The Role of inoculation with *Rhizobium leguminosarum* in properties of plant (*Vicia faba*) under different levels of salinity

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Abstract. An experiment was conducted to study the role of inoculation with *Rhizobium leguminosarum* and soil salinity levels (4, 6, 8 or dsm⁻¹). Their interrelationship with the chlorophyll content and the weight and number of root nodes of the *Vicia Faba L.* plant. *Rhizobium leguminosarum* bacteria were isolated from soil grown in the previous season with *Rhizobium* with salts 4, *Rhizobium* with salts 6, *Rhizobium* with salts 8, and Without fertilization. The experiment results showed that the isolates were superior to the rhizobia isolates, it showed also that RS1 isolates were superior to RS2 and RS3 in most studied traits, with the highest rate of root nodules (22.33 node. Plant⁻¹) and the dry weight of root nodules (157.53 mg.plant⁻¹) salinity levels were significantly higher than the lowest level of S1 salinity. The results showed that the levels of 4ds⁻¹ at high levels (6 and 8 ds.m⁻²) were higher than those of chlorophyll content, number of root nodes, The chlorophyll content (20.32 mg.gm⁻¹ fresh weight), The number of root nodules and the dry weight of the root nodule (22.50 nodules.Plant⁻¹ and 159.06 mg.plant⁻¹). The interaction between *Rhizobium leguminosarum* and 4ds.m⁻¹ salinity was higher in all studied traits. Interference between isolation R and S1 was highest in dry weight of root nodule, number of root nodules and chlorophyll content (184.30 mg.plant⁻¹, 27.00 node. Plant⁻¹, 22.15 mg.gm⁻¹) respectively.

1. Introduction

Bacterial inoculation is one of the most important techniques used in agriculture because it is important to improve the agricultural production of leguminous crops. The process of fixation of atmospheric nitrogen by rhizobia bacteria is the most important biological process of the reduces atmospheric nitrogen to [12]. The greater part of the plant's need for nitrogen can be represented by atmospheric nitrogen fixation in legume plants. The amount of bio accumulated nitrogen is estimated at 114 million tons per year and the amount of legumes proven by about 80 million tons per year [3]. The symbiotic relationship between rhizobia and plant depends on many vital and non-vital factors. Salinity is one of the most important problems in arid and semi-arid regions. It affects the growth and production of agricultural crops. It causes high osmotic pressure outside the absorption area, which is in turn affects in the absorption of water and nutrients for plant growth [7] and because of increased
water scarcity and soil salinity, the researchers have adopted different concepts and strategies to eliminate the salinity effect, namely foliar application, [4].

Legumes are important crops that contain high protein content, as well as contain many vitamins, such as thymine, riboflavin [6], and because of Water scarcity in Iraq There is an accumulation of salts, particularly in the southern regions This study was aimed at:

Isolation and diagnosis of rhizobia bacteria from Karbala governorate and its efficiency in nitrogen fixation under salinity levels and study of the interaction between salinity levels and isolates of rhizobia.

2. Materials and Methods

2.1. Isolation and diagnosis of Rhizobium bacteria

Rhizobium isolates were isolated from soil grown in the previous season from different areas of Karbala governorate, Husseiniya district. The dilution method was used in isolating bacteria. present to soil mitigation by adding 10 g of selected soil samples to 90 ml of sterilized water. A 250 mL flask was well mixed and cleaned in sequence 10-1 – 10-7. Take 1 mL of each dilution and test tubes containing 9 ml of Food medium (Yest extract Mannital broth) described by [3], With three replicates per dilution, incubate the tubes at 28 °C for 48 hours. Take 0.1 mL of tubes and spread on a surface of agar Yest extract Mannital incubated at 28 °C for 48 hours. The colonies appear to be white, convex, mucous. The congo red test was tested in other soil bacteria. The colonies appeared in a snowy white color for lack of dye absorption. The color of the medium changed from green to yellow at the time of the examination of Bromothmeol blue [3].

2.2. Preparation and loading of the vaccine on the Betemus:

A inoculum of nitrogen-fixing rhizobia was prepared by isolating a 500 ml flask containing the nutritional environment (Yest extract Mannital broth), autoclave sterilized at 121 °C and 15 a pound / ing 2 for 20 minutes and incubated at 28 °C for 3 days and then 50 MI of the inoculum and add to the carrier (peatmoss) and move the contents well by ensuring the distribution and homogeneity of the vaccine with the peatmoss [10].

2.3. Soil and seed preparation:

Soil was brought from the field of the College of Agriculture plant located on the Husseiniya River in the city of Karbala note the soil dried aerobically and sifted using a sieve diameter of its openings (2 mm), Table (1) shows some of the chemical, physical and biological characteristics of the soil, obtained the required salinity levels 4, 6 8 dSm-1 according to the method shown by [2], where the soil was placed in a barrel and at the end of the hole for the purpose of water out, and the water was added to the barrel several times and the salinity of the water was measured for the purpose of making sure the salinity took a pattern of The soil of the drum was taken to the laboratory and its salinity was measured. Tah medical cotton saturated with alcohol ethyl, . Planted seeds Vicia faba local variety was obtained from specialized in the sale of seeds and agricultural offices..

2.4. The experiment of pots:

An experiment was carried out with the design Randomized Complete Block Design (RCBD) with 3 replicates. The number of samples used in the experiment was 18 seeds. A quantity of other seeds was placed in the peatmoss containing the inoculum after moisturizing with 20% arabic gum solution. 12 seeds Potted seeds were planted with pollinated and non-pollinated seeds and watered with water. After a week of germination, the plants were reduced by 5 plants per pot. Plant growth was monitored. The end of the season was taken at the end of the agricultural season, ie at harvest on 29/3/2019.

2.5. Attributes measured:
1. Chlorophyll content: measured by chlorophyll.
2. Number of root nodes.
3. dry weight of the root nodules: and has been calculated by the balance sensitive.

Table 1. some chemical, physical and biological properties of soil pre planting:

| property          | measuring unit | Value   |
|-------------------|----------------|---------|
| PH                | ----           | 7.4     |
| Ec                | dSm-l          | 3.3     |
| O.M               | g.m. kg -l     | 1.2     |
| N Available       | mg kg -l soil  | 32.7    |
| P Available       | mg kg -l soil  | 13.6    |
| K Available       |                | 111.2   |
| Clay              |                | 124     |
| Silts             | g.m. kg -l     | 706     |
| Sand              |                | 188     |
| Total bacteria    | gm-l.soil C.F.U| 2.2*10^7|
| Rhizobia bacteria |                | 3.4*10^3|

3. Results and Discussions

3.1. Effect of Inoculation on Rhizobia isolates and salinity levels in total chlorophyll content (Vicia faba)
The results of Table [2] indicate that the treatment of bio-fertilization had a significant effect on the leaves content of the total chlorophyll plant. The treatment Rhizobia (R) showed the highest chlorophyll content in the leaves of plant (19.63 mg.gm^{-1} fresh weight) compared to the non-fertilization comparison (C0) recorded (15.96 mg.gm^{-1} wet weight), the reason for increased chlorophyll content in the plant may be due to the fact that good processing of nitrogen increases the duration of the representation and makes it more effective, Increased chlorophyll content in leaves [14].

The same table showed significant differences in the effect of salinity levels of S1, S2 and S3 in total plant chlorophyll leaf content, where the salinity level of S1 exceeded the other levels in the chlorophyll plant leaf content, which was 20.32 mg.gm^{-1}) followed by treatment of salinity level S2 and S3 (17.43 and 15.63 mg.gm^{-1} wet weight), respectively. The low chlorophyll content may be due to salinity, which reduced nitrogen content in the soil and inhibited the efficacy of rhizobia bacteria and Reducing their numbers, which affected their effectiveness and efficiency in the conversion of nitrogen to the plant. The interaction between rhizobia isolates and salinity levels showed that the overlap between isolation of rhizobia (R) and salinity level S1 significantly exceeded the other interactions, which gave the highest chlorophyll content in the leaves of the plant (22.15 mg.gm^{-1} wet weight) (C0) at the level of salinity S3 was the lowest total chlorophyll content (13.58 mg.gm^{-1} wet weight), which did not differ significantly with salinity level S2.
Table 2. Effect of inoculation with rhizobia isolates and salinity levels on total chlorophyll content (mg.gm$^{-1}$ wet weight):

| Salinity levels dsm-1 | mean     |
|-----------------------|----------|
| Rhizobia isolates     | S1       | S2       | S3       | 15.96 | 13.58 | 15.58 | 15.96 |
| Without fertilization (C0) | 18.50 | 15.80 | 13.58 | 20.32 | 17.43 | 20.32 | 15.63 |
| Rhizobia (R)          | 22.15    | 19.06    | 17.68    | 19.63 |
| mean                  | 20.32    | 17.43    | 15.63    |
| LSD                   | 1.4447   | 1.7693   | 2.5022   |

Table 3. Effect of Inoculation with rhizobia isolates and salinity levels in the number of root nodules of the pods (Vicia faba) (nodules.Plant$^{-1}$) at 50%:

| Salinity levels dsm-1 | mean     |
|-----------------------|----------|
| Rhizobia isolates     | S1       | S2       | S3       | 13.00 |
| Without fertilization (C0) | 18.00 | 13.66 | 7.33    | 13.00 |
| Rhizobia (R)          | 27.00    | 21.33    | 18.66    | 22.33 |
| mean                  | 22.50    | 17.50    | 13.00    |
| LSD(0.05)             | 2.1922   | 2.6849   | 3.797    |

3.2 Influence of inoculation with rhizobia isolates and salinity levels in the number of root nodules of (Vicia faba) at the 50%

The results of Table (3) indicate that the treatment of biofertilization had a significant effect on the number of root nodules of pea (nodules.Plant$^{-1}$) at 50% flowering stage. Compared with the comparative treatment without fertilization recorded (13.00 nodules.Plant$^{-1}$), the reason for the superiority of bacterial insemination treatments in increasing the number of root nodes is due to the increase in the number of active rhizobias in the soil that are able to infect and penetrate the roots of the host plant and this is consistent with the findings [5].

The same table also showed significant differences in the effect of different salinity levels S1, S2 and S3 on the number of root nodules of pea (nodules.Plant$^{-1}$). Then, the salinity level S2 and S3 were recorded (17.50 and 13.00 nodules.Plant$^{-1}$), respectively. This reduced the formation of root nodes formed on the roots of the host plant.

The interaction between isolation of rhizobia and the salinity level of S1 was significantly higher than the rest of the interactions, which gave the highest number of root nodes of plant (27.00 node.plant$^{-1}$), while the comparison treatments were recorded without Fertilizer (C_0). The lowest value of this characteristic at the level of Salinity S3 was (7.33 nodules.Plant$^{-1}$).

3.3 Effect of inoculation with rhizobia isolates and salinity levels in the dry weight of the root nodule of (Vicia faba) (mg.plant$^{-1}$) at 50%

The results of Table (4) indicate that the treatment of bio-fertilization had a significant effect on the dry weight of the root nodule of salts (mg.plant$^{-1}$) at 50% flowering stage. The root of the plant was (157.53 mg.plant$^{-1}$) compared with the control treatment without fertilization (C_0), which recorded the lowest dry weight of the root nodule (124.73 mg.plant$^{-1}$). The reason for increasing the dry weight of
the root nodules may be due to the inoculation of rhizobia leads to an increase in their numbers in the soil and thus the possibility of injury and the formation of root nodules (8).

The same table also showed significant differences in the effect of different salinity levels S1, S2 and S3 on the dry weight of the root nodule of pea (mg.Plant^{-1}). It was (159.06 mg.Plant^{-1}) and followed by the treatment of salinity level S2 and S3 (139.95 and 124.38 mg.Plant^{-1}). To reduce the efficiency of rhizobia in root injury and the formation of root nodes or the formation of small and low-weight nodes[13], Regarding the effect of the interaction of rhizobia isolates and different salinity levels, the interaction between Rhizobia isolation (R) and salinity level S1 significantly exceeded the other interventions, which gave the highest dry weight of root nodes (184.30 mg.Plant^{-1}). Without fertilization (C0) the lowest value of this trait at the salinity level of S3 was (112.93 mg.Plant^{-1}) which did not differ significantly with the salinity level S2.

Table 4. Effect of Inoculation with rhizobia isolates and salinity levels in the dry weight of the root contract of (Vicia faba) (mlgm.plant^{-1}) at 50% :

| Salinity levels dsm-l | Average isolation |
|-----------------------|-------------------|
| Rhizobia isolates     | S1    | S2    | S3    |                      |
| Without fertilization (C0) | 133.83 | 127.43 | 112.93 | 124.73              |
| Rhizobia (R)          | 184.30 | 152.46 | 135.83 | 159.06              |
| Salinity rate         | 159.06 | 139.95 | 124.38 |                      |
| LSD (0.05)            | Rhizobia isolates Salinity Interference |
|                       | 9.3846 | 11.494 | 16.255 |

4. Conclusions
We conclude from the results shown in Table 2, 3 and 4 that the inoculation of rhizobia isolates with bacterial biomass has achieved an increase in both leaf content of total chlorophyll and number of root nodules and dry weight of the root nodules of the Vicia faba plant compared to the control the reason for this to the ability of bacterial bio-fertilizer to enrich the soil with nutrients and easily absorbed by the plant by interfering with the roots of the plant by accelerating some vital processes, as well as its important role in improving growth by increasing the readiness of the necessary elements necessary for the growth of plants such as nitrogen and phosphorus, which works Bacteria on the soil. [8, 9, 11].

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