Usage of some Halo bacteria species to alleviate sodium chloride toxicity in *Vigna radiate* L. cuttings in terms rooting response

K A Hussein¹, N H Kadhum¹ and M R Sharqi¹

¹Department of Biology, College of Science, University of Kerbala, Kerbala, Iraq
Email: khalid.a@uokerbala.edu.iq

Abstract. Four 1-aminocyclopropane-1-carboxylate (ACC) deaminase enzyme-producing halotolerant bacterial isolates were used to remove toxic concentration of NaCl salt (75 mM) for mung bean as an experimental system in terms of the rooting response by regulating ethylene levels in mung bean. Mung bean was supplied by combination (inoculated DF media with all isolates + Hoagland solution) before and after exposure to toxicity and at the same time with toxicity. The post-treatment was the best throughout decrease the cell membranes damage, MDA levels, stress intensity in mung bean and increased tolerance index of the mung bean and the mung bean leaves of auxin content.

1. Introduction
Soil salinity represents one of the obstacles that limited expansion of agricultural areas or increasing in agricultural production of many crops. So, support for security food is directed towards reducing the damage to environmental stresses. Known, the salt stress of soil inhibits plant growth [1]. Microorganisms are forming intracellular or endocellular colonies in natural conditions with plant [2]. These microorganisms in the Rhizosphere, especially bacteria and fungi can improve plant under stress condition directly or indirectly. Many PGPB isolated from soil and plant roots were observed to improve plant growth and productivity [3]. Several strategies have been developed to mitigate the toxic effects of high salinity on plant growth; including the use of bacteria PGPRs. Plant Growth Promoting Bacteria (PGPB) promotes growth of plant in many ways including aerobic nitrogen fixation and hormone synthesis and excretion such as IAA [4], synthesis of ACC deaminase enzyme has role in reducing response for stress ethylene plants during work 1-Aminocyclopropane-1-carboxylic acid (ACC) precursor to synthesis ethylene and convert to ammonia and α-Ketobutyrate, therefore, controlling in level of ethylene hormones in plant has the ability to produce an enzyme which is used as precursor to synthesis of ethylene (Gaseous plant hormone is a regulator and development plant growth) [5]. *Bacillus* can increase tolerance to high temperatures and high salinity concentrations on the plant uses a sustainable and environmentally friendly solution designed to use. Previous studies have indicated that some bacteria producing to ACC deaminase enzyme and interfere with plant are *Burkholderia* spp., *Klebsiella* spp., and *Pseudomonas* spp.[6], *Bacillus* spp. [7], *Enterobacter* spp. and *Ochrobactrum* spp. [8]. So, the
use bacteria producing enzyme in improving the rooting response of mung bean exposure to sodium chloride toxicity, to understand the role of these bacteria in controlling and eliminating the oxidative damage of sodium chloride toxicity and its production of ethylene stress. Finally, this study offers a new method as a commercial inoculation suitable for many stressful plants and environmentally friendly.

2. Materials and methods

2.1. Collection of soil samples
Salinity-affected soil samples were collected from various areas of holy Kerbala province and its environs and samples were taken from a depth of 5-15 cm from the area perimeter the roots of different plants [9].

2.2. Culture media and solution used in isolate of bacteria

**Nutrient Agar**: use this medium to isolate, purify and preserve bacteria prepare with 5% NaCl.

**Nutrient Broth**: prepare the medium according to the manufacturer's instructions, and this medium has been used for the purpose of re-culturing and growth bacteria.

**Normal saline**: prepare the solution by dissolving 0.85 g of NaCl in a quantity of distilled water after completing the dissolution, and then complete the volume to 100 ml. The method of isolate and purification bacteria was followed [10].

2.3. Screening bacterial isolates producing ACC deaminase enzyme

**Primary screening (Quality)**: all bacterial isolates obtained from saline soils were tested for their ability to produce ACC deaminase enzyme by growing on DF salt minimal media followed according to [11].

**Secondary screening (Quantity)**: All the positive bacterial isolates tested for primary screening were subjected to secondary screening, the ability of bacteria to produce ACC deaminase enzyme was detected according to the method [12].

2.4. Detection of Bacteria Growth in Different Concentrations of sodium chloride (NaCl)
Bacterial isolates that showed their production of ACC deaminase enzyme were grown on medium containing different concentrations of NaCl (5%, 10%, 15%, 20%) and incubated at 37 °C for 4 days and the growth of the colonies on the medium is a positive test [13].

2.5. Experiments of ACC deaminase producing bacteria on rooting response under NaCl toxicity
*Vigna radiate* L. seeds were soaked with tap water after overnight, cultured with homogeneous lines on sawdust as a medium for cultivation and watering Hoagland solution in growth cabinet (Binder KBW Plant Growth Chambers) it is characterized standard conditions (temperature 25±1, Intensity of light 1600-1800 Lux and relative humidity 60-70%). Hoagland solution was added according to the requirement until 10-day. Cuttings were prepared according to [14]

2.6. Determination of NaCl toxicity to Mung bean in terms of rooting response
The basal parts of the cutting were treated with test solutions by placing the basal parts of the cutting in glass vials. Each treatment included three replicates (vials), each vial contain four
cutting, as (12 cuttings per treatment). The hypocotyl of cuttings with length of 3 cm after removing of root system. These cuttings were treated with different concentrations of NaCl (25-300) mM for 24 h. then transferred to Boric acid (5 µg/L) as rooting medium for 6-days under the same conditions that used for seedlings development. Thereafter, the average of root number was calculated per cutting.

2.7. Determine the optimum treatment in the cutting rooting response

The hypocotyl was treated with test solution for 24 hours, distilled water (control), Hoagland solution, DF Media, DF inoculated bacteria, Hoagland solution with DF inoculated bacteria, and then transferred to medium containing boric acid solution for six days. Thereafter, the average of root number was calculated per cutting.

2.8. Determine the optimum treatment and use it in NaCl detoxification

Mung bean cuttings were treated with distilled water (control) and the optimum treatment of the rooting and toxic concentration of NaCl for 24 hours after which it was transferred to the rooting medium for six days and counting the number of roots in the cutting, and also the cutting was treated with the optimum treatment of rooting (within the first 24 hours) before the toxic NaCl concentration (within the second 24 hours) on the other hand, the cutting was treated together with the optimum treatment and the toxic concentration of NaCl for 24 hours and then transferred to the rooting medium for 5 days. Thereafter, the average of root number was calculated per cutting.

2.9. Effect of treated treatment on NaCl stress in terms of rooting response, Stress intensity and endurance evidence, membrane damage, Malondialdehyde and auxin content

Measuring the intensity of stress according to [15], measurement of electrolyte leakage in [16]. Malondialdehyde content was estimated by [17]. Determination of auxin, it was determined by the method in [18].

2.10. Statistical analysis

completely randomized design (CRD) was employed statistically and LSD was depended for comparison between the treatment’s mean for 3 replicates on P<0.05 level [19].

3. Results and Discussion

3.1. Screening Quality and Quantity

Seven soil samples collected in this study from saline areas, 32 saline tolerant bacterial isolates were obtained by testing their growth on nutrient agar medium with 5% NaCl. Primary screening results showed that the growth of bacterial colonies on the DF media. It was observed that only 9 isolates were positive for this test, i.e., using ACC as a source of nitrogen. Based on that isolates no-growth on DF salt media were excluded while primary screening test for secondary screening for the purpose of determining the most efficient ones in ACC deaminase enzyme production by determining its quantity by color method. Table (1) shows that.
Table 1. Secondary screening for the production of ACC deaminase by PGPB.

| No. | Symbol | Enzyme activity |
|-----|--------|-----------------|
| 1   | 35     | 0.62            |
| 2   | 9      | 0.87            |
| 3   | 31     | 0.52            |
| 4   | B      | 0.91            |
| 5   | A      | 0.37            |
| 6   | 55     | 0.83            |
| 7   | 50     | 0.61            |
| 8   | 46     | 0.90            |
| 9   | D      | 0.28            |
| L.S.D. at 0.05 |        | 0.027          |

Effectiveness was highest in isolates (B, 46) (0.91, 0.90) unit respectively, followed by isolation 9, which gave an activity of 0.87, and then recorded isolation 55, which recorded an enzymatic activity of 0.83. The most effective isolates (four isolates) were selected in subsequent experiments while the other isolates were excluded.

3.2. The ability of bacteria to grow in different concentrations of NaCl salt

The effect of several concentrations of NaCl (0, 5, 10, 15, 20)% on the growth of bacterial isolates was shown to contain the ACC deaminase enzyme (ACC +). The results showed that the salt tolerance of the bacteria was different for bacterial isolates. The results show in table (2). The growth of all bacterial isolates at low concentrations of 5% NaCl was noticeable but less compared to NaCl (0.0). It was observed that the isolates (B, 46) were able to grow very little at the concentration of 15% NaCl compared to other isolates that could not grow at the same concentration. It can be said that the two isolates (46, B) of the most isolates are resistant to salinity, while bacterial isolates (9, 55) were considered isolates tolerant to salinity, which grew at a concentration of 10% NaCl (Siddikee et al., 2010). No growth was observed for all bacterial isolates in the NaCl 20% saline concentration.
Table 2. The ability of bacterial isolates to grow in different concentrations of NaCl.

| No. | Symbol | 0.0% | 5%  | %10 | 15% | 20% |
|-----|--------|------|-----|-----|-----|-----|
| 1   | 55     | +    | +   | -   | -   | -   |
| 2   | 9      | +++  | ++  | +   | -   | -   |
| 3   | A      | ++   | +   | -   | -   | -   |
| 4   | B      | ++++ | +++ | ++  | +   | -   |
| 5   | 31     | ++   | +   | -   | -   | -   |
| 6   | 50     | ++   | +   | -   | -   | -   |
| 7   | 35     | ++   | +   | -   | -   | -   |
| 8   | 46     | ++++ | +++ | ++  | +   | -   |
| 9   | D      | ++   | +   | -   | -   | -   |

+ growth - non growth.

3.3. Determine the toxic concentration of NaCl in the cutting of Mung bean

Table (3) indicates the effect of different NaCl salt concentrations on rooting responses of mung bean cuttings which taken from the seedling growing in light for ten days. The control sample (distilled water) cuttings developed 11 roots / cutting.

The cutting treated with different concentrations of NaCl (25, 50, 75, 100, 150, 200, 300) m mol for 24 hours was inhibitory for rooting except the low concentration 25 m mol was a catalyst for rooting 13.33 with rate an increase of 21.18%, as for concentration from 50 up to 100 had a toxic effect on growth indicators in the mung bean cuttings, and concentrations from 150 upwards were lethal and caused 100% inhibition. On this basis, the low concentration of 75 m mol was considered the toxic concentration (reduced in terms of rooting response to approx. 50%). was considered in subsequent experiments.

Table 3. Determination of sodium chloride toxicity in terms of rooting response.

| NaCl Conc. m mol/ml For 24 hr. | Mean Root number/cutting |
|-------------------------------|--------------------------|
| 0.0 (D.W.)                    | 11.00                    |
| 25                            | 13.33                    |
| 50                            | 7.33                     |
| 75                            | 5.33                     |
| 100                           | 2.67                     |
| 150                           | 0.00                     |
| 200                           | 0.00                     |
| 300                           | 0.00                     |
| L.S.D. at 0.05                | 0.76                     |

Table (4) indicates the effect of bacterial isolation, components of DF media and Hoagland solution at half strength in the rooting response of mung bean cuttings. The cutting of the control sample (distilled water treatment) revealed (9, 8.5, 9.5, 10.5) roots/ cutting for
followed isolate (9, 46, 55, B), respectively. When treating the cutting for 24 h. with the toxic concentration of NaCl, the number of roots was approximately half the number compared to control. The cuttings treated with a Hoagland solution during 24 h. revealed 13.5, 14.5, 13, 15.5 roots/cutting for each of the following isolates (9, 46, 55, B) respectively and was a significant stimulus in the rooting response. Providing the cuttings with the nutrition medium inoculated with bacteria is a catalyst for response rooting. The number of roots was rounded to the number of roots in the Hoagland solution. The effect of the enrichment medium did not significantly affect the mean number of transverse roots when compared statistically at a probability level of 0.05 with the control cutting and the optimum response was in the combination (Hoagland's half-force solution and DF media inoculated with bacteria giving (22.5, 19, 17.5 and 19.5) roots at rate increase of (150, 123.53, 78.95, 85.71) % over the comparative treatment which was subsequently adopted by subsequent experiments.

Table 4. Determine the optimum treatment in the Mung bean cutting rooting response.

| Treatment with d/H2O for 24h | Isolate B | Isolate 55 | Isolate 46 | Isolate 9 |
|------------------------------|-----------|------------|------------|-----------|
| Toxic NaCl                   | 6.5       | 5.5        | 4.5        | 5         |
| Hoagland solution            | 15.5      | 13         | 14.5       | 13.5      |
| DF media                     | 10.5      | 10         | 10.5       | 11        |
| DF inoculated bacteria       | 16.5      | 13.5       | 15         | 14.5      |
| Hoagland solution +DF inoculated bacteria | 19.5 | 17.5 | 19 | 22.5 |
| L.S.D at 0.05               | 1.12      | 0.90       | 2.59       | 2.55      |

3.4. Determine the best way to remove NaCl toxicity

3.4.1. Hoagland solution half strength and DF media inoculated with bacterial isolation B
The results in table 5 show the effect of the combination (Hoagland's half-strength solution and DF media inoculated with bacterial isolation B when prepared before, after and together with the toxic concentration of NaCl in rooting the mung bean cutting. The number of roots treated with the toxic concentration of NaCl developed half the number of roots in the control cutting, which developed 13.5 roots/cutting, while the effect of the combination was significant and stimulating when processed alone for 24 hours as the number of roots of 22 roots. The processing of the cutting with the treated treatment (24 h) after the toxic concentration of NaCl (24 h) was more stimulating than the opposite treatment, ie before the toxic NaCl (24 h) the first was 19 roots and in the second case 17.5, both of which had a significant effect when compared with a cutting of control. The processing of the cutting with the treated treatment and the toxic concentration of NaCl together (for 24 hours) there is no significant difference when compared with the control cutting. Therefore, the optimum treatment method is when the combination is processed after NaCl, which has an increase of 171.43% compared to the control cutting.

3.4.2. Hoagland solution half strength and DF media inoculated with bacterial isolation 55
The results in same table show that treatment of cattle brains for 24 hours with distilled water revealed (12.5 roots). The processing of the treatment individually within 24 hours, Hoagland's half-force + DF media inoculated with bacteria 55 had a catalytic effect (18 roots) and for toxic NaCl it had inhibitory effect (6.5), both of which were statistically significant. On the other hand, the processing of the treated treatment during the first 24 hours before NaCl during the second 24 hours, or vice versa, the effect was catalytic in both cases 15 roots (The first case) and 16.5 (in the second case) became close to treatment. However, when processing NaCl and treated together, it removes the toxicity of NaCl by raising the rooting response from 6.5 (toxic concentration) to 13.5 root/ cutting by an increase of 107.69%. But it did not reach the optimum treatment used in the treatment, which raised the rooting response to an increase 176.92% compared to the toxic concentration of NaCl which was adopted in detoxification in subsequent experiments.

3.4.3. Hoagland solution half strength and DF media inoculated with bacterial isolation 46
Table (5) shows the effect of treatment with Hoagland solution with half strength and DF media inoculated with bacterial isolation 46 when prepared before, after and together with the toxic concentration of NaCl in rooting the mung bean cutting. The control cutting treated with distilled water revealed 9.5 root/ cutting to nearly double the number of roots treated in the toxic concentration of NaCl, which revealed 5.5 roots per cutting. The treatment of the cuttings with the treated treatment (within the second 24 hours) after exposure to the toxic concentration of NaCl (during the first 24 hours) had a significant stimulating effect in the rooting response at a probability level of 0.05 for the treated treatment before exposure to the toxic concentration of NaCl and inhibit the number of roots when compared statistically, by treatment with 16.5 roots/ cutting. In addition, equipping the cutting together with the treated treatment and toxic concentration of NaCl, it removes the toxicity of NaCl and there is no statistical difference when compared to the control cutting. Therefore, all pre-treatment and post-treatment simultaneously removes NaCl toxicity and raises the rooting response, but post-treatment after NaCl toxicity outweighed the treatment coefficients and raised the rooting response to a higher level. So this treatment was used to detoxify in subsequent experiments.

3.4.4. Hoagland solution half strength and DF media inoculated with bacterial isolation 9
The results in Table (5) show that the highest rooting response was in the treated treatment (Hoagland solution with half strength + DF media inoculated with bacteria) 9 for 24 hours after transfer to boron 5 mg/ ml for 6 days used alone and number of roots 21.5 roots. Since we are in the process of using the combination to remove the toxicity of NaCl, which gave a higher number of roots after exposure to the toxic concentration of NaCl (for the first 24 hours) in which the number of roots revealed 17 roots, an increase of 240% compared with the cutting treated with toxic concentration of NaCl by an increase of 61.90 % compared with cutting control while the effect of the combination before exposure to toxic concentration NaCl was also catalytic, but a lower rate of increase of 38.09% compared to the control cutting, which has a number of roots of 10.5 roots. The processing of the cutting together with the toxic synthesis of NaCl was inhibited by the number of roots compared to the treatment cutting before and after NaCl.
Table 5. The effect of bacterial isolates, Hookland solution and DF media components on NaCl detoxification when processed before, after and together.

| Treatment for 24 h with | Subsequent treatment | Average number of root with B isolate | Average number of root with 55 isolate | Average number of root with 46 isolate | Average number of root with 9 isolate |
|------------------------|----------------------|--------------------------------------|---------------------------------------|----------------------------------------|---------------------------------------|
| d/H2O                  | 5 mg/ml boron for 6 days | 13.5                                 | 12.5                                  | 9.5                                    | 10.5                                  |
| Toxic Conc. of NaCl    | 5 mg/ml boron for 6 days | 7                                    | 6.5                                   | 5.5                                    | 5                                     |
| Hoagland solution half strength and DF media inoculated with bacterial isolation | 5 mg/ml boron for 6 days | 22                                   | 18                                    | 16.5                                   | 21.5                                  |
| Hoagland solution half strength and DF media inoculated with bacterial isolation | NaCl toxic (75mM) for 24 h 5mg/ml boron for 5 days | 17.5                                 | 15                                    | 11                                     | 14.5                                  |
| Toxic NaCl (75mM)      | Hoagland solution half strength and DF media inoculated with bacterial isolation for 24 h 5 mg/ml boron for 5 days | 19                                   | 16.5                                  | 13.5                                   | 17                                    |
| Hoagland solution half strength and DF media inoculated with bacterial isolation + Toxic NaCl | 5 mg/ml boron for 6 days | 13                                   | 13.5                                  | 10.5                                   | 10                                    |
| L.S.D. at 0.05         |                      | 1.2                                  | 1.09                                  | 2.28                                   | 1.5                                   |

3.5. Detoxification of sodium chloride in terms of stress intensity.

Figure 1 shows the stress intensity of the toxic NaCl toxic concentration in the mung bean cutting, where the intensity of the cutting stress exposed to the toxic concentration was NaCl 0.51 when treating the cutting with treatment DF media + Hoagland solution + bacteria (within the second 24 hours) after exposure to toxic concentration (during the first 24 hours), where the severity of stress in the cutting treated with the following isolates (46, 55, 9, B) respectively and reached (0.29, 0.27, 0.21, 0.18) with a decrease of 43.14, 47.06, 58.82, 64.71) % for each of the above isolates. So bacterial isolation B is superior to the rest of the isolates, which reduced the stress intensity to 0.18.
The intensity of stress in the Mung bean cuttings treated with the toxic concentration of NaCl and the combination of DF media + Hoagland solution + bacteria.

3.6. Detoxification of sodium chloride in terms of tolerance index

The results in Fig. 2 indicate tolerance index of sodium chloride stress and treated treatment (Hoagland’s half-strength solution and DF media inoculated with bacterial isolates (B, 9, 55, 46) within 24 hours after exposure to the toxic concentration of NaCl (within the first 24 hours) for the cutting of the mung bean, the value of stress tolerance in the brain exposed to treat DF media+ Hoagland solution+ bacteria were highest. This indicates the role of ACC deaminase-producing bacteria in tolerating saline stress. The lowest evidence in the cutting was treated with isolation 46, where it reached 1.08 which gave the highest stress while the tolerance index in the exposed brain was 9 (within the second 24 hours) after exposure to the toxic concentration of NaCl during the first 24 hours, it was nearly the same when compared statistically at a probability level of 0.05.

3.7. Remove NaCl toxicity as a function of plasma membrane damage in the mung bean cutting

Table (6) indicates that the percentage of relative damage in the plasma membrane caused by NaCl in terms of electrical conductivity (EC) in leaf tissue cells where the rate of damage was 47.16% in the leaves of cutting treated with toxic NaCl and the increase was statistically significant at a significant level 0.05 for control (22.13% Treatment of DF media + Hoagland solution + bacteria after exposure to the NaCl toxic concentration caused a reduction in the plasma membrane damage rate from 47.16% to (23.88, 22.57, 25.57, 22.47) % for each of the following isolates (9, B, 46, 55), respectively. There are not significant differences when compared statistically with the control cutting, in other words, the values of (EC) in treatment...
Bacteria + Hoagland solution + DF media was almost equal to their values in the control cutting.

Table 6. Relative damage to the plasma membrane Electrolytic leakage caused by NaCl

| Treatment for 24h with                        | Electrolytic leakage% |
|---------------------------------------------|-----------------------|
| Cutting in d.w for 24h                      | 22.13                 |
| Cutting in NaCL 75mM for 24 h               | 47.16                 |
| NaCL for 24hr.→ Cutting in(9+ Hoagland solution+ DF media) for 24 hr. | 23.88                 |
| NaCL for 24hr.→ Cutting in(B+ Hoagland solution+ DF media) for 24 hr. | 22.51                 |
| NaCL for 24hr.→ Cutting in(46+ Hoagland solution+ DF media) for 24 hr. | 22.57                 |
| NaCL for 24hr.→ Cutting in(55+ Hoagland solution+ DF media) for 24 hr. | 22.47                 |
| L.S.D. at (0.05)                            | 3.73                  |

3.8. The role of better treatment (Bacteria + Hoagland solution + DF media) in the removal of NaCl toxicity in terms of MDA content

The results indicated in table (7) show the effect of Hoagland solution and DF media inoculated with bacteria, reducing the effects of NaCl toxicity on the content of mung bean cutting leaves in light for 10 days. The results showed that MDA content increased in the leaves of the cutting treated with the toxic concentration of NaCl to 47.69µg/g tissue plant compare with cutting treatment at concentration 26.25 µg/g of wet weight with a statistical difference of 0.05% and rate increase of 81.68%. In order to treat NaCl toxicity, the combination of Hoagland solution and DF media inoculated with bacteria after exposure to the NaCl toxic concentration decreased the MDA content in the leaves of cutting to (29.32, 27.36, 27.10, 28.46), respectively for each treatment treated with the following isolates (55, 46, B, 9), respectively. MDA content is come close to its value in the control treatment with the survival of statistical differences indicating the role of the bacteria producing ACC deaminase enzyme in inhibiting lipid oxidation in cellular membranes and thus decreasing final lipid oxidation products in terms of MDA content, thus maintaining the integrity of biological membranes.
Table 7. Effect of NaCl toxicity on MDA content of cattle cutting and treatment with Hoagland solution and DF media inoculated with bacteria.

| Treatment for 24h with | mg/g F.W |
|-----------------------|----------|
| Cutting in d.w for 24h | 26.25    |
| Cutting in NaCL 75mM for 24 h | 47.69    |
| NaCL for 24hr.→ Cutting in(9+ Hoagland solution+ DF media) for 24 hr. | 28.46    |
| NaCL for 24hr.→ Cutting in(B+ Hoagland solution+ DF media) for 24 hr. | 27.10    |
| NaCL for 24hr.→ Cutting in(46+ Hoagland solution+ DF media) for 24 hr. | 27.36    |
| NaCL for 24hr.→ Cutting in(55+ Hoagland solution+ DF media) for 24 hr. | 29.32    |
| L.S.D. at (0.05) | 2.86     |

3.9. The role of the Bacteria + Hoagland solution + DF media) in removing NaCl toxicity in terms of Indole Acetic Acid (IAA) content

It is noted in table (8) that the content of acetic acid indole decreased significantly and statistically difference on the level of probability p 0.05 leaves Mung bean cutting exposure to sodium chloride toxicity 1.78 µg per g of wet weight and the decrease is estimated at (41.83%) compared to control cutting (3.06) µg per g of wet weight. The IAA content of the cutting leaves treated with Hoagland solution and DF media inoculated with bacterial isolates increased after exposure to NaCl to (3.95, 4.07, 3.18, and 3.45) when isolates related to (9, B, 46, 55) respectively. The IAA content increased significantly and overcame on the control cuttings, that is, the cutting in a treatment responded as if the cutting was not stressful and restored content, but increased significantly from the auxin IAA.

Table 8. Effect of NaCl toxicity on IAA content of Mung bean cutting and treatment Bacteria + Hoagland solution + DF media.

| Treatment for 24h with | IAA µg/g F.W |
|-----------------------|--------------|
| Cutting in d.w for 24h | 3.06         |
| Cutting in NaCL 75mM for 24 h | 1.78         |
| NaCL for 24hr.→ Cutting in(9+ Hoagland solution+ DF media) for 24 hr. | 3.95         |
| NaCL for 24hr.→ Cutting in(B+ Hoagland solution+ DF media) for 24 hr. | 4.07         |
| NaCL for 24hr.→ Cutting In (46+ Hoagland solution+ DF media) for 24 hr. | 3.18         |
| NaCL for 24hr.→ Cutting in(55+ Hoagland solution+ DF media) for 24 hr. | 3.45         |
| L.S.D. at (0.05) | 0.17        |

3.10. Diagnosis of bacteria

The results of the diagnosis and through the characteristics of cultural and microscopic and biochemical tests showed that all the bacteria obtained that producing ACC deaminase enzyme and saline tolerant belong to the genus Bacillus spp.. The results of isolation 46 indicate that they are related to Bacillus licheniformis, whereas the characteristics of isolates B and 55 are closer to Bacillus subtilis. The results show in the table 9.
Table 9. Results of biochemical tests to diagnose isolates B, 55, 46 and 9

| Test                              | 55·B | 46  | 9  |
|-----------------------------------|------|-----|----|
| Motility test                     | +    | +   | +  |
| Catalase test                     | +    | +   | +  |
| Oxidase test                      | +    | +   | +  |
| Growth in anaerobic               | +    | +   | +  |
| Casein hydrolysis test            | +    | +   | +  |
| Nitrate reduction test            | +    | +   | +  |
| Gelatin liquefaction test         | +    | +   | +  |
| Urease test                       | -    | -   | +  |
| Methyl red test                   | +    | -   | -  |
| Voges-proskauer test              | -    | +   | +  |
| Citrate utilization test          | +    | +   | -  |
| Indole test                       | -    | -   | -  |
| Sugar fermentation test (Mannose, Maltose and Glucose) | +    | +   | +  |
| Mannitol, xylose and arabinose    | +    | +   | -  |

Bacteria stimulate of plant growth, PGPR are beneficial bacteria that have the ability to forming colonies with plant roots and improve plant growth with direct and indirect mechanics, which is associated with their ability to produce IAA and ACC deaminase enzyme.

Salinity is one of the most important environmental stresses that negatively effect on plant growth and productivity. One of the modern and appropriate solutions to the problem of salinity is the use of the positive bacteria of ACC (+). The study found that 9 isolates obtained from saline soils were positive (ACC +). It was able to grow on the DF medium containing ACC as a carbon source, which was tested for tolerance to NaCl salt levels. It was found that all the positive isolates of ACC were able to grow at a concentration of 5%, and 44.44% of the isolates able to grow in concentration 10% and only two isolates were able to grow in concentration 15%, in the rate of 16%, while isolates growing in concentration was 20% was 0%. Bacterial isolation of high salinity levels may have resulted from the synthesis of protection and adaptation factors to environmental conditions. [13] found only 6 isolates from 132 salinity-tolerant isolates growing in concentration 20%. Mentioned [20] the salinity-tolerant bacteria that can challenge the high salt concentrations of NaCl 1.75 M and can improve plant growth in the presence of growth-inhibiting concentrations. Soil salinity plays an important role in microbial diversity and environmental stress leading to the reduction of bacterial diversity [21].

The level of NaCl toxicity in the Mung bean cutting was determined based on the reduction of growth indicators by the number of transverse roots detected in the cutting to a half as well as the emergence case of poisoning through morphological symptoms. The results showed that the number of roots was reduced by approximately half in the toxic concentration of NaCl (75 μg /ml) 5.33 cutting/ root compared to control cutting, which detected 11 cutting/ root with a rate decrease of 51.54%. The decrease in rooting response is attributed to the decrease in the essential growth requirements of growth regulators, proteins and carbohydrates, and the lack of water absorption due to saline stress [22]. Due to the oxidative
processes of free radicals (ROS) that increased when exposed to the toxicity of NaCl which negatively reflected in the rooting response.

PGPR and ACCD-producing bacteria stimulate plant growth and development under stress conditions by reducing ethylene stress levels [23]. In this study four saline-tolerant ACCD-producing isolates (high NaCl concentrations) were selected for experiments to detoxify or reduce the toxicity of NaCl in Mung bean cutting and biochemical tests showed that the isolates are on the other hand, the control of the phenomenon of poisoning with NaCl has been prepared with the cutting treatment with DF inoculated with bacterial isolation + Hoagland solution) as the optimum for the treatment of toxicity.

When the treatment was processed before and after and together with the toxic concentration of NaCl (at the same time) table (5), it was found that the combination of the four isolates had a catalytic effect compared to the control (distilled water treatment) and that the toxic concentration of NaCl had an inhibitory effect compared to the control and combination affecting NaCl toxicity when Pre- and post-NaCl or at the same time but capable of better detoxifying NaCl when processed after NaCl (ie a therapeutic role) as well as inducing an increase in rooting response where an increase of rooting % from control is estimated table (5). So isolatesboron toxicity when prepared after toxic exposure (post-treatment) preparation, the combination was depending on the detoxification experiments.

An important indicator of damage to NaCl toxicity is broken to biofilms due to membrane lipid oxidation processes and thus increased MDA, which results from lipid peroxidation and this oxidation, indicators of damage to membranes under stress conditions [24]. In particular, metal stress where free radicals break down biofilms, and cutting exposed to sodium chloride toxicity showed an increase in MDA levels.

When the combination inculcated with the ACC deaminase bacterial isolation after exposure to NaCl toxicity, prevented the oxidation of lipids by MDA and thus maintained its level as unstress in the cutting, ie the bacteria reduced the MDA content. Any that treatment with these isolates reduced the effects of oxidative stress induced by saline stress through the decrease of these indicators of low MDA table 7 and decrease electrical conductivity table 6 and decrease the intensity of stress figure 1 and increase the tolerance index figure 2 and maintain the content of auxin table 8 (rooting hormone), and this indicates that the bacterial isolates producing the enzyme ACCD have maintained the integrity of the plasma membranes, this is positively reflected on the mind rooting response.

4. Conclusion
NaCl toxicity caused great changes in the studied indicators that are correlated with physiological and biochemical results and when inoculation of the cutting with saline-tolerant bacterial isolates producing ACCD inhibited the adverse effects of toxicity NaCl, this is clear by reducing damage in cell membranes and levels MDA and the intensity of stress in the cutting and increase the guide endurance of the cutting and the content of the leaves of the cutting of auxin.

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