

Drought survival test of eight fen moss species

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Abstract – Eight fen moss species (*Aulacomnium palustre*, *Bryum pseudotriquetrum*, *Calliergonella cuspidata*, *Campylium stellatum*, *Climacium dendroides*, *Hamatocaulis vernicosus*, *Plagiomnium elatum* and *Tomentypnum nitens*) were studied in order to assess their ability to survive long-term drought. The mosses differed significantly in their desiccation resistance. The highest survival rate was shown in the hummock moss species *Climacium dendroides*, *Aulacomnium palustre* and *Tomentypnum nitens*, with more than 10% of stems surviving after 20 weeks of desiccation. On the other hand, in *Campylium stellatum* and *Plagiomnium elatum*, almost no stem survived after 6 weeks without water supply. The remaining mosses (*Hamatocaulis vernicosus*, *Calliergonella cuspidata* and *Bryum pseudotriquetrum*) showed medium desiccation resistance, which differed little among species. After 12 weeks of desiccation, less than 10% of their stems were able to restore their growth. In general, most species displayed remarkable desiccation resistance, unexpected for species from permanently wet fen habitats.

Desiccation tolerance / bryophyte / fen / survival / regeneration capacity

INTRODUCTION

Survival in conditions of intermittent water supply has been a major challenge of the life on Earth for land plants since their colonization of dry land in the Paleozoic (Bartels *et al.*, 2011). The first land plants probably developed the strategy of poikilohydry – a drastic reduction of metabolic activity during the dry period, before means to prevent water losses evolved. This strategy has to the present generally been used by bryophytes, in contrast to most other representatives of embryophyte lineages. Despite the general characteristic of mosses being desiccation tolerant, it is known that some species such as *Sphagnum* do not tolerate desiccation very well (Shipperges & Rydin, 1998). Usually, the mosses from aquatic habitats have reduced ability to tolerate dehydration (Glime, 2007). Nevertheless, all fen mosses in the temperate European climate, despite the overall humidity of the habitat, face desiccation several times during the year. Kooijman & Whilde (1993) observed a notable desiccation resistance of the fen moss *Scorpidium scorpioides* during particularly dry summer months, even though its growth was drastically reduced. The ability of mosses to withstand prolonged periods of drought was already studied by Schröder (1886) but the first large-scale study on desiccation resistance was realized by Irmscher (1912). He conducted a series of observations, in which he described the maximum resistance of 47 mosses from different habitats including streams, meadows, forests or dry rocks to

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uninterrupted drying. The resistance was defined as the survival ability of cells tested by observations of plasmolysis of leaf cells. The resistance to desiccation was shown to be related to the habitat of each species, with increased ability to withstand desiccation in plants from drier habitats. The remarkable range of Irmscher's observations on species from different habitats did not, on the other hand, allow focusing on fine-scale comparison of species from individual habitats.

Drought resistance in poikilohydric species is a very complex matter and also the terminology in this field is ambiguous. The ability of plants to withstand the drought period by interrupting the metabolism and its resuming after rewetting is usually labelled as a desiccation tolerance. This is usually measured by tests of physiological activity, such as the plasmolysis test (Irmscher, 1912), or photosynthesis or respiration recovery after rewetting (Bewley, 1995). The plants can, however, resume their growth even after no signs of physiological activity are detectable using the above named conventional tests (Wagner & Titus, 1984; Cleavitt, 2002; Bader, 2013). Even if the original stem tissue is dead, mosses can resume growth from meristematic cells (usually called dormant buds), shielded in the most protected parts of the stem (Schröder, 1886; Irmscher, 1912; Watson, 1914; Stark *et al.*, 2013). In our experiment, we have selected eight fen moss species for comparison of their long-term desiccation resistance. We have tested the overall regeneration ability after variously long periods of desiccation, rather than recovery of original leaf tissue, which happens only in the first few weeks. Hence, in describing our results, we prefer the more general term desiccation resistance, which combines the desiccation tolerance and the desiccation avoidance, to avoid confusion with the desiccation tolerance, as understood in plant physiology.

MATERIALS AND METHODS

Eight fen moss species were selected to represent the typical range of microhabitats, life forms and growth forms commonly occurring at rich fens of Central Europe: *Climacium dendroides* (Hedw.) F. Weber & D. Mohr, which occurs in loose turf or wefts at the driest parts of fens, *Aulacomnium palustre* (Hedw.) Schwägr. and *Tomentypnum nitens* (Hedw.) Loeske, growing in tall turfs in drier parts of the fen, *Calliergonella cuspidata* (Hedw.) Loeske and *Plagiomnium elatum* (Bruch & Schimp.) T. J. Kop., growing in loose turfs to wefts at variously wet parts, and *Bryum pseudotriquetrum* (Hedw.) P. Gaertn., B. Mey. & Scherb., *Campylium stellatum* (Hedw.) Lange & C. E. O. Jensen and *Hamatocaulis vernicosus* (Mitt.) Hedenäs inhabiting the wettest places and growing in tall turfs, and loose turfs to wefts, respectively.

The species were collected in November 2008 from three localities to reduce potential random influences such as decreased vitality of some populations, or genetically fixed variability. As all species did not occur at each locality in sufficient quantity for removal, missing species were sampled from two additional localities with large populations. The shoots were cut to the length of 5 cm to ensure their equal access to water table after rewetting. Eleven equal sets, comprising 45 shoots of every studied species (three groups of five shoots of each species selected from each of the three localities, see the diagram of the experiment in Fig. 1), were prepared and placed into perforated plastic pots. These sets were used for the individual drying/rewetting treatments. All sets were

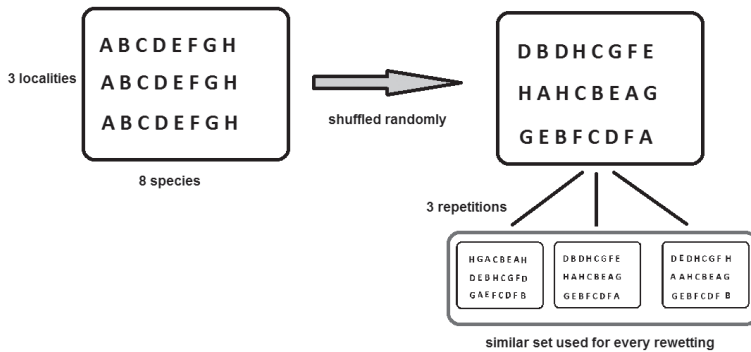


Fig. 1. The desiccation experiment arrangement.

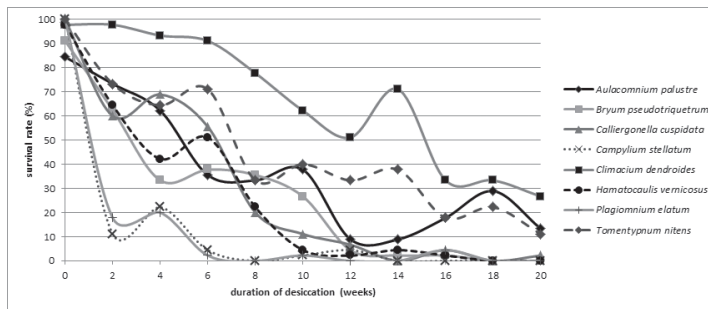


Fig. 2. Mean survival rate of individual cohorts (15 stems from each of three localities) of eight studied moss species after variously long desiccation time (0-20 weeks).

left to dry spontaneously in a growth chamber at 18°C under 12 h light period ($ca\ 60\ \mu\text{mol photons m}^{-2}\text{s}^{-1}$). Once in every two weeks, one set of 45 plants for each species (a cohort) was rewetted upon placing the pots into a plastic dish filled with tap water, and grown in a stable water level of $ca\ 3.5\ \text{cm}$ below the shoot apex for five weeks under the temperature and light period as described above. This created 11 cohorts experiencing the desiccation period of 0 to 20 weeks (Fig. 2). The survival rate was expressed as the percentage of living stems counted five weeks after rewetting. The stem of the moss was considered alive when it had at least one green growing branch (the original or a new one). The differences in desiccation resistance among species were tested by general linear models (GLM) – factorial ANOVA and Tukey's HSD test using the program Statistica 9.0 (StatSoft, 2010).

RESULTS AND DISCUSSION

The results of factorial ANOVA showed that the desiccation resistance differed significantly among species ($p < 0.001$, $F = 11.831$, $Df = 7$). As apparent from Fig. 2, three groups formed among the cohorts, as is also supported by

Table 1. Multiple comparisons of desiccation resistance among species (Tukey HSD). The pairs printed in bold differ significantly at $\alpha = 0.05$. Used abbreviations: Ap – *Aulacomnium palustre*, Bp – *Bryum pseudotriquetrum*, Cc – *Calliergonella cuspidata*, Cs – *Campyllum stellatum*, Cd – *Climacium dendroides*, Hv – *Hamatocaulis vernicosus*, Pe – *Plagiomnium elatum*, Tn – *Tomentypnum nitens*.

Species	Hv	Cc	Tn	Cd	Pe	Cs	Bp	Ap
Hv		0.999696	0.000032	0.000032	0.004062	0.002296	1.000000	0.004836
Cc			0.000032	0.000032	0.000551	0.000299	0.999749	0.027019
Tn				0.005528	0.000032	0.000032	0.000032	0.186418
Cd					0.000032	0.000032	0.000032	0.000032
Pe						1.000000	0.003852	0.000032
Cs							0.002172	0.000032
Bp								0.005096
Ap								

Tukey’s multiple comparison test (Table 1). It also shows that the higher survival rate of *Climacium dendroides* differs significantly from that of all other tested species. Whereas in *Climacium dendroides* no significant decrease of recovering ability occurred in the course of the first 8 weeks, the survival rate had already decreased below 20% after the first 2 weeks of drought in *Campyllum stellatum* and *Plagiomnium elatum*. The response to drought exposure in the remaining species oscillated between these extremes; however, all of them showed a survival rate exceeding 20% after 8 weeks of desiccation. The situation changed rapidly after 10 weeks, when the survival rate of another two species of the medium-resistant group (*Hamatocaulis vernicosus*, *Calliergonella cuspidata*) dropped under 10%. After 12 weeks only two species, *Climacium dendroides* and *Tomentypnum nitens*, showed a notable ability for recovery. In these species, the limited ability to resume growth existed even after 20 weeks of desiccation (with survival rate of nearly 30% in the former).

Our results seem generally to confirm that similar desiccation resistance is largely shared by species inhabiting similar microhabitat conditions (Abel, 1956; Lee & Stewart, 1971; Seel *et al.*, 1992; Oliver *et al.*, 1993; Šinžar-Sekulić *et al.*, 2005). The lawn species *Climacium dendroides* tolerated desiccation significantly better than *Hamatocaulis vernicosus*, which occurs at much wetter parts of fens. On the other hand, the survival was very similar in *Hamatocaulis vernicosus* and *Calliergonella cuspidata*, although the latter shows much broader ecological amplitude, tolerating also sites with a much lower water table. Nevertheless, a broader range of ecological conditions tolerated by *Calliergonella cuspidata* might refer to long-time habitat adaptation. It has been reported that stems of *Calliergonella cuspidata* sampled at differently wet places differed significantly in their desiccation tolerance (Lee & Stewart, 1971), but all our studied mosses were sampled in places with rather high water levels. Some of the unexpected results could perhaps also be attributed to the effect of hardening by previous mild desiccation. A recent study, which tested the desiccation tolerance of *Sphagnum cuspidatum* growing in dry and wet microhabitats, reported no significant difference when plants were de-hardened, having been grown under standardized conditions (Hájek & Vicherová, 2013).

Interestingly, the last three cohorts of *Aulacomnium palustre* showed significantly better survival rates than the two preceding ones. The inconsistent

results for the individual cohorts of *Aulacomnium palustre* might have resulted either from the above-mentioned hardening in mild desiccation stress experienced at localities with non-identical environmental conditions, genetic variability of the individuals or the poor health of some *Aulacomnium* stems in time of sample collecting in combination with suboptimal conditions during the experiment. Some cohorts of the moss might have not fully tolerated the experimental conditions, as evidenced by non-zero mortality in the control (not desiccated) group. The mortality of *Aulacomnium palustre* in the control was highest among the studied species despite the generally high desiccation resistance of the moss. Considerable decrease of the desiccation tolerance resulting from suboptimal experimental conditions was reported also by Hájek & Beckett (2008).

The maximum leaf cell survival limit for similar fen species *Sarmentypnum exannulatum*, *Warnstorfia fluitans* and *Philonotis fontana*, reported by Irmischer (1912), was 15–20 weeks. However, the same species were able to form protonemata, which formed gametophores after prolonged cultivation, even after 9 months of desiccation (Irmischer, 1912). We cannot rule out that the seemingly dead plants from our experiment would also form protonemata if planted in suitable conditions long enough, but our experimental design did not test this. The slightly different results obtained in our study are probably caused by slightly different experimental conditions in combination with different site history, age and phenology of individuals or genetic differences. The desiccation tolerance is highly influenced by desiccation speed, temperature or light intensity (Krochko *et al.*, 1978; Bohnert, 2000), and season (Proctor *et al.*, 2007).

The desiccation *in situ* may also differ from conditions of our experiment. It may be faster in direct sun or when a single stem is detached from the tuft, but normally the desiccation *in situ* is much slower, due to the buffering effect of dense turfs. This life form allows capillary rise of water to considerable height and it also slows evaporation significantly (Vitt & Wieder, 2008). However, as shown by Irmischer (1912), the desiccation tolerance of stems from the turf centre does not differ from those at the edge, which are more directly exposed to environmental influences. The fen mosses often face the negative effect of water table fluctuation, as shown by Štechová *et al.* (2012). The problem occurs when a sudden water level drop reveals stems acclimated to living in a higher water level, as such stems tend to grow in sparse turfs. Another difference between experimental and *in situ* conditions is that in the field, the drought period is rarely uninterrupted for several weeks. Moreover, even if no rain occurs, horizontal precipitation such as mist or dew can slow down the desiccation process.

The water stress involves not only mere water deficiency, but also the competition changes and vegetation replacements on a larger time scale (Mälson *et al.*, 2008). While the stem is regenerating, its competitive ability is probably impaired and at the same time, the lowered water table can pose the competitive advantage for tracheophytes since high water table suppresses the growth of tracheophytes. This situation occurs especially when the water regime of the locality is disturbed.

Our study demonstrated that most fen mosses, despite being usually labelled as desiccation intolerant, particularly with respect to stem fragments (Pépin *et al.*, 2013), can mostly survive at least eight, and some of them even 20 weeks, without water. This ability, however, mostly refers to resprouting from dormant buds after the drought period exceeds a few weeks. On the other hand, the mortality which occurred in nearly all species already after 2 weeks of drought indicates that desiccation is a serious stress. This shows that the physiological tests of tissue vitality based on plasmolysis or photosynthesis restart should be

complemented by the regeneration test to reflect the actual recovery and survival of the mosses. Contrary to the contention of Pépin *et al.*, the desiccation resistance of these fen mosses appears to be more than sufficient for the reproduction and dispersal by stem fragmentation.

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