# RESPONSE OF WHEAT TO *AZOTOBACTER* - ACTINOMYCETES INOCULATION AND NITROGEN FERTILIZERS

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## ABSTRACT

**The aim of this research was to investigate the effect of inoculation (*Azotobacter chroococcum* and actinomycetes) and nitrogen mineral fertilizers (doses of 60 kg N ha$^{-1}$ and 120 kg N ha$^{-1}$) on the yield of wheat and on the number and activity of certain microorganisms in rhizospheric soil. The investigation was carried out in humogley soil with three wheat varieties. The variants of the experiment were the following: 1) control (without fertilization or inoculation); 2) *A. chroococcum* + actinomycetes, ratio 1:1; 3) *A. chroococcum* + actinomycetes + 45 kg P ha$^{-1}$ + 45 kg K ha$^{-1}$ + 60 kg N ha$^{-1}$); 4) *A. chroococcum* + actinomycetes + 45 kg P ha$^{-1}$ + 45 kg K ha$^{-1}$ + 120 kg N ha$^{-1}$ ). P, K and 60 kg N were applied in autumn whereas the rest of N was added in spring. The wheat seed was inoculated before sowing. The yield of wheat, the number of azotobacter and actinomycetes, and dehydrogenase activity were determined at the end of the vegetation period.**

**Depending on the variety and type of treatment, the increase of yield was 8-11%. Inoculation and nitrogen fertilizers contributed to the increased number of actinomycetes and azotobacter with all three varieties. Dehydrogenase activity significantly increased in inoculated variants with two varieties (Pobeda and Bg Maksima).**

**Key words**: inoculation, nitrogen, microorganisms, wheat, yield

## INTRODUCTION

Soil is an ecosystem inhabited by various groups of microorganisms, which are its living component. Active microbiological processes in soil increase the speed of synthesis and mineralization of organic matter which then leads to better plant nutrition. Knowledge about microbiological processes and factors on which they depend makes possible the use of many microorganisms in agriculture. Symbiotic nitrogen fixators have a wide use in legume production and there is an ever growing interest for the use of free nitrogen fixators in non-legume production (Okon and Labandera-Gonzales, 1994; Casanovas et al., 2000).

Among the free-living nitrogen-fixing bacteria, those from genus *Azotobacter* have an important role, being broadly dispersed in many environments such as soil, water and sediments. Many authors have shown the positive effect of inoculation of wheat with these bacteria (Elshanshoury, 1995; Pati et al., 1995; Jarak et al., 1977).

There are many microorganisms in rhizospheric soil that decompose complex organic matter. Synthesis and transformation of organic matter in soil are constant processes. As a result of those, plants are supplied with a part of necessary nutrients (Alexander, 1977; Paul and Clark, 1989).

Actinomycetes also take part in this process. Actinomycetes are hemoorganotrophs and have an oxydative type of metabolism and use a wide range of organic components as a source of carbon and energy. They produce various antibiotics (chloramphenicol, neomycin, streptomycin), biologically active matter such as B vitamins, auxines and others. They also have a good effect on physiological processes of plants. The use of actinomycetes in plant production has been only sporadically investigated, first and foremost with the aim of protection from pathogens (Klokocar-Smit et al., 2002). In contrast to this, the use of *Azotobacter* in plant production has often been investigated.

This investigation focused on both application of *Azotobacter* (*Azotobacter chroococcum*) and actinomycetes. The aim was to investigate their effect, as well as the effect of two different doses of nitrogen on the yield of wheat and on the activity of certain microorganisms in rhizospheric soil.

## MATERIAL AND METHODS

The investigation was carried out in humogley soil, with three varieties of wheat (Pobeda from Institute of Field and Vegetable Crops, Novi Sad (Serbia), Bg Maksima from PKB INI Agroekonomik, Belgrade (Serbia) and Kg-A-54 from Center for Small Grains, Kragujevac (Serbia). Microorganisms where from Faculty of Agriculture, Novi Sad (Serbia).

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The variants of the experiment were the following:

1. control (without fertilization or inoculation);
2. *Azotobacter chroococcum* + actinomycetes, ratio 1:1;
3. *Azotobacter chroococcum* + actinomycetes + 45 kg P $ha^{-1}$+ 45 kg K $ha^{-1}$ + 60 kg N $ha^{-1}$);
4. *Azotobacter chroococcum* + actinomycetes + 45 kg P $ha^{-1}$+ 45 kg K $ha^{-1}$ + 120 kg N $ha^{-1}$.

Potassium (45 kg $ha^{-1}$), phosphorus (45 kg $ha^{-1}$) and half the nitrogen (urea) were applied in autumn. The rest of nitrogen was added in spring ($NH_4NO_3$).

*Azotobacter chroococcum* was grown on a liquid medium by Fjodorov (Anderson, 1965). After three days of incubation, a suspension was made ($10^6$ cells in 1 ml). Actinomycetes were grown on a solid medium by Krasiljnikov (Scharlau Microbiology, 1999). After five days of incubation, the spores were washed away with sterile water and their number reduced to $10^6$ in 1 ml.

The seed was inoculated immediately before sowing. 300 g seed of every variety was mixed with 50 ml of inoculum (25 ml of *Azotobacter chroococcum* strains and 25 ml of actinomycete strains). The size of the experimental plots was 5 $m^2$. Each plot was sown with 600 seeds of wheat.

The yield of wheat (t $ha^{-1}$), the number of *Azotobacter* and actinomycetes (MPN, Benson, 2000) and dehydrogenase activity (spectrophotometric method by Thalman, 1968) were determined at the end of the vegetation period. The effect of the treatments on the investigated parameters was determined by ANOVA. The difference between average values was determined by least significant difference method (LSD, P>0.01, P>0.05). The dependence of yield upon microbiological activity was determined by the coefficient of correlation (r).

## RESULTS AND DISCUSSION

Yield and quality of wheat is directly related to the characteristics of variety and soil, crop management and characteristics of microbiological processes (Protić et al., 1998; Jarak et al., 2004). The soil in which the investigation was conducted had good chemical characteristics (pH - 7.2; $CaCO_3$ - 2.55 %; humus - 2.55 %; total nitrogen - 0.192 %; 9.6 mg of available phosphorus in 100 g of soil; 22.3 mg of available potassium in 100 g of soil) and good conditions for the growth of wheat and microbiological activity (Jarak et al., 1997; Protić et al., 2004).

The yield of wheat was high in all variants (Table 1). Treatments increased yield by 320-1000 kg $ha^{-1}$ in Pobeda variety, by 470-980 kg $ha^{-1}$ (p>0.05) in Bg Maksima and by 90-610 kg $ha^{-1}$ in Kg-56-A. Relative yield increase was 8-11% in all varieties.

*Table 1*. The yield of wheat (t $ha^{-1}$)

| Variants | Varieties of wheat | | |
|---|---|---|---|
| | Pobeda | Bg Maksima | Kg-56-A |
| Control | 8.39 | 9.58 | 7.89 |
| *Azotobacter chroococcum* + actinomycetes | 8,71 | 10,05 | 8,19 |
| *Azotobacter chroococcum* + actinomycetes + P+K + 60 kg N | 9.34 | 10.56 | 7.98 |
| *Azotobacter chroococcum* + actinomycetes + P+K + 120 kg N | 9.36 | 10.54 | 8.50 |
| LSD > 0.01<br>> 0.05 | 1.412<br>0.983 | 1.089<br>0.758 | 1.264<br>0.880 |

The amount of nitrogen in soil can increase by 20-60 kg N $ha^{-1}$ as the result of introduction of free nitrogen fixators and mineralizers (Peoples et al., 1995; Shabev et al., 1991).

In this investigation, the amount of fixed nitrogen was around 60 kg. The yield in the inoculation + 60 kg N $ha^{-1}$ variant was the same as in the inoculation +120 kg N $ha^{-1}$. This shows that there is a possibility for mineral nitrogen to be partially replaced by biological nitrogen.

Other field experiments have also shown that inoculation with azotobacter had beneficial effects on the yield of plants. Jagnow (1987) claims that the inoculation of wheat and maize with *Azotobacter* increases both the mass of the above ground part of the plant (26-50%) and the yield (19-30%).

According to Zahir et al. (1996) and Kumar et al. (2001), wheat which has been inoculated with free nitrogen fixators grows more evenly and has a higher yield. Apart from producing plant nutrients, about 66% of actinomycetes, fungi and bacteria produce growth substances which effect the growth of plants (Martinez Toledo et al., 1990; De la Vega et al., 1991). The introduction into the soil of microorganisms which produce growth substances (*Azotobacter*, actinomycetes) leads to the increased length and mass of a plant and to an increased yield (Maltseva et al., 1995; Govedarica et al., 2002).

Introduction of a new amount of microorganisms in soil causes a change in microbiological activity (Paul and Clark, 1989; Jarak et al., 2005). Field and laboratory experiments have shown that after inoculation, microorganisms firmly settle on the root surface and remain there, surrounded by the natural soil microflora. Then they take part in the processes of humification, dehumification, nitrogen fixation, nitrification and other processes, and supply the plant with nutrients (Kloeper et al., 1991). Good plant nutrition leads to an increased amount of plant exudates which then additionaly activates microbiological processes (Arsac et al., 1990; Belimov et al., 1995; Govedarica et al., 1997).

In this investigation (Table 2), the number of microorganisms was characteristic of the investigated type of soil (Jarak et al., 1997, 2005). Inoculation and nitogen fertilizers had a significant effect on the number of actinomycetes ($p > 0.05$) with all three wheat varieties.

Dehydrogenase activity significantly increased in all inoculated variants of Pobeda and Maksima varieties ($p > 0.01$) and, only in the variant: inoculation + 120 kg N/ha ($p > 0.01$) in Kg-56-A variety.

Microbiological activity of soil is one of the most important factors that affects the fertility of soil. Therefore, microorganisms also influence the success of production (Alexander, 1977). The yield of plants is usually in a positive correlation with the number of microorganisms which produce plant nutrients (Paul and Clark, 1989; Jarak et al., 2004).

In this investigation, there was a trend for positive association between yield of wheat and the number of actinomycetes, Azotobacter and dehydrogenase activity, although correlation was significant in only two cases (Table 3).

The results show that the use of microorganisms in plant production can lead to higher yield, less frequent use of mineral fertilizers and to a higher microbiological activity of soil. Although it is symbiotic nitrogen fixators that are most widely used in the production of legumes, free nitrogen fixators in the production of non-legume also show good prospect.

Our results confirm the results of many previous investigations, that biofertilization activates microbiological processes in soil and that part of nitrogen fertilizers in wheat production can be replaced by microbiological fertilizers (Okon and Itzigson, 1995; Ozturk et al., 2003; Fares, 1997).

*Table 2.* The number (log $N_0$) of actinomycetes (act.), *Azotobacter* (azb.) and dehydrogenase activity (DHA-μg TPF/g of soil) in the rhizospheric soil of wheat

| Variants | Varieties of wheat | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pobeda | | | Bg Maksima | | | Kg-56-A | | |
| | act. | Azb. | DHA | act. | Azb. | DHA | act. | Azb. | DHA |
| Control | 4.02 | 2.10 | 930 | 4.14 | 2.31 | 745 | 3.87 | 2.34 | 782 |
| *A. chroococcum* + actinomycetes | 5.88 | 3.75 | 1156 | 5.30 | 3.47 | 985 | 4.94 | 3.69 | 972 |
| *A. chroococcum* + actinomycetes + P, K + 60 kg N | 5.54 | 3.70 | 1178 | 5.75 | 3.75 | 1230 | 5.63 | 3.50 | 1091 |
| *A. chroococcum* + actinomycetes + P, K + 120 kg N | 6.37 | 2.90 | 1570 | 5.90 | 3.17 | 1311 | 5.84 | 3.10 | 1391 |
| LSD: P > 0.01 | 0.28 | 0.10 | 20.60 | 0.14 | 0.24 | 13.81 | 0.17 | 0.30 | 520.56 |
| P > 0.05 | 0.18 | 0.06 | 13.60 | 0.09 | 0.16 | 9.12 | 0.11 | 0.20 | 343.62 |

*Table 3*. The correlation between the yield and microbiological activity

| Microorganisms | Coefficient of correlation (r) | | |
|---|---|---|---|
| | Varieties of wheat | | |
| | Pobeda | Bg Maksima | Kg-56-A |
| Actinomycetes | 0.75 | 0.97* | 0.66 |
| *Azotobacter* | 0.49 | 0.80 | 0.30 |
| Dehydrogenase activity | 0.79 | 0.98* | 0.86 |

## CONCLUSIONS

The use of microorganisms in plant production can result in higher yields, lower use of mineral fertilizers and higher microbiological activity of soil.

The yield of wheat was high in all variants. Treatments increased yield by 320-1000 kg $ha^{-1}$ in Pobeda variety; by 470-980 kg $ha^{-1}$ in Bg Maksima and by 90-610 kg $ha^{-1}$ in Kg-56-A, (8-11% in all varieties).

The number of microorganisms was characteristic of the investigated type of soil. Inoculation and nitrogen fertilizers had a significant effect on the number of actinomycetes and *Azotobacter* with all three wheat varieties. Dehydrogenase activity increased significantly in all inoculated variants with Pobeda and Maksima variety. With Kg-56-A variety, it increased in the variant with inoculation + 120 kg N $ha^{-1}$

## REFERENCES

Alexander, M., 1977. Introduction to Soil Microbiology. John Wiley and Sons. Inc.New York, London.

Anderson, G.R. 1965. Ecology of *Azotobacter* in soil of the palouse region I. Occurence. Soil Sci., 86: 57-65.

Arsac, J.F., Lamothe, C., Mulard, D., Fages, J., 1990. Growth enhancement of maize (*Zea mays* L.) trough *Azospirillum lipoferum* inoculation: effect of plant genotype and bacterial concentration. Agronomie, 10: 649-654.

Belimov A.A., Kunakova, A.M., Vasileva, M.D., Grudzeva, E.V., Varobier, N.J., Kozenjakov,A.P., Khamova.F., Postaovskaja, S.M., Sokova, S.A., 1995. Relationship between survival rates of associative nitrogen-fixers on roots and yield response of plants to inoculation. FEMS Microbial Ecology, 17:187-196.

Benson, J.H., 2002. Microbiological applications.Laboratory Manual in General Microbiology. Eight Edition. Ed. Mc Grow Hill, Boston.USA.

Casanovas, E. M., Barassi, C. A., Sueldo, R. J., 2000. *Azospirillum* inoculation of maize seed during imbibition. Cereal Research Communications 28, 1-2:25-32.

De la Vega, M.G., Cejudo, F.J. and Paneque, A., 1991. Production of exocellular polysaccharide by *Azotobacter chroococcum*. Applied Biochemistry and Biotechnology 30: 273-84.

Elshanshoury, A.R., 1995. Interaction of *Azotobacter chroococcum*, *Azospirillum brasilense* and *Streptomyces mutabilis*, in relation to their effect on wheat development. Journal of Agronomy and Crop Science, 175: 119-127.

Fares, C.N., 1997. Growth and yield of wheat plant as affected by biofertilization with associative, symbiontic $N_2$-fixers and endomycorrhizae in the presence of the different P-fertilizers. Annals of Agriculture Science, 42: 51-60.

Gonzales-Lopez, J., Martinez Toledo, M.V., Reina, S. and Salmeron, V., 1991. Root exudates of maize on production of auxins, gibberellins, cytokinins, amino acids and vitamins by *Azotobacter chroococcum* chemically defined media and dialysed soil media. Toxicology and Environmental Chemistry 33: 69-78.

Govedarica, M., Milosevic, N., Jarak, M., Jelicic, Z., Protic, R., 1997. Diazotrophs and their activity in maize and wheat. 11th Intern. Congress of Nitrogen Fixation. 20-25. Jul, Paris, France. Proceedings: 408-409.

Govedarica M., Milošević N., Jarak M., Đurić S., Hajnal T., Jeličić Z., Kuzevski, J., 2002. Application of biofertilizers in agriculture production. 6th International Symposium Interdisciplinary Regional Research Hungary-Romania-Yugoslavia. Novi Sad, 3rd-4th October. Proceedings: 407.

Hajnal T., Jarak M.,Milošević N., Jeličić Z., 2005. Influence of bacterization on the number of microorganisms in the rhizospheric soil and the length of the above ground parts of maize plant. Savremena poljoprivreda, (Yu), 1-2:77-84.

Jagnow G., 1987: Inoculation of cereal crops and forage grasses with nitrogen-fixing rhizosphere bacteria: possible causes of success and failure with regard to yield response. A review - Z. Pflanzenernahrbodenkd. 150:361-368.

Jarak, M., Protić, R., Govedarica, M., Milosevic, N., Djuric, N., 1997. Microbiological activity in soil under wheat inoculated with diazotrophs. A periodical of Scientific Research PKB INI Agroeconomic, Belgrade, Yugoslavia, 3, 1: 39-45.

Jarak, M., Djukic, D., Djuric, S., Stevovic, V., Djalovic, I., 2004. The activation of microbiological processes in soil with the aim of increasing the yield of forage legumes. Acta Agriculture Serbica, 9, 17: 221-228.

Jarak, M., Hajnal, T., Djuric, S., Zurkic, J., 2005. Survival of rhizobia, *Azotobacter* and actinomycetes in soils of different pH values. Annals of scientific work. Faculty of Agriculture, Novi Sad (Yu) 29. 1: 41-50.

Lynch, J.M., 1983. Biotechnology. Microbial Factors in Crop productivity. Blackwell Scientific Publications. London, Boston, Melbourne.

Kloepper, W., Mahaffee, W., McInroy, A., Backman, P.A., 1991. Comparative analysis of methods for recovering rhizobacteria from cotton roots. Can. J. Microbiology 37, 953-957.

Kumar, V., Kumar, B.V., Narula, N.,2001. Establishment of phosphate-solubilizing strains of *Azotobacter chroococcum* in the rhizosphere and their effect on wheat cultivars under green house conditions. Microbiological Research 156, 2: 87-97.

Klokocar-Smit, Z., Jarak, M., Mitar, M., Pericevic, D., 2002. Relationship between alfalfa, *Rhizobim meliloti, Fusarium oxysporum* f. sp. *medicaginis* and actinomycetes. XII Symposium of pesticide application, Zlatibor (Yu) 25-29. November, Abstract book: 51.

Maltseva, N.N., Nadkernichnaya, E.V. and Kanivets, N.A., 1995. Associations of nitrogen-fixing bacteria with winter rye. Proceedings of the 10th International Congress on Nitrogen Fixation, St Petersburg, Russia, 28.05.-3.06: 614 .

Martinez Toledo, M.V., Moreno, J., De la Rubia, T. and Gonzalez-Lopez, J., 1989. Root exudates of *Zea mays* and production of auxins, gibberellins and cytokinins by *Azotobacter chroococcum*. Plant and Soil 110: 149-152.

Milosevic, N., Govedarica, M., Jarak, M., 1997. Soil microbes: importance and possibilities. Papers of the 9th Congress of the Yugoslav Society of Soil Science. Novi Sad, Yugoslavia, 23- 27 June: 389-399.

Mrkovacki, N., Mezei, S. and Kovacev, L., 1996. Effect of *Azotobacter* inoculation on dry matter mass and nitrogen content in the hybrid varieties of sugar beet. A Periodical of Scientific Research on Field and Vegetable Crops 25: 107-113.

Okon,Y., Itzigson, R., 1995. The development of *Azospirillum* as commercial inoculant for improving crop yields. Biotech. Adv. 13: 415-424.

Okon,Y., Labandera-Gonzalez, C., 1994. Agronomic applications of *Azospirillum*: an evolution of 20 year worldwide field inoculation. Soil Biol. Biochem 26: 159-1601.

Ozturk, A., Caglar, O., Sahin, F., 2003. Yield response of wheat and barley to inoculation of plant growth promoting rhizobacteria at various level of nitrogen fertilization. Journal of Plant Nutrition and Soil Science 166, Iss. 2: 262-266.

Pati, B.R., Sengupta, S. and Chjandra, A.K., 1995. Impact of selected phyllospheric diazotrophs on the growth of wheat seedlings and assay of the growth substances produced by the diazotrophs. Microbiological Research 150: 121-127.

Paul, E. A., Clark, E. F., 1989. Soil Microbiology and Biochemistry. Academic Press, Inc. San Diego, California.

Peoples M.B., Herridge D.F., Ladha J.K., 1995. Biological nitrogen fixation: an efficient source of nitrogen for sustainable agricultural production. Plant and Soil 174: 3-28.

Protić, R., Sarić, M., Protić N., Janković, S., 1998. Production and processing values of different genotypes of wheat grown in Yugoslavia. Acta Agronomica Hungarica 46, 1: 149-155.

Protić, R., Rajković S., Janković S., Protić, N., 2004. Grain yield of stubble crops in the Republic of Serbia and some more important agricultural measures. A Periodical of Sci. Res. PKB INI Agroeconomic 10, 1: 79-90.

Scharlau Microbiology: Handbook of Microbiogical Culture Media. (1999): Ref.1-219: 346, Fifth International Edition, Barcelona.

Shabev, V.P., Smolin,V.Y., Strekozova, V.I., 1991. The effect of *Azospirillum brasiliense* sp.7 and *Azotobacter chroococcum* on nitrogen-balance in soil under cropping with oats (*Avena sativa* L.). Biol. Fert. Soils 10: 290-292.

Vande Broek, A., Vanderleyden, J., 1995. The role of bacterial motility, chemotaxis, and attachment in bacteria-plant interactions. Mol. Plant-Microbe Interact., 8: 800-810.

Thalman, A., 1968. Zur Methodik der Bestimmung der Dehydrogenase Activitat in Boden Mittels Triphenyltetrazoliumhlorid (TTC).Landw. Forsch. 21: 249-257.

Zahir, Z.A., Arshad, M., Hussain, A. and Sarfraz, M., 1996. Improving wheat yield by inoculation with *Azotobacter* under optimum fertilizer application. Pakistan Journal of Agricultural Science 11: 129-131.