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Isolation of Multi-Trait Plant Growth-Promoting *Serratia marcescens* and Evaluation of Growth-Promoting Effects on Wheat Plant under Salinity Stress

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Abstract: A total of 135 soil samples were collected during the period between December 201) to January 2020. Twenty of them were identified as S. marcescens according to 16S rRNA gene sequence analysis. The promising characterized and identified isolate was selected for further screening and evaluation of wheat growth in pots experiment. S marcescens isolates were applied alone and in combination with varying levels of salt (0, 100, 200, and 300 mM NaCl) to determine its tolerance to salinity and its role to mitigate the effect of salt on wheat growth, activities of enzymatic antioxidants, and oxidative damage. In vitro tests showed that this bacterium was able to fix nitrogen, solubilize phosphorus, production of siderophores, and synthesize indole acetic acid. The presence of the gene for ACC deaminase production was confirmed using the DegACC primer designed to amplify the accdS (ACC deaminase) gene. The AH-20 isolate showed growth at high salt (NaCl) concentration of up to 11%, indicating its potential to survive and interact with plants growing in the saline soil. The inoculation by S. marcescens significantly promoted the growth of wheat plants under salt stress (100–300 mM), and generated changes in antioxidative enzyme activities (Superoxide dismutase, Peroxidase, and Catalase) under different salt levels, reducing salinity-induced oxidative damage to the plants.

Keywords: *Serratia marcescens*, wheat, salinity, PGPR.

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Introduction

Salinity has been a serious problem for plant growth and a global reduction in crop yield. Salt stress is responsible for some physiological and metabolic alterations in plants, such as nutritional imbalances, water uptake inhibition, germination of seeds, photosynthesis, and reduced growth. In the natural environment, microbes colonize plants. Root-associated microbes work closely with plants and mediate significant physiological and metabolic processes, enhancing the plant's stress tolerance to salinity (1). Plant growth-promoting bacteria (PGPB) provide many benefits to plants such as induction of abiotic and biotic stress tolerance; improved nutrient absorption, growth, and development (2). PGPB's applications in agriculture have increased in recent years, thereby encouraging sustainable development practices. Thus, PGPR's management of stress either directly or indirectly is productive and a viable alternative in the agricultural sector (3). PGPR can promote growth directly through secretion of indole-3-acetic acid (IAA), phosphorus dissolution, and

production of siderophore, nitrogen fixation, and indirectly through increased tolerance to stress of the environment, phytopathogen control, and induce systemic resistance (4).

Materials and methods

Isolation of Plants Growth-Promoting bacteria

Ten grams of each soil sample was suspended into 90 mL of Nutrient Broth (NB) and mixed thoroughly, and then it was allowed to settle. The samples were then serially diluted to the appropriate dilutions (10-2, 10-3, and 10-4). The samples were then spread on Nutrient Agar (NA) plates and incubated at 30º C for 24 h.

Identification of PGPB Strains Using 16S rRNA Sequencing

The genomic DNA was extracted from the isolate using the bacterial genomic DNA isolation Kit (Promega, USA). The 16S rRNA gene was amplified using primers 27F (5`-AGAGTTTGATCCTGGCTCAG-3`) and 1492R (5`-TACGGTTACCTTGTTACGACTT-3`) (5). The polymerase chain reaction (PCR) was performed in 25µL reactions containing 1µl for each primer, 12.5µl master mix, 3µl template DNA, and 7.5µl Nuclease Free Water. The PCR conditions were 95 °C for 5 min, 30 cycles of 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 60 s, followed by a final extension at 72 °C for 7 min. The PCR products were qualified by 2 % agarose gel electrophoresis and then sequenced using ABI3730XL, an automated DNA sequences, by Macrogen Corporation – Korea.

Screening for Plant Growth Promoting Traits

Salinity Tolerance

The ability of the bacteria to grow in the presence of different concentrations of Sodium Chloride (NaCl) carried out in the liquid culture of nutrient broth (pH7) with 0, 250, 500, 1000, 1500, and 2000 mM NaCl. The cultures were grown for 5 days at 30º C, and the bacterial growth was measured using a spectrophotometer at 600 nm at the end of incubation.

Indole Acetic Acid (IAA) Production

The test bacteria were inoculated into 10 mL culture tubes by adding 50 uL of the cell suspension to 5 mL of sterile nutrient broth supplemented with 0.2% tryptophan and incubating for 72 hours at 30 C in the dark. Then, 1.5 mL of this broth was centrifuged at 12000×g for 10 min, followed by the addition of 1 mL of Salkawaski reagent (50 mL, 35% of perchloric acid, 1 mL 0.5 M FeCl3 solution) to the 1 mL of the supernatant in 2 mL eppendorf tube. The culture tubes were then incubated at 37 ◦ C in the dark for 1 h. The formation of red color in the medium indicated that the bacteria were producing IAA (6). The red color was estimated using a spectrophotometer at 535 nm and comparison with a standard curve.

Phosphate Solubilization

Bacterial inoculate were grown for 24 h on the nutrient broth media at 30 °C. To carry out a qualitative P solubilization assay, 10 µl of the

bacterial suspension were spread to petri dishes containing Pikovskayas (PVK) Agar medium at pH 7 and incubated at 30 °C for 7 days. Positive P solubilization phenotypes were based on halo formation around bacterial colonies and results were expressed in the form of a Solubilization Index (SI). The phosphate solubilization index (PSI) was calculated by the following formula(7).

Solubilization Index (SI) = (colony dimeter + halo dimeter) / colony dimeter.

ACC Deaminase Activity

DNA was extracted using a DNA Kit. Amplification of ACC deaminase gene was carried out using 1 cycle at 95°C for 5 min; 30 cycles at 95°C for 30 sec, 55°C for 30 sec, and 72°C for 30 sec; and a final extension at 72°C for 7 min using a Thermal Cycler. The content of the PCR mixture was 2 µL of DNA extract; 1 µl each of 10-mM primer, and 10 µL of Master Mix. The PCR product was later run through gel electrophoresis, stained, and observed for the DNA bands.

Nitrogen fixation test

The test was carried out by inoculating the bacteria in the sterile nitrogen-free Jensen's medium, and the plate was incubated for 10 days at 30°C and observing growth. The ability to fix atmospheric nitrogen for bacterial growth is demonstrated by the appearance of bacterial growth on media.

Siderophore Production

The isolation efficiency test of iron-chelating compounds was performed by growing bacteria on nutrient agar medium supplemented with 0.2 ml of 2-2-dipyridyl which was prepared from dissolving 2 mg of 2-2-dipyridyl in 10 ml sterile d.w and incubated at 30 ° C for 72 hours. The bacterial colonies producing Siderophores were identified by the appearance of weak (+), moderate (++), or intensive (+++) growths (8).

Effect of *Serratia* marcescens on The Growth of The Wheat Plant under Salinity Stress

Plant growth was evaluated in a pot analysis with the effects of NaCl and bacterium inoculation. The soil was sterilized by autoclaving for 20 min at 121°C to remove all microorganisms, and 8 kg of sterilized soil was filled into plastic pots with no drainage holes. Wheat seeds were surface sterilized by soaking in 1% Sodium hypochlorite (NaOCl) solution for 5 min and 70% ethanol for 1 min followed by three times washing with sterilized water. For inoculum preparation, the bacterial isolate was grown in nutrient broth for 24 h at 30° C. Cells were collected following centrifugation at 3000 gt for 5 min. Pellets were then re-suspended in sterile distal water, and wheat seeds were inoculated with a bacterial suspension.

Immediately after 10 days of germination, the plants were irrigated with a pure solution of 0, 100, 200, and 300 mM NaCl for the duration of the experiment (30 days). Pots were placed in a completely randomized block design way. The treatments used in the experimental design were as follows:

- T1 = non-inoculated control plants ؛
- T2 = *Serratia marcescens* - inoculated plants؛
- T3 = 100 mM NaCl-treated plants؛
- T4 = plants primed with 100 mM NaCl and *Serratia marcescens*؛
- T5= 200 mM NaCl-treated plants؛

- T6= plants primed with 200 mM NaCl and *Serratia marcescens*؛
- T7= 300 mM NaCl-treated plants؛
- T8= plants primed with 300 mM NaCl and *Serratia marcescens*.

Wheat plants were harvested and used for further analysis after 30 days of growth under salt stress, each replicate's growth parameters, such as shoot length, root length, fresh weight, and dry weight, were measured. Shoots and roots were oven-dried separately at 60°C for 48 hours to calculate the dry weight .

The chlorophyll content of wheat leaves was determined as previously described (9). Pigments were extracted and measured from fresh leaf samples of 0.5 g homogenized in 10 ml of 80% acetone. To estimate total chlorophyll, optical density was measured at 663 and 645 nm respectively, by spectrophotometer using 80% acetone as a blank. The concentrations were calculated as mg g-1 fresh weight using the formulas below:

Total Chlorophyll = (20.2 × D645 – 8.02 × D663) × V/W × 1000

Antioxidant Enzyme Activities

To evaluate the activity of antioxidant enzymes superoxide dismutase (SOD), Peroxidase (POD), and Catalase (CAT), 1 g of fresh leaves was homogenized with 10 ml of 100 mM phosphate buffer solution (pH7.8). Then the homogenate was centrifuged at 10000g for 15 min (10).

Activity Catalase enzyme (CAT)

The activity of the enzyme was estimated using a spectrophotometer depending on the change in absorbance at 240 nm for 30mM hydrogen peroxide solution and 50mM phosphate buffer solution (pH7) (11).

0.1 ml of enzyme extract was added to 1.9 ml of 50 mM phosphate buffer (pH7). Then add one ml of 30 mM hydrogen peroxide and mix well. After 1 min the absorbance was measured at 240 nm and the time required for the decrease in the absorbance was noted. Enzyme solution containing hydrogen peroxide and phosphate buffer was used as a control. The activity of the enzyme was calculated according to the following equation:

Enzyme activity (unit.ml-1) = (ΔAb /ΔAt) / 0.1 × 0.01

Enzyme activity (unit.gm-1) = CAT (unit.ml-1) × 1/C

Where Ab is the absorbance of sample, At is the time, and C is the concentration of phosphate buffer.

Activity of Peroxidase enzyme (POD)

The activity of the POD enzyme was measured according to the method described by (12). One ml of 0.1% hydrogen peroxide solution, 1 ml guaiacol pigment were mixed. Then 0.1 ml of the enzyme extract was added. The absorbance was then measured directly at 420 nm and the change in absorbance was followed every 30 seconds for 3 minutes. The activity of peroxidase was calculated using the formula below:

CAT activity (Unit.gm-1) = Ab / (Ws/ Vs) × Vt

Where Ab is the absorbance of the sample, Ws is the weight of the sample, Vs is the volume of sample, and Vt is the volume of the reading sample.

Superoxide Dismutase (SOD) Activity

The activity of the enzyme was estimated according to (13), depending on the ability of the enzyme to inhibit the photochemical reaction that leads to the reduction of nitro blue tetrazolium. Put 1.5 ml of the working mixture

(18.35ml of 50 mM phosphate buffer solution (pH7.8), 1.5 ml of 14mM methionine, 0.75 ml of 1% triton X-100, and 1 ml of nitro blue tetrazolium) into the test tubes and then add 500 µl of distilled water. Then 40 µl of the enzyme extract was added to the tubes. The blank tube was prepared in the same way, but by adding distilled water instead of the sample. Then 40 µl of riboflavin solution was added. The contents were mixed well and the absorbance was measured at 560 nm. The tubes were exposed to light for seven minutes using two 18-watt fluorescent lamps. The absorbance was measured directly at 560 nm. The standard curve for the enzyme showing the percentage of inhibition was prepared by taking different volumes of the enzyme extract. The SOD activity was estimated as follows:

- SOD (inhabitation %)=((A2S-A1S)-(A2B-A1B))/((A2B-A1B)) ×100
- Where:
- A1S = absorbance of the sample before lighting
- A2S = absorbance of the sample after lighting
- A1B=absorbance of blank before lighting
- A2B = absorbance of blank after lighting
- SOD activity (unit.ml-1) = (sample inhibition%)/max〖inhibition%〗 × (D.F)/Vs

Where D.F is the factor dilution (2000), and Vs is the volume of sample (µl).

Measurement of Oxidative Damage Induced Salinity

Detection of Hydrogen Peroxide (H2O2)

Hydrogen peroxide was detected according to the method described by (14), 0.5 g of fresh leaves was homogenized with 2 ml of 0.1% trichloroacetic acid (TCA). Then they were placed in sterile test tubes and centrifuged at 10000g for 15 min. the extracted solution (0.5 ml) was then mixed with 0.5 ml of 10 mM phosphate buffer (pH7) and 1 ml of 1M potassium iodide (KI). The blank tube was prepared in the same way, but by adding phosphate buffer instead of the sample. The contents were mixed well and the absorbance was measured at 390 nm. The amount of hydrogen peroxide (µmol.g-1) was calculated from the standard curve by using dilute solutions of hydrogen peroxide.

Estimation of Membrane Stability Index (MSI)

The membrane stability index was calculated using two sets of 0.5 g leaf tissue in 20 ml of deionized distilled water (DDW). One set was heated in a water bath at 40°C for 30 minutes, and the electrical conductivity (C1) was measured using a conductivity meter. The second set was boiled for 10 minutes at 100°C in a water bath, and its conductivity was also measured using the conductivity meter (C2) (15). MSI was estimated with the following formula:

$$MSI = 1- (C1/C2) \times 100$$

Statistical analysis

The Statistical Analysis System-SAS (2012) program was used to detect the effect of different factors in study parameters. Least significant difference –LSD test was used to significantly compare between means. Chi-square test was used to significantly compare between percentage (0.05 and 0.01 probability) in this study.

Results

Identification of the isolate

Based on the difference in morphology, we preliminarily identified a pigmented bacterium that was characterized as *S. marcescens* by microscopy, biochemical tests, and 16S rRNA gene sequencing. The 16S rRNA gene was amplified successfully and the identity of the *S. marcescens* AH-20 was confirmed as shown in Fig 1. The isolate was named *S. marcescens* AH-20 and was screened for a variety of plant growth-promoting traits. The sequencing of the 16S rRNA gene showed 100% sequence similarity with *S. marcescens* strain JW-CZ2 and *S. marcescens* strain ADS-6. The AH-20 sequence has been submitted to Genbank under the accession number MZ413658. Phylogenetic analysis of strain AH-20 showed that it is linked to many other *Serratia sp*. Strains (Figure 2).

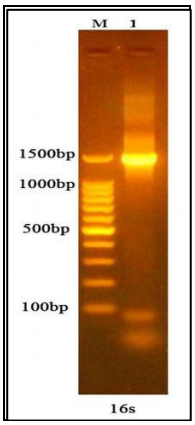


Figure (1): **Amplification of *16SrRNA* gene on 1% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker. Lane 1 resembles 1500bp PCR products**.

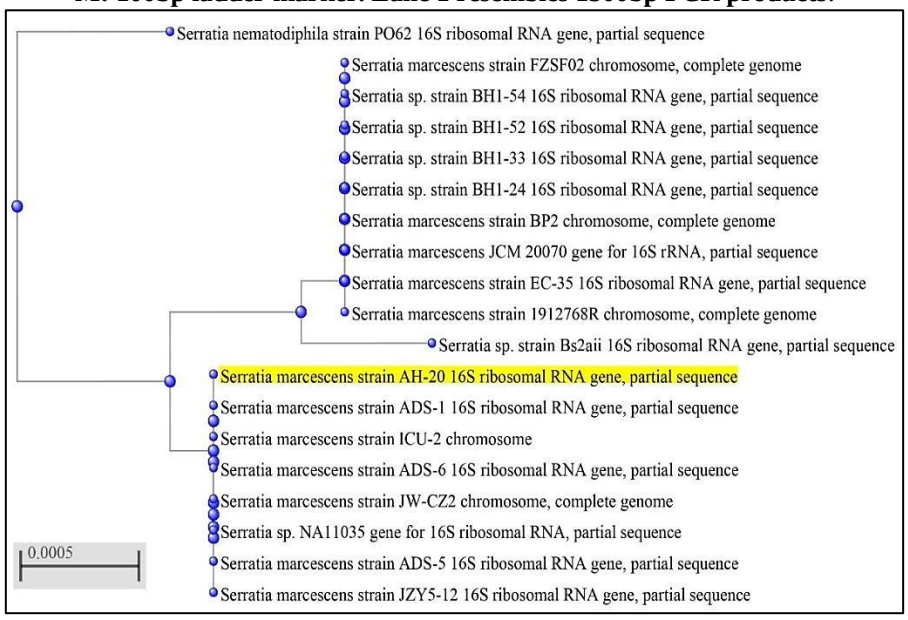


Figure (2): The phylogenetic tree based on sequencing of *16SrRNA* gene showing the relationship of *S. marcescens* AH-20 with other closely related bacterial strains and species.

Serratia marcescens Tolerance to Salinity

The AH-20 strain growth showed an inverse linear relationship with the NaCl concentration down to the 2000 mM concentration (Figure 3). The isolate was found to grow in medium supplemented with up to 11% NaCl. As a result, the rhizosphere of typical wheat plants demonstrates the ability to recruit specific strains and aid plant growth in salinity stress; nonetheless, salinity tolerance is a rare characteristic (16).

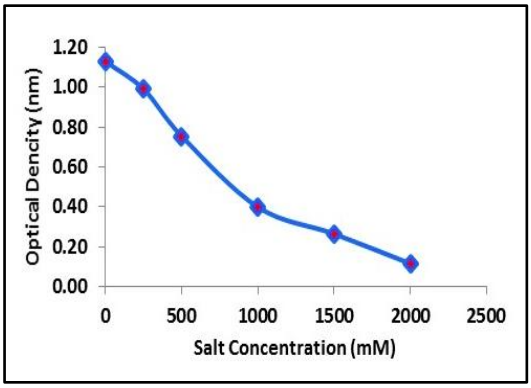


Figure (3): Salt tolerance curve of *Serratia marcescens* strain AH-20.

The ability of the isolate to survive in a saline environment was used to examine the functioning of the bacteria based on their adaptability to the target habitat. This study tested salinity tolerance with more than 300 *mM* NaCl concentration and found that the AH-20 strain was observed to be tolerant and could grow very well in the salinity level applied. Similar results were obtained by (16).

Screening for Plant Growth Promoting Traits

Detection of *DegACC* Gene Using Conventional PCR

The increase in plant growth reported by ACC deaminase-producing *S. marcecens* is supported since ACC deaminase-producing bacteria have been found to improve plant growth by decreasing ethylene levels. The presence of the gene for ACC deaminase production was confirmed using the *DegACC* primer designed to amplify the *accdS* (ACC deaminase) gene. Using this primer, a 750 bp fragment of the expected size was also amplified for the isolate (Figure 4A). The production of ACC deaminase regulates the ethylene level in plants, which contributes to stress tolerance. The test isolate had a positive result for the Accd gene, which helps to alleviate salt stress. This is confirmed by the fact that the isolate can affect plant growth promotion by decreasing the ethylene produced under salt stress (17).

Indole Acetic Acid Production (IAA)

Bacterial production of IAA is considered a major factor in enhancing plant growth (18). Indole-3-acetic acid (IAA), one of the most physiologically active auxins, is produced by 80% of PGPR which promotes several growths and developmental events, such as cell

division, elongation, and differentiation (19). Therefore, the current study tested the ability of *S. marcescens* AH-20 to synthesize indole compounds *in vitro* and verified that it produces IAA in the presence of tryptophan that resulted in the appearance of pink color in the medium (Figure 4B), and the production of IAA by the isolate was 38.23µg/ml. It is well known that IAA production is widespread among soil bacteria that inhabit the rhizosphere of plants, and IAA production promotes growth and development. Accordingly, IAA indirectly increases water and nutrient supplies leading to higher root exudation and biomass production (20).

Phosphate Solubilization

Phosphate solubilizing bacteria (PSB) play an important role in releasing phosphates from organic molecules or to solubilize insoluble inorganic phosphate. Plants absorb phosphate only as monobasic ($HPO4^{-}$) and dibasic ($H2PO42^{-}$) ions (21). The S. marcescens AH-20 showed phosphate solubilization efficiency in the qualitative assay. In the qualitative experiment, the solubilization efficiency was measured by the solubilization index (SI). As a positive result for the solubilization of the phosphate, utilized the formation of a halo. A solubilization index (SI) was calculated using the halo and colony dimensions, which is useful for estimating the phosphate solubilization capacity (Figure 4C). After 96 hours, the isolate's phosphate solubilization index was determined to be 4.88. (22) stated that the isolate S. marcescens S2I7 was found to be best as it could solubilize inorganic phosphate in Pikovaskay's agar medium. Also, (23) investigated the ability of S. marcescens to solubilize phosphate in vitro.

Nitrogen Fixation

Nitrogen is among the most limiting and critical macronutrients for plant growth and development (24). Nitrogen is converted into ammonia (plant utilizable forms) by nitrogen-fixing organisms using a complex enzyme system called nitrogenase (25). The ability of the test isolate to fix atmospheric nitrogen exhibited significant rich growth on the nitrogen-free agar medium, indicating a positive result for nitrogen fixation (Figure 4D), suggesting that the AH-20 can be beneficial in improving the growth of wheat and other plants by providing nitrogen nutrition.

Siderophores Production

Iron is a necessary component for the growth and development of all living organisms. Bacteria create siderophores and absorb iron from the environment during iron deficiency conditions. As a result, siderophores are increasingly being used in agriculture to promote plant development and protect plants against infections (26). The results of the qualitative siderophores production test revealed that the AH-20 isolate was positive for siderophore production (Figure 4E). The results are in agreement with previous studies (27, 22) that found that *S. marcescens* exhibited the production of siderophore. And with a previous study that found the ability of another type of bacteria *P. aeruginosa* KH-K10 isolate to the production of siderophore (28).

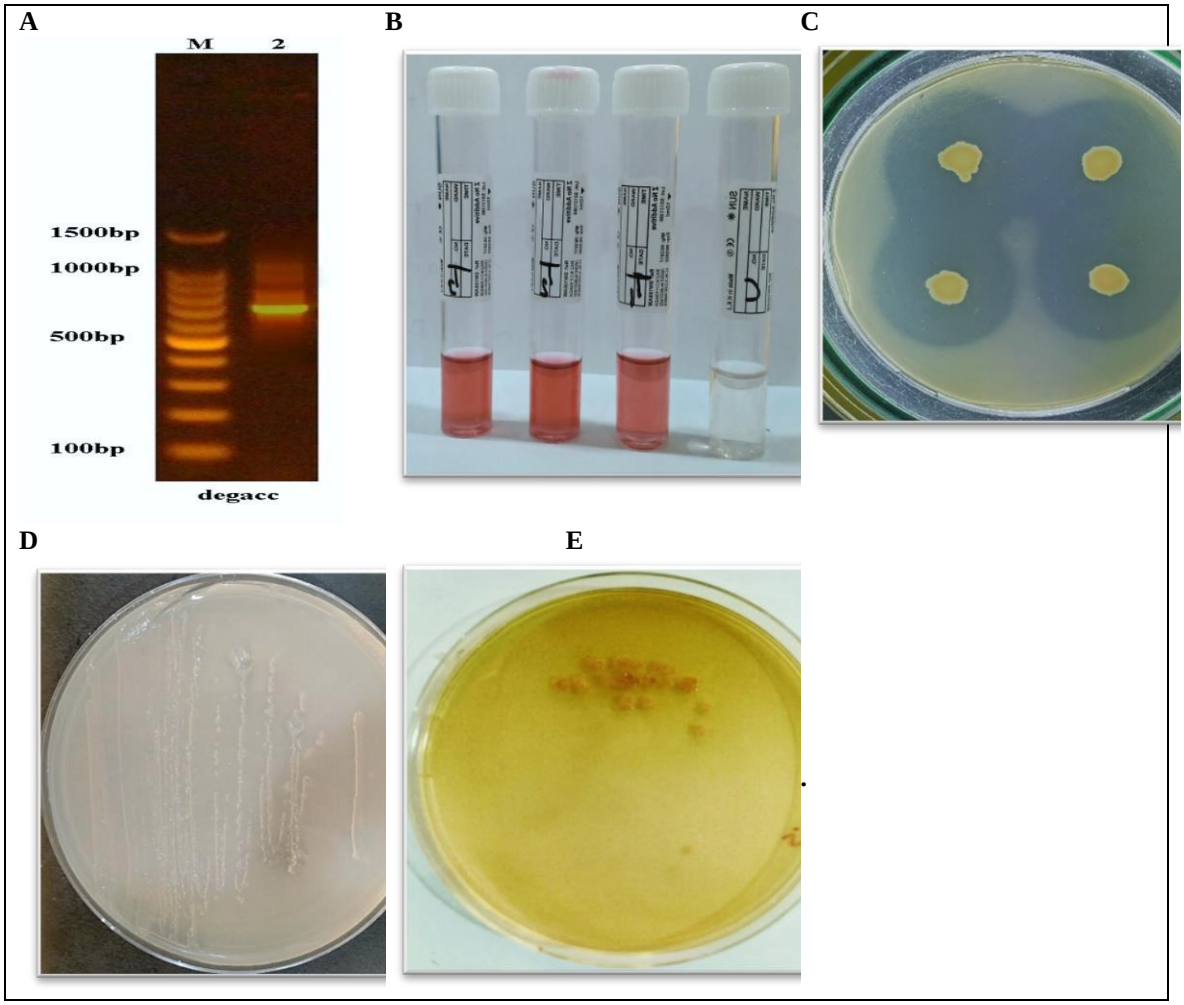


Figure (4): Plant growth-promoting production by the isolate AH-20: (A) Amplification of the *accdS* gene using the DegACC primer (B) Production of IAA (C) Phosphate solubilization (D) Nitrogen fixation (E) Siderophores production.

Effect of AH-20 Isolate on Wheat Growth under Salt Stress

The growth of wheat plants was evaluated under salt stress by pot experiments. As a result, inoculation with S. marcescens AH-20 was used to alleviate salt stress in the wheat plant. Inoculation with the test isolates promoted the growth of wheat plants exposed to various concentrations of salt (NaCl) stresses. The growth of the wheat plant was measured using various morphological and physiological parameters.

Morphological Characters

A pots experiment was conducted to evaluate whether S. marcescens AH-20 can promote plant growth, which is the overall effect of the beneficial properties of a PGPR on the host plant. Inoculation with S. marcescens AH-20 increased root and shoot plant height and root length significantly (P=0.05). Under salinity stress minimum shoot and root length (18.1 and 8.5 cm) was observed at 300 mM of NaCl stress and uninoculation with bacterial inoculation, while the maximum shoot and root

length (26.0 and 13.07cm) were observed at 100 mM NaCl and inoculation with bacterial, which not differ significantly from unstressed control, with a decrease of 30.38 and 34.97%, respectively (Figure 5A and 5B). The results revealed that salt stress reduced the shoot and root length of wheat plants, and the effect became more pronounced as salt concentrations increased, which is in line with the results of (29) who stated that wheat had slower root growth with the increase of salt concentrations, and with (30) who observed increased wheat root and shoot highest and root length per plant as compared with the un-inoculated control soil. Also, the results of the current study indicated that inoculation mitigated the effect of salt stress. Previous studies have shown beneficial effects of S. marcescens isolates on plants, such as in the mitigation of salt stress in wheat (31). From the results of this study can observe that the stress NaCl concentrations >200mM had significant effects on plant growth (Figure 4.17), which corresponded with the results of (32) who reported that the stress NaCl concentrations >200mM showed significant effects on plant height. Interestingly, soil inoculation with S. marcescens under control conditions (without salt stress) resulted in a high increase in shoot height and root length compared to uninoculated soil (Figure 5), these effects promoted by S. marcescens isolates were may be due to its ability to synthesize phytohormones that in certain amounts can stimulate or inhibit the root development of plants and as a consequence, increase the concentration of certain nutrients in the plant (33).

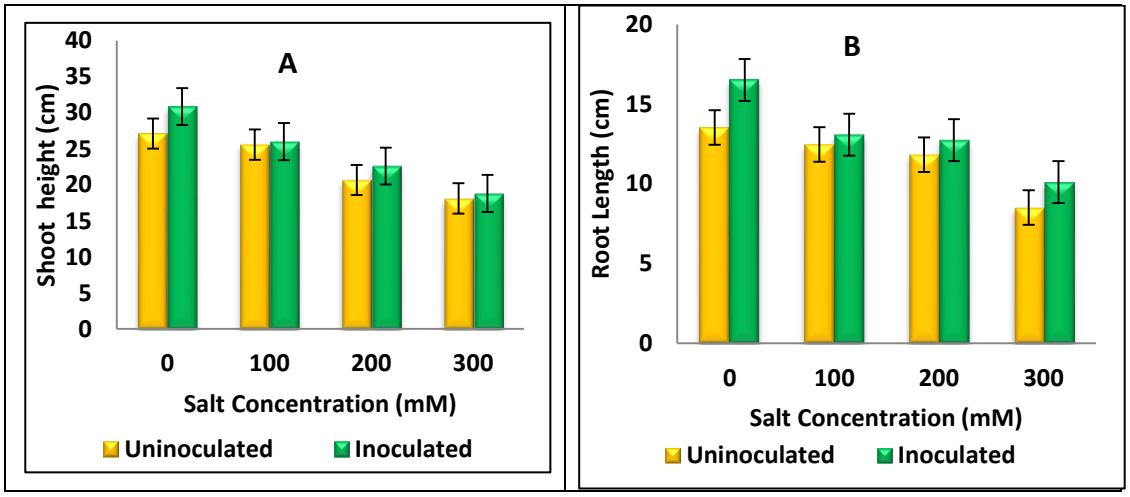
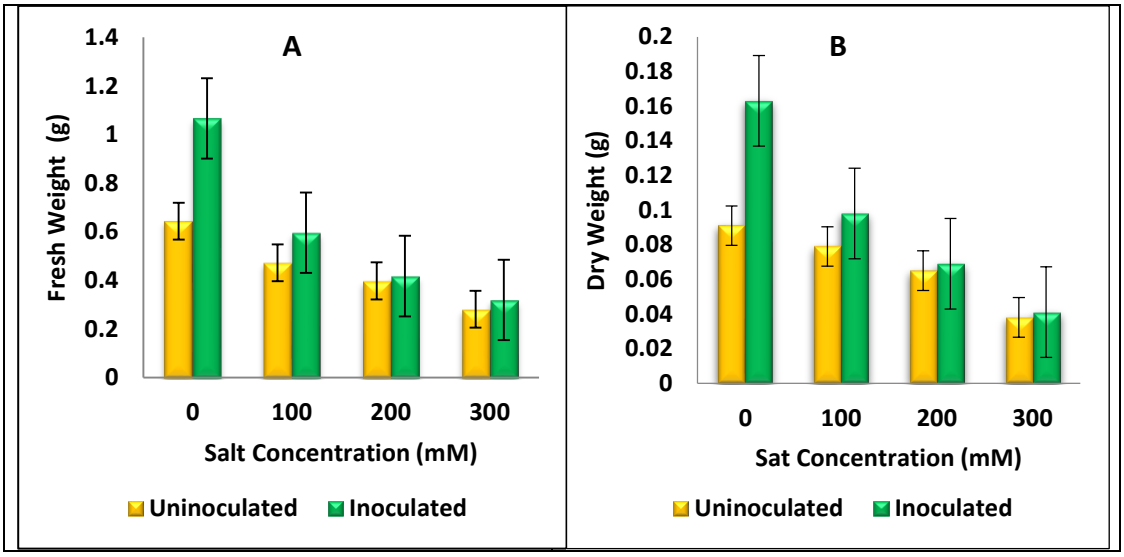


Figure (5): Effect of inoculation with AH-20 isolate on (A) shoot height and (B) root length under different concentrations of NaCl.

Salinity stress has a deleterious impact on the fresh and dry weight of wheat plants. Under 300mM NaCl stress, both un-inoculated control and bacterial treatments showed significant reductions in fresh and dry weight of wheat. The AH-20 bacterial strain was once again found to be resistant to salt stress and a growth promoter. Inoculation with the isolate AH-20 enhanced the growth of wheat plants treated with different levels of salt (NaCl) stressors. (Figure 6) demonstrates inoculation with

bacterium AH-20 significantly enhanced fresh and dry weight of wheat plants (approx. two folds) (1.066 and 0.163g) as compared to control plants (0.644 and 0.091g), respectively.

Furthermore, the growth of plants inoculated with AH-20 was evaluated under salt stress by pot experiment. In pot studies, bacterium inoculation resulted in a significant increase in fresh and dry weight of wheat plant by 42.58 and 111.35, and 50.77 and 157.89%, respectively at 100mM compared to 200 and 300*mM* (Figure 6). Similar results were observed by (34) stated that salinity (50, 75, and 100 *mM*) of NaCl stress has increased the dry weight of the aerial part. (15) found in a pot study, bacterium inoculation resulted in the highest increase in fresh weight of wheat plants. Also (30) observed an increase in root and shoot fresh and dry biomass per wheat plant as compared with the un-inoculated control soil. Drastic reduction in the fresh and dry matter by 56.21and 58.24% of wheat plants was observed due to the salt stress (Figure 6). However, the reduction in fresh weight was comparatively less. (32) observed a high reduction in shoot dry matter of the wheat due to the stress, while the reduction in root dry matter was less. Inoculation with bacteria increased the shoot dry matter, phosphorus, and nitrogen concentration (35). PGPR are capable of enhancing plant growth either directly or indirectly through multifarious ways (19).



Figure(6): Effect of inoculation with AH-20 isolate on (A) fresh weight and (B) dry weight under different concentrations of NaCl.

Leaf Chlorophyll Content

In plants, chlorophyll content is regarded as a biological marker and an indicator of salt tolerance. Under salt stress, salt-tolerant plants show maintained or increasing chlorophyll levels, but salt-sensitive plants show decreased chlorophyll levels (36). Stress condition's effects on chlorophyll content were similar to that seen in other morphological parameters of wheat plants. The total chlorophyll content of leaves of plants grown in soil

uninoculation with AH-20 isolates decreased as the salt concentration increased but did not reach the significant levels as given 1.79, 1.71, 1.64, and 1.47 mg.g-1 for control, 100, 200, and 300mM, respectively. Whereas the results showed a significant (P=0.05) increase in total chlorophyll content of leaves of plants grown in soil inoculation with AH-20 isolate (Figure 7). The biomass increment was 70.76, 78.05, and 98.64% compared with 100, 200, and 300mM NaCl, respectively. The results were in agreement with the results of (37) who revealed a significant decrease were recorded in chlorophyll and cell membrane stability with salt stress up to EC 8 dSm-1. High accumulation of sodium in plant tissues has been reported as one of the effective factors in the reduction of photosynthetic pigments and rate of photosynthesis (38, 32), due to the adverse effect on chlorophyll biosynthesis and photosynthesis process (particularly photosystem-II) in plants (39).

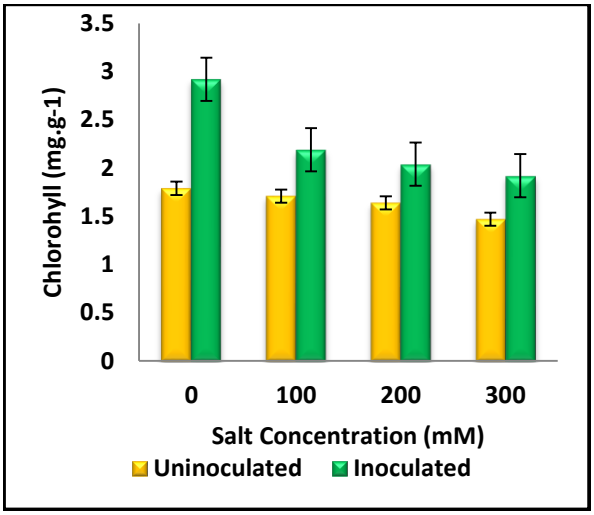


Figure (7): Effect of inoculation with AH-20 isolate on leaf chlorophyll content under different concentrations of NaCl.

In the current study, the chlorophyll contents significantly decreased under elevated salt stress concentrations >200mM either in inoculated or uninoculated soils (1.92 and 1.47 mg.g-1), respectively (Figure 7), as the chlorophyll contents are sensitive to salt exposure and a reduction in chlorophyll levels due to salt stress has been reported in previous studies (40, 37, 29).

Antioxidant Enzyme Activities

The activity of SOD in plants was increased significantly with the increase of salt levels in both inoculated and uninoculated soil. The increase was by 29.05, 96.82, and 129.11% in uninoculated soil treated with 100, 200, and 300 mM NaCl, respectively, and by 74.25, 103.30, and 138.75% in inoculated soil treated with 100, 200, and 300 mM NaCl compared with control (uninoculated and untreated with salt stress) (Figure 8A). But following the inoculation by the isolate AH-20, there was a significant increase ($P \leq 0.05$) in the anti-oxidative enzyme than in uninoculated soil at 100 mM NaCl by 35.02%. A similar increase of 34% was observed by (15), where

increased SOD activity in bacterium-treated plants can enable plants to scavenge superoxide radicals during salt stress. The SOD plays a critical role in ROS defense by converting the dismutation of O2 to H2O2 and O2, whereas the POD enzyme catalyzes the H2O2 to water (41). Interestingly, even in the absence of salinity (control), the inoculation with AH-20 isolates led to a significant increase in enzyme activity by 25.81% (Figure 8).

The activity of POD in plants was increased significantly ($P \leq 0.05$) with the increase of salt levels in inoculated soil treated with 200 and 300 mM by 11.32 and 12.20%, while the increase did not reach the significant levels in uninoculated soil treated with 100, 200 and 300 mM when compared with control treatment (uninoculated and untreated with salt stress) (Figure 8B). A significant increase ($P \leq 0.05$) in the POD enzyme was observed following inoculation of the AH-20 isolate. As shown in the figure the level of SOD increased by 8% in inoculated soil than in uninoculated soil at 200 mM NaCl. The increase in POD activity in inoculated plants was probably because the bacterial inoculation stimulated the synthesis of the anti-oxidative enzyme (15). POX activity increases contribute significantly to plant growth because the POX enzyme is involved in lignin synthesis throughout plant growth and defense mechanisms (42).

As similar to the activity of the POD enzyme, the activity of CAT also increased (Figure 8C). Where the activity of CAT in plants was increased significantly ($P \leq 0.05$) with the increase of salt levels in inoculated soil treated with 200 and 300 mM by 23.77 and 24.15%, while the increase did not reach the significant levels in uninoculated soil treated with 100, 200 and 300 mM when compared with control treatment (uninoculated and untreated with salt stress). Endophytic bacteria may alleviate salt stress in inoculated plants by altering the expression of stress-related genes (43).

(15) reported that treatment of the wheat plants with S. macescens caused a change in the activity of the oxidative enzymes (superoxide dismutase, peroxidase, and catalase) at different salinity levels, thus reducing the salinity induced oxidative damages to the plants. Many beneficial bacteria activate cellular antioxidant systems such as POD and SOD in the mitigation of oxidative damages induced by salinity has been reported (44, 45, 46).

In this study, salt stress on wheat plants increased studied antioxidant enzymes SOD, POD, and CAT, but their activities were higher when inoculated the soil with PGPB. The results showed that inoculation with S. marcescens alleviated salt-induced damage in plants by increased antioxidant enzyme activity, maybe by elimination of toxic reactive oxygen (ROS), as reported in a previous study that plant antioxidant enzymes (SOD, POD, and CAT) can eliminate oxygen radicals from intracellular membranes, minimize membrane peroxidation, stabilize membrane permeability, improved water use efficiency and promote plant photosynthesis (47).

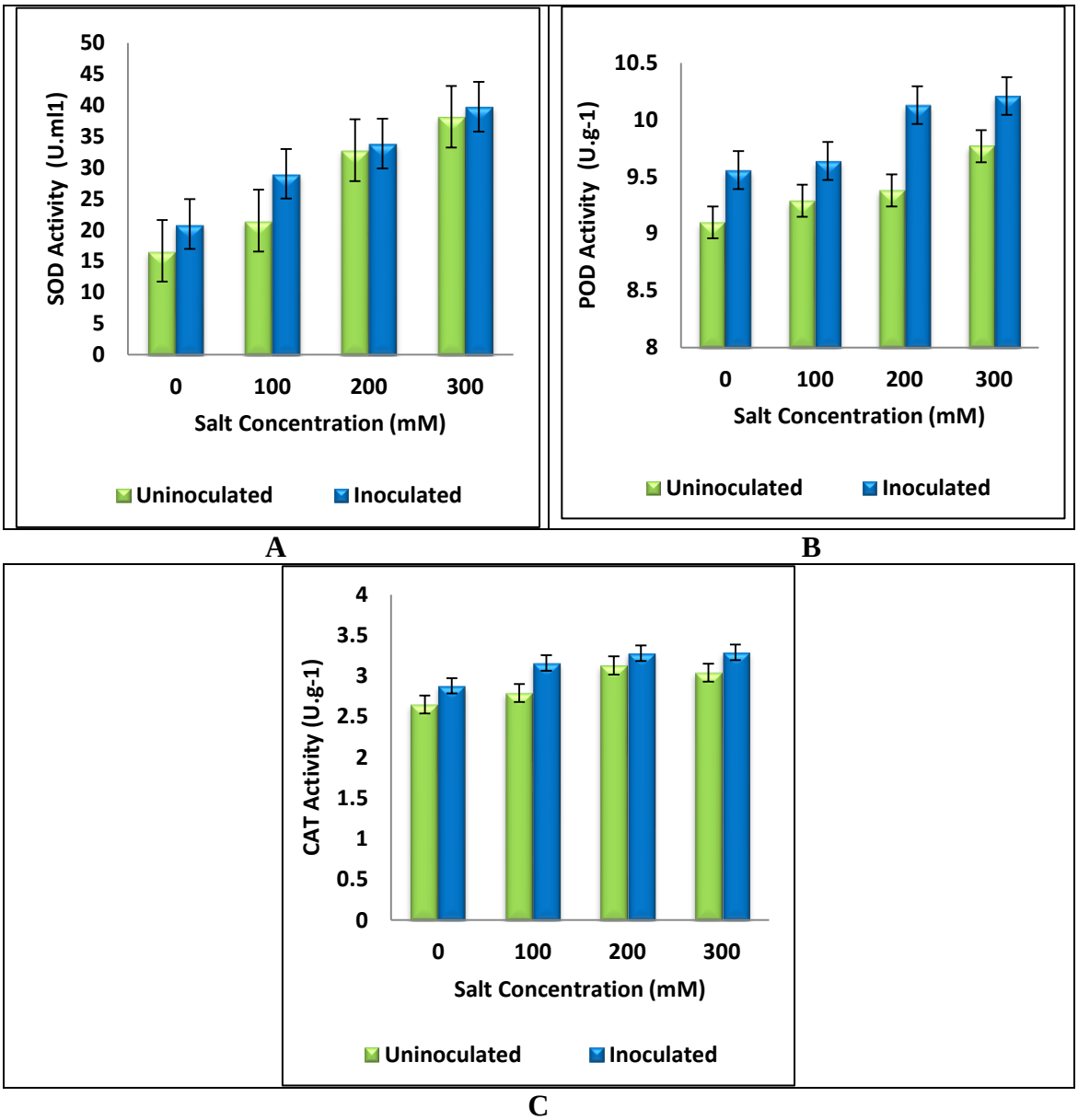


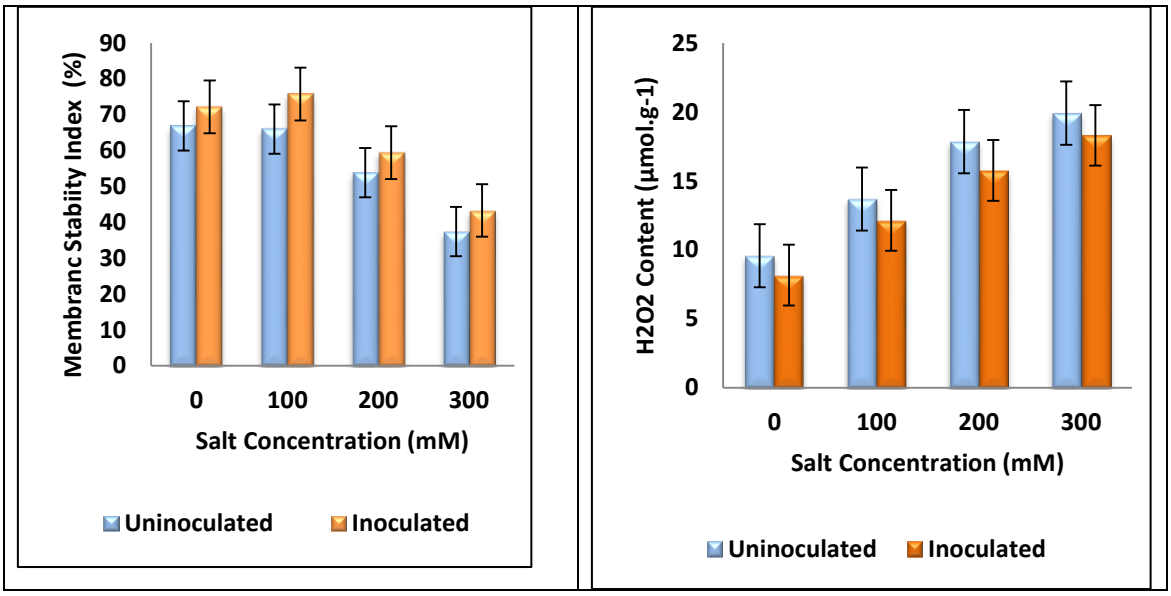
Figure (8): Effect of inoculation with isolate AH-20 on antioxidative enzymatic activities in bacterium treated and control under-tested salt stress conditions. (A) SOD activity (B) POD activity (C) CAT activity.

Oxidative Damage

The MSI of the wheat genotypes under the stress was assessed by measuring electrical conductivity/electrolyte leakage. Figure 9A releases that saline environment caused a significant ($P \leq 0.05$) decrease in membrane stability index in the leaves of wheat. The decrease reaches 38.47 and 168.52% at 200 and 300 mM. The results are following the findings of (37) with salt stress up to EC 8 dS.m-1, and with (40) who demonstrated that in most cases the EC% of wheat and bean cultivars was significantly stimulated by salt stress and EC% increased with the rise of salt level.

In comparison to bacterium-inoculated wheat plants, wheat plants

treated with various concentrations of NaCl showed a higher accumulation of H2O2. With a rise in salinity from 0 to100, 200, and 300 mM (Figure 9B), the content of H2O2 increased significantly by 42.95, 86.52, and 108.05%, respectively. These results support the results obtained by (15) that the content of H2O2increased by 39 to 62% when the salt increased to 200 mM. ROS are not dangerous for plant cells as long as their generation and neutralization in plant cells are balanced. However, abiotic stresses such as salinity upset this balance and increase ROS accumulation. The increasing H2O2content under salt stress conditions has been reported previously (48, 49, 46). On the other hand, inoculation with AH-20 isolates decreased the H2O2 concentration, especially at the treatment of 100 mM NaCl which did not differ significantly from untreated control. These data show that ROS scavenging became more efficient after bacterial treatment for improving salt tolerance in wheat plants, which comes in the line with the obtained by (15) that bacterium inoculation reduced the H2O2content in the range of 10–57%.



A B

Figure (9): Effect of inoculation with isolate AH-20 on salinity-induced oxidative damages in wheat plants with respect to generation of (A) Membrane stability index (B) H_2O_2content.

Conclusions

This research supports the hypothesis that employing the abilities of specific soil microbes may be an important contribution to enhancing plant growth, and has the potential for the betterment of salinity stress in wheat plants. Here S. marcescens AH-20 isolated from wheat rhizosphere soil can able to tolerate NaCl concentrations up to 11 %. Serratia marcescens can be used in the enrichment of substrates for plant growth promotion or as part of bio inoculants for agriculture. The oxidative stress induced by salt stress adversely impacted the wheat plants. In contrast, the implementation of S. marcescens increased the relative tolerance of the wheat plants to salt stress by decreasing

the content of H2O2, and improved the membrane stability and pigments concentration, the activity of antioxidant enzymes, and the transcription level of genes than observed in untreated.

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