How drought stress and CO₂ concentration influence stomatal conductance and photosynthesis?

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Abstract

Major aspects of climate change include increased temperature and CO_2 concentrations, as well as decreased water availability (i.e. drought). How plants respond to these changes and what would be the further concequence remians a major topic to study. Stomata present the « gateway » between plants and ambient air and are responsible for gas exchange, transpiration and carbon assimilation. Therefore the role of stomata in the response of to the changing environment is a crucial one to be understood. We aimed at studying the stomatal responce with two model species (sedge and mint) from contrasting habitats that were exposed to control and drought treatments. We measured stomatal conductunce, assimilation rate, stomatal shape, size and density in three CO_2 concentrations.

Key words : climate change, drought, mint, sedge, stomata

Introduction

Drought stress, temperature rise and increase in ambient CO_2 are combined evidences of the pronounced changes in climatic conditions. One of the fundamental and still unsolved questions is the response of plants to the changing environment. Stomata represent the connection between plant and atmosphere and are responsible for both gas exchange and transpiration, and thus fundamental processes such as photosynthesis. Stomatal conductance therefore plays an important role in how plants cope with changing conditions. However, how stomatal conductance and photosynthesis of different plant species will respond to changing environmental conditions remains a topic to be investigated and question to be answered. The aim of our project was to study stomatal responses of plants to drought stress.

For our study we set up some hypotheses. First hypothesis was that stomatal conductance should decrease with drought stress. We also expected that when CO_2 concentration is increased, stomata would close and when decreased stomata would open. Finally, we expected CO_2 assimilation rate (photosynthesis) and stomatal conductance to be correlated positively.

Stomata are small pores on the top and bottom of a leaf that are responsible for taking in and expelling CO_2 from and to the outside air as well as for transpiration. The rate of stomatal conductance, or its inverse, stomatal resistance, is directly related to the boundary layer resistance of the leaf and the absolute concentration gradient of water vapor from the leaf to the atmosphere. It is under direct biological control of the leaf through the use of guard cells, which surround the stomatal pore (Taiz/Zeiger 1991).

Materials and Methods

Stomatal conductance and photosynthesis

Measurements of stomatal conductance (g_s ; mol m⁻² s⁻¹) and photosynthesis A (µmol⁻² s⁻¹) were measured with Portable Photosynthesis and Fluorescence System (LI-COR, LI-6400XT) with a chamber of 6 cm². Each measurements was carried out on two models species: *Carex acuta* (sedge) and *Mentha piperita* (mint). Two treatments were applied on each species. The first simulates drought stress conditions by exposing plants whithout watering under high solar irradiance during one week. The second (named « control » in the following study) represents the blank sample of plants that were grown in conditions whithout drought stress and watered every day. Measurements were taken on one green leaf per each subject at 3 p.m. under three CO₂ concentration (100, 400 and 1200ppm). Other environmental conditions within the chamber were stable throught all measurements, namely light at 1000 µmol.m².s⁻¹, air humidity at 50% and temperature at 25°C. These conditions aimed to mimic optimal growing conditions for plants.

Stomatal size and density measurement

Stomatal size, density, and type were determined by microscope observations. The method consisted of the usage of nail varnish to obtain stomata « negatives » and leaf surface « impressions » (Weyers and Johansen, 1985). Fingernail polish imprints were obtained from leaves epidermal impressions of both adaxial and abaxial surfaces and then clear tape was used to transfer the « impression » to a microscope slide. Stomatal size (μ m²) and density (stomata

mm⁻²) were determined by taking pictures with a camera attached to a microscop (Olympus BX61, magnification x40) and then with using free software Image J-ga. Two images of each imprint were taken, ten stomata were measured and stomatal density was determined directly by counting the number of stomata per image.

Results

Mentha piperita (Mint)

The stomatal conductance of the control plant of *Mentha piperita* was 90.0 mmol H₂O m⁻² s⁻¹ at a CO₂ concentration of 400 parts per million (ppm) (Table 1, Figure 1a), which is the usual CO₂ concentration in the atmosphere in this time. It decreased to 80.0, when the CO₂ concentration was decreased. When the CO₂ concentration was increased, the stomatal conductance decreased stronger to 62.3 mmol H₂Om⁻² s⁻¹. The stomatal conductance of the stressed plant stayed consistently at 27 H₂O m⁻² s⁻¹, there was no influence from the change of the CO² concentration.



Figure 1: Stomatal conductance (a) and photosynthetic rate (b) in the experimental conditions.

The photosynthesis of both plants decreased with a decrease of the CO₂ concentration and increased with an increase of the CO₂ concentration. The measured values at a CO₂ concentration of 400 ppm were 8.18 μ mol CO₂ m⁻² s⁻¹ for the control plant and 2.77 μ mol CO₂ m⁻² s⁻¹ for the stressed plant (Table 1, Figure 1b). With the decrease of the CO₂ concentration on 100 ppm the values dropped to $0.82 \ \mu mol \ CO_2 \ m^{-2} \ s^{-1}$ for the control plant and to $0.02 \ \mu mol \ CO_2 \ m^{-2} \ s^{-1}$ for the stressed plant. At the CO₂ concentration of 1200 ppm the photosynthesis of the control plant doubled, compared to the standard value (400 ppm CO₂) to 16.7 $\mu mol \ CO_2 \ m^{-2} \ s^{-1}$, while the value of the stressed plant went up to 8.94 $\mu mol \ CO_2 \ m^{-2} \ s^{-1}$, which is more than three times higher than for the usual CO₂ concentration.

Table 1: Stomatal conductance and photosynthesis of *Mentha piperita* control and stressed plant. Both values were measured at three different CO₂ concentrations (100, 400 and 1200 ppm).

	[CO ₂]	control plant	stressed plant
Stomatal	100	80.0	27
conductance (gs)	400	90.0	27
$[\text{mmol H2O m}^{-2} \text{ s}^{-1}]$	1200	62.3	27
Photosynthesis (A)	100	0.82	0.02
[µmol CO ₂ m ⁻² s ⁻¹]	400	8.18	2.77
	1200	16.7	8.94

The measured stomata density of *Mentha piperita* was 80 stomata per mm^2 and the average size of the stomata was 1354 μm^2 . The stomata of *Mentha piperita* are kidney-shaped stomata (Figure 2).



Figure 2: Stomata of Mentha piperita with magnification 500.

Carex acuta (Sedge)

The stomatal conductance of the control plant of *Carex acuta* at the CO₂ concentration of 400 ppm was 9.3 mmol H₂O m⁻² s⁻¹, the value of the stressed plant was 1.26 mmol H₂O m⁻² s⁻¹ (Table 2). When the concentration of CO₂ was reduced to 100 ppm, gs decreased in the control plant to 7.0 H₂O m⁻² s⁻¹, while no stomatal conductance could be measured at the stressed plant. At a CO₂ concentration of 1200 ppm, gs decreased to 6.0 mmol H₂O m⁻² s⁻¹ for the control plant and to 0.5 mmol H₂O m⁻² s⁻¹ for the stressed plant.

The photosynthesis at a concentration of CO₂ of 400 ppm was 0.517 μ mol CO₂ m⁻² s⁻¹ for the control plant and 0.2 μ mol CO₂ m⁻² s⁻¹ for the stressed plant (Table 2). After the decrease of the CO₂ concentration, both plants showed 0.2 μ mol CO₂ m⁻² s⁻¹, so there was a little decrease for the control plant, but the value of the stressed plant did not change. But both plants increased their photosynthesis, at the CO₂ concentration of 1200 ppm. A of the control plant rised to 2.0 μ mol CO₂ m⁻² s⁻¹ and A of the stressed plant to 0.29 μ mol CO₂ m⁻² s⁻¹.

Table 2: Stomatal conductance and photosynthesis of the *Carex acuta* control plant and the stressed plant. Both values were measured at three different CO₂ concentrations (100, 400 and

	[CO ₂]	Control plant	stressed plant
Stomatal	100	7.0	0
conductance gs	400	9.3	1.26
$[\text{mmol H2O m}^{-2} \text{ s}^{-1}]$	1200	6.0	0.5
Photosynthesis	100	0.2	0.2
$[\mu mol CO_2 m^{-2} s^{-1}]$	400	0.517	0.2
	1200	2.0	0.29

1200	ppm).
1200	ppin.

For *Carex acuta*, the stomata density was 146 stomata per mm², the size of the stomata was $339 \ \mu m^2$. *Carex acuta* has Dumbbell-shaped stomata (Figure 3).



Figure 3: stomata of Carex acuta with magnification 500.

Discussion

Stomatal conductance and photosynthetic rate with increasing plant drought

The experiment showed that the stomatal conductance and the photosynthetic rate decrease with an increasing plant drought. Considering *Mentha piperita*, the stomatal conductance and the photosynthetic rate of the control plant is higher for all three different CO_2 concentrations in comparison with the dry plant. *Carex acuta* also provided this result for every CO_2 concentration investigated in the experiment. However, the values for Carex acuta measured in our experiment were exceptionally low, especially considering that previous measurements for both species show much higher values. This might have resulted from the sedge individuals beeing either stressed (even the control plants) or senescent..

Relation between stomatal conductance and CO₂ concentration

A decrease in stomatal conductance is expected when the CO_2 concentration is increased at the same time. This effect is caused by a CO_2 saturation with a higher concentration. In the same moment the plant tries not to lose too much water. The result is a slight closure of the stomata. Although the control plant of *Mentha piperita* showed a decrease in stomatal conductance with an increasing CO_2 concentration, the decrease in CO_2 concentration led to a decrease in stomatal conductance. The control plant of *Carex acuta* provided a contrary result. An increase in CO_2 concentration results in a higher value for stomatal conductance. Stomata of the plant opened by decreasing the CO_2 concentration. The decrease in stomatal conductance with a decreasing CO_2 concentration of the control plant of *Mentha piperita* as well as the increase in stomatal

conductance with an increasing CO₂ concentration of the control plant of *Carex acuta* may be caused by other parameters that influenced the plant or a wrong measurement during the experiment. The stressed plant of *Mentha piperita* kept a constant stomatal conductance during increase and decrease of CO₂ concentration. A possible reason for the lack of response to the change in CO₂ concentration could be the growing stress due to drought. The stressed plant of *Carex acuta* decreased its stomatal conductance with the increase or decrease of CO₂ concentration. Thus, due to the same response to the change in CO₂ concentration in the control plant and the stressed plant of *Carex acuta* the same reasons for the constant stomatal conductance can be assumed. A further reason for the stressed plant is the increase of drought stress that may affect the stomatal behaviour.

Relation between photosynthetic rate and CO₂ concentration

A positive correlation between the photosynthetic rate and the CO_2 concentration is generally detectable. The control plant and the stressed plant of *Mentha piperita* as well as the control plant of *Carex acuta* show this correlation. There is no positive correlation for the stressed plant of *Carex acuta* because of an unchanged photosynthetic rate at a decrease of CO_2 concentration.

Relation between stomatal size and stomatal density

A negative relation between size and density of stomata is expected for the different species in the experiment. The results of the measurement confirm this expectation. While the stomata size of *Mentha piperita* is larger than the size of stomata of *Carex acuta* the stomata density is less. As a conclusion it could be obtained that with larger stomatal size the number of stomata decreases and the other way round.

Prospects

To investigate and clarify the expectations and results of the experiment better more repetitions in measurement and plant preparation should be run. The conditions for the plants before and during the experiment have to be clarified, too. Further investigations might help to learn more about the drought stress of different plant species.

References

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