Theses of the university doctoral (PhD) dissertation

DIFFERENT RESPONSES OF SUNFLOWER HYBRIDS AS A RESULT OF BACTERIA-BASED BIO-FERTILISER TREATMENTS

by

László Nagy doctoral candidate

Supervisor:

Dr. Szilvia Veres associate professor



UNIVERSITY OF DEBRECEN Kerpely Kálmán Doctoral School

Debrecen, 2017

1. PRELIMINARIES AND OBJECTIVES OF THE DOCTORAL THESIS

Sunflower production is of great significance both on global and domestic scale. In Hungary, 90% of the field crop production is covered by the cultivation area of five species (maize, wheat, sunflower, barley, rape). Demand for sunflower is continuously increasing, partly as a result of rising quantitative expectations, partly because of the more multilateral utilisation. Diverse utilisation of oil crops turned this group into one of differentiated significance. Sunflower is of determining importance within the Hungarian oil crop production, however due to the limitations related to crop rotation the primary emphasis is required to be put on the qualitative development of cultivation technology instead of increasing the cultivation area. One of the influencing factors of cultivation efficiency is the nutriment supply of plants. Besides the quantitative characteristics of products intended for human consumption their quality is also particularly important. Application of useful, plant growth promoting soil microorganisms (PGPR=Plant Growth Promoting Rhizobacteria) as bio-fertilisers is becoming one of the major tools of sustainable and green agriculture. As a result of the above, the amount of applied chemical fertilisers can be decreased, which is essential due to economic, nature- and environment preservation standpoints; it is the base principle of sustainable agriculture. Independent or complementary bio-fertilisation applied in the course of cultivation might constitute both qualitative and quantitative further development.

The test plants of our controlled trials carried out under hydroponic condition have been sunflower (Helianthus annuus L.) hybrids. In the course of our work the effects of 3 microorganism-based bio-fertilisers have been analysed in the early developmental stage of sunflower on parameters influencing their performance. The objective was to find out whether the analysed bio-fertilisers have growth-promoting effect in the early developmental stage of the plants, namely whether it is reasonable to utilise them as soon as possible within the growing period or not. Potential differences amongst hybrids in terms of their sensitivity to the application of bio-fertilisers have also been analysed, as well as the extent of differences in terms of responses to the application of certain bio-fertilisers. Our objective was to measure the maximum growth-promoting effect achievable within the early developmental stages by means of the application of bio-fertilisers. Moreover, it was considered important to determine if any age dependence is experienced in the plant-microorganism response reactions. For achieving the objective, the following parameters (related to floral production) have been measured: dry matter content and proportion of shoots and roots, specific leaf area (SLA) of younger and older leaves, relative (SPAD index) and actual chlorophyll content, the amount of chlorophyll-a, -b and carotenoids also in terms of age dependence and the pH change of the growth conditions.

2. MATERIAL and METHOD

2.1. Experimental conditions

2.1.1. Materials

The trial plants have been hybrids of sunflower (*Helianthus annuus* L.) (PR63E82, Heliasol RM, P64LE25, Barolo RM, P63LE13, NK Alego, NK Brio, Alexandra PR, NK Kondi,

PR64H42, NK Oktava, NK Stradi, NK Tristan, NK Neoma, Tutti HO, NK FERTI, Talento CL (NX 99338), Mv Lolita, Sunflora CL, Paraiso 1000 CL PLUS, Paraiso 102 CL, Morena CL, Meridies CL, NK Estiva). Sterilisation of seed surfaces has been carried out with 30% H₂O₂. The sterilized seeds were flushed several times with distilled water, and then soaked for 2 hours for the sake of better germination. The seeds were germinated between wet blotting paper sheets so as to make the polarity of the seedlings natural. Seeds wrapped into the blotting paper have been placed into a thermostat which operated at a temperature of 22°C. The 3.5-4 cm seedlings have been placed onto a nutrient solution. A nutrient solution with the following composition has been used for the growing of the seedlings: 2.0mM Ca(NO₃)₂, 0.7mM K₂SO₄, 0.5mM MgSO₄, 0.1mM KH₂PO₄, 0.1mM KCl, 10μM H₃BO₃, 1μM MnSO₄, 1μM ZnSO₄, 0.2 μM CuSO₄, 0.01μ M(NH₄)₆Mo₇O₂₄. The plants received iron in the form of 100 μM Fe(III)-EDTA. The nutrient solution was replaced every second day, while its aeration was continuous. The growing bowls have been 1.7 litres in terms of volume; 170 ml of nutrient solution has been diluted to 1.7 litres to which bio-fertilizers were added. Four plants were grown in each bowl and 3-3 bowls have been used per treatment and hybrid.

2.1.2. Growing conditions

Growing conditions were controlled; the experiments took place at the climatic room of the Department of Agriculture, Botany Plant, Physiology and Biotechnology of the Institute of Crop Sciences. Environmental conditions were regulated: light intensity 300 µmol m⁻²s⁻¹, temperature periodicity 25/20°C (day/night), relative humidity (RH) 65-75%, lighting/dark period 16 hrs/8 hrs.

2.1.2.1. Applied treatments

Three commercially available bio-fertilisers were used in the trials. In the case of the first 6 hybrids of the analysis bio-fertilisers 'A' and 'B' have been applied, and this has been completed with bio-fertiliser 'C' in the case of the rest of the hybrids.

Composition of the bio-fertiliser indicated with 'A':

- Azotobacter chroococcum
- Bacillus megaterium
 Total population: (5x10⁹ pcs. cm⁻³)

Composition of the bio-fertiliser indicated with 'B':

- Azospirillum lipoferum
- Azotobacter vinelandii
- Bacillus circulans
- Bacillus megaterium
- Bacillus subtilis
- Micrococcus roseus
- Pseudomonas fluorescens

Total population: 5.2x10⁹ pcs. cm⁻³

Composition of the bio-fertiliser indicated with 'C':

• Azotobacter chroococcum

- Azospirillum lipoferum
- Bacillus megaterium
- Bacillus subtilis

Total population: $1x10^9$ pcs. cm⁻³ + $3x10^7$ pcs. cm⁻³

The bio-fertilisers have been added to the nutrient solution at a concentration of 1 ml dm⁻³.

2.2. Applied methods

2.2.1. Determination of dry matter content

Dry matter weight of floral parts – root and shoot – has been determined by means of a thermogravimetric method. At the end of the experiments shoots and roots have been placed into oven at a temperature of 65°C and they have been dried until reaching constant weight. Following cooling down, the weight of dry samples has been measured at a four decimal accuracy. OHAUS Explorer (Switzerland) analytical balance has been utilised for the measurements. Additionally, shoot/root ratio has been calculated for each treatment.

2.2.2. Determination of the specific leaf area

For the quantitative characterisation of the dry matter content of the plant and leaf structure, the value of specific leaf area (SLA) has been applied. Specific leaf area is the leaf range in comparison with the dry weight of the leaf. The value shows the extent to which the product produced by the photosynthesising surface is utilised for the dry matter accumulation of the plant.

For the determination of the specific leaf area 7 mm diameter leaf circles have been sampled from younger (level 2) and older (level 1) leaves, 4 pieces for each sample. The circles have been wrapped into numbered tinfoil and they have been dried in an oven at 104°C until reaching constant weight. The samples have been left to cool down in an exicator on CaCl₂, and after cooling down dry samples have been measured at a four decimal accuracy OHAUS Explorer (Switzerland) analytical balance has been utilised for the measurements.

2.2.3. Measurement of relative chlorophyll content

The relative chlorophyll content (SPAD value) was measured with SPAD-502 (MINOLTA, Japan) chlorophyll meter. The device shows relative chlorophyll content as a SPAD (Soil Plan Analysis Development) value, which is calculated form the intensity of the red and infrared light

passing through the leaves. The device provides information about the relative total chlorophyll content. Our measurements have been carried out in 40 repetitions in the case of both young and older leaves.

2.2.3.1. Evaluation of the nitrogen remobilising ability

Nitrogen remobilisation ability of the hybrids, namely their ability to recycle nitrogen into younger leaves has been characterised by means of the nitrogen remobilisation ratio (NRR) on the basis of the following:

```
NRR = 1 - (SPAD_o - SPAD_y) / SPAD_o
```

where SPAD_y= relative chlorophyll content of the younger leaf (level 2) SPAD_o= relative chlorophyll content of the older leaf (level 1)

2.2.4. Qualitative and quantitative determination of photosynthetic pigments

For the determination of photosynthetic pigments, namely the chlorophyll-a, -b and carotenoid content, 0.05 grams of leaf circles have been sampled from fresh leaves – younger and older leaves – from which photosynthetic pigments have been extracted in conformity with the method of Moran and Porath (1989). 5 ml of N,N-dimethylformamide has been applied on the leaf samples and they have been stored for 72 hours at a temperature of 4°C, in order for the photosynthetic pigments to be entirely dissolved from the leaf circles. The samples have been measured by means of a METERTEK SP-830 type spectrophotometer at 480, 647 and 664 nm. The amount of chlorophyll-a, -b and carotenoid has been calculated on the basis of the formulae of Wellburne (1994).

```
Chlorophyll-a content = 11.65*a664-2.69*a647

Chlorophyll-b content = 20.81*a647-4.53*a664

Carotenoid content = ((1000*a480-1.28*chlorophyll-a) -56.7* chlorophyll-b)) /100
```

Parameters used for the measurements:

a480: absorbance measured at 480 nm a647: absorbance measured at 647 nm a664: absorbance measured at 664 nm

Total chlorophyll/carotenoid ratio has been calculated; this value provides information about the plasticity of the plant against stress and its stress handling ability.

2.2.5. Measurement of the pH of the nutrient solution

The pH-change of the nutrient solution of sunflower hybrids grown under hydroponic circumstances has also been monitored. The pH values of the prepared nutrient solution and the replaced (3^{rd} day) nutrient solution have been measured for every treatment and every repetition. Measurement of pH has been carried out by means of an OPTIMA 200A (USA) device. For the evaluation of the change Δ pH has been used and it was calculated from the difference of the pH of the fresh (pH_{fresh}) and the replaced, used (pH_{used}) nutrient solution:

$$\Delta pH = (pH_{used} - pH_{fresh})$$

2.2.6. Applied statistical methods

For the processing of the results Microsoft® Excel 2003 software has been used, while for the statistical analysis of the significant differences the SigmaPlot 12.0 software was applied. The minimum number of repetitions was 3 in the case of each analysis and measurement; in the figures and tables average values and standard deviation have been indicated (s.e.=standard error). Indication of significant differences: *p≤0.05, **p<0.01, ***p<0.001. There is a significant difference amongst the different letter indications on the p≤0.05 level within the figures. Normality of the data was analysed by means of the Kolmogorov-Smirnov test. If the result belonging to the samples had normal distribution, one-, two- or three-factor analysis of variance has been applied for the demonstration of the differences. In the latter case, Tukey-test has been applied for the separation of the groups. For the comparison of the pairs showing normal distribution, t-test was applied. When the normality analysis of the data provided negative results, Mann-Whitney test and Kruskal-Wallis test have been applied from among the non-parametric tests.

3. RESULTS AND DISCUSSION

3.1. Change of dry matter increase as a result of bio-fertiliser treatments

In the course of our trials, dry matter content of the analysed hybrids have been compared in the case of the control plants as a result of the bio-fertiliser treatments. The results are summarised in *Table 1*.

Table 1 Change of dry matter (g plant⁻¹) content of the analysed sunflower hybrids as a result of microorganism-based bio-fertiliser treatment ('A', 'B', 'C') \pm s.e.; n=9. Indication of the significant difference among the average values of the control: p \leq 0.05*, p \leq 0.01***, p<0.001***The different indications (a, b,c) represent the significant

difference among the control dry matter values.

difference among the	control dry matter valu			
	control	'A' bio-	'B' bio-	'C' bio-
		fertiliser	fertiliser	fertiliser
Barolo	1.062 ± 0.05^{a}	$0.879 \pm 0.06*$	1.069 ± 0.08	
Heliasol	0.631 ±0.05 b	0.785 ± 0.06 *	0.684 ± 0.04	
Alego	0.922 ±0.10 a	0.723 ±0.06*	0.970 ± 0.09	
PR63E82	0.806 ±0.11°	0.854 ± 0.04	0.845 ± 0.10	
P63LE13	0.806 ±0.01 °	0.851 ± 0.04	0.855 ± 0.11	
P64LE25	0.974 ±0.06 a	0.819 ± 0.07	0.874 ± 0.11	
Brio	0.918 ±0.05 a	0.991 ± 0.05	1.091 ±0.11	1.169 ±0.08*
Alexandra	0.867 ±0.09 a	0.835 ± 0.04	0.940 ±0.06*	1.022 ±0.11*
Kondi	1.025 ±0.04 a	1.109 ± 0.07	1.145 ±0.06	1.284 ±0.10*
PR64H42	0.975 ±0.03 a	0.836 ±0.06*	1.015 ±0.06*	0.917 ± 0.08
Estiva	0.861 ±0.08 a	0.972 ±0.10*	0.849 ± 0.07	0.787 ±0.04*
Morena	1.282 ±0.09 d	1.139 ± 0.14	1.543 ±0.08*	1.508 ±0.09*
Paraiso 102	1.164 ±0.14 a	1.095 ± 0.08	1.433 ±0.14*	1.582 ±0.13**
Paraiso	1.282 ±0.11 ^d	1.136 ± 0.05	1.487 ± 0.12	1.402 ±0.12*
1000				
Sunflora	0.999 ±0.05 a	1.051 ± 0.07	1.128 ±0.10*	1.391 ±0.11*
Lolita	0.846 ± 0.09 a	0.895 ± 0.05	0.845 ± 0.06	0.757 ± 0.05
Talento	0.895 ±0.06 a	0.954 ± 0.06	1.049 ±0.03*	0.988 ± 0.07
Ferti	1.072 ±0.06 a	0.945 ± 0.01	1.300 ±0.12**	1.185 ± 0.16
Tutti	0.843 ±0.08 a	0.652 ± 0.06 *	$0.637 \pm 0.02*$	0.631 ±0.04*
Oktava	0.998 ±0.06 a	0.864 ± 0.10	0.554 ±0.02**	0.571 ±0.03**
Stradi	0.861 ±0.11 a	1.311 ±0.10*	1.066 ± 0.08	1.208 ±0.07**
Meridies	1.219 ±0.08 a	1.089 ±0.11	1.110 ± 0.04	1.206 ± 0.09
Tristan	0.896 ±0.05 a	0.986 ± 0.09	1.059 ±0.09*	1.060 ± 0.09
Neoma	1.097 ±0.09 a	1.139 ± 0.09	1.166 ± 0.08	1.143 ± 0.10

The highest dry matter content was characteristic for the Morena (1.282 ± 0.09) and Paraiso 1000 (1.282 ± 0.11) hybrids. Dry matter content of the hybrids Meridies, Barolo, Ferti and Neoma are less by some percentage, but they are also high $(Table\ I)$. Heliasol had the lowest dry matter content (0.631 ± 0.05) ; its 50% less compared to hybrid with the highest dry matter content

(Morena). Dry matter content is also lower in the case of the hybrids PR63E82, P63LE13, Lolita and Tutti.

Change of dry matter content as a result of bio-fertilisers is diverse. Bio-fertiliser 'A' resulted in a 34% increase in the case of Stradi, but the dry matter content of Heliasol (which is characterised by low dry matter content) also increased by 20% due to the effect of bio-fertiliser 'A'. Bio-fertiliser 'B' caused dry matter content increase (above 10%) for multiple hybrids: Kondi, Morena, Paraiso 1000, Sunflora, Talento, Ferti, Stradi, Tristan. The highest of these increases (20%) was achieved by Stradi. Bio-fertiliser 'C' resulted in dry matter increase above 20% in the case of multiple hybrids: Brio, Kondi, Paraiso 102, Sunflora, Ferti, Stradi, Meridies. However, there was no dry matter content increase in the case of other hybrids (PR64H42, Estiva, Lolita, Talento, Tutti, Oktava). All three bio-fertilisers resulted in increased dry matter content in the case of the following hybrids: Brio, Kondi, Sunflora, Stradi, Tristan. Among these, Stradi achieved a 20-34% dry matter content increase within this early developmental stage as a result of the bio-fertiliser treatment. However, the cause of the total dry matter content increases above 10% is the intensive growth of roots, which is a basis of better nutrient supply in the future of the plant.

3.2. Change of specific leaf area values as a result of microorganism-based bio-fertiliser treatments

In the course of our trials the effect of treatments on the change of specific leaf area has also been measured. Specific leaf area (SLA) can be utilised for the quantitative characterisation of floral production, dry matter content of the plant and leaf structure. Its value is the ratio of the area and dry matter content of the fresh leaf (mm² mg⁻¹ or dm² g⁻¹). SLA is an attribute of the leaf; its value is determined by leaf thickness and the ratio of mechanical e.g. sclerenchyma tissues. The value of SLA is an important and sensitive parameter; it is connected to the extent to which fixed chemical energy is utilised for floral production.

According to our results, there is a difference among certain hybrids of the analysed species in terms of SLA. This finding extends the observation of Kalapos (1994) according to which there can be significant differences among the SLA values of different plant species. As the results of *Table 1* show, there are differences among the hybrids as well. Comparing the SLA values of older and younger leaves (*Fig 1*) no significant difference is experienced in the case of the control plants, except for the Stradi hybrid (indicated as no.21). The high SLA value indicates the relatively large area of thin leaves, while it indicates the opposite in the case of lower values. Kondi and PR64H42 can be characterised by relatively low values; an average value interval of 60-80 dm² g⁻¹ is characteristic to those hybrids.

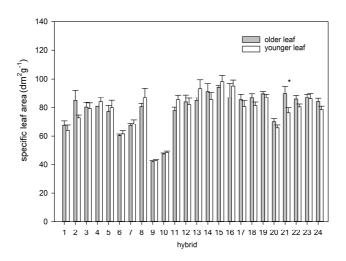


Figure 1 Value of specific leaf area (SLA) (dm² g⁻¹) in the case of older (level 1) and younger (level 2) leaves of the control plants (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14. Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma.) n=6±s.e., p<0.05*, p<0.01***, p<0.001*** in terms of differences based on age.

In the case of plants treated with bio-fertilisers the tendency is that younger leaves can be characterised by lower SLA values (Figures 2, 3, 4).

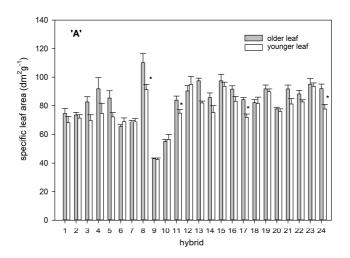


Figure 2 Value of specific leaf area (SLA) (dm² g⁻¹) in the case of older (level 1) and younger (level 2) leaves as a result of bio-fertiliser 'A' (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14. Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma. n=6±s.e., p<0.05* in terms of differences based on age.

As a result of the application of bio-fertiliser 'A' 2-27% higher SLA values have been recorder in comparison with the control. In the case of bio-fertiliser 'B' this difference is 2-18%, while the effect of bio-fertiliser 'C' increased the value of specific leaf are by 2-10% in comparison with the control.

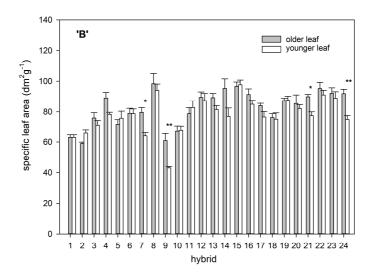


Figure 3 Value of specific leaf area (SLA) (dm² g⁻¹) in the case of older (level 1) and younger (level 2) leaves as a result of bio-fertiliser 'B' (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14. Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma. n=6±s.e., p≤0.05*, p<0.01** in terms of differences based on age.

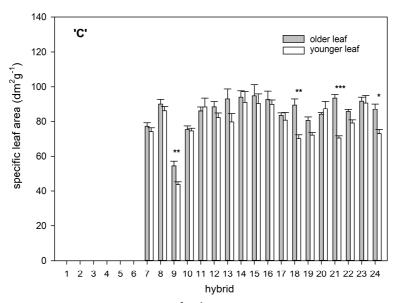


Figure 4 Value of specific leaf area (SLA) (dm² g⁻¹) in the case of older (level 1) and younger (level 2) leaves as a result of bio-fertiliser 'C' (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14.

Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma. n=6±s.e., p<0.05*, p<0.01**, p<0.001*** in terms of differences based on age.

A relatively large leaf area might be advantageous for higher photosynthetic efficiency. The value of photosynthetic activity is primarily determined by the quantitative and qualitative parameters of photosynthetic pigments. The value of SLA shows the difference among hybrids as a result of the treatment as well. The value of specific leaf area can be utilised well for the characterisation of the adaptation of certain species to different environmental factors; a higher SLA value indicates higher N content within the dry matter content and also higher photosynthetic capacity. In the case of the lower, older leaves the amount of photosynthetically active light is less due to the shadowing effect of the upper leaves, but the qualitative parameters of the incoming light are also different. The leaves situated in shaded circumstances are relatively thin, leaf volume density is low. The amount of palisade parenchyma cells per area unit is low. The value of SLA decreases with the increasing amount of light.

3.3. Change of relative chlorophyll content as a result of bio-fertiliser treatments

The SPAD-502 is a device for the determination of relative chlorophyll content. SPAD value is in close connection with the chlorophyll content and nitrogen content of leaves and the amount of yield, therefore on the basis of the regression equations determining the connection between SPAD value and the measured biological parameters (chlorophyll content, nitrogen content, yield amount) allow the estimation of nitrogen supply, chlorophyll content and yield amount. The change of chlorophyll content and the connection between chlorophyll concentration and SPAD-values is an important index which changes together with the age of the plant.

In the course of our analyses relative chlorophyll content of the older and younger leaves of sunflowers treated with bio-fertilisers (and also the control) has been measured (*Figure 5*).

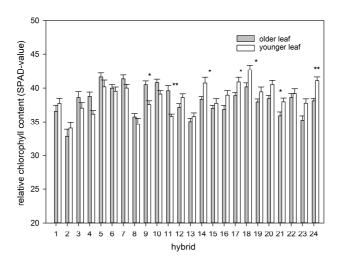


Figure 5 Change of relative chlorophyll content (SPAD value) in the case of older (level 1) and younger (level 2) leaves for the control plants (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14.

Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma.) n=6±s.e., p<0.05*, p<0.01** in terms of differences based on age.

SPAD values are between 32 and 42, the highest value was measured in the case of the P63LE13 and Brio control plants, but SPAD-values are also high – similarly to dry matter content – in the case of the Ferti and Neoma hybrids. In the case of most of the analysed hybrids younger leaves can be characterised with higher SPAD values, the difference proved to be significant for onethird of the hybrids. The difference is 5-7%, the hybrid showing the highest difference is the Neoma. As a result of the bio-fertiliser 'A' treatment higher SPAD values have been measured, in the case of the young and older leaves the difference is usually larger (mainly in the case of P63LE13, Paraiso 1000, Neoma) and in many cases this difference is significant (Figure 5). The effect of bio-fertiliser 'B' is less significant, but in this case also the Paraiso 1000 and Neoma hybrids have been the most sensitive and the change was also significant. As a result of the 'B' bio-fertiliser there was no significant difference between young and older leaves in the case of most hybrids. As a result of the bio-fertiliser 'C', SPAD value of the young leaves was significantly higher in the case of the following hybrids: Estiva, Paraiso 1000, Sunflora, Talento, Ferti, Stradi, Stradi, Neoma. The difference was around 10% in these cases. From among the younger leaves of the hybrids Tutti, Oktava, Stradi, Meridies, Tristan, and Neoma, relative chlorophyll content has increased only in the case of Stradi for all three bio-fertilisers. Biofertiliser 'C' has been the most effective. Bio-fertilisers 'A' and 'B' increased the relative chlorophyll content of the young leaves by 2-5%, while bio-fertiliser 'C' increased it by 9%. SPAD value of the control plants of the hybrids is between 32 and 42. The highest relative chlorophyll content has been measured in the case of the P63LE13 hybrid (41.63±0.67), while the lowest was recorded in the case of Heliasol (32.87±0.47).

3.4. Applicability of the remobilisation ratio

Since large differences have been experienced between the responses given by younger and older leaves to bio-fertiliser treatments, it is assumed that there is a difference among the analysed hybrids in terms of to what extent and how fast they are able to remobilise elements. Among the elements, nitrogen has a major role, because it is recyclable for the younger organs, it is a major component of chlorophylls and it is also a main component of the ribulose-1,5-diphosphate-carboxylase-oxygenase enzyme which is a key enzyme of photosynthesis. Without the proper amount of nitrogen, the photosynthetic system is damaged both structurally and functionally, which is the main obstacle of dry matter content increase. Nitrogen is a mobile element within the plant; if there is insufficient amount of it within the nutrient solution, it might be mobilised or recycled from the older plant parts for the young developing organisms. An important element of floral nitrogen utilisation is the remobilisation ability. There might be major differences among varieties and species in terms of to what extent and how fast is the plant able to recycle or remobilise. It is important to look for varieties and hybrids, which utilise the nutrient stock of the soil as effective as possible, resulting in larger economic benefit and advantageous environmental effects.

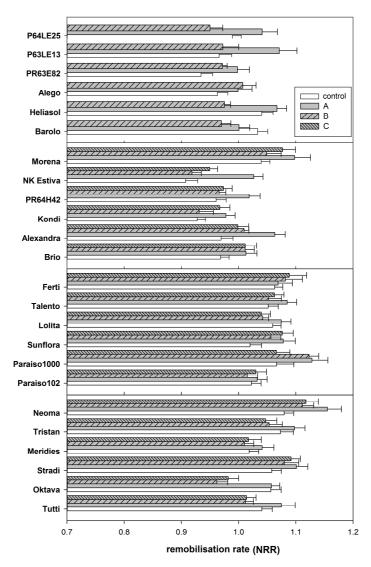


Figure 6 Effect of microorganism-based bio-fertiliser on the nitrogen remobilisation ratio (NRR) of sunflower hybrids (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14. Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma). There is no significant connection between the hybrid and the treatments. The effect of the treatment is significant on the level of $p \le 0.05$. $n = 12, \pm s.e$.

As a result of a stress situation – biotic and abiotic environmental stress e.g. the lack of nutrients – plants start decomposing proteins and chlorophylls first; therefore chlorophyll loss is by all means expectable. The introduced nitrogen remobilisation ratio (NRR) considers the change of the SPAD values of older and younger leaves compared to each other. If the value is above 1, it indicates the higher nitrogen content of the younger leaf; remobilisation ability is better than if the value is closer to 0. In the case of the analysed hybrids, remobilisation ratio has been

compared for the control plants, analysing the effect of the bio-fertiliser (*Figure 6*). According to our results, bio-fertiliser treatments had a positive effect on the remobilisation ratio. The values are above 1, and they mostly increased as a result of the 'A' bio-fertiliser. The effect of the 'A' bio-fertiliser resulted in a 10-15% increase in the case of the hybrids P63LE13, P64LE25, Alexandra and Neoma. In terms of the analysed parameter, the Barolo hybrid was the less sensitive.

3.5. Qualitative and quantitative change of photosynthetic pigments as a result of different bio-fertiliser treatments

3.5.1. Change of chlorophyll content

From the point of view of dry matter increase, the most important metabolic process is photosynthesis. The basis of proper photosynthesis is provided by the adequate amount and quality colour materials, photosynthetic pigments. From among photosynthetic pigments chlorophylls are the major pigments, which are able to transform light energy to chemical energy. SPAD-value is perfectly suitable for the characterisation of relative chlorophyll content; however the qualitative and quantitative cognition of photosynthetic pigments provides more exact information about the structure and operation of the photosynthetic system. *Figures 7, 8, 9* and *10* show the change of the amount of chlorophyll-a molecules as a result of the bio-fertiliser treatments in the case of the analysed hybrids.

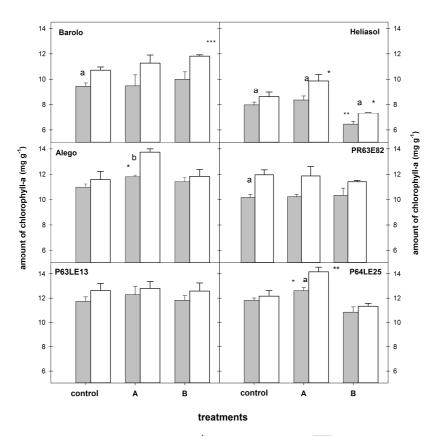


Figure 7 Change of chlorophyll-a content (mg g⁻¹) of older (level 1 \blacksquare) and younger (level 2 \square) leaves in the case of the 'A' and 'B' bio-fertiliser treatments (Barolo, Heliasol, Alego, PR63E82, P63LE13, P64LE25) n=3±s.e., p≤0.05*, p<0.01**, p<0.001***, compared to the control, p≤0,05a, p<0.01b, significant difference between old and young leaves

Similarly to relative chlorophyll content, chlorophyll-a content is higher in younger leaves (2-5%). Depending on the hybrid, bio-fertiliser treatments also increased chlorophyll-a content at this extent (*Figure 7*). Chlorophyll-a contents of the young and old leaves of the hybrids Brio, Alexandra, Kondi, PR64H42, Estiva and Morena and their change are shown in *Figure 8*.

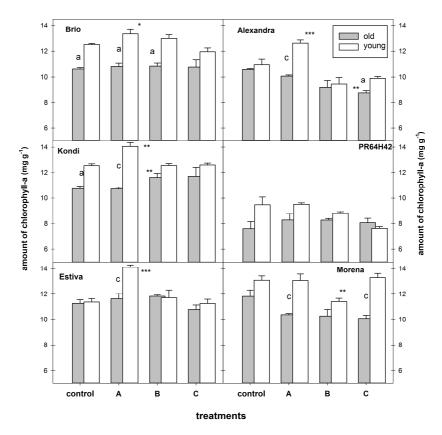


Figure 8 Change of chlorophyll-a content (mg g⁻¹) of older (level 1 \blacksquare) and younger (level 2 \square) leaves in the case of the 'A' and 'B' bio-fertiliser treatments (Brio, Alexandra, Kondi, PR64H42, Estiva, Morena) n=3±s.e., p≤0.05*, p<0.01***, p<0.001****, compared to the control, p≤0,05a, p<0.01b, significant difference between old and young leaves

Besides the fact that chlorophyll-a content of young leaves is higher in this case as well, it needs to be pointed out that there is a significant change in the case of the 'A' bio-fertiliser when comparing both to the control and by age. Chlorophyll-a content of the young leaves is 15-20% higher than that of the older leaves, besides except for the PR64H42 and Morena hybrids chlorophyll-a content has increased by 8-17% as a result of the 'A' bio-fertiliser compared to the control. Change of the chlorophyll-a content of the hybrids Paraiso 102, Paraiso 1000, Sunflora, Lolita, Talento and Ferti is summarised by *Figure 9*.

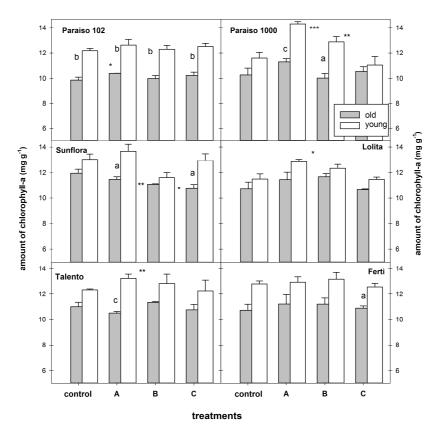


Figure 9 Change of chlorophyll-a content (mg g⁻¹) of older (level 1 \blacksquare) and younger (level 2 \blacksquare) leaves in the case of the 'A' and 'B' bio-fertiliser treatments (Paraiso 102, Paraiso 1000, Sunflora, Lolita, Talento, Ferti) n=3±s.e., p \le 0.05*, p<0.01**, p<0.001***, compared to the control, p \le 0,05a, p<0.01b, significant difference between old and young leaves

The higher chlorophyll-a content of the younger leaves is as a result of the 'A' bio-fertiliser is significant in the case of the Paraiso 1000, Lolita and Talento hybrids, but the tendency is the same for every hybrid. In comparison with the control, the chlorophyll-a increasing effect of the bio-fertilisers is mostly effective in younger leaves. Chlorophyll-a contents of the young and old leaves of the hybrids Tutti, Oktava, Stradi, Meridies, Tristan and Neoma and their change are shown in *Figure 10*.

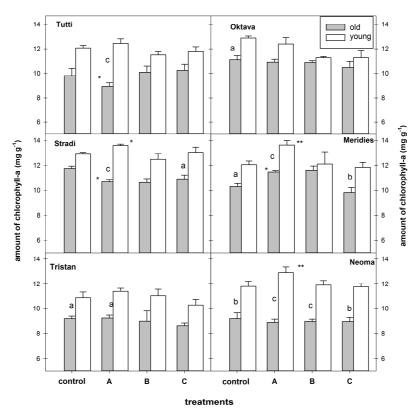


Figure 10 Change of chlorophyll-a content (mg g⁻¹) of older (level 1 \blacksquare) and younger (level 2 \square) leaves in the case of the 'A' and 'B' bio-fertiliser treatments (Tutti, Oktava, Stradi, Meridies, tristan, Neoma) n=3±s.e., p≤0.05*, p<0.01**, p<0.001***, compared to the control, p≤0,05a, p<0.01b, significant difference between old and young leaves

The positive effect of bio-fertilisers on chlorophyll-a content is not is not convincing, there is no significant difference among the chlorophyll-a content of the older leaves of the control and treated plants. Chlorophyll-a content of the young leaves is higher than that of the older leaves, just like it was experienced in the case of the rest of the hybrids (Figure 8, 9, 10). In the case of the young leaves, the effect of the 'A' bio-fertiliser significantly increased chlorophyll-a content in the case of the Stradi, Meridies and Neoma hybrids. Among the analysed hybrids PR64H42 and Heliasol have the lowest chlorophyll-a content (6-8 mg g⁻¹). None of the hybrids have outstandingly high chlorophyll-a content; chlorophyll content of the older leaves is 7-9 mg g⁻¹, while that of the younger leaves is 11-13 mg g⁻¹. The effect of bio-fertilisers on the chlorophyll-a content is generally not significant, except for the effect of bio-fertiliser 'A' on the younger leaves of the Stradi, Meridies, Neoma, Paraiso 1000, Lolita, Talento, Brio, Alexandra, Kondi, Estiva, P64LE25 and Helisaol hybrids.

In the course of the trials the value and change of the chlorophyll-a/chlorophyll-b ratio has been analysed as a result of the bio-fertiliser treatments in the case of both older (*Figure 11*) and younger (*Figure 12*). The value of the ration is around 3 in the case of the analysed control hybrids; it is the lowest in the case of the PR64H42 hybrid (2.43 ± 0.12). From among the control values it was higher than 3 in the case of four hybrids: Neoma (3.01 ± 0.16), Talento (3.02 ± 0.12), Tutti (3.05 ± 0.08) and Tristan (3.17 ± 0.11). In the case of these hybrids the relatively low value of

chlorophyll-b causes the ratio to be above 3, however the values of deviation are also relatively high. No significant change was experienced for the older leaves as a result of bio-fertilisers; in terms of tendency, the values of the ratio are lower which is resulted by the higher chlorophyll-b amount. The Estiva, Oktava, Brio and Paraiso 102 hybrids are exceptions as their chlorophyll-a/chlorophyll-b ratio is higher as a result of the bio-fertiliser treatment, but the change is non-significant. Change of the chlorophyll-a/chlorophyll-b ratio in younger leaves is shown by *Figure 13*. In terms the above ratio in the case of younger leaves, the proportion of chlorophyll-a and chlorophyll-b pigments is similar. The value of chlorophyll-a/chlorophyll-b ratio is around 3, just like it has been in the case of the older leaves.

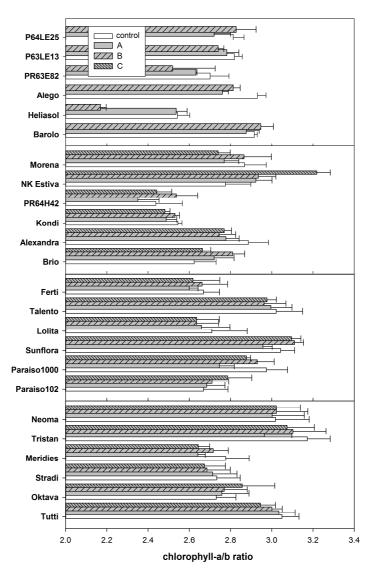


Figure 11 The effect of microorganism-based bio-fertilisers on the change of the chlorophyll-a/chlorophyll-b ratio in the case of the older (level 1) leaves of sunflower hybrids (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14. Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma). There is no significant connection

between the hybrid and the treatments. The treatment and the hybrid effect are also non-significant. n=3, $\pm s.e.$

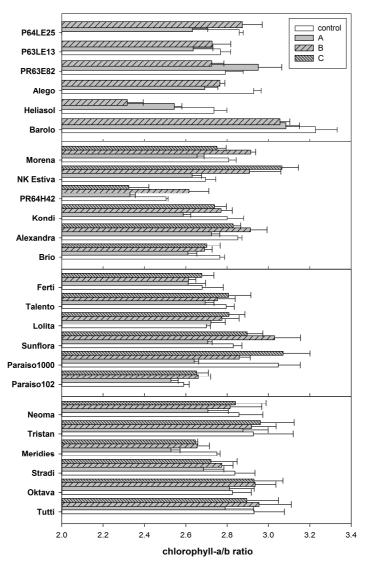


Figure 12 The effect of microorganism-based bio-fertilisers on the change of the chlorophylla/chlorophyll-b ratio in the case of the younger (level 2) leaves of sunflower hybrids (1. Barolo, 2. Heliasol, 3. Alego, 4. PR63E82, 5. P63LE13, 6. P64LE25, 7. Brio, 8. Alexandra, 9. Kondi, 10. PR64H42, 11. Estiva, 12. Morena, 13. Paraiso 102, 14. Paraiso 1000, 15. Sunflora, 16. Lolita, 17. Talento, 18. Ferti, 19. Tutti, 20. Oktava, 21. Stradi, 22. Meridies, 23. Tristan, 24. Neoma). There is no significant connection between the hybrid and the treatments. The treatment and the hybrid effect are also non-significant. n=3, ±s.e.

Usually the value of the chlorophyll-a/chlorophyll-b ratio of the treated hybrids is lower than the control values, this is generally below 10%, however deviation is relatively high, and therefore the difference is non-significant. In the case of the Oktava, Lolita, Estiva hybrids the 'B' and 'C' bio-fertiliser increases chlorophyll-a/chlorophyll-b ratio in comparison with the control.

3.5.2. Quantitative change of carotenoids

Although the change of the chlorophyll-a/chlorophyll-b ratio in plants is a good stress indicator, carotenoids have an important role in the light-harvesting complex and the protection of the photosynthetic system. It is proven that these – beyond their antioxidant properties – these compounds participate in the protection of the photosynthetic apparatus against photo-oxidation. Change of the total carotenoid content in the case of young and old leaves of the analysed hybrids as a result of bio-fertiliser treatments and in the case of the control is shown in *Figures 13*, *14*, *15*, *16*.

Carotenoid content of the Heliasol hybrid (Figure 14) is relatively low (2.1-2.4 mg g⁻¹). The carotenoid increasing effect of the 'A' bio-fertiliser is prominent (5-15%) in the case of young leaves, except for the Heliasol hybrid. Younger leaves have higher carotenoid content (5-50%).

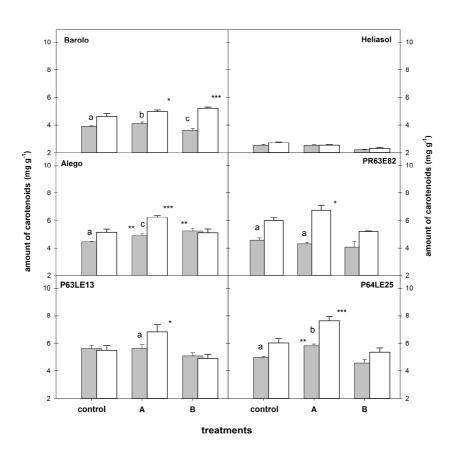


Figure 13 Change of carotenoid content (mg g⁻¹) in the case of older (level 1 \square) and younger (level 2 \square) leaves for the control and the 'A' and 'B' bio-fertiliser treatments (Barolo, Heliasol, Alego, PR63E82, P63LE13, P64LE25) n=3±s.e., p≤0,05*, p<0.01**, p<0.001***, compared to the control, p≤0,05°, p<0.01°, significant difference between old and young leaves

Figure 14 represents the change of the carotenoid content within the old and young leaves of hybrids Brio, Alexandra, Kondi, PR64H42, Estiva and Morena in the case of the control plants and the ones treat with 'A', 'B' and 'C' bio-fertilisers. Younger leaves if these hybrids contain more carotenoid, like the ones represented in Figure 14. Carotenoid content of the young leaves of the Kondi hybrid is especially high in comparison with the rest of the hybrids as a result of the control (7.521±0.1 mg g⁻¹) and the bio-fertiliser treatments (7.75-7.9 mg g⁻¹). Carotenoid content is also high in the case of the young plants of the Estiva hybrid as a result of the 'A' bio-fertiliser (8.12±0.09), as well as for Morena (8.22±0.11) (Figure 14), Paraiso 1000 (9.8±0.11), Sunflora (10.2±0.15) (Figure 15), Stradi (9.8±0.15), Meridies (9.8±0.05) (Figure 16).

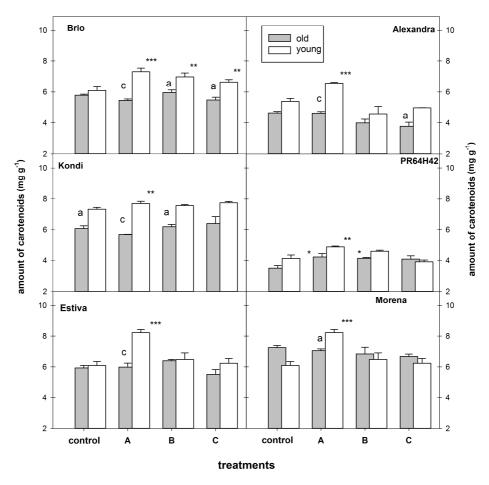


Figure 14 Change of carotenoid content (mg g⁻¹) in the case of older (level 1 \square) and younger (level 2 \square) leaves for the control and the 'A' and 'B' bio-fertiliser treatments (Brio, Alexandra, Kondi, PR64H42, Estiva, Morena) n=3±s.e., p≤0,05*, p<0.01***, p<0.001***, compared to the control, p≤0,05*, p<0.01b*, significant difference between old and young leaves

There was no significant difference in terms of the carotenoid content of older leaves; however the effect of the 'A' bio-fertiliser resulted in significant increase in the case of younger leaves, except for the Brio hybrid, where carotenoid content in comparison with the control has been significantly increased by all three bio-fertilisers. *Figure 15* represents the carotenoid content of the following six hybrids: Paraiso 102, Paraiso 1000, Sunflora, Lolita, Talento, Fertias a result of the bio-fertiliser effect. Carotenoid content of the younger leaf of Paraiso 1000 increased by 13% (9.8±0.11) as a result of the 'A' bio-fertiliser (*Figure 15*). This 10-15% increase is characteristic for the rest of the plants shown in *Figure 15* 'with regards to the 'A' bio-fertiliser.

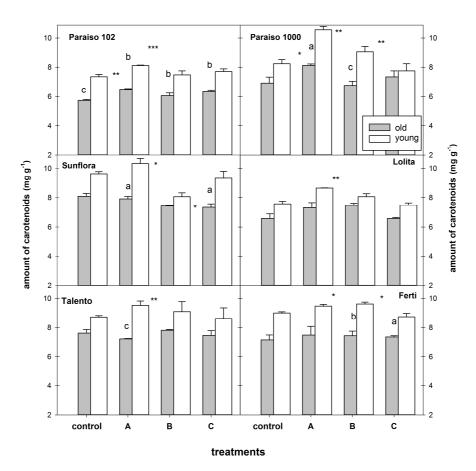


Figure 15 Change of carotenoid content (mg g⁻¹) in the case of older (level 1 \square) and younger (level 2 \square) leaves for the control and the 'A' and 'B' bio-fertiliser treatments (Paraiso 102, Paraiso 1000, Sunflora, Lolita, Talento, Ferti) n=3±s.e., p≤0,05*, p<0.01***, p<0.001****, compared to the control, p≤0,05*, p<0.01b*, significant difference between old and young leaves

Figure 16 represents the carotenoid content of the young and old leaves of hybrids Tutti, Oktava, Stradi, Meridies, Tristan, Neoma as a result of the bio-fertiliser effect.

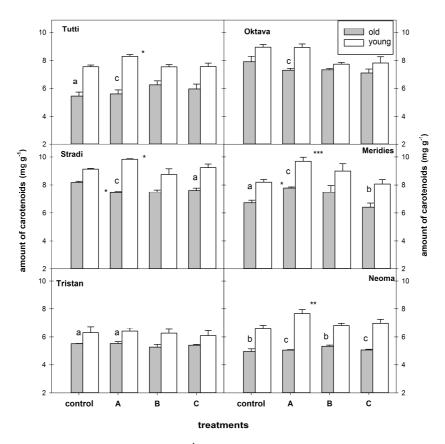


Figure 16 Change of carotenoid content (mg g⁻¹) in the case of older (level 1 \square) and younger (level 2 \square) leaves for the control and the 'A' and 'B' bio-fertiliser treatments (Tutti, Oktava, Stradi, Meridies, Tristan, Neoma) n=3±s.e., p≤0,05*, p<0.01**, p<0.001***, compared to the control, p≤0,05a, p<0.01b, significant difference between old and young leaves

The analysed hybrids can be classified in three groups in terms of the control of their older leaves. The older leaves of the Barolo, Helisaol and PR64H42 hybrids have a carotenoid content around 2-4 mg g⁻¹, Tristan, Tutti and Neoma have a carotenoid content around 4-6 mg g⁻¹, while the rest of the hybrids have 6-8 mg g⁻¹ carotenoid content. Higher carotenoid content of younger leaves is general, as is the carotenoid increasing effect of the 'A' bio-fertiliser. However, there are hybrids which are less sensitive in terms of this analytical aspect, therefore the carotenoid content of Tristan and Heliasol – which is the less carotenoid containing hybrid – has not increased significantly as a result of the bio-fertiliser treatments.

3.6. Change of the nutrient solution pH as a result of bio-fertiliser treatment

Nutrient uptake is influenced by numerous abiotic factors. One of the most important factors influencing the absorbability of nutrients is the pH of the nutrient solution. Plants are able to modify the acidity of the solution by extracting organic materials through their roots into their environment. However, microorganisms living in the growing substance – soil, nutrient solution

– are also able to extract materials which have direct influence on the pH. Additionally, the microorganisms indirectly influence the extraction ability of the roots as well.

In the course of our trials the pH change of the nutrient solution as growing substance has been continuously monitored; pH of the used and fresh nutrient solution has been measured throughout the different development stages, namely as a function of age. If the value of pH change (Δ pH) is negative, pH of the used nutrient solution will be lower, which means that solution acidifies due to the materials extracted by the microorganisms and the plant. If it is positive, pH increases and the system becomes more alkaline. Growing substance of the microorganisms, the composition of the bio-fertiliser might influence the pH of the nutrient solution.

According to the statistical evaluation, there is no significant difference in terms of either the joint effect of the hybrid and the treatment, or their separated influence. However, it can be laid down as a fact that the largest change in terms of the pH of the fresh and used nutrient solution was recorded in the case of the 'A' bio-fertiliser; it is higher than in the case of the control. In comparison with the control, the difference is similar or lower in the case of the 'C' bio-fertiliser. The smallest difference between the pH of the fresh and used nutrient solutions was recorded in the case of the 'B' bio-fertiliser. In comparison with the control, the extent of differences is diverse, e.g. the changes in the case of hybrids Sunflora and Estiva are close to the control, while for the hybrids Barolo, P64LE25 and PR64H42 the difference is larger compared to the control.

The value of pH as well as ΔpH in terms of the growing substance are changing with time and the age of the plant; no significant difference can be detected here either, however it is clear from the figures that in parallel with the development stage of the plant diverse differences can be measured between the pH of the fresh and used nutrient solution. Namely, the pH modifying effect is not only elicited by the applied bio-fertiliser itself, but the more developed plant and its more developed root system. As a matter of fact, the root of the plant is the main barrier against the boundless infiltration of the less useful and toxic elements.

4. NOVEL SCIENTIFIC RESULTS OF THE THESIS

- 1. On the basis of our trials it was found that the bio-fertilisers involved into the trials have growth promoting effect during the early developmental stage of the analysed sunflower hybrids in terms of both roots and shoots. However, in the case of hybrids where the total dry matter increase of the plant was above 10% in comparison with the control as a result of the treatments, the larger increase was caused by the weight increase of the root.
- 2. There is a difference among the hybrids in terms of the sensitivity to the applied bio-fertiliser and the same hybrid reacted differently to every bio-fertiliser treatment with different composition. Among the analysed hybrids, all three bio-fertilisers caused the increase of dry matter content for Brio, Kondi, Sunflora, Stradi and Tristan.
- 3. Bio-fertiliser treatments applied within the early developmental stage are able to increase dry matter content even by 30-34%. The largest dry matter content increase occurred in the case of the Stradi hybrid, where as a result of the treatment indicated with ,A' a 34% increase has been recorded.
- 4. Bio-fertiliser treatments have an increasing effect in both relative and absolute chlorophyll content, which induces more active photosynthesis, thus increasing dry matter production. Chlorophyll-a content of the young leaves was 15-20% higher than that of the older leaves; and except for the hybrids PR64H42 and Morena, chlorophyll-a content has increased by 8-17% in comparison with the control as well as a result of the 'A' bio-fertiliser.
- 5. According to our results the introduced nitrogen remobilisation ratio (NRR) is a suitable indicator for the evaluation of the growth promoting effect of bio-fertilisers. In the case of hybrids with lower nutrient element recycling ability e.g. Barolo, the growth promoting effect of the bio-fertiliser is also lower.
- 6. In the course of our trials it has been proven that certain bio-fertilisers have carotenoid increasing effect, which determines the stress resistance ability of the given hybrid. Among the analysed bio-fertilisers, a 10-15% increase has been recorded in terms of the carotenoid content of the younger leaves in comparison with the control as a result of the 'A' bio-fertiliser.
- 7. Age dependence has been experienced in terms of the responses of plant-microorganism, which drew the attention to the importance of applying the bio-fertiliser on time. For the majority of the analysed hybrids the favourable effects of bio-fertilisers a realised significantly in the younger leaves, in the course of the change of SPAD value, chlorophyll-a content and specific leaf area.

PRACTICAL APPLICABILITY OF THE RESULTS

- 1. All of the applied microorganism-based bio-fertilisers are suitable for the nutrient supply of the plants. On the basis of parameters directly characterising floral production dry matter content increase their application is justified in agricultural practice.
- 2. According to our results, it is reasonable to apply bio-fertilisers as soon as possible in the course of sunflower production in order for it to contribute to intensive growth during the early development stage. Since both the shoots and the roots have grown more intensively in the case of the sensitive hybrids, it is recommended to apply the bio-fertiliser together with sowing or during the first week after germination.
- 3. On the basis of the results of the test involving a high number of analysed hybrids and the applied microorganism-based bio-fertiliser it has been found that the potential effect of other bio-fertilisers (that have not been analysed in the course of this study) with different species composition is highly dependent on the genotype (beyond their nutrient supply ability). Therefore in practice it is recommended to proceed carefully in terms of the selection of the bio-fertiliser to be applied.
- 4. The analysed hybrids have different sensitivity for bio-fertiliser treatments. Successful applicability of the analysed bio-fertilisers is genotype dependent, responses of a resistant variant of the given hybrid might by different, however similar in terms of tendency.
- 5. Our analytical results contribute to the improvement of sustainable sunflower production, since the application rational application of bio-fertilisers containing less chemicals decreases environmental pressure, potential health risks and unnecessary energy consumption, in compliance with the National Agri-Environmental Programme of Hungary.

5. LIST OF PUBLICATIONS



UNIVERSITY OF DEBRECEN UNIVERSITY AND NATIONAL LIBRARY



Registry number: Subject: DEENK/76/2017.PL PhD Publikációs Lista

Candidate: László Nagy Neptun ID: CLCA9F

Doctoral School: Kálmán Kerpely Doctoral School

MTMT ID: 10054887

List of publications related to the dissertation

Hungarian conference proceedings (1)

 Hankovszky, G., Bojtor, C., Nagy, L., Gombás, D., Tóth, B.: Komplementációs vizsgálatok a biogáz üzemi présvíznél.

In: 56. Georgikon Napok, PE Georgikon Kar, Keszthely, 144-152, 2015.

Foreign language conference proceedings (7)

2. Tóth, B., Nagy, L. G., **Nagy, L.**, Veres, S., Lévai, L.: Is there any possible way of the use of industrial wastes in crop croduction?

In: Legislation, Technology and Practice of Mine Land Reclamation: Proceedings of the Beijing International Symposium on Land Reclamation and Ecological Restoration (LRER 2014), Beijing, China, 16-19 October 2014. Ed.: Zhenqi Hu, CRC Press, London, 243-246, 2015. ISBN: 9781138027244

3. Bodnár, K. B., Nagy, L. G., Nagy, L., Hankovszky, G., Makleit, P., Lévai, L., Veres, S., Tóth, B.: Are there different effects of biofertilizers on maize hybrides? Research People and Actual Tasks on Multidisciplinary Sciences. 4, 11-15, 2013. ISSN:

Research People and Actual Tasks on Multidisciplinary Sciences. 4, 11-15, 2013. ISSN 1313-7735.

4. Nagy, L., Nagy, L. G., Tóth, B., Makleit, P., Veres, S.: Improving soil fertility by applying biofertilizer.

Növénytermelés. 62 (Suppl.), 281-284, 2013. ISSN: 0546-8191.

 Tóth, B., Nagy, L. G., Nagy, L., Lévai, L., Fodor, F., Solti, Á., Veres, S.: Possible recycling of industrial wastes and by-products in agriculture.

Proc. Env. Sci. 18, 737-741, 2013. ISSN: 1878-0296.

DOI: http://dx.doi.org/10.1016/j.proenv.2013.04.100



UNIVERSITY OF DEBRECEN UNIVERSITY AND NATIONAL LIBRARY



- Bodnár, K. B., Nagy, L. G., Nagy, L., Hankovszky, G., Veres, S., Tóth, B.: Role of biofertilizers in sustainable agriculture.
 - In: Book of Abstracts : Krakow Conference of Young Scientists. Krakow, September 26-28, 2013, KKMU, Krakow, 62-66, 2013.
- Nagy, L. G., Nagy, L., Bodnár, K. B., Veres, S., Tóth, B.: Suitable nutrient supply good fertility. Növénytermelés. 62 (Suppl.), 409-412, 2013. ISSN: 0546-8191.
 DOI: http://dx.doi.org/10.12666/Novenyterm.62.2013.suppl
- Tóth, B., Bodnár, K. B., Nagy, L. G., Nagy, L., Hankovszky, G., Lévai, L., Veres, S.: Use of by-products of lime factory in the maintenance of soil pH.
 Research People and Actual Tasks on Multidisciplinary Sciences. 4, 31-35, 2011. ISSN: 1313-7735.

Foreign language abstracts (6)

- 9. **Nagy, L.**, Nagy, L. G., Makleit, P.: Cyclic hydroxamic acid content of maize hybrids measured by HPLC-method
 - In: 48th Croatian and 8th International Symposium on Agriculture: Book of Abstracts. Ed.: Sonja Marić, Zdenko Lončarić, Faculty of Agriculture, University of Josip Juraj Strossmayer in Osijek, Dubrovnik, Croatia, 192, 2013.
- 10. Makleit, P., Bodnár, K. B., Hankovszky, G., Tóth, B., Lévai, L., Veres, S., Nagy, L., Nagy, L. G.: Dimboa- and Diboa-content of various maize (Zea Mays L.) hybrids.
 In: Research People and Actual Tasks on Multidisciplinary Sciences: fourth Conference: Volume 1: Agriculture and Veterinary medicine. Technical sciences, Processing & Post Harvest Technology and Logistics, Power and machinery: Lozenec, Bulgaria, 12-16 June, 2013, Bulgarian National Multidisciplinary Scientific Network of the Professional Society for
- 11. Tóth, B., **Nagy, L.**, Nagy, L. G., Veres, S., Lévai, L.: Lead pollution and living bacteria containing fertilizers.

Research Work, Lozenec, 120-124, 2013.

- In: Phytotecnologies, International Phytotechnology Society, New York, 177, 2013.
- 12. Nagy, L., Nagy, L. G., Tóth, B., Makleit, P., Veres, S.: Sensitivity of different sunflower hybrids to biofertilizer treatments.
 - In: Research People and Actual Tasks on Multidisciplinary Sciences: fourth Conference: Volume 1: Agriculture and Veterinary medicine. Technical sciences, Processing & Post Harvest Technology and Logistics, Power and machinery: Lozenec, Bulgaria, 12-16 June, 2016, Bulgarian National Multidisciplinary Scientific Network of the Professional Society for Research Work, Lozenec, 262-265, 2016.



UNIVERSITY OF DEBRECEN UNIVERSITY AND NATIONAL LIBRARY



- 13. Nagy, L., G., **Nagy, L.**, Bodnár, K. B., Lévai, L., Veres, S., Tóth, B.: Sustainable plant nutrition the global challenge.
 - In: 3rd AGRIMBA-AVA Congress, Agribusiness and Rural Development as a Global Challenge, Programme and abstract book. Ed.: Wim Heijman, University of the Mediterranean in Podgorica, Budva, Montenegro, 21, 2013.
- 14. Nagy, L. G., Nagy, L., Makleit, P., Lévai, L., Hankovszky, G., Bodnár, K. B., Veres, S., Tóth, B.: Use of living bacteria containing fertilizer for the good nutrient supply. Research People and Actual Tasks on Multidisciplinary Sciences. 1, 21-25, 2013. ISSN: 1313-7735.

List of other publications

Hungarian scientific articles in Hungarian journals (1)

 Nagy, L. G., Nagy, L., Bodnár, K. B., 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 articles in international journals (1)

- 16. 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. DOI: http://dx.doi.org/10.12720/jomb.3.4.288-291

Hungarian conference proceedings (2)

- 17. Hankovszky, G., **Nagy, L.**, Nagy, L. G., Juhász, C., Veres, S., Lévai, L., Tóth, B.: A biogáz üzemi présvíz lehetséges alkalmazása a növénytáplálásban.
 - In: Alap és alkalmazott kutatások eredményei a növénytudományokban. Szerk.: Szabó András, Debreceni Egyetemi Kiadó, Debrecen, 174-180, 2012. ISBN: 9786155183171
- 18. Bojtok, K., **Nagy, L.**, Nagy, L. G., Juhász, C., Veres, S., Lévai, L., Tóth, B.: A fa-és szalmahamu vizsgálata, mint lehetséges növényi tápanyagforrás.
 - In: Alap és alkalmazott kutatások eredményei a növénytudományokban, Szerk.: Szabó András, Debreceni Egyetemi Kiadó, Debrecen, 149-155, 2012. ISBN: 9786155183171



UNIVERSITY OF DEBRECEN UNIVERSITY AND NATIONAL LIBRARY



Foreign language conference proceedings (1)

19. Tóth, B., **Nagy, L.**, Nagy, L. G., Ványiné Széles, A., Lévai, L.: Agricultural use of sewage sludge to reduce environmental pollution.

Int. Proc. Chem., Biol. Envir. Eng. 44, 34-37, 2012. ISSN: 2010-4618.

Hungarian abstracts (4)

- 20. Makleit, P., Nagy, L., Nagy, L. G., Veres, S.: Az együttes nevelés hatása a napraforgó (Helianthus annuus L. cv.: NK Brio) és a bojtorján szerbtövis (Xanthium strumarium L.) növekedésére és fotoszintetikus pigment tartalmára.
 In: LV Georgikon napok: 55th Georgikon Scientific Conference: Kivonat-kötet: Programfüzet, valamint az elhangzó és poszter előadások rövid kivonatainak gyűjteménye, Universitas Pannonia, Keszthely, 68, 2013. ISBN: 9789639639522
- 21. Nagy, L. G., **Nagy, L.**, Lévai, L., Veres, S., Bodnár, K. B., Tóth, B.: Baktérium tartalmú biotrágyák hatása a növénytermesztésben.

In: LV Georgikon napok : 55th Georgikon Scientific Conference : Kivonat-kötet : Programfüzet, valamint az elhangzó és poszter előadások rövid kivonatainak gyűjteménye, Universitas Pannonia, Keszthely, 73, 2013. ISBN: 9789639639522

- 22. **Nagy, L.**, Nagy, L. G., Tóth, B., Makleit, P., Veres, S.: Biotrágyák hatása napraforgó (Helianthus Annuus L.) fiziológiai paramétereire.
 - In: LV. Georgikon napok: Georgikon Scientific Conference : Kivonat-kötet : Programfüzet, valamint az elhangzó és poszter előadások rövid kivonatainak gyűjteménye, Universitas Pannonia, Keszthely, 74, 2013. ISBN: 9789639639522
- 23. Veres, S., Malik, I., Tóth, B., Makleit, P., Nagy, L., Nagy, L. G., Rengel, Z.: Búza genotípusok fotokémiai aktivitásának változása eltérő adagú nitrogéntáplálásnál.
 In: LV Georgikon napok: 55th Georgikon Scientific Conference: Kivonat-kötet: Programfüzet, valamint az elhangzó és poszter előadások rövid kivonatainak gyűjteménye, Universitas Pannonia, Keszthely, [1], 2013. ISBN: 9789639639522

Foreign language abstracts (3)

- 24. Nagy, L. G., **Nagy, L.**, Tóth, B., Lévai, L., Veres, S.: Biofertilizer application as a tool of suistainable and economical agriculture.
 - In: 3rd AGRIMBA-AVA Congress, Agribusiness and Rural Development as a Global Challenge, Programme and abstract book. Ed.: Wim Heijman, University of the Mediterranean in Podgorica, Budva, Montenegro, 16, 2013.
- Hankovszky, G., Bodnár, K. B., Nagy, L. G., Nagy, L., Lévai, L., Veres, S., Tóth, B.: Effect of fluid by-product of biogas factory on Helianthus annuus.
 Research People and Actual Tasks on Multidisciplinary Sciences. 1, 41-45, 2013. ISSN: 1313-7735.