Theses of the university doctoral (PhD) dissertation

THE EFFECT OF VARIOUS BIOFERTILIZERS ON THE GROWTH OF MAIZE HYBRIDS IN THE SCOPE OF A WATER CULTURE EXPERIMENT

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INTRODUCTION

One of the fundamental elements of the general expectations in environmental protection is the reduction of the quantities of chemicals used for agricultural production. Such reduced application of chemical fertilizers has a double effect: on the one hand, the volume of the expected crop decreases, whereas on the other the quality improves, and consequently the contamination of the environment and surface waters is mitigated. Soil life has an essential role in the mobilization and accessibility of nutrients. Besides other favourable effects, free N_2-fixing organizations increase the quantity of N that is present the soil. Microorganisms enhance the decomposition of organic residues, and therefore increase the quantity of nutrients that are available for the plants. Owing to the respiration of the microorganisms in the soil, CO_2 concentration in plants increases, which has a yield-increasing effect because of the more effective photosynthesis. Beyond the applied tilling and crop production techniques, a further procedure of biological crop production is the detailed investigation of soil fertility in order to achieve more favourable yields.

The characteristics of the rhizosphere significantly differ from the properties of the soil areas that are farther from the roots. The research of the rhizosphere microorganisms that have favourable effect and favourably influence the crop growth is intensive. Having positive effects on plant growth, symbionts and free microorganisms can be regarded. Some of the microorganisms of the rhizosphere directly stimulate plant growth, and similarly crop quantity also increases owing to their effects. On the other hand, there are also rhizosphere bacteria known that indirectly influence the vital processes of the plants. There exist bacterial strains that have already been used in the agricultural practice to inoculate the soil. Soil conditions determine the growth and general life conditions of microorganisms. Soil conditions were classified by Bowen and Rovira (1976). In order for a microorganism to be classified as a plant growth-promoting bacterium (PGPB), the most important requirement is to be permanently viable in the soil. Another rather important expectation for PGPBs is to be able to colonize the surface of the root in order to exercise their effect directly. At the same time, a significant proportion of PGPBs are not suitable for colonization, but have positive effects just indirectly (Suslow, 1982).

2. SUBJECT OF THE THESIS

At the present and expectedly in the future, one of the key aspects of sustainable agriculture is whether there are technologies available for the reduction of the use of chemicals. Requirements relating to food quality raise the issue of plant nutrition that determines the quality of end products. We can use our soil optimally, and manage it properly for the purpose of crop production in case we do not consider it only as a lifeless mass of purely physical and chemical properties, but as a living material whose composition changes continuously due to the cyclic process of metabolism.

One of the key issues of crop production and forestry on acidic soils is whether plants can adjust themselves to low soil pH, and if they are capable of tolerating potentially toxic metals, the uptake of which is a potential source of hazard.

The applied agricultural techniques are partly responsible for the acidity of the soils. The acidifying effects of certain chemical fertilizers are well known.

Specific micro-organisms play a key role – both in aerobe and anaerobe soil layers – in turning certain micro- and trace elements (which are essential in the immunology of warm-blooded vertebrates) water soluble and absorbable for plants. Among these the most important are Selenium (Se) as the ancient natural inhibitory element of cancer diseases, as well as Chromium (Cr) as the predisposed regulator of our sugar (glucose) balance. As a result of the chemicals applied in agriculture, the Se and Cr mobilising activity of the soil micro-flora has decreased. This might be the reason for the quantitative decrease of the above elements within the food chain.

Although the use of chemical fertilizers has been reduced recently, the primary underlying causes are of economic nature. The mildly acidic or acidic pH favours the uptake of most toxic metal ions. There is a seemingly antagonistic relationship between the quality and quantity of end products from crop production. Procedures that ensure sustainable crop production in any area by exploiting the local natural endowments, circumstances have special significance. One example for such natural constituents is the present of bacterium-based bio-fertilizers. Nevertheless, the heavy metal content of soils determines not only the success of crop production. The human health aspects become increasingly important. The strict requirements set out by quality assurance systems encompass the entire food chain from production through processing to shop shelves. Therefore, I regard the use of formulas containing biological or organic ingredients base materials instead of chemical materials and fertilizers to be essential.

2. 1. Objectives

The objective is to prove the diverse effect of the biofertilizers in the involved maize hybrids. The following comparative analyses have been the tasks of this objective:

- to clarify if the effect of different biofertilizers is identical, therefore 3 different commercial products have been involved in the comparative studies.
- to clarify if effect of a biofertilizer containing more component is significantly more advantageous in comparison with the products consisting of less bacterial strains, therefore fertilizers containing 2, 4 and 6 microbe species have been applied.
- to clarify if the involved hybrids react differently to biofertilizers, therefore 31 different hybrids have been involved which originate from different breeding houses and they possess different FAO numbers.

In order to clarify the difference or identity of the effects, the measurements took place on two different age maize leaves. The presence of the differences or standard nature of physiological processes is observed through specific analyses (chlorophyll and carotene). The analyses provide a comprehensive picture of the effects of biofertilizers on the cultivated crops in order to support cultivation-related decisions.

3. MATERIAL AND METHOD

3. 1. Cultivation of the test plants

Maize *(Zea mays L.)* was used as test crop. Name, FAO number, maturity time and other information are shown in Table 1.

Seed surfaces were sterilized with 30% H_2O_2. The sterilized seeds were flushed several times with distilled water, and then soaked in 10 mM $CaSO_4$ solution for 4 hours. The seeds were germinated between wet blotting paper sheets so as to make the polarity of the seedlings natural.

The temperature of the thermostat used for germination was 22 °C. The maize seedlings with 4-cm coleoptile were placed in medium. We used the following nutrient solution for the growing of the plants: 2.0 mM $Ca(NO_3)_2$, 0.7 mM K_2SO_4, 0.5 mM $MgSO_4$, 0.1 mM KH_2PO_4, 0.1 mM KCl, 10μM H_3BO_3, 1μM $MnSO_4$, 1μM $ZnSO_4$, 0.2 μM $CuSO_4$, 0.01μM$(NH_4)_6Mo_7O_{24}$. The plants received iron in the form of 100 μM Fe(III)-EDTA (Lévai, 2004).

The nutrient solution was replaced in every second day, while aeration of the medium was continuous.

The test plants were grown in 1.7 litre bowls; 170 ml culture medium was diluted to 1.7 litres to which bio-fertilizers were added. Four plants were grown in each bowl. Three bowls have been applied for every treatment.

The test plants were grown in 1.7-liter bowls; 170 ml culture medium was diluted to 1.7 litres to which bio-fertilizers were added. Four plants were grown in one bowl.

The plants were grown in the air-conditioned room of the Faculty of Agricultural Crop Sciences, Plant Physiology and Biotechnology. The environmental conditions were regulated: light intensity 300 μmol $m^{-2}s^{-1}$, temperature periodicity 25/20°C (day/night), relative humidity (RH) 65-75%, lighting/dark period 16 hrs/8 hrs.

3. 2. Measuring the relative chlorophyll value (SPAD value)

We used the second and third newest, but fully developed leaves of 4-leaf plants for measuring chlorophyll content. The relative chlorophyll value was measured with SPAD-502 (MINOLTA, Japan) Chlorophyll Meter on 12 plants, with 60 repetitions in each treatment.

The essence of this measurement is the fact that light characterised by different wavelengths is absorbed differently by chlorophylls (chlorophyll-a and -b) situated within the chloroplasts. The degree of light absorption is closely related to the chlorophyll content of leaves. The SPAD-502 device uses red light for the measurements, because its absorption is not influenced by the carotene content of the leaf. There are two photodiodes within the illumination system of the device, a red (650 nm max. value) and an infrared (940 nm max. value).

The two photodiodes emit light alternately, with the same intensity. The illuminated area is 6 mm^2. The two kinds of light reach the leaf-blade, some of it is reflected, some is absorbed and some passes through the leaf. The passed through light is intercepted by a sensor made of a silicone photodiode and converted to an analogue electronic signal. The electronic signal is amplified by the device and converted to numbers.

3. 3. Spectrophotometric determination of chlorophyll a and b, as well as carotenoid

In the course of the determination of photosynthetic pigments, namely chlorophyll- a, -b and carotenoid content a 0.05 g leaf-disc was sampled from which the photosynthetic pigments have been extracted. 5 ml N,N dimethyl-formamide was applied on the leaf samples and they were stored cooled for 72 hours in order for the photosynthetic pigments to be entirely extracted from the leaves. The leaves have been measured with a METERTEK SP-830 spectrophotometer at 480, 647 and 664 nm wavelength. Three leaf samples have been used from each treatment for the measurements with the device. The amount of chlorophyll-a, -b and carotenoid was calculated on the basis of the formula of Wellburn (1994).

3. 4. Measuring of dry weight

To measure the dry weight, 9 samples were dried for each treatment to constant weight at 65°C, then cooled to room temperature and measured on analytical balance (OHAUS).

3. 5. Applied biofertilizers

Three commercially available biofertilizers were used in the experiments.

One of the applied biofertilizer (marked "A") is a viscous liquid containing two bacteria: *Azotobacter chrococcum* (1-2x10^9 / cm^{-3}) and *Bacillus megaterium* (1-2x10^8 / cm^{-3}); its use is recommended for biofarming as well.

The other biofertilizer (marked "B") is a viscous liquid, which contains the following bacteria, altogether six different strains: *Azospirillum brasilense, Azotobacter vinelandii, Bacillus megaterium, Bacillus polymyxa, Pseudomonas fluorescens, Streptomyces albus*. The total population is: 4,3x10^9 db cm^{-3}.

The third biofertilizer ("C") contained the following bacteria: *Azotobacter chroccoccum, Azospirillum* ssp., *Bacillus megaterium, Bacillus subtilis.*

The biofertilizers were added to the nutrient solution in a concentration of 1 ml dm^{-3}

Table 1: Maize hybrids utilised in the course of the experiments and the most important information related to them

FAO No.	Hybrid name	Producer	Seed hl weight kg hl^{-1}	Yield t ha^{-1}	Plant density (thousand)
Very early maturity maize					
290	Karnevalis	KWS			62-65
Early maturity maize					
300	Clemenso	KWS			70-80
300	NK Kansas	Syngenta	73	8-10	72-78
320	DKC 590	Monsanto	75	9.5	67-69
330	NK Lucius	Syngenta	74	10.5	65-75
330	Shakira	Agromag	77-79	8.5	60-70
340	Eric	Agromag	77-79	8.5-9	60-70
350-a	NK Octet	Syngenta	74	12	65-80
350-b	NK 37219	Syngenta	73	11	68-71
350-c	MV 350	Martonvásár	74	9.5	60-70
360-a	SC 3510	Syngenta	76	9	71-74
360-b	NK 3850	Syngenta	77	11	72-73
360-c	MV 343	Martonvásár	80	12	65-72
370-a	DKC 4490	Monsanto	74	11.5	68-75
370-b	Kamaria	Martonvásár	79	12	60-70
370-c	GK Boglár	Gabonakutató	75	8.5	55-70
370-d	Temes	Agromag	78-80	8	60-70
380	MV Tarján	Martonvásár	79	11.57 (ethanol)	60-70
390-a	SY Flovita	Syngenta	75	9.5	65-70
390-b	Szegedi 386	Gabonakutató	77	(silo)	65-70
Mid-early maturity maize					
400-a	DKC 4717	Monsanto	78	10.5	70-76
400-b	SE 4410	Syngenta	76	11	72-74
410-a	Mikolt	Martonvásár	77	13	60-70
410-b	Kenéz	Gabonakutató	77	9.5	55-65
420	MV Koppány	Martonvásár	76	13.19 (ethanol)	60-70
450-a	NK Columbia	Syngenta	73	9.5	65-75
450-b	Jennifer	Agromag	75	(silo)	65-85
470	NX 47279	Syngenta	75	10.5	66-72
480	DKC 5222	Monsanto	74	11.5	62-72
490	Szegedi 475	Gabonakutató		(silo)	55-65
Late maturity maize					
560	Szegedi 521	Gabonakutató	72	silo	70-75

3. 6. Statistical evaluation of the results

Microsoft Excel 2003 and Sigma Plot 8.0 have been used for the evaluation of the results. For the analysis of the significance level, a “t” test has been performed.

4. RESULTS AND EVALUATION

4. 1. Dry weight of the shoots and roots of maize hybrids as a result of the biofertilizer treatments

The effect of the three biofertilizers on the biomass production of maize hybrids produced by different breeding houses has been analysed. It was found that the fertilizers had diverse effects on the dry weight even amongst the hybrids of the same breeding house. As an example, the weight increase of maize hybrids produced by different breeding houses is demonstrated. The order of the hybrids was determined according to the different breeding houses.

4. 1. 1. Dry weight of the shoots and roots of maize hybrids from the MONSANTO breeding house as a result of biofertilizer treatments

Four hybrids have been analysed from the ***Monsanto*** breeding house, these are DKC 4490, 4717, 590 and 5222. It has been found that the weight of the hybrids changed differently as a result of the bacterial treatments. The less reaction was provided by the ***DKC 590*** hybrid; its plant biomass weight was 50% less in average. The plant biomass weight provided a more balanced reaction than the root weights where a higher standard deviation was experienced (Figures 1. A and 1. B).

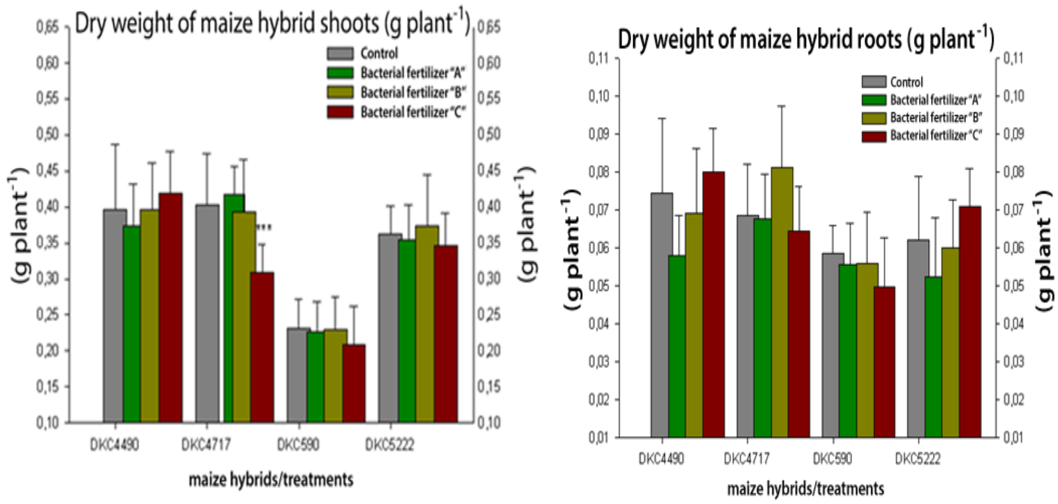


Figure 1. A and B: Dry weight of the maize hybrid shoots (Figure A) and roots (Figure B) from the MONSANTO plant breeding house (g plant-^{1}) n=9± S.D. Significant difference compared to the control: ***p<0.001

4. 1. 2. Dry weight of the shoots and roots of maize hybrids from the KWS breeding house as a result of biofertilizer treatments

Two hybrids have been analysed from the ***KWS*** breeding house. Dry weight of the shoots and roots of these maize hybrids are shown by Figures 2. A and 2. B. It is found that the two analysed hybrids reacted differently for the bacterial treatments. In the case of the ***Clemenso*** hybrid, significantly lower biomass weight was measured as a result of the "A" labelled biofertilizer than the root weight. Root weights showed larger differences in this case as well.

The dry shoot weight of the ***Karnevalis*** hybrid significantly increased (27 %) in comparison with the control. This tendency can be experienced in the case of the root as well; however the increase is not significant there. The dry shoot weight of Karnevalis increased by 8% as a result of the "B" fertilizer and by 11% as a result of the "C" fertilizer.

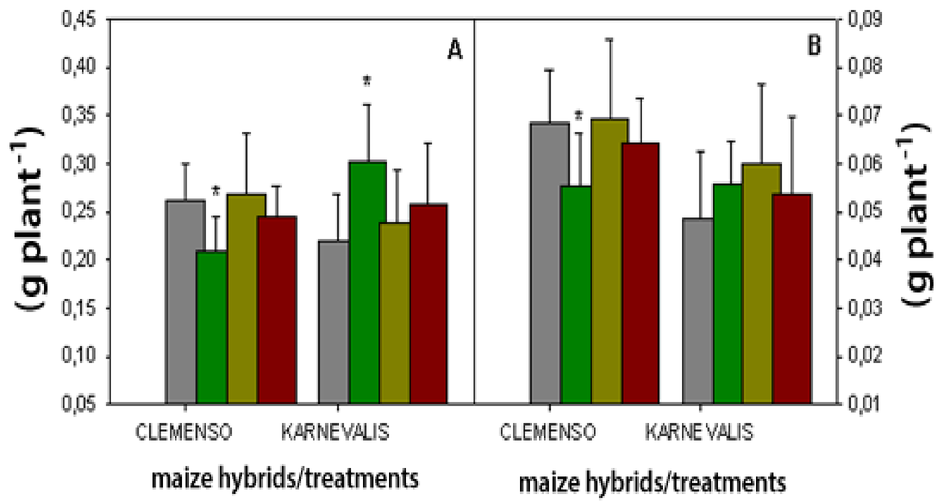


Figure 2. A and B: Dry weight of the maize hybrid shoots (Figure A) and roots (Figure B) from the KWS plant breeding house (g plant-^{1}) n=9± S.D. Significant difference compared to the control: *p<0.05 (indications are based on Figure 1)

4. 1. 3. Dry weight of the shoots and roots of maize hybrids from the SYNGENTA breeding house as a result of biofertilizer treatments

Ten hybrids of ***Syngenta*** breeding house were involved in the analyses: NK Kansas, NK Lucius, NK Octet, NK 37219, SC 3510, NK 3850, SY Flovita, SE 4410, NK Columbia, NX47279.

Similarly to the previously introduced results, the biofertilizers had diverse effects on the maize hybrids of the Syngenta breeding house.

The three analysed biofertilizers did not have a significant effect on the shoot weight of the maize hybrids. As a result of the biofertilizer “A” a slight increase is experienced in the dry weight of the ***NK Lucius***, ***NK Octet, SE 4410*** and ***NK Columbia*** hybrids. Fertilizers “B” and “C” had different effects on each hybrid in terms of shoot weight, the differences are not significant here either (Figure 3).

In the case of the rest of the analysed hybrids the analysis of the dry root weight provided values close to the control for every treatment (Figure 4).

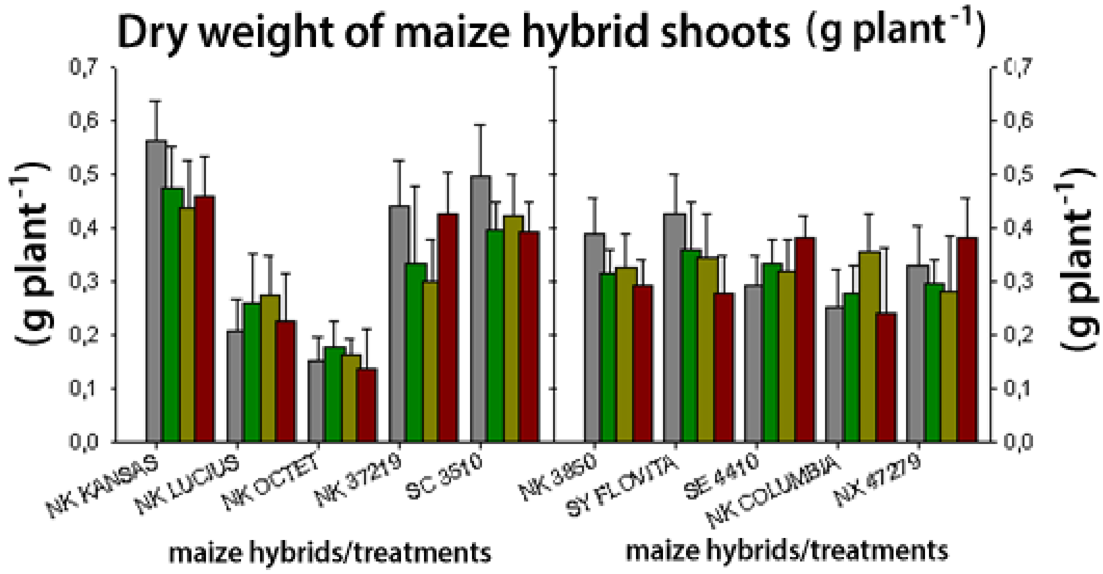


Figure 3: Dry weight of the maize hybrid shoots from the Syngenta plant breeding house (g plant-[1]) n=9± S.D. Significant difference compared to the control: *p<0.05 (indications are based on Figure 1)

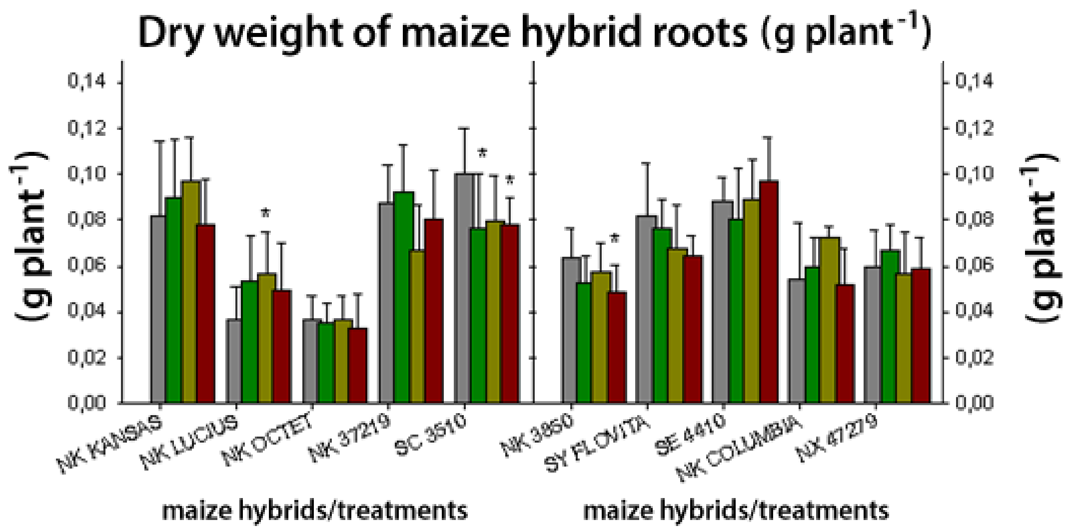
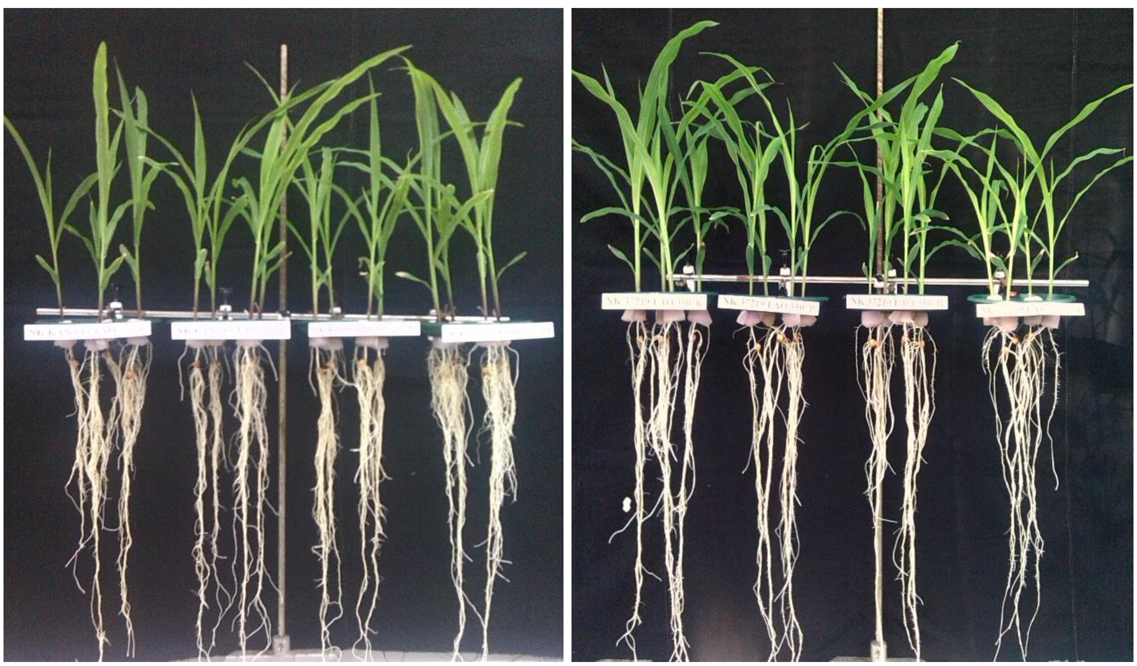


Figure 4: Dry weight of the maize hybrid roots from the Syngenta plant breeding house (g plant-[1]) n=9± S.D. Significant difference compared to the control: *p<0.05 (indications are based on Figure 1)

In the course of further analyses it has been found that the "A" biofertilizer increased the dry shoot weight of the ***NK Lucius*** hybrid, which became 20% larger than the control. The dry weight of the shoot and root of the NK Lucius hybrid increased as a result of every treatment in the case of the "B" biofertilizer. In terms of the root, the increase significant, 34 %.

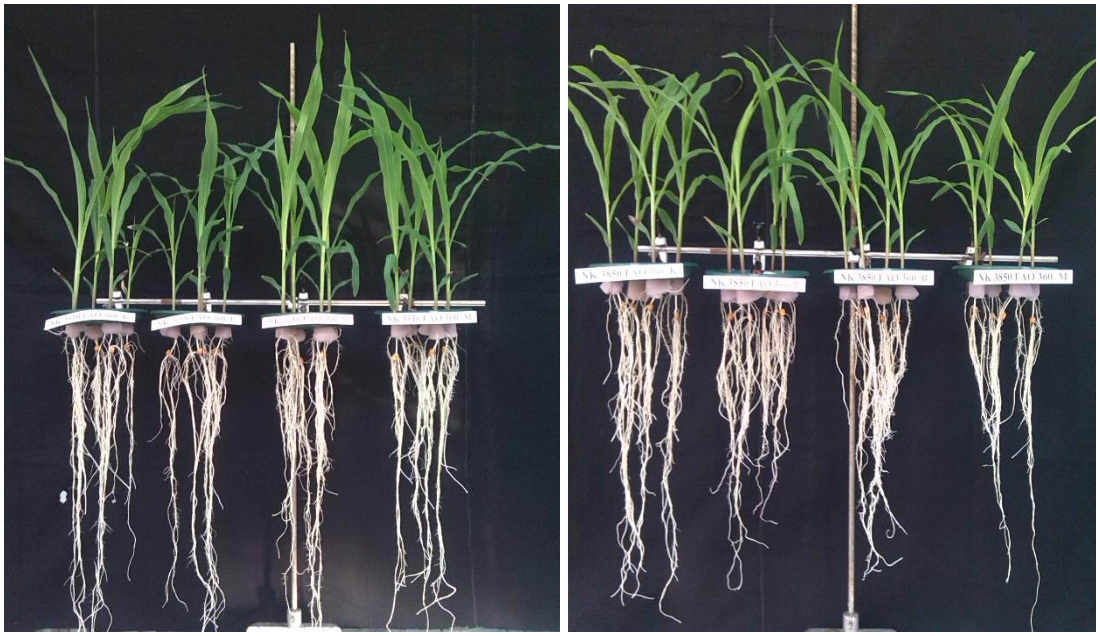
The dry shoot weight of the ***NK 37219*** hybrid decreased for all three biofertilizer treatments compared to the control. The dry weight of the root increased as a result of the “A” treatment, and it decreased as a result “B” and “C” treatments. The changes are non-significant.

In the case of the ***NK Kansas*** maize hybrid the dry weight of the shoot significantly decreased for every biofertilizer treatment, compared to the control. However, the “A” and “B” biofertilizers increased the dry weight of the root. The dry shoot weight of the ***NK 37219*** hybrid decreased as a result of all three biofertilizer treatments, compared to the control. Dry root weight increased for the “A” treatment and decreased as a result of the “B” and “C” treatments. The changes are non-significant (Picture 1 A and B).



Picture 1 A and B: Growth of the NK Kansas and NK 37219 hybrids as a result of biofertilizers (“A”, “B”, “C”) (Photo: Nagy L.G., 2012) (treatments from left to right: 1. control, 2. “A” biofertilizer, 3. “B” biofertilizer, 4. “C” biofertilizer)

The dry root weight of the ***SC 3510*** hybrid decreased as a result of all three treatments compared to the control. The dry shoot and root weight of the ***NK 3850*** hybrid decreased after every biofertilizer treatment compared to the control. The dry root weight decreased significantly as a result of the “C” treatment. The results are shown in pictures 2 A and B.



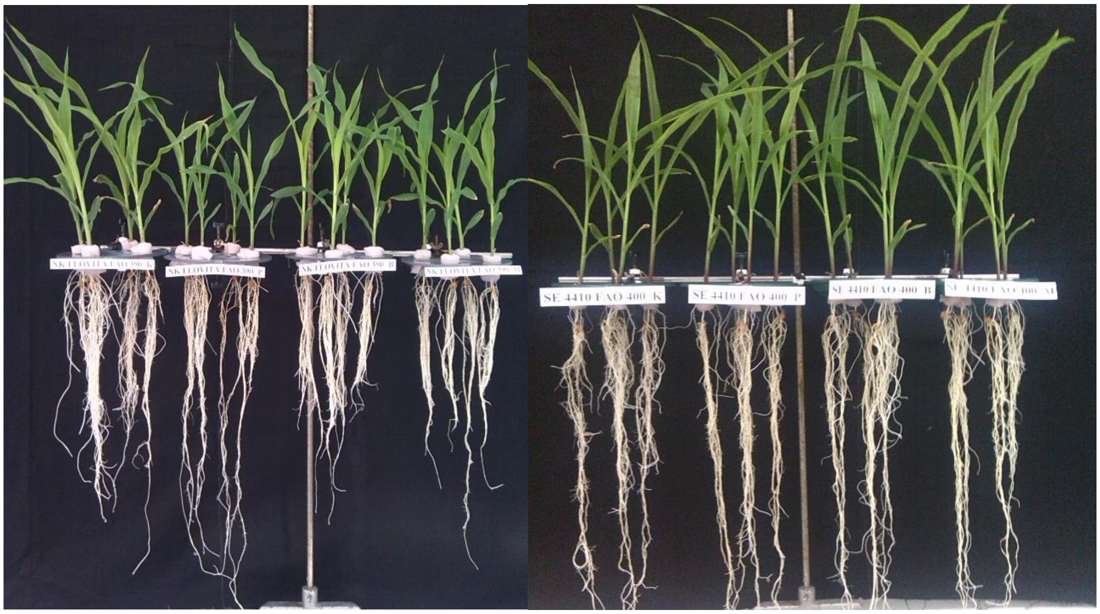
Picture 2 A and B: Growth of the SC 3510 and NK 3850 hybrids as a result of biofertilizers (“A”, “B”, “C”) (Photo: Nagy L.G., 2012) (treatments from left to right: 1. control, 2. “A” biofertilizer, 3. “B” biofertilizer, 4. “C” biofertilizer)

The dry shoot and root weights of the ***SY Flovita*** decreased as a result of the three treatments compared to the control. The largest decrease was caused by the “C” biofertilizer.

Relative chlorophyll content measured in the 2nd leaf increased as a result of every treatment, the “A” biofertilizer caused significant increase compared to the control.

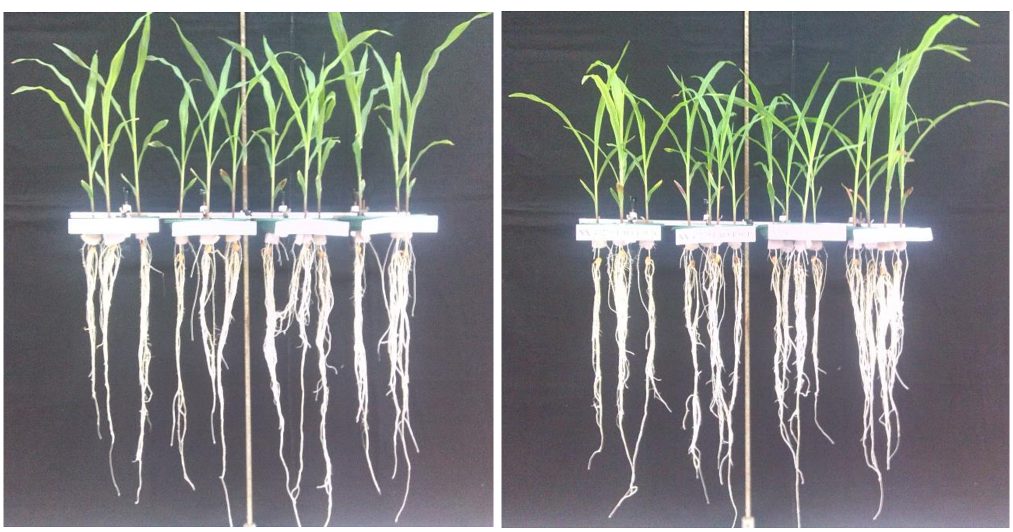
Significant decrease was recorded in the 3rd leaf in the case of the “C” treatment.

The dry shoot weight of the ***SE 4410*** hybrid increased for every biofertilizer compared to the control. The dry root weight decreased for the “A” and “B” treatments but it increased as a result of the “C” treatment (Picture 3 A, B).



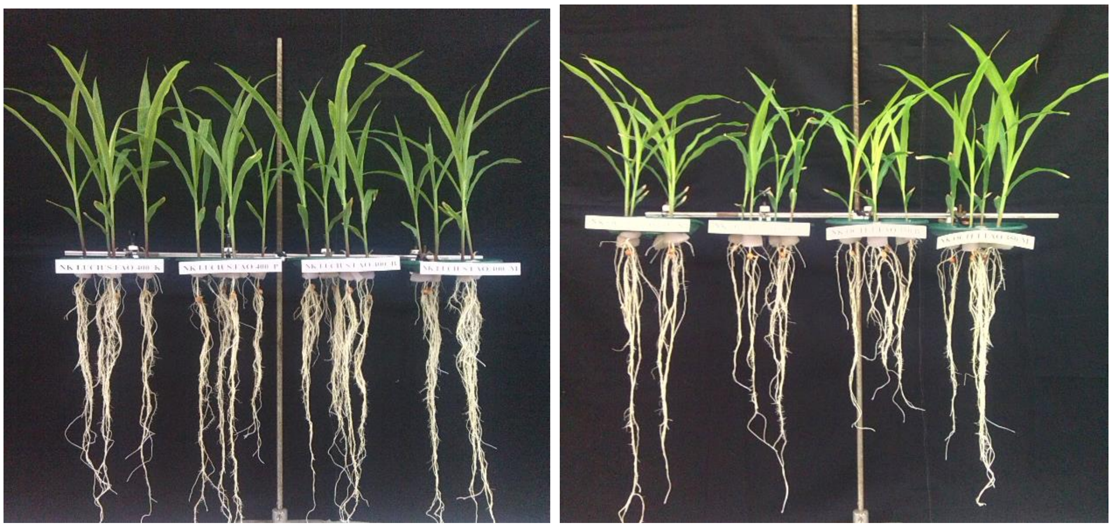
Picture 3 A and B: Growth of the SY Flovita and SE 4410 hybrids as a result of biofertilizers (“A”, “B”, “C”) (Photo: Nagy L.G., 2012) (treatments from left to right: 1. control, 2. “A” biofertilizer, 3. “B” biofertilizer, 4. “C” biofertilizer)

As a result of the “A” and “B” treatments, the dry shoot and root weight of ***NK Columbia*** increased compared to the control. The “B” biofertilizer caused larger increase. The dry shoot weight of the ***NK 47279*** maize hybrid increased for the “C” biofertilizer treatment. The dry root weight increased for the “A” treatment and it decreased for the rest of the treatments compared to the control (Picture 4 A, B).



Picture 4 A and B: Growth of the NK Columbia and NX 47279 hybrids as a result of biofertilizers (“A”, “B”, “C”) (Photo: Nagy L.G., 2012) (treatments from left to right: 1. control, 2. “A” biofertilizer, 3. “B” biofertilizer, 4. “C” biofertilizer)

In the case of the ***NK Octet***, “A” and “B” biofertilizers increased the dry weight of the shoot, while the “C” biofertilizer decreased it. The analysis of the dry root weight resulted in values close to the control for every treatment (Picture 5 A-B).



Picture 5 A and B: Growth of the NK Lucius and NK Octet hybrids as a result of biofertilizers (“A”, “B”, “C”) (Photo: Nagy L.G., 2012) (treatments from left to right: 1. control, 2. “A” biofertilizer, 3. “B” biofertilizer, 4. “C” biofertilizer)

4. 1. 4. Dry weight of the shoots and roots of maize hybrids from the MARTONVÁSÁRI breeding house as a result of biofertilizer treatments

Six hybrids of the ***Martonvásári*** breeding house have been analysed: MV 350, MV 343, Kamaria, Tarján, Mikolt, Koppány. The analysed hybrids reacted differently to the biofertilizers in this case as well. There was an average 100% difference between the lowest and highest weight hybrids in this case as well in terms of shoot biomass weight. Compared to the lowest 0.2 g (MV 350) even 0.8 g could be measured in the case of the ***Mikolt*** hybrid, where no bacterial treatment has been applied. The Mikolt hybrid reacted negatively to every bacterial treatment, namely the biomass (both shoot and root weight) decreased. (Figures 5 and 6).

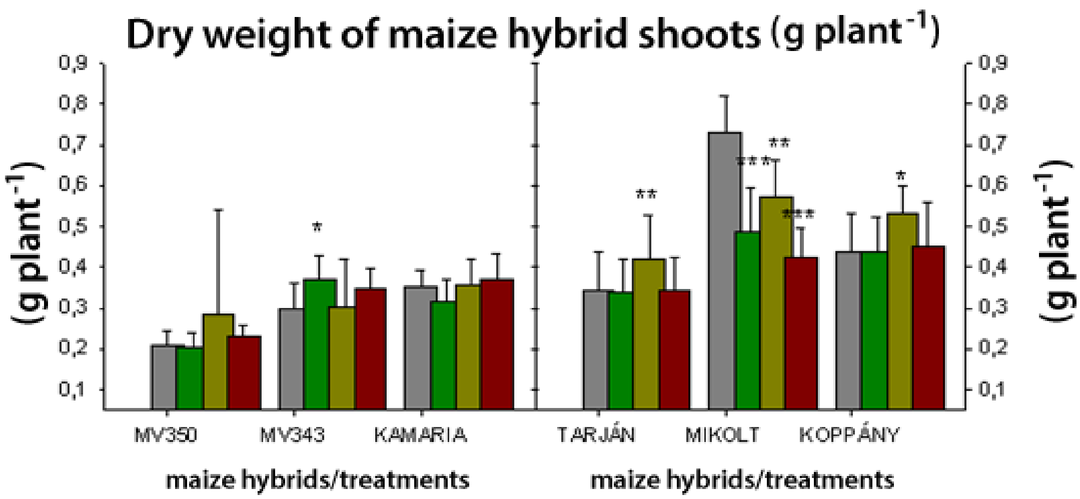


Figure 5: Dry weight of the maize hybrid shoots from the Martonvásári plant breeding house (g plant-[1]) n=9± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (indications are based on Figure 1)

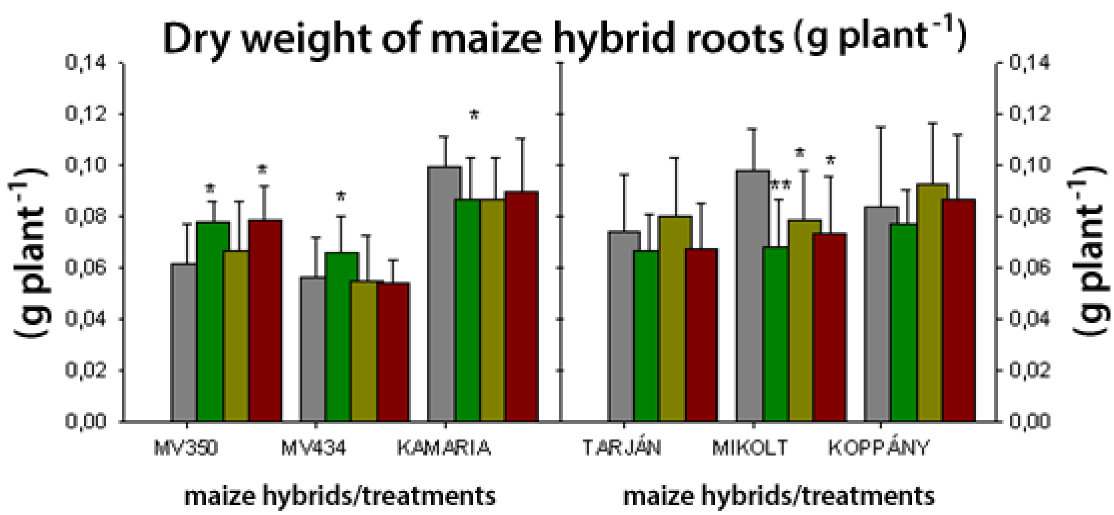


Figure 6: Dry weight of the maize hybrid roots from the Martonvásári plant breeding house (g plant-[1]) n=9± S.D. Significant difference compared to the control: *p<0,05; **p<0,01 (indications are based on Figure 1)

4. 1. 5. Dry weight of the shoots and roots of maize hybrids from the SZEGEDI GABONAKUTATÓ breeding house as a result of biofertilizer treatments

Five hybrids of the ***Szegedi Gabonakutató*** have been analysed: GK Boglár, Szegedi 386, 475, 560 and Kenéz. The lowest biomass weight was recorded for the ***Szegedi 521*** hybrid; the value of this was only fourth of the rest of the analysed hybrids, namely 0.1-0.2 g in average for each plant. This tendency appeared for root weights as well. Significantly negative and positive reactions to the biofertilizers have been experienced amongst the hybrids of Szegedi Gabonakutató as well (Figures 7. A and 7. B).

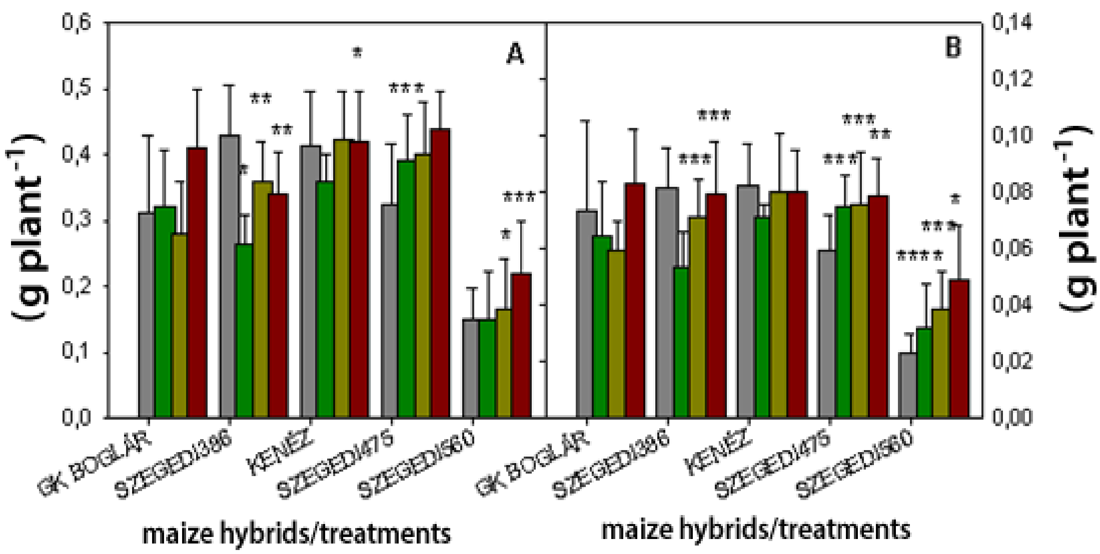


Figure 7: Dry weight of the maize hybrid shoots (A) and roots (B) from the Szegedi Gabonakutató plant breeding house (g plant-1) n=9± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (indications are based on Figure 1)

4. 1. 6. Dry weight of the shoots and roots of maize hybrids from the AGROMAG SZEGED breeding house as a result of biofertilizer treatments

Four hybrids of the ***Agromag Szeged*** breeding house have been analysed: Shakira, Eric, Temes, Jennifer.

Amongst the four hybrids, the lowest plant production was provided by the Eric hybrid. There was a twofold difference between the lowest and highest shoot biomass weights. The tendency appeared for both shoot and root weights (Figures 8. A and B).

The dry weight of shoots and roots of the ***Shakira*** hybrid decreased as a result of the biofertilizer treatments in comparison with the control. The decrease was 25% for shoots, 31% for roots in the case of the “A” treatment. These reductions are significant compared to the control values. Shoot weight decreased significantly, by 29% in the case of the “C” biofertilizer. As a result of the “B” and “C” biofertilizer treatments the dry root weight showed values close to the control.

Decrease was recorded for the ***Eric*** and ***Jennifer*** hybrids in terms of both shoot and root weights as a result of the treatments. The dry shoot and root weights of the Eric hybrid decreased by 36%

as a result of the “A” biofertilizer in comparison with the control. The dry root weight of the Jennifer hybrid decreased by 28% as a result of the “A” treatment compared to the control. In the case of the “B” treatment the dry shoot weight decreased significantly by 33%.

The “A” biofertilizer significantly reduced both the dry shoot weight and dry root weight of the ***Temes*** hybrid.

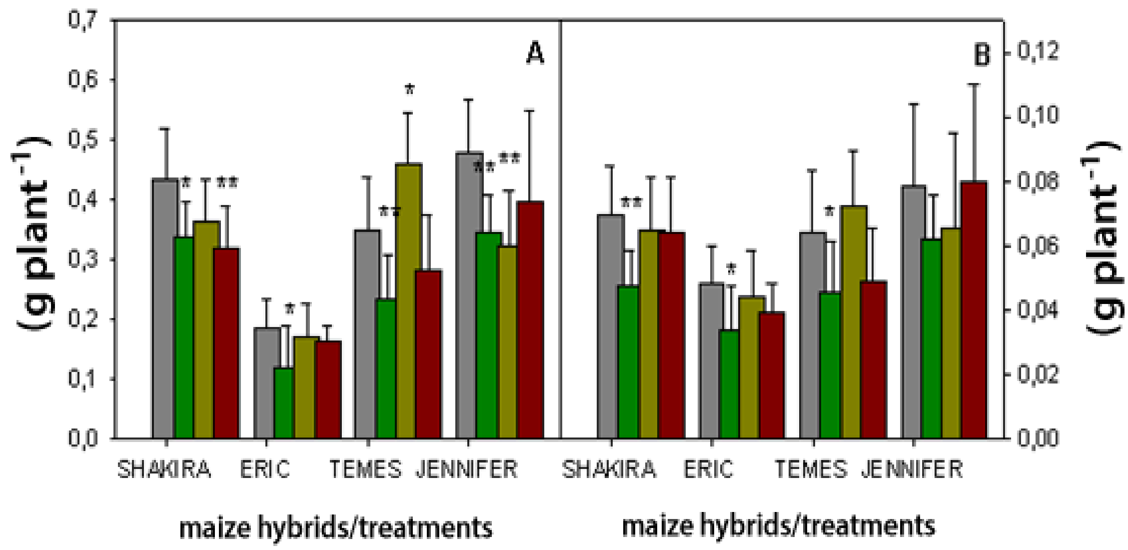


Figure 8: Dry weight of the maize hybrid shoots (A) and roots (B) from the Agromag Szeged plant breeding house (g plant-1) n=9± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (indications are based on Figure 1)

The effect of the three analysed biofertilizers on the shoot and root weight of the analysed maize hybrids is summarized in the following table (Table 2).

Table 2: The effect of the involved three biofertilizers on the shoot and root weight of maize hybrids

ns=non-significant; *↑= significant increase; *↓= significant decrease; A; B; C= biofertilizer

No., FAO No.	**Hybrid name**	**Producer**	**Dry shoot weight**			**Dry root weight**		
			A	B	C	A	B	C
Very early maturity maize								
290	Karnevalis	KWS	*↑	ns	ns	ns	ns	ns
Early maturity maize								
300	Clemenso	KWS	*↓	ns	ns	*↓	ns	ns
300	NK Kansas	Syngenta	ns	ns	ns	ns	ns	ns
320	DKC 590	Monsanto	ns	ns	ns	ns	ns	ns
330	NK Lucius	Syngenta	ns	ns	ns	ns	*↑	ns
	Shakira	Agromag	*↓	ns	*↓	*↓	ns	ns
340	Eric	Agromag	*↓	ns	ns	*↓	ns	ns
350-a	NK Octet	Syngenta	ns	ns	ns	ns	ns	ns
350-b	NK 37219	Syngenta	ns	ns	ns	ns	ns	ns
350-c	MV 350	Martonvásár	ns	ns	ns	*↑	ns	*↑
360-a	SC 3510	Syngenta	ns	ns	ns	*↓	ns	*↓
360-b	NK 3850	Syngenta	ns	ns	ns	ns	ns	*↓
360-c	MV 343	Martonvásár	*↑	ns	ns	*↑	ns	ns
370-a	DKC 4490	Monsanto	ns	ns	ns	ns	ns	ns
370-b	Kamaria	Martonvásár	ns	ns	ns	*↑	ns	ns
370-c	GK Boglár	Gabonakutató	ns	ns	ns	ns	ns	ns
370-d	Temes	Agromag	*↓	*↑	ns	*↓	ns	ns
380	MV Tarján	Martonvásár	ns	*↑	ns	ns	ns	ns
390-a	SY Flovita	Syngenta	ns	ns	ns	ns	ns	ns
390-b	Szegedi 386	Gabonakutató	*↓	*↓	*↓	ns	*↓	*↓
Mid-early maturity maize								
400-a	DKC 4717	Monsanto	ns	ns	ns	ns	ns	ns
400-b	SE 4410	Syngenta	ns	ns	ns	ns	ns	ns
410-a	Mikolt	Martonvásár	*↓	*↓	*↓	*↓	*↓	*↓
410-b	Kenéz	Gabonakutató	ns	ns	*↑	ns	ns	ns
420	MV Koppány	Martonvásár	ns	ns	*↑	ns	ns	ns
450-a	NK Columbia	Syngenta	ns	ns	ns	ns	ns	ns
450-b	Jennifer	Agromag	*↓	*↓	ns	ns	ns	ns
470	NX 47279	Syngenta	ns	ns	ns	ns	ns	ns
480	DKC 5222	Monsanto	ns	ns	ns	ns	ns	ns
490	Szegedi 475	Gabonakutató	*↑	ns	ns	*↑	*↑	*↑
Late maturity maize								
560	Szegedi 521	Gabonakutató	ns	*↑	*↑	*↑	*↑	*↑

4. 2. The effect of biofertilizer treatments on the SPAD-value of maize hybrids measured in two different age leaves

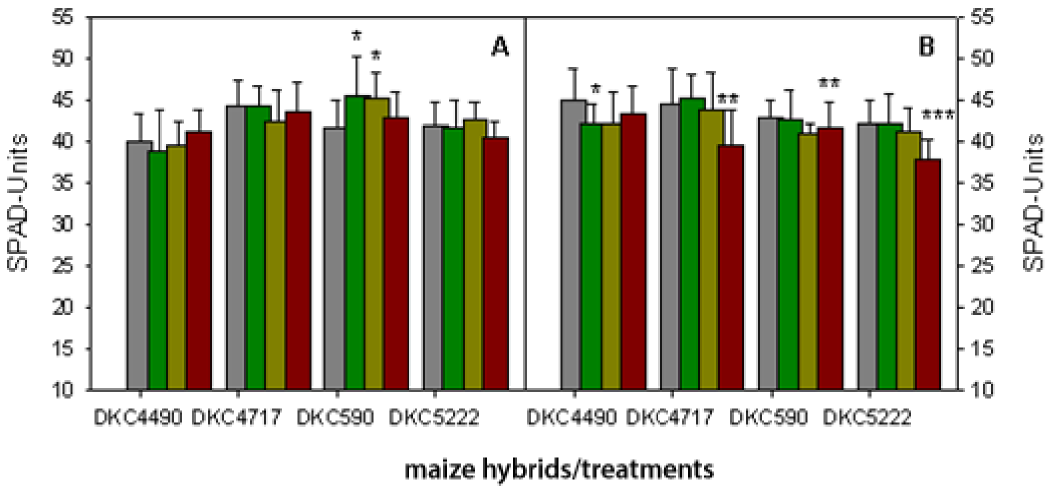
The result of the bacterial treatment can not only be indicated by the measurement of floral dry weight, but by SPAD-value (relative chlorophyll-value) as well. During its development cycle, the plant reacts differently to the effect of biofertilizer treatments.

It has been found that similarly to the experiences of dry mass measurements, biofertilizer treatments also affected differently the SPAD-value of the maize hybrid leaves.

4. 2. 1. Change of the SPAD-value as a result of biofertilizer treatments measured in the leaves of maize hybrids of the MONSANTO breeding house

SPAD-value of the 2^{nd} and 3^{rd} leaves has been measured on four ***Monsanto*** hybrids. The SPAD-value increased compared to the control in the 2^{nd} leaf as a result of the “C” biofertilizer treatment in the case of ***DKC 4490*** hybrid. However, the SPAD-value of the 3^{rd} leaf significantly decreased as a result of the “A” biofertilizer treatment.

SPAD-value measured on the 3^{rd} leaf of maize increased in comparison to the control in the case of the ***DKC 4717*** hybrid as a result of the “A” biofertilizer. The “C” biofertilizer reduced the SPAD-value compared to the control in the case of ***DKC 4717*** hybrids. The „C” biofertilizer significantly reduced the SPAD-value of the ***DKC 5222*** hybrid compared to the control (Figure 9. A and B). The relative chlorophyll value in the 2^{nd} leaf of the ***DKC 590*** hybrid significantly increased as a result of the „A” and „B” biofertilizer treatments. Significant reduction was experienced in the 3^{rd} leaf as a result of the “C” biofertilizer.



Figures 9. A and B: Relative chlorophyll value measured on the 2nd (A) and 3rd (B) leaf of Monsanto maize hybrids (SPAD-Units) n=60± S.D. Significant difference compared to the control: *$p<0,05$; **$p<0,01$; ***$p<0,001$ (Indications are based on Figure 1).

4. 2. 2. Change of the SPAD-value as a result of biofertilizer treatments measured in the leaves of maize hybrids of the KWS breeding house

SPAD-value of the 2nd and 3rd leaves has been analysed on two ***KWS*** hybrids.

Relative chlorophyll value significantly decreased in the 2nd leaf of the ***Clemenso*** hybrid as a result of the "A" biofertilizer compared to the control. As a result of the "B" treatment the measurement results were close to the control, while a non-significant reduction was recorded in the 2nd leaf as a result of the "C" biofertilizer. SPAD-value of the ***Karnevalis*** hybrids significantly decreased in the 2nd leaf as a result of the "A" biofertilizer. The "A" biofertilizer reacted identically measured on the 2nd leaf in the case of both analysed hybrids.

4. 2. 3. Change of the SPAD-value as a result of biofertilizer treatments measured in the leaves of maize hybrids of the SYNGENTA breeding house

SPAD-value of the 2nd and 3rd leaves has been analysed on ten ***SYNGENTA*** hybrids.

In the case of the ***NK Kansas*** hybrid, the SPAD-value decreased in both analysed leaves for every treatment. However, the decrease is not significant. In the case of the ***NK Lucius*** hybrid there was not significant difference between the control and treated plants in terms of the values measured on the 2nd leaves. However, the SPAD-value measured on the 3rd leaf significantly decreased as a result of the “C” biofertilizer.

In the case of the ***NK Octet*** maize hybrid, the SPAD-value significantly increased in both the 2nd and 3rd leaves compared to the control as a result of the “B” and “C” treatments. As a result of the “B” biofertilizer, there was an approximately 6.5 SPAD-value increase in the 2nd leaf and 5 in the 3rd leaf. As a result of the “C” treatment, the values increased by 6 in both the 2nd and the 3rd leaf compared to the control. In the 2nd leaf of ***NK 37219*** the SPAD-value increased significantly, while in the 3rd leaf non-significantly compared to the control. As a result of the “C” treatment, the SPAD-value decreased in both the 2nd and the 3rd leaves. (Figures 10-11).

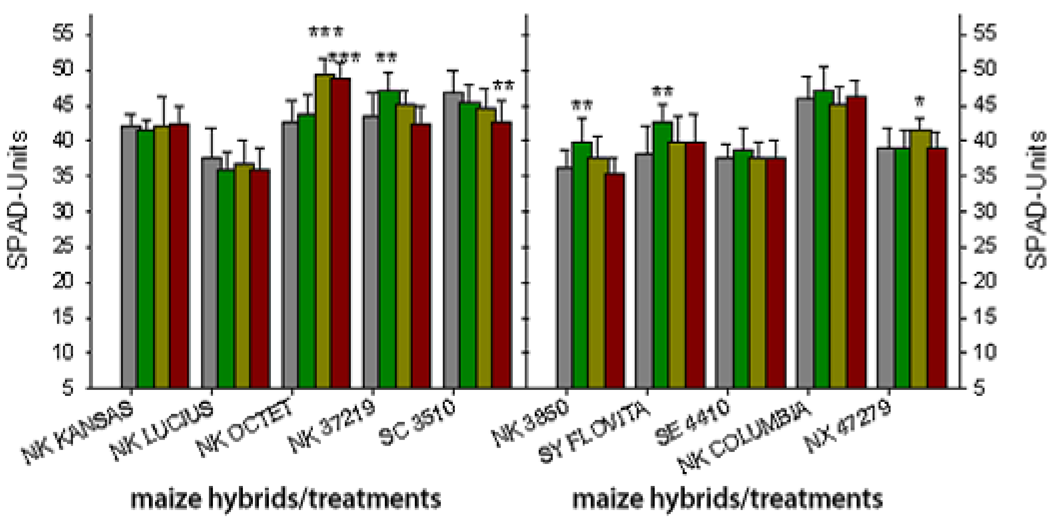


Figure 10: Relative chlorophyll value measured on the 2nd leaf of Syngenta maize hybrids (SPAD-Units) n=60± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (Indications are based on Figure 1).

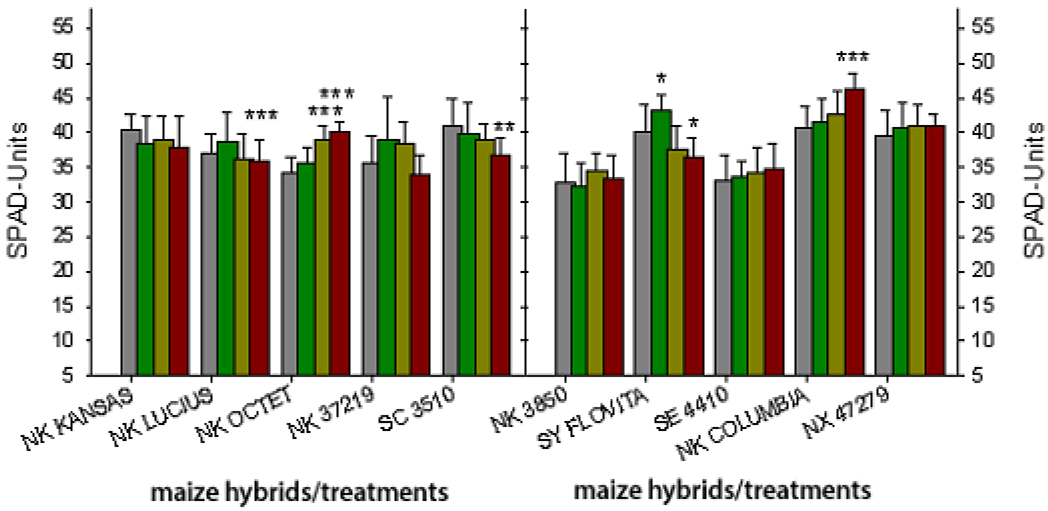


Figure 11: Relative chlorophyll value measured on the 3rd leaf of Syngenta maize hybrids (SPAD-Units) n=60± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (Indications are based on Figure 1).

The SPAD-value decreased in both the 2nd and 3rd leaves of the ***SC 3510*** maize hybrid as a result of the analysed three biofertilizers. The decrease resulted by the "C" treatment is significant.

The SPAD-value increased in the 2nd leaf of the ***NK 3850*** as a result of the "A" and "B" treatments. The decrease resulted by the "A" treatment is significant compared to the control.

As a result of the "A" treatment, The SPAD-value decreased in the 3rd leaf, while it increased in the case of the "B" and "C" treatments.

The SPAD-value in the 3rd leaf of the ***SE 4410*** hybrid increased for all three treatments. In the 2nd leaf, SPAD values increased as a result of the "A" treatment, while values close to the control have been measured for the "B" and "C" treatments.

Absolute amount of photosynthetic pigments increased in the 2nd leaf of the ***SE 4410*** hybrid as a result of the "A" biofertilizer treatment. In the case of the "C" treatment, the measured values have been around or slightly above the control.

A significant increase was recorded in the 3rd leaf of the ***NK Columbia*** hybrid as a result of the "C" biofertilizer. The SPAD-value significantly decreased for the "B" biofertilizer treatment in the 2nd and 3rd leaves.

SPAD-value of the ***NX 47279*** hybrid increased significantly in the 2^{nd} leaf as a result of “B” treatment, while the rest of the treatments resulted in values close to the control. SPAD-value measured in the 3^{rd} leaf increased non-significantly for every treatment compared to the control.

4. 2. 4. Change of the SPAD-value as a result of biofertilizer treatments measured in the leaves of maize hybrids of the MARTONVÁSÁRI breeding house

The effect of biofertilizers on the SPAD-values of the 2^{nd} and 3^{rd} leaves has been analysed on six ***MARTONVÁSÁRI*** hybrids.

In the 2^{nd} leaf of the ***MV 350*** hybrid the amount of the measured SPAD-value slightly decreased compared the control. The SPAD-value measured in the 2^{nd} and 3^{rd} leaves of the ***MV 343*** maize hybrid increased significantly as a result of the “A” and “B” treatments, while the “C” treatment caused a non-significant increase compared to the control.

There was a significant increase in the 2^{nd} and 3^{rd} leaves of ***Kamaria*** as a result of the “A” treatment, while “B” and “C” treatments induced significant increase only in the 3^{rd} leaf.

As a result of the “A” treatment, in the 2^{nd} leaf of ***Tarján*** the SPAD-value increased significantly by 3.4 units and by 2.5 units in the 3^{rd} leaf. The “B” treatment resulted in a 2.7 SPAD unit increase in the 2^{nd} leaf and a 3.5 SPAD unit increase in the 3^{rd} leaf.

The amount of relative chlorophyll value measured in the 2^{nd} leaf of the Tarján maize hybrid slightly increased compared to the control.

In the case of the ***MV Mikolt*** hybrid, the SPAD-value decreased significantly as a result of the “B” and “C” biofertilizers in the 2^{nd} and 3^{rd} leaves compared to the control. An increase of the SPAD-value is recorded for the “A” treatment compared to the control.

The SPAD-value measured in the 2^{nd} leaf of ***GK Boglár*** increased slightly as a result of the “C” treatment, while other treatments caused values close to the control. The SPAD-value measured in the 3^{rd} leaf increased in all three treatments. There was no significant difference amongst the SPAD-values measured in the 2^{nd} leaf of ***MV Koppány***, however the value measured on the 3^{rd} leaf was significantly lower than the value measured on the control plant (Figures 12 and 13).

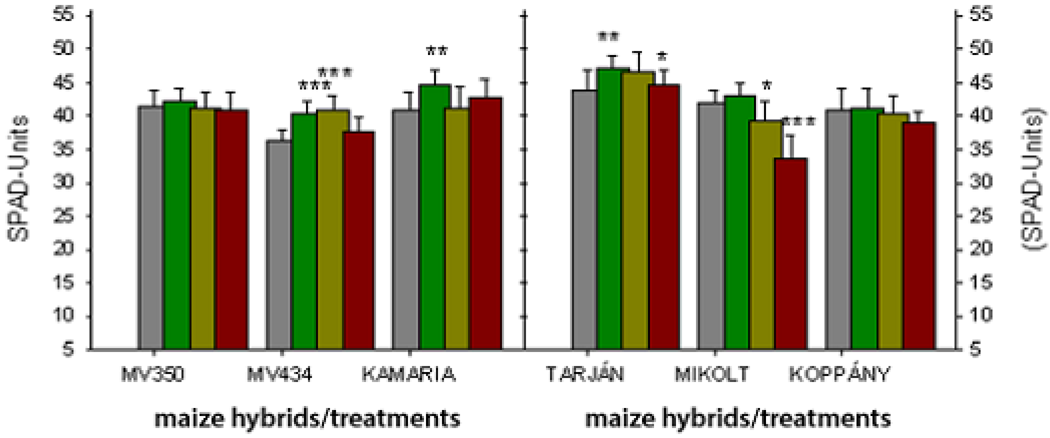


Figure 12: Relative chlorophyll value measured on the 2nd leaf of Martonvásári maize hybrids (SPAD-Units) n=60± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (Indications are based on Figure 1).

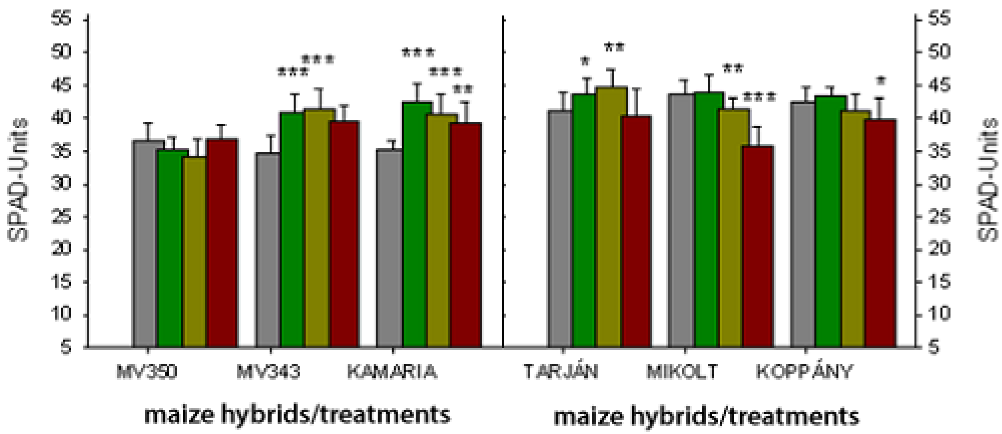


Figure 13: Relative chlorophyll value measured on the 3rd leaf of Martonvásári maize hybrids (SPAD-Units) n=60± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (Indications are based on Figure 1).

4. 2. 5. Change of the SPAD-value as a result of biofertilizer treatments measured in the leaves of maize hybrids of the SZEGEDI GABONAKUTATÓ breeding house

Relative chlorophyll value (SPAD-value) of the 2nd and 3rd leaves has been analysed on five **SZEGEDI GABONAKUTATÓ** hybrids as a result of the three biofertilizer treatments (Figure 14).

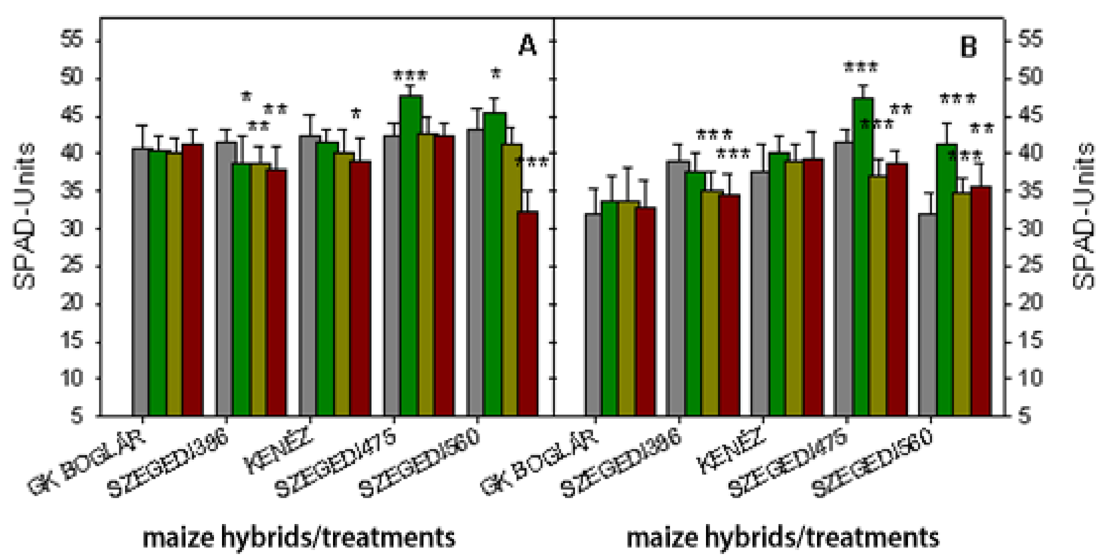
The SPAD-value increased in the 3rd leaf of the ***GK Boglár*** hybrid as a result of the treatments compared to the control, however the increase was non-significant.

Relative chlorophyll value measured on the 2nd and 3rd leaves of the ***Szegedi 386*** maize hybrid decreased as a result of the biofertilizer treatments. The changes on the 2nd leaf are significant in the case of all three biofertilizers. On the 3rd leaf, the “B” and “C” biofertilizers caused significant decrease.

The SPAD-value in the 3rd leaf of the ***Kenéz*** maize hybrid increased for every biofertilizer, while it decreased in the 2nd leaf compared to the control.

Relative chlorophyll value in the 2nd leaf of the ***Szegedi 475*** hybrid increased for every treatment compared to the control.

In the 3rd leaf of the ***Szegedi 521*** (FAO 560) relative chlorophyll value increased for all three treatments compared to the control (Figure 14).



Figures 14. A and B: Relative chlorophyll value measured on the 2nd (A) and 3rd (B) leaf of Szegedi Gabonakutató maize hybrids (SPAD-Units) n=60± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (Indications are based on Figure 2).

4. 2. 6. Change of the SPAD-value as a result of biofertilizer treatments measured in the leaves of maize hybrids of the AGROMAG SZEGED breeding house

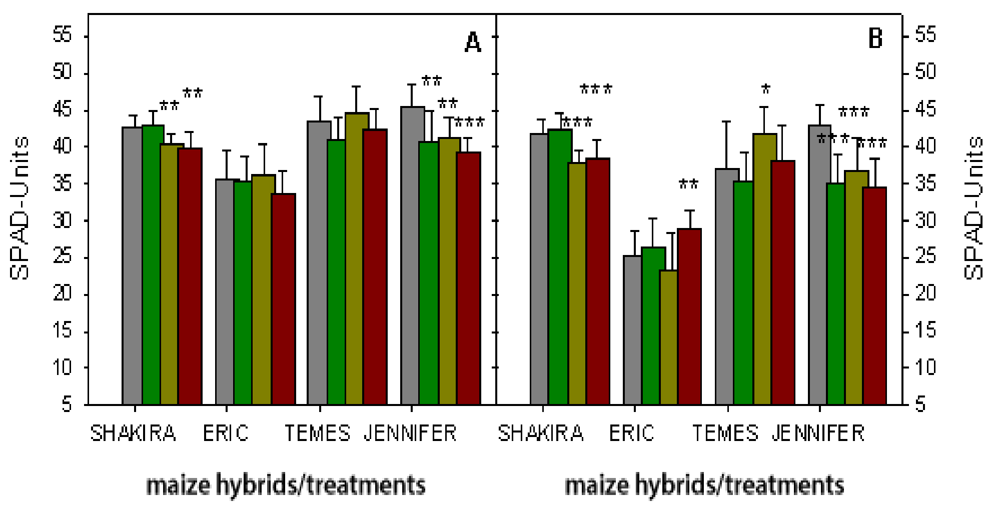
Relative chlorophyll value (SPAD-value) has been analysed in the 2nd and 3rd leaves of four ***AGROMAG SZEGED*** hybrids.

Figures 16 A and B shows the relative chlorophyll value measured in the in the 2nd and 3rd leaves of the maize hybrids originating from the ***AGROMAG SZEGED*** breeding house.

In the course of the analysis of the relative chlorophyll value measured in the 2nd leaf of the maize hybrids, the value in the 2nd leaf of ***Shakira*** was close to the control for the "A" treatment, and decreased significantly for the "B" and "C" treatments.

In the case of the ***Eric*** hybrid, relative chlorophyll value increased compared to the control as a result of the "B" treatment, but the difference is non-significant. The ***Jennifer*** hybrid reacted similarly to the biofertilizers on the basis of the measurements of both the 2nd and the 3rd leaves; however every treatment decreased the SPAD-value.

There was a significant SPAD-value increase in the case of the 3rd leaf of the ***Temes*** hybrid, caused by the "B" biofertilizer treatment (Figure 15).



Figures 15. A and B: Relative chlorophyll value measured on the 2nd (A) and 3rd (B) leaf of Agromag Szeged maize hybrids (SPAD-Units) n=60± S.D. Significant difference compared to the control: *p<0,05; **p<0,01; ***p<0,001 (Indications are based on Figure 1).

Summarizing the above, it has been found that SPAD units measured on the 2nd and 3rd leaves did not show identical tendencies for most of the hybrids. Consequently, in most of the cases it is very important which leaf is the subject of the measurement of biofertilizer effects (Table 3).

Table 3: Comparative evaluation of the effect of the three biofertilizers on the SPAD unit of the 2nd and 3rd leaves. ns=non-significant; *↑= significant increase; *↓= significant decrease; A; B; C= biofertilizer

No., FAO No.	**Hybrid name**	**Producer**	**SPAD unit of the 2nd leaf**			**SPAD unit of the 3rd leaf**		
			A	B	C	A	B	C
Very early maturity maize								
290	Karnevalis	KWS	*↓	ns	ns	ns	ns	ns
Early maturity maize								
300	Clemenso	KWS	*↓	ns	ns	ns	ns	ns
300	NK Kansas	Syngenta	ns	ns	ns	ns	ns	ns
320	DKC 590	Monsanto	*↑	*↑	ns	ns	ns	*↓
330	NK Lucius	Syngenta	ns	ns	ns	ns	ns	*↓
	Shakira	Agromag	ns	*↓	*↓	ns	*↓	*↓
340	Eric	Agromag	ns	ns	ns	ns	ns	*↑
350-a	NK Octet	Syngenta	ns	*↑	*↑	ns	*↑	*↑
350-b	NK 37219	Syngenta	*↑	ns	ns	ns	ns	ns
350-c	MV 350	Martonvásár	ns	ns	ns	ns	ns	ns
360-a	SC 3510	Syngenta	ns	ns	*↓	ns	ns	*↓
360-b	NK 3850	Syngenta	*↑	ns	ns	ns	ns	ns
360-c	MV 343	Martonvásár	*↑	*↑	ns	*↑	*↑	ns
370-a	DKC 4490	Monsanto	ns	ns	ns	*↓	ns	ns
370-b	Kamaria	Martonvásár	*↑	ns	ns	*↑	*↑	*↑
370-c	GK Boglár	Gabonakutató	ns	ns	ns	ns	ns	ns
370-d	Temes	Agromag	ns	ns	ns	ns	*↑	ns
380	MV Tarján	Martonvásár	*↑	ns	*↑	*↑	*↑	ns
390-a	SY Flovita	Syngenta	*↑	ns	ns	*↑	ns	ns
390-b	Szegedi 386	Gabonakutató	*↓	*↓	*↓	ns	*↓	*↓
Mid-early maturity maize								
400-a	DKC 4717	Monsanto	ns	ns	ns	ns	ns	*↓
400-b	SE 4410	Syngenta	ns	ns	ns	*↑	ns	ns
410-a	Mikolt	Martonvásár	ns	*↓	*↓	ns	*↓	*↓
410-b	Kenéz	Gabonakutató	ns	ns	*↓	ns	ns	ns
420	MV Koppány	Martonvásár	ns	ns	ns	ns	ns	*↓
450-a	NK Columbia	Syngenta	ns	ns	ns	ns	ns	*↑
450-b	Jennifer	Agromag	*↓	*↓	*↓	*↓	*↓	*↓
470	NX 47279	Syngenta	ns	*↑	ns	ns	ns	ns
480	DKC 5222	Monsanto	ns	ns	ns	ns	ns	*↓
490	Szegedi 475	Gabonakutató	ns	ns	*↓	*↑	*↓	*↓
Late maturity maize								
560	Szegedi 521	Gabonakutató	*↑	ns	*↓	*↑	*↑	*↑

4. 3. Chlorophyll content and the amount of carotenoids

The relative chlorophyll content of maize hybrids is only a relative value, therefore the absolute amount of photosynthetic pigments (chlorophyll-a chlorophyll-b, carotenoids) was also measured in the 2^{nd} and 3^{rd} leaves of the maize plants. The results were determined by the diversity of the hybrids. Clear tendencies in terms of the chlorophyll and carotenoid contents measured in the 2^{nd} and 3^{rd} leaves of the plants could not be determined. It has been found that the biofertilizers had the most positive effect on the amount of carotenoids amongst the photosynthetic pigments measured in the 2^{nd} leaves of the maize hybrids.

5. NEW AND NOVEL SCIENTIFIC RESULTS

1. The hybrids involved in the analyses had diverse reactions to the different biofertilizer treatments. This interaction was not influenced by the maturity group of the involved maize hybrids. This proves the specific effect between hybrids and the biofertilizers.

2. It has been proven that as a result of biofertilizer treatments the dry weight of maize shoots provided a more balanced reaction than the dry root weight. A higher standard deviation was experienced in the case of dry root weight.

3. It has been found that the applied biofertilizer products do not provide the same effect on the chlorophyll-value (SPAD-value) of the 2^{nd} and 3^{rd} leaves of maize, which proves that the nature and method of the plant-bacteria interaction changes with the growth of the plant.

4. It has been proven that in the early stage of maize hybrid growth, biofertilizers had a statistically verified positive effect on the amount of photosynthetic pigments (namely carotenoids) measured in the 2^{nd} leaf of the plants. However, their amount is a unique property and depends on the given hybrid

5. It has been found that the utilisation of the biofertilizer containing more bacterial strains does not result in the proportional increase of the dry shoot and root weight of maize plants.

6. RESULTS TO BE APPLIED IN PRACTICE

Aspiration for higher yields and better quality with the use of fewer chemicals and through environmentally aware cultivation are equally characteristic to today's agriculture. New hybrids continuously appear in production; their reactions to environmental factors might be different. The complex effect of environment is a widely researched field of science, especially in terms of nutriment supply, irrigation and technological solutions. The scope of available bio-fertilizers is growing and all of them are considered as miraculous products by their manufacturers. However, there are no trials/experiments which focus on the hybrid specific effect of the biofertilizers. This thesis aims to complement this insufficiency. The work needs to be continued, because the task is wider than the scope of present thesis.

Amongst the numerous favourable effects of biofertilizers one of the important properties is that they support nutriment uptake of the plants by enhancing the absorbability of nutriments which are difficult to mobilise; this results in more harmonic nutriment supply. However, it is a known fact that there might be differences amongst hybrids in terms of nutriment requirement and nutriment utilisation. It is without doubt that in a system where the interaction of soil-root-bacteria is analysed, every component is important, but the improvement of the nutriment uptake of roots is the result of the soil-bacteria interaction. When the amount, utilisation of artificial fertilizers applied in the soil is an important aspect, the mobilisation of the nutriment reserves of the soil might be at least of the same importance. The amount of absorbable nutriments increases as a result of both process, however plants and different hybrids have different reactions.

The practical usefulness of present work is to raise awareness to the complexity of the nutriment supply of plants and the hybrid-specific effects of bio-fertilizers. Undoubtedly, continuous research is required, because the number of hybrids is in constant change and soil types can significantly influence the effect of the applied bacteria. The thesis is an initial step on the way on which research activities related to the utilisation of conventional fertilizers reached a great distance and achieved outstanding results. Within the building of the improvement of nutriment supply, utilisation of less chemicals and environmental friendly cultivation present thesis is such a brick which might contribute to a more successful agricultural production.

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Candidate: László Géza Nagy
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List of publications related to the dissertation

Hungarian scientific article(s) in Hungarian journal(s) (1)

1. **Nagy L.G.**, Nagy L., Bodnár K., Makleit P., Lévai L., Veres S., Tóth B.: Biotrágyák hatása kukoricahibridek főbb növényfiziológiai paramétereire.
   Növénytermelés. 62 (5), 67-78, 2013. ISSN: 0546-8191.

Foreign language scientific article(s) in international journal(s) (1)

2. Tóth, B., **Nagy, L.G.**, Veres, S., Nagy, L., Lévai, L.: Industrial by-products: stress factors or nutrients?
   J. Med. Bioeng. 3 (4), 288-291, 2013. ISSN: 2301-3796.
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Hungarian conference proceeding(s) (7)

3. Bojtor C., Gombás D., **Nagy L.G.**, Hankovszky G., Tóth B.: A mangán-toxicitás mérséklése baktérium tartalmú trágyákkal.
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7. **Nagy L.G.**, Nagy L., Lévai L., Veres S., Bodnár K., Tóth B.: Baktérium tartalmú biotrágyák hatása a növénytermesztésben.
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