

**THESIS OF DOCTORAL (PhD) DISSERTATION**

**THE EFFECT OF pH, Fe- AND Zn-SUPPLY, AS WELL AS A  
BIOFERTILIZER TREATMENT ON THE MORPHOLOGICAL AND  
PHYSIOLOGICAL PROPERTIES OF YOUNG MAIZE, CUCUMBER AND  
BEAN**

**Nóra Bákonyi**

*Supervisor: Dr. László Lévai*



**UNIVERSITY OF DEBRECEN**

**Hankóczy Jenő Doctoral School of Plant Production, Horticulture and Food  
Science**

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## 1. INTRODUCTION

The most of soils on Earth are zinc-deficient in greater or lesser proportion. According to an examination concerned 298 soils in different countries the zinc deficiency is the most commonly widespread micronutrient deficiency (SILLANPÄÄ, 1990). According to GRAHAM and WELCH (1996) the plant available zinc is low in case of 50% of the soils use to cereal production. In accordance with KALOCSAI et al. (2006) the zinc supply of soils of Hungary is low in international comparison as well. The zinc supply of the Hungarian soils is medium or rather small. The largeness of the zinc-deficient soils exceeds the 50% of the cultivated areas. According to KÁDÁR (2008) statement 46% of the examined Hungarian soils had low zinc content. This ratio is 85-87% in case of the soils from the counties Békés and Fejér. The zinc content of the plants cultivated in zinc-deficient soils have low zinc content, which is dangerous in aspect of food production, because in zinc deficiency nervous system, muscle and skin tissue disorders are becoming frequent. Particularly the food production based on cereals can cause serious human health problems mainly in zinc-deficient areas (CAKMAK, 2006). The zinc deficiency is a yield-limiting factor.

The sensitivity of plants to zinc deficiency is different and there are visual zinc deficiency symptoms only in case of serious zinc deficiency. The crop yield can be reduced by hidden, latent or subclinical zinc deficiency without the appearance of any obvious visible symptoms, or with visible zinc deficiency symptoms in case of optimal zinc supply. This rate of the zinc deficiency remain unobserved without soil and plant diagnostic examinations in many cases, which cause significant economic loss and decrease in quality of crop production (IZA, 2011).

The complex, dynamic relationship of the soil (rhizosphere) and the plant affect the solubility and uptake of nutrients. The uptake and integration of nutrients into the plant cells are influenced by several factor, such as physical and chemical soils characteristics e.g. pH, high clay contents, buffer capacity or ion antagonism (KÁDÁR, 1995; KÁDÁR, 2008; LINGLE et al., 1963; OLSON et al., 1965; ALAM et al., 1999).

The unfavourable environmental factors reduce the solubility and uptake of nutrients, the biomass production of plants thereby the achievable crop yield. The soil life can be a solution to solubilize the sparingly soluble nutrient or moderate the latent nutrient deficiency of plants, thus the living bacteria PGPB (Plant Growth Promoting Bacteria) capable to stimulate the growth of host plants by excreting e. g. organic acids,

phytohormons (BLOEMBERG and LUGTENBERG, 2001). Nowadays, the importance of soil life is getting valuable, because the animal husbandry decrease and less organic fertilizer produced, which involve the decrease of humus content, and useful bacteria of soil. This problem gives opportunities to investigate and utilize effectively bacteria based biofertilizers in the 21<sup>st</sup> century.

The effect of bicarbonate given to the nutrient solution and infiltrated into the apoplast, the absolute zinc deficiency and NAA treatment, the non optimal Fe/Zn ratio, the lime Beremendi treatments applied in soils with high clay and organic matter contents, as well as a biofertilizer were examined on pH, nutrient uptake and physiological parameter of experimental plants, the excreted organic acids by roots and on the relationship of these in the evolution of latent zinc deficiency.

## **2. MATERIALS AND METHODS**

In the experiments the environmental conditions were controlled: the photosynthetic photon flux density  $300 \mu\text{mol m}^{-2} \text{s}^{-1}$ , the temperature periodicity 25/20 °C (day/night), 65-70% relative humidity and the light/dark regime 16/18 h.

### **2.1. Preparation of the experimental plants**

#### *2.1.1. The applied experimental plants*

Maize (*Zea mays L. cv. Reseda SC.*, *Zea mays L. Dekalb DKC 4490*), cucumber (*Cucumis sativum L. cv. Rajnai fűrtös*, *Cucumis sativus L. cv. Delicatess*), and bean (*Phaseolus vulgaris L. cv. Debreceni Tarka*) were used as experimental plant considering the different nutrient uptake mechanism of mono- and dicotyledonous plants (MARSCHNER et al., 1986).

#### *2.1.2. The sterilization of the seeds*

The surface of the maize seeds was sterilized by 25 min long 18%- H<sub>2</sub>O<sub>2</sub> treatment. The traces of H<sub>2</sub>O<sub>2</sub> were removed by multiple rinsing with sterile distilled water. The last rinsing solution the  $5 \times 10^{-3} \text{M}$  CaSO<sub>4</sub> was in case of maize. The seeds were soaked in this for four hour. The cucumber and bean seeds were placed on the filter paper

without any sterilization. The seeds were geotropically stimulated vertically and germinated on moistened, sterile H<sub>2</sub>O filter paper at 24°C, in climate room.

### *2.1.3. The conditions of the nutrient solution culture*

The plants were grown on nutrient solution of the following composition: 2.0 mM Ca(NO<sub>3</sub>)<sub>2</sub>, 0.7 mM K<sub>2</sub>SO<sub>4</sub>, 0.5 mM MgSO<sub>4</sub>, 0.1 mM KH<sub>2</sub>PO<sub>4</sub>, 0.1 mM KCl, 1 μM (maize) or 10 μM (cucumber) H<sub>3</sub>BO<sub>3</sub>, 1 μM MnSO<sub>4</sub>, 1 μM ZnSO<sub>4</sub>, 0,25 μM CuSO<sub>4</sub>, 0.01 μM (NH<sub>4</sub>)<sub>6</sub>Mo<sub>7</sub>O<sub>24</sub>. The iron was added to the nutrient solution in complex form of 10<sup>-4</sup>M FeEDTA.

### *2.1.4. The applied treatments in case of the nutrient solution experiments*

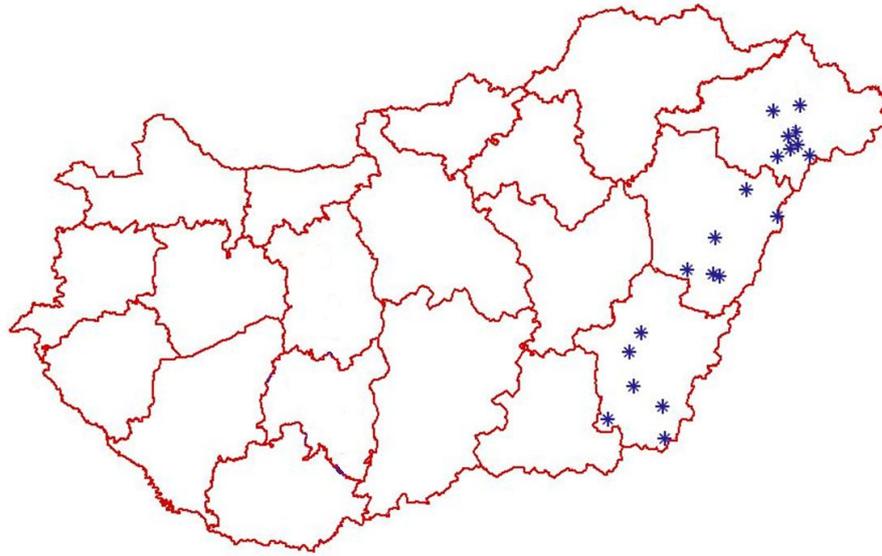
In case of the examination of the effect of bicarbonate given to the nutrient solution (ion uptake by roots) and infiltrated into the apoplast (uptake from the apoplast of leaf) the bicarbonate was added in the form of NaHCO<sub>3</sub>. The bicarbonate concentration of the nutrient and infiltration solution was 10-, 20-, 40- and 80 mM.

In case of the examination of the effect of total zinc deficiency and NAA (naphthyl -acetic acid) the following treatments were applied: control, -Zn (totally Zn deficient), -Zn+NAA (totally Zn deficient and NAA treatment). The auxin was used in synthetic form (NAA) to the top of the stem on every 3<sup>th</sup> day. One fold treatment: 1 drop NES (0, 03369g=5,37x10<sup>-1</sup>M) on the top of stem<sup>-1</sup> per plant.

In case of the examination of the effect of non optimal Fe/Zn ratio 15 different doses of Fe and Zn treatment was applied. The nutrient solution does not contain zinc and iron in case of the -Zn and -Fe treatments. The single Fe dose was 10<sup>-4</sup>M FeEDTA dm<sup>-3</sup> and the single Zn dose was 1 μM ZnSO<sub>4</sub> dm<sup>-3</sup>. The control contained single Fe and Zn doses.

### *2.1.5. The origin of the soils applied in the rhizobox experiments*

The soils used in rhizobox experiments were taken from three counties (Szabolcs-Szatmár-Bereg, Hajdú-Bihar and Békés) according to coordinates and data of the Hungarian Soil Information and Monitoring System (TIM) (**Figure 1**). It was also tested a calcareous chernozem soil originated from the Research Station Látókép of the University of Debrecen.



**Figure 1.** The selected low zinc containing topsoils in Szabolcs-Szatmár-Bereg, Hajdú-Bihar and Békés counties.

#### *2.1.6. Properties of soils applied in the rhizobox experiments*

The physical and chemical properties of soil samples were determined, such as Arany type of plasticity index, humus content and salt %,  $H_2O$  and  $CaCl_2$  pH, as well as the  $CaCl_2$ -extractable N forms and organic N contents. The clay % of soils was calculated by Arany type of plasticity index. Furthermore the ammonium lactate-extractable P, K, Ca and Mg contents were measured and the Zn, Fe and Mn contents of the soils were determined by Lakanen Erviö (LE) extractant.

#### *2.1.7. The conditions in case of the rhizobox experiments*

Three, about the same size seedling were planted into one rhizobox. In the experiments 1 cm deep 255 cm<sup>3</sup> size rhizoboxes were used. The number of the replicates was 3. Filter paper moistened by distillate water was put into rhizoboxes before the soils were placed in to be ensured the consistent water uptake for the plants. The rhizoboxes were placed in special rhizobox rack in 45 ° angle; therefore the roots were grown along the clear side of the rhizobox, which made to continuous examination of roots possible. The weight of the rhizoboxes was measured daily and missing water amount of rhizoboxes was filled.

### 2.1.8. The applied treatments in case of the rhizobox experiments

The previously described soils (19 TIM sample and one from the Research Station Látókép of the University of Debrecen) were incubated with the biofertilizer - two weeks before the experiment started - in the concentration of 1 ml dm<sup>-3</sup>. The soils were moistened to 50% water-holding-capacity (WHC). The biofertilizer was added to the distilled water used to make the soil moist.

After the above described examinations high organic matter and available zinc content soils were selected from the 20 soil mentioned before. Based on this seven soil was selected (8, 10, 15, 16, 18, 19, 20). The selected soils were treated with 1 g kg<sup>-1</sup> lime (pH 9), 2 g kg<sup>-1</sup> lime (pH 10), and 7 g kg<sup>-1</sup> lime (pH 11) doses. The number of the replicates was 3. The soils were moistened to have crumb structure - close to 50% WHC -.

### 2.2. The applied biofertilizer

The biofertilizer used in the experiments contains *Bacillus megaterium* var. *phosphaticum* (1-2×10<sup>8</sup> db cm<sup>-3</sup>) and *Azotobacter chroococcum* (1-2×10<sup>9</sup> db cm<sup>-3</sup>) bacteria stains. The biofertilizer was applied in the concentration of 1 ml dm<sup>-3</sup>.

### 2.3. The applied lime

The applied lime originated from the factory of Carmeuse Hungarya Ltd. Beremend. Regarding the quality of lime is side product, which is perfectly able to increase the pH of the soils, because rich in potassium and sodium (**Table 1.**), therefore the formed KOH and NaOH able to alkalizing the pH significantly, which enables to examine the effect of extreme high pH on the physiological parameters of experimental plants in a provocative way. In the followings in case of the description and evaluation of experiments with lime from Beremend the expression „lime” and "liming” will be used for simplicity.

**Table 1.** The contents of measured elements of applied lime from Beremend (mg kg<sup>-1</sup>).

Elements	Al	B	Ba	Ca	Cd	Cr	Cu	Fe
mg kg <sup>-1</sup>	1070	3.72	4.41	321500	1.08	4.32	1.42	377
Elements	K	Mg	Mn	Na	Ni	P	S	Zn
mg kg <sup>-1</sup>	1116	2372	16.8	254	<1	23.2	567	12

## **2.4. Methods of measurements**

### *2.4.1. Measurement of the pH of nutrient solution and apoplastic fluid*

The pH of nutrient solution was measured with Optima 200A. The pH of apoplast fluid was measured with Orion Micro Combination 12 cm pH electrode connected to the previous device. The plants were placed in a space with high relative humidity and after the appearance of guttation drops I measured their pH.

### *2.4.2. Determination of absolute and relative chlorophyll contents*

The relative chlorophyll contents were measured in different stages of development (1, 2 and 3), but fully developed leaves with SPAD 502 Minolta. The absolute chlorophyll contents was determined by the method of MORAN and PORATH (1980), VIDICIAN and CACHITA-COSMA (2010). The samples were taken from second and third leaves depending on the development of the plant. 5 ml N,N-dimethylformamid (DMF) was added to 0.5 g leaf disc. The samples were soaked in this solvent for 72 h in 4 °C. After 72 h the discs were removed and the content of chlorophyll-*a*,-*b*, total carotenoids was measured by METEREK SP-830 spectrophotometer.

### *2.4.3. Determination of the volume of the shoots and roots*

It was measured the effect of the treatments on the volume of the shoot of maize and the volume of roots of maize and cucumber. Measuring cylinder filled with distilled water was used for the measurements. The volume was determined by the water displacement - by reading the scale of measuring cylinder -.

### *2.4.4. Infiltration*

In case of the infiltration it was made 400-405 mbar vacuum in the space where the plant located. As a result of vacuum the air is head out of the stomata of mesophyll. With the careful decrease of the atmospheric pressure the infiltration solution is pushed into the apoplast. The bicarbonate concentration of the infiltration solution was 10-, 20-, 40-, 80 mM NaHCO<sub>3</sub>, over and above in case of the control distilled water was the infiltration solution.

#### *2.4.5. Determination of the root growth*

The day (6 a.m.) and night (10 p.m.) root growth were measured according the dark light regime in the climate room. The diurnal root growth was followed by drawing and measured with ruler.

#### *2.4.6. Preparation of the agar medium and the bromcresol purple indicator*

It was prepared agar medium containing special bromcresol purple indicator (pH 6.0) to visualizing pH changes by excreted organic acids by roots. The indicator solution contained 1.25% bromcresol purple and the agar medium contained 1.25 g agar-agar in 100 ml. The indicator containing agar plates were removed from the roots after 24 h.

#### *2.4.7. Determination of dry weight and the element contents of the plant and soil samples*

The plant samples were placed in preheated 85 °C MEMMERT UIM 400 oven and dried for 2 days until constant weight. When the samples cooled down to room temperature the dry weight of the samples were measured with OHAUS analytical balance. 1 g of the samples was measured after drying and grind. In case of the predigestion 10 cm<sup>3</sup> HNO<sub>3</sub> was added to the plant samples and 5 cm<sup>3</sup> HCO<sub>3</sub> to the soil samples for 30 min in 60 °C. When the samples cooled down 10 and 5 cm<sup>3</sup> H<sub>2</sub>O<sub>2</sub> was added to the plant and soils samples, respectively. The digestion kept for 90 and 270 min in 120 °C in case of the plant and soil samples, respectively. When the digested samples cooled down the tubes were completed to 50 cm<sup>3</sup> and filtered with MN 640 W (plant sample), Filtrak 388 (soil sample) filter paper. Perkin Elmer made OPTIMA 3300 DV type ICP-OES spectrometer was used to element analysis.

#### *2.4.8. The evaluation of the experimental data*

Microsoft® Excel 2007 and SigmaPlot 8.0 and 11.0 (T-test, One Way ANOVA) were used for the statistical evaluation of the data.

### 3. RESULTS AND DISCUSSION

#### 3.1. The plant physiological examination of the effect of bicarbonate and a biofertilizer given to the nutrient solution

The pH is one of the most important environmental factor affecting the solubility and uptake of nutrients. In the experiments nutrient were provided in optimal amount thus their uptake depended on the environmental factors. The NaHCO<sub>3</sub> applied in the experiments alkalizes the pH of the nutrient solution in all cases (**Table 2.**). The added biofertilizer moderated the alkalizing effect of bicarbonate. The bicarbonate retarded the solubility and uptake of nutrients by making the pH of nutrient solution alkaline. The retarded nutrient uptake resulted decrease in the shoot and root growth, which continued to decrease with the increasing bicarbonate concentration. As the effect of biofertilizer the decrease in growth moderated. The bicarbonate decreased the total root length by 16%-64%, the total shoot length of maize by 27%-64%, as well as the number of the cucumber leaves by 32%-79%. The biofertilizer added to the 80 mM bicarbonate treatment moderated the bicarbonate caused decrease in shoot and root growth by 9% and 15%, respectively. The biofertilizer could moderate the inhibitory effect of bicarbonate on the number of the cucumber leaves and on the total root length by 11% and 40% respectively.

**Table 2.** The effect of treatments on the pH of nutrient solution in case of 2 and 8-day-old maize grown in nutrient solution (n=3±s.e.). Significant different in comparison to the control: \*p <0.05, \*\*p<0.01, \*\*\*p<0.001. Significant different in comparison the pH of the fresh nutrient solution: <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001.

Treatments	2 <sup>nd</sup> day		8 <sup>th</sup> day	
	basic pH	changed pH	basic pH	changed pH
	in 0 <sup>th</sup> hour	in 72 <sup>th</sup> hour	in 0 <sup>th</sup> hour	in 72 <sup>th</sup> hour
control	7.06±0.23	6.44±0.16 <sup>b</sup>	4.86±0.06	7.04±0.24 <sup>c</sup>
control+ biofertilizer	6.94±0.45	5.75±0.12 <sup>**b</sup>	4.85±0.03	7.01±0.45 <sup>c</sup>
10mM NaHCO <sub>3</sub>	6.50±0.43	8.08±0.50 <sup>**b</sup>	6.86±0.14 <sup>***</sup>	7.53±0.15 <sup>*b</sup>
10mM NaHCO <sub>3</sub> + biofertilizer	6.43±0.42	7.71±0.46 <sup>***a</sup>	6.84±0.10 <sup>***</sup>	7.53±0.03 <sup>*c</sup>
20mMNaHCO <sub>3</sub>	7.89±0.09 <sup>**</sup>	8.08±0.03 <sup>***b</sup>	7.74±0.03 <sup>***</sup>	8.23±0.63 <sup>*</sup>
20mM NaHCO <sub>3</sub> + biofertilizer	7.81±0.08 <sup>**</sup>	8.06±0.34 <sup>***</sup>	7.70±0.06 <sup>***</sup>	8.18±0.32 <sup>**</sup>
40mM NaHCO <sub>3</sub>	8.21±0.07 <sup>***</sup>	8.65±0.38 <sup>***</sup>	8.09±0.01 <sup>***</sup>	8.31±0.04 <sup>***c</sup>
40mM NaHCO <sub>3</sub> + biofertilizer	8.20±0.07 <sup>***</sup>	8.03±0.34 <sup>***</sup>	8.06±0.04 <sup>***</sup>	8.25±0.17 <sup>**</sup>
80mM NaHCO <sub>3</sub>	8.22±0.02 <sup>***</sup>	8.70±0.10 <sup>***c</sup>	8.36±0.03 <sup>***</sup>	8.96±0.31 <sup>***a</sup>
80mM NaHCO <sub>3</sub> + biofertilizer	8.18±0.21 <sup>***</sup>	8.43±0.31 <sup>***</sup>	8.33±0.07 <sup>***</sup>	8.80±0.52 <sup>**</sup>

The bicarbonate decreased the volume of roots by 30%-75% in case of maize and by 57%-99% in case of cucumber. The biofertilizer added to the 40 mM bicarbonate treatment moderated the root volume decreasing effect of bicarbonate by 21% and 71% in case of maize and cucumber respectively. The bicarbonate reduced the dry matter production (**Table 3**).

**Table 3.** The effect of treatments on the dry matter production in case of 8-day-old maize and 24-day-old cucumber grown in nutrient solution (maize n=10±s.e., cucumber n=8±s.e.) (g plant<sup>-1</sup>). Significant different in comparison to the control: \*\*p<0.01, \*\*\*p<0.001. Significant different in comparison the treatments without biofertilizer: <sup>a</sup>p<0.05, <sup>c</sup>p<0.001.

Treatments	<i>maize</i>		<i>cucumber</i>	
	shoot	root	shoot	root
control	0.2211±0.06	0.0636±0.01	2.3515±0.56	0.4075±0.19
control+ biofertilizer	0.1810±0.02	0.0481±0.01**	2.4236±0.66	0.4390±0.16
10mM NaHCO <sub>3</sub>	0.1345±0.04**	0.0467±0.01***	0.8803±0.25***	0.1634±0.07**
10mM NaHCO <sub>3</sub> + biofertilizer	0.1135±0.06**	0.0421±0.01***	0.9745±0.52**	0.1730±0.05**
20mMNaHCO <sub>3</sub>	0.0319±0.02***	0.0432±0.01***	0.3327±0.14***	0.0761±0.04***
20mM NaHCO <sub>3</sub> + biofertilizer	0.1138±0.04** <sup>c</sup>	0.0783±0.13	0.4196±0.08***	0.0842±0.02***
40mM NaHCO <sub>3</sub>	0.0785±0.03***	0.0369±0.01***	0.1060±0.09**	0.0069±0.00***
40mM NaHCO <sub>3</sub> + biofertilizer	0.0684±0.02***	0.0378±0.01***	0.2189±0.02*** <sup>a</sup>	0.0130±0.00**
80mM NaHCO <sub>3</sub>	0.0436±0.02***	0.0360±0.01***	-	-
80mM NaHCO <sub>3</sub> + biofertilizer	0.0477±0.03***	0.0363±0.01***	-	-

The shoot dry weight of maize and cucumber decreased by 39%-95% on average, the root dry weight of maize and cucumber decreased by 27%-98% depending on the bicarbonate concentration. The 40 mM caused decrease in shoot and root dry weight of cucumber was moderated by the biofertilizer addition by 52% and 47%, respectively.

It was observed, that the effect of the investigated environmental factor is more complex than it was expected. The pH-dependent nutrient uptake influenced the synthesis of the photosynthetic pigments. The bicarbonate treatment reduced the relative chlorophyll contents of 2<sup>nd</sup> and 3<sup>rd</sup> leaves of maize and cucumber by 10%-64% and 5%-47%, respectively (**Table 4**). When biofertilizer was added to the treatments the SPAD index of the 2<sup>nd</sup> and 3<sup>rd</sup> leaves of maize and cucumber was higher by 3%-50% and 1%-30% in comparison the treatments without biofertilizer, respectively. Decrease was observed in case of the examination of the chlorophyll-*a*, chlorophyll-*b* and carotenoids contents as the effect of bicarbonate (which reduced the chlorophyll-*a*, chlorophyll-*b* and carotenoids contents on average by 65%, 67% and 53%, respectively in case of the maize (80 mM) and cucumber(40mM)), what the biofertilizer treatment could compensate (by 1.5%-4.7% in case of maize, 44%-124% in case of cucumber). The maize respond to the

bicarbonate treatments was more sensitive, because bicarbonate caused bigger reduction in the SPAD index and in the contents of chlorophyll-*a*, chlorophyll-*b* and carotenoids in case of maize than in case of cucumber.

**Table 4.** The effect of bicarbonate and a biofertilizer on the relative chlorophyll contents of 8-day-old maize and 24-day-old cucumber (SPAD units) (n=30±s.e.). Significant different in comparison to the control: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Significant different in comparison the treatments without biofertilizer: <sup>a</sup>p<0.05, <sup>b</sup>p<0.01, <sup>c</sup>p<0.001.

Treatments	<i>maize</i>		<i>cucumber</i>	
	2 <sup>nd</sup> leaf	3 <sup>rd</sup> leaf	2 <sup>nd</sup> leaf	3 <sup>rd</sup> leaf
control	44.17±1.62	41.15±1.97	44.44±1.75	40.36±1.60
control+ biofertilizer	45.78±2.38	42.42±1.43	46.20±2.93	43.90±4.01
10mM NaHCO <sub>3</sub>	35.17±5.49**	36.88±3.21**	41.70±5.87	38.48±2.03
10mM NaHCO <sub>3</sub> + biofertilizer	42.30±3.17 <sup>a</sup>	38.63±3.53	42.58±1.29	38.80±0.44
20mMNaHCO <sub>3</sub>	35.35±4.15***	31.38±3.80***	37.98±4.21**	-
20mM NaHCO <sub>3</sub> + biofertilizer	41.47±3.14 <sup>b</sup>	39.40±1.60 <sup>c</sup>	44.12±3.09 <sup>a</sup>	-
40mM NaHCO <sub>3</sub>	30.27±5.32***	31.38±3.66***	23.70±0.99***	-
40mM NaHCO <sub>3</sub> + biofertilizer	36.37±1.19*** <sup>a</sup>	35.78±3.47*** <sup>a</sup>	34.03±0.78*** <sup>c</sup>	-
80mM NaHCO <sub>3</sub>	16.10±4.82***	18.40±5.70***	-	-
80mM NaHCO <sub>3</sub> + biofertilizer	32.33±3.86*** <sup>c</sup>	26.88±6.73*** <sup>a</sup>	-	-

The bicarbonate treatments significantly increased the amount of the excreted organic acids by roots, what was moderated in case of the biofertilizer treatments, because the microorganism able to relieve the plant under unfavourable environmental factors thus the organic acid excretion of roots moderated (**Figure 2.**). The bicarbonate retarded the development of lateral roots and root hairs of maize and cucumber, what play important role in uptake of nutrients. As the effect of biofertilizer the roots were more differentiated morphologically, which enables a more effective nutrient uptake under high pH as well.

It can be concluded that the bicarbonate increased the pH of the nutrient solution, what resulted reduction in solubility and uptake of nutrients, therefore the growth and length of shoots and roots, the number of the leaves, the volume of the roots, the absolute and relative chlorophyll contents of leaves and the dry weight of the shoots and roots decreased. The bicarbonate reduced the morphological differentiation of the roots and enhanced the excretion of organic acids of roots by the increase of pH. The biofertilizer addition moderated the bicarbonate stress caused organic acid excretion and enhanced the differentiation of the lateral roots and root hairs.

The respond of cucumber to the bicarbonate treatments was more sensitive the respond of maize, because the rate of the retardation was higher the in case of cucumber than in case of maize in the examined parameters except the absolute and relative chlorophyll contents, which can be explained by the different nutrient uptake mechanism of mono- and dicotyledonous plants.

According to the results the stress effect caused by bicarbonate was moderated with the use of the applied bacteria based biofertilizer. It is supposed that the reason of this favourable effect is the similarities of nutrient uptake of bacteria and plants.

### 3.2. The plant physiological examination of the effect of bicarbonate infiltrated into the apoplast

The bicarbonate infiltrated into the apoplast influenced its pH, thus determined the solubility and uptake of nutrients by the plant cell. The shoot and root growth of maize were reduced in line with the increasing concentration of the bicarbonate. The bicarbonate decreased length of the shoot and root of maize by 11%-17% and 30%-44%; the root length of cucumber decreased by 38%-51% in comparison to the control. The bicarbonate infiltrated into the apoplast decreased the dry weight of shoots by 18%-70% and the dry weight of roots by 8%-68% in the function of the bicarbonate concentration (Table 5.)

**Table 5.** The effect of treatments on the dry matter production of 8-day-old maize and 24-day-old cucumber (maize n=12±s.e., cucumber n=4±s.e.) (g plant<sup>-1</sup>). Significant different in comparison to the control: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

Treatments	<i>maize</i>		<i>cucumber</i>	
	shoot	root	shoot	root
control	0.7121±0.25	0.0895±0.02	3.5546±0.56	0.8968±0.14
inf. with H <sub>2</sub> O	0.5746±0.12*	0.0905±0.04	1.5101±0.33*	0.4882±0.11*
inf. with 10 mM NaHCO <sub>3</sub>	0.5648±0.23*	0.0728±0.03*	1.3552±1.16*	0.4795±0.81
inf. with 20 mM NaHCO <sub>3</sub>	0.5804±0.23*	0.0804±0.02*	1.3196±0.13**	0.4713±0.17*
inf. with 40 mM NaHCO <sub>3</sub>	0.5572±0.22*	0.0797±0.04	1.0791±0.35**	0.2895±0.07**
inf. with 80 mM NaHCO <sub>3</sub>	0.5355±0.17**	0.0563±0.01***	-	-

The cucumber reacted more sensitive to the infiltration with bicarbonate, than the maize - like in the nutrient solution experiments -, because the rate of the inhibition of bicarbonate in case of cucumber in the examined parameters was two or more times large as in comparison the maize.

**Table 5.** The effect of infiltrated bicarbonate on the relative chlorophyll contents of 8-day-old maize and 24-day-old cucumber seedlings (SPAD units) (maize: n=20±s.e., cucumber: n=25±s.e.). Significant different in comparison to the control: \*p <0.05, \*\*p<0.01, \*\*\*p<0.001.

Treatments	<i>maize</i>		<i>cucumber</i>	
	2 <sup>nd</sup> leaf	3 <sup>rd</sup> leaf	2 <sup>nd</sup> leaf	3 <sup>rd</sup> leaf
control	42.88±2.48	43.55±1.07	49.88±4.47	51.84±4.77
inf. with H <sub>2</sub> O	43.55±1.07	39.33±1.04*	43.73±3.21	42.04±6.04
inf. with 10 mM NaHCO <sub>3</sub>	41.13±4.72	39.85±3.77	33.69±2.48**	40.88±2.20*
inf. with 20 mM NaHCO <sub>3</sub>	39.05±1.36*	33.63±5.57**	23.60±3.76***	35.56±1.20**
inf. with 40 mM NaHCO <sub>3</sub>	29.38±8.47*	38.73±2.50*	24.10±7.49***	34.24±2.00**
inf. with 80 mM NaHCO <sub>3</sub>	28.50±2.68***	28.25±9.57**	-	-

As the photosynthesis determines the dry matter production the relative chlorophyll contents were measured, what decreased by 4%-52% on average in the 2<sup>nd</sup> and by 8%-35% in the 3<sup>rd</sup> leaves in comparison the control (**Table 6**). The bicarbonate increased the pH of the mesophyll, thus retarded the uptake of nutrients (Fe) by plant cells needed to the photosynthesis. As a consequence of this the intensity of photosynthesis decreased, therefore the organic matter production decreased, what resulted retarded plant growth. It can be concluded that the bicarbonate given to the nutrient solution and infiltrated into the apoplast affected the examined physiological parameters similarly. According the results it was concluded that nutrient uptake mechanism of root and mesophyll cells take place by the similar mechanism.

### **3.3. The plant physiological examination of the effect of bicarbonate given to the nutrient solution and infiltrated into the apoplast and a biofertilizer on the pH of the apoplastic fluid**

It was examined the effect of bicarbonate given to the nutrient solution and infiltrated into the apoplast, as well as the effect of a biofertilizer on the pH of the apoplast fluid. To the examination the plant were forced to guttate and the pH of the guttation drops were measured. According to the data the pH of the plants cultivated with optimal nutrient supply was between 5.45- 5.65, which is appropriate for the mobilization and uptake of nutrient. The bicarbonate infiltrated into the apoplast increased the pH of apoplast fluid by 10%-38% in the function of the bicarbonate concentration of the infiltration solution, what the original apoplast fluid of the plant could buffer (**Table 7**).

**Table 7.** The pH of apoplast fluid of maize seedlings infiltrated in the age of 4, 6 and 8 days and cultivated in control nutrient solution (n=6±s.e.). Significant different in comparison to the control: \*p <0.05, \*\*p<0.01, \*\*\*p<0.001.

Treatments	pH of the infiltration solution	infiltrated		
		4-day-old	6-day-old	8-day-old
control	-	6.11±0.39	4.69±0.82	5.66±0.16
inf. with H <sub>2</sub> O	5.80	5.88±0.41	5.55±0.56	6.87±0.34***
inf. with 10 mM NaHCO <sub>3</sub>	8.54	6.75±0.26**	5.65±0.26*	7.93±0.82***
inf. with 20 mM NaHCO <sub>3</sub>	8.77	7.68±0.57***	5.11±0.54	7.66±0.25***
inf. with 40 mM NaHCO <sub>3</sub>	8.81	8.65±0.20***	6.91±0.35***	8.05±0.61***
inf. with 80 mM NaHCO <sub>3</sub>	9.04	8.99±0.09***	7.00±0.02***	9.12±0.22***

The pH of the apoplast fluid measured in the evening was less, the one measured in the morning by the reason of the fact that the excretion of organic matter or organic acids produced by the light phase of photosynthesis presumably decreased the pH of apoplastic fluid (**Table 8**). It was observed that the oldest plants have less - by 8%-15% - buffer effect than the younger have.

**Table 8.** The pH of apoplast fluid in different times of the day in case of infiltrated 4 and 8-day-old maize seedling cultivated in control nutrient solution (n=8±s.e.). Significant different in comparison to the control: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

Treatments	infiltrated in the age of 4 days		infiltrated in the age of 8 days	
	day (8 a.m.)	night (8 p.m.)	day (8 a.m.)	night (8 p.m.)
control	6.11±0.39	6.48±0.29	6.03±0.75	5.87±0.29
inf. with H <sub>2</sub> O	5.88±0.41	5.59±0.43**	6.03±0.57	6.12±0.33
inf. with 10 mM NaHCO <sub>3</sub>	6.75±0.26**	6.11±0.16*	7.03±0.32*	6.82±0.22***
inf. with 20 mM NaHCO <sub>3</sub>	7.68±0.57***	6.63±0.31*	7.58±0.14***	7.19±0.16***
inf. with 40 mM NaHCO <sub>3</sub>	8.65±0.20***	8.06±0.61***	7.98±0.35***	7.48±0.21***
inf. with 80 mM NaHCO <sub>3</sub>	8.99±0.09***	8.91±0.33***	8.23±0.64***	7.78±0.42***

**Table 9.** The effect of bicarbonate and biofertilizer given the nutrient solution on the pH of apoplastic fluid in case of 4-day-old maize and 15-day-old cucumber seedlings (maize: n=10±s.e.; cucumber: n=6±s.e.). Significant different in comparison to the control: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001. Significant different in comparison the treatments without biofertilizer: <sup>b</sup>p<0.01.

Treatments	maize	cucumber
control	8.00±0.30	7.55±0.49
control+ biofertilizer	8.20±1.27	8.60±0.16** <sup>b</sup>
10mM NaHCO <sub>3</sub>	7.35±0.43*	8.06±0.34
10mM NaHCO <sub>3</sub> + biofertilizer	8.62±0.03**	8.12±0.44
20mMNaHCO <sub>3</sub>	7.71±0.11*	8.23±0.35
20mM NaHCO <sub>3</sub> + biofertilizer	7.73±0.43*** <sup>b</sup>	-
40mM NaHCO <sub>3</sub>	6.48±0.61***	-
40mM NaHCO <sub>3</sub> + biofertilizer	6.95±0.37***	-
80mM NaHCO <sub>3</sub>	6.13±0.20***	-
80mM NaHCO <sub>3</sub> + biofertilizer	6.78±0.52***	-

According to the data the pH of the nutrient solution and the microorganism living in the nutrient solution able to influence the pH of the apoplast fluid (**Table 9.**) The bicarbonate given to the nutrient solution decreased the pH of the apoplast fluid by 4%-23%. As the effect of biofertilizer addition the pH of the apoplast fluid increased by 1%-17% on average in comparison to the treatments without biofertilizer.

### 3.4. The plant physiological examination of the effect of absolute zinc deficiency and NAA treatments

The role of zinc in the growth processes is important. The shoot and root growth of plants cultivated in zinc deficient nutrient solution was retarded. The zinc deficiency decreased the growth of shoots and roots, the dry weight (in case of shoots by 34%-36%, in case of roots by 29%-64%) in comparison to the control (**Table 10.**) The NAA treatment increased the root dry weight by 1% in case of maize and by 92% in case of cucumber in comparison the -Zn treatment, which shows that the role of zinc is significant in growth processes through auxin syntheses.

**Table 10.** The effect of treatments on the dry matter production of 15-day-old maize and 30-day-old cucumber (maize: n=27±s.e., cucumber: n=12±s.e.) (g plant<sup>-1</sup>). Significant different in comparison to the control: \*p <0.05, \*\*\*p<0.001.

Treatment	<i>maize</i>		<i>cucumber</i>	
	shoot	root	shoot	root
control	0.57±0.15	0.18±0.06	3.55±0.56	0.86±0.11
- Zn	0.37±0.12***	0.12±0.04***	2.46±0.06*	0.32±0.10***
- Zn+NES	0.37±0.12***	0.13±0.04***	2.50±0.36*	0.61±0.13*

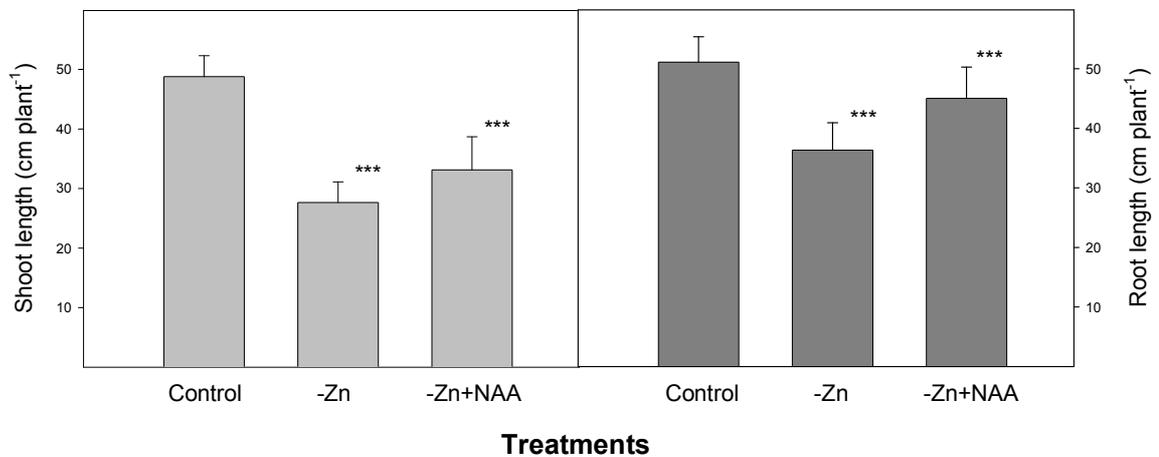
The chlorophyll syntheses were influenced by the zinc deficiency significantly in the experiments. According the data the relative chlorophyll contents of the 2<sup>nd</sup> and 3<sup>rd</sup> leaves decreased by 13%-45% in case of zinc deficiency (**Table 11.**) The NAA treatment increased the SPAD index of leaves by 9%-44% in case of maize and by 30%-35% in case of cucumber in comparison the zinc deficient treatment. The zinc deficiency decreased the total chlorophyll contents of 1<sup>st</sup> and 2<sup>nd</sup> leaves of cucumber by 57% and 63%.

The contents of chlorophyll-*a*,-*b* and carotenoids decreased in zinc deficiency on average by 11%-62%, 30%-71%, 19%-70% in the 2<sup>nd</sup> and 3<sup>rd</sup> leaves of maize and cucumber in comparison to the control. The NAA increased the contents of the

investigated photosynthetic pigments by 17%-72% in the 2<sup>nd</sup> and 3<sup>rd</sup> leaves of maize in comparison to the -Zn treatment. The effect of NAA was more effective in case of cucumber, because the increase was 15%-54% in the 2<sup>nd</sup> and 3<sup>rd</sup> leaves in comparison to the zinc deficient treatment.

**Table 11.** The effect of treatment on the relative chlorophyll contents of leaves (maize: n=475±s.e., cucumber: n=250±s.e.) (SPAD units). Significant different in comparison to the control: \*p <0.05, \*\*\*p<0.001.

Plant	Day of me.	Place of me.	Treatments		
			control	- Zn	- Zn+NES
maize	Day 7	2 <sup>nd</sup> leaf	46.5±4.41	36.0±7.14***	44.2±5.29*
	Day 14		45.1±4.50	37.5±6.48***	47.1±5.12
		3 <sup>rd</sup> leaf	45.3±3.84	39.5±6.04*	48.9±4.25*
cucumber	Day 12	2 <sup>nd</sup> leaf	53.7±5.64	41.4±4.83***	45.5±6.51*
	Day 16		50.8±1.19	31.6±3.00***	48.7±2.97
	Day 22		52.6±0.79	35.9±1.73***	51.0±4.09
		3 <sup>rd</sup> leaf	52.9±2.37	29.0±5.89***	51.6±4.25



**Figure 2.** The effect of zinc deficiency and NAA treatment on the total shoot and root length of 15-day-old maize cultivated in nutrient solution (cm plant<sup>-1</sup>), (n=30±s.e.). Significant different in comparison to the control: \*\*\*p<0.001.

The zinc deficiency decreased the total shoot length of maize by 41%, what shows that the plant population fall behind in the normal high expected by the certain phonological stage (**Figure 2.**). The total length of the zinc deficient maize roots were shorter by 29% and 41% in case of maize and cucumber respectively, than the control one. The NAA addition moderated by 14% the decrease of shoot length and by 18%-24% the root

length. The number of the cucumber leaves decreased by 35% in case of zinc deficiency in comparison to the control.

The number of the internodes decreased by 25%-26% in case of the treatments -Zn and -Zn+NES in comparison to the control (**Table 12.**). The internodes get shorter in zinc deficiency. According to the data as the effect of -Zn treatment the length of internodes decreased by 68% and 55% in comparison to the control. When the NAA was applied the length of internodes increased by 29% in comparison to the zinc deficient treatment. The roots of zinc deficient plants were less differentiated, than the control. The NAA addition enhanced the growth of the later roots and root hairs.

**Table 12.** The effect of zinc deficiency and NAA treatment on the number (piece plant<sup>-1</sup>) and length (cm plant<sup>-1</sup>) of internodes in case of 30-day-old cucumber (n=5±s.e.). Significant different in comparison to the control: \*\*\*p<0.001.

Treatments	Number of internodes	Length of internodes
control	13.80±3.42	5.80±1.06
- Zn	10.40±2.07	1.84±0.54***
- Zn+NES	10.20±1.92	2.60±0.38***

It was observed that the zinc deficiency affected negatively the investigated physiological parameters via the retardation of auxin synthesis. It was concluded that the dicotyledonous cucumber reacted more sensitive to the zinc deficiency than the maize.

### 3.5. The plant physiological examination of the effect of non optimal Fe/Zn ratio

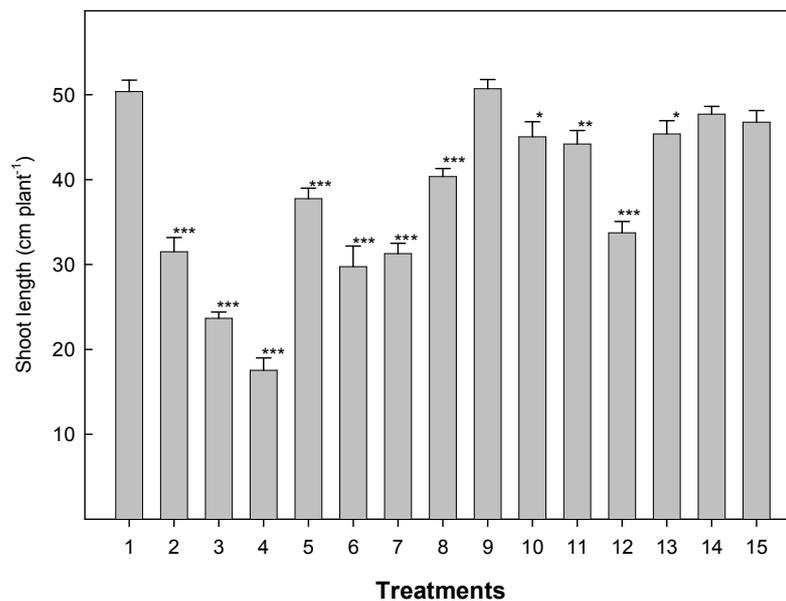
In accordance with the literature the high Fe contents of plant tissues reduce the uptake and transport of zinc. In the experiments the iron content of zinc deficient cucumber shoots increased by 42% in comparison to the control (**Table 13.**). The iron and zinc ratio of the zinc deficient leaves was 9.22, while in case of the control this value was 2.41.

**Table 13.** The Zn contents and Fe/Zn ratio of 23-day-old cucumber shoots cultivated in control and totally zinc deficient nutrient solution (mg kg<sup>-1</sup>) (n=3±s.e.). Significant different in comparison to the control: \*p <0.05, \*\*\*p<0.001.

Treatments	Fe	Zn	Fe/Zn ratio
control	99.40±1.04	41.60±4.55	<b>2.41</b>
- Zn	141.25±35.70*	15.58±2.90***	<b>9.22</b>

The growth of shoots and roots decreased in case of the non optimal zinc and iron treatments with the increase of the iron concentration in comparison to the control. The extreme high - tenfold - iron concentration significantly decreased the root growth of maize. The different concentration iron and zinc treatments caused significant differences in the root growth of cucumber. It was observed iron deficiency symptoms in the maize and cucumber leaves in case of the iron deficient treatments.

The non optimal zinc and iron supply reduced the total shoot and root length of maize by 3%-57% and 2%-50% in comparison to the control (**Figure 3.**). The roots of cucumber decreased (by 5%-24%) or increased (by 5%-19%) in the function of the treatments in comparison to the control. The increasing given doses given to the zinc deficient nutrient solution and the one-, five- and tenfold zinc treatment significantly reduced the number of cucumber leaves by 38%-60% and 14%-49% in comparison to the control.



**Figure 3.** The effect of different zinc (Zn) and iron (Fe) treatments on the total shoot length of 14-day-old maize (cm plant<sup>-1</sup>) (n=8±s.e.). 1: control, 2: - Zn+1xFe, 3: - Zn+5xFe, 4: - Zn+10xFe, 5: 1xZn+ -Fe, 6: 1xZn+5xFe, 7: 1xZn+10xFe, 8: 5xZn+ -Fe, 9: 5xZn+1xFe, 10: 5xZn+5xFe, 11: 5xZn+10xFe, 12: 10xZn+-Fe, 13: 10xZn+1xFe, 14: 10xZn+5xFe, 15: 10xZn+10xFe. Significant different in comparison to the control: \*p<0.05, \*\*p<0.01, \*\*\*p<0.001.

It was examined the number and the length of internodes, because the internodes get shorter in zinc deficiency and it may occur zinc deficiency in case of optimal zinc supply when the Fe/Zn ration is not optimal in the tissues. The number and the length of

internodes decreased by 19%-43%, 71%-90% when the zinc deficient plants were treated with increasing iron doses in comparison to the control. The number of the internodes decreased by 14%-43% in case of different zinc (Zn) and iron (Fe) treatments in comparison to the control (**Table 14.**)

The volume of the shoots decreased by 15%-67% in case of the zinc deficient plants treated with different iron doses in comparison to the control. The volume of the roots decreased by 1%-87% on average in case of the non optimal zinc (Zn) and iron (Fe) treatments in comparison to the control.

**Table 14.** The effect of different zinc (Zn) and iron (Fe) treatments on the number (piece plant<sup>-1</sup>) and length (cm plant<sup>-1</sup>) of internodes of 23-day-old cucumber (n=6±s.e.). Significant different in comparison to the control: \*p <0.05, \*\*p<0.01, \*\*\*p<0.001.

Treatments	Number of internodes	Length of internodes
control	7.0±1.0	2.38±0.78
- Zn+1xFe	5.7±0.5	0.68±0.32***
- Zn+5xFe	5.0±1.0*	0.35±0.13***
- Zn+10xFe	4.0±1.0**	0.24±0.04***
1xZn+ -Fe	8.0±2.0	1.84±1.03
1xZn+5xFe	6.0±0.0	0.72±0.32***
1xZn+10xFe	7.0±1.7	1.38±0.70**
5xZn+ -Fe	4.7±0.5*	0.59±0.04***
5xZn+1xFe	5.0±1.0*	0.81±0.54***
5xZn+5xFe	6.0±1.0	1.04±0.05***
5xZn+10xFe	4.7±1.5*	0.44±0.36***
10xZn+ -Fe	4.0±0.0**	0.30±0.18***
10xZn+1xFe	6.0±0.0	0.90±0.19***
10xZn+5xFe	5.3±1.5	0.59±0.30***
10xZn+10xFe	5.0±1.0*	0.50±0.20***

As a result of the non optimal iron and zinc supply the dry weight of the shoots and roots decreased by 1%-69% and 8%-73% on average in comparison to the control. The relative chlorophyll contents of the 2<sup>nd</sup> leaves of maize decreased by up to 50% in case of the absolute iron deficient treatments (**Table 15.**) The SPAD index decreased by 5%-25% in the 2<sup>nd</sup> and 3<sup>rd</sup> leaves in case of the increasing iron doses added to the zinc deficient nutrient solution. The one and multiple doses of zinc treatments decreased the relative chlorophyll contents by 28%-43% and by 47%-72% in the 2<sup>nd</sup> and 3<sup>rd</sup> leaves in comparison to the control. The relative chlorophyll contents by 3%-70% and 2%-69% on average in the 2<sup>nd</sup> and 3<sup>rd</sup> leaves of cucumber on the 18<sup>th</sup> and 22<sup>nd</sup> measuring days. The absolute chlorophyll-*a* contents decreased by 46%-87%, the chlorophyll-*b* 52%-

83%, the carotenoids by 45%-82% in case of the iron deficient treatments in the 2<sup>nd</sup> and 3<sup>rd</sup> leaves of maize in comparison to the control. The absolute contents of chlorophyll-*a*, the chlorophyll-*b* 52%-83% and the carotenoids of the 2<sup>nd</sup> and 3<sup>rd</sup> leaves of cucumber decreased by 1%-58%, 3%-64% and 4%-58% in case of the different zinc (Zn) and iron (Fe) treatments and by 32%-58%, 40%-64% and 33%-58% in case of the iron deficient treatments in comparison to the control.

**Table 15.** The effect of different zinc (Zn) and iron (Fe) treatments on the relative chlorophyll contents of 2<sup>nd</sup> and 3<sup>rd</sup> leaves of 18 and 22-day-old cucumber (SPAD units) (n=20±s.e.). Significant different in comparison to the control: \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

Treatments	Day 18.		Day 22.	
	2 <sup>nd</sup> leaf	3 <sup>rd</sup> leaf	2 <sup>nd</sup> leaf	3 <sup>rd</sup> leaf
control	41.28±1.64	37.40±1.57	43.53±0.51	42.80±3.12
- Zn+1xFe	43.90±3.87	39.45±2.89	42.10±4.96	43.50±1.15
- Zn+5xFe	48.13±2.60**	19.13±22.31	42.17±3.92	39.70±5.41
- Zn+10xFe	22.30±5.75***	20.95±4.19	43.43±3.10	38.73±4.80
1xZn+ -Fe	36.73±2.16**	32.78±6.44	34.73±1.26***	28.57±1.47**
1xZn+5xFe	35.03±3.37	30.60±20.41	39.97±1.32**	41.17±0.58
1xZn+10xFe	12.43±4.85***	31.68±3.35	44.27±2.84	38.10±5.72
5xZn+ -Fe	28.65±0.61***	30.18±4.80*	28.60±1.73***	21.43±3.59***
5xZn+1xFe	44.98±2.02*	43.65±2.38**	43.60±1.05	41.60±3.36
5xZn+5xFe	00.00±0.00***	0.00±0.00***	47.97±2.84*	47.60±1.42
5xZn+10xFe	21.95±5.40***	30.25±3.38	46.20±1.93	42.63±0.84
10xZn+-Fe	27.48±1.72***	26.45±3.61***	24.10±2.36***	22.37±3.06***
10xZn+1xFe	45.00±3.53	39.75±1.31	46.40±4.10	43.53±2.92
10xZn+5xFe	32.7±2.16	30.38±3.63	45.00±3.68	41.80±1.80
10xZn+10xFe	42.50±2.02	40.0±3.30	42.43±7.77	39.20±140

The non optimal Fe/Zn ratio resulted differences in the excretion of organic acids by roots. The iron deficient maize roots excreted the biggest amount of organic acids, what can be explained by the increasing phytosiderophore excretion of grasses in case of iron deficiency. As a result of the non optimal iron and zinc supply the excretion of cucumber roots was similar to the control in case of all treatments except one (5xZn+5xFe).

The size of the roots decreased by the increasing iron doses added to the zinc deficient nutrient solution (treatments 2., 3., 4.) in line with the increase of iron concentration. It was observed decrease in the differentiation of maize roots in case of the treatments where the iron was applied in tenfold concentration. The differentiation of cucumber roots

were retarded mostly by the iron deficient and extreme high iron concentration treatment.

In summary, the non optimal Fe/Zn ratio cause difficulties if the metabolism, which affect several physiological parameter. It can be concluded that the higher iron content in the tissues than zinc can cause latent zinc deficiency.

### **3.6. The plant physiological examination of the effect of low zinc supply and biofertilizer treatment**

It was examined the effect of biofertilizer treatment on the growth of plants cultivated in low - under  $1.5 \text{ mg kg}^{-1}$  KCl-EDTA extractable Zn (MÉM NAK, 1979) - zinc supply soils with different soil properties. The shoot growth of plants grown in soils treated with  $1 \text{ ml dm}^{-3}$  biofertilizer increased in the most cases in comparison to the treatments without biofertilizer. The biofertilizer addition increased root growth of maize - except the treatments numbered 9 and 11 (sandy loam soils), as well as the 18 (clay soil) - in comparison to the control. The biofertilizer treatment affected the day and night root growth favourably (**Table 16.**).

On the 1., 2. and 3. days the day root growth was higher by 2%-71% than the control in case of the biofertilizer treated plants mostly in case of the soils 16 (clay) and 17 (sandy loam). The night root growth was higher by 1%-78% than the control in case of the biofertilizer treatments on the 1., 2. and 3. days. The favourable effect of biofertilizer on night root growth was the most pronounced in case of the soils 17 (sandy loam) and 18 (clay). Accordingly, the total daily root growth increased - by 1%-65% on average - in case of the biofertilizer treatment in the most cases in comparison to the control.

When the biofertilizer was applied differences were observed in the dry weight of the shoots and roots between the soil types. The biofertilizer treatments caused significant differences. The dry weight of maize shoots decreased (by 1%-36%) in case of some treatments or increased (by 2%-26%) in case of other treatments in comparison to the control. The dry weight of roots of biofertilizer treated plants was higher then the control by 12%-35% in some cases and it was similar or less (by 2%-70%) in some cases. The differences measured in the dry weight show the diversity of soil characteristics.

The PGPR containing biofertilizer affected the root growth, in some cases the dry weight and growth of shoots and roots positively indicating the possible positive effect of bacteria on the solubility, mobility and uptake of nutrients.

**Table 16.** The effect of low zinc supply and biofertilizer treatment on the day and night root growth of 1, 2 and 3-day-old maize seedlings cultivated in rhizoboxes (n=9±s.e.) (cm root<sup>-1</sup>). +P: biofertilizer addition. Significant different in comparison to the untreated soil: p\* < 0.05, p\*\* < 0.01, p\*\*\* < 0.001.

Treat.	Day 1.		Day 2.		Day 3.	
	day	night	day	night	day	night
<b>1</b>	1.28±0.21	1.57±0.31	2.77±0.25	2.01±0.46	2.40±0.68	2.01±0.47
<b>1P</b>	1.36±0.32	2.13±0.54*	3.10±0.35	2.25±0.37	3.06±0.48	2.36±0.58
<b>2</b>	1.11±0.46	1.66±0.34	2.65±0.38	2.27±0.34	2.92±0.15	2.17±0.26
<b>2P</b>	1.40±0.31	1.66±0.16	3.04±0.37	2.36±0.63	2.91±0.56	2.34±0.41
<b>3</b>	1.42±0.47	1.72±0.61	2.88±0.90	2.32±0.84	2.54±0.54	2.12±0.40
<b>3P</b>	1.85±0.22*	2.16±0.30	3.23±0.20	2.62±0.47	2.64±0.82	2.26±0.34
<b>4</b>	1.53±0.32	1.94±0.42	3.12±0.23	2.29±0.31	2.42±0.89	1.66±0.59
<b>4P</b>	1.79±0.20	2.18±0.28	3.51±0.23**	2.56±0.30	2.94±0.70	2.12±0.73
<b>5</b>	1.60±0.25	1.71±0.31	2.78±0.64	2.30±0.36	3.02±0.61	2.15±0.55
<b>5P</b>	2.19±0.28**	2.32±0.28***	3.33±0.27**	2.37±0.21	2.73±0.56	2.20±0.45
<b>6</b>	2.08±0.08	2.19±0.22	2.72±0.23	2.18±0.33	2.69±0.51	2.01±0.18
<b>6P</b>	2.21±0.14	2.26±0.19	2.85±0.23	2.21±0.32	2.69±0.76	2.09±0.22
<b>7</b>	1.61±0.26	1.88±0.39	2.52±0.43	2.11±0.55	2.26±1.18	1.62±0.76
<b>7P</b>	1.99±0.16*	2.14±0.32	3.10±0.41*	2.48±0.13	2.66±0.12	2.78±0.67*
<b>8</b>	1.57±0.18	1.58±0.52	2.69±0.23	2.14±0.13	2.82±0.62	1.69±0.48
<b>8P</b>	1.71±0.21	1.71±0.52	2.84±0.11*	2.33±0.18*	3.24±0.29	1.99±0.41
<b>9</b>	1.60±0.43	1.72±0.23	2.67±0.77	2.25±0.09	3.21±0.10	2.33±0.25
<b>9P</b>	1.99±0.34	1.89±0.25	3.46±0.96	2.25±0.22	3.58±1.95	2.25±0.15
<b>10</b>	1.93±0.09	1.87±0.23	2.29±0.33	2.17±0.31	2.42±0.52	1.75±0.34
<b>10P</b>	1.99±0.12	1.97±0.22	2.83±0.17*	2.25±0.23	2.55±0.31*	1.75±0.53
<b>11</b>	1.00±0.41	1.11±0.30	1.88±0.43	1.68±0.75	2.42±0.51	2.00±0.30
<b>11P</b>	1.16±0.31	1.11±0.26	2.08±0.14	1.81±0.68	2.47±0.24	2.10±0.51
<b>12</b>	1.39±0.24	1.31±0.18	1.94±0.43	1.83±0.78	2.26±0.75	1.89±0.40
<b>12P</b>	1.61±0.22	1.33±0.30	2.34±0.24	1.91±0.63	2.37±0.66	2.34±0.30*
<b>13</b>	1.44±0.39	1.44±0.23	2.19±0.72	2.28±0.49	2.58±0.10	2.16±0.29
<b>13P</b>	1.49±0.26	1.44±0.22	2.30±0.40	2.31±0.32	2.52±0.10	2.15±0.57
<b>14</b>	1.39±0.33	1.36±0.22	2.06±0.21	1.99±0.30	2.24±0.81	1.84±0.20
<b>14P</b>	1.89±0.24**	1.60±0.21	2.25±0.25	2.06±0.31	2.38±0.23	2.00±0.44
<b>15</b>	1.64±0.37	1.45±0.16	2.38±0.34	2.26±0.41	2.33±0.35	2.14±0.21
<b>15P</b>	1.88±0.41	1.48±0.35	2.38±0.23	2.38±0.27	2.45±0.17	2.15±0.33
<b>16</b>	0.77±0.24	1.34±0.32	2.13±0.66	1.04±0.32	1.93±0.54	1.49±0.49
<b>16P</b>	1.13±0.34*	1.53±0.32	2.33±0.46	1.41±0.36	2.61±0.50*	2.41±0.18**
<b>17</b>	0.64±0.24	0.54±0.31	0.80±0.43	0.95±0.27	1.50±0.00	1.29±0.63
<b>17P</b>	0.73±0.59*	2.49±0.55***	2.77±0.50***	2.26±0.58**	2.43±0.87*	1.30±0.30
<b>18</b>	0.72±0.25	1.54±0.19	2.24±0.43	1.26±0.57	2.18±0.27	1.08±0.48
<b>18P</b>	0.97±0.32	1.58±0.30	2.36±0.68	1.51±0.34	2.18±0.32	1.93±0.68
<b>19</b>	1.36±0.11	1.73±0.22	2.79±0.27	1.43±0.54	2.82±0.39	1.98±0.33
<b>19P</b>	1.51±0.27	1.95±0.06**	2.92±0.37	1.87±0.24	3.16±0.45	2.36±0.51
<b>20</b>	0.87±0.28	1.26±0.47	2.30±0.73	1.28±0.53	1.85±0.96	1.44±0.53
<b>20P</b>	1.17±0.25	1.58±0.19	2.57±0.38	1.74±0.16*	2.40±0.42	1.86±0.32

### 3.7. The plant physiological examination of the effect of lime treatments

It was observed decrease of growth in case of the treatments with different concentration 1, 2, 7 g kg<sup>-1</sup> lime. The lime caused decrease in growth continued to decline in line with the increase of the lime concentration. The total shoot length decreased by more than 15% proportionately with the increase of lime doses in comparison to the control.

The different doses of lime caused decrease in the length of the internodes (**Table 17.**). The length of the hypocotyls was shorter by close to 3 cm and the 1<sup>st</sup> internodes by more than 1 cm than the control one in case of the biggest lime doses (7 g lime kg<sup>-1</sup> soil). One of the zinc deficiency symptoms is the shortening of shoot length and internodes. According to the literature the mobility and uptake of zinc decrease with the increase of the pH, the clay and lime content, as well as the phosphate supply of soil. In the experiments the lime treatments caused increase of soil pH reducing the uptake of nutrients (Zn), what caused shortening of the internodes.

**Table 17.** The effect of treatments on the length of hypocotyls and epicotyls of 13-day-old bean (n=9±s.e.) (cm plant<sup>-1</sup>). Significant different in comparison to the control: p\* < 0.05.

Number of soils	Hypocotyls			
	control	1 g lime kg <sup>-1</sup> soil	2 g lime kg <sup>-1</sup> soil	7 g lime kg <sup>-1</sup> soil
8	4.38±1.90	3.39±1.31	3.28±1.79	3.02±1.54
10	4.57±0.42	4.50±0.42	4.38±1.07	3.07±1.72
15	3.73±0.55	4.31±0.69	3.72±1.03	3.53±1.12
16	5.44±0.92	4.40±0.76*	4.22±0.13*	4.05±0.71*
18	3.94±1.85	3.36±1.52	3.22±1.59	2.97±1.46
19	4.20±1.03	3.44±2.26	3.34±0.47	3.09±0.47
20	4.47±1.16	2.91±1.77	2.89±1.91	1.67±1.32*
Number of soils	Epicotyls			
	control	1 g lime kg <sup>-1</sup> soil	2 g lime kg <sup>-1</sup> soil	7 g lime kg <sup>-1</sup> soil
8	3.42±0.69	2.86±1.51	2.79±1.72	2.50±1.30
10	3.27±0.50	3.12±0.63	3.11±0.52	2.63±1.66
15	3.43±1.58	2.91±0.95	2.44±1.51	2.37±1.18
16	3.56±0.51	2.74±1.19	2.73±1.18	2.53±0.51*
18	3.60±0.80	3.11±2.21	2.59±1.00	2.11±1.59
19	2.91±0.43	2.19±1.21	2.08±1.39	2.07±1.07
20	2.73±1.01	2.17±1.64	2.06±1.38	0.99±1.13

The relative chlorophyll contents of maize and bean decreased by 3%-20% and 7%-75% in case of the lime treatments in comparison to the control (**Table 18.**). The

intensity of photosynthesis determines the dry matter production. According to the results the dry matter production of shoot and roots significantly decreased (by 45%-60%) in case of maize. The dry weight of shoot and root of bean decreased in the function of the lime concentration by 12%-29% and 17%-23% in comparison to the control. The lime treatments caused decrease in the dry matter production can be explained by the correlation of high pH and retarded nutrient uptake.

**Table 18.** The effect of different dose of lime on the relative chlorophyll contents of 2<sup>nd</sup> leaves of 10-day-old maize and 1<sup>st</sup> leaves of 13-day-old bean (SPAD units) (maize: n=30±s.e., bean: n=45±s.e.). Significant different in comparison to the control: p\* < 0.05, p\*\* < 0.01.

Plant	Number of soils	control	1 g lime kg <sup>-1</sup> soil	2 g lime kg <sup>-1</sup> soil	7 g lime kg <sup>-1</sup> soil
<i>maize</i>	8	33,76±4,48	32,34± 7,43	32,12± 4,07	30,37± 1,72
	10	35,50±4,58	33,13± 3,30	31,88± 2,70	30,62± 6,45
	15	37,76±1,69	37,44± 3,15	35,74± 1,53	32,28± 7,52
	16	46,02±1,11	36,00± 0,52*	36,90± 1,06	36,27± 3,11
	18	42,12±2,50	35,44± 1,46*	34,38± 4,69*	37,85± 0,07
	19	42,83±3,99	32,78± 6,16*	32,24± 4,69*	31,60± 1,41*
	20	42,75±1,42	33,20± 1,95*	34,15± 1,52*	32,28± 3,51*
<i>bean</i>	8	45,27±2,43	41,93±15,81*	33,18±13,94**	33,03±14,62**
	10	46,66±3,53	42,58± 5,28	41,16± 2,63	30,03±17,64*
	15	48,17±3,71	43,98± 5,99	40,67± 5,60	33,10±13,62*
	16	49,38±3,98	41,71±15,73	40,75± 7,69	35,08±19,88*
	18	47,10±3,17	45,43± 5,04	39,11± 6,06	26,32±19,92*
	19	47,08±2,56	40,34± 2,54*	40,12± 2,42*	39,38± 3,29*
	20	51,10±3,29	33,47±23,03	32,98± 19,33*	13,41±20,62*

According the data the total daily root growth decreased by 2%-45% on the 1., 2. and 3. day in case of the different dose of lime treatments in comparison to the control. The day and night root growth decreased by 6%-43% and 2%-47% on the 1<sup>st</sup> day, by 9%-59% and 4%-37% on the 2<sup>nd</sup> day, by 8%-50% and 5%-35% on the 3<sup>rd</sup> day on average in case of maize in comparison to the control. In case of bean the day and night root growth also decreased. The biggest lime doses caused 29% and 53% decrease in the day and night root growth.

The increasing lime doses decreased the root growth, which directly influences the nutrient uptake capacity of plant, because less roots able to uptake less amount of water and nutrient from the soil.

#### 4. NEW AND NEW-TYPE SCIENTIFIC RESULTS

1. The pH has significant importance in solubility and uptake of nutrients. The slightly acidic (pH 6.0-7.0) soils are the most favourable for the most crop plants, because the availability of the most nutrients is the biggest in soils with slightly acid or neutral pH. **It was concluded that bicarbonate applied in the experiments as a stressor basified the pH, thereby retarded the growth of maize and cucumber, influenced the synthesis of photosynthetic pigments in the leaf of maize and cucumber, thus the intensity of photosynthesis. The bicarbonate caused alkaline pH increased the organic acid excretion of roots of maize and cucumber and retarded the morphological differentiation of roots.**
2. The bicarbonate infiltrated into the apoplast - like in case of the pH of nutrient solution - basified the pH of apoplast fluid of maize mesophyll as well. **It was concluded that the alkaline pH - with the increase of pH - can cause retardation in growth and in the relative chlorophyll contents like in case of the alkaline pH of nutrient solution, because the nutrient uptakes of root and mesophyll cells take place according to the same mechanism.**
3. From the content, the pH of apoplastic fluid can be inferred to the processes take place in the apoplast. **It was examined first the pH of apoplast fluid by guttation under the effect of bicarbonate given to nutrient solution and infiltrated into the apoplast and biofertilizer treatment. Based on the results of these investigations it was concluded that the pH (bicarbonate concentration) of the nutrient solution affects the pH of apoplastic fluid of maize and cucumber.**
4. The biofertilizer treatment applied in the nutrient solution increased the pH of apoplastic fluid of maize and cucumber, while modified (moderated) the pH (alkalinity) of nutrient solution in an opposite way. **It was concluded that bacteria (*Bacillus megaterium var. phosphaticum* and *Azotobacter chroococcum*) living in the nutrient solution able to modify the pH of the apoplast fluid of maize and cucumber under stress conditions (alkaline pH) as well, thus enhance the solubility of nutrients and their uptake by plant cells. It was concluded that the apoplast fluid of plants has buffer ability - like the soil has - thus the plant can**

**modify the pH of mesophyll thereby moderate the effects of unfavourable environmental factors.**

- 5. The role of zinc is important in the growth processes - via auxin syntheses -. It was concluded that the absolute zinc deficiency can decrease the total length of maize shoots, the number and length of internodes of cucumber (thus the high of the plant population), the relative chlorophyll contents of maize and cucumber leaves, the absolute contents of photosynthetic pigments. The zinc deficiency can reduce the differentiation of roots of maize and cucumber. It was concluded that NAA treatment applied in zinc deficiency compensated for the zinc deficiency caused growth retardation, eliminated the decrease of photosynthetic pigments and improved the differentiation of lateral roots and root hairs.**

## 5. PRACTICAL RESULTS

1. The PGPB microorganisms living in the nutrient solution able to stimulate the growth of plants. **It was concluded that the applied *Bacillus megaterium* var. *phosphaticum* and *Azotobacter chroococcum* containing biofertilizer moderated the pH increasing effect of bicarbonate, thus the unfavourable effect of bicarbonate, such as decrease of growth and content of photosynthetic pigments. In addition the biofertilizer affects the morphological differentiation of roots positively (thus enhance the effectiveness of nutrient uptake), as well as compensated the organic acid excretion of roots (thus help to achieve more economic yield under stress condition as well), therefore necessary to apply biofertilizer in soils with slightly alkaline, alkaline pH to improve nutrient uptake.**
2. It was demonstrated, that the applied *Bacillus megaterium* var. *phosphaticum* and *Azotobacter chroococcum* containing biofertilizer improve the evaluation of the optimal pH via moderating the pH of the nutrient solution or increasing the pH of the apoplast fluid with a new type of mechanism of action thus make the nutrients soluble and the nutrient uptake more effective.
3. According to the literature the maize and the bean belong to the species most sensitive to the zinc deficiency. **It was pointed out that the cucumber responded more sensitive than the maize in case of the examined parameters (length of shoots and roots, number of leaves, root dry weight, relative chlorophyll contents, the contents of chlorophyll-*a*, -*b*, carotenoids of 2<sup>nd</sup> leaves).**
4. The non optimal Fe/Zn ratios of tissues cause difficulties in the metabolism of plants. **It was concluded that the non optimal Fe/Zn ratio decrease the growth of shoots and roots in case of maize and cucumber, the number and length of internodes in case of cucumber, this is the reason why supposed to determine the Fe/Zn ratio in case of the soil analysis completed with plant analysis purposed diagnostic.**

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