

# Computerised experiments on photosynthesis in plants biology

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**Abstract** – Photosynthesis is the most important photochemical process of conversion of light energy to chemical energy and storing it in the bonds of sugar in plants. The photosynthesis takes place in the chloroplast, where four main photosynthetic pigments called chlorophyll a, chlorophyll b, xanthophylls, and carotenes are found. All these pigments absorb light and utilize the light energy in photosynthesis. Chlorophyll is the dominant pigment in plants which absorb blue and red light. Laboratory experiments with equipment produced in ComLab project for improvement knowledge about photosynthesis process are proposed. In the first presented experiment students investigate influence of color of lighting (red, blue, green and yellow) to the amount of oxygen produced by plant. In the second experiment the chlorophyll concentration in the plants grown in the light of different color (red, blue, green, white, infrared and in the dark) are estimated. Laboratory works were implemented using hardware usually applied in computerized laboratory – data acquisition device, oxygen sensor or carbon dioxide sensor, light emitting diodes of different color, spectrophotometer and a standard software for data analysis. First experiment takes one lesson, and second one takes about one week. Experimental results help students to see influence of the lighting color to photosynthesis processes in the plants.

**Keywords** – Plant biology, photosynthesis, lighting color, chlorophyll content.

## 1. INTRODUCTION

Oxygen is one of the most important materials for all living organism, therefore humans have to understand how plants produce oxygen. Through a process known as photosynthesis, plants use the energy of sunlight to convert atmosphere carbon dioxide and water into simple sugars and oxygen [1]. Therefore, the rate of photosynthesis is determined by number of photons between 400 and 700 nm that the plant absorbs [2]. The process of photosynthesis takes place in the chloroplasts, specifically using green pigments chlorophyll a and chlorophyll b [3]. Chlorophyll looks green because it absorbs red and blue light, so these colors aren't reflected and seen by our eyes. However, it is often thought that the color of light does influence the rate of photosynthesis. But chlorophyll is not the only pigment playing a role in photosynthesis, the most important process for plant growth. Other pigments (carotenes and xanthophylls) are also involved. Research has shown that photosynthesis is the most efficient under red orange light. Lighting with balanced specter is important for normal plant and leaves color development. Blue color is

important for correct regulation of the stomata, but shortage of blue and far-red light causes stem growth and yellowing of leaves [2].

These laboratory experiments give possibility to examine how photosynthesis rate depends on different color of lighting. It is a supplement for other learning methods that improves knowledge of the photosynthesis processes and of factors that contribute to proper plant functioning. In addition, students learn how to use a oxygen sensor, to measure chlorophyll content and to get better understanding of scientific methods.

Photosynthesis rates can be easily measured by an oxygen sensor. Students use equipment and software to measure, calculate and organize data. They frame arguments in quantitative terms when it is possible, they question claims and understand that findings may be interpreted in more than one acceptable way.

## 2. LABORATORY EXPERIMENTS

Our computerized experiments series on photosynthesis in plants biology comprise of two experiments. The goal of first experiment is to investigate photosynthesis rate by measuring oxygen concentration change when plants are illuminated with lighting of different color. Second exercise comprises of two parts: (1) to measure plants leaves color differences when plants are grown in lighting of different color, and (2) to investigate dependency of leaves chlorophyll content on lighting color. These experiments help students to improve their knowledge in plants biology and process of oxygen production.

### 2.1. Materials and methods

#### 2.1.1. Growth condition

Cress (*Lepidium sativum*) seeds were sown in pots filled with natural soil and were grown in laboratory room. The plants which were used to measure photosynthesis rate were illuminated with outside light from windows (without additional lighting). The plants which were used to measure content of chlorophyll were illuminated with different color (white, blue, green, red, IR and dark) diodes. The air temperature was about 25 °C during the day and 20 °C at night. Plants were watered daily with fresh water from tap without additional nutrients. The plants were grown for 6-7 days, and fully developed mature plants were used for experiments.

### 2.1.2. Photosynthesis rate

For measurement of oxygen concentration in air O<sub>2</sub> Gas Sensor (order code 02-BTA or 02-DIN) [4] was used. The Vernier O<sub>2</sub> Gas Sensor is used to monitor gaseous oxygen levels in a variety of biology and chemistry experiments.

Photosynthesis rate was measured as an oxygen concentration change. The plants that were used to measure photosynthesis rate were grown in a laboratory room with natural lighting through windows. The plants were put into plastic transparent containers with airtight lids. The oxygen sensor and four different color (red, blue, green and yellow) diodes were inserted into lid of vessel. An oxygen sensor was connected to channel of e-BioL data acquisition device. This multifunction data acquisition and control device is designed as a computer interface for input of analogue and digital electrical signals and for generation of digital and analogue signals for control.

The oxygen concentration measurement is started when container with plants is covered with lid, but without lighting at beginning (for measurement of initial oxygen concentration). After few minutes the plants are illuminated by two color light diodes (blue and red or green and yellow) and the measurement is continued. The oxygen concentration was measured about 1 hour and measurement is finished when the oxygen concentration achieves stable level. The same work procedures are done with other colors lighting.

The oxygen concentration was calculated as:

$$C (\%) = C_a - C_b$$

where C<sub>b</sub> is O<sub>2</sub> concentration without lighting and C<sub>a</sub> is maximal O<sub>2</sub> when plant is lighted by light diode of specified color.

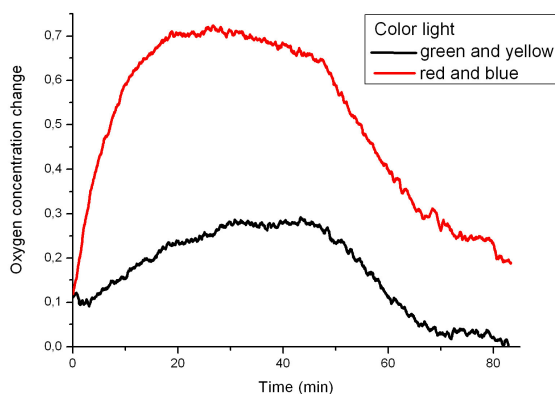


Fig. 1. Oxygen concentration changes when plants are lighted by diodes pairs of different colour..

Results (see Fig. 1) show that oxygen concentration change was maximal when plants were illuminated with red and blue diodes pair. That confirms that chlorophyll absorption maximum is in red and blue light.

### 2.1.3. Comparison of chlorophyll content and leaves color in plants grown in lighting of different colour

This experiment consists of 2 parts. In the first part the leaves color was measurement by using digital camera PANASONIC DMC-FZ5EG. Pictures were made three times: (1) when plants were fully developed and mature on 6th day after sowing and additional pictures were taken at (2) 8th and (3) 10th days.

For identification of color from photo simple software for digital photo (Irfanview, ImageJ, Image Analyzer) processing and RGB converter were used. The fragments with plants leaves only were excised from photo of leaves. These fragments were blurred (Gaussian blurring) by ImageJ program. After that the photos were converted to three RGB color photos that were converted to grey scale (Image Analyzer). Then ImageJ program was used to measure intensities of RGB colors. The values of RGB colors were converted to CIE scale by using RGB converter [5].

Fig. 2 shows how leaves colors of plants grown in blue light and in dark (without light) change. The leaves color of plants grown in blue light was yellowish green and the one in dark (without light) was yellow green.

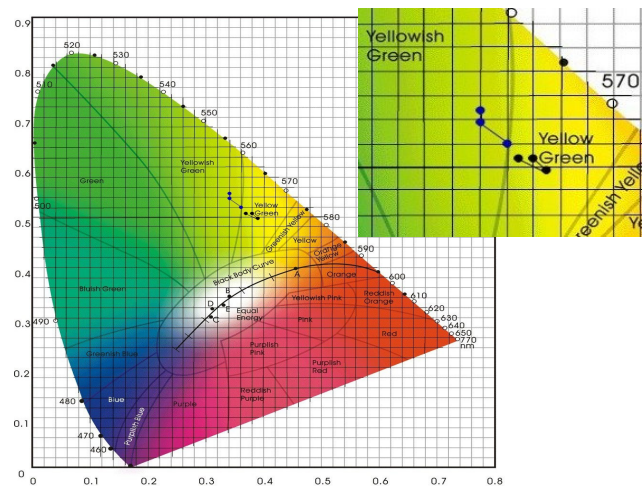


Fig. 2. Leaves color change in plants grown in blue light and in dark.

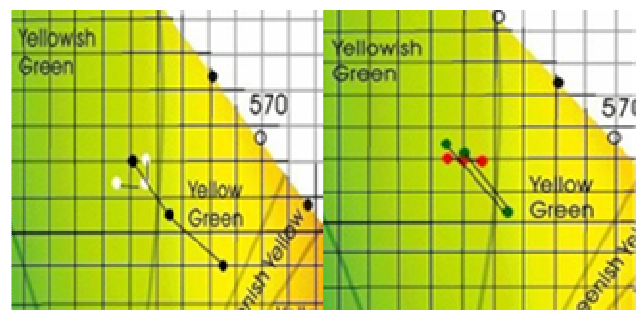


Fig. 3. Leaves color change: left – plants grown in white and IR light; right – plants grown in red and green light.

Data processing demonstrates that in plants grown in IR and green lighting (see Fig. 3.) leaves color changed from

yellowish green to yellow green, but in other cases leaves color was stable.

Second part of experiment was the measurement of chlorophyll content. It was measured by Perkin Elmer Lambda 20 spectrophotometer. Plants grown in lighting of different color (white, blue, red, green, IR) and without light were used in this part. At first suspension of the plants leaves was prepared. 2 g of fresh cress (*Lepidium sativum*) leaves after petioles removing were grinded into homogeneous mass and mixed with 3 ml of ethyl alcohol. Chlorophylls solution must be transparent thereto suspension was centrifuged. If laboratory has not centrifuge the suspension can be filtered through filter paper. The suspension of plants grown in different lighting was poured to different cuvettes. Then ready solution of chlorophyll was diluted by ratio 1:9 (1 part of chlorophyll solution and 9 parts of ethyl alcohol). The chlorophyll concentration was measured on wavelength from 350 to 700 nm, because chlorophyll absorbs light in visible spectrum.

Chlorophyll content was calculated by using the Beer-Lambert law that relates the absorption of light to the properties of the material through which the light is traveling.

$$A = \varepsilon \cdot c \cdot l;$$

then chlorophyll concentration is  $c = A/(\varepsilon \cdot l)$ , where A – the absorbance of the sample. In this experiment A was taken from chlorophyll absorbance graph on wavelength 663-664 nm.

$\varepsilon$  – the absorption coefficient or the molar absorptivity of the absorber ( $\varepsilon = 34,5 \text{ mM}^{-1}\text{cm}^{-1}$ ),

c – the concentration of absorbing species in the material,

l – the distance that the light travels through the material (the path length) (0,5 cm).

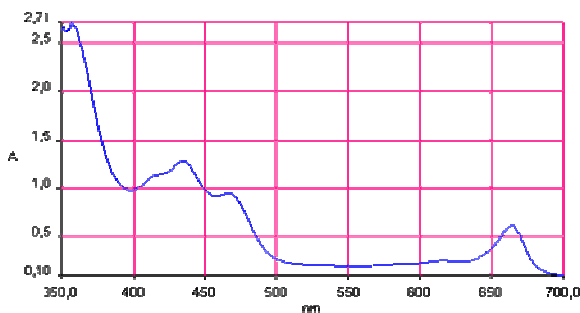


Fig. 4. The absorbance dependency on wavelength.

The graph shows chlorophyll absorbance on 663-664 nm. From such curve students calculate chlorophyll concentration and then converts to comparative value.

Students can see what colour of light is the best for plants growth and in what conditions the chlorophyll content is maximal. Such data are presented in Table 1. The experiment shows that the chlorophyll content was maximal in plants grown in red and white color lighting, and the chlorophyll content was minimal in plants grown without light and in IR light. It shows also that IR light stops chlorophyll production in plants.

Diode color	A $\lambda=664$	C (comparative value)
Red	0,6120	1
White	0,4345	0,78
Blue	0,1990	0,37
Green	0,2940	0,36
Dark	0,2769	0,34
IR	0,0131	0,15

Table 1. Chlorophyll content in plants grown in different lighting colors

### 3. CONCLUSION

The experiments series "Computerized experiments on photosynthesis in plants biology" consists of two experiments. Simple exercises help students to understand photosynthesis rate dependence on lighting spectre and to improve knowledge in plants biology. Students learn to work with computerized devices and software for data analyses. These skills later are used in other areas of their study. Experiments are done with simple devices that are in every laboratory room.

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