

MASTERARBEIT | MASTER'S THESIS

Titel | Title

The influence of drought stress on mosses and liverworts

verfasst von | submitted by

Stefanie Wernig BSc

angestrebter akademischer Grad | in partial fulfilment of the requirements for the degree of
Master of Science (MSc)

Wien | Vienna, 2025

Studienkennzahl lt. Studienblatt |
Degree programme code as it appears on the
student record sheet:

UA 066 832

Studienrichtung lt. Studienblatt | Degree
programme as it appears on the student
record sheet:

Masterstudium Botany

Betreut von | Supervisor:

ao. Univ.-Prof. Mag. Dr. Ingeborg Lang Privatdoz.

Index

Aknowledgements	1
Summary	2
Zusammenfassung	3

1. Introduction

1.1. Bryophytes	4
1.1.1. Life cycle.....	5
1.1.2. Morphology.....	5
1.1.3. Phylogeny.....	5
1.1.4. Anatomy.....	6
1.2. Significance of water to bryophytes	7
1.2.1. Poikilohydry.....	8
1.2.2. Water content, - potential, & osmotic potential.....	9
1.2.3. Structure adaptations.....	10
1.2.4. Cytorrhysis.....	11
1.2.5. Molecular mechanisms.....	11
1.3. Photosynthesis	12
1.3.1. Effect of dehydration on photosynthesis.....	13
1.3.2. Effect of rehydration on photosynthesis.....	13
1.4. Hypothesis and aims	14

2. Materials and Methods

2.1. Plant species	15
<i>Atrichum undulatum</i>	15
<i>Plagiomnium affine</i>	16
<i>Physcomitrium patens</i>	17
<i>Funaria hygrometrica</i>	18
<i>Marchantia polymorpha</i>	19
2.2. In vitro culture	
2.2.1. Medium preparation.....	20
2.2.2. Sterile culture.....	21
2.2.3. Transplantation.....	21
2.2.4. Growth conditions for sterile cultures.....	22
2.2.5. Moss cultivation and contamination.....	22

2.3. Photosynthetic parameters	
2.3.1. Quantum yield (Φ)	24
2.3.2. Pulse Amplitude Modulated (PAM) Fluorometry.....	25
2.4. Dehydration	
2.4.1. Photosynthetic activity & weight loss.....	26
2.5. Rehydration	28
2.6. Light microscopy	
2.6.1. Sample preparation & setup.....	30
2.6.2. Cell area.....	31
2.6.3. Hydrated cell area.....	31
2.6.4. Leaf area.....	31
2.7. Stereomicroscopy	
2.7.1. Sample preparation.....	32
2.7.2. Gametophyte time-lapse.....	32
2.8. Confocal laser scanning microscopy (CLSM)	
2.8.1. Sample preparation.....	33
2.8.2. Leica SP8 settings.....	33
2.9. Statistical analysis	35

3. Results

3.1. Dehydration	36
3.1.1. Weight loss.....	36
3.1.2. Photosynthetic activity.....	41
3.1.3. Combined weight loss & photosynthetic activity.....	46
3.1.4. Photosynthetic activity (x-axis) & weight (y-axis).....	50
3.2. Rehydration	
3.2.1. Photosynthetic activity.....	54
3.3. Cell area	58
3.4. Hydrated cell area	67
3.5. Leaf area	70
3.5.1. Microscopic images.....	71
3.5.2. Mean leaf area.....	74
3.6. Gametophyte dehydration	77
<i>Atrichum undulatum</i>	80
<i>Plagiomnium affine</i>	81
<i>Physcomitrium patens</i>	82
<i>Marchantia polymorpha</i>	83

3.6.1. Gametophyte dehydration (30 min).....	84
3.6.2. Gametophyte dehydration (120 min).....	87
3.7. Confocal laser scanning microscopy (CLSM)	92
3.7.1. Cell wall and chloroplasts.....	93
3.7.2. Leaf diameter.....	98
4. Discussion	101
4.1. Weight loss and photosynthetic activity	101
4.2. Species-specific effects	103
4.3. Impact of dehydration on the cytological level	108
5. Conclusion	112
5. Sources	113
6. Appendix	124

Acknowledgments

Firstly, I want to thank my parents for providing for me throughout my studies, my sister for giving me emotional support, and my aunt for always having an open ear and motivating me. Further, I want to mention my grandparents, who passed away recently and who contributed a lot to my interest in nature and plants.

I would like to express my gratitude to my supervisor and group leader, ao. Univ.-Prof. Mag. Dr. Ingeborg Lang, Privatdoz., for her continuous support with all concerns and questions during the writing process. My regard extends to all the members of the Lang group, which provided an abundance of supportive feedback in a friendly atmosphere.

Furthermore, I want to express my gratitude to the members of CIUS (Cell Imaging and Ultrastructure Research) for giving access to their facility and their support, including Helmuth Goldammer, who was involved in the initial set up of the light microscopy set up and an introduction to the NIS Elements Software, the Lasso Tool used for cell area measurement.

Further, I want to thank ao. Univ.-Prof. Mag. Dr. Michael Schagerl for providing me with an introduction to photosynthetic parameters and kindly providing the PSI AquaPen.

Summary

In their natural habitat, mosses have adapted to repeated drought and wet cycles, which makes them excellent model species. This study aimed to give insights into the response of mosses to dehydration, - and rehydration series on a morphological, physiological, and cytological level. Further, it revealed how drought stress caused by prolonged dehydration impacts the photosynthetic efficiency, weight, cell area, leaf area, and gametophyte area. Five species have been used for the dehydration and rehydration series, *A. undulatum*, *P. patens*, *F. hygrometrica*, *M. polymorpha* were grown in *vitro culture*, and *P. affine* under greenhouse conditions.

Weight loss was measured at regular intervals in parallel with the quantum yield (Φ), a key indicator of photosynthetic efficiency. Measurements were taken during dehydration for 48 hours, and rehydration for 120 minutes to track changes in photosynthetic activity. Further, cell and leaf areas were evaluated using light microscopy to assess structural changes during dehydration and confocal laser scanning microscopy (CLSM) to determine the cell diameter. This approach allowed for a comparative analysis of species-specific desiccation tolerance and photosynthetic recovery under controlled conditions.

A weight decline of 50 % was seen in *A. undulatum*, *P. affine*, *P. patens*, and *F. hygrometrica* at one hour and 2 hours, 68-84 %. *M. polymorpha* diverged, showing a decline of 36 % at 1 hour, and 66 % at 2 hours. All species showed a loss of 90 % at 3-4 hours and remained at this level until 48 hours. Quantum yield responses to dehydration showed a decline at various species-specific patterns, with fluctuations. Upon rehydration, photosynthetic efficiency initially increased but then steadily decreased. These results highlight the diverse physiological strategies mosses employ to cope with water loss, offering valuable insights into mechanisms of desiccation tolerance that could inform drought resilience in higher plants.

Zusammenfassung

In ihrer natürlichen Umgebung haben sich Moose an wiederkehrende Trocken,- und Nasszyklen angepasst, was sie zu ausgezeichneten Modellorganismen macht. Ziel dieser Arbeit war, zu untersuchen, wie Moose auf morphologischer, physiologischer, und zellulärer Ebene, auf Austrocknung,- und Rehydrierungsreihen reagieren. Es wird darauf eingegangen, wie Trockenstress, bedingt durch lang andauernde Austrocknung, die Photosynthetische Aktivität, Gewicht, Zellfläche, Blattfläche, Gametophyt-Fläche beeinflusst. 5 Arten, die für die Dehydrierung- und Rehydrierung verwendet wurden, sind *A. undulatum*, *P. patens*, *F. hygrometrica*, *M. polymorpha*, welche *in vitro* Kultur und *P. affine* unter Gewächshausbedingungen gezogen wurden.

Der Gewichtsverlust wurde in regelmäßigen Abständen gleichzeitig mit der Quantum yield (Φ), einem wichtigen Indikator der photosynthetischen Effizienz gemessen. Die Dehydratationsserie erstreckte sich über 48 Stunden und die Rehydration über 120 Minuten. Die Reaktion des Quantum yield auf Dehydration zeigte einen mit artspezifischen Rückgang mit Schwankungen. Zell- und Blattflächen wurden mithilfe der Lichtmikroskopie untersucht, um strukturelle Veränderungen während der Dehydration zu analysieren. Die Zell-Durchmesser wurden mithilfe von Confocal Laser Scanning Microscopy (CLSM) bestimmt. Dieser Ansatz ermöglichte einen vergleichenden Überblick über artspezifische Trockentoleranz unter kontrollierten Bedingungen.

Ein Gewichtsverlust von 50 % wurde bei *A. undulatum*, *P. affine*, *P. patens* und *F. hygrometrica* nach einer Stunde und zwei Stunden bei 68-84 % beobachtet. *M. polymorpha* wich ab, mit einem Rückgang von 36 % nach einer Stunde und 66 % nach zwei Stunden. Alle Arten zeigten nach 3-4 Stunden einen Verlust von 90 % und blieben auf diesem Niveau bis 48 Stunden. Nach der Rehydrierung nahm die photosynthetische Effizienz zunächst zu, fiel anschließend ab. Diese Ergebnisse verdeutlichen die unterschiedlichen physiologischen Strategien, die Moose entwickeln, um mit Wasserverlust umzugehen, und bieten wertvolle Einblicke in die Mechanismen der Desikationstoleranz, die die Trockenheitsresistenz in höheren Pflanzen zu implementieren.

1.Introduction

Due to their sessile life strategy, plants are confronted with a lot of challenges for surviving and acclimatizing to environmental stressors. Recently, drought has become one of the main abiotic factors constricting crop productivity (Ansari 2020). During adaptation to drought, land plants implemented several structural changes, such as reduction of leaf area and modification of the stem. Besides morphological changes, physiological, biological, and molecular processes were implemented to improve their capability to cope with drought stress (Ghosh et al., 2021).

The global presence of mosses, inhabiting almost all ecosystems, points to the evolutionary success of this life form. As a consequence, they have evolved to utilize water, which is essential for their metabolism, when it is available above ground. Their main strategy, **poikilohydry**, includes decreasing the amount of metabolism when water is declining, surviving without water, and resuming photosynthesis and growth after rehydration (Oliver et al., 2000).

1.1. Bryophytes

1.1.1. Life cycle

Characteristic for bryophytes is a multicellular, haploid, phototrophic gametophyte generation, on which a short-lived diploid sporophyte develops. After germination of the spore, protonema develops and produces buds, which develop into leafy or thalloid plants. Contrary to most land plants, the haploid phase is dominant (Fig. 1.).

The leaves of the mature gametophyte structures

are involved in sexual reproduction, including archegonia, containers for egg production, and

antheridia, containers for male gamete production. One archegonium produces a single egg cell, while one antheridium produces multiple sperm (Fig. 1). Asexual reproduction is done via caducous leaves, stems, and rhizoids, as well as morphologically specialized brood bodies. Fragments of gametophytes, if dispersed to a suitable substrate, produce protonema from which new shoots will develop (Crandall-Stotler & Bartholomew-Began, 2007).

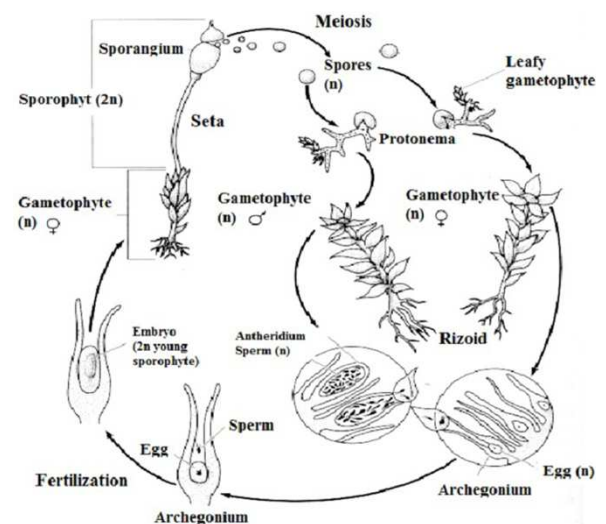


Fig. 1. Life cycle of bryophytes, by Ursavas Serhat and Cetin Barbaris (2012).

1.1.2. Morphology

The gametophytes of mosses are small, perennial plants with a branched or unbranched shoot system, bearing spirally arranged phyllids. In nature, they are found in colonies with special growth forms, including mats, cushions, wefts, and tufts. The group of the true mosses is divided into two morphological groups: **ac-rocarps**, which are unbranched to sparsely branched shoots, and **pleuro-carps**, characterized by creeping shoots with extensive lateral branching (Crandall-Stotler & Bartholomew-Began, 2007).

1.1.3. Phylogeny

Bryophytes are amongst the **embryophyta**, with plant tissues and enclosed reproductive tissues. Since they lack lignin and true xylem and phloem, they are not considered vascular plants. Bryophytes are part of a paraphyletic group, containing 3 divisions, Marchantiophyta, Anthocerotophyta, and Bryophyta, characterized by an abundant variety of morphologies from thalloid to leaf-bearing structures (Shaw et al., 2000). In this study, 4 bryophyta, *Atrichum undulatum*, *Plagiomnium affine*, *Physcomitrium patens*, and *Funaria hygrometrica*, and one species of the Marchantiophyta, *Marchantia polymorpha*, will be investigated.

The historical concept of dividing bryophytes into 3 groups, Marchantiophyta (liverworts), Bryophyta (mosses), and Anthocerotophyta (hornworts), is misleading due to the fact that both leafy and flattened thalloid forms are found in the liverworts and hornworts. Bryophytes are the only group made with unlobed leaves that are arranged in spirals, whereas foliose liverworts are more complex with 2-3 lobes. A characteristic of liverworts is that they can be distinguished from bryophytes by their lack of midribs and unicellular rhizoids, whereas bryophytes have midribs and multicellular rhizoids. The simple liverwort sporophyte produces a delicate elongated seta and lacks stomata, operculum, and columella (Shimamura, 2016).

1.1.4. Anatomy

In contrast to seed plants that differentiated layers of tissue with upper, and lower epidermis, vascular tissue, spongy and palisade parenchyma, the haploid **bryophyte gametophytes** consist of stems, leaves, and roots. Stem cross sections reveal, in most cases, a rather undifferentiated central parenchyma in the interior, consisting of large parenchyma cells, surrounded by a uni- to multistratose cortex (Büdel et al., 2024). In younger stems, the epidermal cells frequently contain chlorophyll and can assimilate. The stem is covered with thin cuticle without stomata (Shaw et al., 2000). Some higher developed bryophytes have conducting tissue at the center of the cortex, with leptoids, conducting assimilates, or hydroids, conducting water. The leaves, or phylloids, cross section reveals a **midrib at the center**, and **blade cells, containing chloroplasts** (Fig.2.). This characteristic bryophyte anatomy is found in *A. undulatum*, *P. affine*, *P. patens*, and *F. hygrometrica*.

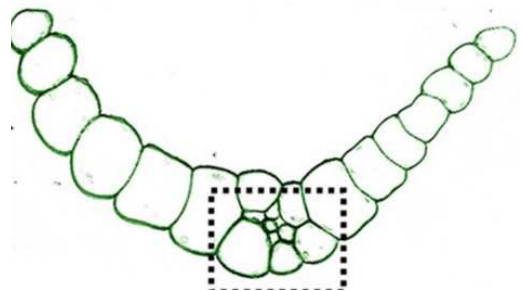


Fig. 2. Cross section of *P. patens* adult leaf, published in Leaf Morphogenesis: Insights from the Moss *P. patens* (Lin et al., 2021).

The cross section of the complex, lobed *M. polymorpha* thallus (Fig. 3) reveals **large parenchyma** cells, containing starch grains, and **rhizoids** at the base of the thallus. The upper thallus layer contains **small parenchyma** cells, which contain **chloroplasts**; photosynthesis takes place (Schweingruber and Börner, 2018).

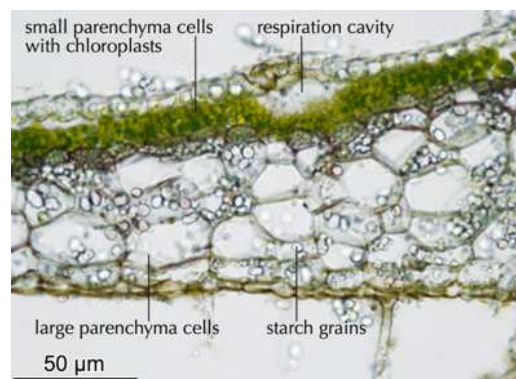


Fig. 3. Cross section of *M. polymorpha* thallus (Schweingruber and Börner, 2018).

1.2. Significance of water to bryophytes

To describe the response to “water deficit,” “drought,” “dehydration,” and “desiccation” correctly, the amount of water loss, the rate of dehydration, and the duration of the stress are important (Bray, 1997).

“**Dehydration**” implies that whole plants or detached organs encounter a steady water loss and are often kept in air to lose water, and “**desiccation**” is the final result of dehydration, and the water status is equilibrated with the air desiccated is equal to extremely dehydrated. It occurs at a water potential below 35 and a relative water content below 50 % (Fig. 4). In fig, the water status “**hydrated**” is defined with a water potential of 0 and relative water content of 80-100 %. “**Mild stress**” occurs at a water potential of 5-10 and a relative water content of 8-10 % (Fig. 4). “**Moderate stress** is” defined at 12-15 water potential, a relative water content of 10-20 %, and “**severe stress**” takes place below a water potential of 15 and a relative water content of 20 % (Zhang & Bartels, 2018).

Water status	Water potential (Ψ)	Relative water contents
Hydrated	0 bar	80–100% ^a
Mild stress	5–10 bars lower	8–10% lower
Moderate stress	12–15 bars lower	10–20% lower
Severe stress	>15 bars lower	>20% lower
Desiccation	>35 bars lower ^b	>50% lower

Fig. 4. Plant water status under various stress conditions by Quingwei Zhang and Dorothea Bartels, in (Zhang & Bartels, 2018).

“**Water deficit**” also referred to as deficiency, is observed if the transpiration rate from the leaf surface is higher than the water uptake by the roots, caused by an imbalance of water uptake and water loss (Salehi-Lisar & Bakhshayegan-Agdam, 2016).

“**Drought stress**” can be described from various perspectives; one is a general term for the description of atmospheric or weather phenomena and is commonly explained as a period without rainfall. From a physiological background, drought stress occurs when the water availability in the soil decreases due to low soil moisture.

“**Desiccation tolerance**” is the ability of bryophytes to equilibrate the internal water potential with that of the dry air of their environment and resume function when rehydrated (Alpert, 2000). Desiccation-tolerant mosses have the ability to dry

completely (i.e., equilibrate their internal water potential to extremely dry air) and resume normal function upon rehydration. More than 200 mosses have been shown to be desiccation tolerant (Gao et al., 2017). It provides ecophysiological advantages that allowed bryophytes to colonize and thrive in extreme environments with little precipitation, in which other plants were unsuccessful. As a result, members of this group are found on all continents, occupying substrates, including rock outcrops, tree trunks, soil, sand, forest floors, and man-made habitats (Longton, 1992). Whereas some species confined to moist habitats can tolerate dehydration over a period of time, others tend to be highly **dehydration tolerant** (Dilks & Proctor, 1979).

Vascular plants reach the limit of their **drought tolerance** and experience cell death at approx. 30 % to 40 % real water content (RWC) and a water potential of 7 MPa. Bryophytes experience a decline of photosynthesis rate at 15-20 % RWC, further, they are able to recover from dehydration after experiencing 5-10 % RWC (Dilks & Proctor, 1979). There is seasonal variance in the desiccation tolerance of bryophytes. Some experience drought hardening while exposed to drought, below a threshold that is not considered critical (Gupta, 1977). The pattern of Central European desiccation tolerance indicates the first peak is between late spring and early summer, and the second one during the coldest part of winter. Low desiccation tolerance was observed in early spring, and the main growth period is summer and autumn (Proctor, 1977).

1.2.1. Poikilohydry

Bryophytes are amongst the first photoautotrophic, multicellular eukaryotes that colonized terrestrial habitats with uncontinuous water supply. They conquered land in the Devonian, one of their difficulties was adjust to a life to under dry atmospheric conditions, due to their lack of differentiated tissues for water absorption and storage. Poikilohydry is a strategy that was developed by primarily water plants of aqueous habitats in response to tidal rhythms or periodic drying out of their habitat (Lüttge et al, 2013). This strategy can be defined as the ability to tolerate desiccation by dormancy and resume the metabolic activity upon rehydration, as consequence the water content is highly influenced by the humidity present in the air. Bryophytes, lichens and fungi are considered **primary**

poikilohydric organisms which express desiccation tolerance in their somatic cells and reproductive structures such as spores, zygotes, and cysts. They are enveloped by a thickened cell wall. Some vascular plants are **secondary poikilohydric** that in which desiccation tolerance has evolved secondarily. Mosses show a high diversity of strategies for water uptake and movement, compared to land plants. In this poikilohydric group, there is further distinction between **endohydric species**, which take up water from the substrate and is conducted to leaves internally. The leaves are covered with a water repellent surface (Buch, 1947). Endohydric species occur on moist, nutrient rich substrates, examples include *Funaria hygrometrica*, Mniaceae, and Marchantiaceae. They have a transpiration system, similar as the one found in vascular plants. In addition, some have root like structures, that provide contact to the soil, which facilitates water uptake from substrate (Grubb, 1970). **Ectohydric** bryophytes are characterized by water absorption and loss over the surface and more diffuse water movement. These species, native to bark and rock (Orthotrichaceae, Hypnaceae), are not connected to the soil and therefore rely on atmospheric water to replenish their internal water storage. In ectohydric species, rhizoids primarily provide stability, since water uptake from the ground is minimal (Odu, 1978). **Mixohydric** bryophytes display a combination of ectohydric and endohydric characteristics, such as the presence of a wettable surface and an internal conduction system.

The size restriction can be explained by the fact that surface-to-volume ratio is one of the factors contributing to the dehydration tolerance. the ability of larger bryophytes to hold water turgor more effectively. Further, microclimatic conditions are of primary importance for the water conduction.

1.2.2. Water content, - potential, & osmotic potential

Water content is a parameter that varies greatly between different bryophyte species. In the hydrated state, at full turgor, mosses and thalloid liverworts have a water content of around 200 % of the dry weight. The water of the moss shoot is comprised of apoplast water in the capillary spaces, symplast water in the cell, and external capillary water. Supersaturation occurs above a certain water content since water limits photosynthesis due to high diffusion resistance (Dilks & Proctor, 1979).

Taking into consideration physiological and morphological factors of non-vascular plants, the parameter gives insights in the status of the cell water during desiccation and rehydration. The **water potential** can be defined as correlation between the hygroscopic force, and the relative water content of the moss. The determination of the water potential is based on the relationship between pressure and volume curves (Proctor et al., 1998).

Solute potential (Ψ_s), also called **osmotic potential**, is negative in a plant cell and zero in distilled water. Typical values for cell cytoplasm are -0.5 to -1.0 MPa. Solutes reduce the water potential resulting in a negative Ψ_w by consuming some of the potential energy available in the water. Estimates for osmotic values are based on the molar concentrations of solutes (Rütten and Santarius, 1993). The osmotic value can be determined with an osmometer, from fresh, or dried plant material. In turgid mosses, the osmotic potential varies widely. Thalloid liverworts have low osmotic potential between 0.5 to 1.5 MPa, species from mesic environments may reach 3 to 6 MPa (Will-Richter, 1949). A high osmotic value found in mosses may reflect an adaptation to maintain high water potential gradients for the water movement, therefore contributing to desiccation tolerance (Renner, 1932). Under field conditions, the osmotic potential, humidity, rate of evaporation, and temperature determine the ability of the bryophytes to maintain their water content. Further, seasonal fluctuations were observed (Proctor et al., 2009).

1.2.3. Structure adaptations

Several structural adaptations by bryophytes include capillary structures involved in water transport. Hydroids are elongated conducting cells that form a strand in the center of the stem. Further, water uptake can take place through the free space of cell walls, from cell to cell via cell membranes, and over external capillary spaces (Héban, 1977). Studies on *Funaria hygrometrica* revealed movement of water stained with a fluorescent dye, rising through the rhizoids to the central strand, and rapidly to the stem apex and to the base of the sporophyte (Bopp and Weniger 1971). The internal conduction of *Plagiomnium undulatum* was demonstrated with dyes (Haberlandt, 1986). *Marchantiales* with rhizoids are rich in capillary conducting spaces (McConaha, 1939).

1.2.4. Cytorrhysis

This term is defined as an osmotic collapse of the cell walls, which can be observed in mosses with thin and flexible cell walls. Cytorrhysis is a cell physiological response that can be initiated under lab conditions by applying a strong hypertonic solution, which can result in plasmolysis. Further, it can be observed in the natural habitat of mosses, which undergo dehydration, contributing to increased drought tolerance. Cytorrhysis does not destroy the cells and is reversible (Url, 1947).

1.2.5. Molecular mechanisms

Dehydrins from the late embryogenesis abundant (LEA) group proteins are able to interact with hydrophilic and hydrophobic cell structures (Tunaclyffe & Wise, 2007) and contribute to the ability of the bryophytes to cope with drought. The desiccation response of proteins includes material and metabolism, defense, cytoskeleton, and signal transduction (Wang et al., 2009). They are required for counteracting oxidative damages such as denatured proteins, pigment loss, photosystem damages, and lipid peroxidation (Tunaclyffe & Wise, 2007). Enzymes and other redox molecules, glutathiones and ascorbates act as antioxidants, supporting thermal energy dissipation. **Osmotically active sugars** prevent the degradation of macromolecules and stabilize membranes and cells. Recent studies showed that bryophytes, including *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, *M. polymorpha* accumulate **abscisic acid** (ABA) as cellular desiccation protection, which initiates the production of genes, stress genes, and osmotically osmotic sugars (Pessarakli, 2016).-The molecular response is initiated minutes to 2 hours until the activation of the metabolism in the form of protein synthesis, respiration at (20 MPa water potential), and finally photosynthesis at 10 MPa water potential (Dilks & Proctor, 1979). After a day the nutrient transport is reestablished, enabling recovery of the cytoskeleton and cell cycle. However, the speed of the recovery process is highly dependent on the degree of desiccation. This metabolic reactivation is a characteristic attribute of the poikilohydric organisms. Remaining photosynthetically active while having a very low water content is a characteristic of mosses.

1.3. Photosynthesis

Photosynthesis is the key physiological process of all green plants and provides the energy source for plant metabolism and is directly related to productivity in terms of primary production of biomass and yield (Persserakli, 2016). Earliest fossils of photosynthesizing organisms, including cyanobacteria, originating in an aquatic environment, 3.5×10^9 years ago.

Phototrophs have the ability to convert solar energy into chemical energy, including photosynthetic bacteria, cyanobacteria, green algae, and land plants. The primary reactions of photosynthesis are mediated by a series of photosynthetic complexes associated with or embedded in the photosynthetic membranes. These pigment–protein complexes can be classified into several groups according to their functions: light-harvesting antenna complexes, photosynthetic reaction centers (RCs), the cytochrome (Cyt) complex, and ATP synthase (ATPase). The photosynthetic apparatus of the higher land plants, including mosses consists of PSI and PSII with membrane-embedded antennae, Light Harvesting Complex I (LHCI) and Light Harvesting Complex II (LHCII) (Persserakli, 2016). As in many other land plants, carbon assimilation of the mosses follows the **C3 metabolic pathway** with Ribulose-Bisphosphate-Carboxylase-Oxygenase (Rubisco) as the enzyme binding CO_2 (Büdel et al., 2024).

Internal parameters that contribute to **photosynthetic activity** are the structure of the leaf, chlorophyll content, accumulation of products within the chloroplast, and availability of minerals. **External factors** that impact the yield of photosynthesis are quality and quantity of light, temperature, and CO_2 and O_2 amounts in the atmosphere, as well as water and nutrient availability (Hall and Rao, 1999). To cope with the rapidly fluctuating light environment, specifically in intensity and wavelength distribution, the phototrophs have evolved complex regulatory systems to adapt to changing light conditions (Persserakli, 2016). The abiotic stresses severely affect photosynthesis in growing plants by influencing different components and phases associated with photosynthesis, such as decreasing utilization of light energy (Plaut, 1995).

1.3.1. Effect of dehydration on photosynthesis

During dehydration, the real water content (RWC) decreases, which leads to osmotic stress. An experiment of *P. patens*, in which osmotic stress was imposed with solution, the chloroplast content, thylakoid membrane, chlorophyll content, and the thylakoid protein composition were affected. In response, the photosynthetic apparatus of the moss increased oxidative stress by nonphotochemical quenching (Azzabi et al., 2012). The degree and the duration of dehydration imposed influence PSII activities (Liu et al., 2014).

Light is the most critical stress factor in the stage of dehydration until the rehydration state takes place. **Homoiochlorophyllous** plants, which maintain light-absorbing pigment complexes during photo destruction recover their photosynthetic activity relatively fast if rehydrated. **Poikilochlorophyllous** plants dismantle systems required for photosynthesis to avoid photo destruction by dehydration. Upon rehydration, more time is needed to resume to photosynthetic activity (Lüttge et al, 2013). Amongst bryophytes, the homoiochlorophyllous strategy is common, which contributes to their ability to withstand longer dehydration periods, recover physiological activity faster, and maintain photosynthesis under drought stress (Tuba & Lichtenthaler, 2011).

1.3.2. Effect of rehydration on photosynthesis

During rehydration, the initial process is the displacement of adsorbed gas, which is a physical reaction, and respiration increases above the average rate of dark respiration. This phenomenon is referred to as a “respiration burst “(Farrar & Smith, 1976). Photosynthesis recovers more slowly during rehydration. Until the compensation point is reached, depending on the species, minutes to hours pass. Over a window of 12–24 h, photosynthesis and respiration rates return to normal. In this initial stage, after remoistening, bryophytes encounter issues such as leakiness of cell membranes, which leads to loss of metabolites and nutrients (Proctor, 1979). With increasing intensity of desiccation, recovery upon rehydration becomes slower and less effective. Death can occur as a consequence of extreme increase in respiration upon remoistening after prolonged dehydration (Gupta, 1977).

1.4. Hypothesis and aims

Aim of this study was to reveal how drought stress caused by prolonged dehydration impacts the photosynthetic efficiency, weight, cell area, leaf area, and gametophyte area. 5 bryophyte specimens were used for the experiments, including *Atrichum undulatum*, *Plagiomnium affine*, *Physcomitrium patens*, *Funaria hygrometrica*, and *Marchantia polymorpha*. The selection of 5 bryophytes from different ecological niches was done with the intent to reveal species-specific dehydration and rehydration behaviors. Further, can general differences between mosses and *Marchantia polymorpha*, a liverwort, be observed?

A dehydration series that extends over 48 hours provided simultaneous measurements of the photosynthetic quantum yield (Φ) and weight loss. The coupled data enabled investigation of the correlation of the two parameters, photosynthesis and weight loss. After having undergone rehydration for 48 hours, rehydration series were conducted, with a focus on to what extent photosynthesis is capable of recovering.

Light microscopy was utilized to determine the effect that dehydration has on a cellular level. The initial, hydrated state of each moss will be investigated to be able to determine the effects of dehydration on the cell, leaf, and gametophyte levels including the average cell area of hydrated cells. Stereomicroscopic investigation of the entire gametophyte provided a series of “30-minute” time-lapse videos depicting the decline in gametophyte surface area. The main intention of confocal laser scanning microscopy (CLSM) was to determine the cell diameter and further reveal ultrastructural details of the cell wall and chloroplasts in the hydrated state.

2. Materials and Methods

2.1. Plant species

The 5 plant specimens included in the dehydration and rehydration experiments are *Atrichum undulatum*, *Plagiomnium affine*, *Physcomitrium patens*, *Funaria hygrometrica*, and *Marchantia polymorpha*. They were selected since they are representatives of bryophytes and liverworts, with adaptations to different ecological niches. 4 mosses and one liverwort were grown in *in vitro* culture, and *P. affine* was cultivated in a glass jar.

***Atrichum undulatum* (Polytrichaceae)**

This species is a basal representative of the moss phylogenetic tree. As all members of the *Polytrichaceae*, this species has leaves with lamellae overlying from the nerve to the mouth of the capsule. Since the nerve in *A. undulatum* is narrow, it has only a few lamellae that are aligned in parallel. Mature *A. undulatum* have transversely undulate leaves, which may not be as

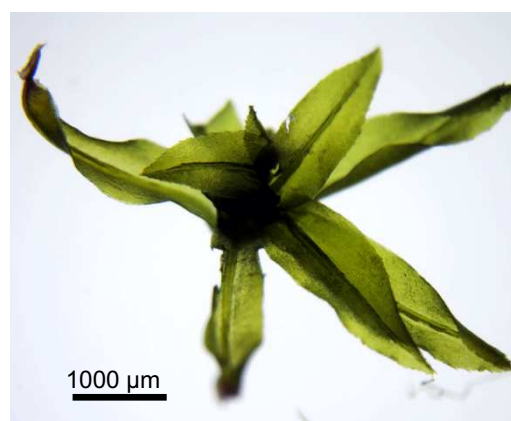


Fig. 5. Stereomicroscopic image of *A. undulatum* shoot

defined in young plants. This acrocarpous, loosely tufted moss with relatively robust species that forms extensive patches. The gametophyte (Fig. 5) has a dark green colour; the erect 7 cm stems have long, narrow, 1 cm long, and distinctively transverse undulate leaves that are pointing downwards. The leaf margins have teeth in pairs from the base, and the back has a rough surface, covered in numerous small teeth (Rumsey, 2014). *A. undulatum* is a monoicous moss that produces spore capsules; male and female gametangia are found on different branches. Capsules are produced abundantly and mature in winter. They have a 3-4 mm inclined cylindrical capsule and a long beak reaching the same length as the capsule, placed on top of a 2-4 cm long, reddish, and thin seta. Characteristic of this species are 32 peristome teeth; the opening is covered by a membrane with a leather texture (Rumsey, 2014). Vegetative reproduction via lateral spread of colonies is achieved by an extensive system of rhizoid wicks (Blockeel, 2014). New colonies can grow from detached leaves (Longton & Schuster, 1983) and the presence of rhizoidal tubers was reported in Austria (Köckinger et al., 1999)

It has a circumpolar boreal-temperate distribution and is widespread in Europe, reaching Iceland, and in the South, spreading to the Mediterranean mountains. Macaronesia, North Africa, Turkey, the Caucasus, temperate zones of Asia, including Japan (Blockeel, 2014). This moss is a common inhabitant of low, lowland woodlands, avoiding soil that is too acidic or highly calciferous. In these areas they will be restricted to overlying clay deposits containing non-calcareous. It occurs in semi-natural, sheltered, and moist habitats. It is found on neutral clay, loamy soils, stream and riverbanks, soil pockets, and landslides. Upland habitats include rocks, scree, moorland, earthy edges, and wet rock surfaces with little to no soil. Further locations include churchyards, parks, and gardens, and temporary soil dumps. The altitudinal range is from 0-840 m (Blockeel, 2014).

***Plagiomnium affine* (Mniaceae)**

P. affine is part of the subgroup of the section *Rossulata*, for which a leaf border with several teeth formed by 2 or more cells is characteristic (Koponen, 1971).

Mature plants have a dark green colour, the stems contain shoots with a length of up to 10 cm and are covered in 5-7 mm long toothed, broadly elliptic leaves with de-



Fig. 6. Stereomicroscopic image of *Plagiomnium affine* shoot, covered with rhizoids

current base, positioned narrowly down the stem (Fig. 6.). The elongated lamina reaches from the base of the leaf to the tip reaches a length of approx. 2 mm. Rhizoids are found on the lower part of the plant and the tip of the shoot. Characteristic for this species is a dimorphism between fertile and infertile plants. Whereas the fertile shoots are growing upright, the sterile shoots lie flat on the substrate and grow in curves (Hofman, 2016). This dioecious moss rarely forms capsules, and the sporophyte contains one or several capsules, located at the tip of the shoot. The long ellipsoid capsule is hanging downwards, and spores with a diameter of 18-25 µm are produced (Hofmann, 2016).

P. affine is a species with European temperate distribution, which occurs abundantly in Europe, from the Mediterranean region to the north of Scandinavia and Russia, and east to the Urals. Further regions include Macaronesia, Northern Africa, Turkey, the Caucasus region, Iran, Kazakhstan, and Kyrgyzstan, to the Western Himalayan Mountain range. The growth form of the species is in a loose patch or as isolated stems and is found in several different habitats, ranging from slightly acidic to alkaline soils. They inhabit well-drained soil, rocks and stones, and logs. Ideal habitats are lightly shaded woods, hedges, lane banks, road riverbanks, coastal turf, and up to the salt marsh zone (Blockeel, 2014).

***Physcomitrium patens* (Funariaceae)**

P. patens is a small moss with a 2.5 mm long shoot and 2 mm leaves (Fig.7.). It has very short setae that keep its lid, partly covered by the lax-celled leaves (Hodgetts, 2014). This paroicous or synoicous species produces numerous capsules that mature in the second half of the year (Blockeel, 2014).



Fig. 7. Stereomicroscopic image of *P. patens* shoot

They are the primary colonists of nutrient-rich mud, abundantly found in the draw-down zone of reservoirs. It can be found on the bottom of dried-up ponds and is widespread but avoids areas that experience disturbance by vehicle rutting or farm animal poaching. *P. patens* inhabit clay in arable fields, streams, wet farm tracks, and woodland rides. It tolerates mildly acidic soils and calcareous soils. In general, bright light is preferred, but in hot summers water dries too fast for the mosses to develop in the open, then they will be found under shade-providing shrubs. Their altitudinal range is from 0-330 m. This species displays Euro Siberian temperate distribution and is found in temperate parts of Europe, Scandinavia, north-west Russia, to north Spain, Italy and north Greece, Siberia Yukata, Western and North America (Blockeel, 2014).

***Funaria hygrometrica* (Funariaceae)**

This moss has short 3-10 mm long shoots arranged in a loose carpet (Fig. 8). The translucent, egg-shaped leaves are 2-4 mm long and contain large, lax cells that become visible under the light microscope with x10 magnification. The gametophyte of *F. hygrometrica* supports the sporophyte, which produces numerous asymmetrical capsules. They are positioned on the top of a long, 3-5 cm long, swan-like seta and delicate, large calyptra covered by a convex-shaped lid (Hodgetts, 2014). This moss is autoicous; arche-

gonia and antheridia are produced on the containing gametes on haploid, gametophytic shoots. Reproductive shoots with determinate growth develop in autumn and produce terminal antheridia arranged in clusters and groups of archegonia positioned on the lateral branches (Shaw et al., 1991), and sporulation can occur all around the year. Under field conditions, the spores are capable of surviving in the substrate for up to 2 years (During, 1986). Asexual reproduction takes place via gemmae produced in young colonies (Duckett et al., 1998).

F. hygrometrica is a cosmopolitan species with a circumpolar, temperate distribution, but it is less frequent in the Southern Hemisphere, reaching Antarctica. (Blockeel, 2014). This species tolerates a wide number of different soil types, from highly alkaline lime to acidic peat containing humus, and prefers nutrient-rich soil with a high concentration of metals, with an altitudinal range from 0-800 m. It is a common inhabitant of lowlands, arable fields, and is famous for recolonizing burned heathland and bonfire sites (Blockeel, 2014). An unusually large population was reported from the Union Copper mine located in North Carolina contaminated with copper, zinc, and cadmium (Shaw et al., 1987). As single, scattered plants or patches, they inhabit bare soil, acting as colonists in disturbed environments, such as waste land, quarries, pits, roadsides, gardens, flowerpots, stream banks, and sometimes walls (Blockeel, 2014).



Fig. 8. Stereomicroscopic image of *Funaria hygrometrica* shoot

***Marchantia polymorpha* (Marchantiaceae)**

The group of Marchantiophyta contains 4500-8500 species (Konrat, 2010) and can be subdivided into the classes *Haplomitriopsida*, *Marchantiopsida*, and *Jungermannniopsida* (Glime et al., 2020). Based on current morphological and molecular studies, this group has diverged earlier than the other land (Crandall-Stotler et. al., 2009). *Marchantiopsida* has around 400 species

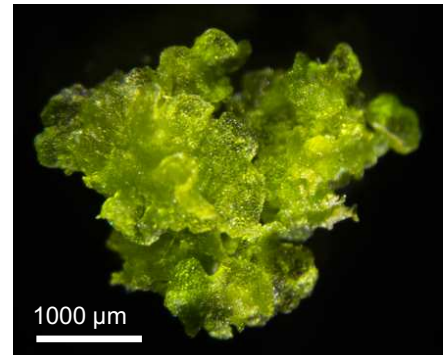


Fig. 9. Stereomicroscopic image of *M. polymorpha* shoot

and its characteristics are complex thalloid form internal pores (Shimamura, 2016). The life cycle of *M. polymorpha* is characterized by a dominant, multicellular haploid gametophyte and a short-lived, multicellular, diploid sporophyte generation. This haploid gametophyte (Fig. 9.) starts as a unicellular spore that germinates and forms an initial short filamentous protonema with rhizoids. The organization of the complex thalloid plant body originates through the highly regulated cell division of an apical cell, which appears in the developing protonema (Shimamura, 2016). It is a dioecious species with antheridia and archegonia on different thalli. An archegonial head contains 30-50 archegonia, with the young ones located at the center, and the mature ones at the edge (Glime et al., 2020). In one archegonium a single egg is produced, while antheridium produces multiple sperm. The motile male gametes reach the gametangium by swimming and fertilizing the egg. Afterward, inside the archegonium, the zygote develops into a sporophyte (Shimamura, 2016). Spores with spiral elaters are produced upon meiosis and they alter their shape if moisture changes. *M. polymorpha* requires more bright light for spore production than for protonema growth (Inoue, 1960) and spore production tends to take place only once and simultaneously in a population (Glime et al., 2020). Asexual reproduction is restricted to the dorsal side of the mature thallus, accommodating numerous multicellular gemmae (Shimamura, 2016). *M. polymorpha* are amongst the complex liverworts, with air chambers with pores, which allow a balance between gas exchange and water vapor loss, as efficient as the stomata in the sporophyte of land plants. Located at the inner region of the thallus, a storage region is found, made up of parenchymatous and oil cells (Shimamura, 2016).

2.2. *In vitro* culture

2.2.1. Medium preparation

The recipe for the medium is based on Benecke and Knopp (Benecke & Knopp, 1924). 4 stock solutions containing salts that dissociate upon hydration: ammonium nitrate (NH_4NO_3), potassium dihydrogen phosphate (KH_2PO_4), the epsom salt magnesium sulfate heptahydrate ($\text{MgSO}_4 \times 7\text{H}_2\text{O}$), and calcium chloride dihydrate ($\text{CaCl}_2 \times 2\text{H}_2\text{O}$) were prepared in the following concentration (Tab. 1.) and stored under cold conditions.

Tab. 1. Components of the Benecke medium

stock solution	per 100 ml
NH_4NO_3	2 g
KH_2PO_4	4 g
$\text{MgSO}_4 \times 7\text{H}_2\text{O}$	1 g
$\text{CaCl}_2 \times 2\text{H}_2\text{O}$	1 g

For the preparation 0,5 l of medium 5 ml of the 4 stock solutions were pipetted into a glass cylinder, with a 1000 μl pipette (Eppendorf Research Plus). Distilled water was filled into the glass cylinder until the 0.5 l mark was reached. In one session this process was done twice to produce 1 l of volume of Benecke medium. The liquid was transferred into two labeled 0.5 l glass bottles and with a long metal spoon/spatula 6g of agar with a gel strength of 600-1200 g/cm^2 (1.5 %, 20 °C) were added. Rotary magnets were sterilized with 96 % EtOH and then placed at the bottom of the bottles. They were placed on a magnetic stirrer (IKA RH basic) at level 3 for 2 minutes, allowing the rotary movement of the magnet to homogenize all components in the liquid medium.

While remaining on the stirrer, the pH was determined with a pH-meter (pH-enominal IS 2100L) device by inserting the electrode arm into the solution after calibration. If agar was added, the initial pH values measured were in the range of 6.0-6.2. To adjust the pH to approx. 5.800 small drops of 1 % HCL solution was added with a glass pipette. Since it took several seconds until the effect of acidification was detected by the device, this step was done carefully at a slow pace. Then the glasses containing Benecke medium were closed tightly and labelled with

autoclave indicator tape. Autoclaving took place during the next days, after which the ready-to-use liquid media were placed into the refrigerator with an estimated expiration date/shelf life of 2-3 months.

2.2.2. Sterile culture

Glass bottles containing Benecke medium were taken from the refrigerator and then placed in a microwave (SHARP). The lid was opened slightly to avoid overheating. On the display, a program with a duration of 30 seconds and medium heat was selected, then the consistency of the medium was checked and heated up repeatedly until the cloudy, solid medium transformed into a clear liquid. In total this step was repeated 3-4 times until the consistency of the medium was liquid.

The surface of the sterile bench for plant samples was disinfected using 96 % EtOH, and then the light and fume hood were switched on. Hot glass bottles were handled using heat-resistant gloves and placed under the lid of the cell culture bank for plant samples. A package with sterile Petri dishes, 60x15 mm (Greiner Bio One), with vents was taken from their plastic envelope, distributed on the surface of the workbench, and opened by removing the lid. Liquid medium was poured in until 2/3 of the height of the dishes was covered. Fresh hot medium in the Petri dishes was left to cool and solidify for 20 minutes, then the mosses were transplanted. All remaining dishes containing Benecke medium were placed in their plastic envelope and stored in the refrigerator for the next weeks.

2.2.3. Transplantation

Before the propagation of the moss gametophytes in the sterile bench, the surface was disinfected with 96 % EtOH. Petri dishes containing the moss cultures were opened by removing the Parafilm® and carefully disinfected on the outside. Empty Petri dishes were opened, with the lid placed at the edge to avoid contamination from the work bank surface. 2 fine freshly autoclaved forceps were used to remove a gametophyte shoot with protonema close to the base and transfer them to a new Petri dish, by gently pushing their base into the medium to give them anchorage. This process was repeated until 9 spots on the fresh Petri dish were inoculated with plant material. They were closed with Parafilm® to prevent water loss and infections, then labeled.

2.2.4. Growth conditions for sterile cultures

The growth chamber (Convicon CMP60) (Fig. 11.A) provides a controlled environment for tissue cultures, including temperature, humidity, and light. For the moss cultures (Fig. 11.B), a day-night cycle of 10-14 hours a temperature of 22 °C and a humidity of 60 % were selected. As a light source, Osram H0 36W/840 Lumilux Cool White LEDs were used. Above each of the 4 shelves, all 4 LED lights were installed, of which only 2 were currently in use, alternating from the outer two to the inner two to maintain uniform light conditions.

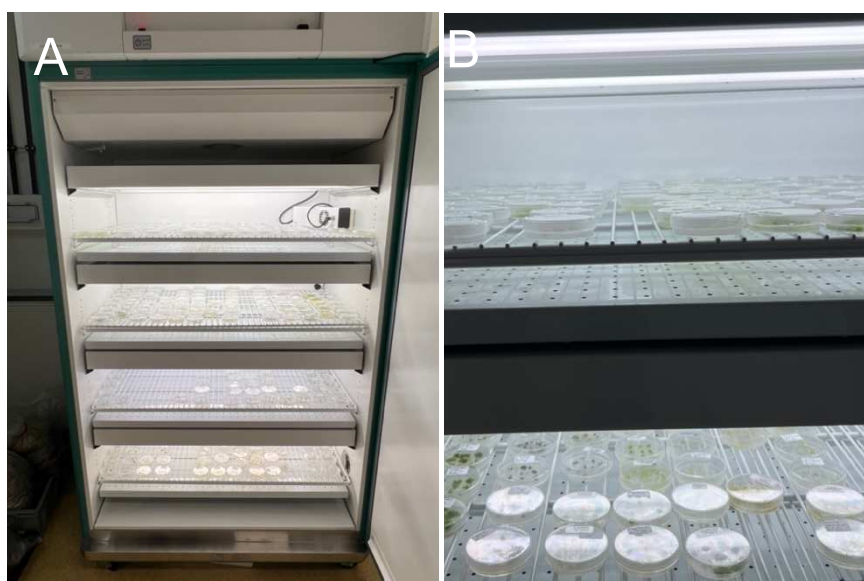


Fig. 11.A) Inside of the Convicon growth chamber. B) *In vitro* culture dishes were distributed evenly on the shelves and the Cool White LED light sources are seen on top.

2.2.5. Moss cultivation and contamination

The mosses were grown successfully in Benecke medium without additional micronutrients for 5 months. A previous comparison between the growth of mosses in MS medium and Benecke without micronutrients revealed that the second method had the best results. This can be explained by the fact that agar has electrical and adsorptive capabilities and contains high amounts of copper and sodium (Bonga and Aderkas, 1992). Further, there is no general guideline of what micronutrient quantities are required for optimal moss growth of different moss species. *P. affine* was the only moss not grown in *in vitro* culture. It was placed in a glass container filled with water up to approx. 3 cm, covered with a lid, and mainly stored in the greenhouse, placed on a shaded bench. According to the gardeners, they provide liquid fertilizer regularly. For practical reasons, it was not possible to return it every day after the experiments were performed. During the dehydration of 48

hours and rehydration, it remained on the windowsill in the lab, for a maximum of 6 weeks. After the sample preparation for the light, - stereo, - and confocal microscopy, the moss was placed close next to a window for around one week. During this period, *P. affine* remained in good condition, despite losing many shoots used for the experiments.

During the lab work, the 3 remaining *in vitro*-grown mosses were propagated regularly, taking into consideration that it took around a month to reach adequate shoot size for the dehydration and rehydration experiments. This process was done in a workbench for plant samples, which were sterilized before transplanting the mosses. Since the UV light was not functional for months, only 96 % ethanol was used to clean the surface of the bench, but the transplanted mosses kept growing without any signs of infection.

The most viable, shoots were selected for microscopic and stereomicroscopic images. The lid of the Petri dish containing the *in vitro* cultures was opened, and using fine scissors, a shoot of the moss was removed. After extraction, the dishes were closed and immediately resealed with Parafilm® and labeled with an x and the date, on which it was opened. The Petri dishes were placed back in the growth chamber and were used if required for further experiments in the future. Some *in vitro* culture dishes remained in good condition, without signs of infection, even after being opened repeatedly over several months. One can conclude that the major origin of infection is during transplantation in the work bench, and not from airborne pathogens, such as fungi to which they may be exposed by unsealing the lid. All Petri dishes containing *F. hygrometrica* showed signs of fungal infection during the end of the lab work, including whitish shoots that remained small and protonema growing over the agar substrate. The species was still included in the “120 minutes shoot dehydration,” but the experiment was redone over a shortened period of 30 minutes, no viable *F. hygrometrica* was available in the growth chamber. As a consequence, the “30-minute shoot dehydration” and time-lapse video includes only 4 species *A. undulatum*, *P. affine*, *P. patens*, and *M. polymorpha*. Further species showing signs of contamination include *P. patens* at the end of the experiment, followed by *P. affine* briefly afterward.

2.3. Photosynthetic parameters

2.3.1. Quantum yield (Φ)

The maximum quantum yield (Φ) is a critical concept in photosynthesis, representing the efficiency with which absorbed light photons are converted into chemical energy. It is defined as the maximum number of oxygen molecules released or carbon dioxide molecules reduced per photon absorbed (Fig.10). This concept has been central to understanding photosynthetic efficiency and has been the subject of significant scientific debate and research (Lawlor, 2013). It can be defined as a measure of the efficiency of photochemical processes, specifically in photosystem II (PSII) of plants and algae. In the context of photosynthesis, it is often used to assess the efficiency of energy conversion in PSII, which is crucial for understanding plant and algal responses to environmental conditions, since the quantum yield is often measured in ecophysiological studies to assess photosynthetic efficiency (Klughammer & Schreiber, 2008).

For a photochemical reaction,

$$\Phi(\lambda) = \frac{\text{amount of reactant consumed or product formed}}{\text{amount of photons absorbed}}$$

Fig. 10. Formula of the quantum yield in a photochemical reaction (Fig. 10). Published by Silvia Braslavsky in Glossary of terms used in photochemistry, IUPAC recommendation 3rd edition, by Braslavski, 2007).

Several external factors influence the quantum yield of photosynthesis, including light intensity, nutrient availability, and water temperature. These lead to deviations from the maximum theoretical yield, particularly under low-light conditions where reductions in quantum yield are not always observable with conventional methods. Accurate estimation of quantum yield requires careful consideration of these factors and the use of appropriate models to account for potential errors (Dera et al., 2002). Drought stress will affect the maximum quantum yield, which is used as a diagnostic tool for assessing plant health (Parkhill et al., 2001).

2.3.2. Pulse Amplitude Modulated (PAM) Fluorometry

PAM fluorometry is a widely used technique to determine the quantum yield of PSII of plants and algae. It provides insights into the photosynthetic activity and stress responses of plants and algae by measuring fluorescence parameters (Snellenburg et al., 2017; Schuurmans et al., 2015; Acuña et al., 2015). Non-Photochemical Quenching (NPQ): PAM fluorometry can be used to study NPQ, a mechanism that protects plants from excess light by dissipating it as heat. This technique allows for the analysis of different states of PSII and their quantum yields, providing a detailed understanding of quenching dynamics and photoinactivation (Snellenburg et al., 2017).

Modern PAM fluorometers, such as the AquaPen-C AP 110-C (Photon Systems Instruments) calculate quantum yields of photochemical and non-photochemical energy conversion in PSII. These are expressed as various parameters $Y(II)$, $Y(NPQ)$, and $Y(NO)$, which together sum to unity, offering a comprehensive view of energy distribution in PSII. In this device, an emitter generates blue LED excitation light at 470 nm, which is optically filtered and precisely focused to achieve PAR values of up to $3,000 \mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The blue excitation light excites the chlorophylls within the measured volume. In the experiment, quantum yield was selected as the photosynthetic parameter, and moss samples were dark-adapted for five minutes before measurements began (<https://psi.cz/sup-port/downloads/hd001/ap001/>).

The maximum photochemical quantum yield of photosystem II (PSII) is often assessed using chlorophyll fluorescence parameters. The F_v/F_m ratio, which reflects the maximum quantum yield, is typically measured after dark adaptation (Xia et al., 2023). It allows comparative studies amongst different species, such as land plants and mosses. However, it may underestimate the photosynthetic efficiency in mosses due to specific physiological characteristics, suggesting that PAM measurements should be interpreted qualitatively (Schuurmans et al., 2015).

2.4. Dehydration

2.4.1. Photosynthetic activity & weight loss

During dehydration, the weight and in photosynthetic activity was measured in parallel. The weight of 3 cellulose discs was determined, and placed on a large glass Petri dish (Fig. 12.A). All mosses were pre-darkened for 5 minutes under a box before the first photosynthetic measurements were taken. An entire moss plant was extracted from the *in vitro* dish, using forceps and micro-scissors, placed on a cellulose disc (Fig. 12.A). They were mounted on trapezoidal plastic holders, to facilitate the transfer of the cellulose discs, from the glass Petri dish to the (Fig. 12.B), fine scale (Sartorius). 200 μ l water were pipetted on the mosses to ensure they were fully hydrated before the start of the measurement. During the first 10 minutes, the weight and photosynthetic activity were determined simultaneously, every 30 seconds. Further measurements were taken 10 min, 15 min, 20 min, 30 min, 60 min, 3 h, 4 h, 5 h, 6 h, 8 h, 10 h, 12 h, 24 h, 48 h after the initiation of the desiccation process (Fig. 13.).

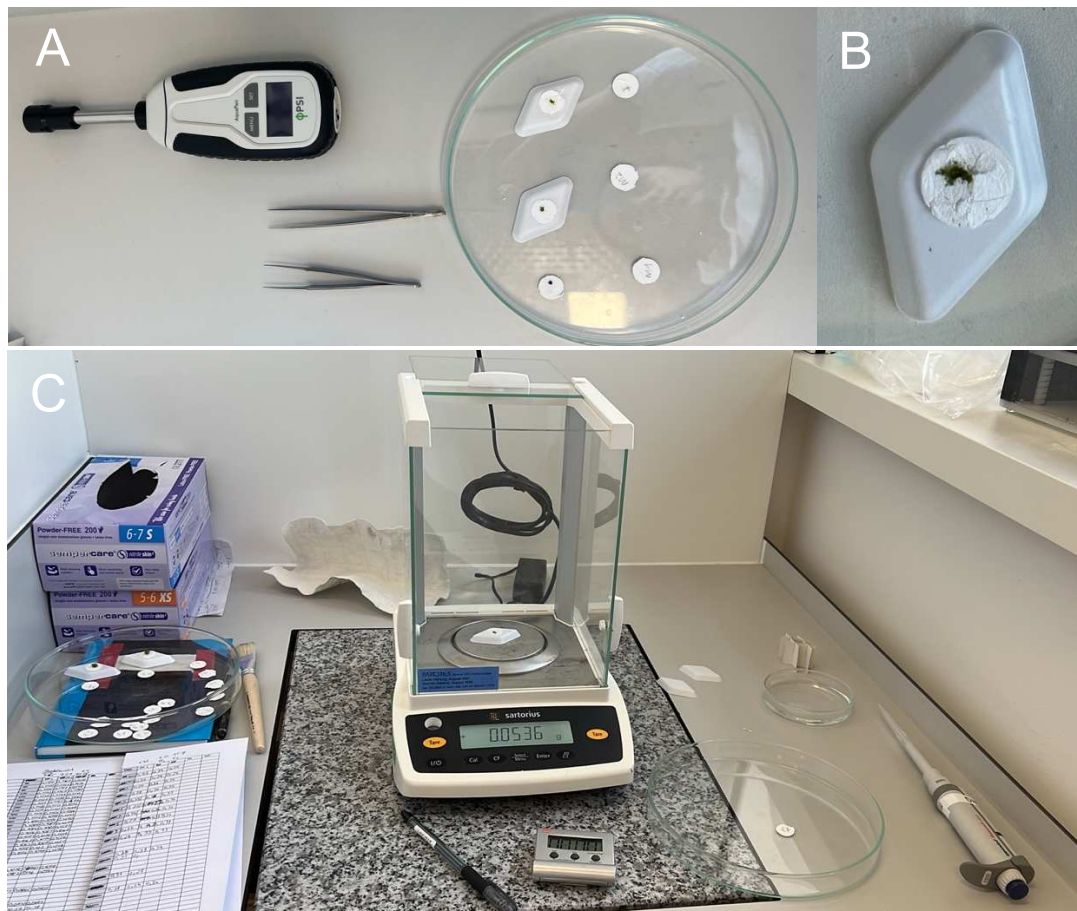


Fig. 12.A) Set up for the dehydration includes PSI AquaPen, forceps for placing the mosses to the scale, large glass dish containing 3 moss samples. B) Detailed image of trapezoid plastic holders with a cellulose disc containing a moss. C) Overview of the lab bench, as seen above, the scale remained open during the dehydration process.

In one setting, water weight loss and photosynthetic activity were determined in parallel for 3 moss specimens. The first moss sample, located at the center of the cellulose disc, remained on the scale for the first 15 minutes, then the second measurement was initiated, and after 15 more minutes, the third moss was placed on the scale. Further measurements after longer time intervals required specimen rotations that were coordinated with the help of several timers.

An AquaPen-P AP 110-P (Photon Systems Instruments) was inserted from the upper scale opening, and the tip of the device was placed on the moss samples at a 90-degree angle until it was fully covered. The quantum yield (Φ), of PSII was measured by pressing the SET button, which made the device emit a short, blue flash that excited chlorophyll molecules. This photosynthetic parameter was selected in the settings. The upper lid of the scale remained open for the duration of the experiment (Fig. 12.C) to allow the water of the mosses to evaporate. After all parameters were measured, the discs containing mosses were placed on the glass dish with an opened lid and remained there for the next 48 hours.

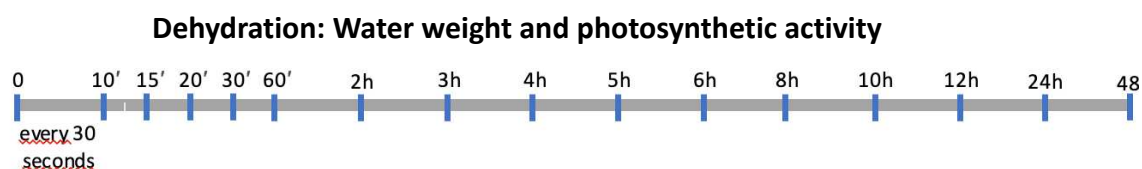


Fig. 13. Experimental set up and timeline of the combined photosynthetic activity and weight measurements.

2.5. Rehydration

All five moss species have previously undergone dehydration for 48 hours (Fig. 12.C). At the end of the dehydration experiment, dry moss shoots remained on their cellulose discs, which were transferred to a large glass dish (Fig. 17.A).

To gain an insight into the effect that rehydration has on the quantum yield (Φ), a key indicator of photosynthetic efficiency, it was analyzed with the pulse amplitude modulated (PAM) fluorometry method after dark adaptation. The device used was AquaPen-C AP 110-C (Photon Systems Instruments), a small, portable PAM fluorimeter. Measurements were done while the mosses recovered from dehydration for 48 hours, and 200 μ l of water was pipetted on the mosses on the cellulose discs (Fig. 17.B). The number of replicates is 6 per species, the same as in the dehydration experiment.

An initial experiment aimed to measure the photosynthetic activity twice, at 48 hours of dehydration, (at the beginning of the rehydration experiment), and 30 minutes after 200 μ l water was added. To cover a longer time span, a **120-minute rehydration** series was performed, by measuring the quantum yield (Φ), using a PAM fluorimeter at 0 min, 15 min, 20 min, 25 min, 30 min, 45 min, 60 min, 75 min, 90 min, and 120 min after initiation of the rehydration.

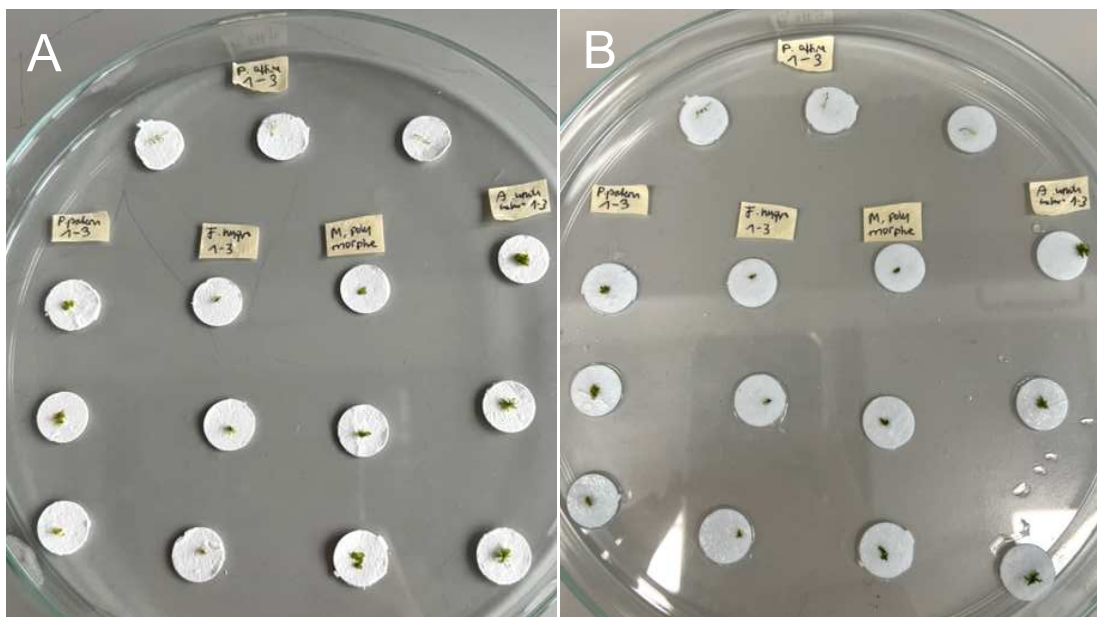


Fig. 17. A) Glass Petri dish with cellulose discs containing the dehydrated moss samples, of *P. affine*, *P. patens*, *F. hygrometrica*, *M. polymorpha*, and *A. undulatum* before water was added, and B) after 200 μ l water was added.

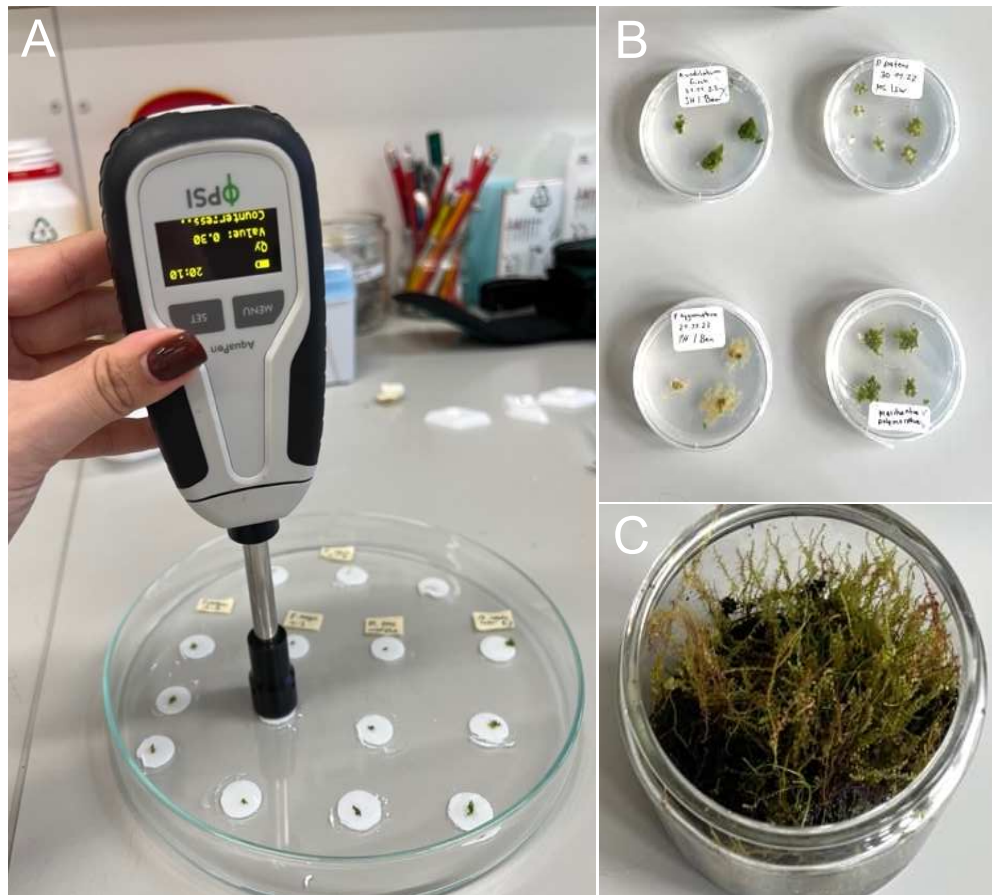


Fig. 18. A) The AquaPen was placed in a 90° over the cellulose discs containing the mosses. B) Species used in the rehydration experiment include *A. undulatum*, *P. patens*, *F. hygrometrica*, *M. polymorpha*, and C) *P. affine* in a glass container.

2.6. Light microscopy

2.6.1. Sample preparation & set up

Under the binocular (Nikon M4) with low magnification, moss leaves were removed using fine scissors (Fig. 14.A) and placed into a drop of water on a glass slide (Fig. 14.B). Leaves of large species, including *P. affine* and *P. patens*, were further dissected into small, square sections. Specimen with small leaves including *A. undulatum* and *F. hygrometrica* required more careful treatment and were cut approximately in the middle and the leaf was then placed on a glass slide with water. Small fractions of the lobed *M. polymorpha* thallus were removed and cut into sections.

For the next step, the binocular was set to higher magnification, and the leaf sections were placed at the center of a fresh, dry glass slide. They were positioned flat on a dry glass slide with fine forceps in one hand and a needle in the other. For the cell area and leaf area, they were transferred into a specimen holder (Nikon Eclipse N) (Fig. 15), equipped with a camera (Nikon DS-Ri2). For further analysis of the images, the computer Software (NIS-Elements BR 5.11.03) was used. The cell area measurement tools were Binary tools and Freehand lasso tool.

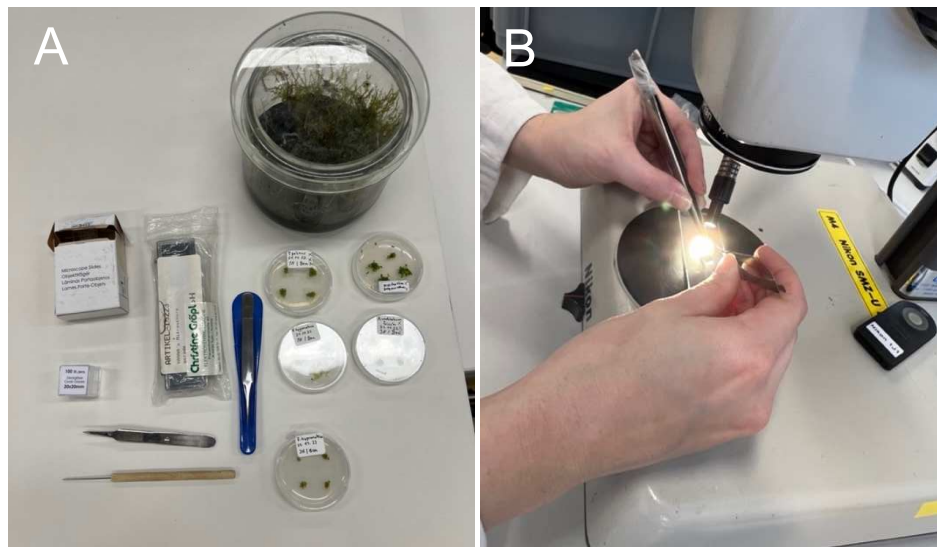


Fig. 14.A) Sample preparation equipment includes glass slides, cover glass, forceps, needle, micro scissors, all 5 moss species. B) Under the binocular, leaves are extracted with combined use of forceps and scissors.



Fig. 15. Light,- and epifluorescence microscope Nikon Eclipse NI and NIS Elements Software.

2.6.2. Cell area

To determine the effect of water loss on a cellular level, the area of three selected cells was measured from initiation of the dehydration to 90 minutes. A series of light microscope images were taken at 0 min, 5 min, 10 min, 15 min, 20 min, 25 min, 30 min, 45 min, 60 min, 75 min, 90 min. For each species, 5 individual moss plants were selected, then on each leaf, 3 distinct cells were selected, and their area was investigated throughout the experiment. Initially, replicates 1 and 2 of each species were done over 30 min, after the third measurement, the time window was elongated to 90 min.

2.6.3. Hydrated cell area

From each of the 5 individual moss plants, 10 randomly distributed cells from 6 different leaves were measured in their initial, hydrated state. Using these data, mean cell area for each species was determined.

2.6.4. Leaf area

For each species, the leaf area was determined at 0 h, 2 h, 4 h, and 6 h into the desiccation process, with 3 replicates. The area of the leaf was measured with the autodetect tool in the NIS Element Software

2.7. Stereomicroscopy

2.7.1. Sample preparation

The *in vitro* culture dishes with *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica* *M. polymorpha*, and the glass container in which *P. affine* was cultivated, were opened. Using fine scissors and forceps, a large fragment of the gametophyte was removed and placed on a dry glass slide and placed on the stage plate of a Stereomicroscope (Nikon SMZ1500) (Fig. 16.A). Further settings include selecting Objective (HR Plan Apo 1.6x) (Fig. 16.B), manually positioning the moss gametophyte in the center by moving the filter, and the focus set with knob, and the brightness was adjusted.

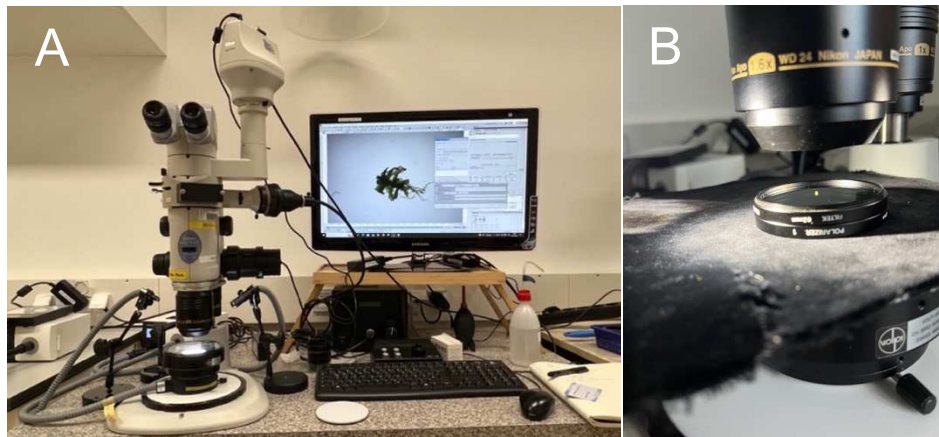


Fig. 16.A) Stereomicroscope Nikon SMZ1500 with computer screen depicting the time-lapse of *P. patens*, B) Set up for improved contrast includes objective HR Plan Apo 1.6x, polarizer filter 62 nm, and black paper.

2.7.2. Gametophyte area time-lapse

With the help of the software NIS Elements BR 5.11.03 a series of images were taken, and the decline in area of the gametophyte area was measured for each time point with the auto-detect function and summarized in a table. For the first measurements, a long time interval was set at **120 minutes** (interval 1 min) and later shortened to **30 minutes** (interval 1 min), based on the observation that the lowest gametophyte area is reached after 20 minutes in all species. An object stage was placed, which was covered in black paper, to provide a contrasting background for the mosses. The gametophyte fragments were placed on a polarizer filter 62 mm, which acted as a moveable stage for them, on top of the black paper. Settings for time-lapse videos include “Acquire” on the top left – “Capture timelapse”- “Capture automatically”. The data was converted into a video format, MP₃, and images depicting the initial size, labeled with (Area 1), and size after 30 minutes of dehydration (Area 2).

2.8. Confocal laser scanning microscopy (CLSM)

2.8.1. Sample preparation

The sample preparation was done under a stereomicroscope, moss leaves were removed by using micro scissors and forceps, then transferred to a wet glass slide and covered with a cover slip (Fig. 19.A). The glass slide was transferred to the specimen holder of a confocal microscope (Leica SP8) (Fig. 19.C).

2.8.2. Leica SP8 settings

In the transmission mode, leaves with intact surface areas were selected, and the area of interest was determined, starting with the smallest objective, then the 60-objective with water immersion was reached. The number of leaves investigated ranged from, 4 in *A. undulatum*, 4 in *P. patens*, 4 in *F. hygrometrica*, to 3 in *P. affine*, and *M. polymorpha*, and per leaf, 3 cells were selected. Species-specific settings for autofluorescence of chlorophyll and the cell wall are described below.

A. undulatum

650-700 nm for chloroplasts fluorescence
440-480 nm cell wall fluorescence

P. affine

650-700 nm chloroplast fluorescence
445-462 nm cell wall fluorescence

P. patens

650-700 nm chloroplast fluorescence
445-462 nm cell wall fluorescence
No transmission available

F. hygrometrica

660-690 nm for chloroplasts fluorescence
With slight adjustments for various leaves
440-480 nm cell wall fluorescence

M. polymorpha

650-710 nm chloroplast fluorescence
440-480 nm cell wall fluorescence
Increased laser power for thicker samples, 20 hz

Leica LAS X software (Fig. 19.D) was used for image capture and processing. Firstly, Lambda scans were made, to get an insight into the species-specific auto-fluorescence. A series of Z-scans were taken, which were used for 3D reconstructions of the investigated leaf areas. Cell walls were depicted in blue and chloroplasts in red. Settings included a speed of 200 hz for a faster scan with lower quality, and 400 hz for a slower scan with better quality.

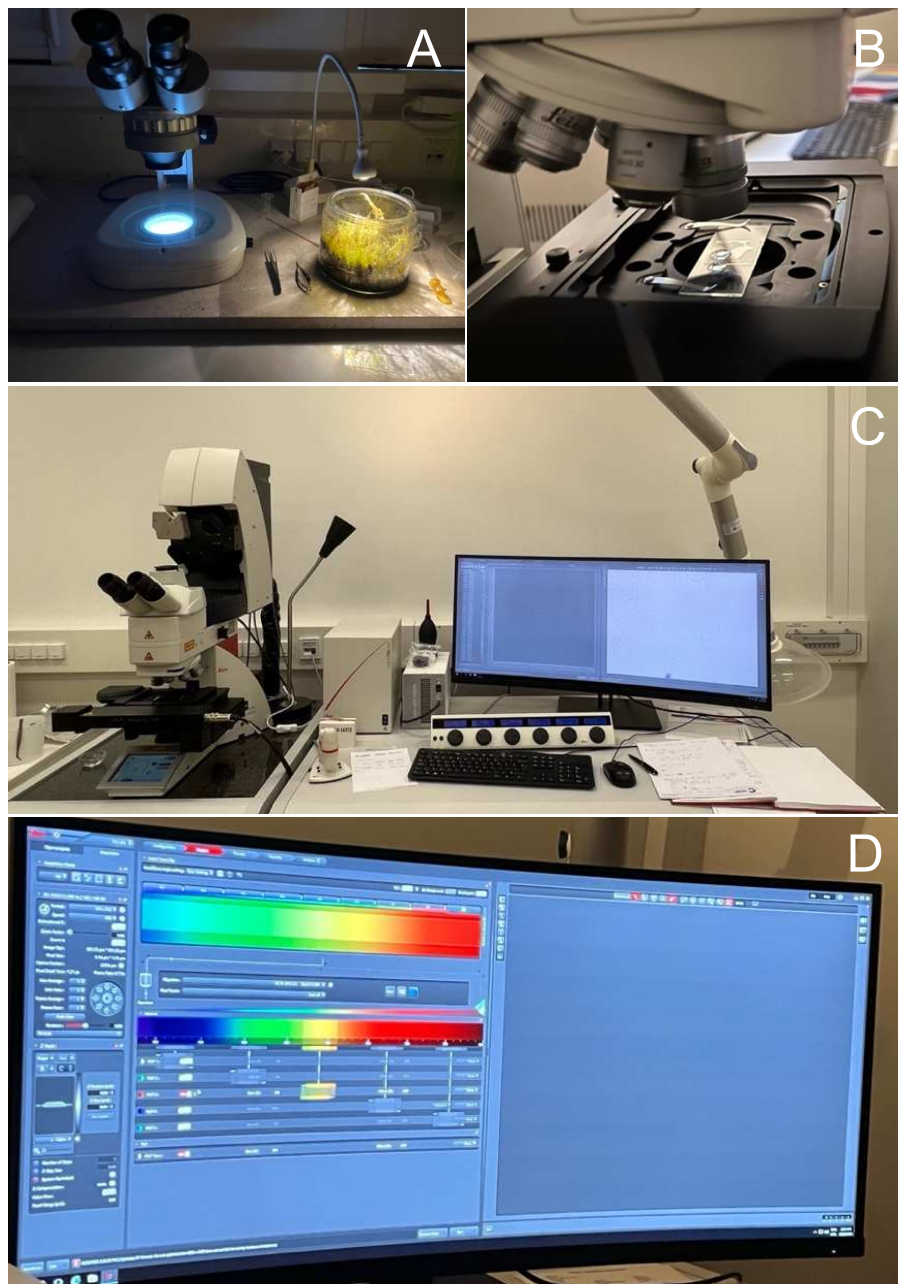


Fig. 19. A) Leaf removal under the binocular of *P. affine* in a glass container. B) Glass slides containing the specimen were placed in the holder of the Leica SP8. C) Confocal microscope (Leica SP8), x and y control, control panels, computer screen with Leica LAS X Software. D) All settings for images were made in the LAS X software.

2.9. Statistical analysis

The raw data of the measurements were summarized in Excel, and the mean weight loss and photosynthetic activity were calculated for each of the 33-time points. For every species and numerical parameter, 6 replicates were done. The weight of the cellulose discs was determined at the start of each measurement. Further, to get an insight into the average weight of the cellulose discs, 20 discs plus the weight of 200 µl water was determined. During this experiment, one specimen of *P. patens* and one *M. polymorpha* was lost at the end of the experiment.

OriginPro software was used to generate dot graphs with lines representing variables such as weight, photosynthetic activity, cell area, leaf area, and shoot area. Box plots were used to visualize the initial hydrated cell area and confocal diameter. Furthermore, a pairwise T-test was performed to assess differences in initial hydrated cell area and leaf diameter. Statistical analysis included the calculation of Pearson's correlation coefficient in OriginPro for all species, assessing relationships between weight, photosynthetic activity, cell area, leaf area, and shoot area.

The correlation between photosynthetic activity and weight was specifically analyzed. Linear regression analysis was conducted in OriginPro (OriginLab) to examine the relationship between weight and photosynthetic activity. Combined graphs were created to show these parameters over time, with time plotted on the x-axis. Additionally, a series of graphs depicting photosynthetic activity on the y-axis and weight on the x-axis were generated. To improve the visibility of the dehydration pattern, the initial 48-hour graphs, which depicted species separately, were shortened to 720 minutes, as the lowest photosynthetic activity and weight were reached before this point in time. The resulting graphs, with all species grouped, include (Fig.21.A & B; Fig.23.A & B; Fig. 29.A – Fig.34.)"

3. Results

3.1. Dehydration

The aim of the cellulose discs was to facilitate the specimen transfer from the glass Petri dish to the scale and provide a wet environment for the mosses during dehydration by retaining the 200 µl of water added at the beginning. Initially, the weight of the cell discs should be deducted from the total weight, which resulted in several negative values at the end of the experiment. The mean weight of 20 cellulose discs plus 200 µl water added, was determined. All weight data provided include the weight of the mosses, 200 µl water, and cellulose disc. The assistance of a colleague was required to measure the weight and photosynthetic activity at the same points in time (Fig. 12). Before the photosynthetic measurements, the mosses were pre-dark adapted for 5 minutes. In one setting, 3 mosses were analyzed which resulted in the occupation of the scales throughout the day, from 9 a.m. to 10 p.m.

3.1.1. Weight loss

The graphs of Fig. (20. & 21.A) illustrate the species specific dehydration patterns over 48 hours. Table 2 summarizes the mean weight, percent, and decline observed during the dehydration of all mosses investigated. The weight loss follows the time curve in a negative linear way in the first 30 minutes in *A. undulatum*, *P. affine*, *F. hygrometrica*, and *M. polymorpha* showed some deviations from the negative linear relationship because of some fluctuations. In all mosses, at one hour. 50 % of the initial weight is lost, at 2 hours, 66-86 %, and at 3 hours, 80-91 % (Tab.2). *M. polymorpha* showed 36 % at 1 hour, 66 % at 2 hours, and at 3 hours, 89 % weight loss. All moss species showed a decline and have lost 90 % at after 3 hours and remain at this level until 48 hours. In *A. undulatum* this decline took place at 4 hours The linear regression of the weight revealed a similar steepness for *A. undulatum*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*. In contrast to the other species, *P. affine* showed a flatter line, with the lowest starting point at the y-axis (Fig. 21.B). A positive Person correlation above 0.99, with a highly significant p-value <0.0001 was documented for all species (Tab. 4.)

Tab. 2. Weight (g), percent, and decline (%) during dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Weight loss (g) during dehydration															
	<i>A.undulatum</i>			<i>P.affine</i>			<i>P. patens</i>			<i>F.hygrometrica</i>			<i>M.polymorpha</i>		
	g	%	decl.	g	%	decl.	g	%	decl.	g	%	decl.	g	%	decl.
0.5 min	0.1080	100.0	0.0	0.1004	100.0	0.0	0.1119	####	0.0	0.1084	100.0	0.0	0.0916	100.0	0.0
1 min	0.1068	98.8	1.2	0.0965	100.0	0.0	0.1100	98.3	1.7	0.1068	98.5	1.5	0.1057	115.3	-15.3
1.5 min	0.1059	98.0	2.0	0.0959	99.4	0.6	0.1091	97.5	2.5	0.1055	97.3	2.7	0.1049	114.5	-14.5
2 min	0.1051	97.3	2.7	0.0950	98.4	1.6	0.1090	97.4	2.6	0.1045	96.4	3.6	0.0952	103.9	-3.9
2.5 min	0.1046	96.8	3.2	0.0947	98.1	1.9	0.1104	98.6	1.4	0.1036	95.5	4.5	0.1031	112.6	-12.6
3 min	0.1033	95.6	4.4	0.0937	97.1	2.9	0.1083	96.8	3.2	0.1023	94.3	5.7	0.1027	112.1	-12.1
3.5 min	0.1030	95.4	4.6	0.0916	95.0	5.0	0.1073	95.9	4.1	0.1004	92.6	7.4	0.1019	111.2	-11.2
4 min	0.1022	94.6	5.4	0.0922	95.6	4.4	0.1070	95.7	4.3	0.1003	92.5	7.5	0.1016	110.9	-10.9
4.5 min	0.1013	93.7	6.3	0.0918	95.2	4.8	0.1065	95.2	4.8	0.1005	92.7	7.3	0.1012	110.4	-10.4
5 min	0.1012	93.6	6.4	0.0912	94.5	5.5	0.1058	94.5	5.5	0.0995	91.7	8.3	0.1003	109.5	-9.5
5.5 min	0.1002	92.7	7.3	0.0907	94.0	6.0	0.1051	93.9	6.1	0.0996	91.9	8.1	0.1003	109.4	-9.4
6 min	0.0998	92.4	7.6	0.0901	93.4	6.6	0.1050	93.9	6.1	0.0989	91.2	8.8	0.0997	108.7	-8.7
6.5 min	0.0991	91.7	8.3	0.0884	91.6	8.4	0.1029	92.0	8.0	0.0981	90.4	9.6	0.0989	107.9	-7.9
7 min	0.0984	91.1	8.9	0.0890	92.3	7.7	0.1017	90.9	9.1	0.0974	89.8	10.2	0.0985	107.5	-7.5
7.5 min	0.0980	90.7	9.3	0.0885	91.7	8.3	0.1014	90.6	9.4	0.0969	89.4	10.6	0.0979	106.8	-6.8
8 min	0.0972	90.0	10.0	0.0872	90.4	9.6	0.1009	90.2	9.8	0.0963	88.8	11.2	0.0973	106.2	-6.2
8.5 min	0.0965	89.3	10.7	0.0869	90.1	9.9	0.1002	89.5	10.5	0.0956	88.1	11.9	0.0969	105.7	-5.7
9 min	0.0956	88.5	11.5	0.0864	89.5	10.5	0.1000	89.4	10.6	0.0963	88.8	11.2	0.0962	105.0	-5.0
9.5 min	0.0948	87.8	12.2	0.0854	88.5	11.5	0.1006	89.9	10.1	0.0945	87.1	12.9	0.0955	104.2	-4.2
10 min	0.0938	86.8	13.2	0.0848	87.9	12.1	0.0978	87.4	12.6	0.0932	85.9	14.1	0.0948	103.5	-3.5
15 min	0.0860	79.6	20.4	0.0766	79.4	20.6	0.0917	81.9	18.1	0.0838	77.2	22.8	0.0870	95.0	5.0
20 min	0.0811	75.1	24.9	0.0739	76.6	23.4	0.0905	80.9	19.1	0.0785	72.4	27.6	0.0833	90.9	9.1
30 min	0.0742	68.7	31.3	0.0685	71.0	29.0	0.0830	74.2	25.8	0.0701	64.6	35.4	0.0742	81.0	19.0
60 min	0.0564	52.2	47.8	0.0472	48.9	51.1	0.0587	52.5	47.5	0.0544	50.1	49.9	0.0588	64.2	35.8
2 h	0.0337	31.2	68.8	0.0147	15.2	84.8	0.0213	19.0	81.0	0.0186	17.2	82.8	0.0313	34.1	65.9
3 h	0.0232	21.5	78.5	0.0100	10.3	89.7	0.0103	9.2	90.8	0.0097	8.9	91.1	0.0104	11.3	88.7
4 h	0.0100	9.2	90.8	0.0096	9.9	90.1	0.0100	8.9	91.1	0.0092	8.5	91.5	0.0098	10.7	89.3
5 h	0.0098	9.0	91.0	0.0097	10.1	89.9	0.0099	8.9	91.1	0.0098	9.0	91.0	0.0096	10.5	89.5
6 h	0.0098	9.1	90.9	0.0091	9.5	90.5	0.0098	8.7	91.3	0.0095	8.7	91.3	0.0090	9.9	90.1
8 h	0.0095	8.8	91.2	0.0098	10.1	89.9	0.0109	9.7	90.3	0.0094	8.7	91.3	0.0092	10.0	90.0
10 h	0.0096	8.9	91.1	0.0097	10.0	90.0	0.0099	8.9	91.1	0.0094	8.6	91.4	0.0096	10.5	89.5
12 h	0.0093	8.6	91.4	0.0095	9.9	90.1	0.0095	8.5	91.5	0.0093	8.6	91.4	0.0243	26.5	73.5
24 h	0.0093	8.6	91.4	0.0090	9.4	90.6	0.0091	8.2	91.8	0.0097	8.9	91.1	0.0092	10.1	89.9
48 h	0.0096	8.9	91.1	0.0095	9.8	90.2	0.0100	9.0	91.0	0.0093	8.6	91.4	0.0094	10.2	89.8

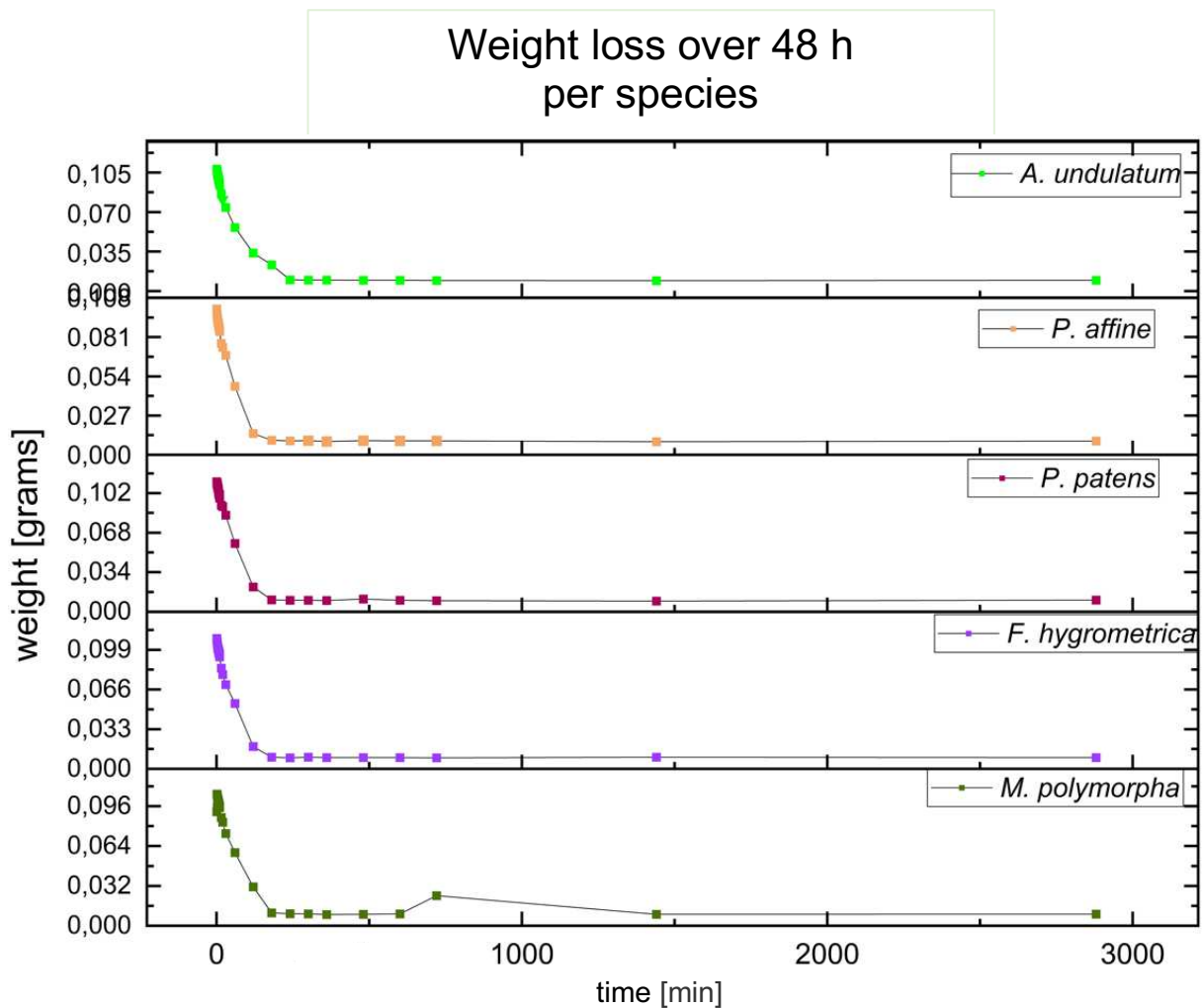


Fig. 20. Graph depicting the of the mean weight in 48 h dehydration, separated for each species, including *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

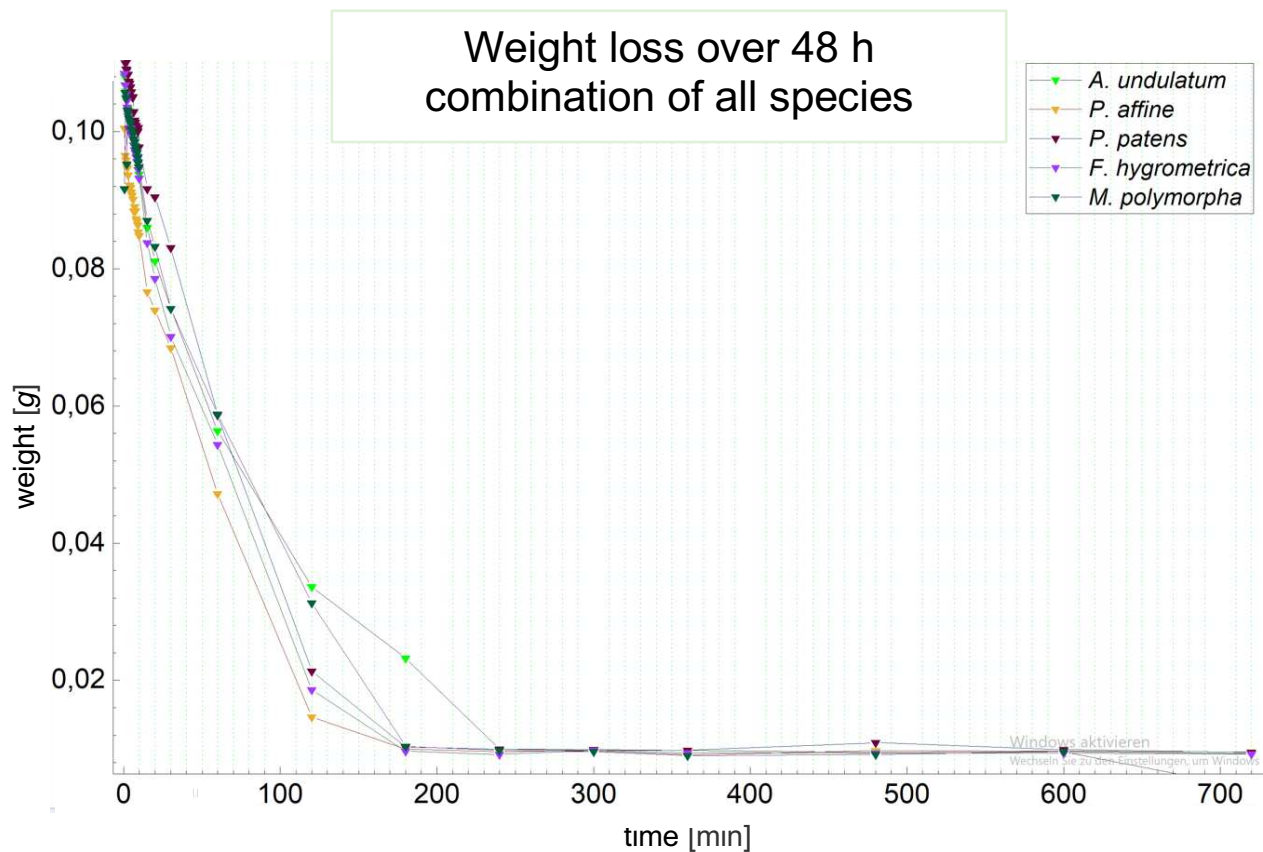


Fig. 21. A) Graph depicting the of the mean weight, in 720 minutes dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

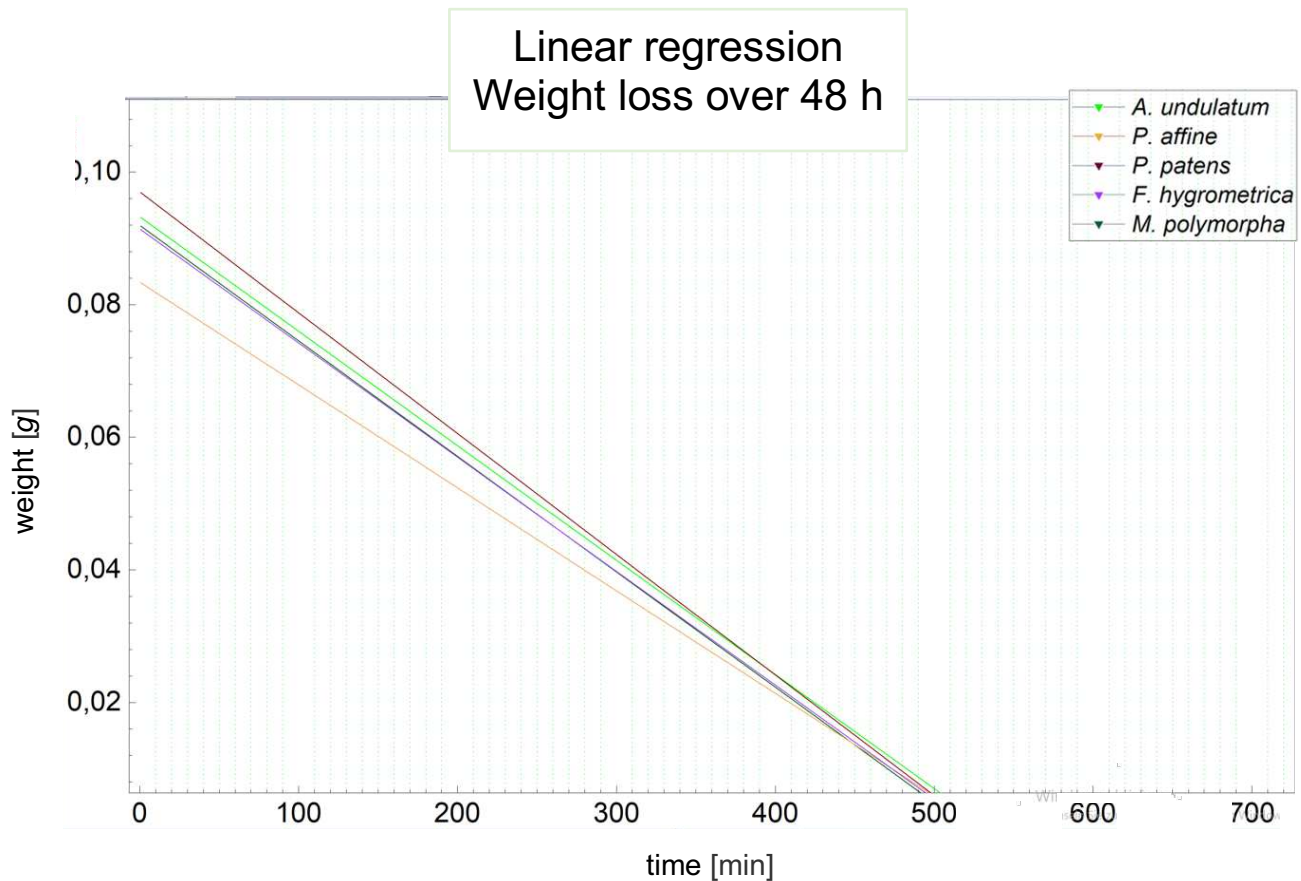


Fig. 21. B) Graph depicting the linear regression lines, generated from the weight loss data, including of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Linear regression of the weight (Fig. 21.B) revealed a similar steepness for *A. undulatum*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*. In contrast to the other species, *P. affine* showed a flatter line, with the lowest starting point at the y-axis.

Tab. 3. Descriptive statistics of the dehydration weight loss (48 hours).

Weight 48 hours	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	34	0,07194	0,03983	2,44605	9E-3	0,108
<i>P. affine</i>	34	0,06432	0,03676	2,18689	9E-3	0,100
<i>P. patens</i>	34	0,07457	0,04271	2,53527	9E-3	0,112
<i>F. hygrometrica</i>	34	0,07026	0,04059	2,3889	9E-3	0,108
<i>M. polymorpha</i>	34	0,07115	0,03921	2,41924	9E-3	0,106

Tab. 4. Pearson correlation coefficient and p-value of the dehydration weight loss (48 hours).

Weight 48 hours		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	0,99576*	0,99642*	0,9967*	0,99275*
<i>A. undulatum</i>	p-value	--	<0.0001	<0.0001	<0.0001	<0.0001
<i>P. affine</i>	Pearson cor.	0,99576*	1	0,99892*	0,99944*	0,99151*
<i>P. affine</i>	p-value	<0.0001	--	<0.0001	<0.0001	<0.0001
<i>P. patens</i>	Pearson cor.	0,99642*	0,99892*	1	0,99847*	0,99392*
<i>P. patens</i>	p-value	<0.0001	<0.0001	--	<0.0001	<0.0001
<i>F. hygrometrica</i>	Pearson cor.	0,9967*	0,99944*	0,99847*	1	0,99285*
<i>F. hygrometrica</i>	p-value	<0.0001	<0.0001	<0.0001	--	<0.0001
<i>M. polymorpha</i>	Pearson cor.	0,99275*	0,99151*	0,99392*	0,99285*	1
<i>M. polymorpha</i>	p-value	<0.0001	<0.0001	<0.0001	<0.0001	--

In Tab.4., the data of the “Weight 48 hours” (Fig. 21.) were summarized and the **Pearson correlation coefficient** indicated a positive correlation > 0.99 in **all species**, including *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*, with highly significant p-value < 0.0001 .

3.1.2. Photosynthetic activity

In Figure 22 and 23.A the photosynthetic activity of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha* during dehydration is depicted (. Table 5 provides data on the quantum yield (Φ), percentage and its decline (%) observed during the dehydration process (Tab.5.). In ***A. undulatum*** the PAM values increased 20 % after the first measurement and continued to fluctuate in the range of 0.312-0.41. The highest quantum yield 0.341 was documented several times at 2, 3, and 3.5 minutes. At one hour, a decline started reaching a loss of 64 %, and from 2-48 hours, and remained at a stable phase, in which 89-99 % are lost. The lowest value 0.01 was seen at 10-12 hours, after this, a slight increase was noted. ***P. affine*** showed the highest increase at 9 minutes, after an increase of 12 % from the initial quantum yield. At 1 hour the decline of 32 %, and from 2 hours to 48 hours, a decline from 80% was documented. At 8 hours and 24 hours, the lowest quantum yield of 0.01 was observed. In ***P. patens*** the highest PAM 0.31 was the second value, measured at 1 minute, with an increase of 82 %. From 2 minutes to 60 minutes, fluctuations between 0.18-0.27 were observed. At one hour, a 56 % decline, and from 2-48 hours, 75-97 % was seen. At 12 hours, the lowest value of 0.01 was documented. ***F. hygrometrica*** was the only moss, in which the first measurement at 0.5 minutes showed the highest photosynthetic activity documented. In the first 20 minutes, a decline of up to 20 % took place, with a lot of fluctuations, and at one hour, 15 % was lost, from 3-48 hours, the loss was in the range of 77-96 %. At 6 hours, the lowest quantum yield of 0.02 was documented. ***M. polyamorpha***, which underwent a gradual increase, reaching the highest value at 6.5 minutes, after a 20 % increase. From 7 to 60 minutes, the values were in the range of 0.37-0.30 and at one hour, a noticeable decline started, with a loss of 34 %. In the last phase from 3 to 48 hours, the decline happens gradually, from 62-96 % (Fig. 22. & 23.A).

Tab. 5. Quantum yield (Φ) percent, and decline (%) during dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Quantum yield (Φ) during dehydration															
	<i>A.undulatum</i>			<i>P.affine</i>			<i>P. patens</i>			<i>F.hygrometrica</i>			<i>M.polymorpha</i>		
	Φ	%	decl.	Φ	%	decl.	Φ	%	decl.	Φ	%	decl.	Φ	%	decl.
0.5 min	0.40	100.0	0.0	0.43	100.0	0.0	0.17	100.0	0.0	0.35	100.0	0.0	0.31	100.0	0.0
1 min	0.34	99.0	1.0	0.41	94.6	5.4	0.31	182.4	-82.4	0.28	81.9	18.1	0.28	91.4	8.6
1.5 min	0.33	97.1	2.9	0.45	104.7	-4.7	0.18	104.4	-4.4	0.27	76.9	23.1	0.33	105.4	-5.4
2 min	0.41	119.6	-19.6	0.42	97.7	2.3	0.22	130.4	-30.4	0.28	80.4	19.6	0.31	100.0	0.0
2.5 min	0.39	115.7	-15.7	0.45	103.9	-3.9	0.17	100.0	0.0	0.28	79.8	20.2	0.33	105.4	-5.4
3 min	0.41	120.1	-20.1	0.44	101.2	-1.2	0.27	157.8	-57.8	0.32	92.5	7.5	0.34	109.7	-9.7
3.5 min	0.41	120.1	-20.1	0.45	105.0	-5.0	0.19	111.8	-11.8	0.31	89.6	10.4	0.36	115.1	-15.1
4 min	0.38	112.7	-12.7	0.42	98.4	1.6	0.25	148.0	-48.0	0.27	79.0	21.0	0.34	108.6	-8.6
4.5 min	0.40	118.1	-18.1	0.44	102.7	-2.7	0.18	105.9	-5.9	0.29	82.7	17.3	0.35	112.9	-12.9
5 min	0.40	116.2	-16.2	0.46	107.0	-7.0	0.25	147.1	-47.1	0.30	86.2	13.8	0.33	105.4	-5.4
5.5 min	0.38	111.3	-11.3	0.45	105.0	-5.0	0.23	136.8	-36.8	0.27	77.5	22.5	0.34	110.2	-10.2
6 min	0.38	112.3	-12.3	0.43	99.2	0.8	0.27	156.5	-56.5	0.26	75.6	24.4	0.35	112.4	-12.4
6.5 min	0.39	115.7	-15.7	0.43	99.6	0.4	0.17	100.0	0.0	0.28	79.8	20.2	0.38	121.0	-21.0
7 min	0.40	116.7	-16.7	0.46	107.4	-7.4	0.24	141.2	-41.2	0.33	94.4	5.6	0.31	101.1	-1.1
7.5 min	0.39	113.7	-13.7	0.41	95.7	4.3	0.21	125.0	-25.0	0.31	88.4	11.6	0.37	119.4	-19.4
8 min	0.38	112.7	-12.7	0.37	86.0	14.0	0.25	144.1	-44.1	0.27	78.0	22.0	0.33	107.5	-7.5
8.5 min	0.37	108.3	-8.3	0.43	99.5	0.5	0.19	111.8	-11.8	0.23	65.3	34.7	0.30	95.2	4.8
9 min	0.38	110.8	-10.8	0.48	112.1	-12.1	0.26	152.9	-52.9	0.29	84.8	15.2	0.34	109.1	-9.1
9.5 min	0.39	114.1	-14.1	0.41	94.9	5.1	0.12	70.6	29.4	0.28	81.5	18.5	0.33	107.0	-7.0
10 min	0.40	118.4	-18.4	0.42	97.7	2.3	0.26	151.0	-51.0	0.30	86.7	13.3	0.32	103.9	-3.9
15 min	0.28	83.3	16.7	0.41	95.7	4.3	0.23	135.3	-35.3	0.27	77.1	22.9	0.30	96.8	3.2
20 min	0.39	114.7	-14.7	0.46	107.0	-7.0	0.23	137.3	-37.3	0.28	81.9	18.1	0.30	97.6	2.4
30 min	0.39	115.2	-15.2	0.45	104.7	-4.7	0.25	144.1	-44.1	0.24	68.9	31.1	0.31	98.9	1.1
60 min	0.32	94.6	5.4	0.29	68.2	31.8	0.18	103.9	-3.9	0.26	75.1	24.9	0.35	111.3	-11.3
2 h	0.12	36.3	63.7	0.09	21.3	78.7	0.08	44.1	55.9	0.30	85.3	14.7	0.21	66.1	33.9
3 h	0.02	6.9	93.1	0.06	13.2	86.8	0.04	25.5	74.5	0.03	9.6	90.4	0.12	37.6	62.4
4 h	0.04	10.8	89.2	0.03	7.4	92.6	0.03	16.7	83.3	0.06	17.3	82.7	0.05	16.7	83.3
5 h	0.03	9.3	90.7	0.09	20.5	79.5	0.04	23.5	76.5	0.03	8.7	91.3	0.08	26.9	73.1
6 h	0.02	4.9	95.1	0.02	3.9	96.1	0.02	11.8	88.2	0.02	4.3	95.7	0.08	24.7	75.3
8 h	0.02	5.9	94.1	0.01	2.7	97.3	0.04	23.5	76.5	0.04	10.6	89.4	0.02	6.5	93.5
10 h	0.01	1.5	98.5	0.02	3.9	96.1	0.05	27.5	72.5	0.08	22.6	77.4	0.01	4.4	95.6
12 h	0.01	3.4	96.6	0.04	10.1	89.9	0.01	2.9	97.1	0.03	8.7	91.3	0.02	5.9	94.1
24 h	0.03	8.3	91.7	0.01	1.9	98.1	0.02	11.8	88.2	0.08	23.1	76.9	0.03	8.6	91.4
48 h	0.03	9.8	90.2	0.04	10.1	89.9	0.05	26.5	73.5	0.07	18.8	81.2	0.03	8.6	91.4

Photosynthetic activity over 48 h per species

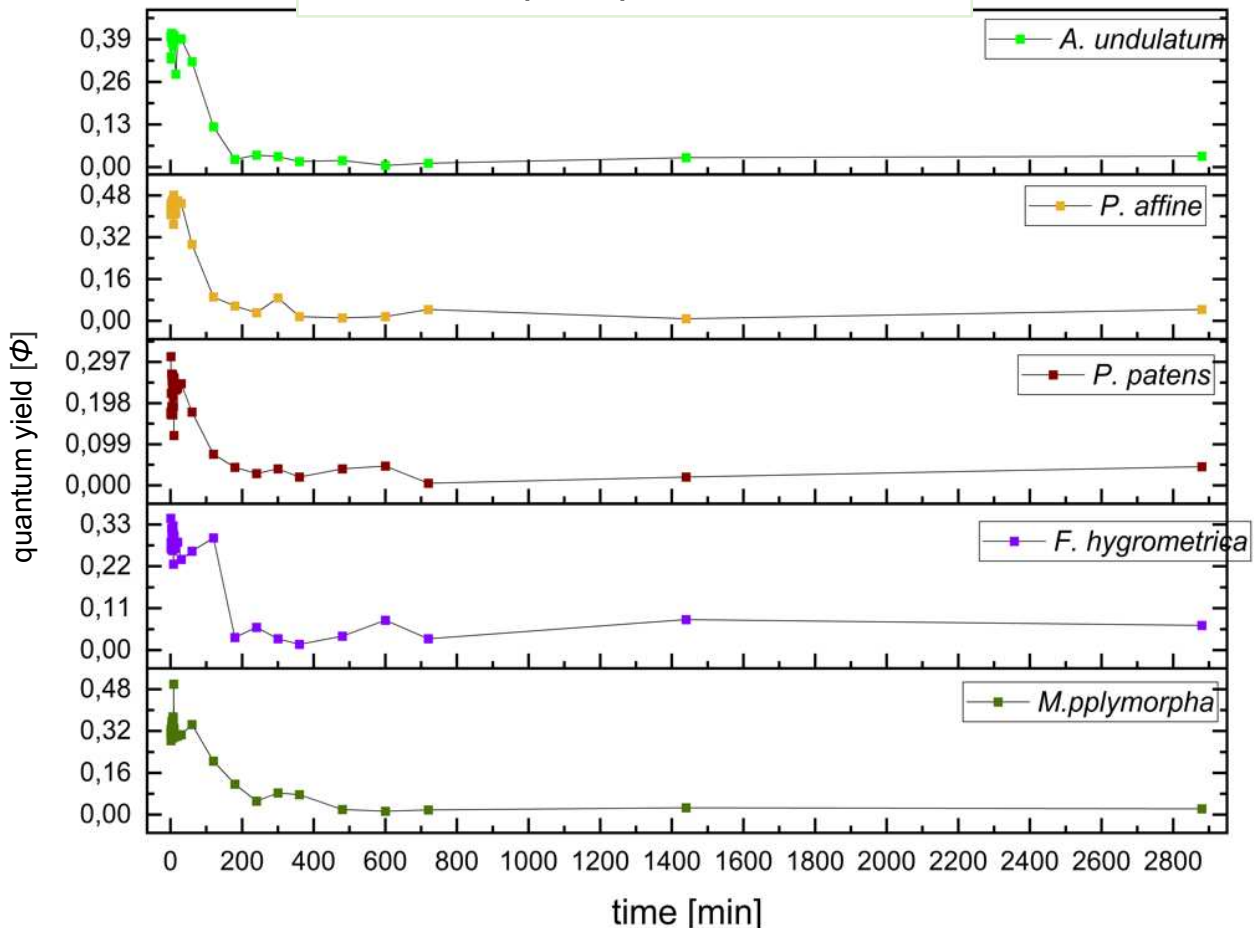


Fig. 22. Graph depicting the of the mean Photosynthetic activity in 48 hours dehydration, separated for each species, including *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Photosynthetic activity over 48 h combination of all species

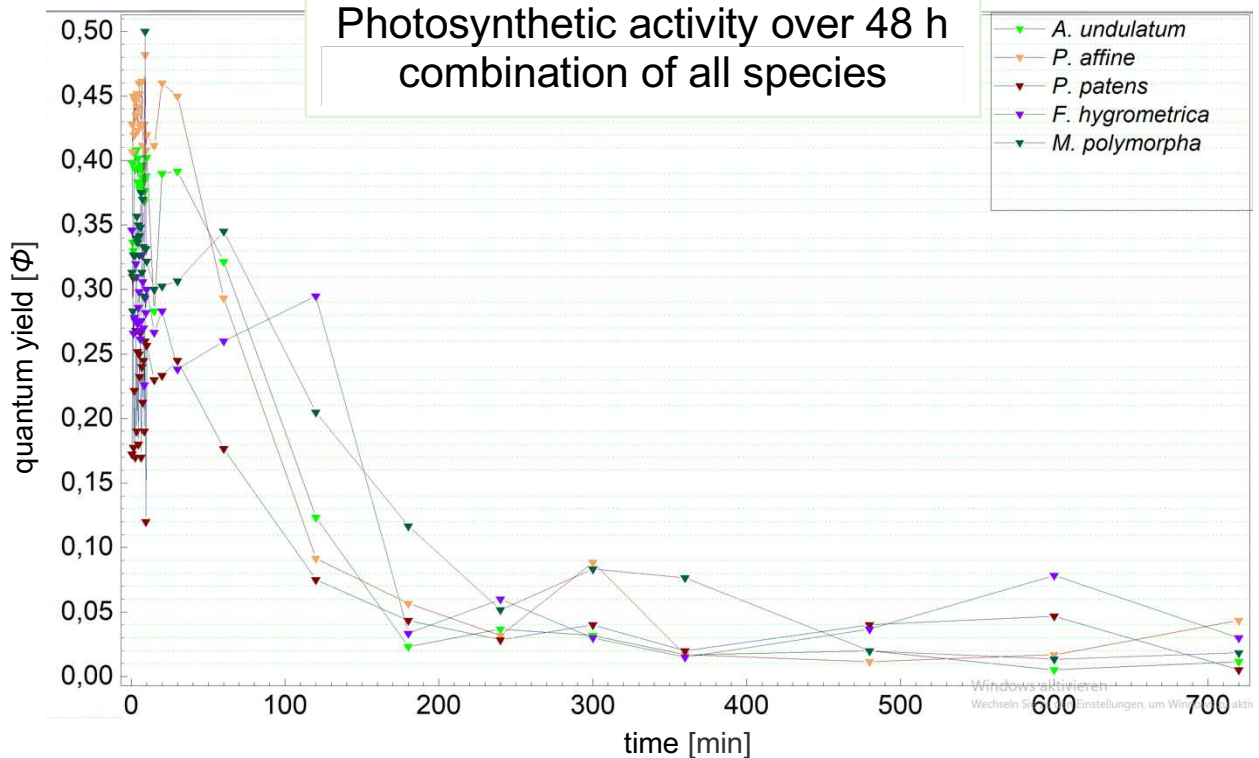


Fig. 23.A) Graph depicting the of the mean photosynthetic activity in 720 minutes dehydration of all species, including *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

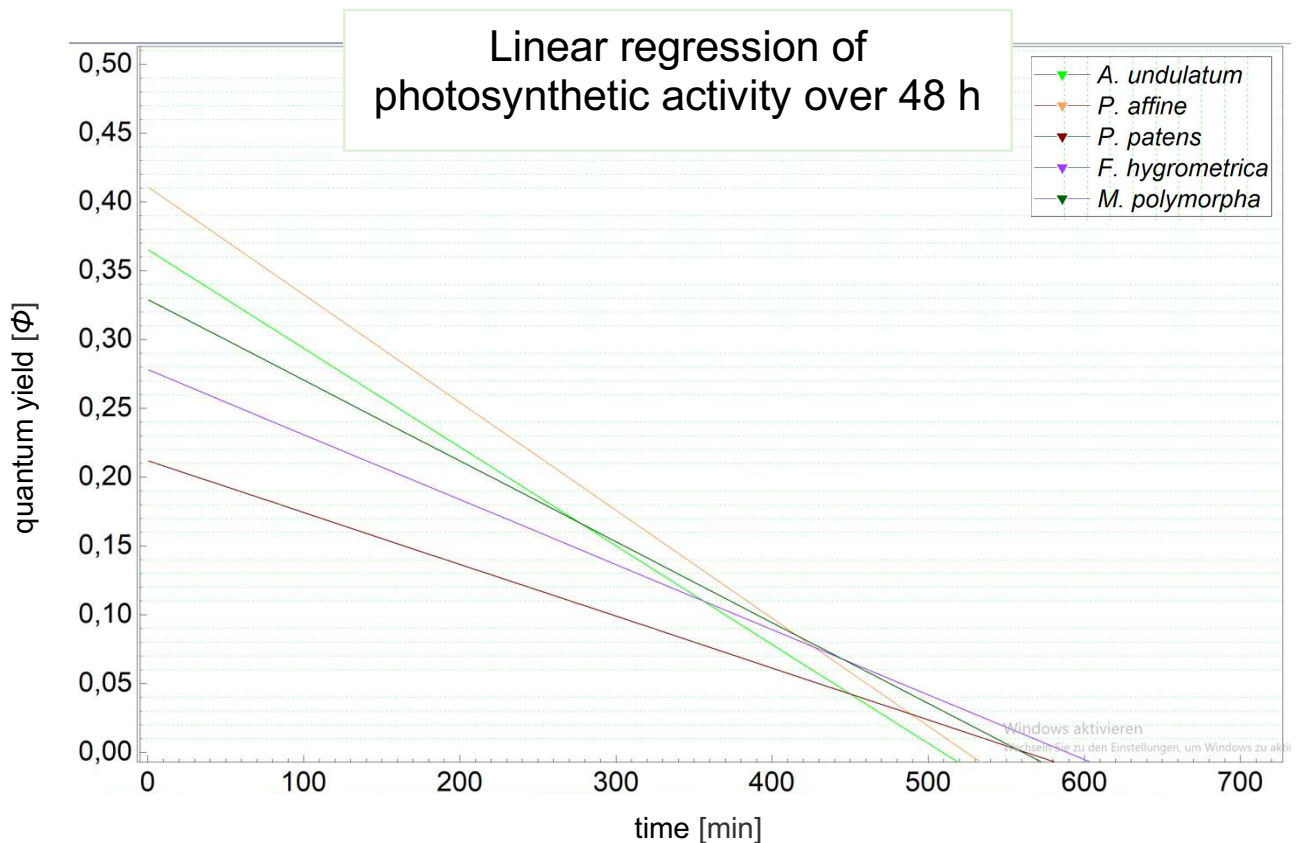


Fig. 23.B) Graph depicting the Linear regression of the photosynthetic activity in 720 minutes dehydration of all species, including *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

The **linear regression** (Fig. 23.B) of *P. affine* with the highest starting point, followed by *A. undulatum*, and both showed lines with similar regression patterns. *M. polymorpha* had the third highest starting point and showed a flatter line than *P. affine* and *A. undulatum*, but steeper than in *F. hygrometrica* and *P. patens*. *F. hygrometrica* and *P. patens* lines with the lowest starting point.

Tab. 6. Descriptive statistics of the photosynthetic activity (48 hours)

PAM 48 hours	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	34	0,27747	0,16318	9,43383	5E-3	0,408
<i>P. affine</i>	34	0,31396	0,18232	10,675	8E-3	0,482
<i>P. patens</i>	34	0,16567	0,0928	5,63267	5E-3	0,31
<i>F. hygrometrica</i>	34	0,22114	0,10868	7,51867	0,02	0,346
<i>M. polymorpha</i>	34	0,25555	0,13443	8,68883	0,01	0,5

Tab. 7. Pearson correlation coefficient and p-value of the photosynthetic activity (48 hours)

PAM 48 hours		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	0,98089	0,90231	0,93285	0,94634
<i>A. undulatum</i>	p-value	--	<0.0001	<0.0001	<0.0001	<0.0001
<i>P. affine</i>	Pearson cor.	0,98089	1	0,91243	0,90517	0,94296
<i>P. affine</i>	p-value	<0.0001	--	<0.0001	<0.0001	<0.0001
<i>P. patens</i>	Pearson cor.	0,90231	0,91243	1	0,85384	0,87061
<i>P. patens</i>	p-value	<0.0001	<0.0001	--	<0.0001	<0.0001
<i>F. hygrometrica</i>	Pearson cor.	0,93285	0,90517	0,85384	1	0,91339
<i>F. hygrometrica</i>	p-value	<0.0001	<0.0001	<0.0001	--	<0.0001
<i>M. polymorpha</i>	Pearson cor.	0,98089	0,94296	0,87061	0,91339	1
<i>M. polymorpha</i>	p-value	<0.0001	<0.0001	<0.0001	<0.0001	--

Analysis of the data of the “Photosynthetic activity (48 h)” during dehydration (Fig. 22. & 23.A). revealed a positive **Pearson correlation coefficient** in all species (Tab.7.) from **0.086** to **0.98**, all with a highly significant p-value <0.0001 (Tab.7.).

3.1.3. Combined weight loss & photosynthetic activity

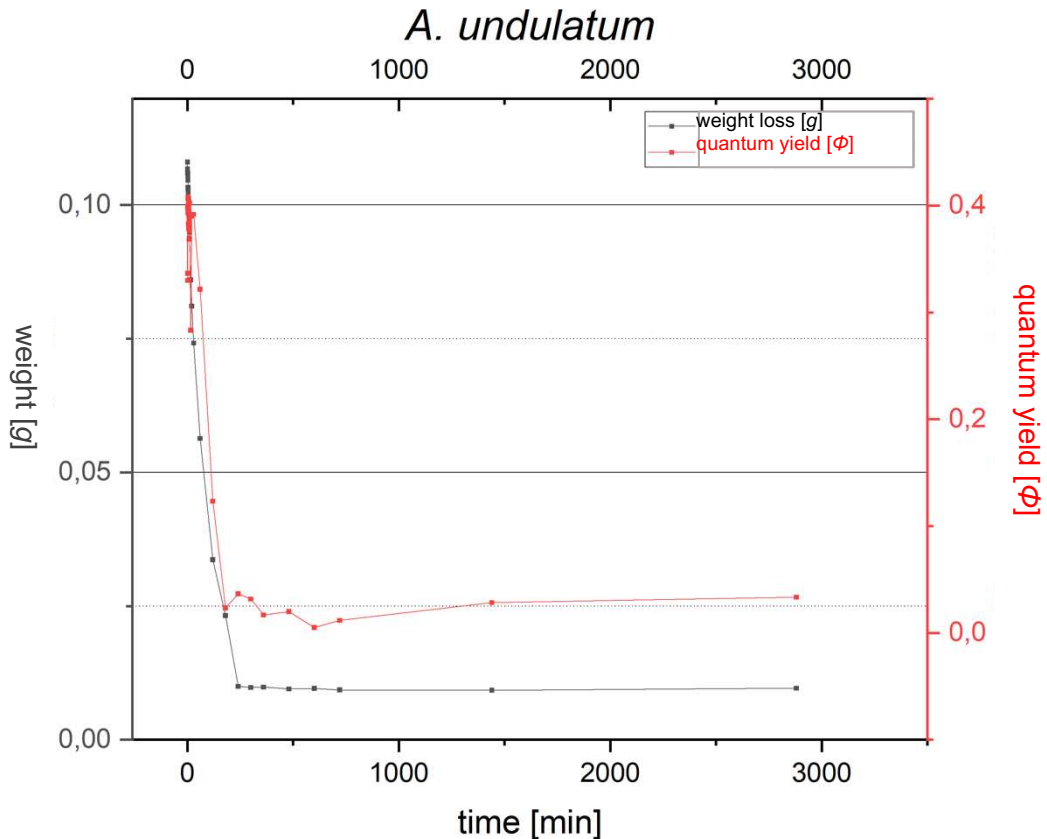


Fig. 24. Graph showing the photosnthetic activity (red) and weight (black) of *A. undulatum* from 0-48 hours.

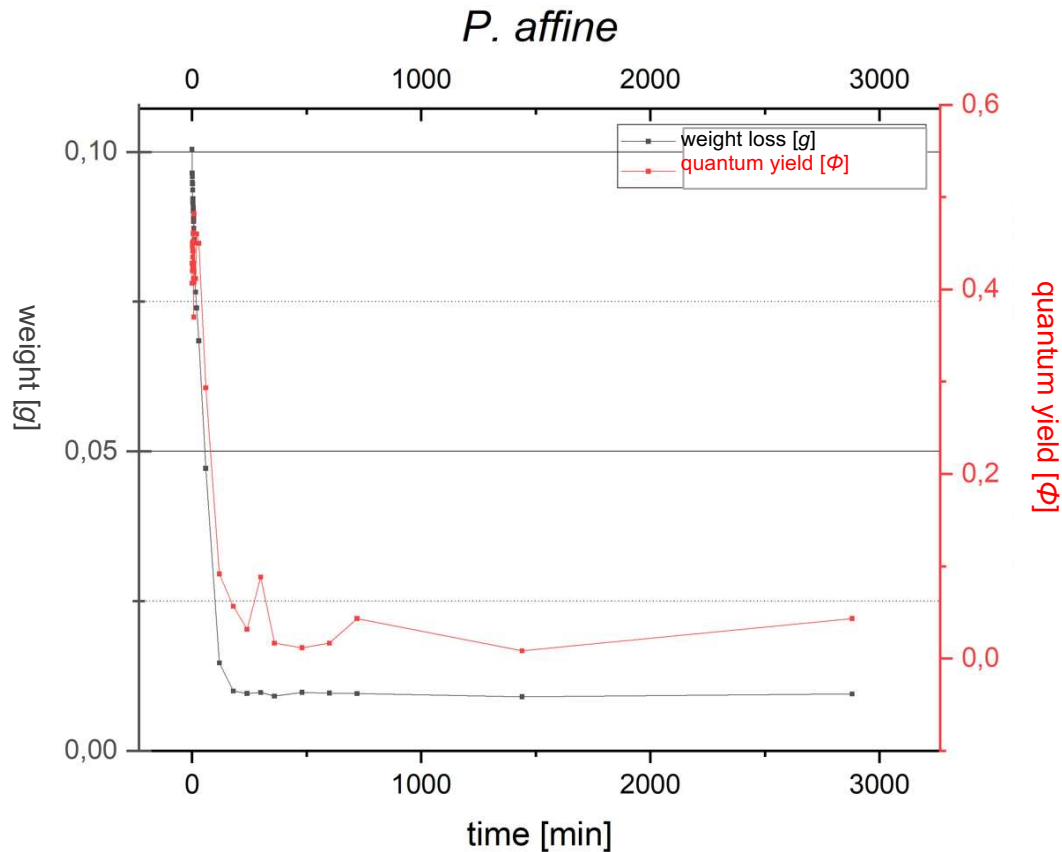


Fig. 25. Graph showing the photosnthetic activity (red) and weight (black) of *P. affine* from 0-48 hours.

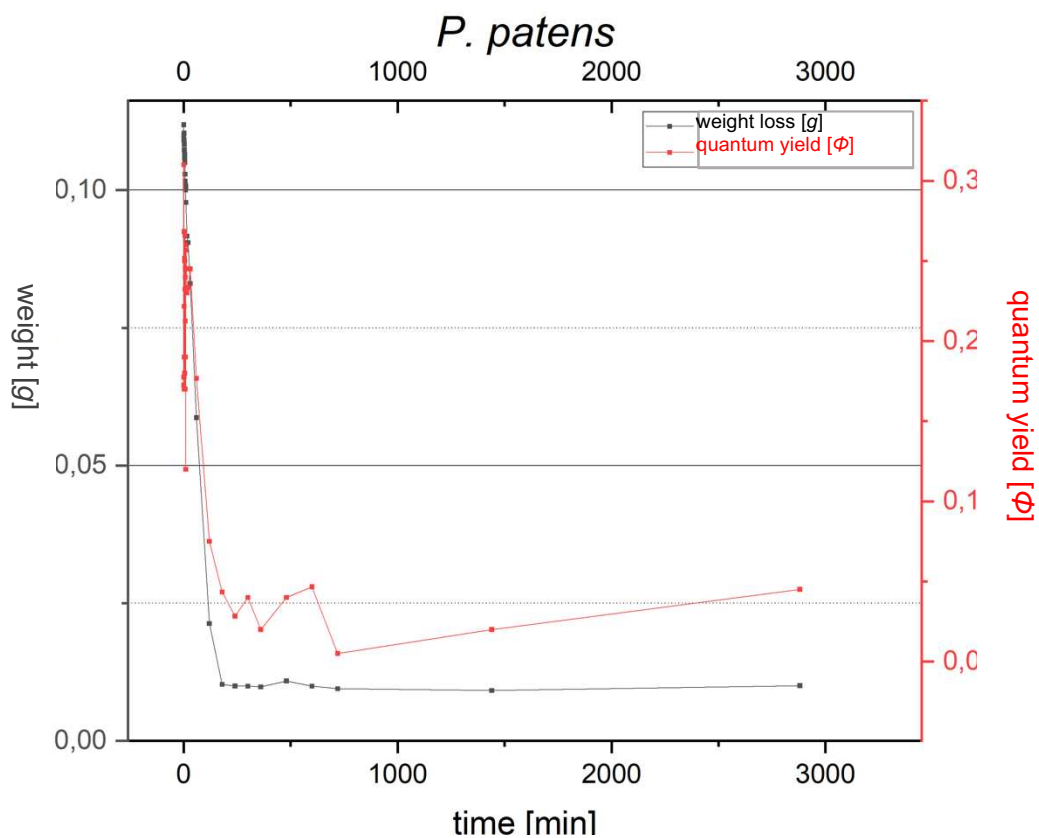


Fig. 26. Graph showing the photosynthetic activity (red) and weight (black) of *P.patens* from 0-48 hours.

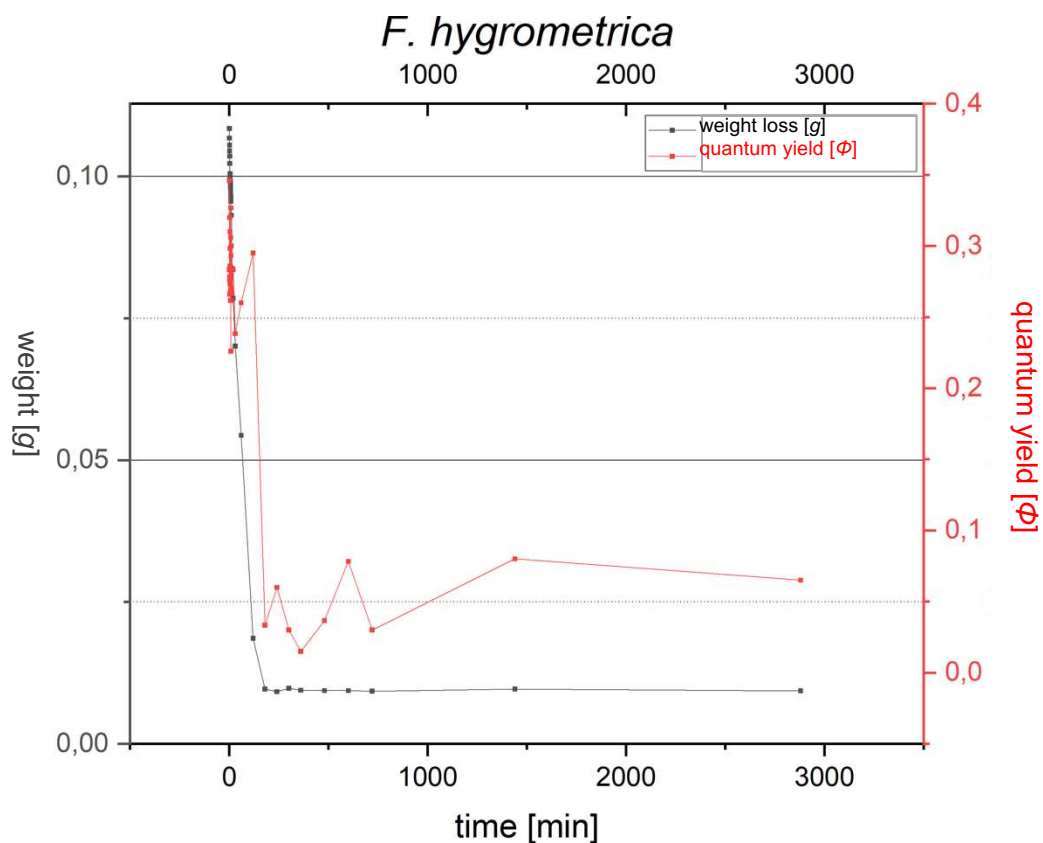


Fig. 27. Graph showing the photosynthetic activity (red) and weight (black) of *F.hygrometrica* from 0-48. hours.

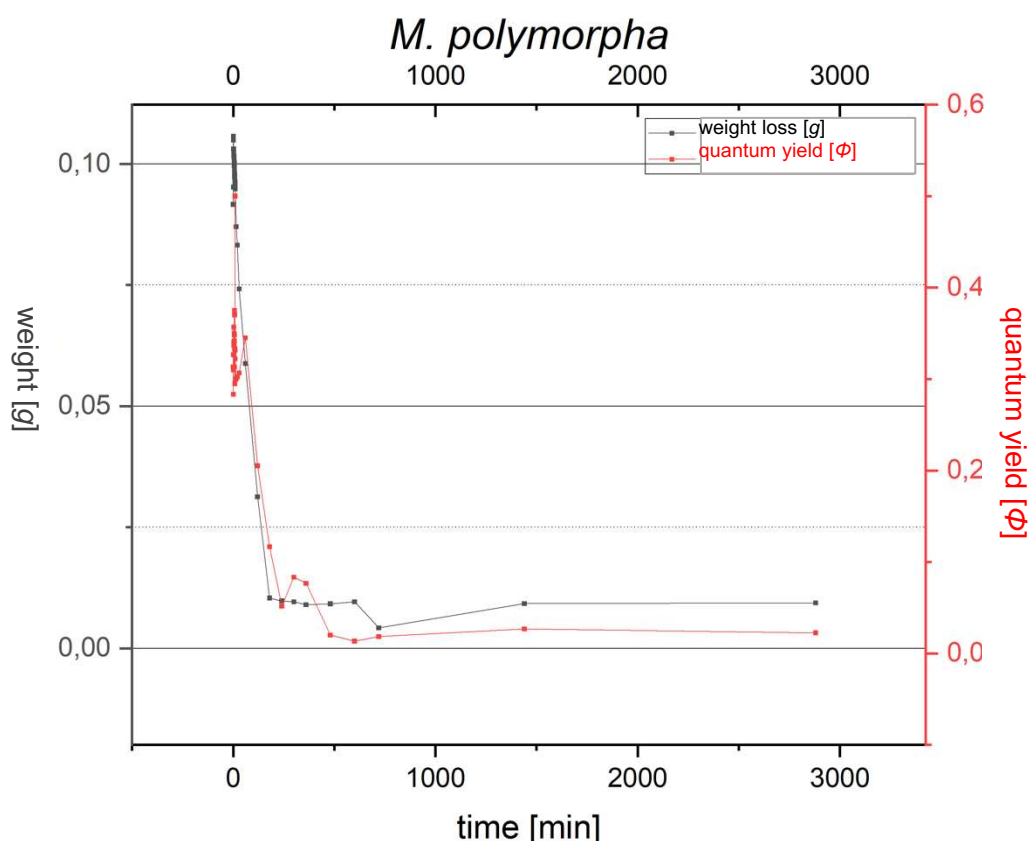


Fig. 28. Graph showing the photosynthetic activity (red) and weight (black) of *M. polymorpha* from 0-48 hours.

The graphs “**Combined weight loss & photosynthetic activity**” revealed, that the decline of the photosynthetic activity in all species remained above the weight line, which was almost linear. *M. polymorpha* (Fig. 28.) deviated from this pattern, since the weight showed fluctuations, and the photosynthetic activity went below the weight line. The bryophytes *A. undulatum* (Fig. 24.), *P. affine* (Fig. 25.), *P. patens* (Fig. 26.), and *F. hygrometrica* (Fig. 27.) showed that the lowest weight point coincided with the minimum photosynthetic activity. This phase revealed a strong increase with fluctuations, and kept on increasing slightly, until 48 hours. In *F. hygrometrica*, a slight decline in photosynthesis was observed. Weight and photosynthetic activity in *M. polymorpha* showed several steps in the dehydration pattern, compared to the other species.

Analysis of the **Pearson correlation coefficient** (Tab. 8.) and of the “combined weight loss & photosynthetic activity graphs revealed in *A. undulatum*, depicted in (Fig. 24.), that the decline in photosynthetic activity follows the weight loss. Characteristics for the quantum yield values were fluctuations, whereas the weight loss

was more stable. A positive correlation of **0.96988** of weight loss and photosynthetic activity with a highly significant p-value < 0.0001 (Tab. 8.) was revealed. In ***P. affine***, the weight loss is almost linear, and the photosynthetic activity showed one strong fluctuation (Fig. 25.). In this species, a positive correlation of **0.97443** was found, indicating a strong correlation between weight loss and photosynthesis, with a highly significant p-value < 0.0001 (Tab. 8.). Characteristic for ***P. patens*** were large fluctuations of the photosynthetic activity (Fig. 26), and a positive correlation **0.9334** was observed between weight loss and photosynthetic activity, with a highly significant p-value < 0.0001 (Tab. 8.). In ***F. hygrometrica***, the weight remained stable, almost linear, and photosynthetic activity showed major fluctuations (Fig. 27.). A positive correlation **0.90282** was observed between weight loss and photosynthetic activity, with a highly significant p-value 0.0001 (Tab. 8.). In ***M. polymorpha***, fluctuations in the photosynthetic activity and weight were noticeable (Fig. 28.). A Pearson correlation of **0.9334** was observed, revealing a positive correlation between weight loss and photosynthetic activity, with a p value 0.0001 (Tab. 8.).

Tab. 8. Pearson correlation coefficient and p-value of the of the weight loss and photosynthetic activity (48 hours).

Weight loss & photosynthetic activity		weight loss	photosynthetic activity
<i>A. undulatum</i>			
Dry weight	Pearson cor.	1	0,96988
Dry weight	p-value	--	<0.0001
Photosynthetic activity	Pearson cor.	0,96988	1
Photosynthetic activity	p-value	<0.0001	--
<i>P. affine</i>			
Dry weight	Pearson cor.	1	0,97443
Dry weight	p-value	--	<0.0001
Photosynthetic activity	Pearson cor.	0,97443	1
Photosynthetic activity	p-value	<0.0001	--
<i>P. patens</i>			
Dry weight	Pearson cor.	1	0,9334
Dry weight	p-value	--	<0.0001
Photosynthetic activity	Pearson cor.	0,9334	1
Photosynthetic activity	p-value	<0.0001	--
<i>F. hygrometrica</i>			
	Pearson cor.		
Dry weight	Pearson cor.	1	0,90282
Dry weight	p-value	--	<0.0001
Photosynthetic activity	Pearson cor.	0,90282	1
Photosynthetic activity	p-value	<0.0001	--
<i>M. polymorpha</i>			
Dry weight	Pearson cor.	1	0,9334
Dry weight	p-value	--	<0.0001
Photosynthetic activity	Pearson cor.	0,9334	1
Photosynthetic activity	p-value	<0.0001	--

3.1.4. Photosynthetic activity (x-axis) & weight (y-axis)

The combined graph (Fig. 29.) depicted the photosynthetic activity on the x-axis and weight on the y-axis over 48 hours for *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*. The flow of time was from the start with the highest weight and photosynthetic activity, at “30 seconds” (top-right corner), to the point with minimal weight and photosynthetic activity (left bottom), at “720 minutes”. The graphs revealed (Fig. 29.-Fig. 34.), that at the beginning of this experiment, within the first 10 minutes, all species were arranged in fluctuating clusters. In *P. patens* (Fig. 32.) the highest weight and lowest photosynthetic activity of all species were revealed, and *F. hygrometrica* showed a lower weight and higher photosynthetic activity. In *M. polymorpha* (Fig. 34, a similar weight and higher photosynthetic activity were seen, *A. undulatum* (Fig. 30.) had a lower weight and increased photosynthetic activity, and *P. affine* (Fig. 31.) showed the lowest weight and highest photosynthetic activity. All species showed strong fluctuations in the first 30 minutes, and *M. polymorpha*, a circular line could be observed, followed by a linear phase, and at the end, more fluctuations were seen.

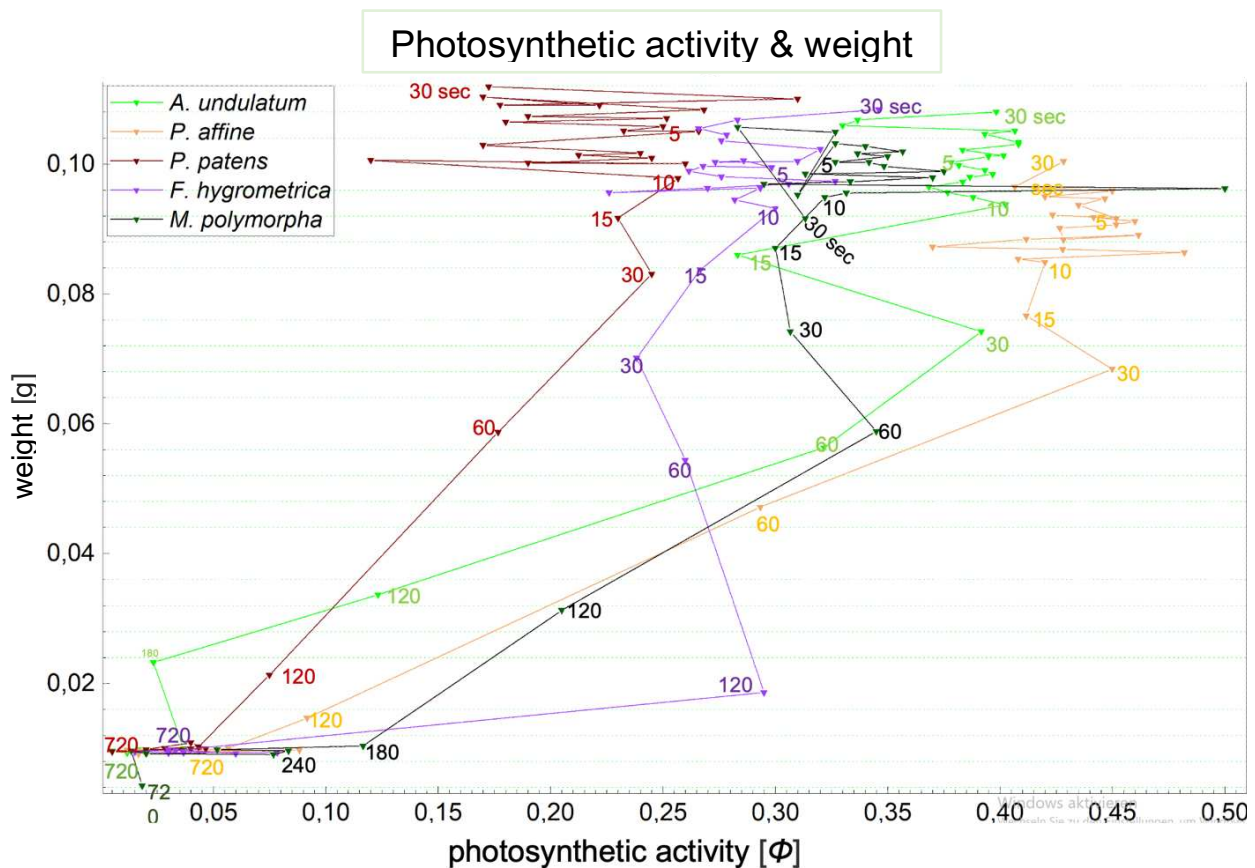


Fig. 29.A) Graph depicting the photosynthetic activity (x-axis), and weight (y-axis) over 48 hours, for *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*. Time points from the start at “30 seconds”, to the final measurement at “720 minutes” were labeled.

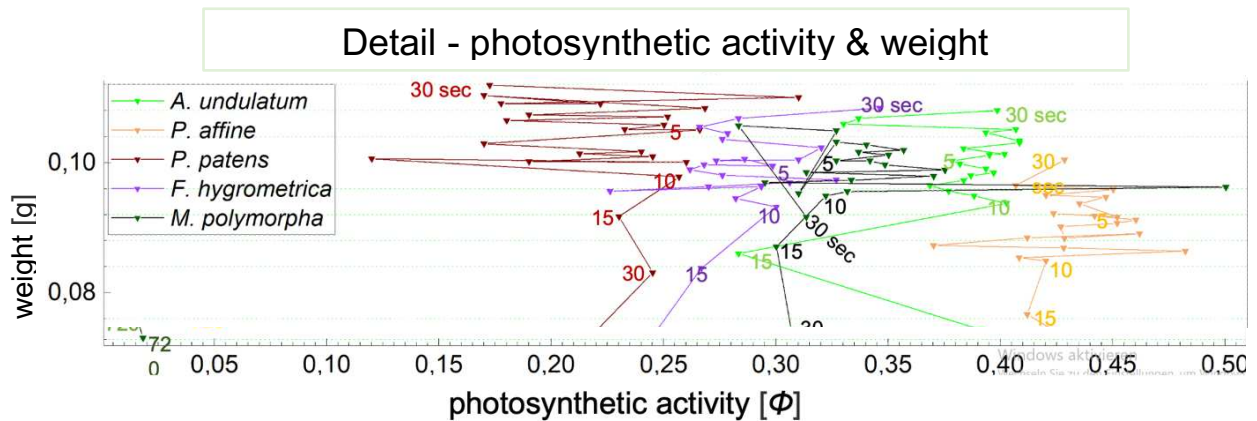


Fig. 29.B) Graph depicting the photosynthetic activity (x-axis) and weight (y-axis) over 48 hours, detailed overview in the first 15 minutes of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*. Time points from the start at "30 seconds", to the final measurement at "15" minutes" were labeled.

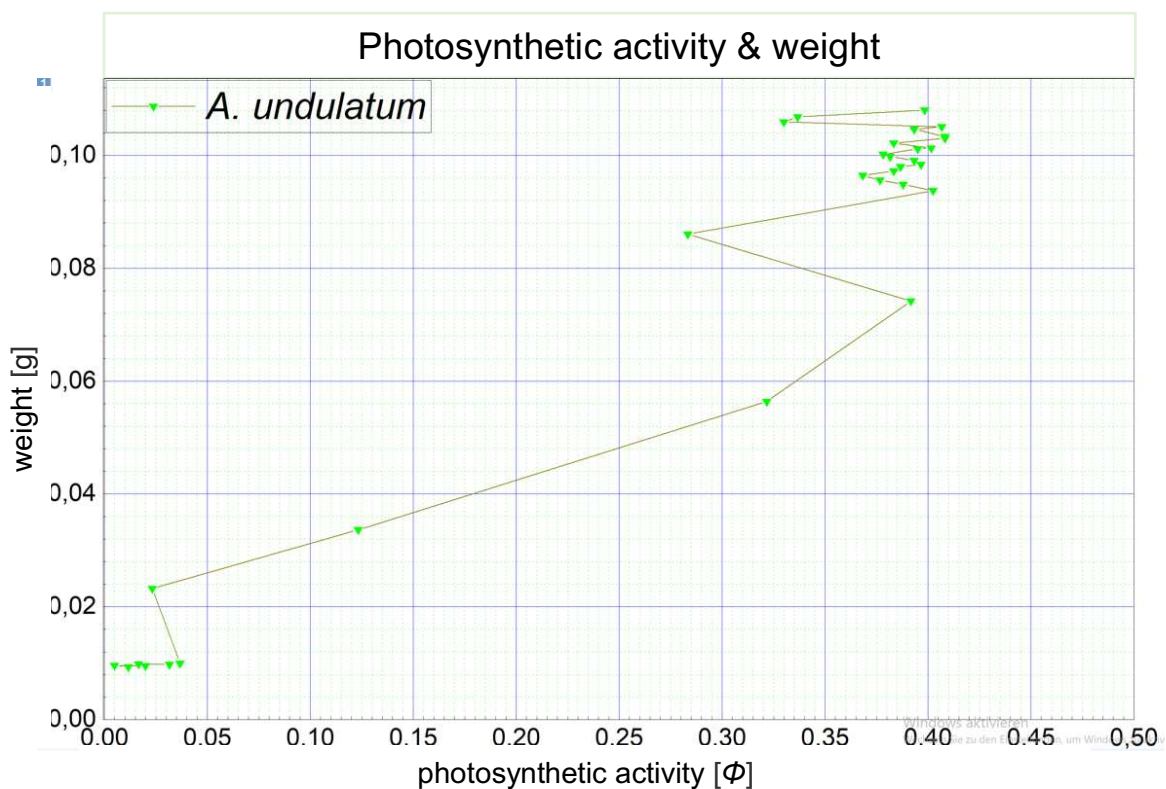


Fig. 30. Graph of *A. undulatum* depicting the photosynthetic activity activity (x-axis), and weight (y-axis) over 48 hours. The time flows from the top right corner to low left bottom.

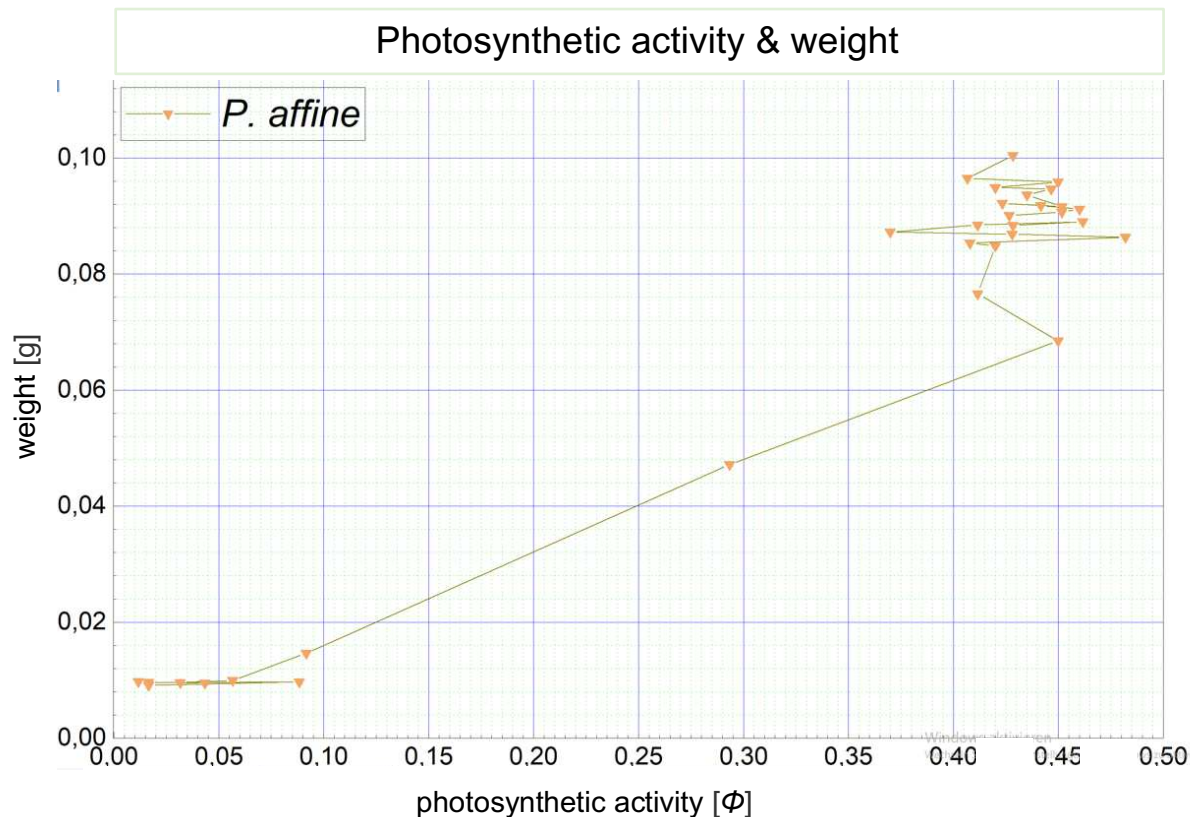


Fig. 31. Graph of *P. affine* depicting the photosynthetic activity activity (x-axis), and weight (y-axis) over 48 hours. The time flows from the top right corner to low left bottom.

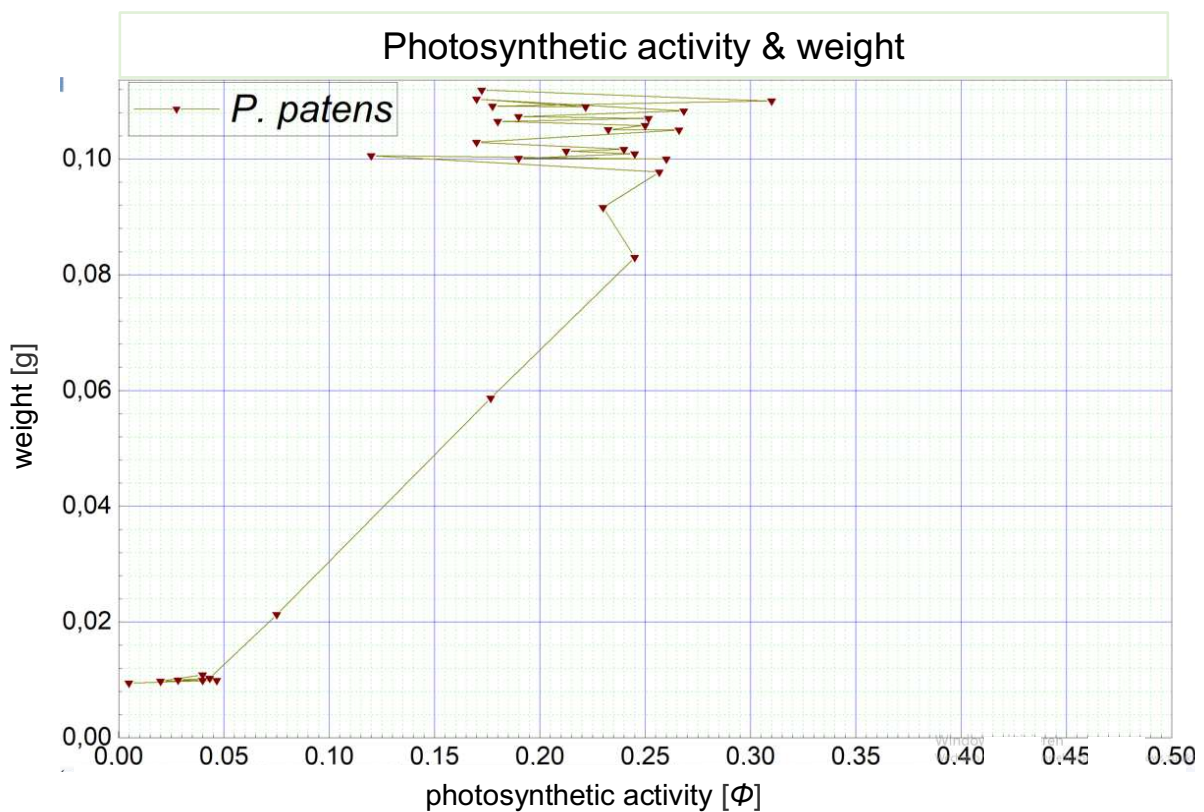


Fig. 32. Graph of *P. patens* depicting the photosynthetic activity (x-axis), and weight (y-axis) over 48 hours. The time flows from the top right corner to low left bottom.

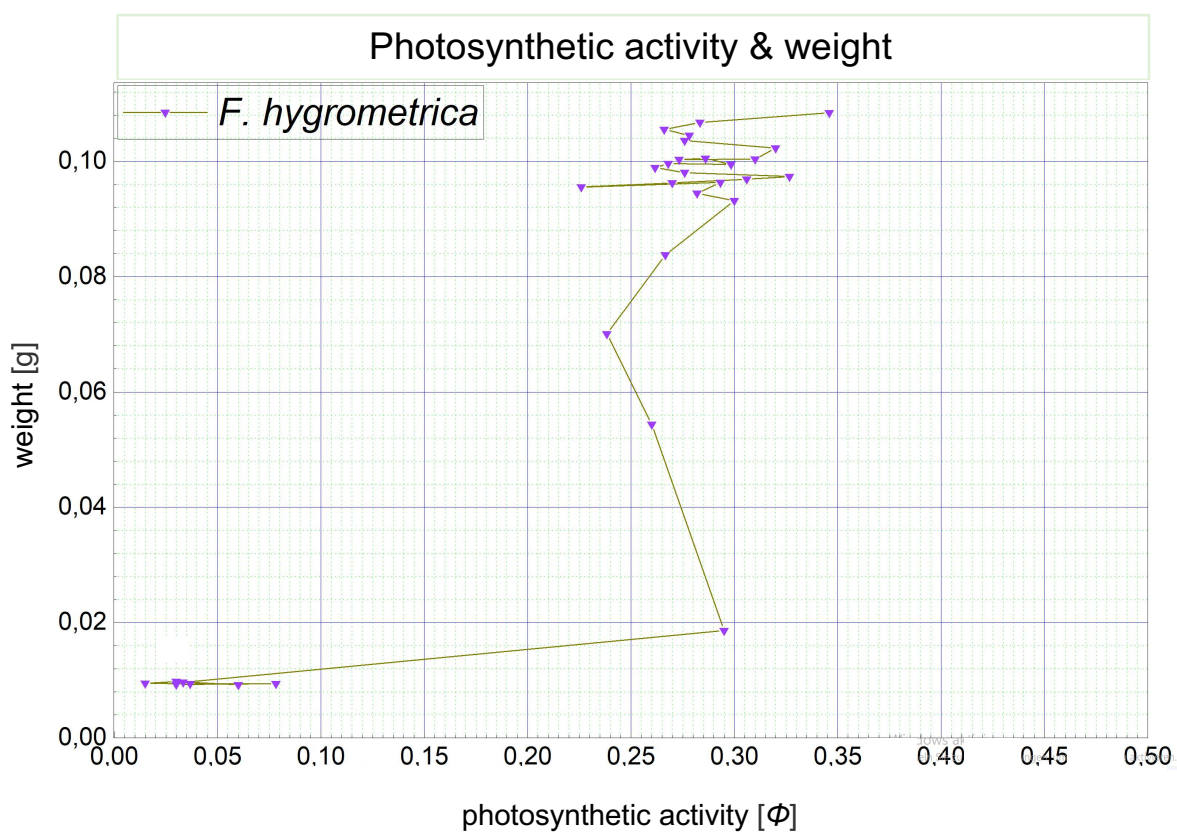


Fig. 33. Graph of *F. hygrometrica* depicting the photosynthetic activity (x-axis), and weight (y-axis) over 48 hours. The time flows from the top right corner to low left bottom.

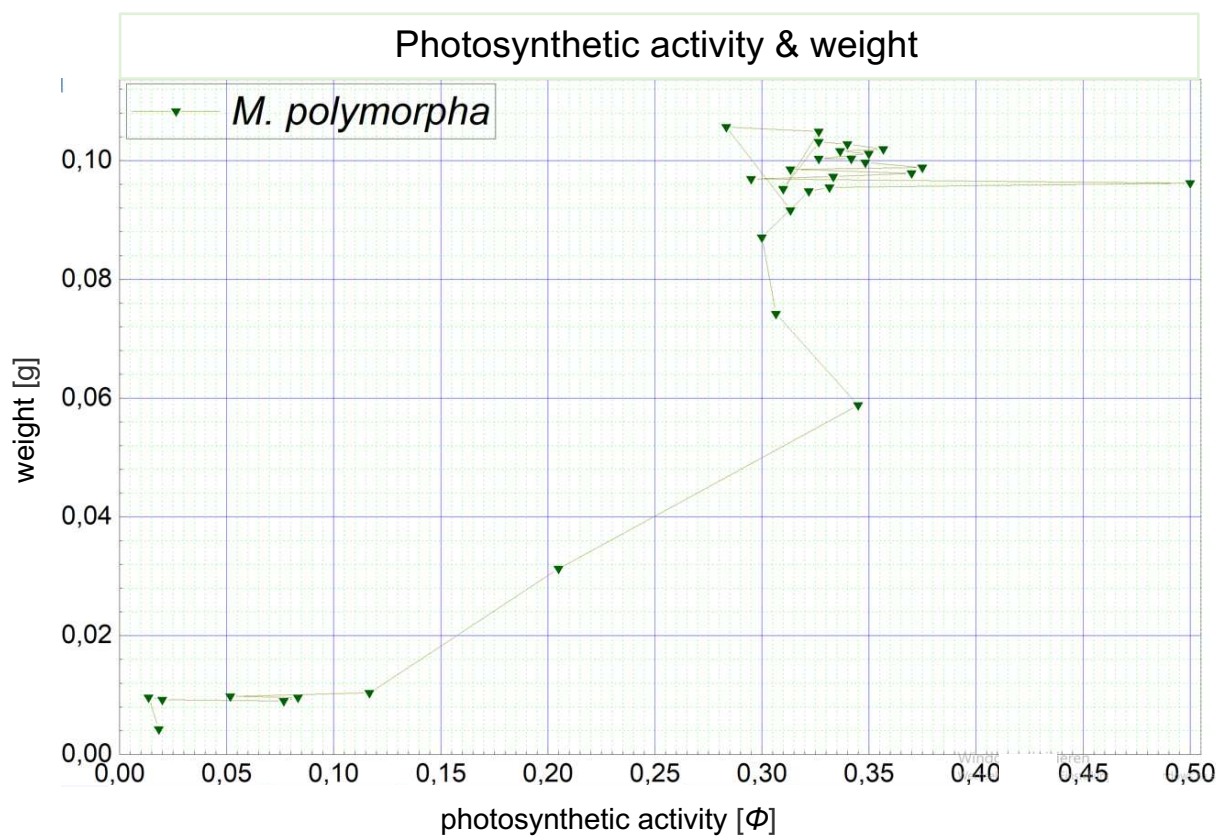


Fig. 34. Graph of *M. polymorpha* depicting the photosynthetic activity (x-axis), and weight (y-axis) over 48 hours. The time flows from the top right corner to low left bottom.

3.2. Rehydration

3.2.1. Photosynthetic activity

The tables “Photosynthetic activity over 120 min” (Fig. 35. & 36.A) provide insights into the rehydration behavior of photosynthetic activity. Table 9 summarizes the quantum yield (Φ) percentage and its decline (%) observed during the rehydration process of all investigated species. The photosynthetic activity was the only parameter investigated during rehydration. During the 120-minute experiment, 200 μ l water was added once at the beginning. In the first 30 minutes, rehydration resulted in a gradual increase in photosynthesis. The fastest increase was observed in *P. affine*, followed by *F. hygrometrica*, *M. polymorpha*, and *A. undulatum*. From 30-90 minutes, dehydration takes place, with a decline in photosynthesis (Fig. 35. & 36.A).

A. undulatum has the highest value with a 140 % increase, compared to the initial photosynthetic activity, measured at 0 minutes. The lowest point was documented after experiencing a loss of 70 %. *P. affine*, the highest value is documented at a 3 % increase, and the lowest after having lost 86 %. In *P. patens*, an increase or 40 % was documented at the highest point, and the lowest point was a 65 % decline at 75 minutes. The highest increase in *F. hygrometrica* was 40 %, was at 30 minutes, much later than in the other mosses. The lowest point of *F. hygrometrica* was at a decline of 75 %. In *M. polymorpha*, the highest increase was 46%, documented at 10 minutes, and the lowest, with a loss of 76 % at 120 minutes (Fig. 35. & 36.A). A positive Pearson correlation (Tab. 11.) between 0.60-0.80 was documented for all species, in *A. undulatum* and *P. affine*, a correlation of 0.77826 with a p-value of 0.00287 was observed. *F. hygrometrica* and *A. undulatum* 0,69086 showed positive correlation and a significant p-value of 0,01286. Positive correlations were observed in *A. undulatum* and *P. affine* 0,7849 and a significant p-value of 0,00249, in *A. undulatum* and *P. patens* 0.60547 with a significant p-value of 0.03695 (Tab. 11.).

Tab. 9. Quantum yield (Φ), percent, and decline (%) during rehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Quantum yield (Φ) during rehydration																
	<i>A. undulatum</i>			<i>P. affine</i>			<i>P. patens</i>			<i>F. hygrometrica</i>			<i>M. polymorpha</i>			
	Φ	%	decl.	Φ	%	decl.	Φ	%	decl.	Φ	%	decl.	Φ	%	decl.	
0min	0.202	100.0	0.0	0.443	100.0	0.0	0.173	100.0	0.0	0.293	100.0	0.0	0.252	100.0	0.0	
5 min	0.288	143.0	-43.0	0.385	86.9	13.1	0.165	95.4	4.6	0.318	108.5	-8.5	0.295	117.1	-17.1	
10 min	0.275	136.4	-36.4	0.392	88.5	11.5	0.172	99.4	0.6	0.320	109.2	-9.2	0.367	145.6	-45.6	
15 min	0.243	120.7	-20.7	0.458	103.4	-3.4	0.168	97.1	2.9	0.277	94.5	5.5	0.253	100.4	-0.4	
20 min	0.213	105.8	-5.8	0.392	88.5	11.5	0.198	114.5	-14.5	0.373	127.3	-27.3	0.322	127.8	-27.8	
25 min	0.225	111.6	-11.6	0.338	76.3	23.7	0.245	141.6	-41.6	0.315	107.5	-7.5	0.243	96.4	3.6	
30 min	0.267	132.2	-32.2	0.410	92.6	7.4	0.222	128.3	-28.3	0.410	139.9	-39.9	0.363	144.0	-44.0	
45 min	0.202	100.0	0.0	0.417	94.1	5.9	0.238	137.6	-37.6	0.320	109.2	-9.2	0.265	105.2	-5.2	
60 min	0.177	87.6	12.4	0.372	84.0	16.0	0.237	137.0	-37.0	0.195	66.6	33.4	0.202	80.2	19.8	
75 min	0.198	98.3	1.7	0.237	53.5	46.5	0.060	34.7	65.3	0.072	24.6	75.4	0.147	58.3	41.7	
90 min	0.160	79.3	20.7	0.148	33.4	66.6	0.112	64.7	35.3	0.123	42.0	58.0	0.080	31.7	68.3	
120 min	0.060	29.8	70.2	0.063	14.2	85.8	0.198	114.5	-14.5	0.115	39.2	60.8	0.060	23.8	76.2	

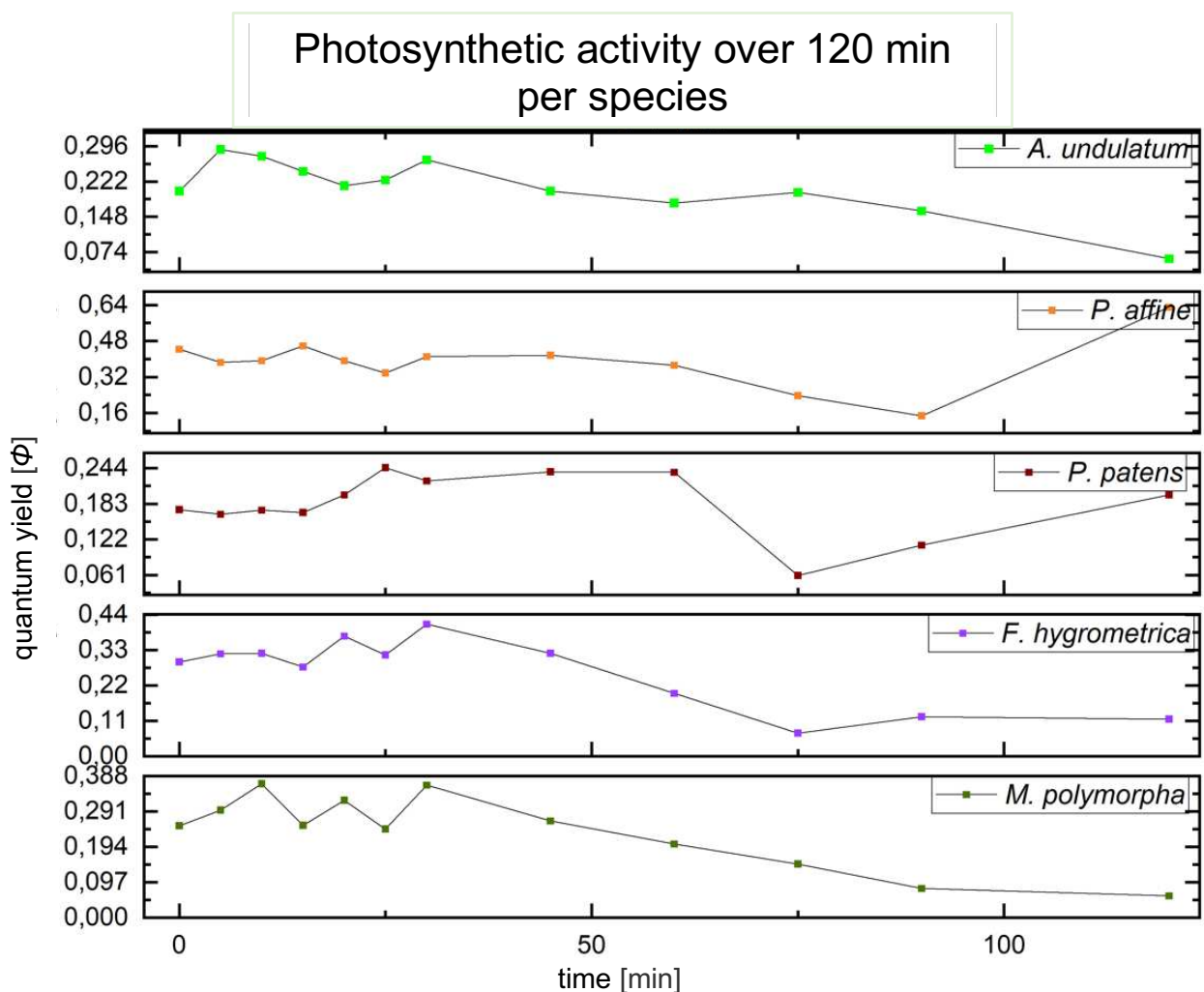


Fig. 35. Graph depicting the of the mean photosynthetic activity (120 minutes rehydration), separated for each species, including *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

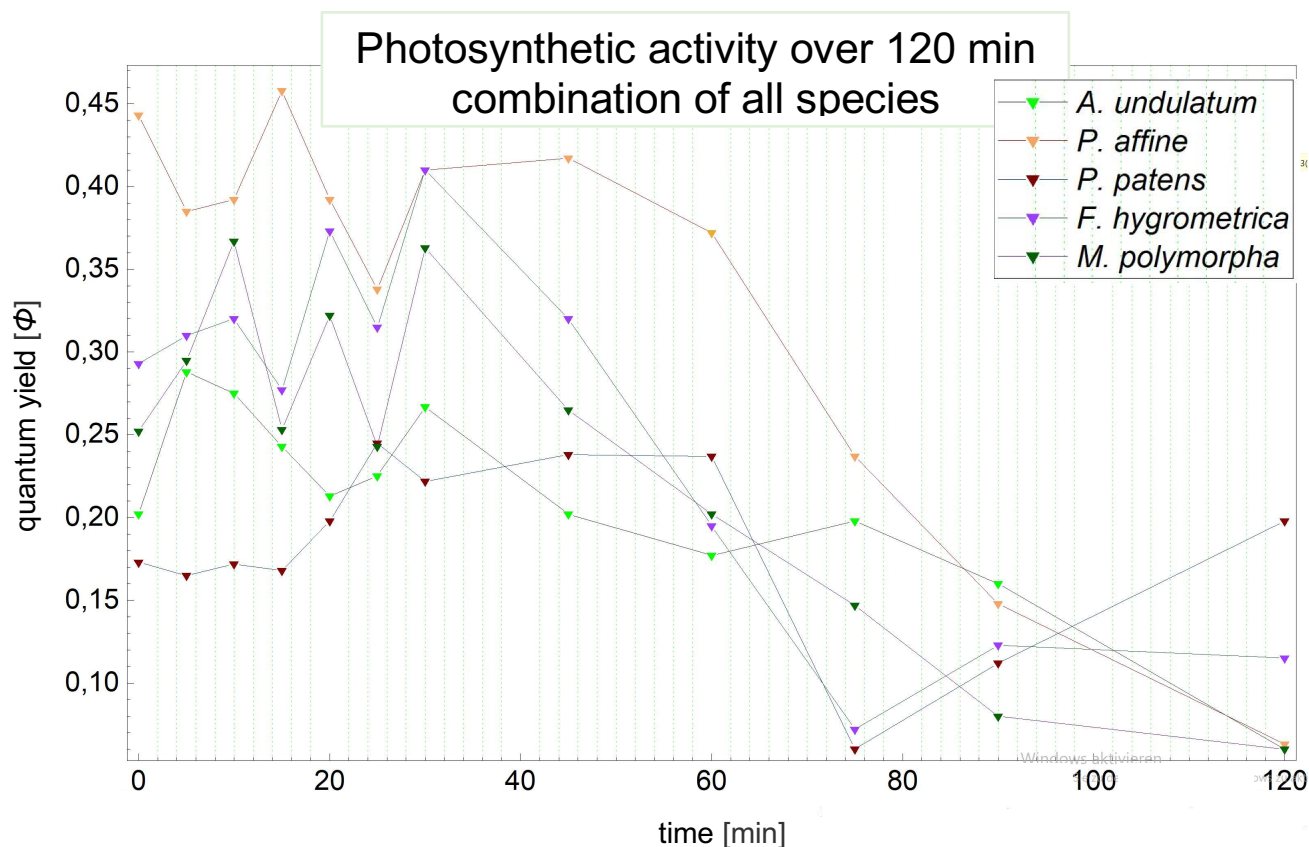


Fig. 36.A) Quantum yield lines for all species *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

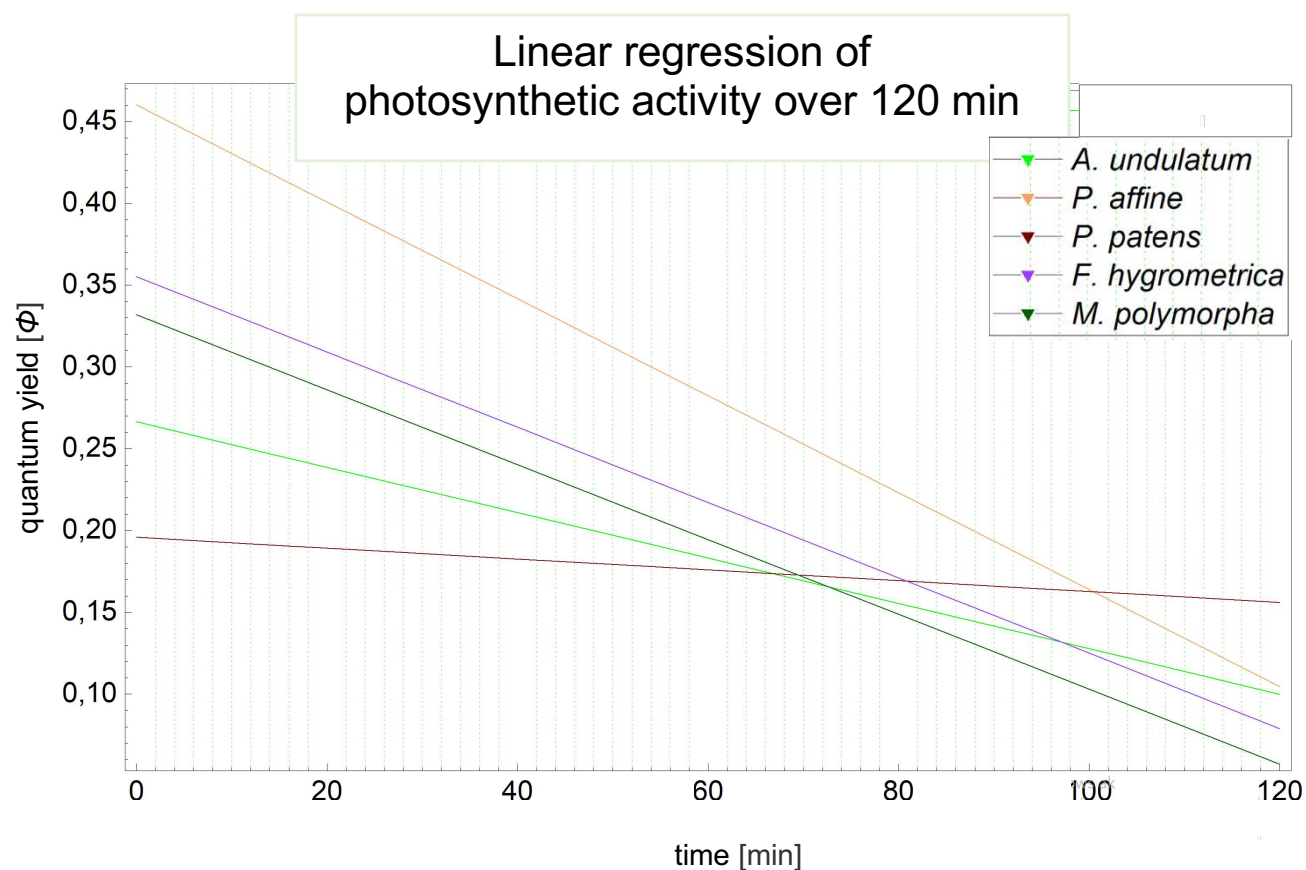


Fig. 36.B) Linear regression lines of the quantum yield for each species *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

The linear regression (Fig. 36.B) of *P. affine* with the highest starting point, followed by *F. hygrometrica*, and *M. polymorpha* which showed a similar steepness of the lines, with large differences in starting points (on the y-axis). *A. undulatum* had the fourth- highest starting point and showed a flatter regression line, compared to the species mentioned above. *P. patens* had the lowest starting point of all species, and the linear regression line is close to parallel to the x-axis.

Tab. 10. Descriptive statistics of the PAM during rehydration (120 min).

PAM 120 minutes	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	12	0,20917	0,06123	2,51	0,06	0,288
<i>P. affine</i>	12	0,33792	0,12357	4,055	0,06	0,458
<i>P. patens</i>	12	0,18233	0,05451	2,188	0,06	0,245
<i>F. hygrometrica</i>	12	0,26025	0,10818	3,123	0,07	0,41
<i>M. polymorpha</i>	12	0,23742	0,09998	2,849	0,06	0,367

Tab. 11. Pearson correlation coefficient and p-value of the rehydration PAM (120 min).

PAM 120 minutes		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	0,77826	0,03299	0,69086	0,8513
<i>A. undulatum</i>	p-value	--	0,00287	0,91892	0,01286	4,43683
<i>P. affine</i>	Pearson cor.	0,77826	1	0,36836	0,7849	0,84998
<i>P. affine</i>	p-value	0,00287	--	0,23873	0,00249	4,62611
<i>P. patens</i>	Pearson cor.	0,03299	0,36836	1	0,60547	0,38214
<i>P. patens</i>	p-value	0,91892	0,23873	--	0,03695	0,22024
<i>F. hygrometrica</i>	Pearson cor.	0,69086	0,7849	0,60547	1	0,91065
<i>F. hygrometrica</i>	p-value	0,01286	0,00249	0,03695	--	3,85486
<i>M. polymorpha</i>	Pearson cor.	0,8513	0,84998	0,38214	0,91065	1
<i>M. polymorpha</i>	p-value	4,43683	4,62611	0,22024	3,85486	--

Analysis of the **Pearson correlation coefficient** (Tab. 11.) and of the “Photosynthetic activity 120 minutes” and the corresponding graph “Photosynthetic activity” (Fig. 35. & 36.A.) revealed 3 positive correlations, with significant p-values, including in *A. undulatum* and *P. affine* **0.77826** and a significant p-value of 0.00287. Further, a positive correlation was found in *P. affine* and *F. hygrometrica* **0.7849**, with a significant p-value of 0.00249. *P. patens* and *F. hygrometrica* **0.60547** showed a positive correlation and a significant p-value of 0,03695. (Tab. 11)

3.3. Cell area

The first step in the preparation was the removal of moss leaves on a drop of water on a glass slide using fine scissors and forceps was done under the binocular (Nikon M4). Each leaf was transferred to a dry glass slide and covered with a cover glass. Images were taken on a Nikon Eclipse NI. The outline of the cells was marked manually, using the Lasso tool of the software (NIS-Elements BR)

The aim of the hydrated cell area was to determine the size of the cells in their hydrated, original state. For each moss, 10 areas on 5 leaves were selected, in total, resulting in 50 cells (Fig. 53.).

Light microscopy images of *A. undulatum* (Fig. 37.), *P. affine* (Fig. 38.), *P. patens* (Fig. 39.), *F. hygrometrica* (Fig. 40.), and *M. polymorpha* (Fig. 41.) depicted 3 cells marked with arrows, observed over 90 minutes of dehydration. The effect of drought on a cellular level, in the form of cytorrhysis was observed in *P. affine* starting at 45 minutes (Fig. 38.), and *F. hygrometrica* at 10 minutes (Fig. 40.). The fact that *A. undulatum*, *P. patens* and *M. polymorpha* did not show signs of cytorrhysis, indicates that they showed reactions to drought stress. Cytorrhysis is defined as an osmotic collapse, which takes place in the natural environment of mosses (Url, 1974).

A maximum cell area of *A. undulatum* was at the initial measurement at zero minutes, and the strongest loss of 14 % was at 30 minutes. In *P. affine*, the second value was the highest, with an increase of 4 %, and the lowest with a 70 % loss. The initial value was highest, and a decline of 31 % was reached at 25 minutes. In *F. hygrometrica* the initial value was the highest, and at 90 minutes, the lowest value after a loss of 32 % In *M. polymorpha*, the maximum cell area was at the first measurement, and the smallest, after losing 30 %, at 30 minutes (Fig. 42.). Statistical analysis revealed positive and negative correlation coefficients, with only one significant negative correlation of *P. affine* and *F. hygrometrica* -0.43766 with a significant p-value of 0.00148 (Tab.14.).

Atrichum undulatum

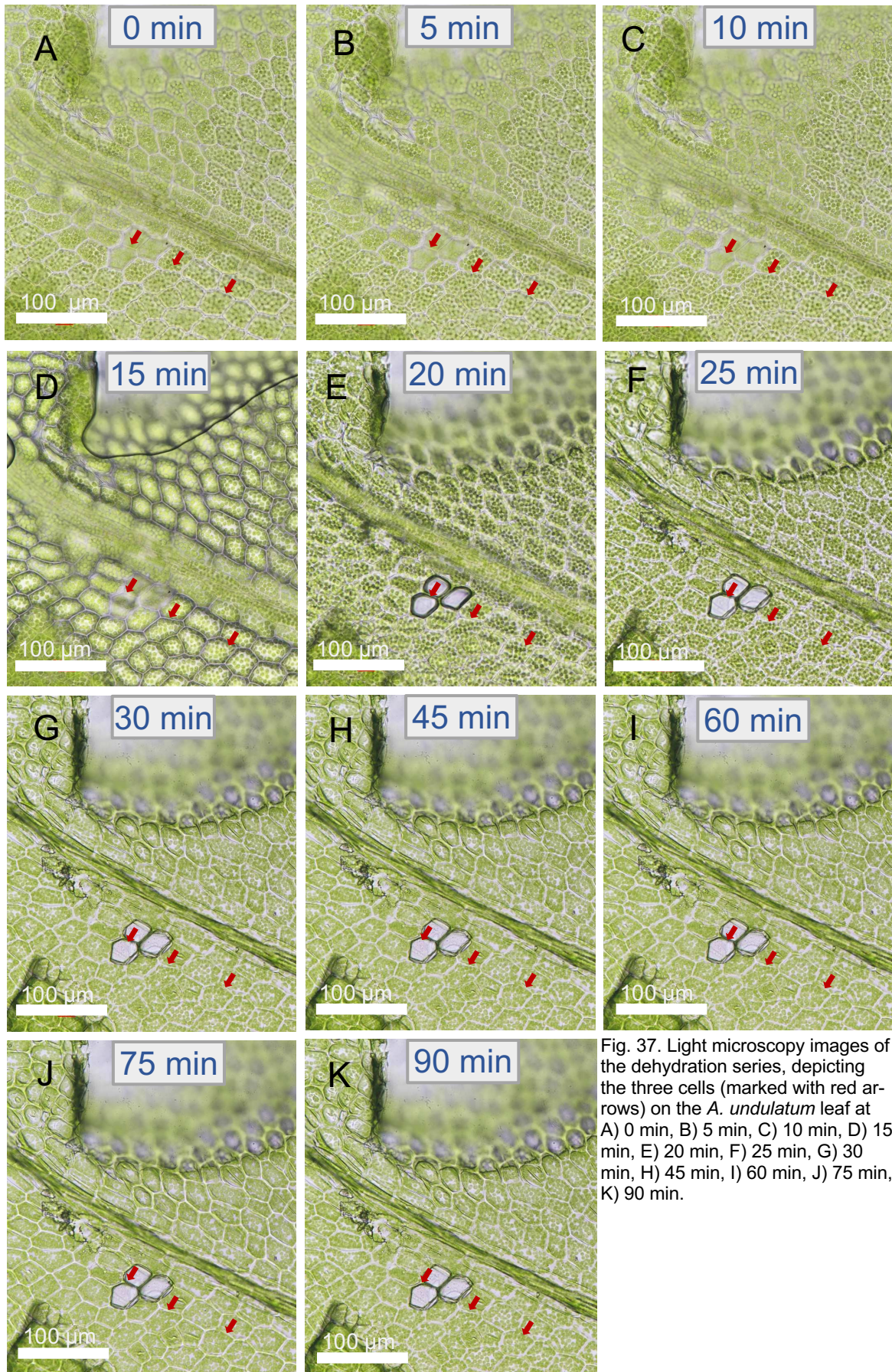


Fig. 37. Light microscopy images of the dehydration series, depicting the three cells (marked with red arrows) on the *A. undulatum* leaf at A) 0 min, B) 5 min, C) 10 min, D) 15 min, E) 20 min, F) 25 min, G) 30 min, H) 45 min, I) 60 min, J) 75 min, K) 90 min.

Plagiomnium affine

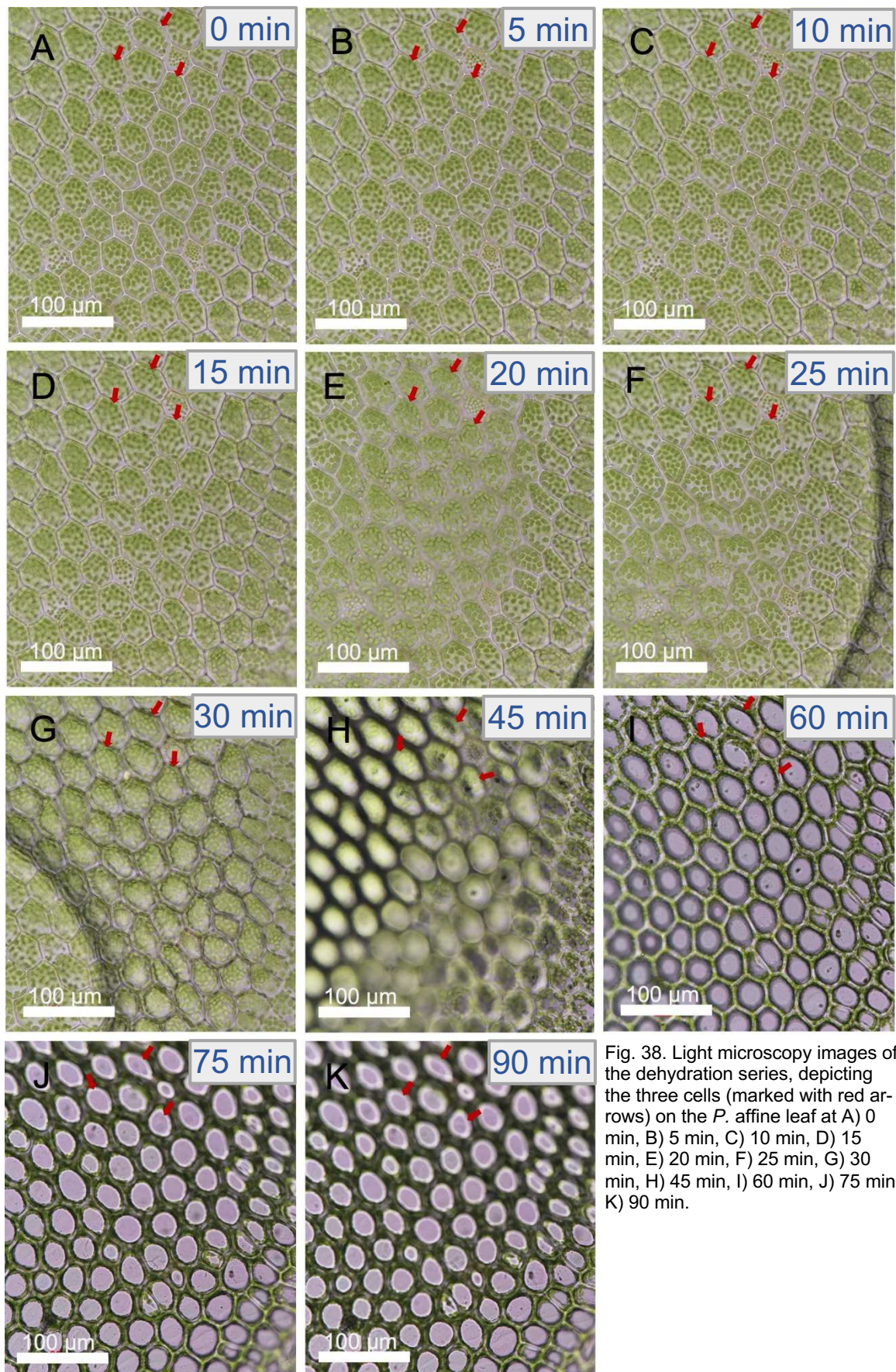


Fig. 38. Light microscopy images of the dehydration series, depicting the three cells (marked with red arrows) on the *P. affine* leaf at A) 0 min, B) 5 min, C) 10 min, D) 15 min, E) 20 min, F) 25 min, G) 30 min, H) 45 min, I) 60 min, J) 75 min, K) 90 min.

Physcomitrium patens

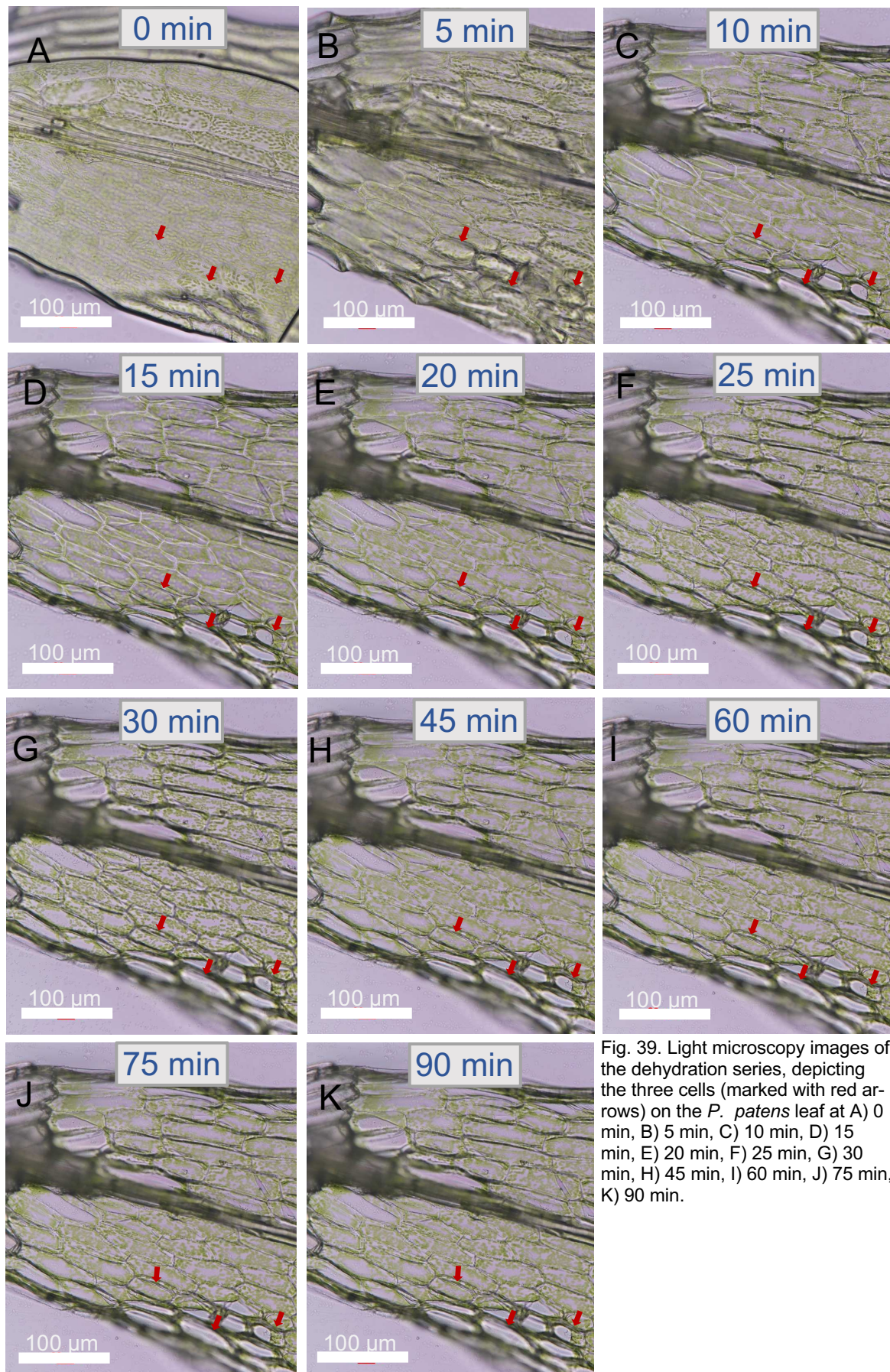


Fig. 39. Light microscopy images of the dehydration series, depicting the three cells (marked with red arrows) on the *P. patens* leaf at A) 0 min, B) 5 min, C) 10 min, D) 15 min, E) 20 min, F) 25 min, G) 30 min, H) 45 min, I) 60 min, J) 75 min, K) 90 min.

Funaria hygrometrica

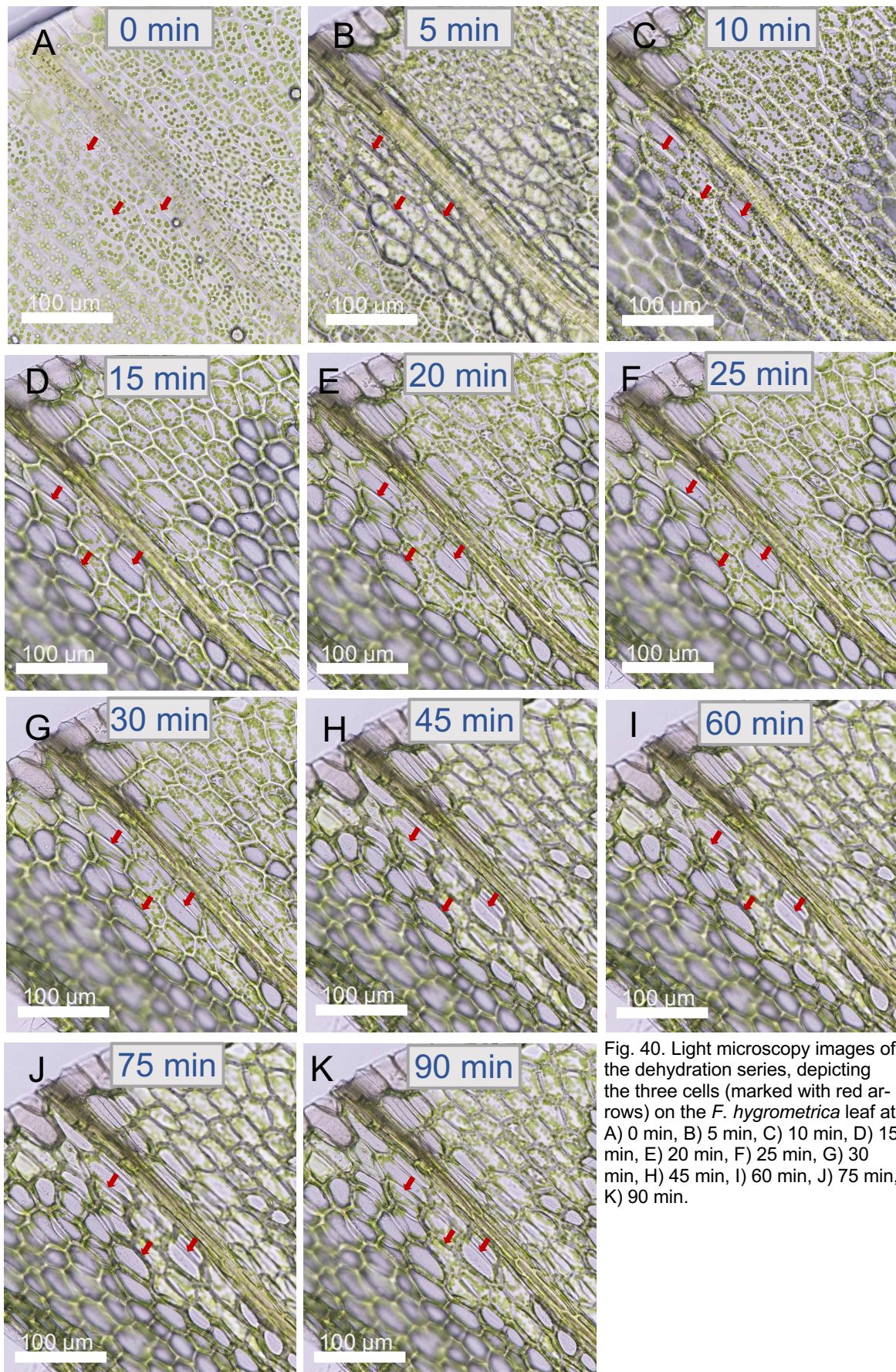


Fig. 40. Light microscopy images of the dehydration series, depicting the three cells (marked with red arrows) on the *F. hygrometrica* leaf at A) 0 min, B) 5 min, C) 10 min, D) 15 min, E) 20 min, F) 25 min, G) 30 min, H) 45 min, I) 60 min, J) 75 min, K) 90 min.

Marchantia polymorpha

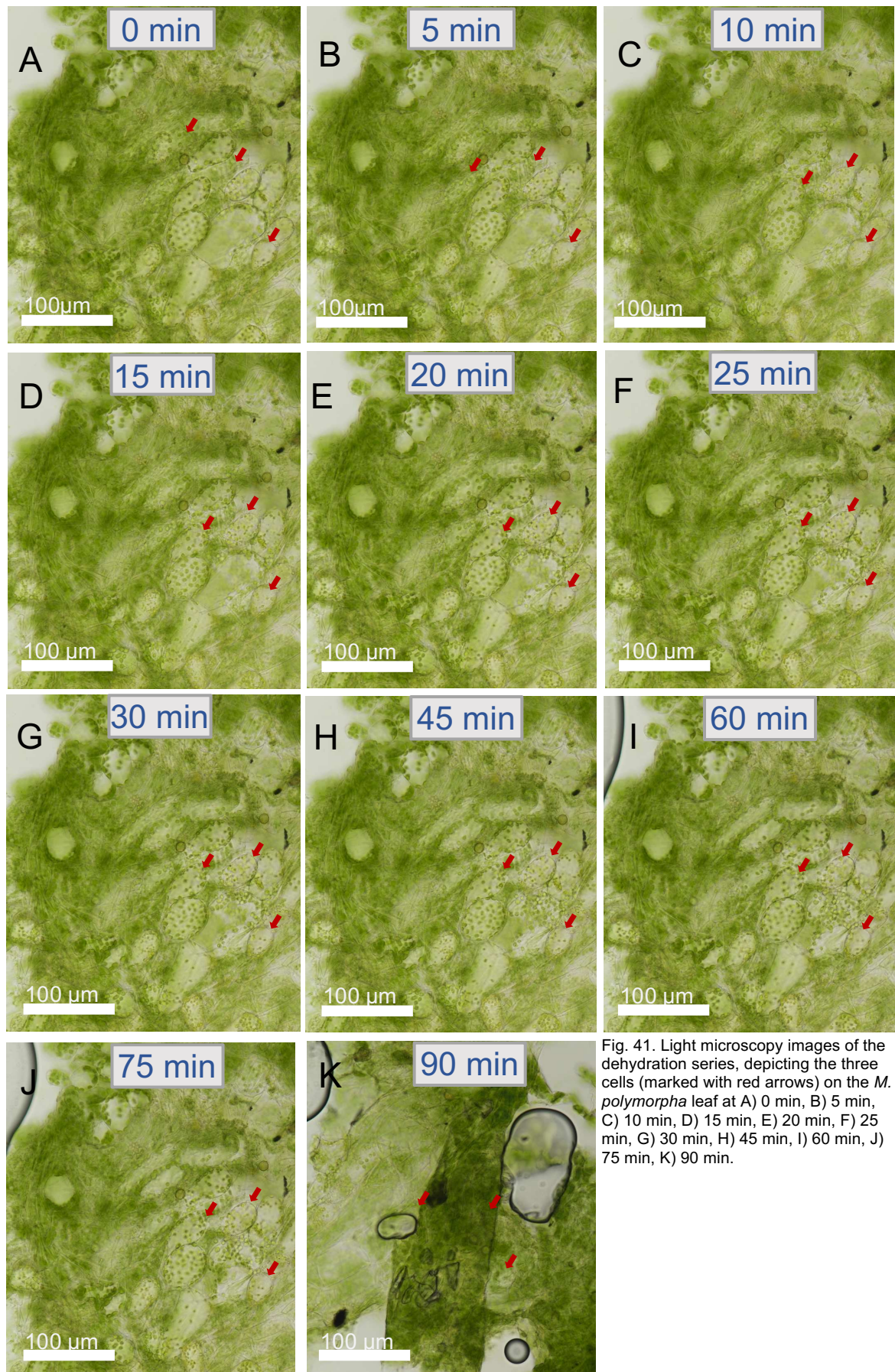


Fig. 41. Light microscopy images of the dehydration series, depicting the three cells (marked with red arrows) on the *M. polymorpha* leaf at A) 0 min, B) 5 min, C) 10 min, D) 15 min, E) 20 min, F) 25 min, G) 30 min, H) 45 min, I) 60 min, J) 75 min, K) 90 min.

The analysis of Table 12. summarizes the mean cell areas, their percentage, and the decline (%) during dehydration. Additionally, the graph “Cell area over 90 min combination of all species” (Fig. 42.) and the corresponding light microscopy images of *A. undulatum* (Fig. 37.), *P. affine* (Fig. 38.), *P. patens* (Fig. 39.), *F. hygrometrica* (Fig. 40.), and *M. polymorpha* (Fig. 41.) reveal that ***A. undulatum*** has the lowest initial cell area among all species. After reaching its highest cell area at the beginning of $605.624 \mu\text{m}^2$, a gradual decrease takes place, until the lowest point of $523.830 \mu\text{m}^2$ is reached at 30 minutes, having lost 13 % of the initial area. At 60 minutes, a small decline in the area took place and remained stable from 75-90 minutes (Fig.42. & 37.).

After ***P. affine*** showed an initial cell area of $1447.814 \mu\text{m}^2$, the peak of $1509.553 \mu\text{m}^2$ is reached at 5 minutes. Between 5-10 minutes, it experiences a distinct decline, losing 76 % of the initial cell area. From 15 - 20 minutes, it remains stable and reaches a low point at 25 minutes, increases slightly at 30 minutes. After undergoing a strong decline at 30-45 minutes, the cell area continues to decrease between 45-90 minutes. The lowest point of $438.832 \mu\text{m}^2$ was observed at 90 minutes, having lost 70 % of the initial area (Fig. 42. & 38.).

In ***P. patens***, the starting cell area of $869.438 \mu\text{m}^2$ was the highest value documented and underwent a decline of 68 % in the first 15 minutes, the lowest value of $594.347 \mu\text{m}^2$ was reached. After experiencing a slight increase between 15-20 minutes, a flat phase from 30-45 minutes was observed. At 60 minutes, a local peak is reached, then continued to decline from 60 -75 minutes, and remained stable from 75 – 90 minutes (Fig.42. & 39.). The initial value $948.724 \mu\text{m}^2$ in ***F. hygrometrica***, was the highest one documented in this species. From 0-30 minutes, it declined gradually and after at 30 minutes, a local minimum was reached, the cell area slightly increased at 45 minutes. From 45 to 90 minutes, it experienced a stable phase, with a minor decline. At 90 minutes, the lowest value of $641.064 \mu\text{m}^2$ is reached, having lost 32.4% of its initial value (Fig. 42. & 40.).

M. polymorpha displayed the highest initial cell area of $1710.644 \mu\text{m}^2$ area from all species. In the first 5 minutes, it underwent a strong decline, losing 86 % of the initial area, reaching $1466.115 \mu\text{m}^2$. Between 5-10 minutes, a 14 % decline was seen and from 10-25 minutes, 24 % were lost. At 30 minutes, the decline of 30%

was documented. From 45-60 minutes, a slight increase took place and at 60 minutes, a local peak was reached. From 75-90 minutes, the cell area continued to increase, reaching the lowest point of 641.064 μm^2 at 90 minutes (Fig. 42. & 41.).

Tab.12. Cell area (μm^2), percent, and decline (%) during dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Cell area (μm^2) during deydration															
	<i>A. undulatum</i>			<i>P. affine</i>			<i>P. patens</i>			<i>F. hygrometrica</i>			<i>M. polymorpha</i>		
	μm^2	%	decl.	μm^2	%	decl.	μm^2	%	decl.	μm^2	%	decl.	μm^2	%	decl.
0 min	605.624	100	0	1447.814	100.0	0	869.438	100	0	948.724	100	0	1710.644	100	0
5 min	583.559	96.4	3.6	1509.553	104.3	-4.3	707.781	81.4	18.6	891.670	94.0	6.0	1466.115	85.7	14.3
10 min	554.061	91.5	8.5	1104.537	76.3	23.7	664.628	76.4	23.6	865.243	91.2	8.8	1401.983	82.0	18.0
15 min	531.396	87.7	12.3	992.162	68.5	31.5	594.347	68.4	31.6	814.256	85.8	14.2	1388.464	81.2	18.8
20 min	537.923	88.8	11.2	985.871	68.1	31.9	600.037	69.0	31.0	745.081	78.5	21.5	1294.239	75.7	24.3
25 min	533.915	88.2	11.8	909.269	62.8	37.2	631.904	72.7	27.3	650.010	68.5	31.5	1291.961	75.5	24.5
30 min	523.830	86.5	13.5	952.439	65.8	34.2	615.873	70.8	29.2	631.092	66.5	33.5	1205.044	70.4	29.6
45 min	554.546	91.6	8.4	687.213	47.5	52.5	619.774	71.3	28.7	689.211	72.6	27.4	1215.221	71.0	29.0
60 min	539.772	89.1	10.9	608.057	42.0	58.0	716.826	82.4	17.6	667.198	70.3	29.7	1253.318	73.3	26.7
75 min	559.822	92.4	7.6	470.631	32.5	67.5	666.048	76.6	23.4	658.972	69.5	30.5	1229.574	71.9	28.1
90 min	559.380	92.4	7.6	438.832	30.3	69.7	667.217	76.7	23.3	641.064	67.6	32.4	1352.963	79.1	20.9

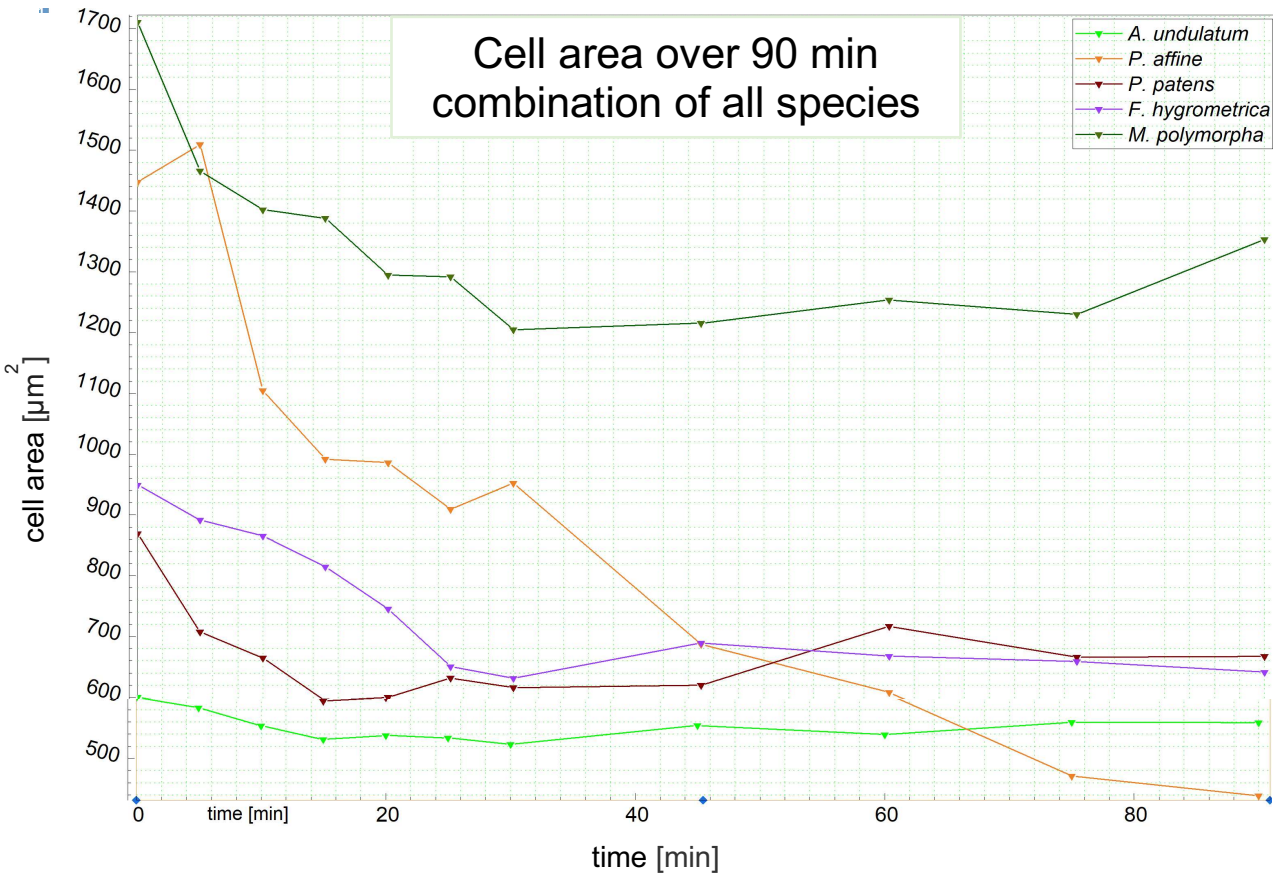


Fig.42. Mean cell area during dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, *M. polymorpha*, measured at 11 points, over 90 minutes.

Tab. 13. Descriptive statistics of the cell area (90 min).

Cell area 90 minutes	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	50	551,0844	184,2521	27554	318	1402
<i>P. affine</i>	50	1426,978	456,19406	71348,9	557	2242
<i>P. patens</i>	50	870,3318	409,90837	43517	269	2277
<i>F. hygrometrica</i>	50	932,6446	437,74715	46632	206	2055
<i>M. polymorpha</i>	50	1498,996	860,42085	74950	461	5020

Tab. 14. Pearson correlation coefficient and p-value of the cell area (90 min).

Cell area 90 minutes		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	-0,05642	0,09604	0,22205	0,006
<i>A. undulatum</i>	p-value	--	0,69716	0,50704	0,12117	0,96701
<i>P. affine</i>	Pearson cor.	-0,05642	1	-0,27252	-0,43766	0,12958
<i>P. affine</i>	p-value	0,69716	--	0,05553	0,00148	0,36979
<i>P. patens</i>	Pearson cor.	0,09604	-0,27252	1	0,25545	-0,27478
<i>P. patens</i>	p-value	0,50704	0,05553	--	0,07338	0,05346
<i>F. hygrometrica</i>	Pearson cor.	0,22205	-0,43766	0,25545	1	0,15079
<i>F. hygrometrica</i>	p-value	0,12117	0,00148	0,07338	--	0,2959
<i>M. polymorpha</i>	Pearson cor.	0,006	0,12958	-0,27478	0,15079	1
<i>M. polymorpha</i>	p-value	0,96701	0,36979	0,05346	0,2959	--

Table (14.) contains the **Pearson correlation coefficient** and p-values for the data presented in “Dehydration Cell Area (90 min)” (Fig. 42). One negative, significant correlation was of **-0.43766** in *P. affine* and *F. hygrometrica* was observed (Tab. 14.).

3.4. Hydrated cell area

The purpose of the hydrated cell area was to measure the size of the cells in their natural, hydrated state. To achieve this, 10 areas on 5 different leaves were chosen, resulting in a total of 50 cells (Fig. 42). Cells in the initial hydrated states are seen in (Fig. 43.A). Characteristic for *A. undulatum* revealed square-shaped cells with broad cell membranes and an abundance of small, round chloroplasts, tightly stacked together (Fig. 43.B). In *P. affine*, larger cells with a round center, with small, with large, almost spherical, chloroplasts were observed (Fig. 43.C).

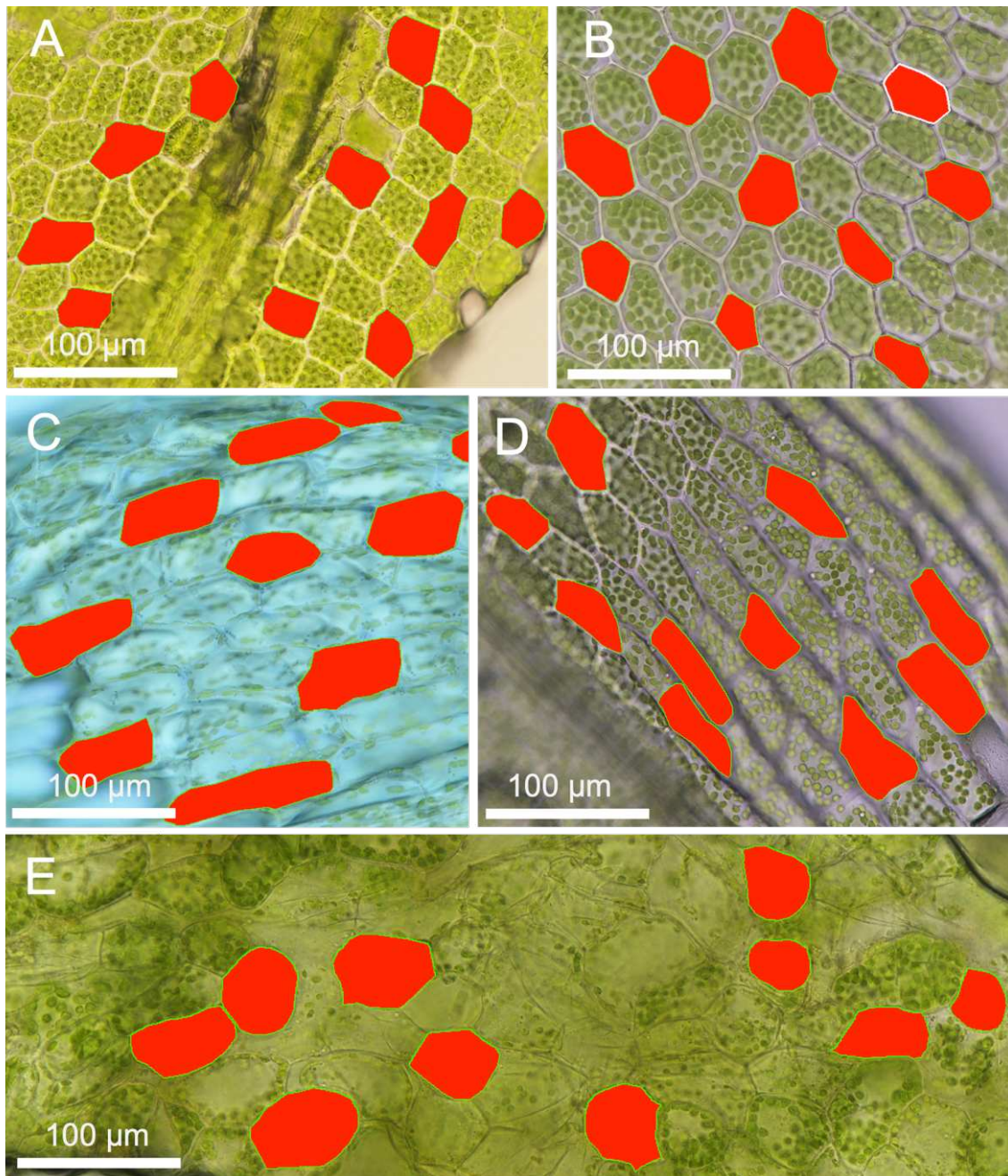


Fig. 43. Cells in their initial hydrated state. Per leaf, 10 cells were marked with the NIS Elements lasso tool and the mean cell area for each species in the turgid state was determined. A) *A. undulatum*, B) *P. affine*, C) *P. patens*, D) *F. hygrometrica*, and E) *M. polymorpha*.

F. hygrometrica has long, spherical cells with almost transparent protoplast, containing very few, small and elongated chloroplasts (Fig. 43.D). In *P. patens*, thin, elongated cells with small, round chloroplasts were seen (Fig. 43.D). In *M. polymorpha*, the cells marked were palisade parenchyma cells. They are large and round with relatively few small round chloroplasts, located on the cell surface (Fig. 53.E).

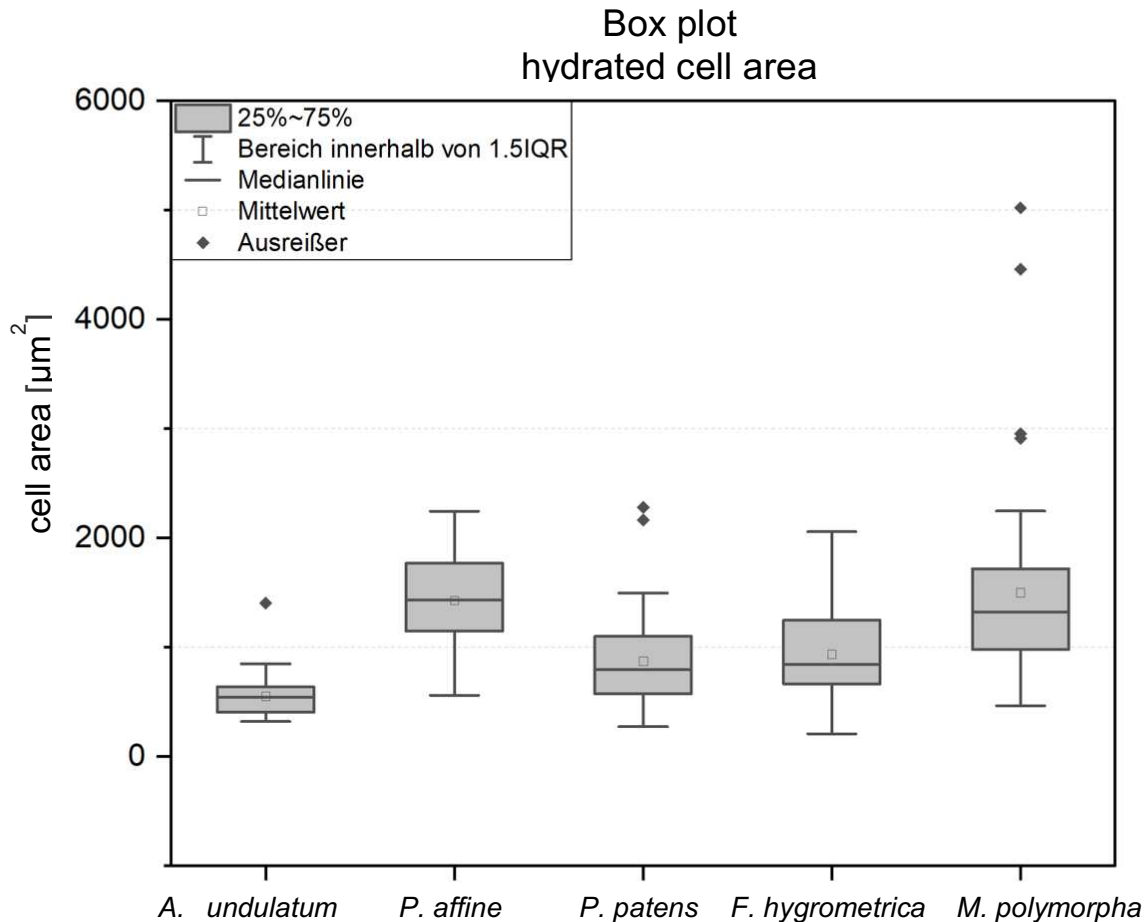


Fig. 44. Box plot depicting the area of 50 initial hydrated cells of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica* and *M. polymorpha*.

The statistical analysis of 50 initially hydrated cells revealed the hydrated cell areas *A. undulatum* (551 µm²), *P. affine* (1427 µm²), *P. patens* (870 µm²), *F. hygrometrica* (933 µm²), and *M. polymorpha* (1499 µm²) (**Fig. 44.**). All data area depicted in the Box plot (Fig. 44.) In Table 15, the mean hydrated cell area and standard deviation (SD) for all species are summarized.

Tab. 15. Mean hydrated cell area (μm^2) and standard deviation (SD) of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Initial, hydrated cell area (μm^2)										
<i>A. undulatum</i>		<i>P. affine</i>		<i>P. patens</i>		<i>F. hygrometrica</i>		<i>M. polymorpha</i>		
μm^2	SD	μm^2	SD	μm^2	SD	μm^2	SD	μm^2	SD	
551.08	182.40	1426.98	451.61	1426.98	405.79	932.64	433.35	1499.00	851.77	

T-test results of the **hydrated cell area** depicted in Table (16.) revealed significant differences in several species. The direction of the t-value (+/-) determines in which species has the larger hydrated cell area. Significant values ($p < 0.05$) confirm meaningful differences, while non-significant values indicate similar cell sizes between species.

A. undulatum exhibited a significantly different cell area compared to *P. affine* ($p = 0.00039$) and *M. polymorpha* ($p = 0.00068$), but not when compared to *P. patens* or *F. hygrometrica*. Similarly, *P. affine* showed a significant difference in cell area compared to *F. hygrometrica* ($p = 0.00742$) and *M. polymorpha* ($p = 8.46\text{E-}06$), but not *P. patens*. *P. patens* and *F. hygrometrica* did not differ significantly, while *P. patens* had a significantly different cell area from *M. polymorpha* ($p = 0.00648$). Finally, *F. hygrometrica* and *M. polymorpha* also showed significant differences ($p = 0.00059$). These findings indicate morphological variations in cell area, particularly between *M. polymorpha* and other species, which may reflect distinct ecological or physiological adaptations (Tab.16).

Tab. 16. T- test of the hydrated cell area (μm^2) and species-comparison of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Species- comparison hydrated cells (μm^2)				
T- test	t-value	df	p-value	($p < 0.05$)
<i>A. undulatum</i> vs <i>P. affine</i>	4.37	98	0.00039	significant
<i>A. undulatum</i> vs <i>P. patens</i>	0.53	98	0.6067	x
<i>A. undulatum</i> vs <i>F. hygrometrica</i>	0.31	98	0.7573	x
<i>A. undulatum</i> vs <i>M. polymorpha</i>	-4.68	98	0.00068	significant
<i>P. affine</i> vs <i>P. patens</i>	-1.36	98	0.1949	x
<i>P. affine</i> vs <i>F. hygrometrica</i>	-2.95	98	0.00742	significant
<i>P. affine</i> vs <i>M. polymorpha</i>	-7.55	98	8.46E-06	significant
<i>P. patens</i> vs <i>F. hygrometrica</i>	-0.33	98	0.7483	x
<i>P. patens</i> vs <i>M. polymorpha</i>	-3.11	98	0.00648	significant
<i>F. hygrometrica</i> vs <i>M. polymorpha</i>	-4.21	98	0.00059	significant

3.5. Leaf area

The area of the leaf was measured with the autodetect tool in the Nis Element Software. A series of dehydration light microscopy images of *A. undulatum* (Fig. 45.), *P. affine* (Fig. 46.), *P. patens* (Fig. 47.), *F. hygrometrica* (Fig. 48.), and *M. polymorpha* (Fig. 49.) of the leaf area were taken at 0, 2, 4, and 6 hours. Table (17.) depicts the data of the stereo microscopic image series, providing a summary of the mean leaf area (mm²), percentage, and decline (%) during dehydration.

The strongest decline was observed in the first 2 hours, in *A. undulatum*. which lost 43 %, *P. affine* and *P. patens* 66 %, and *M. polymorpha* 55 %. In *F. hygrometrica* the lowest decrease in leaf area of 25 % was observed. A stable phase from 2-6 hours was revealed in 3 species and *A. undulatum* reached the lowest are, having lost 45 %, *P. affine* 67 %, and *P. patens* 65 %. *F. hygrometrica* was the only bryophyte that deviated from this pattern, with a loss of 25 % from 2-4 hours, and a further decline of 56 % at 6 hours. *M. polymorpha* reached the lowest area from 2-5 hours with a 53 % decline, and experienced a noticeable increase at 6 hours, reaching 45 % (Fig. 49.).

3.5.1. Microscopic images

Atrichum undulatum

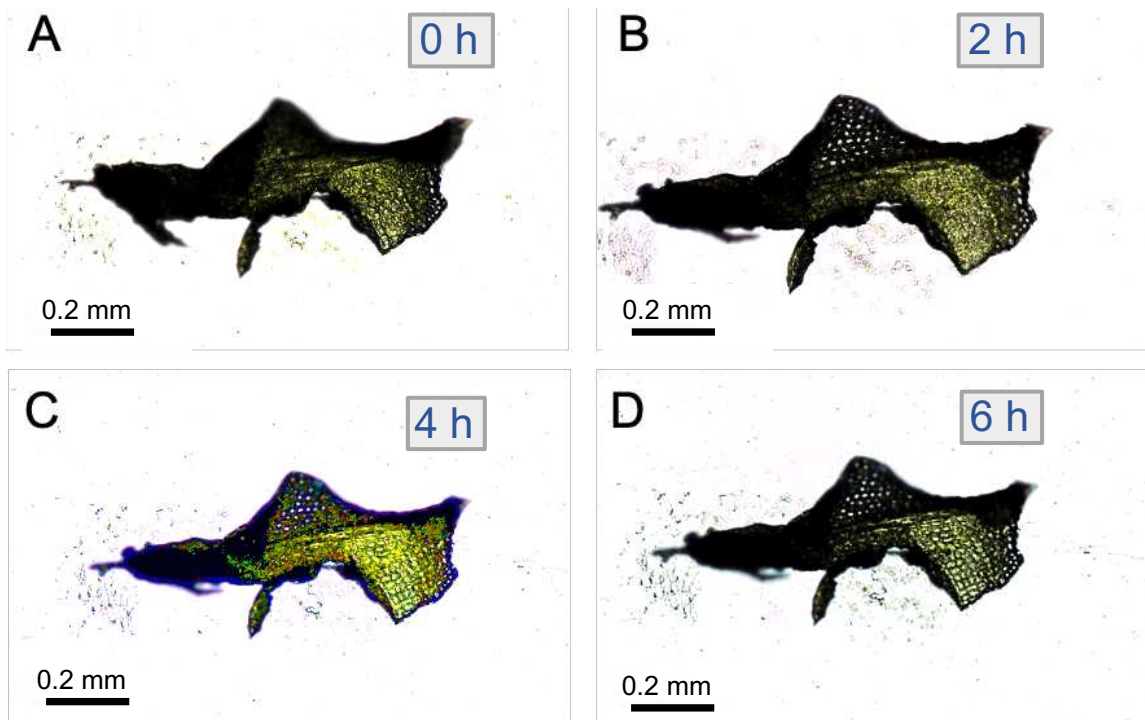


Fig. 45. Light microscopy images show the leaf area of the *A. undulatum* at A) 0 hours, B) 2 hours, C) 4 hours, D) 6 hours after initiation of the dehydration. In total, an area loss of 1.3 % was observed.

Plagiomnium affine

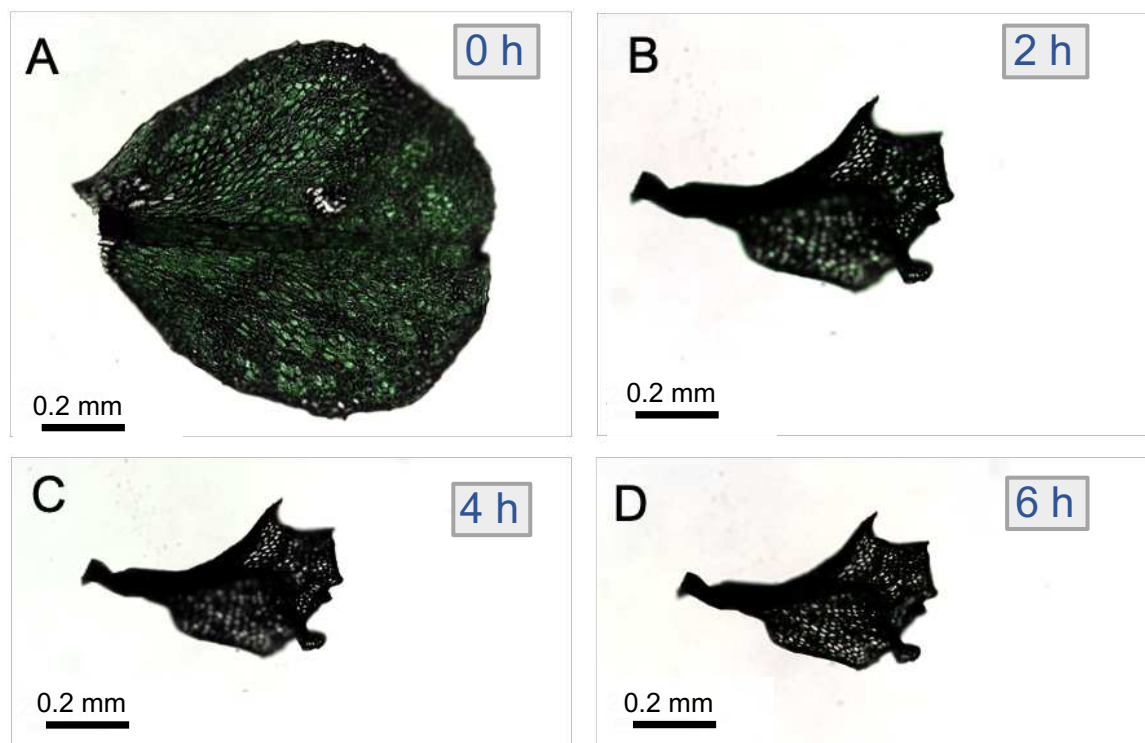


Fig. 46. Light microscopy images show the leaf area of *P. affine* at A) 0 hours, B) 2 hours, C) 4 hours, D) 6 hours after initiation of the dehydration. In total, an area loss of 75 % was observed.

Physcomitrium patens

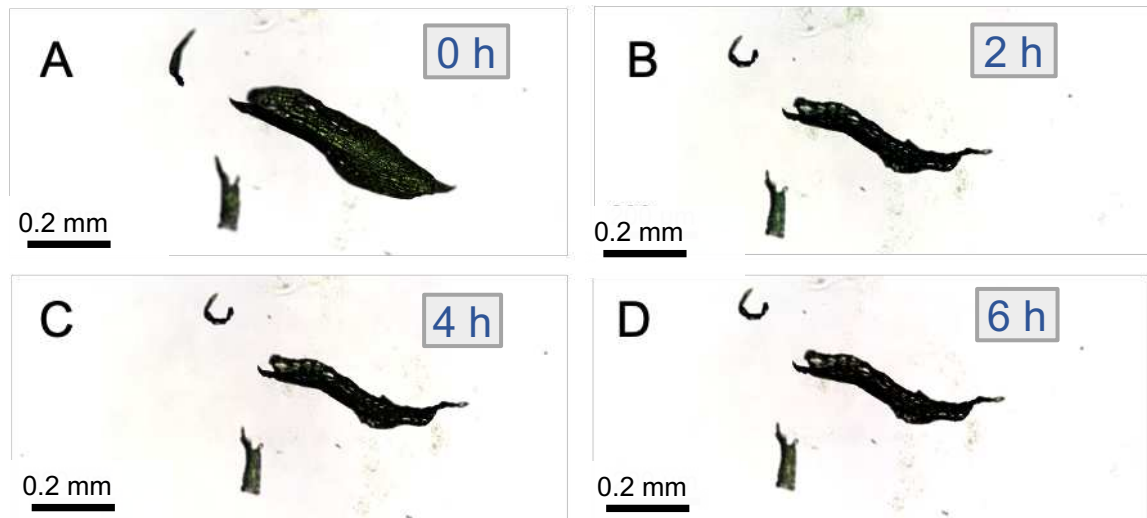


Fig. 47. Light microscopy images show the leaf area of *P. patens* at A) 0 hours, B) 2 hours, C) 4 hours, D) 6 hours after initiation of the dehydration. In total, an area loss of 5.3 % was observed.

Funaria hygrometrica

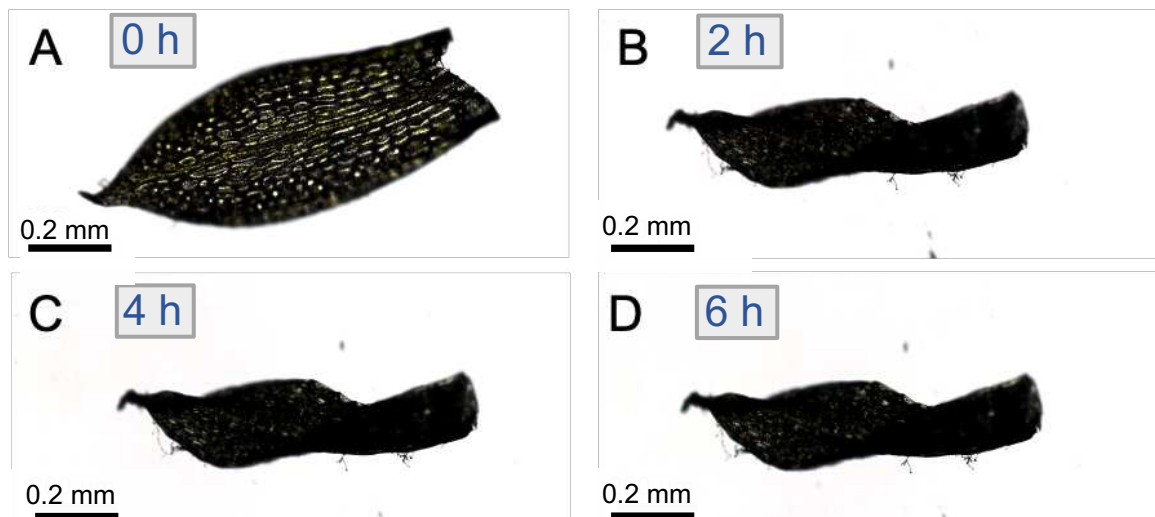


Fig. 48. Light microscopy images show the leaf area of the *F. hygrometrica* leaf at A) 0 hours, B) 2 hours, C) 4 hours, D) 6 hours after initiation of the dehydration. In total, an area loss of 75 % was observed.

Marchantia polymorpha

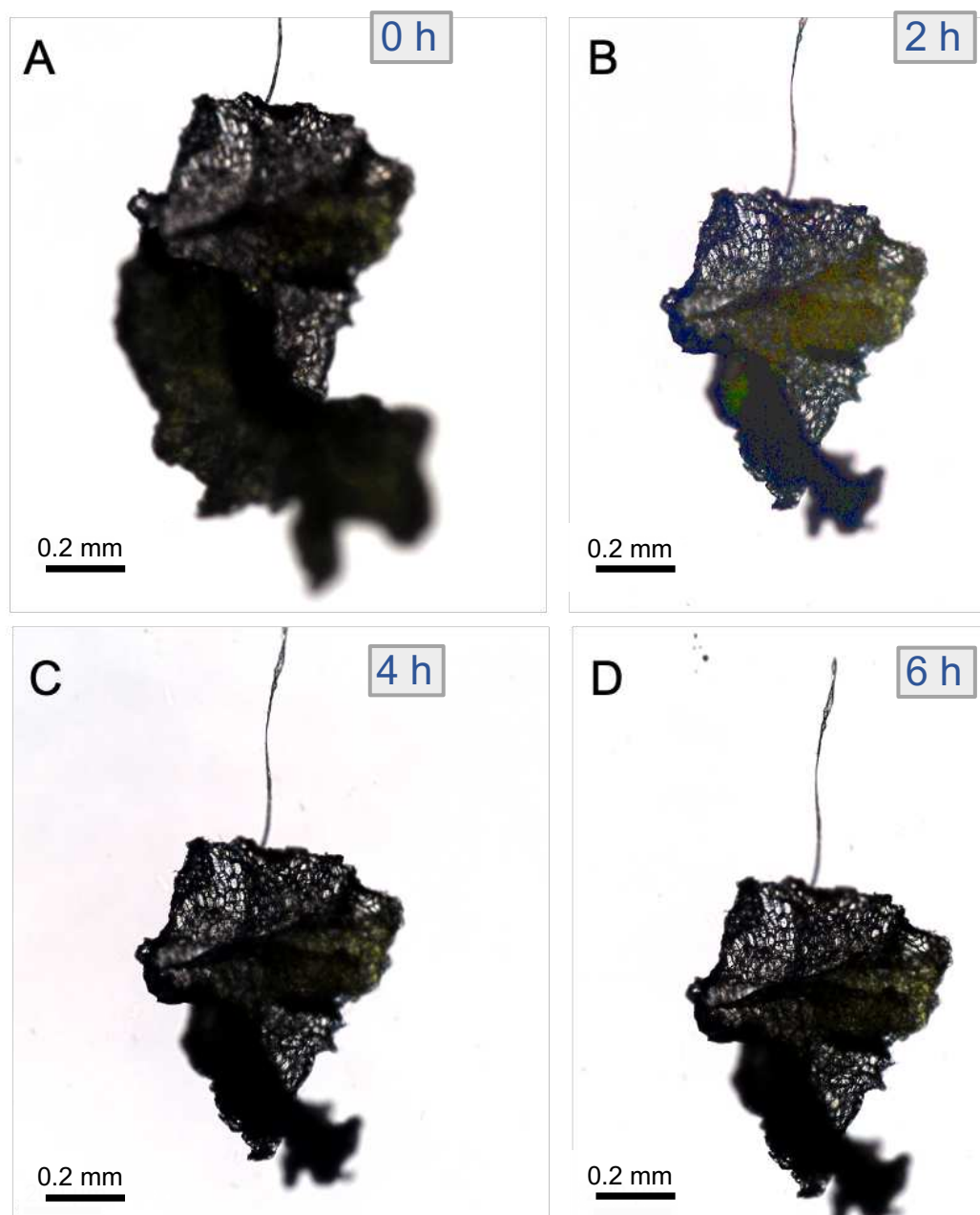


Fig. 49. Light microscopy images show the leaf area of the *M. polymorpha* at A) 0 hours, B) 2 hours, C) 4 hours, D) 6 hours after initiation of the dehydration. In total, an area loss of 75 % was observed.

Tab. 17. Leaf area (mm²), percent, and decline (%) during dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Leaf area (mm ²) depicted macroscopic images															
	A. undulatum			P. affine			P. patens			F. hygrometrica			M. polymorpha		
	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.
0 h	0.1710	100.0	0.0	1.6507	100.0	0.0	0.0581	100.0	0.0	0.2914	100.0	0.0	0.2883	100.0	0.0
2 h	0.1706	99.7	0.3	0.4277	25.9	74.1	0.0371	63.9	36.1	0.2864	98.3	1.7	0.0717	24.9	75.1
4 h	0.1701	99.4	0.6	0.4297	26.0	74.0	0.0384	66.1	33.9	0.2863	98.3	1.7	0.0720	25.0	75.0
6 h	0.1688	98.7	1.3	0.4031	24.4	75.6	0.0550	94.7	5.3	0.0732	25.1	74.9	0.0722	25.0	75.0

3.5.2. Mean Leaf area

Analysis of the graph “Leaf area over 6 h combination of all species” (Fig. 50.) and the corresponding “light microscopy images” (Fig. 45., Fig. 46. Fig. 47., Fig. 48., Fig. 49.,) revealed in ***A. undulatum***, the first value of 0.4743 mm² at 0 hours, which was the highest leaf area observed. At 2 hours, a strong decline was seen, with a decline of 44 %. In table (18.), the mean leaf area (mm²), percentage, and decline (%) during dehydration is summarised. From 2-6 hours, the values continued to remain steady, with leaf area loss between 43-45 %, with the lowest leaf area of 0.2587 mm² documented at 6 hours (Fig. 50.). From all moss species, ***P. affine*** showed the initial, maximum leaf area 1.4643 mm² at 0 hours. it underwent a major decline of 67% in the first 2 hours.

Tab. 18. Mean leaf area (mm²), percent, and decline (%) during dehydration of in *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Mean leaf area (mm ²)															
	<i>A. undulatum</i>			<i>P. affine</i>			<i>P. patens</i>			<i>F. hygrometrica</i>			<i>M. polymorpha</i>		
	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.
0 h	0.4743	100.0	0.0	1.4643	100.0	0.0	0.2669	100.0	0.0	0.2211	100.0	0.0	0.7310	100.0	0.0
2 h	0.2668	56.2	43.8	0.4838	33.0	67.0	0.0919	34.4	65.6	0.1664	75.3	24.7	0.3439	47.0	53.0
4 h	0.2650	55.9	44.1	0.4772	32.6	67.4	0.0920	34.5	65.5	0.1671	75.6	24.4	0.3447	47.1	52.9
6 h	0.2587	54.5	45.5	0.4715	32.2	67.8	0.0975	36.5	63.5	0.0962	43.5	56.5	0.3988	54.6	45.4

At 4 hours, a stable phase with 67 % loss was observed, and at 6 hours, the lowest value of 0.4715 mm² was reached, after losing 68 % of the leaf area (Fig. 50.). An

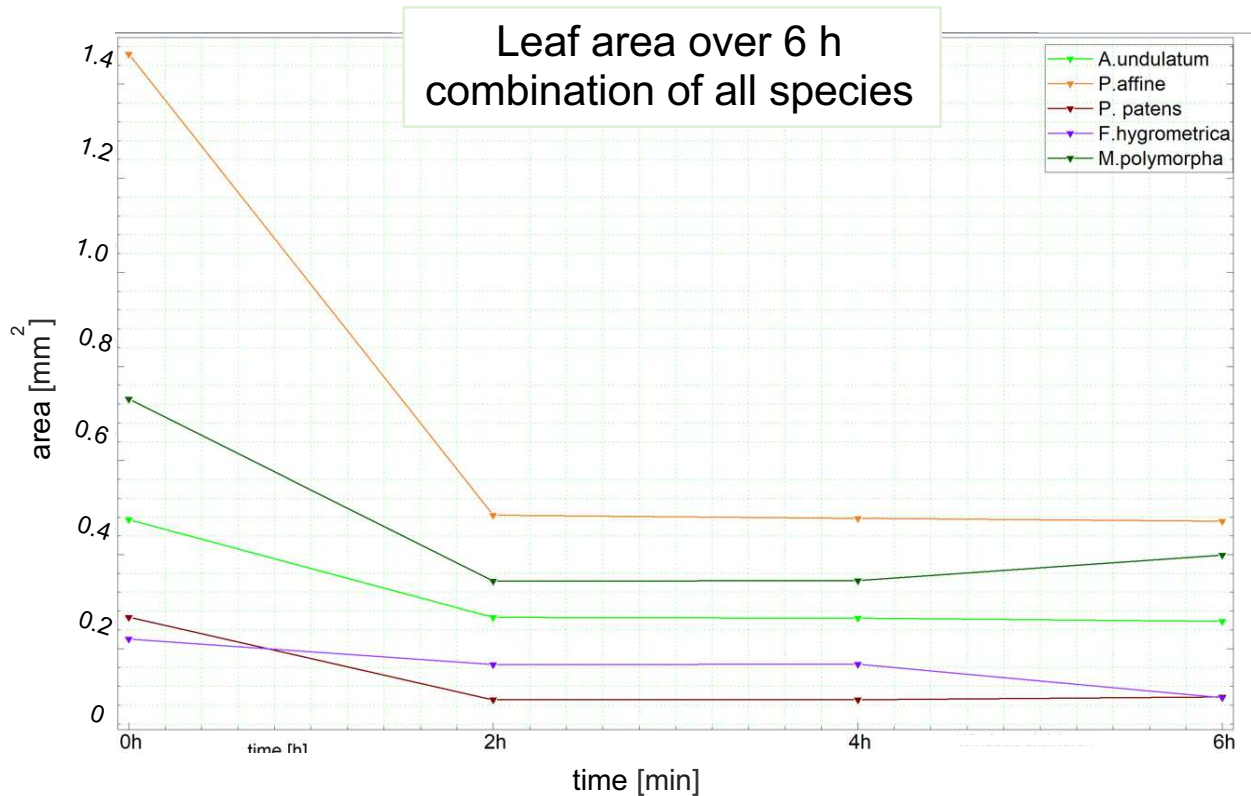


Fig. 50. Graph of the leaf dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha* from 0-6 hours.

initial leaf area of 0.2669 mm² was observed in ***P. patens*** and at 2 hours, 66 % decline took place. At 4 hours, the lowest leaf area 0.0920 mm², with a leaf area loss of 65 % could be observed. At 6 hours, a decline of 63 % could be observed (Fig. 50.). In ***F. hygrometrica***, the initial value 0.221 mm² at zero hours was the highest one documented. From 2-4 hours, a decline of 24-25 % took place. At 6 hours, the lowest value of 0.0962 mm² was reached, having experienced a loss of 57 %, compared to the initial value (Fig. 50.). The highest recorded leaf area in ***M. polymorpha*** was the initial value of 0.7310 mm² at 0 hours. At 2 hours, it underwent a decline of 53 %, reaching the lowest value of 0.3439 mm². At 4 hours, an area loss of 53 % took place. Form 4- 6 hours, a slight increase was observed, having reached 46 % loss at 6 hours (Fig. 50.).

Tab. 19. Descriptive statistics of the leaf area measurement (6 h).

Leaf area 6 hours	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	4	316173,8	105466,4	1,3E6	3E5	5E5
<i>P. affine</i>	4	724174,3	493412,0	2,9E6	5E5	1E6
<i>P. patens</i>	4	137069,5	86597,70	548278	9E4	3E5
<i>F. hygrometrica</i>	4	162704	51199,06	650816	1E5	2E5
<i>M. polymorpha</i>	4	454597	186073,9	1,8E6	3E5	7E5

Tab. 20. Pearson correlation coefficient and p-value of the leaf area measurement (6 h).

Leaf area 6 hours		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	0,99972	0,99801	0,78047	0,98539
<i>A. undulatum</i>	p-value	--	2,82481	0,00199	0,21953	0,01461
<i>P. affine</i>	Pearson cor.	0,99972	1	0,99922	0,76543	0,98916
<i>P. affine</i>	p-value	2,82482	--	7,76884	0,23457	0,01084
<i>P. patens</i>	Pearson cor.	0,99801	0,99922	1	0,73981	0,99415
<i>P. patens</i>	p-value	0,00199	7,76884	--	0,26019	0,00585
<i>F. hygrometrica</i>	Pearson cor.	0,78047	0,76543	0,73981	1	0,66283
<i>M. polymorpha</i>	p-value	0,21953	0,23457	0,26019	--	0,33717
<i>M. polymorpha</i>	Pearson cor.	0,98539	0,98916	0,99415	0,66283	1
<i>M. polymorpha</i>	p-value	0,01461	0,01084	0,00585	0,33717	--

The **Pearson correlation coefficient** of the “Leaf area 6 h” (Tab. 20.) revealed 4 positive correlations with significant p-values. A positive correlation of **0.99801** was observed in *A. undulatum* and *P. patens*, and a significant p-value of **0.00199**. In *A. undulatum* and *M. polymorpha*, **0.98539**, and a significant p-value of **0.01461**. *P. patens* and *M. polymorpha* had **0.99415**, with a significant p-value of **0.00585**. A positive correlation was documented in *M. polymorpha* and *P. affine* **0.98916**, with a significant p-value of **0.00585**.

3.6. Gametophyte dehydration

Under a stereo microscope (Nikon SMZ1500), on a dry glass slide, a fragment of the moss shoot was extracted. The gametophyte dehydration was documented as a series of images with defined time frames, taken at 1-minute intervals automatically. An initial gametophyte dehydration was done over 120 minutes, and after the first analysis, it was concluded the lowest points in the gametophyte area was reached around 20 minutes and reduced to 30 minutes. A timelapse video of the gametophyte dehydration over 30 minutes was made, including *A. undulatum* (Fig. 52.), *P. affine* (Fig. 53.), *P. patens* (Fig. 54.), and *M. polymorpha* (Fig. 54.), but all *F. hygrometrica* plants were not viable anymore. The area was measured with the auto-detect function and summarized in a table. Images aimed to give an insight into the “Area 1” initial area, and the “Area 2” area after 30 minutes. The data were summarized in the graphs “gametophyte dehydration 30 minutes” (Fig. 56.), and “gametophyte dehydration 120 minutes” (Fig. 57.).

30 minutes

In all species, the first measurement at 0 minutes revealed the highest gametophyte area. In *A. undulatum*, 13 % were lost, at 9 minutes, 43 %, lowest gametophyte, area at 23 minutes, and 70 % of the initial area lost. *P. affine* showed a decline of 22 % in one minute, after 2 minutes 43 %, and at 23 minutes, the lowest area, with a loss of 70 % was documented. *M. polymorpha*, after 5 minutes, 26 % loss, lowest documented area at 8 and 18 minutes, having lost 62 % (Fig. 56.). The correlation coefficient showed a positive Pearson correlation in *A. undulatum* and *P. affine* 0.8709, *A. undulatum* and *P. patens* 0.7151, *A. undulatum* and *M. polymorpha* 0.70006. In *P. affine* and *A. undulatum* 0.8709, *P. affine* and *P. patens* 0.89024, *P. affine* and *M. polymorpha*, 0.79858. In *P. patens* and *M. polymorpha* 0.8200, all with a highly significant p-value < 0.0001 (Tab. 23)

120 minutes

In *A. undulatum*, a gradual decrease was seen, at the lowest point at 93 minutes, 82 % lost. In *P. affine*, the lowest detected gametophyte area in this phase lost 42 % at 108 minutes. In *P. patens*, major fluctuations were observed, and the lowest point was at 117 minutes, after having lost 58 %. The lowest value of *F. hygrometrica* was documented at 19 minutes, having lost 6 %, and in *M. polymorpha*, at 42 minutes, after losing 56 % of the initial area (Fig. 57.).

Pearson correlation coefficient (Tab.26.) showed a positive correlation between *A. undulatum* and *P. affine* with 0.87558, *A. undulatum* and *P. patens* 0.54707, *A. undulatum* and *M. polymorpha* 0.90933, *P. affine* and *P. patens* 0.64022, *P. affine* and *M. polymorpha* 0.67088, *P. patens* and *M. polymorpha* 0.46103, *F. hygrometrica* and *M. polymorpha* 0.20016, all with a significant p-value (Tab. 26).

Gametophyte dehydration

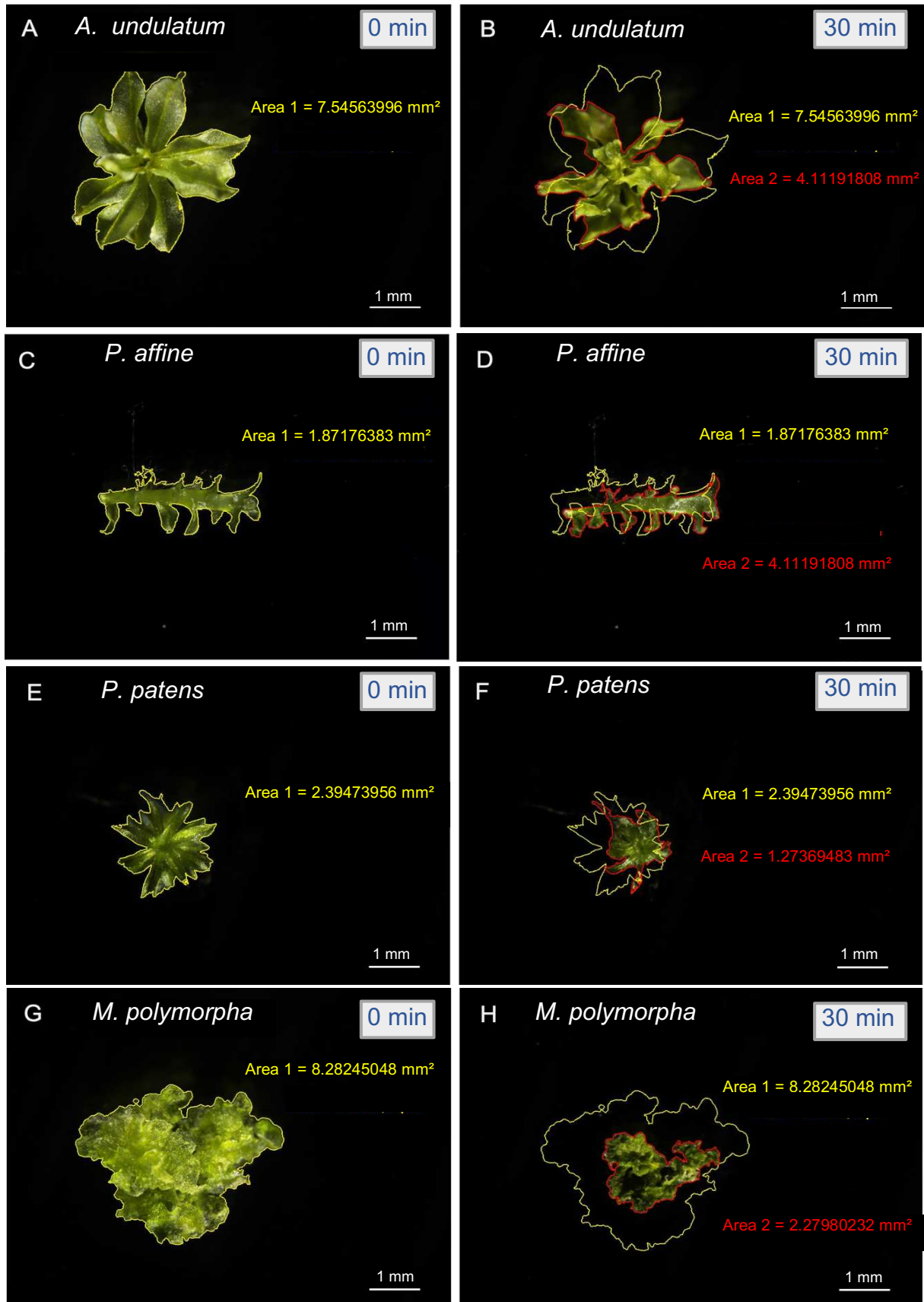


Fig. 51. Overview of gametophyte stereo images, including *A. undulatum*, with the initial gametophyte surface area seen in A) and area after 30 minutes of dehydration B). *P. patens* gametophyte before C) and after 30 minutes of dehydration D). *P. affine* gametophyte before E) and after 30 minutes of dehydration F). The lobed, heart-shaped gametophyte of *M. polymorpha* before dehydration G) and after 30 minutes of dehydration H).

Atrichum undulatum

Stereo images depicted the *A. undulatum* shoot at 0 minutes, before dehydration, with an initial area of 7.54563996 mm² and labeled with “Area 1 (yellow)” (Fig. 52.A). In the first 30 minutes of the gametophyte dehydration an area of 4.11191808 mm² seen in “Area 2 (red)” (Fig. 52.B) was documented. *A. undulatum* lost 46 % of the initial area.

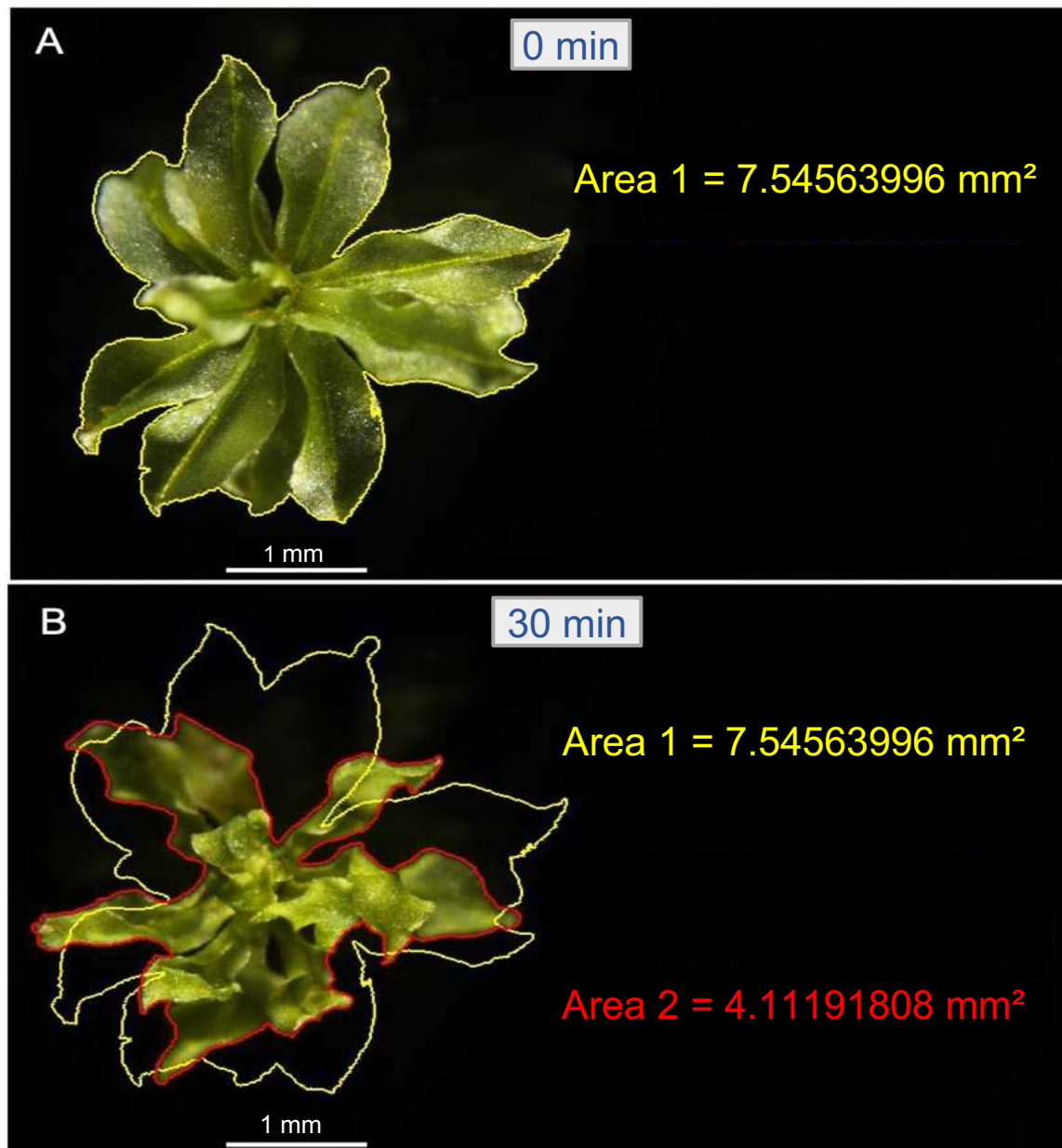


Fig. 52.A) Stereo image of the area of the *A. undulatum* gametophyte in hydrated state. B) The gametophyte after 30 minutes of dehydration shoot area loss of 46 % was observed.

Plagiomnium affine

A stereo image showed the *P. affine* shoot with fine rhizoids, before the initiation of the dehydration, at 0 minutes an area of 1.87176383 mm² labeled with “Area 1 (yellow)” was observed (Fig. 53.A). 30 minutes into the dehydration, the *P. affine* gametophyte area of 1.43765344 mm² seen in “Area 2 (red)” (Fig. 53.B) was documented, after losing 23 % of the initial area.

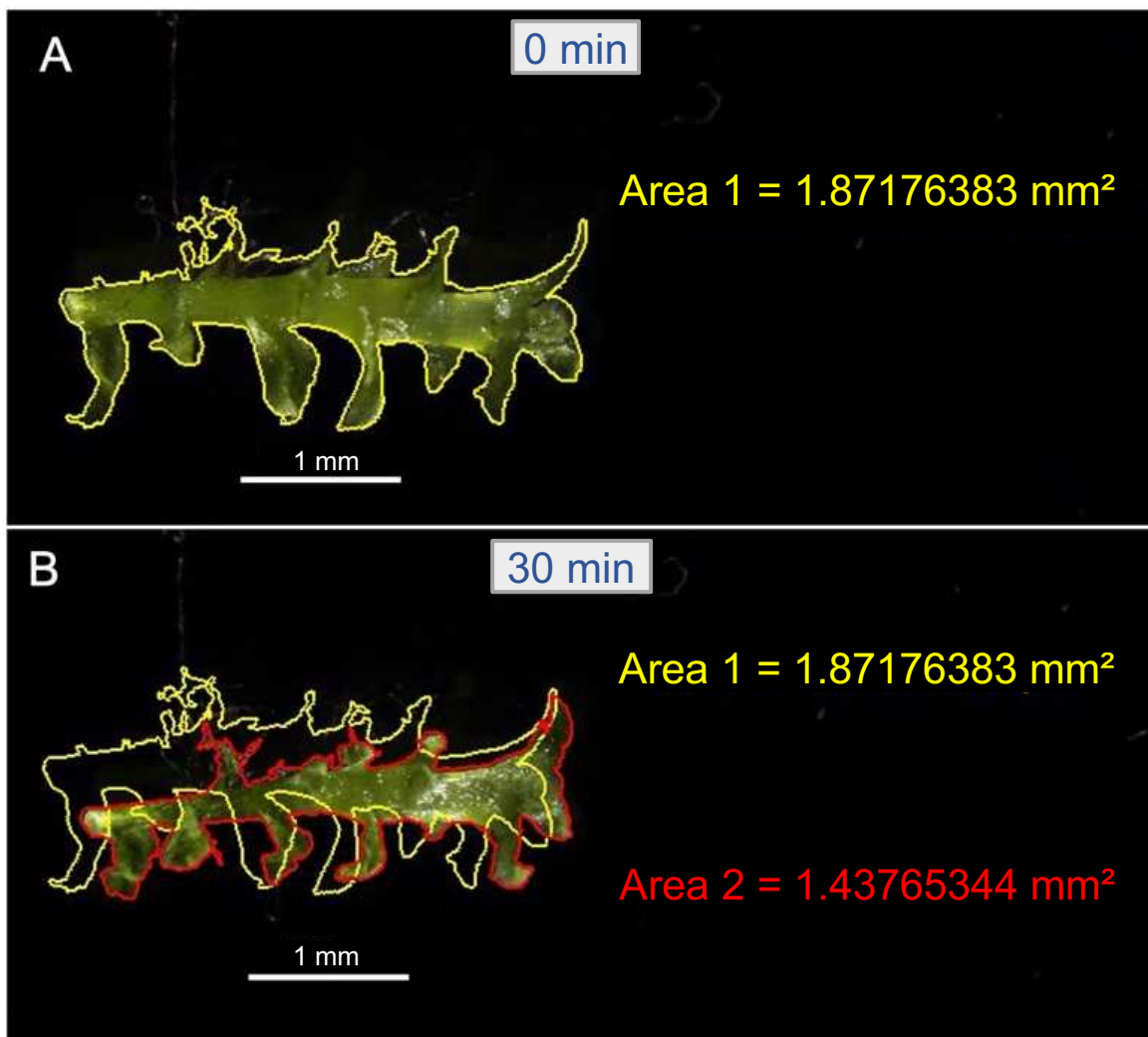


Fig. 53. A) Stereo image of the area of the *P. affine* gametophyte in hydrated state B) The gametophyte after 30 minutes of dehydration an area loss of 23 % was observed.

Physcomitrium patens

A stereo image of *P. patens* revealed the shoot before dehydration, at time point 0, with an area of 2.39473956 mm² labeled with “Area 1 (yellow)” (Fig. 54.A). After a 30-minute dehydration, the gametophyte area measured was 1.27369483 mm² as seen in “Area 2 (red)” (Fig. 54.B), after experiencing a decline in area of 47%.

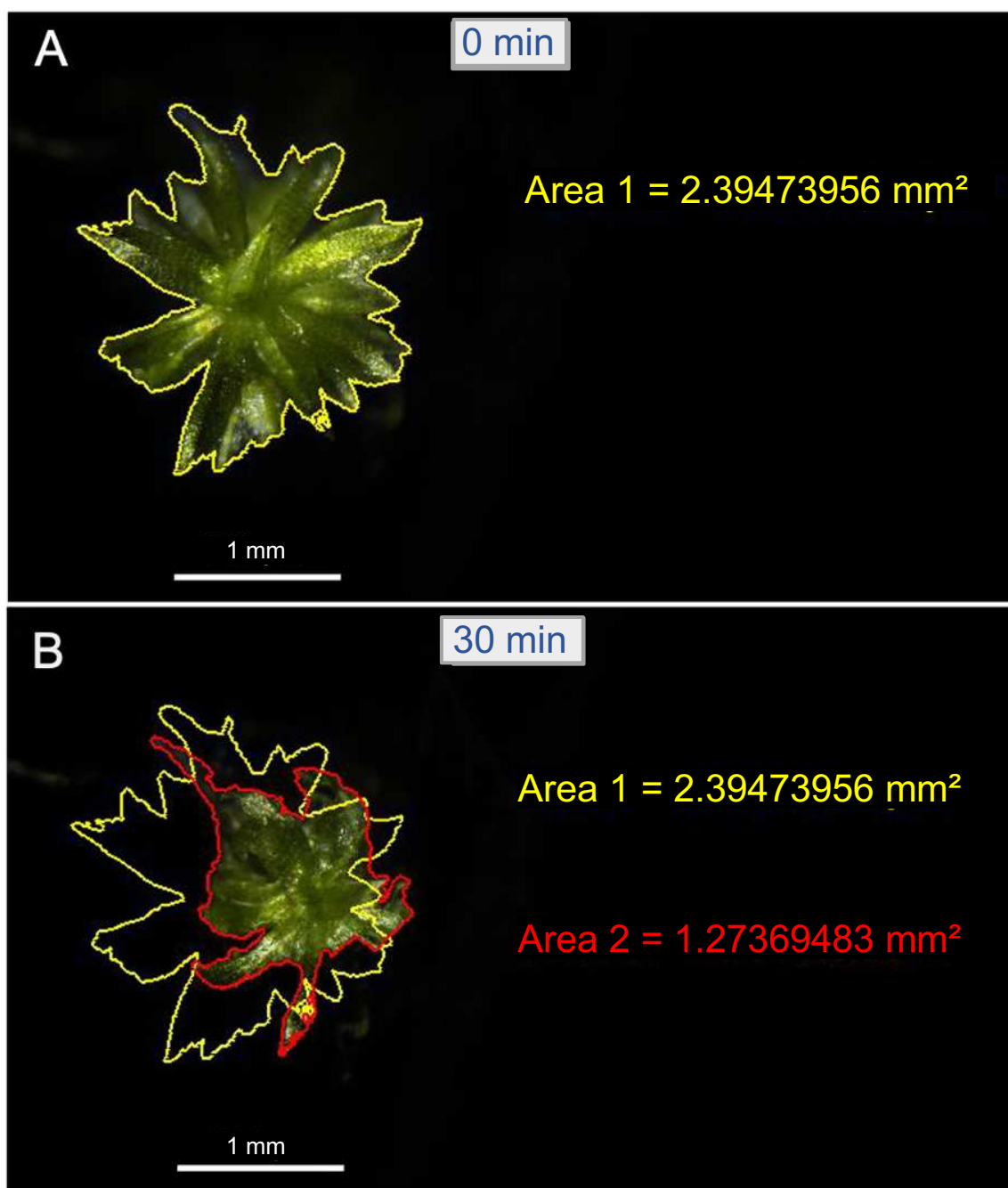


Fig. 54. A) Stereo image of the area of the *P. patens* gametophyte in the hydrated state. B) The gametophyte after 30 minutes of dehydration an area loss of 47% was documented.

Marchantia polymorpha

The *M. polymorpha* shoot is depicted in the stereo image, before dehydration at 0 minutes, an area of 8.28245048 mm² was seen, with the description “Area 1 (yellow)”, (Fig. 55. A). After 30 minutes dehydration, Time-lapse images revealed an area of 2.27980232 mm² seen in “Area 2 (red)” (Fig. 55.B), after experiencing a decline of 72%

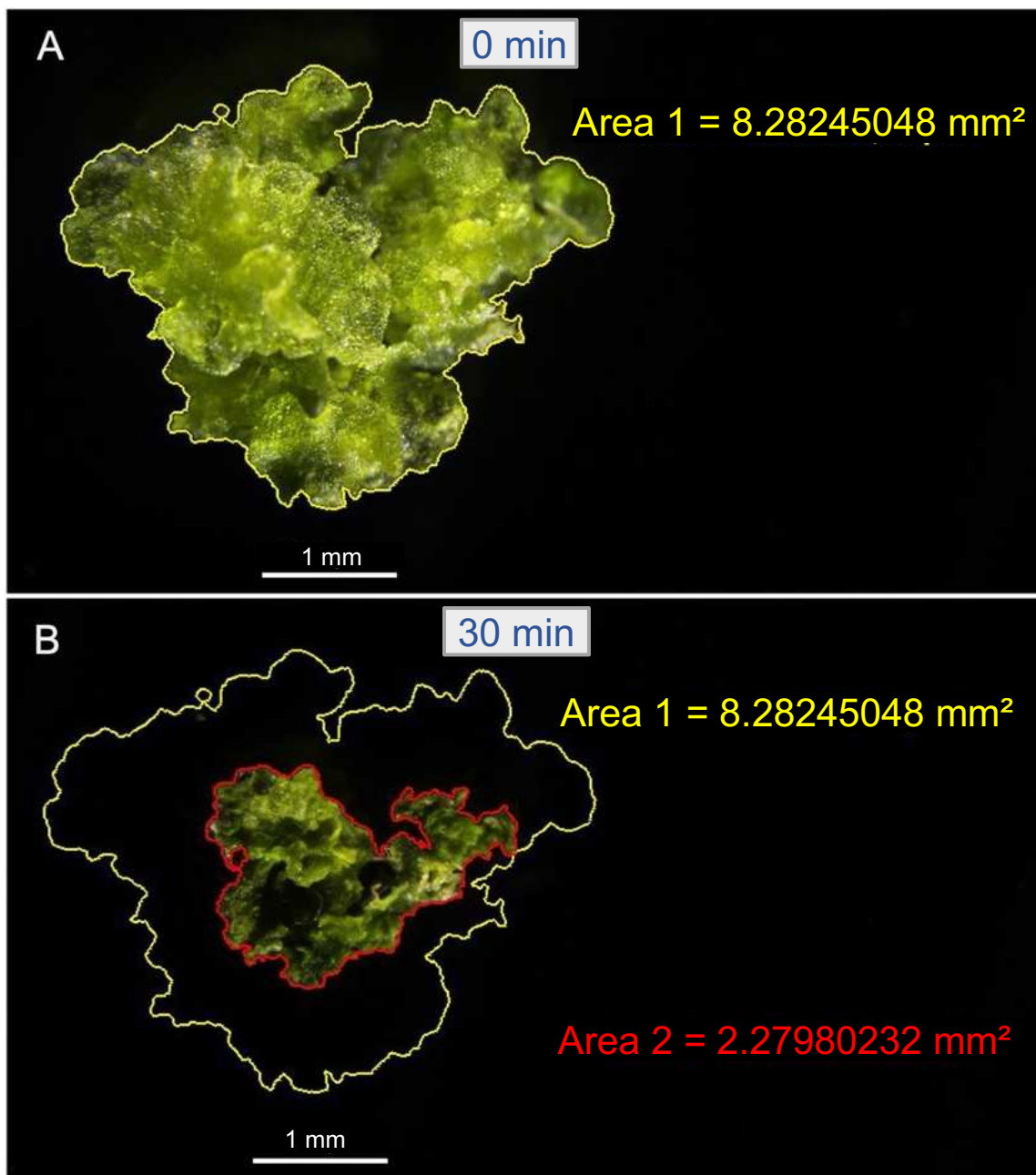


Fig. 55 A) Stereo image of the gametophyte area of the lobed *M. polymorpha* in the hydrated state). B) Gametophyte after 30 minutes of dehydration, an area loss of 72% was documented.

3.6.1. Gametophyte dehydration (30 minutes)

The graph “Gametophyte area over 30 min combination of all species” (Fig. 56.), the “Stereo images” (Fig. 52-55) and Table (21.) uncovered that ***A. undulatum*** had the highest area at the beginning of the dehydration. The initial gametophyte area of 5.418 mm² was the highest found in this species. At one minute, 13 % were lost and after continued decline, at 9 minutes, 43 % were lost. A decline of 26-44 % was documented at 10 to 30 minutes, reaching the lowest point of 2.940 mm² at 29 minutes, having lost 46 % of the initial area (Fig. 56.).

In ***P. affine***, the starting gametophyte area of 3.969 mm² was the highest found in this species. After one minute, 22 % were lost, and after two minutes 43 %. After a further decline, at 5 minutes, 48 % of the initial area are lost. A local minimum was found at 8 minutes, and at 10 minutes, 57 % were lost. From 11-30 minutes, small fluctuations were observed, varying from 59-69 % decline. At 23 minutes,

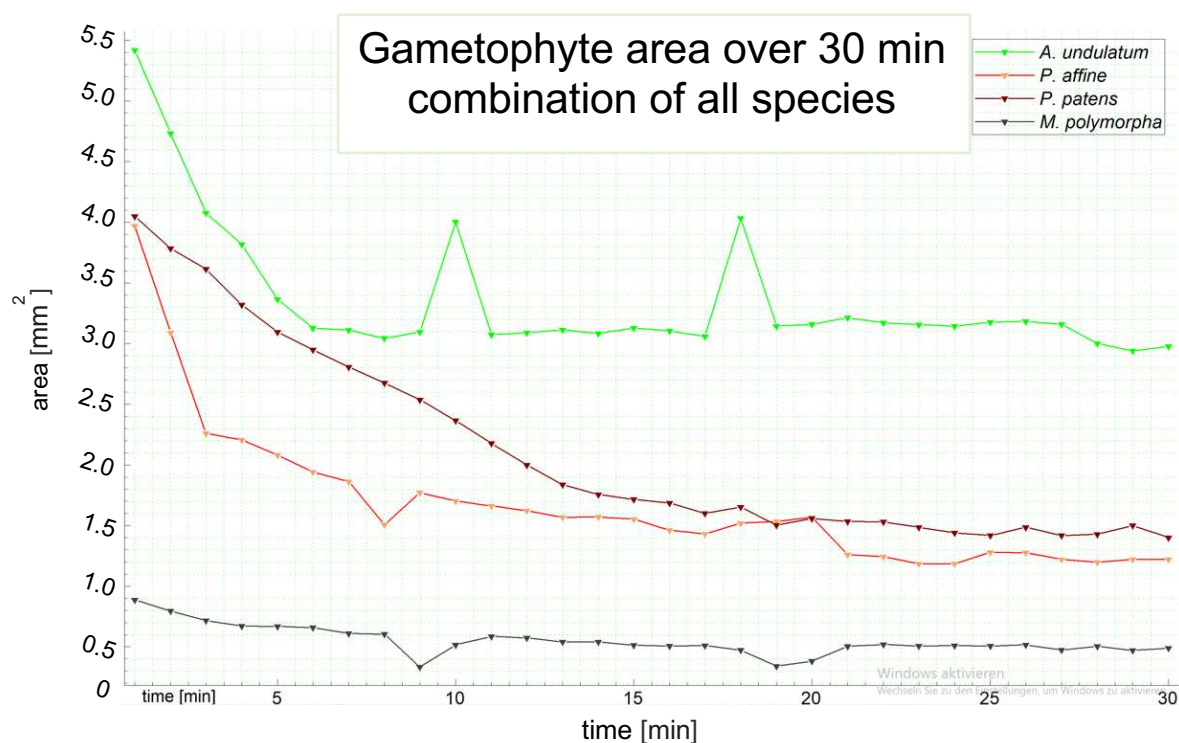


Fig. 56. Dehydration over 30 minutes of *A. undulatum*, *P. affine*, *P. patens*, and *M. polymorpha*.

the lowest area of 1.186 mm² was documented, having experienced a decline of 70 % (Fig. 56.). The initial gametophyte area of ***P. patens*** was 4.049 mm², which was the highest value documented. From 0-5 minutes, a gradual decrease takes place, leading to a loss of 26 % at 5 minutes. At 10 minutes, a decline of 46 % was observed, and after a further gradual decline, from 14-30 minutes, a stable phase with a decrease of 58-65 % was reached, with the lowest value of 1.401 mm² at

30 minutes, having lost 65 % of the initial gametophyte area (Fig. 56.). In *M. polymorpha*, the first value 0.888 mm² was the highest leaf area documented. A decline of 10 % was documented at one minute, at 5 minutes, 26 %, from 6-7 minutes, 31-32 %, and from 8 to 30 minutes. The curve remained stable, with a decline between 39-47 %, except for the lowest gametophyte area of 0.341 mm², documented at 9 minutes and, having lost 62 % of the initial gametophyte area (Fig. 56.).

Tab. 21. Gametophyte area (mm²) percent, and decline (%) during dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Gametophyte area (mm ²) during dehydration												
	<i>A. undulatum</i>			<i>P. affine</i>			<i>P. patens</i>			<i>M. polymorpha</i>		
	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.
1 min	5.418	100.0	0.0	3.969	100.0	0.0	4.049	100.0	0.0	0.888	100.0	0.0
2 min	4.731	87.3	12.7	3.090	77.8	22.2	3.784	93.4	6.6	0.795	89.5	10.5
3 min	4.076	75.2	24.8	2.261	57.0	43.0	3.613	89.2	10.8	0.718	80.8	19.2
4 min	3.818	70.5	29.5	2.205	55.6	44.4	3.321	82.0	18.0	0.672	75.7	24.3
5 min	3.366	62.1	37.9	2.079	52.4	47.6	3.094	76.4	23.6	0.669	75.3	24.7
6 min	3.128	57.7	42.3	1.942	48.9	51.1	2.948	72.8	27.2	0.661	74.4	25.6
7 min	3.110	57.4	42.6	1.862	46.9	53.1	2.806	69.3	30.7	0.610	68.6	31.4
8 min	3.044	56.2	43.8	1.506	37.9	62.1	2.676	66.1	33.9	0.604	68.0	32.0
9 min	3.095	57.1	42.9	1.773	44.7	55.3	2.537	62.7	37.3	0.335	37.7	62.3
10 min	4.003	73.9	26.1	1.701	42.9	57.1	2.366	58.4	41.6	0.517	58.2	41.8
11 min	3.073	56.7	43.3	1.662	41.9	58.1	2.177	53.8	46.2	0.587	66.1	33.9
12 min	3.090	57.0	43.0	1.622	40.9	59.1	1.998	49.3	50.7	0.575	64.7	35.3
13 min	3.112	57.4	42.6	1.568	39.5	60.5	1.839	45.4	54.6	0.542	61.1	38.9
14 min	3.082	56.9	43.1	1.569	39.5	60.5	1.755	43.3	56.7	0.542	61.0	39.0
15 min	3.127	57.7	42.3	1.551	39.1	60.9	1.718	42.4	57.6	0.513	57.7	42.3
16 min	3.106	57.3	42.7	1.462	36.8	63.2	1.686	41.6	58.4	0.508	57.2	42.8
17 min	3.059	56.5	43.5	1.431	36.1	63.9	1.600	39.5	60.5	0.511	57.5	42.5
18 min	4.035	74.5	25.5	1.520	38.3	61.7	1.652	40.8	59.2	0.475	53.4	46.6
19 min	3.142	58.0	42.0	1.534	38.6	61.4	1.505	37.2	62.8	0.341	38.4	61.6
20 min	3.160	58.3	41.7	1.569	39.5	60.5	1.559	38.5	61.5	0.382	43.0	57.0
21 min	3.212	59.3	40.7	1.261	31.8	68.2	1.533	37.9	62.1	0.506	56.9	43.1
22 min	3.172	58.5	41.5	1.244	31.3	68.7	1.531	37.8	62.2	0.521	58.6	41.4
23 min	3.157	58.3	41.7	1.186	29.9	70.1	1.484	36.7	63.3	0.506	57.0	43.0
24 min	3.145	58.0	42.0	1.185	29.9	70.1	1.441	35.6	64.4	0.510	57.5	42.5
25 min	3.176	58.6	41.4	1.278	32.2	67.8	1.420	35.1	64.9	0.506	57.0	43.0
26 min	3.183	58.7	41.3	1.276	32.1	67.9	1.486	36.7	63.3	0.517	58.2	41.8
27 min	3.158	58.3	41.7	1.221	30.8	69.2	1.418	35.0	65.0	0.475	53.5	46.5
28 min	3.000	55.4	44.6	1.198	30.2	69.8	1.427	35.2	64.8	0.505	56.9	43.1
29 min	2.940	54.3	45.7	1.222	30.8	69.2	1.501	37.1	62.9	0.470	53.0	47.0
30 min	2.977	54.9	45.1	1.221	30.8	69.2	1.401	34.6	65.4	0.490	55.2	44.8

Tab. 22. Descriptive statistics of the gametophyte dehydration (30 min).

Gametophyte dehydration 30 minutes	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	30	3363173	565947,2	1,0E8	3E6	5E6
<i>P. affine</i>	30	1672234	598755,1	5,0E7	1E6	4E6
<i>P. patens</i>	30	2110822	807193,7	6,3E7	1E6	4E6
<i>M. polymorpha</i>	30	548436,6	118747,3	1,6E7	3E5	9E5

Tab. 23. Pearson correlation coefficient and p-value of the gametophyte dehydration (30 min).

Gametophyte dehydration 30 minutes		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	0,8709	0,71751	0,70006
<i>A. undulatum</i>	p-value	--	<0.0001	<0.0001	<0.0001
<i>P. affine</i>	Pearson cor.	0,8709	1	0,89024	0,79858
<i>P. affine</i>	p-value	<0.0001	--	<0.0001	<0.0001
<i>P. patens</i>	Pearson cor.	0,71751	0,89024	1	0,82004
<i>P. patens</i>	p-value	<0.0001	<0.0001	--	<0.0001
<i>M. polymorpha</i>	Pearson cor.	0,70006	0,79858	0,82004	1
<i>M. polymorpha</i>	p-value	<0.0001	<0.0001	<0.0001	--

In Tab.23., the **Pearson correlation coefficient** of the gametophyte dehydration area, over the duration of 30 minutes was determined. Analysis revealed 6 positive correlations, all with highly significant p-value < 0.0001, including *A. undulatum* and *P. affine* **0.8709**, *A. undulatum* and *P. patens* **0.71751**, *A. undulatum* and *M. polymorpha* **0.70006**, *P. affine* and *P. patens* **0.89024**, *P. affine* and *M. polymorpha*, **0,79858**, *P. patens* and *M. polymorpha* **0.82004** (Tab. 23.).

3.6.2. Gametophyte dehydration (120 minutes)

The graph “Gametophyte dehydration 120 minutes” (Fig. 57.) and Table (24.), summarising the gametophyte area (mm^2), percentage, and decline (%) during dehydration revealed that *A. undulatum* has an initial gametophyte area at time point 1, which equates to 0 minutes, of 6.596 mm^2 , which was the highest observed in this species. At one minute, 23 % are lost, and at 2 minutes, 40 %. From 3 to 9 minutes, a gradual decrease takes, and from 10 to 120 minutes, a steady phase was documented, with a gametophyte area loss of 80 %. The lowest area was reached at 93 minutes, with 1.229 mm^2 , having lost 82 % of the initial value (Fig. 57.). At zero minutes, the initial value of *P. affine* was 2.098 mm^2 and after an increase of 2 %, the highest value 2.132 mm^2 within the first minute was documented. From 2-3 minutes, a decline of 6 % was observed. From 4-10 minutes, further decline took place, at 10 minutes, 36 % were lost, and at 20 minutes, 39 % were lost. From 20 to 120 minutes, a stable phase with minor fluctuations took place, maintaining an area loss of 39-41 %. The lowest detected gametophyte

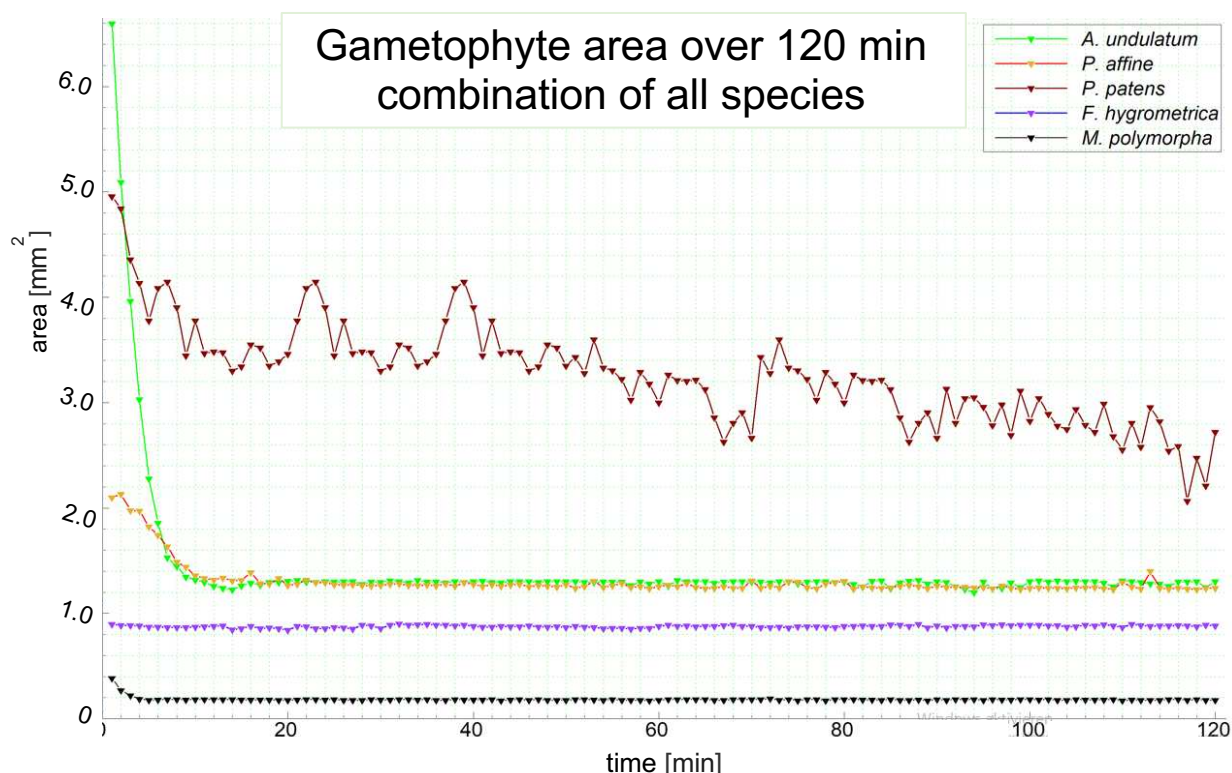


Fig. 57. Initial dehydration over 120 minutes of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, *M. polymorpha*.

area in this phase was 1.237 mm^2 , having lost 42 % at 108 minutes (Fig. 57.).

The peak of the gametophyte area in *P. patens* is the initial value of 4.957 mm^2 at 0 minutes. From 0 to 4 minutes, a decline of 24 % of the initial area. From 5 -6 minutes 16 %, at 7 minutes is a local maximum, with 21 % loss was reached. From

8- 13 minutes a decline with major fluctuation was documented, and at 14 minutes a local low point is seen, having lost 34 %. From 13 to 19 minutes, a stable phase with loss of 33-30 % takes place. From 24 to 35 minutes, the area declined by 24–33 %. From 36 to 46 minutes, the reduction was 18–33 %, and from 47 to 57 minutes, 29–40 % was lost. At 117 minutes, the lowest recorded value was 2.065 mm² reflecting a total loss of 58 %. The gametophyte dehydration pattern of *P. patens* is characterized by numerous fluctuations, local peaks, and local low points (Fig.57.). In *F. hygrometrica* the initial area was, 0.898 mm². From 0 to 10 minutes, a gradual decline takes place, reaching 2.8 % loss at 10 minutes. 11 to 120 minutes, the curve remains stable with small fluctuations, losing gametophyte area loss in the range of 4.5 and 0.8 %. The highest value of 0.900 mm² was documented at 32 minutes, with a decline of 0.3 %, compared to the initial area (Fig.57). In *M. polymorpha*, the highest value 0.379 mm² was recorded at the timepoint 1, at zero minutes. A major decline of 70 % took place in the first 4 minutes, at 2 minutes, 58 %, at 3 minutes, 49 %, and at 4 minutes, 46 %. From 5-120 minutes, the gametophyte area remains stable, fluctuating between 44 % and 49 %, a local maximum, which was observed at 71 minutes. The lowest value was 0.179 mm² at 42 minutes, with a 56 % decline, compared to the initial area (Fig. 57.).

Tab. 24. Gametophyte area (mm²) percent, and decline (%) during dehydration of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Gametophyte area (mm ²) during dehydration															
	<i>A. undulatum</i>			<i>P. affine</i>			<i>P. patens</i>			<i>F. hygrometrica</i>			<i>M. polymorpha</i>		
	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.
1 min	6.596	100	0	2.098	100	0	4.957	100	0	0.898	100	0.0	0.379	100	0
2 min	5.091	77.2	22.8	2.132	101.6	-1.6	4.841	97.7	2.3	0.884	98.5	1.5	0.263	69.5	30.5
3 min	3.964	60.1	39.9	1.975	94.1	5.9	4.358	87.9	12.1	0.884	98.5	1.5	0.216	57.0	43.0
4 min	3.029	45.9	54.1	1.972	94.0	6.0	4.133	83.4	16.6	0.883	98.4	1.6	0.183	48.5	51.5
5 min	2.277	34.5	65.5	1.822	86.9	13.1	3.777	76.2	23.8	0.871	97.1	2.9	0.173	45.6	54.4
6 min	1.858	28.2	71.8	1.741	83.0	17.0	4.087	82.5	17.5	0.871	97.1	2.9	0.176	46.5	53.5
7 min	1.529	23.2	76.8	1.633	77.8	22.2	4.146	83.6	16.4	0.867	96.6	3.4	0.178	47.0	53.0
8 min	1.448	21.9	78.1	1.487	70.9	29.1	3.903	78.7	21.3	0.867	96.6	3.4	0.179	47.4	52.6
9 min	1.347	20.4	79.6	1.440	68.7	31.3	3.445	69.5	30.5	0.867	96.6	3.4	0.176	46.4	53.6
10 min	1.319	20.0	80.0	1.359	64.8	35.2	3.778	76.2	23.8	0.870	97.0	3.0	0.177	46.8	53.2
11 min	1.296	19.6	80.4	1.331	63.5	36.5	3.468	70.0	30.0	0.874	97.3	2.7	0.178	46.9	53.1
12 min	1.259	19.1	80.9	1.319	62.9	37.1	3.481	70.2	29.8	0.878	97.8	2.2	0.177	46.8	53.2
13 min	1.238	18.8	81.2	1.334	63.6	36.4	3.477	70.1	29.9	0.884	98.5	1.5	0.179	47.2	52.8
14 min	1.223	18.5	81.5	1.309	62.4	37.6	3.299	66.6	33.4	0.845	94.1	5.9	0.179	47.1	52.9
15 min	1.262	19.1	80.9	1.313	62.6	37.4	3.341	67.4	32.6	0.856	95.4	4.6	0.176	46.6	53.4
16 min	1.288	19.5	80.5	1.388	66.1	33.9	3.551	71.6	28.4	0.879	97.9	2.1	0.176	46.4	53.6
17 min	1.267	19.2	80.8	1.284	61.2	38.8	3.519	71.0	29.0	0.858	95.5	4.5	0.174	45.8	54.2
18 min	1.297	19.7	80.3	1.282	61.1	38.9	3.350	67.6	32.4	0.864	96.2	3.8	0.176	46.6	53.4
19 min	1.295	19.6	80.4	1.330	63.4	36.6	3.390	68.4	31.6	0.857	95.5	4.5	0.172	45.5	54.5
20 min	1.307	19.8	80.2	1.264	60.3	39.7	3.461	69.8	30.2	0.842	93.8	6.2	0.171	45.2	54.8
21 min	1.314	19.9	80.1	1.275	60.7	39.3	3.777	76.2	23.8	0.879	97.9	2.1	0.176	46.4	53.6
22 min	1.298	19.7	80.3	1.316	62.7	37.3	4.087	82.5	17.5	0.875	97.5	2.5	0.179	47.4	52.6
23 min	1.296	19.6	80.4	1.291	61.5	38.5	4.146	83.6	16.4	0.857	95.5	4.5	0.177	46.7	53.3
24 min	1.304	19.8	80.2	1.293	61.6	38.4	3.903	78.7	21.3	0.857	95.5	4.5	0.174	45.8	54.2
25 min	1.300	19.7	80.3	1.274	60.7	39.3	3.445	69.5	30.5	0.865	96.4	3.6	0.178	47.0	53.0
26 min	1.303	19.8	80.2	1.267	60.4	39.6	3.778	76.2	23.8	0.863	96.2	3.8	0.170	44.9	55.1
27 min	1.302	19.7	80.3	1.274	60.7	39.3	3.468	70.0	30.0	0.850	94.7	5.3	0.171	45.2	54.8
28 min	1.277	19.4	80.6	1.266	60.3	39.7	3.481	70.2	29.8	0.891	99.2	0.8	0.171	45.2	54.8
29 min	1.300	19.7	80.3	1.263	60.2	39.8	3.477	70.1	29.9	0.882	98.3	1.7	0.177	46.9	53.1
30 min	1.296	19.6	80.4	1.266	60.4	39.6	3.299	66.6	33.4	0.857	95.5	4.5	0.176	46.6	53.4
31 min	1.308	19.8	80.2	1.276	60.8	39.2	3.341	67.4	32.6	0.891	99.2	0.8	0.175	46.2	53.8
32 min	1.301	19.7	80.3	1.278	60.9	39.1	3.551	71.6	28.4	0.900	100.3	-0.3	0.178	47.1	52.9
33 min	1.291	19.6	80.4	1.263	60.2	39.8	3.519	71.0	29.0	0.888	99.0	1.0	0.180	47.4	52.6
34 min	1.313	19.9	80.1	1.263	60.2	39.8	3.350	67.6	32.4	0.893	99.4	0.6	0.173	45.8	54.2
35 min	1.298	19.7	80.3	1.270	60.5	39.5	3.390	68.4	31.6	0.897	100.0	0.0	0.178	47.0	53.0
36 min	1.299	19.7	80.3	1.261	60.1	39.9	3.461	69.8	30.2	0.888	99.0	1.0	0.170	44.8	55.2
37 min	1.293	19.6	80.4	1.279	61.0	39.0	3.777	76.2	23.8	0.888	98.9	1.1	0.173	45.7	54.3
38 min	1.304	19.8	80.2	1.258	59.9	40.1	4.087	82.5	17.5	0.884	98.4	1.6	0.177	46.8	53.2
39 min	1.296	19.6	80.4	1.293	61.6	38.4	4.146	83.6	16.4	0.888	98.9	1.1	0.174	45.8	54.2
40 min	1.301	19.7	80.3	1.276	60.8	39.2	3.903	78.7	21.3	0.879	97.9	2.1	0.174	45.9	54.1
41 min	1.305	19.8	80.2	1.256	59.9	40.1	3.445	69.5	30.5	0.871	97.1	2.9	0.174	45.8	54.2
42 min	1.294	19.6	80.4	1.272	60.6	39.4	3.778	76.2	23.8	0.869	96.9	3.1	0.179	47.2	52.8
43 min	1.291	19.6	80.4	1.259	60.0	40.0	3.468	70.0	30.0	0.879	97.9	2.1	0.168	44.3	55.7
44 min	1.302	19.7	80.3	1.270	60.5	39.5	3.481	70.2	29.8	0.875	97.5	2.5	0.176	46.6	53.4
45 min	1.304	19.8	80.2	1.278	60.9	39.1	3.477	70.1	29.9	0.873	97.3	2.7	0.180	47.5	52.5
46 min	1.301	19.7	80.3	1.250	59.6	40.4	3.299	66.6	33.4	0.883	98.3	1.7	0.172	45.4	54.6
47 min	1.294	19.6	80.4	1.267	60.4	39.6	3.341	67.4	32.6	0.869	96.8	3.2	0.177	46.7	53.3
48 min	1.303	19.8	80.2	1.257	59.9	40.1	3.551	71.6	28.4	0.871	97.0	3.0	0.171	45.1	54.9
49 min	1.302	19.7	80.3	1.250	59.6	40.4	3.519	71.0	29.0	0.873	97.3	2.7	0.172	45.4	54.6
50 min	1.303	19.8	80.2	1.271	60.6	39.4	3.350	67.6	32.4	0.867	96.6	3.4	0.172	45.3	54.7
51 min	1.302	19.7	80.3	1.240	59.1	40.9	3.431	69.2	30.8	0.879	97.9	2.1	0.178	46.9	53.1
52 min	1.294	19.6	80.4	1.255	59.8	40.2	3.278	66.1	33.9	0.873	97.2	2.8	0.180	47.4	52.6
53 min	1.294	19.6	80.4	1.306	62.2	37.8	3.598	72.6	27.4	0.865	96.4	3.6	0.175	46.2	53.8
54 min	1.298	19.7	80.3	1.247	59.5	40.5	3.332	67.2	32.8	0.856	95.3	4.7	0.170	44.9	55.1
55 min	1.298	19.7	80.3	1.260	60.1	39.9	3.303	66.6	33.4	0.861	96.0	4.0	0.178	46.9	53.1
56 min	1.300	19.7	80.3	1.286	61.3	38.7	3.221	65.0	35.0	0.861	95.9	4.1	0.172	45.5	54.5
57 min	1.264	19.2	80.8	1.247	59.5	40.5	3.023	61.0	39.0	0.853	95.1	4.9	0.172	45.3	54.7
58 min	1.299	19.7	80.3	1.255	59.8	40.2	3.291	66.4	33.6	0.859	95.7	4.3	0.170	45.0	55.0
59 min	1.278	19.4	80.6	1.237	58.9	41.1	3.179	64.1	35.9	0.860	95.8	4.2	0.169	44.6	55.4
60 min	1.302	19.7	80.3	1.253	59.7	40.3	2.998	60.5	39.5	0.880	98.1	1.9	0.172	45.5	54.5
61 min	1.262	19.1	80.9	1.270	60.5	39.5	3.263	65.8	34.2	0.889	99.0	1.0	0.178	47.1	52.9

	<i>A. undulatum</i>			<i>P. affine</i>			<i>P. patens</i>			<i>F. hygrometrica</i>			<i>M. polymorpha</i>		
	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.	mm ²	%	decl.
62 min	1.312	19.9	80.1	1.255	59.8	40.2	3.211	64.8	35.2	0.878	97.8	2.2	0.179	47.3	52.7
63 min	1.303	19.8	80.2	1.284	61.2	38.8	3.205	64.6	35.4	0.880	98.1	1.9	0.178	47.1	52.9
64 min	1.302	19.7	80.3	1.247	59.4	40.6	3.215	64.9	35.1	0.874	97.4	2.6	0.178	46.9	53.1
65 min	1.302	19.7	80.3	1.236	58.9	41.1	3.124	63.0	37.0	0.878	97.8	2.2	0.179	47.4	52.6
66 min	1.287	19.5	80.5	1.239	59.1	40.9	2.858	57.6	42.4	0.878	97.8	2.2	0.170	44.9	55.1
67 min	1.297	19.7	80.3	1.253	59.7	40.3	2.628	53.0	47.0	0.884	98.5	1.5	0.172	45.4	54.6
68 min	1.303	19.8	80.2	1.241	59.1	40.9	2.804	56.6	43.4	0.888	98.9	1.1	0.179	47.3	52.7
69 min	1.302	19.7	80.3	1.240	59.1	40.9	2.904	58.6	41.4	0.878	97.8	2.2	0.179	47.2	52.8
70 min	1.298	19.7	80.3	1.310	62.4	37.6	2.664	53.7	46.3	0.878	97.8	2.2	0.179	47.3	52.7
71 min	1.298	19.7	80.3	1.241	59.1	40.9	3.431	69.2	30.8	0.866	96.5	3.5	0.179	47.2	52.8
72 min	1.292	19.6	80.4	1.256	59.9	40.1	3.278	66.1	33.9	0.866	96.5	3.5	0.187	49.4	50.6
73 min	1.303	19.8	80.2	1.243	59.3	40.7	3.598	72.6	27.4	0.870	96.9	3.1	0.170	44.8	55.2
74 min	1.300	19.7	80.3	1.303	62.1	37.9	3.332	67.2	32.8	0.863	96.1	3.9	0.179	47.3	52.7
75 min	1.284	19.5	80.5	1.298	61.9	38.1	3.303	66.6	33.4	0.876	97.6	2.4	0.169	44.5	55.5
76 min	1.299	19.7	80.3	1.244	59.3	40.7	3.221	65.0	35.0	0.874	97.4	2.6	0.178	47.0	53.0
77 min	1.303	19.8	80.2	1.234	58.8	41.2	3.023	61.0	39.0	0.876	97.6	2.4	0.179	47.4	52.6
78 min	1.302	19.7	80.3	1.28	61.0	39.0	3.291	66.4	33.6	0.870	96.9	3.1	0.168	44.4	55.6
79 min	1.294	19.6	80.4	1.292	61.6	38.4	3.179	64.1	35.9	0.868	96.7	3.3	0.179	47.4	52.6
80 min	1.299	19.7	80.3	1.307	62.3	37.7	2.998	60.5	39.5	0.880	98.0	2.0	0.179	47.2	52.8
81 min	1.277	19.4	80.6	1.23	58.6	41.4	3.263	65.8	34.2	0.880	98.0	2.0	0.179	47.2	52.8
82 min	1.250	19.0	81.0	1.256	59.9	40.1	3.211	64.8	35.2	0.881	98.2	1.8	0.174	46.0	54.0
83 min	1.306	19.8	80.2	1.242	59.2	40.8	3.205	64.6	35.4	0.877	97.8	2.2	0.176	46.4	53.6
84 min	1.310	19.9	80.1	1.238	59.0	41.0	3.215	64.9	35.1	0.879	98.0	2.0	0.171	45.2	54.8
85 min	1.237	18.8	81.2	1.243	59.2	40.8	3.124	63.0	37.0	0.894	99.6	0.4	0.180	47.5	52.5
86 min	1.290	19.6	80.4	1.258	60.0	40.0	2.858	57.6	42.4	0.890	99.1	0.9	0.171	45.2	54.8
87 min	1.306	19.8	80.2	1.265	60.3	39.7	2.628	53.0	47.0	0.879	97.9	2.1	0.170	45.0	55.0
88 min	1.313	19.9	80.1	1.253	59.7	40.3	2.804	56.6	43.4	0.897	99.9	0.1	0.174	46.0	54.0
89 min	1.280	19.4	80.6	1.236	58.9	41.1	2.904	58.6	41.4	0.864	96.2	3.8	0.169	44.7	55.3
90 min	1.302	19.7	80.3	1.26	60.0	40.0	2.664	53.7	46.3	0.881	98.1	1.9	0.180	47.5	52.5
91 min	1.296	19.6	80.4	1.245	59.3	40.7	3.130	63.1	36.9	0.862	96.0	4.0	0.169	44.7	55.3
92 min	1.252	19.0	81.0	1.25	59.6	40.4	2.806	56.6	43.4	0.877	97.7	2.3	0.180	47.4	52.6
93 min	1.229	18.6	81.4	1.251	59.6	40.4	3.042	61.4	38.6	0.877	97.7	2.3	0.176	46.5	53.5
94 min	1.199	18.2	81.8	1.242	59.2	40.8	3.049	61.5	38.5	0.875	97.5	2.5	0.179	47.3	52.7
95 min	1.300	19.7	80.3	1.245	59.3	40.7	2.957	59.7	40.3	0.894	99.6	0.4	0.171	45.1	54.9
96 min	1.235	18.7	81.3	1.237	59.0	41.0	2.783	56.1	43.9	0.885	98.6	1.4	0.180	47.5	52.5
97 min	1.237	18.8	81.2	1.263	60.2	39.8	2.980	60.1	39.9	0.894	99.6	0.4	0.174	45.9	54.1
98 min	1.291	19.6	80.4	1.237	59.0	41.0	2.690	54.3	45.7	0.885	98.6	1.4	0.175	46.2	53.8
99 min	1.246	18.9	81.1	1.229	58.6	41.4	3.113	62.8	37.2	0.889	99.1	0.9	0.178	47.1	52.9
100 min	1.304	19.8	80.2	1.237	59.0	41.0	2.825	57.0	43.0	0.889	99.1	0.9	0.180	47.4	52.6
101 min	1.311	19.9	80.1	1.244	59.3	40.7	3.039	61.3	38.7	0.889	99.0	1.0	0.173	45.8	54.2
102 min	1.297	19.7	80.3	1.24	59.1	40.9	2.893	58.4	41.6	0.882	98.3	1.7	0.178	47.0	53.0
103 min	1.311	19.9	80.1	1.239	59.0	41.0	2.779	56.1	43.9	0.886	98.7	1.3	0.170	44.8	55.2
104 min	1.302	19.7	80.3	1.237	59.0	41.0	2.746	55.4	44.6	0.872	97.1	2.9	0.173	45.7	54.3
105 min	1.307	19.8	80.2	1.244	59.3	40.7	2.934	59.2	40.8	0.874	97.3	2.7	0.177	46.8	53.2
106 min	1.303	19.8	80.2	1.241	59.2	40.8	2.785	56.2	43.8	0.888	98.9	1.1	0.174	45.8	54.2
107 min	1.303	19.8	80.2	1.248	59.5	40.5	2.721	54.9	45.1	0.881	98.2	1.8	0.174	45.9	54.1
108 min	1.289	19.5	80.5	1.237	58.9	41.1	2.987	60.3	39.7	0.893	99.5	0.5	0.174	45.8	54.2
109 min	1.255	19.0	81.0	1.227	58.5	41.5	2.680	54.1	45.9	0.881	98.2	1.8	0.179	47.2	52.8
110 min	1.310	19.9	80.1	1.296	61.8	38.2	2.553	51.5	48.5	0.867	96.6	3.4	0.168	44.3	55.7
111 min	1.304	19.8	80.2	1.25	59.6	40.4	2.805	56.6	43.4	0.895	99.7	0.3	0.176	46.6	53.4
112 min	1.295	19.6	80.4	1.232	58.7	41.3	2.580	52.1	47.9	0.885	98.6	1.4	0.171	45.1	54.9
113 min	1.279	19.4	80.6	1.398	66.6	33.4	2.955	59.6	40.4	0.877	97.7	2.3	0.172	45.4	54.6
114 min	1.278	19.4	80.6	1.24	59.1	40.9	2.819	56.9	43.1	0.883	98.4	1.6	0.172	45.3	54.7
115 min	1.257	19.1	80.9	1.23	58.6	41.4	2.542	51.3	48.7	0.883	98.4	1.6	0.178	46.9	53.1
116 min	1.299	19.7	80.3	1.24	59.1	40.9	2.585	52.1	47.9	0.885	98.7	1.3	0.180	47.4	52.6
117 min	1.300	19.7	80.3	1.231	58.7	41.3	2.065	41.7	58.3	0.883	98.4	1.6	0.172	45.4	54.6
118 min	1.301	19.7	80.3	1.229	58.6	41.4	2.476	50.0	50.0	0.873	97.3	2.7	0.177	46.7	53.3
119 min	1.246	18.9	81.1	1.24	59.1	40.9	2.211	44.6	55.4	0.890	99.2	0.8	0.172	45.5	54.5
120 min	1.301	19.7	80.3	1.238	59.0	41.0	2.722	54.9	45.1	0.883	98.4	1.6	0.170	45.0	55.0

Tab. 25. Descriptive statistics of the gametophyte dehydration (120 minutes).

Gametophyte dehydration 120 minutes	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	120	1419676,54608	662297,26086	1,7E8	1199395,59	6596495,63
<i>P. affine</i>	120	1305852,98192	161708,28748	1,6E8	1226959,26	2131718,5
<i>P. patens</i>	120	3268049,20892	482171,72019	3,9E8	2065011,25	4956967,8
<i>F. hygrometrica</i>	120	876323,0815	11789,8676	1,1E8	842021,44	900051,7
<i>M. polymorpha</i>	120	177972,84833	20809,0135	2,1E7	167649,25	378683,45

Tab. 26. Pearson correlation coefficient and p-value of the gametophyte dehydration (120 minutes).

Gametophyte dehydration 120 minutes		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	0,87558	0,54707	0,17225	0,90933
<i>A. undulatum</i>	p-value	--	<0.0001	<0.0001	0,05994	<0.0001
<i>P. affine</i>	Pearson cor.	0,87558	1	0,64022	0,05215	0,67088
<i>P. affine</i>	p-value	<0.0001	--	<0.0001	0,57161	<0.0001
<i>P. patens</i>	Pearson cor.	0,54707	0,64022	1	-0,17134	0,46103
<i>P. patens</i>	p-value	<0.0001	<0.0001	--	0,06132	<0.0001
<i>F. hygrometrica</i>	Pearson cor.	0,17225	0,05215	-0,17134	1	0,20016
<i>F. hygrometrica</i>	p-value	0,05994	0,57161	0,06132	--	0,02838
<i>M. polymorpha</i>	Pearson cor.	0,90933	0,67088	0,46103	0,20016	1
<i>M. polymorpha</i>	p-value	<0.0001	<0.0001	<0.0001	0,02838	--

Tab.26. contained the **Pearson correlation coefficients** generated from the data of the “gametophyte dehydration” over 120 minutes (Fig.57.). 7 positive correlations with significant p-values were found, in *A. undulatum* and *P. affine* **0.87558**, in *A. undulatum* and *P. patens* **0.54707**, in *A. undulatum* and *M. polymorpha* **0.90933**, all with a highly significant p-value < 0.0001. Further positive correlations included *P. affine* and *P. patens* **0.64022**, *P. affine* and *M. polymorpha* **0.67088**, in *P. patens* and *M. polymorpha* **0.46103**, with a highly significant p-value < 0.0001. *F. hygrometrica* and *M. polymorpha* revealed a correlation of **0.20016** with a significant p-value of 0.2838 (Tab.26.).

3.7. Confocal laser scanning microscopy (CLSM)

A series of Z images were taken with a laser speed of 200 hz, resulting in a faster scan with lower quality, and a 400 hz for slower and higher quality scan. From the stacks, 3D reconstructions were generated, depicting the cell wall in frontal and side view. Settings included 650-700 nm to excite the chloroplasts, and 440-649 nm the cell wall. The images relied on the autofluorescence of cell wall and chloroplasts, without dyes. The blue cell wall, and red chloroplasts are false colors, selected to be able to distinguish between the cell components visually. Due to the multiple cell thick thallus, for confocal images of *M. polymorpha* smaller magnification was used, and only side view images were made. The smallest mean cell diameter was found in *P. affine*, 10.17 μm followed by *P. patens* 15.85 μm , *A. undulatum* 17.83 μm , *F. hygrometrica* 17.17 μm , and the largest in *M. polymorpha* with 28.67 μm (Fig. 67.).

3.7.1. Cell wall and chloroplasts

A. undulatum in side-view revealed (y-axis on the left - the z-axis below) blue cell wall fragment (Fig. 58.A) and the nicely resolved, red chloroplasts (Fig.57.B). Frontal view images showed (y-axis on the left - x-axis below) images of the cell wall (Fig. 58.A) and the chloroplasts (Fig. 59.B). A **cell diameter of 16 μm** was estimated. The frontal overlay image (Fig. 59.C) showed the blue cell wall in the background, with the chloroplast is in a circular arrangement, accumulating at the edge of the cell

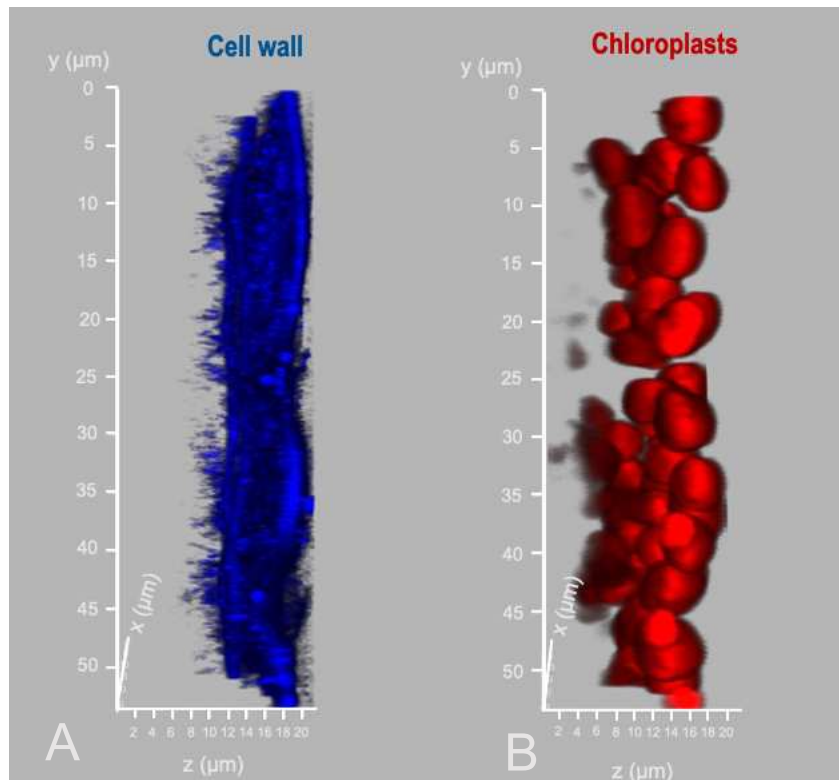


Fig. 58. Confocal side view image of *A. undulatum* shows A) cell wall (blue), B) chloroplasts (red).

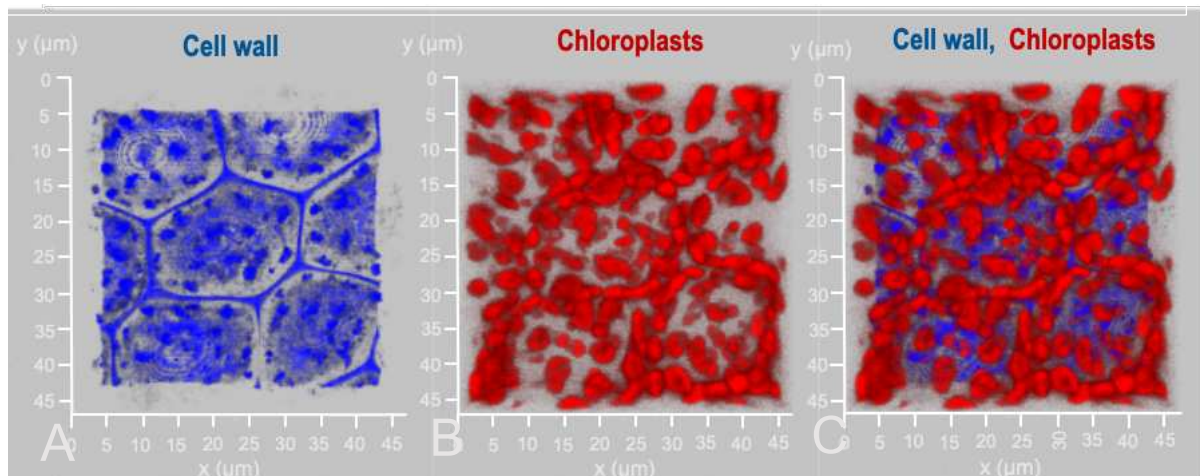


Fig. 59. Confocal frontal view image of *A. undulatum* A) cell walls are in blue, B) chloroplasts in red, C) overlay of chloroplasts and cell wall.

P. affine side view image depicted (y-axis on the left - the z-axis below) blue cell wall fragment (Fig. 60.A), and large, round chloroplasts (Fig. 61.B). The side view overlay image of *P. affine* reveals large, chloroplasts, located in the background, and the cell wall in front encircles, and partially covers them. Frontal view (y-axis on the left - x-axis below) images of the cell wall outline, with a pointed end (Fig. 61.A). The chloroplasts are arranged in a cluster, at the center of the cell (Fig. 61.B) and their shape resembles a peanut. A **cell diameter** of **12 μm** was documented (Fig. 61.C).

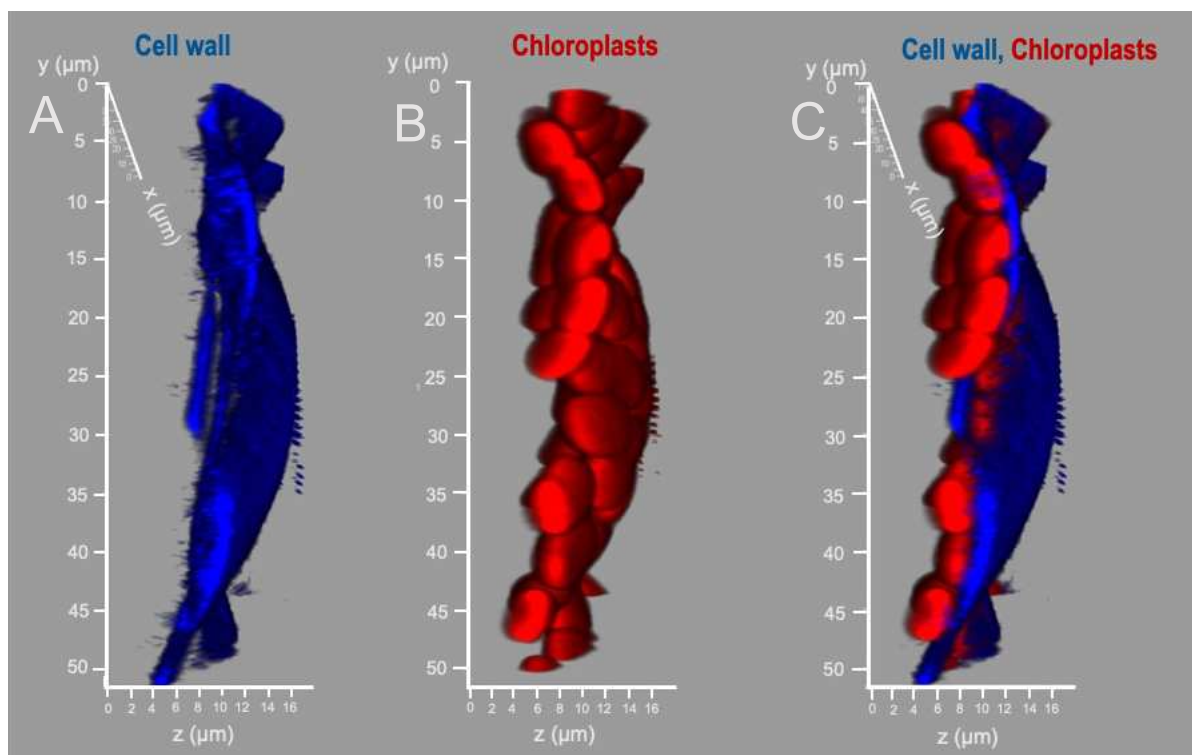


Fig. 60. Confocal side view image of *P. patens* shows A) cell wall blue, B) chloroplasts red.

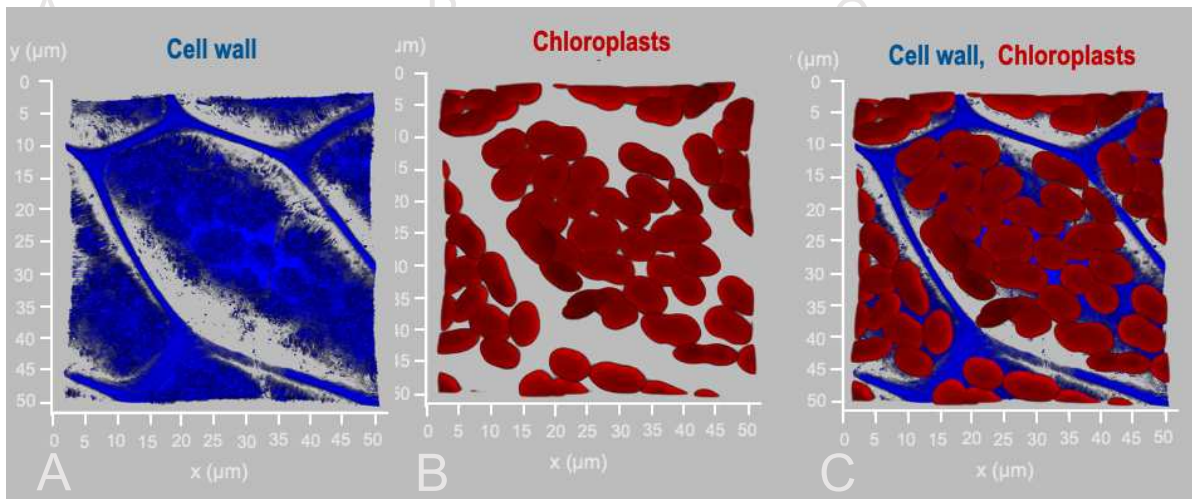


Fig. 61. Confocal frontal view image of *P. affine* depicts A) cell wall in and B) chloroplasts (red), C) and an overlay image of chloroplasts and cell wall is seen.

P. patens side view revealed, (y-axis on the left - the z-axis below) the cell wall is made up of disconnected fragments, partially overlapping. (Fig. 62.A). In the chloroplast sideview, they were overlapping and arranged in a line (Fig. 63.B). The overlay image revealed that the cell area revealed a **cell diameter** of **5,5 μm** (Fig. 63.C) The frontal view (y-axis on the left - x-axis below) images shows a cell wall made up disconnected, lines (Fig.64.A), and small chloroplasts, arranged in lines, in front of the cell wall (Fig.61.B, and C).

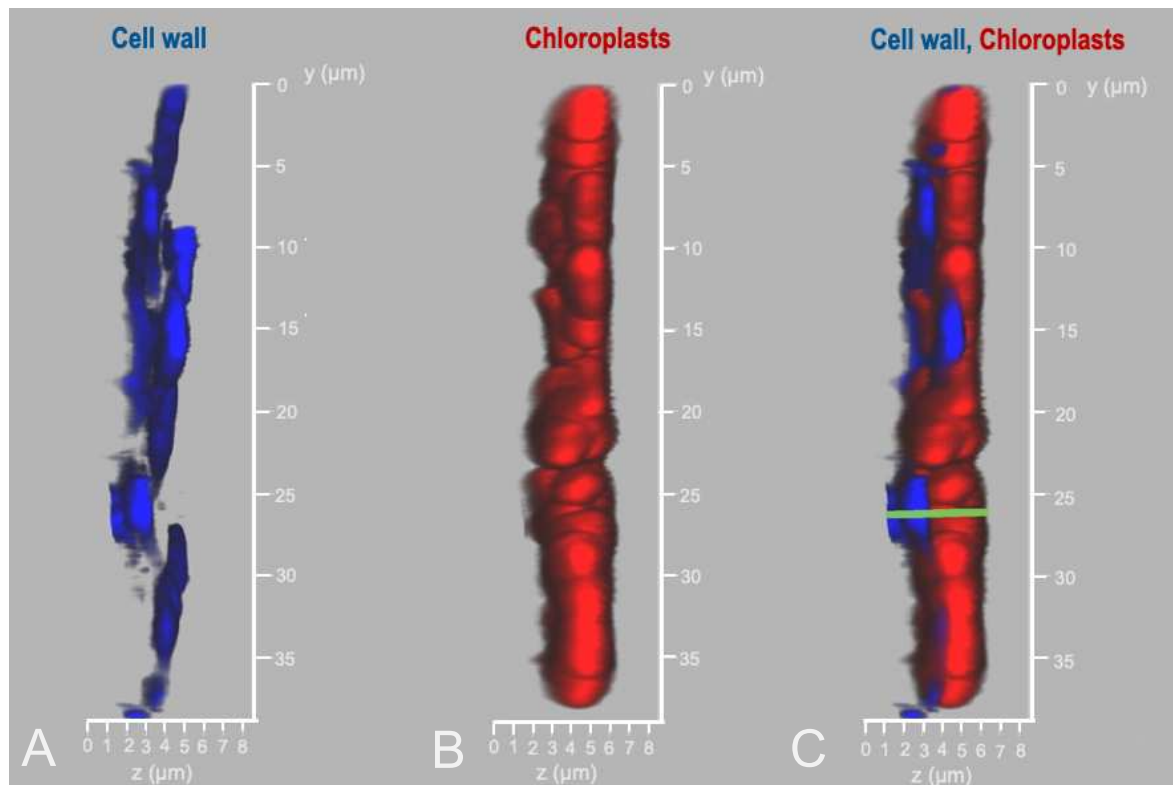


Fig. 62. Confocal side view image of *P. patens* shows A) cell wall in blue and B) chloroplasts in red, C) and overlay of chloroplasts and cell wall.

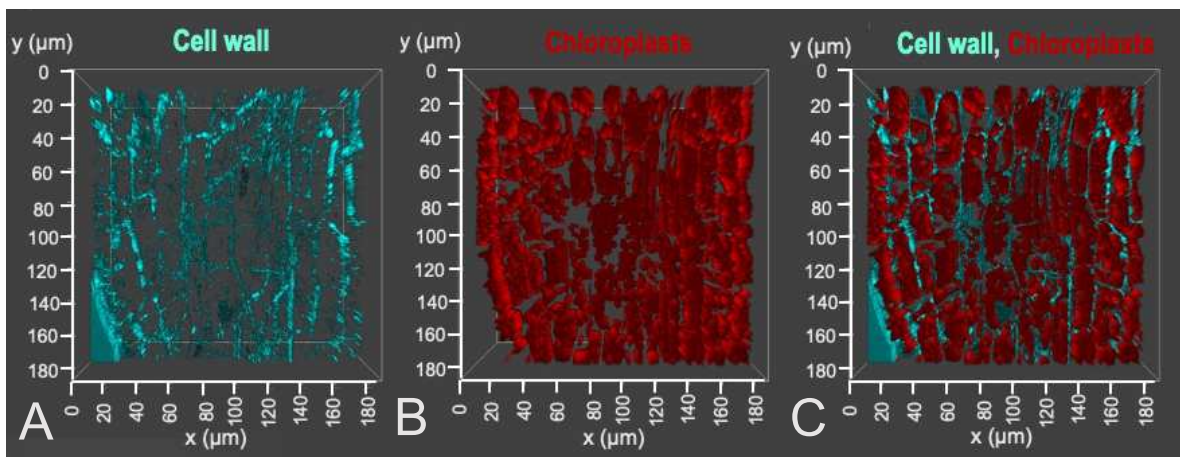


Fig. 63. Confocal frontal image view of *P. patens* depicts A) cell wall in blue and B) chloroplasts in red and C) an overlay image of chloroplasts and cell wall.

The side view of *F. hygrometrica* (y-axis on the left - the z-axis below) shows a line of cell wall (Fig.63.A) and small, round chloroplasts, densely arranged at the center (Fig.63.B), an overlay image (Fig.63.C) showed a **cell diameter** of **23 μm** . In the frontal view (y-axis on the left - x-axis below) images showed a smooth cell wall surface (Fig.64.A), a few, small, round chloroplasts (Fig.64.B), clustered lines at the edge of the cell (Fig.64.C).

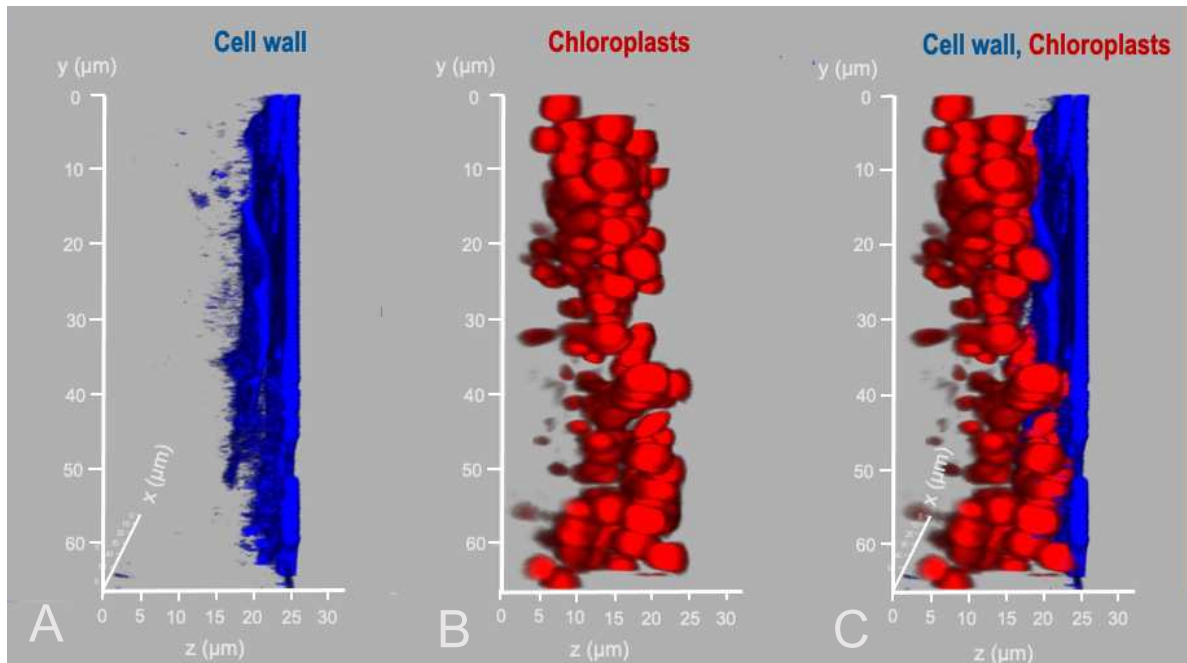


Fig. 64. Confocal side view image of *F. hygrometrica*, shows A) cell wall in blue and B) chloroplasts in red, C) and overlap of chloroplasts and cell wall.

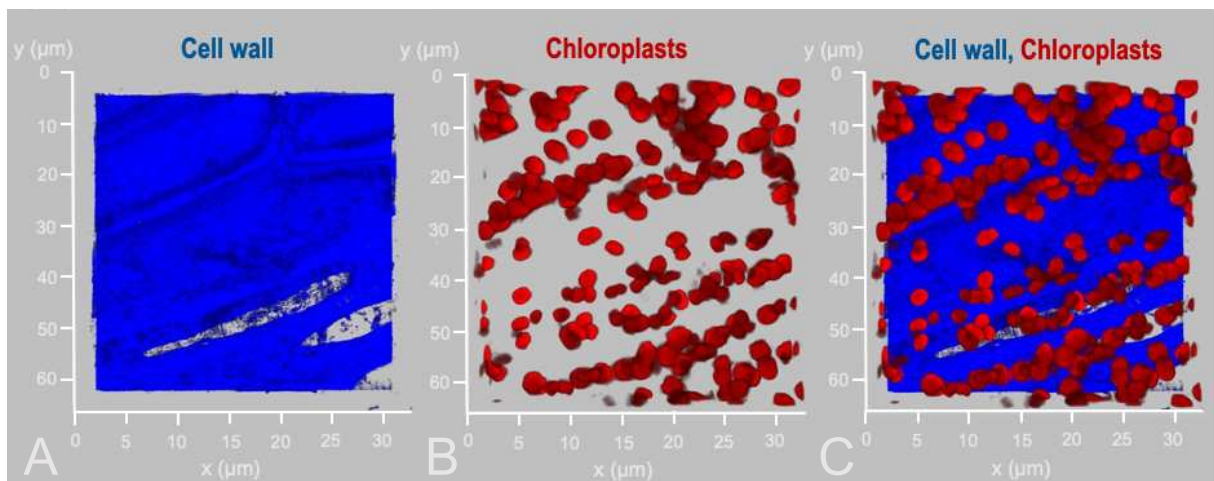


Fig. 65. Confocal frontal view image of *F. hygrometrica*, reveals A) cell wall in blue and B) chloroplasts in red, and C) an overlay image of chloroplasts and cell wall is seen.

Due to the multiple-cell thick thallus, Confocal images of *M. polymorpha* were made under smaller magnification, and only side-view images were made. The side view (y-axis on the left - the z-axis below) shows a line of the cell wall (Fig. 66.A), a large number of small, tightly clustered chloroplasts (Fig. 66. B), and a **cell diameter** was determined **30 μm** (Fig. 66.C).

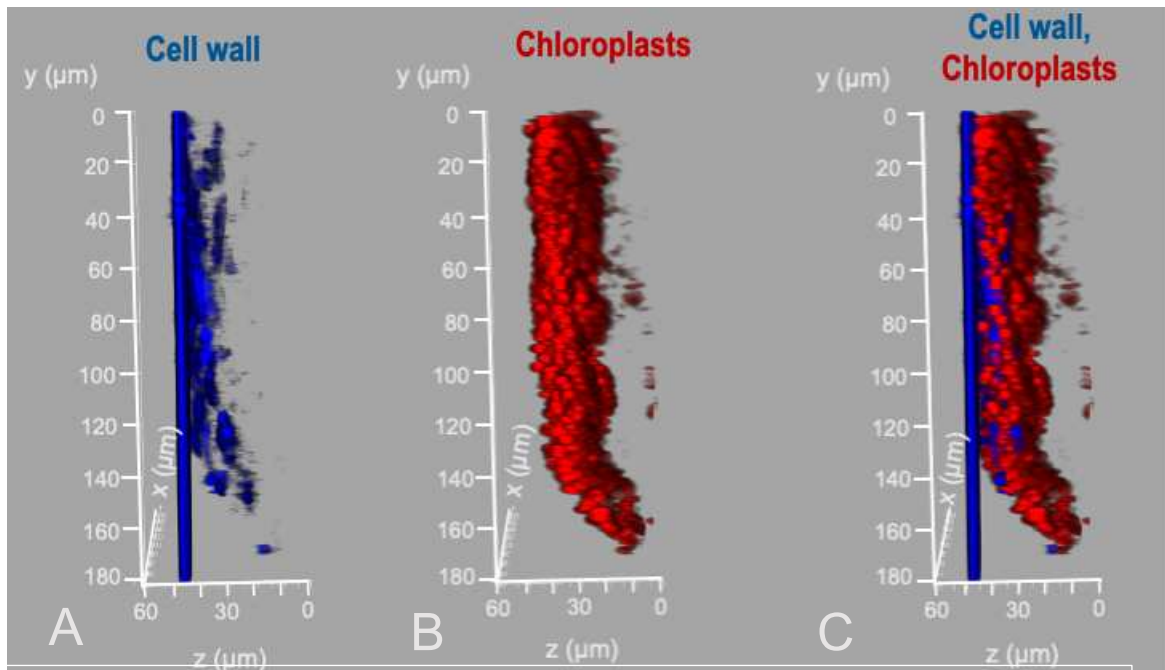


Fig. 66. Confocal side view image of *M. polymorpha* A) cell wall in blue and B) chloroplasts in red, and C) an overlay of chloroplasts and cell wall.

3.7.2. Leaf diameter

The confocal images revealed a **mean leaf diameter** in *A. undulatum* 17.83 μm , *P. affine* 10.17 μm , *P. patens* 15.85 μm , *F. hygrometrica* 17.17 μm , and the largest in *M. polymorpha* with 28.67 μm . The leaf diameter data area depicted in the box plot (Fig. 67) and further descriptive statistics, including the median, average, and standard deviation (SD), are found in Tab. 27."

All leaf diameter raw data can be found in the Appendix (p.145)

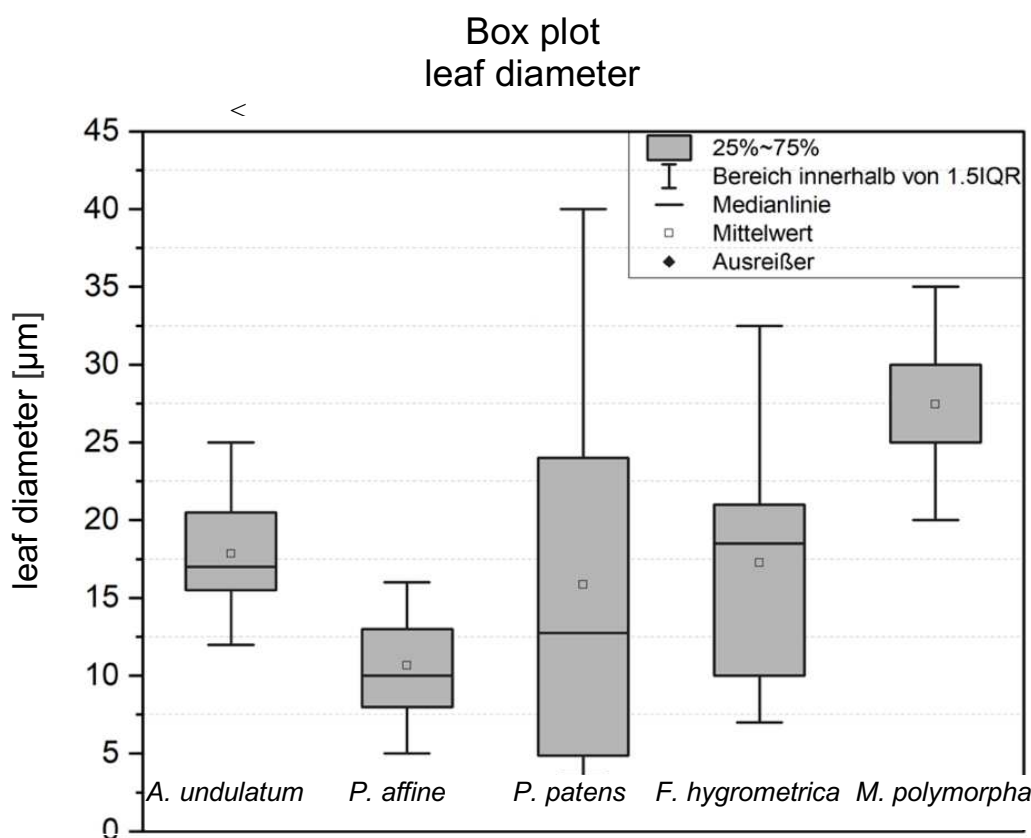


Fig. 67. Box plot depicting the data of the leaf diameter measurements of *A. undulatum*, *P. patens*, *F. hygrometrica*, *M. polymorpha*.

Tab. 27. Mean leaf diameter of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Leaf diameter (μm)			
	Median	Average	SD
<i>A. undulatum</i>	17	17.83	3.79
<i>P. affine</i>	10	10.17	3.71
<i>P. patens</i>	12.75	15.85	12.46
<i>F. hygrometrica</i>	18	17.17	7.09
<i>M. polymorpha</i>	25	28.67	5.54

A **T-test** of the **leaf diameter** revealed highly statistically significant differences in the leaf diameter of most species (Tab.28), indicated by extremely small p-values ($p < 0.05$) and marked as "significant". *A. undulatum* vs. *P. affine* has a p-value of 2.47E-19, which is far below 0.05, confirming a strongly significant difference in leaf diameter between these two species. Similarly, *A. undulatum* vs. *M. polymorpha* ($p = 8.87\text{E-}10$) and *P. patens* vs. *F. hygrometrica* ($p = 0.0000179$) also exhibit significant differences.

Two observations were not statistically significant is *P. affine* vs. *M. polymorpha* ($p = 0.5637$). Since $p > 0.05$, indicates that the leaf diameters of these two species do not differ significantly. *P. patens* vs. *M. polymorpha* ($p = 0.3659$), close to the significance threshold is which is also not significant.

Positive t-values (*A. undulatum* vs. *P. patens* = 4.83) can be interpreted that the first species in the comparison has a larger leaf diameter than the second species. Negative t-values (e.g., *A. undulatum* vs. *P. affine* = -12.26) indicate that the first species has a smaller leaf diameter than the second species.

Tab. 28. T- test of the leaf diameter (μm) and species-comparison of *A. undulatum*, *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha*.

Species- comparison leaf diameter (μm)				
T- test	t-value	df	p-value	($p < 0.05$)
<i>A. undulatum</i> vs <i>P. affine</i>	-12.26	19	2.47E-19	significant
<i>A. undulatum</i> vs <i>P. patens</i>	-4.83	22	8.36E-06	significant
<i>A. undulatum</i> vs <i>F. hygrometrica</i>	-5.81	25	2.04E-07	significant
<i>A. undulatum</i> vs <i>M. polymorpha</i>	-7.46	18	8.87E-10	significant
<i>P. affine</i> vs <i>P. patens</i>	6.26	19	1.1E-08	significant
<i>P. affine</i> vs <i>F. hygrometrica</i>	5.27	22	8.29E-07	significant
<i>P. affine</i> vs <i>M. polymorpha</i>	0.58	15	0.5633	x
<i>P. patens</i> vs <i>F. hygrometrica</i>	-0.91	25	0.3659	x
<i>P. patens</i> vs <i>M. polymorpha</i>	-4.61	18	0.0000179	significant
<i>F. hygrometrica</i> vs <i>M. polymorpha</i>	-4.01	21	0.000148	significant

Tab. 29. Descriptive statistics of the leaf diameter.

<i>Confocal diameter</i>	N	Mean	SD	Sum	Min	Max
<i>A. undulatum</i>	12	17,83333	3,78594	214	12	25
<i>P. affine</i>	9	10,66667	3,67423	96	5	16
<i>P. patens</i>	12	15,85	12,46074	190,2	3,5	40
<i>F. hygrometrica</i>	14	17,25	7,3452	241,5	7	32,5
<i>M. polymorpha</i>	9	27,44444	5,36449	247	20	35

Tab. 30. Pearson correlation coefficient and p-value of the leaf diameter.

<i>Confocal diameter</i>		<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
<i>A. undulatum</i>	Pearson cor.	1	-0,14565	-0,0688	0,00635	0,08031
<i>A. undulatum</i>	p-value	--	0,70847	0,83176	0,98436	0,83727
<i>P. affine</i>	Pearson cor.	-0,14565	1	-0,23768	-0,1349	0,19237
<i>P. affine</i>	p-value	0,70847	--	0,53803	0,72932	0,61999
<i>P. patens</i>	Pearson cor.	-0,0688	-0,23768	1	-0,62399	0,70074
<i>P. patens</i>	p-value	0,83176	0,53803	--	0,03012	0,03549
<i>F. hygrometrica</i>	Pearson cor.	0,00635	-0,1349	-0,62399	1	-0,60724
<i>F. hygrometrica</i>	p-value	0,98436	0,72932	0,03012	--	0,08287
<i>M. polymorpha</i>	Pearson cor.	0,08031	0,19237	0,70074	-0,60724	1
<i>M. polymorpha</i>	p-value	0,83727	0,61999	0,03549	0,08287	--

In table (30). the data of “Box plot - leaf diameter” (Fig. 67.) have undergone statistical analysis, and revealed one positive, and one negative **Pearson correlation coefficient** with significant p-values. A negative correlation of **-0.62399** was observed in *P. patens* and *F. hygrometrica* with a significant p-value of 0.03012. Further, a positive correlation of **0.70074** in *P. patens* and *M. polymorpha*, with a significant p-value of 0.03549 was documented.

4. Discussion

Mosses exhibit remarkable resilience to desiccation-rehydration cycles, with their photosynthetic efficiency typically recovering after rehydration (Orekhova & Hájek, 2022; Csintalan et al., 1999). Desiccation tolerance and sensitivity are influenced by various interacting factors, including genotypic differences (Proctor et al., 2007), ecotypic plasticity, the physiological history of the sample, microclimatic conditions, and the rate of desiccation or dehydration, all of which can interact and modify the plant's response. While experiencing dehydration, the quantum yield of PSII (FV/FM) and effective quantum yield (Φ PSII) decline, but in desiccation-tolerant species, they quickly rebound upon rehydration (Orekhova & Hájek, 2022; Csintalan et al., 1999). In the rehydration series, all species that were investigated here showed an almost instant increase in the photosynthetic activity upon remoistening (Fig.35 & 36.). This supports the finding that all investigated species can be considered drought tolerant. Moss species from hydric environments showed limited recovery potential and may experience photoinhibition when exposed to drying under light conditions (Seel et al., 1992). While the moss specimens were selected based on ecological differences, they can all be considered inhabitants of wet environments. The dehydration series was performed under room light conditions (Fig.12.). Consequently, the observed decline in photosynthesis is likely not due to damage to the photosynthetic apparatus, but rather a result of insufficient water availability.

4.1. Weight loss and photosynthetic activity

The concept of inducible dehydration tolerance is found in many bryophytes (Beckett 1999; Brinda et al. 2016; Bu et al. 2017; Cruz de Carvalho et al. 2014; Greenwood & Stark 2014; Guschina et al. 2002; Hajek & Vicherova 2014; Pressel & Duckett 2010; Werner et al. 1991). These studies suggest that inducible dehydration tolerance in bryophytes is a common trait, facilitated by mechanisms such as slow drying, abscisic acid application, and environmental cues, allowing rapid recovery of photosynthesis and cellular structures upon rehydration. Leaf curling in desiccated mosses reduces photon absorption by 50-60 %, yet this alone does not fully explain the complete loss of photosynthetic activity during dehydration. In our experiments, light microscopy images of the leaves during dehydration for all species (Fig. 45–Fig. 49) revealed that curling took place within

the first 2 hours, which coincided with the phase of major leaf area loss, followed by a stable phase from 2 - 6 hours (Fig.50). A protective mechanism is the Non-photochemical quenching (NPQ) often reaching its peak during the initial phase of rehydration (Csintalan et al., 1999; Giudici, 2021). NPQ was not further investigated in this study, since the only photosynthetic parameter was the quantum yield.

In the present experiment, dehydration series revealed over 48 hours general trends in **weight** loss behavior. In all bryophytes investigated, at one hour, 50 % of the initial weight is lost, at 2 hours, 68-84 %, and at 3 hours, 80-91 %. Species-specific differences include a 36 % decline in *M. polymorpha* at 1 hour, at 2 hours, 66 %, and at 3 hours, 89 %. *P. affine*, *P. patens*, *F. hygrometrica*, and *M. polymorpha* revealed a decline of 90 % after 3 hours and remained at this level until 48 hours, except in *A. undulatum*, in which 90 % were reached at 4 hours. *P. patens* the weight loss showed a gradual decline in the first 2 hours, a strong decline, reaching 90 % weight loss. The species are discussed separately below (Fig.20. & 21.).

To summarize the observations of the **photosynthetic activity** measurements, *P. affine* showed a 12 % increase, *A. undulatum*, *M. polymorpha*, and an increase of 20 % when the highest photosynthetic activity value was documented. *P. patens* an 82 %, increase, and *F. hygrometrica*, the first measurement was the highest photosynthetic activity documented as an initial value and afterward declined 20 %. One general pattern could be observed, that at 48 hours, around 90 % of the initial quantum yield was lost (Fig. 22. & 23.A). Pearson correlation analysis of the quantum yield (Tab. 5.) revealed positive correlation values from 0.89-0.98 and a highly significant p-value <0.0001 was observed in all species. Further, a combined correlation of "Weight loss and photosynthetic activity" (Tab. 6.) indicates a positive correlation in *A. undulatum* 0.97, *P. affine* 0.97, in *P. patens* 0.93, in *F. hygrometrica* 0.90, and in *M. polymorpha* 0.93, with a highly significant p-value <0.0001.

For **rehydration**, 200 µL of water was added at the beginning, followed by gradual rehydration over the first 30 minutes, which led to an increase in photosynthesis. In *P. affine*, *F. hygrometrica*, *M. polymorpha*, and *A. undulatum*, dehydration occurred from 30 to 90 minutes, resulting in a decline in photosynthesis (Fig.35. & 36.). This is an effect of the dehydration because water was only added once (at

the beginning) and after this was used up at room temperature, the plantlets went into a new dehydration. It is particularly interesting that during dehydration, the quantum yield typically drops to around 0.05. In contrast, during rehydration, it begins at a considerably higher level but then declines over time. Notably, at the onset of rehydration, the quantum yield tends to fluctuate rather than show a consistent increase, indicating a delayed or unstable recovery of photosynthetic activity.

4.2. Species-specific effects

Physcomitrium patens, a model moss species, demonstrates a high degree of abiotic stress tolerance in a study, (Frank, Raknadevi, and Reski, 2005) investigating the effect of drought, salt, and osmotic stress on this species. Mosses cultivated in *in vitro* culture were left to dehydrate for up to 48 hours. After rehydration in water, they were re-transferred to their standard growth medium. Despite losing 92 % of their initial weight at 20 hours of dehydration, after 2 weeks they recovered under standard growth conditions and a large part of their biomass. Another parameter investigated was the chlorophyll content, which reached a higher level after dehydration and decreased gradually after 2 weeks under normal conditions. The high degree of tolerance found in *P. patens* makes it a valuable source for the characterization of genes involved in the stress adaptation response (Frank, Raknadevi, and Reski, 2005). Further, the application of external abscisic acid (ABA) allowed *P. patens* to endure surviving desiccation beyond -13 MPa water potential generally (Koster et al., 2010; Xiao et al., 2017). However, when dried gradually, gametophores can withstand severe desiccation without exogenous ABA, highlighting the importance of drying rate in tolerance acquisition (Xiao et al., 2017). Experiments demonstrated that cellulose discs played a role in water retention during dehydration and rehydration, mimicking a natural moist environment. This setup facilitated a gradual, nearly linear dehydration pattern over 48 hours. From all species, *P. patens* had the lowest starting quantum yield of 0.17 (Fig. 22. & 23.) and the flattest curve during both dehydration and rehydration (Fig. 35. & 36.) Endogenous levels of ABA increase during dehydration, however, they do not directly influence the desiccation tolerance (Xiao et al., 2017). Desiccation-induced cellular damage primarily impacts plasma membranes, causing electrolyte leakage upon rehydration (Koster et al., 2010).

Additionally, dehydration and rehydration generate calcium waves that travel from the base to the top of the gametophore, though at slower speeds compared to vascular plants, suggesting a role in stress signaling (Storti et al., 2018). During dehydration, it suppresses the expression of photosynthesis-related proteins while increasing the production of defense proteins, such as late embryogenesis abundant (LEA) proteins (Cui et al., 2011). The moss utilizes rapid protein response mechanisms similar to those found in angiosperm seeds (Cui et al., 2011).

Dehydration tolerance is a key adaptation in plants and has been extensively studied in mosses like *Atrichum undulatum*. In a recent study by Hu and Co. authors conducted a dehydration experiment, in which *A. undulatum* capsules were captured from the wild, and then were cultivated from spores, on Benecke medium for 4 weeks, under fixed light and temperature conditions (85 % Relative water content RH). The dehydration for 3 days took place under the following conditions (30 % RH, 25/20 °C day/night temperature, a 14-h/10-h light-dark cycle, and illumination at 100 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$). Rehydration was done in liquid MS medium for 1 hour, supplemented with 2 % glucose, followed by transfer to solid MS medium, for the recovery process. Increased electrolyte leakage was documented during dehydration and the first 24 h of rehydration, the respiration rate was reduced by 50 % and increased twofold during rehydration. During dehydration, a decrease in net photosynthesis (P_n) of 50 % during the first day, and remained low during the first day of rehydration, and a decline in the PSII potential activity (F_v/F_0), effective PSII quantum yield declined, and Proline accumulation could be observed. (Hu, et al., 2016).) This finding supports the fact that *Atrichum undulatum* is commonly defined as a desiccation-tolerant plant (Beckett et al. 2000). Maintenance of membrane stability is critical for plant survival under environmental stress. It employs strategies to maintain membrane integrity, protect cellular structures, and regulate reactive oxygen species during dehydration and rehydration cycles. Curled leaves, plasmolysis, and increased electrolyte leakage during dehydration reversed during rehydration. The observations suggest that drought stress-induced membrane damage in *A. undulatum* cells is neither severe nor irreparable. (Hu et al., 2016). Water loss in the tissues reduces the area available for CO_2 uptake, resulting in a lower P_n . In

A. undulatum, all photosynthetic parameters F_m , F_v/F_m , and F_v/F_0 were constrained during dehydration, as were $Y(PSII)$, ETR (E), and $Y(NPQ)$. These findings led to the conclusion that dehydration stress significantly affected PSII function in *A. undulatum* (Hu, et al., 2016). The desiccation tolerance in *A. undulatum*, tolerating 90 % water loss, displays a level of desiccation tolerance intermediate between those of *Syntrichia ruralis* and *P. patens*. (Wood, 2007) –A comparison of our findings revealed that *P. patens* lost 90 % of its weight within 3 hours, while *Atrichum undulatum* reached 91 % weight loss at 4 hours. Further analysis of all investigated species showed that the 90 % weight loss threshold was reached at 4 hours for *A. undulatum*, *P. affine*, and *F. hygrometrica*; at 3 hours for *P. patens*; and at 6 hours for *M. polymorpha* (Tab.2). Recent studies suggested that the closely related species, *Atrichum androgynum*, can - stand equilibrium at nearly 0 % relative humidity (RH), corresponding to a relative water content (RWC) of 4 %, with a series of physiological changes (Beckett and Hoddinott 1997; Guschina et al. 2002; Mayaba et al. 2001, 2002; Mayaba and Beckett 2003).

The moss ***Funaria hygrometrica*** has developed specialized mechanisms to withstand dehydration stress. Its calyptra, with a multilayered cuticle, serves as a protective covering that prevents excessive water loss in developing sporophytes, improving their chances of survival (Budke et al., 2013). Mature sporophytes exhibit homoiohydric characteristics, regulating internal water balance through structural modifications such as reduced water loss and specialized stomatal adaptations (Duckett & Pressel, 2022). While poikilohydry is one of the main characteristics of mosses and lichens, physiological traits, including the ability of sporophytes to exhibit homoiohydricity, reduce water loss through structural reinforcements like additional cell wall material around stomata (Duckett & Pressel, 2022). These mechanisms enable *F. hygrometrica* to survive prolonged dehydrated phases and quickly resume photosynthesis, upon water becomes available. A recent publication by McLetchie and Sark (2019) on the photosynthetic activity during dehydration in *Funaria hygrometrica* focused on the desiccation tolerance. These single-clone lines were cultured in Petri dishes placed in a growth chamber set to a 12-hr photoperiod (20°C lighted, 8°C darkened, noting actual temperatures ranged from 16–19°C lighted and 6–9°C darkened due to the use of a lower shelf the chamber) at ~90 $\mu\text{mol m}^{-2} \text{s}^{-1}$

photosynthetic active radiation (PAR). Shoot apices were exposed to a range of drying times, from 10 min to 40 hrs from full turgor to desiccation morphology (curling of leaves at the crown of the shoot apex) at 5 different equilibrating RHs (RHeq: 12, 33, 54, 75 and 93 %) in an environmental chamber at controlled light and humidity parameters. Control plants were assessed directly from the cultures (never dried). Dark-adapted chlorophyll fluorescence was assessed 30 min and 24 h post rehydration (McLetchie and Stark, 2019). Immediately upon rehydration (30 min post rehydration), recovery, as assessed with Fv/Fm, exhibited values that were 40–80 % of control levels. *Funaria hygrometrica* like other desiccation-tolerant plants utilize adaptive strategies to protect and remain photosynthetic active under drought conditions. During dehydration, on the molecular level, it maintains cellular redox balance and modifies its photosynthetic system to reduce stress-related damage. The drying rate is a crucial factor in survival, with slower dehydration improving tolerance to low humidity levels (McLetchie & Stark, 2019).

Desiccation tolerance in bryophytes involves complex physiological and cytological changes. In *Plagiomnium affine*, cryoprotection is enhanced by compatible solutes both inside and outside the cell (Rütten & Santarius, 1993). Hájek and Beckett (2008) demonstrated that chlorophyll fluorescence techniques, including potential (FV/FM) and effective quantum yield (Φ PSII), can be used to evaluate the critical relative water content (RWC) and/or water potential of a moss thallus. While most of the mosses included in this study are well-studied and even considered model organisms, limited literature (mentioned above) is available on the dehydration behavior of *P. affine*. *P. affine*, *P. patens* belong to the family Funariaceae. Considering that *P. affine* and *P. patens* are closely related species phylogenetically (Rahmatpour et al., 2020) this may explain their similar performance in most experiments."

Dehydration in liverworts causes vacuole fragmentation, organelle rounding, and cytoskeleton depolymerization, with oil bodies collapsing during rehydration (Pressel et al., 2009). Recovery of photosynthesis and respiration begins rapidly after rewetting, with full recovery taking about 24 hours (Proctor et al., 2007). These findings suggest that the basic mechanisms of desiccation tolerance are ancestral in land plants, involving physical reactivation of conserved systems and coordinated changes in cellular components (Pressel et al., 2009; Proctor et al., 2007).

The liverwort ***Marchantia polymorpha*** serves as an important model for studying early land plant adaptations to drought stress (Ghosh et al., 2021). A study by Giudici et al, (2018) of several liverworts, including *M. polymorpha* investigated the relationship between the relative water content (RWC) and chlorophyll fluorescence parameters during dehydration and rehydration cycles. *M. polymorpha* thalli were collected from moist soil surfaces in ornamental flower gardens (Brno, South Moravia, Czech Republic). Samples undergoing measurement of *F. hygrometrica* chlorophyll fluorescence parameters experienced desiccation from fully hydrated to fully dry, under laboratory conditions. During the dehydration, room temperature was kept constant (23°C, 40 % RH). At the RWC ranges decreasing from 100 to 60 % in *M. polymorpha*, $\Phi PSII$ decreased at a slow rate. It reached the critical low point of photosynthetic activity ($\Phi PSII = 0$) at RWC of 5 %. In our study, the experiment showed that *M. polymorpha* had a quantum yield of 91 % (Table 5) and experienced a 90 % weight loss after 6 hours (Tab 2.).

Weight loss progressed more slowly, reaching 10 % decline at ten minutes, while *A. undulatum* showed a 20 % reduction, *P. affine* 31 %, *P. patens* 42 %, and *F. hygrometrica* 25 %. During dehydration, a clear pattern emerged: All species exhibited their lowest photosynthetic activity between 10 and 12 hours. However, a slight increase in photosynthetic activity was observed in all species at 24 and 48 hours (Tab.5.).

Research on *M. polymorpha* gemmae exposed to osmotic stress has revealed significant physiological and biochemical changes. Drought conditions resulted in growth inhibition, membrane destabilization, and a decline in photosynthetic pigments, while also increasing oxidative stress markers such as hydrogen peroxide and malondialdehyde (Ghosh et al., 2021). In response, *M. polymorpha* activated antioxidant enzymes, including superoxide dismutase, catalase, and ascorbate peroxidase, and accumulated proline as an osmoprotectant (Ghosh et al., 2021). The presence of the proline biosynthesis gene *P5CS1* suggests that *M. polymorpha* developed early evolutionary mechanisms to cope with drought stress (Ghosh et al., 2021). Additionally, the application of exogenous abscisic acid (ABA) mitigated drought-induced damage by enhancing antioxidant enzyme activity and promoting proline accumulation, emphasizing ABA's crucial role in drought tolerance in early land plants (Ghosh et al., 2021). While most mosses are generally considered desiccation-tolerant,

certain species, such as the liverwort *Marchantia polymorpha*, are classified as desiccation-sensitive (Nabe et al., 2007).

4.3. Impact of dehydration on the cytological level

Dehydration significantly impacts the cell structure of mosses, leading to various cytological changes that are crucial for understanding how mosses survive desiccation and recover upon rehydration. During dehydration, the cytoskeleton undergoes significant changes, with the disappearance of endoplasmic microtubules, leading to a loss of the longitudinal alignment of organelles such as the nucleus, mitochondria, and plastids. This results in the rounding of these organelles, although some cytoplasmic polarity is retained (Pressel et al., 2006; Chen et al., 2003).

The membranes and organelles also undergo alterations during dehydration. The endoplasmic reticulum forms membranous tubules at right angles to the main cellular axis, and the plasmalemma develops labyrinthine tubular extensions. These structural changes are accompanied by the formation of small vacuoles within the cytoplasm (Pressel et al., 2006; Singh et al., 1984). In leptoids, plasmodesmata become plugged with electron-opaque material, and starch deposits in adjacent parenchyma cells are depleted during dehydration (Pressel et al., 2006).

Chloroplasts and mitochondria experience morphological changes, such as rounding, but maintain their structural integrity to some extent. Upon rehydration, these organelles return to their normal state (Proctor et al., 2007; Yuanming, 2010). Despite dehydration, some mosses are able to maintain membrane stability, which is critical for rapid recovery during rehydration. This stability is often supported by the presence of protective substances such as soluble sugars and proline (Wu et al., 2012; S. et al., 2021). Dehydration in moss protonemata causes profound cytological changes, including vacuolar fragmentation, reorganization of endomembrane domains, changes in the cell wall, and controlled dismantling of the cytoskeleton. (Pressel, & Duckett (2010).

According to the Flora of North America (online source), the leaf cells of *A. undulatum* are irregularly angled to hexagonal, typically measuring 20–32 μm in diameter, and no data on the cell area was available. However, in our study, a hydrated cell area of 551 μm^2 (Fig. 44) and a leaf diameter of 17.83 μm (Fig. 67) were recorded, indicating a lower measurement, compared to the literature.

P. affine has been described by Petschinger et al. (2021) as having hexagonal cells with a cell area of 1431 μm^2 . Our study yielded highly consistent results, with a measured cell area of 1427 μm^2 (Fig. 44), with diameter of 10.17 μm (Fig. 67).

In the case of *P. patens*, previous studies report rectangular lamina cells with a cell area of 1979 μm^2 (Petschinger et al., 2021). In contrast, this study recorded a smaller cell area of 870 μm^2 (Fig. 44) and a leaf diameter of 15.85 μm (Fig. 67).

Funaria hygrometrica is characterized by oblong-hexagonal, lax upper laminal cells, with diameters typically ranging from 30–36 μm (NZ Flora, online). In this experiment, a cell area of 933 μm^2 and a leaf diameter of 17.17 μm were observed (Fig. 44 and Fig. 67).

M. polymorpha, the only liverwort investigated features ovoid cells with diameters of 15–30 μm (Mache & Loiseaux, 1973). It exhibited the largest cell area among all studied species, at 1499 μm^2 , along with a leaf diameter of 28.67 μm (Fig. 44 and Fig. 67), indicating that the data of our leaf area were consistent with findings in literature.

Differences in cell area may be explained by the region of origin within the leaf, considering that the referenced studies primarily focused on lamina cells. Several cells measured where close to the mid rib. At the time of microscopic analysis, some mosses, such as *F. hygrometrica* showed signs of infection, which may have contributed to the smaller cell area observed compared to the values reported in the literature. In *M. polymorpha*, the measured cell sizes varied considerably between specimens; however, the average value closely aligned with previously published data.

Conducting a T – test uncovered that *A. undulatum* exhibits significantly distinct hydrated cell size differences ($p > 0.05$) when compared to *P. affine* and *M. polymorpha*. These results indicate that *A. undulatum* has notably different cell sizes compared to the other two species. In the comparison between *P. affine* and the other species, significant differences in hydrated cell sizes are observed. *P. affine* differs from *F. hygrometrica* and *M. polymorpha*, suggesting that

P. affine has unique cell size characteristics when compared to both species. *M. polymorpha* shows the most significant differences compared to other species, suggesting distinct cell morphology. *P. patens* and *F. hygrometrica* exhibit similarities with multiple species, implying some overlap in hydrated cell structure. These observations can be implemented on the dehydration and rehydration behaviour of *P. affine* and *P. patens*, and *A. undulatum* with a flat curve. *F. hygrometrica* had a rehydration and dehydration curve with numerous fluctuations and *M. polymorpha* showed a plateau phase in both dehydration and rehydration.

Species comparison in regard to the leaf diameter revealed the smallest values in *P. affine* 10.17 μm , and *A. undulatum*, *P. patens*, *F. hygrometrica*, in the range from 15.85 μm -17.83 μm . Compared to the other species, *M. polymorpha* has the highest cell area of 28.67 μm . In contrast, the initial hydrated cell area did vary distinctly from species to species. All species, except *P. patens* showed similar initial cell area values, and the cell area at 0 minutes in the dehydration experiment, because they were all taken in the hydrated state.

A T-test revealed highly significant differences ($p > 0.05$) of the **mean leaf diameter** for all moss species, except *P. affine* vs. *M. polymorpha* and *P. patens* vs. *F. hygrometrica*. Findings of the T-test can be interpreted as follows: *A. undulatum* shows significant differences in leaf diameter when compared to all other species, *P. affine* also differs significantly from most species except *M. polymorpha*. *P. patens* and *F. hygrometrica* do not show significant differences when compared to each other. *M. polymorpha* shares similarities in leaf diameter with *P. affine* but differs significantly from *P. patens* and *F. hygrometrica*.

In contrast to the weight loss measurements, the **cell area** decline was not completely linear and is characterised by fluctuations. In some species, the maxima and lowest points are not restricted to first and last measurement, *A. undulatum* has the slowest dehydration with 8.4 % loss, followed by *P. patens*, *F. hygrometrica*, and *M. polymorpha*, with 27-29 %, and *P. affine* the fastest with 52.5 % loss at 45 minutes. After 6 hours, a decline of 7.6 % was seen in *A. undulatum*, *P. affine* 69.7 %, *P. patens*, 23.2 %, *F. hygrometrica* 32.4 %. and *M. polymorpha* 20.9 %. An examination of the light microscopic image of *A. undulatum* (Fig. 37) showed that there was still a film of water between the coverslip and the leaf during

the experiment; the air bubble is only visible at the top of the image. This may explain the relatively uniform trend in cell size.

5. Conclusion

Dehydration on the leaf level resulted in curling of the several hours, resulting in a change in 3-d structure, which means not all leaf area can be accounted for. Concluding from this observation, the cell area represents a more reliable measurement of dehydration, due to the one-cell-layer-thick leaves and thin cell walls.

During the dehydration and rehydration experiments, mosses were placed on cellulose discs to imitate the humidity conditions of their natural environment. Similarities between *P. affine* and *P. patens* suggest a closer evolutionary or functional relationship, possibly indicating shared physiological responses to hydration.

The photosynthetic activity was the only parameter investigated during rehydration. During the 120-minute experiment, 200 µl of water was added once at the beginning. In the first 30 minutes, a gradual rehydration increased photosynthesis. The fastest increase was observed in *P. affine*, followed by *F. hygrometrica*, *M. polymorpha*, and *A. undulatum*. From 30-90 minutes, dehydration takes place, with a decline in photosynthesis. Extending the rehydration series with additional parameters—such as chlorophyll fluorescence, gas exchange measurements, and pigment analysis—over an extended timeframe could provide deeper insights into the mechanisms of desiccation tolerance and recovery.

Further research is needed to investigate the effects of prolonged drought stress on moss cultures, particularly in relation to their physiological and structural adaptations to dehydration. Alternative experimental set ups include exposing the mosses to specific relative humidities, for certain amount of time. An approach with step wise dehydration has shown to impact the dehydration tolerance. The rate at which drying occurs is a key factor conclusion in its ability to endure low moisture levels, with slower dehydration enhancing survival under reduced humidity (McLetchie & Stark, 2019). The recovery process after rehydration can be assessed by replanting the mosses into *in vitro* dishes and assessing their culture for several weeks.

6. Sources

Acuña, L. G., Hernández, M., & Cuevas, E. (2015). *Application of PAM fluorometry to understand photosynthetic responses in aquatic ecosystems. Hydrobiologia*, 742(1), 135-150.

Alpert, P. (2000). The discovery, scope and puzzle of desiccation tolerance in plants. *Plant Ecology* 151: 5–17.

Azzabi, G., Pinnola, A., Betterle, N., Bassi, R., Alboresi, A. (2012). Enhancement of Non-Photochemical Quenching in the Bryophyte *Physcomitrella patens* during Acclimation to Salt and Osmotic Stress. *Plant and Cell Physiology*, Volume 53, Issue 10, October 2012, Pages 1815-1825. <https://doi.org/10.1093/pcp/pcs124>

Beckett, R. P. (1999). Inducible dehydration tolerance in bryophytes. *Journal of Experimental Botany*, 50(334), 1919–1926.

Beckett, R. P., & Hoddinott, J. (1997). Partial dehydration and abscisic acid-induced ion leakage tolerance in *Atrichum androgynum*. *Plant, Cell & Environment*, 20(4), 453-461. <https://doi.org/10.1023/A%3A1026546707649>

Blockeel, T. L. (2014). *Atrichum undulatum*: Distribution from the Atlas (The British Bryological Society) <https://www.britishbryologicalsociety.org.uk/wp-content/uploads/2020/12/Atlas-of-British-and-Irish-Bryophytes-V1-410.pdf>

Blockeel, T. L. (2014). *Funaria hygrometrica*, Distribution from the Atlas (The British Bryological Society) <https://www.britishbryologicalsociety.org.uk/wp-content/uploads/2020/12/Atlas-of-British-and-Irish-Bryophytes-V1-441.pdf>

Blockeel, T. L. (2014). *Physcomitrella patens*, Distribution from the Atlas (The British Bryological Society) <https://www.britishbryologicalsociety.org.uk/wp-content/uploads/2020/12/Atlas-of-British-and-Irish-Bryophytes-V1-451.pdf>

Blockeel, T. L. (2014). *Plagiomnium affine*: Distribution from the Atlas (The British Bryological Society) <https://www.britishbryologicalsociety.org.uk/wp-content/uploads/2020/12/Atlas-of-British-and-Irish-Bryophytes-V2-404.pdf>

Bonga, J. M., von Aderkas, P. (1992). *In vitro* Culture of trees, in Springer science and Business media, Forestry science, volume 38.

Bopp, M., Weniger, H. P. (1971) Wassertransport von Gametophyten zum Sporophyten bei Laubmoosen. *Z. Pflanzenphysiol.* 64, 190–198

Braslavsky, S. E. (2007). Glossary of terms used in Photochemistry 3rd edition (IUPAC Recommendations 2006). Pure Appl. Chem., Vol. 79, No. 3, pp. 293–465, 2007. doi:10.1351/pac200779030293.

Bray, E. A. (1997). Plant Responses to Water Deficit. Trends in Plant Science, 2, 48-54.

Brinda, J. C., & Mishler, B. D. (2016). Bryophyte desiccation tolerance: A review of mechanisms and ecological implications. *Moss Science*, 1(1), 1–15.

Bu, W., Zhang, L., & Li, D. (2017). Physiological and biochemical responses of mosses to desiccation and rehydration. *Plant Biology*, 19(6), 1013–1020.

Buch, H. (1947). Über die Wasser- und Mineralstoffversorgung der Moose II. *Commentat. bioi.* 9, 20.

Büdel, B., Friedl, T., Beyschlag, W. (2024). ISBN 978-3-662-65711-9, ISBN 978-3-662-65712-6 (eBook). <https://doi.org/10.1007/978-3-662-65712-6>

Budke, J. M., Rojas, D., & Reski, R. (2013). Development of a multilayered cuticle in the calyptra of *Funaria hygrometrica* as an adaptation to prevent excessive water loss in developing sporophytes. *Plant, Cell & Environment*, 36(8), 1413–1422. <https://doi.org/10.1111/pce.12112>

Crandall-Stotler, B., Stotler R. E., Long, D.G. (2009). Phylogeny and classification of the Marchantiophyta. *Edinb. J. Bot.* 66: 155–198.

Crandall-Stotler, B.J., & Bartholomew-Began, S. E. (2007). Morphology of Mosses (Phylum Bryophyta).

Cruz de Carvalho, M. H., & Gomes, S. L. (2014). Molecular and physiological responses to desiccation indicate the involvement of abscisic acid in the moss *Sphagnum*. *Journal of Experimental Botany*, 65(13), 4576–4591.

Csintalan, Z., Tuba, Z., & Proctor, M. C. F. (1999). Rapid recovery of photosystems on rewetting desiccation-tolerant mosses. *Journal of Experimental Botany*, 51(351), 1695–1700.

Cui, H., et al. (2011). "Molecular responses to dehydration and desiccation in desiccation-tolerant bryophytes." *Journal of Experimental Botany*, 69(13), 3211–3224.

Dera, J., Sadowski, T., & Wachowicz, S. (2002). *Theoretical aspects of estimating the quantum yield in photosynthesis under variable light conditions.* *Plant, Cell & Environment*, 25(9), 1245-1257.

Dilks, T. J. K., Proctor, M. C. F. (1979). Photosynthesis, Respiration, and water content in bryophytes. <https://doi.org/10.1111/j.1469-8137.1979.tb07564.x>

Duckett, J. G., & Pressel, S. (2022). *Funaria hygrometrica* and other mosses: An overview of their desiccation tolerance and physiological adaptations. *Plant Biology*, 25(2), 312–325. <https://doi.org/10.1111/plb.13450>

Duckett, J. G., Schmid, A. M, Ligrone R. (1998). Protonemal morphogenesis in: Bates J. W., Ashton N. W., Duckett J. G., editors. *Bryology for the Twenty-first Century*. Leeds, UK: Maney and the British Bryological Society; 1998. pp. 223–246.

Farrar, J.F., Smith, D. C. (1976). Ecological physiology of the lichen *Hypogymnia physodes* III. The importance of the rewetting phase. <https://doi.org/10.1111/j.1469-8137.1976.tb01505.x>

Flora of North America. (n.d.). *Atrichum undulatum*. Flora of North America. Retrieved March 18, 2025, from http://floranorthamerica.org/Atrichum_undulatum

Frank, M., Raknadevi, M., & Reski, R. (2005). *Physcomitrella patens* response to dehydration stress: Gene expression and physiological mechanisms. *Planta*, 221(3), 486-494. <https://doi.org/10.1007/s00425-004-1484-5>

Frank, W., Ratnadewi, D., & Reski, R. (2005). "Physcomitrella patens is highly tolerant against drought, salt and osmotic stress." *Planta*, 220(3), 384–394. [Springer Link](#)

Gao, B., Li, X., Zhang, D., Liang, Y., Yang, H., Chen, M., Zhang, Y., Wood, A.J. (2017). Desiccation tolerance in bryophytes: The dehydration and rehydration transcriptomes in the desiccation-tolerant bryophyte *Bryum argenteum* DOI: [10.1038/s41598-017-07297-3](https://doi.org/10.1038/s41598-017-07297-3)

Ghosh, R., Nayak, S. R., & Roy, S. (2021). Osmotic stress and drought tolerance in *Marchantia polymorpha*: Physiological and biochemical responses. *Frontiers in Plant Science*, 12, 625-637. <https://doi.org/10.3389/fpls.2021.625877>

Ghosh, T. K., Tompa, N. H., Rahman, M. M., Mohi-Ud-Din, M., Al-Meraj, S. M. Z., Biswas, M. S., & Mostofa, M. G. (2021). Acclimation of liverwort *Marchantia polymorpha* to physiological drought reveals important roles of antioxidant enzymes, proline and abscisic acid in land plant adaptation to osmotic stress. *PeerJ*, 9, e12419. <https://doi.org/10.7717/peerj.12419>

Giudici, M. (2021). Non-photochemical quenching during rehydration and the impact of dehydration stress on photosynthesis in mosses. *Frontiers in Plant Science*, 12, 654321.

Giudici, M., Lazzari, P., & Pressel, S. (2018). Relationship between relative water content and chlorophyll fluorescence parameters in liverworts during dehydration and rehydration cycles. *Bryology*, 39(2), 98-106. <https://doi.org/10.1111/BRY.12320>

Glime, J. M. and Chavoutier, L. (2020). Glossary. In: Glime, J. M. Bryophyte Ecology. Last updated 16 July 2020. <http://digitalcommons.mtu.edu/bryophyte-ecology/>

Greenwood, J. M., & Stark, L. R. (2014). Desiccation tolerance in bryophytes: A review of mechanisms and ecological implications. *Moss Science*, 1(1), 1–15.

Grubb, P. J. (1970). Observations on the structure and biology of *Haplomitrium* and *Takakia*, hepatics with roots. *New Phytol.* 69, 303-26.

Gupta, R. K. (1977). A study of photosynthesis and leakage of solutes in relation to desiccation effects to bryophytes. *Canadian Journal of Botany*, 55, 1186.

Guschina, I. A., & Beckett, R. P. (2002). Desiccation tolerance in mosses: *Atrichum androgynum* and related species. *Journal of Experimental Botany*, 53(373), 161-168. <https://doi.org/10.1093/jxb/53.373.161>

Guschina, I. A., & Harwood, J. L. (2002). Lipids and desiccation tolerance in bryophytes. *Phytochemistry*, 61(1), 3–13.

Haberlandt, G. (1886). Über die physiologische Funktion des Centralstranges in Laubmoosstämmchen. *Ber. dt. bot. Ges.* 1, 263-68.

Hájek, J., & Vicherová, E. (2014). Photosynthetic responses of mosses to desiccation and rehydration: A review. *Biologia Plantarum*, 58(4), 621–630.

Hájek, T., & Beckett, R. P. (2008). Evaluation of water content and photosynthetic performance in mosses: The use of chlorophyll fluorescence

for assessing desiccation tolerance. *Plant Biology*, 10(6), 762-768. <https://doi.org/10.1111/j.1438-8677.2008.00172.x>

Héban, C. (1977). *The conducting tissues of bryophytes*. Vaduz, Liechtenstein: J. Cramer.

Hodgetts, N. G. (2014). *Funaria hygrometrica*, Field guide account (**The British Bryological Society**) <https://www.britishbryologicalsociety.org.uk/wp-content/uploads/2020/12/Funaria-hygrometrica.pdf>

Hodgetts, N. G. (2014). *Physcomitrella patens*, Field guide account (**The British Bryological Society**) <https://www.britishbryologicalsociety.org.uk/wp-content/uploads/2020/12/Aphanorrhegma-patens.pdf>

Hofmann, H. (2016). *Plagiomnium affine* (Funck) T.J.Kop. In: Swissbryophytes Working Group (Hrsg.), Moosflora der Schweiz. www.swissbryophytes.ch:

Hu, Y., Liu, W., & Xu, X. (2016). Dehydration and rehydration in *Atrichum undulatum*: Effects on photosynthesis, membrane stability, and metabolic changes. *Physiologia Plantarum*, 157(2), 160-174. <https://doi.org/10.1111/ppl.12439>

Inoue, H. (1960). Studies in spore germination and the earlier stages of gametophyte development in the Marchantiales. J. Hattori Bot. Lab. 23: 148–191.

Iqbal, M., Singh, A., Ansari, M. I. (2020). Effect of Drought Stress on Crop Production (Springer). 10.1007/978-981-15-1322-0_3. journals.muni.cz

Klughammer, C., & Schreiber, U. (2008). *The P700 redox state and the quantum yield of photosystem I: Theoretical and practical aspects. Journal of Photochemistry and Photobiology B: Biology*, 92(2), 81-87. <https://doi.org/10.1016/j.jphotobiol.2008.07.004>

Köckinger, H., Krisai, R., Schriebl, A., Suanjak, M., Zechmeister, H., Ehrendorfer F. (1999). Catalogus Florae Austria, II. Teil, Bryophyten (Moose), Heft 1, Musci (Laubmoose)

Koponen, T. (1971). A monograph of *Plagiomnium* sect. Rosulata (Mniaceae). Ann. Bot. Fennici 8: 305 —367.

Koster, K. L., & Xiao, L. (2010). "Desiccation tolerance in *Physcomitrella patens*: Rate of dehydration and the involvement of endogenous abscisic acid." *Plant, Cell & Environment*, 33(1), 35–46.

Lawlor, D. W. (2013). *Photosynthesis: Energy conversion by plants*. In *Photosynthesis: From Light to Life* (pp. 165-185). Springer.

Lin, W., Wang, Y., Coudert, Y., Kierzkowski, D. (2021). Leaf Morphogenesis: Insights From the Moss *Physcomitrium patens*. *Frontiers in Plant Science*. 12. 736212. 10.3389/fpls.2021.736212.

Liu, M., Zhang, Z., Gao, H., Yang, C., Fan, X., & Cheng, D. (2014). Effect of leaf dehydration duration and dehydration degree on PSII photochemical activity of papaya leaves. *Plant physiology and biochemistry : PPB*, 82, 85–88. <https://doi.org/10.1016/j.plaphy.2014.05.003>

Longton, R. E. (1992). The Role of Bryophytes and Lichens in Terrestrial Ecosystems. In: Bates, J., Farmer, A., Eds. *Bryophytes and Lichens in a Changing Environment*, Oxford University Press, New York.

Longton, R. E., Schuster, R.M. (1983). Reproductive biology. In: Schuster, R. M. (ed.), *New manual of bryology*. Hattori Bot. Lab., Nichinan, pp. 386-462.

Lüttge, U. (2013). The planet Earth: Can it feed nine billion people? *Nova Acta Leopoldina* 114(391):345–364.

Mayaba, N. R., & Beckett, R. P. (2003). Desiccation-induced changes in the physiological response of *Atrichum androgynum*. *Plant Physiology and Biochemistry*, 41(5), 383-393. [https://doi.org/10.1016/S0981-9428\(03\)00048-7](https://doi.org/10.1016/S0981-9428(03)00048-7)

McCohana, M. (1939). Ventral surface specialisations of *Conocephalum conicum*. <https://doi.org/10.1002/j.1537-2197.1939.tb09286.x>

McLetchie, D. N., & Stark, L. R. (2019). Photosynthetic recovery in *Funaria hygrometrica* during dehydration: The role of dehydration rate in survival under low humidity. *Journal of Experimental Botany*, 70(18), 5075–5086. <https://doi.org/10.1093/jxb/erz267>

Nabe, T., Shimada, T., & Yamada, T. (2007). Desiccation tolerance in *Marchantia polymorpha*: A comparative study. *Journal of Plant Physiology*, 164(5), 583–591. <https://doi.org/10.1016/j.jplph.2006.08.010>

New Zealand Flora. (n.d.). *Funaria hygrometrica*. Flora of New Zealand. Retrieved March 18, 2025, from <https://www.nzflora.info/factsheet/Taxon/Funaria-hygrometrica.html>

Odu, E. A. (1978). The effect on rhizoid growth of the occurrence of mosses with vascular plants. *Journal of Bryology*, 10(2), 183–189.

<https://doi.org/10.1179/jbr.1978.10.2.183>

Oliver, M., Tuba, Z., Mishler, B. (2000). The evolution of vegetative desiccation tolerance in land plants. *Plant Ecology*. 151. 85-100.
10.1023/A:1026550808557.

Orehova, A., & Hájek, J. (2022). Responses of primary photosynthetic processes to repetitive rehydration differ in two representatives of Svalbard moss flora. *Czech Polar Reports*, 12(1), 103–114.

[Oxford Academic](#)

Parkhill, J. P., Mason, D., & Parry, M. A. J. (2001). *The effect of drought on photosynthesis and quantum yield in agricultural crops. Environmental and Experimental Botany*, 46(3), 229-237.

Pessarakli, M., (Ed.). (2016). Handbook of Photosynthesis (3rd ed.). CRC Press. <https://doi.org/10.1201/9781315372136>

Photon Systems Instruments. (n.d.). *AquaPen-C AP 110-C User Manual*. Retrieved from <https://psi.cz/support/downloads/hd001/ap001/>

Plaut, Z. (1995). Photosynthesis in plants/crops under water and salt stress. In: *Handbook of Plant and Crop Physiology*, ed. M. Pessarakli, 587–603. New York.

Pressel, S., & Duckett, J. (2010). Cytological insights into the desiccation biology of a model system: moss protonemata.. *The New phytologist*, 185 4, 944-63 . <https://doi.org/10.1111/j.1469-8137.2009.03148.x>.

Pressel, S., & Duckett, J. G. (2010). Physiological mechanisms in bryophytes related to dehydration tolerance. *Functional Plant Biology*, 37(7), 583–594.

Pressel, S., Duckett, J. G., & Renzaglia, K. S. (2009). Desiccation tolerance in liverworts: Vacuole fragmentation, organelle rounding, and the role of oil bodies in rehydration. *Plant Biology*, 11(1), 79-86. <https://doi.org/10.1111/j.1438-8677.2008.00167.x>

Proctor, M. C. F. (1983). Sizes and Growth-Rates of Thalli of the Lichen Rhizocarpon Geographicum on the Moraines of the Glacier De Valsorey, Valais, Switzerland. *The Lichenologist*. 1983;15(3):249-261.

[doi:10.1017/S0024282983000389](https://doi.org/10.1017/S0024282983000389)

Proctor, M. C. F., Nagy, Z., Csintalan, Z., Takács, Z. (1998). Water-content components in bryophytes: analysis of pressure–Volume relationships. *Journal of Experimental Botany* 49: 1845 – 1854.

Proctor, M. C. F., Oliver, M. J., Wood A. J., Alpert P., Stark L. R., Cleavitt N. L., Mishler B. D., (2007). Desiccation-tolerance in bryophytes: a review. *Bryologist* 110: 595–621.

Proctor, M. C. F., Tuba, Z., & Lüttge, U. (2007). The evolution of desiccation tolerance in land plants: Insights from liverworts and mosses. *Plant Ecology*, 192(1), 1-17. <https://doi.org/10.1007/s11258-007-9243-x>

Proctor, M. C. F., Tuba, Z., & Stark, L. R. (2007). Desiccation-tolerance in bryophytes: A review. *Bryologist*, 110(4), 595–621.

Rahmatpour, N., Perera, N., Singh, V., Wegrzyn, J., & Goffinet, B. (2020). High gene space divergence contrasts with frozen vegetative architecture in the moss family Funariaceae. *Molecular phylogenetics and evolution*, 106965 . <https://doi.org/10.1016/j.ympev.2020.106965>.

Reißen, W., Dux, F. M., Möschke, M., Hofmeister, M. (2019). Pflanzenanatomischer Grundkurs, Module für die differenzierte Gestaltung, 2. Auflage ISBN 978-3-662-58718-8, ISBN 978-3-662-58719-5 (eBook), <https://doi.org/10.1007/978-3-662-58719-5>

Renner, O. (1932). Zur Kenntnis des Wasserhaushaltes javanischer Kleinpflanzen. *Planta* 18: 215-287.

Rumsey, F. (2014). *Atrichum undulatum*: Field guide account (**The British Bryological Society**) <https://www.britishbryologicalsociety.org.uk/wp-content/uploads/2020/12/Atrichum-undulatum.pdf>

Rütten, T., & Santarius, K. A. (1993). Cryoprotection in *Plagiomnium affine* by compatible solutes: Implications for desiccation tolerance in bryophytes. *Journal of Experimental Botany*, 44(12), 1427-1434. <https://doi.org/10.1093/jxb/44.12.1427>

Rütten, T., & Santarius, K. A. (1993). Osmotic potential and its impact on the water

Salehi-Lisar, S. Y., Bakhshayeshan-Agdam, H. (2016). Drought Stress in Plants: Causes, Consequences, and Tolerance. DOI:[10.1007/978-3-319-28899-4_1](https://doi.org/10.1007/978-3-319-28899-4_1)

Schuurmans, J. M., van Kooten, O., & Polle, A. (2015). *Fluorescence-based estimation of photosynthetic performance and stress in plants. Plant Physiology and Biochemistry*, 96, 32-43.

Schweingruber, F.H., Börner, A. (2018). The Plant Stem: A Microscopic Aspect. Springer ebook ISBN 978-3-319-73523-8, ISBN 978-3-319-73524-5
<https://doi.org/10.1007/978-3-319-73524-5>

Seel, W. E., Pressel, S., & Duckett, J. G. (1992). Leaf curling in desiccated mosses reduces photon absorption by 50–60 %, yet this alone does not fully explain the complete loss of photosynthetic activity during dehydration. *Functional Ecology*, 6(5), 629–636.

Shaw, A. J. (1991). The Genetic Structure of Sporophytic and Gametophytic Populations of the Moss, *Funaria hygrometrica* Hedw. Society for the Study of Evolution. 45(5), 1991, pp. 1260-1274, Department of Biology, Ithaca College, Ithaca. <https://doi.org/10.2307/2409732>

Sheffield, L. (2001). Shaw AJ, Goffinet B, eds. (2000). Bryophyte biology. 476 pp. Cambridge: Cambridge University Press. *Annals of Botany*. 87. 703–704. 10.1006/anbo.2001.1376.

Shimamura, M. (2016). *Marchantia polymorpha* : Taxonomy, Phylogeny and Morphology of a Model System Plant and Cell Physiology, Volume 57, Issue 2, February 2016, Pages 230–256, <https://doi.org/10.1093/pcp/pcv192>

Snellenburg, J. J., van der Veen, A., & van Oosterwijk, P. (2017). PAM fluorometry and its application in studying plant stress and photosynthetic activity. *Environmental Monitoring and Assessment*, 189(12), 573.

Storti, G., et al. (2018). "Drying without dying: A genome database for desiccation-tolerant plants." *Plant Physiology*, 194(4), 2249–2263.

Tuba, Z., Lichtenthaler, H. K. (2011). Ecophysiology of Homoiochlorophyllous and Poikilochlorophyllous Desiccation-Tolerant Plants and Vegetations. In: Lüttge, U., Beck, E., Bartels, D. (eds) *Plant Desiccation Tolerance*. Ecological Studies, vol 215. Springer, Berlin, Heidelberg.
https://doi.org/10.1007/978-3-642-19106-0_9

Tunnacliffe, A., Wise, M. J. (2007). The continuing conundrum of the LEA proteins. *Die Naturwissenschaften*, 94(10), 791–812.
<https://doi.org/10.1007/s00114-007-0254-y>

Url, W. (1974). Institut für den wissenschaftlichen Film C1144/1974, Plasmolyse und Cytrorrhise, Begleitveröffentlichung von Prof. Dr. W. Url, Wien

Wang, X. Q., Yang, P. F., Liu, Z., Liu, W. Z., Hu, Y., Chen, H., Kuang, T. Y., Pei, Z. M., Shen, S. H., He, Y. K., (2009). Exploring the Mechanism of *Physcomitrella patens* Desiccation Tolerance through a Proteomic Strategy, *Plant Physiology*, Volume 149, Issue 4, April 2009, Pages 17391750. <https://doi.org/10.1104/pp.108.131714>

Werner, O., & Huth, H. (1991). Desiccation tolerance in mosses: A comparative study. *Plant Ecology*, 95(1), 1–8.

Will-Richter, G. (1949). Der osmotische Wert der Lebermoose. S.B. Wien. Akad. Wiss., math.-nat. Kl., Abt. I, 158, 431.

Wood, A. J. (2007). Invited Essay: New Frontiers in Bryology and Lichenology: The Nature and Distribution of Vegetative Desiccation-Tolerance in Hornworts, Liverworts and Mosses. *The Bryologist*, 110(2), 163–177. <http://www.jstor.org/stable/20110849>

Xia, F., Zhang, T., & Liu, H. (2023). *The role of chlorophyll fluorescence parameters in assessing photosystem II quantum yield in plants. Photosynthetica*, 61(1), 1-15.

Xiao, L., Koster, K. L., & Oliver, M. J. (2017). "Desiccation tolerance in *Physcomitrella patens*: Rate of dehydration and the involvement of endogenous abscisic acid." *Plant, Cell & Environment*, 41(1), 275–284. [Wiley Online Library](#)

Xiao, X., Wang, S., & Koster, K. L. (2017). Exogenous abscisic acid increases dehydration tolerance in *Physcomitrella patens*. *Plant, Cell & Environment*, 40(7), 1313-1324. <https://doi.org/10.1111/pce.12854>

Zhang, Q., Bartels, D. (2018). Desiccation in desiccation-tolerant angiosperm plants. *Journal of Experimental Botany*, Vol. 69, No. 13 pp. 3211–3222, 2018.

Figure sources

(Fig.1) “Moss life cycle image” by Ursavas S., Cetin, B. (2012)

(Fig.2) “*P. patens* cross section” by Lin et al., (2021)

Lin, W., Wang, Y., Coudert, Y., Kierzkowski, D. (2021). Leaf Morphogenesis: Insights From the Moss *Physcomitrium patens*. *Frontiers in Plant Science*. 12. 736212. [10.3389/fpls.2021.736212](https://doi.org/10.3389/fpls.2021.736212).

(Fig.3) “*M. polymorpha* cross section” by Schweingruber and Börner, (2018).

Fritz H. Schweingruber, Annett Börner (2018). The Plant Stem: A Microscopic Aspect. <https://doi.org/10.1007/978-3-319-73524-5>

(Fig.4) Table “Definitions water status” by Zhang and Bartels, (2018)

Zhang, Q., Bartels, D. (2018). Desiccation in desiccation-tolerant angiosperm plants. *Journal of Experimental Botany*, Vol. 69, No. 13 pp. 3211–3222, 2018. <https://doi.org/10.1093/jxb/erx489>

(Fig.10) “Photosynthetic parameters, quantum yield” by Braslavsky, S.E. (2007).

Glossary of terms used in Photochemistry 3rd edition (IUPAC Recommendations 2006). *Pure Appl. Chem.*, Vol. 79, No. 3, pp. 293–465, 2007. doi:10.1351/pac200779030293

7. Appendix

Poster “The influence of desiccation on mosses”

Specific research project

presented at

ATSPB 2023: Plants in the context of food security,
biodiversity and climate change.

13th to 15th April 2023 in Hall in Tirol, Austria

The influence of dessication on mosses

Stefanie Wernig, Michael Schagerl, Ingeborg Lang
Department of Functional and Evolutionary Ecology, University of Vienna
Djerassiplatz 1, 1030 Vienna

Summary

This study focuses on the cytological, and morphological aspects of how mosses cope with drought stress. A measured numerical parameter is the weight of the water lost from 0 to 48 hours, after initiation of the dehydration.

Under the light microscope, the cell area size was determined, to learn how cells of moss leaves react to a reduced water content from 5 to 20 minutes.

Furhtermore, to visualize the the photosynthetic activity on a cellular level, an epifluorescence microscope including a PAM system was used.

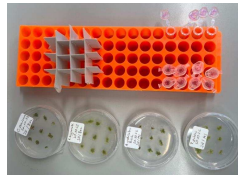


Fig. 1: 4 moss species grown in vitro culture and the set up for water weight loss experiment.

Material and Methods

3 mosses *Physcomitrella patens*, *Funaria hygrometrica*, *Atrichum undulatum* and 1 liver wort *Concepalum conicum* were used to give insight in the various dehydration patterns of different species and groups.

All mosses used for the water weight measurement were grown in invitro culture under sterile conditions in petri dishes containing Benecke medium and cultivated for one month. Firstly, the fresh weight was measured, and then after 30 min, 2h, 24h, and 48h

For the cell area loss they were placed on a light microscope Nikon Eclipse N. A series of pictures was taken every five minutes from 5 to 20 min, using the with the free hand Lasso of NIS elements, the same three cells of were marked in each picture, to determine their surface area.

A Zeiss AxioScope A1 epifluorescence microscope combined with a PAM system was used for analysis of photosynthetic activity.

Results

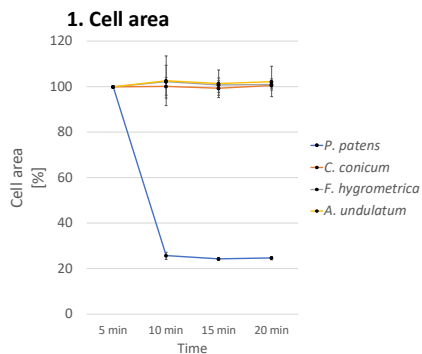


Fig. 2: Area measurement of the 3 selected cells, *C. conicum*, *F. hygrometrica* and *A. undulatum* have a similar pattern with no distinct dehydration, *P. patens* showed a decline in cell surface area.

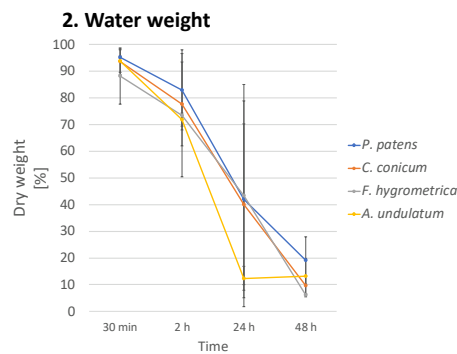


Fig. 3: Comparison of the water loss revealed that *P. patens*, *C. conicum*, *F. hygrometrica* overall follow a similar dehydration pattern, *A. undulatum* Shows a strong decline from 2-24 hours.

3. PAM-Microscopy

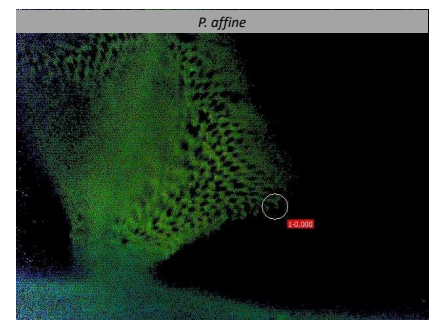


Fig. 4: *Plagiommium affine* leaf under a Zeiss AxioScope A1 epifluorescence microscope combined with a PAM system. Low photosynthetic activity and no cells without chloroplasts were observed.

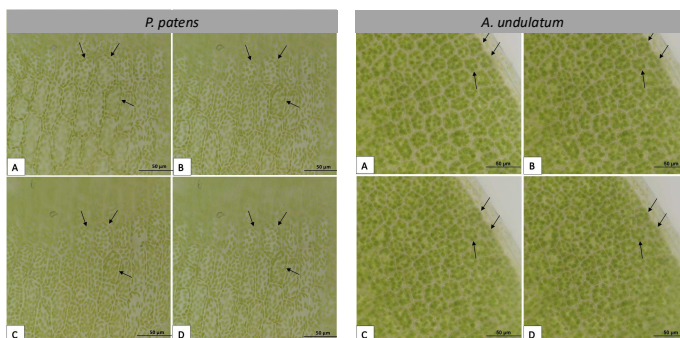


Fig. 5: Light microscopy images, Light microscope Nikon DS-Ri2, 20x magnification, Cells of *P. patens* and *A. undulatum* after A) 5 min B) 10 min C) 15 min D) 20 min dehydration time. The 3 arrows mark cells analyzed in the cell area loss measurement.

4. Further Studies

P. patens PAM and weight

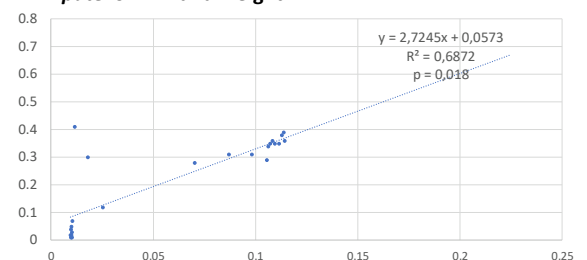


Fig. 6: Graph of a Linear Regression generated from PAM and weight measurements. The $R^2 = 0,6872$ indicates a correlation.

Introduction

Basic cryptogamic land plants such as bryophytes, lichens and fungi are considered primary poikilohydric organisms which express dessication tolerance in their somatic cells and reproductive structures (Lüttge et al., 2011). Poikilohydry is the ability to tolerate desiccation by dormancy and resume the metabolic activity upon rehydration. Consequently, the water content is highly influenced by the humidity present in the environment (Lüttge et al., 2011). Desiccation can be defined as more severe form of dehydration.

Discussion

1. Cell area

- the pattern of cell area loss in *C. conicum*, *F. hygrometrica* and *A. undulatum* is similar, showing slight increase from 5 to 10 minutes, lowest is reached at 15 min, then a slight decline could be observed after 20 min.
- The moss with a distinct decline is *P. patens*, losing 83 % of its area in the first 10 min, maintaining their cell size from 10 to 20 min.

2. Water weight

- P. patens*, *C. conicum*, and *F. hygrometrica* follow a similar pattern, whereas the weight loss of *A. undulatum* was distinct -> 60 % of their initial fresh weight was lost.
- A. undulatum* lost 95% of its original water weight after 24 hours.

3. PAM-Microscopy of *P. affine* leaf revealed a lack of chloroplasts and a no photosynthetic activity.

4. Further studies are performed with combined measurements of PAM and Water weight loss have revealed interspecific differences between *A. undulatum* and *P. patens*.

48 h dehydration - weight (g)

A. undulatum

	1	2	3	4	5	6	Mean	SD
0,5 min	0.1116	0.12	0.1033	0.1139	0.1	0.1	0.11	0.01
1min	0.1094	0.117	0.1031	0.1113	0.0997	0.101	0.11	0.01
1,5min	0.1095	0.117	0.1015	0.1096	0.0981	0.1	0.11	0.01
2min	0.108	0.116	0.1018	0.1082	0.0973	0.099	0.11	0.01
2,5min	0.1076	0.116	0.1001	0.109	0.097	0.098	0.10	0.01
3min	0.1069	0.114	0.0986	0.1075	0.0951	0.098	0.10	0.01
3,5min	0.1061	0.114	0.0979	0.107	0.0958	0.097	0.10	0.01
4min	0.1039	0.113	0.0968	0.1068	0.0954	0.097	0.10	0.01
4,5min	0.1014	0.113	0.097	0.1061	0.0951	0.096	0.10	0.01
5 min	0.1018	0.113	0.097	0.1062	0.0942	0.095	0.10	0.01
5,5min	0.1016	0.112	0.0942	0.1047	0.0938	0.095	0.10	0.01
6min	0.1016	0.112	0.094	0.1045	0.0929	0.094	0.10	0.01
6,5min	0.1013	0.11	0.093	0.1041	0.092	0.094	0.10	0.01
7min	0.0991	0.11	0.093	0.1035	0.0924	0.093	0.10	0.01
7,5min	0.0997	0.109	0.092	0.1031	0.0917	0.092	0.10	0.01
8min	0.0994	0.107	0.092	0.1027	0.0913	0.091	0.10	0.01
8,5min	0.0978	0.107	0.091	0.1022	0.0907	0.09	0.10	0.01
9min	0.0976	0.106	0.09	0.1009	0.0895	0.09	0.10	0.01
9,5min	0.0969	0.104	0.089	0.101	0.0892	0.088	0.09	0.01
10min	0.096	0.104	0.087	0.0998	0.0883	0.087	0.09	0.01
15min	0.0905	0.094	0.081	0.0874	0.0834	0.081	0.09	0.01
20min	0.0823	0.089	0.077	0.084	0.0774	0.077	0.08	0.00
30min	0.0761	0.081	0.0709	0.0801	0.0673	0.07	0.07	0.01
60min	0.0586	0.069	0.0573	0.0671	0.0432	0.043	0.06	0.01
2h	0.025	0.032	0.0277	0.0096	0.0097	0.098	0.03	0.03
3h	0.0095	0.011	0.09	0.0094	0.0099	0.01	0.02	0.03
4h	0.0106	0.01	0.0097	0.0099	0.0097	0.01	0.01	0.00
5h	0.0099	0.01	0.0103	0.0104	0.0091	0.009	0.01	0.00
6h	0.0097	0.01	0.0099	0.009	0.0102	0.01	0.01	0.00
8h	0.0097	0.01	0.0092	0.0097	0.009	0.01	0.01	0.00
10h	0.0093	0.01	0.0091	0.0095	0.0097	0.01	0.01	0.00
12h	0.0091	0.01	0.0092	0.0096	0.0096	0.009	0.01	0.00
24h	0.0091	0.009	0.0085	0.0095	0.0099	0.009	0.01	0.00
48h	0.01	0.011	0.0086	0.0091	0.0092	0.01	0.01	0.00
Cell disc	0.0091	0.009	0.0093	0.0093	0.0094	0.009	0.01	0.00

48 h dehydration - weight (g)

P. affine

	1	2	3	4	5	6	Mean	SD
0.5 min	0.1016	0.099	0.099	0.1003	0.101	0.1019	0.10	0.00
1 min	0.0832	0.098	0.096	0.0998	0.0999	0.1012	0.10	0.01
1.5 min	0.0823	0.098	0.096	0.0997	0.0993	0.1004	0.10	0.01
2 min	0.082	0.098	0.095	0.0993	0.0972	0.099	0.09	0.01
2.5 min	0.0815	0.097	0.093	0.0993	0.0979	0.0993	0.09	0.01
3 min	0.081	0.096	0.093	0.0986	0.0956	0.0979	0.09	0.01
3.5 min	0.0798	0.096	0.092	0.088	0.0963	0.0979	0.09	0.01
4 min	0.0795	0.094	0.09	0.0971	0.0954	0.0973	0.09	0.01
4.5 min	0.0797	0.093	0.09	0.0972	0.0948	0.096	0.09	0.01
5 min	0.0785	0.092	0.09	0.0967	0.0945	0.0953	0.09	0.01
5.5 min	0.0778	0.092	0.09	0.0962	0.0945	0.0946	0.09	0.01
6 min	0.0774	0.091	0.088	0.095	0.0941	0.0952	0.09	0.01
6.5min	0.0764	0.091	0.089	0.0945	0.0871	0.0929	0.09	0.01
7 min	0.076	0.09	0.087	0.0942	0.0926	0.0937	0.09	0.01
7.5 min	0.0749	0.09	0.087	0.094	0.0926	0.092	0.09	0.01
8 min	0.0738	0.089	0.084	0.0933	0.0914	0.092	0.09	0.01
8.5 min	0.0736	0.088	0.084	0.0923	0.0908	0.0925	0.09	0.01
9 min	0.0732	0.088	0.085	0.0917	0.0896	0.0911	0.09	0.01
9.5 min	0.0728	0.087	0.083	0.0904	0.0886	0.0902	0.09	0.01
10 min	0.0723	0.086	0.083	0.0904	0.0878	0.0895	0.08	0.01
15 min	0.0706	0.078	0.077	0.0779	0.0771	0.0793	0.08	0.00
20 min	0.0671		0.072	0.0758	0.0778	0.0766	0.07	0.00
30 min	0.0575	0.067	0.075	0.0718	0.07	0.0704	0.07	0.01
60 min	0.0388	0.047	0.044	0.05	0.0534	0.0502	0.05	0.01
2 h	0.0094	0.01	0.01	0.0301	0.0211	0.008	0.01	0.01
3 h	0.0092	0.008	0.012	0.0093	0.0112	0.0102	0.01	0.00
4 h	0.0093	0.01	0.01	0.0091	0.0095	0.0096	0.01	0.00
5 h	0.0099	0.009	0.01	0.0102	0.0104	0.0094	0.01	0.00
6 h	0.0088	0.009	0.01	0.0089	0.0095	0.0093	0.01	0.00
8 h	0.0087	0.01	0.01	0.0097	0.0105	0.0104	0.01	0.00
10 h	0.0096	0.01	0.01	0.0095	0.0093	0.0095	0.01	0.00
12 h	0.009	0.01	0.01	0.0098	0.0098	0.0094	0.01	0.00
24 h	0.009	0.009	0.008	0.0094	0.0094	0.0086	0.01	0.00
48 h	0.0091	0.009	0.01				0.01	0.00
Cell disc	0.0093	0.008	0.009	0.0092	0.0097	0.0099		

48 h dehydration - weight (g)

P. patens

	1	2	3	4	5	6	Mean	SD
0.5 min	0.1189	0.1167	0.11	0.1076	0.1152	0.1034	0.09	0.0060
1 min	0.115	0.1153	0.109	0.1073	0.1127	0.1011	0.11	0.0055
1.5 min	0.1135	0.1147	0.108	0.1065	0.1117	0.1003	0.10	0.0054
2 min	0.1142	0.1147	0.108	0.1057	0.1112	0.1006	0.10	0.0054
2.5 min	0.124	0.1142	0.107	0.1064	0.1107	0.0999	0.10	0.0082
3 min	0.1137	0.1139	0.106	0.1059	0.1101	0.0999	0.10	0.0054
3.5 min	0.1134	0.1125	0.106	0.1038	0.11	0.0985	0.10	0.0057
4 min	0.113	0.1113	0.105	0.1042	0.1092	0.0992	0.10	0.0051
4.5 min	0.1118	0.111	0.105	0.1036	0.1091	0.0987	0.10	0.0051
5 min	0.1114	0.1103	0.103	0.1031	0.1081	0.0983	0.10	0.0050
5.5 min	0.1112	0.1098	0.103	0.1016	0.1076	0.0977	0.10	0.0053
6 min	0.1095	0.1094		0.1013	0.1077	0.0972	0.10	0.0055
6.5min	0.1089	0.1089	0.095	0.101	0.1067	0.0965	0.10	0.0061
7 min	0.1084	0.1085	0.091	0.1004	0.1054	0.096	0.10	0.0071
7.5 min	0.1076	0.1081	0.091	0.1004	0.1064	0.095	0.10	0.0073
8 min	0.1071	0.1078	0.091	0.0996	0.1054	0.0947	0.10	0.0071
8.5 min	0.1067	0.1071	0.09	0.0978	0.1052	0.094	0.10	0.0072
9 min	0.1063	0.1067	0.091	0.098	0.1041	0.0938	0.10	0.0067
9.5 min	0.1058	0.1063	0.097	0.0975	0.1037	0.0933	0.10	0.0054
10 min	0.1056	0.1044	0.084	0.0965	0.1028	0.0931	0.09	0.0082
15 min	0.0991	0.099	0.082	0.0926	0.0918	0.085	0.09	0.0069
20 min	0.0982	0.0946	0.081	0.0888	0.0955	0.0844	0.08	0.0067
30 min	0.0871	0.0865	0.075	0.0809	0.091	0.0777	0.07	0.0062
60 min	0.0702	0.071	0.049	0.0606	0.0813	0.02	0.06	0.0219
2 h	0.0254	0.0334	0.017	0.0189	0.0219	0.0109	0.03	0.0077
3 h	0.0097	0.0106	0.01	0.0098	0.0111	0.01	0.01	0.0005
4 h	0.0096	0.0102	0.009	0.0102	0.011	0.0099	0.01	0.0007
5 h	0.0104	0.01	0.009	0.0113	0.0102	0.0089	0.01	0.0010
6 h	0.0099	0.0101	0.008	0.0095	0.0095	0.0113	0.01	0.0009
8 h	0.0102	0.0102	0.008	0.012	0.015	0.01	0.01	0.0024
10 h	0.0103	0.01	0.008	0.0108	0.0105	0.0096	0.01	0.0009
12 h	0.0097	0.0099	0.009	0.0091	0.0096	0.0098	0.02	0.0004
24 h	0.0099	0.0101	0.008	0.0091	0.0092	0.0087	0.01	0.0008
48 h	0.0099	0.0101	0.009	0.01	0.0112	0.01	0.01	0.0007
Cell disc	0.0086	0.0089	0.009	0.0093	0.0095	0.0092		

48 h dehydration - weight (g)

F. hygrometrica

c	1	2	3	4	5	6	Mean	SD
0.5 min	0.142	0.11	0.106	0.101	0.1	0.092	0.11	0.017
1 min	0.142	0.11	0.102	0.101	0.098	0.09	0.11	0.018
1.5 min	0.138	0.11	0.102	0.101	0.096	0.088	0.11	0.017
2 min	0.135	0.11	0.103	0.1	0.096	0.088	0.10	0.016
2.5 min	0.134	0.11	0.101	0.098	0.095	0.087	0.10	0.016
3 min	0.13	0.11	0.1	0.098	0.095	0.085	0.10	0.015
3.5 min	0.128	0.1	0.093	0.097	0.094	0.086	0.10	0.015
4 min	0.127	0.1	0.095	0.097	0.094	0.085	0.10	0.015
4.5 min	0.127	0.1	0.098	0.097	0.094	0.085	0.10	0.014
5 min	0.127	0.1	0.098	0.096	0.091	0.083	0.10	0.015
5.5 min	0.129	0.1	0.098	0.094	0.092	0.083	0.10	0.016
6 min	0.129	0.1	0.097	0.094	0.091	0.082	0.10	0.016
6.5min	0.128	0.1	0.097	0.092	0.09	0.082	0.10	0.016
7 min	0.127	0.1	0.096	0.092	0.089	0.081	0.10	0.016
7.5 min	0.127	0.1	0.095	0.091	0.089	0.081	0.10	0.016
8 min	0.126	0.1	0.094	0.091	0.088	0.081	0.10	0.016
8.5 min	0.126	0.1	0.093	0.09	0.089	0.078	0.10	0.016
9 min	0.125	0.1	0.1	0.089	0.088	0.079	0.10	0.016
9.5 min	0.125	0.1	0.091	0.089	0.087	0.078	0.09	0.016
10 min	0.123	0.1	0.091	0.088	0.086	0.076	0.09	0.016
15 min	0.106	0.08	0.085	0.071	0.08	0.078	0.08	0.012
20 min	0.098	0.08	0.081	0.063	0.074	0.075	0.08	0.012
30 min	0.091	0.08	0.07	0.06	0.064	0.052	0.07	0.014
60 min	0.069	0.07	0.053	0.04	0.049	0.05	0.05	0.011
2 h	0.031	0.03	0.017	0.009	0.014	0.013	0.02	0.008
3 h	0.01	0.01	0.01	0.009	0.009	0.009	0.01	0.001
4 h	0.01	0.01	0.009	0.009	0.008	0.008	0.01	0.001
5 h	0.01	0.01	0.009	0.009	0.009	0.011	0.01	0.001
6 h	0.01	0.01	0.009	0.009	0.009	0.008	0.01	0.001
8 h	0.01	0.01	0.009	0.01	0.009	0.009	0.01	0.000
10 h	0.01	0.01	0.009	0.01	0.008	0.01	0.01	0.001
12 h	0.01	0.01	0.009	0.009	0.009	0.009	0.01	0.001
24 h	0.01	0.01	0.01	0.008	0.009	0.009	0.01	0.001
48 h	0.01	0.01	0.01	0.007	0.009	0.009	0.01	0.002
Cell disc	0.009	0.01	0.008	0.01	0.01	0.009	0.01	0.000

48 h dehydration - weight (g)

M. polymorpha

	1	2	3	4	5	6	Mean	SD
0.5 min	0.1204	0.1095	0.105	0.0102	0.1015	0.1036	0.09	0.04
1 min	0.1199	0.108	0.106	0.0989	0.1016	0.1	0.11	0.01
1.5 min	0.1195	0.1078	0.106	0.0982	0.0972	0.1004	0.10	0.01
2 min	0.1169	0.1068	0.105	0.0464	0.097	0.0996	0.10	0.02
2.5 min	0.1164	0.1069	0.105	0.097	0.0956	0.098	0.10	0.01
3 min	0.1166	0.1063	0.105	0.0957	0.0954	0.0976	0.10	0.01
3.5 min	0.1162	0.1058	0.103	0.0949	0.0936	0.0979	0.10	0.01
4 min	0.1164	0.104	0.103	0.0955	0.094	0.0965	0.10	0.01
4.5 min	0.1155	0.1042	0.104	0.0941	0.0932	0.0959	0.10	0.01
5 min	0.116	0.1035	0.101	0.0936	0.0918	0.0958	0.10	0.01
5.5 min	0.1148	0.1032	0.103	0.093	0.0919	0.0958	0.10	0.01
6 min	0.1148	0.1024	0.102	0.0925	0.0912	0.0946	0.10	0.01
6.5min	0.1142	0.1025	0.102	0.0914	0.0886	0.0946	0.10	0.01
7 min	0.1139	0.1017	0.102	0.0906	0.089	0.0941	0.10	0.01
7.5 min	0.1137	0.1016	0.101	0.0896	0.0882	0.0933	0.10	0.01
8 min	0.1131	0.1002	0.101	0.0899	0.0871	0.0929	0.10	0.01
8.5 min	0.1124	0.0999	0.1	0.0895	0.0863	0.093	0.10	0.01
9 min	0.1121	0.0991	0.1	0.0888	0.0859	0.0913	0.10	0.01
9.5 min	0.1119	0.0972	0.1	0.0875	0.0852	0.0918	0.10	0.01
10 min	0.1112	0.098	0.0987	0.087	0.0843	0.0898	0.09	0.01
15 min	0.1072	0.0881	0.0871	0.0788	0.076	0.085	0.09	0.01
20 min	0.104	0.0852	0.083	0.0743	0.0714	0.0815	0.08	0.01
30 min	0.0922	0.0787	0.0766	0.0671	0.062	0.0686	0.07	0.01
60 min	0.0765	0.0624	0.0674	0.0451	0.0474	0.054	0.06	0.01
2 h	0.045	0.0283	0.0764	0.0097	0.0094	0.0189	0.03	0.03
3 h	0.0166	0.0103	0.0098	0.0095	0.0075	0.0086	0.01	0.00
4 h	0.0101	0.0108	0.0087	0.0093	0.0107	0.0092	0.01	0.00
5 h	0.0099	0.0106	0.0086	0.0094	0.0092	0.0099	0.01	0.00
6 h	0.0103	0.0095	0.009	0.0083	0.0082	0.0089	0.01	0.00
8 h	0.01	0.0095	0.0088	0.0092	0.0091	0.0086	0.01	0.00
10 h	0.01	0.0104	0.0089	0.0091	0.0087	0.0105	0.01	0.00
12 h	0.099	0.0099	0.009	0.0092	0.0095	0.0089	0.02	0.04
24 h	-sample	0.0109	0.0085	0.0092	0.0089	0.0087	0.01	0.00
48 h	-lost	0.0102	0.0088	0.0097	0.0088		0.01	0.00
Cell disc	0.0093	0.0093	0.0094	0.0091	0.0092	0.0089		

48 h dehydration – quantum yield (Φ)

A. undulatum

	1	2	3	4	5	6	Mean	SD
0.5 min	0.43	0.37	0.44	0.5	0.31	0.34	0.40	0.07
1 min	0.46	0.36	0.41	0.15	0.31	0.33	0.34	0.11
1.5 min	0.47	0.3	0.41	0.34	0.18	0.28	0.33	0.10
2 min	0.46	0.3	0.37	0.46	0.38	0.47	0.41	0.07
2.5 min	0.46	0.3	0.37	0.44	0.32	0.47	0.39	0.07
3 min	0.46	0.48	0.48	0.41	0.34	0.28	0.41	0.08
3.5 min	0.58	0.4	0.41	0.41	0.35	0.3	0.41	0.09
4 min	0.52	0.38	0.41	0.35	0.34	0.3	0.38	0.08
4.5 min	0.52	0.49	0.36	0.4	0.35	0.29	0.40	0.09
5 min	0.49	0.46	0.38	0.44	0.31	0.29	0.40	0.08
5.5 min	0.5	0.39	0.36	0.41	0.3	0.31	0.38	0.07
6 min	0.47	0.42	0.38	0.34	0.36	0.32	0.38	0.06
6.5min	0.45	0.42	0.37	0.42	0.38	0.32	0.39	0.05
7 min	0.5	0.42	0.45	0.37	0.31	0.33	0.40	0.07
7.5 min	0.5	0.42	0.36	0.41	0.29	0.34	0.39	0.07
8 min	0.52	0.33	0.36	0.45	0.33	0.31	0.38	0.08
8.5 min	0.51	0.37	0.33	0.32	0.36	0.32	0.37	0.07
9 min	0.54	0.37	0.4	0.29	0.34	0.32	0.38	0.09
9.5 min	0.54	0.36	0.32		0.38	0.34	0.39	0.09
10 min	0.51	0.38	0.36			0.36	0.40	0.07
15 min	0.54	0.41	0.36	0	0	0.39	0.28	0.23
20 min	0.52	0.5	0.4	0.32	0.25	0.35	0.39	0.11
30 min	0.52	0.48	0.47	0.31	0.22	0.35	0.39	0.12
60 min	0.51	0.5	0.36	0.36	0.12	0.08	0.32	0.18
2 h	0.17	0.13	0	0.36	0.05	0.03	0.12	0.13
3 h	0	0.02	0	0.08	0.04	0	0.02	0.03
4 h	0	0	0.14	0.04	0	0.04	0.04	0.05
5 h	0	0.06	0.04	0	0.04	0.05	0.03	0.03
6 h	0	0.06	0.01	0.03	0	0	0.02	0.02
8 h	0.03	0.05	0	0	0.04	0	0.02	0.02
10 h	0.02	0	0	0	0	0.01	0.01	0.01
12 h	0	0	0	0.04	0	0.03	0.01	0.02
24 h	0.01	0.02	0.08	0	0.05	0.01	0.03	0.03
48 h	0.01	0.02	0.06	0	0.04	0.07	0.03	0.03

48 h dehydration – quantum yield (Φ)

P. affine

	1	2	3	4	5	6	Mean	SD
0.5 min	0.52	0.6	0.61	0.15	0.23	0.46	0.43	0.19
1 min	0.54	0.57	0.61	0.14	0.12	0.46	0.41	0.22
1.5 min	0.56	0.6	0.6	0.18	0.23	0.53	0.45	0.19
2 min	0.56	0.62	0.58	0.09	0.21	0.46	0.42	0.22
2.5 min	0.55	0.61	0.59	0.2	0.25	0.48	0.45	0.18
3 min	0.54	0.58	0.6	0.18	0.25	0.46	0.44	0.18
3.5 min	0.56	0.68	0.63	0.16	0.23	0.45	0.45	0.21
4 min	0.56	0.61	0.57	0.17	0.17	0.46	0.42	0.20
4.5 min	0.59	0.58	0.57	0.25	0.16	0.5	0.44	0.19
5 min	0.57	0.6	0.56	0.15	0.32	0.56	0.46	0.18
5.5 min	0.63	0.57	0.55	0.21	0.26	0.49	0.45	0.17
6 min	0.63	0.57	0.58	0.2	0.05	0.53	0.43	0.24
6.5min	0.63	0.55	0.53	0.18	0.17	0.51	0.43	0.20
7 min	0.63	0.55	0.59	0.27	0.19	0.54	0.46	0.18
7.5 min	0.44	0.57	0.59	0.21	0.24	0.42	0.41	0.16
8 min	0.6		0.63	0.13	0.12	0.37	0.37	0.25
8.5 min	0.62		0.59	0.25	0.18	0.5	0.43	0.20
9 min	0.58		0.62	0.32	0.33	0.56	0.48	0.14
9.5 min	0.59		0.58	0.22	0.2	0.45	0.41	0.19
10 min		0.59	0.59	0.26	0.12	0.54	0.42	0.22
15 min	0.61	0.6	0.56	0.21	0.04	0.45	0.41	0.24
20 min	0.73	0.58	0.52	0.16	0.38	0.39	0.46	0.20
30 min	0.76	0.6	0.5	0.15	0.37	0.32	0.45	0.22
60 min	0.42	0.59	0.34	0.12	0.25	0.04	0.29	0.20
2 h	0	0.3	0	0.07	0.18	0	0.09	0.12
3 h	0.06	0.12	0.04	0.05	0.01	0.06	0.06	0.04
4 h	0.02	0.12	0	0	0.02	0.03	0.03	0.04
5 h	0.07	0.08	0	0.11	0.22	0.05	0.09	0.07
6 h	0	0.03	0.05	0.02	0	0	0.02	0.02
8 h	0.01	0	0.06	0	0	0	0.01	0.02
10 h	0.05	0.03	0.01	0	0.01	0	0.02	0.02
12 h	0.06	0.08	0.08	0.01	0	0.03	0.04	0.04
24 h	0	0	0.05	0	0	0	0.01	0.02
48 h	0.11	0.02	0				0.04	0.06

48 h dehydration – quantum yield (Φ)

P. patens

	1	2	3	4	5	6	Mean	SD
0.5 min			0.2	0.24	0.16	0.09	0.17	0.0640
1 min	0.41	0.34	0.3	0.25	0.49	0.07	0.31	0.1446
1.5 min			0.18	0.25	0.19	0.09	0.18	0.0660
2 min	0.36	0.34	0.24	0.29	0.1	0	0.22	0.1429
2.5 min			0.24	0.25	0.19	0	0.17	0.1163
3 min	0.39	0.36	0.34	0.25	0.13	0.14	0.27	0.1134
3.5 min			0.25	0.21	0.16	0.14	0.19	0.0497
4 min	0.38	0.34	0.22	0.27	0.16	0.14	0.25	0.0964
4.5 min			0.22	0.26	0.16	0.08	0.18	0.0783
5 min	0.35	0.36	0.22	0.26	0.25	0.06	0.25	0.1088
5.5 min			0.26	0.24	0.23	0.2	0.23	0.0250
6 min	0.35	0.34		0.24	0.2	0.2	0.27	0.0740
6.5min			0.24	0.25	0.19	0	0.17	0.1163
7 min	0.36	0.34	0.24	0.25	0.21	0.04	0.24	0.1144
7.5 min			0.27	0.25	0.21	0.12	0.21	0.0665
8 min	0.35	0.27	0.24	0.28	0.21	0.12	0.25	0.0771
8.5 min			0.23	0.28	0.16	0.09	0.19	0.0829
9 min	0.34	0.35	0.24	0.34	0.2	0.09	0.26	0.1037
9.5 min			0.23	0.2	0.05	0	0.12	0.1122
10 min	0.29	0.35	0.32	0.32	0.26	0	0.26	0.1294
15 min	0.3	0.31	0.23	0.19	0.28	0.07	0.23	0.0906
20 min	0.31	0.24	0.23	0.14	0.32	0.16	0.23	0.0742
30 min	0.31	0.28	0.27	0.3	0.15	0.16	0.25	0.0712
60 min	0.28	0.24	0.11	0.24	0.06	0.13	0.18	0.0882
2 h	0.12	0.15	0	0.09	0.02	0.07	0.08	0.0575
3 h	0.04	0.02	0	0	0.2	0	0.04	0.0784
4 h	0.02	0.09	0.03	0.03	0	0	0.03	0.0331
5 h	0.07	0.06	0.07	0.01	0.03	0	0.04	0.0310
6 h	0.05	0.02	0	0	0.03	0.02	0.02	0.0190
8 h	0.03	0.02	0	0.03	0.14	0.02	0.04	0.0502
10 h	0.01	0	0	0.04	0.23	0	0.05	0.0911
12 h	0.01	0	0	0.02	0	0	0.01	0.0084
24 h	0.01	0.08	0	0.01	0.02	0	0.02	0.0303
48 h	0.02	0	0.06	0.01	0.12	0.06	0.05	0.0446

48 h dehydration – quantum yield (Φ)

F. hygrometrica

	1	2	3	4	5	6	c	SD
0.5 min		0.23	0.43	0.45	0.24	0.38	0.346	0.105
1 min	0.34	0.23	0.45	0.24	0.26	0.18	0.283	0.097
1.5 min		0.23	0.22	0.4	0.14	0.34	0.266	0.103
2 min	0.24	0.27	0.4	0.21	0.35	0.2	0.278	0.080
2.5 min		0.23	0.36	0.27	0.27	0.25	0.276	0.050
3 min	0.24	0.21	0.33	0.35	0.47	0.32	0.320	0.092
3.5 min		0.23	0.42	0.35	0.32	0.23	0.310	0.082
4 min	0.35	0.23	0.45	0.16	0.34	0.11	0.273	0.129
4.5 min		0.23	0.31	0.41	0.2	0.28	0.286	0.081
5 min	0.35	0.23	0.38	0.28	0.26	0.29	0.298	0.056
5.5 min		0.23	0.26	0.24	0.35	0.26	0.268	0.048
6 min	0.36	0.23	0.34	0.16	0.28	0.2	0.262	0.079
6.5min		0.26	0.34	0.19	0.28	0.31	0.276	0.057
7 min	0.36	0.26	0.4	0.22	0.37	0.35	0.327	0.070
7.5 min		0.32	0.33	0.31	0.16	0.41	0.306	0.091
8 min	0.39	0.26	0.24	0.29	0.15	0.29	0.270	0.078
8.5 min		0.27	0.24	0.24	0.18	0.2	0.226	0.036
9 min	0.31	0.27	0.28	0.24	0.32	0.34	0.293	0.037
9.5 min		0.27	0.37	0.36	0.17	0.24	0.282	0.084
10 min	0.33	0.33	0.38	0.3	0.12	0.34	0.300	0.092
15 min	0.39	0.31	0.13	0.29	0.31	0.17	0.267	0.098
20 min	0.35	0.34	0.24	0.28	0.26	0.23	0.283	0.051
30 min	0.22	0.29	0.23	0.23	0.36	0.1	0.238	0.086
60 min	0.36	0.32	0.1	0.26	0.23	0.29	0.260	0.091
2 h	0.48	0.33	0.03	0.25	0.36	0.32	0.295	0.150
3 h	0.01	0.05	0	0.06	0.05	0.03	0.033	0.024
4 h	0.17	0.01	0.17	0	0.01	0	0.060	0.085
5 h	0.04	0.02	0	0	0.05	0.07	0.030	0.028
6 h	0.03	0	0	0	0	0.06	0.015	0.025
8 h	0.03	0.04	0	0	0.15	0	0.037	0.058
10 h	0.02	0.1	0.06	0.14	0.15	0	0.078	0.062
12 h	0.05	0.06	0.04	0	0.03	0	0.030	0.025
24 h	0.03	0	0.07	0.17	0	0.21	0.080	0.090
48 h	0.02	0	0.03	0.25	0	0.09	0.065	0.096

48 h dehydration – quantum yield (Φ)

M. polymorpha

	1	2	3	4	5	6	Mean	SD
0.5 min	0.34	0.32	0.3	0.23	0.38	0.31	0.31	0.05
1 min	0.23	0.32	0.31	0.2	0.36	0.28	0.28	0.06
1.5 min	0.23	0.34	0.39	0.33	0.35	0.32	0.33	0.05
2 min	0.23	0.31	0.39	0.16	0.38	0.39	0.31	0.10
2.5 min	0.32	0.39	0.32	0.27	0.35	0.31	0.33	0.04
3 min	0.34	0.34	0.37	0.18	0.36	0.45	0.34	0.09
3.5 min	0.35	0.39	0.4	0.29	0.31	0.4	0.36	0.05
4 min	0.35	0.39	0.33	0.34	0.28	0.33	0.34	0.04
4.5 min	0.35	0.38	0.35	0.33	0.33	0.36	0.35	0.02
5 min	0.32	0.38	0.26	0.32	0.4	0.28	0.33	0.05
5.5 min	0.32	0.35	0.31	0.32	0.38	0.37	0.34	0.03
6 min	0.33	0.35	0.36	0.31	0.38	0.36	0.35	0.02
6.5min	0.29	0.36	0.39	0.34	0.45	0.42	0.38	0.06
7 min	0.32	0.36	0.3	0.25	0.26	0.39	0.31	0.06
7.5 min	0.35	0.39	0.36	0.37	0.31	0.44	0.37	0.04
8 min	0.35	0.32	0.34	0.35	0.42	0.22	0.33	0.07
8.5 min	0.32	0.35	0.32	0.35	0.36	0.07	0.30	0.11
9 min	0.31	0.42	0.32	0.31	0.32	0.35	0.34	0.04
9.5 min	0.31	0.41	0.33	0.28	0.28	0.38	0.33	0.05
10 min	0.32	0.36		0.3	0.27	0.36	0.32	0.04
15 min	0.34	0.41	0.32	0.3	0.09	0.34	0.30	0.11
20 min	0.37	0.41	0.31	0.28	0.27	0.35	0.30	0.04
30 min	0.41	0.4	0.29	0.2	0.28	0.26	0.31	0.08
60 min	0.37	0.4	0.29	0.3	0.33	0.38	0.35	0.05
2 h	0.28	0.28	0.17	0.03	0.29	0.18	0.21	0.10
3 h	0.36	0	0.02	0.13	0.1	0.09	0.12	0.13
4 h	0.02	0.1	0.03	0.11	0.05	0	0.05	0.04
5 h	0.19	0.04	0.06	0	0.2	0.01	0.08	0.09
6 h	0	0	0.12	0.23	0	0.11	0.08	0.09
8 h	0.06	0.01	0.05	0	0	0	0.02	0.03
10 h	0	0	0.07	0	0.01	0	0.01	0.03
12 h	0	0	0.11	0	0	0	0.02	0.04
24 h	0	0	0	0	0	0.16	0.03	0.07
48 h	-	0.03	0	0.06	0		0.03	0.03

Gametophyte area dehydration (120 min)

One measurement per species

area (μm^2)

	<i>A. undulatum</i>	<i>P. patens</i>	<i>P. affine</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
1.	6596495.63	2098017.02	4956967.8	897526.65	378683.45
2.	5091049.76	2131718.5	4840515.28	883863.12	263142.08
3.	3963803.53	1974653.28	4357545.47	883824.28	215678.18
4.	3028658.92	1971775.07	4132639.37	883163.88	183488.2
5.	2276780.02	1822246.16	3776868.54	871220.21	172794.7
6.	1858437.37	1740600.49	4087184.92	871114.27	176146.13
7.	1529446.21	1632888.54	4145766.1	867060.06	178088.48
8.	1447927.69	1487470.33	3902524.25	866975.3	179381.02
9.	1347031.6	1440416.1	3444677.81	866869.36	175778.85
10.	1318570.92	1359395.52	3778252.91	870344.39	177212.65
11.	1296113.86	1331210.3	3468448.6	873741.73	177544.62
12.	1259195.15	1319068.87	3481296.34	877979.58	177163.21
13.	1237967.06	1334095.57	3476751.25	883725.39	178575.83
14.	1222534.24	1309427.77	3299093.62	844641.85	178522.86
15.	1262002.72	1312687.38	3340532.7	855812.11	176411
16.	1287733.52	1387655.83	3550754.66	878703.55	175711.75
17.	1267413.04	1284156.07	3518561.15	857570.81	173617.55
18.	1296806.04	1281570.98	3349524	863736.88	176312.11
19.	1295019.09	1330433.36	3389596.38	857450.74	172243.78
20.	1307125.2	1264238.69	3460799.28	842021.44	171325.58
21.	1314290.7	1274525.56	3776868.54	878760.05	175884.8
22.	1298317.54	1315749.22	4087184.92	874882.42	179455.18
23.	1295778.37	1290516.37	4145766.1	856931.6	176916.01
24.	1304119.86	1293066.14	3902524.25	856931.6	173518.66
25.	1299913.8	1274281.89	3444677.81	865255.44	177834.21
26.	1303063.93	1267331.82	3778252.91	863111.8	169976.53
27.	1301619.53	1274080.59	3468448.6	850370	171272.61
28.	1276937.6	1266109.9	3481296.34	890788.47	171166.66
29.	1300376.43	1262899.73	3476751.25	882358.69	177481.05
30.	1295965.54	1266318.26	3299093.62	857030.49	176435.72
31.	1308315.33	1276033.53	3340532.7	890714.31	175037.23
32.	1300743.71	1278445.57	3550754.66	900051.7	178484.01
33.	1290851.87	1263450.65	3518561.15	888185.73	179501.09
34.	1312846.3	1263478.91	3349524	892564.84	173274.99
35.	1297667.74	1269818.02	3389596.38	897141.71	178035.5
36.	1299066.23	1260943.26	3460799.28	888108.04	169799.95
37.	1292783.62	1279211.91	3776868.54	887843.17	172946.55
38.	1303787.9	1257560.05	4087184.92	883517.03	177036.08
39.	1295993.79	1292670.61	4145766.1	887744.29	173614.02

40.	1300683.68	1275553.24	3902524.25	879127.33	173642.27
41.	1305055.72	1255677.74	3444677.81	871149.58	173614.02
42.	1294160.92	1271802.74	3778252.91	869313.18	178702.96
43.	1290707.08	1259392.91	3468448.6	878943.69	167649.25
44.	1302128.07	1269786.24	3481296.34	875090.78	176329.77
45.	1303660.76	1277707.48	3476751.25	872961.26	179889.56
46.	1300560.07	1250362.77	3299093.62	882658.87	171943.6
47.	1293941.97	1266526.62	3340532.7	868960.03	177004.29
48.	1303392.37	1257461.16	3550754.66	870757.58	170658.12
49.	1301916.18	1249631.74	3518561.15	873240.25	171985.98
50.	1303364.11	1271114.09	3349524	867360.24	171671.67
51.	1301605.41	1240276.69	3430908.33	878601.13	177682.35
52.	1293581.75	1255116.22	3277586.54	872530.41	179529.34
53.	1293793.64	1305719.65	3598045.51	865456.74	175005.44
54.	1298462.34	1247477.5	3332004.04	855586.09	170114.26
55.	1298483.53	1260120.41	3303179.61	861395.47	177675.29
56.	1300005.62	1285508.65	3220852.36	861077.63	172272.03
57.	1263757.9	1247403.34	3023432.24	853251.74	171615.16
58.	1298635.38	1254773.66	3291158.25	858605.55	170435.63
59.	1278410.26	1236723.96	3179183.73	859756.84	169030.08
60.	1301781.98	1252877.22	2997909.8	880034.94	172247.31
61.	1262055.7	1270326.56	3263093.11	888517.69	178395.72
62.	1312118.8	1254544.11	3211225.38	878177.35	179140.88
63.	1303035.68	1283598.09	3204670.85	880260.95	178406.32
64.	1301573.62	1246940.71	3214834.62	874260.87	177742.39
65.	1301633.66	1236473.22	3124356.57	878071.4	179398.68
66.	1286610.49	1239033.59	2857682.96	877735.9	169934.15
67.	1296862.55	1252743.03	2627857.43	883806.62	172042.48
68.	1303166.35	1240817.02	2804497.97	887945.58	179077.31
69.	1301838.49	1240350.85	2904454.67	877527.54	178748.87
70.	1298381.11	1310010.47	2663578.95	877608.77	179151.47
71.	1298331.67	1240598.06	3430908.33	866191.3	178763
72.	1291950.18	1256263.97	3277586.54	866138.33	187122.15
73.	1303191.07	1243345.6	3598045.51	869867.63	169778.76
74.	1300228.11	1303258.17	3332004.04	862755.11	179109.09
75.	1283612.21	1297717.18	3303179.61	875906.57	168631.01
76.	1298758.99	1244228.48	3220852.36	874066.63	177954.28
77.	1303244.04	1234371.96	3023432.24	875737.05	179451.65
78.	1301693.7	1280253.72	3291158.25	869832.32	168030.65
79.	1293610	1292116.16	3179183.73	867762.84	179398.68
80.	1298578.88	1306563.69	2997909.8	879635.87	178589.96
81.	1276912.88	1230229.46	3263093.11	879540.52	178582.89
82.	1250143.81	1255776.62	3211225.38	881401.64	174239.1
83.	1306337.67	1241615.14	3204670.85	877375.69	175521.05

84.	1309547.84	1238069.48	3214834.62	879173.24	171156.06
85.	1236942.92	1242773.49	3124356.57	894365.92	180048.48
86.	1290445.74	1257817.85	2857682.96	889541.84	171106.62
87.	1306309.42	1265445.97	2627857.43	879095.55	170241.4
88.	1312602.62	1252598.23	2804497.97	896679.08	174179.06
89.	1280479.74	1236007.06	2904454.67	863556.77	169118.37
90.	1301951.5	1259795.51	2663578.95	880787.15	179709.45
91.	1295524.1	1244970.11	3130314.28	861868.7	169393.83
92.	1252414.59	1250062.59	2806316.72	876853.02	179684.73
93.	1228933.39	1250910.16	3041997.54	876800.05	176234.42
94.	1199395.59	1242021.27	3049050.02	874917.74	179006.68
95.	1300482.38	1244825.31	2957152.3	893843.26	170838.23
96.	1234710.98	1237260.76	2783209.85	884890.8	180055.54
97.	1237366.7	1263302.33	2980156.75	893977.45	173716.43
98.	1290671.76	1237359.64	2689931.3	884908.46	175037.23
99.	1245694.07	1228954.58	3112635.39	889315.82	178484.01
100.	1303805.56	1237334.92	2825461.19	889471.21	179501.09
101.	1311154.69	1244383.87	3038960.42	888870.85	173274.99
102.	1297364.03	1240163.68	2892652.26	881987.88	178035.5
103.	1311306.55	1238747.53	2779243.93	886155.09	169799.95
104.	1301919.71	1236784	2746181.66	871919.46	172946.55
105.	1307418.32	1244242.61	2934395.06	873713.48	177036.08
106.	1303148.69	1241353.81	2785300.52	887889.08	173614.02
107.	1303212.26	1247526.94	2721436.16	881419.3	173642.27
108.	1288651.72	1236759.28	2987361.09	892670.78	173614.02
109.	1254893.73	1226959.26	2679838.16	881263.91	178702.96
110.	1309551.37	1296248.06	2553260.72	867353.18	167649.25
111.	1303653.7	1250062.59	2805366.73	894980.41	176329.77
112.	1295174.47	1232468.46	2580354.69	885388.75	170658.12
113.	1279173.07	13967.24	2955043.97	877181.45	171985.98
114.	1278290.18	1239796.4	2819436.39	883280.42	171671.67
115.	1257372.87	1230183.55	2542185.81	883199.2	177682.35
116.	1298656.57	1239965.92	2584885.66	885455.85	179529.34
117.	1299836.11	1230706.22	2065011.25	883478.19	171943.6
118.	1301135.71	1228636.74	2476025.95	873455.68	177004.29
119.	1246449.82	1239690.46	2210983.9	890029.19	172272.03
120.	1301033.3	1238154.24	2721559.77	883411.09	170435.63

Gametophyte area dehydration (30 min)

Mean values from 3 repetitions

	A(X)	B(Y)	C(Y)	D(Y)	E(Y)
Langname	time	A. undulatum	<i>P. affine</i>	<i>P. patens</i>	<i>M.</i> <i>polymorpha</i>
Einheiten	ninute:	µm ²	µm ²	µm ²	µm ²
Kommentare					
F(x)=					
1	1	5418078,14	3969183,617	4049362,117	888303,0467
2	2	4731177,44	3089716,19	3783583,347	795327,8733
3	3	4076294,78	2260907,303	3613419,403	717531,63
4	4	3818183,643	2204972,207	3320707,97	672062,1033
5	5	3365686,77	2079333,927	3094185,48	669276,0367
6	6	3127861,697	1942424,73	2948025,66	660870,3367
7	7	3110128,207	1861812,733	2806070,667	609599,14
8	8	3044240,04	1505793,413	2676089,047	604282,2567
9	9	3094579,25	1772537,807	2537373,26	335050,5833
10	10	4003418,68	1701202,16	2365826,17	517158,225
11	11	3072878,38	1661813,31	2177331,153	587494,6033
12	12	3089673,95	1621528,087	1997851,287	575010,7167
13	13	3112029,78	1567561,843	1839253,09	542466,34
14	14	3081812,403	1569281,37	1754970,53	541710,46
15	15	3127186,947	1551031,423	1717508,973	512733,7533
16	16	3105519,717	1462380,833	1686242,863	507982,7933
17	17	3059153,82	1431103,797	1600102,267	510569,01
18	18	4034530,445	1520070,04	1652218,387	474648,8967
19	19	3142324,33	1533828,24	1504631,893	341289,5633
20	20	3160419,93	1568822,303	1559098,76	382105,0833
21	21	3212441,07	1260755,83	1533181,19	505863,56
22	22	3172246,873	1243914,75	1530775,04	520723,9167
23	23	3157384,54	1186058,297	1484375,503	506381,99
24	24	3144635,5	1185470,61	1440578,02	510381,03
25	25	3175788,823	1277622,63	1419595,433	506223,69
26	26	3182928,127	1276059,42	1485869,453	517049,39
27	27	3157904,947	1220710,037	1417507,857	474961,5367
28	28	2999934,017	1197610,197	1427324,4	505376,7867
29	29	2940207,647	1222330,63	1500907,897	470365,91
30	30	2976541,32	1221178,997	1400698,44	490298,7667

Leaf area (6 hours)

Raw data

<i>A. undulatum</i> (μm^2)				<i>P. patens</i> (μm^2)		
	1080881.8					
0h	1	170957	171023.19	370924.43	58096.14	371698.64
2h	450667.14	179052.6	170578.25	183541.77	37141.99	54989.09
4h	447286.24	177640.3	170051.53	182532.45	38377.28	54995.01
6h	437101.05	170051.5	168802.25	184062.57	54995.01	53485.33
<i>P. affine</i> (μm^2)				<i>F. hygrometrica</i> (μm^2)		
	1113889.1					
0h	4	1650663	1628212.3	309966.05	291397.47	61834.28
2h	612113.63	427728.8	411433.34	145409.28	286374.54	67496.91
4h	598833.22	429660.3	403091.39	141900.87	286298.15	73217.64
6h	608529.9	403091.4	402853.04	143864.63	73217.64	71481.46
<i>M. polymorpha</i> (μm^2)				Tab. Columns of the raw data marked in grey were shown as images in the “leaf area” chapter.		
0h	288336.69	733566.6	1171188.5			
2h	71741.86	395203	564660.54			
4h	72038.84	393884.3	568107.62			
6h	72213.7	568107.6	556121.14			

Mean leaf area

mean	<i>A. undulatum</i>	<i>P. patens</i>	<i>P. affine</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
0h	474287.3	266906.4	1464254.7	221065.93	731030.6
2h	266766	91890.95	483758.587	166426.91	343868.46
4h	264992.7	91968.247	477194.96	167138.89	344676.92
6h	258651.6	97514.303	471491.443	96187.91	398814.15

Cell area (90 minutes)

<i>A. undulatum</i>	min 0	min 5	min 10	min 15	min 20	min 25	min 30	min 45	min 60	min 75	min 90
Cell 1	515.07	289.9	312.1	289.93	307.55	356.09	327.64				
Cell 2	565.14	620.02	531.02	482.6	484.8	518.64	557.78				
Cell 3	604.76	502.42	555.56	542.23	541.51	549.03	469.52				
Cell 4	664.65	626.26	533.3	572.44	532.06	495.82	471.42				
Cell 5	525.32	568.64	519.94	511.7	499.84	452.56	439.89				
Cell 6	660.12	637.73	631.3	524.97	507.24	529.33	545.97				
Cell 7	540.64	527.86	491.36	485.34	503.26	547.38	508.47	501.87	318.52	315.6	330.34
Cell 8	326.11	354.29	299.84	314.37	307.03	298.45	325.41	305.68	579.41	595.94	527.43
Cell 9	598.97	622	596.67	574.22	583.85	606.74	614.12	556.89	499.82	513.42	615.61
Cell 10	753.41	678.8	703.1	714.8	754.1	651.34	740.17	800.51	652.45	701.77	679.17
Cell 11	878.03	853.87	874.38	833.51	885.48	840.93	765.02	863.77	814.04	833.4	873.8
Cell 12	951.77	963.85	886.31	918.25	919.83	914.61	846.92	809.8	857.36	816.88	805.95
Cell 13	509.47	582.8	627.67	504.57	505.33	533.85	523.84	462.13	445.84	481.68	494.45
Cell 14	522.91	468.3	303.89	305.38	434.61	275.22	281.97	284.93	271.47	367.02	328.31
Cell 15	467.99	456.65	444.48	396.63	302.36	438.73	439.31	405.33	419.04	412.69	379.36
Mean	605.62	583.56	554.06	531.40	537.92	533.91	523.83	554.55	539.77	559.82	559.38
SD	159.47	171.64	179.73	180.97	188.14	175.27	163.80	220.65	205.03	190.10	200.21

<i>P. affine</i>	min 0	min 5	min 10	min 15	min 20	min 25	min 30	min 45	min 60	min 75	min 90
Cell 1	1454.03	1687.7	1614.91	1347.2	1525.69	114.66	1192.8				
Cell 2	1551.92	1734.8	1841.53	1142.9	1288	1099.5	1129.8				
Cell 3	1555.76	1683.6	1749.6	1315	1162.45	1067.7	1188.4				
Cell 4	1456.09		1479.51	1468.8	1492.88	1506.8	1396.7				
Cell 5	1456.09	1895.2	1762.17	1706	1667.46	1558.9	1661				
Cell 6	1431.31	1394	1191.55	1416.3	1398.33	1825.9	1289.8				
Cell 7	1348.65	1308.7	711.68	554	639.12	588.57	550.92	689.63	666.5	617.3	636
Cell 8	1528.29	1488.6	657.26	550.14	592.11	577.76	518.89	739.42	522.4	535.9	557.5
Cell 9	1314.24	1301.5	391.55	322.32	406.67	307.75	390.87	538	465.8	325.5	321.7
Cell 10	1661.59	1635.5	541.87	465.95	379.85	334.25	404	377.47	358.9	396.3	358.9
Cell 11	1410.01	1519.4	182.77	155.22	142.47	202.14	160.69	153.38	167.6	187.4	151.7
Cell 12	1287.82	1403.6	225.85	248.9	162.94	223.44	213.93	171.92	203.7	196.4	201.7
Cell 13	1459.84	1384	1409.83	1379.9	1440.29	1398.2	1285.4	1247.6	996.4	665.6	602.2
Cell 14	1437.47	1416.3	1421.96	1440.5	1185.9	1438.2	1479.3	994.37	1048	667.7	523
Cell 15	1364.1	1388.7	1386.01	1369.4	1303.91	1395.3	1424.2	1273.2	1043	643.7	597
Mean	1447.81	1517.25	1104.54	992.16	985.87	909.27	952.44	687.21	608.06	470.63	438.83
SD	98.95	180.67	590.08	536.46	535.85	594.18	514.55	421.77	350.78	198.28	183.89

	min	min	min	min	min	min	min	min	min	min	min
<i>P. pat-ens</i>	0	5	10	15	20	25	30	45	60	75	90
Cell 1	1048.8	637.26	666.25	649.82	739.39	753.26	861.56				
Cell 2	649.35	487.37	570.6	472.23	514.49	500.73	534.48				
Cell 3	1033.6	754.19	785.34	746.14	784.82	799.36	737.95				
Cell 4	473.61	473.9	476.9	519.64	502.32	500.06	480.49				
Cell 5	240.18	262.1	269.09	253.55	268.32	281.57	245.23				
Cell 6	399.11	335.53	342.82	331.84	327.5	363.75	324.66				
Cell 7	1772.2	1075.66	1404.65	967.64	842.44	904.52	745.49	716.99	1031.63	951.28	760.29
Cell 8	1030.4	1029.69	1087.38	717.35	886.25	767.95	1057.91	890.1	911.36	917.64	877.42
Cell 9	1341.4	1190.23	1093.39	754.36	775.27	790.19	836.68	853.42	1022.9	865.69	1097.2
Cell 10	490.08	408.61	324.4	327.86	371.66	400.04	357.25	351.08	344.8	356	357.08
Cell 11	531.88	436.96	363.31	463.17	435.19	525.97	434.85	489.43	481.99	468.95	395.06
Cell 12	569.45	394.08	395.83	435.85	446.07	479.75	440.96	411.5	489.54	432.29	428.38
Cell 13	1061.9	1035.61	839.81	870.1	663.02	1025.3	714.25	761.23	829.09	792.75	803.85
Cell 14	1688.9	1152.61	881.46	874.6	934.36	917.17	996.89	670.25	859.45	808.7	817.46
Cell 15	710.71	942.92	468.19	531.05	509.46	468.99	469.45	433.97	480.67	401.13	468.17
Mean	869.44	707.78	664.63	594.35	600.04	631.90	615.87	619.77	716.83	666.05	667.22
SD	464.96	331.79	340.58	220.98	215.99	230.01	252.12	202.10	265.65	245.13	261.23

<i>F. hygromet-rica</i>	min	min	min	min	min	min	min	min	min	min	min
	0	5	10	15	20	25	30	45	60	75	90
Cell 1	398.8	312.83	255.37	249.72	206.79	224.38	223.24				
Cell 2	772.72	644.04	486.5	568.46	556.48	487.52	548.31				
Cell 3	735.63	401.64	339.81	290.17	299.04	285.09	270.49				
Cell 4	776.04	653.97	734.09	691.29	626.01	705.71	669.17				
Cell 5	604.59	483.34	403.05	465.4	420.54	361.02	394.26				
Cell 6	1497.1	1304.1	1284.09	1382.3	1397.9	1216.2	1235.3				
Cell 7	1001	1017.2	1052.99	862.22	680.26	734.8	701.51	826.2	721	769.7	753
Cell 8	769.3	691.88	663.43	701.76	839.95	760.85	643.58	586.9	673.1	602.4	551
Cell 9	794.15	887.34	828.16	743.34	824.05	805.46	689.98	768.6	804	766.2	751
Cell 10	997.21	1068.4	1091.87	712.85	630.95	615.4	648.17	630.9	790	869.5	665
Cell 11	1275.3	1101	1023.02	1136	913.37	791.41	862.84	752	629.9	647.2	783
Cell 12	1462.3	1347.2	1154.84	1134.8	797.88	936.82	1007	1015	800.8	818.2	801
Cell 13	887.35	942.79	1011.3	1018.5	707.99	461.03	331.05	248.4	286.4	230.3	288
Cell 14	1045.9	1072.3	1377.94	979.97	1283.1	472.72	381.42	524.1	445.5	384.3	359
Cell 15	1213.6	1447.1	1272.18	1277	991.87	891.72	860.14	850.8	854.1	843	820
Mean	948.72	891.67	865.24	814.26	745.08	650.01	631.09	#####	667.20	#####	#####
SD	309.69	349.67	366.94	341.55	325.72	270.00	284.66	#####	188.76	#####	#####

	min	min	min	min	min	min	min	min	min	min	min
<i>M. poly-</i> <i>morpha</i>	0	5	10	15	20	25	30	45	60	75	90
Cell 1	1348.56	1009.47	1118.19	1126.55	1300.26	1151.99	1245.89				
Cell 2	1061.5	1030.65	838.83	1188.15	1011.29	900.18	927.24				
Cell 3	1846.99	1483.83	657.42	1203	632.73	942.89	650.04				
Cell 4	3424.6	2022.87	1939.93	1953.58	1900.47	1995.11	1915.8				
Cell 5	1981.21	1539.14	1602.09	1533.51	1473.02	1466.86	1454.18				
Cell 6	772.43	733.51	680.3	774.87	711.19	665.63	430.41				
Cell 7	1067.51	1147.7	1115.37	892.74	942.35	931.29	967.25	831.71	892.98	837.33	910.63
Cell 8	1442.38	1392.43	1511.22	1067.94	1138.72	1177.91	1179.89	1106.74	1430.4	1205.72	1225.13
Cell 9	1606.54	1567.42	1597.61	1007.25	981.3	1026.41	1036.41	890.35	1042.5	1003.9	1005.94
Cell 10	2376.16	2103.75	2073.24	2075.78	1233.59	1207.53	929.99	901.8	989.82	926.9	892.85
Cell 11	2505.16	2327.07	2319.15	2325.33	2369.49	2294.36	2123.47	1615.13	1597.6	1600.36	1646.42
Cell 12	3041.13	2591.63	2492.14	2514.79	2462.7	2466.89	2111.39	2402.76	2046.2	2316.7	2436.81
Cell 13	1068.73	1082.66	1115.43	1108.18	1126.98	1114.52	1070.28	1046.32	1021.1	1024.63	
Cell 14	1287.69	1255.25	1309.55	1329.81	1321.6	1250.18	1258.1	1360.23	1342.8	1354.94	
Cell 15	829.07	704.34	659.28	725.48	807.9	787.67	775.32	781.95	916.46	795.69	
Mean	1710.64	1466.11	1401.98	1388.46	1294.24	1291.96	1205.04	1215.22	1253.32	1229.57	1352.96
SD	809.37	570.34	601.02	566.57	553.78	541.54	506.11	520.83	386.91	482.48	600.83

Initial, hydrated cells

5 leaves were investigated per species, the same shown in the cell area dehydration.

Cell area (μm^2)

1		638.48	341.63	538.88	552.87	349.61	585.41	403.79	403.8	318.06
2	326.04	477.7	446.96	684.78	584.26	428.14	381.16	334.74	388.6	457.2
3	1402.34	583.08	787.78	683.12	670.15	505.22	707.39	539.54	473.5	600.98
4	402.78	537.02	543.68	592.18	602.52	633.43	738.4	748.67	782.2	845.59
5	487.52	499.73	401.93	549.76	587.94	633.05	365.42	724.55	522.9	421.25
<i>P. affine</i>										
1	1742.33	2113.83	2241.7	1955.2	1815.5	2142.5	1127.7	566.34	1344	1603.2
2	751.87	1005.74	1405.7	1441.4	1560	1618.6	1765.4	1838.1	1904	2144.8
3	1280.87	1337.71	1549.5	1334.7	1146.7	1588.8	881.64	1671.2	1422	557.39
4	1280.88	2034.29	1950.2	1292.9	1555.9	2102	1470.4	1766	1766	1514.2
5	1401.07	1279.22	901.75	1276.7	1159.8	958.93	760.12	754.84	592.3	672.68
<i>P. pat-</i> <i>ens</i>										
1	987.77	737.71	849.4	1115.7	648.57	673.5	1023	296.18	941.2	816.08
2	506.33	582.72	690.03	654.45	514.77	667.52	628.27	463.52	269.5	568.12
3	1279.67	1270.66	1134.2	934.44	1051.1	1328.8	1078.8	983.9	1182	875.21
4	514.29	769.67	532.98	626.17	459.28	407.37	463.72	512.41	592.5	570.83
5	2161.27	2277.31	1493.2	655.94	1111.6	1438.6	1123.2	1011.1	1098	944.1
<i>F. hygrometrica</i>										
1	297.23	404.53	255.36	684.13	473.76	616.32	205.88	476.87	670.2	840.79
2	479.1	686.72	833.91	1252.6	440.59	692.16	393.05	426.1	1245	575.1
3	1143.08	969.08	696.68	1144.1	845.37	828.53	1066.5	1378.8	888.8	1361.4
4	704.51	1317.38	902.59	1147.1	773.41	1410.5	663.07	773.02	1157	825.15
5	791.69	1022.73	1407.7	1138.6	1656.4	1626	1801.7	2055.3	1282	1904.7
<i>M. polymorpha</i>										
1	1350.02	933.87	1446.8	797.48	985.5	1133.7	1292.5	461.22	1091	796.16
2	1961.83	1330.16	1962	1434.7	859.39	1545.9	1716.6	1644.8	1359	1466.3
3	979.06	1497.77	651.84	1295.6	646.39	1247.7	976.93	1107	1228	1670.1
4	2910.82	747.13	1882.7	1882.7	2245	4459.7	825.08	5020.5	2952	634.32
5	1027.87	1074.99	2023.9	1312.7	1894.3	783.83	2199.2	1658.1	1451	1094.6

Leaf diameter (CLSM)

	<i>A. undulatum</i>	<i>P. affine</i>	<i>P. patens</i>	<i>F. hygrometrica</i>	<i>M. polymorpha</i>
A1	17µm	8µm	40µm	11µm	35µm
A2	25µm	10µm	13,5 µm	8µm	25µm
A3	20µm	12µm	20 µm	7µm	35µm
B1	15µm	5µm	5,5 µm	21µm	22µm
B2	16µm	16µm	4,2 µm	16µm	25µm
B3	17µm	10µm	3,5 µm	20µm	30µm
C1	23µm	13µm	12 µm	25µm	30µm
C2	12µm	15µm	4 µm	23µm	25µm
C3	21µm	7µm	7,5 µm	32,5µm	20µm
D1	18µm		18 µm	18µm	
D2	16µm		28 µm	10µm	
D3	14µm		34 µm	10µm	
E1				19µm	
E2				21µm	
E3				16µm	

	Median	Mittelwert	STABW
<i>P. patens</i>	12.75	15.85	12.4607
<i>A. undulatum</i>	17	17.83	3.7859
<i>M. polymorpha</i>	25	28.67	5.5377
<i>P. affine</i>		10.17	3.71035
<i>F. hygrometrica</i>	18	17.17	7.09

Tab. Cell diameter (µm)

Rehydration – Quantum yield (Φ)

<i>A. undulatum</i>	1	2	3	4	5	6	<i>F. hygrometrica</i>	1	2	3	4	5	6
0min	0.27	0.06	0	0.23	0.34	0.31	0min	0.18	0.35	0.29	0.38	0.24	0.32
5 min	0.36	0.33	0.1	0.34	0.31	0.29	5 min	0.52	0.39	0	0.38	0.24	0.38
10 min	0.17	0.55	0	0.29	0.32	0.32	10 min	0.45	0.3	0.19	0.34	0.32	0.32
15 min	0.29	0.12	0.1	0.33	0.34	0.28	15 min	0.32	0.22	0.13	0.32	0.36	0.31
20 min	0.05	0.27	0.02	0.34	0.33	0.27	20 min	0.38	0.45	0.28	0.34	0.31	0.48
25 min	0.1	0.11	0.2	0.32	0.33	0.29	25 min	0.4	0.4	0.24	0.25	0.31	0.29
30 min	0.44	0.13	0.09	0.33	0.37	0.24	30 min	0.66	0.62	0.18	0.35	0.35	0.3
45 min	0.13	0.06	0.04	0.39	0.34	0.25	45 min	0.36	0.31	0.41	0.34	0.27	0.23
60 min	0.13	0.21	0.1	0.03	0.25	0.34	60 min	0.53	0.16	0.25	0.06	0.1	0.07
75 min	0.16	0.18	0.08	0.28	0.24	0.25	75 min	0.04	0.05	0.08	0	0.16	0.1
90 min	0.02	0.03	0.08	0.3	0.3	0.23	90 min	0.05	0.12	0.17	0.08	0.24	0.08
120 min	0.03	0.05	0.04	0.1	0.06	0.08	120 min	0.15	0.06	0.12	0.13	0.22	0.01
<i>P. affine</i>	1	2	3	4	5	6	<i>M. polymorpha</i>	1	2	3	4	5	6
0min	0.42	0.06	0.57	0.55	0.62	0.44	0min	0.11	0.18	0	0.49	0.36	0.37
5 min	0.16	0.37	0	0.61	0.61	0.56	5 min	0.37	0.1	0.05	0.48	0.37	0.4
10 min	0.02	0.48	0.25	0.5	0.59	0.51	10 min	0.4	0.24	0.23	0.52	0.33	0.48
15 min	0.27	0.3	0.54	0.54	0.54	0.56	15 min	0	0.04	0.18	0.5	0.31	0.49
20 min	0.43	0.32	0.12	0.42	0.53	0.53	20 min	0.24	0.39	0.15	0.44	0.28	0.43
25 min	0.26	0.5	0	0.41	0.33	0.53	25 min	0.1	0	0.21	0.41	0.3	0.44
30 min	0.36	0.39	0.3	0.42	0.48	0.51	30 min	0.11	0.15	0.27	0.48	0.28	0.89
45 min	0.29	0.39	0.35	0.4	0.55	0.52	45 min	0.2	0.1	0.22	0.45	0.29	0.33
60 min	0.47	0.16	0.33	0.27	0.45	0.55	60 min	0.17	0.38	0.1	0.31	0.14	0.11
75 min	0.18	0.09	0.11	0.28	0.51	0.25	75 min	0.11	0.07	0.16	0.28	0.13	0.13
90 min	0.19	0.01	0.1	0.07	0.43	0.09	90 min	0.15	0.06	0.05	0.16	0.05	0.01
120 min	0.05	0.03	0.13	0	0.12	0.05	120 min	0.04	0.07	0	0.05	0.15	0.05
<i>P. patens</i>	1	2	3	4	5	6							
0min	0.09	0.16	0.21	0.15	0.17	0.26							
5 min	0.18	0.02	0.17	0.24	0.12	0.26							
10 min	0.12	0.3	0	0.2	0.15	0.26							
15 min	0.2	0.17	0.13	0.21	0.08	0.22							
20 min	0.3	0.04	0.02	0.37	0.14	0.32							
25 min	0.14	0.18	0.39	0.3	0.14	0.32							
30 min	0.22	0.18	0.19	0.28	0.19	0.27							
45 min	0.21	0.07	0.34	0.27	0.24	0.3							
60 min	0.31	0.26	0.21	0.32	0.16	0.16							
75 min	0.1	0.12	0	0	0.06	0.08							
90 min	0.15	0.09	0.06	0.17	0.07	0.13							
120 min	0.4	0.1	0.2	0.23	0.09	0.17							