

## COMPARISON OF DESICCATION TOLERANCE AMONG MOSSES FROM DIFFERENT HABITATS

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**Abstract** - Three moss species from the karst region were compared to establish their respective patterns of desiccation tolerance. Different life forms of bryophytes were chosen to obtain evidence of their life strategies during drought conditions. Comparative analyses of electrolyte leakage were performed to screen for tolerance of the membrane to water stress and for signs of damage to the fine structure of the protoplasm. The experiments were carried out by exposing the plants to water stress caused by PEG 600. The results show that the most desiccation tolerant species is *Thamnobryum alopecurum*, less but fairly tolerant is *Anomodon viticulosus*, while the aquatic *Rhynchostegium riparioides* is intolerant of desiccation.

**Key words:** Desiccation tolerance, mosses, *Thamnobryum alopecurum*, *Anomodon viticulosus*, *Rhynchostegium riparioides*

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### INTRODUCTION

Mosses are often highlighted as plants with a capacity for revival and an extreme tolerance of drought, i.e., the specific ability to pass in and out of anabiosis. However, not all moss species are equally tolerant of water loss, and a significant number of bryophytes die when exposed to desiccation (Bewley and Krochko, 1982). Important differences exist among various moss species in their levels of morphological and physiological as well as biochemical organization. For example, *Tortula ruralis* (Hedw.) Gaertn., Meyer & Scherb. can tolerate severe water deficit and great extremes of temperature at the same time, while some other species, such as many leafy hepatics, are unable to tolerate even small fluctuations in the water balance of their habitats. *Tortula ruralis* even appears to survive better at lower than at higher humidities (Proctor, 1982).

During water stress the changes in permeability of the cellular membrane are one of the first signs of disturbance to the fine structure of the protoplasm (Senaratna and McKersie, 1983). This results in an increase in electrolyte leakage (Levitt, 1980).

With this in mind, the aim of the present study was to establish the difference in resistance to water stress between some moss species from different habitats. Ion conductivity measurements were used as a screening test to determine the extent of damage to the cellular membrane, as well as the stress-survival capacity of the cells in reconstituting the integrity of the membrane.

### MATERIAL AND METHODS

Three moss species: *Thamnobryum alopecurum* (Hedw.) Gang, *Anomodon viticulosus* (Hedw.) Hook & Tayl., and *Rhynchostegium riparioides* (Hedw.) Card. were collected in the karst region of Petnica in West Serbia. *Thamnobryum alopecurum* was collected from dry, vertical limestone rock by the river Banja, while *Anomodon viticulosus* was gathered from shaded sites on rock outcrops in a *Quercus-Carpinetum moesiicum* Rudski forest, and *Rhynchostegium riparioides* from a shaded waterfall of the river Banja. The collections were made just an hour before the beginning of the experiment. *Thamnobryum alopecurum* and *A. viticulosus* were in a naturally dried and partially dried condition respectively, while the aquatic *R. riparioides* was fully hydrated. Their water states were calculated as relative water

content (RWC):

$$\text{RWC} = (\text{fresh weight} - \text{dry weight}) / ((\text{saturated weight} - \text{dry weight}) \times 100)$$

The degree of resistance to water stress in these species was investigated by measuring the quantity of electrolytes that permeated through the membranes after subjection to additional water deficit.

Ion conductivity was determined according to the method of Vasquez-Tello *et al.* (1990), modified for poikilohydrous plants (Stevanović *et al.* 1997/98) by extending the time of exposure to water stress in view of their high desiccation tolerance. Moreover, only young apical shoots, 3 mm long, with total mass of 0.300 g were taken as specimens for the experiments.

The moss specimens were kept in polyethylene-glycol (PEG 600) solution for 24 hours and then rinsed in distilled water. Subsequently the specimens were placed in 10 cm<sup>3</sup> of distilled water and the ion conductivity of the solution measured at 25°C at hourly intervals. After a 24-hour period, the samples were dried to 80°C in a dry oven for 4 hours and the total amount of electrolytes released was measured. Control samples were treated with distilled water only. Electrolyte conductivity in  $\mu\text{S}$  was detected with an HI 8733 (Hanna, Tokyo) conductometer.

Using the data obtained for ion conductivity, injury indices ( $I_d$ ) were calculated according to the equation proposed by Flint *et al.* (1966):

$$I_d = R_t/R_o \times 100; \text{ where}$$

$R_t = \text{EC}/\text{EC}_{\text{total}}$  for PEG treated specimens;  $R_o = \text{EC}/\text{EC}_{\text{total}}$  for control specimens; EC = ion conductivity at hourly intervals,  $\text{EC}_{\text{total}}$  = ion conductivity after a 24-hour period.

The results represent the mean value of three successive measurements.

## RESULTS

The investigated species were differentiated by their RWC values, owing chiefly to their diverse hydric states. The relative water content of completely dehydrated *T. alopecurum* was only 4.5%, that of partially dehydrated

*A. viticulosus* was 25.6 %, while the value for aquatic *R. riparioides* was 82.0%.

Additional water deficit, provoked by the osmotically active substance PEG 600, brought about different degrees of functional changes in cell membranes in the mosses examined. These changes resulted in electrolyte leakage that could be measured and expressed as an injury index ( $I_d$ ).

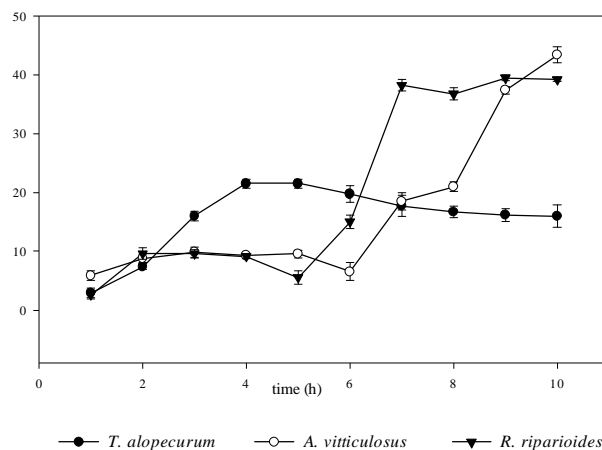


Fig. 1. Electrolyte leakages of stressed mosses *Thamnobryum alopecurum*, *Anomodon viticulosus* and *Rhynchostegium riparioides* (Values of Injury index are given as percentage  $\pm$  Standard Error)

As evident from Fig. 1, the desiccation tolerant species *T. alopecurum* had the most balanced values of  $I_d$ , indicating the stable state of the cell membranes. Specifically, over the first five hours of rehydration  $I_d$  gradually increased from 2.9 to 21.5%. From the fifth hour  $I_d$  decreased slightly, reaching a value of 16.0% at the tenth hour after rehydration.

Conversely, the two other species, *A. viticulosus* and *R. riparioides*, exhibited a different pattern of  $I_d$  changes during rehydration, indicative of greater changes in integrity of the cell membrane. During the first five hours of rehydration,  $I_d$  values in *A. viticulosus* were around 9%. At the sixth hour,  $I_d$  slightly decreased (6.6%) and over the subsequent four hours rose sharply to 43.4%. *Rhynchostegium riparioides* showed similar results. During the first four hours,  $I_d$  values of this species increased from 2.6 to 9%, at the fifth hour  $I_d$  decreased (5.6%), and subsequently it increased sharply, reaching 39.2% at tenth hour.

## DISCUSSION

The investigated bryophytes show different degrees of desiccation tolerance, as expected, in accordance with their various types of habitat. *Thamnobryum alopecurum* which occupied insulated vertical rocks in naturally dry conditions, had a RWC of only 4.2%. Its  $I_d$  values were more or less stable during all ten hours of measurements. This indicates cell membrane stability in *T. alopecurum*, as well as the consequently significant capacity to survive stress predominantly by rapid reconstitution of cell constituents and of the whole cell.

*Anomodon viticulosus* from limestone outcrops in forest was distinguished by a RWC of 25.6%. The  $I_d$  values of this species demonstrated temporary stability of the cell membranes (for up to six hours), with rapid increases in  $I_d$  after that time. This desiccation pattern might be a consequence of collecting the samples in a partially hydrated state. A similar reaction was observed in the desiccation tolerant fern *Asplenium ceterach* L. (Stevanović *et al.* 1997), which grew in a habitat analogous to that of *A. viticulosus*. These experiments confirmed the well known fact that the partially hydrated state of poikilohydrous plants is the most unstable one.

*Rhynchostegium riparioides*, a hydrophilous moss, was fully hydrated with a RWC of 82.0%. It retained membrane stability during dehydration for five hours, as judged by ion leakage measurements. However, after five hours, membrane stability decreased rapidly and very excessive electrolyte leakage was detected. Deltoro *et al.* (1998) demonstrated a similar pattern of potassium leakage in *R. riparioides* during desiccation. These results agree with the ecological requirements of this species, as it grows on limestone rocks in the riparian zone with wide and frequent fluctuations of water level.

According to the data obtained, *Thamnobryum alopecurum* is the most desiccation tolerant species,

*Anomodon viticulosus* is less but fairly tolerant, and the aquatic *Rhynchostegium riparioides* is intolerant of desiccation.

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## ПОРЕЂЕЊЕ ТОЛЕРАНЦИЈЕ ИСУШИВАЊА КОД МАХОВИНА СА РАЗЛИЧИТИХ СТАНИШТА

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Упоредна испитивања феномена толеранције исушивања ("desiccation tolerance") обављена су на појикилохидричним маховинама *Thamnobryum alopecurum* (Hedw.) Gang, *Anomodon viticulosus* (Hedw.) Hook & Tayl. и хомојохидричној маховини *Rhynchostegium riparioides* (Hedw.). Истраживане врсте прикупљене су са различитих станишта у околини Петнице, са кречњачке геолошке подлоге. Као тест за утврђивање степена толерантности еколошки диференцираних врста маховина коришћена је компаративна анализа пропуштања електролита након излагања биљака водном стресу. Овим тестом могуће је установити степен оштећења, односно очувања интегритета ћелијских мембрана

биљака у стресним условима средине.

Резултати су показали да се врста *Thamnobryum alopecurum* која насељава отворене, вертикалне кречњачке литице, одликује највећим степеном толерантности. Изразитије промене, сагледане на основу интензивнијег испуштања електролита, констатоване су код врсте *Anomodon viticulosus*, која насељава кречњачке камењаре у оквиру шумског склопа, док је хомојохидрична, хидрофилна маховина *Rhynchostegium riparioides* веома брзо испољила неповратна оштећења.