Effect of Biological and Mineral Nitrogen on Soil And Maize (*Zea mays*) Yield

Gorica Cvijanović, Gordana Dozet
Megatrend University, Faculty of Biofarming, M. Tita 39, 24300 Bačka Topola, Serbia
e-mail: cvijagor@yahoo.com

Abstract

The use of biological and mineral fertilizers used separately or in different combinations should be oriented towards the protection of primary agricultural land resources. The conventional maize fertilizing questions the sustainability of the production within the concept of sustainable agriculture. The experimental and theoretical studies on the application of biofertilizers as replacements or supplements to mineral nutrients in the crop production show economic and ecological justification of their broad practical application and commercialisation of biofertilizers. Nitrogen fixators are very important for sustainable agriculture because their application increases soil fertility and nitrogen balance in the soil, reducing the consumption of mineral fertilizers. This study analyzes the importance of combined use of biological and mineral fertilizers, the yield of dried maize kernels and the main parameters of soil fertility in maize hybrids FAO maturation group 600, 400. Prior to sowing, maize seed was treated with liquid inoculates containing pure species (*Azotobacter chroococcum* and *Azotobacter vinelandii*) and their mixture with the addition of humic acid. Mineral nitrogen was applied at doses 60, 90, 120 and 150 kgN ha^{-1}. The results of the study showed certain regularity in the increase of soil fertility parameters at low concentrations of mineral nitrogen. The highest levels of biogenicity parameters studied were found at the fertilization dosages of 90 and 120 kgN ha^{-1}. The highest average yields were obtained by fertilization with 90 kgN ha^{-1}, which was statistically significant.

Keywords: nitrogen fixators, fertilization, yield, maize

Introduction

Maize is the most important grain crop that has great economic importance and is grown worldwide. The largest areas under maize are in the regions with mild and moderate climate. The selection for highly productive plants has been a constant process since the discovery of maize. Due to the lack of manpower and small plots, during the initial period of maize growing, only the highly productive plants were selected and individually cultivated to achieve maximum yield. The successful breeding along with the introduction of heterosis hybrids and intensive crop management based on biological sciences led to even greater productivity of maize in modern conditions and intensive production. Large green mass production and grain yield require the complete use of agrotechnics, especially essential nutrients (NPK). Numerous studies conducted in different soil-climatic conditions, showed that nitrogen fertilizers increase the yield and protein and fat content of maize grain (Tolstunov, 1974). The nutritive value of maize changes with the nitrogen dose applied. Knowing its requirements for nitrogen fertilizers, and in line with environmental protection, a growing number of studies on biological nitrogen fixation have been performed during the last twenty years.

As a macronutrient, nitrogen is the most important amongst the biogenic elements in plant nutrition, being the key factor of the yield and element in the first minimum. The vast majority of plants synthesize amino acids and proteins using nitrates in soil and water, implying the necessity of

transformation of atmospheric nitrogen into the form accessible to plants. Among the many factors affecting the increase of organic matter production, nitrogen will have an even more important place in the future than it has today.

Nitrogen is a gas that acts like a precious metal being very stable, it is less soluble in water than oxygen, and does not produce compounds with any element at room temperatures; hence it is called the "aristocrat" among the elements. Nitrogen molecule consists of two nitrogen atoms (N_2) linked with triple covalent bond, whose termination requires a large amount of energy. At high temperatures it combines with hydrogen and oxygen building ammonia (NH_4) and nitric oxide (NO). This occurs in natural conditions during electric discharge and water vapour passing through the electric arc, creating reactive oxygen ions that produce compounds with nitrogen. Elemental nitrogen constitutes 78.09% by volume of Earth's atmosphere, which is one of its main sources (atmosphere $3.9x10^{21}$ g, lithosphere $2.3x10^{19}$ g; hydrosphere $1x10^{23}$ g) (Paul and Clark, 1996).

The process of biological nitrogen fixation is of greatest importance for plants (Cvijanovic, 2012). During this process, nitrogen is reduced to ammonia that is built into plant proteins, and consequentially used by all living beings. About 178 million tons of nitrogen is believed to be fixed on Earth through biological fixation (Babeva and Zenova, 1983).

From the moment nitrogen-fixing organisms were discovered many hypotheses on the possible means and ways of nitrogen fixation were made. The central place in biological nitrogen fixation belongs to prokaryotic microorganisms (bacteria, cyanobacteria, actinomycetes and archaebacteria). These microorganisms transform atmospheric nitrogen (N_2) into organic compounds and integrate it into the soil, independently (directly) or in symbiosis with higher plants (via plant proteins). Major portion of nitrogen in crop yield (60-90%) is fixed by microorganisms or originates from mineralized soil organic matter. So, it can be concluded that the basic amount of nitrogen required by plants is provided by microorganisms, whose activities make up to 60-90% of the total soil metabolic activity. The application of nitrogen fixation is a high priority issue in modern agricultural production, because the rational use of mineral nitrogen fertilizer is feasible with the correction and combination with biological preparations (Graham and Vance, 1995; Cvijanovic, 2010).

By virtue of their interactions with plants, microorganisms that carry out nitrogen fixation are divided into two main groups: symbiotic and non-symbiotic. Associative nitrogen fixing microorganisms make a special part of the non-symbiotic group that live in the rhizoplane, phylloplane or on the surface of root hair. During associative nitrogen fixation microorganisms form a fine thin membrane on the root surface, acting as powerful filter of roots excretes transported into the rhizosphere.

Johan Döbereiner (1966) studied the host plant specificity in associating with soil bacteria and thus opened the way for further studies of associative nitrogen fixation. Great genetic variability of plants makes defining the genetic contribution of a plant to the formation of specific interaction with bacteria impossible. Lately, a number of studies was carried out investigating the association of nitrogen-fixing microorganisms on the roots of non-leguminous plants, such as maize (Cvijanovic et al. 2007a), sorghum, sunflower, wheat (Cvijanović et al. 2007, 2008; Mićanović 1997; Mićanović et al., 2008), sugar beet (Mrkovački et al., 2010), fodder (Jarak et al. 1999) as well as in some vegetable crops. The use of associative nitrogen-fixing bacteria (*Azotobacter, Azospirillum, Derxia* etc.) in the production of wheat, corn, sugar beet, sunflower and some vegetable crops, show that depending on the strain there is a possibility of replacing up to 60 kg N ha^{-1}, and possibly even up to 150 kg N ha^{-1} of mineral fertilizer.

Associative nitrogen fixing bacteria are present in all soils in unequal numbers. Their numbers depend on physical and chemical properties of the soil, the presence of oxygen, the presence and absence of Ca and P, trace elements content, organic matter content, the presence of the antagonists and toxic chemicals, and can also be affected by different plant species. Associative nitrogen fixing bacteria are most abundant in plant rhizosphere and within root hairs zone (Malik et al. 2005). Number of root hairs is different depending on the plant species and quite large in maize with 425 per mm^2 (Marinovic, 1979). Bacteria accumulate at the base of root hair. Root hairs are almost impregnated by the gel they produce and by rhizosphere soil thus creating rhizosheaths (Foster, 1986.) Main root produced compounds which attract bacteria are amino acids, sugars and organic acids.

Analyzing maize root exudates Kraffczyk (1984) found 65% sugar, 33% organic acids and only 2% amino acids. A number of researchers found different genera of associative nitrogen-fixing bacteria in the rhizosphere of maize roots. Govedarica (1986) found *Azotobacter, Klebsiella, Derxia, Beijerinckia,* and *Azospirillum*. The majority of studies carried out in Serbia dealt with the identifying the dynamics of the number and activity of *Azotobacter* genus (Govedarica, 1986; Saric, Cvijanovic, 2002; Mićanović, 1997; Mrkovački et al., 2010). After three years of research on soybean, Cvijanovic et al. (2011) concluded that the number of *Azotobacter* decreased very significantly with increasing amounts of nitrogen applied. Associative nitrogen fixing bacteria are capable of rapid proliferation and colonization of roots making firmer contact with the plant, therefore enabling better exploitation of biological nitrogen.

Associative nitrogen fixing bacteria have an important place in non-leguminous plants fertilization in modern agricultural production. They can be used as a substitute or supplement to mineral fertilizers either as individual strains of certain species or strain mixture of one or more species in various forms (liquid, wet, dry). They are most commonly applied as seed treatment (seed inoculation) immediately before planting, by irrigation through drip system or application into the soil.

Nitrogen fixing bacteria used must have the ability to survive in the new environment, enter appropriate competitive relationships with host plant and indigenous microbial populations in soil. For those reasons, the selection of nitrogen-fixing bacteria is carried out in laboratories, based on years of research in both laboratory and field conditions.

Given the aforementioned, the aim of this study was to investigate the effectiveness of mixtures of two representatives of the genus *Azotobacter* (*Azotobacter chroococcum* and *Azotobacter vinelandii*) and the genus *Azospirillum* supplemented with 0.75% humic acid.

Material and methods

The study was conducted on weakly carbonated chernozem soil type in 2007. All agrotechnical measures were carried out in optimum quality and timing. Immediately before planting, FAO maize seed maturing groups 400 (ZP 434) and 600 (ZP 633) were inoculated with a mixture of associative nitrogen-fixing bacteria. The nitrogen fixing bacteria used were from the *Azotobacteraceae* family, genus *Azotobacter*. The mixture was prepared as liquid inoculum, (cell titre 15 x 10^8 ml^{-1}), comprising of the selected highly effective strains of different associative nitrogen-fixing bacteria species:

1. *Azotobacter chroococcum,*
2. *Azotobacter chroococcum+Azotobacter vinelandii*
3. *Azotobacter vinelandii*+ humic acid 0.75%
4. *Azotobacter chroococcum*+ humic acid 0.75%
5. genus *Azospirillum*
6. Control (without inoculation)

Representatives of *Azotobacter* genus do not require growth substances for their development, but fix atmospheric nitrogen better in the presence of small quantities of trace elements. Some strains produce mucus and pigments. To date, a number of representatives of the genus *Azotobacter* were described: *A. chroococcum, A. beirinckii, A. vinelandii, A. agilis, A. nigricans, A. macrocytogenes, A. galophilum, A. micellum,* etc.

Basic fertilization was carried out in autumn by complex NPK fertilizers (15:15:15) and in spring by Urea nitrogen (46% N). Before the spring fertilization, the reserve of 25 kg ha^{-1} residual nitrogen was found in the soil. The quantities and types of nitrogen fertilizer applied reflected clear nutrient values: N_{60}- 60 kgN ha^{-1}, N_{90}-90 kgN ha^{-1}, N_{120}- 120 kg ha^{-1}, N_{150}- 150 kg ha^{-1}. The basic plot was 9.800 m^2 and fertilization combination subplots were 600 m^2. The effect of the applied procedures was examined on plant height in tassel formation stage and on the yield at 14% grain moisture level.

In addition to the yield, the parameters of soil biogenicity were analyzed (total number of bacteria and dehydrogenase enzymes). The total number of microorganisms was determined by standard microbiological methods on agar solid medium, and calculated as 10^7 number per g of absolutely

dry soil (Pochon and Tardieux, 1962). The intensity of the oxidation-reduction processes, or dehydrogenase activity (DHA) was determined by the modified Thalman (1968) method, which is based on measuring the absorbance of triphenyl formazan (TPF), obtained by triphenyltetrazolium chloride (TTC) reduction, expressed in μg10 TPF g^{-1} soil.

Average monthly temperature for the Belgrade area in the growing season of 2007 was 24.9^0C, which was by 3.9^0 higher than the twelve-year average (1998-2010) (Table 1).

Table 1. Average monthly temperature in the growing season of 1998-2010 (^{0}C)

Year	IV	V	VI	VII	VIII	IX	X	Average
1998	14.9	17.0	25.0	23.8	23.7	17.6	14.6	19.5
1999	14.0	17.7	20.9	22.2	23.0	20.5	13.5	18.8
2000	16.3	19.8	22.8	23.3	25.7	18.6	15.8	20.3
2001	11.0	17.6	18.1	22.4	23.3	15.9	13.9	17.5
2002	11.6	19.5	22.0	23.4	21.6	16.5	15.0	18.5
2003	11.5	20.9	24.6	22.6	24.7	19.2	10.6	19.2
2004	17.6	21.1	25.6	28.1	27.7	22.7	20.2	23.3
2005	17.4	22.8	25.3	27.8	25.9	23.5	17.8	22.9
2006	18.4	22.6	24.8	30.0	26.3	24.7	21.4	24.0
2007	20.7	24.8	29.0	32.0	30.2	21.8	15.7	24.9
2008	18.8	24.8	28.2	29.1	30.3	21.8	20.8	24.8
2009	16.2	19.8	21.1	24.0	24.5	21.0	14.0	20.1
2010	14.0	18.2	21.8	24.0	24.4	18.4	11.9	18.9
Mean	15.6	20.5	23.8	25.6	25.5	20.2	15.8	21.0

In addition to agricultural practices, maize growth in vegetative season also depends on climate (temperature and precipitation). Although maize is considered a plant that uses water economically, because of its long growing seasons, large vegetative mass and high-yield, it requires large quantities of water. The total amount of water required from sowing to wax maturity ranges from 500 to 600 mm. Total precipitation during the entire experimental year was 694.8 mm, and during the growing season only 392.6 mm. The water need in the developmental stage until tassel formation is relatively low, because it is met using water from winter moisture which was abundant, hence precipitation did not affect the plant height significantly (Table 2).

Table 2. The total rainfall in the growing season of 1998-2010 (mm)

year	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	Sum
1998	86.0	0	16.6	16.1	46.5	52.5	27.5	17.5	74.5	81.5	26.1	21.3	466.1
1999	47.9	45.0	12.7	50.7	86.1	101.6	185.1	44.2	63.4	47.3	80.6	117.1	881.7
2000	24.9	18.9	21.8	31.9	29.4	22.2	14.0	5.2	52.2	15.6	18.3	28.7	283.1
2001	46.2	12.8	68.0	148.8	46.2	168.0	41.8	35.0	70.8	20.2	62.4	20.2	740.4
2002	11.2	13.4	17.0	54.8	29.4	65.0	34.8	105.2	55.4	93.0	49.2	42.0	570.4
2003	50.8	17.6	3.4	18.1	35.3	9.4	102.3	9.5	35.4	123.0	32.3	4.5	442.2
2004	38.3	45.2	8.4	65.7	50.7	149.4	55.7	64.7	41.4	35.7	95.7	28.5	679.1
2005	3.5	68.0	41.7	35.9	36.4	98.3	58.5	126.2	32.1	23.6	17.7	72.2	613.9
2006	33.1	47.9	81.4	93.1	33.3	143.6	27.3	109.0	10.8	17.7	15.5	48.7	661.4
2007	22.5	64.1	93.2	11.0	49.6	86.0	18.9	51.6	73.0	102.5	102.0	20.4	694.8
2008	42.8	5.4	56.2	27.3	39.7	36.3	46.2	19.7	55.4	10.5	35.6	68.9	443.0
2009	90.1	108.7	47.4	44.0	86.2	180.7	45.0	54.0	51.1	47.9	40.2	78.9	871.2
2010	54	84	63	6	34	153	79	45	4	101	62	122	807.0

The soil was analyzed according to the methods described in the Manual of Yugoslav Society of Soil Science (JDPZ, 1966): pH was determined in a suspension (10 g : 25 cm^3) of soil with potassium chloride (potential acidity) and suspension (10 g : 25 cm^3) of soil with water (active acidity), potentiometrically using pH meter. $CaCO_3$ content - volumetrically, using Scheibler calcimeter, ISO 10693:1995. The humus content - by Tjurin method of organic matter oxidation, DM 8/1-3-017. Total nitrogen content by CHNS analyzer; AOAC Method 972.43. Easily accessible phosphorus (extraction with ammonium lactate) by AL method, spectrophotometrically. Easily accessible potassium (extraction with ammonium lactate) by AL method, determination by flame photometry. NO_3-N and NH_4-N in the soil by Scharpf and Whermann method for N-min method, DM 8/1-3-019.

Results and discussion

In Serbia, maize is grown on a third of arable land, making it one of the most important agricultural crops. Its high genetic yield potential is limited by agro-ecological conditions, the agricultural practices applied and appropriate selection of hybrids. The interaction between genotype and environmental conditions affect the adaptability and stability of the variety (Borojević, 1991). The experiment was conducted on weakly carbonated chernozem soil with less than 5% $CaCO_3$ in humus accumulative layer, resulting in a neutral to slightly alkaline reaction of the soil solution (Table 3). In the interim AhC horizon, $CaCO_3$ content reached the value of 18.12% and increased with depth as a result of loess substrate that is rich in lime. The total nitrogen in soil is optimal (Ah horizon average 0.201%). C: N ratio is important because it determines the inclusion of ammonia in the nitrogen cycle. This relationship is quite good (average 9.39), meaning that ammonia is removed from the microbial cells and available for plants' nutrition.

Table 3. Basic agrochemical properties of the soil

Depth (cm)	pH		$CaCO_3$ (%)	Humus (%)	N (%)	C:N	mg/100 g of soil	
	KCl	H_2O					P_2O_5	K_2O
0-20	6.85	7.75	3.46	3.33	0.209	1.93	14.8	32.4
20-30	6.95	7.90	3.12	3.25	0.206	1.89	12.5	30.1
30-50	7.30	8.25	4.88	3.06	0.173	1.78	10.2	28.6
50-70	7.35	8.20	5.96	2.11	-	1.23	-	-
70-90	7.30	8.25	18.12	1.78	-	1.03	-	-

Numerous results indicate a wide distribution *Azotobacter chroococcum* in soil and rhizosphere, but do not discuss their relationship with other soil bacteria (Balandreau et al. 1988). This species can fix 10 mg nitrogen per 1 gram of carbon. *Azospirillum* genus is represented by microaerophilic free living soil bacteria that can also be found associations with grasses and grains. Both types of bacteria are sensitive to high concentrations of H^+ ions, hence are most abundant and active in neutral soils with adequate water and air regimes. The agrochemical properties of the soil on which the research was carried out were very suitable for the development and activities of these bacteria.

Nitrogen fixing bacteria used for seed inoculation (bacterisation) have the advantage in creating associations compared with the rest of the microflora in the soil, because of their contact with the germinating seeds. After entering the soil, their propagation and relationships with microbes already present in the soil are different. Inoculated nitrogen fixing bacteria enter into competitive relationships for space and food with microorganisms from the microbial communities, causing changes in these communities. Their effect on the dynamics of the increase in the total number of

bacteria and some systematic and physiological groups of microorganisms (fungi, actinomycetes, Azotobacter, ammonifiers) and total oxidizing and reducing processes, e.g. dehydrogenase activity in soil is influenced by the amounts of mineral nitrogen in the soil (Cvijanovic et al., 2008a, 2008b). The increase in numbers and levels of enzymatic activity of useful microorganisms leads to the increase in the intensity of biochemical reactions and consequentially to more intensive extraction of plant assimilatives in the root zone. This certainly affects the intensity of plant growth.

The total number of microorganisms and their activity are balanced and characteristic for each soil type. The implementation of certain agrotechnical practices (cultivation, fertilizing, crop protection, etc.) changes their numbers and activity. Therefore, the number of microorganisms (total, certain systematic and physiological groups) and enzymatic activity are used as indicators of changes in soil. The soil on which this research was conducted contains over 3% humus in the surface layer up to 30 cm, a neutral reaction (pH 6.90 in KCl) indicating favourable conditions for the development of predominantly heterotrophic microorganisms. It certainly is an advantage in the mineralization of organic compounds and humification processes.

Within ZP 434 rhizospheres, the greatest abundance was found (average 610.10^7 g^{-1} soil) when fertilized with 90 kgN ha^{-1}. Treatment combination 2 across all levels of fertilizing resulted in the highest number of total microorganisms (503.10^7 g^{-1} soil) (Table 4).

Table 4. The total number of microorganisms (10^7 g^{-1} soil) in ZP 434 rhizopshere

Fertilisation (kgN.ha^{-1})		Bacterisation						
		1	**2**	**3**	**4**	**5**	**Average**	**6**
0	Number	154	441	309	39	396	339	285
	Index level	54	154	108	138	139	119	100
60	Number	595	431	512	667	365	514	286
	Index level	205	150	178	232	127	179	100
90	Number	549	956	595	631	320	610	236
	Index level	232	404	251	267	135	258	100
120	Number	439	388	522	357	375	416	449
	Index level	97	86	116	79	83	92	100
150	Number	404	300	366	407	614	417	368
	Index level	109	81	113	110	166	113	100
Average	Number	428	503	459	491	414	459	405
	Index level	105	124	113	121	102	113	100

The total number of microorganisms in ZP 633 rhizosphere (436.10^7 g^{-1} soil) was lower compared with ZP434 hybrid (459.10^7 g^{-1} soil). On average, the largest number of microorganisms (450.10^7 g^{-1} soil) was found in treatment combination 4 samples (bacterisation with *Azotobacter chroococcum* plus 0.75% humic acid). The amount of mineral nitrogen also affected the number of microorganisms. The highest numbers were found in samples fertilized with 120 kgN ha^{-1} (513. 10^7 g^{-1} soil) (Table 5). Similar results where different levels of nitrogen applied resulted in different total number of microorganisms in soybean have been reported by Dozet (2009).

In addition to total number of microorganisms, their dehydrogenase activity is another parameter which can be used for determination of rhizosphere conditions. Dehydrogenases enzymes transfer hydrogen from the donor to the acceptor in the process of respiration. All soil organisms have these enzymes that are mostly of microbial origin. High dehydrogenase activity indicates intensive redox and humification processes. This parameter also indicates the soil fertility level.

Table 5. The total number of microorganisms (10^7 g^{-1} soil) in ZP 633 rhizopshere

Fertilisation (kgN.ha^{-1})		Bacterisation						
		1	2	3	4	5	Average	6
0	Number	231	381	518	439	402	394	151
	Index level	60	108	147	125	114	112	100
60	Number	499	509	345	361	551	453	405
	Index level	123	125	85	89	136	111	100
90	Number	445	321	637	531	483	483	465
	Index level	95	68	136	113	103	103	100
120	Number	433	671	325	569	564	513	507
	Index level	85	132	64	112	111	101	100
150	Number	445	276	405	351	201	336	332
	Index level	134	83	122	105	60	101	100
Average	Number	411	432	446	450	440	436	412
	Index level	99	104	108	102	106	105	100

Dehydrogenase activity (DHA) was most intense in samples fertilized by 120 kgN ha^{-1} (273. µg10 TPF g $^{-1}$ soil), while decreased at the maximum fertilizer of 150 kgN ha^{-1} (Table 6). The highest DHA intensity was recorded in treatment combination 4 samples (*Azotobacter chroococcum* plus 0.75% humic acid).

Based on the results, it can be concluded that a large amount of mineral nitrogen had negative effect on microbial niches in the rhizosphere of maize roots.

Table 6. Dehydrogenase activity in ZP 434 rhizosphere (µg10 TPF g $^{-1}$ soil)

Fertilisation (kgN.ha^{-1})		Bacterisation						
		1	2	3	4	5	Average	6
0	Number	235	217	222	280	272	245	195
	Index level	120	111	114	144	140	126	100
60	Number	301	259	301	261	235	271	201
	Index level	149	129	149	129	117	135	100
90	Number	233	235	296	328	266	271	259
	Index level	89	91	114	126	103	105	100
120	Number	348	290	237	256	235	273	248
	Index level	140	117	95	103	95	110	100
150	Number	151	182	177	156	154	164	230
	Index level	66	79	77	68	67	71	100
Average	Number	253	237	245	256	232	244	226
	Index level	112	104	109	113	103	108	100

Dehydrogenase activity in ZP 633 hybrid (238. µg10 TPF g $^{-1}$ soil) was lower than in ZP 434 (244. µg10 TPF g^{-1} soil), which is positively correlated with the total number of microorganisms. The highest DHA values were found in samples fertilized with 90 (238.µg10 TPF g $^{-1}$ soil) and 120 kgN ha^{-1} (248.µg10 TPF g $^{-1}$ soil), although both values were lower compared with the control (no fertilizer).

The combination 4 samples that were inoculated with *Azotobacter chroococcum* and 0.75% humic acid had the highest DHA intensity (244.µg10 TPF g $^{-1}$ soil) (Table 7).

Table 7. Dehydrogenase activity in ZP 633 rhizosphere (µg10 TPF g $^{-1}$ soil)

Fertilisation (kgN.ha^{-1})		Bacterisation						
		1	2	3	4	5	Average	6
0	Number	156	122	248	256	187	213	240
	Index level	65	92	103	106	77	89	100
60	Number	256	280	282	256	272	26	217
	Index level	118	129	129	118	125	124	100
90	Number	250	243	190	303	206	238	248
	Index level	100	97	76	122	83	96	100
120	Number	240	243	250	235	261	245	253
	Index level	94	96	98	92	102	97	100
150	Number	246	246	200	171	263	225	214
	Index level	114	114	93	79	122	105	100
Average	Number	229	246	234	244	237	238	234
	Index level	97	105	99	104	101	102	100

The soil biogenicity parameters indicated possible high yields in both hybrids. The plant growth until the tassel formation phenophase is in stronger correlation with temperature than with any other single climate parameter, therefore high temperatures are relevant to rapid growth before the tasselling but not to the ripening speed.

At the tassel formation phenophase, the average ZP 433 plant height was 256.8 cm. The greatest measured plant height (in all types of bacterisation) was in the variants of 90 kgN ha^{-1} (276.0 cm), which was statistically significant ($p<0.01$) compared with: 0, 60 and 150 kgN ha^{-1} fertilization, while 120 kgN ha^{-1} fertilization produced no statistically significant results.

Seed inoculation treatments (factor B) affected the plant height, but were not statistically significant. The greatest plant height measured (259.2 cm) was recorded at treatment combination 4 (*Azotobacter chroococcum* plus 0.75% humic acid), but was not statistically significant, nor was AB interaction (Table 8).

Table 8. Effects of hybrid ZP 434, bacterisation and fertilisation on plant height at tassel formation stage

Hybrid	Factor A	Factor B: Bacterisation						Mean
	Fertilisation (kgN ha^{-1})	plant height at tassel formation stage (cm)						$\bar{x}$A
		1	2	3	4	5	6	
ZP434	0	224.6	221.7	225.8	224.4	222.9	217.3	222.8
	60	241.7	252.5	244.2	257.3	252.9	246.9	249.3
	90	275.0	275.4	279.2	271.6	278.7	275.8	276.0
	120	266.2	272.1	276.9	275.4	275.5	263.9	271.7
	150	259.2	266.7	262.5	267.3	263.3	266.9	246.3
	$\bar{x}$B	253.3	257.7	257.3	259.2	258.7	254.2	a$_1$$\bar{x}$256.8
Statistical analysis								
LSD 5%								
LSD 1%								

ZP 633 hybrid had an average plant height of 277.2 cm at tassel formation stage, which is 7.94% higher compared with ZP 433 hybrid, which is its varietal characteristic. Greatest plant height was measured in samples fertilized with 90 kgN ha^{-1} (285.1 cm), which was statistically significant at $p<0.01$ compared to non-fertilized control and significant at $p<0.5$ compared with samples fertilized 120 kgN ha^{-1} (Table 9).

Table 9. Effects of hybrid ZP 633 bacterisation and fertilisation on plant height at tassel formation stage

Hybrid	Factor A	Factor B: Bacterisation						Mean
	Fertilisation (kgN.ha^{-1})	plant height at tassel formation stage (cm)						$\bar{x}$A
		1	2	3	4	5	6	
ZP633	0	258.3	260.8	253.3	262.1	257.9	259.4	258.6
	60	286.0	285.8	274.9	283.9	284.8	283.4	283.1
	90	285.0	283.9	280.8	286.7	288.7	285.6	285.1
	120	277.3	280.4	271.9	278.7	276.7	278.3	277.2
	150	281.5	280.0	276.5	283.3	286.5	282.7	281.8
	$\bar{x}$**B**	277.3	278.2	271.2	278.9	278.9	277.8	$a_1\bar{x}$277.2
Statistical analysis								
LSD 5%								
LSD 1%								

Maize yield is directly dependent on the amount of available nitrogen. The results of the interaction analysis revealed that the ZP 434 obtained up to 1.84% (i.e. 210 kg ha^{1}) higher yield (11.84 t ha^{-1}), which was statistically significant at $p<0.05$ compared to the ZP 633 yield (11.63 t ha^{-1}) (Table 10). Such a difference in the yields affected the statistical significance of the AxB interaction.

The highest ZP 434 yield at AxB interaction was achieved by fertilization with 120 kg N ha^{-1} (13:18 t ha^{-1}), which was statistically significant at the level of $p<0.01$ compared to fertilizations with 150 kgN ha^{-1} and the control (the amount of nitrogen in the soil). The highest ZP 633 yield was obtained by fertilization with 90 kgN ha^{-1} (13.43 t ha^{-1}).

Table 10. Effects of hybrids, bacterisation and fertilisation on *Zea mays* yield

Factor A	Factor B	Factor C: Bacterisation						Mean
Hybrid	Fertilisation (kgN ha^{-1})	Yield maize (t.ha^{-1}) AC interaction						$\bar{x}$**AB**
		1	2	3	4	5	6	
ZP434	0	8.92	9.32	9.07	9.91	9.35	8.64	9.20
	60	12.70	11.43	11.30	12.72	12.72	12.52	12.23
	90	12.96	12.83	12.44	13.42	13.04	12.63	12.89
	120	13.22	13.19	12.97	13.88	12.96	12.86	13.18
	150	12.38	11.36	12.02	11.25	11.88	11.70	11.77
	$\bar{x}$**AC**	12.03	11.62	11.56	12.23	11.99	11.67	$a_1\bar{x}$ 11.84
ZP633	0	9.79	9.56	10.80	9.78	11.58	9.82	10.23
	60	12.53	11.68	12.32	12.58	12.19	12.01	12.22
	90	13.39	12.25	11.98	13.19	12.02	11.83	12.43
	120	12.27	11.77	11.87	11.61	12.38	11.76	11.94
	150	11.22	10.51	11.37	11.79	11.26	11.27	11.35
	$\bar{x}$**AC**	11.84	11.15	11.67	11.79	11.89	11.33	$a_2\bar{x}$ 11.63
BC interaction								
	0	9.35	9.44	9.95	9.845	10.46	9.23	$b_1\bar{x}$ 9.23
	60	13.04	11.84	11.64	12.95	12.37	12.17	$b_2\bar{x}$12.17
	90	12.74	12.25	12.38	13.00	12.61	12.32	$b_3\bar{x}$12.32
	120	12.74	12.48	12.42	12.74	12.67	12.31	$b_4\bar{x}$12.31
	150	11.80	10.93	11.69	11.52	11.88	11.48	$b_5\bar{x}$11.48
Effects of factor $\bar{x}$**C**		11.93	11.38	11.61	12.01	11.94	11.93	$\bar{x}$ 11.73

Statistical analysis	A	B	**C**	AB	AC	BC	ABC
LSD 5%	0.168	0.537	0.528	0.800	0.795	1.390	2.367
LSD 1%	0.221	0.701	0.696	1.085	1.085	2.023	3.926

The highest yield (12.32 t ha^{-1}) was obtained by fertilization with 90 kgN ha^{-1}, which was statistically significant ($p<0.01$) compared to fertilization with 150 kgN ha^{-1} and the non-fertilized control, while no statistically significant increase was found when compared with the samples fertilized with 60 and 120 kgN ha^{-1}.

Bacterisation also statistically significantly affected the yield at $p<0.05$. The highest yields (12.01 t ha^{-1}) were achieved by the application of treatment combination 4 (mixture of *Azotobacter chroococcum* with 0.75% humic acid). Based on correlation analysis, it can be concluded that the bacterisation significantly affected the yield at lower doses of mineral nitrogen (Fig. 1 and 2).

Figure 1. Bacterisation and mineral nitrogen dose dependent yield levels of ZP 434 maize hybrid

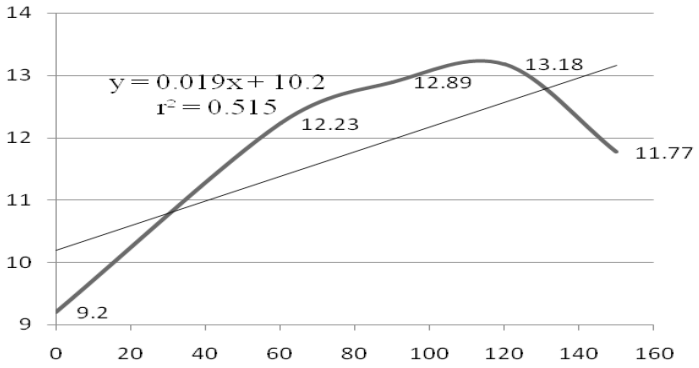
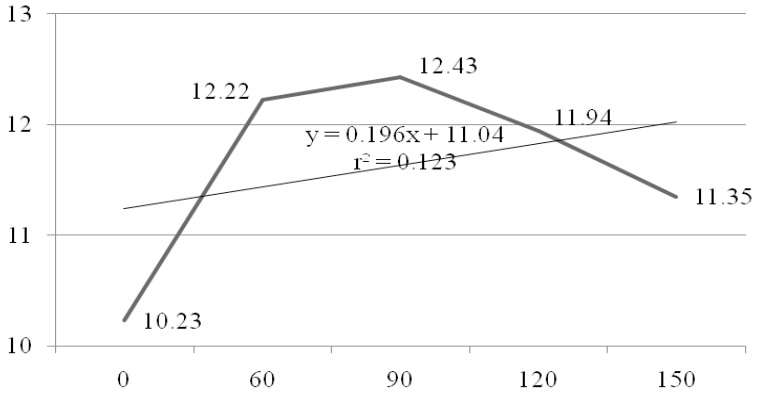


Figure 2. Bacterisation and mineral nitrogen dose dependent yield levels of ZP 633 maize hybrid



ZP 434 hybrid achieved higher yields than ZP 633. This yield difference of 201 kg ha^{-1}, can be explained by the fact that ZP 434 is the mid-early hybrid (FAO group 400) that reaches the maturity over fewer days from emergence (116-120 days), hence is highly adaptable to different agro-ecological conditions, especially high temperatures and lack of precipitation (Tolimir et al., 2003).

Inoculation affected both varieties, regardless of their belonging to different maturing groups. Given that in 2007 the amount of rainfall during the growing season was only 392.6 mm, and temperatures high during the phases of rapid and maximum growth, the yield obtained proves that plants were constantly provided with nitrogen and compatible with nitrogen-fixing bacteria used for inoculation.

Research should primarily focus on the production technology of quality microbiological fertilizer that should contain effective microorganisms able to trigger certain microbiological processes, to be highly competitive, provide plant with assimilatives and promote its growth. In order to fulfil these criteria, the research should be focused on the selection of microorganisms for specific plant genotypes, the area which is not sufficiently exploited today.

Conclusions

The effectiveness of the application of nitrogen-fixing inocula depended on the amount of nitrogen applied and the types of inocula. Soil biogenicity parameters in the rhizosphere of each strain were higher with the inoculation. In ZP 434 rhizosphere the highest numbers of microorganisms (average 610.10^7 g^{-1} soil) were found in samples fertilized with 90 kgN ha^{-1}. In ZP 633 rhizosphere the highest numbers of microorganisms (513. 10^7 g^{-1} soil) were found in samples fertilized with 120 kgN ha^{-1}. In ZP 633 hybrid rhizosphere the total number of microorganisms was 436.10^7 g^{-1} soil, less than in ZP 434 hybrid rhizosphere (459.10^7 g^{-1} soil). Dehydrogenase activity in both varieties was correlated with the total number of microorganisms.

Greatest ZP 633 plant height was found in fertilization with 90 kgN ha^{-1} (285.1 cm), which was statistically significant at $p<0.01$ compared with the control, and at $p<0.05$ compared with fertilization with 120 kgN ha^{-1}. Greatest ZP 433 plant height was found in fertilization with 90 kgN ha^{-1} (276.0 cm), which was statistically significant at $p<0.01$ compared with 0, 60 and 150 kgN ha^{-1} fertilizations, while fertilization with 120 kgN ha^{-1} produced no statistically significant results. The yield, as the ultimate production goal, with bacterisation was on average for 201 kg ha^{-1} higher in ZP 434 compared with ZP 633. The highest yields (12.01 t ha^{-1}) were obtained by the application of treatment combination 4 (mixture of *Azotobacter chroococcum* species with 0.75% humic acid). The application of treatment combination 4 (mixture of *Azotobacter chroococcum* with 0.75% humic acid) could be used as fertilizing supplement in both hybrids inoculation to ensure economic production while maintaining a stable yield and environmental protection in sustainable agriculture.

On the basis of these results it can be concluded that free and associative microorganisms can successfully be used as biofertilizers in the form of microbial fertilizers. Research should primarily focus on the production technology of quality microbiological fertilizer that should contain effective microorganisms able to trigger certain microbiological processes, to be highly competitive, provide plant with assimilatives and promote its growth. In order to fulfil these criteria, the selection of microorganisms for specific plant genotypes should be performed.

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