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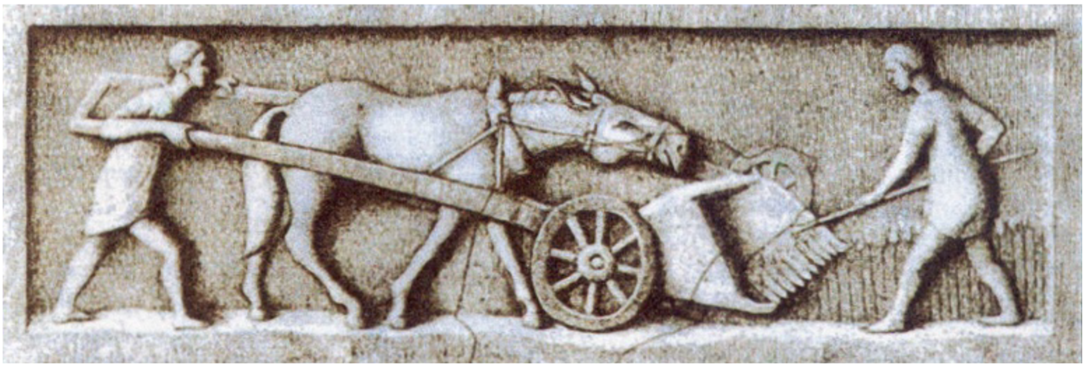

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MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

Columella

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Journal of Agricultural and Environmental Sciences

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 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 $Ca(NO_3)_2$	potassium nitrate KNO_3	nitric acid (59%) HNO_3
62 kg	26 kg	13.3 l
complex fertilizer	mono-potassium phosphate	
21 kg	KH_2PO_4	
	17 kg	
nitric acid (59%) HNO_3	magnesium-sulfate $MgSO_4$	
1.0 liter	21 kg	
pH 6.5	nitric acid (59%) HNO_3	
EC 1.8 mS/cm	12.3 liter	
	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,

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.

- studies on the utilization of potassium in relation to sugar and capsaicin content 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.

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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.

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

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.

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

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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.

then compare these deviations with a critical value in order to determine whether these deviations 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

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.

be retrieved from a table. MSw is the mean squared deviation within the group, 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

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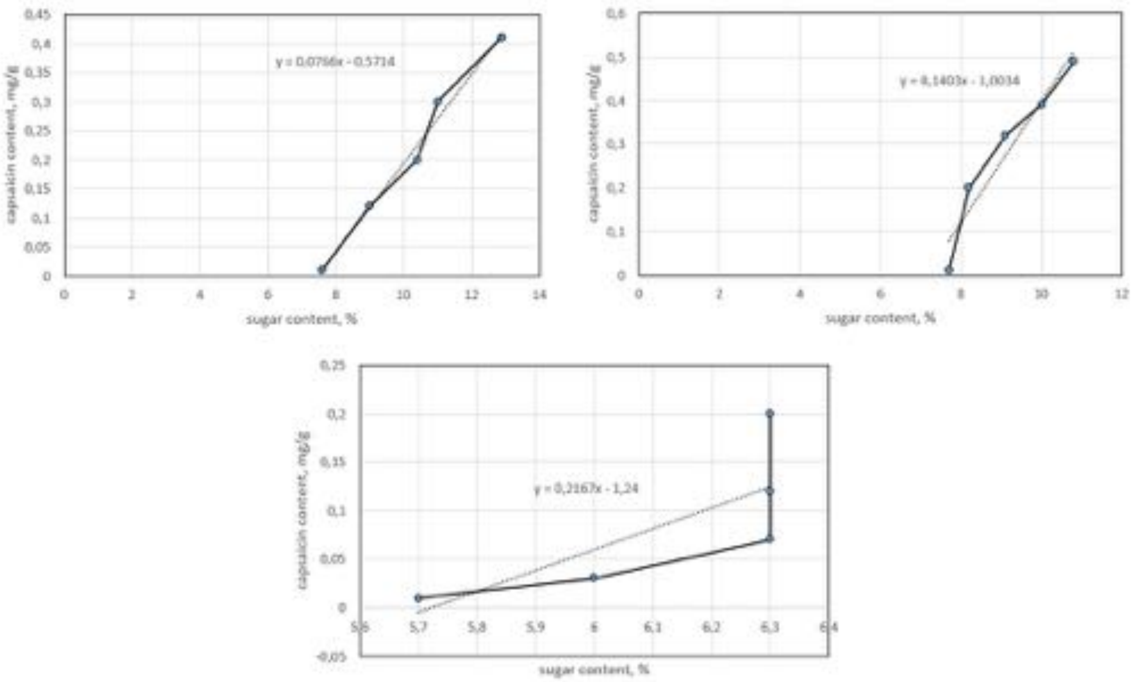

Figure 1: Relationship between sugar and capsaicin content of the studied pepper varieties.

difference in the average SPAD index values of the leaves of sweet peppers – Bogyiszló 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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Assessment of measured and estimated meteorological data in terms of sorghum production on the example of Hamelmalo, Eritrea

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Abstract: Eritrea is exposed to climate variability and extreme events like drought and precipitation variability. Hamelmalo, a sub region in Eritrea, suffers from all the problems brought by climate change, especially because local people mainly depend on rainfed agriculture. I it is difficult to conduct climate related research activities for the region due to the shortage of meteorological data. However, in 2015, a new, complete meteorological station was established providing the chance of the first observations for practical and scientific purposes. The main objective of this study was to evaluate some climatic parameters from crop productional point of view by comparing the observed values with ones calculated by the Local Climate Estimator (LCE) model. Chi-square test was used to statistically analyse the differences. Based on the results, all the studied climatic parameters, except for precipitation, were almost on a par, which means there were no statistically significant differences between the observed and the estimated values. It can be concluded that the most variable climatic parameter in Hamelmalo is precipitation and this also affects the climatic water balance hence the need for irrigation if higher yields are wanted to be achieved. Sufficient water is vital in the mid-season and the late developmental stage of sorghum. Therefore, sowing time is advised to be adjusted to early July to ensure the maximum vegetative growth and seed setting period to be reached at the end of August in order to take the advantage of the positive climatic water balance of these two months.
Keywords: climate change, meteorological data, sorghum production, Eritrea
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Introduction

Since its existence, the Earth's climate has been changing. This change is sometimes faster, sometimes slower. Nowadays human activities influence not only the micro- and macroclimate, but the global climate as well (Harnos, 2003). If we want to understand the climatic status of a region, precise observations and measurements in sufficient spatial and temporal resolution are crucial (WMO, 2010). The determination of the tendencies of the changes is of great importance besides the characterization of the climatic status of a region. Sufficiently long data series as the bases of the assessments are necessary to monitor the changes and determine their tendencies (Konkolyné Bihari et al., 2008).

Humans cannot feel climate change tendencies directly as they are relatively slow. Nevertheless, the increasing frequency of the extreme meteorological events have direct and indirect impacts on society and agriculture. Not only the frequency and intensity of weather extremes are increasing, their spatial and temporal occurrence is getting more

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and more variable, one of the biggest challenges of our society is to accommodate to them (Zsembeli, Kovács, et al., 2019).

Just like many African countries, Eritrea is highly vulnerable to climate change (UNFCCC, 2001), especially water is the most limiting factor for crop growth in the sub-Saharan Africa (Lal, 1991). Agriculture is the top priority for economic development in the whole country as 80% of the population depend on agricultural farming and livestock (IPCC, 2007). Agriculture is the sector highly influenced by climate variability with important involvements for agricultural efficiency and food security, mainly in the developing countries like Eritrea (FAO, 2017).

As reported by Niang et al. (2014), the countries of East Africa, including Eritrea, are especially exposed to the climate variability and extreme events like drought and precipitation variability, and specifically there is a rise in minimum and maximum temperature trends with extremes and high variation in precipitation both daily and seasonally (Cattani et al., 2018). The high variation in precipitation in East Africa is due to the complicated relations of forced and free atmospheric variations (Mutai & Ward, 2000) and this is common in both Eritrea and Ethiopia, which makes the people waiting humanitarian and other organizations assistance (Sidahmed, 2017). Besides the large unevenness in precipitation, the lack of improved technologies, poor management ability, and low agricultural efficiency are characteristic in Sub-Saharan Africa (Calzadilla et al., 2009).

Hamelmalo, a sub region in Eritrea, also suffers from all the problems mentioned above where people mainly depend on rainfed agriculture for their living. In Hamelmalo, just like all over the country, sorghum is the top crop followed by pearl millet (UNFCCC, 2001). Yields are very low, for instance, the average yield of sorghum in Hamelmalo in a period with good rainfall is 0.2-0.6 t ha^{-1} (MoA, 2005). This may be due to several factors, but one of them is climate variability that can cause variation in precipitation and evapotranspiration, hence the climatic water balance. The impacts of climate change are clearly visible on the environment manifested in land degradation, desertification, worsening food security, loss of biodiversity and elongated drought, all are common in the country. The elongated droughts have a great impact on water resources, especially on drinking water, and this leads to water scarcity in the country (Eritrea, 2004).

In Eritrea, precipitation is the most variable climatic parameter with erratic, torrential, short duration, high intensity features from year to year. Two rainy seasons are characteristic in the whole country: June-September (extends from highlands to western region of the country) and October-March (covers eastern escarpments) and also there are short precipitations in April and May along with the highest precipitation records in July and August (FAO, 1994). The mean annual rainfall ranges from less than 200 mm (in the semi-desert) to 1,100 mm (in the sub-humid zone) (UNFCCC, 2001), and in the most part of Eritrea, south-western monsoon winds are the main source of precipitation during the summer and spring seasons (FAO, 1994). Mean annual temperature varies from 18 °C in the moist and arid highlands to 35 °C in the semi-desert (FAO, 2005a).

In conjunction with the shortage of precipitation, it is already proven that supplying additional irrigation water increases the yields in Hamelmalo. As reported by Tripathi et al. (2015), sorghum yield lifted to over 3.9 t ha^{-1} by applying 70 mm irrigation for 21 days after the cut-off of precipitation in September, which is much above the usual without irrigation. This observation is an accurate estimation of water and the appropriate date to save water during irrigation as an important factor for the increase of the production.

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The forecast or measurements of meteorological data used to estimate crop evapotranspiration can also provide important information for having better irrigation scheduling and controlling extreme conditions. Irrigation scheduling uses meteorological data in addition to soil and crop data for determining evapotranspiration of a crop and water balance of the soil (Todorovic, 2006). The Penman Monteith equation method also uses meteorological data from meteorological stations to calculate reference evapotranspiration (Allen et al., 1998).

In the study area of Hamelmalo, there is agricultural college with ongoing research activities concerning climate and agriculture. Nevertheless, there is a shortage of meteorological data due to the lack of meteorological stations, therefore it is difficult to conduct climate related research activities. In general, long-term climate related studies were not common in Eritrea. However, our recent study can help the researchers to have general understanding about the climate and the weather of the region as a basic reference not only for local level, but due to the similarities (Camberlin & Philippon, 2002), for the countries of the Horn of Africa.

The general aim of this study was to assess meteorological parameters if there are significant differences between the data recorded at the meteorological station of Hamelmalo and the relevant ones estimated by means of climate estimating models. As the preconception (hypothesis) of our research work, we assumed that climate is a really complex issue and cannot be definitely quantified locally only on the base of estimated meteorological data. The meteorological data were assessed from the point of view of sorghum production. Cereal crops, inclusive of sorghum, are the most influential crops for food security in the world as a whole and in particular in Eritrea, which is a food insecure country. In Eritrea, the increase of the efficiency of sorghum production will help to reduce food insecurity and improve quality of life, because sorghum accounts 50% of the total cereal production (MoA, 2010).

Materials and Methods

The study site

The study is relevant to Hamelmalo Agricultural College located at 15°52′16″ N latitude and 38°27′44″ E longitude, and at 1,278 m elevation with semi-arid climate. In Hamelmalo, 80% of its population depends on agricultural farming and livestock, which are highly sensitive to the disturbances in the climate system (IPCC, 2007). The most common crops grown in Hamelmalo, in the order of their importance, are sorghum, pearl millet, barley, maize, ground nut, wheat, and finger millet.

Terrestrial, short-duration rainfalls are characteristic that vary from year to year. So far, there was no data about the average precipitation and mean temperature for Hamelmalo. Although precipitation seems to be sufficient for resistant crops, especially for sorghum, but the yields are low owing to the serious shortage of water during the flowering or seeding period (September-October), when additional irrigation is highly needed to increase yield (Tripathi & Ogbazghi, 2010). Agriculture is mainly rainfed and exposed to climatic variability and low agricultural productivity and land degradation have become the major features of this sub-region.

Remarkable degradation including serious soil erosion, poor rain water storage, inadequate vegetation, compaction and structural degradation in over 90% of the agricultural land are characteristic to the area. Most part of the land in this region is not cultivated owing to steep slopes. The soils of Hamelmalo are loamy sand and sandy soils with dominant sand and some percentage of silt, clay, cobbles and boulders forming a porous bed, some organic matter and average bulk density of soil surface ranged from 1.54 Mg m^{-3} to 1.69 Mg m^{-3} (Tripathi et al., 2015). In general, the soils in Hamelmalo have dominantly light and medium texture.

The meteorological station of Hamelmalo has been working since 2009, but first it was only measuring the amount of rainfall, hence they were not sufficient for extended meteorological analyses. However, in 2015, a new, complete meteorological station was established according to the standards providing the chance of the first observations for scientific purposes as well as practical ones.

Acquisition of meteorological data

The data collected from the weather station of Hamelmalo cover five years of the 2015-2019 period for all the computations in this study. The data were recorded hourly except for a few months. The observed meteorological data include precipitation, temperature (mean, maximum, minimum), relative humidity, wind speed, and sunshine hours. The evaluation of the available data includes all data that are equally related to the estimated ones. The observed values of all the recorded meteorological parameters are the averages of five years measured during the investigated period of 2015-2019.

The possibility of utilizing the meteorological data recorded in Hamelmalo for any research activities, planning and designing projects is limited as short period records may not be fully representative of the prevailing climate. With the absence of long period records, Local Climate Estimator (LCE) model by FAO was used to crosscheck the observed values with the expected ones. LCE is a free online tool that can be used to estimate local climatic data for any location in the world. Climate data recorded nearly 30,000 stations worldwide are available for the estimations (Grieser et al., 2006). The expected values were obtained from the LCE after feeding the data of Hamelmalo location (latitude, longitude and altitude) into the software, which automatically calculates the estimated values of weather variables such as the ones assessed in this study: air temperature (mean, maximum, minimum), amount of precipitation, relative humidity, number of sunshine hours, and potential evapotranspiration (PET).

The model delivers three alternative values of the estimation (the best, the minimum and the highest) of the given observatory station based on the records of at least ten neighbouring observatory stations that are maximum 1,000 km away. In the case of Hamelmalo, they are Keren, Gheleb, Nakfa, Barentu, Massawa, Asmera, Adikeyih, Agordet, Faghena, Filfil, Fishey Merara, Adinfas, Mendefera in Eritrea, and Adwa and Sheraro in Ethiopia.

Calculation of potential evapotranspiration

Generally, PET can be computed by different methods. By means of precision weighing lysimeters actual evapotranspiration (ETact) can be calculated quite accurately (Zsembeli, Czellér, et al., 2019). However, these devices are not available in most of the developing countries owing to their high price and lack of expertise. In this study, observed PET values were estimated from pan evaporation because that is the instrument installed and operated in the meteorological station of Hamelmalo and there was no gap in the measurements during the investigation period (2015-2019). Pan reading can be used to calculate PET using the pan coefficient according to Equation 1.

$$PET = K_p \times E_p$$

where K_p = pan coefficient (mm day^{-1}) determined according to Doorenbos (1976); E_p = pan evaporation (mm day^{-1}).

Calculation of climatic water balance

Climatic water balance is the difference of precipitation and potential evapotranspiration determined for a defined period of time and can be calculated according to Equation 2 (Gebreyesus et al., 2021).

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$$CWB = P - PET$$

where: CWB = climatic water balance (mm); P = precipitation (mm); PET = potential evapotranspiration (mm).

Statistical analysis

Chi-square test was used to see if there is statistical similarity between the meteorological data observed in Hamelmalo and the estimated values by the LCE model. The advantages of this method are its robustness with respect to data distribution, simple calculation, data handling flexibility from two groups and complete information obtained from the test. In this study the degree of freedom was 11 (11df), which is based on the Row-1 and Column-1 system. P-value approach was compared with α. According to the rule for p-value approach, the hypothesis (H) was rejected if H value was less or equal to α and was not rejected if it was greater than α (0.05). Alternatively, it could also be compared with the critical value: if the result was above the critical value, H could be rejected, while accepted if it was less than the critical value. The analyses were performed in Microsoft Excel software by comparing the values to accept or reject H.

Results

Air temperature

Temperature is one of the most important factors determining the success of crop production, especially in terms of the frequency and duration of extremes. In Table 1, the observed and the estimated air temperature values determined for Hamelmalo are listed.

For the mean monthly maximum temperature values, based on the test, the probability level was 0.992, which means no convincing statistically proven difference among the observed and estimated values. Nevertheless, all the monthly values were overestimated by the LCE model resulting in an average mean of 36 °C contrary to the observed 34 °C.

The probability level for the mean monthly temperatures was 1, which means that statistically there were no remarkable differences between the observed and expected values (Table 1). The LCE model slightly overestimated the monthly values for January, April, May, June, August, October, November, and December, while underestimation was found for February, March, July, and September resulting in only a little difference between the observed and the estimated average means (0.2 °C).

Probability level of 0.93 was found when the mean monthly minimum temperatures were tested, which also indicates no considerable statistically proven difference between the observed and estimated values (Table 1). All the monthly values were underestimated by the LCE model resulting in an average mean of 9.9 °C contrary to the observed 11.6 °C.

Precipitation

Natural precipitation is the only water input for sorghum if it is grown under rainfed conditions. Therefore, the amount and temporal distribution of precipitation basically determines the yields of sorghum. Furthermore, the time and amount of irrigation can be planned easier if the probability of rainfall can be estimated in advance. The observed and the estimated monthly amounts of rainfall determined for Hamelmalo are compared in Table 2.

The probability level for the mean monthly amounts of precipitation was under 0.05, which means a high variation among the observed and expected values, even the average values were very similar (Table 3). The LCE model overestimated the monthly amount of precipitation for April, June, August, and October, while underestimated for March, July, September, and November. Similar values were found for May, January, February, and December, in the latter three months actually no precipitation can be expected.

Table 1: Observed monthly air temperature values in Hamelmalo, Eritrea compared to the ones estimated by the Local Climate Estimator (LCE) model developed by FAO.

Month	Mean monthly maximum temperature		Mean monthly temperature		Mean monthly minimum temperature	
	observed	estimated	observed	estimated	observed	estimated
January	32.0	35.1	20.2	20.5	8.5	7.5
February	34.6	36.1	22.0	21.3	9.3	8.1
March	36.2	38.2	23.6	23.5	11.0	10.9
April	37.0	40.0	25.0	25.3	13.0	12.0
May	37.0	40.3	25.4	26.2	14.0	12.4
June	35.5	38.7	24.8	25.2	14.0	10.4
July	34.0	34.4	24.3	22.7	14.6	11.0
August	31.2	32.6	21.4	21.7	11.6	10.6
September	34.2	35.6	22.8	22.7	11.3	8.9
October	34.0	37.7	22.6	23.6	11.2	9.7
November	32.5	36.2	21.9	22.2	11.3	9.6
December	30.2	35.6	19.9	21.1	9.7	8.4
Average	*34*	*36.7*	*22.8*	*23.0*	*11.6*	*9.9*
	Probability level (p) = 0.992		Probability level (p) = 1.00		Probability level (p) = 0.953	

Underestimated values are underlined

Table 2: Observed monthly precipitation values in Hamelmalo, Eritrea compared to the ones estimated by the Local Climate Estimator (LCE) model developed by FAO.

Month	Mean monthly precipitation	
	observed	estimated
January	0.0	0
February	0.0	0
March	8.9	4
April	4.2	16
May	29.5	30
June	57.7	62
July	135.3	124
August	156.8	178
September	68.6	40
October	8.8	14
November	2.9	2
December	0.2	0
Average	*39.4*	*39*
Annual	472.9	470
	Probability level (p) < 0.05	

Underestimated values are highlighted with underlining

Number of sunshine hours

Sorghum is a short-day plant, the optimum photoperiod of sorghum production is 10-11 hours. Photoperiods longer than 12 hours stimulate only vegetative growth. In terms of day length, flower initiation is the most crit-

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Table 3: Observed daily number of sunshine hours in Hamelmalo, Eritrea compared to the ones estimated by the Local Climate Estimator (LCE) model developed by FAO.

Month	Mean daily sunshine hours (h)	
	observed	estimated
January	9.4	11.0
February	9.2	10.6
March	9.1	10.9
April	8.7	10.2
May	7.7	9.6
June	7.0	9.0
July	7.5	6.7
August	5.3	5.8
September	7.3	7.9
October	8.8	9.9
November	9.3	9.9
December	9.5	10.7
Average	8.2	9.4
	Probability level (p) = 0.996	

Underestimated values are highlighted with underlining

ical period. In Table 3, the observed and the estimated sunshine hours per day determined for Hamelmalo are compared.

The probability level for the mean monthly sunshine hours was 0.996, which means that statistically there were no considerable differences between the observed and expected values (Table 3). Nevertheless, the LCE model overestimated the monthly number of sunshine hours for all the months, except for July, resulting in a 1.2 h day^{-1} average mean difference.

Relative humidity

If relative humidity of the air surrounding the crops is high, the transpiration rate falls as it is inversely proportional to humidity. Therefore, having data about the temporal dynamics of relative humidity provides information on ETact and indirectly on crop water demand. The observed and the estimated monthly relative humidity values determined for Hamelmalo are compared in Table 4.

The probability level for the mean monthly relative humidity of the air was 0.999, which means that statistically there were no differences between the observed and expected values (Table 4). Nevertheless, the LCE model overestimated the monthly number of sunshine hours for all the months, except for June, July, and September resulting in a 2.4% average mean difference.

Potential evapotranspiration

As its definition says, PET determines the sum of water that could be evaporated from the soil surface and transpirated by the crops if water is not a limiting factor. It provides information on the theoretical water demand of crops. On the base of PET, the gap between water demand and water supply can be calculated. This gap is an important issue and generally increases under climate change (Whetton & Chiew, 2021). In Table 5, the monthly PET values calculated from the observed pan evaporation data and by means of the LCE model are compared.

For the mean monthly PET values, based on the test, the probability level was 0.96,

Table 4: Observed monthly relative humidity values in Hamelmalo, Eritrea compared to the ones estimated by the Local Climate Estimator (LCE) model developed by FAO.

Month	Mean monthly relative humidity (%)	
	observed	estimated
January	78.2	81.0
February	74.0	81.0
March	71.5	76.0
April	70.3	75.0
May	67.3	70.0
June	66.7	<u>65.0</u>
July	78.0	<u>77</u>
August	83.1	84
September	75.4	<u>74</u>
October	70.8	73
November	75.0	76
December	76.4	84
Average	73.9	76.3
	Probability level (p) = 0.999	

Underestimated values are highlighted with underlining

Table 5: Monthly potential evapotranspiration (PET) values calculated from pan evaporation in Hamelmalo, Eritrea compared to the ones estimated by the Local Climate Estimator (LCE) model developed by FAO.

Month	Mean monthly PET (mm)	
	observed	LCE
January	114.7	122.2
February	121.9	129.2
March	163.5	<u>162.2</u>
April	171.4	172.1
May	178.0	<u>173.4</u>
June	159.6	163.0
July	121.5	130.9
August	91.8	100.1
September	114.0	121.4
October	138.3	149.5
November	127.4	128.0
December	118.8	<u>117.4</u>
Average	135.0	139.1
Annual	1620.9	1669.4
	Probability level (p) = 0.96	

Underestimated values are highlighted with underlining

which indicates no statistically proven difference among the observed and estimated values. Nevertheless, we detected overestimated values for 9 month, only slight underestima-

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tion was found for March, May, and December resulting in 48.5 mm annual difference, which cannot be considered too big.

Monthly climatic water balance

Negative CWB is characteristic to a region when PET is lower than the amount of precipitation (Gebreyesus et al. 2021). Under the conditions of Hamelmalo, crops definitely suffer from the shortage of water as drought events are common. The drought tolerance of sorghum is better than of most other grain crops. It is due to the fact that it has an exceptionally well developed and finely branched root system, which is very efficient in the absorption of water. Furthermore, the leaf area per plant of sorghum is small limiting transpiration very effectively. The monthly and annual CWB values calculated as the difference of PET and natural precipitation determined for Hamelmalo on the base of the five-year averages are shown in Table 6.
The annual climatic water deficit (negative CWB) was found to be huge. There are only two months (July and August), when CWB is positive. These results indicate when irrigation is necessary to ensure yield safety and also provide rough quantified estimation on the irrigation water needed. For more precise calculations, information on the relevant soil properties is essential.

Discussion

In this study, we evaluated some climatic parameters from crop productional point of view by comparing the observed values with ones calculated by the LCE model on the example of Hamelmalo, Eritrea. Our results are especially of great importance for that location as there was a lack of locally measured meteorological data until 2015, when a new, complete meteorological station was established there. On the other hand, Hamelmalo, just like many other places of the world, is highly vulnerable to climate change. The main meteorological parameters were analysed, which basically determine the success of crop production.

Comparing the observed and estimated mean, maximum, and minimum air temperature values, we found them to be quite similar but in most of the cases overestimated by the LCE model. Sorghum requires high temperatures for germination and growth. The mean monthly temperatures were in the range of 19.9–25.4 ℃, which can be considered is a bit low as for optimum growth and development of sorghum, air temperature of 27 to 30 ℃ is required, even under 21 ℃, its growth is limited. Nevertheless, the mean monthly temperature is under this threshold in Hamelmalo only in December based on the observed data. The maximum temperatures varied between 30.2 and 37 ℃ calling the attention to the fact that exceptionally high temperatures also cause yield depression. In this respect, the period between March and June can be considered risky as the mean maximum temperature is above 38 ℃. For the germination of sorghum seeds, the minimum temperature must be 7–10 ℃. The minimum temperatures observed in Hamelmalo varied between 8.5 and 14.6 ℃, which means that temperature is not likely to be a limiting factor in this respect.

Mean monthly precipitation was the only climatic parameter which showed convincing difference among the observed and estimated values. Precipitation variability is common in East Africa including Eritrea (Lim & Hendon, 2017). Precipitation data were available in Hamelmalo before 2015, so based on the data of ten years and by incorporating standard deviation, we could estimate the mean annual rainfall as 445 $\pm$ 23.9 mm. Within these ten years in Hamelmalo, the lowest precipitation was recorded in 2015 and the highest in 2019, which shows increment with some variation. According to the UNFCCC (2001) in the whole country of Eritrea there

Table 6: Climatic water balances (CWB) in Hamelmalo.

Month	PET	Precipitation	CWB
January	114.7	0.0	-114.7
February	121.9	0.0	-121.9
March	163.5	8.9	-154.6
April	171.4	4.2	-167.2
May	178.0	29.5	-148.5
June	159.6	57.7	-101.9
July	121.5	135.3	+13.8
August	91.8	156.8	+65.0
September	114.0	68.6	-45.4
October	138.3	8.8	-129.5
November	127.4	2.9	-124.5
December	118.8	0.2	-118.6
Annual	1620.9	472.9	-1148.0

is an increasing trend in terms the amount of annual precipitation.

On the base of the observed precipitation data, in the months of January and February, no rainfall can be expected in the studied region. Practically the same is valid for December and October, almost no effective rainfall is characteristic during these months. These results suggest that additional irrigation is important to acquire the daily crop water needs if any crop is supposed to be grown in these four months. Rainfed sorghum is endangered by the risks of water stress during critical growth stages and droughts. Sorghum yields of about 4 t ha^{-1} can be assured only by irrigating once in terraced plots with high amount of residual soil moisture (Tripathi & Ogbazghi, 2010; Weldeslassie et al., 2016).

In terms of sunshine hours, it can be concluded that the observed mean daily sunshine hours, with their range of 5.3–9.5 h day^{-1}, do not reach the optimal photoperiod of 10–11 hours in neither of the months. Nevertheless, this range is also under the critical value of 12 hours when only vegetative growth is stimulated.

Potential evapotranspiration in inverse conjunction with relative humidity of the air determine transpiration of crops. Annual PET rates range from 1,700–2,000 mm in the northern highland of Eritrea (FAO, 2005b) where Hamelmalo is found. On the base of the observed meteorological data, we calculated 1620.9 mm of annual PET, which is a bit less than the general trend, but close to the estimated value by LCE (1669.4 mm). Crop ETact increases with increasing air temperature and solar radiation, the two primary drivers of evapotranspiration (Irmak, 2009). The annual ETact of the sorghum was reported as 450–650 mm by Doorenbos and Kassam (1979) and 210–293 mm by Kuo et al. (2006). There was also a study about crop water requirement in Ethiopia, which is not far from Eritrea, and the result was 500.4 mm during the growing season (Shenkut et al., 2013). So, the demand for water was less in Eritrea than in Ethiopia may be due to difference in rainfall variation between the two countries. Generally, this indicates that ETact is very much dependent on the atmospheric conditions experienced at a particular location.

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The monthly climatic water balances were figured out to be strongly negative in ten months of the year, only July and August can be characterized with positive CWB. The results indicate that irrigation is critical in the mid-season stage and late developmental stage of sorghum crop. To avoid yield reductions in sorghum cultivation more water should be applied in the late developmental stage. This is probably because large quantities of water are needed for grain formation (Abou Kheira & Atta, 2009). In areas where water is a limiting factor in crop production like in Eritrea, deficit irrigation may be preferable. It is believed to be the least sensitive to water stress (Fereres & García-Vila, 2018).

For sorghum, early sowing dates start from 1st April, while late sowing dates last from mid-May till mid-June (Haile & Hofsvang, 2001). Based on our results, sowing time and early growth period can be adjusted from around 20th June up to early July to take the advantage of the positive CWB of July. The maximum vegetative growth and seed setting period should be adjusted from the first week of July up to the end of August as the latter month has the most positive CWB. The first two weeks of September is the suitable period for ripening. Otherwise, supplementary irrigation is a must, especially for sorghum as it has a short growing season of around 125 days (Smith et al., 2002). All these should be considered by the farmers to adjust the planting and harvesting time if the amount of water is limited.

As a conclusion it can be stated that sufficient water is vital in the mid-season and the late developmental stage of sorghum, more than for other developmental stages. Especially in the initial and development stages, irrigation is not definitely needed. This indicates that proper planning and timing of planting dates to coincide the late developmental stage with high rainfall months is vital in water resource management, especially under the semi-arid climate of Eritrea. Up-to-date, accommodating crop and soil water management practices need to be adopted for the production to be economically sustainable. Therefore, it is recommended to shift planting dates to coincide with the rainy season and also to allocate water according to the critical developmental stages of sorghum in order to maximize yields without adverse implications for water resources.

The evaluation of the climatic parameters shows that the LCE model we used to observe the studied variables is functional. There were no any significant differences ($p = 0.05$) between data of the observed and the estimated data except for rainfall. Naturally, our results can be extended to other locations with similar agri-ecological conditions, and the methods we used can be applied for similar studies in terms of the comparison of observed and estimated meteorological data. Therefore, we encourage researchers to carry out similar studies based on the approaches we used.

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In silico promoter analysis and expression of the *BIG BROTHER* gene in different organs of potato

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Abstract: The ubiquitin E3 ligase *BIG BROTHER/ENHANCER OF DA1 (BB)* gene encoding a RING finger protein was identified as a central growth regulator in *Arabidopsis thaliana*. It was found that *BB* restricts cell proliferation and promotes leaf senescence. Besides of *Arabidopsis*, however, the role and regulation of *BB* in other plant species is only sparsely known. Supposing that the *BB* gene, like in *Arabidopsis*, has an important role in the development of potato we aimed to analyse a 3.0-kb promoter sequence of the potato *BB* gene, *StBB*, *in silico* and study the level of *StBB* expression by quantitative reverse transcription PCR in different organs. A total of 48 binding sites for 15 transcription factor (TF) families were predicted. Most of them were located in the -1.5-kb promoter region. The dominating family of TFs was DOF. It was found that 20 out of the 24 TFs with known functions are involved in developmental processes such as for example, the flower-, leaf-, stem- and root development or cell cycle regulation. In line with this finding, the *StBB* mRNA was detected in each organ tested with the largest amounts in petal and stamen. These results suggest a similar function of *StBB* in potato than that is of *BB* in *Arabidopsis*, i.e., restriction of organ overgrowth during development and limitation of the plant growth.

Keywords: *BIG BROTHER* gene, *cis*-acting regulatory elements, *Solanum tuberosum* L., transcription factors

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Introduction

Although plant growth is a complex, dynamic process, it is divided into the following phases: cell proliferation, elongation, and differentiation, which are regulated by both genetic (internal) and environmental (external) factors (Müller & Sheen, 2008; Bögre et al., 2008; Denay et al., 2017; Zluhan-Martínez et al., 2021). Hence leaves are photosynthetic organs, their size is pivotal for plant growth. During leaf development, the size is controlled by an organ-wide mechanism that coordinates the cell proliferation with cell expansion (Horiguchi et al., 2005). Numerous genes have been identified as cell proliferation regulators in *Arabidopsis thaliana* including the ubiquitin receptor *DA1–ENHANCER OF DA1 (DA1–EOD1)* module, which is identified as a negative regulator of leaf growth (Du et al., 2014; Vercruysse et al., 2019). DA1 is activated by the E3 ligases *BIG BROTHER/ENHANCER OF DA1 (BB)* and *DA2* (Peng et al., 2013; Xia et al., 2013; Dong et al., 2017; Vercruysse et al., 2019).

The RING finger protein *BIG BROTHER (BB)* was identified as a repressor of plant organ growth in 2006. The homozygous *bb-1* mutants, which lack *BB* mRNA, form larger petals and sepals, as well as thicker stems than wild-type (Disch et al., 2006)). Vanhaeren et al. (2016) found that single mutations such as *da1-1* and *bb/eod1-2* increase the leaf size in *Arabidopsis*. The double mutation *da1-1_bb/eod1-2* cause the synergistic enlargement of both the first leaf pair and younger rosette leaves. Furthermore, depending on the *BB* expression, plants start to die prematurely. Thus, it was concluded that ectopic expression of *DA1* or *BB* restricts cell proliferation and promotes leaf senescence. Cattaneo and Hardtke (2017) reported that *bb2* loss-of-function mutations prolong the cell proliferation and uncouple cell proliferation from elongation in the root meristem. They evidenced that *BB* acts similarly in leaf (-like) organs and the primary roots. Downstream transcriptional effects of *DA1* and *BB* were also tested in the young, proliferating leaves within different induction time frames. It was found that both *DA1* and *BB* trigger molecular changes shortly after induction of their expression, but the expression of *BB* is higher than that of *DA1* and rapidly stimulates the expression of senescence markers.

To identify the connection between the individual factors and larger regulatory pathways, expression of *BB* was investigated by a combination of promoter deletion analysis and a phylogenetic footprinting approach. It was shown that removing 150 bp from the 5′ non-transcribed promoter sequence resulted in a 40% increase in petal size in the transgenic lines. Alignment of the isolated *BB* coding sequence from *A. thaliana* and seven other species from the Brassicaceae family showed a high degree of conservation within all genera (Breuninger & Lenhard, 2012).

Besides of *Arabidopsis*, however, the role and regulation of *BB* is only sparsely known. In *Saltugilia*, four candidate genes, including *BB*, underpinning of flower size were identified and down-regulation of *BB* in synthetic polyploids of *Nicotiana tabacum* with increased corolla tube size was demonstrated (Landis et al., 2017, 2020). According to our knowledge, however, no study on *BB* gene in potato has been reported thus far.

Potatoes are the fourth most consumed crop in the world, behind rice, wheat and corn (Birch et al., 2012) and the consumption is even increasing especially in Asian countries (http://www.fao.org/faostat). Therefore, productivity is still a key issue of potato breeding.

In our previous study, we found a positive correlation between the growth rate of the leaves and the time of tuber initiation (Odgerel & Bánfalvi, 2021). Supposing that the *BB* gene is involved in the regulation of leaf growth also in potato, the aim of our study was the analysis of the *BB* promoter sequence in silico and the level of *BB* expression in different organs of *Solanum tuberosum* cv. 'Désirée'. Here we show that the promoter region of the potato *BB* gene (*StBB*) carries several *cis*-regulatory elements (CAREs) for transcription factors (TFs) involved in the regulation of plant development and *StBB* is expressed in each organ of *S. tuberosum* cv. 'Désirée'.

Materials and Methods

Plant materials and growth conditions

Potato (*Solanum tuberosum* L.) cv. 'Désirée' plants were propagated *in vitro* in solid (0.8% agar) RM culture medium (MS medium without vitamins; (Murashige & Skoog, 1962)) supplemented with 2% (w/v) sucrose. Plants were grown in a growth room at 24°C with 16-h day/8-h dark cycles at a light intensity of 75 μmol m^{-2} s^{-1}. Plantlets were continuously cultured from stem segments in fresh medium every month. One-month-old plantlets were transferred into the sterile soil A200 (Stender GmbH, Schermbeck, Germany) and grown in a greenhouse under standard conditions with a 10-12 h light/26-28°C and 10-12 h dark/18-22°C cycle. In order to provide optimal growth condition in the greenhouse, plants were treated with fungicides at the be-

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ginning of acclimatisation and weekly with the pesticide Mospilan (Nippon Soda Co., Ltd., Tokyo, Japan). The plants were watered twice a week. For the *StBB* expression analysis, 'Désirée' root, stolon, tuber, stem, petiole, leaf, sepal, petal and stamens of greenhouse-grown plants were used. Tubers were harvested at the end of the vegetation period.

In silico DNA sequence analysis of the StBB promoter

Three thousand-bp sequences upstream from the translational start sites of the *S. tuberosum* Group Phureja *BB* gene (chromosome 11, from 37009829 bp to 37012829 bp, reverse complement) was retrieved from the Potato Genomic Resource Spud DB (http://solanaceae.plantbiology.msu.edu/).

The binding sites of transcription factors (TFs) were predicted by the plant regulatory data and analysis platform The Plant Transcriptional Regulatory Map (http://plantregmap.gao-lab.org/). The latest, upgraded version PlantTFDB v5.0 (Tian et al., 2019) was used at a threshold p-value $\leq$ $1e^{-5}$.

RNA extraction, reverse transcription PCR (RT-PCR) and reverse transcription – quantitative PCR (RT-qPCR)

Total RNAs from plant tissues were extracted by using the method of Stiekema et al. (1988). One μg of RNA was reverse transcribed into first-strand cDNA using the Maxima H minus First Strand cDNA Synthesis Kit with dsDNase (Thermo Scientific Molecular Biology, Waltham, MA, USA). RT-PCR was conducted in T100 Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) for checking the cDNA quality using a primer pair for the cytoskeleton component gene *ACTIN*, a commonly used reference gene for RT-qPCR in potato (Nicot et al., 2005). RT-qPCR assays were performed using a Light Cycler-96 thermal cycler (Roche Diagnostics GmbH, Mannheim, Germany) and the Luminaris Color HiGreen Flourescein qPCR Master Mix (Thermo Scientific Molecular Biology, Waltham, MA, USA). Data were analysed with the Light Cycler-96 Software version 1.1 (Roche Diagnostics GmbH, Mannheim, Germany). Expression analysis of the *StBB* gene was carried out using the primer pair StBB Fw and StBB R. Parallel reactions to amplify *ACTIN* and *EF1α*, the commonly applied reference genes (Nicot et al., 2005), were used to normalize the amount of template. Relative expression was calculated based on both reference genes. Non-reverse transcriptase control without enzyme mixture and a negative control without cDNA were always included, with three technical replicates in each experiment. All primers were designed using the NCBI primer designing tool (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) and their sequences are presented in Table 1.

Results

In silico prediction of TFs binding to the StBB promoter

The prediction of transcriptional binding sites by The Plant Transcriptional Regulatory Map (PlantRegMap) resulted in identification of 48 binding sites for 29 TFs in the *StBB* promoter region. These TFs belonged to 15 families. Eighteen out of the 48 binding sites served for the DOF family TFs. The other dominating families were BBR-BPC, bHLH, M-type MADS and MIKC-MADS (Table 2). Location of the TF binding sites with the indication of TF families is presented in the top of Figure 1.

The predicted TFs are involved in a wide range of biological processes and respond to different internal and external stimuli (Table 2). Nevertheless, we found that 20 out of the 24 TFs with known functions are related to developmental processes as for example, the

Table 1: Primer sequences used in RT-qPCR

Name	Forward sequence ($5' - 3'$)	Reverse sequence ($5' - 3'$)
StBB	TCCATCAGCACCAATCCATAC	CATGCTCCTCGATTCCAGATAC
Actin	TGGACTCTGGTGATGGTGTG	GGTTTCAAGTTCCTGTCTGT
EF1	GACAAGCGTGTTATTGAGAGG	CACAGTGCAGTAGTACTTAGTG

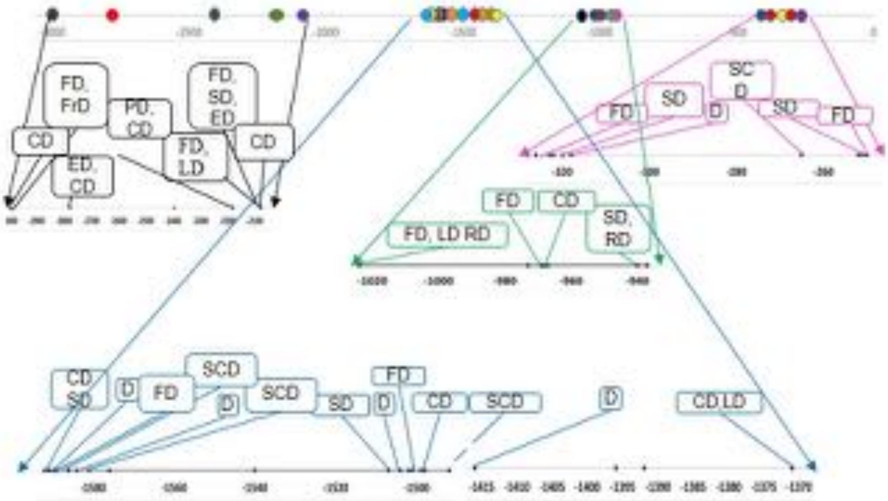

Figure 1: Predicted binding sites of TFs in the promoter region of the *S. tuberosum* Group Phureja *StBB* gene. Thin line represents the promoter region from the translation start site to -3000 bp. Round shapes in different colour represent predicted TF families: red, DOF; light blue, BBR-BPC; yellow, C2H2; green, M-type MADS; purple, MIKC-MADS; pink, B3; light green, LBD; grey, HB other; white, WRKY; orange, GRAS; dark green, ERF; black, RAV; brown, bHLH; red circle, MYB; squash, MYB-related. TFs involved in developmental processes are illustrated by boxes. The coloured arrows indicate zooming in the different regions of the promoter. Abbreviations: CD, cell development; D, development; ED, embryo development; FD, flower development; FrD, fruit development; LD, leaf development; RD, root development; SD, stem development; SCD, seed and seed coat development; PD, pollen development.

flower-, leaf-, stem- and root development or cell cycle regulation (Fig. 1).

Identification of the main CAREs in the StBB promoter

The binding sequences and locations of TFs with known functions are listed in Table 2. The BARLEY B RECOMBINANT/BASIC PENTACYSTEINE (BBR/BPC) is a plant-specific transcription factor family (Meister et al., 2004). BBR/BPC TFs bind to a GA-rich motif and this motif was found at around -1.5 kb in the *BB* promoter. BPC6 and BPC1, which bind to this motif, both respond to ethylene. In addition, BPC1 is involved in the regulation of plant development (PlantRegMap prediction).

The CACGTG motif representing the binding site of the basic helix-loop-helix (bHLH) family proteins was identified at the very distal end of the *StBB* promoter. The second largest class of the plant TFs, the bHLH fam-

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Table 2: Transcription factors with known functions binding to the *StBB* promoter

Family	Name	Position	Matched sequence	Function*
BBR-BPC	BPC6	-1589 -1540	ACTTTTTTCTCTCTCTCTCTC TTCATCTTCTCTCTCTCATGC	Response to ET
	BPC1	-1591 -1416 -1502	AGGAGAGAGAGAGAGAAAAAAGTG AAGAAAGGAAGAATAATAAAGAGA GAGAAAAAAAGAAGAAGAAGAG	Response to ET, regulation of development
bHLH	PIF3	-2996	GTCCACGTGG	De-etiolation, GA and far-red light signalling, regulation of anthocyanin metabolism
	SPT	-2994	ACCACGTGG	Circadian rhythm, response to cold and red light, fruit and carpel development, negative regulation of seed germination
	PIF3	-2997	GGTCCACGTGGT	Response to CK, cell growth
C2H2	IDD1	-265	AATTAGAAGACAAAAAT	Regulation of GA signalling, seed germination and maturation
	REF6	-1371	GAAAACAGAGTG	Response to BR, cell growth, flowering, histone modification, leaf development
DOF	DOF1.5	-1584	AGAGAGAGAAAAAAGTGAAAA	Seed coat development
	DOF1	-323 -1581 -973 -1504	AAAAGGGAAAAGCAAAGAAAA GAGAGAAAAAAGTGAAAAAAA CTGAACAAAAAGGAAAACAAA AGGAGAAAAAAGAAGAAGAAG	Response to chitin
	DOF5.9	-320 -1507 -251	AACAAAAGGGAAAAGCAAAGA CAAAGGAGAAAAAAGAAGAAG AACCAAGCCAAAAAGGAAAAT	Phloem or xylem histogenesis
	OBP3	-1586 -318	TTCACTTTTTTCTCTCTCTCT TTTGCTTTTCCCTTTTGTTAA	Photomorphogenesis
	OBP1	-1498 -967	TTCTCTCTTCTTCTTCTTTTT ATTCCCTTTGTTTTCCTTTTT	Response to AUX and SA, cell wall modification, positive regulation of cell cycle
GRAS	GAI	-1589 -1492	GAGAGAGAGAGAGAAAAAAG AAGAAGAAGAGAGAACAACT	Regulation of N utilization, protein catabolism, seed dormancy and ROS, response to ET, ABA, SA, JA, far-red light and salt, negative regulation of GA signalling, seed germination, phloem transport
HB_other	KNAT1	-1592	TTTTCTCTCTCTCTCTCCTC	Cell fate specification, xylem and phloem development
LBD	LBD18	-940	ATTCTGCCGGTTTTTATGG	Xylem development, lateral root formation
M-type_MADS	AGL20	-1590 -1501 -969 -326	CTTTTTTCTCTCTCTCTCTCC TCTCTTCTTCTTCTTTTTTCT TCCCTTTGTTTTCCTTTTTGT TTTTTTTCTTTGCTTTTCCCT	Translocation, response to cold and GA, positive regulation of flower development
MIKC_MADS	PI	-250	GCCAAAAAGGAAAA	Specification of floral organ identity
	AGL15	-2078	CTTTCCACATTTAGGAATT	Somatic embryogenesis, negative regulation of SD photoperiodism and seed maturation, flowering, negative regulation of floral organ and fruit abscission, cellular response to AUX
	AG	-2080	CACTTTCCACATTTAGGAA	Leaf development, maintenance of floral organ identity
	AP3	-2077	TCCTAAATGTGGAAA	Specification of floral organ identity
MYB	MYB124	-2778	CGTAAACGCTCCACA	Embryo sac development, guard cell differentiation
MYB_related	MYBL2	-1392	CACCTCCTTATCTTC	Response to salt, AUX, JA and Cd
RAV	RAV1	-1024	GTGGTAATTTCTGTTGA	Response to BR, negative regulation of flower development, leaf and lateral root development
WRKY	WRKY2	-2181	GGGTCAAC	Pollen development, longitudinal axis specification, establishment of cell polarity

*Abbrevations: ABA, abscisic acid; AUX-auxin; BR, brassinosteroid; Cd, cadmium; CK, cytokinin; ET, ethylene; GA, gibberellic acid; JA, jasmonic acid; N-nitrogen; ROS, reactive oxygen species; SA, salicylic acid; SD, short day

ily proteins are involved in ethylene and gibberellin signalling pathways and are identified as positive regulators of carpel and fruit development, light signalling, flavonoid biosynthesis, anthocyanin metabolic process and repression of seed germination (Feller et al., 2011).

The binding sites of C2H2 family zinc finger proteins including IDD1 and the RELATIVE OF EARLY FLOWERING 6 (REF6) were found at -265 bp and -1371 bp, respectively. IDD1 is involved in gibberellin signalling, seed germination and maturation (Feurtado et al., 2011), while REF6 responds to brassinosteroids and regulates the cell growth, flowering and leaf development (C. Li et al., 2016).

Eighteen CAREs recognised by seven DOF TFs were predicted, however, some of them were overlapping and only five CAREs were unique. The predicted DOFs are involved in different biological processes. DOF1.5 is involved in seed coat development, DOF1 responds to chitin and DOF5.9 has a role in phloem or xylem histogenesis. The OBP-type DOF family TF, OB3, is involved in photomorphogenesis, while OBP1 is involved in cell wall modification and cell cycle regulation and respond to auxin and salicylic acid. The core sequence recognised by DOFs is the AAAG motif (Yanagisawa & Schmidt, 1999) and this motif or its reverse sequence CTTT was present in all predicted DOF family TF binding sites.

The GAc/gAAA core motif that previously proposed to be the binding site of the GRAS family proteins, which play role in nitrogen utilisation, hormone and red-light response, seed germination and dormancy (Hakoshima, 2018) is located at -1492 bp and -1589 bp upstream from the translation start site of the *StBB* gene. The HB-other and LBD family TFs, KNAT1 and LBD18, both play a role in xylem development. In addition, KNAT1 is important for cell fate specification, while LBD18 is involved in lateral root development (Liebsch et al., 2014; Lee et al., 2009).

The M_type_MADS TFs have four binding sites, while the MIKC_MADS TFs have only two binding sites in the *StBB* promoter as three out of the four predicted sites are overlapping. These TFs belong to the large group of TFs, the MADS-domain family. The MADS-domain proteins are involved in diverse plant developmental processes including embryogenesis, flower development, maintenance of floral organ identity, flowering time, response to cold and gibberellic acid (Theißen et al., 2016; Borner et al., 2000). According to PlantRegMap AGAMOUS like-20 (AGL20s) responds to cold and gibberellic acid and regulates flower development as well. AGAMOUS like-15 (AGL15) is found to be the regulator of somatic embryogenesis and negative regulator of short day photoperiodism, seed maturation, floral organ and fruit abscission. AG15 responds to auxin. AGAMOUS (AG) is involved in leaf development and maintenance of floral organ identity. The binding sites of AGL15 and AG are overlapping and are located at around -2 kb in the *BB* promoter.

Binding sites of MYB and MYB-related family proteins, which regulate various developmental processes and salt and drought stress responses (X. Li et al., 2019), were detected at two different sites in the *StBB* promoter region, at -2278 bp and -1392 bp. The CARE recognised by RAV1, which belongs to RAV TF family, was found at -1024 bp. RAV1 negatively regulates the flower, leaf and root development and responds to brassinosteroids (Hu et al., 2004).

Although WRKY is a large family of TFs it was represented only by WRKY2. The binding site at -2181 bp carries the characteristic GGTCAA motif found also in the WRKY2 binding site of tomato, a relative of potato (PlantRegMap prediction).

Expression of the StBB gene in different organs of S. tuberosum cv. 'Désirée'

Expression of *StBB* in root, stolon, tuber, stem, petiole, source- and sink leaf, petal, sepal and stamen is shown in Figure 2. Expression of *StBB* was detected in all organs tested. The highest level of expression was found in petal followed by the reproductive organ, stamen. *StBB* mRNA level in petal was 7.5-fold higher than in root and stem. Medium level of *StBB* expression was detected in stolon, tuber, source- and sink leaves and in sepal. The lowest *StBB* transcript levels were found in root and stem.

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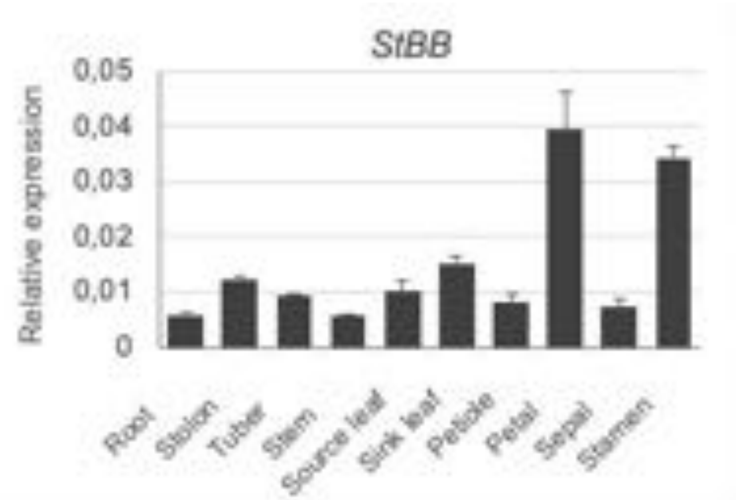

Figure 2: Expression profile of the *StBB* gene in different organs of *S. tuberosum* cv. 'Désirée' determined by RT-qPCR. Bars indicate mean relative expression values of *StBB* gene compared to the mean expression values of *ACTIN* and $EF1\alpha$ + SE ($n = 3$ technical replicates). Samples were collected from organs of 3-5 plants.

Discussion

Analysis of promoters and CAREs is important for genetic engineering of crops. In this study, CAREs in the *StBB* promoter of potato were identified. A 3-kb region upstream from the translation start site was tested *in silico* and 48 TF binding sites of 15 TF families were predicted. We assume that the 3-kb fragment carries all the important regulatory elements. This assumption is based for example, on the publication of Lang et al. (2007), who studied the promoter of the *SBgLR* gene in *S. tuberosum* and showed that a 2.3-kb DNA sequence upstream from ATG contains all regulatory motifs that are likely to be required for the high-level of gene expression, specifically, in pollen. In another study, it was demonstrated that the majority of the discovered common motifs in the promoters of *GLUCAN ENDO-1,3-BETA-GLUCOSIDASE* genes of *S. tuberosum* cv. DM 1-3 516 R44 are concentrated between +1 and -500 bp of the transcription start site (Kebede & Kebede, 2021).

In a previous study, Breuninger and Lenhard (2012) analysed a region located at 1035 bp upstream from the *BB* start codon for identification of upstream regulators that promote or inhibit *BB* expression in *A. thaliana*. Based on a promoter deletion assay by complementing of a *bb* mutant with the *BB* cDNA fused to the *BB* promoter region they found that with the exception of the distal 100 bp the other part of the fragment contains important positively acting promoter elements. Searching for binding sites for TFs in the PLACE database full matches were found to the AUXIN REPONSE FACTOR (ARF) binding site (TGTCTC) and to that of a MYB TF. However, using a luciferase assay it turned out that the ARF is most likely not functional in the *BB* promoter of *Arabidopsis*. Using the PlantRegMap prediction tool, we identified three TFs responding to auxin, namely OBP1, AGL15 and MYBL2. Besides of the MYB-related MYBL2, the MYB TF family protein MYB124 also was predicted to have a binding site in the *StBB* promoter at -2778 bp (Table 2). Nevertheless, additional experiments are needed to decide whether the predicted CAREs are functional or not in the *StBB* promoter.

In the current study, binding sites of 20 TFs involved in developmental processes such as flower, fruit, leaf, stem and root development or cell cycle regulation were predicted to be located in the *StBB* promoter, which is in line with the expression of *StBB* gene in each tested organ, i.e., root, stolon, tuber, stem, source leaf, sink leaf, petiole, petal, sepal and stamen. Disch et al. (2006) also detected *BB* mRNA in all organs with highest amounts in proliferating tissues including shoot, root and floral meristems, vasculature, young organs and developing embryos of *Arabidopsis*. Disch et al. (2006) examined not only the *BB* expression level in *Arabidopsis* but also tested a series of genotypes that expressed increasing amounts of *BB* mRNA from the endogenous promoter ranging from 0% to 600% of the wild-type level and concluded that *BB* is both necessary and sufficient to limit *Arabidopsis* floral organ size, floral biomass accumulation and stem thickness. We detected the highest level of *StBB* expression in petals followed by the reproductive tissue, stamen. Thus, we hypothesise that the function of *StBB* in potato may be similar to that found for *BB* in *Arabidopsis*, i.e., restricting organ overgrowth and especially, the overgrowth of petal and stamen.

On the basis of current research of *in silico* and expression analysis, *StBB* could be a promising target for potato crop improvement as repression of *StBB* may result in accelerated plant growth and early tuber bulking. A future analysis of *StBB* will be required to understand how the level of *StBB* expression is determined and how *StBB* influences organ growth at the molecular level.

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Effect of different irrigation regimes on the early development of pot-grown black locust saplings

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Abstract: Black locust currently is considered to be the most important tree species of short-rotation forests in Hungary with the purpose of either woody biomass or industrial wood. Despite the general supposition on the drought tolerance of the species, water availability seems to be a more limiting factor to exploit the growing potential of highly productive new varieties than nutrient amendments. Preliminary measurements of the current study were made on the connection between the depth of the water-retaining soil layer and the growth of saplings on black locust plantations. A significant negative correlation was found between the depth of the water-retaining layer, the stem diameter and the height of the saplings. To investigate the phenomenon, a model experiment was launched with loamy sand soil in the pots. During six weeks, pots were watered every morning up to the weight referring to the 30, 40, 60 and 80% of field capacity (FC). Our results showed that 30% FC was only sufficient for the survival of the saplings, growth was only noticeable at plants with 40% FC or more. During the first 4 weeks, differences in growth and cumulative evapotranspiration between the 60% and 80% FC treatment were not considerable. However, in the last two weeks, saplings with the highest FC produced substantially higher biomass, resulting in a one-third higher final weight than those of 60% FC. Even with the limited soil capacity of the pots, water use of these saplings of 1 m height and 1 cm stem diameter exceeded 1.5 L per day. Our results confirmed that black locust is a water-intensive species with a high water use potential, which emphasizes the importance of irrigation on nurseries and the first years of plantations.
Keywords: Turbo Obelisk clone, plant development, relative chlorophyll content, plant mass, water use efficiency

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Introduction

Black locust (*Robinia pseudoacacia* L.), belonging to the Fabaceae family, is considered to be one of the most commonly cultivated broad-leaved tree species in the World (Schwärzel et al., 2018). Due to its high adaptability, the species is one of the three most widespread non-native tree species in Europe (Lambdon et al., 2008). Black locust is a widely cultivated tree especially on the Chinese loess Plateau (Jiao et al., 2018), or on the Great Plain in Hungary (Rédei et al., 2008). Currently, the black locust is the most important tree species in Hungary with a total surface of 445 000 hectares, representing 24% of the Hungarian forest area (KSH, n.d.).

Black locust is diversely utilized for building, firewood or as an exceptional honey plant for bees (Kurtz & Hansen, 2017), even successfully applied in the reclamation of lignite opencast areas in Germany (Grünewald et al., 2009). The tree is commonly used in short rotation coppice forests in Hungary and Germany (Grünewald et al., 2009; Rédei & Veperdi, 2009).

A suitable environment, selected varieties and intensive cultivation technology can help to exploit its intensive growing potential and high-quality timber (Keresztesi, 1988; Rédei & Veperdi, 2009). The yield on a biomass energy plantation was mainly affected by soil water availability (Megyes, 2013). Our first observations made on young, fertilized plantations established with high-potential clones seem to confirm the above findings (unpublished observations).

Black locust originates from the humid and sub-humid regions of the Appalachia mountains and the Ozark Plateau with annual rainfall between 800–1800 mm (Gaffin & Hotz, 2000; Kurtz & Hansen, 2017). Therefore, the species is considered to be a water-demanding and drought-sensitive plant (Wu, Huang, & Warrington, 2015; Yan, Yamanaka, Yamamoto, & Du, 2009). Still, black locust is proved to be a highly adaptable species to drought compared to other broad-leaved species by numerous studies (Han & Kakubari, 1995; Li et al., 2018; Rolbiecki et al., 2019; Veste & Kriebitzsch, 2010). The plasticity of the species makes the tree suitable for biomass plantations throughout the arid regions of Central and Eastern Europe (Mantovani et al., 2014b). Black locust either can reduce leaf size and transpiration (Mantovani et al., 2014a) or enhance its nitrogen fixation (Mantovani et al. 2015b) under long-term drought stress, however, the growth rate and biomass production of the trees decline considerably (Mantovani et al., 2013, 2014b; Veste et al., 2013).

Under well-watered conditions, transpiration of the tree increases without an improvement in photosynthetic activity, for this reason, black locust cannot be treated as a water-saving tree species (Mantovani et al., 2011, 2014a). The water use efficiency (WUE) value of the black locust ranges on a wide scale from 0.03 kg m^{-3} in humid to 0.74 kg m^{-3} in semi-arid areas (Hu et al., 2001). Yet, Mantovani et al. (2014b, 2014a, 2015) measured remarkably higher values of 2.3-2.8 kg m^{-3} with three-year-old saplings under arid conditions. Nevertheless, those WUE values of black locust were still markedly lower than the 4-6 kg m^{-3} values of willow and poplar trees, both widely used as biomass producers. Contrary to poplar, precipitation can affect the growth of black locust not only in the storage (Nov–Apr) and the main growth (May–June) period but also in the late growth period (Aug–Nov)(Manninger, 2008). Black locust can exploit the whole water resource of the soil even under humid circumstances (Veste & Kriebitzsch, 2010), resulting in the drying out of plantation soil (Jiao et al., 2019; Y.-L. Wang et al., 2010; Wu et al., 2015).

Saplings are sensitive to spring/summer drought after the establishment of a plantation, so suitable water management is necessary in dry areas (Rolbiecki et al., 2019; Veste & Kriebitzsch, 2010). A former study proved that irrigation in the first year can improve growth by 80%, while biomass increase dropped to 50% by the third year. Addition of 200–500 mm water, representing the half of the in-season precipitation enhanced biomass production by 75%. In the same experiment, the growth-stimulating effect of irrigation and nitrogen fertilization accumulated (Bongarten et al., 1992). Yet, irrigation under humid environment of the Appalachian did not affect plant survival in a two-year-experiment (Brinks et al., 2011). Note that in lower, water-affected spots, 100% plant mortality was registered (Bongarten et al., 1992). Airy environment around the roots is necessary with a soil water level not higher than 150 cm (Megyes, 2013).

Kurtz and Hansen (2017) estimated black locust yearly water demand between 400 and 1650 mm. Daily evapotranspiration (ET) of the species is calculated 3–4 mm for the summer season (L. Wang et al., 2011), confirmed by a Polish value of 3.5 mm. However, due to global climate change, yearly ET increased by 9 mm in a decade during a period of 1980–2010 (Rolbiecki et al., 2019). Above

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the potential evapotranspiration value (PET) of 3.2–4.0 mm, depending on soil type, ET of the black locust would not grow higher (Wu et al., 2015). In Hungary, black locust is generally planted on looser soils. Under optimal water circumstances, ET of black locust was higher and more dependent on weather in sandy soils than on loamy, clayey ones (Wu et al., 2015).

High survival rate and fast early growth of young black locust saplings is essential in intensive short rotation woody plantations. Literature findings and the authors' own observations both confirm that water availability can even have higher impact on biomass production than variety or nutrient content of the soil. Our objective was to run a simple experiment to investigate the connection between soil hydrology parameters and the initial sapling growth in a newly established black locust plantation. Effect of soil water content on early (first 3 months) growth of black locust saplings 'Turbo Obelisk' variety was also investigated in a pot experiment.

Materials and Methods

Examination on the plantation

The investigated plantation of 1.3 ha was planted on the outskirts of Tápiószele (N 47.306484, E 19.885437) on 7 April 2018, for nutrient supplementation experiment and variety comparison. After planting, the seedlings were cut back to trunk. The clones of the fast growing cultivar 'Turbo Obelisk' OBE-01, OBE-34, OBE-53, OBE-69 were planted in 2m x 2m spacing. The area is characterised by an altitude of 95 m above sea level, an average annual air temperature of 10.0–10.3°C and an annual rainfall of 530–550 mm. The area is dry and poorly drained, with groundwater depths of between 2 and 4 m. Soil analysis carried out during the pre-planting survey shows that the soil is sandy, alkaline, high in lime (5–10%) and low in organic matter (less than 1%).

In some parts of the plantation, there were serious differences in size between adjacent seedlings of the same clone and fertilization treatment, which could not be explained by plant health reasons. In mid-April 2020, in five such areas, we made 6 m long and 2 m deep narrow trenches next to 3–3 trees, in which we measured the depth of the aquifer layer under the trunks of the trees to an accuracy of 5 cm. In addition, we measured the stem diameter of the trees above ground with a caliper to the nearest millimetre and the height of the trees with a tape measure to the nearest 10 cm. The 15 trees measured were clones OBE-01 (3), OBE-34 (3) and OBE-53 (9). Correlation analyses between aquifer layer depth and plant characteristics were performed to find correlations, without separating data from different clones during this data analysis.

Container experiment

The containerized water supply experiment was conducted at the Department of Horticulture, Szent István University, Gödöllő-Szárítópuszta, in an unheated, plastic covered multi span greenhouse equipped with automatic roof ventilation. The temperature was recorded every half hour with a thermometer (TR 71-S, T & D Corporation, Japan) placed between the containers. The data showed that the average air temperature during the experiment was 26.4°C, which is about the same as the temperature on a hot summer day in the Hungarian Great Plain, a typical area of black locust plantations. Irradiance data were obtained from the Priva-type climate computer of a glasshouse, located 20 m from the experiment site. The daily average solar irradiance was 269 W m^{-2}.

For the experiment, micropropagated clone of the very intensive growing cultivar Turbo Obelisk, OBE-01 was used. The small plants, initially grown in $25 \times 25 \times 40$ mm rockwool starter plugs, were transplanted into 12 cm diameter polypropylene pots on 8 June 2020. The pots were filled with the same soil - fertilizer mixture that was later used in the container phase. We used 370 g of dry soil per pot. The seedlings were grown in the pots until 30 June, after which 40 seedlings of average height were selected and transplanted into pots for the experiment. The soil used for the experiment was collected from a loamy sand top soil layer of one of the farm's fields. The laboratory analysis showed that the soil was slightly alkaline (pH_{KCl} = 7.4), with low humus (0.83%), salt (<0.02%) and lime (<0.2%) content. Although the soil contained moderate amounts of nutrients, to ensure that nutrient levels, especially nitrogen, did not limit seedling growth, a controlled release Osmocote fertilizer (8–9 months 12:11:17 + microelements) was mixed into the soil. The dose applied was 2.5 kg m^{-3}, which is the same rate used for black locust seedlings in the nurseries. The field capacity of the soil was measured as 33.2 % gravimetric water content.

The soil to be used for the experiment was air-dried, crushed to pass through a 2-mm sieve, mixed with the fertilizer, and then poured into 12 L polypropylene containers. The amount of soil loaded in one container corresponded to 11.28 kg of soil with 0% water capacity, dried at 105 °C to constant weight. We planted a seedling in the centre of each container, then watered each with 0.5 litres of water to ensure safe rooting.

Treatment started 3 days after the transplant. Four water availability levels were applied, maximum 30, 40, 60 and 80% of the field capacity (FC), corresponding to successively 10.0, 13.3, 20.0 and 26.6 wt% water content. 10–10 containers of each treatment were set up. Based on our preliminary studies, using a baseline water capacity level below 30% would no longer have ensured the safe survival of the seedlings. We achieved the specified water capacity levels in the experiment by measuring the weight of each container on a digital balance to the nearest 10 gram from 8:00 to 9:00 every morning from July 3 until the end of the experiment, and then watering to the weight required to achieve the specified water capacity level. The required amount of water was slowly poured onto the surface of the soil, allowing time for infiltration without the water staying on the surface. The soil surface was regularly loosened to avoid soil compaction. The daily ET rate was calculated based on the weight measurements, then daily values were summed up. Due to the continuous weight gain of the growing black locust seedlings, a weight correction was applied to the measurements at increasing rates throughout the experiment. At the very end of the experiment, the rates of correction in the 30, 40, 60 and 80% water capacity treatments were 10, 50, 150 and 200 g, respectively.

From planting to the end of the experiment, the stem diameter, height and relative chlorophyll content of the leaves of each seedling were measured approximately every two weeks (0, 16, 30, 43 days after planting on 30 June, 16 July, 30 July and 13 August 2020). Stem diameters were measured with a digital caliper, to the nearest tenth of a millimetre, perpendicular to the stem axis, 3–4 cm above the soil surface. Plant height was measured from the soil surface to the growing tip using a measuring rod, with an accuracy of centimetres. The relative chlorophyll content of the leaves was measured to the nearest tenth of a SPAD value using a Minolta SPAD 502. The measurement was performed on the middle leaflets of the youngest mature leaves, 4 leaves per plant.

At the end of the experiment (14 August 2020), the fresh and dry weight and dry matter content of the above-ground parts of the plants were also determined. The fresh plant mass was obtained by cutting the stems at ground level two hours after morning watering and immediately measuring their mass on a digital precision balance, to the nearest

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hundredth of a gram. The cut stem sections were then dried in a drying oven at 70°C until constant weight was reached. The dry mass was also measured to the nearest hundredth of a gram. The dry matter content was calculated as the ratio of fresh to dry weight, expressed as a percentage.

Statistical evaluation of the data was carried out using a one-way analysis of variance to examine the effect of water supply level on each of the characteristics examined. For mean separation of the treatment averages statistically, the Fisher's least significant difference test was used as a post-hoc test with a 5% level of significance (LSD5%).

Results and discussion

Results of the plantation survey

The results of the survey confirmed the exceptional growth vigour of the 'Turbo Obelisk' clones, as they were able to reach trunk diameters of over 40 mm and heights of up to 5 m two years after pruning back to trunk. While the least developed specimens were around 2.5 m tall and 10–15 mm stem diameter. The depth of aquifer layer in the trenches varied between 40 and 130 cm (Figure 1).

Despite the fact that data from individuals of the three different clones included in the study were analysed together, a significant negative correlation between aquifer depth and tree size was found. The correlation was slightly stronger for plant height than for trunk diameter (Figure 1). Thus, during the first two years, the depth of the aquifer layer beneath a given plant was a major determinant of its development. Differences of up to 100% in stem thickness and height could occur between neighbouring individuals. Where the plantation had a loamy aquifer in the sandy soil at a depth shallower than 80 cm, a markedly better growth was observed due to better water supply. This result is consistent with literature claims that black locust is a water-demanding tree species (Kurtz & Hansen, 2017; Wu et al., 2015; Yan et al., 2009).

Results of the container experiment

Trends in water consumption

The daily weight loss of the containers consisted of two factors, evaporation of the soil surface and transpiration of the seedlings. In the treatments with higher FC, evaporative water loss was higher due to a more wet soil surface. This is evidenced by the results of the first days, when there was no significant difference in plant size and thus presumably transpiration between treatments, yet the daily water consumption of the 60 and 80% treatments (0.35–0.50 L per container) was much higher than that of the 30 and 40% treatments (0.08–0.25 L per container).

In the 30% maximum FC treatment, the daily ET was very low throughout the experiment, between 0.08 and 0.30 L, and did not increase much with the progress of the experiment. Accordingly, the cumulative ET of this treatment showed a linear trend (Figure 2). The tendency was similar for the 40% treatment, except that the average daily ET value over the whole period was 50% higher (0.25 L) than for the 30% treatment (0.16 L). Daily ET values in the 40% treatment had a wider range, with values between 0.12 and 0.39 L. By the end of the experimental period, the cumulative ET per container for the 30% treatment was 6.61 L and for the 40% treatment 10.41 L. This difference between the two treatments increased steadily throughout the experimental period (Figure 2).

During the first four weeks of the experiment, the daily ET per container of the 60% and 80% treatments varied between 0.34 and 0.44 L and did not show a large increase during this period. The cumulative water consumption of the two treatments was then still completely the same (Figure 2). We attribute this to the fact that, based on the results of

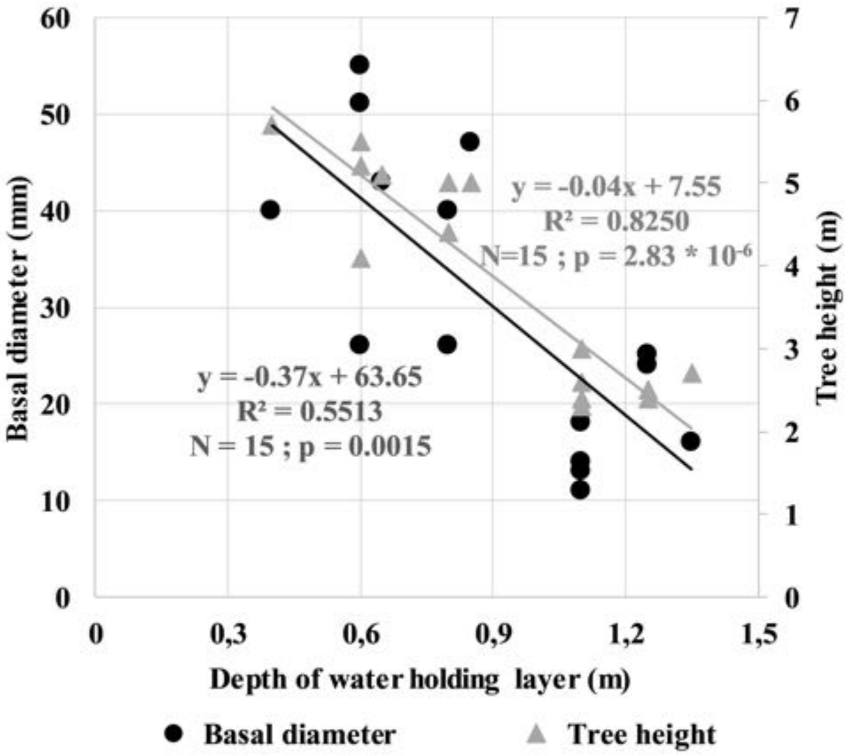

Figure 1: Correlation between aquifer level depth and trunk diameter and height of 'Turbo Obelisk' black locust trees next to the sample trenches in a plantation, two years after planting (Tápiószele, 2020)

Wu et al. (2015), transpiration of black locust seedlings in sandy soils above 60% water capacity is no longer limited by soil water content. It is a well-known phenomenon that transpiration of plants only starts to decline below a critical soil water capacity level (Sadras & Milroy, 1996). In the first month of the study, there was not yet a difference in seedling size and thus canopy area between 60 and 80% treatments that would have created a significant difference in ET. During this period, the cumulative ET of these two treatments also followed a linear trend (Figure 2).

However, during the last two weeks of the experiment, in the 60 and 80% treatments, seedling growth accelerated greatly, with increasing canopy area and a large increase in transpiration water loss. The daily ET values increased dramatically, always being above 0.7 L, and in the 80% treatment they even exceeded 1.5 L per day on several occasions at the end of the experiment. This also meant that the 80% field water capacity value achieved in these containers at morning irrigation had dropped to around 30–40% after 24 h. This justified ending the experiment just six weeks after it started. During these final two weeks, a significant difference in ET between the 60 and 80% treatments had already developed. A similar trend was observed by Mantovani et al. (2011). In their lizymetric experiment, the cumulative water consumption of the low water supply (35% FC) black locust seedlings was already at the very beginning largely below that of the medium and high water supply treatments, but only after a month did a difference start to emerge between the latter two. In our study, by the end of the experimental period, the cumulative ET was 23.88 L, or 16% less in the 60% FC treatment than that of the 28.44 L of

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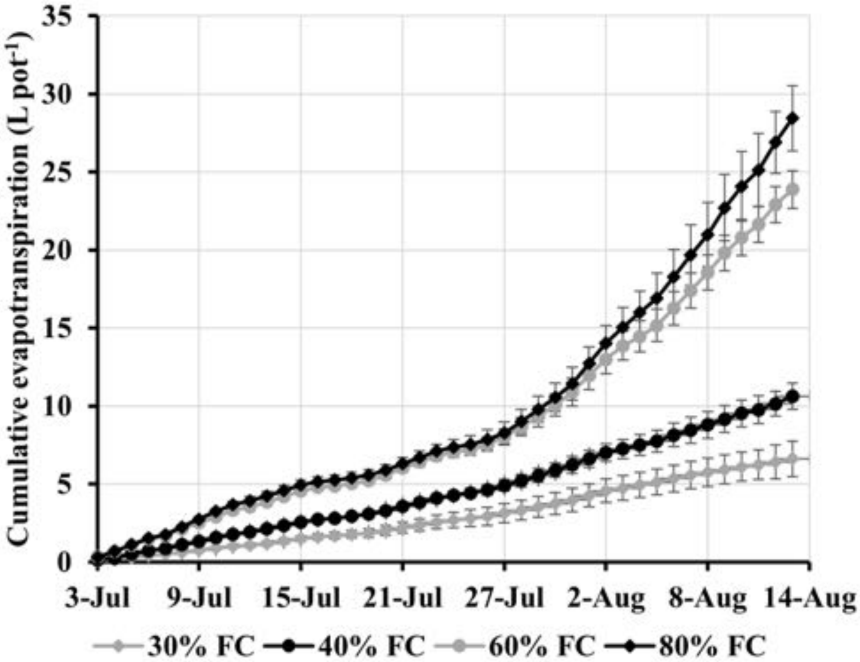

Figure 2: Evolution of the summed evapotranspiration values of containerized 'Turbo Obelisk' black locust seedlings under different maximum water capacity rate treatments (FC = Field Capacity)

the 80 % FC treatment. The water consumption of the 40% treatment (10.41 L) was 63% lower than that of the 80% treatment, almost identical to the 61% difference between transpiration of well and scarcely watered black locust seedlings recorded in the experiment of Mantovani et al. (2013).

Plant height and stem diameter

At the end of the potted growing period, at the first measurement after transplanting, there was no significant difference in the mean height of the seedlings. After almost two weeks, at the 2nd measurement (16 July 2018), the effect of improved water supply was already evident, despite the slow initial development. The plants in the 60% FC and 80% FC treatments were significantly taller than the seedlings in the two lower FC treatments (Table 1). By the time of the third measurement, one month after the start of the treatment, there was also a significant difference in plant height between the 30 and 40% FC treatments. Plants in the 30% FC treatment grew significantly slower and overall grew only 7 cm taller during the study. In contrast to the 30% FC treatment, the 40% water capacity was already a high enough water supply level for the seedlings to not only survive but also grow significantly. From mid-July onwards, their height growth accelerated and by the end of the experiment they had reached an average height of nearly 60 cm. There was no significant difference in height between plants in the two higher water treatments in any of the measurements (Table 1). In these two treatments, seedlings that were initially 12 cm tall grew to an average height of 1 m during the month and a half of the experiment.

Based on measurements taken immediately after planting, there were statistically significant differences in stem diameter between treatment averages, but these were small and did not affect the final results of the experiment. As in the height measure-

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Table 1: Effect of soil water content on certain growth characteristics of 'Turbo Obelisk' black locust seedlings (mean ± SD)

Plant height (cm)	30.06.	16.07.	30.07.	13.08.
30% FC	14 ± 1	16 ± 2[b1]	19 ± 3[c]	21 ± 6[c]
40% FC	12 ± 2	17 ± 4[b]	34 ± 5[b]	58 ± 5[b]
60% FC	12 ± 3	24 ± 4[a]	60 ± 7[a]	100 ± 5[a]
80% FC	12 ± 2	26 ± 6[a]	61 ± 7[a]	105 ± 5[a]
P-value	0.0687	1.28×10^{-5}	7.79×10^{-13}	5.34×10^{-24}
LSD5%	N.S.	4.2	7.4	5.3
Basal diameter (mm)	**30.06.**	**16.07.**	**30.07.**	**13.08.**
30% FC	2.2 ± 0.4[a]	2.4 ± 0.2[c]	2.8 ± 0.3[c]	3.4 ± 0.5[d]
40% FC	2.0 ± 0.1[ab]	2.9 ± 0.4[b]	3.9 ± 0.5[b]	5.9 ± 0.5[c]
60% FC	2.1 ± 0.2[a]	3.3 ± 0.3[b]	6.0 ± 0.6[a]	9.7 ± 0.9[b]
80% FC	1.8 ± 0.0[b]	3.7 ± 0.4[a]	6.6 ± 0.8[a]	11.0 ± 0.9[a]
P-value	0.0334	1.52×10^{-5}	1.07×10^{-10}	1.11×10^{-18}
LSD5%	0.2	0.4	0.8	0.7
Relative chlorophyll content (SPAD value)	**30.06.**	**16.07.**	**30.07.**	**13.08.**
30% FC	33.3 ± 2.7	36.8 ± 3.8	34.8 ± 2.8	39.0 ± 2.0[b]
40% FC	31.4 ± 3.7	39.1 ± 4.3	37.3 ± 3.4	41.6 ± 2.1[a]
60% FC	34.5 ± 2.0	38.8 ± 4.9	33.8 ± 1.6	40.1 ± 1.3[ab]
80% FC	31.5 ± 3.6	37.3 ± 4.7	34.4 ± 3.2	38.2 ± 3.3[b]
P-value	0.1579	0.6750	0.0876	0.0316
LSD5%	N.S.	N.S.	N.S.	2.3

[1]Average values of the same parameter, date and letter mean no significant differences at 95% probability based on Fischer's least significant difference test

ment, the effect of different watering levels was already evident in the second measurement two weeks later. Significantly the largest stem diameter was obtained in the 80% FC treatment and the smallest in the 30% FC treatment, while no significant difference in stem diameter was observed between the 40% FC and 60% FC treatment seedlings (Table 1). Thus, the developmental difference between the 30% FC and 40% FC seedlings based on stem diameter was already apparent at this time, earlier than based on height. At the third measurement, the difference between the mean stem diameter of 80% FC and 60% FC increased further in numerical terms, but due to the larger deviations, this difference did not prove to be significant at this time. Similar to the trend in the height data, the development of the 30% FC and 40% FC seedlings was increasingly lagging behind the two treatments with higher water availability and the difference between the two was also increasing in favour of the 40% FC. At the end of the experiment, the four treatments became statistically completely distinct, with each treatment having a significantly different stem diameter from the other ones. The average stem diameter of the 80% FC seedlings exceeded 1 cm, and the average of the 60% FC was close to this value (Table 1). In the 40% FC treatment, the average stem diameter was 6 mm, which was the same level reached by plants in the two better water supply levels

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two weeks earlier. In the 30% FC treatment, stem diameter increased to only one and a half times during the month and a half of the study, but for this parameter a greater improvement was observed in this treatment than for height.

Relative chlorophyll content

The relative chlorophyll content results did not show as clear a trend as for plant height and stem diameter. The order of treatments differed between measurement times and significant differences were only found for the last measurement time (Table 1). Even then, there was only a verifiable difference between the highest SPAD mean of 40% FC and the mean values of 30% FC and 80% FC (Table 1). All containers were supplied with the same amount of nutrients, including nitrogen. Relative chlorophyll content is positively correlated with nitrogen supply (Li et al., 2018) and negatively correlated with foliage volume at the same nitrogen dose. Improved water supply can promote more efficient nitrogen uptake and stimulate better vegetative growth and canopy development. In turn, more intensive growth requires more nitrogen and consequently reduces the amount of nitrogen available for uptake in the soil. As a result of these relationships, the relative chlorophyll content was highest in the 40% FC treatment and lowest in the 80% FC treatment.

Plant mass

During the experiment, we were able to monitor the development of the black locust seedlings by measuring height and stem diameter, but the most reliable way to characterise the degree of development of the vegetative parts is by the production of plant mass. In our experiment, different water availability levels had a strong influence on plant mass production. There was a complete statistical separation between the treatment averages for both fresh and dry mass, with the average value of each treatment being significantly different from all others (Table 2). The results showed a clear trend, the higher the water availability rate, the higher the plant mass. Plant weight showed a strong positive correlation with the amount of water used in the experiment (Figure 3), in agreement with the results of Mantovani and coworkers (2013, 2015a). The water demand of black locust is clearly shown by the fact that the fresh and dry plant mass was one third higher in the 80% FC treatment than in the 60% FC treatment. The difference between the plants in the two treatments was not in height but in stem diameter (Table 1) and lateral shoot development. Seedlings in the 80% FC treatment increased their fresh above-ground plant weight from 1-2 grams to 190 grams over a period of one and a half months. However, in terms of mass production in grams, the largest jump was observed between the 40% FC and 60% FC treatments, and in terms of proportions between the 30% FC and 40% FC treatments, where plant mass increased more than sixfold with only a 10% increase in water capacity (Table 2). The above ground dry matter content of young, leafy seedlings ranged between 27 and 33%. As expected, the results were significantly higher in the low water treatments with 30 and 40% FC than in the 60 and 80% FC treatments.

WUE was calculated based on the above mentioned plant mass and the cumulative ET data. On fresh weight basis WUE was 0.81, 3.11, 6.14 and 6.93, while on dry weight basis it was 0.26, 0.93, 1.70 and 1.87 kg m^{-3} for the 30, 40, 60 and 80% FC treatments, respectively. With the exception of the 30% FC treatment these values can be considered high. This is probably mainly due to the very young age of the saplings and the protected conditions.

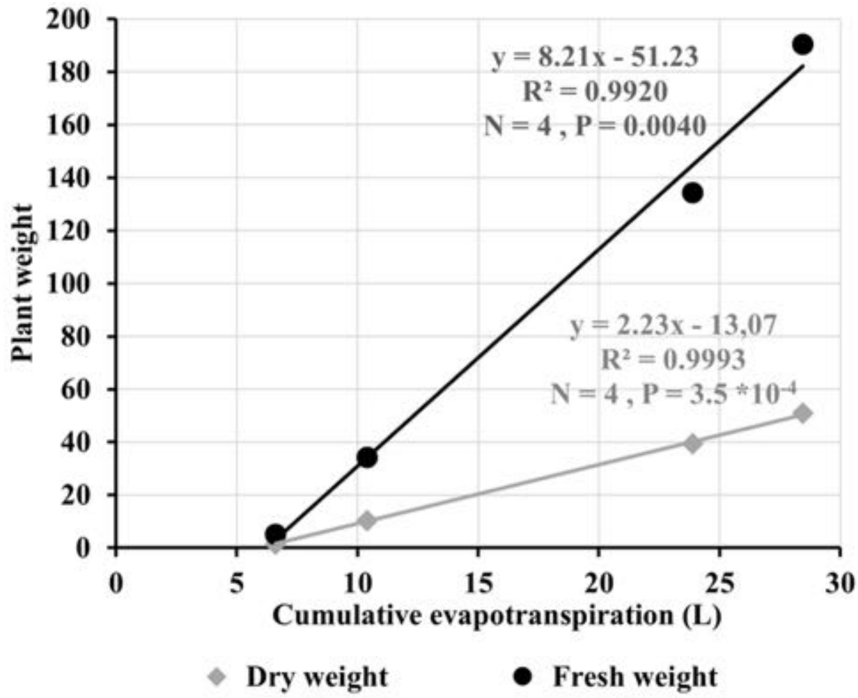

Figure 3: Correlation between evapotranspiration and the above-ground plant mass of the containerized 'Turbo Obelisk' black locust seedlings summed over the duration of the experiment

Table 2: Effect of soil water content on weight of 'Turbo Obelisk' black locust seedlings (mean ± SD)

	fresh weight (g)	dry weight (g)	dry matter content (%)
30% FC	5.2 ± 3.3^{d1}	1.6 ± 1.0^{d}	33.0 ± 2.3^{a}
40% FC	34.2 ± 4.5^{c}	10.4 ± 1.6^{c}	30.5 ± 1.3^{b}
60% FC	143.4 ± 19.3^{b}	39.3 ± 6.9^{b}	27.2 ± 1.4^{c}
80% FC	190.4 ± 19.4^{a}	50.9 ± 7.5^{a}	26.6 ± 1.4^{c}
P-value	1.01×10^{-21}	7.84×10^{-18}	6.88×10^{-6}
LSD5%	14.3	5.3	2.2

[1]Average values of the same parameter, date and letter mean no significant differences at 95% probability based on Fischer's least significant difference test

Conclusions

Both our plantation survey and our container experiment showed that soil water availability has a strong influence on the initial growth of black locust seedlings and saplings. It is therefore advisable to pay close attention to the hydrological conditions and the water holding capacity of the soil when selecting the area for planting. In areas with less favourable conditions, irrigation may be considered after economic calculations. It would be advisable to carry out irrigation experiments in plantations to find out the level of water supply which will still result in a

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satisfactory growth rate without adversely affecting the wind and winter hardiness of the seedlings and the quality of the wood product.

In nursery production. irrigation is essential during drier summers. Our results show that in loamy sandy soils, a soil water capacity level of 30% was only sufficient for the survival of the trees, not for the initiation of growth. For this, a water capacity level of at least 40% is required. During the first month, it is enough to maintain a soil water content of 60% to ensure a sufficient growth rate. After that, however, it is advisable to maintain an even higher water capacity level of 80% to better exploit the growth potential of fast-growing varieties. At the end of our study, the daily water consumption of the 1 m tall and 1 cm stem diameter black locust seedlings reached one and a half litres even with the limited soil volume of the containers. It can be assumed that in a nursery or plantation the daily water consumption of plants of similar size would exceed even this level.

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MAGYAR AGRÁR- ÉS ÉLETTUDOMÁNYI EGYETEM

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GIS in the service of plant breeding in Karcag

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Abstract: Plant varieties bred by the Hungarian Breeding Institutes in different agro-ecological conditions can bear the unfavourable factors of the regions with greater tolerance, so they provide advantages and yield stability for the farmers who choose from these varieties. Farmers can contribute to the genetic potential of the planted seeds with the applied cultivation technology. The stable genetical background (the high quality pre-basic, basic and certified seeds) is provided by plant breeding to the farmers. Breeding is a long and tiring task: the classical breeding process, which usually takes 8–10 years, starts with selecting variety assignments and its growing. Finally new, stable varieties are produced which can provide balanced, high yield and also have good or significant qualitative features among extreme conditions. They can bear the unfavourable conditions of the region with greater tolerance, so provide significant yield stability for the farmers. Space technology supported IT solutions (remote sensing, precision farming and soil-friendly agro-technics) has been introduced into plant breeding methods in Karcag, which greatly support the aims of breeding. The main goal is to provide harmonical growing of the nursery, the large punctuality and to decrease the number and cost of agricultural operations. In this study, the new methods and technologies applied in plant breeding in Karcag are introduced.
Keywords: site-specific plant breeding, GIS methods, remote sensing, precision farming (PF), climate change
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Introduction

The aim of plant breeding is to create new crop varieties which have bigger productivity, increased disease-resistance and provide safe biological base for crop production (Braun et al., 1997). Breeding varieties which can adapt to the local agroecological conditions means the smallest environmental load, the land variety/race can be grown economically and with large stability. Breeding the land varieties which belong to a special landscape also contributes to the environmental sustainability. The comparative advantage of breeding and producing Hungarian wheat is that the growing conditions are appropriate for reaching high yields and good quality (Bedő & Láng, 2019). Globally, sustainable intensification is expected to spread, which reduces GGE[1] with the specific yield-growing because it contributes to the increase of biodiversity on the cultivated area (Popp, 2020). In the decades of climatic change, only the varieties with large plastic-

[1]GGE: Greenhouse Gas Emission

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ity, high abiotic and biotic stress-resistance are perspective, so breeding such varieties is a big challenge of our present and must be the long-term aim of breeders (Á. Czimbalmos, 2016). The status of the Hungarian plant breeding and seed production has significantly changed in the last three decades. The seed production area was 313 thousand hectars in 1980, this decreased under 150 thousand hectars by the turn of the Millenium (Bedő, 2004). Although the area decreased, it is good that the share of the certified seed slightly increased, which indicates the quality improve of seed production and propagation. Preserving and improving seed sector is a national interest, it deserves high attention. The interoperability of the markets has extended the opportunities of this sector, but the competition has also grown. The multinational companies with their breeding farms and seed factories are strongly presented in our country. In this taut market situation only those Hungarian breeding houses and seed producers can remain on their feet which can use their comparative advantages well in the production of new varieties. The 21st century has also brought economic seed production and genetically modified plant varieties besides the traditional seed production. From these three tendencies, the genetically modified plant varieties is forbidden by moratorium in Hungary. This situation should be preserved for as long time as possible because the multinational companies can beat the Hungarian plant breeding and seed production industry with their GMO[2] maize and crop hibrides within some years (Heszky, 2008). If the living moratorium ends, only in the seed market of hibrid maize the sector can expect the loss of 300 billion forints (Balla, 2010). What are the comparative advantages which we must build upon? At first, the favourable ecological conditions, the land varieties, the Hungarian experiences and knowledge of breeders and farmers gained over centuries. There are also the latest production systems, geostatistics and GIS applications (Mesterházi, 2018) and Precision Farming (later PF!) and technologies in connection with this new tillage systems which preserve soil moisture (Berényi & Tánczné Óvári, 2018) and decrease drought damage (R. Czimbalmos, 2017; R. Czimbalmos & Kovács, 2017). Agriculture sector agrees that PF became unavoidable in plant breeding and plant production (Takácsné, 2015). The effect of climate change is more and more measurable for decades in our country too. Where does this lead to? The productivity of the soil injures (Stefanovics et al., 1999), our soil is eroded, wind storms erode the surface soil, the organic compounds of the soil decrease (Mátai, 2016). Besides fencing the negative effects of climate change, the role of precision methods, engineering solutions based on computers, sensors and drones will further increase (Szellő, 2018). Based on the satellite signals we can conclude the health condition of the plants from the changing chlorophyll content (NDVI[3]). From the satellite records after adequate transformations the expected product can be evaluated very punctually (Harangi, 2017). We must maximize the yield along the „golden rule"[4] 40–30–30 because the genetic potential of the valuable seed of a breeding variety can be approached only by this method. A breeding institute must also apply the GIS inventions from the last two decades for the complex breeding process (producing

[2]GMO: Genetically Modified Organisms

[3]In remote sensing the Normalised Vegetation Index (NDVI - Normalised Difference Vegetation Index) is a first generation index, shows the photosynthetically active vegetation. For spectral analyse of vegetation between 400 and 700 nm of the visible light and 700–1300 nm of infrared light are the most suitable because of the tissue features of the plants.

[4]The yields of a crop-growing farm are determined 40% by the ecological conditions, 30% by the genetic background of the seed used, and 30% by the applied agrotechnics.

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core material, variety-preserving, breeding, tillage and harvest, properly handling and storing of the core material and the candidate varieties, multiplication of core mixture, pre-basic and basic seed). A production supported by PF technology can lead to give 7-10% additional yield, and the properly chosen variety of seed can still bring additional 25-30% yield (Csurja, Zs., 2020). The proper expertise, knowledge is essential, the complex database of all the breeding varieties must be created, because breeding and cultivation success can depends on the skilled or unskilled humanside (Gönczi, n.d.). Despite the fact that for example in Jász-Nagykun-Szolnok County over one-fourth of the farmers have the workmachines and tractors for the introduction and application of PF and mulch cultivation method, they do not use these systematically (R. Czimbalmos & Kovács, 2017). Our aim is to introduce the primary and secondary data sources, GIS applications which we use and largely contribute to plant breeding, regarding the process of production of pre-basic and basic seed materials. Although the technical – software and hardware – background has been intensively developed for two decades, their explosive spread in daily implementation has not happened yet. The narrow section is not the entry value of these systems, but the lack of proper knowledge and targeted national and EU supports (Takácsné, 2015).

Materials and Methods

The nursery gardens of the plant breeding and variety maintenance department in MATE (Hungarian University of Agriculture and Life Sciences) Karcag Research Institute are found in the most extreme region of the Great Hungarian Plain, with highly dry climate, where the average yearly amount of precipitation is 400–550 mm. The maintenance and breeding of winter wheat, winter barley, winter triticale and alternative plants, production of core mixtures and seed with high productivity take place on the better quality plots (B-1, B-2, I-2, G-2, G-3 and G-4) of the institute, on solonetz meadow chernozem soil (Figure 1 and Figure 2). The area demand of breeding is about 10–12 hectars, beacuse of the isolation distance sorghum plants require larger area every year. The area demand of pre-basic, basic, and certified seed is bigger, between 5–30 hectars. Data collections took place between 2013–2016 (NDVI index) and 2018–2021. The primary aim of breeding is to achieve excellent adaptibility and favourable content parameters (high protein content, favourable amino acid- composition), untimeliness and an excellent drought- and freeze-resistance. Our breeding program is a pedigree selection based on ear selection.

The breeding aim is to create early, high yielded varieties with good stem strength and resistance to unfavourable climatical and soil conditions. With the use of excellent raw materials we create tribes which meet the requirements of the farmers (Á. Czimbalmos et al., 2019). In Karcag breeding the variety sortiment (14 species, 38 variety) is managed with traditional methods, modest area and human resources. High importance of good agrotechnique is given and it is also important to use the scarce resources as efficiently as possible. This is helped by besides breeding and the Accredited Laboratory of the Institute the special agrotechnique of seed production, and by GIS (remote sensing, PF) tools built up at the University of Debrecen. GIS applications and RTK-controlled tractors are the machine backgound which is suitable for GIS software in the office (ArcGIS 9.2, WayQuest, Digiterra Explorer v4, GoogleMaps), on the field, (Thales MobileMapper, Trimble RTK) and in the cultivation system without rotation in the Institute (R. Czimbalmos et al., 2017).

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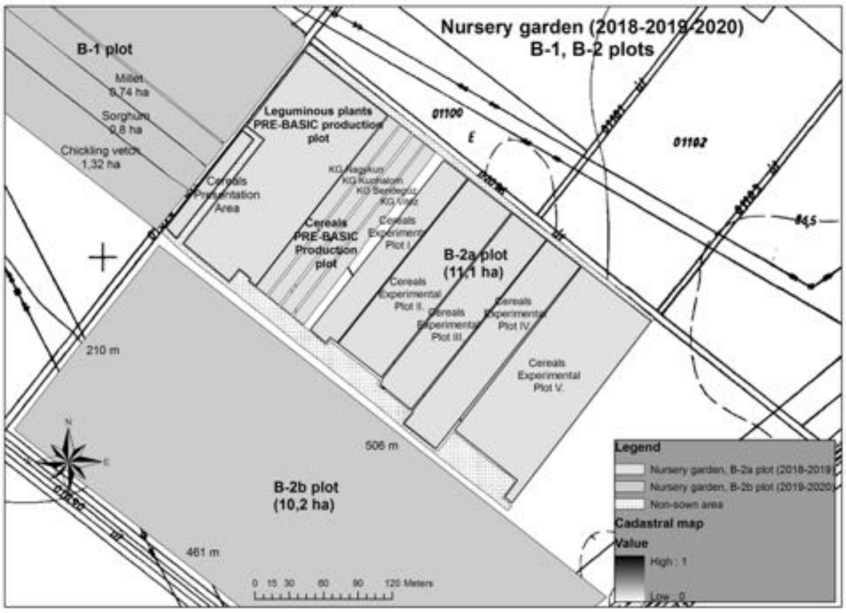

Figure 1: Nursery gardens in B-1, B-2 plots, 2018-2019-2020 (Source: own editing)

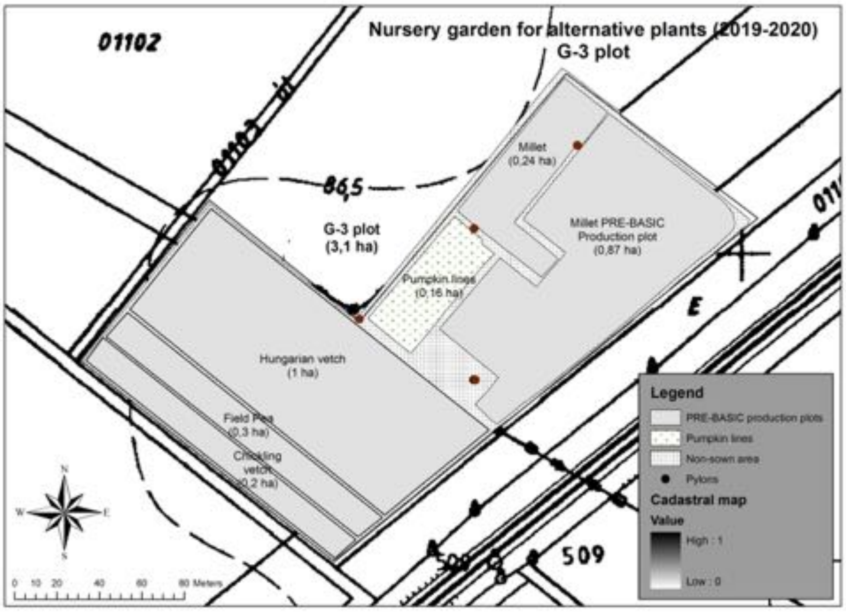

Figure 2: Nursery garden in G-3 plot, 2019/2021 (Source: own editing)

Results

PF applies GIS, geostatistical and remote sensing innovative results in an adaptive way. The results of the research have been applied at operating level and appeared in breeding: very punctual (Differential GPS) soil sample taking, examinations, then the designed differentiated nutrient replenishment plan, within the plot heterogen periodical deep-cultivation, sowing supported with RTK, plant care, cultivation system without rotation (mulch cultivation). The paradigm shift of plant growing – cultivation technol-

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ogy change, digitalisation and PF– was not unexpected for GIS research of the Institute. Only with this complex technology the need of fuel, fertilizer, seed and live labor use for area unit can be decreased without yield and quality loss. Breeding starts with choosing the perfect nursery garden, then the demanding soil preparation considering the suitable isolation distances and pre-crops. During data collection and data processing we use the *primary* (own measurements, that are more expensive) and the much cheaper, *secondary sources* (GIS databases, satellite records, attributive databases). During design of nursery garden the field measures, measurement of parcels with traditional method are time-consuming besides local knowledge and observing professional aspects.

- This is specified – within *the primary data collection* – by the high-punctual hand GPS devices, which can do the following exercises: measure regular and irregular poligons, fixing corner points, measure field barriers, point-like establishments, control length- and area measures, survey of areas exposed by vis maior events, designing and fixing soil sample taking points, soil sample taking, making nutrient supply and other thematic maps, navigation onto specified points, yearly area measures etc. After submetre-punctual field measure, making the soil plans of the nursery gardens happens in the office with the use of field data with centimetre accuracy. At first, we make a digital sketch of the nursery gardens of all the species and varieties with field barriers, borders, cultivation roads and high-voltage columns. The cadastral map give the basic to create the map of the agricultural parcels, on which we overlay our own new thematic maps. Using ArcGIS, we edit the actual nursery garden with the correct size for the given year, the new thematic map (in this study the map and attributive database of the 2019/2020 and 2020/2021 growing season). After the official planning the thematic map is the raw material for the scientists and assistants, who measure the parcels on the field and do the breeding tasks. After the necessary soil tillage operation winter species are sowed with a special small parcel, computer-controlled, high-accuracy, self-propelled seeding machine. In the phenophase the corner points and poligons of the parcels are re-fixed with GPS, edited and we get the final thematic map databases of the nursery gardens (Figure 1). Here parcels, rotation areas, not cultivated areas, and the parcels of summer species, the poligons of the new experiments with necessary basic information (name, measure, treatments, databases of photos and videos, the primary data sources) appear in detailed and cm-punctual. The autumn and spring soil cultivation, soil preparation minimized turns of machinery use, according to the protocol of cultivation system without rotation (mulch cultivation).
- Within *the secondary data collection* in late-spring early-summer when the vegetative mass production are in the most intesive phase, we can start NDVI examination on the plots with LANDSAT satellite images. The aim of image analysis is to evaluate the amount of yield and the loss of yield. Rouse et al. (1973) developed an index, which characterizes the biophysical condition of plant cover (Gulácsi – Kovács 2015). NDVI correlates with the specific chlorophyll content of the plant cover on the area (Mika et al. 2011). The higher value of the index

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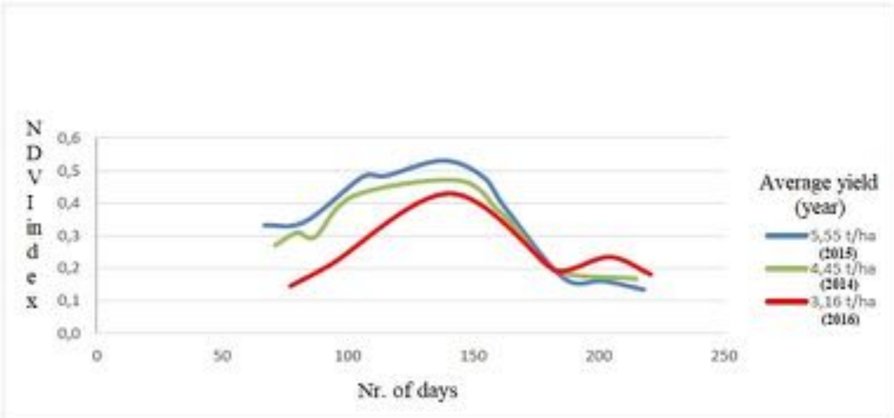

Figure 3: The correlation between different average wheat yields and yearly NDVI (Source: Harangi)

shows the higher vitality and photosynthetic capacity of the leaves, so the healthy, larger biomass vegetation has larger ratio between the near infrared and red reflectancy values than the smaller biomass vegetation (Burai – Tamás 2005). Accordingly this vegetation index can be used well for biomass examinations. This kind of research of our Institute – with the instructors of University of Debrecen – began in 2016 and mainly concentrated to the NDVI examination.

After processing wheat yield data between 2013-2016, the annual meteorological data, the processed satellite records, according to a method in a thesis (Harangi, 2017) there are the following results: we can follow the lifecycle of plants from stem grow to harvest with derived reflectance curves of NDVI values. The highest values of the wheat curves indicate the start of the total ripening phase, when plants have the highest water content. At this point the expected yield can be concluded. Decrease of the NDVI values on the day 150 of the year (the end of May – the beginning of June) shows the gradual reduction of the chlorophyll content, when the plant loses its moisture content. The curves are falling until the harvest, then after the harvest the different curves show similar values with some smaller differences. In the compared period the average yields of wheat varieties of our Institute indicate similarity to the measured NDVI values (Figure 3).

Discussion

During the classical breeding methods with the applied GIS methods, soil-friendly-, moisture retaining cultivation methods without rotation, plant breeding is done in better quality, with less human source and lower cost. Our conclusions:

- Besides good genetical seeds Precision Farming can increase the yield, crop safety, decrease producing costs and contribute to the long-term sustainable farming.
- The primary – more expensive, bigger human source-need – data collection methods are unavoidable, but these must be completed with secondary data collection methods.
- One of the secondary methods is to use satellite images for evaluating yield: before harvest the expected average yield can be evaluated with the unique NDVI reflectance curve which is specific for the plant species. The condition of the evaluation punctuality s

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to have weather data on the day of the orthoimagery. On the studied field the weeds and the amount of the precipitation can influence the reflectance curves of evaluation. Since the reflectance curves of the different plants are different, also the curves of the wheat and maize, besides the health condition of the plants we can determine what kind of plant can be found on the field.

- Within our class more data is needed for improving the reliability of GIS system, for doing more punctual measures, the sources can be provided by the secondary data collection methods. The Landsat system measures every eight days on the same area, and recordings of the Sentinel-2 system can be included.
- The right GIS human background with professional knowledge is needed for creating and operating GIS system, processing geometrical and attributive databases.

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Source of the graphics

Front cover:
Gallo-Roman harvesting machine, called Vallus. Source: U. Troitzsch - W. Weber (1987): Die Technik : Von den Anfangen bis zur Gegenwart

Rear cover:
Portrait of Columella, in Jean de Tournes, Insignium aliquot virorum icones. Lugduni: Apud Ioan. Tornaesium 1559. Centre d'Études Supérieures de la Renaissance - Tours

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Lucius Junius Moderatus Columella

(AD 4 – 70) is the most important writer on agriculture of the Roman empire. His De Re Rustica in twelve volumes has been completely preserved and forms an important source on agriculture. This book was translated to many languages and used as a basic work in agricultural education until the end of the 19th Century.