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PS Chougule
Department of Biochemistry,
Post Graduate Institute,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

AA Kale
Department of Biochemistry,
Post Graduate Institute,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

PK Lokhande
Department of Biochemistry,
Post Graduate Institute,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

RM Naik
Department of Biochemistry,
Post Graduate Institute,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

BM Bhalerao
Department of Biochemistry,
Post Graduate Institute,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

Corresponding Author:
PS Chougule
Department of Biochemistry,
Post Graduate Institute,
Mahatma Phule Krishi
Vidyapeeth, Rahuri,
Ahmednagar, Maharashtra,
India

Effect of endophytic bacteria on physiological parameter's under induced salt stress in sugarcane varieties

PS Chougule, AA Kale, PK Lokhande, RM Naik and BM Bhalerao

Abstract

Sugarcane (*Saccharum officinarum* L., Family: Poaceae) is one of the most important crop for sugar production in India. Salinity one of the major problems for the losses of sugarcane productivity and endophytic bacteria not only enhance plant growth but also provide tolerance against salt. The potential endophytic bacteria such as *Azospirillum*, *Acetobacter*, *Herbaspirillum*, *Azoarcus* etc. isolated from saline soil were exploited to enhance the growth of sugarcane crop under salt stress conditions.

In the present study two sugarcane varieties viz. COC-671 susceptible and another tolerant COM-265 were used for comparative studies of salt stress. Endophytic bacteria can improve the plant tolerance against salt stress via the enhancing morphophysiological parameters. The current outcome focus on role of isolated endophytic bacteria and their consortium were investigated for their potential to ameliorate plant salinity stress. The sugarcane varieties COM-265 and COC-671 were subjected to four levels of salt stress (2, 4, 8ds/m and sodic soil) under glasshouse conditions by using a CRD.

Analyses of various plant growth parameters were conducted to investigate the stress tolerance induced by the endophytic bacteria on the contrary; treatment with endophytic bacteria significantly increased the resistance of sugarcane plants to salt stress by increasing morphophysiological parameters. High levels of NaCl exhibited negative effects in both varieties. However, inoculation with bacterial endophytes increased the morphophysiological traits of the plants in all treatments compared to uninoculated treatment. The endophytic bacterial strains consortium performed significantly better in all morphophysiological parameters such as leaf area, root and shoot length and total chlorophyll compared with various endophytic bacterial strain viz. *Gluconacetobacter* (GA), *Azospirillum* (ASP), *Herbaspirillum* (HERB) and *Azoarcus* (AAR) in the was higher than the control in the tissues of the bacterially treated plants, likely to keep the balance of nutrients. The results of the current investigation showed that the inoculation of plants with EB has the potential to improve plant growth and stress tolerance. Additionally, endophytic bacteria associated with host plants can interact with plants very well and can withstand highly salinized circumstances.

Keywords: Sugarcane, entophytic bacteria, morphophysiological, biochemical, salinity stress

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) [56] for sugar, ethanol, bagasse or lignocellulose and has been subjected to extensive multidimensional research (Gupta *et al.*, 2022) [36].

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) [42].

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) [56]. Thus, it is estimated that 80% of sugar production worldwide comes from sugarcane (Otto *et al.*, 2022) [63]. Sugarcane is highlighted for its role as a feedstock for both sugar and ethanol production.

India is the second major country in the world commercial crop, cultivated in an area of 5.042 mha with the production of 411.0 MT and productivity of 83.5 t ha⁻¹ in 2020-2021

(Indiastat 2021-2022) area under sugarcane cultivation increased substantially from 1.71 mha in 1950-51 to 4.86 mha in 2020-21 (Directorate of Economics & Statistics, DA & FW). In India, the major sugarcane growing states are Uttar Pradesh, Maharashtra, Karnataka, Tamil Nadu, Andhra Pradesh and Gujarat. Among the list 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 (Maharashtra state sugarcane commission report 2021). These six states together accounted for about 84.06 per cent of India's total area in 2020-21. Maharashtra is the largest producer of sugar contributes about 34% of sugar in the country followed by Uttar Pradesh 30% (India stat 2020-21).

According to the Sugarcane Vision 2050, the second-largest sugar industry in the world, which is based in India, employs nearly 4% of the population directly or indirectly. The industry is inherently inclusive, supporting over 6 million farmers and their families as well as the employees and business owners of over 550 sugar mills, in addition to a variety of wholesalers and distributors located all over the nation (Solomon, 2016) [84].

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. One of the main agro-based sectors in India is the sugar industry, which has a production capacity of more than 25 MT per year. India currently makes up 13% of the world's sugar production and 15% of the world's sugar consumption (Solomon, 2016; Rao, 2021) [84, 69].

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.*, 2015) [7].

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 (Rao *et al.*, 2021) [69].

In India, 3.77 million ha area is sodic and 2.96 million ha 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) [69].

Sugarcane cultivation in regions with great production potential may be compromised due to soil degradation by salinity and sodicity (Asfaw *et al.*, 2018) [14]. 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) [20].

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 (Taiz *et al.*, 2017; Zuffo *et al.*, 2020) [87, 95]. The salinity effect on sugarcane varies and can promote reduced stomatal conductance and, consequently, decreased photosynthetic rates (Simoes *et al.*, 2018; Simoes *et al.*, 2016) [80, 79]. 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) [65].

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 (Chaum *et al.*, 2012, Rao *et al.*, 2021) [21, 69].

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 (Devi *et al.*, 2017) [25]. Cumulatively these attributes ultimately leads to reduced yield and sucrose accumulation (Patade *et al.*, 2011; Rao *et al.*, 2021) [65, 69].

Growing salt-tolerant cultivars developed by traditional breeding and transgenic procedures can also help in sustainable food production during the process of reclaiming salt-affected arable lands. However, field evaluation and the use of bacterial endophytes for boosting defence mechanisms may be a more economical and environmentally friendly way to lessen the negative effects of salinity on crop growth and productivity (Fouda *et al.* 2019) [29].

Regardless of the consequence of the association, bacterial endophytes are defined as strains that have their complete or partial life cycle inside the plant and are found in intra- and intercellular spaces or in the vascular tissue (Kandel *et al.* 2017) [44].

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; Khan *et al.*, 2017) [49, 45]. 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 *et al.*, 2018) [94].

Endophytic bacteria naturally have the capacity to colonise every interior surface of a root, stem, leaf, flower, fruit, or seed (Kumar *et al.* 2017) [48]. However, depending on the tissue type, stage of plant growth, and ecological niches, their number may vary (Kuklinsky-Sobral *et al.* 2004) [47]. Additionally, they have an impact on the physiology of plant cells (Santoyo *et al.* 2016) [73]. Additionally, because of their superior resistance to biotic and abiotic stressors, they are superior to bacteria that promote plant growth (Ding *et al.* 2011, Lata *et al.* 2018) [96, 49]. Typically, rhizospheric bacteria and endophytic bacteria use similar strategies to encourage plant growth. 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.
- 2) Biosynthesis of anti-stress biochemical by endophytes (Lata *et al.*, 2018) [49].

Salt tolerant bacteria *viz.*, *Rhizobium*, *Azospirillum*,

Pseudomonas, *Flavobacterium*, *Arthrobacter* and *Bacillus*) have a positive impact on crops productivity in stressed environments (Almaghrabi *et al.* 2014) [1] However, Souza *et al.* (2015) [85] reported that among the important endophytic bacterial genera, the study is concentrated on *Azospirillum*, *Gluconacetobacter*, *Herbaspirillum* and *Azoarcus* and application of these endophytic bacteria enhanced the salinity tolerance (Souza *et al.*, 2015) [85].

Materials and Methods

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 & broth, Semi-solid LGI-P media, N-free malate medium, Rennies media, *Azoarcus* media.

Preparation of media

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.

Isolation of endophytic bacteria

The isolation of endophytic bacteria was carried out on selective media by enrichment culture technique and serial dilution method described by with some modification. The fresh stem sugarcane plant samples were first washed under tap water. These samples surface sterilized with 0.1% HgCl₂ solution with successive 3 to 4 washings of sterilized distilled water. 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. The outer portion of cane node was removed with sterilized scalpel and small pieces were made. These pieces were then grinded in presterilized electric grinder-mixer and juice was extracted. The extracts of leaf sheath and root cortex and juice was homogeneously mixed together, strained through muslin cloth and collected in 100ml conical flasks. 1-2 ml sugarcane plant extracts were then transferred to sterilized petri plates of respective selective medium. 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. 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. Then plates were kept at 28±2 °C in BOD incubator for 4-5 days. All plates were observed for the appearance isolated bacterial colony.

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.

Selection of *Herbaspirillum* colony

Herbaspirillum cells grown slimy, colonies on Rennies

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.

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 Rennies medium plate for single colony. These purified cultures of *Azoarcus* were maintained on the slants of *Azoarcus* medium for storage and characterization.

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.

The endophytic bacteria isolated were screened for salt stress tolerance with the protocol described by Gayathri, *et al.* (2010) [97]. The ability of crop plant endophytic bacteria isolates to tolerate a high concentration of sodium chloride was tested on NA solid medium. NA medium was prepared and supplemented with 2 dSm⁻¹, 4 dSm⁻¹ and 8 dSm⁻¹ of NaCl for salt tolerance limit. The media was autoclaved at 121 °C temperature and 15 lbs pressure for 20 min and poured into sterilized petri plates under aseptic condition. A loopful culture of all endophytic bacterial isolates were streaked onto the different NaCl supplemented nutrient agar plates. The inoculated petri plates were incubated at 28-30 °C for 7 days.

Plant material and Salinity (NaCl) stress induction

Sugarcane [*Saccharum officinarum* L.] varieties identified as salinity stress tolerant CoM 0265 and salinity stress susceptible CoC 671 was collected from Central Sugarcane Research Station, Padegaon, Dist. -Satara. 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. For control plants it was irrigated with distilled water. Then 105 days after planting third leaf sample was taken for further analysis.

Pot culture experiment treatment details

1. Control, 2ds/m, 4 ds/m and 8 ds/m salt stress.
2. Consortia + control, Consortia + 2,4 and 8 ds/m salt stress.

3. GA + control, GA + 2 ds/m, GA + 4 ds/m, GA + 8 ds/m salt stress.
4. ASP + control, ASP + 2 ds/m, ASP + 4 ds/m, ASP + 8 ds/m salt stress.
5. HERB + control, HERB + 2 ds/m, HERB + 4 ds/m, HERB + 8 ds/m salt stress.
6. AAR + control, AAR + 2 ds/m, AAR + 4 ds/m, AAR + 8 ds/m salt stress.

Optimization of the soil salinity with NaCl solution

Electrical conductivity of the soil was optimized at ~ with 2, 4 and 8 dSm⁻¹ with NaCl solution. Electrical conductivity of given soil samples was measured by electrical conductometer method given by Jackson (1973). Soil was obtained from the Post Graduate Institute Research Farm, MPKV, Rahuri to grow the sugarcane plants in pots. Fifty g of soil was measured and 100 mL distilled water was added into it and stirred at regular interval for 1 hour. The content was allowed to settle for 30 minutes and electrical conductivity was measured by dipping electrode into the supernatant and the procedure repeated twice to confirm the EC. The standard NaCl stock solution of 32 dSm⁻¹ and 62 dSm⁻¹ was used to optimize desired EC. It was observed that to obtain 2 dSm⁻¹ EC of the soil 4 ml of 32 dSm⁻¹ standard stock was required, similarly for 4 dSm⁻¹ EC of soil 8 ml of 32 dSm⁻¹ stock was required and for 8 dSm⁻¹ EC of soil 9 ml 62 dSm⁻¹ standard NaCl stock solution was required.

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 hours 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 density (10⁸CFU/ml) all these bacterial culture spray on sugarcane plants at an interval of 15 days.

Leaf area (cm²), Shoot height and Root length (cm)

Leaf area were measured by leaf area meter, shoot and root lengths measured for each plant by using measuring tape.

Relative leaf water content (mg g⁻¹ FW)

Relative leaf water content (RLWC) was measured by the method described by Henderson and Davies (1990). Relative leaf water content was determined by taking known weight of fresh leaves grown under stressed and unstressed conditions. Initially midribs from 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 90 °C to obtain dry weight (DW). The RLWC was calculated by using the following formula.

$$RLWC = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Total chlorophyll content

Total leaf chlorophyll content was determined by method described by Arnon (1949) [13]. The leaf samples were cut into small pieces and known weight (0.2 g) of fresh leaf sample was macerated in a mortar and pestle and extracted with 20 ml of 80 percent acetone. The contents were centrifuged at

5000xg for 10 min and the supernatant was collected. The final volume of extract was made to 5 ml. The extinction of chlorophyll extract was recorded at 645 and 663 nm on a Spectrophotometer and a blank was run with 80 percent acetone.

The amount of total chlorophyll content was calculated by using the following formula and expressed in mg g⁻¹ FW.

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 g.

Result and discussion

Leaf area

The effect of different level of salt stress on leaf area of salt resistant & salt susceptible varieties is depicted in table No. 1. The results revealed that there was significant decrease in the leaf area of both varieties with increasing salt conc. as compare to control.

From the result obtained it was noticed that leaf area was 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 more decrease was observed in salt susceptible COC-671 variety (83.6 cm²) than salt tolerant COM-0265 variety (94.1 cm²). By application of the different endophytes along with consortia it was noticed that the shoot length was increased over the control (without application of endophytes) in each of salt stress treatment in both the varieties. However, more increase was observed in control plants (112.08 cm²) (without salt stress) than the salt stressed plants. Among the various salt stress levels the plants grown under sodic soil had more shoot length (108.12 cm²) than imposed salt stress levels in both the varieties treated with endophytic bacteria. Further, least decrease in shoot length with imposition salt stress was observed in CEB treatment 115 cm² and 110.4 cm² followed by GA treatment 111.3 cm² and 108.86 cm² in COM-0265, salt tolerant variety and COC-671 salt susceptible variety respectively.

Aini *et al.* 2021 [5] studied that application of consortium saline-tolerant bacteria isolates with different soil ameliorants on growth and yield of tomato grown saline lands, they observed that application of saline-tolerant bacteria significantly improved leaf area (96.49%).

Moncada *et al.* (2020) [98, 101] reported that total leaf area of leaf lettuce plants grown during autumn and spring in nutrient solution containing different levels of NaCl, with (+B) or without (-B) bacterial biostimulant, the result showed that the highest leaf area per plant was measured in bacterial biostimulant compare to control.

Bacilio *et al.* (2016) [16] reported humic acids and inoculation with *Pseudomonas stutzeri* was used alone and combined to mitigate negative effects of soil salinity pepper. Leaf surface area, were measured in a salt-tolerant and a salt-susceptible pepper. In summary, under increased salt stress, application of the PGPB or humic acids improved leaf area.

From the above results and review its reveals that the result obtained in present are inconformity with the literature cited.

Shoot length

The effect of different level of salt stress on shoot length of

salt resistant & salt susceptible varieties is depicted in table No. 2. The results revealed that there was significant decrease in the shoot length of both varieties with increasing salt conc. as compare to control.

From the result obtained it was noticed that shoot length was decreased with increase in imposed salt stress levels in both the varieties COM-0265 and COC-671 in both endophyte untreated plants. The more decrease was observed in salt susceptible COC-671 variety (83.50 cm) than salt tolerant COM-0265 variety (94.3 cm). By application of the different endophytes along with consortia it was noticed that the shoot length was increased over the control (without application of endophytes) in each of salt stress treatment in both the varieties. However, more increase was observed in control plants (112.17 cm) (without salt stress) than the salt stressed plants. Among the various salt stress levels the plants grown under sodic soil has more shoot length (108.53 cm) than imposed salt stress levels in both the varieties with endophytic

bacteria. Further, least decrease in shoot length with imposition salt stress was observed in CEB treatment 115 cm 110.48 cm followed by GA treatment 111.14 cm and 109.04 cm COM-0265, salt tolerant variety and COC-671 salt susceptible variety respectively.

Bacilio *et al.* (2016) [16] reported humic acids and inoculation with *Pseudomonas stutzeri* was used alone and combined to mitigate negative effects of soil salinity pepper. Plant height, were measured in a salt-tolerant and a salt-susceptible pepper. In summary, under increased salt stress, application of the PGPB or humic acids improved leaf area.

Aini *et al.* 2021 [5] studied that application of consortium saline-tolerant bacteria isolates with different soil ameliorants on growth and yield of tomato grown saline lands, they observed that application of saline-tolerant bacteria significantly improved plant height (23.36%).

From the above results and review its reveals that the result obtained in present are inconformity with the literature cited.

Table 1: Effect of endophytic bacteria on leaf area (cm²) at tillering stage of sugarcane varieties grown under induced salt stress conditions

Parameter	Leaf area (cm ²)											
	COM 265						CoC 671					
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	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)
EBT	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 parenthesis is % decrease over control.

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

Table 2: Effect of endophytic bacteria on shoot length (cm) at tillering stage of sugarcane varieties grown under induced salt stress conditions

Parameter	Shoot length (cm)											
	COM 265						CoC 671					
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	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)
EBT	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 parenthesis is % decrease over control.

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

Root length

The effect of different level of salt stress on root length of salt resistant & salt susceptible varieties is depicted in table No. 3. The results revealed that there was significant decrease in the root length of both varieties with increasing salt conc. as compare to control.

From the result obtained it was noticed that root length was decreased with increase in imposed salt stress levels in both the varieties COM-0265 and COC-671 in both endophyte untreated plants. The more decrease was observed in salt susceptible COC-671 variety (47 cm) than salt tolerant COM-0265 variety (50 cm). By application of the different endophytes along with consortia it was noticed that the root length was increased over the control (without application of endophytes) in each of salt stress treatment in both the varieties. However, more increase was observed in control plants (56 cm) (without salt stress) than the salt stressed plants. Among the various salt stress levels the plants grown under sodic soil has more root length (52.4 cm) than imposed salt stress levels in both the varieties with endophytic bacteria. Further, least decrease in root length with imposition salt stress was observed in CEB treatment 54 cm 53 cm followed by GA treatment 52 cm and 51 cm COM-0265, salt tolerant variety and COC-671 salt susceptible variety respectively.

Bacilio *et al.* (2016) [16] reported humic acids and inoculation with *Pseudomonas stutzeri* was used alone and combined to mitigate negative effects of soil salinity pepper. Root length, were measured in a salt-tolerant and a salt-susceptible pepper. In summary, under increased salt stress, application of the PGPB or humic acids improved root length.

The reports showed in inoculated plants with bacteria, root length and nutrient absorption such as phosphorus, potassium, and nitrogen (Babalola 2010 growth rate, and dry mass production were higher than non-inoculated plant under stress and control conditions (Gupta *et al.* 2015, Liu *et al.* 2019) [35, 51].

According to Ansari *et al.* (2019) [11], earlier research on root growth under salt demonstrated that increased ethylene and reactive oxygen species synthesis could result in shorter and lighter roots (Habib *et al.* 2016) [37]. Additionally, nutrition availability via photosynthetic products, nodule metabolism, atmospheric nitrogen diffusion, and root hair deformation could all result in a reduction in the number of nodules under salt (Gopalakrishnan *et al.* 2015; Egamberdieva *et al.* 2019) [33, 27]. According to their research, bacterial inoculation reduced the negative effects of salinity on root length, weight and nodule number up to 10 dSm⁻¹, but it had no discernible effect at 20 dSm⁻¹ the aforementioned results and review its

reveals that the result obtained in present are inconformity with the literature cited.

Total chlorophyll

The effect of different level of salt stress on total chlorophyll of salt resistant & salt susceptible varieties is depicted in table No. 4. The results revealed that there was significant decrease in the root length of both varieties with increasing salt conc. as compare to control.

From the result obtained it was noticed that total chlorophyll content was decreased with increase in imposed salt stress levels in both the varieties COM-0265 and COC-671 in both endophyte untreated plants. The more decrease was observed in salt susceptible COC-671 variety () than salt tolerant COM-0265 variety (83.6 mg/g fr.wt.). By application of the different endophytes along with consortia it was noticed that the total chlorophyll content was increased over the control (without application of endophytes) in each of salt stress treatment in both the varieties, However, more increase was observed in control plants (112.08 mg/g fr.wt.) (without salt stress) than the salt stressed plants. Among the various salt stress levels the plants grown under sodic soil has more total chlorophyll content (108.12 mg/g fr.wt.) than imposed salt stress levels in both the varieties with endophytic bacteria. Further, least decrease in total chlorophyll content with imposition salt stress was observed in CEB treatment 115 mg/g fr.wt. and 110.4 mg/g fr.wt. followed by GA treatment 111.3 mg/g fr.wt. and 108.86 mg/g fr.wt. COM-0265, salt tolerant variety and COC-671 salt susceptible variety respectively.

Bacilio *et al.* (2016) [16] reported humic acids and inoculation with *Pseudomonas stutzeri* was used alone and combined to mitigate negative effects of soil salinity pepper. Plant height, were measured in a salt-tolerant and a salt-susceptible pepper. In summary, under increased salt stress, application of the PGPB or humic acids improved total chlorophyll.

According to Afridi *et al.* 2019 [4], the application of salt stress resulted in a considerable drop in the amount of chlorophyll in the leaves. But when *K. rhizophila* and *C. sakazakii* were applied, Pasban 90's and Khirman's leaf chlorophyll contents were increased by 10-17% and 11%, respectively, in comparison to controls.

Aini *et al.* (2021) [5] studied that application of consortium saline-tolerant bacteria isolates with different soil ameliorants on growth and yield of tomato grown saline lands, they observed that application of saline-tolerant bacteria significantly improved total chlorophyll content (11.86%). Sofy *et al.* 2021 [99, 100] reported that 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*, *P. fluorescens*) leads to

an increase in the content of total pigment content in both saline-stressed and non-saline-stress plants. The most pronounced effect was recorded in plants applied with *B.*

subtilis under normal and salt-stress conditions.

From the above results and review its reveals that the result obtained in present are inconformity with the literature cited.

Table 3: Effect of endophytic bacteria on root length (cm) at tillering stage of sugarcane varieties grown under induced salt stress conditions

Parameter	Root length (cm)											
	COM 265						CoC 671					
Varieties	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
EB												
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)
EBT	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)	54 (-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 parenthesis is % decrease over control.

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

Table 4: Effect of endophytic bacteria on total chlorophyll (mg/g fr. wt.) at tillering stage of sugarcane varieties grown under induced salt stress conditions

Parameter	Total chlorophyll (mg/g fr. wt.)											
	COM 265						CoC 671					
Varieties	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean	Control	Sodic soil	2dSm ⁻¹	4dSm ⁻¹	8dSm ⁻¹	Mean
EB												
Control	100 (0)	96 (-3.47)	93.5 (-8.53)	91.5 (-13.60)	89.5 (-21.87)	94.1 (-10.13)	90 (0)	85 (-9.49)	83.5 (-11.19)	80.5 (-12.41)	79 (-22.14)	83.6 (-13.81)
EBT	120.5 (0)	117 (-6.08)	114.5 (-11.65)	113 (-14.18)	110 (-16.71)	115 (-12.15)	115 (0)	112 (-4.90)	110.5 (-10.23)	108.5 (-13.01)	106 (-15.14)	110.4 (-10.82)
GA	117.5 (0)	113.5 (-7.46)	111.5 (-12.85)	109 (-15.42)	105 (-17.48)	111.3 (-13.30)	114.5 (0)	111 (-6.40)	109 (-11.48)	106.3 (-13.69)	103.5 (-16.34)	108.86 (-11.98)
ASP	114.5 (0)	110.5 (-8.36)	108.5 (-13.05)	104.5 (-16.19)	102 (-18.02)	108 (-13.90)	113.5 (0)	110 (-7.21)	107.7 (-12.33)	104.5 (-14.19)	102 (-17.44)	107.54 (-12.79)
HERB	110.5 (0)	106.5 (-8.99)	104 (-13.48)	100.4 (-17.42)	98 (-19.38)	103.88 (-14.82)	109.5 (0)	106 (-8.94)	103.5 (-12.47)	100.2 (-14.82)	98 (-18.59)	103.44 (-13.71)
AAR	109.5 (0)	105.2 (-9.17)	102 (-14.07)	98.5 (-18.04)	97 (-20.18)	102.44 (-15.37)	99.5 (0)	96 (-9.48)	93 (-12.80)	91 (-14.93)	89 (-19.67)	93.7 (-14.22)
Mean	112.08 (0)	108.12 (-7.80)	105.67 (-12.27)	102.82 (-15.81)	100.25 (-18.94)		107 (0)	103.33 (-7.74)	101.20 (-11.75)	98.50 (-13.84)	96.25 (-18.22)	
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 parenthesis is % decrease over control.

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

Conclusion

Environmental change has a significant impact on plants, which results in poorer yields and growth. The relationship between plant stress tolerance and the accompanying microorganisms is well documented. An environmentally friendly strategy to improve growth and crop yield in plants is endophytic bacteria's ability to withstand stress. A better alternative to the chemical fertilisers and insecticides now utilised in agriculture is beneficial bacterial endophytes. Understanding the individual mechanisms inside a given plant-microbe interaction is challenging due to the enormous diversity of plant-associated microorganisms. The advantage of endophytes over rhizospheric bacteria is that they are shielded from biotic and abiotic environmental stresses.

Endophytes have thus become a potential tool for improving plant growth and output in challenging settings. The diversity of endophytic populations at various stages of the plant and their roles in fostering plant growth and stress tolerance must be the main focus of our research. The mechanism of interaction with plants has to be thoroughly studied. Endophytic bacteria are present in halophytic plants and, unlike rhizospheric bacteria, can endure high salt concentrations and interact with their host plants rather successfully. Future saline agriculture has a lot of potential thanks to these techniques. All of the aforementioned study fields seek to improve plant performance overall through a better understanding and application of the endophyte-plant symbiosis. Given the foregoing, it is very clear. Considering the above, it is very clear that we are heading in a right direction to achieve our goal of sustainable food production under constantly changing climate.

Endophytes have thus become a potential tool for improving plant growth and output in challenging settings. The diversity of endophytic populations at various stages of the plant and their roles in fostering plant growth and stress tolerance must be the main focus of our research. The mechanism of interaction with plants has to be thoroughly studied. Endophytic bacteria are present in halophytic plants and, unlike rhizospheric bacteria, can endure high salt concentrations and interact with their host plants rather successfully. Future saline agriculture has a lot of potential thanks to these techniques. All of the aforementioned study fields seek to improve plant performance overall through a better understanding and application of the endophyte-plant symbiosis. Considering the exploitation of bacterial endophytes in enhancing crop production under salt-affected agricultural fields have convincing evidence for commercialization of microbe-based formulations for salinity tolerance.

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