Abscisic-acid-induced drought tolerance in *Funaria hygrometrica* Hedw.

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**Abstract.** Three-week-old protonemata of *Funaria hygrometrica* cultivated in Petri dishes tolerate slow drying (24 h to complete dryness) but not rapid drying (1 h to complete dryness). Slowly dried mosses show, on a dry-weight basis, a sixfold increase in abscisic-acid (ABA) contents during the drying process. Rehydrated, slowly dried protonemata have the ability to tolerate subsequent rapid drying. When ABA is added to three-week-old protonemata at a concentration of 10 μM for 16 h, tolerance to rapid drying is induced. These data indicate that the induction of drought tolerance in *Funaria hygrometrica* is mediated by ABA. Mosses treated with ABA loose their water as fast as controls do; therefore, ABA does not act via reduced water loss. However, induction of synthesis of new proteins by ABA may form an important part of the drought tolerance because 10 μM cycloheximide inhibits the ABA-mediated tolerance to rapid drying.

**Key words:** Abscisic acid (desiccation tolerance) – Desiccation tolerance – *Funaria*

**Introduction**

It is generally accepted that all higher plants contain abscisic acid (ABA) and that ABA plays an important role in the stress reactions of these plants (for a review, see Zeevaart and Creelman 1988; Creelman 1989). There are a few reports that the same is true for lower plants as well, e.g. for Hepaticae and Anthocerotae (Hartung et al. 1987) and Algae (Hirsch et al. 1989). However, our knowledge of the role of ABA in mosses is very limited (Bopp 1990). The ABA-mediated reactions of higher plants are much better understood than those of lower plants; the reactions of higher plants include stomata closure (reviewed in Zeevaart and Creelman 1988) and protein synthesis (Gomez et al. 1988; Mundy and Chua 1988; Harada et al. 1989; Bartels et al. 1990). With regard to water stress, poikilohydric or resurrection plants exhibit unique features (Walter 1955; Gaff 1977). They can tolerate complete drying and exist in a nearly water-free state for a long time. After rehydration, they recover completely within a few hours. This phenomenon is found in only a few vascular plants, but is a normal feature in mosses (Bewley 1979). For the resurrection plant *Craterostigma plantagineum* (Scrophulariaceae), it was reported that ABA induces the formation of a set of new proteins that may be responsible for the extreme desiccation tolerance (Bartels et al. 1990; Piatkowski et al. 1990). The possibility that the stomataless gametophytes of mosses react in a similar way prompted us to investigate whether ABA is involved in the induction of desiccation tolerance in *Funaria hygrometrica*. 

**Material and methods**

*Plant material and culture conditions.* Spores of *Funaria hygrometrica* from the collection of the Botanical Institute Heidelberg, harvested in October 1987, were sown under aseptic conditions on Knop-agar plates (2% agar, w/v) covered with cellophane sheets and maintained at 20 ± 1 °C at 1.5 W·m⁻² and 20 h light per day. After 7 d the young protonemata were transplanted to new plates. All experiments were repeated at least three times.

**Application of ABA and-or cycloheximide CHI.** The cellophane sheets carrying the protonemata were transferred to Knop agar plates containing the various amounts of ABA and-or CHI given in the Results. 2-cis-(R,S)-abscisic acid and CHI (both from Sigma, München, Germany) were added as sterile solutions after cooling the autoclaved agar down to approx. 50°C. An ABA content of 10⁻⁵ M changes the pH of the Knop-containing agar only very slightly, not affecting the growth of the mosses.

**Drying conditions.** Slow drying consisted of transferring the cellophane sheets to empty Petri dishes. The Petri dishes were main-
tained closed – but not sealed – in a growth chamber under the conditions given above. Mosses to be dried rapidly were put with their cellophane sheets in a laminar air flow and incubated for different times under a maximum stream of air.

**Determination of water loss.** The relative water loss (RWL, %) was calculated as $100-[FW_x-DW]x100/(FW_0-DW)$ where $FW_0$ and $FW_x$ indicate the fresh weight at the beginning and end of the experimental time, respectively. Dry weight (DW) was determined after drying the mosses at 100°C for 24 h or after freeze drying, when the mosses were used for ABA estimation.

**Rehydration of dried mosses.** The cellophane sheets were dipped into sterile water until the protonemata were apparently soaked. The cellophane was then transferred to a new Knop-agar plate and the mosses maintained under standard conditions.

**Extraction of ABA and purification.** Mosses were frozen in liquid nitrogen and then freeze dried. The weight before and after freeze drying was determined to calculate the RWL. [H] Abscisic acid was added to methanolic extracts (70%, v/v) of the freeze-dried mosses as internal standard. The extracts were passed through C18 columns (Baker, Groß-Gerau, Germany) to remove pigments and other interfering substances. The methanol was removed by evaporation and 5 ml water added. The solvent was acidified to approx. pH 1.5 by the addition of HCl and then partitioned three times against ethyl acetate. The combined organic fractions were reduced to dryness. Finally, the sample was redissolved in Tris-buffered saline (50 μM Tris-HCl; pH 7.8; 100 mM NaCl) containing 5% methanol (v/v). The recovery was in the range of 80% as calculated from the internal standard.

**Determination of ABA.** Abscisic acid was analyzed by enzyme-linked immuno-sorbent-assay (ELISA) using monoclonal antibodies specific for 2-cis-(S)-ABA provided by E. Weiler (Lehrstuhl für Pflanzenphysiologie, Bochum, Germany). The protocol given by Weiler (1986) was followed. For Funaria extracts, high-performance liquid chromatography (HPLC) purification prior to ABA-ELISA could be omitted since the values obtained by ABA analysis of the semipurified extracts as described above were in the same range as HPLC-purified extracts (data not shown).

**Results**

Slowly dried, three-week-old protonemata of Funaria lost 50% of their initial water content within the first 4 h of the drying process. After 24 h, they had lost almost all of their water (Fig. 1). Rapidly dried mosses lost more than 90% of their water within 0.5 h and more than 95% within 1 h (Fig. 1). The mosses recovered completely after slow drying when they were rehydrated. Rapid drying within 0.5 h did not have irreversible effects. After 1 h of rapid drying the cells of the outer filaments of the protonemata that grow as circular areas died, while the central (older) parts were not severely affected. Rapid drying within 2 h killed the whole protonema (Table 1). The difference between living and dead cells can be seen very easily. All killed cells appear completely white and collapsed after a few hours whereas the living cells are green and turgescent.

The ABA content of slowly dried mosses increased from 1.7 nmol · g⁻¹ DW in the control to 10.5 nmol · g⁻¹ DW after 20 h of water loss (Fig. 2) which is approxi-

![Fig. 1. Relative water losses of slowly dried protonemata ×--×, rapidly dried control protonemata (*) and rapidly dried protonemata that had been incubated for one week on 10⁻⁵ M (R,S)-ABA plates (—–). Three-week-old Funaria protonemata were taken. The insert shows in more detail the situation of the rapidly dried protonemata during the first 2 h](image)

**Table 1. Survival of three-week-old Funaria protonemata after rapid drying.** Moss plants were grown for two weeks on (R,S)-ABA of the given concentrations. 2, all cells (100%) recovered after rehydration; 1, only central parts of protonemata survived, all peripheral cells dead; 0, all protonema cells dead; –, not tested.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Drying time (h)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>0.5 1 2 3 4 8 24</td>
</tr>
<tr>
<td>Control</td>
<td>2 1 0 0 0 0 0</td>
</tr>
<tr>
<td>10⁻⁷ M ABA</td>
<td>– – – 0 – – –</td>
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<tr>
<td>10⁻⁶ M ABA</td>
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<td>10⁻⁵ M ABA</td>
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![Fig. 2. Increase of 2-cis-(S)-ABA levels in slowly dried Funaria plants mg⁻¹ DW) (•-•) in comparison to the relative water loss (○-○)](image)