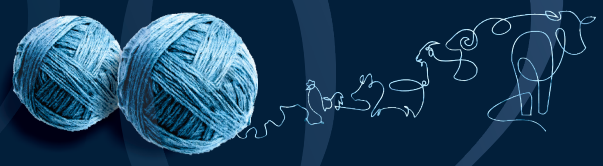
# 13th INTERNATIONAL SYMPOSIUM

# MODERN TRENDS IN LIVESTOCK PRODUCTION

2021



# PROCEEDINGS

6 - 8 October 2021, Belgrade, Serbia

Institute for Animal Husbandry
Belgrade - Zemun, SERBIA

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## ORAL PRESENTATION



## POSTER PRESENTATION

Proceedings of the 13th International Symposium
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# INOCULATION OF MAIZE WITH PGPR

**Violeta Mandić[1], Snežana Đorđević[2], Zorica Bijelić[1], Vesna Krnjaja[1], Aleksandar Simić[3], Marija Gogić[1], Maja Petričević[1]**

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Invited paper

**Abstract:** Maize is a highly productive crop intended for human and animal nutrition, and industrial processing. The standard agrotechnical measures are used in its production. However, the utilization of bacterial inoculants is becoming more popular due to their effect on increased soil quality, plant performance, and yield while preserving and enhancing the agroecosystem. In addition, bacterial inoculants can be applied together with pesticides used in seed treatments. Our earlier results showed that the inoculation of maize seed with plant-growth-promoting rhizobacteria (PGPR) increased the number of diazotrophs bacteria in the rhizosphere, morphological and productive traits, and yield of maize. Accordingly, seed inoculation may represent an important strategy to improve commercial maize production and reduce the environmental impact of maize production processes.

**Key words:** N-fixing bacteria, nitrogen, maize, seed inoculation, yield

## Introduction

Maize (*Zea mays* L.) is the most important crop in Serbia. In 2019, it was cultivated in a 962.000 ha area with a production of 7.3 million tons and with an average yield of 7.6 t $ha^{-1}$ *(Statistical Yearbook of the Republic of Serbia, 2020)*. The average maize yield in Serbia is considered still very low (for 27.6%) compared to the USA average (10.5 t $ha^{-1}$), the largest maize producer in the world *(FAO, 2021)*. The maize grain yield depends on the performance of the hybrid, characteristics of soil, applied agrotechnical measures, and climatic factors *(Mandić et al., 2013; Mandić et al., 2020)*. Mostly the farmers in Serbia use available superior maize genotypes of domestic and foreign selection in combination with appropriate usual production technology. However, the use of microbial seed inoculation in maize production is not common practice, as is the

case with soybeans. In Serbia, seed inoculation of maize and other non-leguminous crops is not as popular as in developed countries. On the other hand, many countries, such as EU members, USA, China, India, and Japan are trying to increase the use of microbiological inoculants through legislation and incentives for agriculture. The use of biofertilisers can increase root microbiome, especially free-living nitrogen (N)-fixing bacteria in the rhizosphere, soil fertility, yield, and biomass of maize, and reduce the use of expensive artificial N fertilizers *(Mandić, 2011)*. This is very important because artificial N fertilizers are known to adversely affect biological balance, soil quality, plant productivity, and ecological balance *(Naher et al., 2016; Vejan et al., 2016)*. Thus, the inoculation of maize seed with *Azospirillum brasilense* reduces the amount of artificial N fertilizers by 25% *(Fukami et al., 2016)*. The mismanagement of artificial N fertilizers contributes to its loss from agroecosystems due to ammonia volatilization, nitrate leaching, and denitrification *(Cui et al., 2010)*. The increase in reactive nitrogen in the atmosphere, soils, and water is expected to come in the near future *(Sutton et al., 2011)*. In general, the chemical N fertilizers do have hidden dangers and represent health hazards to both humans and animals *(Alori and Babalola, 2018)*. Accordingly, the use of biofertilizers is one of the basic and best strategies to reduce the use of synthetic N fertilizers and preserve the environment.

In the present study, we will consider only the effect of inoculation of seed with an individual strain and in a consortium of PGPR. The effect of PGPR mutants on maize growth will not be considered.

## PGPR in maize and biological N fixation

The beneficial rhizobacteria are called plant growth-promoting bacteria (PGPR). In addition to symbiotic N-fixing bacteria which are associated with legumes, various PGPR are associated with non-leguminous plants, especially with cereal grasses. The bacteria responsible for N fixation are called diazotrophs. These bacteria reside at the surface or interior of the root of non-legume crops *(de Bruijn, 2015)*.

PGPR such as *Bacillus*, *Azospirillum*, *Azotobacter*, *Pseudomonas*, *Streptomyces*, *Enterobacter*, *Rhizobium*, *Agrobacterium*, *Arthrobacter*, *Achromobacter*, *Micrococcus*, *Pantoea*, and *Serratia* are present in the rhizosphere of different crops *(Olanrewaju and Babalola, 2019; Verma et al., 2019)*. Their strains are good for the improvement of the growth and productivity of crops in a sustainable way.

The PGPR in biofertilizers improves plant growth primarily by synthesizing phytohormones (indole-3-acetic acid, gibberellic acid, cytokinins, and ethylene), fixing atmospheric N, and solubilising phosphates and other mineral

nutrients *(Kudoyarova et al., 2015)*. PGPR has a role in the biocontrol of pathogenic microorganisms and the prevention of the development of plant diseases because of produced bacteriocins and antibiotics *(Beneduzi et al., 2012)*. Example, *Bacillus* sp. strain B25 is a biocontrol agent of maize pathogen *Fusarium verticillioides (Douriet-Gámez et al., 2018)*. The riboflavin, thiamin, auxin, and gibberellin of *Azotobacter* may accelerate seed germination and improve the control of maize plant diseases *(Baral et al., 2013)*.

Also, PGPR produce extracellular enzymes which decompose organic and non-organic matter in the soil increasing its fertility *(Alexander, 1977)*. *Ahemad and Kibret (2014)* and *Olanrewaju et al. (2019)* emphasize that the PGPR help maize production by nitrogen fixation, increasing the availability of phosphorus, and control pests with different mechanisms. The root system uptake properties improve when the PGPR colonize it allowing easier adsorption of ion nitrate, solubilization of phosphate, and chelation of iron *(Islam et al., 2009)*. Furthermore, the inoculation of maize with a PGPR improves plant tolerance to water deficit *(Pereira et al., 2020)*, high salt concentration *(Chen et al., 2016)*, and high concentrations of heavy metals *(Hassan et al., 2014)*.

Nitrogen input from the air ($N_2$) through the process of biological $N_2$-fixation in agricultural soils is about 50-70 million tonnes of N per year *(Matiru and Dakora, 2004; Herridge et al., 2008)*. $N_2$-fixing bacteria synthesize enzyme nitrogenase which converts inert $N_2$ into biologically useful $NH_3$. These bacteria isolated from root, stem, and leaf tissues of maize belong to different genera *Pantoea*, *Pseudomonas*, *Rhanella*, *Herbaspirillum*, *Azospirillum*, *Rhizobium* (*Agrobacterium*), and *Brevundimonas*, and may contribute to biological nitrogen fixation which ranges from 12 to 33% *(Montañez et al., 2009)*. The bacteria can fix from 30 to 90 kg N $ha^{-1}$, and even up to 150 kg N $ha^{-1}$ *(Milošević et al., 1994)*. *Van Deynze et al. (2018)* point that the atmospheric N fixation contributes to 29–82% of the N nutrition of maize. *Kuan et al. (2016)* report that the $N_2$-fixing bacteria are able to fix N from the air up to 304 mg N $plant^{-1}$. *Baral et al. (2013)* concluded that the *Azotobacter* fixes about 20 kg N $ha^{-1}$ $year^{-1}$.

*Zahir et al. (2004)* report that the fresh biomass and weight of the ear of maize are significantly higher in treatment with inoculation of seed with *Azotobacter* sp. compared to the control. *Adjanohoun et al. (2011)* show that the *Azospirillium lipoferum*, *Pseudomonas fluorescens*, and *Pseudomonas putida* isolated from the rhizosphere of maize significantly increase grain yield compared to *Pseudomonas aeruginosa*, *Bacillus coagulans*, *Bacillus thurengensis*, *Bacillus pumilus*, *Bacillus poly*, *Bacillusfimosus*. Therefore they can be used to produce biofertilizers for maize. Similarly, *Kang et al. (2010)* demonstrate that the *Bacillus* spp., *Pseudomonas* spp., and *Azospirillum lipoferum* improve plant growth and maize yield. *Nezarat and Gholami (2009)* show that the inoculation of maize seed

with strains of *P. putida* (R-168 and DSM291) and *P. fluorescens* (R-93 and DSM 50090) significantly increases germination and growth of maize compared to an uninoculated seed. Also, *Biari et al. (2008)* find that the *Azospirillum lipoferum* DSM 1691, *Azospirillum brasilense* DSM 1690, *Azotobacter chroococcum* DSM 2286 increase yield and growth parameters of maize. According to *Olanrewaju and Babalola (2019)*, the six indigenous isolates (A1 - *Bacillus subtilis*, A18 and A29 - *Pseudomonas* sp., NWU4 - *Streptomyces globisporus*, NWU14 - *Streptomyces griseoflavus*, and NWU198 - *Streptomyces heliomycini*) and their consortium of two or three organisms, enhance maize growth, but the effect of the consortia was greater. *Azospirillum brasiliense* increases the grain yield of maize *(Lana et al, 2012; Oliveira, 2018). Arruda et al. (2013)* have isolated 292 bacterial isolates from five maize crop areas and concluded that the dominant genera were *Klebsiella* and *Burkholderia*, but strains, identified as *Achromobacter*, *Burkholderia*, and *Arthrobacter*, were effective as PGPR in two investigated maize cultivars. *Akhtar et al. (2018)* report that the inoculation of maize seed with a consortium of beneficial bacteria significantly increases root length and biomass and improves root architecture. Therefore, the root absorbs N, P, and K better due to the solubilization of nutrients *(Ranjan et al., 2013)*. The inoculation with strains of *Azotobacter* and *Azospirillum* significantly increased the dry matter accumulation and grain yield of maize *(Naserirad et al., 2011; Sharifi et al., 2011)*. The inoculation treatment of seeds with consortia of six PGPR strains (*Pseudomonas putida* strain R-168, *P. fluorescens* strain R-93, *P. fluorescens* DSM 50090, *P. putida* DSM291, *Azospirillum lipoferum* DSM 1691, and *A. brasilense* DSM 1690) increases nitrogen content in the rhizosphere, germination, plant height, 100-grain weight, number of seed per ear, leaf area, grain yield and quality *(Gholami et al., 2009). Hungria et al. (2010)* have found increases of up to 30 % in the grain yields of maize, inoculated with *A. brasilense* due to improved N nutrition and increased nutrient absorption. Inoculation of maize with strains *A. brasilense* Az39 increases yield from 13 to 33% *(Cassán and Diaz-Zorita, 2016).*

It must be pointed out that the species and strains of PGPR colonize differently the roots of maize hybrids and influence soil biogeny (*Gałązka et al., 2017; Walters et al., 2018)*. That primarily depends on root exudates of which are under host-genetic control *(Bulgarelli et al., 2013)*. For that reason, it is necessary to select strains of PGPR according to the plant genotype.

## The inoculation of maize with PGPR in Serbia

Previous researches on chernozem in the Srem District (Vojvodina province, northern Serbia) shows the significant effect of the inoculation of rhizobacteria on maize growth and yield. *Mandić et al. (2016)* report that the maize

seed inoculated with consortia of *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* has significantly increased nitrogen content, the total number of microorganisms, number of azotobacter and aminoheterotrophs in the soil and grain yield of Serbian maize hybrid ZP 684 during 2006-2008. Also, maize seed inoculation with *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* has increased the stem diameter, grain yield, and rain-use efficiency, and decreased the stem lodging and percentage of barren plants in maize hybrid Dijamant 6 belong to FAO 600 maturity group (*Mandić et al., 2018a)*. Accordingly, the seed bacterial inoculation reduces the risk of stem lodging due to the accumulation of soluble solids in the stem *(Fancelli and Dourado Neto, 2000)*. It is known that the lodging of plants ranges from 5% to 20% annually worldwide *(Flint-Garcia et al., 2003)* with losses of grain yield from 40% *(Ransom, 2005)* to 75% *(Van Dyk, 2001)*. Thus, bacterial inoculation of maize seed can reduce the risk of lodging, especially at high plant densities, and contribute to high grain yields. *Mandić et al. (2018b)* find that the higher nitrogen amount, total number of microorganisms, number of azotobacters, number of aminoheterotrophs, number of oligotrophic and grain yield of two maize hybrids NS 6010 and Dijamant 6 are obtained in the treatment with inoculation of seed with *Azotobacter chroococum*, *Azotobacter vinelandii*, *Bacillus megaterium*, and *Bacillus licheniformis* than in untreated treatment. Also, the individual strains of *Azotobacter chroococcum* and *Bacillus megaterium* and their combination increased nitrogen content in the rhizosphere of two maize hybrids PKB-509 and Srećko-5 *(Hajnal et al., 2001)*. The consortia of *Azotobacter vinelandii*, *Azospirillum lipoferum*, *Bacillus megaterium* and *Bacillus suptilis (Govedarica et al., 2002)*, *Azotobacter chroococcum*, *Azotobacter vinelandii*, *Azospirillum lipoferum*, *Bacillus megatherium*, and *Bacillus subtilis (Cvijanović et al., 2007)* and *Azotobacter chroococcum* and *Bacillus megaterium (Hajnal and Govedarica, 2004)* significantly increase microbial activity in the soil and maize production in Vojvodina Province. Similarly, *Jarak et al. (2011)* find that the *Azotobacter chroococcum* significantly increases microbiological activity, early plant growth, and grain yield of maize. In essence, the higher number and biomass of soil microorganisms increase microbial activity, mineralization of organic matter, and concentration of mineral nutrients available for maize plants *(Mandić, 2011; Mrkovački et al., 2012)*. In addition, according to *Govedarica et al. (1999)* and *Bjelić et al. (2010)* the total number of microorganisms and the number of azotobacters increases in the course of the growing season. *Bjelić et al. (2010)* concluded that the *Azotobacter chroococcum*, *Bacillus subtilis*, and *Pseudomonas fluorescens* applied individually and in mixture increased the number of microorganisms in the rhizosphere and height and weight of maize plants. *Jarak et al. (2012)* found that the individual strains *Pseudomonas sp*. Q4b, *Bacillus sp*. Q5a

and *Azotobacter chroococcum* strain 8 and their combinations increased the number of azotobacters, pseudomonads, and aerobic spore-forming bacteria, plant height, dry weight, and yield of maize compared to control. These authors got the highest yield in the treatment with a mixture of three strains. *Govedarica et al. (1992)* find that the mixture of two strains of *Beijerinckia derx* increases the yield of maize hybrid NSSC-606, while one strain of *Azotobacter chroococcum* increases the yield of maize hybrid PKB-624.

In general, these results demonstrate that the PGPR can improve maize production, microbial abundance in the rhizosphere, and soil fertility because they increase the content of available nitrogen in the soil. However, it is necessary to constantly expand knowledge about the mutual interactions of plants and diazotrophic bacteria.

## Commercialization of bacterial inoculants

The cited researches report that the use of PGPR bacteria in the cultivation of maize can be beneficial to improve yield. All this has led to the development of biofertilizers or bio-inoculants. Biofertilizers are eco-friendly organic agro-input intended to improve the growth and yield of crops and soil health and thus are considered as the best alternative to synthetic fertilizers. The form of the inoculant depends on the carrier and can be solid or liquid. There is a wide range of commercial preparations (monovalent, bivalent, and polyvalent) for the inoculation of maize seeds. In Europe and South America, the most represented is the monovalent inoculum containing *Azospirillum* sp. *(Dobbelaere et al., 2001)*. The genus *Azospirillum* is able to improve the grain yield of maize, wheat, and rice *(Steenhoudt and Vanderleyden, 2000)*. *Santos et al. (2019)* state that about 70 million doses of *A. brasilense* inoculants, for maize and wheat, are sold annually in Brazil. Nowadays, global interest has increased for the development of new microbiological inoculants, the identification of new strains, and new inoculation methods. Scientists are constantly making great innovative efforts to find new microbiological inoculants and this is an inexhaustible area for investing knowledge, techniques, and skills to improve crop yields. China has registered 800 patents related to inoculation, while India has about 100 *(Santos et al., 2019)*. There is a tendency to discover new inoculants with a wide range of applications consisting of a consortium of bacteria.

## Conclusions

In the near future, the development of agriculture must be based on the application of crop cultivation technology and plant breeding that contribute to better establishment of ecological balance and stability of natural resources in the agroecosystem. In doing so, the method of growing crops must be based on an economically effective basis. Biofertilization fits into this concept. The components of microbiological fertilizers are various bacteria, mostly from the group of N-fixing bacteria that deliver nitrogen to plants (*Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, and others), but also translate inaccessible phosphorus, potassium, iron, and sulfur into an accessible form for plants. In addition, these bacteria synthesize plant hormones (auxin and gibberellins) affecting plant growth and induce plant resistance and increase the free-living root microbiome. Accordingly, the application of microbiological fertilizers containing mixed populations of microorganisms can improve the growth and development of plants through the supply of plants with essential nutrients while increasing yields and preserving the environment, and producing safe food.

## Inokulacija kukuruza sa PGPR

*Violeta Mandić, Snežana Đorđević, Zorica Bijelić, Vesna Krnjaja, Aleksandar Simić, Marija Gogić, Maja Petričević*

## Rezime

Kukuruz je visokoproduktivni usev namenjen za ishranu ljudi i životinja i industrijsku preradu. U njegovoj proizvodnji koriste se standardne agrotehničke mere. Međutim, upotreba bakterijskih inokulanata postaje sve popularnija zbog povećanja kvaliteta zemljišta, performansi biljaka i prinosa. Pored toga, bakterijski inokulanti se mogu primeniti zajedno sa pesticidima koji se koriste u tretiranju semena. Naši raniji rezultati pokazali su da inokulacija semena kukuruza sa rizobakterijama koje podstiču rast biljaka (PGPR) povećava brojnost diazotrofa u rizosferi, morfološke i produktivne osobine i prinos kukuruza uz očuvanje i unapređenje agroekosistema. Shodno tome, inokulacija semena može predstavljati važnu strategiju za poboljšanje komercijalne proizvodnje kukuruza i smanjuje uticaj procesa proizvodnje kukuruza na životnu sredinu.

**Ključne reči:** N-fiksirajuće bakterije, azot, kukuruz, inokulacija semena, prinos

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