REVIEW PAPER

# Can mosses serve as model organisms for forest research?

**Stefanie J. Müller · Desirée D. Gütle ·
Jean-Pierre Jacquot · Ralf Reski**

**Abstract**
· *Key message* **Based on their impact on many ecosystems,
we review the relevance of mosses in research regarding
stress tolerance, metabolism, and cell biology. We intro-
duce the potential use of mosses as complementary model
systems in molecular forest research, with an emphasis on
the most developed model moss *Physcomitrella patens*.**
· *Context and aims* Mosses are important components of sev-
eral ecosystems. The moss *P. patens* is a well-established non-
vascular model plant with a high amenability to molecular

S. J. Müller · D. D. Gütle · R. Reski (✉)
Plant Biotechnology, Faculty of Biology, University of Freiburg,
Schaenzlestr.1, 79104 Freiburg, Germany
e-mail: ralf.reski@biologie.uni-freiburg.de

D. D. Gütle · R. Reski
Spemann Graduate School of Biology and Medicine (SGBM),
University of Freiburg, Albertstr. 19A, 79104 Freiburg, Germany

D. D. Gütle · J.-P. Jacquot
Interactions Arbres—Microorganismes, UMR1136, Université de
Lorraine, 54500 Vandoeuvre-lès-Nancy, France

J.-P. Jacquot
Interactions Arbres—Microorganismes, UMR1136, INRA,
54280 Champenoux, France

R. Reski
BIOSS Centre for Biological Signalling Studies, Schaenzlestr. 18,
79104 Freiburg, Germany

R. Reski
FRIAS Freiburg Institute for Advanced Studies, Albertstr. 19,
79104 Freiburg, Germany

R. Reski
USIAS Strasbourg Institute for Advanced Study, Strasbourg, France

biology techniques and was designated as a JGI plant flagship
genome. In this review, we will provide an introduction to
moss research and highlight the characteristics of *P. patens*
and other mosses as a potential complementary model system
for forest research.
· *Methods* Starting with an introduction into general moss
biology, we summarize the knowledge about moss physiology
and differences to seed plants. We provide an overview of the
current research areas utilizing mosses, pinpointing potential
links to tree biology. To complement literature review, we
discuss moss advantages and available resources regarding
molecular biology techniques.
· *Results and conclusion* During the last decade, many fun-
damental processes and cell mechanisms have been studied in
mosses and seed plants, increasing our knowledge of plant
evolution. Additionally, moss-specific mechanisms of stress
tolerance are under investigation to understand their resilience
in ecosystems. Thus, using the advantages of model mosses
such as *P. patens* is of high interest for various research ap-
proaches, including stress tolerance, organelle biology, cell
polarity, and secondary metabolism.

## 1 Introduction

Often, the ability to disproof or to support a scientific hypoth-
esis in an experiment depends on the choice of the biological
model system. In life sciences, several model species have
been used in relation with the different advantages they offer
in terms of generation time, growth, evolutionary position, or
amenability to molecular biology techniques (Jansson and
Douglas 2007; Müller and Grossniklaus 2010; Reski 1998).

In plant sciences, several seed plant model species are established, whereas lycophytes and ferns are more problematic model organisms due to their low transformability or large genome size (Plackett et al. 2014). Liverworts, mosses, and hornworts constitute the paraphyletic group of bryophytes and represent early diverging lineages of land plants with an intermediate position between vascular plants and algae (Kenrick and Crane 1997; Lang et al. 2010). Many moss features are regarded as ancestral, though their special life style has led to the evolution of many moss-specific morphological and physiological traits, which support alternative survival strategies among land plants (Mishler and Oliver 2009). While the simple morphological structure of mosses is largely conserved since at least 330 million years (Hubers and Kerp 2012), they colonize an extensive spectrum of diverse habitats (Turetsky 2003; Turetsky et al. 2012). Their occurrence in many extreme habitats such as the Antarctic tundra and deserts implies the existence of many yet unknown moss-specific survival mechanisms (Oliver et al. 2005; Roads et al. 2014). The resilience of mosses could recently be demonstrated with the successful regeneration of subglacial bryophytes following 400 years of ice entombment (Farge et al. 2013) and even by the regrowth of over 1500-year-old moss from the Antarctic permafrost (Roads et al. 2014).

However, besides these extreme examples, mosses are also important components of tropical systems (Gradstein et al. 2001), boreal forests, and woodlands or unshaded habitats in temperate zones (Turetsky 2003). Despite their small size, mosses have a huge impact on various ecosystems and are essential contributors to complex biological cycles. Besides the prevention of soil erosion, the development of microtopography, and the regulation of soil climate and water availability, main aspects are nitrogen fixation and carbon cycling.

For instance, mosses are important contributors to bog formation and carbon trapping (Jones et al. 1994; Shaw et al. 2003) in peat lands, where almost 100 % of the ground layer is covered by mosses (Vitt and Wieder 2009) such as *Sphagnum* species. The relevance of biotechnology approaches regarding the production and cultivation of peat mosses is increasing due to the rising demand of peat as, e.g., substrate for horticulture (Beike et al. 2015a).

In forest ecosystems such as rain forests, in which mosses live in immediate vicinity to vascular plants, they can serve as water reservoir (Gradstein et al. 2001) as well as bio-indicators for environmental changes and air pollution (Bates 2009).

The role of mosses for nitrogen fixation in boreal forests is a research area of high interest, as a substantial amount of nitrogen fixation can rely on feather moss-cyanobacteria associations (DeLuca et al. 2002; Rousk et al. 2013).

Hence, mosses represent an omnipresent organism group whose impact is seldom taken into account in forest research and which deserve attention as attractive model systems regarding ecology as well as regarding fundamental processes and evolution of land plants. Moss model species include, e.g., the Funariaceae *Funaria hygrometrica* and *Physcomitrella patens*, the Ditrichacea *Ceratodon purpureus*, and peat moss (*Sphagnum*) species. Moreover, some model mosses provide extensive advantages for research projects employing molecular biology. The currently most developed model moss is *P. patens*, a moss of the temperate zones which can be found on soil exposed by falling water levels or on fields (Cove 2005). It provides a fully sequenced genome (Rensing et al. 2008), many resources for -omics techniques, a high amenability to microscopy, and a valuable experimental platform for comparative studies with other model organisms, as, e.g., trees. Therefore, the *Physcomitrella* genome was designated, like poplar, as a "JGI plant flagship genome" (http://jgi.doe.gov/our-science/science-programs/plant-genomics/plant-flagship-genomes/). Potential research areas include general moss physiology, moss-specific stress resistance mechanisms, putatively interesting for the transfer to seed plants, as well as research on fundamental mechanisms which evolved early and are conserved in many land plants. In addition, the technical advantages of the moss system can offer complementing approaches to many open questions in seed plant research by, e.g., enabling the manipulation of the genome down to the single base pair level by homologous recombination.

This review gives a basic introduction into moss biology and the current reference model *Physcomitrella* and its advantages, and provides an overview of the current use of mosses to gain knowledge in different research areas, with an emphasis on potential links with molecular tree research.

## 2 The moss model: particularities and advantages

Plants started to colonize the land mass of Earth about 450–500 million years ago (Kenrick and Crane 1997; Lang et al. 2010), shaping the new environment and establishing the basis of land ecosystems, paving the way for the colonization by animals. During land plant evolution, many adaptations occurred, some of them leading to large radiation of new groups, as, e.g., the flowering plants. Ancestral features of land plants can be either reconstructed from fossils or by the investigation of early diverging land plant groups, as mosses. Though for some aspects moss biology is similar, in others it is fundamentally different from flowering plants.

### 2.1 Mosses are haploid dominant

All plants undergo an alternation of generations between a diploid sporophyte and a haploid gametophyte. Whereas in all vascular plants the sporophyte is the dominant generation, in bryophytes the gametophyte forms the main photosynthetically active plant body (see Fig. 1). Hence, in a
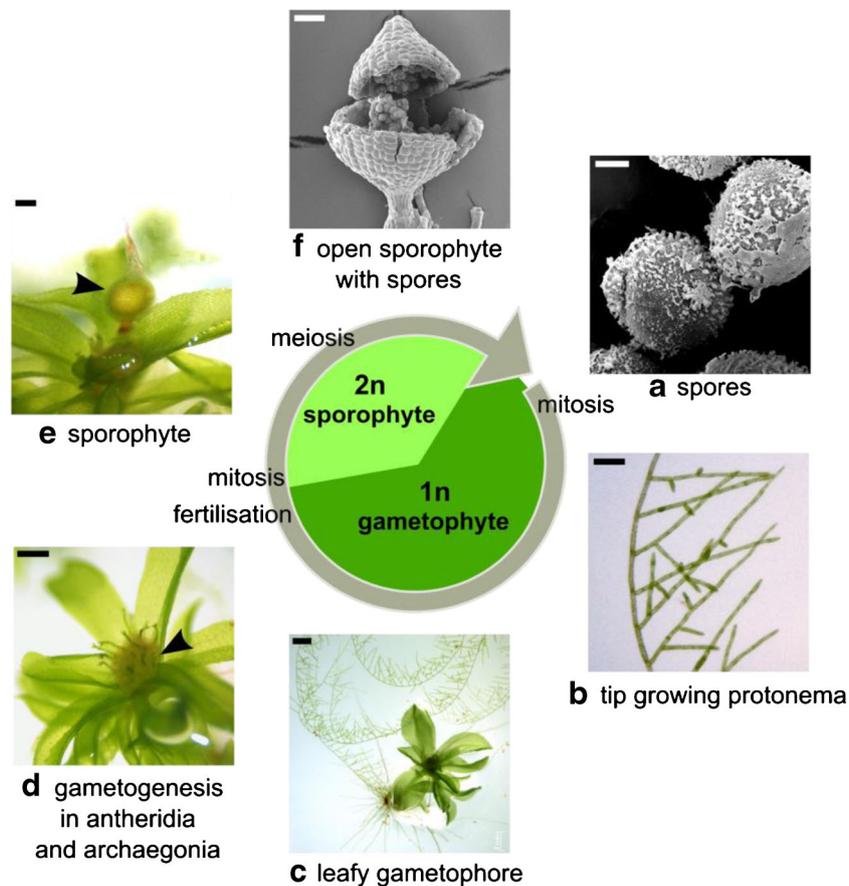
**Fig. 1** The life cycle of *Physcomitrella patens*. Development in *Physcomitrella patens* starts from the haploid spore, which forms protonema, a tip growing filamentous tissue. During the juvenile-to-adult transition of the moss gametophyte, the protonema forms buds which contain a three-faced apical meristematic cell (Harrison et al. 2009) able to generate the leafy gametophore. Within several weeks of inducing conditions (15 °C short day), male and female sexual organs develop on the apex of the gametophores. Male gametangia (antheridia) release flagellate gametes that need a water film to travel to the female gametangia (archaegonia). From the diploid (2n) zygote, a sporophyte is formed on top of the gametophyte, which in turn forms spores (1n) by meiosis. Ripe sporophytes open by rupture and release spores which germinate to generate protonema. *Scale bars*: **a** 6 μm, **b** 50 μm, **c** 1 mm, **d** 500 μm, **e** 200 μm, **f** 100 μm

developmental comparison of homologous structures between non-vascular and vascular plants, moss gametophytes correspond to flowering plant gametophytes, i.e., the pollen and the embryo sac. At the molecular level, the developmental programs underlying the gametophyte and sporophyte body plans are often not synapomorphies of land plants and rely on different mechanisms (Sakakibara et al. 2013).

Though mosses are haploid during a large fraction of their life, i.e., possess just one allele of each gene, they can often cope with higher amounts of toxic or mutagenic substances compared to flowering plants (Charlot et al. 2014; Harrison et al. 2009). Considering the absence of a second allele which could balance somatic mutations, this drawback is compensated by a high metabolic redundancy (Lang et al. 2005), whole genome duplications (Rensing et al. 2007), and the arrest of the cell cycle after DNA synthesis in the G2 phase (Schween et al. 2003), combined with efficient DNA repair mechanisms such as homologous recombination (Kamisugi et al. 2006).

### 2.2 *P. patens* exhibits several growth modes and a high regeneration capacity

The juvenile stage of gametophyte development, the filamentous protonema (Fig. 1b) is growing by perpetually dividing meristematic tip cells, whereas the adult stage, the gametophores (Fig. 1c), possess a three-faced apical meristematic cell which supports the formation of leafy shoots (Aoyama et al. 2012; Harrison et al. 2009). The role and response of hormones during the different developmental stages and possible culture conditions are well characterized and reviewed elsewhere (Bonhomme et al. 2013; Decker et al. 2006; Kofuji and Hasebe 2014; Strotbek et al. 2013). For research, moss tissue can easily be cultivated either on agar plates where gametophores are formed within few weeks or as protonema in liquid medium. Cells from a detached leaflet, a protoplast, or a wounded protonema filament undergo reprogramming to a meristematic protonema tip cell without the addition of exogenous hormones (Nishiyama et al. 2012).

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This high regeneration capacity also supports the cultivation of moss tissue in liquid medium using weekly disruption cycles and the easy regeneration of plants from protoplasts. The molecular mechanisms underlying this pluripotency have been investigated and unraveled several key players including a basic helix loop helix transcription factor and two WUSCHEL-related homeobox 13-like genes (Busch et al. 2013; Nishiyama et al. 2012; Sakakibara et al. 2014).

## 2.3 Mosses are poikilohydric

In addition to the distinct dominant generations, mosses and vascular plants differ also in the morphology and consequently in resulting features such as water balance and body height. Moss tissues are mostly only one cell layer thick which makes them poikilohydric organisms. Mosses equilibrate to the humidity of the surrounding environment, in contrast to endohydric vascular plants (Mishler and Oliver 2009). In consequence to changes of humidity, wind, illumination, or temperature, bryophyte species often have to deal with high fluctuations in water content with most species, including *P. patens*, being dehydration tolerant, whereas some species even being desiccation tolerant (Oliver et al. 2005).

## 2.4 Gene targeting in *P. patens* is facilitated by homologous recombination

*P. patens* is amenable to several transformation techniques to generate transient or stable transgenic plants, with polyethylene glycol mediated protoplast transformation being the most commonly used (Cove et al. 2009; Strotbek et al. 2013). RNAi-based procedures and inducible expression systems are established and available (reviewed in Strotbek et al. 2013). However, one of the original reasons to choose *P. patens* as a model organism was its exceptionally high rate of homologous recombination enabling gene targeting strategies as a tool for reverse genetics (Reski 1998). Similarly, an increased gene targeting efficiency compared to vascular plants, but lower than in *P. patens*, was reported for the moss *C. purpureus* (Trouiller et al. 2007).

DNA double strand breaks are in general repaired either by non-homologous end joining (NHEJ) or by homologous recombination (HR). The balance between these two mechanisms can shift, depending on the species and/or the developmental state. In *P. patens*, the integration of foreign DNA via homologous recombination can occur in frequencies of up to 100 % of successful transformants (Kamisugi et al. 2005). Hereby, the addition of 500–1000 bp of homologous sequence is sufficient to target a DNA construct to a specific genomic locus (Kamisugi et al. 2005). In reverse genetics, this technique is employed to disrupt or completely delete the target gene, resulting in a knock-out, but it can also easily be used to knock-in a reporter at the endogenous locus (Mosquna et al.

2009; Mueller et al. 2014; Schaefer 2002) or to modify a gene on the single base pair level. Thus, proteins with altered amino acid sequence can be generated from the endogenous genomic locus, enabling techniques such as the mutation of specific disulfide bridges or phosphomimetic mutants, similar to techniques used in the model yeast *Saccharomyces cerevisiae*.

The molecular basis of the high rate of homologous recombination of the "green yeast" *P. patens* has been the subject of several studies, identifying some important factors (Charlot et al. 2014; Kamisugi et al. 2012), although a complete mechanistic model is still lacking.

## 2.5 Extended moss resources: bioinformatics and high throughput data

Other benefits of the moss model are the increasing resources on the annotation and bioinformatic level. Gene models for *P. patens* are available since the sequencing of the genome (Rensing et al. 2008) and implemented in two organism-specific databases: www.cosmoss.org and http://moss.nibb. ac.jp/. Genome annotation is permanently improved (Zimmer et al. 2013). In addition, moss gene models are well integrated in orthology and phylogenomics databases such as PLAZA , KEGG, and OrthoMCL (Van Bel et al. 2012; Chen et al. 2006; Kanehisa et al. 2014). Comparative studies are also supported by the integration of moss into the Plant Ontology, providing a controlled and structured plant term vocabulary (Cooper et al. 2013). A curated list of ortholog groups of *P. patens* and 28 plant species was published with the current genome annotation (Zimmer et al. 2013), enabling the simultaneous search for homologs in many species, including poplar and papaya.

In recent years, several -omics techniques have added a considerable amount of new data concerning the *P. patens* metabolome and proteome (Erxleben et al. 2012; Mueller et al. 2014), as well as the transcriptome (Beike et al. 2015b; Cuming et al. 2007; O'Donoghue et al. 2013; Richardt et al. 2010), which can be screened on the Genevestigator transcriptomics platform (Hiss et al. 2014).

At the transcriptome level, additional moss data is available for *C. purpureus* (Szövényi et al. 2015), and the 1000 Plants (1KP) consortium will add RNA-seq data for 41 moss species in the near future (Matasci et al. 2014).

Moss data on metabolic pathways have been integrated into the Plant Metabolic Network database (Caspi et al. 2007), generating the MossCyc database (http://www.plantcyc. org/). Furthermore, mutants and ecotypes of *P. patens* as well as *F. hygrometrica* can be stored and are available from the International Moss Stock Center (IMSC; http://www. moss-stock-center.org/), employing cryo-preservation (Schulte and Reski 2004).

Thus, the search for moss mutants and for the homologs of genes of interest is facilitated and complemented by

information on tissue- and development-specific expression as well as functional annotation. Whereas *P. patens* serves as a reference model, the increasing amount of sequence data on other moss species enables broader analyses including different habitats.

## 2.6 *P. patens* can serve as a production platform

An additional use of *P. patens* is its establishment as an expression system for recombinant proteins, particularly with respect to glycosylated biopharmaceuticals. The potential of cultivation in photo-bioreactors under low-cost and standardized conditions with volumes of up to 100 l guarantees a high yield of proteins according to good manufacturing practice guidelines (Decker and Reski 2007; Fischer et al. 2012). Many important recombinant biopharmaceuticals need to undergo post-translational modifications, e.g., glycosylation, which are important for their stability and function in the human body. Therefore, bacterial systems are often excluded for expression (Sola and Griebenow 2010). Plants show a similar glycosylation pattern to humans, though the few occurring differences can be immunogenic in humans. Due to the fast and precise gene manipulation technique based on homologous recombination in *P. patens*, the humanization of the moss glycosylation pattern is feasible and glyco-engineering in moss plants is already quite advanced (reviewed in Decker et al. 2014). To date, several human proteins are produced in moss, e.g., vascular endothelial growth factor (Baur et al. 2005), erythropoietin (Weise et al. 2007), and human complement factor H (Buettner-Mainik et al. 2011). In addition, moss is used as production platform for secondary metabolites such as terpenoids (Bach et al. 2014).

## 3 Current moss research: unraveling plant evolution and utilizing advantages of moss systems

In this section, we would like to illustrate specific examples for the study of ancestral traits of land plants and comparative analyses employing mosses, as well as highlight some research on moss-specific features (see Fig. 2). Most of the presented studies listed below use techniques such as phylogenomics, gene knock-out, and overexpression or complementation experiments between different species. In order to facilitate to start working with moss, we provide an overview of the current knowledge regarding moss physiology, especially integrating research areas with potential links to tree physiology. Thus, we present examples of how mosses were used to establish evolutionary relationships in gene families and how special molecular biology techniques applicable to mosses can support the characterization of target proteins in single protein and high-throughput studies.

## 3.1 Several regulatory pathways evolved in the common ancestor of land plants

Many hormone systems were already established in early land plants and subsequently adapted, and are thus conserved on the molecular level in mosses and vascular plants. The ancient pathways for auxin (Bennett et al. 2014a, 2014b; Finet and Jaillais 2012; Paponov et al. 2009), cytokinin (Lindner et al. 2014; Pils and Heyl 2009; von Schwartzenberg et al. 2007), and ethylene (Yasumura et al. 2012) were investigated with respect to their corresponding transporters, biosynthesis, and signaling pathways. Further, moss is lacking essential characteristics of the classical gibberellin signaling pathway, although it responds to external gibberellin treatment (Vandenbussche et al. 2007). The abiotic stress response is highly conserved within land plant plants, employing abscisic acid and orthologous transcriptional regulators (Beckett et al. 2000; Khandelwal et al. 2010).

Moreover, the conservation of certain developmental processes, which seem to be associated to early land plant evolution, is a topic of current research. The development of chloroplasts is for example regulated by the GOLDEN2-LIKE transcription factors in angiosperms as well as in mosses, but cannot be found in *Chlamydomonas reinhardtii* (Yasumura et al. 2005).

In land plants, mutations in the organellar genomes are counterbalanced by the "RNA-editing" mechanism which mediates base exchanges on organellar transcripts. The number of editing sites per gene and specificity of the editing factors, the pentatricopeptide repeat proteins (PPR), can vary between even closely related (moss) species and seems to follow a dynamic evolutionary process (O'Toole et al. 2008; Rüdinger et al. 2012). In flowering plants, up to 500 RNA editing sites were identified in their organelle transcripts, whereas in contrast only 11 sites were found in *P. patens* mitochondria (Rüdinger et al. 2009).

Interestingly, the gene regulation system including NAC transcription factors which are responsible for the formation of water-conducting cells can be found in vascular and non-vascular plants, but operating in the sporophytic (Yamaguchi et al. 2011) or in the gametophytic generation (Xu et al. 2014), respectively.

In contrast, stomata are present only in the sporophytic generation of mosses and seed plants and operate with a highly conserved guard cell control mechanism, as the involved moss homolog can complement the *Arabidopsis thaliana* mutant (Chater et al. 2011).

## 3.2 Moss is amenable to fundamental research in cell biology

The amenability of *P. patens* to microscopy coupled with the knock-in of fluorescent reporters via gene targeting to specific loci can be used for the investigation of fundamental cell
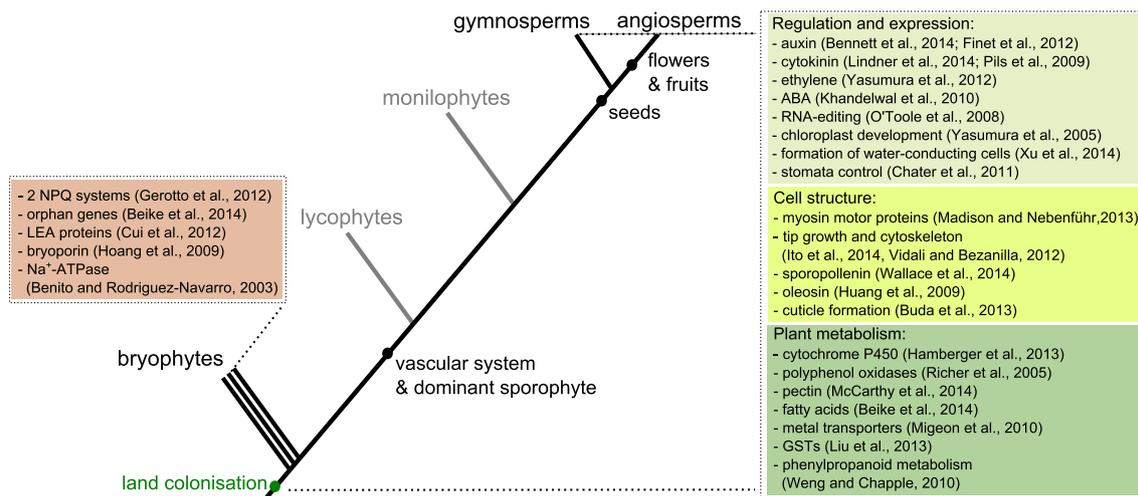
**Fig. 2** Evolutionary novelty and conservation investigated using *Physcomitrella patens*. The main lineages of land plants are depicted in an evolutionary tree. Bryophytes are a paraphyletic group, depicted as three separate lineages in the figure, encompassing the liverworts, mosses, and hornworts. *Physcomitrella patens* is a model moss. On the one hand, several studies investigate moss-specific traits (*red box*) often related to the resilience of moss to environmental conditions. On the other hand, early adaptations of land plants and functional evolution of gene families can be analyzed by comparative studies between mosses and vascular plants (*green boxes*)

biological questions. As moss protonema filaments elongate by tip growth, as root hairs and pollen tubes in flowering plants, the role of the cytoskeleton and associated factors has been investigated intensively (Finka et al. 2008; Furt et al. 2013; Vidali and Bezanilla 2012). The function of different types of myosin motor proteins were recently analyzed, confirming their conserved function in growth, organelle shape, and actin organization (Madison and Nebenführ 2013), but also identifying a novel role in cell division (Wu and Bezanilla 2014). In addition, the role of the conserved Rop/RAC signaling module in moss tip growth was established (Ito et al. 2014).

Furthermore, the post-translational modification prenylation was shown to be necessary for moss cell differentiation and the establishment of polarity (Thole et al. 2014). Cell polarity as well as organelle morphology and dynamics were also investigated at the subcellular level (Furt et al. 2012; Mueller et al. 2014), including the FtsZ protein family of plastid division factors (Martin et al. 2009). By employing quantitative proteomics techniques such as metabolic labeling with a heavy nitrogen isotope, organellar proteomes were characterized (Mueller et al. 2014). In organelle biology and protein targeting, mosses serve as valuable reference (Xu et al. 2013).

In addition to intracellular communication, intercellular communication via plasmodesmata was visualized by stable transgenic moss lines expressing a photoconvertible fluorescent protein (Kitagawa and Fujita 2013).

Moreover, *P. patens* is to date the most basal land plant possessing oil bodies for energy storage, reflected by the presence of oleosin genes (Huang et al. 2009).

Thus, quantitative high-throughput analyses for metabolites or proteins are readily available for moss cultures. Moreover, the one-cell-layer-thick moss tissues offer a unique microscopy platform coupled with ease of protein tagging via homologous recombination. These features and techniques could be used to investigate questions of molecular forest research, such as protein modifications and stability, the regulation of cell and organelle signaling, and redox homeostasis.

3.3 Mosses harbor novel enzymatic functions and can be a model for the early adaptations of metabolism to the life on land

While development and body plan underwent large modifications during land plant evolution, many metabolic pathways evolved early and are already present in mosses. The partitioning of metabolism between cell organelles is largely conserved between *P. patens* and flowering plants (Mueller et al. 2014), with some exceptions (Birke et al. 2012). Sometimes even algal and vascular plant features are combined in mosses, as it is the case for the mechanism of non-photochemical quenching (NPQ), used in plants for converting excess energy produced by photosynthesis (Gerotto et al. 2012).

In early land plants, several cell structures and their corresponding biosynthetic pathways were adapted to the altered environment with increased abiotic stress frequency. Thus, a waxy cuticle with a similar composition protects mosses and seed plants from dehydration, with the plasma membrane transporter involved in its formation being functionally conserved during evolution (Buda et al. 2013). Reproductive propagules in land plants, i.e., spores and pollen, are protected by a tough sporopollenin wall. The respective moss and angiosperm homologs of the sporopollenin biosynthesis gene *MALE STERILITY2* (*MS2*) exhibit a very similar function in

spore and pollen formation, respectively. However, the moss *MS2* gene did not complement the angiosperm mutant, implying functional specialization after the transfer of sporopollenin from spores to pollen (Wallace et al. 2014).

In addition, the genes responsible for the biosynthesis and modification of the cell wall component pectin share a common ancestor in mosses and vascular plants (McCarthy et al. 2014). In order to protect the early land plants from UV radiation, secondary metabolism diversified and phenylpropanoid metabolism emerged (Weng and Chapple 2010). In vascular plants, the phenylpropanoid polymer lignin enabled the evolution of large plant bodies and tracheids by reinforcing and waterproofing the cell wall. In mosses, only soluble phenylpropanoids are known, though homologs of the biosynthetic enzymes producing the lignin monomers *p*-coumaryl alcohol and coniferyl-alcohol are present (Weng and Chapple 2010). Among other reactions, enzymes of the cytochromes P450 enzyme family participate in monolignol biosynthesis by catalyzing the hydroxylation of the aromatic ring. The important role of the P450 enzyme family, contributing to the diversity of plant compounds, is reflected in the increasing number of encoded genes during plant evolution, e.g., in *P. patens* with 71 and *A. thaliana* with 245 genes (Hamberger and Bak 2013). An opposite effect was reported for putative genes encoding polyphenol oxidases (PPO) which are responsible for the browning reactions in fruits. Starting in 2005 with the first characterization of a single PPO gene in *P. patens* (Richter et al. 2005), the release of the genome resulted in 13 putative genes encoding for PPOs, whereas for example in *A. thaliana*, no homologs were identified (Tran et al. 2012).

Polyunsaturated fatty acids (PUFAs) are important components in the cell for signaling, hormone biosynthesis, and membrane formation. Compared to higher plants, *P. patens* and other mosses possess high amounts of long PUFAs, whose profile is depending on the developmental stage of the plant (Beike et al. 2014). This difference is based on the existence of several desaturases as well as elongases in moss which were lost in angiosperm evolution and catalyze alternative pathways in the biosynthesis of these fatty acids (Eiamsa-ard et al. 2013; Girke et al. 1998; Kaewsuwan et al. 2006; Zank et al. 2002).

In order to track gene family evolution and function on the sequence level, phylogenomics, i.e., phylogenetic analyses based on sequence comparisons between many plant genomes, is frequently used. Thus, two novel classes of the large gene family of Glutathione-S-Transferases were identified in *P. patens*, whereas one of the most abundant classes in vascular plants, tau GSTs, was not found (Liu et al. 2013). These enzymes are important players in oxidative stress metabolism and detoxification (see review Jacquot et al. in the same issue).

Furthermore, the types and subfamilies of the land-plant-specific transcription factor AT-Hook Motif Nuclear Localized (AHL) gene family (Zhao et al. 2014) or metal transporters (Migeon et al. 2010) were analyzed, including

sequences of *Physcomitrella*, *Arabidopsis*, poplar, and many other species. Hence, phylogenomic studies including non-vascular plants give insights into the mechanisms of gene family evolution leading to functional and regulatory diversification (Mueller et al. 2014; Rensing et al. 2007). This approach can be extended to the analysis of subcellular protein compartmentation and protein targeting (Mueller et al. 2014; Xu et al. 2013).

### 3.4 Mosses employ several strategies to increase stress tolerance

Form and function of mosses entail survival strategies which are thought to be similar to the early land plants and have been lost or adapted in endohydric vascular plants (Oliver et al. 2005). Research on vegetative and seed drought resistance has used mosses such as the desiccation-tolerant *Tortula ruralis* as reference (Farrant and Moore 2011). Thus, the ecophysiology of mosses is interesting at the molecular, biochemical, and whole plant level in order to identify alternative stress resistance mechanisms. Especially with regard to the progressing climate change, it is crucial to get insights into the resilience mechanisms of mosses (Peñuelas et al. 2013).

In the last decade, several studies have investigated the response of *P. patens* to abiotic stresses such as light quality and quantity (Alboresi et al. 2010; Wolf et al. 2010), temperature stress (Beike et al. 2015b; Bhyan et al. 2012; Chang et al. 2014), and osmotic stress (Cuming et al. 2007; Frank et al. 2005; Khandelwal et al. 2010). *P. patens* is dehydration-tolerant but can become desiccation- and freezing-tolerant by pre-treatment with ABA (Khandelwal et al. 2010; Nagao et al. 2005). Whereas abiotic stress signaling seems largely conserved between mosses and flowering plants (Khandelwal et al. 2010; Komatsu et al. 2013; Richardt et al. 2010), several studies report on so-called orphan, non-characterized stress responsive moss genes and proteins (Beike et al. 2015b; Cui et al. 2012).

To date, most attention has been paid to the mechanisms by which mosses master the cellular challenges during dehydration/rehydration and freezing, sometimes also employing ABA or salt treatment (Cui et al. 2012; Cuming et al. 2007; Hiss et al. 2014; Nagao et al. 2005; Oliver et al. 2005). Results include the avoidance of stress by downregulation of photosynthesis (Cuming et al. 2007; Hiss et al. 2014) and the rapid upregulation of protective mechanism for photosynthesis, membranes and protein folding (Azzabi et al. 2012; Hiss et al. 2014; Ruibal et al. 2013). At the metabolic level, free soluble sugars, such as trehanderose, are accumulated to increase the intracellular osmolarity and counterbalance water-loss (Nagao et al. 2005, 2006). At the transcript as well as at the protein level, chaperones such as LEA (*late embryogenesis abundant*) proteins, also called dehydrins or rehydrins, play prominent roles, though their exact molecular

function is often still unclear. Later during land plant evolution, LEA protein function was probably adapted to the desiccation of seeds (Cui et al. 2012; Oliver et al. 2005). Functional studies of mutants confirmed the importance of some dehydrins in the moss response to salt and osmotic stress (Ruibal et al. 2012; Saavedra et al. 2006). Two moss dehydrins did also confer increased stress tolerance to *Arabidopsis*, when overexpressed (Ruibal et al. 2012). In the desiccation-tolerant moss *T. ruralis*, rehydrin mRNAs stored in messenger ribonucleoprotein particles (mRNPs) during drying enable a fast recovery from desiccation (Wood and Oliver 1999).

A remnant of a horizontal gene transfer of metazoans to early land plants may be the presence of a "bryoporin" in *P. patens*, a putative pore-forming stress-induced actinoporin (Hoang et al. 2009). The high salt tolerance in moss was shown to involve specific $Ca^{2+}$ signatures (Qudeimat et al. 2008). Further, in contrast to flowering plants, mosses possess $Na^+$-ATPases to limit $Na^+$ toxicity (Benito and Rodríguez-Navarro 2003; Lunde et al. 2007). Further, the function of a $Na^+/H^+$ antiporter present in seed plants and mosses was clarified using targeted knock-outs in *P. patens* (Fraile-Escanciano et al. 2010).

In order to fully understand protective mechanisms against abiotic stress in moss, the increasing amount of transcriptome and proteome data needs to be complemented by functional studies on the contribution of single genes and by the characterization of moss-specific orphan genes. However, it is already clear that moss cells respond specifically, and according to their developmental state, at many different levels to adjust cell morphology and metabolism to the corresponding stress challenge. Thus, the ecophysiology of mosses from different habitats and the key pathways of their stress tolerance are still under investigation with mosses providing morphologically simple model systems for comparative and biotechnological studies.

## 4 Conclusion

Mosses are omnipresent extant relatives of early land plants whose ecological significance is often not well characterized. Thus, the investigation of moss biology offers insights into plant evolution, plant survival strategies as well as an often simpler experimental system with valuable molecular biology tools.

At the moment *P. patens* constitutes the most developed model moss regarding molecular biology techniques, bioinformatics as well as available physiology data. With the amount and quality of genome annotation, transcriptomics, and proteomics data still increasing, this model moss will serve as a platform for many exciting de novo studies, as well

as a reference for many other sequencing projects of mosses from different habitats, as, e.g., peat mosses.

The amenability of several bryophytes to single-base-pair exact changes and knock-in of tags in the genome is still a major advantage for many studies envisaging custom mutations and tracking of tagged proteins. With more and more puzzle pieces of land plant evolution unraveled, the right choice of complementary model systems to seed plants becomes easier. Especially organelle biology, cell polarity, stress tolerance, and secondary metabolism represent shared topics in moss and forest research. Thus, mosses represent alternative model systems for many molecular biology studies.

By studying moss biology, we can learn more about fundamental questions of plant physiology and evolution and will further strive to integrate this knowledge on the macro-scale in the study of ecosystems.

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