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Metabolic activity of *Sphagnum recurvum* under different environmental conditions

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Although peatlands dominated by *Sphagnum* mosses cover about 3% of the Earth's land surface and *Sphagna* are amongst the most important carbon fixers in these habitats, studies of their physiology are scarce. Since in Hungary *Sphagnum* bogs are very rare and represent glacial relicts with special microclimatic characteristics, they are strictly protected. Because of their unique ecological niche, *Sphagna* can easily disappear if environmental factors change unfavourably. Therefore it is important to know their physiological responses under different environmental conditions and to determine their stress tolerance. Among the various groups of mosses, *Sphagnum* is unique in many respects. The most distinctive feature of peat moss morphology is the leaf with an unusual arrangement of two different kinds of cells: large hyaline cells (dead at maturity) and small slender chlorophyllose cells, which together have a great impact on the metabolic activity.

This paper reports on experiments we have done so far, and shows some physiological properties of *Sphagna* (exemplified by *S. recurvum*) which has made them succesful and helped them survive in their exceptional habitat. Our data focus on the pigment composition, chlorophyll fluorescence induction parameters, the influence of temperature and tissue water status on the dependence of net phyotosynthesis (Pn), non-structural carbohydrate pool and acid invertase activity in *S. recurvum.*

Materials and Methods

S. recurvum originated from north-east Hungary and were acclimatised at 100% RH in desiccators at 16ºC, at a PPFD of 100 µmol m^{-2} s^{-1}, and a photoperiod of 12/12 light/dark for 1 month before treatments.

In vivo chl fluorescence was measured using a PAM fluorometer. The experimental protocol, terminology and calculations followed Schreiber et al. (1986) and Van Kooten and Snel (1990). CO_2 assimilation was measured in normal air by infrared gas analyzer in an open gas-exchange system using a temperature controlled leaf chamber. Assimilation rates (*A*) were calculated after von Caemmerer and Farquhar (1981). Photosynthetic pigments were extracted from fresh material homogenizing twice with ammoniacal acetone (80%). The concentration of chl-*a*, -*b* and total carotenoids were determined according to Lichtenthaler and Wellburn (1983).

Temperature dependence of P_n was measured at 5, 10, 16, 20 and 25 C at PPFD of 100-1200 µmol m^{-2} s^{-1} in plants at full turgor. Measurements of water status dependence on P_n were carried out at 16∞C, at a PPFD of 600 µmol m^{-2} s^{-1} on samples with known RWC. These experiments started at full turgor of the plants (~1400% RWC dw.), followed by "natural drying" (at 16ºC, at a PPFD of 100 µmol m^{-2} s^{-1} and 35% RH), or controlled drying (in desiccators at 54% RH and at 16ºC, at a PPFD of 100 µmol m^{-2} s^{-1}) or controlled levels of osmotic stress by exposure to PEG (4000) solutions.

In sugar feeding experiments plants were placed on filter papers moistened with 10 mol m^{-3} glucose, fructose and sucrose solutions (and with distilled water for control) in Petri dishes for 7 d in light and dark at 16ºC at a PPFD of 100 µmol m^{-2} s^{-1}. For the carbohydrate determinations tissue was extracted with 80% ethanol followed by hot water and with boiling in 3% HCl for 3 h for starch. Fructans were quantified using a ketose-specific method with resorcinol and separated by TLC on silica gel plates and detected with urea-phosphoric acid stain. Total soluble sugars were detected by the Dubois method. Sucrose was quantified by a combined enzymatic assay (invertase-glucose-oxidase-peroxidase) of glucose release. Invertase was assayed by enzymatic assay of glucose after incubation of sucrose (Marschall et al. 1998).

Results and Discussion

Bryophytes, even those of open, exposed habitats, have been regarded as showing shade plant-like characteristics, on evidence including fine-structural features of the chloroplasts and typically low chl a/b ratios. Total chl concentration in *S. recurvum* was ~2.0 mg g^{-1} (dw.) as in other mosses of sun-exposed habitats (Marschall and Proctor, unpublished data). Chl a/b ratio was similar as found in most of the mosses (2.65). The ratio of total chls to total carotenoids was typically ~4.0 as in species of sun-exposed habitats. Many bryophytes (and also *S. recurvum*) of sun-exposed habitats show two remarkable features in their PPFD-response curves. First, electron flow does not saturate, but continues to rise almost linearly with increasing irradiance. Second, these species show extraordinarily high levels of *NPQ*, which often also continues to rise almost linearly to irradiances equivalent to full sunlight. At the same time, *1-qP* generally stabilises at around 0.3 to 0.4. The high *NPQ* is suppressed by DTT, so it is likely that it reflects an extreme development of xanthophyll cycle-dependent photoprotection similar to that of higher plants. Comparing RETR and IRGA CO_2-uptake curves for *S. recurvum*, the data suggest that CO_2-uptake accounts for ~60% of the low-PPFD "saturation" value, and

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that all the rest goes to other electron sinks. Responses of this kind are found in a taxonomically and ecologically diverse range of bryophytes of sun-exposed habitats (Marschall and Proctor, unpublished data).

Temperature response of P_n was investigated in the interval of 5-25ºC at full turgor. A linear relationship was found between temperature and A (μmol CO_2 kg^{-1} dw. s^{-1}) measured at 600 μmol m^{-2} s^{-1} (r=0.9532), temperature and A_{max} (r=0.9771) and PPFD (μmol m^{-2} s^{-1}) and A_{max} (r=0.9263). The annual temperature fluctuations of the peat bog water surrounding the *Sphagna* are minimal. Populations of thermally fluctuating environments show greater levels of photosynthetic acclimation than do species from thermally stable environments (Oechel 1976). According to this the temperature response of *S. recurvum* above 20ºC is in the unexpected direction and perhaps caused simply by physical factors (*e.g.* dead tissue CO_2-absorption, or alteration of CO_2 diffusion) at higher temperatures.

In the course of "natural drying" the P_n of *S. recurvum* falled to 0 in 90 mins, while RWC (as % dw.) decreased to ~25% from full turgor. *A* was stable until RWC = 0.4 (as % of WC at full turgor) and then continued to decrease almost linearly to 0.1. Applying controlled drying at RH 54% *A* declined slowly and after 5 h-desiccation it reached ~40% of its value at full turgor. -0.2481 MPa osmotic stress resulted in 9% decrease in *A* after 1.5 h and 50% after 24 h exposure.

Many of the bryophytes are desiccation-tolerant, so their carbohydrate composition and metabolism are of interest. Leafy liverworts (*Jungermanniales*) contain a diverse range of soluble carbohydrates, including sucrose, fructan and polyols such as mannitol, sorbitol and volemitol. Mosses have a simple soluble carbohydrate pool consisting of sucrose. *Sphagnum* species - as an exception amongst mosses - synthetise fructan. In both leafy liverworts and mosses starch and reducing sugars such as glucose and fructose are present at relatively low concentration. Although sucrose and fructans are storage compounds it is also likely that they have other functions. Accumulation of soluble carbohydrates in higher plants is linked to desiccation and freezing resistance and also to osmotic adjustment and turgor maintenance during water deficit. The latter function is unlikely to be important to poikilohydric plants, so it is possible that these compounds have other protective effects such as stabilization of macromolecules or scavenging of hydroxyl radicals.

Sucrose and fructan are the major soluble carbohydrates of the examined species. Starch was present in less than 1% of the total soluble sugars. TLC showed that fructans form a homologous series of increasing DP in a similar manner to fructans in Angiosperms. The trisaccharide has the same mobility as 1-kestose in the *Helianthus* standard suggesting that the fructan could be of the inulin type.

Exogenous sucrose and glucose had very little effect on the soluble carbohydrate pool of the leaves at the relatively low (10 mol m^{-3}) concentration employed. Glucose, fructose and sucrose feeding resulted in a significant increase in the soluble sugar concentration after a week. Fructan content also increased in glucose- and sucrose-treated leaves. The sucrose content did not change. Dark starvation even after a week did not cause a significant decrease in the non-structural carbohydrates. Overall *S. recurvum* contrasts with higher plants in which soluble sugars fall to very low levels after shorter exposure to the dark. It could be related to the very small proportion of non-photosynthetic sink tissue.

Exogenous sugars supplied for 7 d either in light or dark caused depression of Pn. Glucose feeding had the most relevant effect on Pn. Photosynthetic capacity was still retained after 7-d-darkness. This contrasts with the loss of photosynthetic capacity and degreening in higher plants exposed to prolonged dark. Dark storage decreased respiration rate by 40%. These responses of photosynthesis and respiration presumably contribute to conservation of resources when photosynthesis is prevented but allows rapid resumption of photosynthesis when the plant is illuminated.

In contrast to liverworts, mosses show very little, or no acid invertase activity (Marschall et al. 1998). *S. recurvum* showed 10% of the "Porella enzyme" activity. The acid invertase activity in *S. recurvum* was found six-fold higher (0.63 ± 0.02 μmol glucose min^{-1} g^{-1} d.w.) than in "real mosses".

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