Biofilm-Producing Bacteria Enhances Sodium Chloride Stress Tolerance in Maize Seedlings

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Abstract- Salinity is an ecological problem, and become a global challenge that affects crop productivity. The current research was designed to evaluate the mediated effects of biofilm-producing bacteria (*Bacillus sonorensis* NCCP-59) on physiological, biochemical, and antioxidant characteristics of maize cultivar (Azam 2000) under saline conditions (0, 50, 100, 150 mM)**.** Salinity significantly reduced the germination percentage with increasing stress while a significant increase was noted in seeds primed with biofilm-producing bacteria. Sodium chloride (NaCl) stress reduced seedling length, fresh/dry weight, ion (Ca^{++}, K^{+}) contents, and photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids). While a significant increase was recorded in sodium ion (Na^{+}) content, proline (Pro) and total soluble sugar (TSS), antioxidants (superoxide dismutase (SOD), peroxidase (POD), hydrogen peroxide (H_2O_2), malondialdehyde (MDA), ascorbate peroxidase (APX) and catalase (CAT) contents. However, the priming of maize seeds with biofilm-producing bacteria (*B. sonorensis)* significantly improved the physiological and biochemical, and antioxidative parameters of the plant by reducing the uptake of Na^{+}. The study concluded that biofilm-producing bacteria (*Bacillus sonorensis* NCCP-59) can enhance (NaCl) stress tolerance in maize by reducing the uptake of Na^{+}.

Index Terms- Biofilm producing Bacteria, Salinity, Maize, Priming, Metabolites

I. INTRODUCTION

It is estimated that the world population will exceed 9.4 billion in 2050 [1]. The increasing population has raised the issue of food safety and security mainly in developing countries. Food safety is the main challenge to these countries, as they trying to improve their economy. There is also a need to increase the production of crops to fulfill the food requirements for the growing population [2]. These crops are facing different abiotic stresses like salinity, drought, and waterlogging which adversely affect the quality and quantity of the crop yield [3]. Salinity is a type of abiotic stress which almost covers 6% of the world's area. Additionally, 19.5% of the irrigated agriculture fields are regarded as salinity-prone. Furthermore, two million acres of the world's agriculture fields deteriorate each year due to salinity, resulting in little or no crop production [4]. The main causes of the rising salinity include irrigation with saline and poor cultural practices and many other factors are involved in salinity which affects the plants in different ways like membrane injury, protein denaturation, and osmotic stress [5]. Maize (*Zea mays* L.) is the third vital cereal crop after rice and wheat and moderately sensitive to salt stress. Salinity affects 37.6% (6.06 million hectares), out of 18 million hectares area of Pakistan [6]. Under saline conditions, sodium plays an important role in toxicity which affects potassium uptake and stromal variations leading to loss of water and cell death [7]. Besides different other techniques used for stress tolerance, microorganisms associated with plants also help to alleviate abiotic stresses including high temperatures drought, and salinity [8]. Bacteria, like PGPR, enhance salinity tolerance through compound production, antioxidant enzymes, and improved nutrient uptake, enabling healthy growth under saline conditions. Studies show that PGPR induces osmoprotectant synthesis, regulates osmotic balance, scavenges reactive oxygen species, and enhances ion balance. For instance, Upadhyay et al. [9] improved wheat salt tolerance with PGPR, while Egamberdieva et al. [10] enhanced soybean salt tolerance. In summary, bacteria, especially PGPR, increase the salinity threshold for healthy plant growth by mitigating stress effects and improving physiological mechanisms.

In agriculture, colonizing happens in plant roots due to PGPR which multiplies further into microcolonies (due to competition with other microbiota or unfavorable conditions), as a result, biofilm is formed which is helpful in plant-microbe interactions [8]. Bacteria that produce biofilms have a protective mechanism against hostile environments [11]. This unique developmental characteristic of biofilms is different from non-biofilm-forming cells. Molecular and genetic studies have identified that biofilms are significantly different from microbes that have a planktonic mode (non-biofilm foaming cells) of growth in physiological functions and gene expression [12]. Moreover, it is suggested that the antimicrobial tolerance ability of bacteria is the result of biofilm structure and the adherent nature of microbial cells [13].

The root system of the plants, which is associated with biofilm, is beneficial for plant growth, yield, and grain quality [14]. When bacterial inoculants are introduced in the form of biofilms, these inoculants protect the environment from adverse conditions such as heavy metals, low pH, high salinity, and drought stress [15]. Bacteria other than *Bacillus sonorensis* can contribute to improved growth of maize in saline conditions. Various species of plant growth-promoting rhizobacteria (PGPR) have been studied for their potential to enhance maize growth and salinity tolerance. For example, Ma et al. [16] showed that *Pseudomonas putida* improved maize growth under saline conditions, while studies by

El-Shabrawy et al. [17] and Egamberdieva et al. [10] also reported positive effects of *Bacillus subtilis* and *Azospirillum brasilense*, respectively [16,17,18]. Biofilms produced by the *Paenibacillus polymyxa* around the root tip act as a root-invading bacterium. Other studies also showed that root colonizing the rhizosphere with the biofilm inocula produced NH^{4+} in the soil solution due to high acidity for plants near root hairs, which helps in plant physiology and growth [19]. These findings demonstrate the potential of different bacterial species in promoting maize growth and salinity tolerance. The literature revealed that halophilic bacteria develop biofilms containing extracellular polymeric substances which help in plant development [20], but the role of certain halophilic bacteria is not defined on plant growth under stress conditions, especially in *Bacillus sonorensis.* Therefore, the current research was planned to observe the role of biofilm-producing bacteria (*Bacillus sonorensis)* in NaCl stress alleviation by studying the physiological, biochemical, and antioxidant characteristics of maize.

II. Methods and Materials

Seed collection

Maize seeds (Cultivar Azam) were collected from the Agriculture Research Station, Serai Naurang (Lakki Marwat) Khyber Pakhtunkhwa, Pakistan. The Azam was developed by backcrossing the F1 of Pirsabak 7930 and Zia to Pirsabak 7930 as the recurrent parent.

Bacterial strains

Two halophilic bacterial strains NCCP -59 (National Culture Collection of Pakistan) (*Bacillus sonorensis*-Biofilm producer) and NCCP-93 (*Bacillus endophyticus*- Non-Biofilm producer) were isolated from the salt mines of Karak, Khyber Pakhtunkhwa Pakistan Roohi et al. [21]. Both *Bacillus endophyticus* and *Bacillus sonorensis* bacterial strains were cultured under sterilized conditions on Luria Bertani media (LB media) modified with 5% (w/v) sodium chloride (NaCl). Culture media was autoclaved at 15 PSI (pounds per square inch) and 121°C for 15-20 minutes. To observe growth, plates were placed on a shaking incubator at 37°C (120 rpm) for 24 hours [21]. To obtain 10^8 CFU/ml in broth before inoculation, liquid culture dilutions were made in autoclaved distilled water for the calculation of the colony formulating unit. An inoculum of 1000 µl (OD600) from each dilution was spread on LB agar plates and incubated for 48 h at 37°C. CFU/g was calculated from the appeared colonies by using the following formula [22].

Colony formulating unit (CFU/g) = number of colonies × dilution factor/ volume of culture plate

Seed priming

Healthy maize seeds were surface sterilized with 3.5% sodium hypochlorite solution for 3-4 min and washed three times with distilled water. Priming with bacterial culture broths (OD600) along with 2% sucrose was carried out in a shaking incubator for 2 hours. The primed seeds were then removed from bacterial culture media and dried on filter paper under sterilized conditions. Hydro-primed seeds and *Bacillus endophyticus* (NCCP-93- non-Biofilm producer) were used as a control.

Physiological study

Primed seeds of maize were sown in Petri plates for 10 days having sand and treated with 0-, 50-, 100-, and 150-mM sodium chloride (NaCl) stress concentrations. Seeds germinated under dark conditions. Germination readings were noted at intervals of 12 hours for 5 days. Shoot and root length/fresh weights were recorded after 10 days. Root and shoot were kept at 80°C for 48 hours to record dry weight.

Greenhouse experiment

Maize primed seeds were transferred in plastic pots having 1 kg well washed, dried sterilized sand. Seeds were sown at a depth of 1 inch having a relative distance of 2 cm apart, nourished with 500 ml of Hoagland solution weekly. The NaCl stress (50, 100, and 150 mM) was given to the maize plants for a period of 1 week in Hoagland solution [23]. Plants were exposed to 1 week of stress and then harvested. Fresh leaves were selected from each treatment, ground in liquid nitrogen, and kept further at -80 °C. The rest of the material was dried in an incubator at 80 °C for 2 days.

Biochemical Analysis

Determination of photosynthetic pigments and ion analysis

Plant's pigments (Chlorophyll "*a*", "*b*" and total carotenoids) were calculated according to the method of Wellburn and Lichtenthaler [24]. Three different wavelengths i.e., 666, 653, and 470 nm were used to find out absorbance readings against a solvent blank in a spectrophotometer (UV-VIS spectrometer) C-7200S double beam UV-visible spectrophotometer. Ions analysis was determined by the method of Joslyn [25] with little modifications and was measured by a flame photometer (Jenway PFP7).

Metabolic analysis

Total soluble sugar and Proline Content

The phenol-sulphuric acid method was used to find total soluble sugar contents as described by Harborne [26] with slight modifications. Proline content was determined by following the methodology described by Bates et al. [27].

Antioxidants enzyme assay

Peroxidase (POD) activity was determined by the method mentioned by Kumar and Khan [28] Using Guaiacol as the substrate in a total volume of three milliliters. Beauchamp and Fridovich's [29] method was used to determine superoxide dismutase (SOD) content. Catalase (CAT) activity was determined using the protocol of Vasconcelos et al. [30]. Ascorbic peroxidase (APX) was determined by a spectrophotometer as described by Nakano and Asada [31]. Velikova and Yordanov [32] protocol was used to measure H_2O_2 activity. MDA contents were determined by using the standard protocols of Daud et al. [33].

Statistical Analysis

Data are presented as a mean of three replicates. Statistical analysis was performed using Statistix-9 software ver. 8 for statistical significance. Differences between treatments were assessed using a one-way analysis of variance (ANOVA). Means were separated by the Least Significance Difference (LSD) test at a 5% level of significance.

III. Results

Screening Test

Two halophilic strains *Bacillus sonorensis* (NCCP-59 Biofilm producer as indicated by black colonies) and *Bacillus endophyticus* (NCCP-93- non-Biofilm producer as indicated by orange colonies), were selected after screening among different halophilic strains on CRA (Congo Red Agar) plates. (Fig. 1). These two strains (NCCP-59 and NCCP-93) were used in further experiments.

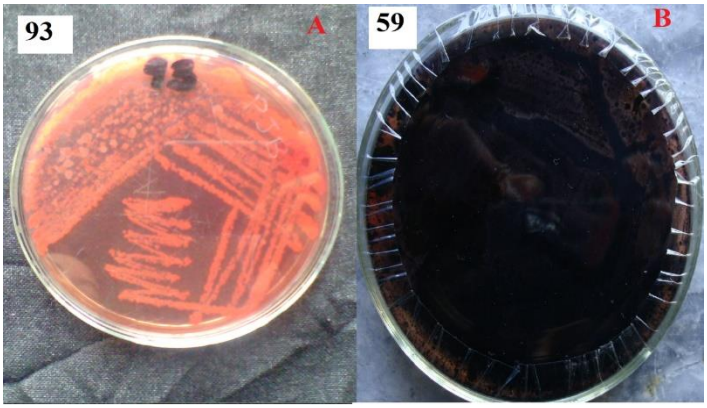

Fig. 1. Two halophilic strains (A) Non-Biofilm producer strain *Bacillus endophyticus* (NCCP-93) and (B) Biofilm producer strain *Bacillus sonorensis* (NCCP-59), were selected after screening among different halophilic strains.

Effect on physiological parameters

Germination percentage

Significant variations were marked in the germination pattern of biofilm-producing bacteria as compared to non-biofilm and hydro-primed seeds of maize cultivar at different saline treatments throughout germination (Fig. 2). Germination percentage was reduced with an increase of salinity concentrations from 0 to 150 mM, however, biofilm priming enhanced seed germination than hydro priming and non-biofilm producing bacteria under normal and stress conditions. Control (0 mM) showed a higher germination percentage (80 %) as compared to 150 mM NaCl stress (30 %). The maximum germination percentage recorded for biofilm-producing bacteria was 99.9 % as compared to non-biofilm-producing bacteria (60 %) and control (80 %) at 0 mM. A similar pattern was observed under saline conditions, particularly at 150 mM NaCl stress, where seeds primed with biofilm-producing bacteria showed 40 % germination while non-biofilm-producing bacteria showed 25 %.

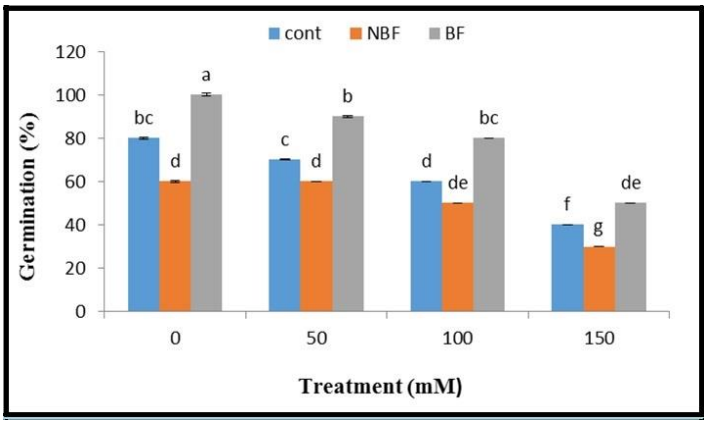

Fig. 2. The effect of non-biofilm and biofilm-producing bacteria on germination percentage of maize seeds under NaCl stress under Mock and Cd^{2+} treatments 50 mM to 150 mM. Results are represented as a mean ± SE (n=3). Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

Seedling growth

Both root and shoot length were improved through seed primed with biofilm-producing bacteria of maize cultivar as compared to non-biofilm-producing bacteria under NaCl stress conditions (Fig 3). High concentration of salt stress reduces the shoot and root length of maize seeds (Fig. 4). Length of root and shoot were recorded as 13.78 and 16.36 cm at control (distilled water) while it was gradually reduced (4, 3.01 cm) under saline condition such as 150mM NaCl stress condition. Primed seeds with biofilm-producing bacteria showed better growth in root and shoot length than hydro-primed and non-biofilm-producing bacteria under normal and stress conditions. At control, observed values of root and shoot length were 17.09, and 24.51 cm in biofilm and in non-biofilm primed seeds were 6 and 5cm while at 150mM NaCl stress, the observed values were decreased up to 10.54, 5 cm as compared to non-biofilm producing bacteria that were 1, 2.5 cm respectively (Fig. 7).

Salinity adversely affected fresh and dry weights of shoot and roots of maize but the priming treatments were able to alleviate the stress (Fig 5). At 150 mM NaCl stress, the fresh shoot weight was 1.055 mg and the root weight was 0.213 mg dramatically decreased to 3.11, 0.645 at control conditions while, primed seeds with biofilm-producing bacteria showed an increase in the fresh shoot 1.405 mg and root weight 0.335 mg as compared to non-biofilm producing bacteria 1, 0.25 mg at 150 mM respectively.

The observed dry weight of root hydro-primed seeds was 0.124mg which was reduced to 0.044mg at 150 mM NaCl stress. The shoot

dry weight of hydro-primed seeds was 0.138 g which was reduced to 0.038 g at 150mM NaCl stress treatment with Biofilm-producing bacteria alleviated the adverse effect of NaCl stress as compared to biofilm-producing bacteria. The root dry weight of the seed primed with biofilm-producing bacteria was 0.073 mg at 150mM concentration while 0.053 mg was observed in biofilm-producing bacteria, shoot dry weight in biofilm-primed seed was 0.075 mg while in the non-biofilm-primed seed it was 0.055 mg at 150 mM NaCl stress condition.

Fig. 3. The effect of non-biofilm and biofilm-producing bacteria on maize seedlings under different salt (NaCl) stress conditions. **A** = Control, **B** =50 mM, **C** = 100 mM, **D** = 150 mM, **E** = Control + NCCP-93, **F** = 50 mM + NCCP-93, **G** = 100 mM + NCCP-93, **H**= 150 mM + NCCP-93, **I** = Control + NCCP-59, **J** = 50 mM + NCCP-59, **K** = 100 mM + NCCP-59, **L** = 150 mM + NCCP-59.

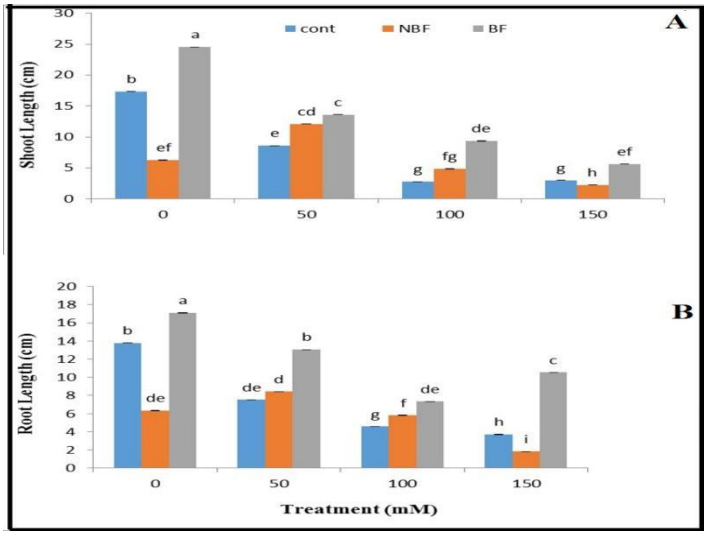

Fig. 4. The effect of non-biofilm and biofilm-producing bacteria on 'the shoot length (A) and 'Root length (B) of maize under 50mM to 150mM. Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

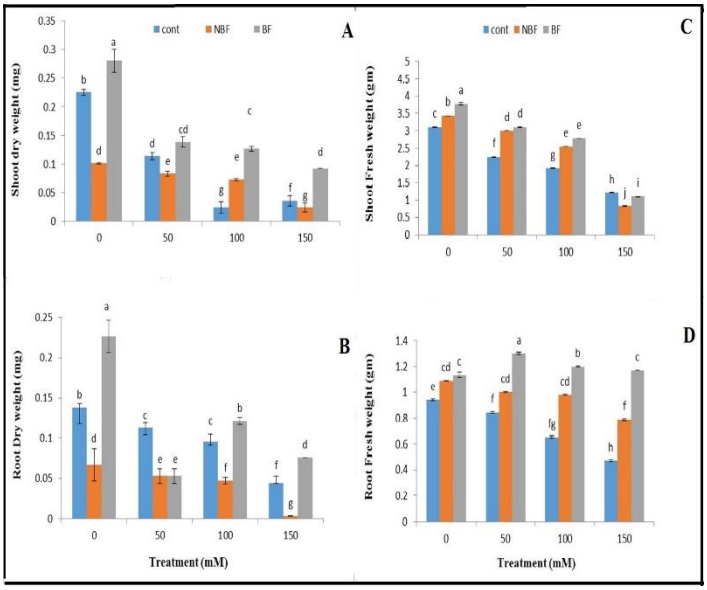

Fig. 5. The effect of non-biofilm and biofilm-producing bacteria on dry shoot weight (A), dry root weight (B), fresh shoot weight (C), and Fresh root weight '(D) of maize under different salt (NaCl) stress conditions. Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

Biochemical analysis

Electrolyte contents (Na^+, K^+ and Ca^{+2})

Significant variations were noted in electrolyte (Na^+, $Ca^{2+,}$ and K^+) content of biofilm and non-biofilm primed seed of maize cultivar at various NaCl concentrations (Fig. 6). Increase in Na^+ content while a decrease in Ca^{2+} and K^+ was observed with an increasing NaCl concentration under saline conditions. At 150 mM NaCl stress, the Ca^{2+} and K^+ content was reduced to 5, 22 and Na^+ was increased to 95 ppm respectively. Maize inoculated with biofilm-producing bacteria has a large amount of K^+, $Ca^{2+,}$ and low Na^+ ions contents under saline conditions in comparison to non-biofilm-producing bacteria. The observed values of Ca^{2+}, $K^{+,}$ and Na^+ in biofilm-producing bacteria were 7, 38, and 77.5 ppm at 150 mM NaCl stress, while in non-biofilm-producing bacteria, these values were 6, 33.5, and 81 ppm respectively.

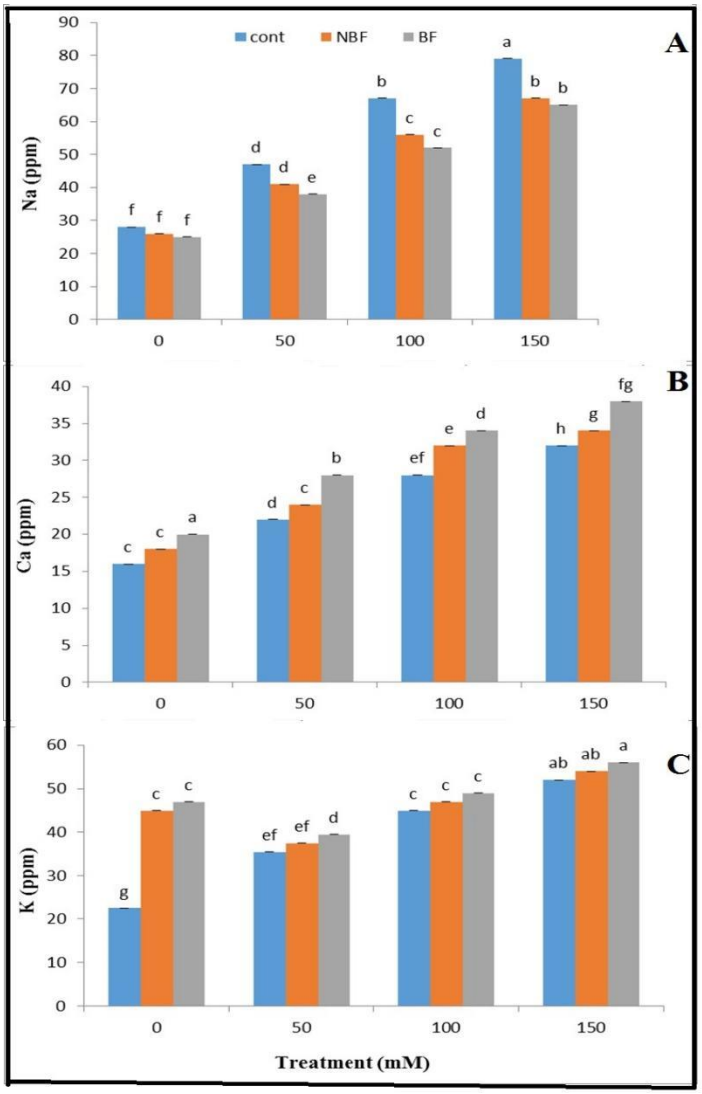

Fig. 6. The effect of non-biofilm and biofilm-producing bacteria on Na, K, and Ca ions of maize under different salt (NaCl) stress conditions. Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

Effect on photosynthetic pigments

Salinity negatively affected chlorophyll ("*a*", "*b*") and carotenoid contents in maize shoots but priming treatments showed a positive effect on these parameters in maize cultivars (Fig. 7). NaCl treatment at 150 mM reduced the content of photosynthetic pigments significantly as compared to control condition. Results showed that at 150 mM chlorophyll ("*a*", "*b*") and carotenoid content reduced to 5.02, 11.12, and 151.25 mg/g respectively as compared to the control treatment i.e., 9.285, 30.49, and 330.12 mg/g. Seeds primed with biofilm-producing bacteria showed a higher number of pigments than non-biofilm-producing bacteria. The recorded values in seed primed with biofilm-producing bacteria were 8.69, 22.86, and 161.44 mg/g at 150 mM NaCl stress as compared to biofilm-producing bacteria that were 8.36, 14.11, and 1212.28 mg/g.

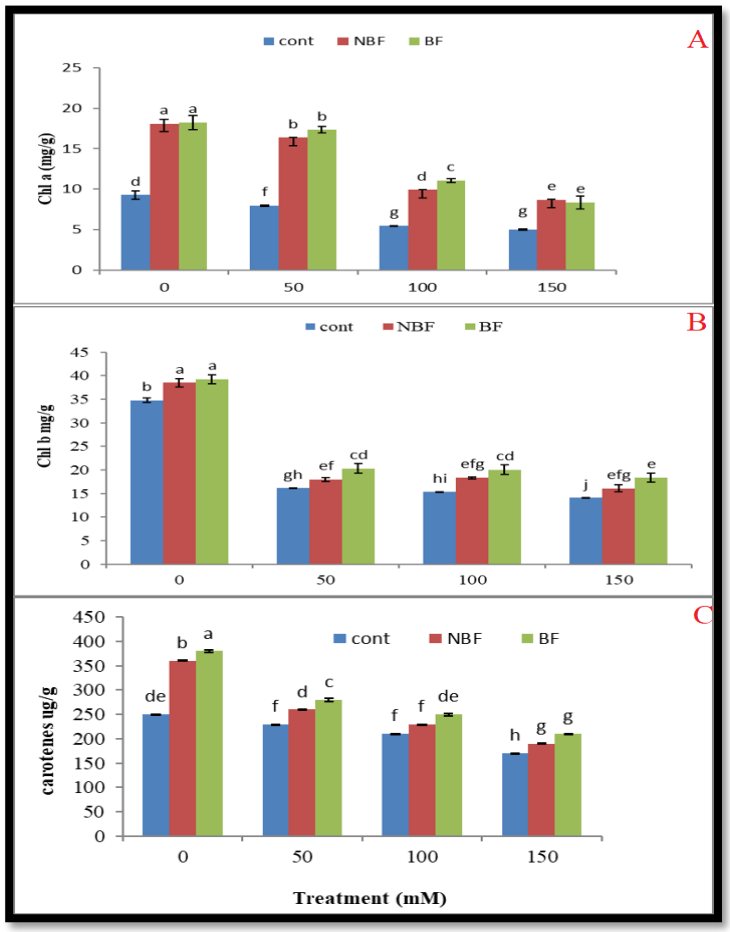

Fig. 7. The effect of non-biofilm and biofilm-producing bacteria on chlorophyll content of maize seeds under different salt (NaCl) stress conditions. Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

Metabolite content

Determination of Total soluble sugar

The content of total soluble sugar was increased by increasing NaCl content (Fig. 8A). The value recorded at high concentrations of NaCl stress-treated plants was 55.87 mg/g FW (Fresh Weight) at 150mM as compared to the control (30.31 mg/g FW) respectively. A significant reduction in total soluble sugar content was observed for all the priming treatments in the case of biofilm-producing bacteria as compared to non-biofilm-producing bacteria under normal and stress conditions. Seed primed with biofilm-producing bacteria showed a maximum value of 15.1 mg/g FW as compared to non-biofilm-producing bacteria 20.6 mg/g FW at control, whereas at 150mM NaCl stress condition, the sugar content was reduced to 32.68 mg/g FW in biofilm-producing bacteria as compared to non-biofilm producing bacteria 43.56 mg/g FW.

Proline content

Considerable changes were observed in proline content when seeds primed with biofilm-producing bacteria as compared to non-biofilm primed seeds of maize cultivar at various saline conditions

(Fig. 8B). Under saline conditions, accumulation of proline increased with increasing concentration of NaCl in treated plants. Proline amount was 33.03 µmol/g FW at control condition which increased up to 61.88 µmol/g FW at 150mM NaCl concentration in maize. Biofilm-primed seeds reduced the amount of proline with increased NaCl concentration from 0 to 150 mM (22.91, 32.70 µmol/g FW). A similar trend was also noted in seeds primed with non-biofilm-primed seeds under saline conditions. Biofilm-primed seeds are more effective than non-biofilm-primed seeds under NaCl.

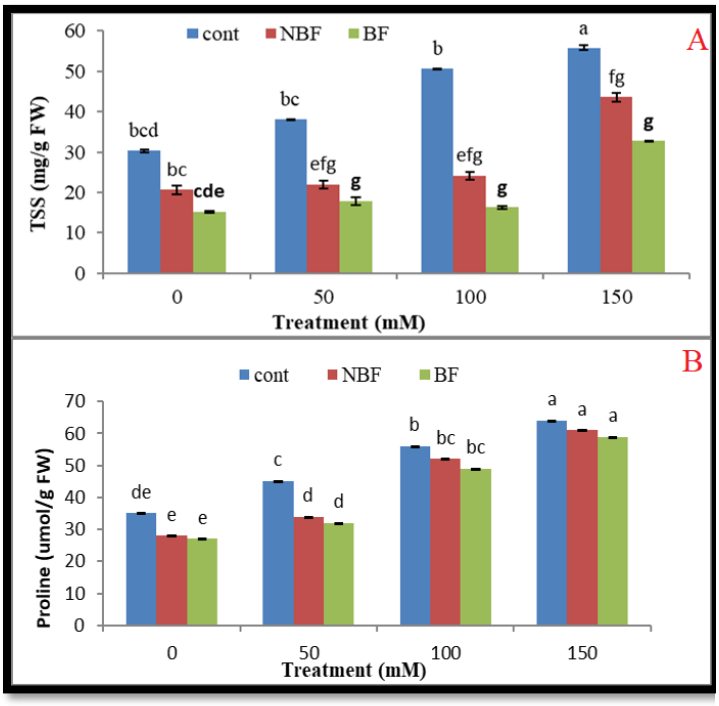

Fig. 8. The effect of non-biofilm and biofilm-producing bacteria on the Total soluble sugar (A) and proline content (B) of maize under different salt (NaCl) stress conditions. Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

Antioxidants

Peroxidase (POD)

At high NaCl stress, a significant increase was observed in POD activity. Still, the priming treatment showed a positive effect on this enzyme activity in maize cultivar (Fig. 9A). The POD content in mock seedling was 3.522 (U min $^{-1}$ g $^{-1}$ FW) which was reduced to 4.466 (U min $^{-1}$ g $^{-1}$ FW) at 150mM NaCl concentration. POD content increased in seeds primed with biofilm-producing bacteria as compared to non-biofilm-producing bacteria. At control, biofilm-producing and non-producing bacteria showed better scavenging content of 1.02 (U min $^{-1}$ g $^{-1}$ FW) and 1.8 9 (U min $^{-1}$ g $^{-1}$ FW) as compared to 150mM NaCl concentration. The recorded values in seed primed with biofilm-producing bacteria was 2.61(U min $^{-1}$ g $^{-1}$ FW) at 150mM as compared to biofilm-producing bacteria 3.56 (U min $^{-1}$ g $^{-1}$ FW).

Superoxide dismutase (SOD)

Variations were observed in the SOD content of biofilm and non-biofilm primed seeds at different saline conditions in maize seeds (Fig. 9C). The SOD content was increased with increasing concentration of NaCl stress from 0 to 150 mM. In control, SOD content was 269.83 µgFW while non-biofilm and biofilm-producing bacteria were 277.10 and 242.75 µgFW respectively, at 150mM concentration the SOD content was 263.22 µgFW which was reduced in biofilm-producing bacteria up-to 109.40 µg/FW and non-biofilm producing bacteria to 171.41 µg/FW. Biofilm-producing bacteria showed a decrease in SOD content as compared to saline conditions and non-biofilm-producing bacteria.

Catalase (CAT)

When compared to non-biofilm primed seeds of maize cultivar at various conditions, the CAT content of seeds primed with biofilm-producing bacteria revealed substantial changes (Fig. 9B). The CAT content increased with increasing concentration of NaCl stress. At 150 NaCl concentration, it increased up to 0.6 (U min $^{-1}$ g $^{-1}$ FW) as compared to control 0.33 (U min $^{-1}$ g $^{-1}$ FW) respectively. A significant reduction in CAT content was observed for all the priming treatments in the case of biofilm-producing bacteria and non-biofilm-producing bacteria in both normal and stress conditions. Seed primed with biofilm-producing bacteria showed a significant value of 0.1 mmolg/FW at control condition as compared to non-biofilm-producing bacteria 0.2 (U min $^{-1}$ g $^{-1}$ FW), whereas a decrease was observed in CAT content of biofilm-producing bacteria and non-biofilm producing bacteria up-to 0.3 (U min $^{-1}$ g $^{-1}$ FW) and 0.5 (U min $^{-1}$ g $^{-1}$ FW) at 150mM NaCl stress.

Ascorbate peroxidase (APX)

A high mean value was observed in APX content at higher NaCl stress conditions. Variations in APX content were observed when primed seeds with biofilm-producing bacteria were compared to non-biofilm-producing bacteria of maize cultivar at various saline conditions (Fig. 9D). APX content was increased at 150 mM NaCl stress 1.5 mMg/FW as compared to control seedlings 0.4 mMg/FW respectively. Reduced values were observed when compared to primed seeds with biofilm-producing and non-biofilm-producing bacteria at 150 mM NaCl stress. Thus, the results conclude that seeds primed with biofilm-producing bacteria are more effective than non-biofilm-producing bacteria under different saline conditions.

Hydrogen peroxide (H_2O_2)

Major changes were observed in the H_2O_2 content of biofilm and non-biofilm primed seeds at several saline conditions of maize cultivar (Fig. 10A). The observed values increased from 0 to 150 mM NaCl stress; however, biofilm-primed seeds showed better response as compared to non-biofilm primed seeds under normal and stress conditions. H_2O_2 content was 1.09 µmolg^{-1} FW at control treatment as compared to 150 mM NaCl stress 1.117 µmolg^{-1} FW. The recorded value of H_2O_2 for biofilm-producing bacteria was 0.84 µmolg^{-1} FW as compared to non-biofilm-producing bacteria 1.035 µmolg^{-1} FW in hydro-primed seedlings.

At 150 mM NaCl stress treatment primed seeds with biofilm-producing bacteria 0.67 µmolg^{-1} FW showed better results as compared to non-biofilm-producing bacteria 0.79 µmolg^{-1} FW.

Malanodialdehyde (MDA)

In maize, modifications were perceived in the MDA content of biofilm and non-biofilm-primed seeds at various saline conditions. (Fig. 10B). The MDA content increased with increasing NaCl concentration. MDA content was increased up to 0.174 mM/g FW at 150 mM NaCl stress as compared to control 0.264 mM/g FW respectively. At control, the recorded value in seed primed with biofilm-producing bacteria was 0.103 mM/gFW and 0.170 mM/gFW in non-biofilm-producing bacteria. In comparison, a similar trend was also observed at 150 mM NaCl stress in both biofilm-producing and non-producing bacteria, MDA content recorded in seed primed with biofilm-producing bacteria was 0.119 mM/gFW and 0.145 mM/gFW in non-biofilm-producing bacteria respectively.

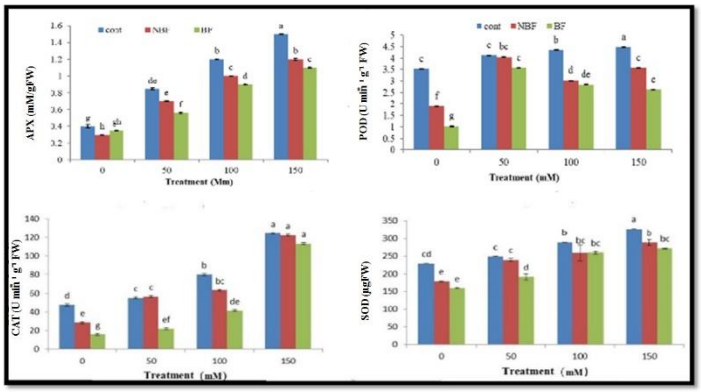

Fig. 9. The effect of non-biofilm and biofilm-producing bacteria on ascorbate peroxidase (A), catalase (B) peroxidase(C), and Super Oxides dismutase (D) contents of maize under different salt (NaCl) stress conditions. Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

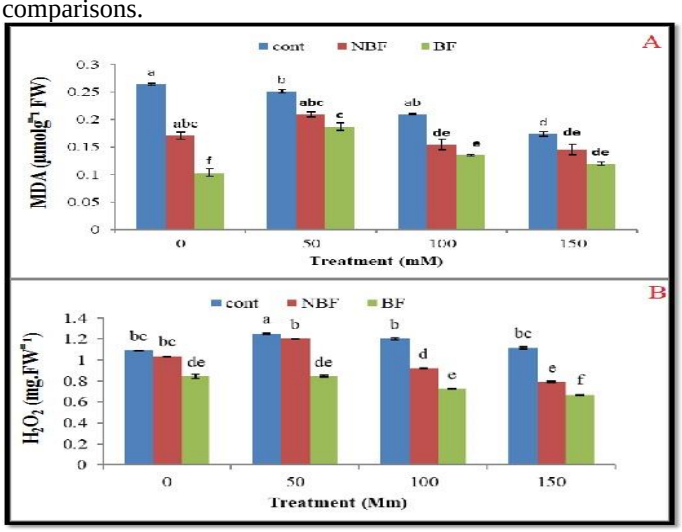

Fig. 10. The effect of non-biofilm and biofilm-producing bacteria on malondialdehyde (A) and hydrogen peroxide (B) contents of maize under different salt (NaCl) stress conditions. Letters indicate significant differences between means; determined using Duncan's multiple range mean comparisons.

IV. DISCUSSION

Sodium chloride stress is the most vital environmental factor which affects crop production around the world. Different techniques were used to reduce the drastic effects of salt stress on plant growth [34] but the use of biofilm-producing bacteria especially *Bacillus sonorensis*-(biofilm-producing bacteria) are limited. Two strains NCCP-59 (*Bacillus sonorensis*-biofilm producing bacteria) and NCCP-93 (*Bacillus endophyticus*- non-biofilm producing bacteria) were used in this study to investigate NaCl stress alleviation by studying physiological, biochemical, and antioxidants parameters. Previous studies suggested that the use of biofilm-producing bacteria has become an auspicious alternative to alleviate NaCl stress [35]. Liu et al. [18] *reported that Bacillus* spp. has been documented to alleviate salt tolerance in *Arabidopsis* (up to 250 mM NaCl), *maize* (up to 100 mM NaCl), *barley* (up to 200 mM NaCl), *rice* (up to 200 mM NaCl), wheat (up to 400 mM NaCl), tomato (up to 300 mM NaCl), chickpea (2 % NaCl), cotton (up to 200 mM NaCl) and soybean (up to 240 mM NaCl).

Seed germination and early seedlings' growth are usually sensitive to environmental stresses. According to our results maize germination percentage was decreased by sodium chloride but biofilm-primed seeds showed increased germination percentage by comparing to non-biofilm-primed seeds (Fig. 2). NaCl stress creates osmotic pressure which in turn decreased water absorption and this phenomenon has negative effects on seed germination [36]. Exopolysaccharides also play a vital role in the adhesive and stability properties of biofilm-producing bacteria on various abiotic surfaces [37]. The exopolysaccharide is an important component of the biofilm [38]. Bhagat et al. [39] also suggested that bacteria-produced exopolysaccharides in saline soil can reduce osmotic stress. Previous reports also showed that exopolysaccharide production and biofilm formation by bacterial strains significantly contribute to improving plant growth and soil fertility [39].]

Salinity mainly affects the physiological growth of the plants including root and shoot length and biofilm-producing bacteria (NCCP-59) to overcome the effect of salinity and improved root and shoot length (Fig. 4&5). Ramasamy et al. [40] also found that NaCl stress in wheat seedlings can be alleviated by the inoculation of halo-tolerant bacterial strains. Our results also strongly agreed with Khan et al. [41] who reported that plant growth-promoting bacteria showed a major increase in shoot length and dry weight as compared to the control. Indole acetic acid produced by bacteria has positive effects on root length, root surface area, and number of root tips leading to higher uptake of nutrients thus refining plant strength under stress [42]. Our result showed similarity to Cardarelli et al. [43], who demonstrated seeds inoculated with micro-organisms enhanced the root and shoot length and biomass of roots and shoots. Jamil et al. [44] reported that enzymes that are required for chlorophyll synthesis are suppressed under higher salinity. Our result showed that chlorophyll and carotenoid contents were decreased in maize as the level of sodium chloride increased (Fig. 7). The results were also similar to the observations of Hameed et al. [45] that salinity greatly influences the

chlorophyll content. Chlorophyll instability and synthesis of the pigment-protein complex are destroyed due to NaCl stress [45]. Jan et al. [46] found that plant growth-promoting rhizobacteria (PGPR) affect seed germination, plant growth, plant biomass, plant height and shoot weight, nutrient content, and photosynthetic pigments. Maize seeds treated with PGPR under saline conditions produced more photosynthetic pigments and decreased the ratio of Na^+ and K^+ ions in the saline environment [47].

Salinity affects the concentration of Ca^{+2}, $K^{+,}$ and $Na^{+,}$ ions in plants. With increasing NaCl stress, Na^+ ions were increased while Ca^{2+} and K+ ions were reduced (Fig. 6). Due to sodium chloride stress, Cl^- and Na^+ are dissociated and their increased availability inhibits the intracellular processes. Ketehouli et al. [48] reported that the accumulation of Na^+ and Cl^- occurs in plant tissue during NaCl stress. Farooq et al. also proved that when the salinity level increases from 0.25M NaCl concentration, it damages maize plants and may cause severe wilting and stunt growth. In maize plants, Sodium (Na^+) ions accumulated which directly enhanced the high NaCl concentration in the growth medium, while K^+ and Ca^{+2} ions were decreased. In salinity K^+ and Ca^{+2} ions, uptake is hindered in the root and cannot be transported to the shoot, causing deficiency in plant tissue [49]. In plants, the formation of calcium bonds provides energy in different cytoplasmic structures and it plays an important role in cell membrane linkage [50]. Potassium development and reproduction are affected due to salinity that increases the level of sodium-potassium ratio which disturbs different metabolic pathways [51]. In comparison with hydro priming, biofilm-producing bacteria reduced the opposing effect of salinity. The negative effects of sodium chloride stress are reduced due to biofilm-producing bacteria (Fig. 7). These biofilm-producing bacteria are closely related to exopolysaccharides, these exopolysaccharides consist of a group of polymers that help offer protection against alleviating Na^+ stress and desiccation [52].

In metabolism, soluble sugars play an important role in osmotic homeostasis and structural components of cells. Like hormones, soluble sugars also act as messenger and control signals that are involved in the expression of various genes [53]. The total soluble sugar content increased with the increase of NaCl stress in the root and shoot of the maize plant (Fig. 8A). Biofilm-producing bacteria decreased the NaCl stress efficiently as compared to non-biofilm-producing bacteria, current findings were similar to Gandonou et al. [54], they observed that NaCl stress increase total soluble sugars and proline level in the plant. In non-inoculated plants, an increase in the amount of total soluble sugars was observed as the NaCl concentration increased. Total soluble sugar increased at a greater level accompanied by the maintenance of the plant turgor, vital for growth below sodium chloride stress [55]. Against abiotic stresses, the natural defense system in the plant is present in the form of secondary metabolites. Proline is one of the most important components of this mechanism of plant-to-sodium chloride stress (Fig. 8B). The synthesis of compatible solutes is an important mechanism of the plant because due to this mechanism, plants can survive in saline areas. In the cytosol, proline can accumulate and contribute significantly to osmotic adjustment in the cytoplasm [56]. According to Maggio et al. [57], proline may proceed with signaling or act as a regulatory molecule and can stimulate various responses in the adaptation process. Compatible solutes help to keep the lower water potential in $CaCl_2$ and as a result, produce the driving force for the uptake of water by plant roots [62]. Results of our study showed that accumulation of proline was observed in maize plants under NaCl stress but the biofilm-producing bacteria lowered the NaCl stress in maize plants as compared to non-biofilm-producing bacteria. Other studies [63, 64] also observed that proline accumulation occurs in NaCl-stressed plants.

Hydroxyl radicals, hydrogen peroxide, and superoxide are reactive oxygen species (ROS) that are generated during NaCl stress, leading to oxidative stress that results in damaged plant growth. Several antioxidant enzymes are produced due to the stress conditions of NaCl [65]. In the case of our study, results showed that increasing the amount of NaCl increased antioxidant enzymes like CAT, sodium oxide dismutase, POD, APX, malondialdehyde, and hydrogen peroxide contents in comparison to other treated plants and control (Figs. 9 & 10). Similar to our results, Azooz al. [62] reported that during salinity stress, antioxidant enzyme activity like peroxidase, SOD, catalase, and APX increases in the salt-tolerant cultivars. Abbasi et al. [63] showed that salinity stress improved the concentration of CAT, POD, and SOD in maize under saline conditions. Treated seeds with biofilm-producing bacteria lowered the SOD, POD, CAT, H_2O_2, APX, and MDA levels under NaCl stress. Our results are in line with Jha and Subramanian [64], according to them when plants were inoculated with *Pseudomonas pseudoalcaligenes*, the enzyme activity was reduced in comparison with control under NaCl stress. Egamberdieva [65] also observed the same kind of results.

V. CONCLUSION

It is concluded that salinity affects plant growth by changing the physiological, biochemical, and antioxidative characteristics of maize seedlings, while primed seeds with biofilm-producing bacteria showed positive effects on the physiological and biochemical profile of maize plants under stress conditions; resulting in lowering the sodium ion uptake in plants. It is also concluded that halophilic bacteria especially *Bacillus sonorensis* play a vital role like other *Bacillus spp*. in improving plant growth under saline conditions. It is suggested that the impact of these biofilm-producing bacteria on maize seeds be assessed at the molecular level to identify the involved genetic mechanisms. Field tests must be conducted as well for effective evaluation.

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REFERENCES

[1] Gu, D., Andreev, K., & Dupre, M. E. Major trends in population growth around the world. China CDC weekly. 3(28), 604 (2021).

[2] Kousar, S., Ahmed, F., Pervaiz, A., & Bojnec, Š. Food insecurity, population growth, urbanization, and water availability: the role of government stability. Sustain. 13(22), 12336 (2021).
[3] Ma, Y., Dias, M. C., & Freitas, H. Drought and salinity stress responses and microbe-induced tolerance in plants. Front. Plant Sci. 11, 591911 (2020).
[4] Ashraf, M.; Foolad, M., Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ. Exp. Bot. 59, (2), 206-216 (2007).
[5] Evelin, H., Devi, T. S., Gupta, S., & Kapoor, R. Mitigation of salinity stress in plants by arbuscular mycorrhizal symbiosis: current understanding and new challenges. Front. Plant Sci. 10, 470 (2019).
[6] Ali, Q., Tariq Javed, M., Haider, M. Z., Habib, N., Rizwan, M., Perveen, R., ... & Al-Misned, F. A. α-Tocopherol foliar spray and translocation mediate growth, photosynthetic pigments, nutrient uptake, and oxidative defense in maize (Zea mays L.) under drought stress. Agron. 10(9), 1235(2020).
[7] Hasanuzzaman, M., Bhuyan, M. B., Nahar, K., Hossain, M. S., Mahmud, J. A., Hossein, M. S., ... & Fujita, M. Potassium: a vital regulator of plant responses and tolerance to abiotic stresses. Agron. 8(3), 31 (2018).
[8] Ma, Y.; Dias, M. C.; Freitas H., Drought and Salinity Stress Responses and Microbe- Induced Tolerance in Plants. Front. Plant Sci. 11:591911(2020).
[9] Upadhyay, S. K., Singh, D. P., & Saikia, R. Genetic diversity of plant growth promoting rhizobacteria isolated from rhizospheric soil of wheat under saline condition. Curr. Microbiol. 59, 489-496 (2009).
[10] Egamberdieva, D., Wirth, S., Bellingrath-Kimura, S. D., Mishra, J., & Arora, N. K. Salt-tolerant plant growth promoting rhizobacteria for enhancing crop productivity of saline soils. Front. Microbiol. 10, 2791 (2019)
[11] Santoyo, G.; Urtis-Flores, C. A.; Loeza-Lara, P.D.; Orozco-Mosqueda, M. D. C. l.; Glick, B. R., Rhizosphere Colonization Determinants by Plant Growth-Promoting Rhizobacteria (PGPR). Biology (Basel). 10(6):475(2021).
[12] Parrilli, E., Tutino, M. L., & Marino, G. (2022). Biofilm as an adaptation strategy to extreme conditions. Rendiconti Lincei. Scienze Fisiche e Naturali. 33(3), 527-536.
[13] Gosset, M., Roques, C., & Taillandier, P. Microbial biofilms in enology. OENO One. 56(3), 167-184 (2022).
[14] Singh, S., Datta, S., Narayanan, K. B., & Rajnish, K. N. Bacterial exo-polysaccharides in biofilms: role in antimicrobial resistance and treatments. J. Gene.t Eng. Biotechnol. 19(1), 1-19 (2021).
[15] Haque, M. M., Mosharaf, M. K., Khatun, M., Haque, M. A., Biswas, M. S., Islam, M. S., ... & Siddiquee, M. A. Biofilm producing Biofilm-producing multiple plant growth-promoting traits promote the growth of tomatoes wunderkinder wtomatoeseficit stress. Front. Microbiol. 11, 542053 (2020).
[16] Khan, N., & Bano, A. Exopolysaccharide producing rhizobacteria and their impact on growth and drought tolerance of wheat grown under rainfed conditions. PLoS One. 14(9), e0222302 (2019).
[17] Ma, Y., Rajkumar, M., Zhang, C., & Freitas, H. Beneficial role of bacterial endophytes in heavy metal phytoremediation. J. Environ. Manage. 174, 14-25 (2016)
[18] El-Shabrawy, E. M., & Shehata, H. S. Controlling maize late-wilt and enhancing plant salinity tolerance by some rhizobacterial strains. Egypt. J. Phytopathol. 46(1), 235-255 (2018).
[19] Liu, Y., Xun, W., Chen, L., Xu, Z., Zhang, N., Feng, H., ... & Zhang, R. Rhizosphere microbes enhance plant salt tolerance: Toward crop production in saline soil. Computat. Struct. Biotechnol. J. (2022).
[20] Saeed, Q., Xiukang, W., Haider, F. U., Kučerik, J., Mumtaz, M. Z., Holatko, J., ... & Mustafa, A. (2021). Rhizosphere bacteria in plant growth promotion, biocontrol, and bioremediation of contaminated sites: A comprehensive review of effects and mechanisms. International J. Mol Sci. 22(19), 10529.
[21] Banerjee, A., Sarkar, S., Cuadros-Orellana, S., & BandopadhyaBandyopadhyay. Exopolysaccharides and biofilms in mitigating salinity stress: The biotechnological potential of halophilic and soil-inhabiting PGPR microorganisms. Microorganisms in saline environments: strategies and functions. 133-153 (2019).
[22] Roohi, A., Ahmed, I., Khalid, N., Iqbal, M., & Jamil, M. Isolation and phylogenetic identification of halotolerant/halophilic bacteria from the salt mines of Karak, Pakistan. Int. J. Agric. Biol. 16(3), 564-570 (2014).
[23] Rani, M. U., Arundhathi, A., & Reddy, G. Screening of rhizobacteria containing plant growth promoting (PGPR) traits in rhizosphere soils and their role in enhancing the growth of pigeon pea. Afri. J. Biotechnol. 11(32), 8085-8091(2012).
[24] Hoagland, D. R.; Arnon, D. I., The water-culture method for growing plants without soil. Circular. Calif. Agric. 347, 2nd edit (1950).
[25] Wellburn, A.; Lichtenthaler, H., Formulae, and program to determine total carotenoids and chlorophylls a and b of leaf extracts in different solvents. Adv. Photosyn. Res. pp 9-12. Springer, (1984).
[26] Joslyn, M. A., Methods in food analysis. Physical, chemical, and instrumental methods of analysis. Methods in food analysis. Physical, chemical, and instrumental methods of analysis. 2nd edition (1970).
[27] Harborne, J. B., Methods in plant biochemistry. Volume 1. Plant Phenolics. Academic Press Ltd.: (1989).
[28] Bates, L.; Waldren, R.; Teare, I., Rapid determination of free proline for water-stress studies. Plant Soil. 39 (1), 205-207. (1973).
[29] Kumar, K.; Khan, P., Peroxidase and polyphenol oxidase in excised ragi (Eleusine corocana cv PR 202) leaves during senescence. Ind. J. Exp. Biol. 20 (5), 412-416 (1982).
[30] Beauchamp, C.; Fridovich, I., Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 44 (1), 276-287 (1971).

[31] Vasconcelos, A. C. F. d.; Zhang, X.; Ervin, E. H.; Kiehl, J. d. C., Enzymatic antioxidant responses to biostimulants in maize and soybean subjected to drought. Sci. Agric. 66 (3), 395-402 (2009).

[32] Nakano, Y.; Asada, K., Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach chloroplasts— Plant Cell Physiol. 22 (5), 867-880 (1981).

[33] Velikova, V.; Yordanov, I.; Edreva, A., Oxidative stress and some antioxidant systems in acid rain-treated bean plants: protective role of exogenous polyamines. Plant Sci. 151, (1), 59-66 (2000).

[34] Daud, M.; Mei, L.; Variath, M.; Ali, S.; Li, C.; Rafiq, M.; Zhu, S., Chromium (VI) uptake and tolerance potential in cotton cultivars: effect on their root physiology, ultra-morphology, and oxidative metabolism. BioMed. Res. I. 2014. (2014).

[35] Alharbi, K., Al-Osaimi, A. A., & Alghamdi, B. A. Sodium chloride (NaCl)-induced physiological alteration and oxidative stress generation in Pisum sativum (L.): A toxicity assessment. ACS Omega, 7(24), 20819-20832 (2022).

[36] Khan, A. R., Mustafa, A., Hyder, S., Valipour, M., Rizvi, Z. F., Gondal, A. S., ... & Daraz, U. (2022). Bacillus spp. as Bioagents: Uses and Application for Sustainable Agriculture. Biol. 11(12), 1763.

[37] Ludwiczak, A., Osiak, M., Cárdenas-Pérez, S., Lubińska-Mielińska, S., & Piernik, A. Osmotic stress or ionic composition: which affects the early growth of crop species more? Agron. 11(3), 435 (2021).

[38] Karygianni, L., Ren, Z., Koo, H., & Turner, T. Biofilm matrixome: extracellular components in structured microbial communities. Trends Microbiol. 28(8), 668-681(2020).

[39] Mahto, K. U., Priyadarshanee, M., Samantaray, D. P., & Das, S. Bacterial biofilm and extracellular polymeric substances in the treatment of environmental pollutants: Beyond the protective role in survivability. J. Clean Prod. 134759 (2022).

[40] Bhagat, N., Raghav, M., Dubey, S., & Bedi, N. Bacterial exopolysaccharides: Insight into their role in plant abiotic stress tolerance (2021).

[41] Ramasamy, K. P., & Mahawar, L. Coping with salt stress-interaction of halotolerant bacteria in crop plants: A mini-review. Front. Microbiol. 14 (2023).

[42] Khan, M. Y., Nadeem, S. M., Sohaib, M., Waqas, M. R., Alotaibi, F., Ali, L., ... & Al-Barakah, F. N. (2022). The potential of plant growth promoting bacterial consortium for improving the growth and yield of wheat under saline conditions. Front. Microbiol. 13.

[43] Gang, S., Sharma, S., Saraf, M., Buck, M., & Schumacher, J. Bacterial indole-3-acetic acid influences soil nitrogen acquisition in barley and chickpea. Plants. 10(4), 780 (2021).

[44] Cardarelli, M., Woo, S. L., Rouphael, Y., & Colla, G. Seed treatments with microorganisms can have a biostimulant effect by influencing germination and seedling growth of crops. Plants. 11(3), 259 (2022).

[45] Jamil, M.; Bashir, S.; Anwar, S.; Bibi, S.; Bangash, A.; Ullah, F.; Rha, E. S., Effect of salinity on physiological and biochemical characteristics of different varieties of rice. Pak. J. Bot. 44, 7-13 (2012).

[46] Hameed, A., Ahmed, M. Z., Hussain, T., Aziz, I., Ahmad, N., Gul, B., & Nielsen, B. L. Effects of salinity stress on chloroplast structure and function. Cells. 10(8), (2023).

[47] Jan, S. U., Rehman, M., Gul, A., Fayyaz, M., Rehman, S. U., & Jamil, M. Combined application of two Bacillus species enhance phytoremediation potential of Brassica napus in an industrial metal-contaminated soil. I. J. Phytoremediation. 24(6), 652-665 (2022).

[48] Widnyana, I. K. PGPR (Plant Growth Promoting Rizobacteria) benefits in spurring germination, and growth and increase the yield of tomato plants. Recent Advances in Tomato Breeding and Production. 17-25 (2018).

[49] Ketehouli, T., Idrice Carther, K. F., Noman, M., Wang, F. W., Li, X. W., & Li, H. Y. Adaptation of plants to salt stress: characterization of Na+ and K+ transporters and role of CBL gene family in regulating salt stress response. Agron. 9(11), 687(2019).

[50] Farooq, M., & Hussain, M. wakeel A, Siddique KHM. Salt stress in maize: effects, resistance mechanism, and management. Agron Sustain Develop, 35, 461-481(2015).

[51] Cooper, D., & Dimri, M. Biochemistry, Calcium Channels. In StatPearls [Internet]. StatPearls Publishing (2021).

[52] Kumari, S., Chhillar, H., Chopra, P., Khanna, R. R., & Khan, M. I. R. Potassium: A track to develop salinity tolerant plants. Plant Physiol. Biochem. 167, 1011-1023 (2021).

[53] Singh, S., Datta, S., Narayanan, K. B., & Rajnish, K. N. Bacterial exo-polysaccharides in biofilms: role in antimicrobial resistance and treatments. J. Genet. Eng. Biotechnol. 19(1), 1-19 (2021).

[54] Afzal, S., Chaudhary, N., & Singh, N. K. Role of soluble sugars in metabolism and sensing under abiotic stress. Plant Growth Regulators: Signalling under Stress Conditions, 305-334 (2021).

[55] Gandonou, C. B.; Bada, F.; Abrini, J.; Skali-Senhaji, N., Free proline, soluble sugars and soluble proteins concentration as affected by salt stress in two sugarcane (Saccharum sp.) cultivars differing in their salt tolerance. Int J. Biol. Chem. Sci. 5 (6), 2441-2453 (2011).

[56] Khatkar, D.; Kuhad, M., Short-term salinity induced changes in two wheat cultivars at different growth stages. Biol. Plant. 43 (4), 629-632 (2000).

[57] Kathuria, S.; Bhardwaj, A., Export quotas and policy constraints in the Indian textile and garment industries. World Bank Publications: Vol. 2012 (1998).

[58] Maggio, A.; Miyazaki, S.; Veronese, P.; Fujita, T.; Ibeas, J. I.; Damsz, B.; Narasimhan, M. L.; Hasegawa, P. M.; Joly, R. J.; Bressan, R. A., Does proline accumulation play an active role in stress-induced growth reduction?. Plant J. 31 (6), 699-712 (2002).

[59] Carpenter, J. F.; Crowe, J. H.; Arakawa, T., Comparison of solute-induced protein stabilization in aqueous solution and the frozen and dried states. J. Dairy Sci. 73 (12), 3627-3636 (1990).

[60] Misra, N.; Saxena, P., Effect of salicylic acid on proline metabolism in lentil grown under salinity stress. Plant Sci. 177 (3), 181-189 (2009).

[61] Shafi, M.; Bakht, J.; Khan, M. J.; Khan, M. A.; Raziuddin, M., Role of abscisic acid and proline in salinity tolerance of wheat genotypes. Pak. J. Bot. 43, 1111-1118 (2011).

[62] Gossett, D. R.; Millhollon, E. P.; Lucas, M., Antioxidant response to NaCl stress in salt-tolerant and salt-sensitive cultivars of cotton. Crop Sci. 34 (3), 706-714 (1994).

[63] Azooz, M.; M. Ismail, A.; F. Abou Elhamd, M., Growth, Lipid Peroxidation, and Antioxidant Enzyme Activities as a Selection Criterion for the Salt Tolerance of Maize Cultivars Grown under Salinity Stress; Vol. 11 (2009).

[64] Abbasi, G.; Javaid, A.; Anwar-ul-Haq, M.; Ali, S.; Zh, C.; Malik, W., Exogenous potassium differentially mitigates salt stress in tolerant and sensitive maize hybrids. Vol. 46 (2014).

[65] Jha, Y.; Subramanian, B., PGPR regulates caspase-like activity, programmed cell death, and antioxidant enzyme activity in paddy under salinity. Vol. 20, p 201-7 (2014).

[66] Egamberdieva, D.; Davranov, K.; Wirth, S.; Hashem, A.; Abd_Allah, E. F., Impact of soil salinity on the plant–growth–promoting and biological control abilities of root-associated bacteria. Saudi J. Biol. Sci. 24 (7), 1601-1608 (2017).