THE FUNCTION OF ABSCISIC ACID IN BRYOPHYTES

WOLFRAM HARTUNG\textsuperscript{1,2}, ELKE M. HELLWEGE\textsuperscript{1} AND O. H. VOLK\textsuperscript{1,3}

ABSTRACT. Abscisic (ABA) acid has been detected in all members of Bryophyta which have been analysed until now. Highest ABA contents were detected in species that were adapted to dry environmental conditions, lowest amounts were found in aquatic and hydrophilic species. Abscisic acid seems to be involved in several ecophysiological and developmental processes. 1) ABA induces stomatal closure in stomata-bearing sporophytes of Anthocerotae and Musci; 2) ABA induces desiccation tolerance in several drought resistant members of the Marchantiales and in Funaria-protone mata. 3) ABA converts the waterform of \textit{Riccia fluitans} L. (submerged living) and \textit{Ricciocarpus natans} L. Corda (floating on the water surface) into the landform. Ecophysiological as well as molecular biological experiments will be discussed.

INTRODUCTION

Abscisic acid (ABA) is a sesquiterpenoid compound which occurs universally in higher plants and which plays an important role as a stress hormone (Hartung and Davies 1991). Under conditions of stress, biosynthesis and release from the site of synthesis of ABA is increased. Having arrived at the stomata and other target tissues ABA, causes physiological changes that improve the performance of plants under stress conditions.

Until recently the existence of ABA in liverworts and Anthocerotae has been questioned. It was suggested that the occurrence of abscisic acid may be linked with the occurrence of stomata, and that in liverworts and algae, lunularic acid regulates processes of stress physiology which are influenced by ABA in higher plants (Pryce 1972). However, since new, highly specific and sensitive assays have become available, ABA has been detected not only in several species of \textit{Anthoceros} but in more than 30 members of the Marchantiales (Hartung et al. 1987; Hartung and Gimmler 1994). Additionally, Werner et al. (1991) detected ABA in the protonema of \textit{Funaria hygrometrica} L.

ABSCISIC ACID IN BRYOPHYTES

In well watered species of the Marchantiales a large variability in ABA content was observed. It ranged from 30 nmol g\textsuperscript{-1} in the extremely xerophytic \textit{Exormotheca} species, followed by a group of liverworts with ABA in the range of 50–150 pmol g\textsuperscript{-1} fr wt, down to 1–10 pmol g\textsuperscript{-1} in species that live submerged (\textit{Riccia fluitans}), float on the water surface (\textit{Ricciocarpus natans}) or grow under very humid conditions (Hartung and Gimmler, 1994). We also observed a large variability in some \textit{Anthoceros} species, dependent on environmental conditions. Significantly higher amounts of ABA were observed when samples

\textsuperscript{1} Julius von Sachs Institut für Biowissenschaften, Universität Würzburg, Mittlerer Dallenbergweg 64, D 97082 Würzburg, FRG.
\textsuperscript{2} To whom correspondence should be addressed.
\textsuperscript{3} Dedicated to Prof. Czygan on the occasion of his 60\textsuperscript{th} birthday.
were collected from dry habitats (Hartung et al. 1987).

**DISTRIBUTION OF ABA WITHIN ONE PLANT**

ABA is unevenly distributed in *Anthoceros*. Highest amounts could be extracted from the stoma-bearing sporophytes. Within the sporophytes a basipetal gradient could be observed. Particularly high concentrations could be detected in the desiccation tolerant tubers of *Anthoceros dichotomus* Raddi, indicating that ABA could play a role in the induction of desiccation tolerance (Hartung et al. 1987).

In the gametophytes of liverworts ABA seems also to be unevenly distributed. Hellwege (unpubl.) found remarkably high ABA amounts in the apical parts of *Riccia fluitans* thalli. This finding was confirmed by autoradiography of *R. fluitans* thallus pretreated with \(^{14}\text{C}\)-abscisic acid (Fig. 1). Radioactivity seems to be concentrated in the apices. This may be explained by the high cytosolic fraction of the meristematic cells which can accumulate ABA by anion trapping (Hartung and Slovik 1991).

**THE PHYSIOLOGICAL ROLE OF ABSCISIC ACID**

Some members of the Marchantiales (*Riccia fluitans*, *Ricciocarpus natans*) which live under or on the surface of water undergo characteristic developmental changes when occupying the land, as can occur when a pond is drying out. Most characteristically the land form exhibits air pores on the thallus surface, an increased number of rhizoids and ventral scales, and a decreased length/width ratio of the thalli. During the first 50 hours after transfer to an agar surface, ABA is increased transiently in these thalli, most probably in the meristems, by a factor of 10–30. Afterwards ABA content decreases again (Fig. 1). The newly developed land form has an ABA content which is 2–3 times higher than that of the submerged form. When thalli of the land form were kept submerged in water, ABA content oscillates during the first 10 hours, then decreases slowly within the following 90 hours until the low level, typical for the submerged form, has been reached (Fig. 3). This may be

![Figure 1](image-url)  
**Fig. 1.** Autoradiograph of thalli of *Riccia fluitans*. Thalli have been incubated in \(10^{-5}\text{M}\) \(^{14}\text{C}\)-labelled abscisic acid for 5 h in the light and exposed to X-ray films for two weeks (Hellwege unpublished). Accumulation of label can be seen clearly in the apical regions of the thalli.
Fig. 2. Time course of endogenous ABA in thalli of *Riccia fluitans*. Thalli of the waterform were transferred to an agar surface in closed petri dishes under light at 22°C. After time intervals as shown in the figure ABA contents were measured using an immunological technique (after Hellwege et al. 1992, with permission).

Fig. 3. Time course of endogenous ABA in thalli of *Riccia fluitans* after transfer of the land form into water in the light. The thalli have been kept submerged during the experimental period. (Unpublished data of Hellwege. Culture of thalli, experimental conditions and techniques have been described earlier by Hellwege et al. 1992).
a result of flooding stress as observed earlier by Jackson for higher plants (Jackson; 1991, more references cited therein). Treatment of the water form with ABA also induces land form characteristics (Hellwege et al. 1992). This process is accompanied by characteristic changes in patterns of polypeptides, which suggests that during transition to land a specific set of proteins is formed under the influence of ABA (Hellwege et al. 1994a). The function of these proteins is unknown. They seem to be different from ABA-induced dehydrins of the resurrection plant *Craterostigma plantagineum* Hochst. and from desiccated corn embryos. On the other hand, however, in both the ABA treated water form and in the land form, antibodies raised against a specific 31 kD tonoplast protein (Betz and Dietz 1991) recognize one of the ABA induceable proteins. The formation of the land form seems to be accompanied by the synthesis of a specific tonoplast protein. It should be emphasised that the stress *Riccia* thalli are exposed to, is very mild. We obviously have an extremely sensitive stress response leading to an ABA accumulation. This differs from higher plant tissues, where threshold levels of stress up to -2.0 MPa often have to be reached to induce ABA-accumulation (Hartung and Heilmeier 1994). A comparable function of ABA as described here for *Riccia fluitans* has been proposed by Kandel (pers.comm.) for *Ricciocarpus natans*.

ABA dependent conversion of water to land form characteristics have been described earlier for the fern *Marsilea quadrifolia* (Lin-Liu 1984; Lin et al. 1993) and for several heterophyllous plants such as *Ranunculus fluviatilis*, *Callitriche* or *Potamogeton* (for references see the review of Trewavas and Jones 1991). Again, endogenous ABA is higher in aereal organs and can be increased in stressed submerged leaves (in the case of *Marsilea*, by salt stress).

Other developmental changes of ABA-treated bryophytes are listed by Hartung and Gimmler (1994). In most cases ABA seems to inhibit growth processes. These data are difficult to discuss. Detailed dose-response curves are lacking, as well as attempts to correlate time courses of endogenous ABA with the physiological phenonema observed.

**STOMATAL REACTIONS**

The stoma-bearing sporophytes of Anthocerotae contain particularly high ABA-amounts that can be further increased under stress conditions. (Hartung et al. 1987). The stomata react to exogenous ABA by closing, whereas fusicoccin causes opening. The ABA dose response strongly resembles those of higher plants, such as *Valerianella locusta* (Hartung 1983). Data about ABA-concentrations of the stoma-bearing sporophytes of Musci are not available. However, Garner and Paolilio (1974) have demonstrated that stomata of sporophytes of *Funaria* respond to ABA in a very similar manner. The physiological significance of the ABA-effect on guard cells of *Anthoceros* is unclear. The guard cells of Anthocerotae are not connected to a water transporting system. Some of them do not even have a significant substomatal cave. Their role for gas exchange must be negligible. Obviously a stress physiological system for ABA was developed at a very early stage of evolution, although no ecophysiological need for such a system existed.
INDUCTION OF DESICCATION TOLERANCE

As pointed out earlier variability of endogenous ABA-contents in Marchantiales can be attributed to differences in desiccation tolerance. The extremely tolerant species of the genus *Exormotheca* have the highest, the extreme hydrophilic species such as *Riccia fluitans* or *Cyathodium africanum* Mitten exhibit the lowest ABA-amounts (Hartung and Gimmler 1994). Similarly, the desiccation tolerant tubers of *Anthoceros dichotomus* which survive longer periods of dryness are particularly rich in ABA, when compared with the thalli and sporophytes of this species (Hartung et al. 1987).

Werner et al. (1991) and Bopp and Atzorn (1992) have shown that protonemata of *Funaria hygrometrica* can survive desiccation when drying out slowly, a condition which permits a significant increase in endogenous ABA. This is not possible during rapid drying. When treated with ABA, non hardened *Funaria* protonemata can also survive a desiccation treatment. The same is true also for the xerophilic species *Exormotheca holstii* St. ABA content fluctuated by 10 to 25 fold during cycles of dehydration and hydration. However, this is only true for hardened thalli which are adapted to the stressful conditions. *Exormotheca* which has been cultured for longer periods under well watered conditions in the glasshouse, as well as the hygrophytic thalli of *Marchantia polymorpha*, do not survive a desiccation treatment. ABA-pretreatment, however, restores desiccation tolerance to non hardened thalli of *Exormotheca holstii*. We could demonstrate by fluorescence techniques (Hellwege et al. 1994b) that vitality of non hardened but ABA pretreated thalli is restored completely within 2–4 h after remoistening.

In desiccated thalli of *Marchantia polymorpha* and non hardened *Exormotheca*, ABA increases only by 2–3 fold which is not sufficient for induction of desiccation tolerance (Hellwege et al. 1994b). Bopp and Werner (1993) and Werner and Bopp (1993) have shown that in desiccated or ABA treated protonemata, several proteins were synthesised which are comparable to the dehydrins of barley and corn, described by Close et al. (1993). These dehydrins are believed to protect desiccation sensitive enzymes (Close et al. 1993). In *Funaria*, ABA stimulated the phosphorylation of these proteins (Werner and Bopp 1993).

Bopp and Werner (1993) have probed the ABA-induced polypeptides of *Funaria hygrometrica* protonemata with antibodies raised by Schneider et al. (1993) against some of the *Craterostigma* dehydrins. They were able to recognise a cytosolic and plastidic 18 kDa dehydrin of *Funaria* indicating that those of both systems are very similar.

Hellwege et al. (1994b) have also detected a group of proteins in ABA treated and desiccated thalli of *Exormotheca*. Unlike in *Funaria*, one of those is recognised only by an antibody to a cytosolic dehydrin of *Craterostigma* but not to the antibody raised against a plastidal dehydrin (Schneider et al. 1993). Drought- and ABA-dependent polypeptides are absent in non hardened thalli of *E. holstii* which were cultivated in the glasshouse for several weeks under moist, unstressed conditions, as long as they are not incubated with ABA. Treatment with ABA restored their desiccation resistance. Our western blot studies (Hellwege et al. 1994b) showed that different from the 18 kDa dehydrins of higher plants (maize embryos, Close et al. 1993; *Craterostigma*, Schneider et al. 1993; *Lindernia intrepidus* (Dinter ex Heil) Oberm., Hellwege (unpubl.) and *Funaria* protonemata (Bopp and Atzorn,
1993), those of the liverwort *E. holstii* have a molecular weight of 40 kDa and those of the stress tolerant green alga *Dunalieila parva* of approximately 60 kDa (Gimmler, pers. comm.). These studies indicate that mechanisms of desiccation tolerance which have been detected in higher plant systems appeared very early during evolution.

**CONCLUSION**

From our data we conclude that ABA occurs universally in bryophytes. During evolution, three well documented physiological functions for ABA have developed. All of them are connected with water relations of the species and enable life in dry habitats. ABA causes (1) stomatal closure of the guard cells of the sporophytes of Anthocerota and Bryidae, (2) developmental changes which can be observed when submerged living species occupy the land and (3) induction of desiccation tolerance, especially in highly xerophytic species such as the desert species *Exorrhettea*. It seems that ABA acts in liverworts and hornworts as a stress hormone, in a mechanistically very similar manner as in the higher plants. We therefore doubt that lunularic acid ever played a significant role in lower plants, as suggested earlier. Abscisic acid is present in all the lower plants including algae (Hirsch et al. 1989). There was no need for a theoretical pioneer plant (Yoshikawa and Doi 1993) to switch from a compound such as lunularic acid to ABA.

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