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SPAD values, as well as sugar- and capsaicin content in different varieties of outdoor peppers

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Abstract: The marketability of the sweet peppers is determined by their quality in Class I, which must also meet the highest standards in terms of the color, shape of the variety and the characteristics of the various flavors. However, the determinants of quality may vary from one pepper type to another. During of our research, we examined the utilization of nitrogen, magnesium and potassium in different types of domestic peppers in the context of the relative chlorophyll content of the foliage and the amount of sugar and total capsaicin in the fruits. We determined that the nutrient solution prepared by Duna-r Ltd. is suitable for achieving the highest sugar and capsaicin content, but their levels can differ significantly. The uptake and utilization of nitrogen, magnesium and potassium of nutrient solution can be checked with the SPAD (Soil Plant Analysis Development) index data of the foliage. We found that there are periods in the phenophase of the pepper cultivars studied when both sugar content and capsaicin content increase significantly. Another major result is that sugar content is a basic determinant of capsaicin content in hot peppers and cherry peppers, while it is not an important factor of capsaicin content for Bogyiszló-type peppers.

Keywords: *Capsicum annuum* L., SPAD index, BRIX%, total capsaicin

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Introduction

In Hungary, pepper cultivation was established during the Turkish occupation of the country in the sixteenth and seventeenth centuries. At the beginning, it was used as a medicinal herb and an ornamental plant. Paprika gained widespread popularity at the late eighteenth century as a spice in Hungarian foods (Allaire et al., 2006). However, pepper breeding in Hungary started countrywide in 1950 (Moór & Zatykó, 1995). Hungarian peppers have become dominant in the European vegetable market. The marketability of peppers are determined by their quality, which can be classified as Class I, which must also meet the highest requirements in terms of color, yield and the characteristics of health and flavors (Terbe, 2003; Angeli, 1959). Nowadays, the variety offer and the cultivation technology are constantly changing and evolving. Quality is a determining factor, therefore economically cost-effective farms with high yields and outstanding quality can only remain competitive in the long run (Kicska, 2016). However, the factors that determine quality can expand by type of pepper. In case of cherry peppers, the outstanding total capsaicin, the carotenoids and colorants measured in the ASTA value in the ground pepper mills, and the high vitamin C and sugar content in the quality of sweet peppers are the most important

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indicators (Mashabela et al., 2015; Caruso et al., 2018). The Hungarian Food Book (*Codex Alimentarius Hungaricus*, 2018) has defined the quality classes, which are special: above 120 ASTA, delicacy: 100-120 ASTA is sweet: 80-100 ASTA, and rose: at least 60-80 ASTA. On the one hand, the amount of capsaicin was determined based on a sensory examination. In evolution of capsaicin the nitrogen has central role. By Medina-Lara et al. (2008) several hot pepper varieties were tested. The nitrogen fertilization regime also affects the concentrations of many secondary metabolites such as alkaloids (e.g., capsaicin), phenols, and others. It can also be characterized by the Scoville Heat Unit (SHU) (Govindarajan & Sathyanarayana, 1991). However, it can also be detected by HPLC measurement, which is expected to be at least 0.2 mg/g in Hungary (Lantos et al., 2017). One of the foundations of their formation is the adequate nutrient supply. In addition to the genetic characteristics of the variety and the cultivation technology, agrochemical factors are also decisive in achieving a high quality crop. During the test production of the varieties, the composition of the nutrient solutions and the timing of their application are an additional examination of the soil's ability to provide nutrients, which provides the basis for the planning of field vegetable production and the expected yield quality (Lantos, 2015). In proper nutrient management, leaf analysis can provide reliable information on both the current state of the plant and the yield quality (Vona, 2020; Miller et al., 1979; Faber, n.d.). The relationship between individual organs, i.e. the effect that one part of a plant has on the development and function of another part, is called correlation. Most correlation effects occur in the differentiation of tissues, in the formation of organs, in the relationship between the part and the whole (Szalai, 1974).

Chlorophyll a and b are the major pigments for the absorption of light energy and synthesis of both pigments requires Mg. According to Tränkner et al. (2018) Mg is the central atom of the chlorophyll pigment and also required for its biosynthesis.

SPAD-502 chlorophyll meter is widely used in the special literature for determining chlorophyll index (e.g. (Yuzhu et al., 2013)) Tang et al. (2016) found significant positive correlations between leaf SPAD value and chlorophyll content (chlorophyll a, chlorophyll b and total chlorophyll) in two pepper varieties. Similarly, Madeira et al. (2003) reported that in situ SPAD readings and extractable chlorophyll content showed significant proportional relationship, both for chlorophyll a and total chlorophyll. Yu et al. (2016) combined hyperspectral imaging with chemometric analysis for determining chlorophyll and SPAD values in pepper leaves during leaf senescence, which provides a reference for monitoring plant growth.

Le Bot et al. (2009) devised a simple model to simulate the trade-off between growth and secondary metabolism in response to N nutrition. N affected growth and metabolite concentrations proportionally in the leaves. Dry biomass, leaf area, and concentrations of nitrate and organic acid (malic, citric) changed proportionally with nitrate concentrations up to a threshold, above which they remained constant. Starch, sucrose, and organic N concentrations were invariant with nitrate concentrations. While, glucose, fructose, and phenolic concentrations were highest at lowest nitrate concentrations. They declined progressively with rising nitrate concentrations until a threshold, above which they remained constant. With the help of the chlorophyll measuring instrument SPAD 502, we can measure the chlorophyll content of the plant, so we can get information about the current N content of the leaves, which can help determine the need for N fertilizers (Tóth et al., 2014).

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Table 1. Nutrient content of the soil of the research area.

EC	pH	NO_3^-	NH_4^+	P_2O_5	K_2O	Ca^{2+}	Mg^{2+}	SO_2^{4-}
0.3	6.5	0.8	-	0.01	0.3	0.9	0.25	0.1
mS/cm					mmol/l			
	Fe	Mn	Zn	B	Cu	HCO_3^-	Cl^-	Na^+
	17	2.6	1.0	17	1.1	2.56	0.2	2.7
	μm/l				mmol/l			

Table 2. Composition of nutrient replenishment in field test production.

nutrient tank A (1000 l water)	nutrient tank B (1000 l water)	tank C (nitric acid)
calcium-nitrate $Ca(NO_3)_2$ 62 kg	potassium nitrate KNO_3 26 kg	nitric acid (59%) HNO_3 13.3 l
complex fertilizer 21 kg	mono-potassium phosphate KH_2PO_4 17 kg	
nitric acid (59%) HNO_3 1.0 liter	magnesium-sulfate $MgSO_4$ 21 kg	
pH 6.5	nitric acid (59%) HNO_3 12.3 liter	
EC 1.8 mS/cm	pH 6.5	
	EC1.8 mS/cm	

Table 3. The total sugar and capsaicin content of the fruits.

Paprika types	Sugar content (BRIX%)		Total capsaicin content (mg/g)
	Fruit setting	Harvest	Powder
Hot red pepper	7.6	12.9	0.41
Sweet red pepper	7.5	12.0	–
Cherry shaped pepper	7.7	10.8	0.49
Cece type sweet pepper	7.5	7.6	–
Bogyiszló type hot pepper	5.7	6.3	0.20

Taking into account the findings discussed above, the aim of our research was based on three years (2018-2020) of test production data:

- preparation of a complex nutrient solution, which is suitable for the growing of outdoor pepper species,
- studies on the utilization of nitrogen and magnesium in relation to the relative chlorophyll content of the foliage,
- studies on the utilization of potassium in relation to sugar and capsaicin con-

Table 4. Average SPAD values for the five measurement with the results of the post-hoc Tukey tests, Hot red pepper (*Capsicum annuum* L. *var. longum*), SZ-84. Comparison of the SPAD index values of 5-5 ripe peppers harvested from pepper stem in four successive periods (A, B, C, D) based on ANOVA and Tukey test.

A	B	C	D
Average SPAD			
80.3	76.7	66.3	70.3
78.4	74.3	70.4	73.8
77.5	71.3	69.9	77.6
74.9	74.3	71.2	75
72.1	76.7	68.2	73.7
[1]76.6	[1]74.7	[1]69.2	[1]74.1
Results of the post-hoc Tukey tests[2]			
Treatments pair	Tukey HSD *p*-value	Tukey HSD interference	X signficantly higher than Y
A vs C	0.0014667	$**p < 0.01$	A than C
B vs C	0.0176992	$*p < 0.05$	B than C
C vs D	0.0361489	$*p < 0.05$	D than C

[1]Mean values; [2]Significant differences between the average SPAD values of 5-5 ripe peppers harvested from pepper stem in the examined four successive periods (A, B, C, D). (*: significant difference at the $p < 0.05$ probability level; **: significant difference at the $p < 0.01$ probability level). Only those treatment pairs are indicated among the results of the post-hoc Tukey tests, SPAD values of which show significant differences.
A, B, C: 2018-2020, three-year period, first decade of August;
D: 2018-2020, three-year period, first decade of September.

tent of the fruits,
- determination of the reference value for the SPAD (Soil Plant Analysis Development) (Colla et al., 2017) index for sweet peppers,
- demonstration of the correlation between foliage and yield.

Materials and Methods

Our research was carried out at the research site of the pepper seed producer Duna-R Ltd. in the Southern Great Plain, HU-6600 Szentes (Hungary). The composition of the applied nutrient solution was compiled based on several years of cultivation and research experience, as well as the annual plant and soil analysis (Table 1, 2). The appropriate pH and EC levels of the nutrient solution and the utilization of nitrogen and magnesium were determined by the change in the relative chlorophyll content of the foliage. For the measurements, a SPAD-502 Plus Chlorophyll Meter instrument (manufacturer: Konica Minolta) was used, which shows the relative chlorophyll content (SPAD index) calculated from the ratio of the amount of red and infrared light passing through the leaf (de Gil et al., 2002). The SPAD index number determines the metabolic process of the plant in the context of the utilization of the absorbed nutrients.

SPAD measurements were performed in all three years, with 5-5 replicates designated

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Table 5. Average SPAD values for the five measurement with the results of the post-hoc Tukey tests, Sweet red pepper (*Capsicum annuum* L. *var. longum*), SZ-102. Comparison of the SPAD index values of 5-5 ripe peppers harvested from pepper stem in five successive periods (A, B, C, D, E) based on ANOVA and Tukey test.

A	B	C	D	E
Average SPAD				
82	57.8	72.2	67.3	67
79.8	59.9	68.2	61.3	68.5
81.9	57.7	69.5	56.6	72.7
72.7	69.2	66.1	67.2	71.1
57	73.5	68	52.6	63.3
[1]74.7	[1]63.6	[1]68.8	[1]61.0	[1]68.5

Results of the post-hoc Tukey tests[2]			
Treatments pair	Tukey HSD *p*-value	Tukey HSD interference	X signficantly higher than Y
A vs D	0.0311040	$^{*}p < 0.05$	A than D

[1]Mean values; [2]Significant differences between the average SPAD values of 5-5 ripe peppers harvested from pepper stem in the examined four successive periods (A, B, C, D, E). (*: significant difference at the $p < 0.05$ probability level.) Only those treatment pairs are indicated among the results of the post-hoc Tukey tests, SPAD values of which show significant differences.
A, B, C: 2018-2020, three-year period, first decade of August;
D, E: 2018-2020, three-year period, first decade of September.

per plants. During our research, we tested 40 pepper plasnts per type. Measurements were usually performed two to three days before harvest.

The sugar content was determined from the juice extracted from the fruits. All measurements were carried out in 3 replicates. The spices and cherry peppers were harvested at full biological maturity, but the Cecei and Bogyiszló type in technological ripeness. A Hanna sugar refractometer instrument was used for measurement (Table 3).

Measurements were performed on the selected plants from the stand, first during the period of fruit set and then at the time of harvest, every two weeks, in five replicates. The sugar content of fruits was determined in BRIX% (Hanna Instruments Refractometer) and the capsaicin content of ripened fruits made from hot peppers was determined in mg/g. The determination of capsaicin concentration was carried out by a local method MSZ 9681-4: 2002 also with Shimadzu UV-1800 spectrophotometer. The determination of the carotene dyestuff of space red peppers was carried out by a local method of MSZ 9681-5:2002 with Shimadzu UV-1800 spectrophotometer. The different dates of the paprika harvest are marked with the letters A, B, C, D, E. Here A, B and C measurements occurred for the three year-year period 2018-2020, first decade of August, while D and E measurements occurred for the three year-year period 2018-2020, first decade of September. The results reported in the manuscript are averages over three years.

One-way analysis of variance (ANOVA) was used to determine if there was a signifi-

Table 6. Average SPAD values for the five measurement with the results of the post-hoc Tukey tests, Cherry shaped pepper (*Capsicum annuum* L. *var. cerasiforme*), candidate variety. Comparison of the SPAD index values of 5-5 ripe peppers harvested from pepper stem in five successive periods (A, B, C, D, E) based on ANOVA and Tukey test.

A	B	C	D	E
Average SPAD				
65.6	73	82	76.2	56.9
55.7	55.3	81.2	75.2	65.7
66.5	78.5	77.7	78.7	65.3
66.6	74.5	71.9	83.1	63.3
69.5	73.6	66.8	77.7	62.8
[1]64.8	[1]71.0	[1]75.9	[1]78.2	[1]62.8

Results of the post-hoc Tukey tests[2]			
Treatments pair	Tukey HSD p-value	Tukey HSD interference	X signficantly higher than Y
A vs C	0.0498104	$*p < 0.05$	C than A
A vs D	0.0136565	$*p < 0.05$	D than A
C vs E	0.0160993	$*p < 0.05$	C than E
D vs E	0.0041721	$**p < 0.01$	D Than E

[1]Mean values; [2]Significant differences between the average SPAD values of 5-5 ripe peppers harvested from pepper stem in the examined four successive periods (A, B, C, D, E). (*: significant difference at the $p < 0.05$ probability level; **: significant difference at the $p < 0.01$ probability level). Only those treatment pairs are indicated among the results of the post-hoc Tukey tests, SPAD values of which show significant differences.
A, B, C: 2018-2020, three-year period, first decade of August;
D, E: 2018-2020, three-year period, first decade of September.

cant difference in the pairwise averages of the SPAD index values determined for 5-5 replicates of different pepper types every two weeks during fruit binding and harvesting. Such an examination may reveal whether a significant change in the relative chlorophyll content of the plant can be detected during the maturation process (Bolla & Krámli, 2012). An F-test was performed to check whether the values of the average SPAD index per pair of 5-5 peppers harvested in 5-5 replicates every two weeks for the given pepper type differ significantly. If the difference is significant, we reject our 0-hypothesis about the similarity of the means. It is then and only then that the Tukey test can be used to determine whether there is a significant difference between the average SPAD values of the pairwise replicates of the particular pepper type (Tukey, 1953). The Tukey test behaves well in terms of both the accumulation of the first type of error and the strength of the test. (If the 0-hypothesis is met by performing a one-way ANOVA, then there is no point in performing the Tukey test.) When performing the post hoc Tukey test, for a given pepper type, we first get the differences of the pairwise mean SPAD index values of the 5-5 replicates of different pepper types harvested every two weeks. We then compare these deviations with a critical value in order to determine whether these de-

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Table 7. Average SPAD values for the five measurement with the results of the post-hoc Tukey tests, Cece type sweet pepper (*Capsicum annuum* L.), BSZ-6. Comparison of the SPAD index values of 5-5 ripe peppers harvested from pepper stem in five successive periods (A, B, C, D, E) based on ANOVA and Tukey test.

A	B	C	D	E
Average SPAD				
65.6	73	82	76.2	56.9
55.7	55.3	81.2	75.2	65.7
66.5	78.5	77.7	78.7	65.3
66.6	74.5	71.9	83.1	63.3
69.5	73.6	66.8	77.7	62.8
[1]64.8	[1]71.0	[1]75.9	[1]78.2	[1]62.8

Results of the post-hoc Tukey tests[2]

Treatments pair	Tukey HSD p-value	Tukey HSD interference	X signficantly higher than Y
A vs B	0.0010053	$^{**}p < 0.01$	A than B
A vs C	0.0010053	$^{**}p < 0.01$	A than C
B vs C	0.0010053	$^{**}p < 0.01$	B than C
B vs D	0.0010053	$^{**}p < 0.01$	D than B
B vs E	0.0219182	$^{*}p < 0.05$	E than B
C vs D	0.0010053	$^{**}p < 0.01$	D than C
C vs E	0.0010053	$^{**}p < 0.01$	E than C

[1]Mean values; [2]Significant differences between the average SPAD values of 5-5 ripe peppers harvested from pepper stem in the examined four successive periods (A, B, C, D). (*: significant difference at the $p < 0.05$ probability level. **: significant difference at the $p < 0.01$ probability level). Only those treatment pairs are indicated among the results of the post-hoc Tukey tests, SPAD values of which show significant differences.
A, B, C: 2018-2020, three-year period, first decade of August;
D, E: 2018-2020, three-year period, first decade of September.

viations exceed a critical level, i.e., are significant. If the difference between the average SPAD index values of every 5-5 peppers per pair harvested exceeds the threshold, then the actual difference is said to be significant. When comparing the average SPAD index values per pair with Tukey test for a given pepper type, then in the differences between the pairwise index values as group averages not only the individual effects (the effect of the current two samples of 5-5 peppers each) but also the common effect (the effect of the remaining three samples, each consisting of 5-5 peppers) are also taken into account. When performing the Tukey test, we first determine the differences between the means of all possible group pairs and then compare them with the following statistics:

$$HSD = q\sqrt{((MSw)/n)}$$

where q is the statistics of the studentized values with the appropriate degree of freedom, the current value of which can be retrieved from a table. MSw is the mean squared deviation within the group,

Table 8. Average SPAD values for the five measurement with the results of the post-hoc Tukey tests, Bogyiszló type hot pepper (*Capsicum annuum* L.) BSZ-27. Comparison of the SPAD index values of 5-5 ripe peppers harvested from pepper stem in five successive periods (A, B, C, D, E) based on ANOVA and Tukey test.

A	B	C	D	E
Average SPAD				
67.3	61.8	56.2	61.4	70.6
88	72.2	59.7	64.9	73.9
78	70.1	72.1	73.6	72.7
74.9	71.9	64.7	74.2	65.9
68.8	67.9	61.3	74.4	67.6
[1]75.4	[1]68.8	[1]62.8	[1]69.7	[1]70.1

Results of the post-hoc Tukey tests[2]			
Treatments pair	Tukey HSD p-value	Tukey HSD interference	X signficantly higher than Y
A vs C	0.0213405	$^{*}p < 0.05$	A than C

[1]Mean values; [2]Significant differences between the average SPAD values of 5-5 ripe peppers harvested from pepper stem in the examined four successive periods (A, B, C, D). (*: significant difference at the $p < 0.05$ probability level). Only those treatment pairs are indicated among the results of the post-hoc Tukey tests, SPAD values of which show significant differences.
A, B, C: 2018-2020, three-year period, first decade of August;
D, E: 2018-2020, three-year period, first decade of September.

which is already known when performing ANOVA, while n is the number of sample elements within the group (Tukey, 1953; Matyasovszky et al., 2011; Makra et al., 2016).

The examined field pepper types are as follows:

- Hot red pepper (*Capsicum annuum* L. *var. longum*), SZ 84
- Sweet red pepper (*Capsicum annuum* L. *var. longum*), SZ 102
- Cherry shaped pepper (*Capsicum annuum* L. *var. cerasiforme*), candidate variety
- Cece type sweet pepper (*Capsicum annuum* L.), BSZ-6
- Bogyiszló type hot pepper (*Capsicum annuum* L.) BSZ-27

Results

Using one-way Analysis of Variance (ANOVA), we found that the mean SPAD index values of the leaves of the hot peppers in periods A and C, as well as in the harvest periods B and C and C and D, respectively, differed significantly (Table 4). However, in the case of non-hot peppers, only the average SPAD index values of periods A and D differ significantly (Table 5). Cherry pepper foliage showed significant differences in periods A-C, A-D, C-E, and D-E (Table 6). For sweet pepper type fruits the same indicator shows a significant difference between the A-B, A-C, B-C, B-D, C-D, and C-E harvest periods (Table 7). There was a significant difference in the average SPAD index values of the leaves of sweet peppers – Bogyiszló

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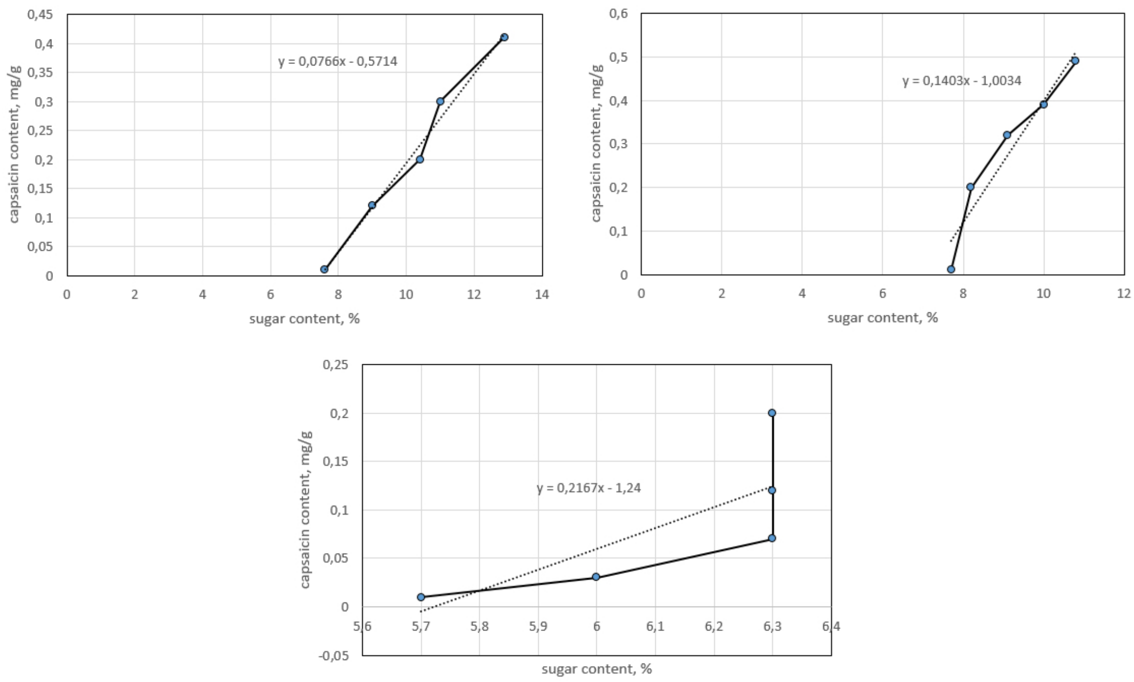

Figure 1. Relationship between sugar and capsaicin content of the studied pepper varieties.

hot peppers only between periods A and C (Table 8).

In order to analyze the utilization of the applied nutrient solution in the plant, correlation analysis was performed that showed a significantly positive relationship between the sugar and capsaicin content in hot peppers ($r = 0.992$, $p < 0.05$) and cherry peppers ($r = 0.963$, $p < 0.05$) respectively, at the 5% probability level. However, in the case of Bogyiszló-type peppers, no relationship was detected between the production of the two substances and nutrient utilization (Fig. 1).

Discussion

Based on the comparison of the SPAD index values of the sugar- and capsaicin content of 5-5 ripe peppers harvested from pepper stem of different types of peppers in successive periods (A, B, C, D, E) showed significant differences in several cases. This can be explained by the fact that in the consecutive periods (A, B, C, D, E) – and in some non-adjacent periods, as well – the SPAD index values show a significant, i.e. statistically significant difference. This indicates that there are periods in the phenophase of the pepper cultivars studied when both sugar content and capsaicin content increase significantly. The tested red pepper varieties SZ 84; SZ 102 reached the requirements 120 ASTA in each measurement.

We found that sugar content is a basic determinant of capsaicin content in hot peppers and cherry peppers, while it is not an important factor for Bogyiszló type BSZ 27 peppers. This result may be related to the fact that bogyiszló peppers are thick-fleshed peppers.

Based on the results of our measurements, we established the optimal SPAD index during the open field cultivation of several peppers. We suggest that they are the referenced values in the future (Table 9). In our opinion, the nutrient solution used corresponds to most pepper types grown in Hungary. A further perspective is extending the scope of the analysis to more refined periods for the studied pepper cultivars.

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