Effects of carbon dioxide concentration on chlorophyll fluorescence of peas „*Pisum sativum* L."

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Abstract: The atmospheric concentration of carbon dioxide increases from decade to decade in increasing pace. In 1957, atmospheric carbon dioxide levels were around 315 ppm, while in 2012 it amounted to 394.49 ppm concentration. The atmospheric concentration of carbon dioxide is expected to reach 550 mol^{-1} to mid-century. In parallel, the global temperature is rising, which is projected to average 1.5-4.5°C. These global environmental changes, directly or indirectly affect plant growth, development, yield and quality of the crop. During the research, in climate chambers, "Irina" pea was sowed, which were tested near 700 and 400 ppm carbon dioxide concentration. Chlorophyll fluorescence was measured both in dark-adapted (F_v/F_m test) and in light-adapted leaf samples (Yield test; Y(II)).

Keywords: carbon dioxide, concentration, pea, chlorophyll fluorescence

Introduction

The rising concentration of atmospheric carbon dioxide (CO_2) contributes to global warming, and thus the changes affect both precipitation and evaporation quantity. Moreover, the concentration of carbon dioxide directly affects the productivity and physiology of plants (Kruijt, 2008). The atmospheric concentration of carbon dioxide is expected to reach 550 ppm in the middle of the century (Carter et al., 2007). In parallel, the global temperature is rising, which is projected to average 1.5-4.5°C. It could be more frequent occurrences of extreme weather events such as heat waves and/or drought (Carter et al., 2007). These global environmental changes, either directly or indirectly affect plant growth and development, yield and quality of the crop (Ainsworth and Rogers, 2007, Seneweera et al., 2005). The carbon dioxide concentration is a key factor that in interaction with the light, affects the plant's photosynthesis. If a particular crop is being tested, the CO_2 concentration may be influenced by the contents of soil organic matter (soil respiration), the type of plants, air movement, etc. The crop itself is an opened ecosystem, which has continous, constant and dynamic interaction with the biotic and abiotic environmental factors. The rate of photosynthesis is affected by a number of external (environmental) factors such as light intensity, CO_2 concentration, temperature, water and nutrient supply, and internal factors such as the plant's age, medical condition, particularly to the leaves. Among the various factors significant interactions prevail: environmental factors affect the growth and development of plants, leaf area and composition, the functioning of the photosynthetic apparatus, the duration and length of growing season.

Materials and methods

Chlorophyll fluorescence was measured in dark-adapted and in light-adapted samples using F_v/F_m (dark-adapted) test to determinate the maximum quantum yield and light adapted yield of photosynthetic efficiency of PSII for the determination of effective quantum photochemical yield. The maximum quantum yield of PSII in the samples was measured after 30 min-long dark adaptation of the leaves by using F_v/F_m protocol. F_v/F_m ratio is used for estimate of the largest proportion of absorbed quanta used in PSII reaction centres. Dark adaptation allows the reoxidation of PSII and to relax nonphotochemical quenching. Minimum and maximum fluorescence (F_0 and F_m) of dark-adapted leaves were measured in the same leaves after 0.8 second of saturation pulse (35W halogen lamp with 690 nm short pass filter) on previously dark-adapted samples. Variable fluorescence (F_v=F_m−F_0) and maximum quantum yield of PSII (F_v/F_m) were calculated by the Fluorometer software, and maximal efficiency of the photochemical process in PSII (F_v/F_0) could be counted. Actual quantum yield of PSII (Y(II)) in the samples was measured using Yield protocol which is a light adapted steady-state test of photosynthesis, measure the ratio of light amount used in photochemistry in PSII, the light amount adsorbed by chlorophylls of PSII. Leaves were tested by steady-state photosynthetic conditions. This protocol shows the achieved efficiency of PSII in addition specific light condition. Steady-state fluorescence (F_s) and maximum fluorescence (F_{ms}) of light-adapted leaves were measured, actual quantum yield of PSII ($Y(II)=(F_{ms}-F_s)/F_{ms})$) and estimated relative electron transport rate (ETR) were calculated by the Fluorometer software. The tests were occured in precision and hermetically sealed air chambers. The soil emissions of carbon dioxide continuously and easily can be measured. In addition to monitoring the reactions of plants, chlorophyll fluorescence measurements were made. We can do statistical comparison of the research and compare the results. Datas were analyzed statistically by Independent-Samples T test for all pair wise comparisons using SPSS for Windows (SPSS®, version 21.0) at $p \leq 0.05$. Experiment details are the following; the plant was Irina pea (3x3 crops / climate chamber), the soil contained N>0.3 w/w%, P_2O_5>0.1 w/w%, K_2O>0.3 w/w%, pH 6.8, the drilling depth was 5 cm, 14 hours of light condition, 21-23°C internal temperature, varying humidity, measurements schedule happened in 4-6 leaf and flowering phenophases.

Results and discussion

Yield Protocol: In terms of steady-state fluorescence there is significant difference between the two concentrations. At higher concentration, higher value can be observed. The efficiency of PSII system was superior to 700 ppm concentration. In case of actual quantum yield of PSII, the difference is significant, as it is with ETR, by the way, the actual quantum yield of PSII was better on lower concentration (Table 1).

Table 1: Differences between 400 and 700 ppm CO_2 concentrations in 4-6 leaf phenophase by Yield protocol

	CO_2 ppm	N	Mean	Std. Deviation	F	Sig.	Sig. (2-tailed)
Fs	700	4	1384	66.79321	2.585	0.159	0.001
	400	4	808	189.910			0.006
Fms	700	4	3874,75	3.86221	7.026	0.038	0.235
	400	4	3410,25	704.583			0.279
Y	700	4	0,6425	0.01748	0.896	0.38	0.000
	400	4	0,7638	0.0096			0.000
ETR	700	4	32,35	0.85829	0.736	0.424	0.000
	400	4	38,475	0.49244			0.000

In phenophase of flowering, steady-state fluorescence shows no significant difference between the two concentrations. Among the PSII efficiency of the systems, there were no significant differences and there is no significant difference in maximum fluorescence too. In case of actual quantum yield of PSII the difference is significant, as it is with ETR, but it is striking that in the case of flowering the actual quantum yield of PSII was better on higher concentration (Table 2).

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Table 2: Differences between 400 and 700 ppm CO_2 concentrations in flowering phenophase by Yield protocol

	CO_2 ppm	N	Mean	Std. Deviation	F	Sig.	Sig. (2-tailed)
Fs	700	4	1241,25	55,61999	22,44	0,00	0,038
	400	4	2164,5	694,79325			0,076
Fms	700	4	3876,75	2,06155	9,66	0,02	0,179
	400	4	3591,5	374,89421			0,225
Y	700	4	0,679	0,01431	23,03	0,00	0,019
	400	4	0,4008	0,17373			0,049
ETR	700	4	34,175	0,73655	22,56	0,00	0,019
	400	4	20,15	8,76907			0,049

F_s: Steady-state fluorescence, F_{ms}: maximum fluorescence, Y: actual quantum yield of PSII, ETR: relative electron transport rate

F_v/F_m protocol: In 4-6 leaf phenophase, minimum fluorescence according to a significant difference but no significant difference was measured by maximum fluorescence. Variable fluorescence shows significant differences too. In case of F_v/F_m ratio, significant differences were observed as a result, and further measurements as well F_v/F_0 (Table 3). In case of atmospheric concentration, in 4-6 leaf stage, the values are higher, and they are significantly different.In flowering phenophase, minimum fluorescence according to a significant difference but no significant difference by maximum fluorescence. Variable fluorescence shows significant differences as it was in 4-6 leaf stage. Here too in F_v/F_m ratio, significant differences were measured as a result, and F_v/F_0 as well. The measured values are higher near 400 ppm concentration in this phenophase too (Table 4).

Table 3: *Differences between 400 and 700 ppm CO_2 concentrations in 4-6 leaf phenophase by F_v/F_m protocol*

	CO2	N	Mean	Std. Deviation	F	Sig.	Sig. (2-tailed)
Fo	700	4	1257.00	61.04	0.26	0.63	0.00
	400	4	973.75	51.14			0.00
Fm	700	4	3871.25	3.50	3.00	0.13	0.47
	400	4	3872.75	1.71			0.48
Fv	700	4	2614.25	60.16	0.24	0.64	0.00
	400	4	2899.00	49.91			0.00
FvFm	700	4	0.68	0.02	0.25	0.64	0.00
	400	4	0.75	0.01			0.00
FvFo	700	4	2.09	0.15	0.26	0.63	0.00
	400	4	2.99	0.20			0.00

Table 4: *Differences between 400 and 700 ppm CO_2 concentrations in flowering phenophase by F_v/F_m protocol*

	CO2	N	Mean	Std. Deviation	F	Sig.	Sig. (2-tailed)
Fo	700	3	1293.67	53.72	0.32	0.60	0.00
	400	4	843.00	43.40			0.00
Fm	700	3	3873.00	5.29	6.04	0.06	0.46
	400	4	3789.25	176.85			0.41
Fv	700	3	2579.33	54.50	2.07	0.21	0.01
	400	4	2946.25	148.90			0.01
FvFm	700	3	0.67	0.01	1.54	0.27	0.00
	400	4	0.78	0.01			0.00
FvFo	700	3	2.00	0.12	1.01	0.36	0.00
	400	4	3.50	0.16			0.00

F_0, F_m: minimum and maximum fluorescence, F_v: variable fluorescence, F_v/F_m: maximum quantum yield of PSII, F_v/F_0: maximal efficiency of the photochemical process in PSII

Conclusions

The photochemical efficiency was measured by fluorescent parameters (F_0, F_v, F_m - minimum, variable and maximum chlorophyll fluorescence) with comparing these ratios. The F_v/F_m ratio is also informative to us, in terms of efficiency. In our experiments, significant differences can be seen in several cases. It causes by interaction between the plant and the environmental factors (CO_2). In Yield protocol, the fluorescence maximum values are higher near 700 ppm CO_2 concentration in the case. In case of F_v/F_m protocol informative indicators were higher in all cases near lower concentrations. It is also important to note that at 700 ppm concentration, the vegetation period is shorter, the plants thrived after 35 days, the 4-6 leaf stage achieved within 16 days. Despite the higher concentration, the green mass of plants grown significantly more than in control plants. Overall, the increased green mass, the shortened growing season are due to the increasing level of CO_2 concentration. I am planning tests under different concentrations, which will be compared with the current results and further examination of plant varieties.

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