

**POSSIBILITIES TO DETERMINE CHANGES
IN CHLOROPHYLL CONTENT IN LEAVES OF SUGAR
BEET (*BETA VULGARIS* L.) BY MINOLTA
CHLOROPHYLLMETER***

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A study of 1995 to 1997 testing of Minolta chlorophyllmeter for orientation measurement of changes in chlorophyll content in leaves of sugar beet throughout the growing season, including an evaluation of the extent of reaction to changes in cultivation technology by changes in chlorophyll content in leaves as well as the possibility and speed of measuring the reaction by chlorophyllmeter and, furthermore, a definition of places on leaves and in sugar beet stands fit for the measurement. The evidence was found with selected plants of Hilma and Edda sugar beet varieties of a relation between the number of chlorophyll units and the age of leaves throughout the growing season. The best choice for field measurement by chlorophyllmeter in sugar beet stands are fully developed visibly lustrous leaves of dark green colour. The choice of the middle (heart) or peripheral leaves as well as those damaged or already getting dry was to be avoided. A series of measurement of individual varieties or selected variants of biogamik fertilizing proved a difference in chlorophyll values.

sugar beet; nitrogenous fertilizing; chlorophyll; sugar beet growth; chlorophyllmeter

INTRODUCTION

A number of cultivation actions in sugar beet cultivation technology have a significant influence on plant metabolism with effect also, e.g. on changes in chlorophyll content. Chlorophylls are considered to be substances sensitive to changes in cultivation technology of most field crops, i.e. also sugar beet.

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Photophysical, photochemical and biochemical processes of photosynthesis in higher plants, i.e. also in sugar beet, run almost exclusively in cells of a photosynthetic system of an organized structure, or leaf (Čatský, Tichá, 1981). According to Larcher (1988), the radiation utilization degree depends on chlorophyll concentration or, more precisely, on the concentration of photosynthetically active pigments. According to Kindl and Wöber (1981), chlorophyll cumulation is closely linked to the synthesis of membrane lipids, structural proteins and membrane structure.

Vegetable pigments, especially chlorophylls, reflect in a sufficiently exact and visible way physiological changes and health condition of sugar beet. This made them a factor used already earlier for determination of diseases caused by lack of some nutrients or virus diseases (Římsa et al., 1978) and, furthermore, for monitoring production processes in application of distance methods to plant production (Petr, 1989) or in application of precision farming (Griepentrog, 1998). At the time being, they also serve to evaluating the condition of stands (Justes, 1994; Guérif et al., 1995). Quantity and quality of chlorophylls as the primary photoreceptors in photosynthesis are critical in the process of photosynthesis. Photosynthesis is closely related to saccharose synthesis in beet leaves (Zalewski, 1992). The content and activity of pigments and their mutual relations depend on the growth and development stage of plants and reflect with sensitivity influences of the changing external environment (Stehlík, 1982; Šebánek et al., 1983).

Chlorophyll content is a critical pre-condition of photosynthesis. An indicator of its quantity is the intensity of colour of leaf or other photosynthesizing bodies (Rubin, 1968). Changes in chlorophyll content in sugar beet leaves may contribute to the explanation of differences in yields and quality under different environmental conditions or to serve as the background data for differentiating cultivation actions with respect to the required quality of beet roots. Guérif et al. (1995) show that the close correlation between chlorophyll content and concentration of nitrogenous substances in leaves allows to determine deficit in nitrogenous nutrition as well as the optimum doses necessary for cultivation. In an attempt to contribute to an explanation of the influence of some factors on chlorophyll content as well as to an improvement or elaboration of new, if possible simple, methods used in evaluation of chlorophyll content, the Czech University of Agriculture, Prague run tests of the chlorophyllmeter SPAD-502.

The presented study aims at evaluating the use of Minolta chlorophyllmeter for orientation measurement of changes in chlorophyll content in sugar beet leaves throughout the growing season in order to find out the extent of reaction of sugar beet to some changes in cultivation technology by changes

in leaf chlorophyll content and the possibility and speed of the measurement; in short, to test the possibility of using chlorophyllmeter for evaluation of some physiological reactions of sugar beet.

MATERIAL AND METHODS

Changes in chlorophyll content in sugar beet leaves throughout the growing season were tested by chlorophyllmeter in the period of 1995 to 1997. Chlorophyll testing was run for comparison also by a standard extraction method (Šesták, Ullmann, 1964) in the first year. Chlorophyllmeter is a light compact device fit for determination of chlorophyll content in vegetable leaves. The value determined by SPAD-502 chlorophyllmeter (introduced on the market by Hydro CZ under the name N Tester) indicates the relative amount of chlorophyll content in vegetable leaves. The new Minolta chlorophyllmeter supplied by Hydro CZ Prague identifies chlorophyll content directly in the field without damaging the tested leaves. The values of SPAD (relative chlorophyll units) defined by Minolta specify the relative amount of chlorophyll content in vegetable leaves. Tested is a surface of 1 by 3 mm. The basis for calculating the values is the transmittance of two wavelengths of light through the leaf with different absorption by chlorophyll. The values identified by receiver (silicon photodiode) are computer-evaluated and displayed. The device calculates an average number of chlorophyll units usually of 30 individual partial measurements. Following a statistical evaluation and potential adding of data, the average of identified chlorophyll units is displayed.

To determine the methodology of measuring chlorophyll content by chlorophyllmeter, individual sugar beet leaves were tested first, followed by testing of categories of leaves. Principles of measurement under field trial conditions were deduced from the evaluation results. Sugar beet stands of different experimental variants of cultivation technology (nitrogenous fertilizing, different varieties) were tested in the next stage.

A statistical evaluation was made of the identified values. The table programmes Excel and Quattro-Pro were used to calculate linear regression and variance analysis of individual trials.

RESULTS AND DISCUSSION

An evaluation was made of chlorophyll content (in relative chlorophyll units) in leaves of selected sugar beet varieties Hilma and Edda (15 plants of each variety). The sequence of measured leaves corresponded to their devel-

opment. Each plant had 20 to 30 leaves at the time of measurement. Only fully developed, healthy, not yet dry leaves were tested. The count and sequence of leaves ran from the oldest to the youngest (heart) ones. Such selected leaves were first tested for chlorophyll content on the periphery (A – periphery of leaf). After cutting a margin of approx. 2 to 3 cm off, chlorophyll content in the middle of the leaf approx. 2 to 3 cm alongside the main vessel fascicle was measured (B – middle of leaf). The next test traced the influence of secondary vessel fascicles on the measured values (C - Vessel fascicle inclusive). A statistical evaluation of data for individual varieties (Hilma, Edda) and measured places was made and the results (Tabs. I to III, Figs. 1 to 3) were used for formulating general principles of selection of places fit for measuring by chlorophyllmeter. For identified and calculated average values of the extensive measurements, see Tab. I. The statistical evaluation shows that fully developed, healthy leaves may be used to measure the involved sugar beet stands (Tab. II and III). Values identified in the youngest as well as the oldest dying leaves are lower. Values identified in young plants have always to be given with the growth stage (Pulkrábek, 1989) or leaf sequence as changes in numbers of chlorophyll units are dramatic and grow rapidly with the growing number of leaves and their surface. The influence of chlorophyllmeter positioning on the leaf is statistically significant only at a significance level alpha 0.05.

Areas of sugar beet stands on the operational plots visually regular and featuring a regular shape and size of leaves but differing from the rest of the stands in colour, damage, variety, etc. were selected. Such selected types of stands could influence the selection of leaves fit for measurement. Hence, the leaves of different varieties and stands of sugar beet tested in the next series of measurement of relative chlorophyll units by chlorophyllmeter were split into four categories (easy to determine at a standard field operational measurement), namely small leaves (the heart of the leaf rosette, young rapidly growing leaves of lettuce-green colour and high lustre); medium leaves (developed yet still growing leaves of a higher intensity of green colour and lustre); large, fully developed middle leaves (of a size corresponding to the growth stage of the plant, finished in growth, dark-green with a visible lustre), and large, fully developed peripheral leaves (gray-green leaves beginning to lose the lustre). Leaves beginning to lose the green colour, usually on the margin of the leaf rosette, were not tested. For the summary data, see Tab. IV. The identified values and statistical results (Tabs. IV to VI) show a relatively small difference between categories of fully developed leaves. This means that the best choice for field measurement by chlorophyllmeter in sugar beet stands are fully developed visibly lustrous leaves of dark green colour (with a regular pattern of colour corresponding to the prevailing colour

I. Influence of leaf sequence on changes in relative chlorophyll units determined by chlorophyllmeter

Leaf No.	Leaf length (cm)	Place or manner of chlorophyll measurement on leaf			average values
		A periphery of leaf	B middle of leaf	C vessel fascicle incl.	
Average values of plants and varieties					
1	25.8	451	469	477	466
2	24.8	500	520	529	516
3	25.3	510	523	515	516
4	22.9	554	558	552	555
5	24.7	547	552	554	551
6	24.3	564	588	581	578
7	23.5	563	574	569	569
8	21.8	563	585	589	579
9	22.3	592	627	613	611
10	21.1	553	587	575	572
11	20.8	553	570	569	564
12	20.0	536	552	558	549
13	20.7	546	551	562	553
14	20.5	554	578	577	570
15	18.9	551	562	567	560
16	18.3	540	557	557	551
17	17.5	542	554	564	553
18	16.5	520	535	540	532
19	16.2	514	536	534	528
20	15.3	499	507	503	503
21	13.6	480	520	503	501
22	14.0	490	510	516	505
23	12.5	477	494	511	494
24	11.0	443	441	443	442
25	10.2	414	404	424	414
Average	19.0	522	538	539	533
Average deviation	3.9	35.7	36.9	35.0	35.9
Standard deviation	4.54	42.87	48.08	43.90	44.65
Linear regression	-0.62	-2.85	-3.11	-2.76	-2.91
Correlation coefficient (leaf length x)	*	0.59	0.58	0.57	0.59

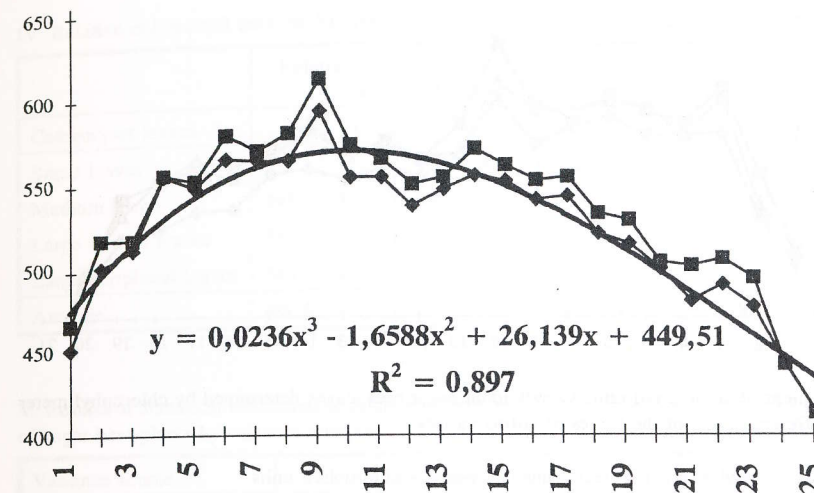
II. Statistical evaluation (*F*-test) of the influence of the manner of determination of relative chlorophyll units in sugar beet leaves by chlorophyllmeter

Level of significance	<i>F</i> -test for sets	<i>F</i> -crit. (1)	<i>F</i>	Statistical significance
Alpha 0.05	periphery of leaf x average value	0.5041	0.9216	yes
	periphery of leaf x middle of leaf	0.5041	0.7949	yes
	periphery of leaf x vessel fascicle incl.	0.5041	0.9533	yes

III. Statistical evaluation of the influence of leaf sequence on changes in relative chlorophyll units determined by chlorophyllmeter in Hilma and Edda varieties

Statistical characteristics	Leaf length (cm)	Periphery of leaf	Middle of leaf	Vessel fascicle incl.	Average values
Hilma variety					
Average	20	506	529	524	520
Average deviation	3.4	41.1	41.0	41.0	40.9
Minimum value	12	364	368	343	358
Maximum value	26	580	625	593	599
Standard deviation	4.02	51.60	56.69	57.10	54.70
Linear regression	-0.65	-4.93	-5.04	-4.63	-4.87
Correlation coefficient (leaf length x)	*	0.67	0.63	0.59	0.63
Edda variety					
Average	20	541	551	556	550
Average deviation	3.6	42.1	46.4	42.3	43.2
Minimum value	10	414	404	424	414
Maximum value	26	604	629	633	622
Standard deviation	4.48	52.91	57.43	52.35	53.74
Linear regression	-0.59	-1.63	-1.85	-1.77	-1.75
Correlation coefficient (leaf length x)	*	0.42	0.42	0.42	0.42

of the stand). The choice of the middle (heart) or peripheral leaves as well as those damaged or already getting yellow and dry was to be avoided as they would critically influence the average identified values.

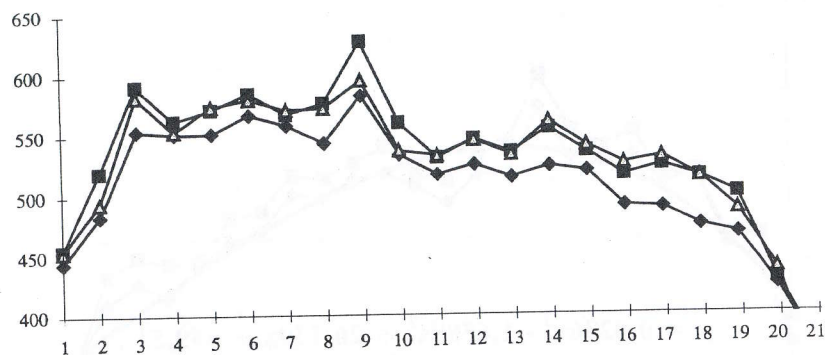


1. Content of chlorophyll units in individual sugar beet leaves determined by chlorophyllmeter on different places of the leaves (average values of individual measurements of Hilma and Edda varieties; peripheral measured places on the leaves (periphery of leaf); a polynomial function for peripheral measurement on the leaves of average values of all measurements)

x-axis – leaf sequence, y-axis – chlorophyll units
 ◆ periphery of leaf, ■ average values, — polynomial (periphery of leaf)

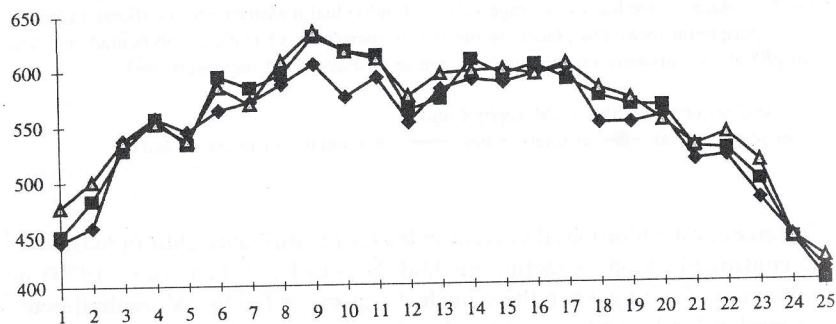
Differences in chlorophyll content in leaves identified by chlorophyllmeter were confirmed by an extraction method (Šesták, Ullmann, 1964) in the first year of testing when the same leaf was measured by chlorophyllmeter and sampled for laboratory test by the above mentioned extraction method. Values for correlation and regression analysis were deduced from the experimental ones ($y = 100.81x + 108.97$; $R^2 = 0.7842$; $R = 0.8856$, see Fig. 4). The measurements confirmed the fitness of chlorophyllmeter for determination of changes in chlorophyll content in sugar beet leaves and comparability between the chlorophyllmeter values and those obtained by the standard extraction method (by recalculation from Fig. 4). Unlike the laboratory determination, measurement by chlorophyllmeter is fast and fits also field measurements in larger trials with ensured higher number of repetitions compared to the traditional extraction method.

In the following testing, chlorophyllmeter was used as a supplement for determination of chlorophyll content in trials identifying the influence of



2. Content of chlorophyll units in individual sugar beet leaves determined by chlorophyllmeter on different places of the leaves of Hilma variety

For Figs. 2 and 3: x-axis – leaf sequence, y-axis – chlorophyll units
 ◆ periphery of leaf, ■ middle of leaf, ▲ vessel fascicle inclusive



3. Content of chlorophyll units in individual sugar beet leaves determined by chlorophyllmeter on different places of the leaves of Edda variety

variety and bioganic on quantity and quality of harvested sugar beet roots. Content of chlorophyll in leaves of individual varieties and fertilized vs. non-fertilized sugar beet was tested by chlorophyllmeter from early May to the harvest, usually fortnightly. For the identified data, see Figs. 5 to 7. A series of measurement of individual varieties or selected bioganic fertilizing variants in 1995 to 1997 proved differences in chlorophyll content in leaves and fitness of chlorophyllmeter for their determination. The determination proved the reaction of sugar beet plants by changes in chlorophyll

IV. Relative chlorophyll units in different categories of leaves of individual sugar beet plants

Category of leaves	Relative chlorophyll units in different categories of leaves of individual types – areas of sugar beet stands							average
	a	b	c	d	e	f	g	
Small leaves	391	321	479	489	456	427	436	428.4
Medium leaves	491	374	505	542	523	487	474	485.1
Large middle leaves	535	461	517	559	536	522	507	519.6
Large peripheral leaves	545	458	507	536	527	511	495	511.3
Average	490.5	403.5	502	531.5	510.5	486.75	478	486.1

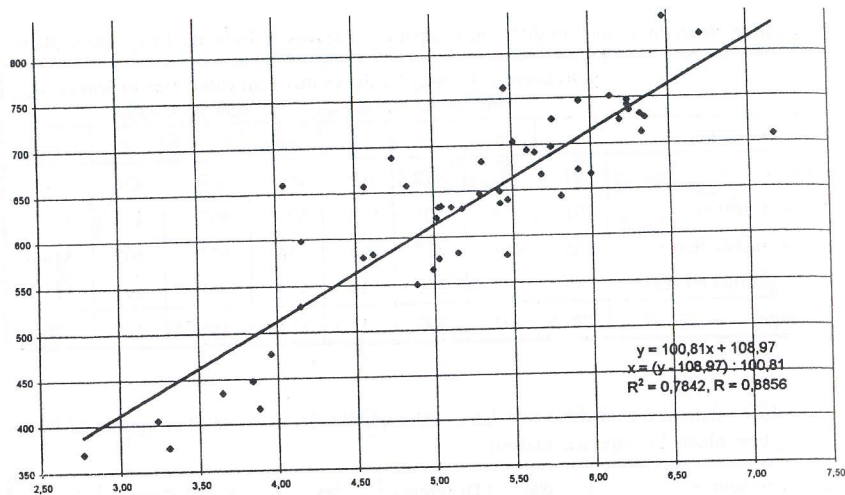
V. Results of statistical evaluation of relative chlorophyll units in different categories of leaves of sugar beet plants by variance analysis

Variance source	SS	Difference	MS	F	P-value	F-crit.
Rows – category of leaves	35571	3	11857	23.5211	1.9E-06	3.15991
Columns – type of stand	39269.9	6	6544.99	12.9835	1.1E-05	2.6613
Error	9073.79	18	504.099			
Total	83914.7	27				

VI. Results of statistical evaluation (F-test) of a set of category of peripheral and middle large leaves

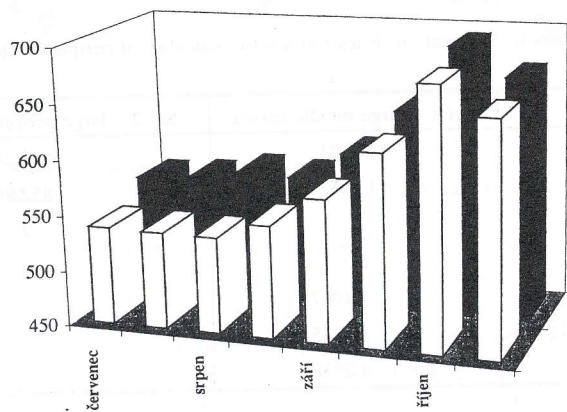
	Set 1 – large middle leaves	Set 2 – large peripheral leaves
Mean value	519.571	511.286
Variance	943.952	852.905
Observation	7	7
Difference	6	6
F	1.10675	
P(F <= f) (1)	0.45258	
F-crit. (1)	4.28386	

content measured in relative chlorophyll units. Differences in chlorophyll content were identified especially in relation to fertilizing (Fig. 5) and selected varieties according to their genetics (Fig. 6) (Pulkrábek, 1993, 1995; Švachula et al., 1997).



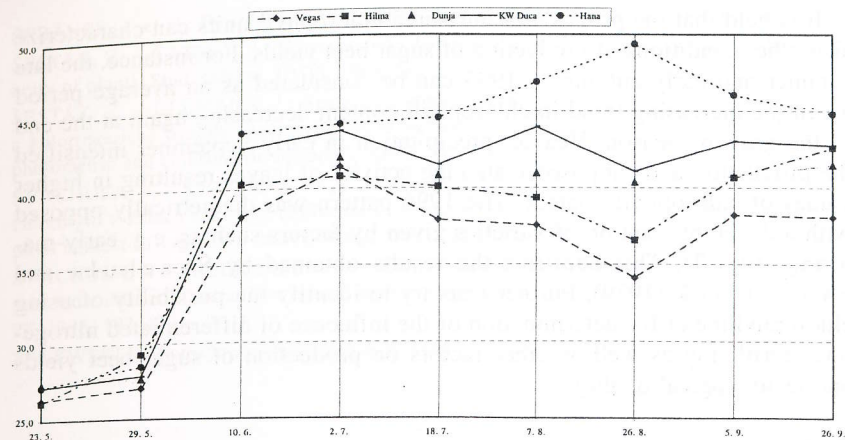
4. Relation between chlorophyll units determined by chlorophyllmeter and those determined by extraction

x-axis – chlorophyll content $a + b$ (mg/dm^2) – sugar beet leaf blades (x), y-axis – chlorophyllmeter units (y)



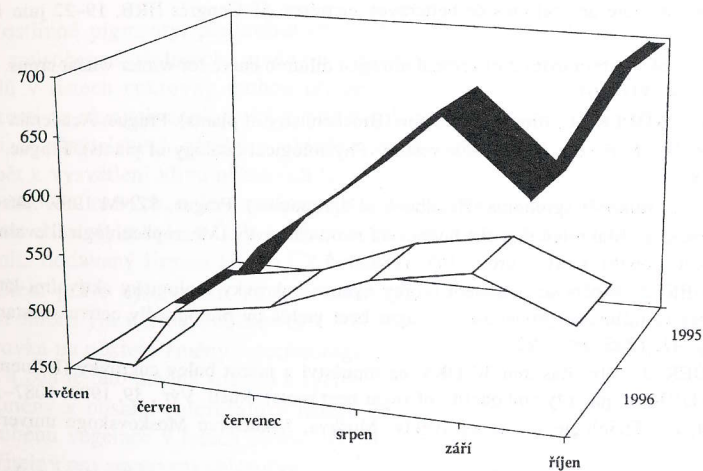
5. Influence of biogonik on chlorophyll units in sugar beet leaves in the 1995 and 1996 growing season

x-axis – July, August, September, October, y-axis – chlorophyll units
 □ check – average of years, ■ biogonik – average of years



6. Determination of chlorophyll units in selected sugar beet varieties by chlorophyllmeter in the growing season

x-axis – measurement dates, y-axis – chlorophyllmeter units



7. Influence of the year on chlorophyll content in sugar beet determined by chlorophyllmeter in the growing season.

measurement dates

x-axis – May, June, July, August, September, October, y-axis – chlorophyll units

It is held that the progress of measured chlorophyll units can characterize also other conditions of production of sugar beet yields. For instance, the late summer and early autumn of 1995 can be considered as an average period given the increasing trend in chlorophyll content decreasing again at the end of the growing season. Heavier precipitation in early September intensified the production and supposedly also the activity of leaves resulting in higher values of chlorophyll content. The 1996 pattern was diametrically opposed with a different reaction of varieties given by factors such as, e.g. early-maturing (Fig. 7). This confirms the results obtained by Švachula and Švachulová (1979). Further tests try to identify the possibility of using chlorophyllmeter for determination of the influence of differentiated nitrogenous fertilizing as well as other factors on production of sugar beet yields and technological quality.

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Možnosti stanovení změn obsahu chlorofylu v listech řepy cukrové (*Beta vulgaris* L.) chlorofylmetrem Minolta.

Scientia Agric. Bohem., 29, 1998: 1–15.

Rostlinné pigmenty, především chlorofyly, odrážejí dostatečně přesně a viditelným způsobem fyziologické změny a zdravotní stav cukrovky. Změny obsahu chlorofylů v listech cukrovky mohou přispět k vysvětlení rozdílů při utváření výnosů a jakosti za různých ekologických podmínek, případně poskytnout podklady k diferenciaci pěstitelských zásahů s ohledem na požadovanou kvalitu bulev. Ve snaze přispět k vysvětlení vlivu některých faktorů na obsah chlorofylů a ke zdokonalení či propracování nových, pokud možno jednoduchých metod používaných při hodnocení obsahu chlorofylu byl na ČZU v Praze ověřován chlorofylmetr – nový přístroj firmy Minolta dodávaný firmou Hydro CZ Praha.

Cílem práce bylo ověřit použití chlorofylmetru Minolta k orientačnímu měření změn chlorofylu v listech cukrovky v průběhu vegetace a zjistit, do jaké míry reaguje cukrovka na některé změny v technologii pěstování změnou obsahu chlorofylů v listech a zda je tato změna snadno a rychle měřitelná.

Změny v obsahu chlorofylu v listech cukrovky byly sledovány chlorofylmetrem v průběhu vegetace v letech 1995–1997. Chlorofylmetr je lehký kompaktní přístroj využitelný pro stanovení chlorofylu přítomného v listech rostliny. Hodnota stanovená přístrojem SPAD-502 ((firmou Hydro CZ je uváděn trh pod označením N tester) poskytuje údaj o relativním množství chlorofylu přítomného v listech rostliny. Pro potřebu zpracování metodiky měření obsahu chlorofylu chlorofylmetrem byly nejprve sledovány jednotlivé listy rostliny cukrovky, následně skupiny listů a z vyhodnocených závěrů byla odvozena pravidla pro měření v polních pokusnických podmínkách.

V další etapě byly hodnoceny porosty cukrovky různých pokusných variant technologie jejího pěstování (hnojení dusíkem, různé odrůdy).

U vybraných rostlin cukrovky odrůdy Hilma a Edda byl hodnocen obsah chlorofylu v relativních chlorofylových jednotkách. Listy byly měřeny v pořadí odpovídajícím jejich vývoji (od nejstarších po listy v srdéčku). U takto připravených listů byly nejprve stanoveny chlorofylové jednotky po obvodu listu (A – celý list), pak byl odříznut obvod (B – vnitřní částí listů), a další stanovení sledovalo vliv cévních svazků (C – měření i s cévními svazky). Údaje zjištěné pro jednotlivé odrůdy a místa měření byly statisticky vyhodnoceny (tab. I až III, obr. 1 až 3). Ze statistického vyhodnocení výsledků vyplývá, že pro měření u zapojených porostů cukrovky lze využít plně vytvořené a zdravé listy (tab. II a III). Nejmladší listy, stejně jako staré, odumírající listy, mají hodnoty nižší. U mladých rostlin je třeba vždy uvádět růstovou fázi (Pulkrábek, 1989) či pořadí listu. Vliv sledovaného umístění chlorofylmetru na list je statisticky významný jen na hladině významnosti α 0,05.

Na provozních plochách cukrovky byly vybrány části porostu, které byly ve tvaru a velikosti listů pravidelné a na pohled vyrovnané, ale odlišovaly se od okolní části porostu barvou, poškozením, odrůdou atd. Takto vybrané typů porostů by mohly mít vliv na výběr vhodných listů pro měření. V další sérii měření relativních chlorofylových jednotek chlorofylmetrem byly proto hodnocené listy rozděleny do čtyř skupin (snadno stanovitelné skupiny při běžném polním provozním měření) na listy malé (srdéčko listové růžice, mladý rychle rostoucí list salátově zelené barvy s výrazným leskem), střední (rozvinutý, ale ještě dorůstající list s intenzivnějším zeleným zbarvením a leskem), velké plně vyvinuté listy – vnitřní (velikostí odpovídající růstové fázi rostliny, list s ukončeným růstem, temně zeleného zbarvení s viditelným leskem) a velké plně vyvinuté – vnější (list se šedozeleň zbarvením, začíná ztrácet lesk) u různých odrůd a částí porostu cukrovky. Listy ztrácející zelenou barvu, zpravidla z okraje listové růžice, nebyly měřeny. Souhrnné údaje jsou uvedeny v tab. IV. Ze zjištěných výsledků a statistických vyhodnocení (tab. IV až VI) je patrná relativně malá diference mezi skupinami listů plně vyvinutých. Z uvedeného vyplývá, že jako nejvhodnější pro polní měření chlorofylmetrem v porostu cukrovky je třeba vybírat listy plně vyvinuté, temně zeleného zbarvení s viditelným leskem (barevně vyrovnané – odpovídajícím převažujícímu zbarvení porostu) a vyvarovat se měření listů ze středu (srdéčka) a na okraji listové růžice, stejně tak jako listů poškozených nebo již žloutnoucích a zasychajících, které by výrazně ovlivnily průměr naměřených hodnot.

V prvním roce sledování byly chlorofylmetrem zjištěné diference v obsahu chlorofylu v listech cukrovky potvrzeny extrakční metodou a odvozeny hodnoty korelační a regresní analýzy ($y = 100,81x + 108,97$; $x = (y - 108,97) : 100,81$; $R^2 = 0,7842$ a $R = 0,8856$, viz obr. 4). Sledovaná měření potvrdila vhodnost použití chlorofylmetru pro sledování změn obsahu chlorofylu v listech cukrovky.

V průběhu dalšího sledování byl chlorofylmetr využit jako doplněk ke stanovení obsahu chlorofylu v pokusech sledujících vliv odrůdy a bioganiku na množství a ja-

kost sklizených bulev cukrovky. Od začátku května do sklizně byl zpravidla ve čtrnáctidenních intervalech hodnocen obsah chlorofylu v listech jednotlivých odrůd nebo hnojené a nehnojené cukrovky pomocí chlorofylmetru. Zjištěné údaje jsou patrné z obr. 5 až 7. Série měření u jednotlivých odrůd či vybraných variant hnojení bioganikem v letech 1995 až 1997 prokázala diference v obsahu chlorofylu v listech a vhodnost použití chlorofylmetru k jejich hodnocení. Hodnocení prokázala reakci rostlin cukrovky změnou obsahu chlorofylu měřeného v relativních chlorofylových jednotkách. Rozdíly v obsahu chlorofylu byly zjištěny především u hnojení (obr. 5) a sledovaných odrůd v závislosti na jejich genetickém základu (obr. 6).

řepa cukrová; hnojení dusíkem; chlorofyl; růst cukrovky; chlorofylmetr

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