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BRIEF COMMUNICATION

# $Ca^{2+}$ reduces the effect of hypoxia in mosses *Mnium undulatum* and *Polytrichum commune*

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## Abstract

Gametophores of mosses *Mnium undulatum* and *Polytrichum commune* were submerged in distilled water or in calcium chloride solution (0.9 mM $Ca^{2+}$) to induce hypoxia. The net photosynthetic ($P_N$) and dark respiration rate ($R_D$) were measured in the air containing 300 - 400 $\mu$mol($CO_2$)$\cdot$mol$^{-1}$(air) and 0.21 mol($O_2$)$\cdot$mol$^{-1}$(air). $P_N$ of *M. undulatum* gametophores decreased to 58 % of the control after 1-h submersion in water, whereas to 80 % of the control in *P. commune* gametophores. A smaller decrease in $P_N$ was observed when the gametophores were immersed in $CaCl_2$ solution. In hypoxia, $R_D$ in the tested mosses species was a little higher than in the control.

*Additional key words*: net photosynthetic rate, dark respiration rate.

In natural conditions plants are exposed to various stresses, one of which is oxygen deficiency leading to changes in the plant metabolism. Mosses with a simple anatomic structure, are transiently flooded in natural conditions. This leads to a decrease in the amount of available oxygen. On the basis of oxygen availability three types of response can be distinguished: normoxia, hypoxia, and anoxia (Bartosz 1997, Mustroph and Albrecht 2003). It has been widely reported that oxygen defiiency affects the profile of protein synthesis and gene expression (Van der Luit *et al.* 1999, for recent reviews see Subbaiah and Sachs 2003). The majority of anaerobic proteins have been identified as enzymes involved in glycolysis or sugar-phosphate metabolism. The oxygen shortage limits the oxidative phosphorylation and consequently leads to a decrease in ATP production. If the glycolysis and fermentation does not provide sufficient amounts of ATP, the concentration of ethanol or lactate increases, which leads to changes in pH and limits the activity of many enzymes (Drew 1997). Adaptative mechanisms enabling anoxia- and hypoxia-tolerance in plants vary. In oxygen stress tolerant plants, an increase in the contents of malate, succinate and alanine, in addition to an increase in the lactate and ethanol contents, are observed (Kennedy 1992). During re-oxygenation, mechanisms preventing a sharp increase in the content of reactive oxygen species (ROS) are important (Drew 1997, Chai *et al.* 2005). Maintaining a low content of ROS plays an important role in the hypoxia- and anoxia-tolerance mechanism (Rzepka *et al.* 2005). Moreover, changes in $Ca^{2+}$ concentration in the cytosol is a signal changing many metabolic pathways, one of which is the expression of genes related to oxygen-shortages in the environment. The flux of $Ca^{2+}$ through the cytoplasmic membranes is an important factor defining the final response of plants to stress (Subbaiah *et al.* 1994, Chung *et al.* 2001).

Studies concerning the effect of hypoxia on photosynthesis and respiration have involved mainly higher plants. Little research has been done on bryophytes, although those organisms are often affected by this type of stress. Determination of their response to hypoxia seems to lead to better understanding of changes observed in natural bryophyte habitats.

*Mnium undulatum* and *Polytrichum commune* gametophores collected in their natural habitat (a forest site near Krakow) were transfered into growth chambers and kept under controlled conditions: temperature of 15 ± 2 °C and 12-h photoperiod with irradiance (at PAR range) of 70 $\mu$mol$\cdot$m$^{-2}$s$^{-1}$. After a 2-month-long adaptation period, gametophores were rinsed in distilled water, dried with blotting paper and mounted in holes drilled in

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*Abbreviations*: $P_N$ - net photosynthetic rate; $R_D$ - dark respiration rate.

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plexiglass boards. Then, the boards with evenly spread gametophores were placed in glass vessels of 1 $dm^3$ volume. The vessels were filled with either distilled water or $CaCl_2$ solution of 0.9 mM $Ca^{2+}$. The concentration of $Ca^{2+}$ was determined as optimal for the studied bryophyte species on the basis of preliminary experiments carried out at concentrations from 0.01 till 10 mM (unpublished data). After 1, 24, 72, 96, 120, 144, 168 h, gametohores, previously flooded, were transfered to the measurement chamber where gas exchange was determined. Carbon dioxide exchange rate was monitored using an infrared gas analyzer (*ADC-225-MK-3*, Hoddesdon, England) at $CO_2$ concentration 300 - 400 $\mu mol(CO_2) \cdot mol^{-1}(air)$ and the $O_2$ concentration 0.21 $mol(O_2) \cdot mol^{-1}(air)$. The volume of the whole system (closed system) comprising a chamber with a water jacket and air humidifying system was 0.644 $dm^3$. The temperature inside the chamber was 25 °C and the irradiance was 70 $\mu mol \cdot m^{-2}\ s^{-1}$. The net photosynthetic rate ($P_N$) and respiration rate ($R_D$) were calculated as a percentage of the respective initial values (0 h) before flooding of the gametophores.

Studies of the effects of low concentrations of $O_2$ (hypoxia) on gas exchange in *M. undulatum* and *P. commune* were carried on totally submerged gametophores. The anaerobic treatment of *M. undulatum* gametophores results in a 42 % decrease of $P_N$ after 1 h. The prolongation of the hypoxia treatment did not cause such significant changes, but $P_N$ decreased steadily and after 168 h it was about 62 % lower than that of the control (Fig. 1*A*).

*P. commune* gametophores show higher tolerance to the low oxygen stress. The $P_N$ was 20 % lower than that of the control under hypoxia lasting 1 h. The prolongation of the hypoxia treatment led to a decrease in $P_N$, but after 168 h it was only about 40 % lower than that of the control (Fig. 1*A*). The exposure of gametophores to the low oxygen stimulated the dark respiration. For *P. commune* gametophores an increase in $R_D$ was especially noticeable after 24 h of the stress treatment. After 168 h, its $R_D$ was similar to that observed for plants not subjected to the hypoxia stress. For *M. undulatum* gametophores under hypoxia, $R_D$ was a little higher than that in the control plants (Fig. 1*C*).

Under prolonged hypoxia the ratio of ATP to NADPH decreases, which can result in plant cell death (Drew 1997). A different response can be observed in photosynthesing plant organs. The disturbances of the metabolism caused by the flooding stress, especially after a prolonged period of its action, can be compensated through the photosynthesis. In the case of bryophytes, the leaf structure which enables a direct contact of assimilating cells with the environment facilitates gas exchange changes (Krupa and Rzepka 1999, Rzepka and Krupa 2002). The measurements of $P_N$ and $R_D$ showed that the addition of $Ca^{2+}$ to the substrate noticeably limited the flooding stress. In the presence of $Ca^{2+}$ in the substrate, the 1-h flooding stress led to only about 20 % reduction $P_N$ of *M. undulatum* gametophores. The influence of $Ca^{2+}$ on $P_N$ in *P. commune* gametophores was weaker than in *M. undulatum* (Fig. 1*B*). After submersion in $CaCl_2$ solution $P_N$ was reduced only by about 16 % of the control value and remained similar till the of 168-h experiment. A change in the cytosolic concentration of calcium is known to be an essential in the signalling pathways induced by various internal and external stimulii. In our experiments the effects of low oxygen can be correlated with the $Ca^{2+}$ efflux from the cell. When *M. undulatum* gametophores were submerged in distilled water the $Ca^{2+}$ efflux from the cell to the environment was observed. However, no $Ca^{2+}$ efflux from leaves was detected for *P. commune* gametophores subjected to hypoxia (data not shown). The results suggest that the studied bryophytes represented different responses to hypoxia.

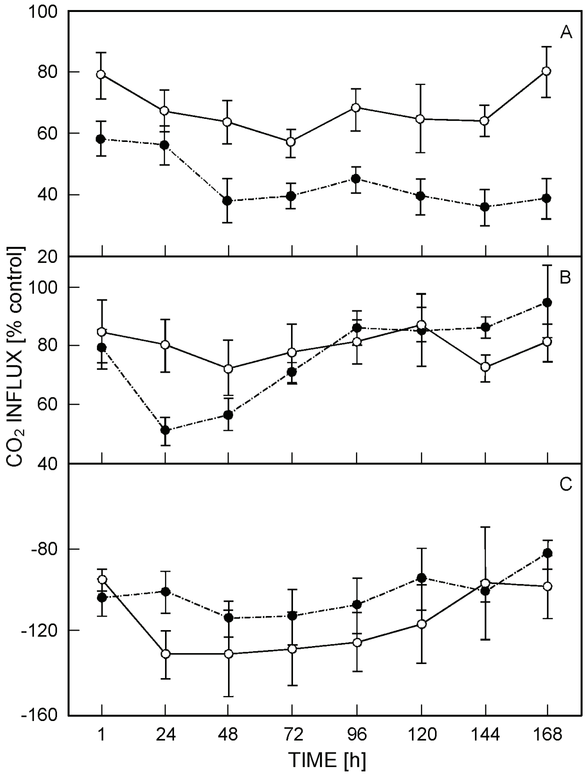

Fig. 1. The net photosynthetic rate ($P_N$) in *M. undulatum* (*closed circles*) and *P. commune* (*open circles*) gametophores immersed in distilled water (*A*) or 0.9 mM $CaCl_2$ (*B*). Dark respiration rate ($R_D$) of gametophores immersed in distilled water (*C*). Values are expressed as percentage of control (before immersion). Means ± SD of 8 independent experiments ($n = 8$).

The $Ca^{2+}$ concentration in the cytosol depends on $Ca^{2+}$ channels present in all cell membranes. The intracellular $Ca^{2+}$ concentration can increase, as a result of the activation of some receptors, a process related to the "calcium induced calcium release" mechanism (Trewavas and Mahlo 1998). Other mechanisms involving the activation of calcium channels are able to release calcium ions from the endoplasmic reticulum (Bartošková *et al.* 1999, Christov and Bakardjieva 1999, Chin and Means

2000, Cousson 2007).

The presented results show that changes in the cytosolic $Ca^{2+}$ affect the reduction in $P_N$ caused by hypoxia, especially noticeable for *Mnium* gametophores. The studied bryophytes exhibit different sensitivities to changes in the $Ca^{2+}$ concentration, but the explanation of the mechanisms responsible for these differences needs further detailed research.

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