Dynamics of CO$_2$ evolution by plants at low pressure

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Abstract

Dynamics of CO$_2$ evolution at low pressure was studied in barley, maize, pea, wheat and pine seedlings using the gas exchange system with laser photoacoustic spectrometer. The CO$_2$ evolution from plant surfaces to environment increased with decreasing air pressure. Simultaneously the changes in activities of phosphoenol-pyruvate carboxylase, glucose-6-phosphate dehydrogenase, glyceraldehyde phosphate dehydrogenase, alcohol-dehydrogenase, isocitrate dehydrogenase, malate dehydrogenase in pea and maize leaves were observed. The response depended on plant species used as well as on air pressure and period of its action.

Additional key words: gas exchange, *Hordeum vulgare*, *Pinus sylvestris*, *Pisum sativum*, *Triticum aestivum*, *Zea mays*.

Introduction

Few works are available treating the influence of low pressure on plant physiological processes, although this effect is rather well known in animal and human physiology. Only isolated data are available on the action of low pressure on photosynthetic and respiratory gas exchange (Gale 1972, Astafurova *et al.* 1990, 1993) and growth and evolution of plants (Costes and Vartapetyan 1978, Musgrave *et al.* 1988). The study of responses to atmospheric pressure changes is of theoretical interest for investigation of plants potentiality as well as mechanisms of their adaptation to extreme conditions.

The characteristics of CO$_2$ exchange serve as an integral index of functional changes. The commercial infra-red gas analyzers (IRGA) with non-laser radiation sources are usually used when studying the dynamics of CO$_2$ evolution and uptake

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by plants. Gas exchange systems with IRGAs are not capable to measure small gas admixtures and to record simultaneously the evolution of C_2H_4 and a number of other volatile metabolites.

This paper describes the gas exchange system using laser photoacoustic spectrometer for measurement of CO_2 contents in real-time scale under different external pressure. We also present the results of investigation of plant CO_2 exchange dynamics as well as activities of enzymes of respiratory metabolism under low pressure.

Materials and methods

Plants: Eight-day-old seedlings of pea (*Pisum sativum* L.), wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) as well as 14-d-old seedlings of maize (*Zea mays* L.) and pine (*Pinus sylvestris* L.) in comparable phase of the primary leaves formation were used. The plants were grown in soil under luminescent lamps (irradiance of 40 W m^{-2}), 12-h photoperiod, at temperature of 22 - 24 °C, and the normal atmospheric pressure of 101 kPa. Before treatment the shoots were cut off and ends put in a cotton. The control and tested groups of the shoots (fresh mass 5 g) were simultaneously put into exposure chambers (volume 1 dm^3).

Experimental setup: Low pressure (54 or 8 kPa, equivalent to altitudes of ~5.1 × 10^3 and 20 × 10^3 m above the sea level) was reached by vacuum pump. The pressure was measured with Hg-pressure meter with an accuracy of ± 0.1 kPa. The partial pressure of O_2 under these conditions was 11.4 and 1.7 kPa. Reference plants were put inside the exposure chamber at normal pressure of the air (101 kPa) with 21 kPa of O_2. The temperature (22 - 24 °C) was the same in both cases. During the course of the experiment the plants under study were in dark. Time of exposure varied from 2 to 48 h. The gas evolution rate by shoots in a closed volume of the exposure chambers was measured with the photoacoustic (PA) spectrometer (Fig. 1). The choice of the radiation source was determined by the fact that some plant volatile metabolites (CO_2, C_2H_4, NH_3) have strong absorption lines near 10 μm region of CO_2-laser radiation. We used the commercial CO_2 laser *ILGN-705* (*NPO Istok*, Fryazino, Russia). For wavelength tuning we replaced the laser output mirror by a combination of diffraction grating of 100 lines mm^{-1} (*3*) and plane 100 % mirror (*4*). The mirror was adjusted so that the first-order reflected radiation should fall back onto the grating and than onto the back spherical mirror with 100 % reflectivity (*1*) tightly welded to the gas-discharge tube of the laser (*2*). Radiation was emitted through grating diffraction of zero order. The radiation wavelength was tuned by the plane mirror swiveling. The resonator construction enable us to obtain the generation at IP(10) - IP(32) lines. Identification of the laser generation lines was carried out with a non-commercial panoramic spectrum analyzer (*10*), whose scale was graduated in absolute wavelength values. Amplitude-modulated radiation was directed with the plane mirror (*6*) through the diaphragm of 3 mm in diameter (*7*) into the cell of the PA detector (*8*) of our design (100 mm in length and 10 mm in diameter, with BaF_2

windows). PA signal arising inside the cell was measured with the plane capacitor microphone also of our own construction (9) mounted into the cell wall. An electric signal from the microphone was preamplified and then recorded by the recording system: selective amplifier V6-4 (17), lock-in amplifier V9-2 (18) (PO Impuls, Krasnodar, Russia) and recorder LKS-4 (19) (Lenteplopribor, St. Peterburg, Russia). The reference signal from the modulator (5) reached the input of the lock-in amplifiers. Similar channel (14, 15, 16) used to record a signal from home made PA calorimeter (11) is located behind the measuring cell (8). The calorimeter measured the radiation power [W] passing through the cell. Reference and experimental groups of plants were placed in the exposure chambers (12) connected with the vacuum system (13) and PA-cell (8).
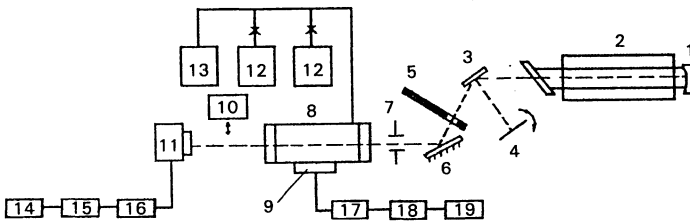

Fig. 1. Block diagram of set-up: 1 - spherical mirror of the resonator; 2 - gas discharge tube; 3 - diffraction grating; 4 - plane mirror of the resonator; 5 - modulator; 6 - take-off mirror; 7 - diaphragm; 8 - PA cell; 9 - microphone; 10 - spectrum analyzer; 11 - PA calorimeter; 12 - exposure chambers; 13 - vacuum system; 16, 17 - selective amplifiers; 15, 18 - lock in amplifiers; 14, 19 - recorders.

During the course of the experiment the gas samples from the exposure chambers were successively put into evacuated measuring PA-cell. When absorbing radiation at a given wavelength, the amplitude of electric signal from PA-detector U is directly proportional to the radiation power absorbed in the gaseous mixture. The ratio A, characterizing the absorptivity of the gas or gaseous mixtures under study, was determined as follows:

$$A = U/W = \alpha K$$

where W is the incident laser power [W], K - absorption coefficient and α is the sensitivity of PA detector. When we measuring gaseous mixtures with small absorption coefficient and using short PA cell the value of incident radiation power is equal to the value of the radiation power passed through the cell (Antipov et al. 1984). α is the function of the total gas pressure P in the cell. For the used PA-detector $\alpha = \alpha_{max}$ at P $\sim$ 8 kPa, therefore all the measurements were carried out at this pressure.

To identify all the gases involved in gas exchange we observed the variation of A with time for the two CO_2 laser wavelengths: $\lambda_1 = 10.591$ μm [P(20)] and $\lambda_2 = 10.532$ μm [P(14)]. Those wavelengths were chosen because carbon dioxide (at λ_1)

and ethylene (at λ_2) are the main contributors to absorption (Hurren 1990). C_2H_4 also absorb at λ_1 but its absorptivity is very low. To estimate its effect the gas samples was passed through the CO_2 chemical absorber ascarite.

Biochemical analysis: The plant material was analyzed just after the experiment completion. Leaf tissue (1 g) was frozen and ground in liquid N_2, extracted in 10 cm^3 of 0.1 M Tris-HCl buffer, pH 7.8, containing 2mM EDTA, 10mM $MgCl_2$ and centrifuged for 15 min at 12 000 *g*. The resultant supernatant was analyzed for activity of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), NAD dependent isocitrate dehydrogenase (EC 1.1.1.41), malate dehydrogenase (EC 1.1.1.37), glyceraldehyde phosphate dehydrogenase (EC 1.2.1.12), alcohol dehydrogenase (EC 1.1.1.1) and phosphoenolpyruvate carboxylase (EC 4.1.31). The activities were measured spectrophotometrically by methods described earlier (Chapman and Osmond 1974, Gavrilenko *et al.* 1975, Möller *et al.* 1977) which were then modified with regard to the objects peculiarities (Verkhoturova and Astafurova 1983). The experiment had 3 fold repetition in 2 biological recurrings and statistically analyzed based on the Student's test. Differences from control values were significant at $P < 0.05$.

Results and discussion

The ratio *A* at two wavelengths under pressure of 8 kPa substantially exceeds the reference one of 101 kPa. This indicated the different rate of release of gases from the plant surfaces at different pressures and the increase of evolution of intracellular gases into the environment at low pressure (Fig. 2). After gas passing through the ascarite, the signal magnitude falls to the background one. So, CO_2 can be considered
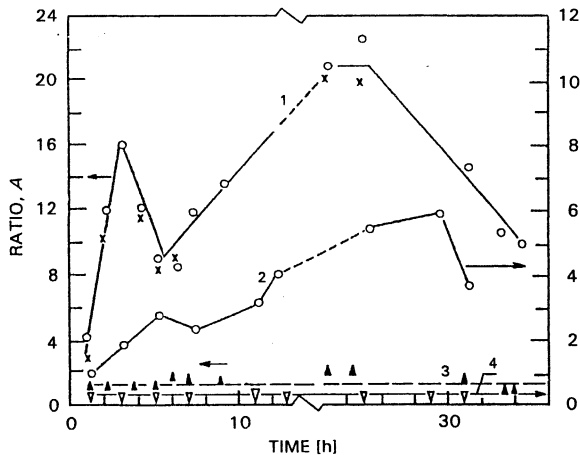

Fig. 2. Ratio *A* vs. time for pea seedlings (dotted line on curves *1* and *2* denotes the night interval without measurements). Curves: *1* - pressure 8 kPa, 2 - 54 kPa, 3 and 4 - 101 kPa (control plants); *open circles* - λ_1 [P(20)], *crosses* - λ_2 [P(14)]

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as the main absorbing component. The ratio between signal values at two generation wavelengths remained approximately the same during the course of the experiment: $A_{P(20)} = A_{P(14)} \cong 1.2$, that also pointed to the fact that plants under lower pressure in the dark mainly evolved CO_2. Apparently, high content of CO_2 counteracted a detection of low concentrations of ethylene and other accompanying gases or their evolution by plants under conditions of anaerobic medium, caused by low pressure (Musgrave *et al.* 1988). At pressure of 54 kPa the curve of CO_2 evolution had two small peaks (Fig. 2). The decrease of pressure to 8 kPa tended to the change of CO_2 evolution rate and two more evident peaks appeared. Maximum evolution of CO_2 was marked 24 h after the test beginning, than its level decreased. The first portion of CO_2 evolution under low pressure was probably connected with the increase of intercellular diffusion rate (Gale 1973, Musgrave *et al.* 1988). The following CO_2 evolution could be explained by amplification of decarboxylation reactions under such conditions (Astafurova *et al.* 1993).
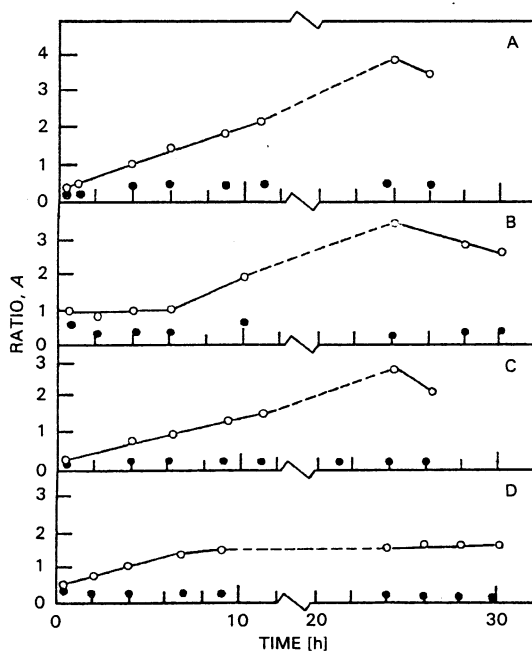

Fig. 3. Ratio *A* vs. time for wheat (*A*), barley (*B*), pine (*C*) and maize (*D*). *Closed circles* - pressure 101 kPa, *open circles* - pressure 54 kPa.

It is known that the capability of an organism to respond to stressors depends on its type, age and individual peculiarities. The evolution of CO_2 by all samples under study was increased with the increased time of plants exposure to low pressure of 54 kPa (Fig. 3). However, differences in curve shape and shift of peaks with time were observed. For example, the relative rate of CO_2 evolution by pea (Fig. 2) is higher than by the other plants. Pea is characterized by a periodical (two peaks) type of reaction. Seedlings of wheat, barley and pine have one pronounced maximum

(Fig. 3). The initial increase of rate of CO_2 evolution by maize seedlings quickly reaches the level of saturation and then remains constant.

The activity of enzymes of main catabolic pathways under normal conditions of aeration had similar values in different plant species (Table 1). The differences were found only for PEPC because of its higher activity in C_4 plants. However, the various metabolic reactions in the plants under analysis manifested themselves in different ways under the low pressure conditions. More activation of GPDH and GAPDH, and lower activation of IDH, MDH and PEPC were noticed in pea. The increase of activity of all above-mentioned enzymes especially PEPC was revealed in maize. The activation degree of ADH was the same for both species. Judging from inhibition of IDH and MDH in pea, the oxidation of carboxydrates was delayed on tricarboxylic acid cycle, and glycolysis turned to fermentation. ADH induction also indicated this. The increase of GAPDH and GPDH activities pointed to the simultaneous activity of glycolysis and oxidative pentose phosphate pathway. Maize accumulated considerable part of carbon as organic acids which could be easily decarboxylated. On the other hand, high carboxylating capability of maize allowed intercellular CO_2 to take and this could explain lesser evolution of CO_2 by maize seedlings as compared to other species. The reactions of decarboxylation of substrates under hypoxia in pea dominated over the carboxylation (Astafurova *et al.* 1993). Developed spongy parenchyma, large intercellular spaces and weak cuticle favoured gas diffusion from the leaf surface of pea seedlings.

Table 1. Effect of low pressure (54 kPa for 48 h) on respiratory metabolism enzymes and PEPC in pea and maize leaves.

Enzymes activity [μmol g^{-1}(f.m.) min^{-1}]	Pea control	54 kPa	Maize control	54 kPa
ADH	0.91 ± 0.06	1.60 ± 0.12	0.65 ± 0.05	1.22 ± 0.08
GPDH	0.40 ± 0.02	1.50 ± 0.10	0.38 ± 0.02	0.59 ± 0.03
GAPDH	1.41 ± 0.09	4.92 ± 0.26	1.23 ± 0.08	2.13 ± 0.16
IDH	0.08 ± 0.005	0.03 ± 0.002	0.09 ± 0.007	0.13 ± 0.01
MDH	10.20 ± 0.9	4.93 ± 0.3	15.30 ± 1.0	17.60 ± 1.2
PEPC	0.98 ± 0.07	0.49 ± 0.03	13.70 ± 0.9	36.20 ± 2.5

The obtained results showed an increase of CO_2 evolution from the surface of plants to the environment under low pressure. The character of response depended on species under study as well as on pressure and period of its action.

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