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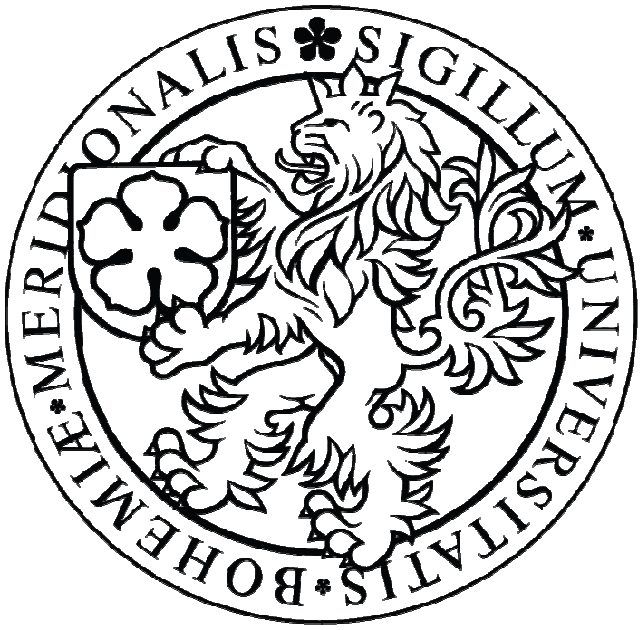

BACHELOR WORK

Oxygen evolution during photosynthesis in sunflower and tobacco using carbon dioxide comprising either ^{12}C or ^{13}C as substrate

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ANOTATION:

This bachelor thesis refers to a study of plant gas exchange measured as oxygen evolution rate and its potential difference in atmosperes with carbon dioxide consisting of either ^{12}C or ^{13}C. In this connection, also other basic plant characteristics were measured. Sunflower, *H. annus* cv. Teddy bear, wild-type and transgenic tobacco, *N. tabacum*, plants were used as an experimental material.

ANOTACE:

Tato bakalářská práce se zabývá studiem výměny plynů u rostlin měřené jako rychlost výdeje kyslíku a její možné rozdíly v atmosférách, ve kterých byl oxid uhličitý obsahující buď uhlík ^{12}C nebo ^{13}C. V této souvislosti byly měřeny také některé další základní charakteristiky rostlin. Jako pokusný materiál byly použity rostliny slunečnice, *H. annus* cv. Teddy bear, divokého a transgenního tabáku, *N. tabacum*.

I hereby declare under oath that the submitted bachelor thesis has been written solely by me without any third-party assistance. Additional sources or aids are fully documented in this paper, and sources for literal or paraphrased quotes are accurately credited.

I hereby declare that, in accordance with Article 47b of Act No. 111/1998 in the valid wording, I agree with the publication of my dissertation thesis, in full / in shortened form resulting from deletion of indicated parts to be kept in the Faculty of Science archive, in electronic form in publicly accessible part of the STAG database operated by the University of South Bohemia in České Budějovice accessible through its web pages.

23rd of May 2010, Budweis
Signature:

Martina Sklenářová

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ABSTRACT

The aim of this work was to answer the question how big differences are in the oxygen evolution in ^{12}C atmosphere and in ^{13}C. The study material was C4 plants represented by *Helianthus annus* cv. Teddy bear, and *Nicotiana tabacum,* wild type and 2 types with changed activity of RuBisCO. Those transformed types had been changed with an antisense gene directed at the mRNA of the RuBisco small subunit. The results were obtained by measuring O_2 evolution using a Clark electrode in leaf disc oxygen electrode unit. In addition other characteristics of those plants were measured.The oxygen evolution appears quicker with ^{13}C substrate in sunflower and opposite in tobacco (proved on control W38, and transformed tobacco SSu1)

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LIST OF ABREVIATIONS

SSu1	type of *N.tabacum* with 30-40 % activity RuBisCO
SSu2	type of *N.tabacum* with 10-15 % amount of RuBisCO of wild type
W38	wild type of *N.tabacum*
chl.	chlorophyll
MgO	magnesium oxide
RuBP	ribulose-1,5-bisphosphate
3-PG	3-Phosphoglyceric acid
cv	cultivar
LDOEU	leaf disc oxygen electrode unit
S.D.	standard deviation
vol.	volumetric
RuBP	ribulose-1,5-bisphosphate
CAM	crassulacean acid metabolism
PEPc	phosphoenolpyruvate carboxylase
C	carbon

1.INTRODUCTION

1.1Photosynthesis

1.1.1. Basics

Photosynthesis is key process on the Earth. Without transformation of light energy into chemical energy, as it is done in photosynthesis, the life wouldn't exist in our known form. This chemical energy is trapped in bonds of carbohydrates. Basic idea behind this principle is summed up in Figure 1. Photosynthesis in different forms occurs in plants, algae, and many species of bacteria excluding archea. Plants convert simple products of carbon fixation into more complex biomolecules, including sugars, polysacharides, and other metabolites. The plants differ in way of fixation CO_2.The most abundant are C3 plants.They are using ribulose-1,5-bisphospate carboxylase oxygenase. The plants which are using enzyme phosphoenolpyruvate carboxylase (PEPc) to bind CO_2 are C4 plants. Other type is crassulacean acid metabolism (CAM).

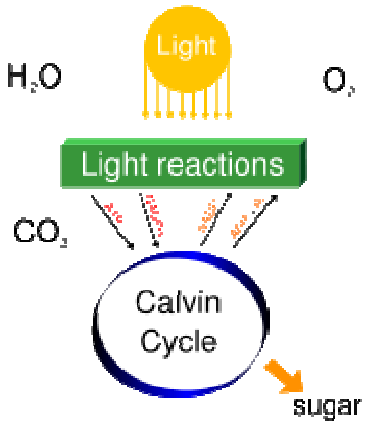


Figure 1. The schematic view of working principle of photosynthesis.(from Daniel Mayer)

1.1.2. Photosynthetic pigments

Pigment is chemical substance capable of reflecting light of certain wavelength. The object, which is reflecting this light, then appears to have colour of this wavelength. On the other hand certain wavelengths are by pigments absorbed. Photosynthetic pigments are chlorophylls, phycobilins, and carotenoids.

The basic structure of chlorophyll is porphyrin ring. The most important from groups of chlorophylls are chl *a* and chl *b*. The difference between chl *a* and *b* is only aldehyde group in second pyrol ring on at the third carbon atom.

Chl *a* is the major photosynthetically active pigment in plants. It absorbs blue and red light of the visible spectrum; hence the green part is reflected. While the photon with energy of the blue light is absorbed, the chl *a* gets excited from its ground state (S0) to the state two (S2). Because the lifetime of this state is short (about ten to thirteen seconds), chlorophyll appears soon in excited state one (S1).The rest of the energy is expelled as a heat. The energy of a "red" photon is only sufficient to excite the chl a to its first excited state (S1).

Carotenoids are isoprenoids, which are made from 40 atoms of carbon atoms. They can be caroten or xantophyls. Their main task is not to catch light energy, but to prevent organs of plant before the photo oxidation as for example in xantophyl cycle.

1.1.3. C3 plants

C3 plants are named according to the first stable three carbon atoms compound; 3-phoshoglycerate.The name for process of carbon fixation and substrate regeneration is called Calvin cycle. With enzyme RuBisCO is CO_2 fixated on ribulose-1,5-bisphosphate(RuBP). This product is labile and is cleaved to 3-phoshoglycerate.By phosphorylation 1,3-bisphosphoglycerate is obtained. This compound is reduced by enzyme using NADPH+ H^+. The glyceraldehyde-3-phosphate is then isomerised to dihydroxy-acetone-phosphate. Both 3 C compounds are transformed than into cytosol. There is synthesized fructose-1,6-bisphospate. Fructose-6-phospate is used for synthesis of starch in chloroplast or it is transported to the cytoplasm and used for the synthesis of sacharose.

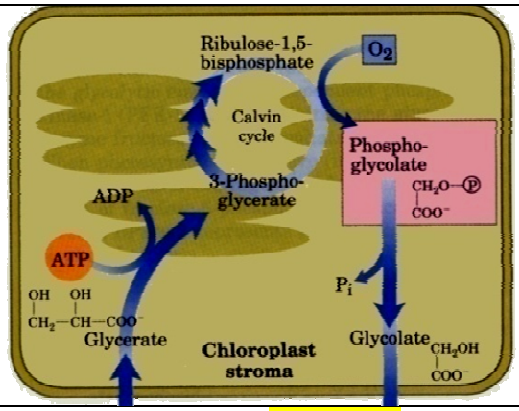


Figure 2. *The schematic process in C3 plants.(Lehninger)*

1.1.4. RuBisCO

RuBisCO is probably most abundant enzyme on the Earth, which is proving its importance. It is an enzyme that catalyzes the first major step of carbon fixation in photosynthesis. In this step ribulose-1,5-bisphosphate reacts (RuBP) with carbon dioxide and by hydrolysis 3-phosphoglycerate is obtained (Figure 3). Carbon dioxide comes through stomata of the leaf from the air and enters the stroma of the chloroplast.

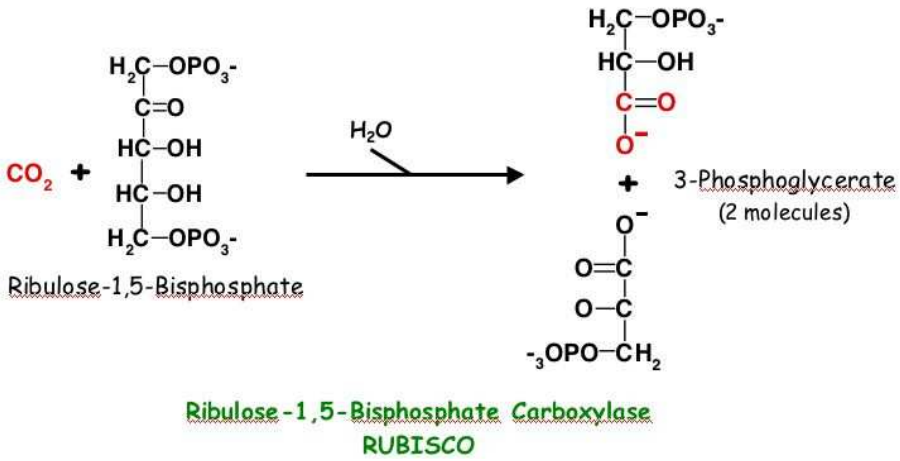


Figure 3. *The reaction of carbon dioxide fixation which is catalyzed by RuBisCo.*

The enzyme RuBisCO has not only carboxylase activity, but it was proved (Gutteridge & Gatenby 1995) that it is capable also of oxygenase action. The oxygenase activity means ,that this enzyme catalyzes fixation of O_2 during the CO_2 consumption on light. Under normal conditions (21 vol. % O_2, 0,035 vol. % CO_2) the ratio of fixation of CO_2 to fixation of O_2 is 3:1.The display of oxygenase activity is creation of glycolate in chloroplasts.

The RuBisCo is inactive enzyme under dark conditions. It is activated under light conditions by substance from stroma of chloroplasts. The inactive enzyme can be recognized by strong bond with riboluse-1, 5-bisphosphate. By activation this bond breaks, and RuBisCo is carbamylated and then bonded with magnesium ions. The activeness of activation is growing with increasing light irradiance. The light illumination is necessary for creation of H^+ and Mg^{2+} gradient in stroma essential for activation of enzyme. Hence, there are other factors like illumination, CO_2 amount and others, which direct the activation of RuBisCo.

1.2 Transgenic tobacco

Transgenic tobacco had been transformed with an antisense gene (produces an mRNA complementary to the transcript of a normal gene-usually constructed by inverting the coding

region relative to the promoter) directed at the mRNA of the Rubisco small subunit (Figure 4). Transgenic tobacco is design to have lower rates of CO_2 assimilation than wild type due to lower content of Rubisco.

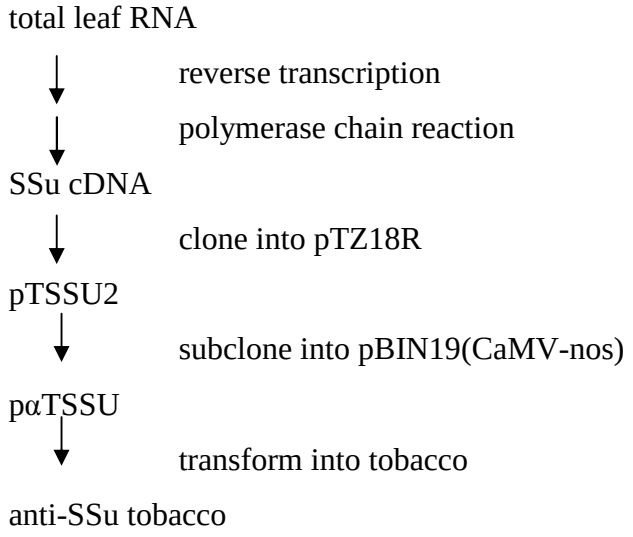


Figure 4.
Scheme for the construction and introduction into tobacco of an antisense gene directed against *rbc*S mRNA for the SSu.

1.3 Clark electrode

The Clark electrode (Clark et al. 1953, Severinghaus & Astrup 1986) (Figure 5) is an electrode, which was design to measure oxygen. The O_2 is measured on catalytic surface of platinum. The net reaction follows:

at the anode: $4\ Ag + 4\ Cl^- \rightarrow 4\ AgCl + 4\ e^-$

at the cathode: $4\ H^+ + 4\ e^- + O_2 \rightarrow 2\ H_2O$

overall : $4\ Ag + 4\ Cl^- + 4\ H^+ + O_2 \rightarrow 4\ AgCl + 2\ H_2O$

Originally it was design to measure oxygen presence in blood. The disagreement between partial pressure of oxygen in blood (pO_2) and gaseous mixture of the same pO_2, led to the improvement of the measuring instrument.

When speaking about polarographic measurement of the oxygen, we are using Clark electrode coupled with monitoring device (a flatbed strip chart recorder, or computer-assisted data acquisition system). The oxygen evolution evoke signal in term of current, which is amplified and converted into a voltage output. This voltage token is noticed by recorder, which moves recorder pen according to the strength of the signal. By moving a paper chart at constant speed and recording voltage changes, oxygen content is recorded as a function of time.

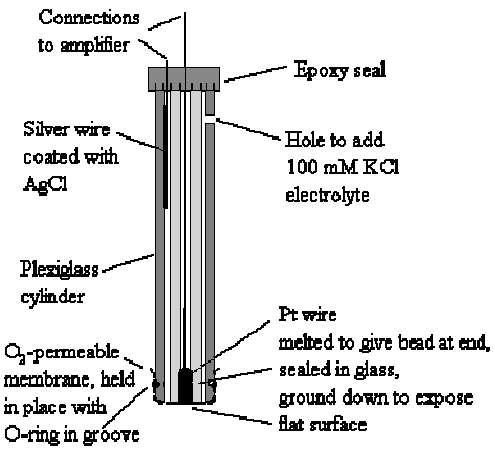


Figure 5. Clark electrode. (www.pharmacology2000.com/822_1/o-electd.gif)

2.AIMS.

The main goals are to obtain information about oxygen evolution in ^{12}C and ^{13}C atmosphere at different plant species. Is any of these substrates preferable to other one? In order to answer this question two types of transgenic tobacco, *Nicotiana tabacum*, one control untransformed tobacco and *Helianthus annuus* cv. Teddy bear were chosen. These species are typical representative of C3 plants, they have sufficient big leaves to accomplish measurement and further researches are done on Plant Physiology Institute of South Bohemian University. In addition, other parameters as chlorophyll *a* and *b*, carotenoids, leaf dry mass, and leaf fresh mass were measured. Later on was oxygen evolution rate related with those parameters.

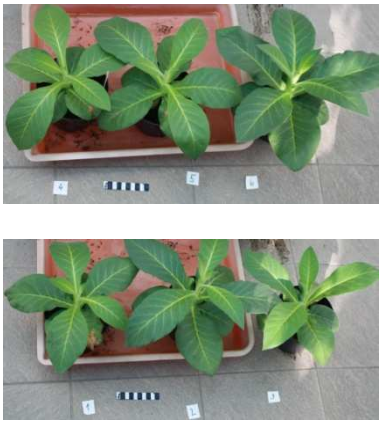
3.MATERIAL AND METHODS

3.1 Plants and growth condition

For this experiment, sunflower, *H. annus* cv. Teddy bear, wild tobacco, *N. tabacum* W38 as control, and transformed tobacco, *N. tabacum* SSu1 and SSu2 were chosen. Gas exchange measurements were made on 9 weeks old tobacco, 5 weeks old sunflower and young fully expanded leaves were chosen. Leaves were divided into 2 groups. The first was group of younger leaves in range 9-12, and second group was older leaves in range 6-8 (counted from the bottom).

I received seeds of both transgenic and wild type tobacco from the Molecular Plant Physiology Group of the Australian National University. The development of this seeds is described by Hudson et al. (1992). The tobacco was grown in 1 l pots in a growth cabinet (25/18 °C, 12 h photoperiod, 350 µbar CO_2, September-October 2009). For the last two weeks before the measurement tobacco was moved in an air glass house with day light, in average 9 hours a day. Two weeks before the measurement transgenic tobacco SSu2 were fertilized with Kristalon Start (Agro CS©) once a week. The sunflower was grown in the growth cabinet at the same conditions as tobacco in the period October-November 2009. Plants can be seen in Figure 6.

Photos of plants at the time of the experiment



N.tabacum cv. W38

N.tabacum cv. SSu1

Figure 6. The pictures of plants exactly before measurement. The transgenic SSu2 appeared lighter by naked-eye.The black stripe in picture is equal to the 10 cm.

H.Annus cv. Teddy bear

N.tabacum cv. SSu2

3.2 Gas exchange measurement

The measurement of oxygen evolution was done with leaf disc oxygen electrode unit (LDOEU) (Hansatech Ltd., Norfolk, U.K.) described by Deliu and Walker (1983). From each plant 2 leaves were chosen for measurement. Out of each picked leaf two discs (tobacco) and one disc (sunflower) were trimmed to fit the leaf chamber (10 cm^2). The water, which thermostated the chamber (Figure 7), had 25°C. Amount of oxygen was measured with Clark-type $Pt/Ag/AgCl_2$ electrode (Deliu and Walker 1972). The chamber was calibrated by flushing it with N_2 and then injecting 1 ml of air of defined temperature by gas-tight syringe while one tap was closed. The difference between the electrical output of the electrode after injection of air and N_2 is a response to the oxygen in 1 ml of air. Before the measurements the chamber was again flushed with N_2. Saturated sodium bicarbonate solution (either with ^{12}C or ^{13}C) was used as a source of CO_2. The signal from electrode was amplified and fed to a chart recorder. Rate of oxygen evolution was calculated as

$$V(O_2) = \frac{2{,}56 \times 10^6}{273{,}15 + t} \times \frac{R_m}{R_c} \times \frac{X}{\tau \times C} \qquad (1)$$

where t means temperature used for calibration in °C, R_m range of chart by time of calibration in V, R_k range of chart by time of measurement in V, X change of concentration of oxygen during time τ recorded by writer in cm, τ time for the concentration change of oxygen was recorded in s, and C calibration in cm.

For the calibration of the irradiation source equipped with a halogen lamp, LI-COR-Quantum sensor was used. The source of irradiation was mounted on the support stand and the window of top section of LDOEU was placed above it. The sensor was mounted to the top of this window. By changing position of this sensor maximum and minimum of irradiance was detected. LI-COR system has shown that the value of light irradiance differed from maximum 1060 to minimum 700 µmol $m^{-2}s^{-1}$.

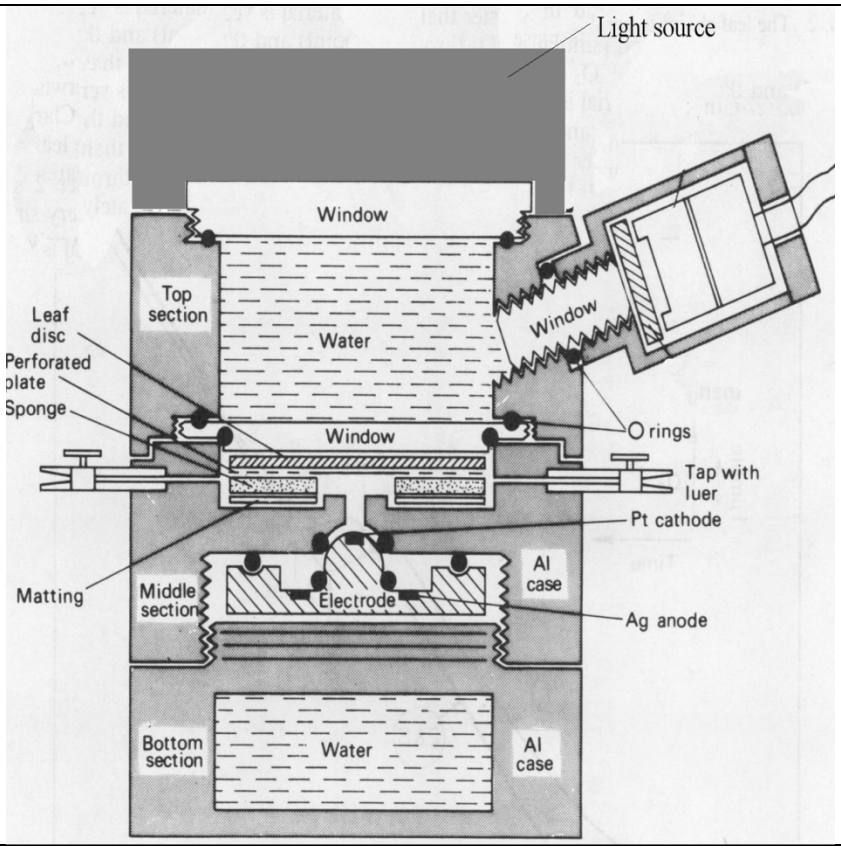


Figure 7. Modified schematic diagram of gas phase 0_2 electrode (from Deliu and Walker 1983).

3.3 Chlorophylls and carotenoids concentrations measurement

Chlorophylls *a* and *b*, and carotenoids were measured using the spectrophotometric method in acetone extract. Three circles with summed up area of 1.5 cm^2 were cut from leaves, frozen in liquid nitrogen and then stored at -75 °C. After 3 months, the samples were mixed with 2 ml of 80 % acetone, a pinch of MgO, and grind in a mortar and a pestle. The extract was filtered through a filtration paper. Then a glass cuvette (1 cm path length) was filled with this solution and it was inserted into a spectrophotometer. Furthermore the absorbances at 470.0, 646.8, 663.2, and 710.0 nm were measured. The absorbance at 710.0 nm is reference, corresponds to the zero absorbance of pigments, and is substracted from all other absorbencies. The concentration of pigments was calculated according to Lichenthaler (1981):

$$c_a = 12{,}25\, A_{663.2} - 2{,}79\, A_{646.8} \quad \textbf{(2)}$$

$$c_b = 21.50\, A_{646.8} - 5.10\, A_{663.2} \quad \textbf{(3)}$$

$$c_{a+b} = 7.15\, A_{663.2} + 18.71\, A_{646.2} \quad \textbf{(4)}$$

$$c_{x+c} = \frac{1000\, A_{470} - 1.82\, c_a - 85.02\, c_b}{198} \quad \textbf{(5)}$$

where c_a stands for the concentration of chl. *a*, c_b for the concentration of chl. *b*, c_c for concentration of the carotenoid, and c_x for concentration of chl. *a*+*b*. A with different indexes is the absorbance at the wavelength which is specified by index

4.RESULTS

In order to find out if oxygen evolution is affected by sequence of substrate the test was run. This test was done on special batch of tobacco W38, which was grown in June-May 2009. First the leaf disc was fed with substrate containing ^{12}C, then with ^{13}C, and the same disc was given substrate in the opposite order. The results of this control measurement have shown that there is no difference between the orders of substrates (Figure 8).

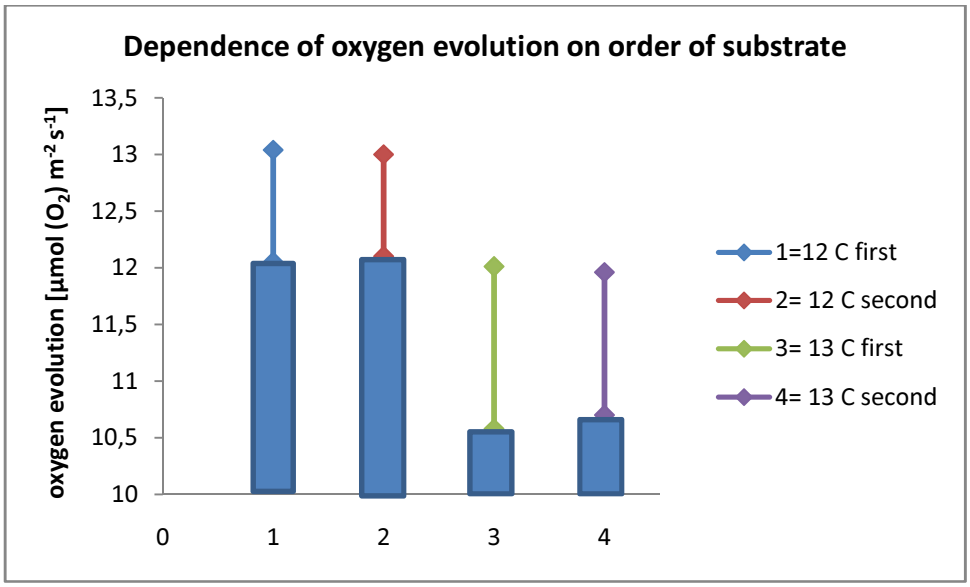


Figure 8. The chart of oxygen evolution dependence on order of substrate. Discs (8) were punched from leaves of tobacco W38.Mean value+ standard deviation.

For purpose of measurement itself, the 3 controls from each sort of plant were chosen. Results supported previous presumption, that amplitude of oxygen evolution rate is not dependent on order of substrate.

The analysis of chl *a*, chl *b*, carotenoids, leaf fresh mass, and leaf dry mass was performed..The mean values determined for the leaves of 6 sunflowers, 5 control plants, 5 SSu1, and 5 SSu2 are presented in Figure 9. The highest amount of chlorophyll had sunflower. The chlorophyll amounts in tobacco W38 were similar to the amounts in tobacco SSu1. One can observe that in SSu2 the content of the chl. and also carotenoids was per unit of leaf area the smallest.

	sunflower		W38		SSu1		SSu2	
	younger	older	younger	older	younger	older	younger	older
Chl *a+b* [mg of pigment/m^2 of leaf]	226,2 ± 18.4	228,6 ± 20.7	202.9 ± 12.1	219.7 ± 11.1	230.1 ± 28.6	214.4 ± 25.4	153.4 ± 14.9	169.9 ± 08.1
Chl *a/b*	3,45 ± 0,93	3,67 ± 1,18	2,73 ± 0,15	2,77 ± 0,50	2,58 ± 0,29	2,59 ± 0,26	2,62 ± 0,10	2,64 ± 0,29
carotenoids [mg /m^2 of leaf]	32.3 ± 3.0	33.1 ± 3.9	25.6 ± 4.4	32.4 ± 2.7	34.4 ± 5.3	32.8 ± 7.0	23.0 ± 2.1	24.7 ± 0.3
leaf fresh mass[g m^{-2}]	156 ± 9	163 ± 17	218 ± 26	254 ± 24	211 ± 30	223 ± 33	198 ± 38	219 ± 14
leaf dry mass[g m^{-2}]	20 ± 4	20 ± 6	26 ± 6	34 ± 6	16 ± 7	15 ± 7	12 ± 3	16 ± 2

Figure 9. Leaf characteristics of sunflower, tobacco control, and two transgenic tobaccos SSu1 and SSu2. Samples were divided into two groups of leaves according to leaf number counted from the bottom. The younger group is from The amount of chl *a+b* was lower in SSu2 than in control and SSu1.The highest ration of chl *a/b* was in sunflower.

The signals from recorder were recalculated to show the oxygen change. The oxygen evolution was then related to the other characteristics of leave. Each oxygen evolution rate was referred to its value of leaf dry mass, leaf fresh mass and chl. content. Then the mean value (M) and standard deviation (S.D.) were calculated. The data were statistically treaded with t-test by independent variables.

The assimilation rates with ^{13}C in sunflower suggest that ^{13}C atmosphere leads in older leaves to the more rapid fixation of CO_2 (Figure 10). This suggestion is confirmed also by relating with fresh mass of leave, but not with dry mass and amount of chlorophyll. The younger leaves haven't shown the same dependency. Even though M of ^{12}C substrate is different from ^{13}C by more than 4 units, the 12C contained values within big range. From those values none could be marked as outlier according to Grubbs test.

The results have shown that assimilation rate with ^{12} C is quicker than with ^{13} C in tobacco (some groups of leaves are not statistically proven to be different). The values for younger leaves of W38 have shown tendency that the $^{12}CO_2$ leads to more rapid oxygen

evolution (Figure 11). In SSu1 the values for older leaves are statistically different and displaying, that $^{12}CO_2$ is substrate leading to quicker oxygen evolution. Neither younger leaf group nor older leaf of SSu2 group has shown significant differences depending on isotope composition of substrate in oxygen evolution (Figure 13).

	sunflower			
	younger		older	
	^{12}C	^{13}C	^{12}C	^{13}C
oxygen evolution [µmol(O_2) s^{-1} m^{-2}]	22.0 ± 5.37^{A}	26.37 ± 2.28^{A}	23.05 ± 2.30^{B}	26.88 ± 3.26^{B}
oxygen evolution /fresh mass [µmol(O_2) s^{-1} m^{-2}/mg of leave]	1426.02 ± 424.32^{A}	1700.44 ± 238.02^{A}	1423.90 ± 145.44^{B}	1664.29 ± 249.96^{B}
oxygen evolution /dry mass [µmol(O_2) s^{-1} m^{-2}/mg of dry leave]	11021.92 ± 1887.99^{A}	13512.67 ± 2421.42^{A}	12583.29 ± 4139.44^{A}	14620.36 ± 4673.38^{A}
oxygen evolution/chl *a*+*b* [µmol(O_2) s^{-1} m^{-2}/ mg of chl *a*+*b*]	1.00 ± 0.35^{A}	1.18 ± 0.20^{A}	1.01 ± 0.83^{A}	1.01 ± 0.08^{A}

Figure 10. Characteristics of younger (4 samples) and older (8 samples)leaves of sunflower. This data were statistical treated with t-test independent variables with level α=0,05. Values with index A are not significantly different, and B are significantly different. (M±S.D.)

	tobacco W38			
	younger		older	
	^{12}C	^{13}C	^{12}C	^{13}C
oxygen evolution [µmol(O_2) s^{-1} m^{-2}]	13.07 ± 3.03^{B}	9.04 ± 2.92^{B}	10.53 ± 2.55^{A}	8.85 ± 3.16^{A}
oxygen evolution /fresh mass [µmol(O_2) s^{-1} m^{-2}/mg of leave]	612.46 ± 182.54^{B}	423.96 ± 151.77^{B}	415.96 ± 92.17.^{A}	348.96 ± 114.40^{A}
oxygen evolution /dry mass [µmol(O_2) s^{-1} m^{-2}/mg of dry leave]	5418.81 ± 1823.04^{B}	3794.54 ± 1630.43^{B}	3268.44 ± 1103.35^{A}	2722.74 ± 1155.49^{A}
oxygen evolution/chl *a*+*b* [µmol(O_2) s^{-1} m^{-2}/mg of chl *a*+*b*]	0.65 ± 0.18^{B}	0.44 ± 0.13^{B}	0.48 ± 0.13^{A}	0.40 ± 0.15^{A}

Figure 11. Characteristics of younger (12 samples) and older (8 samples) leaves of control W38 tobacco. This data were statistical treated with t-test independent variables with level α=0,05. Values with index A are not significantly different, and B are significantly different. (M±S.D.).The values for younger leaves suggest that the $^{12}CO_2$ leads to more rapid oxygen evolution.

	tobacco SSu1			
	younger		older	
	^{12}C	^{13}C	^{12}C	^{13}C
oxygen evolution [µmol(O_2) s^{-1} m^{-2}]	5.28 ± 1.64^{A}	5.62 ± 2.32^{A}	10.67 ± 2.13^{B}	6.27 ± 0.57^{B}
oxygen evolution /fresh mass [µmol(O_2) s^{-1} m^{-2}/mg of leave]	254.83 ± 92.94^{A}	271.72 ± 117.22^{A}	488.20 ± 114.80^{B}	287.20 ± 50.52^{B}
oxygen evolution /dry mass [µmol(O_2) s^{-1} m^{-2}/mg of dry leave]	3975.80 ± 2310.09^{A}	4189.30 ± 2249.66^{A}	8442.13 ± 3045.99^{B}	5073.21 ± 2038.14^{B}
oxygen evolution/chl *a*+*b* [µmol(O_2) s^{-1} m^{-2}/ mg of chl *a*+*b*]	0.23 ± 0.07^{A}	0.24 ± 0.10^{A}	0.50 ± 0.09^{B}	0.30 ± 0.04^{B}

Figure 12. Characteristics of younger (14 with outliers) and older leaves (6 samples) of SSu1 transgenic tobacco. This data were statistical treated with t-test independent variables with level α=0,05. (M±S.D.).Two values were left out as outliers according to Grubbs test. Values with index A are not significantly different, and B are significantly different.

	tobacco SSu2			
	younger		older	
	^{12}C	^{13}C	^{12}C	^{13}C
oxygen evolution [µmol(O_2) s^{-1} m^{-2}]	4.95 ± 1.57^{A}	4.39 ± 1.45^{A}	3.93 ± 1.42^{A}	4.34 ± 1.05^{A}
oxygen evolution /fresh mass [µmol(O_2) s^{-1} m^{-2}/mg of leave]	261.78 ± 107.86^{A}	230.07 ± 93.21^{A}	199.67 ± 53.00^{A}	181.31 ± 69.20.^{A}
oxygen evolution /dry mass [µmol(O_2) s^{-1} m^{-2} /mg of dry leave]	4522.52 ± 2503.81^{A}	3960.25 ± 2086.62^{A}	2676.38 ± 653.21^{A}	2427.10 ± 875.91.^{A}
oxygen evolution/chl *a*+*b* [µmol(O_2) s^{-1} m^{-2}/ mg of chl *a*+*b*]	0.28 ± 0.07^{A}	0.24 ± 0.06^{A}	0.25 ± 0.04^{A}	0.23 ± 0.07^{A}

Figure 13. Characteristics of younger (12 samples) and older (8 samples) leaves of SSu2 transgenic tobacco. This data were statistical treated with t-test independent variables with level α=0,05. Values with index A are not significantly different, and B are significantly different. (M±S.D.).Neither younger leaf group nor older leaf group has shown significant differences depending on isotope composition of substrate in oxygen evolution.

5.DISCUSSION

The aim of this study was to examine oxygen evolution rates in several species together with characterization of those plants. Here, oxygen evolution rate of sunflower, *H. annus* cv. Teddy bear, wild tobacco, *N.tabacum* W38, and 2 transformed tobaccos, *N.tabacum* SSu1 and SSu2, under atmosphere of substrate with different carbon isotope composition was determined. The measurements were done on leaves discs (punched from leaves) in either $^{13}CO_2$ or $^{12}CO_2$ atmosphere.

The pre-tests have shown that oxygen evolution is not dependent on order of the substrate. Because of that first was leaf disc in ^{12} C atmosphere and then in ^{13} C without affecting the results.

The activity of RuBisCO depends on activation (Procházka et al. 1998) by light illumination. In my opinion this activation was sufficient, because the similar values for irradiance were used as in Hudson(1991)

"The major difference between the wild-type and transgenic tobacco leaves was the altered Rubisco content." (Evans et al., 1994). This fact probably affected the oxygen evolution rate as described by von Caemerrer et al.(2004), with difference they measured proportional CO_2 assimilation.

Caemmerer et al. (2004) claim that RuBisCO content in SSu2 corresponds to the 10-15 % of wild tobacco. By this fact the oxygen evolution rate can be affected and they also claim that stomatal conductance does not correlate with photosynthetic capacity in transgenic tobacco

According to Hudson et al. (1992), who analyzed wild tobacco and transgenic tobacco, the chl. amounts were slightly higher in W38 than in SSu1. The transgenic tobacco SSu2 had the content of chl. also slightly lower than SSu1. I observed with naked-eye, that leaves of wild tobacco were darker than transformed, which would suggest higher chl. content in W38 per area unit of leaf. In addition Evans et al. (1994) is talking about similar chl. content.

For measuring of the chlorophyll amount should be rather used units of mass then area unit for better referring. On the other hand Evans et al.(1994) and Hudson et al.(1991) used for pigments units mmol m^{-2} .

6. CONCLUSION

The chlorophyll and carotenoid amount is better to refer to also to the mass of leaf and not only to the area of leaf.

The oxygen evolution with ^{13}C is quicker in sunflower (older leaves).This finding is also the same when referencing this value with mass of fresh leave. The tobacco's oxygen evolution on the other hand is quicker in $^{12}CO_2$ atmosphere (W38-older leaves, SSu1-younger leaves), even though both species are C3 plants with the same way of carbon fixation. Those findings were also confirmed when referring with fresh mass of leave, leaf dry mass, and chl content.

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