of sugar production worldwide comes from sugarcane (Otto et al., 2022). Globally, it is cultivated in an area of 26.52 mha with a production of 1877.10 MT of cane and 172.36 MT of sugar (Mohanraj et al., 2021).

India is the second major country in the world growing sugarcane as commercial crop and cultivated in an area of 5.042 mha with the production of 411.0 MT and productivity of 80.10 t ha⁻¹ in 2020–2021 (Anonymous, 2022). Area under sugarcane cultivation increased substantially from 1.71 Mha in 1950-51 to 5.61 Mha in 2020-21 (Anonymous, 2022). Uttar Pradesh rank first in sugarcane production followed by Maharashtra and Karnataka. In Maharashtra state Kolhapur rank first in sugarcane production (14 %) followed by Ahmednagar (13.40 %) and Pune (12.54 %) during 2020-2021 (Anonymous, 2022). In India, the major sugarcane growing states are Uttar Pradesh, Maharashtra, Karnataka, Tamilnadu, Andhra Pradesh and Gujarat. These six states together accounted for about 84.06 per cent of India's total area in 2020- 21 (Fig.1). Maharashtra is the largest producer of sugar contributes about 34 % of sugar in the country followed by Uttar Pradesh 30 % (Anonymous, 2022).

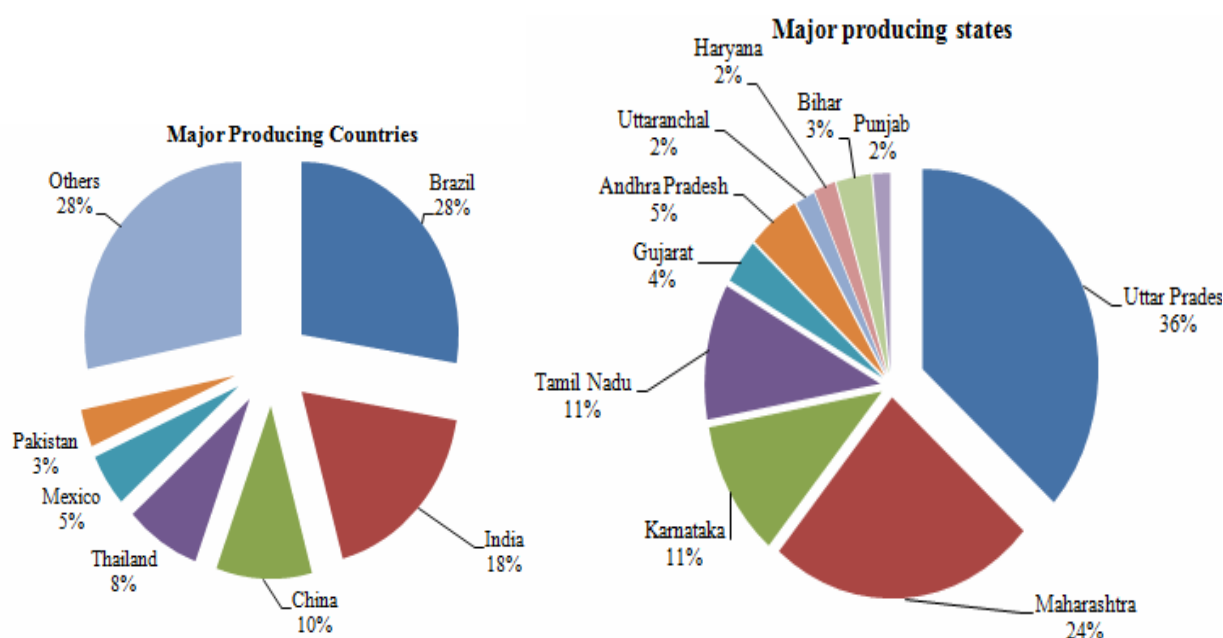


Fig .1. Major sugarcane producing countries worldwide and major producing states india
(Source: USDA & Ministry of Agriculture, GOI)

The Indian sugar industry, second largest in the world, is a key driver of rural development provides employment for nearly 4% of the population directly or indirectly (Anonymous, 2015), the industry is inherently inclusive supporting over 6 M farmers and their families, along with workers and entrepreneurs of over 550 sugar mills, apart from a host of wholesalers and distributors spread across the country (Solomon, 2016).

After textile, sugar industry is the second largest agro based industry in the India that shares two percent of gross domestic product (GDP) which is significant considering that the crop is grown only in 2.81% of the gross cropped area. Sugar industry, with an annual production capacity of more than 25 MT is one of the largest agro-based industries of India. Presently, India contributes 15% towards the global sugar production and 13% towards global sugar consumption (Solomon, 2016; Rao et al., 2021). Instead of such an important cash crop, sugarcane production has been hampered by the different biotic and abiotic stresses around the globe. Multiple biotic and abiotic stresses, including drought, salinity, and cold, adversely affect sugarcane growth and result in yield losses of 50–60% (Ali et al., 2019).

1.1 Effect of salinity stress on sugarcane production and biochemical events

Among the several constrains, salt stress is a major abiotic factor affecting sustainability of agriculture, mainly for high water demanding crops like sugarcane over a vast area in the world. It has been estimated that 20% of total cultivated lands and 33% of irrigated arable land is affected with high salinity worldwide Fig. 2 (Rao et al., 2021).

In India, 3.77 Mha areas is sodic and 2.96 Mha is saline soil and every year more and more land becomes non-productive because of salt accumulation. Human population is on the rise and projected to reach 9.1 billion by 2050 (Rao et al., 2021).

Sugarcane cultivation in regions with great production potential may be compromised due to soil degradation by salinity and sodicity (Asfaw et al., 2018). Increased soil salinity may result from anthropogenic activities, including irrigation with saline water and poorly conducted irrigation and drainage practices, especially in regions with low rainfall associated with shallow soils (Castro and Santos, 2020). Salt stress leads to physiological disorders due to the increase in salts in the root zone, reducing the osmotic potential of the soil and water uptake (Zuffo et al., 2020). The salinity effect on sugarcane varies and can promote reduced stomatal conductance and, consequently, decreased photosynthetic rates (Simoes et al., 2018; Simoes et al., 2019). Sugarcane exhibit stunted growth under saline conditions with yield falling to 50% or less than its inherent potential depending upon the degree of salt stress and genetic constitution of the genotype (Patade et al., 2011).

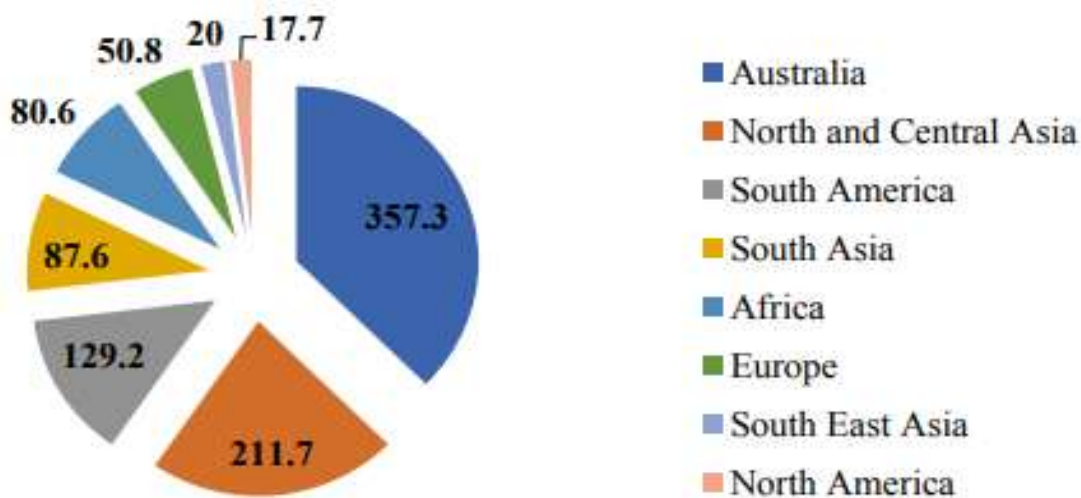


Fig 2. Global area under salinity

Increasing exposure of soil salinity to sugarcane plants leads to cascades of responses at morphophysiological and molecular levels due to salt induced osmotic and ionic stress. Plants have developed certain defense mechanisms to cope with biotic and abiotic stresses such as; ion homeostasis, osmoregulation, antioxidant biosynthesis and accumulation of plant growth regulators facilitating halophytic plant species to adapt and defense in salt and metal excess (Rao et al., 2021). Salinity stress responses are multigenic in that the tolerance mechanism is altered by changes in the expression levels of various compatible solutes/osmolytes and reactive oxygen species (ROS), and these changes adversely affect all levels of plant organization, including the morphological, physiological, biochemical and molecular levels (Hossain, et al., 2021). With respect to the above stress factors, crops respond to salt stress by accelerating a variety of key physiological parameters, such as ionic imbalances due to the accumulation of salt ions by glycophytes, decreases in biomass and photosynthetic metabolism limitations (Chaum et al., 2012).

Glycophyte plant species like sugarcane is sensitive to high salt contents and symptoms are appeared in the form of reduced leaf margins, pigment level, chlorophyll contents which leads to lethal effects viz; wilting, chlorosis and necrosis, leaf drying, and senescence. Cumulatively these attributes ultimately leads to reduced yield and sucrose accumulation (Rao et al., 2021). ROS are produced by electron transport activities in chloroplasts, mitochondria and the plasma membrane or as a by-product of various metabolic pathways. ROS play divergent roles in plants, depending on their concentrations. At low concentrations, ROS act as signaling molecules, mediating the induction of defense genes; however, under prolonged stressful conditions, the level of ROS is enhanced, leading to the disruption of cellular homeostasis and ultimately cell death (Sharma et al., 2012).

To maintain ROS at levels that do not cause cellular damage, plants have developed an efficient scavenging mechanism of defense by which ROS are neutralized by antioxidant enzymes and non-enzymatic defense systems that protect them from stress factors (Medeiros et al., 2014).

Several studies have reported that increase in antioxidant enzyme activities to salt tolerance in plants (Pagariya et al., 2012) however, the relative importance of this process in plant salt tolerance has been demonstrated to be somewhat contradictory (Farhangi-Abriz, 2020). Such associations are also dependent on the plant species, tissues, subcellular

localizations (Mittova et al., 2004), the severity and time of exposure to the salinity (Munns and Tester, 2008) and other environmental stresses. Understanding the mechanisms that coordinate plant responses at all levels of organization, including the morphological, physiological, biochemical and molecular levels, is still a difficult task that requires multidisciplinary efforts (Pagariya et al., 2012). Various chemical and physical options to reclaim arable lands affected with salts, during which growing salt tolerant cultivars produced through traditional breeding and transgenic strategies can also aid in sustainable food production. However, field appraisal and use of bacterial endophytes, for stimulating defense mechanisms can be more ecofriendly and profitable solution to reduce the harmful effect of salinity on crop growth and yield (Fouda et al., 2019).

1.2 Amelioration of salt stress in crop plants with endophytes

Different and numerous metabolic and genetic strategies used by plant-associated microbes can reduce the impact of salt stress and other abiotic stresses arising from extreme environmental conditions (Gopalakrishnan et al., 2015). Induced Systemic Tolerance (IST) is the term used to describe microbe-mediated induction of abiotic stress responses (Meena et al., 2017). In these beneficial situations, rhizosphere microorganisms not only perceive and respond to signal molecules secreted by plant roots, they also release diverse signaling molecules that influence plants, resulting in increased biotic and abiotic stress resistance or tolerance, as well as root development and plant growth (Zhang et al., 2017). Microbial interactions with plants induce several local and systemic responses that improve the metabolic capacity of plants to respond to salt stress (Nguyen et al., 2016).

Bacterial endophytes defines to those strains which have their life-cycle partly or entirely inside the plant and are located in intra-and inter-cellular spaces or in the vascular tissue regardless of the outcome of the association (Kandel et al., 2017). Endophytes are microorganisms including bacteria, fungi, and actinomycetes that survive within healthy plant tissues and promote plant growth under stress. The property of endophytes to induce stress tolerance in plants can be applied to improve adverse effect of salinity and increase crop yields (Lata et al., 2018). Therefore, the evaluation of plant growth-promoting abilities of new and beneficial endophytic microorganisms is a significant area of research for the improvement of plant health and stress resistance (Zhu and She, 2018).

Generally, endophytes have the ability to colonize every internal part of root, stem, leaf, flower, fruit, and seed (Kumar et al., 2016). However, their population may differ with tissue type, plant growth stage and ecological niches (Kuklinsky-Sobral et al., 2004). Moreover they also influence the physiology of plant cell (Santoyo et al., 2016). In addition, they are more advantageous over plant growth promoting bacteria because of their better adaptation against biotic and abiotic stresses (Lata et al., 2018). Usually the mechanisms employed by endophytic bacteria to promote plant growth are analogous to those of rhizospheric bacteria. Yet recent works on the rhizosphere and endophytic microbial communities revealed that the rhizosphere bacteria are significantly distinct from the endophytic bacteria in a few traits. Pinni et al. (2011) documented that endophytic bacteria have large genome (6.73 ± 1.26 Mbp) as compared to rhizosphere bacteria (4.34 ± 0.99 Mbp). Both active and passive mechanisms are involved in the translocation processes of endophytic bacteria inside their plant hosts permitting them to move from the rhizoplane to the cortex of the root system. However, in passive mechanism, bacteria enter at the time of disruption of endodermal cell layer, mostly, during the growth of secondary roots (Gregory, 2006) and during the time of invasion of phytopathogenic bacteria, which helps endophytic bacteria at the same time to penetrate into the central cylinder and move to the pericycle and ultimately reach the root xylem vessels of their hosts (Compant et al., 2010).

Symbiotic endophytes conferred abiotic stress tolerance to plants via at least two mechanisms: (1) activation of host stress response systems soon after exposure to stress allowing the plants to avoid or mitigate the impacts of the stress and (2) biosynthesis of anti-stress biochemical by endophytes (Lata et al., 2018). Colonization of several crops with endophytic fungi has been reported to induce systemic resistance to pathogens, mitigate stress by increasing the levels of protective metabolites and osmoprotectant, activate antioxidant systems to prevent damage caused by ROS, decreasing salt induced root respiration and modulate the phytohormone profile to minimize salt effects on growth of plants, polyamine biosynthesis, uptake and transport regulation of ions (Zhang et al., 2019). These effects are in coordinated to improve plant growth and strength to salinity stress. These constructive effects can be evaluated in terms of improved plant growth exhibited by endophyte colonized plants in comparison to non-endophytic colonized plants (Fig. 3). Salt tolerant bacteria viz., *Rhizobium*, *Azospirillum*, *Pseudomonas*, *Flavobacterium*, *Arthrobacter*,

and *Bacillus*) have a positive impact on crops productivity in stressed environments (Almaghrabi et al., 2013) However, Souza et al. (2015) among important endophytic bacterial genera, concentrated on the study of *Azospirillum*, *Glucanoacetobacter*, *Herbaspirillum* and *Azoarcus* inoculation found that these endophytic bacteria enhanced the salinity tolerance. Maize seedlings inoculated with the *B. amyloliquefaciens* bacterial strain showed enhanced salinity stress tolerance with increased chlorophyll, improved peroxidase and catalase activities along with enhanced glutathione content (Chen et al., 2016). Thus from the earlier reports, it was proven that microorganisms-based plant biotechnology is more efficient in many cases than plant breeding and genetic modification approaches (Smith, 2014).

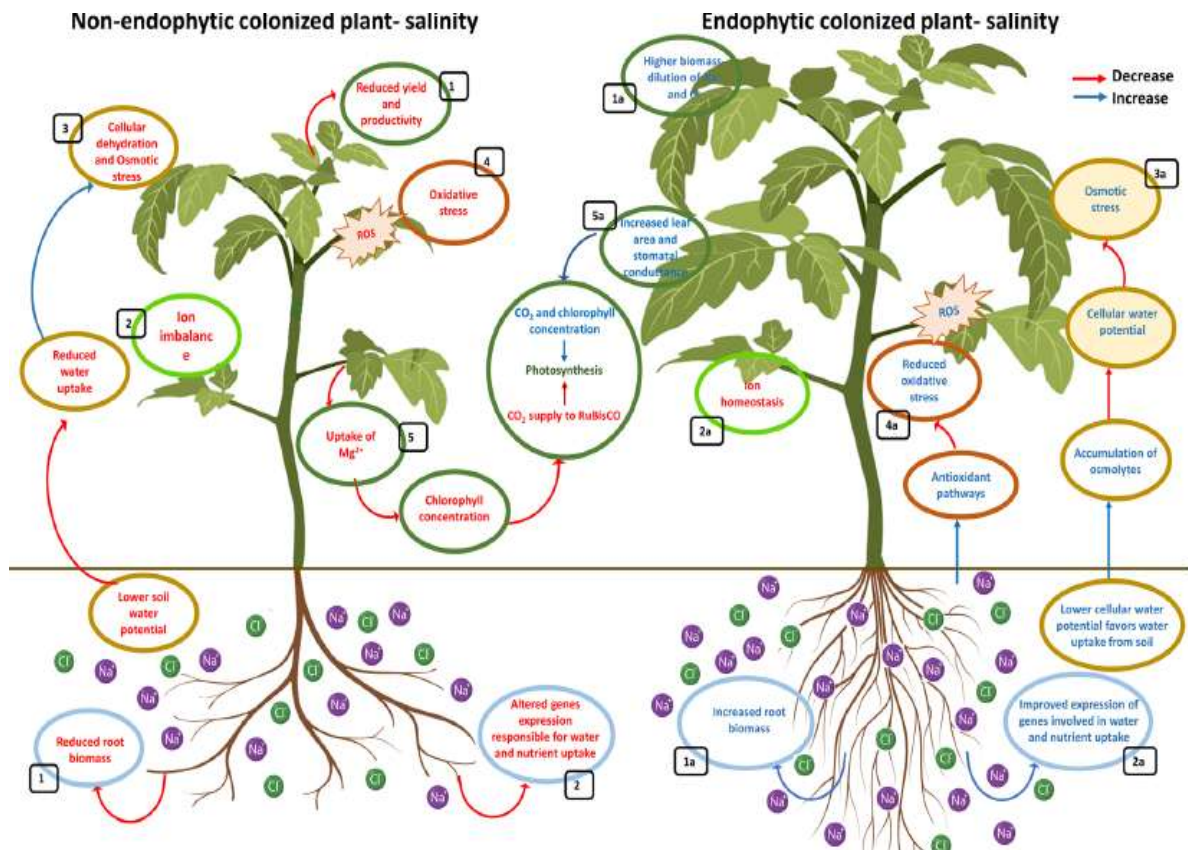


Fig 3. Potential beneficial effects of root colonization of plants by endophytes in saline soil conditions (Source: Gupta et al., 2020)

Similarly, it was revealed that endophytes play a key role in protecting plants against various environmental stresses and therefore, the use of bacterial endophytes in agriculture is a low-

cost and eco-friendly technology to enhance crop productivity in salt affected areas (Vaishnav et al., 2018). In this context the present investigation on biochemical parameters was carried out in contrasting sugarcane varieties viz., CoM-265 (Salt Tolerant) and CoC-671 (Salt Susceptible) grown under different levels of salt concentration and the application of different types of endophytic bacteria to assess the alleviation of salt stress in sugarcane with following specific objectives:

Objectives:

1. To isolate and characterize the endophytic bacteria from sugarcane,
2. To study the effect of endophytic bacteria on physiological and histological properties under induced salinity stress,
3. To study the effect of endophytic bacteria on the osmoprotectant and antioxidant enzymes under induced salinity stress.

2. REVIEW OF LITERATURE

Increasing human populations demand more crop yield for food security while crop production is adversely affected by abiotic stresses like drought, salinity, high temperature. Development of stress tolerance in plants is a strategy to cope with the negative effects of adverse environmental conditions. Endophytes are well recognized for plant growth promotion and production of natural compounds. The property of endophytes to induce stress tolerance in plants can be applied to increase crop yields. With this review, we intend to promote application of endophytes in biochemistry, biotechnology and genetic engineering for the development of stress tolerant plants.

2.1 Worldwide status of agriculture crop productivity

Agriculture plays an important role in the economy of any country. This sector faces many challenges which are responsible for the worldwide loss of crop productivity. These challenges include continuous increase in population, urbanization with decrease in availability of agricultural lands, climatic change, and excess use of chemicals as fertilizers/pesticides in fields, thereby threatening food security and sustainability (Sharma et al., 2020). World's population was estimated to be approximately 7.8 billion people and is projected to rise up to 9.8 billion by the year 2050 (Anonymous, 2017). The drastic increase in world population has raised the demand of food products (Mesa-Marín et al., 2019). The crop production per unit of land cultivated is not sufficient to meet the predicted demand for food. Moreover, change in climate, loss of soil structure, nutrient degradation, drought, and soil salinity contribute to the decreased crop yields (Khan et al., 2019; Ma et al., 2019). Also, global warming, depletion of water resources (drought stress), poor quality of irrigation water, improper soil water-crop management etc. lead to severe issue of saline soil (salt concentration more than 200 mM) (Zafar-ul-Hye et al., 2019). Food and Agricultural Organization (FAO), has estimated a loss of 50% land of total land mass by the year 2050 globally. Excess salt in soil leads to the decreased plant growth and adverse effect on plant metabolic pathways (El-Ramady et al., 2019). Besides high salt concentration, free radical generation such as superoxide radical, hydrogen peroxide (H_2O_2), and singlet oxygen, decrease in plant defensive enzymes, imbalance in sodium homeostasis, decreased iron uptake, phenols and other trace elements are the manifestations seen in the saline stress (Mesa-Marín

et al., 2018). The continuous increase in country's population will put pressure on the requirement of the food and shelter. It is estimated that India will need around 311 MT of food grains during the year 2030 for approximately 1.43 billion people, the food requirement would reach to 350 MT by the year 2050 when the country's population will be 1.8 billion (Kumar and Sharma, 2020). In order to meet the demand of food there must be great attention on expansion of agricultural land along with increase in crop productivity. Indian government needs to focus on restoration of around 26 Mha of lands degraded due to various factors including saline stress. It is estimated that every year nearly 10% area is getting affected with salts, and by the year 2050, approximately 50% of the arable land would be affected with saline stress (Kumar and Sharma, 2020).

2.2 Current Status of Sugarcane cultivation

There are two distinct agro-climatic regions of sugarcane cultivation in India, viz., tropical and subtropical. Tropical region has about 45% area and contributes 55% of the total sugarcane production in the country. Thus, sub-tropical region accounts for 55% area and shares 45% of total production of sugarcane (Shukla et al., 2017; Mohanraj et al., 2021).

Sugarcane (*Saccharum spp.*) is an important industrial crop, ranking among the ten most planted crops in the world. Besides being the major sugar contributor with more than 70% of the world's sugar, sugarcane is important as the raw material for sugar producing and allied industries. India is the largest single producer of sugar including traditional cane sugar sweeteners, khandsari and gur equivalent to 26 MT raw values (Otto et al., 2022).

India is the second position in sugarcane crop area, production and productivity in the world next to Brazil. Globally in world area during 2020-2021 was 20.42 Mha, production 1333 MT and productivity 83.2 tons ha⁻¹ respectively. In India total area of cultivation of sugarcane is 45.7 lakh ha, production is about 355.70 MT and average yield of sugarcane is 80.89 ton ha⁻¹ during the year 2020-21. Particularly in Maharashtra the total area under cultivation of sugarcane is 0.88 Mha and the production is 69.31 MT. The average yield of sugarcane is 84 tons ha⁻¹ (Anonymous, 2021).

2.3 Status of soil salinity

In India the total salt affected area is 6.74 Mha out of which 1.71 Mha is a saline soil, 3.79 Mha is alkali soils and 1.25 Mha is saline soils (Anonymous, 2018). Salinity results by high accumulation of soluble salt, especially NaCl in soil and water. Salinity hampers the

growth and survival of many field crops such as wheat, maize, cotton, sugarcane, sorghum and horticulture crops. Soil salinity threatens agricultural productivity globally in 77 Mha of agricultural land, including 20% of irrigated area and 2.1% of unirrigated field area all over the world (Rao et al., 2015). Among the abiotic stresses, salinity accounts for major portion of yield loss. It restricts yield on almost 40 Mha of irrigated land, which is approximately one-third of the irrigated land on the earth. It is estimated that about 50% of the arable land will be affected by salinity stress by the year 2050 (Shrivastava and Kumar, 2015). High salinity is one of the most severe abiotic environmental factors at a global scale, which has a deleterious effect on crop plant causes restricting plant growth and development. Thus, salt tolerance in glycophytic crop species has become a major issue in the last several years (Himabindu et al., 2016). Despite several efforts, little progress has been made to develop salinity tolerant crop plants by conventional breeding and biotechnological approaches to address the adverse consequences of salinity stress. In recent years, a novel approach of using bacterial endophytes has been proposed to mitigate the salinity stress (Egamberdieva et al., 2019). Soil salinity is emerging as one of the major agricultural limitation worldwide. It is believed that about 62 Mha or approximately 20 percent of the world's irrigated land is affected with salinity. The deposition of an excess amount of soluble salt in cultivable land directly affects crop yields. The addition of high amount of salt affects various physiological and metabolic processes of plants and their overall growth. As of 2019, 1,125 Mha area is salt affected, out of which salinity in 76 Mha is human-induced (Sayyed et al., 2019). There are about 831 to 932 Mha of salt affected soils which may be classified into saline soils, saline-sodic soils and sodic soils. The salinity and sodicity of the soils is based on the measurement of electrical conductivity and exchangeable sodium percentage. Saline soils have high soluble salts and low exchangeable sodium, whereas sodic soils have low soluble salts and high exchangeable sodium. These types of soils occur mainly in arid and semi-arid areas, where precipitation to evapotranspiration ratio is quite low and in coastal areas due to continuous overflow of sea water. Such soils may also occur in the area irrigated due to upraise of water level and in humid areas due to lack of leaching (Osman, 2018).

Arid and semi-arid areas have high evaporation rates, which results in the accumulation of high levels of salts in the plant's root zone. In India, according to Mandal et al. (2018) in climate change scenario with changing precipitation and temperature patterns,

Salt affected soils in Maharashtra are 0.61 Mha and salt-affected soils into three categories. According to these workers sodic, saline, and coastal saline soils are spread over 56, 25, and 19% area in the country. The salt affected soils have been divided into two type's viz., saline and sodic soils, which further can be differentiated based on the preponderance of chlorides and sulphates over sodium ions. In the saline soil, the concentration of sodium is more than chlorides and sulphates with pH less than 8.2, whereas, in the sodic soils, the concentration of sodium is less than chlorides along with pH above 8.2 (Chaudhary and Kharache, 2018). For practical usage, salt-affected lands have been identified as saline, alkaline, sodic, saline-alkaline (Kumar et al., 2018) or saline sodic (Pankaj et al., 2019). Saline soil has an excess of soluble salt in the soil solution, the liquid located between aggregates of soil (Lamizadeh et al., 2019). Sodic soil has too much sodium associated with the negatively charged clay particles (Shahnaz and Manjurul, 2018).

2.4 Emerging technology to overcome salt stress

In the recent years, many approaches have been used to manage the salinity in soil (Acuna Rodriguez et al., 2019; El-Ramady et al., 2019; Gangwar et al., 2020). Development of salt-tolerant crops through breeding, physical removal of salts from the surface of soil and chemical treatment of soil are some of the techniques for reclamation of saline soils (Safeena and Zakeel, 2020). Salt effected soils can be managed through various methods such as selection of salt tolerant crops, salt scraping, flushing and leaching through irrigation and drainage. Chemical treatment with sulphuric acid, substances containing gypsum, CaCl_2 and sulphuric acid are also used prior to leaching. Some studies also demonstrated that phytoremediation is successful on several occasions (Osman, 2018). Calcium added through gypsum stimulates the flocculation and permeability of the soils to facilitate leaching of exchangeable sodium and other salts out of the root zone (Chaudhary and Kharache, 2018). The crop productivity under saline stress conditions can also be maximized using salt tolerant plants. These crops will help in removing salts due to their ability to accumulate salts. Phytoremediation using halophytes is an important method for removal of salts from soil due to its simplicity. The halophytic plant species such as grasses, shrubs, and trees can be helpful in removing salt from different salt-affected soils through salt exclusion, excretion, or accumulation through various mechanisms such as morphological, anatomical, physiological adaptation at organelle and cellular level (Hasanuzzaman et al., 2014). It is

generally accepted that plant cells must maintain a high ratio of K^+ : Na^+ ions in their cytoplasm if they are to grow successfully in saline environments (Wu, 2018; Yun et al., 2018).

Salinity affects physiology of plant through changes of the ionic status in the cells (Parasuraman et al., 2019). Thus, it is vital for the plant to reestablish cellular ion homeostasis for metabolic functioning and growth and to adapt to the saline environment (Ren et al., 2018; Wu et al., 2018). The growth of plant gets affected under salinity stress due to many factors such as decrease in nutrient mobilization, hormonal imbalance, and formation of reactive oxygen species (ROS), ionic toxicity, and osmotic stress. Also, soil salinity has role in modulation of physicochemical properties and reduction of the microbial flora of soil. Studies have demonstrated the benefits of novel microbial assisted techniques and their role in management of salt tolerance and increase in crop yield under saline conditions (Sharma et al., 2021).

The plant growth promoting bacteria produce various compounds such as 1-aminocyclopropane-1-carboxylate (ACC) deaminase, indole-3-acetic acid (IAA), antioxidants, extracellular polymeric substance, and volatile organic compounds which help to protect the plant from salinity stress. Recent researches have shown various strategies via which novel microbial assisted techniques can increase plant salt tolerance and crop yield under saline conditions (Kumar et al., 2016).

2.5 Salinity stress and its effect on plant metabolism

Salt stress occurs in areas where soils are naturally high in salts and/or where soil is irrigated using salty underground water. Inundation of seawater in coastal areas also causes salinity stress to plants. Salt tolerance is a polygenic, highly intricate and complex trait which firmly depends on genotype and plant developmental stages (Kumar et al., 2017). The maintenance of low levels of Na^+ in the cell cytosol is essential for plants. In general, salt tolerance in both dicot and monocot plants is highly dependent on the activity of Na^+/H^+ antiporters (Liu et al., 2008).

Plants have evolved mechanisms that allow them to perceive the incoming stresses and rapidly regulate their physiology and metabolism to cope with them. The mechanisms for salt tolerance can be classified into three main categories. The first one is osmotic stress tolerance, which is regulated by long distance signals that reduce shoot growth and involves

biosynthesis and accumulation of compatible solutes to maintain water uptake. The second mechanism is ion exclusion, in which Na^+ transporters reduce the accumulation of toxic Na^+ within roots and leaves. This system operates by controlling the Na^+ loading into the xylem and Na^+ retrieval from the xylem, before reaching the photosynthetic tissues in the shoot. The third mechanism is tissue tolerance, in which high salt concentration is found in leaves, but Na^+ is compartmentalized at the cellular and intracellular level (especially in the vacuole) reducing Na^+ concentration by specific transporters (Suzuki et al., 2016) and thereby nullifying the deleterious effect of Na^+ in the cytosol and driving water uptake to cells (Roy et al., 2014).

The physiological responses of a plant to salinity are often complex and multifaceted, which makes experiments difficult to design and interpret. At a basic level, the response of plants to salinity can be described in two main phases: the shoot ion-independent response occurs first, within minutes to days, and is thought to be related to Na^+ sensing and signaling. In this first phase, effects of salinity on water relations can be important, causing stomatal closure and the inhibition of leaf expansion. The second phase, the ion dependent response to salinity, develops over a longer period (days to weeks) and involves the build-up of ions in the leaves to toxic concentrations, particularly in old leaves, causing premature senescence of leaves and ultimately reduced yield or even plant death (Negrao et al., 2017).

In plant salt stress regulations and responses include feed-forward mechanisms for stress reduction that are seen after stresses have caused irreversible damage to physiological functions. A good example of such a feed-forward mechanism is the ability of plants to regulate their water loss through partial closure of stomata and/or reduced leaf development, long before there is a substantial loss of their leaf turgor or some irreversible damage to inner membrane systems. In salt-stressed plants, in addition to osmotic effects it is also affected by toxic damages as function of nutritional disequilibrium and high salt levels uptake for plants. Thus, salinity inhibition of plant growth is the result of osmotic and ionic effects and the different plant species have developed different mechanisms to cope with these effects (Munns and Tester, 2008). Salinity stress has significant effect on plant signaling and metabolism (Kumar et al., 2016). The death of plant cells exposed to saline conditions is caused by a high ratio of Na^+ and K^+ ions in the cytoplasm, which is due to drastic increases in the influx of Na^+ ions into the cells and efflux of K^+ ions from the cells (Rubio et al.,

2020). Under saline conditions, the large electrochemical Na^+ gradient results in passive Na^+ uptake into root cells (Singh et al., 2019). The essential role of Ca^{2+} ions have been extensively documented with respect to various cellular functions that are associated with the growth and development of plants (Parvin et al., 2019). In particular, regulation of membrane functions is thought to be one of the most important roles of Ca^{2+} ions in plant cells (Da Wei et al., 2019). Calcium is known to have beneficial effects on plants under high-salinity conditions by decreasing low-affinity Na^+ uptake (Hadi and Karimi, 2012).

A recognizable indication of salinity stress is a reduction in shoot growth, which, in turn, can change the allocation of biomass between roots and shoots. Salinity stress also affects cell expansion in young leaves, generally causing a decrease in leaf area (Negrao et al., 2017).

Many studies confirm the inhibitory effect of salinity on biochemical processes, of which photosynthesis is the most important. The effect on photosynthesis can be gauged from the effect on the photosynthetic pigments (Amira and Qados, 2011; Kumar et al., 2020). The deleterious effects of salinity are considered to be as a result of water stress due to salt, ion toxicities, ionic imbalance or a combination of these factors. The deleterious effects of salt stress on agricultural yield are significant, mainly because crops exhibit slower growth rates, reduced tillering and reproductive development is affected (Roy et al., 2014).

Salinity occurs when soluble salts (usually NaCl) are elevated in soil and affects plants in two ways. High concentration of salts within the plants can be toxic (ion-specific effects), harming plant growth and development, while salts on the outside of roots have an immediate effect on cell growth and associated metabolism due to the increased difficulty in extracting water (Guerzoni et al., 2014).

During the initial phases of salinity stress, water absorption capacity of root systems decreases and water loss from leaves is accelerated due to osmotic stress of high salt accumulation in soil and plants and therefore salinity stress is also considered as hyperosmotic stress. Osmotic stress in the initial stage of salinity stress causes various physiological changes, such as interruption of membranes, nutrient imbalance, impairs the ability to detoxify reactive oxygen species (ROS), differences in the antioxidant enzymes and decreased photosynthetic activity and decrease in stomatal aperture (Gupta and Huang, 2015).

Shoot growth is more sensitive than root growth, a phenomenon that also occurs in drying soils and for which there is as yet no mechanistic explanation. The teleological explanation is that a reduction in leaf area development relative to root growth would decrease the water use by the plant, thus allowing it to conserve soil moisture and prevent an escalation in the salt concentration in the soil (Munns and Tester, 2008).

The salinity causes osmotic stress to the plants. However, certain plants or genotypes can overcome this stress by the production of certain metabolites. Among these osmolytes viz., proline, glycine betaine, sugars, amino acids are accumulated in the plants and overcome the osmotic stress imposed due to salinity and thereby ameliorate the salinity stress (Miller et al., 2010).

2.6 Endophytic bacteria

The application of beneficial microbes in agricultural practices is an integral component that should be validated to improve crop productivity under various abiotic stresses in a defensible manner by treating crop seeds or the foliar spraying of plants with plant growth promoting bacteria (PGPB) (Haitao et al., 2007). PGPB can help plants tolerate salinity through several synergistic mechanisms (Etesami, 2018). The first is relieving osmotic stress by causing osmolyte accumulation and phytohormone signals, for example, indole-3-acetic acid (IAA) and gibberellic acid (GA3), or by reducing the activity of 1-aminocyclopropane-1-carboxylic acid deaminase (ACC) (Zhao et al., 2018). The second is minimizing ion stress and nutrient deficiency by realizing ion homeostasis and improving nutrient uptake (Kumar and Sharma 2020). The third objective is to reduce oxidative stress by increasing the potential for photosynthesis (Bharti et al., 2016). PGPB can improve plant performance in abiotic stress situations by maintaining a compatible ROS level of cellular function (Brilli et al., 2019).

Endophytic bacteria are those bacteria that colonize the internal tissue of the plant showing no external sign of infection or negative effect on their host (Ryan et al., 2008). These bacteria significantly affect plant growth by increasing nutrient cycling, suppressing pathogens by producing antibiotics and siderophores or bacterial and fungal antagonistic substances and/or by producing biologically active substances such as auxins and other plant hormones. The researches revealed that endophytes are colonising several plant tissues, including roots, stems, leaves, flowers, fruits, and seeds, in addition to the rhizosphere.

Furthermore, it has been shown that bacterial endophytes are associated with specialised rhizosphere-dwelling bacteria because of their potential competitiveness with other rhizosphere-dwelling microbes and their very effective interactions with their plant hosts (Ali et al., 2014).

The main entry points of the endophytic bacterial community into plant tissues are via primary roots and associated lateral roots and consequently tissue wounds (Sorensen and Sessitsch, 2015). Firstly, bacteria enter into the root then colonize the stem followed by leaf colonization via the transpiration stream. Hence, population densities of endophytic bacteria vary from tissue to tissue. Another study reported that the association between the plant and the endophytic bacterial community is mediated through the action of various chemical compounds, generally produced by the microorganisms and the host cells (Reinhold-Hurek and Hurek, 2011).

Majority of these include *Achromobacter xylosoxidans* (Jha et al., 2009), *Azoarcus* sp. (Krause et al., 2006), *Azospirillum* (Thakuria et al., 2004), *Azotobacter* (Zahir et al., 2004), *Bacillus* (Cakmakci et al., 2007), *Burkholderia* (Kennedy et al., 2004), *Cronobacter sakazakii* (Afridi et al., 2019), *Enterobacter cloacae* (Liu et al., 2012), *Gluconacetobacter diazotrophicus* (Alqueres et al., 2010).

Hecht-Buchholz (1998) reported that bacteria which have been found in the apoplast of non-leguminous plants include *Azospirillum* spp. *Acetobacter diazotrophicus*, *Herbaspirillum* spp. and *Azoarcus* spp. Cavalcante and Dobereiner (1988) successfully isolated a new nitrogen fixing entophytic bacteria *A. diazotrophicus* from homogenized root, stem and leaf of sugarcane by using diluted cane juice semisolid LGIP medium, acidified LGIP semisolid medium and further purified on acidified LGIP and potato agar plates. Sathyan and Thangaraju (2003) isolated eighteen strains of *Gluconacetobacter diazotrophicus* from the root, stem, leaves and tubers of sweet potato varieties. Elbeltagy et al. (2001) isolated endophytic bacteria from the stems of wild and cultivated rice on a modified Rennies medium which were motile, gram negative, vibriode to slightly curved rods, oxidase and catalase positive. The diazotrophic isolates were close to four genera *Herbaspirillum* spp., *Ideonella*, *Enterobactoe* and *Azospirillum* spp. James (2000) reported the presence of *Herbaspirillum* spp. in sugarcane, rice and *Azoarcus* spp. in rice and Kallar grass. These microbes are found in intercellular spaces of roots, stems, and leaves. *Azoarcus*

spp. are present in high density in juices of stems, roots and leaves of sugarcane plants which showed optimum growth at pH 6.6 to 7.0 and 25⁰C. They were isolated first time from salt tolerant plant. These bacteria belonged to proteobacter beta super family and are aerobic in nature (Baldani et al., 1992). *Azoarcus* invade roots and shoots of grasses without causing symptoms of plant disease (Reinhold-Hurek and Hurek, 1998). Elbeltagy et al. (2001) reported that endophytic diazotrophs such as *Acetobacter diazotrophicus* and *Herbaspirillum* spp. were found in the intercellular space of stem in sugarcane and rice. Singh, et al. (2013) isolated endophytic bacterial isolates from native sugarcane varieties at hilly areas namely Berinag, Champawat and Didihat of Uttarakhand state in northern Himalaya region. *Azospirillum brasilense* and *Gluconacetobacter diazotrophicus* were evaluated for their morphological, biochemical and molecular characteristics. Morphologically all the isolates were rod shaped, gram negative, H₂S producing and none of the isolates hydrolysed gelatine and starch and produced cellulose. Suman, et al. (2001) isolated twenty two endophytic bacteria from the roots of sugarcane and compared morphologically, biochemically and genetically. The cells of all 22 bacterial isolates were microscopically observed to be gram negative, straight rods of $0.8 \pm 1.0 \times 1.5 \pm 2.2 \mu\text{m}$ in size. All isolates were catalase positive and majority of these bacteria (15) were oxidase positive on the semi-solid LGI medium with 10% sucrose and identified as *G. diazotrophicus*, *Azospirillum*. Mehnaz and George, (2006) isolated bacteria from corn and identified on biochemical characterization as *Azospirillum*. The cells of all three isolates (N7, N8, and CQ179) were gram-negative, motile rods, semi helical shape, showing oxidase and catalase positive. Tejera, et al. (2005) reported bacterial strains isolated from roots and rhizosphere of sugarcane which can grow on NFM medium under aerobic conditions were oxidase +ve, catalase +ve can utilize mannitol as carbon source and classified as *Azospirillum* spp. according to Bergey's manual of determinative bacteriology (1983). Wong and Stenberg (1979) reported five isolates obtained from macerated roots of harvested sorghum plant were oxidase positive, catalase positive can utilize glucose and galactose as carbon source and classified as *Azospirillum brasilense*.

2.7 Screening of endophytes for salt stress

Bidgoli et al. (2019) have investigated the effects of soil salinity and the application of bacterial stimulus on the essential oil yield of *Rosmarinus officinalis*. They observed that the percent yield of essential oil was directly proportional to the salinity up to 10 g/L NaCl

either alone or with PGPR inoculation treatments. Halophilic microorganisms produce various enzymes (halozymes) that helps these microbes to survive in high concentrations of salt, in wide range of pH and temperature (Gupta et al., 2016). Sharma et al. (2016) reported that the diazotrophic salt-tolerant bacterial strains of *Klebsiella*, *Agrobacterium*, *Pseudomonas* and *Ochrobactrum* isolated from the roots of a halophytic plant, *Arthrocnemum indicum* showed salinity tolerance ranging from 4 to 8% NaCl, and improved the productivity of peanut in saline as well as in control conditions. Yaish et al. (2015) isolated endophytic bacteria from date palm (*Phoenix dactylifera* L.) and tested their ability to help plants to grow under saline conditions. Arora et al. (2014) Endophytic bacteria were isolated from leaves of four dominant halophyte and salt tolerant plant species of coastal Gujarat. The bacterial counts on nutrient agar were found to be maximum in *Spharanthus indicus* (40%) were minimum in *Salicornia brachiata* (10%). Twenty bacterial isolates were selected and were characterized through morphological characters and biochemical tests. Three were pigmented and 17 were non-pigmented and 50% isolates exhibited amylase activity and only 15% isolates showed urease activity. Six (30%) and two (10%) isolates showed positive results for ammonia production and phosphate solubilization activity. Salt tolerance of the endophytes was also tested. Of the 20 endophytic bacteria, seventeen (85%) isolates showed growth at 7.5% NaCl and fifteen (75%) tolerated upto 10% NaCl concentration. Kothari et al. (2013) reported that *Bacillus safensis* VK from the desert of Gujrat, India, showed salt tolerance up to 14% NaCl and pH ranging from 4 to 8. Further, study of salt tolerant *Klebsiella* sp. IG 3 isolated from the rhizosphere of wheat showed salt tolerance up to 20% NaCl. Gayathri, et al. (2010) isolated 104 bacterial isolates from the leaf samples of mangrove plants. They screened thirty six potentially fast growing endophytic isolates and screened for biological activities from leaves of 5 mangrove and 2 salt-marsh plant species. 34 (94.4%) and 31 (86.6%) endophytic isolates exhibited growth on a medium containing up to 7.5 and 10% NaCl concentrations, respectively. Upadhyay et al. (2009) explored the genetic diversity of salt tolerant bacteria isolated from the wheat rhizosphere. They found that most of the isolates were able to tolerate up to 8% NaCl and belong to the genus *Bacillus*.

2.8 Mechanisms of salt tolerance in endophytes inoculated plants

2.8.1 Chlorophyll and accessory pigments and chlorophyll stability index

Salt stress hinders photosynthesis resulting in an enormous loss in crop productivity (Sudhir and Murthy, 2004). Salt stress has been shown to degrade D₁ and D₂ proteins of the photosystem II reaction centre. These proteins play crucial roles in protein phosphorylation coupled with the flow of electrons (Jansen et al., 1996). Salt stress also results in decreased photosynthetic pigments by reducing the activity of enzymes that synthesize them. Osmotic shock resulted from salt stress lead to reduced leaf area and decrease in stomatal and mesophyll conductance (Chaves et al., 2009). This limits CO₂ availability and assimilation which consequently affect RuBisCO (Seemann and Critchley, 1985). Decreasing CO₂ assimilation also increases the risk of the accumulation of electrons in thylakoid membranes and predisposes the photosynthetic apparatus to increased energy dissipation. Thus, to dissipate this energy, photosystem II loses excess electrons causing injury to photosynthetic tissues and affecting the net photosynthetic rate (Gomez et al., 2010). Plants can protect the photosystems from light induced inhibition and damage in several ways such as minimizing harvesting of light and dispersion of excess energy by non-photochemical quenching (NPQ) (Lima Neto et al., 2015). An increase in NPQ can limit quantum yield (Baker, 2008) but inoculated plants are reported to have lower NPQ, therefore symbiosis enhances photosynthetic efficiency by proficient conversion of harvested light into chemical energy and minimizing NPQ (Pehlivan et al., 2017). Endophytic fungi are also known to reinforce these mechanisms and reduce the negative effects of salinity on plant photosynthetic capacity (Jogawat et al., 2013). Endophytic symbiosis combats the negative effects of salt stress on photosynthesis in several ways. Inoculated plants have shown improved water status resulting in larger leaf area and higher stomatal conductance and eventually better assimilation of carbon dioxide (Zarea et al., 2012). Magnesium (Mg) is one of the essential macronutrients for plant growth and is involved in numerous physiological and biochemical processes such as photosynthesis, enzyme activation and synthesis of nucleic acids and proteins (Chen et al., 2018). It is the central atom of the tetrapyrrole ring of chlorophyll *a* and *b* molecules, which are the major pigments for photosynthetic light absorption (Wilkinson et al., 1990). Salt reduces uptake of Mg²⁺ thus also reducing the concentration of chlorophyll in leaves (Sudhir and Murthy, 2004). Inoculated plants maintain higher chlorophyll

concentration by improving the uptake of Mg^{2+} (Jogawat et al., 2013; Yin et al., 2014) and this leads to maintenance of plastid integrity and enhanced photosynthetic efficiency (Johnson et al., 2014). Another way in which endophytes induce defence systems in plants under saline conditions is by upregulating the ascorbate-glutathione (ASH-GSH) cycle Kumar et al., (2012) reported that during salt stress, the endophytic fungus *P.indica* maintains a high antioxidative environment by defense system priming, especially the ascorbate-glutathione (ASH-GSH) cycle leading to maintenance of plastid integrity and therefore enhanced photosynthetic efficiency in colonized plants during abiotic stress (Johnson et al., 2014). Inoculated plants also confer the benefit of maintaining the integrity of photosystem II by repairing salt-induced degradation of D₁/D₂/Cyt b 559 complexes by the accumulation of glycine betaine in inoculated plants (Rivero et al., 2014).

Omer et al. (2022) reported that results of the mean comparisons recorded showed that increased in all photosynthetic pigments of flax plants under salinity stress conditions. It was concluded that an increase in the chlorophyll content of endophytic bacteria treated plants could be due to the development of a variety of microbial metabolites, such as auxin, gibberellin and cytokinin, arising throughout the absorption of water and minerals (Cassan et al., 2014) which help in facilitate photosynthetic materials in plant cells. Results from this and previous studies indicated that growth-stimulating bacteria, by producing of auxin tend to help plants in root development in order to provide more water and nutrients to vegetative parts and thus increased the amount of pigment production as well as facilitated the transfer of photosynthetic materials in the plant Ahmed and Hasnain (2014), Fasciglione et al. (2015).

Bashan et al. (2006) and Enebe and Babalola (2018) found that wheat plants cultivated under salt stress and inoculated with *Azospirillum brasilense* resulted in increased development of photosynthetic pigments, which would, in turn, led to a significant increase in photosynthetic rates and CO₂ fixation. Also, Omar et al. (2009) and Bhat et al. (2020) noticed that application of endophytic bacterial strains under salt stress enhanced the production of auxiliary photo protective pigments such as zeaxanthin, and beta carotene which may protect Chlorophyll *a* and Chlorophyll *b* from oxidation during exposure to salt stress.

Omer et al. (2022) found that chlorophylls and carotenoids were varied in their contents in salinity stressed flax plants due to the inoculation with the endophytic bacterial

strains *A. brasilense* and *P. geniculata* (individually or in co-inoculation). Flax plants that were grown under salinity stress conditions and inoculated with the bacterial isolate *A. brasilense* observed significant increase in photosynthetic pigments, e.g., Chl. *a*, Chl. *b*, Chl. *a* + *b* and carotenoids by 9.5%, 48%, 24.4%, 84.6% respectively, when compared to uninoculated and salinized control plants. Moreover, treating stressed flax plants with endophytic bacterial strain *P. geniculata* displayed significant enhancements in Chl. *a*, Chl. *b*, Chl. *a* + *b* and carotenoids by 6.47%, 42.9%, 23.29% and 61.54%, respectively. Dual inoculation with the two endophytic bacteria (*A. brasilense* and *P. geniculata*) significantly increased the contents of Chl. *a* (1.36%), Chl. *b* (20.47%), Chl. *a* + *b* (10.23%) and carotenoids (55.38%).

Khalid et al. (2017) observed that leaf chlorophyll content in inoculated plants was increased to 61.5% in non-saline condition. Under appropriated saline condition, this ratio was 63.6, 75 and 0.62% grew at NaCl concentration of 40, 80, and 120 mM, accordingly.

Sofy et al. (2021) studied the photosynthetic pigment content, i.e., Chl *a*, Chl *b*, carotenoids, and total pigment in pea plants treated with endophytic bacteria (i.e., *B. subtilis*, *P. fluorescens*) in the absence or presence of NaCl. In general, all photosynthetic pigment levels (Chl *a*, Chl *b*, carotenoids, and total pigment content) decreased when pea plants were irrigated at different NaCl solution concentrations. However, the treatment of plants with endophytic bacteria (i.e., *B. subtilis* and *P. fluorescens*) leads to an increase in the content of all photosynthetic pigments in both saline-stressed and non saline stressed plants. The most pronounced effect was recorded in plants applied with *B. subtilis* under normal and salt-stress conditions. Further, it was reported that disorder in these biochemical processes and properties in plants grown under salt stress and photosynthesis mechanism and its efficacy inevitably leads to impairment and damage. As revealed by increased in the biosynthesis of photosynthetic pigments in salt-stressed radish plants (Mohamed and Gomaa, 2012). A positive influence from *B. subtilis* for net photosynthesis, Chl *a*, Chl *b*, carotenoids, and complete photosynthetic pigments (Abd_Allah et al., 2018) has previously been recorded in salt-stressed chickpea plants.

Abd_Allah et al. (2016) studied the stress-adaptive mechanisms of chickpea plants mediated by *Bacillus subtilis* (BERA 71) under saline conditions and observed that *B. subtilis* inoculation resulted in a significant increase in the synthesis of photosynthetic pigments,

including chlorophyll a, chlorophyll b and carotenoids. *B. subtilis* supplementation in soil alleviated the NaCl-induced decline in pigment synthesis and led to an increase in the levels of chlorophyll a (17.3%), chlorophyll b (34.1%) and total photo-synthetic pigments (21.0%) compared with the salt-affected uninoculated plants. However, soil salinity led to a significant decrease in the chlorophyll a, chlorophyll b and total chlorophyll content, which decreased by 41.3%, 47.5% and 28.3%, respectively, compared to the controls. Further, soil salinity reduced the ratio of chlorophyll a/b, carotenoids and total chlorophyll.

Abd_Allha et al. (2018) observed exhibited different chlorophyll content to non-inoculated controls that plants inoculated with *B. subtilis* BERA 71 in chickpea, however high salinity stimulates chlorophyllase activity, which degrades pigment proteins. Lower levels of chlorophyll due to NaCl treatment have been detected in *C. arietinum* (Rasool et al., 2013), *Ephedraalata* (Alqarawi et al., 2014) and *Sesbania sesban* (Abd_Allah et al., 2016). Abd_Allha et al. (2018) also observed that an increase in the uptake of magnesium can support pigment synthesis and might be one of the prime reasons for the PGPR-induced increase in chlorophyll content and mitigate the toxicity under saline condition. PGPR have the potential to enhance crop resilience to stresses and are believed to regulate key growth-promoting pathways in the host plants, thereby resulting in proper growth maintenance (Stefan et al., 2013). Mohamed and Gomaa (2012) and Stefan et al. (2013), who reported a significant improvement in the growth of *Raphanus sativus* and runner bean due to inoculation with PGPR, which was caused by its stimulatory effect on the synthesis of chlorophylls by increasing uptake of Mg^{2+} which assists in pigment synthesis. *B. subtilis* protected *C. arietinum* plants from the deleterious effects of NaCl on chlorophyll pigments, which might contribute to the enhancement of the efficiency of photosynthesis by maintaining the function of the pigment-protein complex (Rasool et al., 2013). A high salt concentration in growth medium leads to stunted plant growth due to the *de novo* synthesis of proteins and chlorophyll components (El-Tayeb, 2005) while inoculation with *B. subtilis* increase chlorophyll synthesis through enhanced metabolism and through the synthesis of photo assimilates. Dawwam et al. (2013) reported that the stimulation of pigment synthesis in potato plants by PGPR might be due to the changes in N, P and K uptake.

Vaishnav et al. (2019) found that, a PGPR *Pseudomonas simiae* AU (MTCC-12057) to successfully inter-colonize with soybean and mungbean under salt stress. Further, they

subjected *P. simiae* AU for different mutagenesis treatments with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG), ethyl methane sulfonates and ultraviolet rays + NTG. Of 25 selected bacterial mutants, one mutant strain, AU-M4, recorded higher ACC deaminase activity (98 nmol mg⁻¹h⁻¹) over the wild strain AU (79 nmol mg⁻¹h⁻¹). The wild AU and mutant AU-M4 bacterial strains were inoculated into mungbean and soybean plants and it was found that AU-M4 inoculation in soybean and mungbean plants led to higher increases in chlorophyll contents as compared to AU inoculation. It was suggested that endophytic bacteria sense physiological changes induced by salt stress in plants and provoke plants to alter gene expression to adapt to stress conditions. Hence, endophytes have to be used as biostimulants in plants as they can regulate plant metabolism under stress conditions.

Afridi et al. (2019) studied effect of *K. rhizophila* and *C. sakazakii* on wheat crop varieties Pasban 90 and Khirman. The result revealed that leaf chlorophyll content significantly decreased under the salt stress condition in wheat. However, the application of *K. rhizophila* and *C. sakazakii* enhanced the leaf chlorophyll content of Pasban 90 by 10–17% and that of Khirman by 11%, compared to control. Higher chlorophyll content (44.65 SPAD) was noted in Pasban 90 than Khirman when inoculated with *C. sakazakii*.

Sampangi-Ramaiah et al. (2020) demonstrated that salt-tolerant endophyte isolated from salt-adapted Pokkali rice, a *Fusarium* sp. colonizes the salt-sensitive rice variety IR-64 and it was revealed that chlorophyll stability index was higher in colonized plants. Suggest that fungus stabilizes the cell membrane under salinity stress condition.

Umapathi et al. (2021) reported that higher CSI played an important role in drought tolerance. They carried out the research on effect of endophytes inoculated in sorghum under drought condition and observed that plants with uninoculated had CSI 68.82 % i.e. below 75 % means plant are susceptible to drought, however bacterial inoculation with *Bacillus* sp. gave tolerance to drought (78.49 %) during drought.

Harish (2008) found that application of endophytic bacterial strains significantly increased chlorophyll stability index in banana plants under greenhouse and field condition. Maximum chlorophyll stability index in peanut inoculated with culturable endophytes was observable from 30 DAS. Chlorophyll stability index is a scale for the integrity of membrane or heat stability of the pigment under stress conditions.

Vibita Bala et al. (2019) reported chlorophyll stability index is the measure of the membrane integrity in the plants under the stressed environment. Further they observed rice seeds inoculated with *Acinetobacter* sp. had 80.45 % CSI under moisture stress condition stabilizing photosynthetic pigments and thereby higher amount of dry matter production.

2.8.2 Shoots and roots

Colonization of several crops with endophytic fungi has been reported to induce systemic resistance against pathogens, mitigate stress by increasing the levels of protective metabolites and osmoprotectants, activate antioxidant systems to prevent damage caused by ROS, decreasing salt induced root respiration and modulate the phytohormone profile to minimize salt effects on growth of plants (Jogawat et al., 2013; Li et al., 2017; Nia et al., 2012; Rewald et al., 2015; Zhang et al., 2019). These effects are in coordinated to improve plant growth and resilience to salinity stress. These ameliorative effects can be evaluated in terms of improved plant growth exhibited by endophyte inoculated plants in comparison to non-endophytic inoculated plants. Salinity triggers a decrease in stomatal conductance, thus decreasing the CO₂:O₂ ratio and increasing photo-respiration (Kangasjarvi et al., 2012). This causes an increase in the rate of tissue respiration. Under these conditions, photosynthetic capacity is limited, and the plant uses its own photo-assimilates, resulting in decreased growth.

Rewald et al. (2015) showed that in inoculated *Ulmus glabra* seedlings there was a significant increase in fine root respiration under salt stress as compared to their non-inoculated counterparts. The colonization by endophytic fungi can prevent a major increase of root respiration under moderate NaCl stress, enabling trees to deploy more assimilated C for growth and, theoretically, improve defense mechanisms against other stress factors occurring in urban environments. The endophytes have been frequently reported to not only protect against plant pathogens and pests but also impart strong tolerance against several abiotic stresses in crops (Gangwar and Singh, 2018).

Under different environmental stresses, plant growth-promoting bacteria with the aid of root growth and development, and in particular hair roots, contribute to increased plant water and nutrient uptake (Kaymak, 2010) and also morphological characteristics and metabolism by generating a range of hormone substances such as auxin, gibberellin and cytokinin resulting in increasing water and nutrient uptake (Cassan et al., 2014). Recently,

Abdel Latef et al. (2021) in their study recorded significant increase in the growth parameters of salinity-stressed canola plants in response to the inoculation with the bacterial isolates *Azotobacter chroococcum* and/or *Alcaligenes faecalis*.

Ethylene is produced endogenously by the plant for the control of plant development (roots, shoots). So when the plant is salinity stressed, the plant synthesizes ethylene, increasing the endogenous concentration of that phytohormone (Shilev, 2020). In ethylene biosynthesis, S-adenosyl-L-Methionine (SAM) is further transformed into 1-aminocyclopropane-1-carboxylate (ACC) through the ACC synthase pathway and followed by conversion to ethylene by ACC oxidase (ACO) enzymes (Glick et al., 1998). Some *B. subtilis* and *P. fluorescens*, PGPBs, such as or generate the enzyme ACC-deaminase which divides the ACC molecule, into α -ketoglutarate and NH_4 the most commonly identified mechanism among PGPB (Glick et al., 2007). The activity of ACS was increased by increasing salt stress independently of microbiological treatments. The concentration of ethylene in plant tissues has increased due to a wide range of abiotic and biotic stresses (Mayak et al., 2004). The ethylene synthesis decreased the germination of the seed and the plant's root *B. subtilis* growth under salt stress or *P. fluorescens* -producing ACC-deaminase also helped reduce ethylene production that converted ACC to α -ketobutyrate, ammonia, and limited the harmful effects of ethylene on plant growth under salinity stress. Thus, inoculation of ACC (*B. subtilis* *P. fluorescens*) deaminase-producing bacteria (or in plants was correlated with salt stress tolerance (Glick, 2010).

Khalid et al. (2017) reported that the shoot height was improved to 60.86% the shoot height was significantly increased in *A. brasilense* inoculated plants than that of uninoculated plants. The enhancement in shoot height was recorded to be 58.82, 57.8 and 70% at 40, 80, and 120 mM NaCl treatments, respectively.

Similarly, Khalid et al. (2017) result revealed that the root length of inoculated plants was improved to 62.06% under non-saline condition. *A. brasilense* significantly increased the root length by 70.83, 58.82, and 63.63% under at experimental concentration of 40, 80, and 120 mM NaCl, respectively.

Omer et al. (2022) reported that under salinity stress conditions, inoculation of flax plants with endophytic bacterial isolates (*A. brasilense* and *P. geniculata*) showed different responses in their root length. Bacterial strain *A. brasilense* inoculation enhanced the root

length, by 36.6%. Additionally, application of the bacterial strain *P. geniculata* significantly increased the aspects of root length 56.6% when compared with control plants grown in salinized soil. Furthermore, co-inoculation bacterial species *A. brasilense* and *P. geniculata* markedly enhanced the growth criteria of salinity-stressed flax plants represented in root length 30 %.

Sofy et al. (2021) observed that the shoot length of pea plants which irrigated with NaCl solution and applied with isolated bacteria, i.e., *Bacillus subtilis*, *Pseudomonas fluorescens*. Shoot length decreased significantly when plants irrigated with 75 and 150 mM of NaCl and it was approximately 24.38% and 41.69%, respectively, compared to non-salt plants. Treatment with endophytic bacteria (*Bacillus subtilis*, *Pseudomonas fluorescens*) has significantly increased vegetative parameters in salt-stressed plants compared to non-stressed plants. The most pronounced increase was recorded in plants treated with *Bacillus subtilis*. Shoot length was increased about 36.94%, 46.52% for 75, and 150 mM in NaCl treated plants by the application *B. subtilis*.

Omer et al. (2022) reported that under salinity stress conditions, inoculation of flax plants with endophytic bacterial isolates (*A. brasilense* and *P. geniculata*) showed different responses in their shoot length. Bacterial strain *A. brasilense* inoculation enhanced the shoot length, by 16.5 %. Additionally, application of the bacterial strain *P. geniculata* significantly increased the aspects of root length 37.2 % when compared with control (plants grown in salinized soil). Furthermore, co-inoculation bacterial species *A. brasilense* and *P. geniculata* markedly enhanced the growth criteria of salinity-stressed flax plants represented in shoot length 47.9 %.

Afridi et al. (2019) studied effect of *K. rhizophila* and *C. sakazakii* on wheat crop varieties Pasban 90 and Khirman under imposed saline condition. Under both normal and saline conditions, the effects of both endophytic *K. Rhizophila* and *C. sakazakii* on morphological attributes were more pronounced than their respective uninoculated control soils. Increase in shoot lengths of Pasban 90 and Khirman with the *K. rhizophila* and *C. sakazakii* treatments were observed and in a range of 10%–27% higher than their respective controls. It was noticed that Pasban 90 inoculated with *C. sakazakii* showed better results than Khirman inoculated with *K. rhizophila*.

Alexander et al. (2020) reported that the potential of halotolerant bacterium *Stenotrophomonas maltophilia* BJ01 isolated from saline-soil was explored to enhance the growth of peanut plants under salt stress conditions. It was observed that the shoot length of the untreated plant was about 13.4 cm whereas the shoot length of the treated plant was about 16 cm.

Afridi et al. (2019) studied effect of *K. rhizophila* and *C. sakazakii* on wheat crop varieties Pasban 90 and Khirman under saline condition. Increase in root lengths of Pasban 90 and Khirman with the *K. rhizophila* and *C. sakazakii* treatments were observed to be in a range of 10%–27% higher than their respective controls. It was found that Pasban 90 inoculated with *C. sakazakii* showed better results than Khirman inoculated with *K. rhizophila*.

2.8.3 Leaf area

Abriz et al. (2020) studied the impacts of plant growth promoting bacteria *Pseudomonas* RS-198 and *Azospirillum brasilense* RS-SP7 and foliar application of plant hormones (salicylic acid 1mM and jasmonic acid 0.5mM) on alleviating the harmful effects of salt stress in rapeseed plants (*Brassica napus* cv. okapi) under greenhouse condition. The interaction of salinity × bacteria × hormones was significant on leaf area were significantly declined by increasing salt stress. Inoculation of rapeseed seeds with *Pseudomonas* and *Azospirillum* increased leaf area under saline and non-saline conditions over the control.

Khalid et al. (2017) ascertained the potential of beneficial soil bacterium *Azospirillum brasilense* to alleviate saline stress in *Trifolium repens* it was observed that under 40mM salt stress conditions. *A. brasilense* alleviate stress more effective than non-saline condition as revealed by increase in leaf area 127% under 40mM saline condition than control (no salt stress) imposed having about 38% increase in leaf area in the inoculated plants.

2.8.4 Total biomass

Total biomass is usually evaluated as an indicator of the plant's ability to tolerate salinity. Several studies have highlighted that endophytic fungi impart salinity tolerance in host plants by virtue of higher biomass as compared to non-inoculated plants. Endophytic fungus colonization has been demonstrated to increase biomass in *Zea mays* L. (Rho et al., 2018), soybean (Hamayun et al., 2017), *Vochysia divergens* Pohl (Farias et al., 2019), *Solarium lycopersicum* (Azad and Kaminskyj, 2016), *Brassica juncea* (Ahmad et al., 2015),

Oryza sativa L. (Saddique et al., 2018) and *Triticum aestivum* L. (Zhang et al., 2019). Balliu et al. (2015) investigated the effects of commercially available AMF inoculant (*Glomus* sp. mixture) on growth and nutrient acquisition in tomato (*Solanum lycopersicum* L.) plants grown in media with different levels of salinity. Salinity stress immediately and significantly reduced the LAR, NAR and RGR_{plant} in non-inoculated as compared to inoculated plants. Similarly, Sallaku et al. (2019) showed that AMF alleviates the salinity stress in cucumber plants by extending their root length and root surface area and even more through enhancing their photosynthetic rate (NAR) as compared to non-inoculated plants.

Root branching and root system architecture play a significant role in determining the composition of exudates (Badri and Vivanco, 2009). Changes in the root system architecture for regulating salt acquisition and translocation are crucial for enhancing plant resistance to salt stress (Jung and McCouch, 2013). Barley plants experienced a decline in primary root growth under saline conditions due to salt-induced inhibition of cell division and elongation of root epidermal cells, while simultaneously stimulating lateral root development (Rahnama et al., 2011). Endophytic fungi can modulate the plants ability to modify root architecture (Salope-Sondi et al., 2015; Vahabi et al., 2016). Yun et al. (2018) observed that the length and volume of roots were greater in inoculated than in non-inoculated maize plants under saline conditions and similar observations have been reported in *Hordeum vulgare* (Waller et al., 2005) and *Oryza sativa* L. (Kord et al., 2019). Improved root systems enable the plant to utilize water and minerals from non-saline areas until exploitation of areas affected by salt cannot be avoided (Jogawat et al., 2013). Though few studies have shown the ability of endophytic fungi to alter root architecture under saline conditions for beneficial purposes, much remains to be investigated on endophytic fungi influenced root architecture for better water and nutrient uptake in saline conditions.

2.8.5 Osmoregulation

Upon exposure to saline environments, plants undergo a reduction in water absorbing capacity from the soil, disrupting cell water relations and inhibiting cell expansion. In order to mitigate these effects, plants employ osmoregulation as a mechanism to tolerate salt stress (Munns and Tester 2008). This is achieved by accumulation of osmolytes in the form of proline, glycine betaine, sugars, organic acids, polyamines, and amino acids, contributing to osmotic adjustment (Hasegawa et al., 2000). These osmolytes, often termed as compatible

solutes, are organic compounds of low molecular weight that are water soluble and non-toxic at high concentrations (Chen and Murata 2011). Under salt stress, endophyte inoculated plants have been shown to possess a higher osmotic potential than non-inoculated plants (Contreras-Cornejo et al., 2014) due to the accumulation of osmolytes (Ahmad et al., 2015; Song et al, 2015). Osmolytes are also involved in quenching reactive oxygen species (ROS), maintaining membrane integrity, and stabilizing enzymes. Osmolytes are also described as osmoprotectants (Azad and Kaminskyj, 2016; Li et al., 2017). Endophytic symbiosis can influence the concentration and profile of polyamines and organic acids in plants (Chen et al., 2019; Zhao et al., 2014). Polyamines help retain ion homeostasis in plant cells by enhancing the uptake of nutrients and water (Pang et al., 2007). Organic acids may increase the availability of nitrogen, phosphorus, and potassium (N, P, and K) in soil (Samolski et al., 2012). The role of specific osmolytes in improving salt tolerance in endophyte inoculated plants is discussed below.

2.8.5.1 Glycine betaine

Many plant species naturally accumulate Glycine betaine-like quaternary ammonium compounds (QACs) abiotic stresses. These compounds are thought to play an adaptive role in mediating osmotic adjustment and protecting subcellular structures in stressed plants (Asharf and Foolad 2007). However, some plant species such as rice, mustard, and arabidopsis naturally do not produce GB under stress or non-stress conditions. In the accumulated GB, these species was much lower than naturally found in GB and QACs accumulating plant species under stress conditions (Rhodes and Hanson 1993). Jha et al. (2011) stated that the action of osmoprotectants against salinity stress, paddy variety GJ-17 was treated with rhizospheric *B. pumilus* and endophytic *P. pseudoalcaligenes* and the mixture of both in this study, the overall expression of QACs was also lower. But PGPRs *B. pumilus* and *P. pseudoalcaligenes* help in accumulation of QACs in paddy rice leaves under saline condition; its level was highest in case of paddy inoculated with *P. pseudoalcaligenes* and *B. pumilus* both than inoculated with either of one at different salinity levels. Because the level of GB and QACs in the plant tissue serves as an index of the internal water status of the plants (Hanson and Nelson, 1978), an increase in the level of GB and QACs under stress conditions was found to increase the sodium-flux from cytoplasm to vacuole and was also known to modify the membrane behavior. In addition to osmoregulation, it stabilizes the oxygen

evolving activity of photo system-II protein complex by protecting against dissociation of regulatory extrinsic proteins and also stabilizes manganese cluster (Yeo, 1998).

2.8.5.2 Proline

Proline accumulated within cells to combat salt stress (Matysik et al., 2002; Gupta and Huang, 2014). Proline is one of the most common osmoprotectants that accumulates in plants during salt stress, thereby ameliorating the negative effects of salinity. Proline has been observed to protect cell walls under osmotic stress, protect protein integrity, and increase enzymatic activity by acting as a molecular chaperone. Proline also has a role in scavenging ROS and shows singlet oxygen quenching ability (Kaur and Asthir, 2015). Despite these benefits, there are conflicting reports on the role of endophytic fungi in proline accumulation in salt stressed plants. Several studies reported that increases in proline content in inoculated plants compared to non-inoculated plants, while others report that lower proline content in inoculated plants. Higher proline content in inoculated plants has been attributed to: (i) favouring a decline in ionic influx inside cellular masses, thus helping plants to maintain their osmotic balance; (ii) increasing the expression of the gene encoding Pyrroline-5-carboxylate synthase (P5CS) enzyme, which is involved in proline biosynthesis; and (iii) increasing the activity of the P5CS enzyme (Rawat et al., 2016). Besides its role as an osmolyte, proline can act as a stress marker. Endophytes inoculated tomato plants; proline accumulation was reduced when the toxic effects of salinity were reduced following colonization by an endophytic fungus, *Piriformospora indica* (Abdel aziz et al., 2019).

Omer et al. (2020) studied the response of salinity-stressed on flax plants inoculated with endophytic bacterial isolates. It was observed that significant increase in the contents of free proline in stressed-flax plants as a result of inoculation with the endophyte *A. brasilense* by 17.46%. *Pseudomonas geniculata* inoculation insignificantly increased significantly increased proline contents by 7.94% in salinity stressed flax plants when compared with the uninoculated plants. Co-inoculation with two bacterial strains (*A. brasilense* and *P. geniculata*) significantly increased the contents free proline by 1.58% in shoots of stressed-flax plants.

Sofy et al. (2021) reported that in salt-stressed plants, the osmolyte proline content increased significantly compared to the non-saline plant. The irrigated pea plants with 75 or 150 mM NaCl solutions show significant increases in proline content of approximately

124.41% 109.68%, respectively, compared to untreated plants. The most pronounced increase in relieving stress was observed when *B. subtilis* treatment was used, followed by treatment with *P. fluorescens*. The treatment of endophytic bacteria (viz., *B. subtilis* and *P. fluorescens*) to the pea plant caused a significant increase in the proline of pea plants under saline-stressed condition compared to untreated plants. The best improvement was observed in proline content when the plants were treated with *B. subtilis* when irrigated with 75 mM NaCl or non-saline solution.

Proline is among the most prevalent amino acids reported to accumulate regularly in plant cells in relation to salinity stress (Mansour and Ali 2017). In this study, *A. brasilense* and *P. geniculata* and their co-inoculation increased proline contents in the flax plants grown in saline soil. These findings are consistent with the results of (Huang et al., 2013; Gharsallah et al., 2016; Abdel Latef et al., 2018, 2019; Badawy et al., 2021). Moslemi et al. (2011) increased proline content and increased plant resistance to dehydration have been reported due to inoculation with microbial strains *Azospirillum lipoferum* and *Pseudomonas putida* bacteria. Moreover, those bacterial strains were challenged with reactive oxygen species by modifying the plant's defensive reaction and, as a result, co-operating plants in rising proline content. Mostly on the positive side, proline accumulation has been shown to enhance salt stress tolerance through leading towards osmotic salt stress adjustment in chickpea (*Cicer arietinum*) and canola (*Brassica napus*) (Ahmad et al., 2015; Li et al., 2017; Abdel Latef et al., 2021).

Vaishnav et al. (2019) studied that, a PGPB *Pseudomonas simiae* AU (MTCC-12057) was found to successfully inter-colonize with soybean and mungbean under salt stress. A study was conducted on a mutation in this bacterium for overproducing ACC deaminase. *P. simiae* AU was subjected to different mutagenesis treatments with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (NTG), ethyl methane sulfonates and ultraviolet rays + NTG. Of 25 selected bacterial mutants, one mutant strain, AU-M4, recorded ACC deaminase activity (98 nmol/mg/h) as compared to the wild strain AU (79 nmol/mg/h). The wild AU and mutant AU-M4 bacterial strains were inoculated into mungbean and soybean plants for induced tolerance against salt stress. In both plants, bacterial inoculation had a positive impact during salt treatment. Since overproduction ACC deaminase activity AU-M4 under saline condition might divide ACC into α -ketoglutarate and NH_4^+ and thereby have higher proline

accumulation in soyabean and mung than other strains and therefore endophytes may be used as osmoprotectant in plants (Vashnav et al., 2019).

Afridi, et al. (2019) studied effect of *K. rhizophila* and *C. sakazakii* on wheat crop varieties Pasban 90 and Khirman. The result revealed that the proline accumulation in leaves of both wheat varieties was significantly increased in both the inoculated and uninoculated plants under salt stress conditions. The accumulation of proline, however, was regulated more by *C. sakazakii* than *K. rhizophila* in both Khirman and Pasban 90 at 80 and 160mM NaCl. The increase in proline contents was upto 72% and 83% in Khirman inoculated with *K. rhizophila* and *C. sakazakii*, respectively.

2.8.5.3 Sugars

In salt stressed plants, the accumulation of total soluble sugars, such as glucose, sucrose, dextrans and maltose, serves as an osmoprotectant as they can stabilize the cell membrane and protoplast. These sugars also protect water soluble enzymes from high intracellular concentrations of inorganic ions (Liang et al., 2018). The synthesis of soluble sugars from starch and sucrose in plants is upregulated by the activities of sucrose anabolizing enzymes such as α and β amylase, which convert starch into dextrans and maltose, respectively (Preiss, 2018). Sucrose phosphate synthase and sucrose synthase catalyse the synthesis of sucrose, while 3-fructofuranosidase catalyses the breakdown of sucrose to glucose and fructose (Peng et al., 2016). In plants grown under saline conditions, sucrose undergoes decomposition in order to meet the requirements for glucose (Munns and Tester 2008). There have been reports that show the role of endophytic fungi in enhancing accumulation of soluble sugars in salt stressed plants (Uma Shaanker 2014; Zhang et al., 2019). These sugars act as chemo attractant signals to soil rhizobia (el Zahar Haichar et al., 2014). These chemo attractants can direct movement to microorganisms in response to chemical gradients a behaviour known as chemotaxis. This chemotactic response of microorganisms to root exudates play key role in initiating communication between plant roots and microbes. Yang et al. (2015) reported that the colonization by *Phomopsis liquidambari* could stimulate sugar secretion from the rhizode position of sloughed off cells and root debris of rice, thereby providing carbon to the endophytic fungi. Another study of *P. liquidambari* on peanut showed increased soluble sugar contents in leaves. This was due to the ability of the fungus to form tripartite symbiotic associations with peanut roots and

rhizobia. This tripartite association significantly enhanced peanut nodulation (Zhang et al., 2017). Here, sucrose derived from photosynthesis was transported to bacterial inoculated root nodules and was hydrolysed by sucrose synthase into UDP-glucose and fructose. This was due to the allocation of more carbon by the endophyte toward peanut and rhizobia symbionts by increased soluble sugar content, leading to more active nodule carbon metabolism in inoculated plants. Furthermore, Sherameti et al. (2005) also suggested that one of the major starch-degrading enzymes, glucan-water dikinase, activated by the fungus in colonized roots, is responsible for the increase in soluble sugars in fungal inoculated plants. Similar results were obtained by Ghabooli (2014) with *Piriformospora indica* increasing the level of soluble sugars, including glucose, fructose, and sucrose, in inoculated plants under salt stress conditions. Recently, Zhang et al. (2019) demonstrated that *T. harzianum* improved salt tolerance of cucumber seedlings by enhancing accumulation of sugars. Results in adjustment of the osmotic potential for cellular water retention and turgor maintenance, thereby minimizing the adverse effects of salt stress by balancing the solute potential (Bai et al., 2019).

Omer et al. (2022) results showed that the response of salinity-stressed flax plants to inoculation with endophytic bacterial isolates. Significant increases were recorded in the contents of soluble sugars, in stressed-flax plants as a result of inoculation with the endophyte *A. brasilense* by 69.5%. *Pseudomonas geniculata* inoculation insignificantly increased sugar contents about 34.6% in salinity- stressed flax plants when compared with the uninoculated plants. Co-inoculation with two bacterial strains (*A. brasilense* and *P. geniculata*) insignificantly increased the contents of sugars by 13.19% while significantly increased the contents of soluble protein and free proline by 48.60% and 1.58% respectively, in shoots of stressed-flax plants.

The endophytic bacterial isolates *A. brasilense* and *P. geniculata* and their co-inoculation enabled flax plants to maintain considerable levels of total soluble sugars when they were grown under salinity stress conditions. These results implied that *A. brasilense* and *P. geniculata* and their co-inoculation assisted elevation of sugar which might have contributed to osmotolerance of salt-exposed flax plants by improving water status, stabilizing membranes and protecting enzymes from denaturation (Singh et al., 2015). Moreover, these increases in the contents of soluble sugars can act as energy resources for plants under abiotic stress (Sami et al., 2016).

Sofy et al. (2021) recorded that in salt-stressed pea plants, the total soluble sugars increased significantly compared to the non-saline plant. The irrigated pea plants with 75 or 150 mM NaCl solutions show significant increases in total soluble sugar of approximately 75.75%, 61.29%, respectively as compared to untreated plants.

2.8.5.4 Lipid peroxidation

Malondialdehyde (MDA), a product of lipid peroxidation, is generally regarded as an indicator of free radical damage to cell membranes caused by oxidative stress. Zhang et al. (2016) reported that salt stressed inoculated plants had a 15% reduction in MDA compared to salt stressed non-inoculated plants. The complex interaction between tolerance and reaction to salt stress is a complex phenomenon that involves dynamic changes in development, physiology, metabolic pathways, and gene expression (Atkinson and Urwin 2012; Munns and Tester 2008). Nitric oxide (NO) and compounds to combat formation of reactive oxygen species (ROS). NO directly or indirectly triggers expression of several redox-regulated genes. NO also reacts with lipid radicals thus preventing lipid oxidation, exerting a protective effect by scavenging superoxide radicals and formation of peroxynitrite that can be neutralised by other cellular processes. Nitric oxide also helps in the activation of many antioxidant enzymes including catalase (CAT), ascorbate or thiol-dependent peroxidases (APX), glutathione reductases (GR) and superoxide dismutase (SOD). Salinity increases the level of lipid peroxidation (Hernandez 2019; Yu et al., 2020) which results in higher membrane permeability and loss of ions from the cells (Gupta and Huang 2014). NaCl treatment of inoculated plants resulted in higher rates of lipid peroxidation in salt-sensitive plants than in salt-tolerant plants suggesting that the rate of lipid peroxidation can be used as an indicator to measure how effectively inoculated plants cope with salt stress (Baltruschat et al., 2008). These changes were consistent with the decrease of leaf lipid peroxidation observed in these experiments (Waller et al., 2005). Similar results were shown by Mastouri et al. (2010) and Zhang et al. (2001) where inoculated plants had significantly reduced accumulation of lipid peroxides than control plants under salt stress.

Omer et al. (2022) reported that application of endophytic bacteria *A. brasilense* significantly decreased the contents of MDA by 40.77% and H₂O₂ by 15.85% in flax plants grown under salinity stress conditions result in decline in the contents of malondialdehyde and hydrogen peroxide in flax plants grown in salinized soil Moreover, salinity-stressed flax

plants that were inoculated with the bacterial strain *P. geniculata* observed significant decrease in the contents of MDA by 42.52% and H₂O₂ by 15.03% when compared to un-inoculated stressed plants. Dual inoculation with two bacterial strains *A. brasilense* and *P. geniculata* diminished salinity stress effects in flax plants by declining MDA and H₂O₂ contents by 23.55% and 13.59%, respectively.

The malondialdehyde content represents as a final metabolite of the lipid peroxidation process, which results in disruption to cell membrane components, especially lipid materials (Attia et al., 2021). These results are in line with other researchers who have documented the role of endophytic bacteria in diminishing oxidative damage caused by salinity stress throughout decreasing MDA and H₂O₂ contents (Bharti et al., 2016; Abdel Latef et al., 2021).

Sofy et al. (2021) revealed that *B. subtilis* and *P. fluorescens* treatment caused highly significant decrease in H₂O₂ and MDA content in pea shoots. Further, these isolate in MSI content of pea shoots under non salt or salt stress condition. Salt stress increased oxidative stress markers including (MDA, H₂O₂) in shoots of pea plant by 69.73%, 58.14%, 182.24%, and 90.70%, compared to non saline stress.

Abd_Allah et al. (2017) noticed that plant growth-promoting endophytic bacteria can stimulate stress tolerance of chickpea plants under saline soil conditions. They investigated the stress-adaptive mechanisms of chickpea plants mediated by *Bacillus subtilis* (BERA 71) under saline conditions. Salinity increases the production of hydrogen peroxide, which has a direct impact on the membrane integrity. The percent increase in hydrogen peroxide and MDA due to NaCl was 62.9%, and 41.05% respectively. In contrast, *B. subtilis* decreased the hydrogen peroxide (23.5%) and MDA (27.5%) levels in plants under non-stress conditions, and it significantly mitigated the detrimental effects of salinity by reducing the hydrogen peroxide and MDA levels by 23.7% and 25.2% respectively.

Other important osmolytes in plants are organic acids such as citric acid and malic acid. They are found in plant vacuoles and the regulation of their metabolism plays a crucial role in providing tolerance to salt stress (Guo et al., 2010). Fungal endophytes have been reported to induce the release of organic compounds by the Plant roots (Yang et al., 2015; Zhang et al., 2014), thus influencing the concentrations and profile of organic acids in plants. One of the major plant nutritional disorders associated with increased salinity in soil is iron

(Fe) deficiency. Endophytes can enhance Fe acquisition by their host through their ability to secrete organic acids which chelate and solubilize iron in the soil (Chen et al., 1998; Khan et al., 2006). A study by Zhao et al. (2014) demonstrated that the release of organic acids from endophytes, resulted in ferric solubilization to form organic ferric salts that can be assimilated directly by plants under saline conditions. It has also been shown that inoculated plants have better nutrient uptake capacity and distribution within plant tissues due to modulation of the root architecture and nutrient availability in the soil. These benefits are imparted by increase in organic acids produced by inoculated plants (Samolski et al., 2012; Zhao et al., 2014).

Polyamines (PA) are low molecular weight nitrogenous aliphatic molecules that participate in physiological processes such as activation of antioxidant systems, cell growth and development, and in cellular osmoregulation in plants under salt stress (Singh et al., 2018). PA also regulates ion channels, either by direct binding or via PA-induced signaling molecules (ROS and NO). PAs also regulate the activity of ion channels indirectly by membrane depolarization. The hyperpolarization-activated Ca^{2+} influx and the NO-induced release of intracellular Ca^{2+} result in a higher cytoplasmic Ca^{2+} concentration, which is a major component in general stress responses such as stomatal movements (Wani, 2018; Williams, 1997). They are either presenting in free, soluble conjugated (covalently conjugated with small molecules such as phenolic acids) or insoluble (bound with macromolecules such as proteins, DNA and RNA) forms. These compatible solutes accumulate under salt stress and include putrescine (Put, diamine), spermidine (Spd, triamine) and spermine (Spm, tetramine) (Minocha et al., 2014; Todorova et al., 2016). Differences in PA (Put, Spd, Spm) responses under salt-stress have been reported in several species (Singh et al., 2018) and it remains unclear which polyamine plays the major role in imparting salt tolerance. Chen et al. (2019) demonstrated that the putrescine content was significantly reduced in inoculated plants compared to non- inoculated plants in high stress conditions whereas spermidine and spermine content showed the opposite pattern. It was suggested that salinity stress tolerance induced by endophytic fungus *Epichloe bromicola* correlated with enhanced conversion of putrescine to spermidine and spermine. The fungus also converted the free forms and soluble conjugated forms of polyamines to insoluble bound forms of polyamines. Modulation of the polyamine pool to help tolerate salt stress by

arbuscular mycorrhizal fungi (AMF) is well explored (Evelin et al., 2009). However, research on polyamine metabolism during the interactions between endophytic fungi and plants under salt stress is underrepresented and many questions remain unanswered. For example, most plant polyamine research relates to changes in free polyamines, and where polyamine conjugates have been measured, substantial changes have been detected. The precise role of polyamines, free or conjugated, in inoculated plants remains unclear. Further investigations, focusing on understanding endophyte-facilitated modulation of polyamines, including the intracellular localization of free polyamines and conjugates associated with salt tolerance in plants, is needed. Already some of the key genes involved in the biosynthetic pathways have been cloned making it possible to manipulate polyamine metabolism using molecular genetic approaches (Malmberg et al., 1998). Hence, genetic manipulation of polyamine levels in inoculated plants may allow valuable insights into the role of these compounds especially in studies of plant tolerance to salt stress.

2.8.5.5 Nutrient acquisition and ionic homeostasis

High salt (Na^+ and Cl^-) in the soil disturbs nutrient availability by imposing competition during uptake, translocation or distribution within the plant. This may suppress nutrient associated activities resulting in undesired ratios of $\text{Na}^+ : \text{K}^+$, $\text{Na}^+ : \text{Ca}^{2+}$ and $\text{Ca}^{2+} : \text{Mg}^{2+}$ (Munns et al., 2011). This in turn results in imbalance among ionic composition of the plant subsequently affecting plants physiological traits (Hasegawa et al., 2000; Munns et al., 2006). However, endophytic symbiosis has been shown to improve assimilation of nutrients and assist in maintenance of ionic homeostasis in host plants grown in saline conditions. Although the effects of AM fungi on plant nutrient acquisition are commonly discussed based on the differences of nutrient concentration in plant tissues, the relative uptake rate of nutrient elements (RUR) has recently been suggested as a better tool to distinguish the differences among treatments over a short period, as the nutrient concentration could be largely influenced by the dilution effect of fast growth in young plants. Balliu et al. (2015) found that RUR values of inoculated tomato plants grown in both non-saline and moderate saline conditions were higher than in non-inoculated seedlings. Similarly, another study showed the enhancement effect of AMF inoculation on the nutrient uptake capacity of cucumber seedlings after salt stress (Sallaku et al., 2019).

2.8.5.6 Na⁺: K⁺ ratio

Increased levels of Na⁺ in cells impair important biochemical mechanisms required for plant growth and survival. Sodium accumulation alters cellular Na⁺: K⁺ ratios thereby reducing the availability of K⁺ that is required for activity of various enzymes and for the regulation of osmotic pressure and stomatal closure. Increased Na⁺ also competes with K⁺, disrupting cellular metabolism in roots and leaf tissues (Abdelaziz et al., 2017). This eventually increases the Na⁺: K⁺ ratios in the cytosol, and subsequently disrupts enzyme activity, protein synthesis, turgor maintenance, photosynthesis and stomatal movement (Evelin et al., 2019). High Na⁺: K⁺ ratios in plants indicate higher levels of stress. Hence, plants must maintain low levels of Na⁺ to be able to resist the deleterious effects of salinity. Inoculated plants consistently have lower Na⁺: K⁺ ratios than non-inoculated plants under saline conditions. Reza Sabzalian and Mirlohi (2010) demonstrated that the toxic effect of Na⁺ was mitigated in grasses inoculated with endophytic fungi by increasing K⁺ concentration and thus maintaining the Na⁺: K⁺ ratio in plants. Similar results were found by Song et al. (2015) and Alikhani et al. (2013) where endophytic fungi modulated ion accumulation in colonized barley plants by decreasing the foliar Na⁺: K⁺ ratio. Restricting the transport of Na⁺ to leaves and ensuring a low cytosolic Na⁺:K⁺ ratio are important ways plants can increase their tolerance to high salt levels (Berthomieu et al., 2003; Cuin et al., 2003). Increase of K⁺ concentration is also related to mechanisms that control turgor pressure (Beckett and Hoddinott, 1997). Song et al. (2015) also showed that the lower Na⁺: K⁺ ratios observed in inoculated plants decreased the level of toxic ions and osmotic influence on plants under salt stress. Another study on barley plants inoculated with endophytic fungi showed a decreased Na⁺: K⁺ ratio compared to uninoculated plants, indicating that this ratio is a reliable indicator of the severity of salt stress, or for screening plant genotypes for high Na⁺ tolerance (Ghabooli, 2014). Plants control Na⁺ homeostasis through a variety of membrane proteins, antiporters, nonspecific cation channels, Na⁺ and K⁺ transporters, vacuolar ATPases and aquaporins, and the plasma membrane (PM) (Grabov, 2007). Recently, Abdelaziz et al. (2017) postulated a molecular basis of establishing a balanced ion homeostasis of Na⁺: K⁺ ratio in ENC plants. Inoculated *Arabidopsis* plants had enhanced transcript levels of the genes encoding the high Affinity Potassium Transporter 1 (HKT1) and the inward-rectifying K⁺ channels KAT1 and KAT2, which play key roles in

regulating Na^+ and K^+ homeostasis. Subsequently, lower $\text{Na}^+ : \text{K}^+$ ratios were confirmed in the Arabidopsis line gl1-HKT: AtHKT1; 1 that expresses an additional AtHKT1; 1 copy driven by the native promoter. This study demonstrated that endophytic colonization promotes plant growth under saline conditions by modulating the expression level of the major Na^+ and K^+ ion channels, which helps in the establishment of a balanced ion homeostasis of Na^+ and K^+ under salt stress conditions (Abdelaziz et al., 2019).

2.8.6 Antioxidative enzymes

Salt stress (osmotic and ionic stress) also interferes with proper cellular functions of plants due to enhanced production of ROS, which can lead to oxidative damage in several cellular components such as lipids, proteins and DNA (Gupta and Huang, 2014). ROS consist of a group of chemically reactive oxygen molecules such as hydroxyl radical ($\text{OH}^\cdot$), H_2O_2 , $\text{O}_2^\cdot$ and O and are produced as a result of interrupted pathways in plant metabolism that cause transfer of high energy electrons to molecular oxygen (Gill and Tuteja, 2010). Broad host range endophytic fungi can confer effective tolerance to ROS under abiotic stress conditions such as salinity (Mastouri et al., 2010) reported that exposure to high salt conditions caused ROS accumulation in tomato, rice, panic grass, and dune grass without endophytic associations, whereas the inoculated plants had decreased ROS accumulation through various pathways. Plants have two ways to counteract the adverse consequences of ROS, mainly enzymatic and non-enzymatic antioxidative systems. The enzymatic system includes catalase (CAT), ascorbate peroxidase (APX) and superoxide dismutase (SOD) that play roles in quenching toxic byproducts of ROS. Baltruschat et al. (2008) reported increased activity of CAT and APX in the root tissues of barley under saline conditions. Azad and Kaminskyj (2016) used H_2O_2 localization as a proxy to assess accumulation of ROS and showed that inoculated plants had lower H_2O_2 levels in their leaves following NaCl-stress, confirming the role of endophytes to reduce stress-induced ROS generation. Also, Zhang et al. (2016) reported that inoculated plants had higher SOD, peroxidase (POD) and CAT activity suggesting that the coordination of POD and CAT activity along with SOD activity played a central protective role in the O_2 and H_2O_2 scavenging process in inoculated plants (Ahmad et al., 2015). Increased activity was a result of increased expression of the genes encoding the enzymes (Zhang et al., 2016).

Abd_Allah et al. (2017) reported that the effect of plant growth-promoting endophytic bacteria can stimulate stress tolerance of chickpea plants under saline soil conditions. They investigated the stress-adaptive mechanisms of chickpea plants mediated by *Bacillus subtilis* (BERA 71) under saline conditions. The activity of antioxidant enzyme SOD was stimulated by 12.2% in plants due to the effect of *B. subtilis*. Salinity stressed chickpea plants exhibited enhancement of SOD (63.7%), activity when compared to their controls. However, the inoculation of NaCl-treated plants with *B. subtilis* led to a further increase in the activities of SOD (5.4%).

The activity of antioxidant enzymes, including CAT was stimulated by 8.7% in plants due to the effect of *B. subtilis* while in uninoculated plants was severely decreased under salinity stress, while bacterial interactions in plants stimulated CAT activity.

Omer et al. (2022) reported that activities of antioxidant enzyme enhanced in salinity-stressed flax plants when inoculated with the endophytes *A. brasilense* and *P. geniculata*. Treating flax plants with *A. brasilense* inoculum enhanced the activity of antioxidant enzyme SOD by 50%, when compared with control plants (grown in salinized soil without bacterial inoculation). Also, endophytic bacterial strain *P. geniculata* significantly increased antioxidant enzyme activities in flax plants such as SOD by 125%. Co-inoculation with the two bacterial strains *A. brasilense* and *P. geniculata* showed insignificant enhancements in the activity of SOD by 8.33%.

Sofy et al. (2021) studied antioxidative enzyme SOD enzyme activity in shoots of pea plants applied with isolated endophytic bacteria *B. subtilis* and *P. fluorescens* under different levels of NaCl. In general, the activity of SOD increased significantly under concentrations of NaCl and *B. subtilis* and *P. fluorescens* treatment alone or in combination with NaCl stress increased the activity of SOD energized the removal of toxic ROS. SOD is a vital antioxidant enzyme used to scavenge superoxide radicals, hydroxyl (OH) radicals and H₂O₂. SOD Higher activity in chickpea has reported in response to salinity (Rasool et al., 2013).

CAT enzyme activity was enhanced in shoots of pea plants applied with isolated endophytic bacteria (viz, *B. subtilis*, *P. fluorescens*) under different levels of NaCl. Excessive CAT activity in tomato plants under saline condition can be attributed to reduce H₂O₂ content (Mayak et al. 2004). Thus CAT play vital role during salt stress and removes H₂O₂ to prevent damage to the membrane and organs (Sofy et al. 2021). Acceleration of

POX activity in *B. subtilis*, *P. fluorescens* inoculated pea plants under salinity condition was observed by Sofy et al. (2021) which lead to improved lignin biosynthesis and other associated antioxidant compounds to reducing oxidative stress (Van holme et al., 2010). Chen et al. (2007) observed that POX activity in *Vigna unguiculata* increased under salt stress. Habib et al. (2016) reported that okra plant inoculation with ACC deaminase-producing PGPR enhanced antioxidant-coding genes, contributing to improved salt stress tolerance. Likewise, Younesi and Moradi (2014) reported improved antioxidant systems caused by PGPR. Maintenance and accumulation of redox components have been reported in stress-resistant plants (Agami et al., 2016). Omer et al. (2022) APX increased treating flax plants with *A. brasilense* inoculum enhanced the activities of antioxidant enzyme APX by 41.91%, when compared with control plants (grown in salinized soil without bacterial inoculation). Also, endophytic bacterial strain *P. geniculata* significantly increased APX activity in flax plants by 49.26%. Co-inoculation with the two bacterial strains *A. brasilense* and *P. geniculata* showed insignificant enhancements in the activity of APX by 18.38%.

Antioxidants involve numbers of antioxidant enzymes that play an effective function in protecting plants from oxidative stress damage (Hahm et al., 2017). Enhancing the activities of antioxidant enzymes has supported plants to face and cope with different stresses such as salinity (Ahammed et al., 2019; Tahjib-Ul-Arif et al., 2019; Osman et al., 2021; Dawood et al., 2021). Abdel Latef et al. (2020) reported that inoculation of salinity-stressed maize plants with *Azospirillum lipoferum* led to enhancements in antioxidant enzyme activities as peroxidases (about 25%) and ascorbate peroxidases (about 41%). In another study, the activity of ascorbate peroxidase was increased in stressed basil (*Ocimum basilicum* L.) plants in response to the inoculation with endophytic bacterial species *Pseudomonas* sp. and *A. brasilense* either individually or in combination (Heidari and Golpayegani, 2012). Liu et al. (2019) they stated that activities of SOD was induced in alfalfa plants grown under saline-alkali conditions about 1.20 more than that in control (untreated plants) as a result of inoculation with *Pseudomonas aeruginosa*. It is well known that antioxidant enzymes possess the ability to scavenge the resulted free radicals in the cell during exposure to different abiotic stresses.

Afridi et al. (2019) inoculated *K. rhizophila* and *C. sakazakii* on wheat crop varieties Pasban 90 and Khirman and that the activities of antioxidants SOD were evaluated in

uninoculated and inoculated plants with different salinity treatments. Lower antioxidant activity was observed in uninoculated plant tissues than the inoculated ones, when salinity stress was applied. The SOD activities were higher in both varieties under salt stress when inoculated with *K. rhizophila* and *C. sakazakii* as compared to the uninoculated plants, although the activity of SOD was higher in Pasban 90 than Khirman at 160mM NaCl. Furthermore, the activities of these antioxidants were higher in Pasban 90 than in Khirman. The overall ratio of antioxidant activities was higher in Pasban 90 than in Khirman. In general, the order of antioxidant activities of the enzymes relative to PGPEs was observed as *C. sakazakii* > *K. rhizophila* > Control. Afridi et al. (2019) the activities of antioxidants CAT was evaluated in uninoculated and inoculated plants with *K. rhizophila* and *C. sakazakii* under different salinity treatments. Lower antioxidant activity was observed in uninoculated plant tissues than the inoculated ones, when salinity stress was applied. The CAT activity was higher in both varieties under salt stress when inoculated with *K. rhizophila* and *C. sakazakii* as compared to the uninoculated plants. Furthermore, the activity CAT was higher in Pasban 90 than in Khirman. The overall ratio of antioxidant activities was higher in Pasban 90 than in Khirman. In general, the order of CAT activity of the enzymes relative to PGPEs was observed as *C. sakazakii* > *K. rhizophila* > Control. Afridi et al. (2019) emphasized that antioxidants are one of the indicators of a plant's tolerance to stresses. Catalase, SOD and POD enzymes are low molecular weight antioxidants produced by plants to confer salt stress tolerance (Xu et al., 2015). A majority plant species, however, do not produce an adequate amount of antioxidants to cope with the drastic effects of salinity stress. The increased enzyme activities of POD, CAT, SOD and proline indicates the mitigation of the oxidative stress caused by high NaCl in maize plants (Akram et al., 2016) Gladiolus plants can tolerate salt stress by increased production of proline, POX and other defense enzymes (Panuccio et al., 2014; Khan et al., 2016).

In conclusion the earlier reports indicated that the endophytes inoculated to crop plants under salinity condition ameliorate the salinity stress by increasing leaf area, root shoot height, chlorophyll contents, proline, glycine betaine, sugars, ascorbate peroxidase, superoxide dismutase, catalase decreasing membrane injury index, chlorophyll stability index and lipid peroxidation.

3. MATERIAL AND METHODS

The present study entitled "Physio-chemical studies on effect of endophytic bacteria in combating induced salinity stress in sugarcane" was conducted at the Department of Biochemistry, MPKV, Rahuri during the year 2016-2018.

3.1 Materials

3.1.1 Glassware and plastic wares

The glassware's and polywares used were of standard make such as Borosil. All were scrubbed and washed thoroughly with the detergent then rinsed with tap water followed by distilled water. Finally, it was dried in an oven before use. All poly wares were thoroughly cleaned air-dried before use. It includes conical flask, beaker, measuring cylinders, bottles, sterile petri plates, culture tubes etc.

3.1.2 Equipment

Different equipment which were used for research work includes laminar air flow, centrifuge, ultracentrifuge, water bath, spectrophotometer, vertical gel electrophoresis unit, Shaker, deep freezer, autoclave, water distillation unit etc.

3.1.3 Culture media

The culture media used in this study includes different type of complex, selective and chemically defined media. These culture media are nutrient agar and broth, semi-solid LGIP media, N-free malate medium, Rennies media and Azoarcus media.

3.1.4 Preparation of media and reagents

The culture media was prepared carefully by weighing the chemicals accurately using electronic balance and were dissolved in sterile distilled water and the same procedure was followed for reagent preparation. The culture media was sterilized by autoclaving process (121°C for 15 minutes) at 15 lbs pressure.

3.2 Soil analysis from salt the affected area

Soil samples depth (0-30cm) were collected from salt affected site, cleaned, filled in polythene bags, sterilized by autoclaving. The soil samples were air dried, sieved and used for analysis of various parameters like pH and Electrical Conductivity (EC).

3.2.1 Salinity stress induction

Procedure

The sets of sugarcane cultivars were obtained from CSRS, Padegaon, washed and sterilized with 0.1% sodium hypochlorite and two eye bud sets of cultivars CoM 0265 and CoC 671 were grown in each pots, containing 5 kg soil. The saturation point was measured which was found to be 1200 mL. As per the saturation point for the induction of salinity stress, standard stock of 32 and 62 dSm⁻¹ was used. After 60 days after planting the NaCl stress of ~ 0 dSm⁻¹, ~ 2dSm⁻¹, ~ 4 dSm⁻¹ and ~ 8 dSm⁻¹ NaCl was imposed to each plant by adding 0 mL as control, 400 mL (32 dSm⁻¹), 800 mL (32 dSm⁻¹) and 900 mL (62 dSm⁻¹) of standards stock solution to obtained the desired EC (Table 1). For control plants it was irrigated with distilled water. Then 105 days after planting third leaf sample was taken for further analysis.

Table 1. Optimization of ECe of the soil.

NaCl solution added (mL)	Distilled water added (mL)	Soil ECe (dSm ⁻¹)
00	1200	0.41
400 (32 dSm ⁻¹)	800	2.31
800 (32 dSm ⁻¹)	400	4.21
900 (62 dSm ⁻¹)	300	8.01

3.2.2 Pot culture experiment treatment details

1. Control , 2 dSm⁻¹, 4 dSm⁻¹ and 8 dSm⁻¹ salt stress
2. Consortia + control, Consortia+2, 4 and 8 dSm⁻¹ salt stress
3. GA + control, GA +2 dSm⁻¹, GA +4 dSm⁻¹, GA +8 dSm⁻¹ salt stress
4. ASP + control, ASP +2 dSm⁻¹, ASP +4 dSm⁻¹, ASP +8 dSm⁻¹ salt stress
5. HERB + control, HERB +2 dSm⁻¹, HERB +4 dSm⁻¹, HERB +8 dSm⁻¹ salt stress
6. AAR + control, AAR +2 dSm⁻¹, AAR +4 dSm⁻¹, AAR +8 dSm⁻¹ salt stress

3.2.3 Bacterial cultures preparation

The isolated salt tolerant bacterial strains selected were used for broth culture. A loop full of freshly grown bacterial culture was inoculated in the flask containing liquid nutrient media under sterile conditions. Each flask containing 60 ml of broth inoculated with bacterial isolates were then incubated for 72 hrs on BOD shaker (100 rpm) at 37°C. Prior to bacterization an O.D of 0.5-0.6 at wavelength 535 nM was achieved to maintain uniform cell

(1×10^8 CFU ml⁻¹) all these bacterial culture spray on sugarcane plants at an interval of 15 days.

3.3 Soil sampling and chemical analysis of sodic soil used in pot culture

The soil samples were air dried, sieved and used for analysis of various parameters like pH, electrical conductivity (EC), soluble cations (Sodium, potassium, calcium, magnesium), Sodium Absorption Ratio (SAR) and Exchangable Sodium Percentage(ESP).

3.3.1 pH

pH was determined by using electric pH meter in 1:2 soil water suspension prepared by taking 40g of soil into a 250ml flask with 80 ml of distilled water and the mixture was shaken for an hour. A clear filtrate was obtained by passing through Whatman filter paper No 1.

3.3.2 Electrical conductivity

Electrical conductivity of the clear filtrate obtained by 1:2 soil water extract after passing through Whatman filter paper no 1 was measured by using an EC meter.

3.3.3 Soluble cations

Calcium and Magnesium were determined by titration with (0.01N) EDTA (Richards, 1954).

For calcium 10 ml of aliquot (1:2 soil water extract) was taken in a flask. Then 2-3 ml of 2N NaOH solution and a pinch of ammonium purpurate were added as an indicator. Finally titration was carried out with 0.01N EDTA till the end point occurs from red to purple. The readings were recorded in triplicates.

$$\text{Ca}^{+2} \text{ g L}^{-1} = \frac{\text{Normality of EDTA} \times \text{Vol of EDTA} \times \text{Eq.wt of Ca}^{2+}}{\text{ml of aliquot taken}}$$

For calcium + magnesium, 10 ml of aliquot used above was taken in a conical flask with 3-5 ml buffer solution and few drops of EBT (Erichrome black-T) indicator were added. Titration with 0.01N EDTA till end point appears from Red to bluish green.

$$\text{Ca}^{+2} + \text{Mg}^{+2} \text{ g L}^{-1} = \text{Normality of EDTA} \times \text{Vol of EDTA} \times \text{Eq.wt of Ca}^{++} \text{ ml of aliquot taken}$$

$$\text{Mg}^{+2} \text{ g L}^{-1} = (\text{Ca}^{++} + \text{Mg}^{++} \text{ g L}^{-1}) - (\text{Ca}^{++} \text{ g L}^{-1}).$$

All the readings were in gm/l. For conversion in Mg/kg of soil following conversion was applied

$$\text{Mg/kg} = \text{Reading in g L}^{-1} \times \text{dilution} \times 2$$

3.3.4 Sodium and Potassium

Sodium and potassium in the samples were determined by flame photometer. 1:2 soil water suspensions was prepared and put on shaker for about an hour followed by filtration through Whatman filter paper No. 1. The filtrate obtained was used for analysis of sodium and potassium ions.

$$\text{Soluble cations (Na, K) mg/kg} = \frac{R \times 50}{\text{Soil wt. in gms}}$$

R = Reading obtained by flame photometer, 50 = water used in soil suspension.

SAR is the index of sodium ions are present in water that can be absorbed by the soil. It was calculated by the formula

$$\text{SAR} = \frac{\text{Na}^+}{\sqrt{\text{Ca}^{+2} + \text{Mg}^{+2}}}$$

3.4 Isolation of endophytic bacteria

The isolation of endophytic bacteria was carried out on selective media (Table 2) by enrichment culture technique described by (Aneja, 2003) with some modification. The composition of chemicals used for preparation of media is tabulated in Table 3.

The fresh stem sugarcane plant samples were first washed under tap water.

1. These samples surface sterilized with 0.1% HgCl_2 solution with successive 3 to 4 washings of sterilized distilled water.
2. The stem samples were cut into small pieces and macerated in sterilized mortar and pestle by adding little quantity of sterilized distilled water and extract was made.
3. The outer portion of cane node was removed with sterilized scalpel and small pieces were made.
4. These pieces were then grinded and juice was extracted.
5. The extracts of fresh stem and juice was homogenously mixed together, strained through muslin cloth and collected in 100ml conical flasks.
6. 2 ml sugarcane plant extracts were then transferred to sterilized petri plates of respective selective medium.
7. After a week period when colour of medium changes and showed turbidity, loopful was transferred to fresh sterilized petriplate. Same procedure was repeated for 3 to 4 times.

8. The sterilized selective medium before solidification (45°C temp) was poured in each petri plate and allowed to solidify. Aseptically streaking were made on respective plates with loopful culture from master plate.
9. Then plates were kept at 28±2°C in BOD incubator for 4-5 days.
10. All plates were observed for the appearance isolated bacterial colony.

Table 2. Endophytic bacteria isolated on selective medium.

SR	Isolates	Endophytic bacteria	Media used	Period of incubation (hrs)
1	Isolate -1	<i>Gluconacetobacter spp</i>	LGIP medium	48-72
2	Isolate-2			
3	Isolate-1	<i>Azospirillum spp</i>	Nitrogen free malate	90-100
4	Isolate -2			
5	Isolate-1	<i>Herbaspirillum spp</i>	Rennies medium	48-72
6	Isolate-2			
7	Isolate -1	<i>Azoarcus spp</i>	Azoarcus medium	100-120
8	Isolate-2			

Table 3. Composition of different growth medium used for isolation of endophytic bacteria.

Chemical composition	Media g L ⁻¹			
	LGI-P (<i>Gluconacetobacter</i>)	N-free malate (<i>Azospirillum</i>)	Rennies (<i>Herbaspirillum</i>)	Azoarcus (<i>Azoarcus</i>)
Malic acid	100.0	5	3	2.5
KOH	0.6		0.2	2.5
KH ₂ PO ₄	0.2	4.0	0.8	1.5
MgSO ₄ .7H ₂ O	0.02	0.1	0.2	1.0
NaCl	0.002	0.002	0.004	1.0
Na ₂ MoO ₄ .2H ₂ O	0.01	-	0.2	0.002
CaCl ₂	0.02		0.04	1.0
MnSO ₄ .H ₂ O	-	-	-	0.01
Fe EDTA	-	-	-	0.066
Biotin	-	-	-	0.001
NH ₄ Cl	-	-	-	0.02
Beef Extract	-	-	-	2.2
Yeast Extract	0.5	0.5	0.5	1.0
Agar	30	30	30.0	30.0
Distilled water	1000	1000	1000	1000 ml
pH	5.5	6.6-7.0	7.0	6.6 -7.0

3.4.1 Selection of *Gluconacetobacter* colony

Gluconacetobacter cells grown slimy colonies on LGIP medium and aged cultures showed orange colouration. A loopful of *Gluconacetobacter* colony was purified by streak plate method on fresh LGIP plate for single colony. These purified cultures of *Gluconacetobacter* were maintained on the slants of LGIP medium for storage and characterization.

3.4.2 Selection of *Azospirillum* colony

The isolates showing characteristic subsurface pellicle formation were selected from nitrogen-free malate semi-solid medium in petri plate. The isolates that formed characteristic subsurface white pellicle in this medium were tentatively considered as *Azospirillum*. These purified cultures of *Azospirillum* were maintained on the slants of nitrogen-free malate medium for storage and characterization. The isolates were sub cultured in LB agar plates at 15 days interval. Working cultures were kept in 4°C. It was observed that the isolates were remained alive at 4°C for about month.

3.4.3 Selection of *Herbaspirillum* colony

Herbaspirillum cells grown slimy, colonies on Renneis medium and aged cultures showed light brownish colouration. A loopful of *Herbaspirillum* colony was purified by streak plate method on fresh Rennies medium plate for single colony. These purified cultures of *Herbaspirillum* were maintained on the slants of *Herbaspirillum* medium for storage and characterization.

3.4.4 Selection of *Azoarcus* colony

Azoarcus cells grown as raised, slight slimy, colonies on azoarcus medium and aged cultures showed light brownish colouration. A loopful of *Azoarcus* colony was purified by streak plate method on fresh *Azoarcus* medium plate for single colony. These purified cultures of *Azoarcus* were maintained on the slants of *Azoarcus* medium for storage and characterization.

3.5 Biochemical characterization of endophytic bacteria

Biochemical characterizations of endophytic bacterial isolates were carried out at Department of Plant Pathology and Agril. Microbiology, MPKV, Rahuri.

The isolates were subjected to biochemical characterization by employing the standard procedures according to Burgey's Manual of Systematic Bacteriology and

Cappuccino and Sherman (1987) with some modifications. The biochemical tests viz., H₂S production, gelatin liquefaction, catalase test, oxidase and urease test performed are briefly outlined below.

3.5.1. Catalase test

A loopful of growth was placed on glass slide and emulsified with drops of 3 per cent of H₂O₂ (v/v). The effervescences caused by liberation of free gas bubbles were considered for the presence of catalase in culture.

3.5.2 Oxidase test

A drop of freshly prepared 1% (W/V) solution of Tetramethyl-para-phenylene diamine dihydrochloride reagent was placed on a piece of filter paper. With a sterile loop a test colony was picked and rubbed it on the paper in the area impregnated with oxidase reagent. The paper was become deep purple blue in colour in a few seconds indicating oxidase test is positive for organism.

3.5.3 Urease test

The presence of enzyme urease was detected by inoculating overnight activated cultures of isolates in 3% (W/V) NaCl supplemented Christensen urea agar slants by stab and streak technique and incubated at a temperature of 35⁰C for 48hrs. Uninoculated slant marked as a negative control and a known standard urease positive culture (*Klebsiella pneumoniae*) as a positive control. The phenol red indicator was used in medium, indicated the positive result as it turned into deep pink colour. No colour changes noted as negative reaction and absence of enzyme urease (Christensen, 1946).

3.5.4 H₂S production

SIM agar stabs were inoculated with actively growing culture(S). Blackening along the line of inoculation shows a positive test after 3-5 days at 30⁰C. Observation for blackening in tubes was taken after three days of incubation (Singh et al., 2013)

3.5.5 Gelatin liquefaction

The medium consisting of nutrient broth supplemented with twelve per cent gelatin was used. The test tubes containing medium was inoculated with the bacterial culture and incubated for 24 hrs at 28±2°C. Following incubation, the cultures were placed in a refrigerator at 4°C for 30 min. The cultures that remain liquefied indicated gelatinase and

demonstrate gelatine hydrolysis, whereas the cultures those solidify on refrigeration lack gelatinase activity and gave negative reaction.

3.6 Screening of endophytic bacteria for salt stress tolerance

The endophytic bacteria isolated were screened for salt stress tolerance with the protocol described by Gayathri et al. (2010).

1. The ability of crop plant endophytic bacteria isolates to tolerate a high concentration of sodium chloride was tested on NA solid medium.
2. NA medium was prepared and adjusted with NaCl solutions 2 dSm⁻¹, 4 dSm⁻¹ and 8 dSm⁻¹ for screening salt tolerance limit.
3. The media was autoclaved at 121°C temperature and 15 lbs pressure for 20 min and poured into sterilized petri plates under aseptic condition.
4. A loopful culture of all endophytic bacterial isolates were streaked onto the different NaCl supplemented nutrient agar plates.
5. The inoculaed petri plates were incubated at 28-30°C for 7 days.
6. Colony growth was measured on CFU count instrument.

3.7 Morphological, physiological and biochemical analysis of sugarcane leaf samples

3.7.1 Leaf area

Leaf area was measured by leaf area meter

3.7.2 Shoot height

The shoot lengths measured for each plant by using measuring tape.

3.7.3 Root length

The process of removing the soil from the roots was performed for each plant. The root lengths were measured for each plant immediately after they had been washed thoroughly.

3.7.4 Relative leaf water content

Relative leaf water content (RLWC) was measured by the method described by Henderson and Davies (1990).

Method

Relative leaf water content was determined by collecting the leaves from the mid-section of the grown sugarcane plants. A sharp razor blade was used to recut the leaf and to remove the mid ribs. Leaves of stress and unstressed were then immediately weight for its fresh weight (FW) the leaflets were removed after excision and fresh weight (FW) was taken.

The leaflets were allowed then to float on the distilled water for three hours blotted dry and again weight taken to obtain turgid weight (TW). The leaves were dried in oven at 80 °C for 48 hrs. to obtain dry weight (DW). All measurement was carried out digital analytical balance.

The RLWC was calculated by using the following formula.

$$RLWC = [(FW-DW) / (TW-DW)] \times 100$$

3.7.5 Total Chlorophyll content:

Total leaf chlorophyll content was determined by method described by Arnon (1949) viz., some modifications (Kamble et al., 2015)

Protocol

The leaf samples were cut into small pieces and known weight (0.2g) of fresh leaf sample was macerated in a mortar and pestle to this with 15 ml of 80 % (v/v) acetone and 0.5 g $MgCO_3$ powder was added to prevent acidification and premature phenophytin formation during assay. The material was homogenized and kept 4 °C for 4 hrs in refrigerator. The contents were centrifuged at 5000xg for 10 min and the supernatant was collected. The procedure was repeated with 15 ml acetone till the residue becomes colourless. The final volume of extract was made to 50 ml. The extinction of chlorophyll extract was recorded at 645 and 663 nm on a Spectrophotometer and a blank was run with 80 % acetone.

Calculation

The amount of total chlorophyll content was calculated by using the following formula and expressed in $mg\ g^{-1}\ FW$.

$$\text{Total chlorophyll, } mg\ g^{-1}\ FW = 20.2(A_{645}) + 8.02(A_{663}) \times V / (1000 \times W)$$

Where,

A= absorbance at specific wavelength.

V= Final volume of chlorophyll extract in 80 percent acetone.

W= Fresh weight of tissue extracted in gram.

3.7.6 Membrane stability index

Membrane stability index of leaf samples were determined according to the method of Sairam (1994).

1. Leaf strips (0.2g) of uniform size were taken in to test tubes containing 10 ml of double distilled water in two sets.

2. Test tube in one set were kept at 40°C in water bath for 30 min. Test tubes were cooled under running water and electric conductivity of the water containing samples was measured (C_1) using conductivity bridge.
3. Test tube in the other set incubated at 100°C in the boiling water bath for 15 minute and after cooling the test tube their EC was measured as above (C_2). Membrane stability index (%) was calculated as the reciprocal of relative leakage using formula $MSI (1 - C_1/C_2) \times 100$.

3.7.7 Lipid peroxidation

Lipid peroxidation is oxidative degradation of lipid fatty acid by reactive oxygen species. The level of lipid peroxidation is measured in terms of thiobarbituric acid reactive substance (TBARS) content

Reagent

1. Trichloroacetic acid (0.1%): Trichloroacetic acid solution was prepared by dissolving 0.1g TCA in distilled water and then the volume was made to 100 ml.
2. Thiobarbituric acid reagent: Five gram TBA was dissolved in small amount of 20% (w/v) TCA and then the volume was made to 100 ml by 20% TCA.

Procedure

1. Sample extraction: Leaf sample 0.2g was homogenized in 4 ml of 0.1% TCA. The homogenate was centrifuged at 15000xg for 15 min and the supernatant was used for the estimation of TBARS content.
2. Assay: Four ml of 0.5 % (w/v) TBA was added in 2 ml aliquot of the supernatant. The mixture was heated at 95 °C for 30 min in the water bath and then cooled in an ice bath. After cooling the aliquot was centrifuged at 10,000xg for 10 min. The absorbance of the clear supernatant was recorded at 532 nm. Values of non-specific absorption recorded at 600 nm and were subtracted from the values recorded at 532 nm. The TBARS content was calculated by using extinction coefficient $E = 155 \text{ mM}^{-1} \text{ cm}^{-1}$.

3.7.8 Proline

Proline content in leaf tissues was determined by using the acid ninhydrin reagent as per the method described by Bates et al. (1973)

Reagents:

1. Acid ninhydrin: Ninhydrin (1.25g) was dissolved in 30 ml of glacial acetic acid and 20

ml of 6 M phosphoric acid with agitation.

2. Sulphosalicylic acid (3% w/v): Sulphosalicylic acid (7.5g) was dissolved in distilled water and the volume made to 250 ml.
3. Stock solution of L-proline (100 mM): Standard L-proline (1.15g) was dissolved in distilled water and the final volume made to 100 ml.
4. Working standard solution of L-proline (1 mM): From the stock solution, 1 ml was again diluted to 100 ml with distilled water. This diluted working solution contained 1 μ mole of proline per ml.

Method

Leaf samples (0.2g) were homogenized with 2 ml of 3% (w/v) aqueous solution of sulphosalicylic acid and the homogenate filtered through Whatman No. 2 filter paper. 0.5 ml volume of the filtrate was reacted with 2 ml of acid ninhydrin and 2 ml of glacial acetic acid in a test tube for 1 h at 100 $^{\circ}$ C and the reaction was terminated by placing the tubes in ice. The reaction mixture was extracted with 4 ml toluene and mixed vigorously for 15 to 20 seconds. The chromophore containing toluene was aspirated from the aqueous phase and the absorbance read at 520 nm using toluene as a blank. The proline concentration was determined from the standard curve and calculated on a fresh weight basis.

Calibration of a standard curve

In a triplicate set of test tubes, the working standard solution of proline was pipetted as 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1ml having the corresponding proline concentration of 0 to 100 η moles. The volume was made to 1 ml with distilled water. The colour was developed as described above. The absorbance was recorded at 520 η m and a standard graph of absorbance against concentration of proline was plotted (Fig. 4).

3.7.9 Glycine betaine

Glycine betaine content in leaves was determined by using the Dragendorff reagent as per the method described by Stumpf (1984). The glycine betaine content was expressed as μ g g $^{-1}$ FW.

Reagents:

1. Dragendorff reagent: This reagent was prepared by mixing the equal volume of 0.35 M bismuth nitrate in 20% acetic acid (v/v) and 2.45 M sodium iodide (NaI) in distilled water.

2. Betaine aldehyde solution: The solution was prepared by dissolving 0.5g of betaine aldehyde in 1 ml distilled water.
3. Bismuth nitrate solution (0.35 M): The solution was prepared by dissolving bismuth nitrate in distilled water and 20 per cent acetic acid. The volume was made to 25 ml with distilled water.
4. Sodium iodide solution (2.45 M): The solution was prepared by dissolving NaI in distilled water and the volume was made to 25 ml.
5. Working solution of sodium iodide (0.49 M): Five ml NaI (2.45 M) solution was diluted in distilled water and the volume made to 25 ml.

Method

Leaf sample of 0.2g was crushed in a mortar and pestle in 2 ml of 80% (v/v) ethanol. It was transferred into the eppendorf tubes which were kept in the hot water bath for 20 min. The tubes were then removed from water bath and cooled at room temperature. It was centrifuged at 10,000xg for 20 min and the supernatant was collected in clean eppendorf tubes.

The supernatant (0.2 ml) was pipetted in the microfuge tubes to which 100 μ l Dragendorff reagent was added. The solution was then centrifuged at 7000xg for one min. After centrifugation, the supernatant was drawn with the help of a syringe and the orange pellet was dried. The pellet was dissolved in one ml solution of 2.45 M NaI. From this 200 μ l aliquot was pipetted in test tubes containing 3 ml diluted 0.49 M NaI solution and mixed. The absorbance was read at 467 nm using 0.49 M NaI solutions as a blank.

Calibration of a standard curve

A standard curve for glycine betaine was made by placing 2.5 to 25 μ l of a working solution of betaine aldehyde in a set of 2 ml micro centrifuge tubes (50 mg ml⁻¹) to which 100 μ l of Dragendorff reagent was added causing the precipitation of the QAC. It was then centrifuged at 7000 xg for one min. The supernatant was completely removed. The pellet was dissolved in 1 ml of 2.45 M NaI solution A known quantity of aliquot (30 μ l) was then added to three ml of 0.49 M NaI solution. The absorbance was read at 467 nm using 0.49 M NaI solution as a blank and a standard curve of absorbance vs concentration of glycine betaine was plotted (Fig.5).

3.7.10 Soluble sugar

Sugars were determined by the arsenomolybdate reagent method of Nelson (1944).

Reagents

1. Reagent A: This solution contained 25 g sodium carbonate (anhydrous) 25 g Rochelle salt (Na-K-tartrate), 20g sodium bicarbonate and 200g sodium sulphate (anhydrous) in 1000 ml.
2. Reagent B: Fifteen g of copper sulphate dissolved in D/W containing 1-2 drops of concentrated H_2SO_4 and volume made to 100 ml with D/W.
3. Alkaline copper sulphate: It was a mixture of 25 parts of reagent A and one part of reagent B (v/v).
4. Arsenomolybdate reagent: It was prepared by dissolving 25g ammonium molybdate in 450 ml distilled water. To this 21 ml of concentrated H_2SO_4 acid were added followed by 3 g sodium arsenate dissolved separately in 50 ml water. The contents was mixed and made a volume to 500 ml with distilled water. This solution incubated at 37°C for 48 hrs.
5. Standard D-glucose: D-glucose (100 mg) was dissolved in 100 ml of distilled water. The solution contained $1 \text{ mg D-glucose m}^{-1}$. From this stock solution 10 ml were diluted to 100 ml with distilled water. This diluted solution contained $100 \mu\text{g of D-glucose ml}^{-1}$.

Procedure

For total soluble sugars and reducing sugars, 1 g of leaf sample was macerated in 25 ml 80 % ethanol. The centrifuge tube containing sample was kept in the hot water bath at 100°C for 10 min. The tubes were removed and cooled at room temperature. It was centrifuged at $5000 \times g$ for 10 min. The supernatant were collected in separate beakers. The above procedure was repeated for 3 times and supernatant was collected. The supernatant was then evaporated to excess alcohol and remain up to 10 to 15 ml. The final volume of 100 ml was made with addition of distill water.

3.7.11. Total sugars and reducing sugar

For total sugar in 5ml diluted extract 5 ml of 5 N HCl was added and kept for 24 hr at room temperature for inversion and diluted to 25 ml with distill water. This diluted was neutralized with 5N NaOH using pH meter and the final volume was made to 50 ml with distilled water. The hydrolyzed sugars were estimated as reducing sugar by Nelson- Somogyi method (Nelson, 1944). For colour development one ml of the appropriately diluted sample

extract and 1 ml of copper sulphate reagent were mixed and heated for 20 min. in a boiling water bath. The tubes were cooled and 1 ml of arsenomolybdate reagent was added in to it. The mixture was diluted to 8 ml and the absorbance was measured at 520 nm.

The total and reducing sugar was calculated from a standard curve prepared by the same procedure using D-glucose as a standard with concentration of 0, 10, 20, and 30..... to 100 $\mu\text{g}^{-1}\text{ml}$. (Fig.6)

3.7.12 Non-reducing sugar

Non-reducing sugar was estimated by subtracting the reducing sugar from total sugar content of the sample.

3.7.13 Estimation of soluble proteins

Soluble protein in leaf samples were estimated as per the method Lowry (1951) using bovine serum albumin as the standard protein.

Reagents

1. Reagent 'A' (alkaline sodium carbonate): Sodium carbonate (2g) was dissolved in 0.1 M sodium hydroxide and the volume made to 100 ml with 0.1 M NaOH.
2. Reagent 'B' (Copper sulphate-sodium potassium tartrate solution, 0.5 % (w/v): Copper sulphate 0.5g was dissolved in 1 % (w/v) sodium potassium tartrate and the volume made to 100 ml with 1 % Na-K tartrate solution.
3. Alkaline copper tartarate reagent: Fresh alkaline copper tartarate reagent was prepared just before use by mixing 50 ml of reagent 'A' and 1 ml of reagent 'B'.
4. Folin-Ciocalteu (phenol) reagent: It was prepared by diluting one part of commercial grade reagent with one part of distilled water on the day of use.
5. Stock solution of bovine serum albumin (BSA): BSA (100 mg) was weighed accurately and dissolved in distilled water and the volume made to 100 ml with distilled water.
6. Working standard solution of BSA: Ten ml of the stock solution was pipetted into 100 ml volumetric flask and the volume made with distilled water ($100\text{ }\mu\text{g of BSA ml}^{-1}$).

Procedure

For preparation of standard curve working solution of BSA was pipetted 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 to 1 ml into a series of test tubes and for estimation of soluble protein from leaf samples 0.1ml extract was pipetted in other set of test tubes. The volume was made to 1 ml of all the test tubes with distilled water. A tube with 1 ml of water serves as the blank. To this 5 ml of alkaline copper tartarate reagent, solution C was added and mixed

well by vortexing. The tube was kept 10 min for incubation. After this 0.5 ml of Folin's reagent (solution D) was added. Mixture was vortexed well with vortex mixer and incubated at room temperature for 30 min. The colour developed during the reaction was read at 660 nm.

A standard curve was drawn and the amount of protein from the leaf samples was calculated from standard protein curve and expressed as mg g^{-1} fr. wt. The estimated protein was used in antioxidative enzyme assay for determination of enzyme activity (Fig. 7).

3.7.14 Preparation of crude enzyme extract:

1. For assays of APX, CAT and SOD leaf samples 0.2g was homogenized with a pestle in a mortar with 2 ml of an ice-cold 50 mM potassium phosphate buffer (pH 7.5).
2. The homogenates were filtered through four layers of cheese cloth and then centrifuged at 4°C for 15 min at 15,000xg.
3. The supernatant fraction was used as crude extract for enzyme activity assays.

3.7.15 Ascorbate peroxidase

Ascorbate peroxidase activity was measured immediately in fresh extract which was assayed as per the method described by Nakano and Asada (1981).

Reagents:

1. Phosphate buffer (100 mM, pH 7.0): It was prepared by mixing 39 ml of 0.1 M KH_2PO_4 and 61 ml of 0.1 M K_2HPO_4 and the final volume made to 200 ml with distilled water.
2. Ascorbic acid (3 mM): It was prepared by dissolving 5.28mg ascorbic acid and the final volume made to 10 ml with distilled water.
3. 30% (v/v) Hydrogen peroxide (1mM): H_2O_2 (10 μl) was dissolved in distilled water and final volume made to 100 ml.
4. Ethylenediaminetetraacetic acid (3 mM): It was prepared by dissolving 37.2mg EDTA in distilled water and the final volume made to 100 ml with distilled water.

Method

Three ml enzyme reaction mixture contained: 1.5 ml 50 mM phosphate buffer (pH 7.0), 0.3 ml 0.5 mM ascorbic acid, 0.1 ml 3 mM EDTA and 50 μl enzyme extract. The reaction was initiated by the addition of 0.3 ml of 1 mM H_2O_2 . The hydrogen peroxide dependent oxidation of ascorbic acid was followed by a decrease in the absorbance measured at 240 nm for three min at the interval of 30 sec. The enzyme activity was expressed as $\mu\text{moles of ascorbate oxidized mg}^{-1} \text{ protein min}^{-1}$.

3.7.16 Catalase

Catalase activity was measured immediately in fresh extract and the assay employed was the one described by Aebi (1984). The hydrogen peroxide dependent oxidation was estimated by the decrease in absorbance at 240 nm.

Reagents:

1. 30% (v/v) Hydrogen peroxide (75 mM): Dissolve 775 μ l H_2O_2 was dissolved in distilled water and the final volume made to 100 ml.
2. Phosphate buffer (100 mM, pH 7.0): It was prepared by mixing 39 ml of 0.1 M KH_2PO_4 and 61 ml of 0.1 M K_2HPO_4 and the final volume made to 200 ml with distilled water.

Method

Three ml enzyme reaction mixture contained: 0.3 ml 50 mM phosphate buffer (pH 7.0) and 50 μ l enzyme extract. The reaction was initiated with addition 0.3 ml of 100 mM H_2O_2 . For measurement of catalase enzyme activity, the decline in absorbance was recorded at 240 nm for three min at an interval of 30 sec. The enzyme activity was expressed as μ moles mg^{-1} protein min^{-1} .

3.7.17 Superoxide dismutase

Superoxide dismutase activity was determined by measuring its ability to inhibit the photochemical reduction of nitrobluetetrazolium (NBT) using the method described by Dhindsa et al. (1981).

Reagents:

1. Nitrobluetetrazolium (2.25 mM): It was prepared by dissolving 0.092g nitrobluetetrazolium in 50 ml distilled water.
2. Riboflavin (60 mM): It was prepared by dissolving 0.047mg riboflavin in 25 ml of 0.1 N NaOH and further 0.3 ml diluted to 25 ml with distilled water.
3. Methionine (200 mM): It was prepared by dissolving 1.492 mg L-methionine in 50 ml distilled water.
4. Ethylenediaminetetraacetic acid (3 mM): It was prepared by dissolving 0.112 g EDTA in 100 ml distilled water.
5. Phosphate buffer (100 mM, pH 7.8): It was prepared by mixing 16 ml of 0.1 M KH_2PO_4 and 84 ml of 0.1 M K_2HPO_4 and final volume made to 200 ml with distilled water.

Method

Three ml enzyme reaction mixture contained: 1.5 ml of 100 mM phosphate buffer (pH 7.8), 0.1 ml 75 μ M NBT, 0.1 ml 2 μ M riboflavin, 0.2 ml 13 mM methionine, 0.1mM EDTA, and 100 μ l enzyme extract. The riboflavin was added at last. The reaction was started by switching on light (two bulbs of 15 w) and after 15 min reaction was terminated by switching off light and covering the tubes with black cloth. The non-irradiated reaction mixture without enzyme extract kept in dark served as control. After 10 min tubes containing reaction mixture was spinned for 5-10 seconds and absorbance read at 560 nm using control as a blank. One unit of SOD was defined as the amount of enzyme required to cause 50 per cent inhibition of NBT reduction per min at 560 nm.

4. Result and Discussion

Sugarcane is the important cash crop in the world. In crop plants including sugarcane abiotic stresses viz., drought (water deficit), excessive watering (water-logging/flooding), extreme temperatures (cold, frost and heat), salinity and mineral (metal and metalloid) toxicity decreased growth, development, yield and seed quality of crop plants. Salt stress affects plants in a variety of ways and results in osmotic, ionic and oxidative stress resulting in lowering the soil water potential, reducing the root water uptake, causing cellular dehydration ultimately inhibiting the cell expansion and cell division, nutritional disruption, membrane disorganization and metabolic cycle changes. In addition salinity decreases photosynthesis resulting in stomatal closure and reduction in intracellular partial CO₂ pressure, a decrease in protein concentration and production of ROS which may damage cellular components viz., proteins, lipids, and DNA, undermining vital cellular functions. Amelioration of salinity stress in crop plants may be possible either by developing salt tolerant varieties through classical plant breeding/biotechnological tools or management practices for soil or plant viz., leaching of salt from root zone, change farm management practices or application of the beneficial microbes. Eco-friendly strategies viz., plant-associated beneficial microbes is one of the options to ameliorate plant growth responses and enhance crop productivity in a sustainable way to mitigate salt stress conditions. However, application of this strategy needs the basic knowledge at biochemical level in plants and microbes. Therefore, the present investigation was carried out in sugarcane and the results obtained are presented and discussed below.

4.1. Characterization of Soil

Soil was collected from saline affected area situated at D-Block, PGI, MPKV, Rauri where sugarcane crop was grown and soil was collected and analyzed for various soil salinity parameters and results obtained are tabulated in Table 4. It was observed that pH of the soil was 8.56, EC 3.45 dSm⁻¹, SAR 13.82 and ESP 18.43. It was revealed from the classification of the salt affected soil given by Natural Resources Conservation Services, U.S. Department of Agriculture (Saha, 2022), the soil analyzed in the present investigation might be sodic soil. Among the soluble cations analyzed sodium was found predominant 21.20 meq L⁻¹, followed by Mg (6.01 meq L⁻¹), Ca (3.11 meq L⁻¹) and K (0.15 meq L⁻¹). Low conc. of Ca and Mg may be due to precipitation at high pH 8.56 (Annoymous, 2022). Earlier Lamizadeh

et al. (2016) reported that saline soil having the pH 9.02, EC 4.7 dSm⁻¹, Ca 16.8 meq L⁻¹ and Mg 10.9 meq L⁻¹. Similarly, Abrol et al. (1980) reported that sodium content in sodic soil at Karnal was 84-85 meq L⁻¹ at the depth of 0-25 cm and potassium in the range of 0.1 -0.2 meq L⁻¹ at the depth of 0-25 cm. The values obtained in the present investigation are at par with these reported values.

Table 4. Chemical analysis of soil.

Sr. No.	Parameters	Value
1	Ph	8.56
2	EC (dS/m ⁻¹)	3.45
3	Calcium (meq L ⁻¹)	3.11
4	Magnesium (meq L ⁻¹)	6.01
5	Potassium (meq L ⁻¹)	0.15
6	Sodium (meq L ⁻¹)	25.20
7	SAR	13.82
8	ESP	18.43

4.2 Characterization of endophytic bacteria

Endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* were isolated from the sugarcane stem which was grown in salt affected area which confirmed as sodic soil by the soil analysis described under 4.1. *Gluconacetobacter* were grown in LGIP media and required about 48-72 hrs for its growth. The formation of orange colour colony on LGIP media was considered as main criteria for identification of *Gluconacetobacter* isolates (Fig. 4). These isolates were further characterized for its specific biochemical tests (Table 5. and Fig. 9). *Gluconacetobacter* showed +ve tests for catalase, oxidase, urease and H₂S production however, gelatin liquefaction test appeared –ve and thus confirmed that isolated bacteria were *Gluconacetobacter*. Earlier, Rao et al. (2017) and Muthukumarasamy (2002) reported similar results in respect of colony colour and biochemical tests for *Gluconacetobacter*. Thus results obtained are in conformity with the earlier reports.

Azospirillum endophytes were multiplied within 90-120 hrs in Nitrogen free malate media and white colonies developed was its preliminary confirmation (Fig. 8). This endophyte was also characterized by biochemical tests and positive tests of catalase, oxidase, and urease and H₂S production confirmed that isolated bacterium was *Azospirillum*. The gelatin

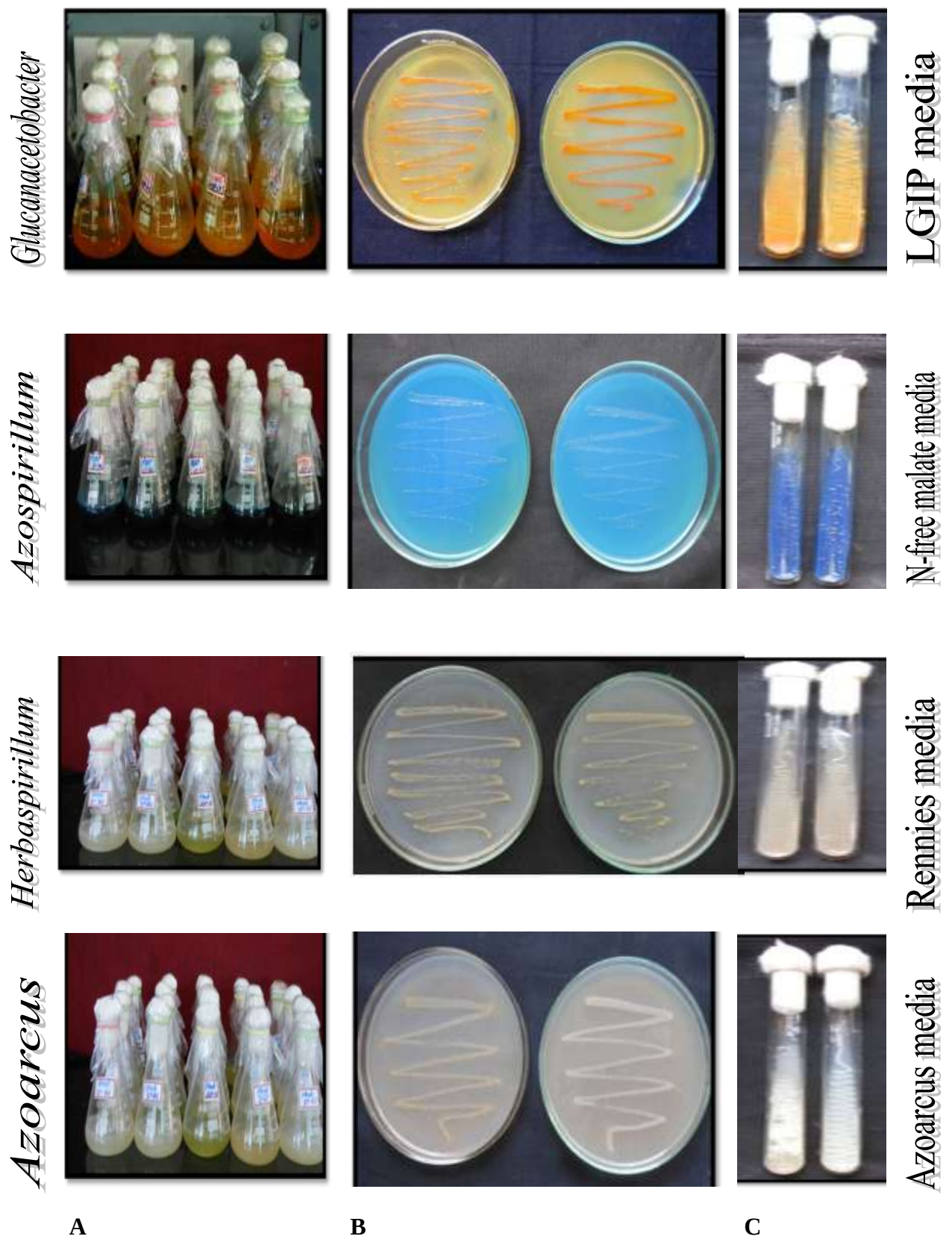


Fig. 8. Isolation of endophytic bacteria from sugarcane grown under salt affected soil.
A:Culture media; B:Culture plate; C:Pure culture

liquefaction test was found –ve in the present study (Table 6 and Fig. 10). Sahoo et al. (2014) and Usha and Kanimozhi (2011) reported the similar results, however, +ve gelatin liquefaction test and -ve H₂S production test was observed by Jhala et al. (2015). The results

Table 5. Biochemical characterization of *Gluconacetobacter* isolates.

Sr. No.	Isolate	Biochemical tests				
		Catalase Test	Oxidase test	Urease test	H ₂ S production	Gelatin liquefaction
1	GA-1	+	+	+	+	-
2	GA-2	+	+	+	+	-

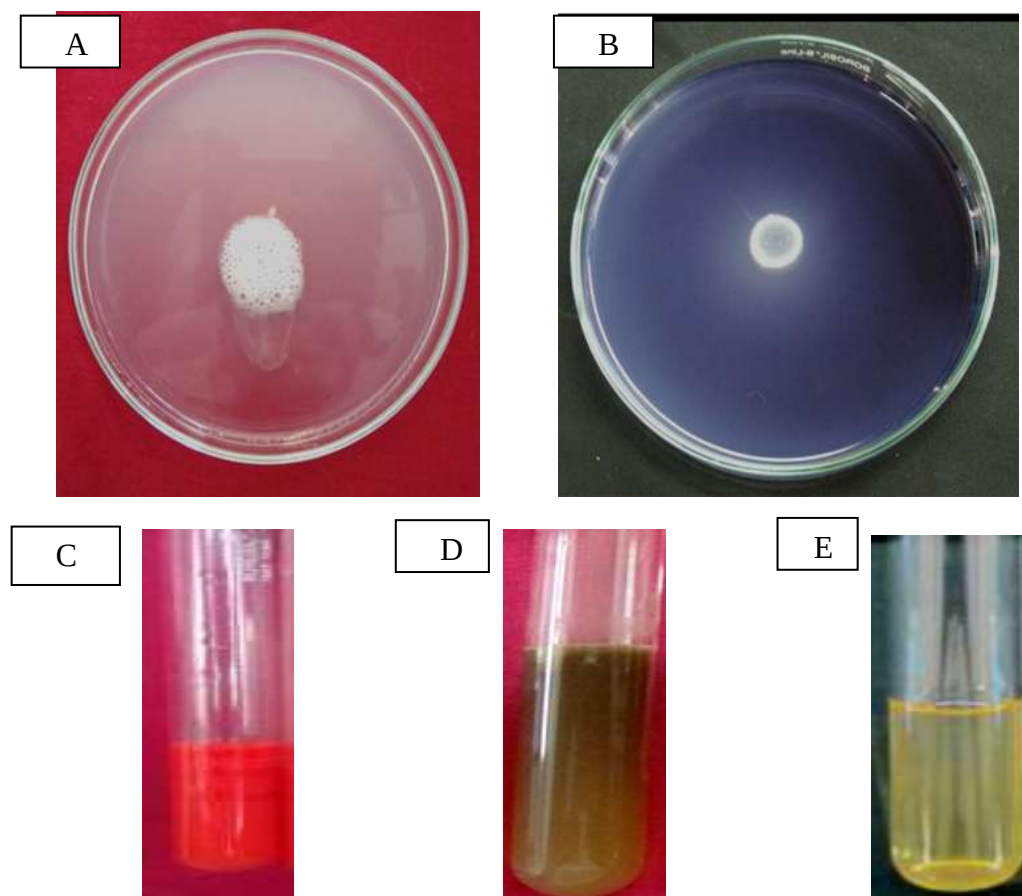


Fig. 9. Biochemical characterization of *Gluconacetobacter* spp.

A : Catalase test; B : Oxidase test; C :Urease test; D : H₂S production; E : Gelatin liquefaction obtained in the present investigation were conformity with the earlier reports for all the tests except reported by Jhala et al. (2015).

Table 6. Biochemical characteristics of *Azospirillum* isolates.

Sr. No.	Isolate	Biochemical tests				
		Catalase test	Oxidase test	Urease test	H ₂ S production	Gelatin liquefaction
1	Asp-1	+	+	+	+	-
2	Asp-2	+	+	+	+	-

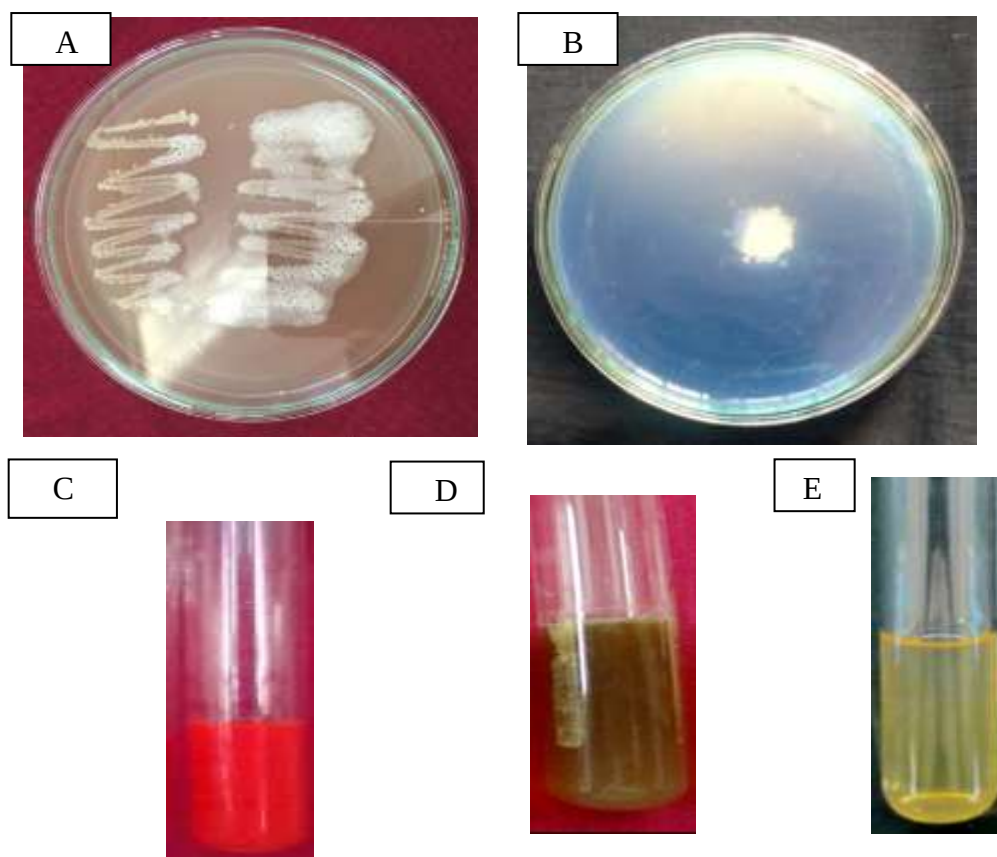


Fig. 10. Biochemical tests *Azospirillum* spp.

A : Catalase test; B : Oxidase test; C : Urease test; D : H₂S production; E : Gelatin liquefaction

Herbaspirillum was grown in Rennis media and it was observed that during its growth, colour of the media was changed from light green to blue in presence of the bromothymol blue indicator within 90-100 hrs (3-4 days) (Fig.10). Initial test of brownish colonies observed on solid media confirmed that isolated bacterium was *Herbaspirillum* (Fig. 8) (Im et al., 2004 and Baldani et al., 1986). The biochemical test of this bacterium was carried out and it was

revealed +ve tests of catalase, oxidase, urease and H₂S production, however, -ve test for gelatin liquefaction (Table 7; Fig. 11).

Table 7. Biochemical characteristics of *Herbaspirillum* isolates.

Sr. No.	Isolate	Biochemical tests				
		Catalase test	Oxidase test	Urease test	H ₂ S production	Gelatin liquefaction
1	Herb-1	+	+	+	+	-
2	Herb-2	+	+	+	+	-

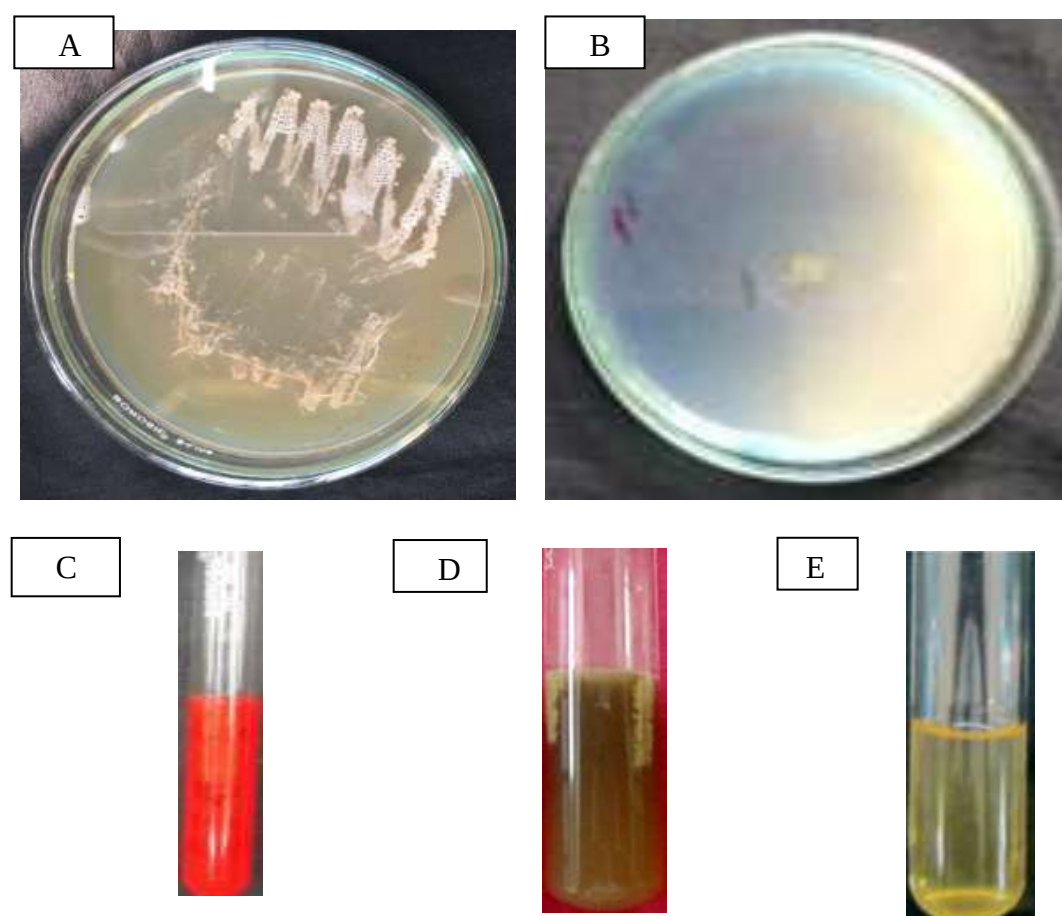


Fig. 11. Biochemical tests *Herbaspirillum* spp.

A : Catalase test; B : Oxidase test; C :Urease test; D : H₂S production; E : Gelatin liquefaction

The results obtained in the present investigation are in conformity with earlier reports of Im et al. (2004); Matteoli et al. (2020); Magnani et al. (2010) and Baldani et al. (1986). Isolation of *Azoarcus* was carried out from sugarcane stems and grown in *Azoarcus* medium

and required 100-120 hrs for its growth. The isolates grown on solid media indicated white colonies (Fig. 8) and +ve biochemical tests were noticed for catalase, oxidase, urease and H₂S (Table 8; Fig. 12).

Table 8. Biochemical characteristics of *Azoarcus* isolates.

Sr. No.	Isolate	Biochemical tests				
		Catalase test	Oxidase test	Urease test	H ₂ S production	Gelatin liquefaction
1	Az-1	+	+	+	+	-
2	Az-2	+	+	+	+	-

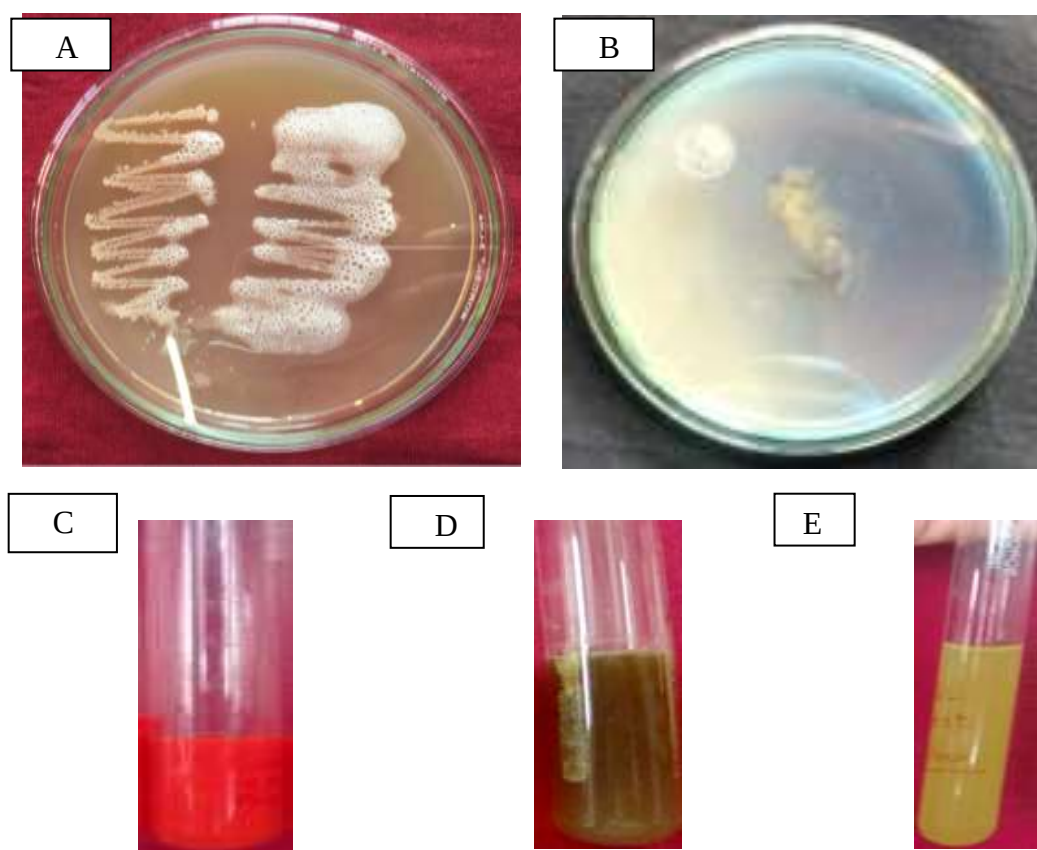


Fig. 12. Biochemical tests *Azoarcus* spp.

A : Catalase test; B : Oxidase test; C :Urease test; D : H₂S production; E : Gelatin liquefaction
The gelatin liquefaction test was found –ve. The results obtained in the present investigation are in conformity with the earlier reports of Hurek et al. (1993).

4.3 Screening endophytic bacteria for various salt concentration

Isolated four endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* were screened on nutrient agar medium with different conc. of NaCl to check their salinity tolerance limit. With increase in salt conc. in the media there was decrease in bacterial growth as revealed from (Fig.13) however; decrease was very negligible as confirmed from CFU count (Table 9). Usha and Kanimozhi (2011) and Jhala et al. (2015) had grown the *Azospirillum* in a media containing different conc. of NaCl solution and reported that these endophyte bacteria tolerate 700 mM and 6% NaCl solution respectively. In the present investigation the 4 endophytic bacteria tolerated salt stress upto 8 dSm^{-1} . It may be because of microorganisms possess multiple strategies to overcome salinity. One of these strategies is the osmoprotectants accumulation in the cytoplasm (Mishra and Sharma, 2012). This important feature demonstrates the potential of endophytic bacteria to alleviate salt stress of host plant by exopolysaccharides production which restricts sodium adsorption by plant (Milosevic et al., 2012) or indirectly by auxin production (Yaish et al., 2015).

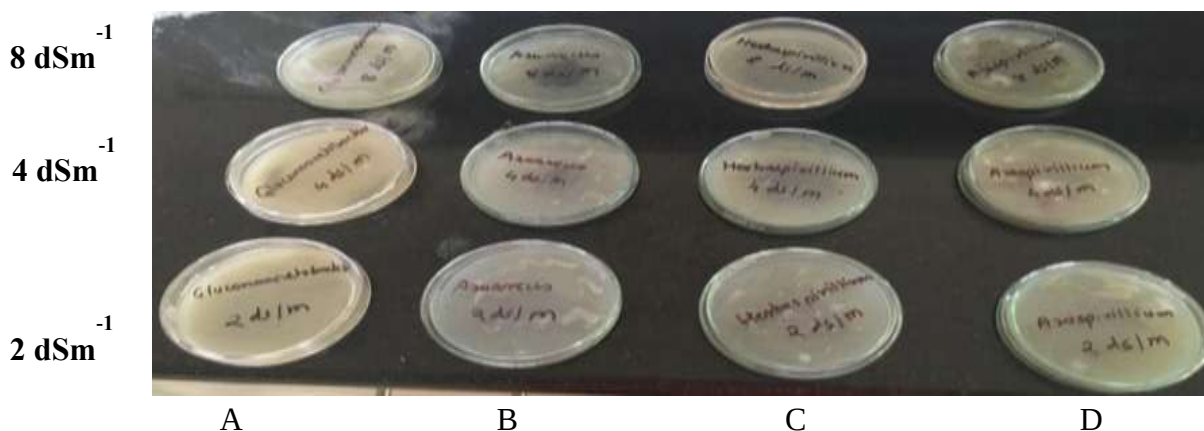


Fig 13. Screening of endophytic bacteria for various salt conc.

A : *Gluconacetobacter*; B : *Azoarcus*; C : *Herbaspirillum*; D : *Azospirillum*

Table 9. Influence of various salt stresses on growth of endophytic bacteria (CFU ml^{-1} broth).

Treatment	<i>Gluconacetobacter</i>	<i>Azospirillum</i>	<i>Herbaspirillum</i>	<i>Azoarcus</i>
Control	7.33×10^5	6.33×10^5	6.00×10^5	5.00×10^5
2 dSm^{-1}	6.66×10^5	5.33×10^5	5.66×10^5	4.66×10^5
4 dSm^{-1}	6.33×10^5	4.33×10^5	4.66×10^5	3.33×10^5
8 dSm^{-1}	5.33×10^5	3.66×10^5	3.33×10^5	2.33×10^5

4.4 Effect of application of endophytic bacteria on leaf area of sugarcane under induced salinity stress

The leaf area at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was measured and illustrated in Table 10 and Fig 14 & 15.

It was observed that the leaf area was in the range of 87 - 120.5 cm² in the CoM 0265 and 79 - 115 cm² in the CoC-671. The maximum leaf area was observed 120.5 cm² in CoM 0265 and 115 cm² in the CoC-671 consortia treated sugarcane plants and grown without imposed salt stress condition.. The minimum leaf area 87 cm² in CoM 0265 and 79 cm² in CoC 671 was observed in without endophyte treated plants and under 8 dSm⁻¹ salt stress conditions. It was noticed that there was gradual decrease in leaf area with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants treated with endophytes of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more decrease was observed in leaf area at 8 dSm⁻¹ (100.25 and 96.25 cm²) followed by 4 (102.82 and 98.50 cm²), 2 (105.67 and 101.20 cm²) dSm⁻¹ salt stress and sodic soil (108.12 and 103.33 cm²) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (112.08 and 107 cm²) treated with the endophytes. Among the different endophyte treated plants, *Azoarcus* treated plants showed more decrease during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Herbaspirillum*, *Azospirillum*, *Gluconacetobacter* and consortia in both the varieties of sugarcane. However, the decrease was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

From the result obtained it was revealed that endophyte treated plant caused increase in leaf area over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Thus at 8 dSm⁻¹ in control condition (without endophyte treatment) leaf area was 87 cm² in CoM 0265 and 79 cm² in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (110 cm²) and (106 cm²) followed by *Gluconacetobacter* (105

Table 10. Effect of endophytic bacteria on leaf area at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Leaf area (cm ²)											
Varieties	COM 265						CoC 671					
SS EB	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	100 (0)	96 (-4)	93.5 (-6.50)	91.5 (-8.50)	87 (-13)	94.1 (-8.00)	90 (0)	85 (-5.56)	83.5 (-7.22)	80.5 (-10.56)	79 (-12.22)	83.6 (-8.89)
CEB	120.5 (0)	117 (-2.90)	114.5 (-4.98)	113 (-6.22)	110 (-8.71)	115 (-5.71)	115 (0)	112 (-2.61)	110.5 (-3.91)	108.5 (-5.65)	106 (-7.83)	110.4 (-5.00)
GA	117.5 (0)	113.5 (-3.40)	111.5 (-5.11)	109 (-7.23)	105 (-10.64)	111.3 (-6.60)	114.5 (0)	111 (-3.06)	109 (-4.80)	106.3 (-7.16)	103.5 (-9.61)	108.86 (-6.16)
ASP	114.5 (0)	110.5 (-3.49)	108.5 (-5.24)	104.5 (-8.73)	102 (-10.92)	108 (-7.75)	113.5 (0)	110 (-3.08)	107.7 (-5.11)	104.5 (-7.93)	102 (-10.13)	107.54 (-6.56)
HERB	110.5 (0)	106.5 (-3.62)	104 (-5.88)	100.4 (-9.14)	98 (-11.31)	103.88 (-7.49)	109.5 (0)	106 (-3.20)	103.5 (-5.48)	100.2 (-8.49)	98 (-10.50)	103.44 (-6.92)
AAR	109.5 (0)	105.2 (-3.93)	102 (-6.85)	98.5 (-10.05)	97 (-11.42)	102.44 (-7.95)	99.5 (0)	96 (-3.52)	93 (-5.51)	91 (-8.54)	89 (-10.55)	93.7 (-7.29)
MEAN	112.08 (0)	108.12 (-3.56)	105.67 (-5.76)	102.82 (-8.31)	100.25 (-11.44)		107 (0)	103.33 (-3.50)	101.20 (-5.45)	98.50 (-8.06)	96.25 (-10.14)	
Factor	SE(d)					CD						
Variety	0.180					0.508						
Salt Stress	0.284					0.803						
Endophytic bacteria treatment	0.311					0.880						
Variety x Salt stress	0.401					1.136						
Variety x Endophytic bacteria	0.440					1.244						
Salt stress x Endophytic bacteria	0.695					1.967						
Variety X Endophytic bacteria X Salt stress	0.983					2.782						

***Figures in paranthesis is % decrease over control.**

SS - Salt stress; EB - Endophytic bacteria; CEB - Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbaspirillum*; AAR - *Azoarcus*.

cm²) and (103.5 cm²); *Azospirillum* (102 cm²) and (102 cm²); *Herbaspirillum* (98 cm²) and (98 cm²); *Azoarcus* (97 cm²) and (89 cm²) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to relieve the stress condition and resulted in increased leaf area over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* endophyte treatments. Earlier decrease in leaf area with increase in salt stress conc. was reported in rice (Sen et al., 2014), pepper (Bacilio et al., 2016), *Arabidopsis thaliana* (Souza et al., 2016; Shi et al., 2022), (Bacilio et al., 2016), lettuce (Moncada et al., 2020), rapeseed (Abriz et al., 2020), tomato (Aini et al., 2021). Treatment/inoculation of endophytes reported

that increased in the leaf area in pepper (Bacilio et al., 2016); maize (Abdel Latef et al., 2020); rapeseed (Abriz et al., 2020) and tomato (Aini et al., 2021) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species. In the present investigation the increase in leaf area in both the varieties of sugarcane under imposed salinity stress condition with the application of endophytes could be due to production of IAA by the endophytes that could cause elongation and proliferation of plant cells under salinity stress condition (Ahmed and Hasnain, 2014). The endophytes treatment to the sugarcane plants may help for root growth and development and especially the hairy roots, lead to more water and nutrient absorption by the plants which resulted in normal plant growth including leaf area under saline condition (Abdel Latef et al., 2020).

4.5 Effect of application of endophytic bacteria on shoot length of sugarcane under induced salinity stress

The shoot length at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was measured and tabulated in Table 11 Fig 16 & 17.

It was noticed that the shoot length was in the range of 89.5 - 120.5 cm observed in the CoM 0265 and 77.5 – 115.4 cm in the CoC-671. The maximum shoot length was 120.5 cm observed in CoM 0265 and 115.5 cm in the CoC-671 sugarcane varieties treated with consortia of endophytes and grown without imposed salt stress condition. The minimum shoot length 89.5 cm in CoM 0265 and 77.5 cm in COC 671 was observed in without endophyte treated plants and under 8 dSm⁻¹ salt stress conditions. It was noticed that there was continuous decrease in shoot length with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants treated with endophytes of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more decrease was observed in leaf area at 8 dSm⁻¹ (100.13 and 96.08 cm) followed by 4 (102.85 and 98.37 cm), 2 (105.87 and 101.53 cm) dSm⁻¹ salt stress and sodic soil (108.53 and 103.25 cm) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the

Table 11. Effect of endophytic bacteria on shoot length at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Shoot length (cm)											
Varieties	COM 265						CoC 671					
SS EB	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	100.5 (0)	96.5 (-3.98)	93.5 (-6.97)	91.5 (-8.96)	89.5 (-10.95)	94.3 (-7.71)	90.5 (0)	85.5 (-5.52)	83.5 (-7.73)	80.5 (-11.05)	77.5 (-14.36)	83.50 (-9.67)
CEB	120.5 (0)	119 (-1.24)	114.5 (-4.98)	113 (-6.22)	108 (-10.37)	115 (-5.71)	115.4 (0)	112 (-2.95)	110.5 (-4.25)	108.5 (-5.98)	106 (-8.15)	110.48 (-5.33)
GA	117.5 (0)	113.5 (-3.40)	111.5 (-5.11)	109.2 (-7.06)	105 (-10.64)	111.14 (-6.55)	114.5 (0)	111 (-3.06)	109.5 (-4.37)	106.7 (-6.81)	103.5 (-9.61)	109.04 (-5.96)
ASP	114.5 (0)	110.5 (-3.49)	108.5 (-5.24)	104.5 (-8.73)	102 (-10.92)	108.22 (-7.10)	113.5 (0)	109 (-3.96)	107.7 (-5.11)	104.5 (-7.93)	102 (-10.13)	107.34 (-6.78)
HERB	110.5 (0)	106.5 (-3.62)	104.2 (-5.70)	100.4 (-9.14)	98 (-11.31)	104.06 (-7.44)	109.5 (0)	106 (-3.20)	103.5 (-5.48)	99.5 (-9.13)	98.5 (-10.50)	103.40 (-7.08)
AAR	109.5 (0)	105.2 (3.93)	103 (-5.94)	98.5 (-10.05)	97 (-11.42)	102.74 (-7.83)	99.5 (0)	96 (-3.52)	94 (-5.53)	90 (-9.55)	89 (-10.55)	93.90 (-7.29)
MEAN	112.17 (0)	108.53 (-3.70)	105.87 (-5.65)	102.85 (-8.41)	100.13 (-10.93)		107.15 (0)	103.25 (-3.28)	101.53 (-5.41)	98.37 (-8.36)	96.08 (-10.55)	
Factor						SE+(d)						CD
Variety						0.103						0.293
Salt Stress						0.164						0.463
Endophytic bacteria treatment						0.179						0.507
Variety x Salt stress						0.231						0.851
Variety x Endophytic bacteria						0.253						0.717
Salt stress x Endophytic bacteria						0.401						1.133
Variety X Endophytic bacteria X Salt stress						0.566						1.603

*Figures in paranthesis is % decrease over control.

SS - Salt stress; EB - Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA - *Glucanacetobacter*; ASP - *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*

control (112.17 and 107.15 cm) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, *Azoarcus* treated plants showed more decrease during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Herbasprillum*, *Azospirillum*, *Glucanoacetobacter* and consortia in both the varieties of sugarcane. The percent decrease was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants however; shoot length found to be more in CoM 0265 than CoC 0671 sugarcane variety.

Further it was noticed that endophyte treated plant caused increase in shoot length over the control (with imposed salt stress and without endophyte treatment) within the

individual salt stress treatment in both the varieties. At 8 dSm⁻¹ in control condition (without endophyte treatment) the values of shoot length was 89.5 cm in CoM 0265 and 77.5 cm in CoC 671 and raised with the application of endophytes and more increase was observed by the application of consortia (108 cm) and (106 cm) followed by *Glucanoacetobacter* (105 cm) and (103.5 cm); *Azospirillum* (102 cm) and (102 cm); *Herbaspirillum* (98 cm) and (98.5 cm); *Azoarcus* (97 cm) and (89 cm) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be better at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to ameliorate the stress condition and boosted in shoot length over respective control followed by *Glucanoacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments both the sugarcane varieties.

Earlier reports indicated that shoot length was decreased with increase in salt stress concentration in cucumber (Kang et al., 2014), lucerne (Ansari et al., 2019), Pea (Sofy et al., 2021). However, treatment/inoculation of endophytes increased the shoot length in cucumber (Kang et al., 2014); lucerne (Ansari et al., 2019); Pea (Sofy et al., 2021) under salt stress condition. The results obtained in the present investigation are concomitant with these earlier reported trends under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species. In the present investigation the increase in shoot length in both the varieties of sugarcane under imposed salinity stress condition by the application of endophytes could be due to production of phytohormones viz., ethylene, IAA and GA by the endophytes resulting in better shoot length in both stress and non-stress situations (Ullah et al. 2013). IAA by the endophytes that could cause elongation and proliferation of plant cells under salinity stress condition (Ahmed and Hasnain, 2014).

4.6 Effect of application of endophytic bacteria on root length of sugarcane under induced salinity stress

The root length at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was measured and depicted in Table 12 and Fig 18 & 19.

Root length was found in the range of 45 - 57 cm in the CoM 0265 and 43 - 56 cm in the CoC-671. The highest root length was observed 57 cm in CoM 0265 and 56 cm in the CoC-671 of sugarcane plants treated with consortia endophytes and grown without imposed

salt stress condition. The lowest root length 45 cm in CoM 0265 and 43 cm in CoC 671 was observed in without endophyte treated plants and under 8 dSm⁻¹ salt stress conditions. From the results obtained in the present study It was observed that there was concomitant decrease in root length with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more decrease was observed at 8 dSm⁻¹ (46.3 and 44.75 cm) followed by 4 (47 and 46.67 cm), 2 (50.4 and 48.67 cm) dSm⁻¹ salt stress and sodic soil (52.4 and 50.18 cm) over the control (54.9 and 52.33 cm) grown plants of CoM 0265 and CoC 671 sugarcane varieties respectively treated with the endophytes. Among the different endophyte treated plants, *Azoarcus* treated plants showed more decrease during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Herbaspirillum*, *Azospirillum*, *Gluconacetobacter* and consortia in both the varieties of sugarcane. The percent decrease in root length was more in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants however, root length was found to be more in each of the treatment in CoM 0265 than CoC 671 sugarcane variety.

It was emphasized that endophyte treated plant caused increase in root length over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. At 8 dSm⁻¹ in control condition (without endophyte treatment) root length was 45 cm in CoM 0265 and 43 cm in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (51.5 cm) and (51 cm) followed by *Gluconacetobacter* (47.5 cm) and (47.5 cm); *Azospirillum* (46 cm) and (45 cm); *Herbaspirillum* (44 cm) and (42 cm); *Azoarcus* (43.5 cm) and (40 cm) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to overcome the stress condition and resulted in increased root length over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments observed by increase in root length in both the sugarcane varieties.

Reduction in root length of the sugarcane plants under salinity stress in the present study was similar to other plants viz., cucumber (Kang et al., 2014); lucerne (Ansari et al.,

Table 12. Effect of endophytic bacteria on root length at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Root length (cm)											
Varieties	COM 265						CoC 671					
EB \ SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	56.0 (0)	52.5 (-6.25)	50.0 (-10.71)	48.0 (-14.29)	45 (-19.64)	50 (-12.72)	53.0 (0)	49.0 (-7.55)	47.0 (-11.32)	44.5 (-16.04)	43.0 (-18.87)	47 (-13.44)
CEB	57.0 (0)	56.0 (-1.75)	53.5 (-6.14)	52.0 (-8.77)	51.5 (-9.65)	54 (-6.58)	56.0 (0)	55.1 (-1.61)	53.0 (-5.36)	52.5 (-6.25)	51.0 (-8.93)	53 (-5.54)
GA	56.0 (0)	53.8 (-3.93)	51.7 (-7.68)	50.0 (-10.71)	47.5 (-15.18)	52 (-9.38)	55.0 (0)	53.0 (-3.64)	51.0 (-7.27)	50.0 (-9.09)	47.5 (-13.64)	51 (-8.41)
ASP	54.0 (0)	51.5 (-4.63)	50.0 (-7.41)	47.0 (-12.96)	46.0 (-14.81)	50 (-9.95)	52.0 (0)	50.0 (-3.85)	49.0 (-5.77)	47.0 (-9.62)	45.0 (-13.46)	49 (-8.17)
HERB	53.5 (0)	51.0 (-4.67)	49.0 (-8.41)	45.0 (-15.89)	44 (-17.76)	49 (-11.68)	50.0 (0)	48.0 (-4.00)	47.0 (-6.00)	44.0 (-12.00)	42.0 (-16.00)	46 (-9.50)
AAR	53.0 (0)	49.4 (-6.79)	48.0 (-9.43)	44.0 (-16.98)	43.5 (-17.92)	48 (-12.78)	48.0 (0)	46.0 (-4.17)	45.0 (-6.25)	42.0 (-12.50)	40.0 (-16.67)	44 (-9.90)
MEAN	54.9 (0)	52.4 (-4.67)	50.4 (-8.30)	47 (-13.27)	46.3 (-15.83)		52.33 (0)	50.18 (-4.13)	48.67 (-6.99)	46.67 (-10.92)	44.75 (-14.59)	
Factor	SE(d)						CD					
Variety	0.224						0.449					
Salt Stress	0.355						0.709					
Endophytic bacteria treatment	0.388						0.777					
Variety x Salt stress	0.501						1.003					
Variety x Endophytic bacteria	0.549						1.099					
Salt stress x Endophytic bacteria	0.868						1.737					
Variety X Endophytic bacteria X Salt stress	1.228						2.457					

*Figures in paranthesis is % decrease over control.

SS - Salt stress; EB - Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*

2019); pea (Sofy et al., 2021); medicago (Zahaf et al., 2012) and legumes (Zahrán, 1991; Manchanda and Garg, 2008). The decrease in root length in the sugarcane plants in the present investigation under salinity stress condition may be due to increased synthesis of ethylene and reactive oxygen species under salinity (Sofy et al., 2021; Habib et al., 2016). The present investigation control (without endophyte treatment) plant ACC was increased by increasing salt stress independently of microbiological treatments (Sofy et al., 2021). In the CoC 671 sugarcane variety in control condition (without application of endophytes) showed higher reduction in root length at 2, 4 dSm⁻¹ and sodic soil grown plants than the CoM 0265 variety indicating CoC 671 is more vulnerable to harmful salinity effects leading to reduction in root length. The increase in root length in both the varieties of sugarcane by the

application of endophytes in the present investigation could be due to production of ethylene, IAA and GA in both stress and non-stress situations (Ullah et al., 2013). The endophyte treatments to the sugarcane plants possibly helped for root growth and development and especially the hairy roots under saline condition (Abdel Latef et al., 2020). The present study showed that application of endophytes ameliorated the salinity stress induced detrimental effect on root length and the results are in line with earlier reports by Kang et al. (2014) in cucumber; Ansari et al. (2019) in lucerne; Sofy et al. (2021) in pea; Noori et al. (2018) in alfalfa. The endophytes used in the present study may produce ACC-deaminase which might help in reduction of ethylene production that converted ACC to α -ketobutyrate ammonia and had limited harmful effect of ethylene on plant root length under salinity stress condition (Sofy et al., 2021).

4.7 Effect of application of endophytic bacteria on total chlorophyll in leaves of sugarcane

The total chlorophyll in leaves at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was estimated and presented in Table 13 and Fig 20 & 21.

It was found that the total chlorophyll in leaves of sugarcane plants was in the range of 2.93 – 4.69 mg g⁻¹ fr. wt. in the CoM 0265 and 1.85 – 3.95 mg g⁻¹ fr. wt. in the CoC-671. The maximum total chlorophyll was observed 4.69 mg g⁻¹ fr. wt. in CoM 0265 and 3.95 mg g⁻¹ fr. wt. in the CoC-671 in leaves of sugarcane plants treated with consortia and grown without imposed salt stress condition. The minimum total chlorophyll 2.93 mg g⁻¹ fr. wt. in CoM 0265 and 1.85 mg g⁻¹ fr. wt. in CoC 671 was observed in leaves without endophyte treated plants and under 8 dSm⁻¹ salt stress conditions. It was revealed that there was gradual decrease in total chlorophyll in leaves with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). It was exhibited from the mean values, more decrease was observed at 8 dSm⁻¹ (3.29 and 2.93 mg g⁻¹ fr. wt.) followed by 4 (3.58 and 3.01 mg g⁻¹ fr. wt.), 2 (3.81 and 3.22 mg g⁻¹ fr. wt.) dSm⁻¹ salt stress and sodic soil (4.00 and 3.27 mg g⁻¹ fr. wt.) over the control (4.42 and 3.69 mg g⁻¹ fr. wt.) grown plants of CoM 0265 and CoC 671 sugarcane varieties respectively treated with the endophytes.

Azoarcus treated plants had more decline in total chlorophyll content in the leaves than the remaining endophytes treatments during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Herbaspirillum*, *Azospirillum*, *Glucanoacetobacter* and consortia in both the varieties of sugarcane. In CoM 0265 decrease was more than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants. Endophyte treated plant caused increase in total chlorophyll over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. At 8 dSm⁻¹ in control condition (without endophyte treatment) total chlorophyll was 2.93 mg g⁻¹ fr. wt. in CoM 0265 and 1.85 mg g⁻¹ fr. wt. in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (3.52 mg g⁻¹ fr. wt.) and (3.29 mg g⁻¹ fr. wt.) followed by *Glucanoacetobacter* (3.42 mg g⁻¹ fr. wt.) and (2.88 mg g⁻¹ fr. wt.); *Azospirillum* (3.29 mg g⁻¹ fr. wt.) and (2.73 mg g⁻¹ fr. wt.); *Herbaspirillum* (3.32 mg g⁻¹ fr. wt.) and (2.61 mg g⁻¹ fr. wt.); *Azoarcus* (3.26 mg g⁻¹ fr. wt.) and (2.35 mg g⁻¹ fr. wt.) in the respective varieties. This trend was similar in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be more effective at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil and mitigated the stress condition and resulted in increased total chlorophyll in leaves of sugarcane plants over respective control followed by *Glucanoacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments. Leaf chlorophyll concentration is an indicator of salt tolerance and responds to increasing salinity (Percival et al., 2003).

Earlier, total chlorophyll content in leaves decreased with increased in salt stress concentration was reported in lucerne (Ansari et al., 2019), pea (Sofy et al., 2021). The results obtained in the present investigation in agreement with results reported earlier under salt stress conditions. The decrease in total chlorophyll content in sugarcane leaves in the present investigation under salinity stress might be due to increased chlorophyllase activity and/or decreased chlorophyll synthesis, and/or destroyed pigment proteins (Santos, 2004; Jaleel et al., 2008; Anower et al., 2013). Further, salinity might resulted in production of reactive oxygen species which may disturbed the cellular metabolism and cause degeneration of cell organelles in the leaf tissue and thereby reduction in chlorophyll content might

Table 13. Effect of endophytic bacteria on total chlorophyll at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Total chlorophyll (mg g ⁻¹ fr. wt.)											
Varieties	COM 265						CoC 671					
SS EB	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	4.38 (0)	3.48 (-20.55)	3.25 (-25.80)	3.04 (-30.59)	2.93 (-33.11)	3.42 (-27.51)	3.60 (0)	3.09 (-14.17)	2.78 (-22.78)	2.66 (-26.11)	1.85 (-48.61)	2.80 (-27.92)
CEB	4.69 (0)	4.46 (-4.90)	4.21 (-10.23)	3.94 (-15.90)	3.52 (-24.95)	4.16 (-14.02)	3.95 (0)	3.71 (-6.08)	3.67 (-7.09)	3.63 (-8.10)	3.29 (-16.71)	3.65 (-9.49)
GA	4.53 (0)	4.24 (-6.40)	4.07 (-10.15)	3.8 (-16.11)	3.42 (-24.50)	4.01 (-14.29)	3.85 (0)	3.6 (-6.49)	3.58 (-7.01)	3.39 (-11.95)	2.88 (-25.19)	3.46 (-12.66)
ASP	4.45 (0)	4.19 (-5.84)	3.96 (-11.01)	3.65 (-17.98)	3.29 (-26.07)	3.91 (-15.2)	3.65 (0)	3.39 (-7.12)	3.44 (-7.75)	3.07 (-15.89)	2.73 (-25.21)	3.26 (-13.49)
HERB	4.25 (0)	3.86 (-9.18)	3.61 (-15.06)	3.6 (-15.29)	3.32 (-21.88)	3.73 (-15.35)	3.55 (0)	3.25 (-8.45)	3.27 (-7.89)	2.86 (-19.44)	2.61 (-26.48)	3.11 (-15.56)
AAR	4.22 (0)	3.78 (-10.43)	3.77 (-10.66)	3.44 (-18.48)	3.26 (-22.75)	3.69 (-15.58)	3.52 (0)	2.60 (-26.14)	2.6 (-26.14)	2.43 (-30.97)	2.35 (-19.89)	3.07 (-15.84)
MEAN	4.42 (0)	4.00 (-9.55)	3.81 (-13.82)	3.58 (-19.08)	3.29 (-25.54)		3.69 (0)	3.27 (-11.41)	3.22 (-12.78)	3.01 (-18.74)	2.93 (-20.39)	
Factor						SE(d)	CD					
Variety						0.013	0.036					
Salt Stress						0.020	0.057					
Endophytic bacteria treatment						0.022	0.062					
Variety x Salt stress						0.028	0.080					
Variety x Endophytic bacteria						0.031	0.088					
Salt stress x Endophytic bacteria						0.049	0.139					
Variety X Endophytic bacteria X Salt stress						0.070	0.197					

*Figures in paranthesis is % decrease over control.

SS - Salt stress; EB - Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbaspirillum*; AAR - *Azoarcus*

observed in sugarcane leaves under control condition (imposed salinity stress and without endophytes treatments) in the present study (Hassine and Lutts, 2010; Abbas et al., 2013).

On the other hand under induced stress condition with application of *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* and its consortia on the sugarcane in the present study exhibited higher chlorophyll content may be because of presence of ACC deaminase activity in endophytes which maintains the photosynthetic efficiency of plants by reducing ethylene biosynthesis (Nadeem et al., 2010 and Habib et al., 2016). Higher chlorophyll content was also reported in endophyte treated plants under salt stressed conditions viz., lucerne (Ansari et al., 2019); pea (Sofy et al., 2021); rice (Bal et al., 2013) and cucumber (Kang et al., 2014) compared with untreated plants. Thus result in the present study showed

endophyte treatment saved the chlorophyll pigments from the harmful effect of salinity. It appeared that significant interaction of variety X endophytic bacteria under salinity condition was effective for protection of chlorophyll pigment. Further, the increase in chlorophyll content in endophyte treated sugarcane plants under salt stress condition over the control (without endophyte treatment) may be due to higher absorption of iron, magnesium and nitrogen (Hossein-Zadah et al., 2011). Moreover, increase in total chlorophyll content in the leaves of sugarcane in the present study with the application of endophytes might be due to higher absorption of iron, magnesium, nitrogen (Hossein-zadah et al., 2011) and less ethylene synthesis (Nadeem et al., 2010; Habib et al., 2016).

4.8 Effect of application of endophytic bacteria on chlorophyll stability index in the leaves of sugarcane under induced salinity stress

The chlorophyll stability index in the leaves at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was calculated and presented in Table 14 and Fig 22 & 23.

It was recorded that the chlorophyll stability index in the leaves was in the range of 71- 91.11 % in the CoM 0265 and 69.87 – 88.90 % in the CoC-671. The highest chlorophyll stability index was noticed 91.11 % in CoM 0265 and 88.90 % in the CoC-671 in sugarcane plant treated with consortia and grown without imposed salt stress condition. The lowest chlorophyll stability index 71 % in CoM 0265 and 69.87 % in CoC 671 was found in without endophyte treated plants and under 8 dSm⁻¹ salt stress conditions. It was noticed that there was regular decrease in chlorophyll stability index in association with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more decrease was observed in total chlorophyll content at 8 dSm⁻¹ (71.57 and 70.98 %) followed by 4 (76.57 and 74.41 %), 2 (84.49 and 80.83 %) dSm⁻¹ salt stress and sodic soil (86.62 and 85.55 %) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (91.02 and 88.99 %) as noticed from mean values of the individual treatment.

In all the endophyte treated plants there was decline in chlorophyll stability index in the leaves of sugarcane varieties with increase in salt stress situation over the control

Table 14. Effect of endophytic bacteria on chlorophyll stability index at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter		Chlorophyll stability index (%)											
Varieties		COM 265						CoC 671					
EB	SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control		90.85 (0)	86.22 (-5.10)	84.17 (-7.35)	76.28 (-16.04)	71 (-21.85)	81.70 (-12.58)	90 (0)	85.26 (-5.27)	80.15 (-10.94)	73.07 (-18.81)	69.87 (-22.37)	79.67 (-14.35)
CEB		91.11 (0)	86.97 (-4.54)	84.77 (-6.96)	76.86 (-15.64)	72.07 (-20.90)	82.36 (-12.01)	88.9 (0)	85.86 (-3.42)	82.05 (-7.71)	74.98 (-15.66)	71.45 (-19.63)	80.65 (-11.60)
GA		91.09 (0)	86.85 (-4.65)	84.67 (-7.05)	76.74 (-15.75)	71.93 (-21.03)	82.26 (-12.12)	88.81 (0)	85.73 (-3.47)	81.85 (-7.84)	74.79 (-15.79)	71.26 (-19.76)	80.49 (-11.71)
ASP		91.07 (0)	86.73 (-4.77)	84.53 (-7.18)	76.63 (-15.86)	71.82 (-21.14)	82.16 (-12.24)	88.77 (0)	85.61 (-3.56)	80.47 (-9.35)	74.67 (-15.88)	71.17 (-19.83)	80.14 (-12.16)
HERB		91.01 (0)	86.54 (-4.91)	84.42 (-7.24)	76.47 (-15.98)	71.41 (-21.54)	81.97 (-12.42)	88.75 (0)	85.48 (-3.68)	80.27 (-9.55)	74.55 (-16.00)	71.12 (-19.86)	80.03 (-12.28)
AAR		90.99 (0)	86.4 (-5.04)	84.35 (-7.30)	76.43 (-16.00)	71.2 (-21.75)	81.87 (-12.52)	88.71 (0)	85.36 (-3.78)	80.21 (-9.58)	74.39 (-16.14)	71.01 (-19.95)	79.94 (-12.36)
MEAN		91.02 (0)	86.62 (-4.84)	84.49 (-7.18)	76.57 (-15.88)	71.57 (-21.37)		88.99 (0)	85.55 (-3.86)	80.83 (-9.16)	74.41 (-16.38)	70.98 (-20.23)	
Factor						SE(d)			CD				
Variety						0.093			0.262				
Salt Stress						0.147			0.415				
Endophytic bacteria treatment						0.161			0.475				
Variety x Salt stress						0.207			0.587				
Variety x Endophytic bacteria						0.227			0.671				
Salt stress x Endophytic bacteria						0.359			1.075				
Variety X Endophytic bacteria X Salt stress						0.508			1.521				

*Figures in paranthesis is % decrease over control.

SS - Salt stress; EB - Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*

(without imposed salt stress and endophytes treated), however, more decrease was observed in *Azoarcus* followed by *Herbasprillum*, *Azospirillum*, *Gluconacetobacter* and consortia in both the varieties of sugarcane. However, the decrease percent was more in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants. It was brought to light that endophyte treated plant caused increase in chlorophyll stability index over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. At 8 dSm⁻¹ in control condition (without endophyte treatment) chlorophyll stability index was 71 % in CoM 0265 and 69.87 % in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (72.07 %) and (71.45 %) followed by *Gluconacetobacter* (71.93 %)

and (71.26 %); *Azospirillum* (71.82 %) and (71.17 %); *Herbaspirillum* (71.41 %) and (71.12 %); *Azoarcus* (71.20 %) and (71.01 %) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be better at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to alleviate the stress condition and resulted in increased chlorophyll stability index over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* endophyte treatments in both the sugarcane varieties.

Chlorophyll stability index is a scale for the integrity of membrane or heat stability of the pigments under stress condition (Prasanna kumar et al., 2022). Umapathi et al. (2021) reported that under drought stress condition uninoculated endophytes treatment had lower CSI than inoculated endophyte sorghum plants. Similar trend was observed in the present investigation that untreated endophyte sugarcane plants had lower CSI value than endophyte treated plants under salinity stress condition. Sampangi-Ramaiah et al. (2020) observed that salt-sensitive rice variety IR-64 colonized with *Fusarium* spp. endophyte had higher CSI under salinity stress condition. Similar results were obtained in the present investigation in sugarcane treated with *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia under salinity stress condition suggesting that endophytes applied stabilizes the cell membrane under salinity stress condition. Previous studies demonstrated that endophyte inoculated plants had higher CSI in sorghum (Umapathi et al., 2021); peanut (Prasanna kumar et al., 2022) and rice (Vibita Bala et al., 2019) under moisture stress condition. The results obtained in the present investigation are in confirmatory with these earlier reports. Thus higher CSI values observed in the sugarcane plants treated with endophytes under salinity stress condition indicating that it stabilizing stress photosynthetic pigments and thereby may results in higher amount of dry matter production.

4.9 Effect of application of endophytic bacteria on relative leaf water content of sugarcane under induced salinity stress

The relative leaf water content at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was measured and showed in Table 15 and Fig 24 & 25.

The relative leaf water content was in the range of 79.02 - 92.46 % in the CoM 0265 and 79.30 – 87.19 % in the CoC-671. The highest values of relative leaf water content was observed 92.46 % in CoM 0265 and 87.19 % in the CoC-671 consortia treated and grown without imposed salt stress condition in sugarcane plant. The lowest values of relative leaf water content 79.02 % in CoM 0265 and 79.30 % in CoC 671 was found in without endophyte treated plants and under 8 dSm⁻¹ salt stress conditions. Increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties associated with gradual decrease in relative leaf water content over the control (without imposition of salt stress). As revealed from the mean values, more decrease was observed in total chlorophyll content at 8 dSm⁻¹ (80.15 and 78.40 %) followed by 4 (84.48 and 82.16 %), 2 (85.62 and 84.54 %) dSm⁻¹ salt stress and sodic soil (87.36 and 85.37 %) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (90.63 and 86.62 %) as noticed from mean values of the individual treatment. Increase in salt stress situation in *Azoarcus* treated plants showed more decline over the control (without imposed salt stress and endophyte treated) followed by *Herbaspirillum*, *Azospirillum*, *Gluconacetobacter* and consortia in both the varieties of sugarcane. In CoM 0265 the percent decline in relative leaf water content was more than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants over the control.

It was disclosed from the results obtained in the present investigation that endophyte treated plant caused increase in relative leaf water content over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Relative leaf water content at 8 dSm⁻¹ in control condition (without endophyte treatment) was 79.02 % in CoM 0265 and 79.30 % in CoC 671 and boosted with the application of endophytes and more increase was observed by the application of consortia (79.02 %) and (79.88 %) followed by *Gluconacetobacter* (81.90 %) and (79.11 %); *Azospirillum* (80.45 %) and (78.25 %); *Herbaspirillum* (79.95 %) and (77.39 %); *Azoarcus* (78.30 %) and (76.45 %) in the respective varieties. The similar trend was observed in all the imposed salinity stress treatments. At different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil grow endophyte treated plants; consortia reduced the stress condition and resulted in increased relative leaf water content over respective control followed by

Table 15. Effect of endophytic bacteria on relative water content content at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Relative water content content (%)											
Varieties	COM 265						CoC 671					
EB \ SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	86.12 (0)	84.51 (-1.87)	84.13 (-2.31)	81.26 (-5.64)	79.02 (-8.24)	83.01 (-4.52)	86.85 (0)	85.1 (-2.01)	82.27 (-5.27)	80.16 (-7.70)	79.3 (-8.69)	82.74 (-5.92)
CEB	92.46 (0)	90 (-2.66)	87.4 (-5.47)	86.99 (-5.92)	81.9 (-11.42)	87.75 (-6.37)	87.19 (0)	86.17 (-1.17)	85.79 (-1.61)	83.98 (-3.68)	79.88 (-8.38)	84.60 (-3.71)
GA	92.16 (0)	89.28 (-3.13)	86.99 (-5.61)	86.42 (-6.23)	81.25 (-11.84)	87.22 (-6.70)	86.85 (0)	85.94 (-1.05)	85.38 (-1.69)	83.22 (-4.18)	79.11 (-8.91)	84.10 (-3.96)
ASP	91.35 (0)	87.64 (-4.06)	85.98 (-5.88)	84.84 (-7.13)	80.45 (-11.93)	86.05 (-7.25)	86.6 (0)	85.44 (-1.34)	85.11 (-1.72)	82.56 (-4.67)	78.25 (-9.64)	83.59 (-4.34)
HERB	90.95 (0)	86.5 (-4.89)	84.89 (-6.66)	84.09 (-7.54)	79.95 (-12.09)	85.28 (-7.80)	86.25 (0)	85.01 (-1.44)	84.55 (-1.97)	81.89 (-5.06)	77.39 (-10.27)	83.02 (-4.68)
AAR	90.71 (0)	86.22 (-4.95)	84.33 (-7.03)	83.25 (-8.22)	78.3 (-13.68)	84.56 (-8.47)	85.97 (0)	84.55 (-1.65)	84.11 (-2.16)	81.15 (-5.61)	76.45 (-11.07)	82.45 (-5.12)
MEAN	90.63 (0)	87.36 (-3.59)	85.62 (-5.49)	84.48 (-6.78)	80.15 (-11.54)		86.62 (0)	85.37 (-1.44)	84.54 (-2.40)	82.16 (-5.15)	78.40 (-9.50)	
Factor	SE(d)						CD					
Variety	0.082						0.232					
Salt Stress	0.130						0.366					
Endophytic bacteria treatment	0.142						0.401					
Variety x Salt stress	0.183						0.518					
Variety x Endophytic bacteria	0.201						0.601					
Salt stress x Endophytic bacteria	0.317						0.953					
Variety X Endophytic bacteria X Salt stress	0.449						1.351					

*Figures in paranthesis is % decrease over control.

SS - Salt stress; EB - Endophytic bacteria; CEB - Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbaspirillum*; AAR - *Azoarcus*

Gluconacetobacter, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties. Earlier relative leaf water content was decreased with increase in salt stress conc. was reported in alfalfa (Ansari et al., 2019; Bertrand et al., 2015); maize (Bano and Fatima, 2009), and pea plants (Ali et al., 2015) However, treatment/inoculation of endophytes increased the leaf water content in alfalfa (Ansari et al., 2019); maize (Bano and Fatima, 2009) and pea plants (Ali et al., 2015) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species. Reduction leaf water potential in the present study in

sugarcane due to salinity may be due to reduction of soil water potential and water absorption of plant roots. Further, the salinity disturbed the balance between transpiration and water uptake by the plants and therefore in the present investigation may cause reduction in the relative leaf water potential in sugarcane (Ansari et al., 2019). Earlier results described under 4.8 revealed that application of endophytes resulted in increase in root length that may cause increased water absorption and thereby increased in relative leaf water potential under salinity stress condition in sugarcane. Further, it may be possible that application of root system architecture and resulted relative leaf water potential (Ansari et al., 2019; Ali et al., 2015; Bertrand et al., 2015; Bano and Fatima, 2009). Thus results obtained in the present study are in agreement with these earlier reports.

4.10 Effect of application of endophytic bacteria on lipid peroxidation of sugarcane under induced salinity stress

The lipid peroxidation content in leaves at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was computed and values obtained are given in Table 16 and Fig 26 & 27.

The lipid peroxidation was in the range of 19.23 -23.05 $\mu\text{mol MDA g}^{-1}$ fr. wt. in the CoM 0265 and 19.85 - 23.12 $\mu\text{mol MDA g}^{-1}$ fr. wt. in the CoC-671 sugarcane varieties. The maximum lipid peroxidation was observed 23.05 $\mu\text{mol MDA g}^{-1}$ fr. wt. in CoM 0265 and 23.12 $\mu\text{mol MDA g}^{-1}$ fr. wt. in the CoC-671 in sugarcane plants grown under imposed salt stress condition without endophytes treatments at 8 dSm^{-1} . The lowest values of lipid peroxidation 19.23 $\mu\text{mol MDA g}^{-1}$ fr. wt. in CoM 0265 and 19.85 $\mu\text{mol MDA g}^{-1}$ fr. wt. in CoC 671 was observed in consortia endophyte treated sugarcane plants under without salinity stress conditions. There was concomitant increase in lipid peroxidation with increase in salt stress levels from 2 dSm^{-1} to 8 dSm^{-1} and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in lipid peroxidation content at 8 dSm^{-1} (22.37 and 22.45 $\mu\text{mol MDA g}^{-1}$ fr. wt.) followed by 4 (22.19 and 22.32 $\mu\text{mol MDA g}^{-1}$ fr. wt.), 2 (21.29 and 21.42 $\mu\text{mol MDA g}^{-1}$ fr. wt.) dSm^{-1} salt stress and sodic soil (20.15 and 20.54 $\mu\text{mol MDA g}^{-1}$ fr. wt.) grown plants in CoM 0265 and CoC 671

sugarcane varieties respectively over the control (19.65 and 20.09 $\mu\text{mol MDA g}^{-1}$ fr. wt.) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, *Azoarcus* treated plants exhibited more increase during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Herbaspirillum*, *Azospirillum*, *Gluconacetobacter* and consortia in both the varieties of sugarcane. In CoM 0265 higher was noticed than CoC 0671 sugarcane variety at each salt stress level of endophyte in at treated plants.

Further, it was exhibited that endophyte treated plant caused decrease in lipid peroxidation over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. In control condition (without endophyte treatment) lipid peroxidation was 23.05 $\mu\text{mol MDA g}^{-1}$ fr. wt. in CoM 0265 and 23.12 $\mu\text{mol MDA g}^{-1}$ fr. wt. in CoC 671 and decreased with the application of endophytes and more decrease was observed by the application of consortia (21.01 $\mu\text{mol MDA g}^{-1}$ fr. wt.) and (21.32 $\mu\text{mol MDA g}^{-1}$ fr. wt.) followed by *Gluconacetobacter* (21.93 $\mu\text{mol MDA g}^{-1}$ fr. wt.) and (21.94 $\mu\text{mol MDA g}^{-1}$ fr. wt.); *Azospirillum* (22.71 $\mu\text{mol MDA g}^{-1}$ fr. wt.) and (22.32 $\mu\text{mol MDA g}^{-1}$ fr. wt.); *Herbaspirillum* (22.73 $\mu\text{mol MDA g}^{-1}$ fr. wt.) and (22.95 $\mu\text{mol MDA g}^{-1}$ fr. wt.); *Azoarcus* (22.76 $\mu\text{mol MDA g}^{-1}$ fr. wt.) and (23.05 $\mu\text{mol MDA g}^{-1}$ fr. wt.) in the respective varieties. In the entire imposed salinity stress treatments similar trend was observed. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm^{-1} and sodic soil to relieve the stress condition and resulted in decreased lipid peroxidation over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties. Lipid peroxidation content was increased with increase in salt stress concentration was reported earlier in pea (Sofy et al., 2021), maize (Li et al., 2017), wheat (Ansari et al., 2019), Rice (Sarkar et al., 2018). However, treatment/inoculation of endophytes decreased the lipid peroxidation in pea (Sofy et al., 2021); maize (Li et al., 2017); wheat (Shahid et al., 2022); wheat (Ansari et al., 2019); Rice (Sarkar et al., 2018) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species.

Table 16. Effect of endophytic bacteria on lipid peroxidation at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Lipid peroxidation ($\mu\text{mol MDA g}^{-1}$ fr. wt.)											
Varieties	COM 265						CoC 671					
EB SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	21.31 (0)	21.95 (3)	22.16 (3.99)	22.92 (7.56)	23.05 (8.17)	22.28 (5.68)	20.15 (0)	21.25 (5.46)	22.41 (11.22)	22.87 (13.50)	23.12 (14.74)	21.96 (11.23)
CEB	19.23 (0)	19.65 (2.18)	20.11 (4.58)	20.71 (7.70)	21.01 (9.26)	20.14 (5.93)	19.85 (0)	20.05 (1.01)	20.75 (4.53)	21.22 (6.90)	21.32 (7.41)	20.64 (4.96)
GA	19.27 (0)	19.75 (2.49)	21.24 (10.22)	21.56 (11.88)	21.93 (13.80)	20.75 (9.60)	19.95 (0)	20.19 (1.20)	20.89 (4.71)	21.74 (8.97)	21.94 (9.97)	20.94 (6.22)
ASP	19.32 (0)	19.81 (2.54)	21.36 (10.56)	22.62 (17.08)	22.71 (17.55)	21.16 (11.93)	20.17 (0)	20.51 (1.69)	21.35 (5.85)	22.24 (10.26)	22.32 (10.66)	21.32 (7.11)
HERB	19.37 (0)	19.86 (2.53)	21.41 (10.53)	22.65 (16.93)	22.73 (17.35)	21.20 (11.84)	20.19 (0)	20.59 (1.98)	21.42 (6.09)	22.85 (13.17)	22.95 (13.67)	21.60 (8.73)
AAR	19.42 (0)	19.89 (2.42)	21.44 (10.40)	22.68 (16.79)	22.76 (17.20)	21.24 (11.70)	20.21 (0)	20.62 (2.03)	21.67 (7.22)	22.99 (13.76)	23.05 (14.05)	21.71 (9.27)
MEAN	19.65 (0)	20.15 (2.53)	21.29 (8.38)	22.19 (12.99)	22.37 (13.89)		20.09 (0)	20.54 (2.23)	21.42 (6.60)	22.32 (11.09)	22.45 (11.75)	
Factor	SE(d)						CD					
Variety	0.028						0.080					
Salt Stress	0.045						0.127					
Endophytic bacteria treatment	0.049						0.139					
Variety x Salt stress	0.063						0.179					
Variety x Endophytic bacteria	0.069						0.196					
Salt stress x Endophytic bacteria	0.110						0.310					
Variety X Endophytic bacteria X Salt stress	0.155						0.438					

*Figures in paranthesis is % increase over control.

SS - Salt stress; **EB** - Endophytic bacteria; **CEB** – Consortia of Endophytic bacteria; **GA** - *Gluconacetobacter*; **ASP** - *Azospirillum*; **HERB** - *Herbasprillum*; **AAR** - *Azoarcus*

Exposure of sugarcane to NaCl may stress resulted in increased production of hydrogen peroxide, which eventually affected the structural integrity of the membrane and exhibited higher values of lipid peroxidation under salinity stress condition. Findings in the present study are consistent with those of Sofy et al. (2021), who found that under NaCl stress, membrane damage resulted in altered membrane function in pea and mustard cultivars respectively. El-Nahrawy et al. (2020) reported that excessive ROS production causes the peroxidation of unsaturated lipids, resulting in loss of membrane integrity, leakage, and desiccation of the membrane. In the present study decrease in lipid peroxidation in sugarcane

with application of endophyte may be because of decreased ROS and regulation of antioxidant system (Sofy et al., 2021). Thus present study corroborated the protective role of endophytes in improving the sugarcane membrane structures under both saline and non-saline condition.

4.11 Effect of application of endophytic bacteria on membrane injury index of sugarcane under induced salinity stress

The membrane injury index at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was evaluated and depicted in Table 17 and Fig 28 & 29.

It was observed that the membrane injury index was in the range of 16.97 - 24.01 % in the CoM 0265 and 17.15 - 27.22 % in the CoC-671. The highest membrane injury index was revealed 24.01 % in CoM 0265 and 27.22 % in CoC 671 grown with imposed salt stress condition at 8 dSm⁻¹ and without endophytes in sugarcane plant. The lowest membrane injury index 16.97 % in CoM 0265 and 17.15 % in CoC 671 was noticed in consortia endophyte treated plants and grown without salt stress condition. It was observed that there was increase in membrane injury index associated with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). Extra increase was observed as revealed from the mean values, more increase was observed in membrane injury index at 8 dSm⁻¹ (22.90 and 23.02 %) followed by 4 (21.16 and 21.51 %), 2 (18.88 and 19.25 %) dSm⁻¹ salt stress and sodic soil (18.52 and 18.68 %) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (17.27 and 17.68 %) as noticed from mean values of the individual treatment. *Azoarcus* treated plants showed more increase in membrane injury index during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Herbaspirillum*, *Azospirillum*, *Gluconacetobacter* and consortia in both the varieties of sugarcane. However, the increase was more in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

From the result obtained it was came to know that endophyte treated plant caused decrease in membrane injury index over the control (with imposed salt stress and without

endophyte treatment) within the individual salt stress treatment in both the varieties. Among the different salt stress levels at 8 dSm⁻¹ in control condition (without endophyte treatment) membrane injury index was 24.01 % in CoM 0265 and 27.22 % in CoC 671 and decreased with the application of endophytes and more decrease was observed by the application of consortia (21.98 %) and (22.10 %) followed by *Gluconacetobacter* (22.34 %) and (22.12 %); *Azospirillum* (22.78 %) and (22.17 %); *Herbaspirillum* (22.89 %) and (22.23 %); *Azoarcus* (23.41 %) and (22.31 %); in the respective varieties. This trend was observed in all the imposed salinity stress treatments.

Table 17. Effect of endophytic bacteria on membrane injury index at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Membrane injury index (%)											
Varities	COM 265						CoC 671					
EB SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	17.66 (0)	19.22 (8.83)	19.89 (12.63)	21.99 (24.52)	24.01 (35.96)	20.55 (20.48)	19.58 (0)	21.58 (10.21)	23.15 (18.23)	25.71 (31.31)	27.22 (39.02)	23.45 (24.69)
CEB	16.97 (0)	17.84 (5.13)	18.12 (6.78)	20.33 (19.80)	21.98 (29.52)	19.05 (15.31)	17.15 (0)	17.99 (4.90)	18.4 (7.29)	20.56 (19.88)	22.10 (28.86)	19.24 (15.23)
GA	17.11 (0)	18.24 (6.60)	18.37 (7.36)	20.65 (20.69)	22.34 (30.57)	19.34 (16.31)	17.25 (0)	18.05 (4.64)	18.44 (6.90)	20.62 (19.54)	22.12 (28.23)	19.30 (14.83)
ASP	17.2 (0)	18.38 (6.86)	18.68 (8.60)	21.05 (22.38)	22.78 (32.44)	19.62 (17.57)	17.31 (0)	18.1 (4.56)	18.48 (6.76)	20.69 (19.53)	22.17 (28.08)	19.35 (14.73)
HERB	17.28 (0)	18.61 (7.70)	19.01 (10.01)	21.41 (23.90)	22.89 (32.47)	19.84 (18.52)	17.35 (0)	18.16 (4.67)	18.52 (6.74)	20.74 (19.54)	22.23 (28.13)	19.40 (14.77)
AAR	17.37 (0)	18.84 (8.46)	19.19 (10.48)	21.54 (24.01)	23.41 (34.77)	20.07 (19.43)	17.45 (0)	18.24 (4.53)	18.56 (6.36)	20.79 (19.14)	22.31 (27.85)	19.47 (14.47)
MEAN	17.27 (0)	18.52 (7.26)	18.88 (9.31)	21.16 (22.55)	22.90 (32.62)		17.682 (0)	18.687 (5.58)	19.258 (8.71)	21.518 (21.49)	23.025 (30.03)	
Factor	SE(d)						CD					
Variety	0.058						0.164					
Salt Stress	0.092						0.259					
Endophytic bacteria treatment	0.100						0.284					
Variety x Salt stress	0.130						0.367					
Variety x Endophytic bacteria	0.142						0.429					
Salt stress x Endophytic bacteria	0.225						0.681					
Variety X Endophytic bacteria X Salt stress	0.318						0.959					

*Figures in paranthesis is % increase over control.

SS - Salt stress; EB - Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbaspirillum*; AAR - *Azoarcus*

Among the different endophyte treatments, consortia of endophytes was found to be better at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to lighten the

stress condition and resulted in decreased membrane injury index over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties. Earlier relative membrane injury index content was increased with increase in salt stress conc. was reported in pea (Sofy et al., 2021), wheat (Ansari et al., 2019). However, treatment/inoculation of endophytes decreased the lipid peroxidation in pea (Sofy et al., 2021); wheat (Ansari et al., 2019) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species. Salinity stress in sugarcane may damage plasmalemma and lipid membrane and these alterations in membrane integrity can lead to increased cell permeability and electrolyte leakage which exhibited higher values of membrane injury index in sugarcane in the present study (Singh et al., 2014).

In the present study sugarcane plant inoculated with endophytes had lower membrane injury index may be because of higher water and nutrient absorption (Werner and Newton, 2005). Membrane injury index in endophyte treated plants was higher than non-inoculated plants may be because of less absorption of Na^+ ions and high relative leaf water potential (Ansari et al., 2019).

4.12 Effect of application of endophytic bacteria on proline of sugarcane under induced salinity stress

The proline content at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was estimated and illustrated in Table 18 and Fig 30 & 31.

It was found that the proline was in the range of $0.63\text{--}1.54\ \mu\text{mol g}^{-1}$ fr. wt. in the CoM 0265 and $0.56\text{--}1.48\ \mu\text{mol g}^{-1}$ fr. wt. in the CoC-671. The maximum proline was observed $1.54\ \mu\text{mol g}^{-1}$ fr. wt. in CoM 0265 and $1.48\ \mu\text{mol g}^{-1}$ fr. wt. in the CoC-671 consortia treated and grown $8\ \text{dSm}^{-1}$ imposed salt stress condition in sugarcane plant. The minimum proline $0.63\ \mu\text{mol g}^{-1}$ fr. wt. in CoM 0265 and $0.56\ \mu\text{mol g}^{-1}$ fr. wt. in CoC 671 was observed in without endophyte treated plants and without salt stress conditions. It was noticed that there was increase in proline with increase in salt stress levels from $2\ \text{dSm}^{-1}$ to $8\ \text{dSm}^{-1}$ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the

control (without imposition of salt stress). As revealed from the mean values, more increase was observed in proline at 8 dSm^{-1} (1.39 and $1.30 \mu\text{mol g}^{-1} \text{ fr. wt.}$) followed by 4 (1.22 and $1.13 \mu\text{mol g}^{-1} \text{ fr. wt.}$), 2 (1.05 and $0.97 \mu\text{mol g}^{-1} \text{ fr. wt.}$) dSm^{-1} salt stress and sodic soil (0.92 and $0.86 \mu\text{mol g}^{-1} \text{ fr. wt.}$) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (0.66 and $0.92 \mu\text{mol g}^{-1} \text{ fr. wt.}$) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more increase during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* in both the varieties of sugarcane. However, the increase was more in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

Further it was brought to light that endophyte treated plant caused increase in proline over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Thus at 8 dSm^{-1} in control condition (without endophyte treatment) proline was $1.29 \mu\text{mol g}^{-1} \text{ fr. wt.}$ in CoM 0265 and $1.12 \mu\text{mol g}^{-1} \text{ fr. wt.}$ in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia ($1.54 \mu\text{mol g}^{-1} \text{ fr. wt.}$) and ($1.48 \mu\text{mol g}^{-1} \text{ fr. wt.}$) followed by *Gluconacetobacter* ($1.45 \mu\text{mol g}^{-1} \text{ fr. wt. m}$) and ($1.43 \mu\text{mol g}^{-1} \text{ fr. wt.}$); *Azospirillum* ($1.41 \mu\text{mol g}^{-1} \text{ fr. wt.}$) and ($1.31 \mu\text{mol g}^{-1} \text{ fr. wt.}$); *Herbaspirillum* ($1.35 \mu\text{mol g}^{-1} \text{ fr. wt.}$) and ($1.26 \mu\text{mol g}^{-1} \text{ fr. wt.}$); *Azoarcus* ($1.32 \mu\text{mol g}^{-1} \text{ fr. wt.}$) and ($1.19 \mu\text{mol g}^{-1} \text{ fr. wt.}$) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm^{-1} and sodic soil to improve the stress condition and resulted in increased proline over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties.

Earlier proline content was increased with increase in salt stress conc. was reported in peanut (Alexander et al., 2020); wheat (Shahid et al., 2022); pea (Sofy et al., 2021); tomato (Vaishnav et al., 2020). However, treatment/inoculation of endophytes increased the proline in pea (Sofy et al., 2021); peanut (Alexander et al., 2020), pea (Sofy et al., 2021) and tomato (Vaishnav et al., 2020) under salt stress condition. The findings in the present investigation are consistent with those reported earlier under salt stress conditions and

application/inoculation of endophytes to the plants under salinity stress condition in various crop species.

The proline content was increased with increase in salt stress in sugarcane in the present study which is the most common osmoprotectant that accumulates during salt stress, thereby ameliorating the negative effect of salinity. Besides its role as an osmolyte, proline acts as a stress marker.

Table 18. Effect of endophytic bacteria on proline at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Proline ($\mu\text{mol g}^{-1}$ fr. wt.)											
Varieties	COM 265						CoC 671					
EB \ SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	0.63 (0)	0.86 (35.86)	0.97 (53.24)	1.14 (80.09)	1.29 (103.79)	0.98 (68.25)	0.56 (0)	0.75 (34.17)	0.83 (48.48)	0.98 (75.31)	1.12 (100.36)	0.847 (64.58)
CEB	0.71 (0)	1.02 (43.66)	1.18 (66.20)	1.33 (87.32)	1.54 (116.90)	1.16 (78.52)	0.67 (0)	0.97 (44.78)	1.12 (67.16)	1.27 (89.55)	1.48 (120.90)	1.102 (80.60)
GA	0.68 (0)	0.96 (41.18)	1.11 (63.24)	1.26 (85.29)	1.45 (112.50)	1.09 (75.55)	0.66 (0)	0.94 (42.42)	1.08 (63.64)	1.23 (86.36)	1.43 (116.67)	1.068 (77.27)
ASP	0.67 (0)	0.93 (38.81)	1.05 (56.72)	1.24 (85.07)	1.41 (110.45)	1.06 (72.76)	0.62 (0)	0.87 (40.32)	0.98 (58.06)	1.15 (85.48)	1.31 (111.29)	0.986 (73.79)
HERB	0.65 (0)	0.90 (38.46)	1.01 (55.38)	1.18 (81.54)	1.35 (108.31)	1.02 (70.92)	0.60 (0)	0.84 (39.17)	0.94 (56.67)	1.10 (83.33)	1.26 (110.00)	0.947 (72.29)
AAR	0.64 (0)	0.88 (36.29)	0.98 (52.65)	1.15 (79.13)	1.32 (104.83)	0.99 (68.22)	0.58 (0)	0.81 (38.79)	0.89 (53.45)	1.04 (79.31)	1.19 (105.17)	0.901 (69.18)
MEAN	0.66 (0)	0.92 (39.04)	1.05 (57.90)	1.22 (83.08)	1.39 (109.46)		0.61 (0)	0.86 (39.94)	0.97 (57.91)	1.13 (83.23)	1.30 (110.73)	
Factor	SE(d)						CD					
Variety	0.007						0.020					
Salt Stress	0.011						0.031					
Endophytic bacteria treatment	0.012						0.034					
Variety x Salt stress	0.016						0.044					
Variety x Endophytic bacteria	0.017						0.053					
Salt stress x Endophytic bacteria	0.027						0.079					
Variety X Endophytic bacteria X Salt stress	0.038						0.116					

*Figures in paranthesis is % increase over control.

SS - Salt stress; EB - Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*

As observed in the present study CoM 0265 had more proline accumulation than the CoC 671 sugarcane variety indicating that CoM 0265 may be sustain the salt stress more effectively than CoC 671. In addition, treatment of endophytes caused further boost in the proline accumulation in both the sugarcane varieties under salinity stress condition led to

increase the stress tolerance in it. In this study result obtained on of endophytes caused increased in proline content in sugarcane plants under salinity condition and alleviate the saline situation are in line with the results by the researchers reported on different crops (Abdel Aziz et al., 2019; Omer et al., 2020; Sofy et al., 2021; Gharsallah et al., 2016; Abdel Latet et al., 2018; 2019; Badawy et al., 2021; Mansour and Ali, 2017; Li et al., 2017; Abdel Latel et al., 2021 and Abd_Allah et al., 2018). Proline has been observed to protect cell walls under osmotic stress, protect protein integrity, and increase enzymatic activity by acting as a molecular chaperone. Proline also has a role in scavenging ROS and shows singlet oxygen quenching ability (Kaur and Asthir, 2015). Despite these benefits, there are conflicting reports on the role of endophytic fungi in proline accumulation in salt stressed plants. Several studies reported that increases in proline content in inoculated plants compared to non-inoculated plants, while others report that lower proline content in inoculated plants. Higher proline content in inoculated plants has been attributed to: (i) favouring a decline in ionic influx inside cellular masses, thus helping plants to maintain their osmotic balance; (ii) increasing the expression of the gene encoding Pyrroline-5-carboxylate synthase (P5CS) enzyme, which is involved in proline biosynthesis; and (iii) increasing the activity of the P5CS enzyme (Rawat et al., 2016).

4.13 Effect of application of endophytic bacteria on glycine betaine of sugarcane under induced salinity stress

The glycine betaine content at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was estimated and recorded in Table 19 and Fig 32 & 33.

The glycine betaine was in the range of 2.19 – 4.28 $\mu\text{mol g}^{-1}$ fr. wt. in the CoM 0265 and 1.80 – 3.57 $\mu\text{mol g}^{-1}$ fr. wt. in the CoC-671. The maximum glycine betaine was observed 4.28 $\mu\text{mol g}^{-1}$ fr. wt. in CoM 0265 and 3.57 $\mu\text{mol g}^{-1}$ fr. wt. in the CoC-671 consortia treated and grown 8 dSm^{-1} imposed salt stress condition in sugarcane plant. The minimum glycine betaine 2.19 $\mu\text{mol g}^{-1}$ fr. wt. in CoM 0265 and 1.80 $\mu\text{mol g}^{-1}$ fr. wt. in CoC 671 was observed in without endophyte treated plants and without salt stress conditions. It was noticed that there was gradual decrease in glycine betaine with increase in salt stress levels from 2 dSm^{-1} to 8 dSm^{-1} and in sodic soil grown sugarcane plants and endophytes

treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in glycine betaine at 8 dSm⁻¹ (3.83 and 3.25 $\mu\text{mol g}^{-1}$ fr. wt.) followed by 4 (3.55 and 2.96 $\mu\text{mol g}^{-1}$ fr. wt.), 2 (3.37 and 2.67 $\mu\text{mol g}^{-1}$ fr. wt.) dSm⁻¹ salt stress and sodic soil (3.12 and 2.42 $\mu\text{mol g}^{-1}$ fr. wt.) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (2.50 and 1.84 $\mu\text{mol g}^{-1}$ fr. wt.) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more increase during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* in both the varieties of sugarcane. However, the increase was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

It was highlighted from the results that endophyte treated plant caused increase in glycine betaine over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Thus at 8 dSm⁻¹ in control condition (without endophyte treatment) glycine betaine was 3.18 $\mu\text{mol g}^{-1}$ fr. wt. in CoM 0265 and 2.76 $\mu\text{mol g}^{-1}$ fr. wt. in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (4.28 $\mu\text{mol g}^{-1}$ fr. wt.) and (3.57 $\mu\text{mol g}^{-1}$ fr. wt.) followed by *Gluconacetobacter* (4.14 $\mu\text{mol g}^{-1}$ fr. wt.) and (3.51 $\mu\text{mol g}^{-1}$ fr. wt.); *Azospirillum* (3.91 $\mu\text{mol g}^{-1}$ fr. wt.) and (3.45 $\mu\text{mol g}^{-1}$ fr. wt.); *Herbaspirillum* (3.80 $\mu\text{mol g}^{-1}$ fr. wt.) and (3.14 $\mu\text{mol g}^{-1}$ fr. wt.); *Azoarcus* (3.67 $\mu\text{mol g}^{-1}$ fr. wt.) and (3.07 $\mu\text{mol g}^{-1}$ fr. wt.) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to reduce the stress condition and resulted in increased glycine betaine over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* endophyte treatments in both the sugarcane varieties.

Table 19. Effect of endophytic bacteria on glycine betaine at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Glycine betaine ($\mu\text{mol g}^{-1}$ fr. wt.)											
Varieties	COM 265						CoC 671					
SS EB	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	2.19 (0)	2.67 (21.92)	2.88 (31.51)	3.01 (37.44)	3.18 (45.21)	2.79 (34.02)	1.80 (0)	2.12 (17.78)	2.28 (26.67)	2.48 (37.78)	2.76 (53.33)	2.29 (33.89)
CEB	2.71 (0)	3.47 (28.04)	3.78 (39.48)	3.98 (46.86)	4.28 (57.93)	3.64 (43.08)	1.87 (0)	2.68 (43.32)	2.91 (55.61)	3.24 (73.26)	3.57 (90.91)	2.85 (65.78)
GA	2.65 (0)	3.35 (26.42)	3.61 (36.23)	3.84 (44.91)	4.14 (56.23)	3.52 (40.94)	1.86 (0)	2.59 (39.25)	2.89 (55.38)	3.18 (70.97)	3.51 (88.71)	2.81 (63.58)
ASP	2.52 (0)	3.14 (24.60)	3.41 (35.32)	3.59 (42.46)	3.91 (55.16)	3.31 (39.38)	1.85 (0)	2.48 (34.05)	2.78 (50.27)	3.03 (63.78)	3.45 (86.49)	2.72 (58.65)
HERB	2.48 (0)	3.07 (23.79)	3.34 (34.68)	3.48 (40.32)	3.8 (53.23)	3.23 (38.00)	1.84 (0)	2.36 (28.26)	2.64 (43.48)	2.98 (61.96)	3.14 (70.65)	2.59 (51.09)
AAR	2.44 (0)	2.99 (22.54)	3.21 (31.56)	3.37 (38.11)	3.67 (50.41)	3.14 (35.66)	1.83 (0)	2.28 (24.59)	2.51 (37.16)	2.87 (56.83)	3.07 (67.76)	2.51 (46.58)
MEAN	2.50 (0)	3.12 (24.55)	3.37 (34.79)	3.55 (41.68)	3.83 (53.03)		1.84 (0)	2.42 (31.21)	2.67 (44.76)	2.96 (60.76)	3.25 (76.31)	
Factor	SE(d)						CD					
Variety	0.002						0.007					
Salt Stress	0.004						0.011					
Endophytic bacteria treatment	0.004						0.012					
Variety x Salt stress	0.005						0.015					
Variety x Endophytic bacteria	0.006						0.016					
Salt stress x Endophytic bacteria	0.009						0.026					
Variety X Endophytic bacteria X Salt stress	0.013						0.037					

*Figures in paranthesis is % increase over control.

SS - Salt stress; EB - Endophytic bacteria; CEB - Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*.

Results obtained in the present that increased levels of glycine betaine in sugarcane plants under salinity stress condition are in confirmatory with the earlier reports on in bean (Abriz and Torabian, 2017), rapeseed (Abriz et al., 2020) and *Oryza sativa* (Jha et al., 2011). Further, enhancement in glycine betaine as observed in the present study by application of endophytes are in agreement with results reported in rapeseed (Abriz et al., 2020), tomato (Vaishnav et al., 2019) under salt stress condition. The enhancement in glycine betaine in sugarcane by application endophytes under salinity stress thought to have an adaptive role in mediating osmotic adjustment and protecting subcellular structures in stress plants (Ashraf and Foolad, 2007). Because of high level of glycine betaine in endophyte treated sugarcane plants may serve as an index of internal water status of plants (Hansari and Nelson, 1978),

and/or it may increase sodium-flux from cytoplasm to vacuole and thereby modify membrane behavior. In addition it may stabilize the oxygen evolving activity of photo system-II protein complex by protecting against dissociation of regulatory extrinsic proteins and may also stabilize manganese cluster under salinity situation (Yeo, 1998).

4.14 Effect of application of endophytic bacteria on reducing sugar of sugarcane under induced salinity stress

The reducing sugar content at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was measured and observed depicted in Table 20 and Fig 34 & 35.

It was observed that the reducing sugar was in the range of 1.77 – 4.14 mg g⁻¹ fr. wt. in the CoM 0265 and 1.72 – 4.05 mg g⁻¹ fr. wt. in the CoC-671. The maximum reducing sugar was 4.14 mg g⁻¹ fr. wt. in CoM 0265 and 4.05 mg g⁻¹ fr. wt. in the CoC-671 consortia treated and grown 8 dSm⁻¹ imposed salt stress condition in sugarcane plant. The minimum reducing sugar 1.77 mg g⁻¹ fr. wt. in CoM 0265 and 1.72 mg g⁻¹ fr. wt. in CoC 671 was observed in without endophyte treated plants and without salt stress conditions. It was noticed that there was gradual decrease in reducing sugar with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in reducing sugar at 8 dSm⁻¹ (3.83 and 3.74 mg g⁻¹ fr. wt.) followed by 4 (2.24 and 2.17 mg g⁻¹ fr. wt.), 2 (2.19 and 2.12 mg g⁻¹ fr. wt.) dSm⁻¹ salt stress and sodic soil (2.05 and 1.97 mg g⁻¹ fr. wt.) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (1.88 and 1.76 mg g⁻¹ fr. wt.) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more decrease during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* in both the varieties of sugarcane. However, the increase was more in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

Further it was emphasized that endophyte treated plant caused increase in reducing sugar over the control (with imposed salt stress and without endophyte treatment) within the

individual salt stress treatment in both the varieties. Thus at 8 dSm⁻¹ in control condition (without endophyte treatment) reducing sugar was 3.63 mg g⁻¹ fr. wt. in CoM 0265 and 3.35 mg g⁻¹ fr. wt. in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (4.14 mg g⁻¹ fr. wt.) and (4.05 mg g⁻¹ fr. wt.) followed by *Gluconacetobacter* (3.96 mg g⁻¹ fr. wt.) and (3.92 mg g⁻¹ fr. wt.); *Azospirillum* (3.93 mg g⁻¹ fr. wt.) and (3.89 mg g⁻¹ fr. wt.); *Herbaspirillum* (3.69 mg g⁻¹ fr. wt.) and (3.67 mg g⁻¹ fr. wt.); *Azoarcus* (3.60 mg g⁻¹ fr. wt.) and (3.57 mg g⁻¹ fr. wt.) in the respective varieties. This trend was observed in all the imposed salinity stress treatments.

Table 20. Effect of endophytic bacteria on reducing sugar at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Reducing sugar (mg g ⁻¹ fr. wt.)											
Varieties	COM 265						CoC 671					
EB SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	1.77 (0)	1.94 (9.60)	1.98 (11.86)	2.03 (14.69)	3.63 (105.08)	2.27 (35.31)	1.72 (0)	1.85 (7.56)	1.91 (11.05)	1.96 (13.95)	3.35 (94.77)	2.16 (31.83)
CEB	1.94 (0)	2.21 (13.92)	2.32 (19.59)	2.37 (22.16)	4.14 (113.40)	2.60 (42.27)	1.81 (0)	2.12 (17.13)	2.29 (26.52)	2.34 (29.28)	4.05 (123.76)	2.52 (49.17)
GA	1.91 (0)	2.15 (12.57)	2.25 (17.80)	2.3 (20.42)	3.96 (107.33)	2.51 (39.53)	1.79 (0)	2.09 (16.76)	2.19 (22.35)	2.24 (25.14)	3.92 (118.99)	2.45 (45.81)
ASP	1.9 (0)	2.05 (7.89)	2.23 (17.37)	2.28 (20.00)	3.93 (106.84)	2.48 (38.03)	1.77 (0)	1.99 (12.43)	2.12 (19.77)	2.17 (22.60)	3.89 (119.77)	2.39 (43.64)
HERB	1.89 (0)	1.99 (5.29)	2.19 (15.87)	2.24 (18.52)	3.69 (95.24)	2.40 (33.73)	1.76 (0)	1.91 (8.52)	2.1 (19.32)	2.15 (22.16)	3.67 (108.52)	2.32 (39.63)
AAR	1.88 (0)	1.96 (4.26)	2.17 (15.43)	2.22 (18.09)	3.6 (91.49)	2.37 (32.31)	1.71 (0)	1.84 (7.60)	2.08 (21.64)	2.13 (24.56)	3.57 (108.77)	2.27 (40.64)
MEAN	1.88 (0)	2.05 (8.92)	2.19 (16.32)	2.24 (18.98)	3.83 (103.23)		1.76 (0)	1.97 (11.67)	2.12 (20.11)	2.17 (22.95)	3.74 (112.43)	
Factor	SE(d)						CD					
Variety	0.012						0.034					
Salt Stress	0.019						0.053					
Endophytic bacteria treatment	0.021						0.058					
Variety x Salt stress	0.027						0.075					
Variety x Endophytic bacteria	0.029						0.082					
Salt stress x Endophytic bacteria	0.046						0.130					
Variety X Endophytic bacteria X Salt stress	0.065						0.184					

*Figures in 96arenthesis is % increase over control.

SS – Salt stress; EB – Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA – *Gluconacetobacter*; ASP – *Azospirillum*; HERB – *Herbasprillum*; AAR - *Azoarcus*.

Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to mitigate the stress

condition and resulted in increased reducing sugar over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* endophyte treatments in both the sugarcane varieties. Earlier reducing sugar content was increased with increase in salt stress conc. was reported in bean (Abriz and Torabian, 2017), rapeseed (Abriz et al., 2020). However, treatment/inoculation of endophytes increased the reducing sugar in rapeseed (Abriz et al., 2020), tomato (Vaishnav et al., 2019) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species.

4.15 Effect of application of endophytic bacteria on non-reducing sugar of sugarcane under induced salinity stress

The non-reducing sugar content at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was quantified and tabulated in Table 21 and Fig 36 & 37.

It was observed that the non-reducing sugar was in the range of 1.82 – 4.14 mg g⁻¹ fr. wt. in the CoM 0265 and 1.75 – 4.05 mg g⁻¹ fr. wt. in the CoC-671. The maximum non-reducing sugar was observed 4.14 mg g⁻¹ fr. wt. in CoM 0265 and 4.05 mg g⁻¹ fr. wt. in the CoC-671 consortia treated and grown 8 dSm⁻¹ imposed salt stress condition in sugarcane plant. The minimum non-reducing sugar 1.82 mg g⁻¹ fr. wt. in CoM 0265 and 1.75 mg g⁻¹ fr. wt. in CoC 671 was observed in endophyte treated plants and salt stress conditions. It was noticed that there was gradual increase in non-reducing sugar with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in non-reducing sugar at 8 dSm⁻¹ (3.82 and 3.70 mg g⁻¹ fr. wt.) followed by 4 (3.10 and 2.97 mg g⁻¹ fr. wt.), 2 (2.22 and 2.13 mg g⁻¹ fr. wt.) dSm⁻¹ salt stress and sodic soil (2.05 and 1.97 mg g⁻¹ fr. wt) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (1.85 and 1.77 mg g⁻¹ fr. wt.) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more increase during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by

Gluconacetobacter, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* in both the varieties of sugarcane. However, the decrease was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

Table 21. Effect of endophytic bacteria on Non-reducing sugar at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter		Non-reducing sugar (mg g ⁻¹ fr. wt.)											
Varieties		COM 265						CoC 671					
EB SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	
	Control	1.82 (0)	1.94 (6.59)	2.14 (17.58)	2.98 (63.74)	3.59 (97.25)	2.49 (46.29)	1.75 (0)	1.85 (5.71)	1.97 (12.57)	2.55 (45.71)	3.1 (77.14)	2.24 (35.29)
	CEB	1.89 (0)	2.21 (16.93)	2.32 (22.75)	3.27 (73.02)	4.14 (119.05)	2.77 (57.94)	1.81 (0)	2.12 (17.13)	2.29 (26.52)	3.19 (76.24)	4.05 (123.76)	2.69 (60.91)
	GA	1.86 (0)	2.15 (15.59)	2.25 (20.97)	3.17 (70.43)	3.96 (112.90)	2.68 (54.97)	1.79 (0)	2.09 (16.76)	2.19 (22.35)	3.09 (72.63)	3.92 (118.99)	2.62 (57.68)
	ASP	1.85 (0)	2.05 (10.81)	2.23 (20.54)	3.09 (67.03)	3.93 (112.43)	2.63 (52.70)	1.77 (0)	1.99 (12.43)	2.12 (19.77)	3.07 (73.45)	3.89 (119.77)	2.57 (56.36)
	HERB	1.84 (0)	1.99 (8.15)	2.19 (19.02)	3.04 (65.22)	3.69 (100.54)	2.55 (48.23)	1.76 (0)	1.91 (8.52)	2.1 (19.32)	2.99 (69.89)	3.67 (108.52)	2.49 (51.56)
	AAR	1.83 (0)	1.96 (7.10)	2.17 (18.58)	3.02 (65.03)	3.6 (96.72)	2.52 (46.86)	1.71 (0)	1.84 (7.60)	2.08 (21.64)	2.93 (71.35)	3.57 (108.77)	2.43 (52.34)
	MEAN	1.85 (0)	2.05 (10.86)	2.22 (19.91)	3.10 (67.41)	3.82 (106.48)		1.77 (0)	1.97 (11.36)	2.13 (20.36)	2.97 (68.21)	3.70 (109.49)	
	Factor					SE(d)				CD			
Variety					0.004				0.012				
Salt Stress					0.007				0.019				
Endophytic bacteria treatment					0.007				0.021				
Variety x Salt stress					0.010				0.027				
Variety x Endophytic bacteria					0.011				0.030				
Salt stress x Endophytic bacteria					0.017				0.047				
Variety X Endophytic bacteria X Salt stress					0.024				0.067				

*Figures in parenthesis is % increase over control.

SS – Salt stress; EB – Endophytic bacteria; CEB – Consortia of Endophytic bacteria; GA – *Gluconacetobacter*; ASP – *Azospirillum*; HERB – *Herbaspirillum*; AAR - *Azoarcus*.

Further it was exhibited that endophyte treated plant caused increase in non-reducing sugar over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Thus at 8 dSm⁻¹ in control condition (without endophyte treatment) non-reducing sugar was 3.59 mg g⁻¹ fr. wt. in CoM 0265 and 3.10 mg g⁻¹ fr. wt. in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (4.14 mg g⁻¹ fr. wt.) and (4.05 mg g⁻¹

fr. wt.) followed by *Glucanoacetobacter* (3.96 mg g⁻¹ fr. wt.) and (3.92 mg g⁻¹ fr. wt.); *Azospirillum* (3.93 mg g⁻¹ fr. wt.) and (3.89 mg g⁻¹ fr. wt.); *Herbaspirillum* (3.69 mg g⁻¹ fr. wt.) and (3.67 mg g⁻¹ fr. wt.); *Azoarcus* (3.60 mg g⁻¹ fr. wt.) and (3.57 mg g⁻¹ fr. wt.) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to overcome the stress condition and resulted in increased non-reducing sugar over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties. Earlier non-reducing sugar content was increased with increase in salt stress conc. was reported in bean (Abriz and Torabian 2017), rapeseed (Abriz et al., 2020). The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species.

4.16 Effect of application of endophytic bacteria on total sugar of sugarcane under induced salinity stress

The total sugar content at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was measured and tabulated in Table 22 and Fig 38 & 39.

It was observed that the total sugar was in the range of 3.49 – 8.28 mg g⁻¹ fr. wt. in the CoM 0265 and 3.34 – 8.10 mg g⁻¹ fr. wt. in the CoC-671. The maximum total sugar was observed 8.28 mg g⁻¹ fr. wt. in CoM 0265 and 8.10 mg g⁻¹ fr. wt. in the CoC-671 Consortia treated and grown with 8 dSm⁻¹ imposed salt stress condition in sugarcane plant. The minimum total sugar 3.49 mg g⁻¹ fr. wt. in CoM 0265 and 3.34 mg g⁻¹ fr. wt. in CoC 671 was observed in without endophyte treated plants and without salt stress conditions. It was noticed that there was gradual increase in total sugar with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in non-reducing sugar at 8 dSm⁻¹ (7.64 and 7.49 mg g⁻¹ fr. wt.) followed by 4 (5.38 and 5.21 mg g⁻¹ fr. wt.), 2 (4.44 and 4.28 mg g⁻¹ fr. wt.) dSm⁻¹ salt stress and sodic soil (4.10 and 3.93 mg g⁻¹ fr. wt) grown plants in CoM 0265 and

CoC 671 sugarcane varieties respectively over the control (3.71 and 3.50 mg g⁻¹ fr. wt.) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more increase during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* in both the varieties of sugarcane. However, the increase was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte in at treated plants.

Table 21. Effect of endophytic bacteria on total sugar at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Total sugar (mg g ⁻¹ fr. wt.)											
Varieties	COM 265						CoC 671					
EB SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	3.49 (0)	3.88 (11.17)	4.32 (23.78)	5.35 (53.30)	7.22 (106.88)	4.85 (48.78)	3.34 (0)	3.7 (10.39)	4.1 (21.66)	4.98 (47.77)	6.75 (100.30)	4.57 (45.03)
CEB	3.83 (0)	4.42 (14.91)	4.64 (24.12)	5.64 (49.86)	8.28 (119.51)	5.36 (52.10)	3.62 (0)	4.24 (18.82)	4.58 (24.73)	5.53 (51.61)	8.10 (122.58)	5.21 (54.44)
GA	3.77 (0)	4.3 (19.43)	4.5 (25.14)	5.47 (52.29)	7.92 (124.00)	5.19 (55.21)	3.58 (0)	4.18 (17.81)	4.38 (23.29)	5.33 (49.86)	7.84 (116.99)	5.06 (51.99)
ASP	3.75 (0)	4.1 (15.70)	4.46 (23.26)	5.37 (52.33)	7.86 (126.16)	5.11 (54.36)	3.54 (0)	3.98 (13.89)	4.24 (23.89)	5.24 (49.17)	7.78 (118.33)	4.96 (51.32)
HERB	3.73 (0)	3.98 (12.02)	4.38 (23.17)	5.26 (50.73)	7.38 (115.25)	4.95 (50.29)	3.52 (0)	3.82 (12.43)	4.2 (23.73)	5.14 (48.59)	7.34 (108.47)	4.80 (48.31)
AAR	3.71 (0)	3.92 (9.52)	4.34 (23.81)	5.21 (50.60)	7.2 (112.50)	4.88 (49.11)	3.42 (0)	3.68 (11.36)	4.16 (23.30)	5.06 (48.01)	7.14 (104.55)	4.69 (46.80)
MEAN	3.71 (0)	4.10 (13.66)	4.44 (23.53)	5.38 (50.60)	7.64 (116.29)		3.5 (0)	3.93 (14.25)	4.28 (23.79)	5.21 (50.90)	7.49 (118.97)	
Factor	SE(d)						CD					
Variety	0.013						0.037					
Salt Stress	0.021						0.058					
Endophytic bacteria treatment	0.023						0.064					
Variety x Salt stress	0.029						0.082					
Variety x Endophytic bacteria	0.032						0.090					
Salt stress x Endophytic bacteria	0.050						0.143					
Variety X Endophytic bacteria X Salt stress	0.071						0.202					

*Figures in paranthesis is % increase over control.

SS - Salt stress; EB - Endophytic bacteria; CEB - Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP - *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*.

Further it was disclosed that endophyte treated plant caused increase in total sugar over the control (with imposed salt stress and without endophyte treatment) within the

individual salt stress treatment in both the varieties. Thus at 8 dSm⁻¹ in control condition (without endophyte treatment) total sugar was 7.22 mg g⁻¹ fr. wt. in CoM 0265 and 6.75 mg g⁻¹ fr. wt. in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (8.28 mg g⁻¹ fr. wt.) and (8.10 mg g⁻¹ fr. wt.) followed by *Gluconacetobacter* (7.92 mg g⁻¹ fr. wt.) and (7.84 mg g⁻¹ fr. wt.); *Azospirillum* (7.86 mg g⁻¹ fr. wt.) and (7.78 mg g⁻¹ fr. wt.); *Herbaspirillum* (7.38 mg g⁻¹ fr. wt.) and (7.34 mg g⁻¹ fr. wt.); *Azoarcus* (7.20 mg g⁻¹ fr. wt.) and (7.14 mg g⁻¹ fr. wt.) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to mitigate the stress condition and resulted in increased total sugar over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments were found to overcome salinity stress as revealed by increase in total sugar in both the sugarcane varieties.

Earlier total sugar content was increased with increase in salt stress conc. was reported in bean (Abriz and Torabian, 2017), rapeseed (Abriz et al., 2020). However, treatment/inoculation of endophytes increased the total sugar in rapeseed (Abriz et al., 2020), tomato (Vaishnavi et al., 2019) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species.

Increase in sugar contents in untreated and treated endophytes to sugarcane plants may serve as an osmoprotection as they can stabilize the cell membrane and protoplast under salt stress condition (Gupta et al., 2020). These sugars also protect water soluble enzymes from high intracellular concentration of inorganic ions (Liang et al., 2013). Increase of total sugar in endophyte treated plants under saline conditions may be because of activation of starch degrading enzyme, glucan water dikinase (Gupta et al., 2021; Ghabooli, 2014; Zhang et al., 2019). Thus due application endophyte resulting in accumulation of sugars may involve in adjustment of the osmotic potential of cellular water retention and turgor maintenance, thereby minimizing the adverse effects of salt stress by balancing the solute potential (Bai et al., 2019).

4.17 Effect of application of endophytic bacteria on catalase of sugarcane under induced salinity stress

The activity of catalase at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was assayed and result obtained are presented in Table 23 and Fig 40 & 41.

It was observed that the catalase activity was in the range of 4.32 – 7.89 $\mu\text{mol H}_2\text{O}_2$ decompose $\text{min}^{-1} \text{mg}^{-1}$ protein in the CoM 0265 and 4.08 – 7.63 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein in the CoC-671. The maximum catalase activity was observed 7.89 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein in CoM 0265 and 6.62 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein in the CoC-671 consortia treated and grown without imposed salt stress condition in sugarcane plant. The minimum catalase 4.32 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein in CoM 0265 and 4.08 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein in CoC 671 was observed in without endophyte treated plants and without salt stress conditions. It was noticed that there was gradual increase in catalase activity with increase in salt stress levels from 2 dSm^{-1} to 8 dSm^{-1} and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in catalase at 8 dSm^{-1} (7.52 and 7.22 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) followed by 4 (6.89 and 6.60 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein), 2 (6.39 and 6.08 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) dSm^{-1} salt stress and sodic soil (5.52 and 5.29 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (4.37 and 4.17 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more increase during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* in both the varieties of sugarcane. However, the increase was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

It was brought to light from the result obtained that endophyte treated plant caused increase in catalase over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Thus at 8 dSm^{-1} in

Table 23. Effect of endophytic bacteria on Catalase peroxidase at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Catalase peroxidase ($\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein)											
Varieties	COM 265						CoC 671					
SS EB	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	4.32 (0)	5.27 (21.99)	6.12 (41.67)	6.49 (50.23)	7.07 (63.66)	5.85 (44.39)	4.08 (0)	4.87 (19.36)	5.65 (38.48)	6.08 (49.02)	6.62 (62.25)	5.46 (42.28)
CEB	4.41 (0)	5.83 (32.20)	6.72 (52.38)	7.28 (65.08)	7.89 (78.91)	6.43 (57.14)	4.24 (0)	5.64 (33.02)	6.52 (53.77)	7.08 (66.98)	7.63 (79.95)	6.22 (58.43)
GA	4.39 (0)	5.69 (29.61)	6.58 (49.89)	7.12 (62.19)	7.76 (76.77)	6.31 (54.61)	4.27 (0)	5.56 (30.21)	6.44 (50.82)	6.94 (62.53)	7.59 (77.75)	6.16 (55.33)
ASP	4.37 (0)	5.53 (26.54)	6.41 (46.68)	6.97 (59.50)	7.56 (73.00)	6.17 (51.43)	4.19 (0)	5.34 (27.45)	6.17 (47.26)	6.71 (60.14)	7.31 (74.46)	5.944 (52.33)
HERB	4.36 (0)	5.42 (24.31)	6.29 (44.27)	6.84 (56.88)	7.47 (71.33)	6.08 (49.20)	4.11 (0)	5.21 (26.76)	5.97 (45.26)	6.49 (57.91)	7.14 (73.72)	5.784 (50.91)
AAR	4.34 (0)	5.36 (23.50)	6.19 (42.63)	6.62 (52.53)	7.37 (69.82)	5.98 (47.12)	4.1 (0)	5.13 (25.12)	5.73 (39.76)	6.29 (53.41)	7.02 (71.22)	5.654 (47.38)
MEAN	4.37 (0)	5.52 (26.36)	6.39 (46.25)	6.89 (57.73)	7.52 (72.25)		4.17 (0)	5.29 (26.99)	6.08 (45.89)	6.60 (58.33)	7.22 (73.23)	
Factor	SE(d)						CD					
Variety	0.002						0.006					
Salt Stress	0.003						0.009					
Endophytic bacteria treatment	0.004						0.010					
Variety x Salt stress	0.005						0.013					
Variety x Endophytic bacteria	0.005						0.014					
Salt stress x Endophytic bacteria	0.008						0.022					
Variety X Endophytic bacteria X Salt stress	0.011						0.035					

*Figures in paranthesis is % increase over control.

SS - Salt stress; EB - Endophytic bacteria; CEB - Consortia of Endophytic bacteria; GA - *Gluconacetobacter*;

ASP - *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*

control condition (without endophyte treatment) catalase activity was 7.07 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein in CoM 0265 and 6.62 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (7.89 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) and (7.63 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) followed by *Gluconacetobacter* (7.76 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) and (7.59 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein); *Azospirillum* (7.56 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) and (7.31 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein); *Herbasprillum* (7.47 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein) and (7.14 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein); *Azoarcus* (7.37 $\mu\text{mol H}_2\text{O}_2$ decomposed $\text{min}^{-1} \text{mg}^{-1}$ protein)

H₂O₂ decomposed min⁻¹ mg⁻¹ protein) and (7.02 µmol H₂O₂ decompose min⁻¹ mg⁻¹ protein) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to alleviate the stress condition and resulted in increased catalase over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties.

Earlier catalase (CAT) was increased with increase in salt stress conc. was reported in wheat (Singh et al., 2017), wheat (Ansari et al., 2019), cucumber (Kang et al., 2014), rice (Khan et al., 2016), wheat (Afridi et al., 2019), tomato (Ansari et al., 2019), Pea (Sofy et al., 2021). However, treatment/inoculation of endophytes increased the catalase in cucumber (Kang et al., 2014); lucerne (Ansari et al., 2019); Pea (Sofy et al., 2021); wheat (Singh et al., 2017); rice (Sarkar et al., 2018); wheat (Ansari et al., 2019) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species.

Increase in catalase activity and further enhancement in it endophyte treatment to sugarcane plants may remove H₂O₂ which generated under salinity stress and prevent damage to the membrane and organs (Sofy et al., 2020; Hashem et al., 2016; Rios-Gonzalez et al., 2002; Kordrostami et al., 2017; Chrysargyris et al., 2018; Ahammed et al., 2019; Rios-Gonzalez et al., 2002; Abdel Latef et al., 2017; D'Amelia et al., 2018).

If the balance between the antioxidant defense system and the production of ROS decreases, the oxidative stress induced weakens the cell membranes and other organelles (Machado and Serralheiro 2017; Abdel Latef et al., 2019; Hasan et al., 2019). Thus, higher enzyme activity in *Azospirillum lipoferum* treatments probably indicated that inoculation of seeds with this bacterium has increased the activity of antioxidant enzymes and subsequently scavenged or reduced ROS in the maize plants and has provided plant's better growth conditions (Jha and Saraf, 2015).

4.18 Effect of application of endophytic bacteria on ascorbate peroxidase of sugarcane under induced salinity stress

The activity of ascorbate peroxidase at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was assayed and illustrated in Table 24 and Fig 42 & 43.

It was observed that the ascorbate peroxidase activity was in the range of 3.32- 6.46 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein in the CoM 0265 and 3.06 - 6.28 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein in the CoC-671. The maximum ascorbate peroxidase activity was observed 6.46 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein in CoM 0265 and 6.28 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein in the CoC-671 in Consortia treated and grown with imposed salt stress condition in sugarcane plant. The minimum ascorbate peroxidase 3.32 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein in CoM 0265 and 3.06 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein in CoC 671 was observed in without endophyte treated plants and without salt stress conditions. It was noticed that there was gradual increase in ascorbate peroxidase activity in salt stress levels from 2 dSm^{-1} to 8 dSm^{-1} and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in ascorbate peroxidase at 8 dSm^{-1} (6.23 and 5.89 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein) followed by 4 (5.69 and 5.41 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein), 2 (5.27 and 5.03 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein) dSm^{-1} salt stress and sodic soil (4.52 and 4.31 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (3.37 and 3.18 μmol ascorbate oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more decrease during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* in both the varieties of sugarcane. However, the increase was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte treated plants.

Further it was highlighted from results that endophyte treated plant caused increase in ascorbate peroxidase activity over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Thus at

Table 24. Effect of endophytic bacteria on ascorbate peroxidase ($\mu\text{mol ascorbate min}^{-1} \text{mg}^{-1} \text{protein}$) at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Ascorbate peroxidase ($\mu\text{mol ascorbate min}^{-1} \text{mg}^{-1} \text{protein}$)											
Varieties	COM 265						CoC 671					
EB \ SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	3.32 (0)	4.39 (32.23)	5.04 (51.81)	5.53 (66.57)	6.03 (81.63)	4.86 (58.06)	3.06 (0)	4.03 (31.70)	4.62 (50.98)	5.07 (65.69)	5.39 (76.14)	4.43 (56.13)
CEB	3.41 (0)	4.68 (37.24)	5.52 (61.88)	5.87 (72.14)	6.46 (89.44)	5.19 (65.18)	3.28 (0)	4.54 (38.41)	5.39 (64.33)	5.75 (75.30)	6.28 (91.46)	5.048 (67.38)
GA	3.39 (0)	4.6 (35.69)	5.44 (60.47)	5.79 (70.80)	6.34 (87.02)	5.11 (63.50)	3.26 (0)	4.46 (36.81)	5.31 (62.88)	5.62 (72.39)	6.14 (88.34)	4.958 (65.11)
ASP	3.37 (0)	4.53 (34.42)	5.32 (57.86)	5.71 (69.44)	6.27 (86.05)	5.04 (61.94)	3.21 (0)	4.38 (36.45)	5.11 (59.19)	5.47 (70.40)	6.02 (87.54)	4.838 (63.40)
HERB	3.36 (0)	4.49 (33.63)	5.19 (54.46)	5.66 (68.45)	6.18 (83.93)	4.98 (60.12)	3.16 (0)	4.29 (35.76)	4.94 (56.33)	5.34 (68.99)	5.84 (84.81)	4.714 (61.47)
AAR	3.34 (0)	4.43 (32.63)	5.13 (53.59)	5.59 (67.37)	6.1 (82.63)	4.92 (59.06)	3.1 (0)	4.18 (34.84)	4.78 (54.19)	5.21 (68.06)	5.68 (83.23)	4.59 (60.08)
MEAN	3.37 (0)	4.52 (34.31)	5.27 (56.68)	5.69 (69.13)	6.23 (85.12)		3.18 (0)	4.31 (35.66)	5.03 (57.98)	5.41 (70.14)	5.89 (85.25)	
Factor	SE(d)						CD					
Variety	0.008						0.024					
Salt Stress	0.013						0.038					
Endophytic bacteria treatment	0.015						0.042					
Variety x Salt stress	0.019						0.054					
Variety x Endophytic bacteria	0.021						0.059					
Salt stress x Endophytic bacteria	0.023						0.069					
Variety X Endophytic bacteria X Salt stress	0.471						1.333					

*Figures in paranthesis is % increase over control.

SS - Salt stress; EB - Endophytic bacteria; CEB - Consortia of Endophytic bacteria; GA - *Gluconacetobacter*;

ASP-*Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*

8 dSm⁻¹ in control condition (without endophyte treatment) ascorbate peroxidase was 6.03 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$ in CoM 0265 and 5.39 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$ in CoC 671 and increased with the application of endophytes and more increase was observed by the application of consortia (6.46 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$) and (6.28 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$) followed by *Gluconacetobacter* (6.34 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$) and (6.14 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$); *Azospirillum* (6.27 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$) and (6.02 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$); *Herbasprillum* (6.18 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$) and (5.84 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$); *Azoarcus* (6.10 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$) and (5.68 $\mu\text{mol ascorbate oxidised min}^{-1} \text{mg}^{-1} \text{protein}$).

oxidised $\text{min}^{-1} \text{mg}^{-1}$ protein) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm^{-1} and sodic soil to reduce the stress condition and resulted in increased ascorbate peroxidase activity over respective control followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties. Earlier increase in ascorbate peroxidase (APX) with increase in salt stress conc. was reported in cucumber (Kang et al., 2014), maize (Abdel Latet et al., 2020), Pea (Sofy et al., 2021). However, treatment/inoculation of endophytes increased the ascorbate peroxidase in cucumber (Kang et al., 2014); lucerne (Ansari et al., 2019); Pea (Sofy et al., 2021); wheat (Singh et al., 2017); wheat (Ansari et al., 2019) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species. The present investigation the increase in ascorbate peroxidase (APX) in both the varieties of sugarcane under imposed salinity stress condition by the application of endophytes could be due to in the plants, antioxidant enzymes control the redox homeostasis by absorbing reducing agents and oxidants (Ahammed et al., 2019). Increasing the activity of antioxidant enzyme APX has helped plants to resist and stabilize against stresses such as salinity (Rios-Gonzalez et al., 2002; Kordrostami et al., 2017; Chrysargyris et al., 2018; Ahammed et al., 2019). Our result of significant increase in the activity of APX enzyme by the application endophytes with increasing salinity level has shown that antioxidant system of the plant has activated against salinity stress and that the plant, by increasing the activity of these enzymes, could resist better against ROS (Rios-Gonzalez et al., 2002).

If the balance between the antioxidant defense system and the production of ROS decreases, the oxidative stress induced weakens the cell membranes and other organelles (Machado and Serralheiro 2017; Abdel Latef et al., 2019; Hasan et al., 2019). Thus, higher enzyme activity in endophyte treatments probably indicated that the bacterium has increased the activity of antioxidant enzymes and subsequently scavenged or reduced ROS in the plants and has provided plant's better growth conditions (Jha and Saraf 2015).

4.19 Effect of application of endophytic bacteria on superoxide dismutase of sugarcane under induced salinity stress

The superoxide dismutase activity at tillering stage of sugarcane varieties viz., CoM 0265 and CoC 671 treated with endophytic bacteria viz., *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, *Azoarcus* and its consortia and grown under induced salt stress condition and sodic soil, was assayed and given in Table 25 and Fig 44 & 45.

It was observed that the superoxide dismutase activity was in the range of 80.67 – 188.17 U min⁻¹ mg⁻¹ protein in the CoM 0265 and 77.88 – 185.62 U min⁻¹ mg⁻¹ protein in the CoC-671. The maximum superoxide dismutase was observed 188.17 U min⁻¹ mg⁻¹ protein in CoM 0265 and 185.62 U min⁻¹ mg⁻¹ protein in the CoC-671 consortia treated and grown with imposed salt stress condition in sugarcane plant. The minimum superoxide dismutase 80.67 U min⁻¹ mg⁻¹ protein in CoM 0265 and 77.88 U min⁻¹ mg⁻¹ protein in CoC 671 was observed in without endophyte treated plants and without salt stress conditions. It was noticed that there was gradual increase in superoxide dismutase activity with increase in salt stress levels from 2 dSm⁻¹ to 8 dSm⁻¹ and in sodic soil grown sugarcane plants and endophytes treated plants of both the varieties over the control (without imposition of salt stress). As revealed from the mean values, more increase was observed in superoxide dismutase at 8 dSm⁻¹ (172.67 and 168.45 U min⁻¹ mg⁻¹ protein) followed by 4 (154.65 and 149.46 U min⁻¹ mg⁻¹ protein), 2 (131.83 and 128.05 U min⁻¹ mg⁻¹ protein) dSm⁻¹ salt stress and sodic soil (109.94 and 107.22 U min⁻¹ mg⁻¹ protein) grown plants in CoM 0265 and CoC 671 sugarcane varieties respectively over the control (83.15 and 80.61 U min⁻¹ mg⁻¹ protein) as noticed from mean values of the individual treatment. Among the different endophyte treated plants, consortia treated plants showed more increase during the increase in salt stress condition over the control (without imposed salt stress and endophyte treated) followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum*, and *Azoarcus* in both the varieties of sugarcane. However, the increase was more prominent in CoM 0265 than CoC 0671 sugarcane variety at each salt stress level of endophyte in treated plants.

Further it was revealed that endophyte treated plant caused increase in superoxide dismutase activity over the control (with imposed salt stress and without endophyte treatment) within the individual salt stress treatment in both the varieties. Thus at 8 dSm⁻¹ in control condition (without endophyte treatment) superoxide dismutase was 165.02 U min⁻¹ mg⁻¹ protein in CoM 0265 and 150.53 U min⁻¹ mg⁻¹ protein in CoC 671 and increased with

Table 25. Effect of endophytic bacteria on superoxide dismutase at tillering stage of sugarcane varieties grown under induced salt stress conditions.

Parameter	Superoxide dismutase (U min ⁻¹ mg ⁻¹ protein)											
Varieties	COM 265						CoC 671					
EB \ SS	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
Control	80.67 (0)	107.43 (33.17)	124.31 (54.10)	149.9 (85.82)	165.02 (104.56)	125.46 (69.41)	77.88 (0)	101.75 (30.65)	116.92 (50.13)	135.12 (73.50)	150.53 (93.28)	116.44 (61.89)
CEB	84.63 (0)	114.64 (35.46)	141.26 (66.91)	164.48 (94.35)	188.17 (122.34)	138.63 (79.77)	82.76 (0)	113.15 (36.72)	138.28 (67.09)	162.03 (95.78)	185.62 (124.29)	136.37 (80.97)
GA	84.06 (0)	111.92 (33.14)	137.48 (63.55)	160.92 (91.43)	180.19 (114.36)	134.914 (75.62)	82.02 (0)	110.53 (34.76)	135.18 (64.81)	157.16 (91.61)	178.31 (117.40)	132.64 (77.15)
ASP	83.65 (0)	110.59 (32.21)	132.68 (58.61)	154.76 (85.01)	174.52 (108.63)	131.24 (71.11)	81.61 (0)	108.26 (32.66)	130.36 (59.74)	152.54 (86.91)	172.18 (110.98)	128.99 (72.57)
HERB	83.07 (0)	108.56 (30.68)	128.7 (54.93)	151.37 (82.22)	168.14 (102.41)	127.968 (67.56)	80.37 (0)	105.82 (31.67)	126.33 (57.19)	148.48 (84.75)	165.15 (105.49)	125.23 (69.77)
AAR	82.83 (0)	106.51 (28.59)	126.56 (52.79)	146.48 (76.84)	159.97 (93.13)	124.47 (62.84)	79.04 (0)	103.78 (31.30)	121.24 (53.39)	141.41 (78.91)	158.89 (101.02)	120.87 (66.16)
MEAN	83.15 (0)	109.94 (33.05)	131.83 (59.15)	154.65 (85.95)	172.67 (109.57)		80.61 (0)	107.22 (32.96)	128.05 (58.72)	149.46 (85.24)	168.45 (108.74)	
Factor	SE(d)						CD					
Variety	0.086						0.243					
Salt Stress	0.136						0.385					
Endophytic bacteria treatment	0.149						0.422					
Variety x Salt stress	0.192						0.544					
Variety x Endophytic bacteria	0.211						0.596					
Salt stress x Endophytic bacteria	0.333						0.943					
Variety X Endophytic bacteria X Salt stress	0.471						1.333					

*Figures in paranthesis is % increase over control.

SS - Salt stress; EB - Endophytic bacteria; CEB - Consortia of Endophytic bacteria; GA - *Gluconacetobacter*; ASP- *Azospirillum*; HERB - *Herbasprillum*; AAR - *Azoarcus*

the application of endophytes and more increase was observed by the application of consortia (188.17 U min⁻¹ mg⁻¹ protein) and (185.62 U min⁻¹ mg⁻¹ protein) followed by *Gluconacetobacter* (180.19 U min⁻¹ mg⁻¹ protein) and (178.31 U min⁻¹ mg⁻¹ protein); *Azospirillum* (174.52 U min⁻¹ mg⁻¹ protein) and (172.18 U min⁻¹ mg⁻¹ protein); *Herbasprillum* (168.14 U min⁻¹ mg⁻¹ protein) and (165.15 U min⁻¹ mg⁻¹ protein); *Azoarcus* (159.97 U min⁻¹ mg⁻¹ protein) and (158.89 U min⁻¹ mg⁻¹ protein) in the respective varieties. This trend was observed in all the imposed salinity stress treatments. Among the different endophyte treatments, consortia was found to be superior at different salinity stress treatment viz., 2, 4, 8 dSm⁻¹ and sodic soil to relieve the stress condition and resulted in increased superoxide dismutase activity over respective control followed by *Gluconacetobacter*,

Azospirillum, *Herbaspirillum*, and *Azoarcus* endophyte treatments in both the sugarcane varieties.

Earlier increase in superoxide dismutase (SOD) with increase in salt stress conc. was reported in wheat (Ansari et al., 2019), soyabean (Kang et al., 2014), pea (Sofy et al., 2021). However, treatment/inoculation of endophytes increased the shoot length in cucumber (Kang et al., 2014); lucerne (Ansari et al., 2019); pea (Sofy et al., 2021) under salt stress condition. The results obtained in the present investigation are concomitant with results reported earlier under salt stress conditions and application/inoculation of endophytes to the plants under salinity stress condition in various crop species. Zhang et al. (2016) and results obtained in the present study exhibited that endophyte inoculated plant had higher SOD activity which played a central role in the O^{-2} and H_2O_2 scavenging process in endophyte treated plants. The upregulated SOD may protect the plants from free radical induced membrane dysfunction (Abd Allah et al., 2018; Omer et al., 2022).

5. SUMMARY CONCLUSION

A pot culture experiment was carried on “Physio-chemical studies on effect of endophytic bacteria in combating induced salinity stress in sugarcane” to study the effect of endophytic bacteria on biochemical parameters in sugarcane grown under imposed salinity stress conditions. Two contrasting varieties viz., CoM-0265 (salt tolerant) and CoC-671 (salt susceptible) were obtained from Central Sugarcane Research Station, Padegaon and planted in pots and salt stress was imposed by irrigating NaCl solutions of 0, 2, 4, 8 dSm⁻¹ and in of the pot containing sodic soil grown under glasshouse conditions using completely RBD. The outcome of research are summarized below in points

1. The endophytic bacteria were isolated from the saline soil grown sugarcane nodes using selective media viz., LGI-P media for *Gluconacetobacter*, Rennies media for *Azospirillum*, nitrogen free malate for *Herbaspirillum* and *Azoarcus* media for *Azoarcus*. These isolated endophytes were confirmed with colony colour and biochemical tests viz., oxidase, gelatin liquefaction, urease, H₂S production and catalase.
2. All the endophytes isolated were screened on respective selective media containing different levels viz., 2, 4, 8 dSm⁻¹ salt concentration. It was observed that all the endophytes tolerated upto 8 dSm⁻¹ salt concentrations.
3. The individual endophytic bacterial culture and consortia was prepared having CFU 1 X 10⁸ and was applied to the sugarcane setts and plants at 15, 30, 45 and 60 DAP and plants samples 105 days after germination i.e. tillering stage.
4. The physiological parameters recorded were leaf area, shoot length, root length and RLWC. From the result obtained it was noticed that leaf area was decreased with increase in imposed salt stress level in both the varieties CoM-0265 and CoC-671 in both endophyte treated and untreated plants. The more decrease was observed in salt susceptible CoC-671 than salt tolerant CoM-0265 variety. By application of the different endophytes along with consortia it was noticed that the shoot length, root length, leaf area and RLWC was increased over the control (without application of endophytes) in each of salt stress treatment in both the varieties. However, more increase in sodic soil, 2 dSm⁻¹, 4 dSm⁻¹ and 8 dSm⁻¹ was observed by the application of consortia followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* under salinity stress condition in both the sugarcane varieties.

5. The chlorophyll content decreased in both the varieties by imposition of various salt stress levels however more decrease was observed in CoC 671 than CoM 0265. The salinity stress was overcome by the treatment of endophytes as revealed by increase in chlorophyll content. Among the various endophyte treatment consortia was found better followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* to ameliorate the stress condition.

6. Due to the imposed salinity stress chlorophyll stability index was decreased. Membrane injury index and lipid peroxidation increased in both varieties of sugarcane. The more decrease in CSI and increase in MII and lipid peroxidation was observed in CoC 671 than CoM 0265 sugarcane variety. However, by the application of endophyte treatment there was increase in CSI and decrease in MII and lipid peroxidation values in both the sugarcane varieties. However, a consortium was found to be superior to the *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus*.

7. Due to the imposed salinity stress osmolytes viz., proline, glycine betaine, reducing sugar, non reducing sugar and total sugar increased in both varieties of sugarcane. The more increase in proline, glycine betaine, reducing sugar, non-reducing sugar and total sugar were observed in CoC 671 than CoM 0265 sugarcane variety. However by the application of endophyte treatment there was increase in proline, glycine betaine, reducing sugar, non reducing sugar and total sugar values in both the sugarcane varieties. However, consortia treatment was found to be the superior than the *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus*.

8. Due to the imposed salinity antioxidative enzymes viz., CAT, APX and SOD increased in both varieties of sugarcane. The more increase in activity of CAT, APX and SOD was observed in CoC 671 than CoM 0265 sugarcane variety. However by the application of endophyte treatment there was increase in CAT, APX and SOD values in both the sugarcane varieties, and treatment with consortia found to be superior to the *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus*.

Conclusion:

From the results obtained in present study it can be concluded that the endophytes viz., *Glucanoacetobacter*, *Azospirillum*, *Herbaspirillum* *Azoarcus* and its consortia play an important role to alleviate the salinity stress by promoting the morphological characters viz., leaf area, shoot length, root length and RLWC under salinity stress condition. Further, endophyte treatment caused reduction in MII and lipid peroxidation and enhanced CSI indicating that under stress condition membrane integrity was protected by endophyte condition. The oxidative stress was relieved by enhancing the activities of antioxidant enzymes viz., CAT, APX and SOD was reduced by the treatments of endophytes to sugarcane plants. The CoC 671 variety responds better to relieve salinity stress condition than CoM 0265 by the application of endophytes. Among the various treatment consortia was found to be the best followed by *Gluconacetobacter*, *Azospirillum*, *Herbaspirillum* and *Azoarcus* to overcome salt stress condition.

6. LITERATURE CITED

- Abbas, S. M. 2013. The influence of biostimulants on the growth and on the biochemical composition of *Vicia faba* CV. Giza 3 beans. *Romanian Biotechnol. Letters*, 18(2):8061-8068.
- Abd_Allah, E. F., Alqarawi, A. A., Hashem, A., Radhakrishnan, R., Al-Huqail, A. A., Al-Otibi, F. O. N. and Egamberdieva, D. 2018. Endophytic bacterium *Bacillus subtilis* (BERA 71) improves salt tolerance in chickpea plants by regulating the plant defense mechanisms. *J. Plant Interact.* 13 (1): 37-44.
- Abd_Allah, E. F., Hashem, A., Alqarawi, A. A., Bahkali, A. H. and Alwhibi, M. S. 2016. Enhancing growth performance and systemic acquired resistance of medicinal plant *Sesbania sesban* (L.) Merr using *arbuscular mycorrhizal fungi* under salt stress. *Saudi. J. Bio. Sci.* 22:274–283.
- Abdel aziz, M. E., Abdelsattar, M., Abdeldaym, E. A., Atia, M. A., Mahmoud, A. W. M., Saad, M. M. and Hirt, H. 2019. *Piriformospora indica* alters Na^+/K^+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress. *Sci. Hort.* 256:108532
- Abdel Latef, A. A. H., Abu Alhmad, M. F. and Kordrostami, M. et al 2020. Inoculation with *Azospirillum lipoferum* or *Azotobacter chroococcum* reinforces maize growth by improving physiological activities under saline conditions. *J. Plant Growth Regul.* 39:1293-1306.
- Abdel Latef, A. A. H., Mostofa, M. G., Rahman, M., Abdel-Farid, I. B. and Tran, L. S. P. 2019. Extracts from yeast and carrot roots enhance maize performance under seawater-induced salt stress by altering physio-biochemical characteristics of stressed plants. *J. Plant Growth Regul* 38:966-979.
- Abdel Latef, A. A. H., Omer, A. M., Badawy, A. A., Osman, M. S. and Ragaey, M. M. 2021. Strategy of salt tolerance and interactive impact of *Azotobacter chroococcum* and/or *Alcaligenes faecalis* inoculation on canola (*Brassica napus* L.) plants grown in saline soil. *Plants* 10 (1):110.

- Abdel Latef, A. A. H., Srivastava, A. K., El-sadek, M.S.A., Kordrostami, M. and Tran, L.S.P. 2018. Titanium dioxide nanoparticles improve growth and enhance tolerance of broad bean plants under saline soil conditions. *L Degrad Dev* 29:1065-1073
- Abdelaziz, M. E., Kim, D., Ali, S., Fedoroff, N. V. and Al-Babili, S. 2017. The endophytic fungus *Piriformospora indica* enhances *Arabidopsis thaliana* growth and modulates Na^+/K^+ homeostasis under salt stress conditions. *Plant Sci.* 263:107-115
- Abdelaziz, M.E., Abdelsattar, M., Abdeldaym, E.A., Atia, M.A., Mahmoud, A.W.M., Saad, M.M. and Hirt, H., 2019. *Piriformospora indica* alters Na^+/K^+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress. *Scientia Horticulturae*, 256, p.108532.
- Abriz, S., Tavasolee, A., Ghassemi-Golezani, K., Torabian, S., Monirifar, H. and Rahmani, H. A. 2020. Growth-promoting bacteria and natural regulators mitigate salt toxicity and improve rapeseed plant performance. *Protoplasma*, 257(4), 1035-1047.
- Abrol, I.P., Yadav, J.S.P. and Massoud, F.I., 1988. *Salt-affected soils and their management* (No. 39). Food & Agriculture Org..
- Acuña-Rodríguez, I. S., Hansen, H., Gallardo-Cerda, J., Atala, C. and Molina-Montenegro, M. A. 2019. Antarctic extremophiles: biotechnological alternative to crop productivity in saline soils. *Front. Bioeng. Biotechnol.* (7): 22. 1-13.
- Aebi, H. 1984. Catalase in vitro. In *Methods in enzymology* (Vol. 105:121-126). Academic press.
- Afridi, M. S., Mahmood, T., Salam, A., Mukhtar, T., Mehmood, S., Ali, J., Khatoon, Z., Bibi, M., Javed, M. T., Sultan, T. and Chaudhary, H. J. 2019. Induction of tolerance to salinity in wheat genotypes by plant growth promoting endophytes: Involvement of ACC deaminase and antioxidant enzymes. *Plant Physiol. Biochem.* 139:569-577.
- Agami, R. A., Medani, R. A., Abd El-Mola, I. A. and Taha, R. S. 2016. Exogenous application with plant growth promoting rhizobacteria (PGPR) or proline induces stress tolerance in basil plants (*Ocimum basilicum* L.) exposed to water stress. *Int. J. Environ. Agri. Res.* 2:78
- Ahammed, G. J., Xu, W., Liu, A. and Chen, S. 2019. Endogenous melatonin deficiency aggravates high temperature-induced oxidative stress in *Solanum lycopersicum* L. *Environ. Exp. Bot.* 161:303-311

- Ahmad, P, Hashem, A, Abd-Allah, E. F., Alqarawi, A. A., John, R., Egamberdieva, D. and Gucel, S. 2015. Role of *Trichoderma harzianum* in mitigating NaCl stress in Indian mustard (*Brassica juncea* L.) through antioxidative defense system. *Front Plant Sci.* 6:868
- Ahmed, A. and Hasnain, S. 2014. Auxins as one of the factors of plant growth improvement by plant growth promoting rhizobacteria. *Pol. J. Microbiol.* 63:261-266
- Aini, N., Dwi Yamika, W. S., Aini, L. Q. and Kurniawan, A. P. 2021. Application of saline tolerant bacteria and soil ameliorants improved growth, yield and nutrient uptake of tomato in saline land. *Aust. J. Crop Sci.* 15(6): 827-834.
- Akram, M.S., Shahid, M., Tariq, M., Azeem, M., Javed, M.T., Saleem, S. and Riaz, S., 2016. Deciphering *Staphylococcus sciuri* SAT-17 mediated anti-oxidative defense mechanisms and growth modulations in salt stressed maize (*Zea mays* L.). *Frontiers in Microbiology*, 7, p.867.
- Alexander, A., Singh, V. K., and Mishra, A. 2020. Halotolerant PGPR *Stenotrophomonas maltophilia* BJ01 induces salt tolerance by modulating physiology and biochemical activities of *Arachis hypogaea*. *Front. Microbiol.* 11:568289.
- Alexander, A., Singh, V.K. and Mishra, A., 2020. Halotolerant PGPR *Stenotrophomonas maltophilia* BJ01 induces salt tolerance by modulating physiology and biochemical activities of *Arachis hypogaea*. *Frontiers in Microbiology*, 11, p.568289.
- Ali ZN, Ullah S, Naseem M, Inam UH, Jacobsen HJ. Soil bacteria conferred a positive relationship and improved salt stress tolerance in transgenic pea (*Pisum sativum* L.) harboring Na⁺/H⁺ antiporter. *Turkish J Bot.* 2015, 39(6):962-972.
- Ali, A., Pan, Y. B., Wang, Q. N., Wang, J. D., Chen, J. L. and Gao, S. J. 2019. Genetic diversity and population structure analysis of *Saccharum* and *Erianthus* genera using microsatellite (SSR) marker. *Sci. Rep.* 9 (1): 1
- Ali, S., Charles, T. C. and Glick, B. R. 2014. Amelioration of high salinity stress damage by plant growth-promoting bacterial endophytes that contain ACC deaminase. *Plant Physiol. Biochem.* 80:160–167.
- Alikhani, M., Khatabi, B., Sepehri, M., Nekouei, M. K., Mardi, M. and Salekdeh, G. H. 2013. A proteomics approach to study the molecular basis of enhanced salt tolerance

- in barley (*Hordeum vulgare* L.) conferred by the root mutualistic fungus *Piriformospora indica*. *Mol Biosyst* 9:1498-1510
- Almaghrabi, O. A., Massoud, S. I. and Abdelmoneim, T. S. 2013. Influence of inoculation with plant growth promoting rhizobacteria (PGPR) on tomato plant growth and nematode reproduction under greenhouse conditions. *Saudi J. Biol. Sci.* 20:57-61.
- Alqarawi, A. A., Hashem, A., Abd_Allah, E. F., Alshahrani, T. S. and Huqail, A. A. 2014. Effect of salinity on moisture content, pigment system, and lipid composition in *Ephedra alata* Decne. *Acta Biol Hungarica*. 65 (1):61–71
- Alquéres, S., Oliveira, J.H.M., Nogueira, E.M., Guedes, H.V., Oliveira, P.L., Câmara, F., Baldani, J.I. and Martins, O.B., 2010. Antioxidant pathways are up-regulated during biological nitrogen fixation to prevent ROS-induced nitrogenase inhibition in *Gluconacetobacter diazotrophicus*. *Archives of microbiology*, 192(10):835-841.
- Amira, M. S. and Qados, A. 2011. Effect of salt stress on plant growth and metabolism of bean plant *Vicia faba* (L.). *J. Saudi Soc. Agric. Sci.* 10: 7–15.
- Aneja, K. R. 2003. Experiments in Microbiology, Plant Pathology and Biotechnology. New Age International Publishers, New Delhi, India.1-607.
- Anonymous, 2015. In: Vision 2050: Sugarcane Breeding Institute Indian Council of Agricultural Research Coimbatore, Tamil Nadu 5-10. www.sugarcane.res.in
- Anonymous, 2017. United Nation Report. World population prospects: The 2017 Revision
- Anonymous, 2018. Salt affected soils: Central Soil Salinity Research Institute, Karnal.
- Anonymous, 2021. Maharashtra State Sugarcane Commission Report 2021.
- Anonymous, 2022. Sugarcane: Area, Production and yield during 2019-20 and 2020-21 in major producing states along with coverage under irrigation. In: Agricultural Statistics at Glance 2021. Ed. by Govt. of India, Ministry of Agriculture and Farmers Welfare, Dept. of Agril. and Farmers Welfare, Directorate of Economic and Statistics. 61-237.
- Anower, M.R., Mott, I.W., Peel, M.D. and Wu, Y. 2013. Characterization of physiological responses of two alfalfa half-sib families with improved salt tolerance. *Plant Physio. and Biochem.* 71:103-111.
- Ansari, F. A., Ahmad, I. and Pichtel, J. 2019. Growth stimulation and alleviation of salinity stress to wheat by the biofilm forming *Bacillus pumilus* strain FAB10. *Appl. Soil Ecol.*143: 45-54.

- Arnon, D. L. 1949. A copper enzyme is isolated chloroplast: polyphenol oxidase in Beta vulgaris. *Plant Physiol.* 24: 1-15.
- Arora, S., 2016. Microbial approach for remediation and health management of salt affected soils. *Natural resource management in arid and semi-arid ecosystem for climate resilient agriculture.*
- Asfaw, E., Suryabhagavan, K. V. and Argaw, M. 2018. Soil salinity modeling and mapping using remote sensing and GIS: The case of Wonji sugar cane irrigation farm, Ethiopia. *J. Saudi Soc. Agril. Sci.* 17(3): 250-258.
- Ashraf, M. F. M. R. and Foolad, M. R. 2007. Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ. Expt. Bot.* 59(2):206-216.
- Atkinson, N. J. and Urwin, P. E. 2012. The interaction of plant biotic and abiotic stresses: from genes to the field. *J. Exp. Bot.* 63:3523-3543
- Azad, K. and Kaminskyj, S. 2016. A fungal endophyte strategy for mitigating the effect of salt and drought stress on plant growth. *Symbiosis* 68:73-78
- Bacilio, M., Moreno, M. and Bashan, Y. 2016. Mitigation of negative effects of progressive soil salinity gradients by application of humic acids and inoculation with *Pseudomonas stutzeri* in a salt-tolerant and a salt-susceptible pepper. *Appl. Soil Ecol.* 107:394-404.
- Badawy, A. A, Alotaibi, M. O. and Abdelaziz, A. M. 2021. Enhancement of seawater stress tolerance in barley by the endophytic fungus *Aspergillus ochraceus*. *Metabolites* 11:428
- Badri, D. V. and Vivanco, J. M. 2009. Regulation and function of root exudates. *Plant Cell Environ* 32:666-681
- Bai, X., Dai, L., Sun, H., Chen, M. and Sun, Y. 2019. Effects of moderate soil salinity on osmotic adjustment and energy strategy in soybean under drought stress. *Plant Physiol. Biochem.* 139:307-313
- Baker, N. R. 2008. Chlorophyll fluorescence: a probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* 59:89-113
- Bal, H..B. Nayak, L., Das, S. and Adhya, T. K. 2013. Isolation of ACC deaminase producing PGPR from rice rhizosphere and evaluating their plant growth promoting activity under salt stress. *Plant and soil*, 366(1):93-105.

- Baldani, V. L. D., Alvarez, M. A. de B., Baldani, J. I. and Döbereiner, J. 1986. Establishment of inoculated *Azospirillum* spp. In the rhizosphere and in roots of field grown wheat and sorghum. *Plant Soil* 90, 35–46.
- Baldani, V.L.D., BALDANI, J.I., Olivares, F.L. and DOBEREINER, J., 1992. Identification and Ecology of *Herbaspirillum seropedicae* and the Closely Related *Pseudomonas rubrisubolbicans*. *Symbiosis*.
- Balliu, A, Sallaku, G. and Rewald, B. 2015. AMF inoculation enhances growth and improves the nutrient uptake rates of transplanted, salt-stressed tomato seedlings. *Sustainability* 7:15967–15981
- Baltruschat, H., Fodor, J., Harrach, B. D., Niemczyk, E., Barna, B., Gullner, G., Janeczko, A., Kogel, K-H., Schäfer, P., Schwarczinger, I., Zuccaro, A. and Skoczowski, A. 2008. Salt tolerance of barley induced by the root endophyte *Piriformospora indica* is associated with a strong increase in antioxidants. *New Phytol.* 180:501–510
- Bano, A. Fatima, M. 2009. Salt tolerance in *Zea mays* (L.) following inoculation with *Rhizobium* and *Pseudomonas*. *Biol Fert. Soils.* 45:405–413.
- Bashan, Y., Bustillos, J. J. and Leyva, L. A. 2006. Increase in auxiliary photoprotective photosynthetic pigments in wheat seedlings induced by *Azospirillum brasilense*. *Biol Fertil Soils* 42:279–285
- Bates, L. S., Waldren, R.P. and Teare, I. D. 1973. Rapid determination of free proline for water stress studies. *Plant Soil.* 39: 205-207.
- Beauchamp, C. and Fridovich, I. (1971). Superoxide dismutase: Improved assays and an assay applicable to acrylamide gel. *Anal. Biochem.* 44: 276- 287.
- Beckett, R., Hoddinott, N. 1997. Seasonal variations in tolerance to ion leakage following desiccation in the moss *Atrichum androgynum* from a KwaZulu-Natal afro-montane forest. *SAfr J Bot* 63:276–279
- Bergey's Manual of Systematic Bacteriology. 1983. Eds. S T Williams, M. E. Sharpe and J. G. Holt. Williams and Wilkins, Baltimore, USA.
- Berthomieu, P., Conéjéro, G., Nublat, A., Brackenbury, W. J., Lambert, C., Savio, C., Uozumi, N., Oiki, S., Yamada, K., Cellier, F., Gosti, F., Simonneau, T., Essah, P. A.,

- Tester, M., Véry, A. A, Sentenac, H. and Casse, F. 2003. Functional analysis of AtHKT1 in Arabidopsis shows that Na⁺ recirculation by the phloem is crucial for salt tolerance. *EMBO J* 22:2004-2014
- Bharti, N, Pandey, S. S., Barnawal, D. et al 2016. Plant growth promoting rhizobacteria *Dietzia natronolimnaea* modulates the expression of stress responsive genes providing protection of wheat from salinity stress. *Sci. Rep.* 6:34768.
- Bhat, M. A., Kumar, V. and Bhat, M. A. 2020. Mechanistic insights of the interaction of plant growth-promoting rhizobacteria (PGPR) with plant roots toward enhancing plant productivity by alleviating salinity stress. *Front Microbiol* 11:1952
- Bibha, R. and Sharma, V. K. 2015. Standardization of methodology for obtaining the desired salt stress environment for salinity effect observation in rice seedlings. *Intl. J. Environ. Sci.* 6(2): 232-236.
- Bidgoli, R. D., Azarnezhad, N., Akhbari, M. and Ghorbani, M. 2019. Salinity stress and PGPR effects on essential oil changes in *Rosmarinus officinalis* L. *Agriculture & Food Security.* 8: 1-7.
- Bienert, M. D., Diehn, T.A., Richet, N., Chaumont, F. and Bienert, G.P., 2018. Heterotetramerization of plant PIP1 and PIP2 aquaporins is an evolutionary ancient feature to guide PIP1 plasma membrane localization and function. *Frontiers in plant sci.* 9:382.
- Brilli, F., Pollastri, S., Raio, A., Baraldi, R., Neri, L., Bartolini, P., Podda, A., Loreto, F., Maserti, B.E. and Balestrini, R. 2019. Root colonization by *Pseudomonas chlororaphis* primes tomato (*Lycopersicum esculentum*) plants for enhanced tolerance to water stress. *J. plant physiol.* 232:82-93.
- Cakmakci, R., Dönmez, M.F. and Erdoğan, Ü., 2007. The effect of plant growth promoting rhizobacteria on barley seedling growth, nutrient uptake, some soil properties, and bacterial counts. *Turkish J. Agril.Forestry*, 31(3):189-199.
- Cappuccino, J. G. and Sherman, N. 1987. Microbiology A Laboratory Manual. The Benjamin/Cummins Publishing Co., USA: 1-458.
- Cappunccino J. C and Sherman N. In: Microbiology: A Laboratory Manual 3rd ed. New York: Benjamin/Cumming Pub. Co. 1992.

- Cassan, F., Vanderleyden, J. and Spaepen, S. 2014. Physiological and agronomical aspects of phytohormone production by model plant-growth-promoting rhizobacteria (PGPR) belonging to the genus *Azospirillum*. *J. Plant Growth Regul* 33:440-459
- Castro, F. O. and Santos, A. M. 2020. Salinity of the soil and the risk of desertification in the semiarid region. *Mercator Magazine* 1:1-12.
- Cavalcante, V. A., and Dobereiner, J. 1988. A new acid-tolerant nitrogen-fixing bacterium associated with sugarcane. *Plant soil*, 108(1):23-31.
- Chaum, S., Chuencharoen, S., Mongkolsiriwatana, C., Ashraf, M. and Kirdmanee, C. 2012. Screening sugarcane (*Saccharum spp.*) genotypes for salt tolerance using multivariate cluster analysis. *Plant Cell Tissue Organ Cult.* 110(1):23-33.
- Chaves, M., Flexas, J. and Pinheiro, C. 2009. Photosynthesis under drought and salt stress: regulation mechanisms from whole plant to cell. *Ann. Bot.* 103:551-560
- Chen T. H. and Murata, N. 2011. Glycinebetaine protects plants against abiotic stress: mechanisms and biotechnological applications. *Plant Cell Environ.* 34:1-20
- Chen, K.M., Gong, H.J., Wang, S.M. and Zhang, C.L., 2007. Antioxidant defense system in *Phragmites communis* Trin. ecotypes. *Biologia plantarum*, 51(4):754-758.
- Chen, L., Dick, W. A., Streeter, J. G. and Hoitink, H. A. 1998. Fe chelates from compost microorganisms improve Fe nutrition of soybean and oat. *Plant Soil* 200:139-147
- Chen, L., Liu, Y., Wu, G., Veronican Njeri, K., Shen, Q., Zhang, N. and Zhang, R. 2016. Induced maize salt tolerance by rhizosphere inoculation of *Bacillus amyloliquefaciens* SQR9. *Physiol. Plant.* 158 (1):34-44.
- Chen, T., Li, C., White, J. F. and Nan, Z. 2019. Effect of the fungal endophyte *Epichloe bromicola* on polyamines in wild barley (*Hordeum brevisubulatum*) under salt stress. *Plant Soil* 436: 29-48
- Chen, Z. C., Peng, W. T., Li, J. and Liao, H. 2018. Functional dissection and transport mechanism of magnesium in plants. *Semin Cell Dev Biol.* 74:142-152
- Choudhary O. P. and Kharche, V. K. 2018. Soil Salinity and Sodicity. In: Soil Science: An Introduction. Ed. by:353-384.
- Choudhary, O.P. and Kharche, V.K., 2018. Soil salinity and sodicity. *Soil science: an introduction*, 12, pp.353-384.

- Christensen, W. B. 1946. Urea decomposition as a means of differentiating *Proteus* and paracolon cultures from each other and from *Salmonella* and *Shigella* types. *J. Bacteriol.* 52 (4):461–466.
- Chrysargyris, A., Michailidi, E. and Tzortzakis, N. 2018. Physiological and biochemical responses of *Lavandula angustifolia* to salinity under mineral foliar application. *Frontiers in plant Sci.* 9:489.
- Compant, S., Clément, C. and Sessitsch, A. 2010. Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biol. Biochem.* 42(5):669–678
- Contreras-Cornejo H. A., Macías-Rodríguez, L., Cortés-Penagos, C. and López-Bucio, J. 2009. *Trichoderma virens*, a plant beneficial fungus, enhances biomass production and promotes lateral root growth through an auxin-dependent mechanism in *Arabidopsis*. *Plant Physiol.* 149:1579–1592
- Cuin, T. A., Miller, A. J., Laurie, S. A., Leigh, R.A. 2003. Potassium activities in cell compartments of salt-grown barley leaves. *J. Exp. Bot.* 54:657–661
- D'Amelia, V., Aversano, R., Chiaiese, P. and Carputo, D. 2018. The antioxidant properties of plant flavonoids: their exploitation by molecular plant breeding. *Phytochemistry Reviews*, 17(3):611-625.
- DA WEI, Z.H.A.N.G., Vu, T.S., Huang, J., Chi, C.Y., Xing, Y., Fu, D.D. and Yuan, Z.N., 2019. Effects of calcium on germination and seedling growth in *melilotus officinalis* L.(Fabaceae) under salt stress. *Pak. J. Bot.* 51(1):1-9.
- Dawood, M. F. A., Sohag, A. A. M., Tahjib-Ul-Arif, M. and Latef, A. A. H. A. 2021. Hydrogen sulfide priming can enhance the tolerance of artichoke seedlings to individual and combined saline-alkaline and aniline stresses. *Plant Physiol. Biochem.* 159:347–362
- Dawwam, G. E., Elbeltagy, A., Emara, H. M., Abbas, I. H. and Hassan, M. M. 2013. Beneficial effect of plant growth promoting bacteria isolated from the roots of potato plant. *Annal Agri Sci.* 58(2):195–201.

- Dhindsa, R. S., Plumb-Dhindsa, P. and Thorpe, T. A. 1981. Leaf senescence: Correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutase and catalase. *J. Expt. Bot.* 32 (126): 93-101.
- Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S. D., Mishra, J. and Arora, N. K. 2019. Salt-tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. *Front. Microbiol.* 10:2791.
- El Nahrawy, A.M., Bakr, A.M., Hemdan, B.A. and Abou Hammad, A.B., 2020. Identification of Fe³⁺ co-doped zinc titanate mesostructures using dielectric and antimicrobial activities. *International Journal of Environmental Science and Technology*, 17(11), pp.4481-4494.
- el Zahar Haichar, F., Santaella, C., Heulin, T. and Achouak W. 2014. Root exudates mediated interactions belowground. *Soil Biol. Biochem.* 77:69-80
- Elbeltagy, A., Nishioka, K., Sato, T., Suzuki, H., Bin Ye, H. T., Isawa, T., Mitsui, H. and Minamisawa, K. 2001. Endophytic colonization and In Planta Nitrogen Fixation by a *Herbaspirillum spp.* isolated from wild rice species. *Applied Env. Microbiol.* 67 (11):5285-5293.
- El-Ramady, H. *et al.* 2019. Soil fertility and its security. In: “The Soils of Egypt” World Soils Book Series, Ed. by El-Ramady, H., Alshaal, T., Bakr, N., Elbana, T., Mohamed, E. and Belal, A. A. Springer Publ. Co., Cham 137–157.
- El-Tayeb, M. A. 2005. Response of barley grains to the interactive effect of salinity and salicylic acid. *Plant Growth Regul.* 45:215–224.
- Enebe, M.C. and Babalola, O.O., 2018. The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. *Applied microbiology and biotechnology*, 102, pp.7821-7835.
- Etesami, H., 2018. Can interaction between silicon and plant growth promoting rhizobacteria benefit in alleviating abiotic and biotic stresses in crop plants?. *Agriculture, Ecosystems & Environment*, 253, pp.98-112.
- Evelin, H., Devi, T. S., Gupta, S. , Kapoor, R. 2019. Mitigation of salinity stress in plants by arbuscular mycorrhizal symbiosis: current understanding and new challenges. *Front Plant Sci.*10:470

- Evelin, H., Kapoor, R. and Giri, B., 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Annals of botany*, 104(7), pp.1263-1280.
- Evelin, H., Kapoor, R., and Giri, B. 2009. Arbuscular mycorrhizal fungi in alleviation of salt stress: a review. *Ann. Bot.* 104:1263-1280
- Farhangi-Abriz, S. and Torabian, S., 2017. Antioxidant enzyme and osmotic adjustment changes in bean seedlings as affected by biochar under salt stress. *Ecotoxicology and environmental safety*, 137, pp.64-70.
- Farias, G. C., Nunes, K. G., Soares, M. A., de Siqueira, K. A., Lima, W. C., Neves, A. L. R., de Lacerda, C. F., Filho, E. G. 2019. Dark septate endophytic fungi mitigate the effects of salt stress on cowpea plants. *Braz. J. Microbiol.* 51:243–253
- Fasciglione, G., Casanovas, E.M., Quillehauquy, V., Yommi, A.K., Goñi, M.G., Roura, S.I. and Barassi, C.A., 2015. Azospirillum inoculation effects on growth, product quality and storage life of lettuce plants grown under salt stress. *Scientia Horticulturae*, 195, pp.154-162.
- Fouda, A., Hassan, S. E. D., Eid, A. M. and El-Din Ewais, E. 2019. The interaction between plants and bacterial endophytes under salinity stress. In: *Endophytes and Secondary Metabolites*. Ed. by Jha M. 591-607.
- Gangwar, O. and Singh, A. P. 2018. *Trichoderma* as an efficacious bioagent for combating biotic and abiotic stresses of wheat- A review. *Agric Rev* 39:49-54
- Gangwar, P., Singh, R, Trivedi, M. and Tiwari, R. K. 2020. Sodic soil: management and reclamation strategies. In: *Environmental Concerns and Sustainable Development*. Springer: 175-190.
- Gayathri, S., Saravanan, D., Radhakrishnan, M., Balagurunathan, R. and Kathiresan, K. 2010. Bioprospecting potential of fast growing endophytic bacteria from leaves of mangrove and salt marsh plant species. *Ind. J. Biotechnol.*, 9:397- 402.
- Ghabooli, M. 2014. Effect of *Piriformospora indica* inoculation on some physiological traits of barley (*Hordeum vulgare*) under salt stress. *Chem. Nat. Compd.* 50:1082-1087
- Gharsallah, C., Fakhfakh, H., Grubb, D. and Gorsane, F. 2016. Effect of salt stress on ion concentration, proline content, antioxidant enzyme activities and gene expression in tomato cultivars. *AoB Plants* 8:055

- Gill, S. S. and Tuteja N. 2010. Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant Physiol. Biochem.* 48(12):909-930.
- Glick, B.R., 2010. Using soil bacteria to facilitate phytoremediation. *Biotechnology advances*, 28(3):367-374.
- Glick, B.R., Penrose, D.M. and Li, J., 1998. A model for the lowering of plant ethylene concentrations by plant growth-promoting bacteria. *Journal of theoretical biology*, 190(1):63-68.
- Glick, B.R., Todorovic, B., Czarny, J., Cheng, Z., Duan, J. and McConkey, B., 2007. Promotion of plant growth by bacterial ACC deaminase. *Critical Reviews in Plant Sciences*, 26(5-6):227-242.
- Gómez, S., Mateos Naranjo, E., Figueroa, M. E. and Davy, A. J., 2010. Salt stimulation of growth and photosynthesis in an extreme halophyte, *Arthrocnemum macrostachyum*. *Plant biology*, 12(1):79-87.
- Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., Varshney, R. K., Gowda, C. .L. and Krishnamurthy, L. 2015. Plant growth promoting rhizobia: challenges and opportunities. *Biotech*, 5(4):355-377.
- Grabov, A. 2007. Plant KT/KUP/HAK potassium transporters: single family-multiple functions. *Ann. Bot.* 99:1035-1041
- Gregory, P. J. 2006. *Plant Roots: Growth, Activity and Interactions with the Soil*. Blackwell, London.
- Guerzoni, J., Belintani, N., Moreira, R., Hoshino, A. A., Domingues D., Filho, J. and Vieira, L. 2014. Stress-induced Δ 1-pyrroline-5-carboxylate synthetase (P5CS) gene confers tolerance to salt stress in transgenic sugarcane, *Acta Physiol. Plant* 8:1573-1579.
- Guo, L., Shi, D., Wang, D. 2010. The key physiological response to alkali stress by the alkali-resistant halophyte *Puccinellia tenuiflora* is the accumulation of large quantities of organic acids and into the rhizosphere. *J. Agron. Crop Sci.* 196:123-135
- Gupta, A., Rai, S., Bano, A., Khanam, A., Sharma, S. and Pathak, N. 2021. Comparative evaluation of different salt-tolerant plant growth-promoting bacterial isolates in mitigating the induced adverse effect of salinity in *pisum sativum*. *Biointerface Res. Appl. Chem.* 11(5):13141-13154.

- Gupta, B. and Huang, B. 2014. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Int. J. Genomics* 2014:701596-701596
- Gupta, B. and Huang, B. 2015. Mechanism of salinity tolerance in plants: physiological, biochemical, and molecular characterization. *Intl. J. Genomics* 3:1-18.
- Gupta, S., Gupta, K., Nehra, C., Gaur, R. K., and Yadav, D. 2022. Biotechnological intervention for sugarcane improvement under salinity. *Sugar Tech.* 1-17.
- Gupta, S., Schillaci, M., Walker, R., Smith, P.M., Watt, M. and Roessner, U., 2021. Alleviation of salinity stress in plants by endophytic plant-fungal symbiosis: Current knowledge, perspectives and future directions. *Plant and Soil*, 461, pp.219-244.
- Gupta, S., Sharma, P., Dev, K. and Sourirajan, A. 2016. Halophilic bacteria of Lunsu produce an array of industrially important enzymes with salt tolerant activity. *Biochem. Res. Int.* 2016.
- Habib, S. H., Kausar, H. and Saud, H. 2016. Plant growth-promoting rhizobacteria enhance salinity stress tolerance in okra through ROS-scavenging enzymes. *Biomed. Res. Int.* 2016:1-10
- Habib, S. H., Kausar, H., Saud, H. M., Ismail, M. R., and Othman, R. 2016. Molecular characterization of stress tolerant plant growth promoting rhizobacteria (PGPR) for growth enhancement of rice. *Int. J. Agric. Biol.* 18, 184–191.
- Hadi, M. R. and Karimi, N. 2012. The role of calcium in plants' salt tolerance, *J. Plant Nutri.* 35(13): 2037-2054
- Hahm, M.S., Son, J.S., Hwang, Y.J., Kwon, D.K. and Ghim, S.Y., 2017. Alleviation of salt stress in pepper (*Capsicum annum* L.) plants by plant growth-promoting rhizobacteria. *Journal of microbiology and biotechnology*, 27(10), pp.1790-1797.
- Haitao Y, Mo W, Li C, Zheng Y and Li H. 2007. The salt stress relief and growth promotion effect of Rs-5 on cotton. *Plant Soil* 297:139–145
- Hamayun, M., Hussain, A., Khan, S. A., Kim, H. Y., Khan, A. L., Waqas, M., Irshad, M., Iqbal, A., Rehman, G., Jan, S. and Lee, I. J. 2017. Gibberellins producing endophytic fungus *Porostereum spadiceum* AGH786 rescues growth of salt affected soybean. *Front. Microbiol.* 8:686

- Hanson, A. D. and Nelson, C. E. 1978. Betaine accumulation and (^{14}C) formate metabolism in water stressed barley leaves. *Plant Physiol.* 62:305–312
- Harish, S., Kavino, M., Kumar, N., Saravanakumar, D., Soorianathasundaram, K. and Samiyappan, R. 2008. Biohardening with plant growth promoting rhizosphere and endophytic bacteria induces systemic resistance against banana bunchy top virus. *Appl. soil ecol.* 39(2):187-200.
- Hasan, U. T. and Bano A. 2019. Construction of IAA-deficient mutants of *Pseudomonas moraviensis* and their comparative effects with wild type strains as bio-inoculant on wheat in saline sodic soil. *Geomicrobiol. J.* 36: 376-384.
- Hasanuzzaman, M., Nahar, K., Alam, M., Bhowmik, P.C., Hossain, M., Rahman, M.M., Prasad, M.N.V., Ozturk, M. and Fujita, M., 2014. Potential use of halophytes to remediate saline soils. *BioMed research international*, 2014.
- Hasegawa, P. M., Bressan, R. A., Zhu, J. K. and Bohnert, H. J. 2000. Plant cellular and molecular responses to high salinity. *Annu. Rev. Plant Biol.* 51:463-499
- Hashem, A., Abd_Allah, E.F., Alqarawi, A. A., Al-Huqail, A.A., Wirth, S. and Egamberdieva, D. 2016. The interaction between arbuscular mycorrhizal fungi and endophytic bacteria enhances plant growth of *Acacia gerrardii* under salt stress. *Frontiers in microbiology*, 7:1089.
- Hassine, A.B. and Lutts, S. 2010. Differential responses of saltbush *Atriplex halimus* L. exposed to salinity and water stress in relation to senescing hormones abscisic acid and ethylene. *J. Plant Physiol.* 167(17):1448-1456.
- Hecht-Buchholz, C. 1998. The apoplast-habitat of endophytic dinitrogen-fixing bacteria and their significance for the nitrogen nutrition of nonleguminous plants. *Zeitschrift für Pflanzenernährung and Bodenkunde*, 161(5):09-520.
- Heidari, M. and Golpayegani, A. 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *J Saudi Soc Agric. Sci.* 11:57-61
- Henderson, J. C. and Davies, Jr. F. T. 1990. Drought acclimation and the morphology of mycorrhizal *Rosa* hybrids L. cv. ‘Ferdy’ independent of leaf elemental content. *New Phytol.* 175 : 503-510.

- Hernández, J. A. 2019. Salinity tolerance in plants: Trends and perspectives. *Int. J. Mol. Sci.* 20:2408
- Himabindu, Y., Chakradhar, T., Reddy, M. C., Kanygin, A., Redding, K. E. and Chandrasekhar, T. 2016. Salt-tolerant genes from halophytes are potential key players of salt tolerance in glycophytes. *Environ. Exp. Bot.* 124:39-63.
- Hossain, A., Skalicky, M., Brestic, M., Maitra, S., Ashraful Alam, M., Syed, M. A. and Islam, T. 2021. Consequences and mitigation strategies of abiotic stresses in wheat (*Triticum aestivum* L.) under the changing climate. *Agron*, 11(2):241.
- Huang, Z, Zhao, L. and Chen, D. et al 2013. Salt stress encourages proline accumulation by regulating proline biosynthesis and degradation in Jerusalem artichoke plantlets. *PLoS ONE* 8:e62085
- Hurek, B.R., Hurek, T., Gillis, M., Hoste, B., Vancanneyt, M., Kersters, K. and De Ley, J. 1993. *Azoarcus* gen. nov. , Nitrogen-Fixing Proteobacteria associated with Roots of Kallar Grass (*Leptochloa fusca* (L.) Kunth), and description of two species, *Azoarcus indigenus* sp. nov. and *Azoarcus communis* sp. nov. *International J. Systematic Bacteriology*. 43(3): 574-584.
- Im, W. T., Bae, H. S., Yokota, A. and Lee, S. T. 2004. *Herbaspirillum chlorophenolicum* sp. nov., a 4-chlorophenol-degrading bacterium. *Int. J. Syst. Evol. Microbiol.* 54(3):851-855.
- Jackson, M. L. 1973 . Soil chemical analysis. Prentice Hall of India Pvt. Ltd, New Delhi:498.
- Jaleel, C.A., B. Sankar, R. Sridharan and R. Panneerselvam. 2008. Soil salinity alters growth, chlorophyll content, and secondary metabolite accumulation in *Catharanthus roseus*. *Turk. J. Biol.*, 32: 79-83.
- James, E. K., Gyaneshwar, P., Barraquio, W. L., Mathan, N. and Ladha, J. K., 2000. Endophytic diazotrophs associated with rice. *The quest for nitrogen fixation in rice*:119-140.
- Jansen, M. A., Gaba, V., Greenberg, B. M., Mattoo, A. K. and Edelman, M. 1996. Low threshold levels of ultraviolet-B in a background of photosynthetically active radiation trigger rapid degradation of the D₂ protein of photosystem - II. *Plant J.* 9:693-699

- Jha, C.K. and Saraf, M., 2015. Plant growth promoting rhizobacteria (PGPR): a review. *Journal of Agricultural Research and Development*, 5(2), pp.108-119.
- Jha, P. and Kumar, A., 2009. Characterization of novel plant growth promoting endophytic bacterium *Achromobacter xylosoxidans* from wheat plant. *Microbial ecol.* 58(1):179-188.
- Jha, Y., Subramanian, R. B. and Patel, S. 2011. Combination of endophytic and rhizospheric plant growth promoting rhizobacteria in *Oryza sativa* shows higher accumulation of osmoprotectant against saline stress. *Acta Physiol. Plantarum*, 33(3):797-802.
- Jhala, Y. K., Shelat, H.N., Vyas, R.V. and Panpatte, D. G. 2015. Biodiversity of Endorhizospheric Plant Growth Promoting Bacteria. *J. Bioferti Biopestici*, 6:1
- Jogawat, A., Saha, S., Bakshi, M., Dayaman, V., Kumar, M., Dua, M., Varma, A., Oelmüller, R., Tuteja, N. and Johri, A. K. 2013. *Piriformospora indica* rescues growth diminution of rice seedlings during high salt stress. *Plant Signal Behav* 8: 268-291
- Johnson, J. M., Alex, T. and Oelmüller, R. 2014. *Piriformospora indica*: the versatile and multifunctional root endophytic fungus for enhanced yield and tolerance to biotic and abiotic stress in crop plants. *J. Trop Agric* 52:103-122
- Jung J. K. H. M. and McCouch, S. R. M. 2013. Getting to the roots of it: genetic and hormonal control of root architecture. *Front Plant Sci.* 4:186
- Kaavya, R., Pandiselvam, R., Kothakota, A., Banuu Priya, E. P. and Arun Prasath, V. 2019. Sugarcane juice preservation: A critical review of the state of the art and way forward. *Sugar Tech.* 21(1):9-19.
- Kamble, P.N., Giri, S.P., Mane, R.S. and Tiwana, A., 2015. Estimation of chlorophyll content in young and adult leaves of some selected plants. *Univers. J. Environ. Res. Technol*, 5:306-310.
- Kandel, S. L., Joubert, P. M., and Doty, S. L. 2017. Bacterial endophyte colonization and distribution within plants. *Microorganisms* 5(4):77.
- Kang, S. M., Khan, A. L., Waqas, M., You, Y. H., Kim, J. H., Kim, J. G. and Lee, I. J. 2014. Plant growth-promoting rhizobacteria reduce adverse effects of salinity and osmotic stress by regulating phytohormones and antioxidants in *Cucumis sativus*. *J. Plant Interact.* 9(1):673-682.

- Kangasjärvi, S., Neukermans, J., Li, S., Aro, E.M. and Noctor, G., 2012. Photosynthesis, photorespiration, and light signalling in defence responses. *Journal of experimental botany*, 63(4), pp.1619-1636.
- Kaur, G. and Asthir, B. 2015. Proline: a key player in plant abiotic stress tolerance. *Biol. Plant* 59:609-619
- Kaymak, H. C. 2010. Potential of PGPR in agricultural innovations. Plant growth and health promoting bacteria. Springer, Berlin: 45-79
- Kennedy, I.R., Choudhury, A.T.M.A. and Kecskés, M.L., 2004. Non-symbiotic bacterial diazotrophs in crop-farming systems: can their potential for plant growth promotion be better exploited. *Soil Biol. Biochem.* 36(8):1229-1244.
- Khalid, M., Bilal, M., Hassani, D., Iqbal, H., Wang, H. and Huang, D. 2017. Mitigation of salt stress in white clover (*Trifolium repens*) by *Azospirillum brasilense* and its inoculation effect. *Botanical studies*, 58(1):1-7.
- Khan, A., Geetha, R., Akolkar, A., Pandya, A., Archana, G. and Desai, A. J. 2006. Differential cross-utilization of heterologous siderophores by nodule bacteria of *Cajanus cajan* and its possible role in growth under iron-limited conditions. *Appl. Soil Ecol.* 34:19-26
- Khan, N. and Bano, A. 2016. Role of plant growth promoting rhizobacteria and Ag-nano particle in the bioremediation of heavy metals and maize growth under municipal wastewater irrigation. *Int. J. Phytoremediation* 18:211–221.
- Khan, N., Bano, A., Rahman, M. A., Guo, J., Kang Z. and Babar, M. A. 2019. Comparative physiological and metabolic analysis reveals a complex mechanism involved in drought tolerance in chickpea (*Cicer arietinum* L.) induced by PGPR and PGRs. *Scientific reports*. 9:1-19.
- Kord, H., Fakheri, B., Ghabooli, M., Solouki, M., Emamjomeh, A., Khatabi, B., Sepehri, M., Salekdeh, G.H. and Ghaffari, M.R. 2019. Salinity-associated microRNAs and their potential roles in mediating salt tolerance in rice colonized by the endophytic root fungus *Piriformospora indica*. *Functional integrative genomics*, 19(4):659-672.
- Kordrostami, M., Rabiei, B. and Hassani Kumleh, H. 2017. Biochemical, physiological and molecular evaluation of rice cultivars differing in salt tolerance at the seedling stage. *Physiol. Molecular Biol. Plants*, 23(3):529-544.

- Kothari, V. V., Kothari, R. K., Kothari, C. R., Bhatt, V. D., Nathani, N. M. and Koringa, P. G. 2013. Genome sequence of salt-tolerant *Bacillus safensis* strain VK, isolated from Saline Desert Area of Gujarat, India. *Genome Announc.* 1:e671-13.
- Krause, A., Ramakumar, A., Bartels, D., Battistoni, F., Bekel, T., Boch, J., Böhm, M., Friedrich, F., Hurek, T., Krause, L. and Linke, B., 2006. Complete genome of the mutualistic, N₂-fixing grass endophyte *Azoarcus* sp. strain BH72. *Nature biotechnol.* 24(11):1384-1390.
- Kuklinsky-Sobral J., Araújo, W. L., Mendes, R., Geraldi, I. O., PizziraniKleiner, A. A. and Azevedo, J. L. 2004. Isolation and characterization of soybean-associated bacteria and their potential for plant growth promotion. *Environ Microbiol.* 6:1244–1251
- Kumar, A., Kumar, A., Kumar, P., Lata, C. and Kumar, S. 2018. Effect of individual and interactive alkalinity and salinity on physiological, biochemical and nutritional traits of marvel grass. *Indian J. Exp. Biol.* 6 (8):573-581.
- Kumar, M., Sharma, R., Jogawat, A., Singh, P., Dua, M., Gill, S. S., Trivedi, D. K., Tuteja, N., Verma, A. K., Oelmuller, R. and Johri, A. K. 2012. *Piriformospora indica*, a root endophytic fungus, enhances abiotic stress tolerance of the host plant. In: Narendra, T., Sarvajeet, G. S., Tiburcio, A. F. and Tuteja, R. (eds) Improving Crop Resistance to Abiotic Stress. Wiley- Blackwell, Weinheim: 543-558
- Kumar, P. and Sharma, P. K. 2020. Soil salinity and food security in india. *Front. Sustain. food syst.* 4:533781.
- Kumar, P., Pandey, P., Dubeya, R. and Maheshwari, D. 2016. Bacteria consortium optimization improves nutrient uptake, nodulation, disease suppression and growth of the common bean (*Phaseolus vulgaris*) in both pot and field studies. *Rhizosphere* 2: 13-23.
- Kumar, S., Kalita, A., Srivastava, R. and Sahoo, L. 2017. Co-expression of *Arabidopsis* nhx1 and bar improves the tolerance to salinity, oxidative stress, and herbicide in transgenic mungbean. *Front. Plant Sci.* 8:215-225.
- Lamizadeh, E., Enayatizamir, N. and Motamedi, H. 2016. Isolation and identification of plant growth-promoting rhizobacteria (PGPR) from the rhizosphere of sugarcane in saline and non-saline soil. *Int. j. curr. microbiol.* 5 (10):1072-83.

- Lamizadeh, E., Enayatizamir, N. and Motamedi, H. 2019. Difference in some biological properties of saline and non-saline soil under sugarcane cultivation. *Eurasian Soil Sci.* 52: 690-695.
- Lata, R., Chowdhury, S., Gond, S. K. and White, J. F. 2018. Induction of abiotic stress tolerance in plants by endophytic microbes. *Lett. Appl. Microbiol.* 66(4):268-276.
- Li, L., Li, L., Wang, X., Zhu, P., Wu, H. and Qi, S. 2017. Plant growth promoting endophyte *Piriformospora indica* alleviates salinity stress in *Medicago truncatula*. *Plant Physiol Biochem.* 225.
- Liang, W., Ma, X., Wan, P. and Liu, L. 2018. Plant salt-tolerance mechanism: A review. *Biochem. Biophys. Res. Commun* 495:286-291
- Lima Neto, M. C., Martins, M. D. O., Ferreira-Silva, S. L. and Silveira, J. A. G. 2015. *Jatropha curcas* and *Ricinus communis* display contrasting photosynthetic mechanisms in response to environmental conditions. *Sci Agr* 72:260-269
- Liu, H., Wang, Q., Yu, M., Zhang, Y., Wu Y. and Zhang, H. 2008. Transgenic salt-tolerant sugar beet (*Beta vulgaris* L.) constitutively expressing an *Arabidopsis thaliana* vacuolar Na⁺/H⁺ antiporter gene, *AtNHX3*, accumulates more soluble sugar but less salt in storage roots. *Plant Cell Environ.* 31:1325–1334.
- Liu, J, Tang, L. and Gao, H. et al 2019. Enhancement of alfalfa yield and quality by plant growth-promoting rhizobacteria under saline-alkali conditions. *J. Sci. Food Agric* 99:281-289.
- Liu, W.Y., Chung, K.M.K., Wong, C.F., Jiang, J.W., Hui, R.K.H. and Leung, F.C.C., 2012. Complete genome sequence of the endophytic *Enterobacter cloacae* subsp. *cloacae* strain ENHKU01.
- Liu, X., Chen, Z. and Gao, Y. et al 2019. A newly discovered ageing-repair bacterium, *Pseudomonas geniculata*, isolated from rescuegrass (*Bromus cartharticus* Vahl) promotes the germination and seedling growth of aged seeds. *Bot.* 97:167-178
- Lowry, O. H., Rosebrough, N. J., Farr, A.L. and Randall, R.J. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193: 265-275.
- Ma, Y., Freitas, H. and Vosatka, M. 2019. Beneficial microbes alleviate climatic stresses in plants. *Front. Plant Sci.* 10: 595.

- Machado, R.M.A. and Serralheiro, R. P. 2017. Soil salinity: effect on vegetable crop growth. Management practices to prevent and mitigate soil salinization. *Horticulturae*, 3(2):30.
- Magnani, G.S., Didonet, C.M., Cruz, L.M., Picheth, C.F., Pedrosa, F.O. and Souza, E.M., 2010. Diversity of endophytic bacteria in Brazilian sugarcane. *Genet. Mol. Res.* 9 (1):250-258.
- Malmberg, R. L., Watson, M. B., Galloway, G. L. and Yu, W. 1998. Molecular genetic analyses of plant polyamines. *Crit. Rev.Plant Sci.* 17:199-224
- Manchanda, G. and Garg, N. 2009. Role of arbuscular mycorrhizae in the alleviation of ionic, osmotic and oxidative stresses induced by salinity in *Cajanus cajan* (L.) Mills (pigeonpea). *J. Agron. Crop Sci.* 195(2):110-123.
- Mandal, S.U.B.H.A.S.I.S., Raju, R., Kumar, A., Kumar, P. and Sharma, P.C., 2018. Current status of research, technology response and policy needs of salt-affected soils in India—A review. *J. Indian Soc. Coast. Agric. Res*, 36:40-53.
- Mansour, M. M. F. and Ali, E. F. 2017. Evaluation of proline functions in saline conditions. *Phytochemistry* 140:52-68
- Mastouri, F., Björkman, T. and Harman, G.E. 2010. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings.
- Matteoli, F. P., Olivares, F. L., Venancio, T.M., da Rocha, L.O., da Silva Irineu, L.E.S. and Canellas, L.P. 2020. *Herbaspirillum*. In *Beneficial Microbes in Agro-Ecology* (493-508). Academic Press.
- Matysik, J, Alia, Bhalu, B. and Mohanty P. 2002. Molecular mechanisms of quenching of reactive oxygen species by proline under stress in plants. *Curr. Sci.* 82:525-532
- Mayak, S. Tirosh, T. and Glick, B. R. 2004. Plant growth-promoting bacteria confer resistance in tomato plants to salt stress. *Plant Physiol. Biochem.* 42:565-572
- Medeiros, C. D., Neto, J. R. F., Oliveira, M. T., Rivas, R., Pandolfi, V., Kido, E. A., Baldani, J. I. and Santos, M. G. 2014. Photosynthesis, antioxidant activities and

- transcriptional responses in two sugarcane (*Saccharum officinarum* L.) cultivars under salt stress. *Acta Physiol. Plant.* 36(2):447–459
- Meena, K. K., Sorty, A. M., Bitla, U. M., Choudhary, K., Gupta, P., Pareek, A. and Minhas, P. S. 2017. Abiotic stress responses and microbe-mediated mitigation in plants: the omics strategies. *Front. Plant Sci.* 8:172.
- Mehnaz, S. and Lazarovits, G., 2006. Inoculation effects of *Pseudomonas putida*, *Gluconacetobacter dazotocaptans*, and *Azospirillum lipoferum* on corn plant growth under greenhouse conditions. *Microbial Ecology*, 51(3):326-335.
- Mesa-Marín, J., Del-Saz, N.F., Rodríguez-Llorente, I.D., Redondo-Gómez, S., Pajuelo, E., Ribas-Carbó, M. and Mateos-Naranjo, E., 2018. PGPR reduce root respiration and oxidative stress enhancing *Spartina maritima* root growth and heavy metal rhizoaccumulation. *Frontiers in plant science*, 9, p.1500.
- Mesa-Marín, J., Mateos-Naranjo, E., Rodríguez-Llorente, I. D., Pajuelo, E. and Redondo-Gómez, S. 2019. Synergic effects of rhizobacteria: increasing use of halophytes in a changing world. Halophytes and climate change: adaptive mechanisms and potential uses. 240. ISBN: 978-1-78639-433-0:386.
- Miller, G.A.D., Suzuki, N., Ciftci-Yilmaz, S.U.L.T.A.N. and Mittler, R.O.N., 2010. Reactive oxygen species homeostasis and signalling during drought and salinity stresses. *Plant, cell & environment*, 33(4):453-467.
- Milosevic N., Marinkovic J B., Tintor B. (2012). Mitigating abiotic stress in crop plants by microorganisms, Proc. Nat. Sci. Matica Srpska Novi. Sad. 123: 17-26
- Minocha, R., Majumdar, R. and Minocha, S.C., 2014. Polyamines and abiotic stress in plants: a complex relationship. *Front.plant sci.* 5:175.
- Mishra I.G., Sharma A. (2012): Exogenously supplied osmoprotectants confer enhanced salinity tolerance in rhizobacteria. *J. Ecobiotechnol.* 4: 11-13
- Mittova, V., Guy, M., Tal, M. and Volokita, M. 2004. Salinity up-regulates the antioxidative system in root mitochondria and peroxisomes of the wild salt-tolerant tomato species *Lycopersicon pennellii*. *J. Expt. Bot.* 55(399):1105-1113.

- Mohamed, H. I. and Gomaa, E. Z. 2012. Effect of plant growth promoting *Bacillus subtilis* and *Pseudomonas fluorescens* on growth and pigment composition of radish plants (*Raphanus sativus*) under NaCl stress. *Photosynthetica* 50:263-272
- Mohanraj, K., Hemaprabha, G. and Vasantha, S. 2021. Biomass yield, dry matter partitioning and physiology of commercial and *Erianthus* introgressed sugarcane clones under contrasting water regimes. *Agril. Water Manag.* 255: 10703-10710.
- Moncada, A., Vetrano, F. and Miceli, A. 2020. Alleviation of salt stress by plant growth-promoting bacteria in hydroponic leaf lettuce. *Agronomy*, 10(10):1523.
- Moslemi, Z., Habibi, D. and Asgharzadeh, A. et al 2011. Effects of super absorbent polymer and plant growth promoting rhizobacteria on yield and yield components of maize under drought stress and normal conditions. *Afr. J. Agric. Res.* 6:4471-4476
- Munns, R. and Tester, M. 2008. Mechanisms of salinity tolerance. *Annu. Rev. Plant Biol.* 59:651-681
- Munns, R., James, R. A., Lauchli, A. 2006. Approaches to increasing the salt tolerance of wheat and other cereals. *J. Exp. Bot.* 57:1025-1043
- Muthukumarasamy, R., Revathi, G., Seshadri, S. and Lakshminarasimhan, C. 2002. *Gluconacetobacter diazotrophicus* (syn. *Acetobacter diazotrophicus*), a promising diazotrophic endophyte in tropics. *Curr. Sci.*:137-145.
- Nadeem, S.M., Zahir, Z.A., Naveed, M. and Ashraf, M. 2010. Microbial ACC-deaminase: prospects and applications for inducing salt tolerance in plants. *Critical Reviews in Plant Sciences*, 29(6):360-393.
- Nakano, Y. and Asada, K. 1987. Hydrogen peroxide is scavenged by ascorbate specific peroxidase in spinach chloroplasts. *Plant Cell Physiol.* 22: 867-880.
- Negrao, S., Schmockel, S. and Tester, M. 2017. Evaluating physiological responses of plants to salinity stress. *Annals Bot.* 119:1-11.
- Nelson, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 15: 375-380.

- Nguyen, D., Rieu, I., Mariani, C. and Dam, N. M. 2016. How plants handle multiple stresses hormonal interactions underlying responses to abiotic stress and insect herbivory. *Plant Mol. Biol.* 91(6):727-740.
- Nia, S. H., Zarea, M. J., Rejali, F. and Varma, A. 2012. Yield and yield components of wheat as affected by salinity and inoculation with *Azospirillum* strains from saline or non-saline soil. *J. Saudi Soc Agric Sci.* 11:113–121
- Noori, F., Etesami, H., Zarini, H.N., Khoshkholgh-Sima, N.A., Salekdeh, G.H. and Alishahi, F. 2018. Mining alfalfa (*Medicago sativa* L.) nodules for salinity tolerant non-rhizobial bacteria to improve growth of alfalfa under salinity stress. *Ecotoxicol. Environ. safety*, 162:129-138.
- Omar, M. N. A., Osman, M. E. H., Kasim, W. A. and Abd El-Daim, I. A. 2009. Improvement of salt tolerance mechanisms of barley cultivated under salt stress using *Azospirillum brasilense*. *Salinity and water stress*. Springer, Berlin: 133-147
- Omer, A. M., Osman, M.S. and Badawy, A. A. 2022. Inoculation with *Azospirillum brasilense* and/or *Pseudomonas geniculata* reinforces flax (*Linum usitatissimum*) growth by improving physiological activities under saline soil conditions. *Botanical Studies*, 63(1):1-15.
- Osman, K.T., 2018. Management of soil problems: an introduction. Ed. by Osman, K. T. In: *Management of soil problems*. 1-14. ISBN: 9783319755274. Springer International Publishing AG, Cham.
- Osman, M. S., Badawy, A. A., Osman, A.I. and Abdel Latef, A. A. H. 2021. Ameliorative impact of an extract of the halophyte *Arthrocnemum macrostachyum* on growth and biochemical parameters of soybean under salinity stress. *J. Plant Growth Regul.* 40 (3):1245-1256.
- Otto, R., Machado, B. A., da Silva, A. C. M., de Castro, S. G. Q. and Lisboa, I. P. 2022. Sugarcane pre-sprouted seedlings: A novel method for sugarcane establishment. *Field Crops Res.* 275:108336-108346.
- Pagariya, M., Suprasanna, P., Patade, V., Desai, N., Devarumath, R., Kavar, P., Ganapathi, A., Manickavasagam, M. and Babu, K. 2012. Biotechnological developments in sugarcane improvement: An Overview. *Sugar Tech.* 3: 2355-2367.

- Pang, X-M., Zhang, Z-Y., Wen, X-P., Ban, Y. and Moriguchi, T. 2007. Polyamines, all-purpose players in response to environment stresses in plants. *Plant Stress* 1:173-188
- Pankaj, U., Singh, D. N., Singh, G. and Verma, R. K. 2019. Microbial inoculants assisted growth of *Chrysopogon zizanioides* promotes phytoremediation of salt affected soil. *Indian J. Microbiol.* 59 (2): 137-146.
- Panuccio, M., Jacobsen, S. E., Akhtar, S. S. and Muscolo, A. 2014. Effect of saline water on seed germination and early seedling growth of the halophyte quinoa. *AoB Plants* 6:47-50.
- Parasuraman, P. and Pattnaik, S. 2019. Chapter 18 - Plant-Microbe Interactions in Ecosystems Functioning and Sustainability. New and future developments in microbial biotechnology and bioengineering. Elsevier 255-266: 10.1016/B978-0-444-64191-5.00018-3.
- Parvin, K., Nahar, K., Hasanuzzaman, M., Bhuyan, M. B. and Fujita, M. 2019. Calcium-mediated growth regulation and abiotic stress tolerance in plants. *Plant Abiotic Stress Tolerance*. 91-331: ISBN: 978-3-030-06117-3. Springer.
- Patade, V. Y., Bhargava, S. and Suprasanna, P. 2011. Salt and drought tolerance of sugarcane under iso-osmotic salt and water stress: growth, osmolytes accumulation, and antioxidant defense. *J. Plant Interact.* 6(4): 275-282.
- Pehlivan, N., Yesilyurt, A. M., Durmus, N. and Karaoglu, S. A. 2017. *Trichoderma lixii* ID11D seed biopriming mitigates dose dependent salt toxicity in maize. *Acta Physiol. Plant* 39:79
- Peng, J., Liu, J., Zhang, L., Luo, J., Dong, H., Ma, Y., Zhao, X., Chen, B., Sui, N., Zhou, Z. and Meng, Y. 2016. Effects of soil salinity on sucrose metabolism in cotton leaves. *PLoS One*, 11(5):e0156241.
- Percival, G. C., Fraser, G. A. and Oxenham, G. 2003. Foliar salt tolerance of Acer genotypes using chlorophyll fluorescence. *Arboriculture & Urban Forestry*, 29 (2):61.
- Pinni, F., Galardini, M., Brzzicalupo, M. and Mengoni, A. 2011. Plant-bacteria association and symbiosis: Are there common genomic traits in Alpha proteobacteria Genes (Basel) 2(4):1017-1032
- Prasanna Kumar, B., Trimurtulu, N., Vijaya Gopal, A. and Nagaraju, Y., 2022. Impact of Culturable Endophytic Bacteria on Soil Aggregate Formation and Peanut (Arachis

- hypogaea L.) Growth and Yield Under Drought Conditions. *Current Microbiology*, 79(10), p.308.
- Preiss, J. 2018. Plant Starch Synthesis. In: Sjööm, Nilsson L (eds) *Starch in food*, 2nd edn. Woodhead Publishing, Elsevier, Cambridge:3-95
- Rahnama, A., Munns, R., Poustini, K. and Watt, M. 2011. A screening method to identify genetic variation in root growth response to a salinity gradient. *J. Exp. Bot.* 62:69-77
- Rao, H. C. and Savalgi, V. P. 2017. Isolation and screening of nitrogen fixing endophytic bacterium *Gluconacetobacter diazotrophicus* GdS25. *Int J Curr Microbiol Appl Sci*, 6(3):1364-1373.
- Rao, V. P., Sengar, R. S. Singh, S. and Sharma, V. 2015. Molecular and metabolic perspectives of sugarcane under salinity stress pressure. *Prog. Agric.* 15 (1):77-84.
- Rao, V. P., Sengar, R. S., and Singh, R. B. 2021. Identification of salt tolerant sugarcane cultivars through phenotypic, physiological and biochemical studies under abiotic stress. *Plant Physiol. Rep.* 26(2): 256-283.
- Rasool, S., Ahmad, A., Siddiqi, T. O. and Ahmad, P. 2013. Changes in growth, lipid peroxidation and some key antioxidant enzymes in chickpea genotypes under salt stress. *Acta Physiol Plant.* 35:1039–1050.
- Rawat, L., Bisht, T., Upadhyay, R. and Kukreti, A. 2016. Selection of salinity tolerant *Trichoderma* isolates and evaluating their performance in alleviating salinity stress in rice (*Oryza sativa* L.). *J Trop Agric*, 34:1869-1875.
- Rawat, L., Bisht, T., Upadhyay, R. and Kukreti, A., 2016. Selection of salinity tolerant *Trichoderma* isolates and evaluating their performance in alleviating salinity stress in rice (*Oryza sativa* L.). *J Trop Agric*, 34, pp.1869-1875.
- Reinhold-Hurek, B. and Hurek, T. 2011. Living inside plants: bacterial endophytes. *Curr. Opin. Plant Biol.* 14:435–443.
- Reinhold-Hurek, B. and Hurek, T., 1998. Life in grasses: diazotrophic endophytes. *Trends in microbial.* 6(4):139-144.
- Ren, M., Zhang, Z., Wang, X., Zhou, Z., Chen, D. and Zeng, H. 2018. Diversity and contributions to nitrogen cycling and carbon fixation of soil salinity shaped microbial communities in Tarim Basin. *Front. Microbiol.* 9: 431.

- Rewald, B., Holzer, L. and Göransson, H. 2015. Arbuscular mycorrhiza inoculum reduces root respiration and improves biomass accumulation of salt-stressed *Ulmus glabra* seedlings. *Urban For Urban Gree* 14:432–437
- Reza Sabzalian, M. and Mirlohi, A., 2010. Neotyphodium endophytes trigger salt resistance in tall and meadow fescues. *Journal of Plant Nutrition and Soil Science*, 173(6), pp.952-957.
- Rho, H., Hsieh, M., Kandel, S. L., Cantillo, J., Doty, S. L. and Kim, S. H. 2018. Do endophytes promote growth of host plants under stress? A meta-analysis on plant stress mitigation by endophytes. *Microb. Ecol.* 75:407-418
- Rhodes, D. and Hanson, A. D. 1993. Quaternary ammonium and tertiary sulfonium compounds in higher-plants. *Annu. Rev Plant Phys.* 44:357–384
- Richards, L.A., 1954. *Diagnosis and improvement of saline and alkali soils* (Vol. 78, No. 2, p. 154). LWW.
- Rios-Gonzalez, K., Erdei, L. and Lips, S. H. 2002. The activity of antioxidant enzymes in maize and sunflower seedlings as affected by salinity and different nitrogen sources. *Plant Sci.* 162(6):923-930.
- Rivero, R. M., Mestre, T. C., Mittler, R., Rubio, F., Garcia-Sanchez, F. and Martinez, V. 2014. The combined effect of salinity and heat reveals a specific physiological, biochemical and molecular response in tomato plants. *Plant Cell Environ* 37:1059-1073
- Roy, S., Negrão, S. and Tester, M. 2014. Salt resistant crop plants. *Curr. Opin. Biotechnol.* 26:115-124.
- Rubio, F., Nieves-Cordones, M., Horie, T. and Shabala, S. 2020. Doing 'business as usual' comes with a cost: evaluating energy cost of maintaining plant intracellular K⁺ homeostasis under saline conditions. *New Phytologist*. 225: 1097-1104.
- Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J. and Dowling, D. N. 2008. Bacteria endophytes: recent developments and applications. *FEMS Microbiol Lett.* 278:1–9
- Saddique, M. A. B., Ali, Z., Khan, A. S., Rana, I. A. and Shamsi, I. H. 2018. Inoculation with the endophyte *Piriformospora indica* significantly affects mechanisms involved in osmotic stress in rice. *Rice*, 11(1):1-12.

- Safeena, M.I.S. and Zakeel, M.C.M. 2020. Nanobiotechnology-driven management of phytonematodes. In *Management of Phytonematodes: Recent Advances and Future Challenges* 1-33. Springer, Singapore.
- Saha, A. K., Acharya, C. L. and Abrol, I. P. 2022. The modifying effect of electrolyte concentration on the effect of exchangeable sodium on some soil physical properties and sodic soil reclamation. *J. Indian Soc. Soil Sci.*, 26 (3):243-253.
- Sahoo, R.K., Ansari, M.W., Pradhan, M., Dangar, T.K., Mohanty, S. and Tuteja, N. 2014. Phenotypic and molecular characterization of native *Azospirillum* strains from rice fields to improve crop productivity. *Protoplasma*, 251(4):943-953.
- Sairam, R. K. 1994. Effect of moisture stress on physiological activities of two contrasting wheat genotypes. *Indian J. Expt. Biol.* 32: 584- 593.
- Sallaku, G, Sandén, H., Babaj, I., Kaciu, S., Balliu, A. and Rewald, B. J. 2019. Specific nutrient absorption rates of transplanted cucumber seedlings are highly related to RGR and influenced by grafting method, AMF inoculation and salinity. *Sci. Hortic.* 243:177-188
- Salope-Sondi, B., Pollmann, S., Gruden, K., Oelmüller, R. and Ludwig- Müller, J. 2015. Improvement of root architecture under abiotic stress through control of auxin homeostasis in *Arabidopsis* and *Brassica* crops. *Endocytobiosis Cell Res.* 26:100-111
- Samolski, I., Rincon, A. M., Pinzón L. M., Viterbo A. and Monte, E. 2012. The *qid74* gene from *Trichoderma harzianum* has a role in root architecture and plant biofertilization. *Microbiol.* 158:129-138
- Sampangi-Ramaiah, M.H., Dey, P., Jambagi, S., Vasantha Kumari, M.M., Oelmüller, R., Nataraja, K.N., Venkataramana Ravishankar, K., Ravikanth, G. and Uma Shaanker, R., 2020. An endophyte from salt-adapted Pokkali rice confers salt-tolerance to a salt-sensitive rice variety and targets a unique pattern of genes in its new host. *Scientific reports*, 10(1):1-14.
- Santos, C. 2004. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. *Sci. Hort.* 103: 93-99
- Santoyo, G., Moreno-Hagelsieb, G., del Carmen Orozco-Mosqueda, M. and Glick, B. R. 2016. Plant growth-promoting bacterial endophytes. *Microbiol Res.* 183:92–99

- Sarkar, A., Ghosh, P. K., Pramanik, K., Mitra, S., Soren, T. and Pandey, S. et al. 2018. A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. *Res. Microbiol.* 169: 20-32.
- Sathyan, R. and Thangaraju, M. 2003. Characterization of endophytic nitrogen fixing bacterium *Gluconacetobacter diazotrophicus* associated with sweet potato (*Ipomoea batatas* L.) Lam). *Madras Agril. J.* 90 (7-9):400-405.
- Sayyed, R., Ilyas, N., Tabassum, B., Hashem, A., AbdAllah, E. and Jadhav, H. 2019. Plausible role of plant growth-promoting rhizobacteria in future climatic scenario, environmental biotechnology: for sustainable future. *Springer*. 175-197.
- Seemann, J. R. and Critchley, C. 1985. Effects of salt stress on the growth, ion content, stomatal behaviour and photosynthetic capacity of a salt-sensitive species. *Phaseolus vulgaris* L *Planta* 164:151-162
- Sen, S. and Chandrasekhar, C. N. 2014. Effect of PGPR on growth promotion of rice (*Oryza sativa* L.) under salt stress. *Asian J Plant Sci Res*, 4(5):62-67.
- Shahid, M., Zeyad, M.T., Syed, A., Singh, U.B., Mohamed, A., Bahkali, A.H., Elgorban, A.M. and Pichtel, J., 2022. Stress-tolerant endophytic isolate *Priestia aryabhatai* BPR-9 modulates physio-biochemical mechanisms in wheat (*Triticum aestivum* L.) for enhanced salt tolerance. *International Journal of Environmental Research and Public Health*, 19(17), p.10883.
- Shahnaz, S. and Manjurul, K. M. 2018. Salinity intrusion and coastal agriculture: adaptation strategies using salt-tolerant plant-growth promoting rhizobacteria for sustainable food security 21(3-1).
- Sharma, A., Dev, K., Sourirajan, A. and Choudhary, M. 2021. Isolation and characterization of salt-tolerant bacteria with plant growth-promoting activities from saline agricultural fields of Haryana, India. *J. Genet. Eng. Biotechnol.* 19:99.
- Sharma, P. J., Dubey, R. S. and Pessarakli, M. 2012. Reactive oxygen species, oxidative damage, and antioxidative defense mechanism in plants under stressful conditions. *J. Bot.* 1–26.
- Sharma, S., Gupta, S., Sourirajan, A. and Dev, K. 2020. Halophilic plant growth promoting bacteria as potential bioinoculants for sustainable agriculture under salt stress. *J. Xian Univ. Arch. Tech.* XII (IV):

- Sharma, S., Kulkarni, J. and Jha, B., 2016. Halotolerant rhizobacteria promote growth and enhance salinity tolerance in peanut. *Frontiers in microbiology*, 7, p.1600.
- Sherameti, I., Shahollari, B., Venus, Y., Altschmied, L., Varma, A. and Oelmüller, R. 2005. The endophytic fungus *Piriformospora indica* stimulates the expression of nitrate reductase and the starch-degrading enzyme glucan-water dikinase in tobacco and Arabidopsis roots through a homeodomain transcription factor that binds to a conserved motif in their promoters. *J. Biol. Chem.* 280:26241-26247
- Shilev, S., 2020. Plant-growth-promoting bacteria mitigating soil salinity stress in plants. *Applied Sciences*, 10(20), p.7326.
- Shrivastava, P. and Kumar, R. 2015. Soil salinity: a serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. *Saudi J. Biol. Sci.* 22(2):123-31.
- Shukla, S. K., Sharma, L., Awasthi, S. K. and Pathak, A. D. 2017. Sugarcane in India: Package of practices for different agro-climatic zones. Published by ICAR all India coordinated Res. Project on Sugarcane, Pub. No. AICRP(S) Tech. Bulletin No. 1. 1-64.
- Simoes, W. L., Calgaro, M., Guimarães, M. J., Oliveira, A. R. D. and Pinheiro, M. P. M. A. 2018. Sugarcane crops with controlled water deficit in the Sub-Middle São Francisco Valley, Brazil. *Revista Caatinga*. 31: 963–971.
- Simões, W. L., Coelho, D. S., Mesquita, A. C., Calgaro, M. and da Silva, J. S. 2019. Physiological and biochemical responses of sugarcane varieties to salt stress. *Rev. Geama, Recife* 3 (4): 184-190.
- Singh, D., Sharma, A. and Saini, G. K. 2013. Biochemical and molecular characterisation of the bacterial endophytes from native sugarcane varieties of Himalayan region. *Biotech.* 3 (3):205-212.
- Singh, L. P., Gill, S. S. and Tuteja, N. 2011. Unraveling the role of fungal symbionts in plant abiotic stress tolerance. *Plant Signal Behav* 6:175-191
- Singh, P., Basu, S. and Kumar, G. 2018. Polyamines metabolism: A way ahead for abiotic stress tolerance in crop plants. In: Wani S H (ed) *Biochemical, physiological and molecular avenues for combating abiotic stress tolerance in plants*. Elsevier: 39-55

- Singh, R. 2014. Microorganism as a tool of bioremediation technology for cleaning environment: a review. *Proc Int Acad Ecol Environ Sci* 4:1
- Singh, R.P. and Jha, P.N., 2017. The PGPR *Stenotrophomonas maltophilia* SBP-9 augments resistance against biotic and abiotic stress in wheat plants. *Frontiers in microbiology*, 8, p.1945.
- Singh, S. K., Singh, P. P., Gupta, A., Singh, A. K. and Keshri, J. 2019. Tolerance of heavy metal toxicity using PRPR strains of *pseudomonas* species. In: PGPR Amelioration in Sustainable Agriculture. Ed. by. Singh, A. K., Kumar A. and Singh, P. K. Elsevier 239-252.
- Smith, P. A. 2014. Foliage friendships. *Sci Am*. 311:24–25
- Sofy, A. R., Hmed, A. A., Alnaggar, A. M., Dawoud, R. A, Elshaarawy, R. F. M. Sofy, M. R. 2020. Mitigating effects of Bean yellow mosaic virus infection in faba bean using new carboxymethyl chitosan-titania nanobiocomposites. *Int J. Biol. Macromol.* 163:1261-1275
- Sofy, M. R., Aboseidah, A. A., Heneidak, S. A. and Ahmed, H. R. 2021. ACC deaminase containing endophytic bacteria ameliorate salt stress in *Pisum sativum* through reduced oxidative damage and induction of antioxidative defense systems. *Environ. Sci. Pollut. Res.* 28(30): 40971-40991.
- Solomon, S. 2016. Sugarcane production and development of sugar industry in India. *Sugar Tech.* 18(6):588–602.
- Song, M., Chai, Q., Li, X., Yao, X., Li, C., Christensen, M. J. and Nan, Z. 2015. An asexual *Epichloë* endophyte modifies the nutrient stoichiometry of wild barley (*Hordeum brevisubulatum*) under salt stress. *Plant Soil* 387:153-165
- Sorensen, J. and Sessitsch A 2015. Plant-associated bacteria lifestyle and molecular interactions. In: van Elsas, J. D. et al (eds) *Modern soil microbiol.* 2nd edn. CRC Press, Boca Raton:211–236
- Souza, R. D., Ambrosini, A. and Passaglia, L. M. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genet. Mol. Biol.* 38:401-419.

- Stefan, M, Munteanu, N. and Mihasan M. 2013. Application of plant growth promoting rhizobacteria to runner bean increases seed carbohydrate and protein yield. *Biol. Moleculara*. (1).14
- Stumpf, D. K. 1984. Quantitation and purification of quaternary ammonium compounds from halophyte tissue. *Plant Physiol*. 75: 273-274.
- Sudhir, P. and Murthy, S. 2004. Effects of salt stress on basic processes of photosynthesis. *Photosynthetica* 42:48-486
- Suman, A., Shasany, A.K., Singh, M., Shahi, H.N., Gaur, A. and Khanuja, S.P.S., 2001. Molecular assessment of diversity among endophytic diazotrophs isolated from subtropical Indian sugarcane. *World Journal of Microbiology and Biotechnology*, 17(1):39-45.
- Suzuki, K., Yamaji, N., Costa, A., Okuma, E., Kobayashi, N.I., Kashiwagi, T., Katsuhara, M., Wang, C., Tanoi, K., Murata, Y. and Schroeder, J.I., 2016. OsHKT1; 4-mediated Na⁺ transport in stems contributes to Na⁺ exclusion from leaf blades of rice at the reproductive growth stage upon salt stress. *BMC plant biology*, 16(1):1-15.
- Tahjib-Ul-Arif, M., Afrin, S. and Polash, M. A. S. et al 2019. Role of exogenous signaling molecules in alleviating salt-induced oxidative stress in rice (*Oryza sativa* L.): a comparative study. *Acta. Physiol. Plant* 41:1-14
- Tejera, N.L.C.M.M.G.J., Lluch, C., Martinez-Toledo, M.V. and Gonzalez-Lopez, J., 2005. Isolation and characterization of *Azotobacter* and *Azospirillum* strains from the sugarcane rhizosphere. *Plant and soil*, 270, pp.223-232.
- Thakuria, D., Talukdar, N.C., Goswami, C., Hazarika, S., Boro, R.C. and Khan, M.R., 2004. Characterization and screening of bacteria from rhizosphere of rice grown in acidic soils of Assam. *Current Sci*.978-985.
- Todorova, D., Talaat, N.B., Katerova, Z., Alexieva, V. and Shawky, B.T., 2016. Polyamines and brassinosteroids in drought stress responses and tolerance in plants. *Water stress and crop plants: a sustainable approach*, 2, pp.608-627.
- Ullah I, Khan A, Park G, Lim J, Waqas M, Lee I, Shin J 2013. Analysis of phytohormones and phosphate solubilization in *Photorhabdus* spp. *Food Sci Biotechnol* 22:25-31

- Uma, Shaanker. R. 2014. Evaluation of endophytic fungal assemblages of salt adapted paddy varieties and their role in imparting salinity tolerance. PhD thesis, University of Agricultural Sciences. Bangalore
- Umapathi, M., Chandrasekhar, C.N., Senthil, A., Kalaiselvi, T., Santhi, R. and Ravikesavan, R., 2021. Effect of bacterial endophytes inoculation on morphological and physiological traits of sorghum (*Sorghum bicolor* (L.) Moench) under drought. *J. Pharmacognosy and Phytochem.* 10(2):1021-1028.
- Upadhyay, S. K., Singh, D. P. and Saikia, R. 2009. Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. *Curr. Microbiol.* 59:489–496.
- Usha, D. K. and Kanimozhi, K. 2011. Isolation and characterization of saline tolerant *Azospirillum* strains from paddy field of Thanjavur district. *Adv. Appl. Sci. Res.* 2(3):239-245.
- Vahabi, K., Dorcheh, S.K., Monajembashi, S., Westermann, M., Reichelt, M., Falkenberg, D., Hemmerich, P., Sherameti, I. and Oelmüller, R. 2016. Stress promotes *Arabidopsis-Piriformospora indica* interaction. *Plant signal. Behave.* 11(5):e1136763.
- Vaishnav, A., Kasotia, A. and Choudhary, D. K. 2018. Role of functional bacterial phylum proteobacteria in *Glycine max* growth promotion under abiotic stress: a glimpse on case study. “In silico approach for sustainable agriculture”. Ed. by Devendra, K., Choudhary, M. Kumar R. P. and Kumar, V. Springer Nature Singapore Pvt. Ltd., 17-49.
- Vaishnav, A., Shukla, A. K., Sharma, A., Kumar, R. and Choudhary, D. K. 2019. Endophytic bacteria in plant salt stress tolerance: current and future prospects. *J. Plant Growth Regul.* 38 (2):650-668.
- Vaishnav, A., Singh, J., Singh, P., Rajput, R.S., Singh, H.B. and Sarma, B.K., 2020. *Sphingobacterium* sp. BHU-AV3 induces salt tolerance in tomato by enhancing antioxidant activities and energy metabolism. *Frontiers in Microbiology*, 11, p.443.
- Vanholme, R., Demedts, B., Morreel, K., Ralph, J. and Boerjan, W. 2010. Lignin biosynthesis and structure. *Plant Physiol.* 153:895-905

- Vibitha Bala, B., 2019. Effect of *Klebseilla oxytoca* and *Acinetobacter* Sp. on Growth of Rice Genotype under Moisture Stress Condition. *International Journal of Agriculture Sciences*, ISSN, pp.0975-3710.
- Waller, F., Achatz, B., Baltruschat, H., Fodor, J., Becker, K., Fischer, M., Heier, T., Hückelhoven, R., Neumann, C., von Wittstein, D., Franken, P. and Kogel, K. H. 2005. The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield. *PNAS* 102:13386-13391
- Wani, S. H. 2018. Biochemical, Physiological and Molecular Avenues for Combating Abiotic Stress in Plants. Elsevier, London:39-55
- Werner, D. and Newton, W.E. eds., 2005. *Nitrogen fixation in agriculture, forestry, ecology, and the environment* (Vol. 4). Springer Science & Business Media.
- Wilkinson, S. R., Welch, R. M., Mayland, H. F., Grunes, D. L. 1990. Magnesium in plants: uptake, distribution, function, and utilization by man and animals. In: Sigel H (ed) *Metal Ions in Biological Systems* Marcel Dekker, New York (26):33-56.
- Williams, K. 1997. Interactions of polyamines with ion channels. *Biochem J.* 325:289-297
- Wong, P.P. and Stenberg, N.E., 1979. Characterization of *Azospirillum* isolated from nitrogen-fixing roots of harvested sorghum plants. *Applied and Environmental Microbiology*, 38(6), pp.1189-1191.
- Wu, H. 2018. Plant salt tolerance and Na^+ sensing and transport. *The Crop J.* 6: 215-225.
- Xu, Y. Raza, W., Zhong, W., Nan, Z., Shen, Q. and Faheem, M., 2015. Evaluation of the biocontrol potential of *Streptomyces goshikiensis* YCXU against *Fusarium oxysporum* f. sp. niveum. *Biological Control*, 81:101-110.
- Yaish M. W. Al-Lawati A, Jana G. A. Vishwas Patankar H. and Glick B. R. 2015. Impact of soil salinity on the structure of the bacterial endophytic community identified from the roots of Caliph Medic (*Medicago truncatula*). *PLoS ONE* 11(7):e0159007
- Yaish, W., Antony, I. and Glick, B. R. 2015. Isolation and characterization of endophytic plant growth promoting bacteria from date palm tree (*Phoenix dactyloifera* L.) and their potential role in salinity tolerance. *Anionic van Leeuwenhoek*, 24: 51-57. (Quoted from

- Yang, B., Wang, X. M., Ma, H. Y., Yang, T., Jia, Y., Zhou, J. and Dai, C. C. 2015. Fungal endophyte *Phomopsis liquidambari* affects nitrogen transformation processes and related microorganisms in the rice rhizosphere. *Front.microbial*.6:982.
- Yeo, A. 1998. Molecular biology of salt tolerance in the context of whole-plant physiology. *J. Expt. Bot.* 49:915–929
- Yin, L., Ren, A., Wei, M., Wu, L., Zhou, Y., Li, X. and Gao, Y. 2014. *Neotyphodium coenophialum*-infected tall fescue and its potential application in the phytoremediation of saline soils. *Int. J. Phytoremed.* 16:235–246
- Younesi, O. and Moradi, A. 2014. Effects of plant growth-promoting rhizobacterium (PGPR) and *arbuscular mycorrhizal fungus* (AMF) on antioxidant enzyme activities in salt-stressed bean (*Phaseolus vulgaris* L.). *Agril.* 60:10–21
- Yu, D, Boughton, B. A., Hill, C., Feussner, I., Roessner, U. and Rupasinghe, T. W. T.2020. Insights into oxidized lipid modification in barley roots as an adaptation mechanism to salinity stress.*Front Plant Sci* 11:1
- Yun, P., Xu, L., Wang, S. S., Shabala, L., Shabala, S. and Zhang W. Y 2018. *Piriformospora indica* improves salinity stress tolerance in *Zea mays* L. plants by regulating Na⁺ and K⁺ loading in root and allocating K⁺ in shoots. *Plant Growth Regul.* 86:323–331
- Zafar-ul-Hye, M. Bhutta, T. S., Shaaban, M., Hussain, S., Qayyum, M. F., Aslam U. and Zahir Z. A. 2019. Influence of multi-strain plant growth promoting rhizobacteria inoculation on wheat crop productivity under soil salinity stress. *Phyton, Int. J. Expt. Bot.* 88: 119-129.
- Zahaf, O. Blanchet, S. De Zélicourt A, Alunni B, Plet, J. and Laffont C 2012. Comparative transcriptomic analysis of salt adaptation in roots of contrasting *Medicago truncatula* genotypes. *Molecular plant.* 5(5):1068–1081.
- Zahir, Z.A., Arshad, M. and Frankenberger, W.T., 2004. Plant growth promoting. *Advances in agronomy*, 81:97.
- Zahran, H. H. 1991. Conditions for successful *Rhizobium*-legume symbiosis in saline environments. *Biol Fertil Soils* 12(1):73–80
- Zarea, M., Hajinia, S., Karimi, N., Goltapeh, E. M., Rejali, F. and Varma, A. 2012. Effect of *Piriformospora indica* and *Azospirillum* strains from saline or non-saline soil on mitigation of the effects of NaCl. *Soil Biol Biochem* 45:139–146

- Zhang F, Meng X, Yang X, Ran W, Shen Q 2014. Quantification and role of organic acids in cucumber root exudates in *Trichoderma harzianum* T-E5 colonization. *Plant Physiol. Biochem.* 83:250-257
- Zhang F., Wang, Y., Liu, C., Chen, F., Ge, H., Tian, F., Yang, T., Ma, K. and Zhang, Y. 2019 *Trichoderma harzianum* mitigates salt stress in cucumber via multiple responses. *Ecotoxicol Environ Saf.* 170:436-445
- Zhang, H. X., Hodson, J. N., Williams, J. P. and Blumwald, E. 2001. Engineering salt-tolerant Brassica plants: characterization of yield and seed oil quality in transgenic plants with increased vacuolar sodium accumulation. *PNAS* 98:12832-12836
- Zhang, R, Vivanco J. M. and Shen, Q. 2017. The unseen rhizosphere root-soil-microbe interactions for crop production. *Curr. Opin. Microbiol.* 37:8-14
- Zhang, S., Gan, Y. and Xu, B. 2016. Application of plant-growth promoting fungi *Trichoderma longibrachiatum* T6 enhances tolerance of wheat to salt stress through improvement of antioxidative defense system and gene expression. *Front. Plant Sci.* 7:1405
- Zhao, L., Wang, F., Zhang, Y. and Zhang, J. 2014. Involvement of *Trichoderma asperellum* strain T6 in regulating iron acquisition in plants. *J. Basic Microbiol.* 54:S115-S124
- Zhao, L., Xu, Y. and Lai, X., 2018. Antagonistic endophytic bacteria associated with nodules of soybean (*Glycine max* L.) and plant growth-promoting properties. *Brazilian journal of microbiology*, 49:269-278.
- Zhu, Y. and She, X. 2018. Evaluation of the plant-growth-promoting abilities of endophytic bacteria from the psammophyte *Ammodendron bifolium*. *Can. J. Microbiol.* 64 (4):253-264.
- Zuffo, A. M., Steiner, F., Aguilera, J. G., Teodoro, P. E., Teodoro, L. P. R. and Busch, A. 2020. Multi-trait stability index: A tool for simultaneous selection of soybean genotypes in drought and saline stress. *J. Agron. Crop Sci.* 206 (6):815-822.

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