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SUMMARY AND CONCLUSION

Legume response to inoculant is measured as the increase in the yield of inoculated over the uninoculated crops. Maximum benefits of N_2-fixation by legumes often requires the inoculation of selected strains of Rhizobium in seed inoculants. The inoculant strain must be effective in its ability to fix N_2 with cultivar concerned and possess the ability to compete for nodulation of the plant with other strains of rhizobia that might be present in the soil. Therefore, the present study dealed with nodulation and N_2-fixation of two important legume crops of soybean and faba bean.

For these reasons, this study was conducted on two levels as follows :

I. Soybean :

A. Greenhouse experiment :

A pot experiment was designed to explore the efficiency of interactions of native rhizobia and four strains of *Bradyrhizobium japonicum* (110, 1477, Sb. 6 and 3432) used to inoculate four cultivars of soybean (Giza 21, Clark, Giza 22 and Giza 83). All possible interactions between the four strains were applied to obtain single, double and quadruple strains inocula to achieve the more active associations between the tested strains and soybean cultivars.

The nitrogen fixation parameters were evaluated after 45 and 75 (DAP) as well as seed yield of soybean and total N_2-accumulation were also determined.

Results of this study could be summarized in the following points :

1. The uninoculated treatments failed to form nodules on all the roots of the tested soybean cultivars, indicating that the soil under investigation is free from native soybean rhizobia.
2. Inoculation with soybean rhizobia enhanced, in general, nodule formation, growth of nodular tissue and the plant growth as well as the fixation of atmospheric nitrogen.
3. Rhizobial strains responded differently with each soybean cultivar.
4. With single strain inoculants, the best combinations were found between (strain 110 x Clark), (strain 1577 x Giza 21), (strain Sb. 6 x Giza 22) and (strain 3432 x Giza 83). Other cross inoculation treatments showed different responses but with less magnitude. The same trend was also, observed for double strains inoculants, but with achieving higher values in all N_2-fixation parameters than did in single strain inoculants. In case of quadruple strains inoculants, the response of soybean cultivars clearly decreased compared to single and double strains inoculants.
5. It is remarkable that every rhizobium strain which can able to form high productive symbiosis with specific cultivar have a tendency to extent this superiority on the same cultivar even if it is incorporated with other tested strain to form mixed inoculants.

B. Field experiment :

To verify the validity of the results obtained under greenhouse conditions, a field experiment with split-plot design was carried out. The same inoculation treatments with two promiscuous cultivars of soybean (Giza 21 and Clark) were applied. Nodule occupancy by the tested strains in relation to soybean cultivars was determined by serological tests including agglutination technique.

From the obtained results the following could be concluded :

1. The tested strains showed variation in their ability in forming nodules on the tested cultivars. On another hand, soybean cultivars exerted different responses in association with Bradyrhizobium strain.
2. Giza 21 and Clark cultivars gave further interesting results under inoculation with strain 1577 and 110, respectively. They achieved the highest N_2-fixation parameters, especially with double strains inoculants that contain each of them in relation with their compatible varieties.
3. A significant increase in seed yield as a result of inoculation with different rhizobia strain. The average increase was 45.49 and 43.3% for soybean cultivars (Giza 21 and Clark, respectively).
4. The nodule occupancy on the roots of the two tested soybean cultivars of non-inoculated control was completely originated from native population indicating that the indigenous of *Bradyrhizobium* spp of unknown serology appear to successfully occupy soybean nodules. The competitive ability percentage of single strain inocula 110, 1577, Sb. 6 and 3432 was

found in respective order 98, 100, 96 and 100 against native rhizobia, while on the other cultivar Giza 21 all nodules were formed (100%) for each of inoculated strain.

5. In double strains inoculants, the soybean cultivar exerted specific preferential or selective effect among nodulating strains. Strain 110 dominate the majority of nodules (83%) on cultivar Clark compared with less than 34.6% on cultivar Giza 21. On contrary greater than 72% of nodules on cultivar Giza 21 cntained strain 1577 with less than 26.5% on cultivar Clark.

II. Faba bean :

A. Greenhouse evaluation of rhizobial strains :

1. Twenty isolates of *Rhizobium leguminosarum* bv. viceae were isolated from root nodules of faba bean plant grown in three Governorates (Kafr El-Sheikh, Gharbia and El-Dakahlia). All the obtained isolates are subjected to purity tests and all the recommended methods used for identification of Rhizobium.
2. Strain identification (strain marker): Intrinsic antibiotic resistance (IAR), as well as serological diagnosis including agglutination and precipitation tests were applied for identification of all the obtained isolates.
3. The symbiotic performance of the obtained isolates were evaluated for their effectiveness and infectiveness on faba bean plants under bacteriological conditions using Leonard jars assembly system (Somasegaran and Hoben 1985).

Results of these studies could be summarized in the following points as follows :

1. The obtained isolates (20) that collected from different locations are so diverse in their response to 5 antibiotics under investigation and showed four different groups according to their IAR pattern. Each group is considered solely for its individual characteristics.
2. The symbiotic performance of the tested isolates on field variety of faba bean (Giza 3) indicate a relative wide range of continuous variation in nitrogen fixation parameters. The differences in number and dry weight of nodules reflect their capacity to supply nitrogen. From the present work, strains 303, 312, 316 and 317 are of particular interest, as they represent the four different groups of IAR and have a tendency to produce highest productive symbiosis with faba bean plants as they fixed more than 3% nitrogen.
3. Serological marker : The cross-agglutination tests conducted between the antisera of the four strains (303, 312, 316 and 317) showed positive reactions with homologous and negative with heterologous antisera. The preception field obtained from double diffusion tests strengthen the results obtained from IAR pattern.

Therefore results obtained from morphological, culture, biochemical, IAR pattern, serological characteristics strongly suggested that all the tested isolates are related to four different groups of *R. leguminosarum* bv. viceae.

B. Field experiment :

A split-plot design with three replicates was carried out to study the response of two faba bean varieties (Giza 3 and Giza blanca) to inoculation with four local strains (303, 312, 316 and 317) used in a form of single, double and quadruple strains inocula. The competitive ability between the tested strains and native rhizobia in relation with the two varieties of faba bean via agglutination tests was performed by heat treated nodules and the antisera prepared against nodulating strains using Microtitre plate technique.

The obtained results could be summarized in the following points :

1. Generally, results showed that infectivity of the tested strains was considerably influenced by nodulating strain or their combination and faba bean variety. Strain 317 and 312 induced the highest levels of nodulation, plant growth, and N_2-fixation with varieties Giza 3 and Giza blanca, respectively.
2. The highest values of N_2-fixation parameters of Giza 3 always correlated with the inoculation with strain 317 in a form of single or in combination with other tested strains. This observation was also achieved by variety Giza blanca and strain 312.
3. The average increase of seed yield was more pronounced in inoculation treatments with double strains than single and quadruple strains inocula. The corresponding values were 26.79, 22.13 and 13.85% in comparison with control treatments.

4. The competitive ability of the tested rhizobial strains were calculated using the equation described by Amarger (1981). In control treatments, the majority of root nodules formation were originated from natural rhizobia present in the soil (72-81%). With respect to single strain inocula, strain 317 and 312 exerted high competitiveness against other tested strains on varieties Giza 3 and Giza blanca, respectively. Also, they show a strong competitor to native rhizobia. In the double and quadruple strains inocula, such strains (317 and 312) still gave higher percentage of nodule occupancy on their compatible varieties indicating that the host variety can determine the success of specific strain in nodulation present among competing rhizobial strains.

Future Prospects :

Rhizobium, although capable of living for long periods in the soil, is primarily a rhizosphere organism. Seeds inoculation, however, introduced vegetative rhizobia into a non-rhizosphere niche. Furthermore, indigenous soil rhizobia, already well adapted to survival in the absence of the host, will have a competitive advantage This is the problem of competition for nodulation of legumes since it involves the plant, the bacteria and the environment, it will not be easy to regulate. Nevertheless, there are ground of optimism in that **a)** it is possible to select rhizobia that are resistant to various unusual environmental factors (e.g. soil, pH, various toxicities, high or low temperatures) and thus to tailor Rhizobium to most agricultural conditions, b) the host specificity of rhizobia is being characterized with long

term goal of developing a very strain-specific host legume genotype, c) the genetic basis of competitiveness in several strains is being examined and isolated and the mechanism for stable integration into the genome of superior nitrogen fixing strains have been developed. This would reduce the likelihood of poorly effective rhizobia forming a high proportion of nodules.