AGRICULTURE APPLICATIONS OF SOME RHIZOBACTERIAL STRAINS ISOLATED FROM MOLDAVIAN PLAINE CAMBIC - CHERNOZEMIC SOILS

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Considering the benefits of intensive agriculture in our time and the negative impact of chemical fertilizers and pesticides against the environment, usage of plant growth-promoting rhizobacteria like biofertilizers is one of the most promising biotechnologies used for increasing the primary production, eliminating the need of chemical fertilizers. In this context, the aim of our work was to study the effects of some rhizobacterial strains isolated from moldavian plaine cambic - chernozemic soils on the development of soybean (Glycine max L.) plants. During the vegetation phases we measured some biometrical parameters (plant height, number of foils, foliar area, and nodulation). Our results suggest that rhizobacteria can stimulate the plant development probably through improvement of nitrogen fixation and radicular nutrients exchanges.

Key words:*soybean, plant growth promoting, rhizobacteria, biometrical parameters, biofertilizers.*

The microorganisms which grow in the rhizosphere (zone surrounding the plant roots) play an important role in plant development. Thus, the complex interrelationships are created, beneficial both for the microorganisms and plants [13].

There are some plant-associated bacteria able to colonize and persist on the roots called plant growth-promoting rhizobacteria (PGPR). These rhizobacteria can stimulate growth and development of soybean plants. The mechanisms by which PGPR have beneficial effects are not fully understood, but are thought to include:

- the ability to produce plant hormones (indoleacetic acid, gibberellic acid, cytokinins [7]);
- dinitrogen fixation [8];
- reducing or preventing the harmful effects of one or more phytopathogenic organisms (e.g., *Fusarium* spp.) [12] by production of siderophores [12], ß-1,3-glucanase (for example an isolate of *Pseudomonas cepacia*, positive for ß-1,3-

glucanase production, decreased the incidence of diseases caused by *Rhizoctonia solani*, *Sclerotium rolfsii*, and *Pythium ultimum*) [5], chitinases [10], antibiotics [13], and cyanide;

- solubilisation of mineral phosphates and other nutrients [3].

Many of the studies involving PGPR shows plant growth promotion effects, but only under gnotobiotic conditions [7] or in potting media [6] where these bacteria do not compete with the normal array of soil microorganisms. Because soybean represent a crop of major economic importance and pesticides are commonly applied to soybean seeds before planting and during vegetation period for crop increase, a study was performed in the field conditions to analyze the impact of some PGPR strains on plant development and the feasibility of using PGPR as an bio-alternative for organic and chemical fertilizers.

MATERIAL AND METHODS

Plant growth promoting rhizobacteria strains

Nine PGPR strains, isolated from soybean rhizosphere, were used in the experiment to investigate their influence on soybean plant growth. The strains solubilised phosphorus and showed the capability to inhibit the growth of some tested bacteria (*Staphylococcus aureus* Atcc - 6538p, *Bacillus cereus* Atcc – 9634, *Pseudomonas aeruginosa* Ip – 5838, *Escherichia coli* Atcc – 10536). In normal conditions there is a possibility for these strains to respond differently to environmental stress (Saleh and Glick, 2001). So, a mixture of the nine strains was used for testing the plant growth promoting effect. The bacteria were cultivated in liquid Bunt Rovira nutrient medium (1955), at 28^0 C for 48 hours.

Plant cultivation and inoculation in field conditions

The experiment in field conditions was carried out with a mixture of nine rhizobacterial strains (OD_{600}=0.509, estimated cells density: 5 x 10^8 CFU/ml according to [1]) used to inoculate the probe beans. The soybeans seeds (Pioneer PR91M10/91M10) coated with or without PGPR mixture were planted using a SPC* 6 sowing machine.

This experiment was realized during 2008 in Ezareni Didactic Farm, USAMV Iasi, on a cambic chernoziom soil with adobe clay texture and good fertility, moderate humus and highly nitrogen content, moderate mobile phosphor supply, highly potassium content and a very low acid reaction, almost neutral.

After sowing, the soybean plants were monitored every week to observe the influence of PGPR strains on plants height, number of foils and foliar area. The plants were growth in ecological conditions, without utilization of organic or chemical fertilizers and pesticides.

Statistical analyses

For each sample the mean, standard deviation and standard error was calculated. The statistical significance of the differences between experimental variants was tested using the T-test and Z-test [4].

RESULTS AND DISCUSSIONS

In order to quantify the effect of rhizobacteria treatment of the seeds on growth and development processes of the soybean plants, some biometrical parameters (plants height, number of foils, foliar area) were investigated. The measurements were taken during the vegetative phase of the plants as follows: 25.06.2008, 07.07.2008 (early stages of flowering), 14.07.2008 (flowering - early stages of fructification) and 30.07.2008 (fructification), 12.08.2008 (fructification).

Plants height

During the vegetation period, activity of inoculated rhizobacteria had determined an increase in the height of treated soybean plants (*fig. 1*). The recorded differences between treated an untreated plants were statistically significant ($p \leq 0.005$) during the whole period of the experiment: $p=0.0004$ (25.06.2008), $p=0.00001$ (07.07.2008), $p=0.002$ (14.07.2008), $p=0.012$ (30.07.2008), $p=0.015$ (12.08.2008).

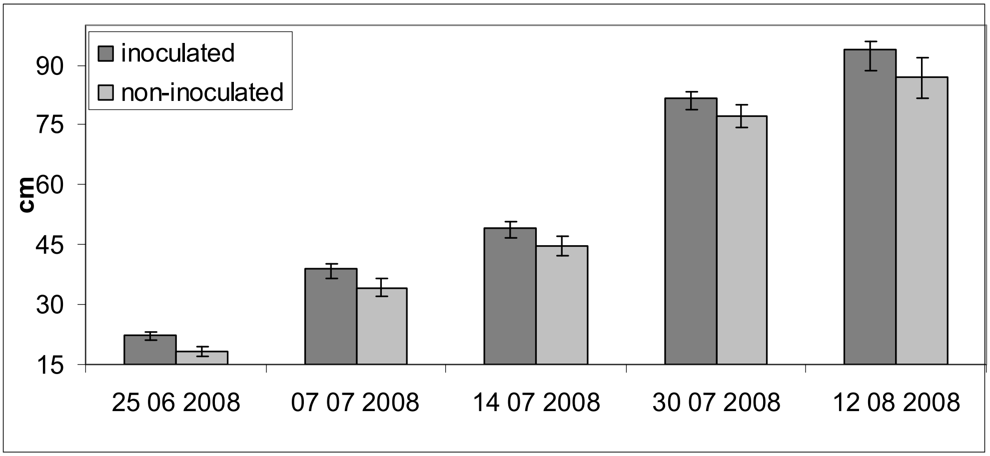

Figure 1 **Variation of soybean plants height**

Foils number

The recorded experimental data regarding the foils number shows that the presence of inoculated rhizobacteria has determined a stimulation of foliar apparatus development (*fig. 2*). Although the foils number developed by the inoculated plants was higher compared to the non-inoculated ones during the whole period of our experiment, the differences were statistically significant only for the early vegetation stages (25.06.2008, 07.07.2008). This could be explained by the fact that the inoculated rhizobacteria mediates radicular exchanges, stimulating the absorption rate and thereby plant development [15].

One field observation made during our study was that in the case of rhizobacteria inoculated plants the flowering process begun earlier compared to the non-inoculated ones. This development delay between the tested plants is due to the beneficial effect of the initial inoculated rhizobacteria. After the flowering begins, the soybean plants development strategy it's modified, focusing all nutrient resources towards flowers and fruits production. In this way the vegetative plant growth is diminished and the foils number remains the same as it's shown in fig. 2. This biometrical parameter is becoming after flowering less important for the interpretation of the beneficial influence of tested rhizobacteria. Supporting this affirmation is the observation that the differences recorded in the later stages (14.07.2008, 30.07.2008, 12.08.2008) of this experiment between inoculated and non inoculated soybean plants are statistically non-significant.

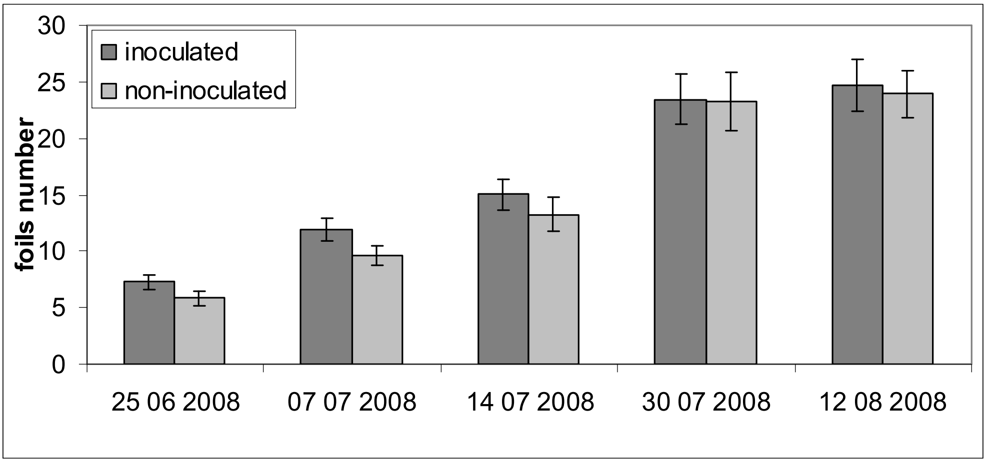

Figure 2 **Variation of foils number**

Foliar area

During the field experiment it was always measured the last complete formed foil. Our records show that the inoculation of the seeds with rhizobacteria before seedling has a positive influence on the foliar area. As it can be seen in fig. 3, in all investigated growth stages, the inoculated plants have a larger foliar surface, up to 38 % compared to non-inoculated plants. All the differences between variants were statistically tested and found to be significant.

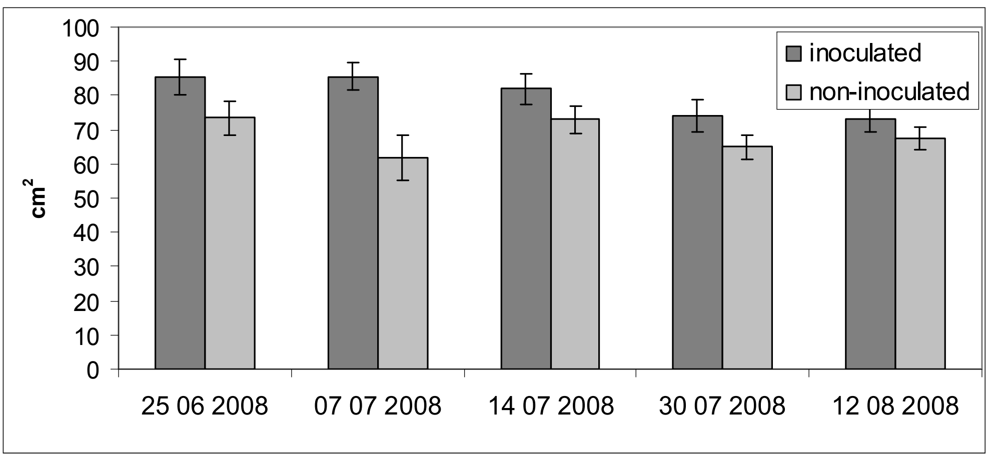

Figure 3 **Variation of foliar area**

All our experimental data recorded so far is in good concordance with the positive effect of rhizobacteria on plants growth reported in the literature by several authors: [3], [15], [14]. During our field experiment, all monitored biometrical parameters shows that tested PGPR strains have the potential to stimulate soybean plants growth: the inoculated plants had a higher height, a bigger foils number and a larger foliar area. In addition to these, the rhizobacteria promoted a faster plant development, the inoculated plants entering the flowering stage earlier compared to the non-inoculated ones. The positive plant growth effect of PGPR was clearly detected only in the vegetative development phases. Once the flowering process starts, the significance of the investigated biometrical parameters for the interpretation of PGPR influence becomes less significant. In this case we have decided to study some biochemical parameters (protein content, assimilatory pigments content, aminoacid-transferase activity, superoxid-dismutase activity, peroxidase activity) which are the subject of undergoing studies. One final parameter considered, which will allow us to make a complete picture on the PGPR effect is the productivity, will be determined.

CONCLUSIONS

The inoculated rhizobacteria strains used in our experiment have clearly showed the capacity to induce a soybean plant growth promoting effect, without any usage of chemical fertilizers or pesticide. In these conditions, we believe that the utilization of PGPR as bio-fertilizers could be viable alternative to the usage of chemical compounds normally used in our days to increase field productivity.

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