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Evaluation of chlorophyll meter method in Egyptian wheat cultivars after Photobiostimulation with Red Polarized Light

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ABSTRACT

The present study has been devoted to compare between the commonly used spectrophotometric method (SPM) for chlorophyll measurement and the portable chlorophyll meter (AtLeaf) method (PCM) for direct reading. Photobiostimulation of chlorophyll has been induced in leaves (30-day-old) of six cultivars of common wheat (*Triticum aestivum* L.) in Egypt, through pre-sowing seed treatments with 9 different doses (exposure times from 1- 1200 seconds) of monochromatic red polarized light (wavelength 632.8 nm and power intensity 0.5 mW.cm^{-2}). The results obtained indicated that polarized light induced a significant increase in chlorophyll contents mostly in all cultivars, as compared to corresponding controls. As a general trend, low intensity red polarized light enhanced the chlorophyll content in leaves of the 6 tested wheat cultivars under study. Both the SPM and the PCM methods were efficient for chlorophyll assessment and the readings of the portable method showed significantly strong correlation with those of the spectrophotometric method. Thus, it might be concluded that easy to use and time rescuing portable chlorophyll meter method can be used efficiently in monitoring the chlorophyll contents of leaves of the 6 tested common wheat cultivars.
Keywords: Chlorophyll, Portable chlorophyll meter, Photobiostimulation, Red polarized light, *Triticum aestivum*, Wheat.

INTRODUCTION

Chlorophyll is a key biochemical component in the molecular apparatus that is responsible for photosynthesis; the critical process in which the energy from sunlight is used to produce life-sustaining oxygen (Hörtensteiner and Kräutler, 2011; Gopal *et al.*, 2002). Determination of chlorophyll concentration is an important aspect for studying photosynthetic characteristics. Currently, the chlorophyll extraction methodologies are mainly based upon solvent extraction method of leaf tissues (e.g. by acetone or methanol). After extraction, the chlorophyll content can be estimated with spectrophotometer. This method is simple and accurate; but unfortunately, extraction may be destructive, time consuming rather painstaking processes (Manetas, 1998). Recently, a rapid, portable, time saving and non-destructive estimation of total chlorophyll is developed and is potentially important in plant and agriculture sciences research.

The so-called Soil Plant Analysis Development (SPAD) can be used for recording chlorophyll meter reading (SCMR) values. It is a simple, portable diagnostic tool for measurement of greenness or relative chlorophyll concentration of leaves (Wang *et al*., 2009). The meter makes instantaneous and non-destructive reading on a plant according to the quantification of light intensity (peak wavelength: approximately 650 nm: red LED) absorbed by the tissue sample. A second peak (peak wavelength: approximately: 940 nm: Infrared LED) is emitted simultaneously with the LED light to compensate the leaf thickness (Netto *et al.*, 2005). Compared to the traditional methods, this equipment might provide a substantial saving in time, space and resources (Netto *et al*., 2005; Chang & Robison, 2003).

Chlorophyll readings on using portable chlorophyll meter (PCM) are mostly positively correlated with the contents of chlorophyll and they provide reliable estimates (Tobias *et al*., 1994). Variations in chlorophyll concentration and the relationship of chlorophyll concentration with PCM readings are important indicators of plant change with age (Wang *et al*., 2009). Chang and Robison (2003) reported a close link between leaf chlorophyll concentration and foliar nitrogen (N), since a pronounced part of foliar N is contained in chlorophyll molecules. Therefore, PCM meters can be also used as an indirect and rapid method for assessing foliar N status.

It should also be added that optical methods of chlorophyll estimation express relative values rather than absolute values of chlorophyll per unit of area or the leaf mass. But, the values obtained on using portable chlorophyll meters are proportional to the concentrations of chlorophyll present in the leaves (Richardson *et al.*, 2002). For this reason, several researches have been attracted to establish the relationship between both methods (Netto *et al.*, 2005; Berg & Perkins, 2004; Cate & Perkins *et al*., 2003; Yamamoto *et al*., 2002).

Electromagnetic radiations including monochromatic red polarized light (low intensity laser) produces photobiostimulation when used on seeds, seedlings and plants (Dziwulska-hunek, 2013; Abu-Elsaoud and Tuleukhanov, 2013; Skvarko and Pochynok, 2010; Perveen *et al*., 2010; Abu-Elsaoud, 2009; Aladjadjiyan, 2007; Chen *et al*., 2005). Numerous studies applying laser in agriculture and these have shown the potentiality of its application in this field.

Previous studies showed that low intensity He-Ne lasers had a positive role in accelerating plant growth and metabolism, improving the concentration of proteins and enzyme activities, thus improving the yield and quality of Chinese traditional herb *Isatis indigotica* Fort and have a positive effect in accelerating plant growth

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and metabolism (Chen *et al*., 2003 & 2005). However, there are numerous studies that show the effects of laser pretreatment in cereals including basic seeds as rice, maize, and in vegetables, such as: tomato, radish, peas, cucumber, lettuce, onion, etc. In this respect, several studies indicated that the seeds of vegetables are more sensitive and susceptible to laser stimulation than cereals (Gladyszewska, 2006). Other researches on laser irradiation in agriculture showed that it could have a potential application in the control of some plant insects (Yao *et al*., 2009), weeds (Mathiassen *et al*., 2006), fungi (Hernández, 2005; Wilczek *et al*., 2005) and pollination incompatibility (Li and Yu, 2006). Wheat (*Triticum aestivum* L.) is one of the main crops consumed by humans and it is cultivated in different environments as one of the most important cereal crops; in many areas of the world, where it represents a principal food (Rauf *et al*., 2007).

Therefore, the present work aimed to analyze the relationship between the extractable chlorophyll content (spectrophotometric method; SPM) and direct readings obtained with the portable chlorophyll meter measurements (PCM) in six selected common wheat (*Triticum aestivum* L.) cultivars cultivated in Egypt, in response to pre-sowing exposure of seeds to monochromatic red polarized light (wavelength 632.8 nm).

MATERIAL AND METHODS

Plant Materials and experimental conditions

Six common cultivars of wheat (*Triticum aestivum* L.) were selected namely: Sakha-93, Sakha-94, Sakha-95, Sids-1, Gemmiza-9 and Giza-168. Seeds were obtained from the National Agricultural Research Center, Egypt (NARC, Egypt). Seeds of each cultivar were randomly divided into two main groups: (1) control group and (2) polarized light group in which seeds were exposed for different times to 632.8 nm monochromatic red polarized light (Table 1).

Experimental wheat plants were cultivated in the field *in vivo* where a plantation site was prepared. 100 seeds in a row represented each treatment or 10 planting pits (hole) 10 cm depth and 15 cm interspace, each planting pit with 10 replicate seeds, plants were irrigated every 3 days. Soil of the cultivation site is silty-clay soil. Leaves were randomly sampled after one month of cultivation samples were exposed to both extraction in organic solvent and spectrophotometric estimation of chlorophyll and using the portable chlorophyll meter.

Table (1): Detailed characteristics of the monochromatic red polarized light applied in the present work for seed pre-sowing irradiation.

Experiment condition	Description
Radiation type	Monochromatic Red Polarized Light (MCRPL)
Wavelength	632.8 nm
Power intensity	$0.5\ mW.cm^{-2}$
Duration of exposition to Polarized light in seconds	0, 1, 5, 10, 30, 60, 120, 600 and 1200 seconds
Wave emission	Continuous (CW)
Irradiation times	Single shot
Beam size	15.5 x 27.7 cm
Laser polarization	Linear
Cultivars treated	*Selected cultivars:* 1. *Triticum aestivum* L. cv. Sakha-93, 2. *Triticum aestivum* L. cv. Sakha-94, 3. *Triticum aestivum* L. cv. Sakha-95, 4. *Triticum aestivum* L. cv. Sids-1, 5. *Triticum aestivum* L. cv. Gemmiza-9, 6. *Triticum aestivum* L. cv. Giza-168.

Estimation of Chlorophyll

a) Spectrophotometric method (SPM)

Total chlorophyll content was determined in the six studied wheat cultivars and different polarized light treatments by the spectrophotometric method recommended by Metzner *et al*. (1965). A known fresh weight of leaves was homogenized in 85% aqueous acetone, the homogenate was centrifuged 3000 rpm for 10 minutes and the supernatant was made to volume with 85% acetone. Absorbance was measured at three wavelengths of A_{644} and A_{663} (nm) using Spekol spectrophotometer VEB Carl-Zeiss.

b) Portable chlorophyll meter method (PCM)

Chlorophyll contents were monitored with a handheld chlorophyll meter using an Atleaf chlorophyll meter (Atleaf-Agri-Lab Supplies, Jay, Maine, USA) for non-invasively measuring the relative chlorophyll content of the control and the treated plant groups. AtLeaf chlorophyll meter (or SPAD meter) measures the optical density of targeted plant leaf at two different wavelengths; 660 nm and 940 nm (Wang *et al.,* 2009). Chlorophyll content was expressed in at Leaf units.

Data analyses

Data collected from both spectrophotometric (SPM) and portable chlorophyll meter (PCM) methods were statistically assessed, using paired sample t-test, to show variations between the two methods. Meanwhile, variations between the control and the corresponding treated groups were tested using two-way-Analysis-of-Variance followed by post-hoc analyses. Simple linear regression analyses were carried out to assess the interrelationship between both SPM and PCM methods in the different wheat cultivars, under study, at different doses of polarized light. Statistical analyses were carried out using IBM SPSS Statistic software release 22.0.

RESULTS

Seed pre-treatment with monochromatic polarized light of wavelength 632.8 nm and power intensity 0.5 $mW.cm^{-2}$ induced a significant increase in chlorophyll content in all cultivars determined by both extractable spectrophotometric method (SPM) and portable chlorophyll meter method (PCM) (Table 2 and Fig. 1A-J).

Total extractable chlorophyll contents (SPM) results indicated that monochromatic red polarized laser light induced a significant increase in chlorophyll at all doses in wheat cultivars Sakha-93, 94, 95 and Giza-168; at all doses except in Sids-1 and 120s in Gemmiza-9. However, in case of portable chlorophyll meter (PCM) red polarized light induced a significant increase in chlorophyll content at all doses (times of exposure) in cultivar Sakha-93; at all doses except 600s in Gemmiza-9, 1200s in Sakha-95 and 10, 300, 1200s in Sids-1, where the results were around the control. The relatively small doses of red polarized light only induced significant increases in chlorophyll content in Sakha-94 (1-120s) and in Gemmiza-168 (1-30s). In general, the results showed that Sakha-94 and Sakha-95 were the cultivars most affected He-Ne laser treatments and that 60- 300s were the most efficient doses.

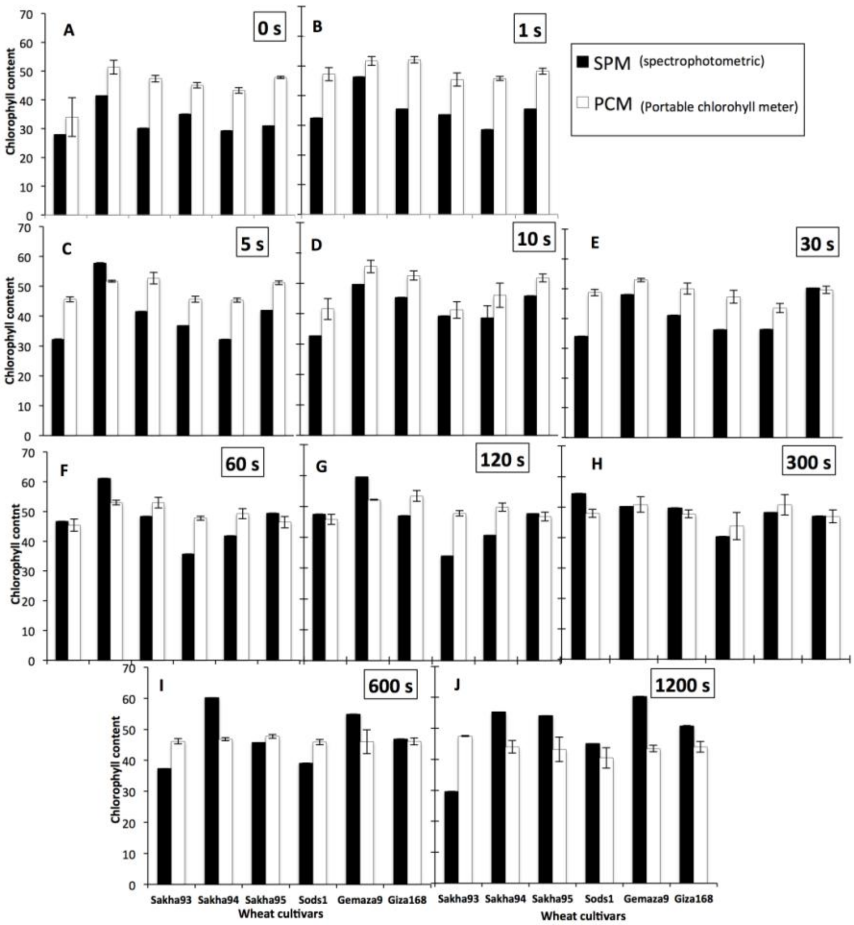

Figure (1 A-J): Chlorophyll contents using the spectrophotometric (SPM) and the portable chlorophyll meter (PCM) methods after seed pre-sowing treatments of six wheat cultivars with different doses (exposure times for 0, 1, 5, 10, 30, 60, 120, 300, 600 AND 1200 seconds) of monochromatic red polarized light Each value is a mean of 5 replicates. The bars on the columns indicate standard errors.

Quantification of chlorophyll contents using portable chlorophyll meter (PCM) method afforded high reading values for total chlorophyll for most of the six wheat cultivars studied throughout the entire experiment in the control groups (34.03, 51.40, 47.40, 45.06, 43.30 and 47.80 for cultivars Sakha-93, Sakha-94, Sakha-95, Sids-1, Gemmiza-9 and Giza-168; respectively). However, spectrophotometric method (SPM) showed less reading values (27.87, 41.41, 30.14, 35.06, 29.20 and 31.0 for the six wheat cultivars, respectively) (Table 2). Pooled data of six wheat cultivars for the ten doses of monochromatic red polarized light are presented in Figure 2. In all cases a strong significant inter relationship (r=0.96, *p*-value=0.000*) were observed between the pooled data of SPM and PCM and the readings of total chlorophyll contents per unit leaf area (Figure, 2). Wheat cultivars except for cultivars Sakha-94 showed strong significant correlation between SPM and PCM assessed by paired sample t-test (t) (Table 3).

Figure (1A–J) represents comparative changes in chlorophyll contents by each of SPM and PCM for the six wheat cultivars after pre-irradiation with monochromatic red polarized light of 632.8 nm. However, Table 2 shows various statistical analyses of variations in SPM and PCM following pre-treatment with polarized light.

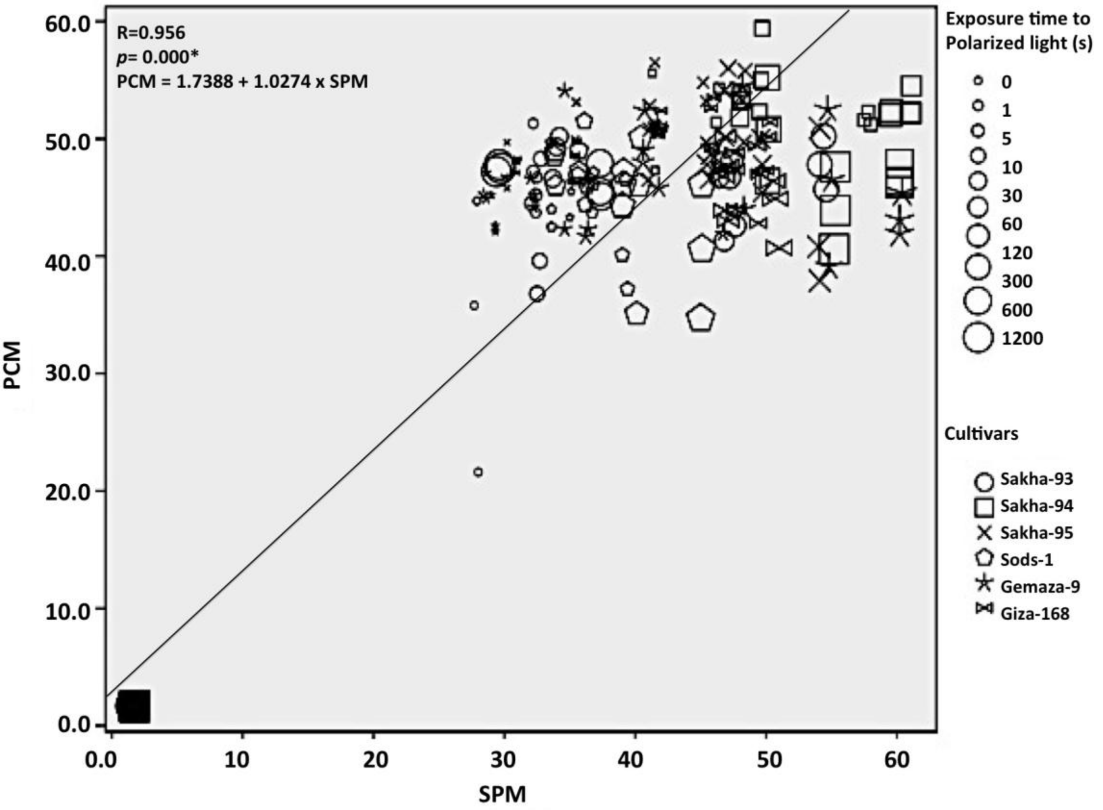

Figure (2): Linear regression trendline showing the interrelationship between the two different methods: the spectrophotometric (SPM) and the portable chlorophyll meter (PCM) for quantification of chlorophyll contents after seed pre-treatments of six wheat cultivars with different doses of (exposure times) of monochromatic red polarized light (data presented based on simple linear regression analyses followed by two-tailed significance test at 0.05 level).

DISCUSSION

Two methods were used, in the present study, for the determination of chlorophyll contents in leaves (from 30-day-old plants) of six cultivars of wheat (*Triticum aestivum*) namely: Sakha 93, Sakha 94, Sakha 95, Sids 1, Gemmiza 9 and Giza 168. Thus, the commonly used spectrophotometric method (SPM) was compared with that of using a direct reading portable chlorophyll meter (PCM) based on SPAD. To expand the range of variables, chlorophyll contents were enhanced in response to pre-sowing seed treatments with monochromatic red polarized light (632.8 nm) applied at different doses (9 different exposure times from 1- 1200 second) and compared with those of corresponding controls (non-irradiated seeds). The results obtained showed that the two applied chlorophyll meter methods showed approximately similar trends in leaves of the six wheat cultivars, under study, either under the influence of different expositions to monochromatic red polarized light or in absence of seed treatment (controls). The accuracy of the portable chlorophyll meter (PCM) was slightly decreased at high chlorophyll contents.

Table(2): Chlorophyll contents of leaves of six *Triticum aestivum* cultivars (30-day-old), obtained by the spectrometric method (SPM expressed as mg/g leaf tissue) and the portable chlorophyll meter (PCM, expressed as AtLeaf "SPAD" units) after pre-sowing seed treatments with different doses (exposure times) of monochromatic red polarized light (632.8 nm). Exposition time 0 represents the controls. Each value is the mean of 5 replicates ±standard error.

Polarized Light Exposure Time (s)	Sakha-93				Sakha-94				Sakha-95				Sids-1				Gemmiza-9				Giza-168			
	SPM		PCM		SPM		PCM		SPM		PCM		SPM		PCM		SPM		PCM		SPM		PCM	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
0	27.8	±0.09	34.0	±6.73	41.4	±0.06	51.4	±2.40	30.1	±0.07	47.4	±1.18	35.0	±0.03	45.0	±0.92	29.2	±0.10	43.3	±0.96	31.0	±0.03	47.8	±0.36
1	32.3*	±0.10	47.2*	±2.21	46.2*	±0.12	51.6	±1.48	35.4	±0.03	52.0	±1.07	33.6*	±0.04	45.4	±2.19	28.5	±0.07	45.7	±0.68	35.5*	±0.03	48.2	±1.03
5	32.2*	±0.12	45.6*	±0.81	57.8*	±0.14	51.7	±0.32	41.5	±0.03	52.7	±1.91	36.8*	±0.04	45.6	±1.01	32.1	±0.09	45.3	±0.7	41.9*	±0.05	51.2	±0.62
10	32.6*	±0.09	41.5	±3.46	49.6*	±0.03	55.6	±2.07	45.3	±0.07	52.6	±1.51	39.2*	±0.12	41.2	±2.75	38.7*	±4.03	46.1	±4.0	45.9*	±0.06	51.8	±1.31
30	33.9*	±0.14	48.7*	±1.08	48.1*	±0.03	52.9	±0.58	41.0	±0.03	50.0	±1.86	36.2*	±0.06	47.3	±2.15	36.3*	±0.07	43.5	±1.51	50.2*	±0.09	49.6	±1.22
60	46.6*	±0.12	45.3*	±2.05	61.1*	±0.04	53.0	±0.77	48.3	±0.06	52.9	±1.77	35.7*	±0.03	47.7	±0.67	41.8*	±0.04	49.2	±1.72	49.4*	±0.05	46.4	±1.93
120	47.4*	±0.12	45.8*	±1.65	59.6*	±0.03	52.2	±0.12	46.9	±0.09	53.4	±1.74	33.8*	±0.03	47.8	±0.91	40.6*	±0.04	49.8	±1.33	47.6*	±0.03	46.7	±1.43
300	54.3*	±0.14	47.9*	±1.30	50.1*	±0.03	50.8	±2.54	49.6	±0.03	47.7	±1.33	40.3*	±0.09	43.7	±4.48	48.2*	±0.09	50.7*	±3.36	46.9*	±0.09	46.9	±2.1
600	37.3*	±0.01	46.2*	±0.87	60.2*	±0.01	46.8*	±0.54	45.6	±0.09	47.7	±0.61	39.1*	±0.04	45.8	±0.85	54.8*	±0.09	46.0	±3.88	46.8*	±0.10	46.1	±1.15
1200	29.5*	±0.09	47.5*	±0.17	55.3*	±0.03	44.0*	±2.02	54.0	±0.03	43.2	±3.94	45.1*	±0.03	40.4	±3.29	60.3*	±0.06	43.4	±1.03	50.8*	±0.13	44.0	±1.71

Tables (3): Paired sample t-test statistic for the values of chlorophyll obtained with both methods (SPM and PCM) for the six studied wheat cultivars.

Cultivars	Mean	SD	SE	t	df	*Sig. (2-tailed)*
1. *T. aestivum* L. cv. Sakha93	-7.55	8.86	1.62	-4.668	29	0.000 *
2. *T. aestivum* L. cv. Sakha94	1.93	8.26	1.51	1.277	29	0.212
3. *T. aestivum* L. cv. Sakha95	-6.15	8.58	1.57	-3.927	29	0.000 *
4. *T. aestivum* L. cv. Sids1	-7.55	6.41	1.17	-6.453	29	0.000 *
5. *T. aestivum* L. cv. Gemmiza9	-5.27	11.03	2.01	-2.616	29	0.014 *
6. *T. aestivum* L. cv. Giza168	-3.29	7.58	1.38	-2.374	29	0.024 *

This conclusion was found to be in accordance with those of Richardson *et al.* (2002) and Monge & Bugbee (1992). However, at low and moderate SPM values, the PCM measures provided a good estimate for chlorophyll contents, these results agreed with those of Van-den-Berg and Perkins (2004). Thus, the present results proved that, on using the PCM method, the relationship between chlorophyll values were linear for the low and moderate concentrations of but were more or less non-linear for the relatively high chlorophyll levels; a conclusion that could be further reinforced by that of Silla *et al.* (2010). One potential limitation of portable chlorophyll-meters or SPAD-meters is that at high levels of chlorophyll, so much of the 650–660 nm light is absorbed by the leaf and little remains to be transmitted and measured by the meter (Richardson *et al.*, 2002; Silla *et al.*, 2010), and likely the values are mainly determined by differences in transmittance at 930–940 nm.

Our data showed that the relationship between PCM and SPM chlorophyll content depended on the exposition dose of monochromatic red polarized light that would be predicted to induce chlorophyll enhancement. Monochromatic red polarized light photobiostimulation was discussed due to the increase in energy supply of seeds. In such cases, the photon energy of laser radiation was absorbed by chlorophyll and directly affected the photosynthetic intensity (Abu-Elsaoud and Tuleukhanov, 2009). The illumination of biological tissue by coherent laser light leads to strong intensity gradients of the radiation in the tissue due to speckle formation, which causes intercellular and intracellular gradient forces whose action may significantly influence the paths and speeds of biological processes (Ruvinov, 2003). However, the results obtained, in the present work, showing the effects of treatments with red polarized light are in agreement with those of Chen *et al.* (2005) on the influence of laser irradiation of 632.8 nm on seeds and seedlings of *Isatis indogotica*. These authors showed significant enhancement and improvement of various seedlings physiological parameters including chlorophyll concentration.

On the bases of the results obtained in the present work it might be concluded that significant differences in PCM values were found between different cultivar leaves as a result of seed pretreated with red polarized light plants. Environmental factors are known to affect leaf morphology, which in turn affects foliar optical properties, and can be expected to affect SPAD values (Pinkard *et al.*, 2006).

Thus, it might be concluded that the use of PCM (SPAD) chlorophyll meters represents an important tool for monitoring chlorophyll contents in time-consuming physiological and agricultural experiments as well as the field of agriculture to monitor crop nutritional status. The use of portable chlorophyll meters (PCM) is limited to our experimental conditions with common wheat cultivars in Egypt.

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تقييم جهاز الكلوروفيل ميتر في تقدير محتوي الكلوروفيل للقمح المصري تحت تأثير الاستحثاث الضوئي بالضوء الأحمر المستقطب

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الملخص العربي

تهدف الدراسة الحالية الي عمل دراسة مقارنة لتقييم طريقتين لقياس الكلوروفيل في أصناف مختارة من القمح المصري تحت تأثير الضوء الأحمر المستقطب وهما: (١) باستخدام إستخلاص الكلوروفيل وجهاز قياس الأطياف (الإسبكتروفوتوميتر)، (٢) بإستخدام جهاز الكلوروفيل ميتر المتنقل. تم معالجة بذور ستة أصناف من القمح قبل زراعتها بجرعات مختلفة (زمن تعريض البذور من ١ الي ١٢٠٠ ثانية) بالضوء الأحمر المستقطب وحيد اللون ذو طول موجي ٦٣٢,٨ نانوميتر وكثافة الطاقة ٠,٥ ملي.وات.سم$^{-٢}$. تم تقدير محتوي الكلوروفيل في أوراق القمح للنباتات المعالجة بالضوء المستقطب والكنترول الغير معالج بعد أسبوعين من الزراعة بإستخدام الطريقتين المقترحتين (الكلوروفيل ميتر والإسبكتروفوتوميتر). وقد أظهرت النتائج المتحصل عليها ب إستخدام الإسبكتروفوتوميتر أن الضوء المستقطب تسبب في زيادة معنوية في محتوي الكلوروفيل في كل أصناف القمح التي تم اختبارها (سخا-٩٣، سخا-٩٤، سخا-٩٥، جميزة-٩، جيزة-١٦٤، سدس-١) مقارنةً بالكنترول الغير معالج بالضوء المستقطب. وقد لوحظ نفس إتجاه النتائج باستخدام الكلوروفيل ميتر وهو زيادة محتوي الكلوروفيل في كل الجرعات في النباتات المعالجة مقارنةً بالكنترول عدا صنفي سخا-٩٤ وجيزة-١٦٨. بصفة عامة ادت الجرعات المختلفة من الضوء الأحمر المستقطب الي زيادة معنوية في عوامل النمو والإنبات ومحتوي الكلوروفيل (بالطريقتين الاسبتكروفوتوميتر والكلوروفيل ميتر) في الاصناف الست المختبرة. وقد أثبتت الدراسة ان كلتا الطريقتين كانتا فعَالتين لتقدير محتوي الكلوروفيل في أصناف القمح التي تم اختبارها و أن العلاقة بينهما كانت معنوية. ويمكن التوصية بإستخدام الكلوروفيل ميتر بشكل فعَال في متابعة أغلب أصناف القمح المعالجة بالضوء المستقطب.