

The ABA-mediated switch between submersed and emersed life-styles in aquatic macrophytes

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Abstract Hydrophytes comprise aquatic macrophytes from various taxa that are able to sustain and to complete their lifecycle in a flooded environment. Their ancestors, however, underwent adaptive processes to withstand drought on land and became partially or completely independent of water for sexual reproduction. Interestingly, the step backwards into the high-density aquatic medium happened independently several times in numerous plant taxa. For flowering plants, this submersed life-style is especially difficult as they need to erect their floral organs above the water surface to be pollinated. Moreover, freshwater plants evolved the adaptive mechanism of heterophylly, which enabled them to switch between a submersed and an emersed leaf morphology. The plant hormone abscisic acid (ABA) is a key factor of heterophylly induction in aquatic plants and is a major switch between a submersed and emersed life. The mechanisms of ABA signal perception and transduction appear to be conserved throughout the evolution of basal plants to angiosperms and from terrestrial to aquatic plants. This review summarizes the interplay of environmental factors that act through ABA to orchestrate adaptation of plants to their aquatic environment.

Keywords Aquatic macrophytes · Hydrophytes · Abscisic acid (ABA) · Heterophylly · Adaptation to a submersed life-style

Introduction

The thriving success of vascular plants on terrestrial ground has widely been associated with their success in pollination and fertilization independent from water. The cutting edge of this evolutionary process can be witnessed in arid habitats like deserts, where plants have evolved highly specialized physiological and morphological mechanisms to survive in a world that lacks water for most of the times. Thick layers of epicuticular waxes and a succulent morphology are obvious characteristics of plants that are well adapted to dry or seasonally dry environments.

At this front line of plant's adaptation to drought they are still able to complete the entire generation cycle with only a little expense of water. This getaway from water towards the dry land had likely begun before the mid-Paleocene in the Silurian about 430 million years ago (Corner 1964; Sculthorpe 1967).

Leafy bryophytes and psilophytes were amongst the first settlers of the green plant lineage to conquer swamps and other humid locations. While gymnosperms and tree ferns (Cyatheaales) dominated land for several million years, the rise of flowering plants pushed them aside during the last 200 million years (Cook 1996; Sculthorpe 1967; Takezawa et al. 2011).

Despite the general trend to establish life on dry ground, several mosses, ferns (pteridophytes) or flowering plants (angiosperms) have ventured back into the fresh water regimes or even marine habitats (Fig. 1a). One can speculate that adaptation to seasonal water logged grounds and flooding tolerance mark the initial steps back to an aquatic life-style (Jackson and Ram 2003; Mommer et al. 2007; Voesnek et al. 2003).

Although gymnosperms successfully adapted to various biomes, one can be quite confident that none of them did

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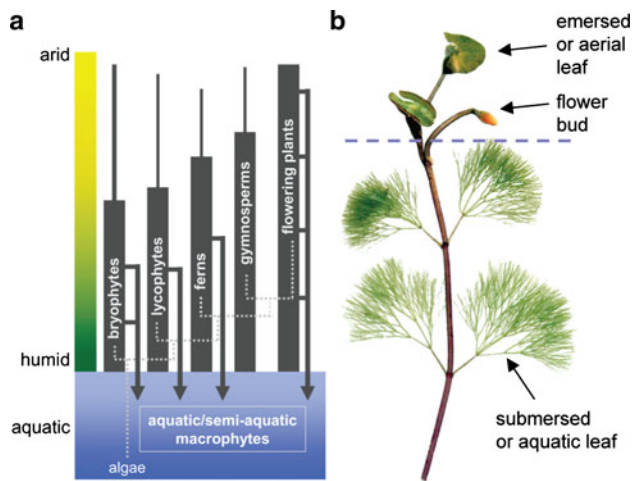


Fig. 1 Aquatic macrophytes evolved from drought adapted terrestrial plants. **a** Members from at least 50 plant genera independently returned to an aquatic life style. Only few species of bryophytes, lycophytes, ferns and gymnosperms successfully settled in arid habitats (*thin lines*). Gymnosperms are an exception as they possibly never evolved an aquatic species. In angiosperms, this return to water has been initiated irrespective from the plant's previous state of adaptation to arid climate and drought or their phylogenetic relatedness. **b** Heterophylly in *Cabomba aquatica*. Submersed and emersed leaves are formed from submerged stems. The submersed leaves show a fan-like outline shape, while the emersed and aerial leaves are entire. After some initial floating or aerial leaves have been formed, the air-adapted flowers emerge over the water surface (*dashed line*)

return to an aquatic life style ever (Cook 1996; Sculthorpe 1967). Aquatic gymnosperms are not found at present and are absent from fossil records. Two reasons why they never returned to an aquatic life style might be on the one hand their lignified primary axis and on the other hand their reproduction that strictly depends on wind pollination (anemophily) (Cook 1996; Sculthorpe 1967).

To date it is assumed that the return to the aquatic life style occurred independently in at least 50 plant genera, including leafy bryophytes (Cook 1996; Sculthorpe 1967). Only few aquatic plants, all of which belong to the *Alismatales*, conquered the marine sea water and it is assumed that they evolved from fresh-water adapted ancestors (Kato et al. 2003; Les et al. 1993; Wissler et al. 2011).

Interestingly, some achievements that adapt to terrestrial ground and to drought could not be overcome so easily: for example, *Crassula helmsii* is an aquatic plant, which possesses obvious succulence and classical Crassulacean acid metabolism (CAM) as their close ancestors (Klavnsen and Maberly 2009, 2010).

The submersed life-style

Living below the water surface requires the ability to cope with a medium of higher density and of different chemical

properties. Compared with our atmosphere, the water sphere has certain advantages and disadvantages (Sculthorpe 1967).

One factor is the high chemical density of water that places physical force on the submerged foliage, which is especially a problem for plants growing in flowing waters.

Therefore, aquatic plants make way for the mechanical stress and evolved a soft, flexible stature with poorly lignified xylem (Rascio et al. 1999; Sculthorpe 1967).

Other factors are fluctuations of the temperature in aquatic habitats, which are much less of a problem than in terrestrial ecosystems. In contrast to land plants, the direct influence of temperature on hydrophytes is buffered by the thermal capacity of water. In contrast, the chemical properties of water and, therefore, the availability of nutrients and gases is immediately affected, as the following example shows: oxygen, which is important for any eukaryote, appears in significantly lower concentrations in aquatic ecosystems compared to air, whereas much more CO_2 is dissolved than in atmosphere. Nevertheless, solubility of gases in water is very much temperature dependent.

At elevated water temperatures the solubility of gases decreases tremendously. Thus, some aquatic plants have adopted a C4/CAM-like metabolism for carbon fixation, which is well known from terrestrial plants that grow in ecosystems with seasonal water deficiency (Hussner 2009; Keeley 1998). The use of a C4-like metabolism has been reported e.g. for the Hydrocharitaceae *Hydrilla verticillata*, *Egeria densa* and *Elodea canadensis* (Casati et al. 2000; Elzenga and Prins 1989; Estavillo et al. 2007; Rao et al. 2006). A temporal separation of carbon fixation like in CAM-plants has been observed in *Isoetes*, *Lobelia* and *Littorella* species (Keeley 1998; Klavnsen and Maberly 2009; Madsen 1987; Rattray et al. 1992; Robe and Griffiths 1990; Sharma and Harsh 1995). In contrast to dissolved gaseous CO_2 , bicarbonate HCO_3^- is the substrate for phosphoenolpyruvate carboxylase and remains dissolved even under elevated water temperatures (Keeley 1998; Mommer and Visser 2005). Therefore, carbon can successfully be fixed and enriched for efficient Calvin cycle, although typical tissues, i.e. bundle sheath cells (C4), or the temporal separation of CO_2 capture and photosynthesis (CAM) are usually lacking in hydrophytes (Estavillo et al. 2007; Keeley 1998; Klavnsen and Maberly 2009; Rattray et al. 1992; Sharma and Harsh 1995).

Adaptation to aquatic habitats

As mentioned above, a submersed life-style is especially difficult for flowering plants as they normally need to erect their floral organs above the water surface to be pollinated.

Therefore, almost all aquatic angiosperms hold the potential to form organs that are able to withstand permanent submergence and cross the water surface to flourish in the air (Cook 1996; Endress 2004, 2010; Lin 2002; Sculthorpe 1967; Soltis et al. 2009). There are also exceptions to this rule: *Ceratophyllaceae* and members of the large *Hydrocharitaceae* family evolved mechanisms for water pollination (hydrophilly) and diaspore dispersal (hydrochory) (Endress 2010; Iwamoto et al. 2003; Meller and van Bergen 2003; Soltis et al. 2009). In addition, some plants have a mechanism to escape from submergence by petiole elongation, which results in emerging their leaves above the water surface. Model species for the analysis of these escape responses have especially been the deepwater rice and *Rumex* species (Chen et al. 2010; Kende 1987; Voesnek et al. 2003).

A general morphological trend in aquatic ferns and angiosperm lineages that are permanently covered by water is the “principle of reduction” (Sculthorpe 1967): compared to characteristic features of terrestrial plants, distinct cell-types (e.g. guard cells), tissues (e.g. epidermis, xylem) or organs (e.g. roots) are reduced or even missing in aquatic macrophytes.

The epidermis of the aquatic foliage misses guard-cells that form the stomata and is thinner than that of terrestrial plants due to a reduction in cell size and number (Gifford and Foster 1988); the waxy cuticle is also reduced or missing. In contrast to emerged leaves, the epidermis cells of submersed leaves have chloroplasts (Gifford and Foster 1988; Prance 1985).

Not all of the present day hydrophytes are rooting in the benthic substrate, but several are floating inside the water or on the surface. The primary root is often shorter and the number of lateral roots is often decreased in hydrophytes when compared to soil grown terrestrial plants (Sculthorpe 1967; Sifton 1945). Several genera retained only small roots, e.g. *Lemna*, or even lost them at all, e.g. *Wolfiella* or *Utricularia* (Albert et al. 2010; Cook 1996; Les et al. 1997; Sculthorpe 1967).

Cross-sections of aquatic roots display air-filled spaces and a central lacuna, which is a source of oxygen for respiration and needed for gas exchange between source and sink organs (Sculthorpe 1967; Sifton 1945; Visser et al. 1997; Voesnek et al. 2003).

A distinct morphological and physiological attribute of the aquatic life cycles in hydrophytes is the development of different types of leaves, known as heterophylly.

Although heterophylly can be observed in many terrestrial plant species (Givnish et al. 1994; Leigh et al. 2011; Mulkey et al. 1992; Winn 1999; Zanewich et al. 1990), the changes in leaf morphology such as submersed, emerged, floating or aerial leaves in aquatic plants are more distinct and conspicuous. Compared to emerged or aerial leaves,

the submersed leaves are often dissected (e.g. *Myriophyllum*), linear (e.g. *Heteranthera*) or filiform (e.g. *Isoetes*) (Horn 1988; Kane and Albert 1989; Lin 2002; Strand and Weisner 2001). In an extreme case, even perforated leaves are found (lace plant; *Aponogeton madagascariensis*), which are secondarily formed by programmed cell death (hypersensitive response) after the initial development of entire leaf laminae (Gunawardena et al. 2004; Lord and Gunawardena 2011). It is not yet clear for all aquatic species whether the submerge leaves of other hydrophytes are also formed by an active reduction process throughout the early leaf development. These distinctive leaf shapes efficiently decrease the mechanical force of the aquatic media and increase the surface area for an optimal uptake of nutrients and gases (Puijalón and Bornette 2006; Puijalón et al. 2005, 2008; Winn 1999).

As introduced above, the ability to form drought resistant terrestrial floral leaves from submerged inflorescence meristems is another aspect of heterophylly in angiosperms (Endress 2004, 2010; Lin 2002; Sculthorpe 1967; Soltis et al. 2009).

Also other specialized tissues or cells are altered (Bruni et al. 1996; Dorken and Barrett 2004; Goliber and Feldman 1990; Kuwabara and Nagata 2006; Santamaria et al. 2003; Sculthorpe 1967): cross section of leaves and stems show premature or absent xylem vessels. Instead, a protoxylem lacuna is built that can form an aerenchyma for buoyancy and gas exchange. Likewise, the cells of the spongy mesophyll parenchyma of the emerged or aerial leaves are transformed into a highly ordered non-spongy mesophyll.

Heterophylly is found in aquatic plants of different lineages and from ferns to angiosperms, but is absent in aquatic bryophytes (Hsu et al. 2001; Lin 2002; Takezawa et al. 2011; Villani and Etnier 2008); A different leaf morphology between submersed and emerged or aerial leaves can be seen in various species, e.g. of the genera *Marsilea*, *Echinodorus*, *Sagittaria*, *Hydrophila*, *Cabomba*, *Hydrocharis*, *Potamogeton*, *Aponogeton*, *Limnophila*, *Nymphaea*, *Myriophyllum* and others. The different outline shapes are immediately obvious, for example in *Cabomba aquatica* (Fig. 1b), where aquatic submersed leaves display fan-shaped laminae and the emerged or aerial leaves show an entire lamina.

Terrestrial plants returned, and possibly will keep on returning in future, into aquatic media from very different developmental backgrounds and, thus, do not form a monophylum (Cook 1996). Also heterophylly is a phenomenon that developed several times by convergent evolution and independently in distantly related taxa (Cook 1996; Lin 2002; Sculthorpe 1967).

Present day hydrophytes as well as all terrestrial plants had once been shaped by the evolutionary processes that have led to the adaptation to drought. Thus, it might not be

farfetched assumption that only a few changes in developmental programs are sufficient to establish a successful submersed life-style.

Induction of heterophylly

Many abiotic factors influence heterophylly formation and the transition from a submersed to an aerial life style. Intriguingly, in some heterophyllous aquatic plants such as *Cabomba*, *Nymphaea* or *Nymphoides* species (Orgaard et al. 1992; Sculthorpe 1967), the induction of aerial leaves is controlled by environmental conditions that predate the time of flowering and are usually characteristic for spring time: light quantity and quality as well as long-day photoperiod or temperature cycles stimulate the formation of floating or aerial leaves in aquatic plants (Minorsky 2003).

The aquatic fern *Marsilea quadrifolia* and the angiosperm *Ludwigia arcuata* are well established aquatic plant models and have been most informative for the analysis of heterophylly (Allsopp 1951; Kuwabara et al. 2003; Kuwabara and Nagata 2006; Kuwabara et al. 2001; Lin and Yang 1999; Sato et al. 2008). Also other plant species such as *Callitriche heterophylla* (Deschamp and Cooke 1985), *Hippuris vulgaris* (Goliber and Feldman 1990; Kane and Albert 1985, 1987a), *Ranunculus flabellaris* (Bruni et al. 1996), *Nymphaea odorata* (Villani and Etnier 2008), *Proserpina* sp. (Davis 1967; Kane and Albert 1987b) and species of the genus *Potamogeton* (Gee and Anderson 1996, 1998; Spencer and Anderson 1987) have been successfully used for the investigation of the mechanisms that control heterophylly.

Investigations on light quality revealed that especially blue light induces the development of emersed leaves on submersed grown plants (Kao and Lin 2010; Lin and Yang 1999). Other reports suggest the involvement of the phytochrome red/far-red light receptors in the initiation of heterophylly (Gaudet 1963; Jo et al. 2010; Lin 2002). This is of special importance as water has a rather low red/far-red light permeability, while wavelength in the blue light range can pass much deeper. It has been observed that hydrophytes can estimate the distance to the surface in respect of elevated fluence-rates of red or far-red light close to the water line (Gaudet 1963; Goliber 1989; Goliber and Feldman 1990), which are possibly perceived by the phytochrome photoreceptors. Recently, it was shown that Phytochrome A (PhyA) in *Arabidopsis thaliana* is not only important for a red/far-red light dependent development of this terrestrial model plant, but also for blue light perception and signaling (Peschke and Kretsch 2011). This finding suggests that also in aquatic plant species only a single photoreceptor is possibly sufficient for both the blue light and the red/far-red light mediated heterophylly induction in

aquatic plants (Lin and Yang 1999; Peschke and Kretsch 2011).

Also long-day photoperiods induce the formation of aerial-type leaves and flowers from a submersed shoot apical meristem (e.g. in *Callitriche* and *Cabomba*) (Deschamp and Cooke 1985; Hotta et al. 2007; Kobayashi and Weigel 2007; Minorsky 2003; Orgaard et al. 1992). One might speculate whether the control of this process might possibly be triggered through mechanisms that are similar to that of terrestrial plants and which enroll a systemic signal that migrates from cell to cell (Hotta et al. 2007; Kobayashi and Weigel 2007).

However, light is not the sole abiotic factor to influence heterophylly in aquatic plants: a reduction in the number of epidermal cells has been used as an indicator for temperature dependent initiation of heterophylly (Madsen and Sandjensen 1994). Also other authors reported a role for relatively high water temperatures in heterophylly formation, e.g. in *Callitriche heterophylla* and *Hippuris vulgaris* (Deschamp and Cooke 1985; Goliber and Feldman 1990). Moreover, it has been mentioned that high salinity stress can also induce the formation of emersed or aerial-type leaves (Deschamp and Cooke 1985; Santos et al. 2011).

Taken together, several abiotic factors appear to form a complex network that integrates in the induction of heterophylly. Interestingly, it has been shown that the exogenous application of the plant hormone abscisic acid (ABA) alone is sufficient to induce the formation of drought tolerant aerial-type leaves (Gee and Anderson 1996; Goliber and Feldman 1989; Kuwabara et al. 2003; Schiller et al. 1997). Therefore, it is not surprising that ABA initiates heterophylly in *Marsilea quadrifolia* even under environmental conditions that are in favor of submerge leaf formation (Lin 2002; Lin et al. 2005; Lin and Yang 1999).

As ABA plays a leading role also in dehydration stress tolerance in many plants (Takezawa et al. 2011), the analysis of ABA-dependent processes is important for our general understanding in the evolutionary adaptation to terrestrial life. Since ABA plays a pivotal role for heterophylly initiation in aquatic plants today, it is plausible that ABA might have played an important role for the adaptation of a submerged life style during their evolution (Lin 2002; Lin et al. 2005; Winn 1999).

Molecular mechanism of heterophylly and ABA-signaling

The involvement of ABA as signaling component was a major evolutionary step for the adaptation to the dry terrestrial land (Cutler et al. 2010; Takezawa et al. 2011). Moreover, the crosstalk of ABA with other developmental

and cellular processes appears to be conserved in all terrestrial macrophytes from bryophytes to ferns, gymnosperms and angiosperms (Takezawa et al. 2011). An interesting notion is that ABA as a signaling molecule is a rather ‘old invention’, as it is not restricted to plants and can be found in all kingdoms of life (Bassaganya-Riera et al. 2010; Cutler et al. 2010; Fujita et al. 2011; Hubbard et al. 2010; Takezawa et al. 2011). Although ancient origin implies the involvement in conserved signaling cascades, this could only be shown for principal processes throughout the green plant lineage (Cutler et al. 2010; Fujita et al. 2011; Takezawa et al. 2011). Moreover, despite their highly conserved upstream signaling components, one can speculate that a wide variety of signaling cascades further downstream probably lead to specific responses, which might differ between species.

Endogenous concentration of ABA increases during a wide variety of environmental stimuli including those mentioned above, which are known to initiate the development of aerial-type leaves on submersed hydrophytes.

About 10% of all protein coding genes were found differentially expressed after ABA treatment in *Arabidopsis thaliana* (Goda et al. 2008; Nemhauser et al. 2006). Most of these genes were also regulated during various biotic and abiotic stresses (Kilian et al. 2007; Wanke et al. 2009). In particular, ABA response-element binding factors (AREB/ABFs) and G-box binding factors (GBFs), which are two subgroups of bZIP transcription factors, have been known as integrators of the ABA signal (Cutler et al. 2010; Fujita et al. 2011; Smykowski et al. 2010; Takezawa et al. 2011); these and other bZIP transcription factors bind to ACGT-like DNA-motifs and orchestrate the downstream gene expression responses (Jakoby et al. 2002).

bZIP proteins have been identified as key regulators for ABA-dependent stress tolerance or resistance. In particular, ABFs and GBFs play pivotal roles in temperature, osmotic, salt and drought stresses, but are also found to influence the circadian clock and senescence (Cutler et al. 2010; Kreps and Kay 1997; Mizuno and Yamashino 2008; Smykowski et al. 2010).

Many ABA-responsive genes follow diurnally oscillating expression trajectories and enroll the elongated Hypocotyl 5 (HY5) transcription factor that acts downstream of the red/far-red light phytochrome photoreceptors by direct activation of ABA-insensitive 5 (ABI5) (Fujita et al. 2011; Kreps and Kay 1997). Interestingly, ABI5 and HY5 are also members of the bZIP transcription factor family (Jakoby et al. 2002). Moreover, the oscillator of the endogenous clock TOC1 functions as a mediator between the circadian clock and the plant’s drought stress response (Legnaioli et al. 2009).

Not surprisingly, orthologs of bZIP transcription factors have also been identified in studies associated with

heterophylly initiation in aquatic plants. The aquatic fern *Marsilea quadrifolia* is one well described model for the analysis of heterophylly formation (Allsopp 1951; Lin et al. 2005; Lin and Yang 1999). ABA functions as a molecular switch to induce aerial leaf morphologies on submersed plants, which is reversible after removal of ABA from the media (Hsu et al. 2001; Lin 2002; Lin et al. 2005). Gene expression studies for ABA-dependent heterophylly revealed changes in gene expression that are clearly associated with aerial-type leaf formation and other ABA-dependent responses (Hsu et al. 2001). The discovery of bZIP class transcription factors in *Marsilea quadrifolia* is consistent with the idea of conserved ABA-dependent signaling cascades between ferns and angiosperms and might provide an important clue to the molecular mechanisms that control heterophylly. Besides other genes, the authors identified a member of the homeotic MADS-box transcription factor family (Hsu et al. 2001; Shan et al. 2009), which is a putative ortholog of AGAMOUS-like 11 (AGL11). In terrestrial plants, AGL11 is a D-function protein that plays pivotal roles in determining floral organ identities, in shaping the leaves of the corolla and in terminating the floral meristem (Shan et al. 2009; Tzeng et al. 2002; Zahn et al. 2006). It is not a farfetched assumption that homeotic MADS-box transcription factors are involved in reprogramming the meristem to form aerial-type leaves. Hence, the transformation of submersed-to-emersed organ identity seems to enroll well known factors that generally give characteristic morphologies to plant organs and that probably can be placed downstream of an ABA-dependent signaling cascade in hydrophytes. It is worthy to mention that an *Eceriferum 1* (CER1)-like gene was found to be upregulated during heterophylly initiation in *Marsilea*. An ortholog of CER1-like has been found essential for the deposition of epicuticular waxes in *Arabidopsis thaliana* (Aarts et al. 1995; Hsu et al. 2001; Lai et al. 2007). The up-regulation of CER1-like proteins might be important to prevent dehydration by a thick layer of waxes on the cuticle of the emersed organs after heterophylly initiation in aquatic plants (Hsu et al. 2001; Lai et al. 2007).

Cross-talk of ABA with other phytohormones

During the last decade we have learned from terrestrial model plants that the changing levels of ABA do not exclusively act through only one specific pathway, but are interconnected with other plant hormones through an interwoven regulatory network (Goda et al. 2008; Nemhauser et al. 2006). Differentiation and stress tolerance are simultaneously controlled through the function of multiple hormones, and recent studies revealed extensive

crosstalk between different signaling pathways and amongst the underlying hormonal pathways (Cutler et al. 2010; Goda et al. 2008; Mizuno and Yamashino 2008; Nemhauser et al. 2006; Wanke et al. 2009). Consequently, experiments with hydrophytes were not conducted exclusively with ABA, but also with other phytohormones.

It is not surprising that gibberillic acid (GA), a well known ABA antagonist in developmental processes, has been found to have an antagonistic effect on heterophylly in aquatic plants, too (Deschamp and Cooke 1985). In *Callitriche heterophylla* GA induced the elongation of cells so that leaves are formed that resemble the submersed leaf morphology (Deschamp and Cooke 1985). Nevertheless, it is speculated that GA induces heterophylly only indirectly through ethylene, which also antagonizes the synthesis of ABA.

Ethylene exposure of aerial shoots of *Ludwigia arcuata* induced the formation of leaves that resemble a submersed leaf morphology (Kuwabara et al. 2003; Kuwabara and Nagata 2006). This gaseous phytohormone is rather hydrophobic and cannot easily evaporate from a submersed plant bodies (Bailey-Serres and Voesenek 2010; Grefen et al. 2008; Jackson 2008; Voesnek et al. 2003). When forcing aerial shoots of *Ludwigia arcuata* to grow below the water surface a strong increase of endogenous ethylene concentration was observed and, subsequently, submersed leaves were formed (Kuwabara et al. 2003; Kuwabara and Nagata 2006). Likewise, the addition of an inhibitor of ethylene perception and signalling to submersed *Ludwigia arcuata* strongly inhibited the outgrowth of submersed leaves (Kuwabara et al. 2003) Hence, ethylene seems to be a key regulator for heterophylly formation in hydrophytes, where it is necessary for the maintenance of a submerge life style.

As ethylene can induce a submerged-type leaf morphology on aerial shoots and, vice versa, ABA initiates the formation of aerial-type leaves on submersed shoots, both hormones must act antagonistically upon each other while they themselves underlie autoregulatory feedback control (Bailey-Serres and Voesenek 2010; Jackson 2008; Kuwabara et al. 2003). As a consequence, the effects of GA, which were mentioned in the beginning of this paragraph, can either be counteracted by ABA or enhanced by ethylene.

One can summarize the findings in a putative model for heterophylly initiation on submersed shoots (Fig. 2), in which experimental data from both terrestrial and aquatic plants have been integrated.

GA acts positively through ethylene on submerge leaf morphology, while ABA antagonizes the function of both GA and ethylene. Thereby, changes in endogenous ABA concentration affect the formation of emersed or aerial-type leaves and immediately feedback on the other two hormones, which otherwise continue the formation of submerge leaves.

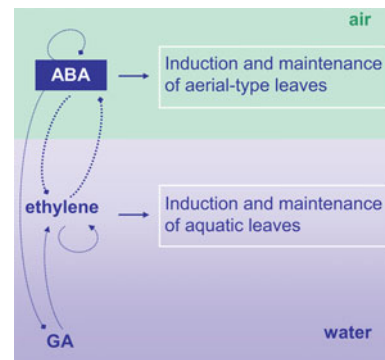


Fig. 2 Schematic model of heterophylly induction. In aquatic plants ABA appears to be sufficient to initiate and maintain the development of aerial-type leaves. The ABA-mediated processes cross-talk with ethylene- and GA-dependent signaling pathways, which initiate and maintain the formation of submerge leaves

When submersed leaves of *Hippuris vulgaris* were brought in contact with air, the endogenous levels of ABA increased, which is the initial step for heterophylly induction (Goliber and Feldman 1989). This increase in ABA concentrations could readily be explained by the model through a possible derepression by ethylene, which is released from the plant during the aerial conditions. Vice versa, when the aerial shoots of *Ludwigia arcuata* were exposed to ethylene, the endogenous concentration of ABA decreased and submersed-type leaves were formed (Kuwabara et al. 2003). Here, the model implies a situation in which the elevated ethylene concentration mimics a submerged environment and either represses ABA synthesis or induces its degradation.

To conclude, the analysis of heterophylly induction in hydrophytes represents a vital model for the investigation of ABA on the developmental processes that were important to successfully establish both terrestrial and aquatic plant life. *Marsilea quadrifolia* and *Ludwigia arcuata* constitute well suited model organisms also for the in depth molecular genetic or genomic analyses. For an even deeper insight, it is feasible to study the role of ABA in heterophylly initiation by next generation sequencing approaches under inducing or inhibiting conditions in future.

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