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Deliverable 4.424 A

Intelligent on-line system for monitoring and controlling the efficiency of the SSL-crop system, Part I

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Deliverable 4.424 A – Intelligent on-line system for monitoring and controlling the efficiency of the SSL-crop system, Part I

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List of abbreviations

ΦPSII:	Quantum yield of Photosystem II
PAR:	Photosynthetically active radiation
Fv/Fm:	Maximum quantum yield of Photosystem II in dark adapted conditions
ETR:	Electron transport rate

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SECTION 1 – Introduction

Measuring photosynthetic efficiency

In plants, light is used in photosynthesis to convert CO_2 into carbohydrates for growth and development. The light use efficiency depends on various factors, such as (a)biotic stresses, light intensity and plant water status. However, it is unknown how efficiently plants use light in general but also different light colours during the day. Given ideal conditions in a controlled greenhouse environment, light intensity and colour are the main factors influencing light use efficiency. To be able to determine whether assimilation light is used with maximum efficiency, continuous monitoring of photosynthetic parameters is necessary.

A commonly used tool to assess light use efficiency, is chlorophyll fluorescence. Fluorescence from Photosystem II (ΦPSII) is an important parameter since it gives an indication how efficient light is used in photosynthesis during the light period, and also provides information about the condition of PSII when measured in the dark period.

This deliverable describes the results of continuous monitoring of the light use efficiency to determine whether the light use efficiency varies over the day. The tool used to do this, is the Micro Moni Set, which uses small Micro Moni PAM sensors which can stay on the leaf to measure continuously.

Photosynthesis can also be assessed with gas exchange measurements. However, the equipment to measure gas exchange is large and complex and takes away light from lower leaves, making it unsuitable for continuous measurements. The Micro Moni PAM sensor measurements were compared to the gas exchange measurements. Since the tomato plants used grow quite fast, the sensors needed to be relocated after a certain period, to avoid a shade effect on the measurements. In addition, leaves may get damaged by the clip of the sensor. A protocol was provided for relocating the sensors in case any of the above applies.

Research questions

1. How efficiently can plants use different colours of light during the day?
2. Does monitoring photosynthetic efficiency with fluorescence techniques provide similar results when compared to gas exchange measurements?
3. What measurement protocol should be used when assessing photosynthetic efficiency with fluorescence measurements?

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SECTION 2 – Materials and methods

Setup of the experiment

In this subtask, 2 experiments were conducted under controlled environmental conditions (see also Deliverable 4.4.21). In the first experiment, tomato (cv. Ingar) plants were treated with different spectral compositions. Under a background of sunlight, additional LED light was provided at different wavelengths. white (day-light spectrum), red (660 nm), blue (450 nm), green (510 nm), amber (580 nm) and red plus 12% blue (commercial reference). White LED contains all colours of the spectrum between 400 and 700 nm in a ratio of 13% blue (400-500 nm), 55% green (500-600 nm) and 32% red (600-700 nm). Plants received 200 µmol m^{-2} s^{-1} LED light during 15 hours per day, under a background of maximum 20% sunlight. On average the plants received 4% sunlight, integrated over the experimental period. The amount of sunlight was controlled by using screens with light transmissions of 30% and 5% (OLS 60 screen and XLS SL 95 Revolux screen, Ludvig Svensson, Sweden). During the experiments, measurements of light levels were done to establish the absolute LED and sunlight levels. The light level of the LEDs was set and checked carefully to be 200±5 µmol m^{-2} s^{-1}. Average day light sums were similar for all treatments.

The treatments were divided over 2 compartments with 3 light treatments each (Figure 1). The climate in both greenhouse compartments was similar. The average temperature was 20.4°C (21.0° C and 19.3° C during light and dark period respectively). CO_2 concentration was on average 540 ppm during the light period and relative humidity was 70%.

The plants were placed on tables measuring 1.45 * 3.5 meter. In each compartment, 6 sensors were used to measure photosynthetic efficiency. The photosynthetic efficiency was monitored for at least two weeks.

In the second experiment the setup is similar, but different light treatments were applied. In this experiment, plants received the same amount of LED and sunlight compared to experiment 1. However, light spectra and treatments are different. In the second experiment, blue light was provided at the start of the light period during 2 hours at 3 different light intensities of 200, 100 and 20 µmol m^{-2} s^{-1} respectively, followed by the standard light treatment of 200 µmol m^{-2} s^{-1} red/amber for the remaining 13 hours of the photoperiod. In the reference treatment, 200 µmol m^{-2} s^{-1} white light was provided for 15 hours. All plants received 1 hour daylight before sunset where the LED light was switched off, resulting in a photoperiod of 16 hours. The climate in both compartments was the same: average temperature was 21.0°C (21.5° C and 19.8° C during light and dark period respectively). CO_2 concentration was 490 ppm during the photoperiod and relative humidity was on average 71%.

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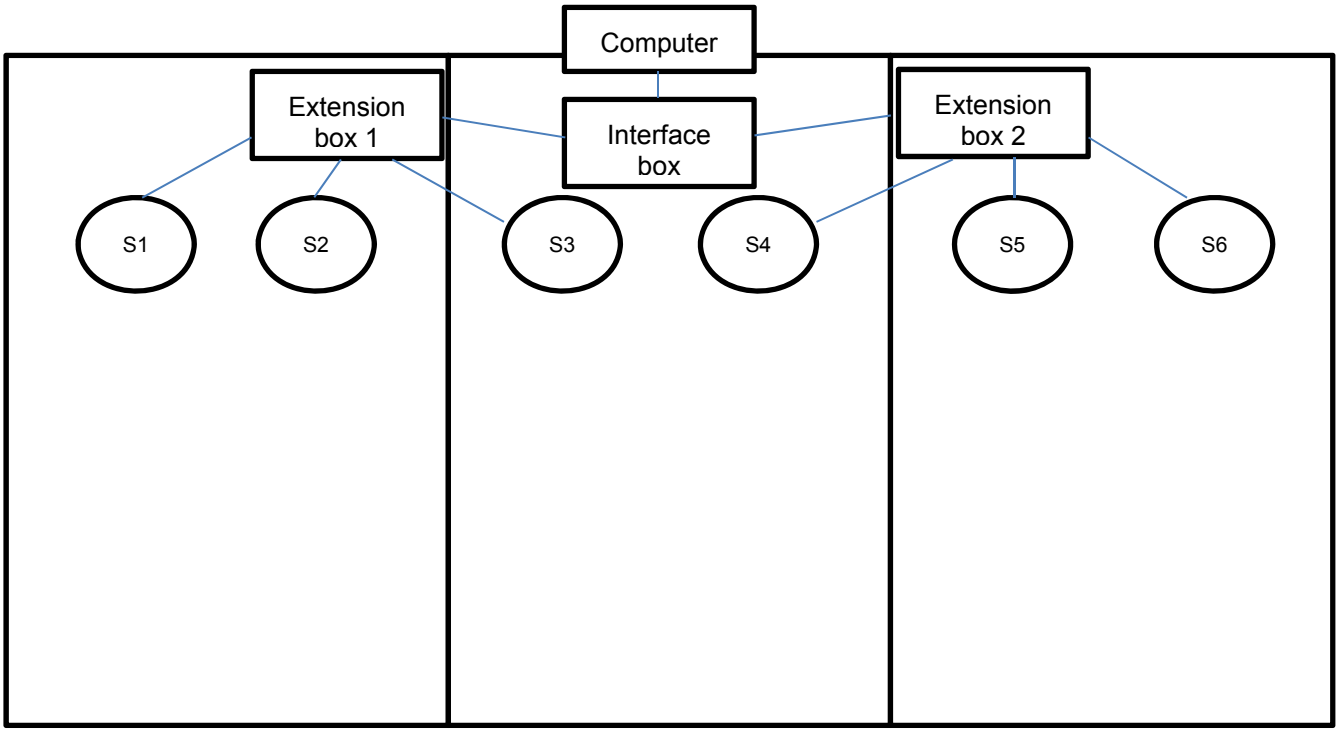

Figure 1. Layout of one of the compartments. 3 sensors are connected to 2 extension boxes, to have 2 sensors in each light treatment.

Fluorescence measurements

Fluorescence measurements were taken continuously for approximately two weeks during the light period in each experiment. From those measurements, different parameters were calculated, such as ΦPSII, which is the quantum yield of photochemistry in PSII [1]; Furthermore, PAR intensity is continuously measured alongside fluorescence to be able to calculate the electron transport rate (ETR). In addition, dark adapted F_v/F_m measurements were taken during night, to assess the maximum PSII quantum yield. In the variable blue experiment, dark adapted fluorescence was measured at 2:00 AM each night.

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Micro-Moni-set

The Micro-Moni-set is used to measure both fluorescence and PAR levels (Figure 2). The sensors were supported by a flexible arm to keep the sensor and leaves in horizontal position towards the light source. In each treatment, 2 sensors were applied.

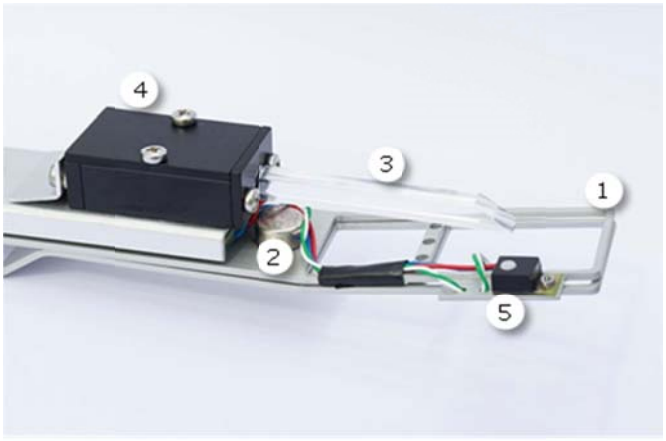

Figure 2. Micro-Moni-PAM: the measuring device or sensor of the Micro-Moni-Set.

The Micro-Moni-PAM consists of:

1. Leaf clip, with a fixed upper part and a removable lower part
2. Magnet to clamp on lower part
3. Light conductor to measure chlorophyll fluorescence
4. Sensor electronics
5. External PAR sensor

Gas exchange measurements:

Leaf photosynthesis was measured in experiment 1 using a portable photosynthesis system (Li-6400XT, LI-COR). Actual leaf photosynthesis was measured using a clear leaf chamber. The leaf chamber was positioned such that 200 µmol m^{-2} s^{-1} of LED light of the treatment fell on the leaf enclosed in the leaf chamber. Measurements were taken when photosynthesis rate and conductance were stable on the fifth leaf of 8 plants per treatment.

Statistical analysis

Data are presented as averages ± standard error of the mean (SEM) per treatment. Fisher's protected least significance test was used to make post-hoc multiple comparisons among means from significant analysis of variance (ANOVA) tests. P-values smaller than 0.05 were considered significant. For the statistical analyses, GraphPad Prism was used (GraphPad Software, Inc., CA, USA).

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SECTION 3 – Results constant spectrum

Fluorescence results

ΦPSII is significantly lower for the continuous red and green treatments at light intensities between 110 and 120 µmol m^{-2} s^{-1} (Figure 3). For red and green light the ΦPSII is 0.65 and 0.63 respectively which is significantly lower compared to the other colours. The other colours showed similar ΦPSII values ranging from 0.69-0.70.

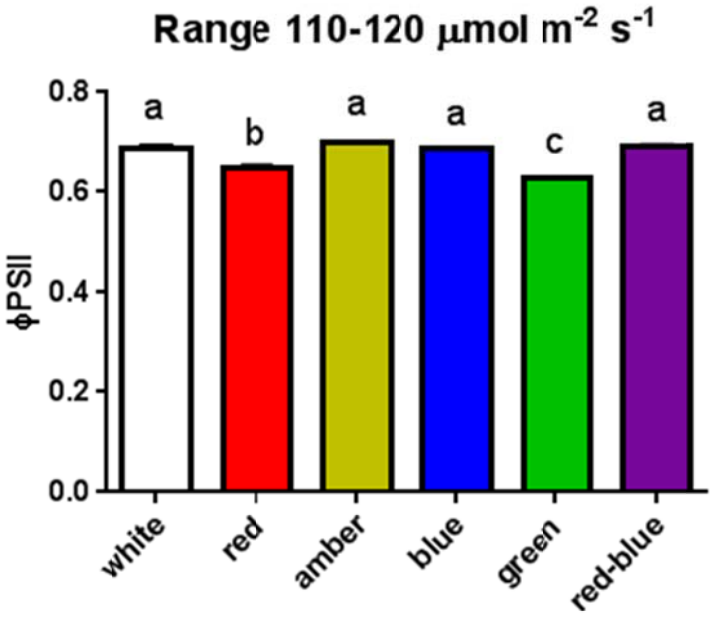

Figure 3. ΦPSII in a low intensity range of 110 to 120 µmol m^{-2} s^{-1}.
Different letters indicate significant differences.

At higher intensity levels, the ΦPSII values are very similar (Figure 4). Both white and green light treated plants have ΦPSII values of 0.65, and the red, blue and red/blue ΦPSII levels were slightly lower around 0.64. The ΦPSII value for amber was the lowest with 0.63.

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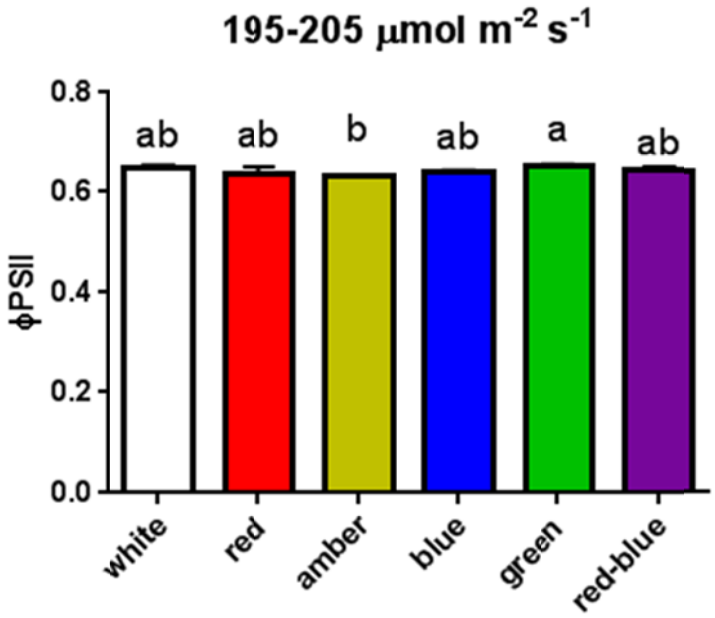

Figure 4. ΦPSII the treatment intensity range of 195 to 210 µmol m^{-2} s^{-1}. Different letters indicate significant differences.

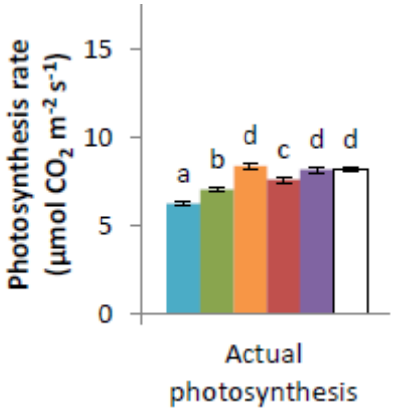

Figure 5. Average photosynthesis rates from gas exchange measurements. Different letters indicate significant differences.

Comparison with gas exchange measurements

Gas exchange measurements differ substantially from the fluorescence measurements. From the gas exchange measurements, clear differences can be observed between treatments. Highest photosynthetic rates were found for the amber, red/blue and white light treatments (Figure 5). Leaves under red light had slightly slower photosynthetic rates, but the leaves under continuous green and blue light had the lowest rates.

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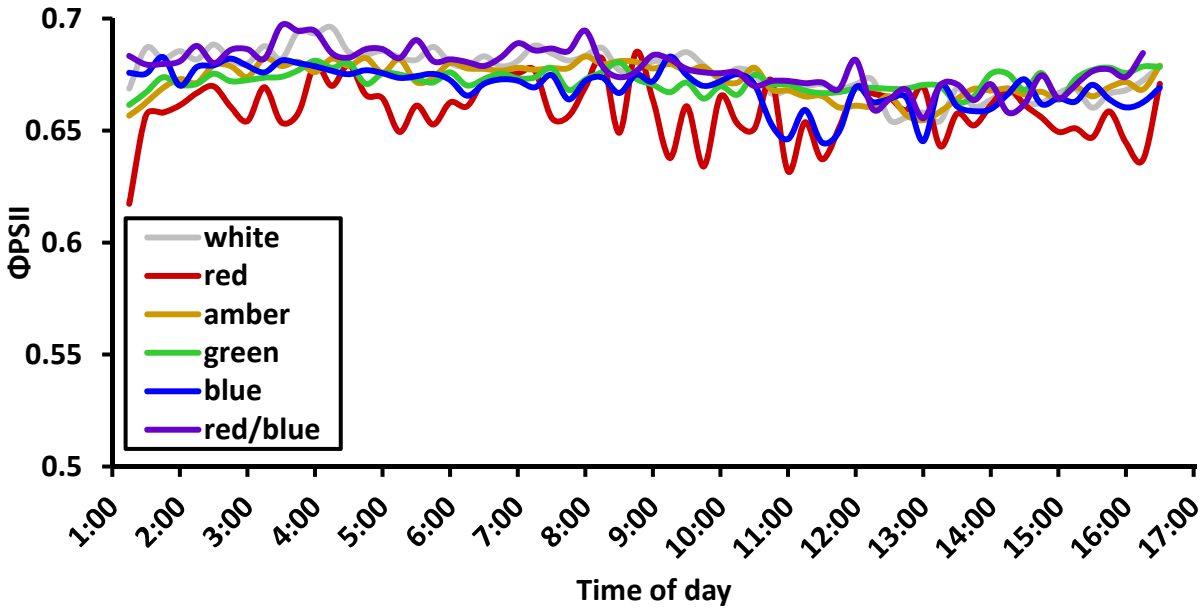

Figure 6. Cyclic average of ΦPSII based on 15 days of continuous measurements during the light period.

Red and red/blue treatments show large variation in ΦPSII during the day (Figure 6). This can be explained by large variation between periods the sensor was attached to a particular leaf (Table 1). On January 15, 20, 22 and 26, the sensors were switched to another leaf. For example, in the first period in the red/blue treatment, the leaf appeared to be damaged resulting in photoinhibition which leads to reduced ΦPSII values (Figure 6). It was found that different leaves have varying ΦPSII levels. Leaves with systematically lower ΦPSII values reduce the cyclic average. While difficult to see in Figure 6, the ΦPSII value decreases slightly after 8:00. To determine whether a leaf is subjected to photoinhibition, F_v/F_m was measured during the night. Typical values of a healthy leaf are above 0.8, and in this experiment healthy leaves showed consistently values around 0.82-0.83. In the period of January 16-20 where the average ΦPSII was 0.61 for red/blue (Figure 7). F_v/F_m values were reduced as well with an average of 0.72.

Table 1. Average ΦPSII values for each period the sensor was attached to a different leaf. Underlined are the values lower than 0.66 possibly indicating a stressed or damaged leaf.

	Treatment					
Period	**white**	**red**	**amber**	**green**	**blue**	**red/blue**
Jan 16-20	0.68	0.65	0.67	0.66	0.68	<u>0.61</u>
Jan 21-22	0.67	0.66	0.68	0.67	0.68	0.67
Jan 23-26	0.67	0.66	0.69	0.68	0.65	<u>0.62</u>
Jan 27-29	0.69	0.70	0.67	0.70	0.69	0.68
average	0.68	0.67	0.68	0.68	0.67	0.64

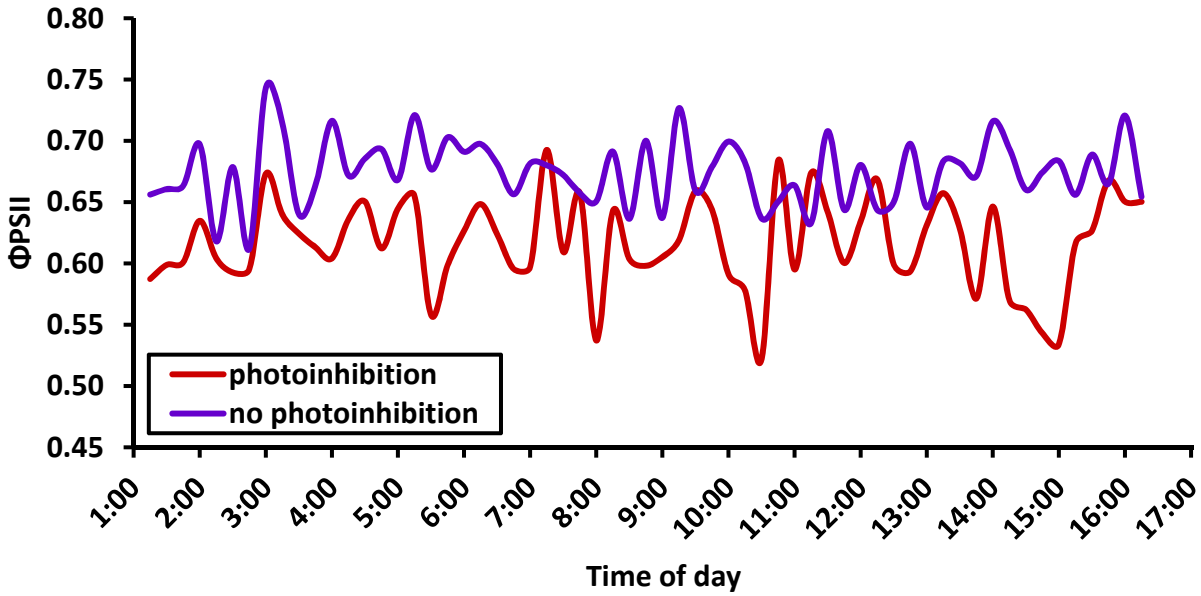

Figure 7. Comparison between a typical pattern of ΦPSII during the light period when photoinhibition occurs due to leaf damage (red line) and a healthy leaf (purple line).

Calculated electron transport rate (ETR) is linearly related to the PAR intensity (Figure 8). All treatments have a similar relationship.

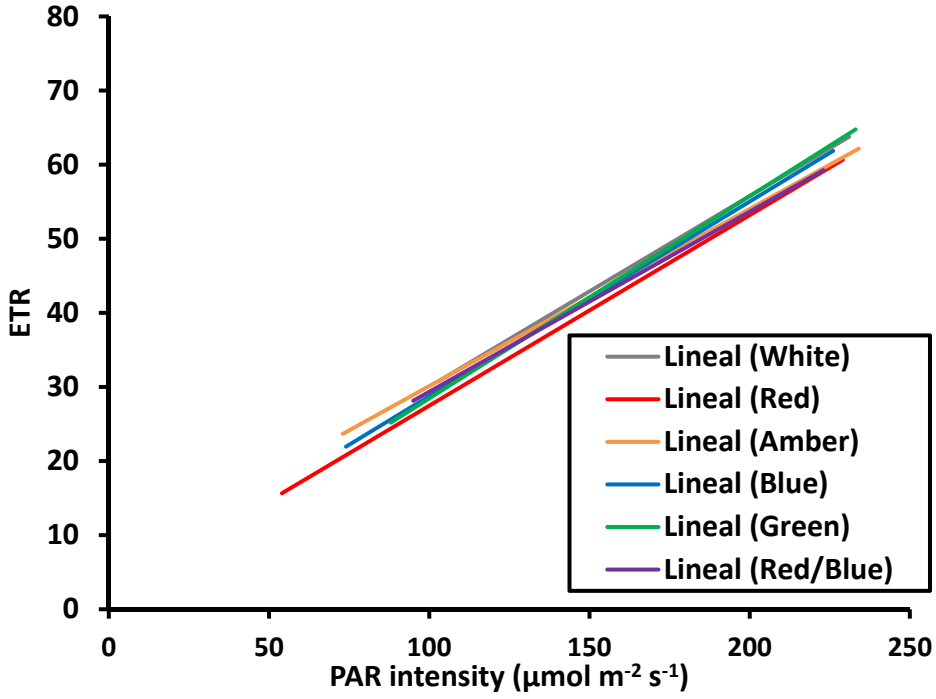

Figure 8. Relationships between electron transport rate and PAR intensity.

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SECTION 4 – Results variable spectrum blue

Fluorescence results

In this experiment, blue light was provided at the start of the light period during 2 hours at 3 different light intensities of 200, 100 and 20 µmol m^{-2} s^{-1} respectively, followed by the standard light treatment of 200 µmol m^{-2} s^{-1} red/amber for the remaining 13 hours of the photoperiod. In the reference treatment, 200 µmol m^{-2} s^{-1} white light was provided for 15 hours.

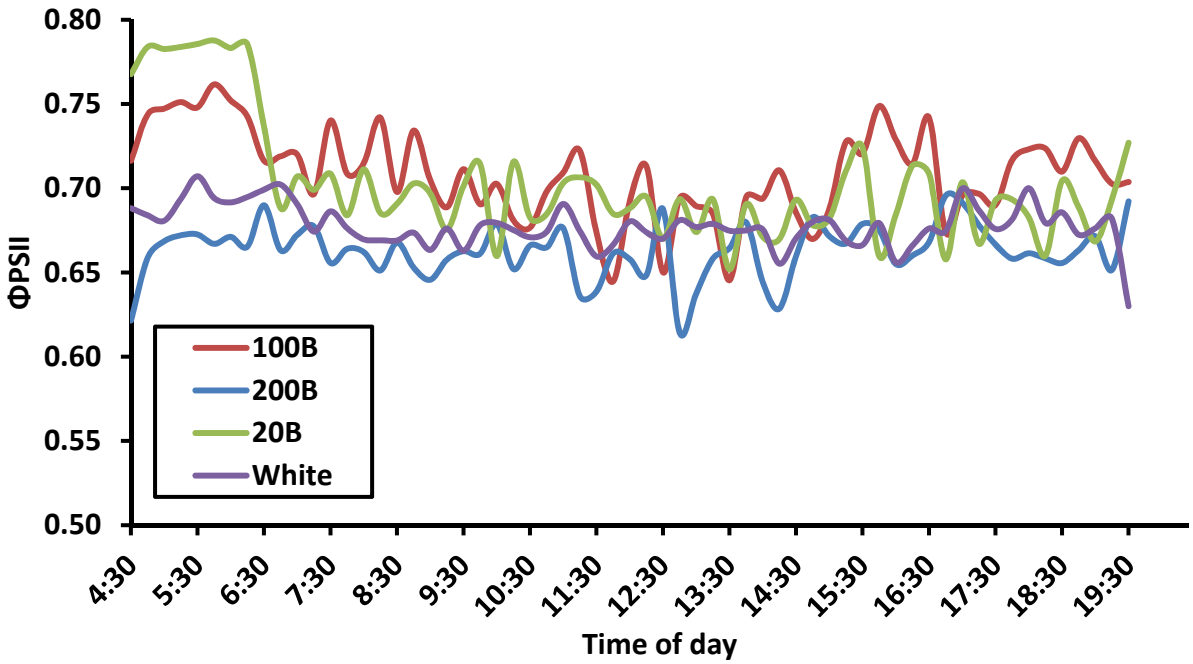

Figure 9. Cyclic averages of ΦPSII for the blue light experiment, monitored for two weeks.

The period of blue light had a clear effect on the ΦPSII (Figure 9). It was highest in the first 2 hours for the leaves under the lowest amount of blue light, followed by the 100 µmol m^{-2} s^{-1} which is expected since ΦPSII depends on the PAR intensity. The leaves under 2 hours of 200 µmol m^{-2} s^{-1} had lower ΦPSII values compared to the reference treatment. Average ΦPSII values were similar for all treatments, except for the 200 blue treatment which showed a decreased ΦPSII in 3 out of 4 periods (Table 2).

Table 2. Average ΦPSII values for each period the sensor was attached to a different leaf.

	Treatment			
Period	**100 blue**	**200 blue**	**20 blue**	**white**
April 8-10	0.68	0.64	0.70	0.68
April 10-13	0.71	0.66	0.70	0.70
April 14-16	0.73	0.65	0.69	0.69
April 17-20	0.70	0.69	0.69	0.68
Average	0.70	0.66	0.70	0.69

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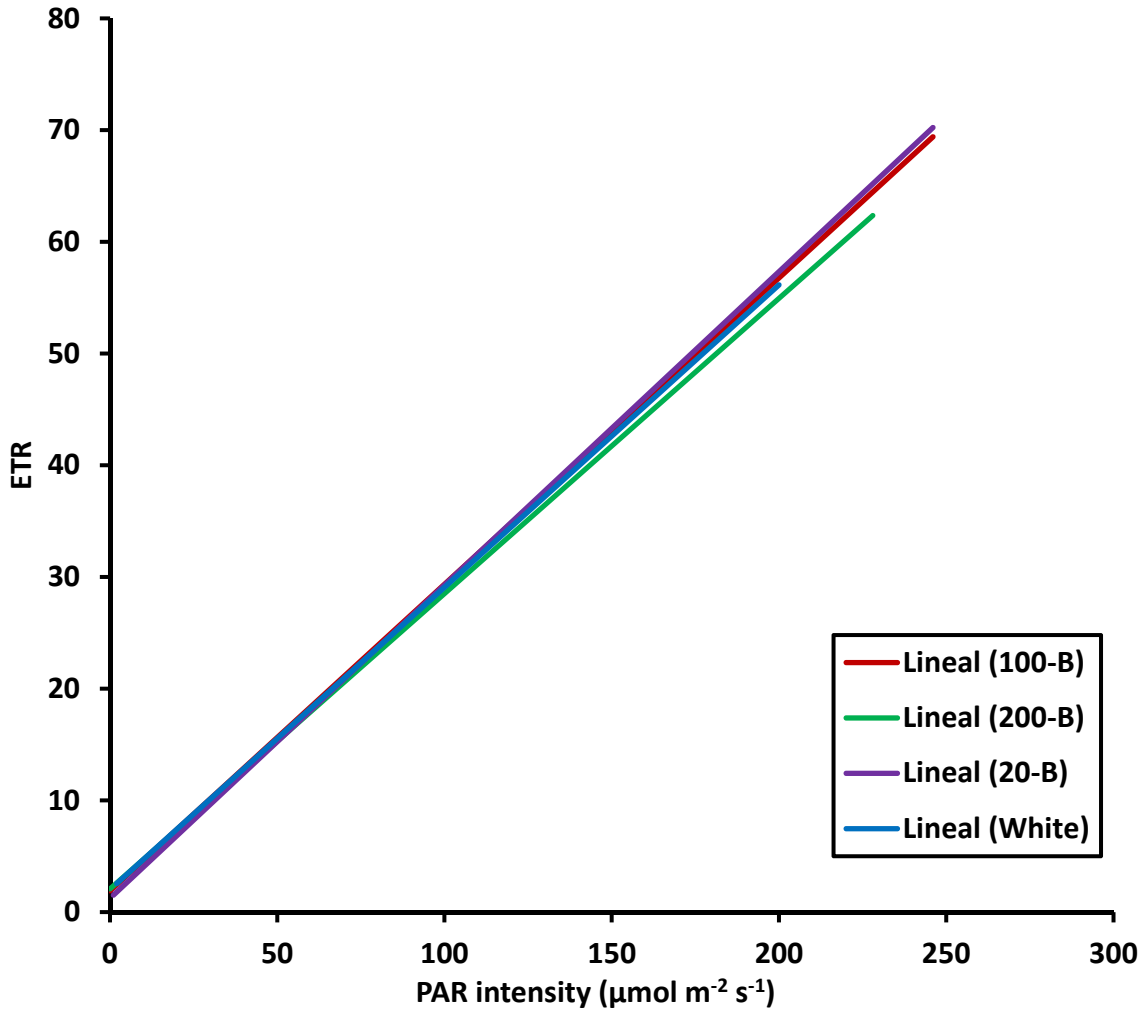

Figure 10. Relationships between electron transport rate and PAR intensity for the variable blue treatment.

F_v/F_m values during night are 0.83 for 100B and 0.82 for 200B, 20B and white light respectively. This indicates that even though the daytime ΦPSII levels are reduced, maximum ΦPSII efficiency was not reduced. The ETR values were similar for all treatments (Figure 10).

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SECTION 5 – Discussion

Constant spectrum

The ΦPSII values in the red/blue treatment show more variation compared to the other treatments. This is mainly due to periods of low ΦPSII caused by leaf damage. It is recommended that the sensors are changed when the dark adapted F_v/F_m measurement is lower than 0.8, since values of around 0.82-0.83 can be expected for healthy leaves.
Overall, the sensors performed well, but careful handling of the leaves is necessary to obtain good measurements.
However, when the Micro-Moni-PAM measurements are compared to gas exchange measurements, a discrepancy is observed. This is due to the fact that ΦPSII is not always the only determining factor of the photosynthetic rate. Some wavelengths are absorbed less efficiently by chlorophyll, resulting in a lower availability of electrons for photosynthesis[2,3]. Red light is most efficient in driving photosynthesis, but a small amount of blue is necessary for normal functioning[4]. It is important to note that the availability of a small amount of sunlight in these experiments already provides some blue light, resulting in good performance under red LED light.
Blue, green and amber light is also absorbed by non-photosynthetic pigments, resulting in lower photosynthetic rates. Fluorescence measurement is not the right tool to determine photosynthetic rates, since information about the amount of absorption of certain wavelengths of light by the leaf, is missing.
In conclusion, measuring solely fluorescence is not sufficient to determine the photosynthetic rates. However, leaf damage and shadowing, which was prevented by switching to different leaves every couple of days, can be measured with the Micro-Moni-PAM sensors. Another important consideration is that in this experiment, light levels were kept constant and between treatments. Since light intensity is the main determinant for ΦPSII, it was expected that ΦPSII values were similar between light treatments.

Variable blue spectrum

The results from the variable blue spectrum treatments were very similar compared to the constant spectrum treatments. A clear effect of blue light intensity at the beginning of the light period was observed. ΦPSII depended mostly on the blue light intensity. In addition it was shown that a blue light intensity similar to the reference white light intensity reduced ΦPSII. This can be explained by the fact that a larger fraction of blue light is absorbed by non-photosynthetic pigments, resulting in a lower ΦPSII.

Measurement protocol

A key element in successful monitoring of ΦPSII, is the proper and careful application of the sensors to the leaves. Tomato plants used in this experiment, has fragile leaves requiring great care. Regarding actual measurements, the following rules are determined based on these experiments:

1. A leaf is damaged or stressed when dark adapted F_v/F_m values are systematically below 0.8. This can be caused by damage due to the sensor, or when the light quantity or quality are not suitable for the leaf.
2. Measuring at least 2 leaves per treatment simultaneously is necessary. During some periods, one sensor showed lower than expected ΦPSII values due to leaf damage.
3. Daily (automated) analysis of the data is necessary to be able to detect deviation from expected fluorescence values due to shadowing by other leaves, or damage inflicted by the sensor or light spectrum or intensity.

4. An average ΦPSII value during daytime lower than 0.65 meant relocation of the sensor was necessary. However, this is light intensity dependent. The ΦPSII will reduce with increasing light intensity. Therefore, establishing a "normal" value is necessary for every new environment.
5. In this context, a value lower than 0.63 meant damage occurred to the leaf. Similar rules apply as under 4.
6. Normal values for tomato leaves under 200 m^{-2} s^{-1} PAR light are around 0.66-0.70. This can be crop dependent.
7. The rate of relocation of the sensors depends on the developmental rate of the plant. Usually, a tomato plant develops around 3 leaves and a truss each week. To avoid shadowing by emerging leaves, the shortest period a sensor can be attached to a leaf is less than a week. In this research, periods ranging from 2-4 days were tested and no difference was found.

SECTION 6 – References

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