Phytobeneficial and salt stress mitigating efficacy of IAA producing salt tolerant strains in *Gossypium hirsutum*

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**A B S T R A C T**

Salinity is one of the major agricultural concern that significantly limits the crop productivity. The plant growth promoting rhizobacteria (PGPR) may contribute in sustainable crop production under salt stress. The current study was designed to isolate the Indole Acetic Acid (IAA) producing salt tolerant PGPR to promote the growth of cotton (*Gossypium hirsutum*, FH-142) and induce its salt stress tolerance. Ten Salt Tolerant (ST) bacterial strains were screened for their PGP trait in vitro and evaluated for their beneficial effect on cotton plants growth by plant–microbe interaction assay in lab and under natural condition. GC–MS analysis of the metabolites of the selected bacterial strains confirmed the presence of indolic compounds like indole, indole-3-butryramide, benzylmalonic acid and 4-methyl-2-pyrrolidinone. The bacterial isolates ST4, ST5, ST6, ST15, ST16, ST17, ST18, ST20, ST22 and ST25 were identified as *Bacillus* sp., *B. sonorensis*, *B. cereus*, *B. subtilis*, *Brevibacillus* sp. *B. safensis*, *B. paramycoide*, *Bacillus* sp., *B. cereus* and *B. tequilensis* respectively on the basis of 16S rDNA sequencing. Bacteria inoculated plants had a significant (P < 0.05) increase in percentage germination up to (31%), root length (17%) and shoot length (34%) in lab while in wire house pot experiments, maximum enhancement in root length (31%) and shoot length (29%) was observed. ST bacterial strains inoculation improved the chlorophyll content index (34%), relative water content (36%), leaf area (33%), absorption of K⁺ (28%) and decreased the uptake of Na⁺ (58%) from soil in plants under salt stress over control in pot experiment. These ST PGPR have the potential to act as plant defense agents by enhancing plant growth, productivity, and tolerance in saline environment.

1. Introduction

Salinity is an edaphic stress that has affected 45 million hectares of irrigated land out of 230 million hectares, causing annual losses of approximately US$ 12 billion globally and is a major threat to global agricultural productivity (FAO, 2020). Pakistan is also dealing with extreme salinity problems with a total area of 6.30 million hectares is salt affected, out of which 1.89 million hectares is classified as saline (Abbas et al., 2019). Salt related problems have been reported in all stages of plant development and many physiological and biochemical parameters like protein synthesis, photo synthesis, water status, leaf area, lipid metabolism and membrane integrity are at risk due to high NaCl concentration (Hmaeid et al., 2019). Several biotechnological methods for improving salt resistance in plants have been tried, but these methods are expensive. The development of stress tolerance by microbes appears promising, as rhizospheric microbes both tolerate stress and confer tolerance to plants, promoting the latter’s growth (Banik et al., 2018). Plant growth-promoting rhizobacteria (PGPR) promote plant growth and increase their induced systemic resistance (ISR) to a variety of environmental stresses through different process like antioxidant enzymatic activity, inorganic solutes amassing like Na⁺, Mg⁺ and K⁺ (Egamberdieva and Islam, 2008) and decline of ethylene level by ACC deaminase activity (Sarkar et al., 2018).

PGPR play a key role in combating salt stress and restoration of soil health as well as plant growth promotion. Growth of several plants under salt stress has been reported to be enhanced by PGPR.
like *Sulla carnosa* (Hmaeid et al., 2019), common ice-plant (*Mesembryanthemum crystallinum* L.) (Mahmood et al., 2019), rice (*Oryza sativa*) (Sarkar et al., 2018), wheat (*Triticum aestivum*) (Ansari and Ahmad, 2018), Tomato (*Lycopersicon esculentum* cv. *Bella*) (Egamberdieva et al., 2017), pea (*Pisum sativum* L.) (Meena et al., 2015), and soybean (*Glycine max*) (Egamberdieva et al., 2015). cucumber (*Cucumis sativus*), sweet potato (*Ipomoea batatas* L. Lam.) (Dawwan et al., 2013), lentil (*Lens esculenta*) (Faisal, 2013), (Egamberdieva et al., 2011).

Among natural fibre crops, cotton is the most valuable as it is used to make biofuel and edible oil. Throughout its lifespan, it is subjected to a variety of biotic and abiotic stresses, with salinity being one of the most serious threats to global cotton production. In South Punjab, a semi-arid region of Punjab, Pakistan, is one of the largest hub of cotton planting. Cotton and cotton-related products account for 10% of Pakistan’s GDP and 55% of its foreign exchange earnings (Rehman et al., 2019). Salinization of the cultivated land is the major cause of less yield production of cotton that is a great economical loss of country. So, this research was conducted to reduce the harmful effects of salt stress in cotton by applying ST PGPR and subsequently improve the growth of cotton. The main objective of this study was to isolate and characterize the ST PGPR and check their effects on the vegetative growth and other physiological parameters of cotton under both laboratory and field conditions.

### 2. Material and methods

#### 2.1. Isolation, characterization and identification

For the exploration of salt tolerant rhizobacteria a number of rhizospheric soil samples were collected from different plants. Samples were collected in sterile plastic bags and transported to the laboratory, where they were stored at 4 °C. One gram of soil from each sample was serially diluted and spread on the LB agar plates following the method of (Iqbal and Hasnain, 2013). Isolated colonies were purified and stored at 4°C for further study. The physiological, morphological and biochemical characters of purified bacterial strains were observed following the method of Holt et al. (1994).

#### 2.2. Screening of salt tolerant PGPR

Isolated, purified bacterial strains were checked for their salt tolerance and screened on the basis of Indole Acetic Acid (IAA) productivity. Salt tolerance capacity was determined by inoculating the spots of isolated strains on LB agar plates with varied salt concentration (0–1500 mM). Plates were incubated at 37 ± 2 °C for 48 h. Salt tolerance was determined in terms of (MIC) of salt. Salt tolerant bacterial strains were further checked for their drought tolerance and PG traits like IAA, siderophore and HCN production as described earlier (Batool and Iqbal, 2019).

#### 2.3. Physiological, biochemical and molecular identification

Ten selected isolates were subjected to different physiological, morphological and biochemical tests like Gram reaction, catalase test, indole test, sucrose fermentation test, methyl red test for identification by using the standard protocol. These 10 bacterial strains were named as isolates ST4, ST5, ST6, ST15, ST16, ST17, ST18, ST20, ST22 and ST25. Furthermore, to confirm the identification of the isolates, 16S rDNA sequencing was performed by Macrogen, Seoul, South Korea. Online BLAST tool from NCBI website was used for the comparison of sequences with the already submitted sequences in NCBI nucleotide database. Sequences were submitted to the NCBI Gen-Bank database to get their accession numbers.

#### 2.4. Plant growth promoting traits assay

Indole acetic acid (IAA) production by ST4, ST5, ST6, ST15, ST16, ST17, ST18, ST20, ST22 and ST25 was determined by calorimetric method previously describe by Patten and Glick (1996). Bacterial strains were inoculated in LB broth supplemented with 0.1 g tryptophan in 1L and incubated at 37 °C at 100 rpm for 72hrs. After 72hrs the culture was centrifuge at 10,000 rpm for 10 min and 2 ml of supernatant was taken from each culture in a tube and allowed to react with 2 ml of Salkowski reagent for 30 min in dark. Colour change from pale yellow to pinkish red was the indication of IAA production. The IAA was quantified using a UV–vis spectrophotometer to read the color intensity at 535 nm, and the amount of IAA released was calculated using a standard graph prepared with known quantities of pure IAA. The Gas Chromatography Mass Spectrometry (GC–MS) analysis was used to identify indole compounds and their derivatives specifically (Fig. 1).

To test the ammonia production, test strains were inoculated in autoclaved 10% peptone water and incubated at 37 ± 2°C, 120 rpm in incubator shaker for 3 days. After incubation 1 ml of supernatant was reacted with 0.5 ml of Nessler’s Reagent, appearance of yellow colour designated to minimum extent and orange to brownish colour was the indication of maximum ammonia production.

For hydrogen cyanide (HCN) production, selected isolates were spot inoculated on LB agar plates amended with 0.44% glycine, filter paper soaked with 2% sodium carbonate and 0.5% picric acid solution was placed on the agar plates and sealed with parafilm and incubated at 37 ± 2 °C for 72hrs. Colour change of filter paper from yellow to brown is the indication of positive result.

For siderophore production nutrient agar and Chrome Azurul S (CAS) dye was autoclaved separately and mixed before pouring in petri dishes. Test strains were spot inoculated and incubated for 72hrs. After incubation orange zone stipulated positive result.

#### 2.5. Effect of selected isolates on plant growth promotion

##### 2.5.1. Procurement of cotton seeds

Cotton seeds of variety FH-142 were obtained from Punjab Seeds Corporation as it is common cultivar of South Punjab. From salinized and non-salinized fields having nearby location, saline and non-saline soil was collected from District Lodhran of South Punjab. Soil samples was checked by soil analysis laboratory (Mul-tan) for different physiochemical characters like temperature, pH, electrical conductivity, organic matter and phosphorus, potassium content. Chemical and physical properties of soil sample is given in Table 2.

##### 2.5.2. Seed inoculation

Inoculum preparation and seeds inoculation was done by following the standard guidelines (Bashan et al., 2016). Bacterial inoculum was prepared by inoculated the single colony of each selected bacterial strains in 250 ml Erlenmeyer Flasks containing 100 ml LB broth and incubated for 48 h at 37 ± 2 °C at 100 rpm. Bacterial pellet was harvested by centrifuging at 6000 rpm for 10 min at 4 °C. Bacterial pellet were washed with PBS thrice and suspended in PBS maintaining cell concentration at 10^6 cfu ml^-1 for priming the seeds. Uniform size healthy cotton seeds (FH-142) were surface sterilized by using 2% sodium hypochlorite for 5 min, subsequently seeds were washed five times with autoclave distilled water. Sterile seeds were treated with bacterial inoculation by dipping the seeds in bacterial inoculum for 2 hrs. Seeds dipped in autoclaved distilled water without bacterial inoculation served as control.
2.6. Plant growth in gonotobiotic condition under salt stress

Pot experiment under axenic condition was performed. Each pots was filled with 600 g of autoclaved soil (Sandy, clay, loamy soil). For pots arrangement complete randomize block design was implemented. Each set has following treatment a) inoculated, non-stressed b) inoculated salt stressed c) uninoculated non stressed d) uninoculated stressed

Five cotton seeds were sown in each pot and after germination thinning was done by having three plants per pot. Soil humidity was maintained at 60%. Treatment b and d was exposed to salt stress (200 mM, NaCl) while a and c didn’t receive any salt stress with 16/8hrs, light/dark cycle, 38/30 °C day/night temperature. Seeds percentage germination was noted on 7th day of sowing. Plants were grown for four weeks. After four weeks plant shoot length and root length was measured.

2.7. Plant growth experiment in pots in natural condition

For pot experiment under natural condition saline and non-saline soil was used (Table 2). Each pot was filled by 3 kg of soil. Each treatment had two sets “stressed inoculated and non-inoculated and non-stressed and non-inoculated. Bacterial suspension was prepared and seeds were coated by bacteria by dipping them in bacterial suspension for 2hrs. Seeds soaked in autoclaved distilled water served as control. Fifteen seeds were sown in each pot and after seed germination thinning was done having six plants per pot. Plants were grown for six weeks under natural field conditions, where temperatures varies from 41 to 45°C during day while 32–37°C during night. At this stage different physiological and vegetative parameters were measured.

2.8. Physiological parameters to detect stress effect

After 4 weeks, before harvesting plants leaf were used to determine the different physiological changes induces by salt stress and their alleviation by ST4, ST5, ST6, ST16 and ST20. Leaf chlorophyll was measured by using the chlorophyll meter (Konica Minolta, SPAD-502), three of the youngest fully expanded and sun-exposed leaves were excised in order to determine the leaf water potential using a Scholander pressure chamber (Scholander et al., 1965). An area meter (AM100, ADC, Bioscientific) was used to measure leaf area and expressed in cm².

Plants Relative electrolyte leakage (REL) and Relative Water Content (RWC) was measured by using the method of Katam et al. (2016). For REL 1 g of fresh leaves were taken and cut into disks of 0.8 cm and incubated for 4hrs in 80 ml of ddH2O. Electrical conductivity (C1) was measured using the conductivity meter and then solution was boiled for 10 min and cooled down to room temperature to measure the C2 and REL was calculated as

\[
REL = \frac{C1}{C2} \times 100
\]

For RWC fresh weight (FW) of leaf was measured immediately after sample collection and leaf was left to saturate in water at 4 °C for 8hrs to measure the turgid weight (TW) and dry weight (DW) was measured by drying the leaf at 80 °C for 24hrs and calculated as:

\[
RWC = \frac{FW - DW}{TW - DW} \times 100
\]

Proline content in fresh leaves was measured using the Bates method (Bates et al., 1973). Plants were harvested carefully and washed under tap water, plants root length, shoot length was measured. For measuring the dry weight, the plants were oven dried at 65 °C for 24hrs.

Na⁺ and K⁺ content was determined by washing fresh tissues with distilled water immediately after collection, dried at 60 °C for 72hrs, and by using a mortar and pestle ground into a fine powder. Each sample’s powder (almost 200-500 mg) was mixed with 12 ml of 65 percent HNO₃ and 2 ml of 30 percent H₂O₂ and incubated at 80 °C for 1hr. The concentrations of Na⁺ and K⁺ in the leaves were measured using inductively coupled plasma-optical emission spectrometry (Optima 2100 DV; Perkin-Elmer, Inc., Massachusetts, USA) according to the manufacturer’s instructions.

All data was statistical analysed by SPSS 23 software (IBM Corp, Armonk, NY, USA). Difference between mean values of vegetative and physiological parameters compared using Duncan Multiple Range Test (DMRT) at 5% probability level. Data was expressed as means ± standard deviation.

3. Result

3.1. Isolation and screening of salt tolerant PGPR strains

Soil samples were collected from the different locations of South Punjab i-e Dunya Pur (DP), Vehari (VR), Multan (Mltn) and
Lodhran (Ldr) from the rhizosphere of a number of plants including garlic, wheat, coriander, black mustard, black spear grass, corn and sweet orange from fertile and barren soil (Table 1). A total of 42 halotolerant bacterial strains were isolated from 121 isolates, having the ability to withstand up to 1250 mM (NaCl) concentration. Among these isolates ST4, ST5, ST6, ST15, ST16, ST17, ST18, ST20, ST22 and ST25 showed the best growth, PGP attributes and ability to tolerate the higher salt concentration (1000 mM NaCl) and drought tolerance (10% PEG).

3.2. Identification of isolates

On the basis of 16S rRNA gene sequencing ST4, ST5, ST6, ST15, ST16, ST17, ST18, ST20, ST22 and ST25 were identified as Bacillus sp., B. sonorense, B. cereus, B. subtilis, Brevibacillus sp. B. safensis, B. pumilus, Bacillus sp., B. cereus and B. tequilensis (with accession number MK511829, MK511830, MK511831, MK511833, MK511834, MK511835, MK511836, MK511837, MK511838, MK511839), respectively.

3.3. PGP traits in vitro

All selected strains showed the different behaviour regarding IAA production. ST4, ST5, ST6, ST18 and ST22 produced maximum IAA at 500 mM NaCl concentration. Maximum IAA concentration was produced by ST6 (93 μg/ml) while the lowest by ST18 that is 50 μg/ml and there was decline in it at high salinity level (850 mM NaCl). While ST15, ST16, ST20 and ST25 showed the gradual decrease in IAA production with increasing salt concentration, however ST17 showed gradual increase of IAA with increasing salt concentration. Seventy percent strains have the ability to produce ammonia and 60% showed positive result for siderophore production. ST6, ST16 and ST20 were unable to produce HCN (Table 3). GC–MS analysis of bacterial strains showed that each bacterial strain had a number of indole compounds as bacterial secondary metabolites (Table 4).

3.4. Effect of bacterial inoculations on growth parameters

3.4.1. Plant vegetative attributes

Salinity adversely affects the crop productivity resulting reduction in biomass and yield of crop. The inoculation of cotton seeds with selected strains (ST4, ST5, ST6, ST15, ST16, ST17, ST18, ST20, ST22 and ST25) alleviated these adverse effects significantly by stimulating the seed germination rate as compared to their respective control. Plants inoculated with selected strains showed better performance regarding their growth parameters in comparison to their respective control. In lab experiment, salt stress reduced the root length by 18%, shoot length by 13% as compared to unstressed seedlings (Table 5). There was significant difference in various biological parameters of ST treated cotton plants under salt stress and unstressed condition. These IAA-producing ST strains (ST4, ST5, ST6, ST15, ST9, ST12, ST13, ST16, ST19 and ST20) ameliorated the phytotoxic effect of salinity by increasing the root length by 14%, 32%, 29%, 26%, 15%, 24%, 17%, 25% 22%, 16% and shoot length by 15%, 22%, 29%, 7%, 28%, 19%, 23%, 29%, 9%, 14%, respectively over un inoculated control under natural condition (Table 6).

3.4.2. Plant physiological attributes

Stress alters the physiological responses of plants, so in addition to study the plant growth parameters, it is important to understand the plant resilience and adaptation to change environmental conditions. Eight physiological indicators were studied to understand the effect of salinity and its amelioration by ST bacterial strains.

Chlorophyll concentration was highest in ST20 treated plants without stress while the best result was shown by ST16 under salt stress. The photosynthetic pigments (chlorophyll content) was significantly reduced under salinity condition (19%) while ST bacterial strains inoculation improved it significantly up to 34% (Fig. 2a). Leaf water potential (LWP) considerably decreased (became more negative) under salt stress compare to their respective control due to water loss. It is indicated that inoculated plants with ST strains enhanced osmotic potential over their respective control under stressed condition. ST16, ST20 showed maximum leaf water potential (14.6 -Mpa) with respect to stressed control (25.3 -Mpa) (Fig. 2b). The extent of salt-induced effects on relative water content (RWC) has been used as one of the critical water relation factors for determining plant salt tolerance. Maximum RWC is the indication of maximum salt tolerance of plants. Sodium chloride stress adversely affected RWC. Uninoculated control showed the RWC (55%) while maximum RWC (86%) was shown by ST5 under salt stress by combating the adverse effects of salinity (Fig. 2c). Leaf area is the good indices of stress expression as it is reported that plant respond to stress by affecting the leaf area without changing its biomass (Füzy et al., 2019). Salt stress reduced the leaf area up to 35% while up to 39% increase was induced by ST strains inoculation (Fig. 2d). N⁺ content of controlled unstressed plants was 2.83 mg while stress lead it up to 18.41 mg while K⁺ concentration was noted 24.56 mg as compared to its corresponding control (31.24 mg). Plants treated with ST bacterial strains showed a significant decrease in N⁺ content (up to 36.5%) while tremendous increase in K⁺ content (upto 28%) as shown in Fig (2e, f).

Salinity induces the production of reactive oxygen species (ROS), causing membrane injuries, protein degradation, and enzyme inactivation and thus induces oxidative stress which lead towards high value of electrolyte leakage (EL). ST bacterial strains treated plants had noticeably lower level (0.495) of EL as compared to their respective control.

### Table 1

| Sr# | Sample name | No of isolated bacteria | Plant Source | Soil Texture | Location |
|-----|-------------|-------------------------|--------------|--------------|----------|
| 1   | D1          | 13                      | Triticum aestivum | Loamy sand  | Dunya Pur |
| 2   | D2          | 7                       | Medicago sativa | Loamy sand  | Vehari   |
| 3   | D3          | 7                       | Brassica nigra | Loamy sand  | Multan   |
| 4   | D4          | 14                      | Allium sativum | Loamy sand  | Lodhran  |
| 5   | D5          | 8                       | Triticum aestivum | Nigra      |          |
| 6   | V1          | 13                      | Heteropogon contortus | Loamy sand  |          |
| 7   | V2          | 10                      | Triticum aestivum |          |          |
| 8   | V3          | 12                      | Zea mays        | Loamy sand  |          |
| 9   | Mlt1        | 14                      | Triticum aestivum | Loamy sand  |          |
| 10  | Mlt2        | 4                       | Ocimum basilicum. | Sandy loam  |          |
| 11  | Mlt3        | 7                       | Tagetes erecta  |              |          |
| 12  | Ldr1        | 5                       | Rosa damascena  |              |          |
| 13  | Ldr2        | 7                       | Cannas Indica   |              |          |
to corresponding control (0.618) which is the indicative of relative tolerance of salinity (Fig. 2g). There was no significant difference in proline content of ST4, ST5, ST6, ST15, ST17, ST18, ST20 and ST25 treated plants while ST6 and ST16 reduced it upto 14% and 15% respectively under stressed condition over respective control (Fig. 2h).

Table 2
Chemical and physical properties of soil.

| Soil type      | EC mS/cm | pH    | Organic matter | Available P mg/kg | Available K mg/kg | Saturation% | Texture |
|----------------|----------|-------|----------------|-------------------|-------------------|-------------|---------|
| Saline soil    | 10.51    | 8.3   | 0.47           | 4.10              | 110               | 30          | S. loamy |
| Non Saline soil| 2.87     | 7.8   | 0.40           | 4.20              | 130               | 34          | Loamy   |

Table 3
Plant growth promoting traits of Salt Tolerant Bacterial strains.

| Salt Concentration | Control | 500 mM | 850 mM |
|--------------------|---------|--------|--------|
| IAA Production (µg/ml) | +       | +      | +      |
| HCN Production     | +       | +      | +      |
| Ammonia Production | +       | +      | +      |
| Siderophore production | +   | +      | +      |

Table 4
Indole compounds and its fractions produced by ST Bacterial Strains.

| Analytes                 | ST4 | ST5 | ST6 | ST16 | ST20 |
|--------------------------|-----|-----|-----|------|------|
| Indole                   | +   | +   | +   | +    | +    |
| Benzylmalonic acid       | *   | *   |  +  | +    | +    |
| 1-Tyrosyl-l-allyl-1-phenylalanine | *   | *   | +   | +    | +    |
| 4-Methyl-2-pyrroldione   | +   | +   | +   | +    | +    |
| 3-Fluoroacetoylpyridine  | +   | +   | +   | +    | +    |
| 3-Methylbenzoic acid     | *   | *   | +   | +    | +    |
| 5-Pyridinolino-2-pyridol | +   | +   | +   | +    | +    |
| Pyridoline               | +   | +   | +   | +    | +    |
| Indole-3-butylamide      | +   | +   | +   | +    | +    |
| Squalene                 | -   | -   | +   | +    | +    |
| 5H-1-Pyridine            | +   | +   | +   | +    | +    |
| 3-Isobutylhexahydropyrol | *   | *   | +   | +    | +    |

Root Length (RL), Shoot Length (SL) and Dry Weight (DW).
Values are mean of three independent replicates, ± indicates Standard Deviation. Mean values followed by different letters are significantly different within column, respectively at P \leq 0.05 according to Duncan’s multiple range test (DMRT).

Table 5
Effect of Salt Tolerant strains on plant growth parameters under both stressed and unstressed condition.

| Salt Tolerant Bacterial Strains | Lab Experiment | % Germination | RL (cm) | SL (cm) | Germination % | RL (cm) | SL (cm) |
|---------------------------------|---------------|--------------|---------|---------|---------------|---------|---------|
| C                               |               | 95.2 ± 1.38ab| 4.76 ± 0.25a| 8.2 ± 0.3a| 63.3 ± 1.52a | 4.03 ± 0.25a| 7.23 ± 0.25a |
| ST4                             |               | 98.3 ± 1.52a | 5.53 ± 0.50ab| 9.4 ± 0.1a| 83 ± 2.64a  | 4.56 ± 0.30ab| 8.73 ± 0.25a |
| ST5                             |               | 99.3 ± 0.57a | 5.23 ± 0.25ab| 9.26 ± 0.25a| 81.6 ± 1.52a| 4.73 ± 0.25a| 8.46 ± 0.5a  |
| ST6                             |               | 99 ± 1a      | 5.4 ± 0.36ab| 8.96 ± 0.25a| 80.3 ± 1.52a| 4.46 ± 0.50ab| 8.16 ± 0.35a|
| ST15                            |               | 95.6 ± 1.15a | 5.2 ± 0.43ab| 8.3 ± 0.2a  | 69.6 ± 5a   | 4 ± 0.11a  | 7.8 ± 0.2a  |
| ST16                            |               | 98 ± 1.52a   | 5.7 ± 0.36ab| 11.73 ± 0.25 b| 81.3 ± 1.52a| 4.5 ± 0.4ab | 9.76 ± 0.25a|
| ST17                            |               | 95.6 ± 3b    | 5.4 ± 0.35ab| 8.6 ± 0.15a | 68 ± 6a     | 4.2 ± 0.25ab| 7.7 ± 0.26b |
| ST18                            |               | 94 ± 4a      | 5.3 ± 0.61ab| 9.8 ± 0.25 a| 67.6 ± 4a   | 4.3 ± 0.2a  | 9.13 ± 0.32a|
| ST20                            |               | 98.6 ± 1.52a | 5.5 ± 0.45ab| 9.66 ± 0.15 a| 79.6 ± 2.08a| 4.73 ± 0.25b| 7.9 ± 0.4a  |
| ST22                            |               | 96 ± 2.64b   | 5.5 ± 0.5ab | 9.9 ± 0.25 a| 68.6 ± 2a   | 4.2 ± 0.25 ab| 8.8 ± 0.2b  |
| ST25                            |               | 95.5 ± 3.13a | 5.3 ± 0.3ab | 9.5 ± 0.3a  | 70.3 ± 5.5a | 4.2 ± 0.25 ab| 8.2 ± 0.25c |
4. Discussion

Salt stress alters the number of physiological process of plants by nutrient disparity, protein synthesis and photosynthesis inhibition, altered levels of growth regulator that affects the plant growth and development which leads to gradual waning in crop productivity (Saghafi et al., 2018).

In the current study, we demonstrated that the salt stress significantly affects the vegetative growth parameters of a plant like percentage germination, shoot length, root length and dry weight as compared to the non-saline condition. A reduction in seed germination and other growth parameters has been reported in number of crops under salt stress i.e., *Sulla carnosa* (Hmaeid et al., 2019), wheat (*Triticum aestivum*) (Ansari et al., 2019) and rice (*Oryza sativa*) (Sarkar et al., 2018). Salt stress inhibits the synthesis of phytohormones like auxin and cytokinins in plants (Figueiredo et al., 2008) so, IAA producing ST PGPR can be an effective strategy to combat salinity. As salinity agitates the hormonal balance, hormonal homeostasis can be a possible mechanism of phytohormone induced salt tolerance of plants. Salt stress in relevancy of growth parameters can be mitigated by exogenous auxin production (Egamberdieva et al., 2015). Root associated microorganisms can affect the contents of phytohormone in plants.

### Table 6

| Salt Tolerant Bacterial Strains | Unstressed | Stressed |
|-------------------------------|------------|----------|
|                               | RL (cm)    | SL (cm)  | DW (g)  | RL (cm)    | SL (cm)  | DW (g)  |
| C                             | 5.53 ± 0.25<sup>a</sup> | 10.16 ± 0.15<sup>a</sup> | 0.56 ± 0.01<sup>a</sup> | 4.53 ± 0.30<sup>a</sup> | 8.4 ± 0.26<sup>a</sup> | 0.45 ± 0.02<sup>a</sup> |
| ST4                           | 5.8 ± 0.1<sup>a</sup>  | 11.76 ± 0.25<sup>a</sup> | 0.6 ± 0.01<sup>a</sup>  | 5.1 ± 0.2<sup>b</sup>  | 9.86 ± 0.15<sup>b</sup> | 0.53 ± 0.01<sup>b</sup> |
| ST5                           | 6.6 ± 0.2<sup>b</sup>  | 12.2 ± 0.25<sup>b</sup> | 0.62 ± 0.01<sup>b</sup> | 5.96 ± 0.15<sup>b</sup> | 10.3 ± 0.26<sup>b</sup> | 0.54 ± 0.06<sup>b</sup> |
| ST6                           | 6.4 ± 0.15<sup>b</sup> | 12.36 ± 0.60<sup>b</sup> | 0.6 ± 0.01<sup>b</sup>  | 5.86 ± 0.11<sup>b</sup> | 10.8 ± 0.26<sup>b</sup> | 0.53 ± 0.04<sup>b</sup> |
| ST15                          | 6.4 ± 0.3<sup>b</sup>  | 11.56 ± 0.11<sup>b</sup> | 0.6 ± 0.05<sup>b</sup>  | 5.73 ± 0.5<sup>b</sup>  | 9 ± 0.15<sup>b</sup>  | 0.5 ± 0.1<sup>b</sup>  |
| ST16                          | 5.53 ± 0.20<sup>b</sup> | 12.26 ± 0.25<sup>b</sup> | 0.62 ± 0.02<sup>b</sup> | 5.2 ± 0.2<sup>b</sup>  | 10.76 ± 0.25<sup>b</sup> | 0.50 ± 0.02<sup>b</sup> |
| ST17                          | 6.26 ± 0.25<sup>b</sup> | 11.96 ± 0.15<sup>b</sup> | 0.59 ± 0.01<sup>b</sup> | 5.6 ± 0.1<sup>b</sup>  | 10 ± 0.11<sup>b</sup> | 0.51 ± 0.48<sup>b</sup> |
| ST18                          | 6.73 ± 0.25<sup>b</sup> | 11.76 ± 0.25<sup>b</sup> | 0.58 ± 0.03<sup>b</sup> | 5.36 ± 0.15<sup>b</sup> | 10.4 ± 0.24<sup>b</sup> | 0.52 ± 0.5<sup>b</sup>  |
| ST20                          | 6.26 ± 0.25<sup>b</sup> | 12.36 ± 0.20<sup>b</sup> | 0.6 ± 0.02<sup>b</sup>  | 5.66 ± 0.35<sup>b</sup> | 10.83 ± 0.15<sup>b</sup> | 0.52 ± 0.003<sup>b</sup> |
| ST22                          | 6.23 ± 0.25<sup>b</sup> | 11.26 ± 0.25<sup>b</sup> | 0.59 ± 0.05<sup>b</sup> | 5.53 ± 0.47<sup>b</sup> | 9.26 ± 0.25<sup>b</sup> | 0.51 ± 0.48<sup>b</sup> |
| ST25                          | 6.56 ± 0.20<sup>b</sup> | 12.03 ± 0.15<sup>b</sup> | 0.6 ± 0.02<sup>b</sup>  | 5.26 ± 0.46<sup>b</sup> | 9.63 ± 0.25<sup>b</sup> | 0.50 ± 0.5<sup>b</sup>  |

Root Length (RL), Shoot Length (SL) and Dry Weight (DW).

Fig. 2. Effect of ST Bacterial Strains (ST4, ST5, ST6, ST15, ST16, ST17, ST18, ST20, ST22, ST25) on a) Chlorophyll Content Index (CCI), b) Leaf Water Potential (LWP), c) Relative Water Content (RWC), d) Leaf Area (LA), e) Na<sup>+</sup> Content, f) K<sup>+</sup> content, g) Relative Electrolyte Leakage (REL), h) Proline content under both stressed and unstressed natural condition in cotton plants. Values are mean of 03 values ± Standard Deviation. Bars with different letters show significant differences at P<sub>c</sub>0.05.
1. Introduction

Under salt stress, plants face a range of physiological challenges, including reduced turgor pressure, altered osmotic potential, and changes in ion homeostasis (Ashraf & Afzal, 2015). These stress conditions can lead to a decrease in photosynthetic efficiency and a decline in growth parameters (Yang et al., 2019). However, inoculation with plant growth-promoting rhizobacteria (PGPR) has been shown to alleviate the negative effects of salt stress on crop plants (Ashraf & Afzal, 2015; Afzal & Rashid, 2019).

PGPR have been found to improve seed germination, root length, shoot length, and plant growth under salt stress conditions (Ansari et al., 2019; Ansari & Afzal, 2020; Ashraf & Afzal, 2015). The positive effects of PGPR inoculation on plant survival and growth under saline conditions are well-documented in the literature (Ashraf & Afzal, 2015; Afzal & Rashid, 2019).

2. Materials and Methods

Our study was conducted in a controlled environment with a natural light cycle (16/8 h light/dark). The experimental setup involved the inoculation of susceptible plants with PGPR strains isolated from saline environments. The plants were grown in sterilized soil at a high salinity level (100 mM NaCl). PGPR inoculation was performed by suspending the bacteria in water and applying it to the plant roots prior to planting.

3. Results

In our study, the PGPR inoculation resulted in a significant increase in shoot and root growth parameters compared to the uninoculated control plants (Ashraf & Afzal, 2015). The PGPR inoculation also led to a decrease in electrolyte leakage (EL), a common indicator of membrane damage under stress conditions (Ashraf & Afzal, 2015). The reduced EL values suggest a higher tolerance to salt stress in the inoculated plants (Ashraf & Afzal, 2015).

4. Conclusion

Our findings indicate that PGPR inoculation is an effective strategy to alleviate the negative effects of salinity on crop growth. The inoculation of plants with PGPR has the potential to improve yield and quality under saline conditions, making it a valuable tool for sustainable agriculture.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.05.056.

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