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Effect of irrigation and water quality on the physiological status of sugar beet and fodder beet using SPAD-502 chlorophyll meter

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Abstract: In Hungary, irrigation determines the success of water-intensive beet cultivation. Taking into account the guidelines of the circular economy, we investigated the effect of irrigation with pre-treated nutrient-rich effluent from an intensive catfish-farm on the growth stages of sugar beet and fodder beet. In the two-year-experiment (2020, 2021), two sugar beet ('Helenika', 'Grandiosa') and two fodder beet ('Rózsaszínű Beta', 'Beta Vöröshenger') cultivars were grown. In addition to the effluent water of the fish farm, the water of the Körös oxbow lake and a mixed water type (1:3 effluent and Körös water, added gypsum) were used for irrigation (sprinkler irrigation methods, 4 replications). The experiment was performed in 64 lysimeter vessels/units (1 m^2) in Szarvas. During the research we sought answers to the following questions: (1) whether the onset and length of sugar growth stage and accumulation stage differ depending on water quality, (2) which beet variety has the highest relative chlorophyll content, (3) whether irrigation water quality affected the relative chlorophyll content of beet cultivars. SPAD values measured with the SPAD-502 chlorophyll meter were used to estimate the relative chlorophyll content of beet leaves. According to our results, in the case of sugar beet, the shifts of the phenological phases due to irrigation were well observed based on the SPAD values. Based on the SPAD values, the quality of the irrigation water had no verifiable effect on the chlorophyll content of the beets.

Keywords: relative chlorophyll content, sugar growth stage, accumulation stage, maximum canopy cover, canopy senescence

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Introduction

An early trace of sugar beet cultivation can be found in Hungary: in 1790, the Hungarian Lutheran pastor Sámuel Tessedik brought from Germany the seeds of the burgundy beet, the ancestor of sugar beet (Rombay, 1914). In 2021, there was sugar beet cultivation in Hungary on an area of 12 173 ha (KSH, 2022). At the national level, the average yield (2019) was 53 t/ha, which corresponds to the Central and Eastern European average. However, it lags far behind the Western European average (61,1–95 t ha^{-1}). Although sugar and fodder beets are currently grown in small areas, crop production has a long history and excellent soil conditions for it in Hungary. The most common limiting factor of the beet production is drought stress, as it is a water-intensive plant and the nitrogen supply because excessive N application results decreased sugar concentration due to increased levels of impurities in the beet (Akeson et al., 1979). Leaf chlorophyll content measurement is a good criterion for estimating nitrogen status of the plant (Ghasemi et al., 2017; Moghaddam et al., 2011). The SPAD chlorophyll meters give immediate results and have been used successfully for determining N status of crops. Aim of our study was to determine the nitrogen status of the sugar and fodder beets under different water quality irrigation using SPAD measurements, in order to examine the different growing periods, the effects of water quality on the plants and to compare 2 fodder and 2 sugar beet cultivars based on SPAD values.

Materials and Methods

Study Site and Climatic Conditions

The experiment was carried out at the Lysimeter Research Station (46°51′49″ N 20°31′39″ E Szarvas, Hungary) of the Hungarian University of Agriculture and Life Sciences (MATE), Institute of Environmental Sciences (IES), Research Center for Irrigation and Water Management (ÖVKI). Sixty-four non-weighing lysimeters (1 m^3) were used to determine the effect of effluent water irrigation on the development of beet cultivars. The lysimeters were 1 m deep and 1 m^2 in surface. Eight plants were sown into each vessel. The soil of the lysimeter is not stratified disturbed soil, where the soil properties in lysimeters where clay loam texture, 0.03% total salinity, 2.1% total carbonate content, and 1.31% total organic carbon content. At the bottom of all lysimeters, a 10 cm layer of fine gravel was placed for the collection of leachate water.

The climate of Hungary is influenced by continental and oceanic effects, the specific area of the experimental site is described as warm dry climate region. The meteorological data during the two years experiment (2020–2021) were collected by an automatic weather station maintained by the MATE ÖVKI in Szarvas. Its distance to the Lysimeter Research Station is 600 m. The year of 2021 was dry, the total precipitation was only 433.9 mm while in the year of 2020, 611.6 mm was measured. The annual average temperature was 12.1 °C in 2020 and 11.6 °C in 2021 (Figure 1).

Plant material

Both fodder beet varieties are on the National List of Varieties according to National Food Chain Safety Office in Hungary (NÉBIH, 2022). The fodder beet 'Beta Vöröshenger' was added to the list on 25 May 1977. The fodder beet 'Rózsaszínű Beta' was added to the list on 31 January 1944. University of West Hungary bred both varieties. 'Grandiosa' was added to our national list on 9 March 2016, the variety belongs to KWS Saat SE, Germany. 'Helenika' was also bred by KWS Saat SE and was added to the list in 2014, however it was cancelled in last year (25/3/2021).

Experimental Design for Effluent Water Irrigation

Two different water types and their combinations were applied for the irrigation experiment of the beet varieties. Untreated effluent water from a local intensive African catfish farm was used directly collected from the outflow of fish rearing tanks. The flow-through system of the fish tanks is supported by a geothermal well from stratified aquifer. This system has the main role of temperature and water quality maintenance since the African catfish are fed high protein diet and need warm water (above 16 °C) to grow. The daily amount of effluent water from the farm exceeds 1000 m^3. That effluent water (EW) contains large amount of metabolites as fish feces, organic materials and rarely chemicals or antibiotics depending on the fish rearing technology (Tóth et al., 2020). Because of the geothermal origin, the effluent water also carrying high content of total salinity including high percent sodium and high concentration of hydrogen-carbonate (Table 1). As an irrigated control treatment, freshwater was applied from the local oxbow lake of the River Körös (KW) (46°51′38.6″ N 20°31′28.0″ E, Szarvas, Hungary). Diluted effluent water with gypsum (DW) was ap-

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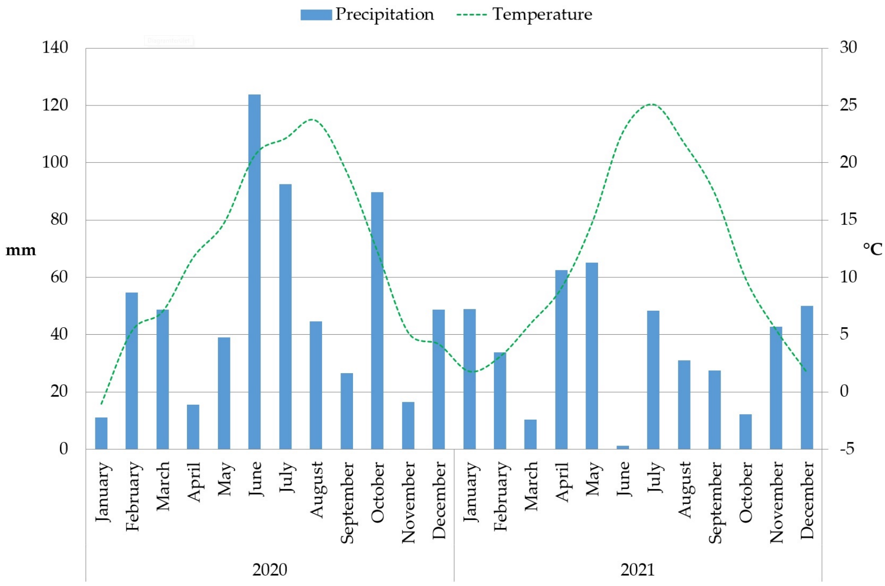

Figure 1: Precipitation and monthly mean temperature of experimental years (2020, 2021)

Table 1: Average major quality parameters of irrigation water used under experiment according to Kolozsvári et al. (2021)

Irrigation waters	EC (μS cm^{-1})	NH4-N (mg l^{-1})	N (mg l^{-1})	P (mg l^{-1})	K (mg l^{-1})	Na (mg l^{-1})	SAR
EW	1306.7	21.9	29	3.9	7.2	273.5	11.9
KW	388.3	0.4	1.2	0.2	4.3	31.3	1.2
DW	1073.0	10.3	13.3	1.7	5.4	132.3	3.5

plied with 1:3 rate of EW and KW + 0,312 kg m^{-3} gypsum. Additionally, a non-irrigated control (C) treatment was also applied with four replications.

The weather in the experimental years differed in 2020 and 2021. There was approximately 200 mm more precipitation in the vegetation period in 2020 than in 2021 (Table 2). To determine the amount of irrigation water, the water demand of sugar beet described earlier was taken into account.

Determination of Phenological Paramteres and Statistical Analyses

SPAD values (Konica Minolta SPAD-502) were measured on a weekly basis during the growing seasons (Table 3). Measurements were performed in 32 replicates per treatment (4 vessels per treatment × 8 plants/vessel). One measurement data is the average of the values measured on the plant at 3 points. (In the first experimental year (2020), leaf senescence was so intense that the measurement had to be stopped in August.)

Statistical analyses were implemented by IBM SPSS Statistics 25.0 software. Applying one-way analysis of variance (ANOVA), we examined the effect of irrigation water quality on the phenological properties of beets per treatment and plant part. The differences were determined significant, where the Tukey's or Dunnett post hoc test were considered significant at $p \leq 0.05$.

Table 2: Precipitation and water amount in the vegetation period of beet. Average water demand of 10 days period according to Posch (1997)

10 days period	Average water demand of beet in the period (mm)	Precipitation in the period (mm)		Irrigation water amount in the period (mm)	
		2020	2021	2020	2021
21-30 April	12	10.6	2.3	10	0
1-10 May	14	7.4	10.0	10	0
11-20 May	17	13.5	52.7	10	0
21-31 May	22	18.0	7.4	4	0
1-10 June	27	56.3	0.0	10	27
11-20 June	35	63.0	0.7	0	35
21-30 June	43	58.2	0.5	0	43
1-10 July	53	32.0	30.5	20	20
11-20 July	56	40.0	16.8	16	60
21-30 July	65	18.5	1.0	46	0
1-10 August	60	27.7	6.7	30	52
11-20 August	53	6.7	0.2	46	53
21-31 August	52	3.7	24.2	48	22
1-20 Sept.	47	0.0	3.9	47	43
Total	556	356	157	297	355

Table 3: Dates of SPAD measurements

Year 1		Year 2	
Day after sowing	Date	Day after sowing	Date
62	17/6/2020	67	14/6/2021
70	25/6/2020	75	22/6/2021
75	30/6/2020	88	5/7/2021
84	9/7/2020	99	16/7/2021
91	16/7/2020	105	22/7/2021
97	22/7/200	113	30/7/2021
106	31/7/2020	120	6/8/2021
117	11/8/2020	139	25/8/2021
		147	2/9/2021
		154	9/9/2021

Results

Effect of irrigation on chlorophyll content of sugar and fodder beet

The SPAD of leaves is presented in Figure 2 and Figure 3. In 2020, chlorophyll content was measured from 17 June till 11 August, this period was from 62nd till 117th days after sowing. The minimum SPAD values were 33.4 and 31.6 and the maximum

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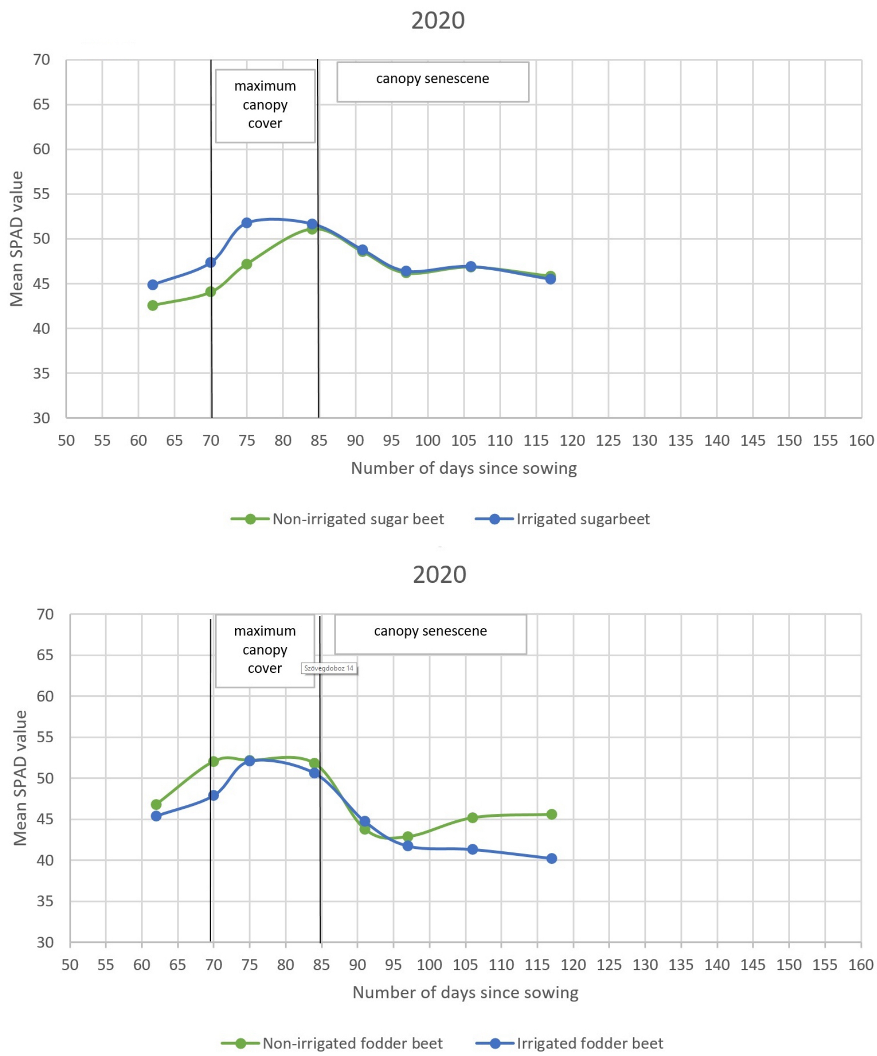

Figure 2: Mean SPAD values in irrigated and non-irrigated beet production in 2020

values were 60.6 and 60.7 for sugar beet and fodder beet, respectively, in the analysed period. The mean value of sugar beet (47.62 ± 4.5) were significantly (Independent T-test, $p < 0.001$) higher than fodder beet (45.49 ± 5.5). Before the 85th day after sowing, the non-irrigated sugar beet has significantly (ANOVA Dunnett *post hoc* test) lower SPAD values (mean 44.62 ± 3.4) than in irrigated treatments (mean 47.94 ± 4.3). After that date, similar chlorophyll content was measured for irrigated (mean SPAD value 46.91 ± 4.7) and non-irrigated sugar beet (mean SPAD value 46.90 ± 3.7). In contrast to sugar beet, between 31 July and 11 August significant differences (ANOVA Dunnett *post hoc* test) were detected between control treatment (mean SPAD value 45.4 ± 4.1) and irrigated treatment (mean SPAD value 40.80 ± 4.3).

In 2021, chlorophyll content was measured from June till 9 September, this period was from 67th till 154th days after sowing. The minimum SPAD values were 30.0 and 30.2 and the maximum values were 77.1 and 74.9 for sugar beet and fodder beet, respectively, in the analysed period. The maximum SPAD values were higher in 2021 than in 2020. There was significant difference between SPAD values in irrigated and non-

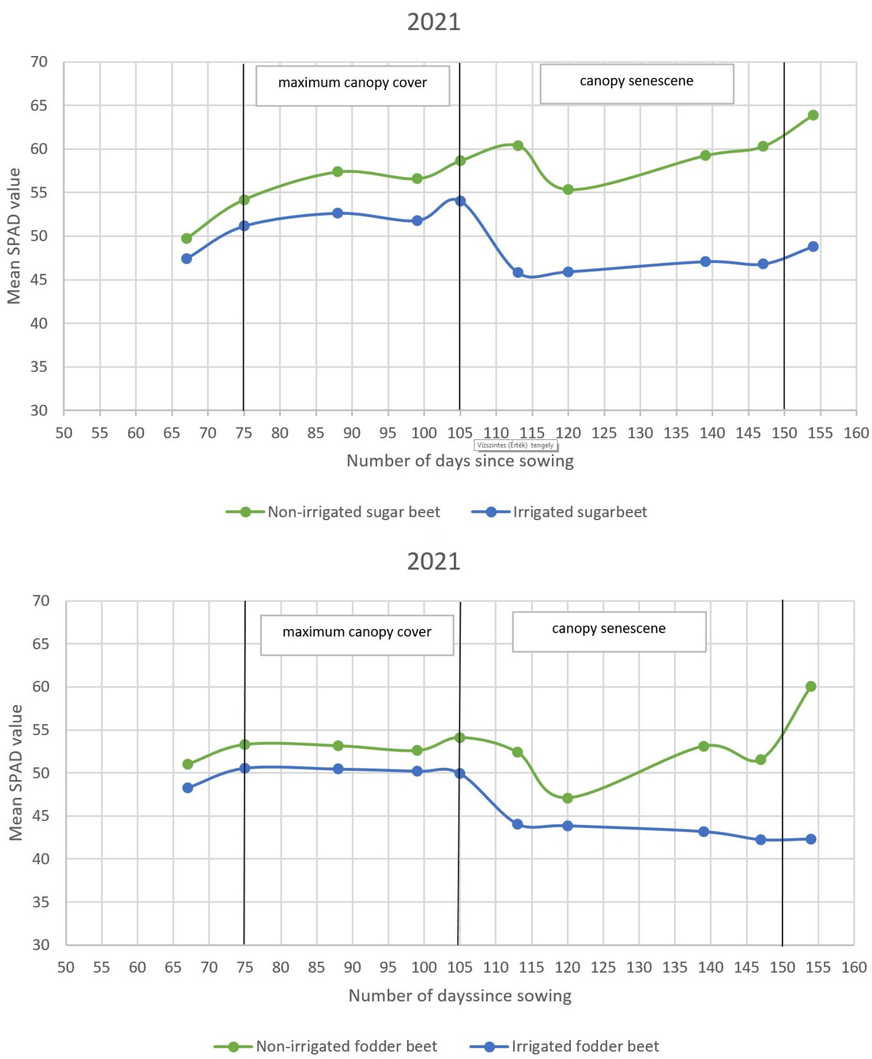

Figure 3: Mean SPAD values in irrigated and non-irrigated beet production in 2021

irrigated treatments over the entire period in the case of both beets. The mean SPAD value of non-irrigated sugar beet and fodder beet (57.5 ± 6.7, 52.8 ± 6.5, respectively) were higher (ANOVA, Dunnett test) than irrigated ones (49.2 ± 5.9, 46.5 ± 6.3, respectively).

Effect of irrigation water quality on chlorophyll content of sugar and fodder beet

When examining growth phases separately, it is generally typical in all treatments and in case of all beet cultivars (Table 4) that in the rapid growth stage of leaf cluster (46.35 ± 3.5) and in the sugar accumulation stage (44.74 ± 5.1) the mean SPAD value is lower than in sugar growth stage (51.3 ± 3.1) in 2020. However, the irrigation water quality has different impact on chlorophyll content in each phases and cultivars. In case of fodder beet ‘Rózsaszínű Beta’, in each phases the highest mean SPAD value was measured in non-irrigated treatment, which means difference was significant compared to irrigated treatments. Comparing water quality, in the DW treatment the mean SPAD value were higher than treatment KW (significant differences in sugar accumulation stage. The mean SPAD differences were significant only between KW and EW treatment in sugar growth stage (Table 4).

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Table 4: Mean SPAD value of beet cultivars in 2020

2020 SPAD (mean±std.dev)		'Rózsaszínű Beta' (fodder beet)	'Beta Vöröshenger' (fodder beet)	'Grandiosa' (sugar beet)	'Helenika' (sugar beet)
Rapid growth stage of leaf cluster	C	49.6 ± 3.4^{c}	49.0 ± 3.1^{c}	44.5 ± 2.7^{ab}	42.2 ± 2.4^{a}
	DW	47.5 ± 3.1^{b}	47.0 ± 3.1^{b}	43.6 ± 1.8^{a}	47.5 ± 4.3^{b}
	KW	47.3 ± 2.8^{ab}	47.8 ± 3.1^{bc}	45.4 ± 2.4^{b}	48.6 ± 3.3^{b}
	EW	45.7 ± 2.6^{a}	44.7 ± 2.3^{a}	43.9 ± 1.7^{a}	47.7 ± 2.6^{b}
Sugar growth stage	C	53.2 ± 3.1^{c}	50.9 ± 2.5^{ab}	50.2 ± 3.9^{ab}	48.9 ± 2.9^{a}
	DW	51.6 ± 3.3^{ab}	52.2 ± 3.1^{c}	50.4 ± 2.3^{ab}	53.8 ± 2.7^{c}
	KW	50.3 ± 1.9^{a}	50.0 ± 2.4^{a}	49.3 ± 2.1^{a}	51.3 ± 2.4^{b}
	EW	52.0 ± 2.9^{b}	51.2 ± 2.2^{ab}	51.4 ± 3.0^{b}	54.3 ± 2.2^{c}
Sugar accumulation stage	C	45.2 ± 4.4^{c}	43.5 ± 4.6^{b}	45.8 ± 4.0^{b}	48.0 ± 2.9^{a}
	DW	43.0 ± 4.8^{b}	42.5 ± 4.8^{ab}	45.9 ± 4.3^{b}	49.7 ± 3.9^{b}
	KW	41.2 ± 4.4^{a}	42.1 ± 4.9^{ab}	44.0 ± 3.2^{a}	48.9 ± 4.2^{ab}
	EW	41.7 ± 4.4^{ab}	41.6 ± 4.3^{a}	44.0 ± 3.8^{a}	49.2 ± 4.6^{ab}

^{a,b,c} indices means the result of Tukey's *post hoc* test of ANOVA.

Table 5: Mean SPAD value of beet cultivars in 2021

2020 SPAD (mean±std.dev)		'Rózsaszínű Beta' (fodder beet)	'Beta Vöröshenger' (fodder beet)	'Grandiosa' (sugar beet)	'Helenika' (sugar beet)
Rapid growth stage of leaf cluster	C	50.8 ± 5.2	51.1 ± 4.5^{b}	48.1 ± 6.3	50.6 ± 4.5
	DW	50.0 ± 5.9	46.9 ± 3.7^{a}	46.7 ± 5.2	48.5 ± 4.9
	KW	49.1 ± 5.2	49.1 ± 4.5^{ab}	46.6 ± 4.6	48.6 ± 4.8
	EW	48.1 ± 4.8	46.9 ± 4.6^{a}	46.0 ± 3.3	48.2 ± 4.2
Sugar growth stage	C	53.4 ± 5.8^{b}	53.0 ± 5.9^{b}	55.6 ± 5.5^{b}	58.4 ± 5.1^{b}
	DW	50.5 ± 6.4^{a}	47.8 ± 6.0^{a}	50.0 ± 5.9^{a}	52.6 ± 5.5^{a}
	KW	49.5 ± 6.2^{a}	48.7 ± 6.3^{a}	49.6 ± 5.2^{a}	52.6 ± 5.3^{a}
	EW	49.8 ± 6.2^{a}	48.4 ± 6.2^{a}	49.8 ± 6.6^{a}	51.9 ± 6.2^{a}
Sugar accumulation stage	C	50.9 ± 6.4^{b}	50.3 ± 6.4^{b}	58.9 ± 7.4^{b}	58.0 ± 5.7^{b}
	DW	43.7 ± 5.3^{a}	43.1 ± 4.1^{a}	45.3 ± 4.6^{a}	47.0 ± 4.8^{a}
	KW	43.2 ± 5.1^{a}	42.7 ± 5.5^{a}	46.3 ± 6.4^{a}	48.6 ± 4.6^{a}
	EW	43.4 ± 4.6^{a}	42.4 ± 4.5^{a}	45.7 ± 5.0^{a}	47.0 ± 4.9^{a}

^{a,b,c} indices means the result of Tukey's *post hoc* test of ANOVA.

In case of fodder beet 'Beta Vöröshenger', only in the sugar growth stage the highest mean SPAD value was not measured in the non-irrigated treatments. In the rapid growth stage of leaf cluster, the mean SPAD value was significantly lower in treatment EW, than in treatment KW and DW, this is also true for sugar accumulation stage, but the difference cannot be statistically justified. In the sugar growth stage, irrigation with used water (treatment DW and EW) resulted in a higher chlorophyll content (Table 4).

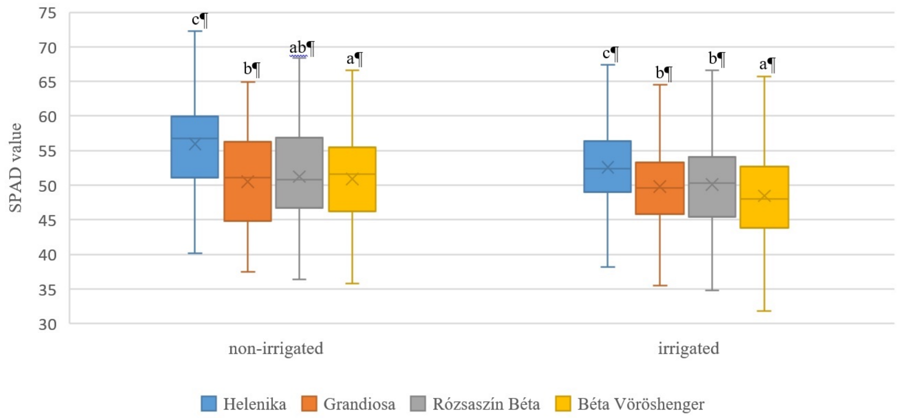

Figure 4: SPAD values of different beet cultivars in sugar growth stage in 2021 ([a,b,c] indices means the result of Tukey's *post hoc* test of ANOVA.)

In case of sugar beet 'Grandiosa', only in sugar accumulation stage was significantly difference between irrigated and non-irrigated treatments (Table 4). Water quality did not have a clear effect in all growth phases, the highest SPAD value was in the first phase in the treatment KW, in the second phase in the treatment EW and in the third phase in the treatment DW.

In case of sugar beet 'Helenika', the lowest mean SPAD value was measured in non-irrigated treatment in each phase (Table 4). Significant differences in mean SPAD values were measured between the irrigated treatments only in sugar growth stage, when the irrigation of the used waters (treatment DW and EW) resulted in a higher SPAD value compared to the Körös River irrigation water.

In 2021, when examining growth phases separately, in the rapid growth stage of leaf cluster (48.45 $\pm$ 4.9) and in the sugar accumulation stage (46.45 $\pm$ 6.8) the mean SPAD value is lower than in sugar growth stage (50.97 $\pm$ 6.5) in 2020, however, it is not true in all treatments and in case of all beet cultivars (Table 5). For all sugar beet cultivars, it can be demonstrated that in the second and third phases, significantly higher SPAD values were measured in the non-irrigated treatments than in the irrigated ones (Figure 2, Table 5). Unlike 2020, significant differences in mean SPAD value were not measured between irrigation treatments in case of any beet cultivars in 2021. In the rapid growth stage of leaf cluster, the chlorophyll content of the fodder beet 'Beta Vöröshenger' was higher in treatment KW than in treatments irrigated with reused waters (Table 5).

Effect of irrigation on chlorophyll content of each beet cultivars

As water quality did not have a significant effect on the SPAD value of any variety in the sugar growth phase of 2021, we examined the differences between the SPAD values of the varieties in this period. In both irrigated and non-irrigated treatment, sugar beet 'Helenika' had significantly higher chlorophyll content (mean SPAD value 52.5 $\pm$ 5.5 and 58.38 $\pm$ 5.1, respectively) than other cultivars (Figure 4). The highest SPAD values were measured also in case of sugar beet of 'Helenika'. The fodder beet 'Beta Vöröshenger' had the lowest mean SPAD values in both treatments (48.28 $\pm$ 6.2 and 52.82 $\pm$ 5.5). However, the lowest SPAD values were measured in case of 'Rózsaszínű Beta' in both treatments. There were no significant differences between mean SPAD

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value of fodder beet 'Rózsaszínű Beta' and sugar beet 'Grandiosa' nor in irrigated and non-irrigated treatment. Each beet cultivars had lower SPAD value in irrigated treatment during this period.

Discussion

According to Zhang et al. (2021), SPAD value in sugar growth stage was relatively higher than in the rapid growth stage of leaf cluster and the sugar accumulation stage. Based on their results and the number of days after sowing in our experiment, developmental stages can be distinguished in both years using SPAD values. According to Sims and Gamon (2002) and Salehi et al. (2016), chlorophyll content declines rapidly when plants are experiencing stress and during leaf senescence. In 2020, between days 85-90 after sowing SPAD values started to decrease in case of both fodder and sugar beets (Figure 2). In 2021, the SPAD value began to decline 15 days later than in 2020 (Figure 3). However, we observed that in 2021, the leaf senescence period started at least 10 days earlier in the irrigated treatments than the non-irrigated treatment in case of sugar beet. In 2020, the sugar growth period began earlier in the irrigated treatments of sugar beet by at least 10 days (Figure 2 and Figure 3). Our result is consistent with J. T. Tsialtas and Maslaris (2012), according to their SPAD readings increased till early July, decrease till early September and then increase toward the end of the season showing maximum at the end of October. Wang et al. (2021) also measured maximum chlorophyll content at harvest time, however, in our experiments SPAD measurements did not occur throughout all leaf senescence period and harvest time because of leaf diseases it was not feasible.

Both irrigation and water quality significantly affect the SPAD values of beets cultivar, but in a different way in the experimental years (Table -5). In 2020, in the rapid growth leaf stage the SPAD values were higher in the irrigated sugar beets than in non-irrigated ones. According to Wang et al. (2021) chlorophyll content was higher in irrigated treatments than in rain-fed treatments in sugar beet. In contrast, in 2021 SPAD values were lower in irrigated treatment throughout the analyses periods in case of all beet cultivars. According to Széles (2008) as a result of irrigation, the leaf area increases and the nitrogen concentration is diluted which caused higher SPAD values in the corn experiment.

Irrigation water quality had significant effect on SPAD values only in rapid growth stage of leaf cluster in case of sugar beet 'Grandiosa' in 2020. We hypothesized that the nitrogen content of the water caused the higher chlorophyll content, several researchers found that a strong correlation could be described between the N content of the soil or fertilizers and the the chlorophyll a, b and total content, petiole NO3-N of the sugar beet (Ghasemi et al., 2017; J. Tsialtas & Maslaris, 2008). In contrast, J. T. Tsialtas and Maslaris (2012) did not found correlation between SPAD value and soil total N, NO_3-N, root alpha-amino-nitrogen and petiole NO_3-N. In conclusion, based only on SPAD value analyses, the effluent water had no clear effect on the chlorophyll content of beets in the years and cultivars studied in this experiment. Among the varieties, 'Helenika' sugar beet had the highest chlorophyll content in both irrigated and non-irrigated treatments. Islam et al. (2020) compared 11 varieties of sugar beet in their research, according to their results 'Helenika' (and 'Recoddina') had significantly higher chlorophyll a, b and total content than other 9 varieties. According to them, 'Helenika' – and six other cultivars – were found best fitted to the given drought condition (Islam et al., 2020).

Conclusion

In the case of sugar beet, the shifts of the phenological phases due to irrigation were well observed based on the SPAD values. Based on the SPAD values, the quality of the irrigation water had no verifiable effect on the chlorophyll content of the beets. The differences between the varieties could be described with great certainty in one vegetation period in 2021 where "Helenika" differed from the other studied varieties. In the continuation of the research, we plan to investigate the relationship between SPAD values and other plant (yield, sugar content, alpha-amino nitrogen content, etc.) and soil (nutrient supply, salinity) parameters.

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