MENOUFIA UNIVERSITY
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RESPONSE OF SOME LEGUMINOUS PLANTS TO SPECIFIC RHIZOBIAL INOCULATION TIMING WITHIN GERMINATION STAGE

A Thesis Submitted

By

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5. SUMMARY

The present work was conducted to: a) Evaluation the effect of some abiotic stress tolerance of the specific rhizobial strains and its phenotypic characterization. b) Evaluation the effect of the timing application of the specific rhizobial liquid inoculation to some leguminous plants on the nodulation process. c) Evaluation the response of some hybrids of the same leguminous plants to the timing application of its specific rhizobial liquid inoculation., and d) Reflection between the response of some leguminous plants and their hybrids to the timing application of its specific rhizobial liquid inoculation on the plant growth and crop quality.

To select the most three efficient strains to employed in the field experiment. Some specific rhizobial strains were subjected to some abiotic stress tolerance and its phenotypic characterization. Antibiotics, temperature, salt (NaCl) lead (Pb) and sodium azide tolerance, IAA, exopolysaccharides (EPS), were determined, as well as morpho-cultural characterization (Gram staining, colony size, shape, border, elevation, color, mucosity, transparency, scanning electron micro graph of the same tested specific rhizobial strains, were performed.

Field Experiment

Three field experiments were conducted at the same site at Om Saber Village-Tahrir regions - Beheira Governorate, Egypt (Latitude 30º.40´N-Longitude 30º.33´E) (sandy soils presented newly reclaimed soil). The first, second and third experiments were carried out on soybean "(Glycine *max*), common bean plants (*Phaseolus vulgaris L.*) and peanut (*Arachis hypogaea* L.), respectively. Each experiment was carried using two cultivars of the cultivated leguminous plants. These treatment of each experiments were

complete randomized block plot design. The planting dates were at 15 April and 15 Mai, 1st September 2019 and for Soybean, common and peanut bean plants, respectively.

Rhizobial specific strains

In this study, the used three rhizobial specific strains were selected among other specific strains, which were subjected to some abiotic stress tolerance and its phenotypic characterization. These strains were: *Rhizobium legumseram var phaseoli*, *Bradyrhizobium japonicum* and *Bradyrhizobium spp* strains for inoculation seeds of common bean (*Phaseolus vulgarisL.*), Soybean (*Glycine max*) and peanut (*Arachis hypogaea* L.), respectively. These strains were kindly provided from Lab of Microbiology, Soil, Water and Environment Research Institute (SWERI), Agriculture Research Center (ARC), Giza, Egypt.

Responses of the nodulation process, plant growth and crop quality to the timing application of their specific rhizobial liquid inoculation was examined in a separately three field experiments. For this propose, the three leguminous plant seeds were continuously inoculated with their specific rhizobial at 0-7th (First Week **"FW"**), 8-14th (Second Week **"SW"**) and 15-21th (third week **"TW"**) days after sowing, through drip irrigation system. Indeed, in these experiments the solid inoculum was applied (at the sowing) as a reference for the liquid inoculation.

a. Inoculum preparations of the rhizobial specific strains.

Inoculum for these experiments was obtained by growing the specific rhizobial strains in **Yeast Mannitol Broth (YMB)** medium. All strains were maintained in **YMB** and cultured routinely at 28℃.

b. Liquid inoculation process

All plots of liquid inoculation of each sub treatment were continuously inoculated with its specific rhizobial strain at 0-7th (First Week **"FW"),** 8-14th (Second Week **"SW"**) and 15-21th (third week **"TW"**) days after sowing, through the dripping system.

c. Solid inoculation (SI) process

All plots of solid inoculation of each sub treatment were inoculated with its solid specific rhizobial strains (Okadin) just before sowing. The solid inoculation were performed by coating the seeds at a rate of 800 gm of solid inoculum/fed.. Three different bags of Biofertilizer (Okadin) (each bag was specific for each leguminous crop).

The experimental treatments

The experimental treatments in this study were arranged within the experimental units in spilt randomized block design in three replicates illustrated in table (1) as follows:

a-Main treatments:

Five main treatments: Control treatment (without any inoculation), solid inoculation **"SI"** at sowing and liquid inoculation which carried out through (via drip irrigation "Fertigation") at three times i.e. at 0-7th (First Week "**FW"**), 8-14th (Second Week **"SW")** and 15-21th (Third Week**" TW")** days after sowing, through the dripping system, were used in this study.

b- Sub-treatments:

These sub treatments represented by the cultured three leguminous plants These plants were sub treatments soybean plants "(Glycine max) "Giza 111 and Giza 135 cultivars" common bean plants (*Phaseolus vulgaris L.*) "Giza 4 and Giza 6 cultivars", and peanut plants (*Arachis hypogaea* L.) "Ponch and NC" Cultivars.

c- Sub sub treatment:

These sub sub treatments were represented by two cultivars of the cultivated crops.

The obtained results could be summarized as follows:

I. Selected three strains that, represent the most efficient of the tested specific Rhizobial and Bradyrhizobial Strains

Some specific rhizobial strains were subjected to some abiotic stress tolerance and its phenotypic characterization, to select the most three efficient strains to employed in the field experiment. These three specific rhizobial strains showed, higher potentials of IAA and EPS production, high survival capabilities at different temperature degrees, salinity levels, lead (Pb), intrinsic sodium azide resistant, intrinsic antibiotic resistance in compared to the other tested specific Rhizobial and Bradyrhizobial strains.

II. . Responses of The Nodulation Process, Plant Growth and Crop Quality of Soybean, Common Bean and Peanut Plants to The Timing Application of Their Specific Rhizobial Liquid Inoculation

A . Soybean Plants

1. Effect of inoculation treatments on nodulation

- The maximum number, weight nodules and nitorgenase activity were observed with the *B. japonicum* liquid application through the first and at the second week **"FW" and "SW"** after sowing compared to the other liquid or solid inoculation treatments.
- The correspondence values of number and weight nodules were 50, 68, 65 and 48 nodule plant^{-1} and 13.05, 42.30, 32.60 and 12,42 mg plant^{-1} for the **"SI", "FW", "SW","SW", and "TW",** after sowing, for the Giza 111 cultivar. whereas, for the Giza 135 cultivar were 64.00, 76.00, 75.00

and nodule plant^{-1} and 13.67, 51.33, 33.08 and 13.12 mg plant^{-1}, with the same abovementioned treatments.

- All values of RC of weight nodules and nitorgenase activity were higher under the treatments of the *B. japonicum* liquid applied at the first week **"FW"** after sowing, with both the two soybean cultivars, than those found with other studied experimental treatments.

2. Fresh and dry matter yield

- The obtained fresh and dry matter yield of Giza 135 soybean cultivar plants were higher than those found of Giza 111 cultivar with all studied inoculation treatments.
- The value of shoots FMY and DMY achieved; 6.483 and 3.848 Mg fed^{-1} for the plants of Giza 135 inoculated with the liquid *B. japonicum* at **"FW"** after sowing, respectively, in compared to 4.078 and 2.305 Mg fed^{-1} for FMY and DMY of Giza 111 soybean cultivar plants, under the same abovementioned liquid inoculation treatment, respectively.

3. Macronutrients contents in shoots of soybean plants.

- It was noticed that nitrogen content (%) and its uptake as Kg fed-1 in shoots were significantly enhanced by the liquid inoculation by B. japonicum at the first "FW" and at the second "**SW**" week in compared to the solid inoculation (**SI**) and the liquid inoculation by B. japonicum at the third week (**TW**).
- In regarding to Giza 111 and Giza135 cultivar soybean plants, inoculated with liquid B. japonicum 3307 at the first week "FW" after sowing, led to maximum values of P uptake by shoots which was: 15.37 and 26.55 Kg fed-1, respectively.
- The inoculation treatments with liquid B. japonicum 3307 at the first "**FW**" and at the second "**SW**"week after sowing, significantly

augmented K uptake in shoots of soybean plants compared to the other solid "**SI**" or liquid inoculation treatments "**TW**".

4. Micronutrient contents in shoots of soybean plants.

- The highest values of Fe content in shoots were: 1080.67 and 707.83 and 1147.67 and 747.00 mg Kg-1 which equal 2490.94 and 1424.25 and 4416.25 and 2699.13 g fed-1, were found in shoot plants inoculated by liquid inoculation at "**FW**" and "**SW**" after sowing, of Giza 111 and Giza 135, respectively.
- The highest promotion of Zn and Mn contents were found in the soybean shoot plants received the liquid inoculum by B. japonicum at the first "FW" and the second "**SW**" week after sowing in Giza 111 and Giza 135 cultivar plants.

5. Grain and straw yield of soybean plants.

- Inoculation with liquid B. japonicum treatments at "FW" and "SW" week after sowing, gained a higher grain yields: 2784.6 and 2687.64 Kg fed-1 of Giza 111. Whereas, the grain and straw yields of Giza 135 cultivars soybean plants were: 2939.3 and 2867.9 Kg fed-1 respectively, with the same aforesaid liquid inoculation treatments.

6. Grain content of macronutrients

- Liquid applications of B. japonicum at "**FW**" and "**SW**" after sowing resulted in a maximum increase in the grain content of N with the Giza 135 it were: 62.77 and 62.77 g Kg-1 and 184.64 180.13 Kg fed-1, compared to the 58.77 57.77 g Kg-1 and 163.8 and 160.98 Kg fed-1 with the Giza 1112.
- The highest grains P and K contents were found in the plants inoculated with the liquid B. japonicum at "**FW**" and "**SW**", after sowing.

compared to the solid inoculation "SI" and the liquid B. japonicum at "**TW**" treatments.

7. Grain content of micronutrients

- The plants successive received the liquid B. japonicum for all first week "FW" and second "**SW**" week after sowing, attained higher values of grain Fe concentration and its uptake by 712.98 and 711.98 mg Kg-1 which equal 1985.37 and 1982.58 g fed-1 of Giza 111, respectively. Whereas, it were 764.98 and 721.98 mg Kg-1 which equal 2248.5 and 2070.57 g fed-1 of Giza 135, respectively.

B. Common Bean Plants

1. Effect of inoculation treatments on nodulation

- The maximum number and weight nodules were observed with the R. phaseoli liquid application the first "**FW**" and the second "**SW**" week after sowing compared to the later application of liquid R. phaseoli or the solid inoculation treatments.
- The correspondence values of number and weight nodules were 68.00 and 65.00 nodule plant-1 which equal 517.83 and 502.17 mg plant-1 for the Giza 4 cultivar. whereas, for the Giza 6 cultivar were 75.00 and 74.00 nodule plant-1 which equal 776.67 and 688.67mg/plant, with the same treatments of R. phaseoli liquid application.
- The data showed in generally increases in nitorgenase activity (symbiotic effectiveness) of nodules formed on common bean root plants inoculated with liquid R. phaseoli at the first "FW" and the second "SW" week after sowing, in both common bean cultivar plants compared to the other applied inoculation treatments.

2. Fresh and dry matter yield

- These data show that, the treatments of inoculation with the *R. phaseoli liquid* application at **"FW"** after sowing was associated by an increase of fresh **(FMY)** and dry **(DMY)** shoots of both studied common bean cultivar plants.
- The obtained fresh and dry matter yield of Giza 6 common bean cultivar plants were higher than those found of Giza 4 cultivar with all studied inoculation treatments.
- The higher nodulation and accumulation of shoot biomass promoted by the liquid inoculation by R. phaseoli at first "FW" and "FW" week after sowing, indicate that these timing applications are promising inoculation methods at the early germination stages of development for common bean growth and productivity.

3. Macronutrient (N, P, K) contents of common bean plants.

- The inoculation treatment with the liquid *R. phaseoli* a at **"FW" and "SW"** week after sowing, induced the maximum values of nitrogen content (%) and Kg fed^{-1} of both cultivars (Giza 4 and Giza 6).
- Nitrogen content (%) and its uptake as Kg fed^{-1} in shoots were significantly enhanced by the early application of liquid inoculation by *R. phaseoli* (**"FW"** and **"SW")** in compared to the late application of the same liquid *Rhizobia* at **"TW"** or solid inoculation **"SI"**.
- The inoculated plants with liquid *R. phaseoli* 301 for **"FW"** and **"SW"** week after sowing appeared a high P concentration, in shoots of the two cultivar common bean plants. In regarding to Giza 4 and Giza 6 cultivar common bean plants, inoculated with liquid *R. phaseoli* 301 at **"FW" and "SW"** week after sowing, led to

maximum values of P uptake by shoots which was: 6.03 and 5.63 and 9.75 Kg fed^{-1} and 9.49 Kg fed^{-1}, respectively.

4. Micronutrient contents in shoots of common bean plants.

- The liquid inoculation with *R. phaseoli* at **"FW" and "SW"**, led to a significant increases of Fe content and its uptake in shoot of common bean plants compared to the **"SI"** and the liquid inoculation with *R. phaseoli* at **"TW"**.
- Moreover, the highest values of Fe content in shoots were: 1950.50 and 2107.67 mg kg^{-1} which equal 2901.75 and 3224.50 Kg fed^{-1}, were observed with treatments of liquid inoculation at "FW" of Giza 4 and Giza 6, respectively.
- The highest promotion of Zn and Mn content were found in the common bean shoot plants received the liquid inoculum by R. phaseoli for successive 7 days during the first ("FW") week after sowing, in Giza 4 and Giza 6 cultivar plants.

5. Grain and straw yield of common bean plants.

- The inoculation treatments by solid or liquid *R. phaseoli 301* significantly augmented grain and straw yields of Giza 4 and Giza 6 cultivars common bean plants, compared to the uninoculated one. Inoculation with liquid *R. phaseoli* treatments at the **"FW"** and **"FW"** week after sowing, gained a significantly higher grain yields: 1401.40 and 1373.4 Kg fed^{-1} and 1684.2 and 1661.1 Kg fed^{-1} of Giza 4 and Giza 6 cultivar common bean plants, respectively.
- Similar increase effect of liquid inoculation **"FW"** and **"FW"** treatments was found with straw yields of the examined common bean cultivar plants. It is clear from of Giza 6 cultivar showed the

highest significantly values of grain and straw yields, compared those with Giza 4cultivar, under all treatments studied.

- In respect to RC %, values of inoculation treatments by liquid *R. phaseoli* at the **"FW"** and **"FW"**, gave the highest RC (%) values of grain yields: 67,25 and 63.91% of Giza 4 cultivar, and 62.02 and 59.80% of Giza 6 cultivar, respectively.

6. Grain content of macronutrients

- Data appeared that**,** there are widely varied according to the planted cultivars and type and applying times of the inoculum. For example, with all inoculation studied treatments N, P and K contents (g/Kg and Kg fed^{-1}) in the grains of Giza 6 cultivars was higher than that found in the grains of Giza 4 plants.
- Generally, applications of liquid inoculation by *R. phaseoli* at the **"FW"** and **"FW"** resulted in a significant increase the grains of common bean plants N, P and K contents (%) and its relative change "RC". These findings related with the effect of liquid inoculation with *R. phaseoli* at **"FW"** and **"FW"** weeks, after sowing, to the establishing the symbiotic relationship between the *R. phaseoli* and the lateral roots in the beginning of the germination stage of common bean plants.

7. Grain content of micronutrients

The data displayed that, the concentrations and uptake of the determined micronutrients, *i.e.* iron "Fe", zinc "Zn" and manganese "Mn" and the rates of their relative changes of their contents "RC, %", in the grains of two common bean cultivars plants grown on the new reclaimed sandy soils, as affected by inoculation with the solid or liquid *R. phaseoli* 3307.

C. Peanut Plants

1. Effect of inoculation treatments on nodulation

- The maximum number and weight nodules were observed with the liquid *Bradyrhizobium spp. 3339*application successively at the first week **"FW"** after sowing compared to the other liquid or solid inoculation **"SI"** treatments. The correspondence values of number and weight nodules were 37.33 nodule plant^{-1} and 660.67 mg plant^{-1} for. liquid *Bradyrhizobium spp* application at first **"FW"** after sowing, for the Ponch cultivar. whereas, for the NC cultivar were 40.0 nodule plant^{-1} and 911.50 mg plant^{-1}, with the same treatments of *Bradyrhizobium spp. 3339*liquid application
- The RC (%) values of nitorgenase activity of nodules formed on peanut root plants received the liquid *Bradyrhizobium spp. 3339*in both peanut cultivar plants, attained higher values of RC of the nitorgenase activity compared with that received the **"SI"** and **"TW"** treatments.

2. Fresh and dry matter yield

- The value of shoots FMY and DMY achieved; 19.63 and 5.79 Mg fed^{-1} for the plants of Ponch inoculated with the liquid *Bradyrhizobium spp.* at **"FW"**, respectively, in compared to 28.61 and 11.37 Mg fed^{-1} for FMY and DMY of NC peanut cultivar plants, under the same abovementioned liquid inoculation treatment, respectively.

3. Macronutrients contents in shoots of peanut plants.

- The inoculation treatment with the liquid *Bradyrhizobium spp. 3339*at **"FW"**, and **"SW"** week after sowing, induced the maximum values of nitrogen content (%) and Kg fed^{-1} of both cultivars (Ponch

and NC). Nitrogen contents of shoots were: 3.02 9 and 2.99%, which equal to 174.96 and 166.17 Kg fed^{-1} of Ponch cultivar, increased to 3.54 and 3.23% which equal to 401.87 and 274.14 Kg fed^{-1} of NC cultivar, respectively. It was noticed that nitrogen content (%) and its uptake as Kg fed^{-1}, in seeds were significantly enhanced by the liquid inoculation at **"FW"**, and **"SW"** in compared to the **"SI"** or **"TW"** treatments.

- In regarding to Ponch and NC cultivar peanut plants, inoculated with liquid *Bradyrhizobium spp.* at **"FW"** week after sowing, led to maximum values of P uptake by shoots which was: 24.03 and 57.98 Kg fed^{-1}, respectively.
- The plants inoculated with the same inoculation treatments **("FW")** gave a higher values of K contents in shoots *i.e.* 0.99 and 1.05 % which equal to 57.32 and 118.80 Kg fed^{-1} of Ponch and NC cultivar plants, respectively.

4. Micronutrient contents in shoots of peanut plants.

- The highest values of Fe uptake by shoots were obtained with the liquid inoculation with *Bradyrhizobium spp*. at **"FW"** and **"SW"** week after sowing were: 3837.0 and 2077.3 and 9286.0 and 4202.8 g fed^{-1} of Ponch and NC, respectively.
- The highest promotion of Zn and Mn content was found in the peanut shoot plants received the liquid inoculum by *Bradyrhizobium spp.* at **"FW"** week after sowing, in Ponch and NC cultivar plants.

5. Grain and straw yields (Kg fed^{-1}) of peanut plants.

- Inoculation with liquid *Bradyrhizobium spp. 3339*treatments at **"FW"** and **"SW"** week after sowing, gained a higher grain yields: 1551.21 and 1438.49 Kg fed^{-1} and 1682.17 and 1483.58 Kg fed^{-1} of Ponch and NC cultivars peanut plants, respectively. Similar increase

effect of liquid inoculation treatments was found with straw yields of the examined peanut cultivar plants.

- It is clear that, NC cultivar showed the highest significantly values of grain and straw yields, compared those with Ponch cultivar, under all treatments studied.

6. Grain content of macro and micronutrients

- Data appeared that**,** there are widely varied according to the planted cultivars and type and applying times of the liquid inoculation.
- For example, with all inoculation studied treatments N, P and K content (g Kg^{-1} and Kg fed^{-1}) in the grains of NC cultivars was higher than that found in the grains of Ponch cultivar.
- Generally, applications of liquid inoculation by *Bradyrhizobium spp. 3339*at **"FW"** and **"SW"** week after sowing, resulted in a significant increase the grins of peanut plants N content (%) and its relative change "RC".
- These findings related with the effect of liquid inoculation at **"FW"** and **"SW"** week after sowing, on the availability of soil N, P and K and its uptake by plants.
- Data recorded manifested that, the concentrations and uptake of the determined micronutrients, *i.e*. iron "Fe", zinc "Zn" and manganese "Mn" and the rates of their relative changes of their contents "RC, %", in the grains of two peanut cultivars plants grown on the new reclaimed sandy soils, were affected by inoculation with the solid or liquid *Bradyrhizobium spp.* 617.

General Conclusion

In these study, data pointed out that, the timing application of specific Rhizobial liquid inoculation (*Bradyrhizobium japonicum* 3307, *Rhizobium legumseram var phaseoli* 301 and Bradyrhizobium spp. 3339) of soyabean,

common bean and peanut plants, respectively, at the first **"FW"** and at the second **"SW"** week after sowing**,** were the most efficient inoculation treatments according to the high values of nodulation efficiency, macro and micronutrients (N, P and K& Fe Mn and Zn) concentrations and uptake by the three tested leguminous plants (high significant) and produced the highest fresh and dry matter yields at all experimental treatments. These findings are due to the successive application of liquid *B. japonicum* 3307, *R. phaseoli* 301 and Bradyrhizobium spp. 3339, at "**FW"** and at "**SW"** week after sowing, gave capability of leguminous plants form a symbiotic relationship (nodule formation) with a host-specific strains during their germination stage, consequently, further nodule development in newly developing root tissue. Meanwhile, the use of liquid inoculation at "**FW"** and at "**SW"** week after sowing, consequently adding huge number of rhizobia on the grains practically at the early germination stage were more effective competitive, and adapted strains to inoculate soybean, common bean and peanut plants, such as *the* specific rhizobial and Bradyrhizobial strains compared to the solid inoculation "**SI"** or the late liquid inoculation at "**TW"**. It is important to mention that, when liquid *B. japonicum* 3307, *R. phaseoli* 301 and *Bradyrhizobium spp. 3339*, applications were applied at third "TW" week after sowing, The seeds had gone through the germination in earlier period and thus, did not affect the nodule numbers and did not affect the symbiotic effectiveness.

In additionally, in this study, drip irrigation allowed us to application of liquid of *B. japonicum* 3307, *R. phaseoli* 301 and *Bradyrhizobium spp*. 617, at the **"FW"**, **"SW"** and **"TW"** week after sowing, consequently, these specific strains maybe play as plant growth promoting rhizobacteria (PGPR).

As an outlook at the results of the nodule number, nitrogenase activity, fresh and dry matter yield N, P, K, Fe, Mn and Zn contents in soyabean,

common bean and peanut shoot plants, and grains (**Tables 11 to 34**), the following orders are inferred, as affected by solid or liquid inoculation by of *B. japonicum* 3307, *R. phaseoli* 301 and *Bradyrhizobium spp*. 617:

- As for inoculation treatments:

  Liquid Inoculation at **"FW"** > Liquid Inoculation at **"SW"** > solid Inoculation **"SI"** > Liquid Inoculation at **"TW"**

  As for soybean cultivars:

  Giza 135 > Giza 111 cultivar**.**

  As for common bean cultivars:

  Giza 6 > Giza 4 cultivar**.**

  As for peanut cultivars:

  NC > Ponch cultivar**.**

Further inoculation studies in leguminous plants with liquid inoculation within this hypothesis. the early 7 days immediately after sowing and responses of the nodulation process, plant growth and rop Quality during vegetative and reproductive developmental stages are needed to test .