



Special issue in honor of Prof. Suleyman I. Allakhverdiev

REVIEW

Chloroplast structure and photosynthetic acclimation in shade-adapted lycophyte *Selaginella*: a window into photosynthesis of ancient vascular plants?

L. FERRONI 

Department of Environmental and Prevention Sciences, University of Ferrara, 44121 Ferrara, Italy

Abstract

Selaginella is a diverse genus of lycophytes, an ancient group of vascular plants that is sister to all other vascular plants (euphyllophytes, including angiosperms). The majority of *Selaginella* species thrive in environments with humid, shaded conditions. This review examines current knowledge and unresolved issues concerning the distinctive photosynthetic adaptations of shade-adapted *Selaginella* species, addressing aspects from cell morphology to thylakoid organisation. Unique is their giant chloroplast, in which the thylakoid system can differentiate into lamellar and granal (bizonoplast). Optimisation of light harvesting and photoprotection is supported by distinctive thylakoid membrane composition and supramolecular organisation of LHCII with PSII and PSI, involving the role of LHCB6 phosphorylation and an outstanding capacity to keep PSI oxidised. *Selaginella* appears to combine ancestral characteristics with highly specialised adaptations to deep shade, offering insight into the evolutionary trajectory of photosynthesis in ancient vascular plants.

Keywords: bizonoplast; excitation spillover; lycophytes; megacomplexes; photoprotection; *Selaginella*.

Introduction

The transition from aquatic to land environments was most likely marked by small plants called eophytes, which evolved from one lineage of charophycean algae during the Ordovician period, approximately 470 million years

ago (Edwards *et al.* 2022). Their subsequent evolution is currently thought to have followed two opposite trajectories: either a structural simplification, leading to bryophytes (mosses, liverworts, hornworts; Donoghue *et al.* 2021); or an increase in sporophyte complexity, leading to vascular plants. During the early Devonian, approximately 415

Highlights

- Epidermal cells and giant chloroplasts in *Selaginella* enhance light harvesting
- Shade-adapted thylakoid composition and function in *Selaginella*
- Flexible supramolecular organisation of LHCII with PSI and PSII in *Selaginella*

Received 6 April 2026

Accepted 4 May 2026

Published online 4 June 2026

e-mail: frrlnz@unife.it

Abbreviations: CEF – cyclic electron flow around PSI; Chl *a(b)* – chlorophyll *a(b)*; *cytb₆f* – cytochrome *b₆f* complex; FLV – flavodiiron protein; F_v/F_m – variable to maximum Chl *a* fluorescence ratio, a proxy of maximum PSII photochemical quantum yield; LCP – light-compensation point; LEF – linear electron flow; NDH – NAD(P)H-dehydrogenase; NPQ – nonphotochemical quenching of Chl *a* fluorescence; P_N – net carbon assimilation; PTOX – plastid (plastoquinol) terminal oxidase; q_E – high-energy quenching; q_I – photoinhibitory quenching; q_{pd} – photochemical quenching in the dark; q_T – state-transition quenching.

Acknowledgements: The support of the University of Ferrara is kindly acknowledged. The author is grateful to Laura Brancaleoni and Federica Grandi for professional collaboration in the maintenance of the *Selaginella* collection at the Botanical Garden of Ferrara, and to Paola Boldrini and Edi Simoni for scientific assistance at the Electron Microscopy Centre of the University of Ferrara.

Dedication: This review is gratefully dedicated to Prof. Suleyman I. Allakhverdiev, who invited the author to lecture on the same subject at the 11th International Conference on Photosynthesis and Hydrogen Research 2023, Istanbul, Türkiye. In the article, the arguments presented in the lecture are enriched by others that emerged, especially during scientific discussions at the conference.

Conflict of interest: The author declares no conflict of interest.

million years ago, the latter diverged into two sister clades: lycophytes and euphyllophytes. Lycophytes develop single-veined microphylls from an intercalary meristem without leaving a leaf gap (though with exceptions, *see e.g.*, Liu *et al.* 2022), while euphyllophytes, through more complex developmental patterns, form megaphylls with multiple veins and associated with a leaf gap (Simpson 2019). The present Earth flora is dominated by euphyllophytes, which include ferns and allies, gymnosperms, and especially angiosperms. However, it was not so until the mid-Carboniferous, when lycophytes thrived in an atmosphere very rich in CO₂. Fossil records tell us a story of an Earth covered by arborescent lycophytes, which in the late Carboniferous formed dense and extensive forests, growing statures of up to 45 m (Thomas and Cleal 2018). The extreme impact of their photosynthetic activity on CO₂ sequestration resulted in the inexorable decline of lycophytes (Beerling and Berner 2005). For lycophytes, which had evolved under an atmosphere with >1,000 $\mu\text{L L}^{-1}(\text{CO}_2)$ at a comfortable temperature of 30°C, the drop in CO₂ concentration down to ~300 $\mu\text{L L}^{-1}$, accompanied by a drastic cooling of the planet – the most significant glacial event in Earth's history – was a catastrophe. The world, which had now become inhospitable for lycophytes, was ready to be populated by the euphyllophytes that until the Carboniferous had mostly lived in the shadow of the former. Only herbaceous lycophytes survived to the present, accounting for only ca. 1% of the extant vascular species with three families: Lycopodiaceae (lycopods), Isoëtaceae (isoëtes), and Selaginellaceae (spikemosses; Pryer *et al.* 2004).

Selaginellaceae include the only genus *Selaginella*, which is monophyletic based on strong molecular evidence and conserved gross morphology (Weststrand and Korall 2016a,b; Fig. 1A,B), which makes recent attempts to split *Selaginella* into more genera inconsistent (*see arguments by Valdespino et al.* 2024). In the history of plants, *Selaginella* is singular in many respects. *Selaginella* is one of the plant genera with the most species in the world (Weststrand and Korall 2016a,b). Approximately 750 species of *Selaginella* have been recorded, and new ones are constantly being discovered and added to the list. Based on retrieved amber fossils, *Selaginella* was already hyperdiverse during the Cretaceous, suggesting that their adaptability and steadily high speciation rate allowed them to colonise the variety of niches emerging upon the rise of angiosperms to ecological dominance, especially in rainforests (Schmidt *et al.* 2020). The greatest diversity of *Selaginella* is found in the tropics and subtropics, and most species are accordingly adapted to warm, humid, and shade conditions. Some arctic-alpine and drought-tolerant xerophytic species also exist, including “resurrection plants” *S. lepidophylla* and *S. tamariscina* (Weststrand and Korall 2016a).

Selaginella species are somehow at the crossroads between being organisms with ancestral traits, reminiscent of the first steps taken by land plants (Spencer *et al.* 2021), and, conversely, the result of a marked adaptation to ecological niches that arose from the radiation of angiosperms. This review focuses on current knowledge and open questions about chloroplast structure and photosynthesis in shade-adapted *Selaginella*. Information

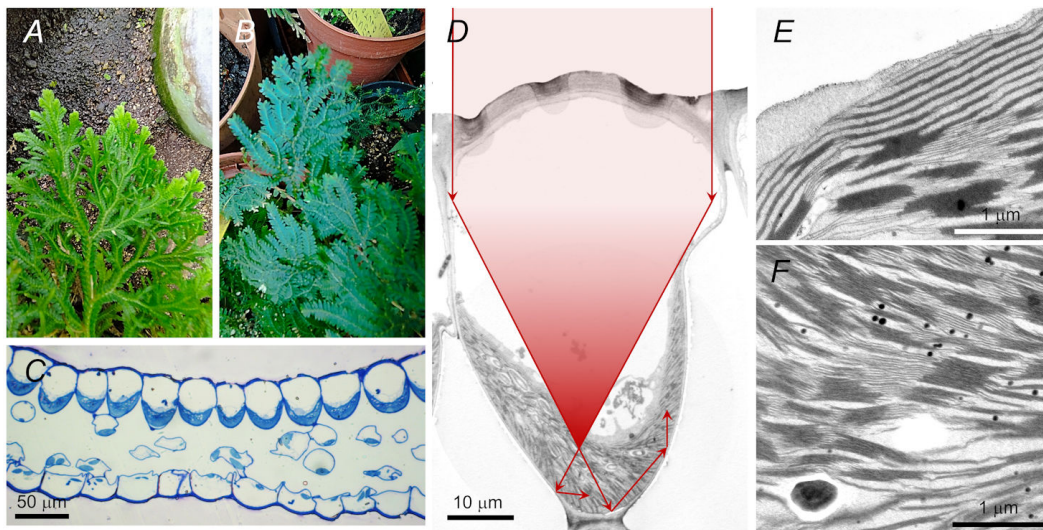


Fig. 1. Morphological aspects of the photosynthetic apparatus of shade-adapted *Selaginella* species. (A) Plant of *Selaginella martensii*, in which iridescence is not particularly evident. (B) Plant of *Selaginella uncinata*, showing evident blue hues caused by iridescence. (C) Cross-section of a ventral microphyll of *S. martensii*, showing the three cell layers: conical upper epidermis cells, sparse irregularly shaped mesophyll cells, flat lower epidermal cells. (D) Electron micrograph of an upper epidermal cell of *S. martensii* with illustration of potential light paths, as per Liu *et al.* (2020). Light coming from above is concentrated in the giant cup-shaped chloroplast lying at the cell's bottom. The upper tangential cell wall and the vacuole are responsible for a lens effect, strengthened by the light-pipe effect due to the conical shape of the cell. Light reaching the oblique cell walls is reflected multiple times inside the cell. (E) In a bizonoplast, the upper region of the chloroplast is occupied by regularly spaced long lamellae formed by 2–4 thylakoids. (F) In a bizonoplast, the lower region of the chloroplast is occupied by grana stacks, which appear quite irregular in shape.

available from a limited number of species illustrates how *Selaginella* chloroplasts combine ancestral characteristics with outstanding specialisation to living in light-limited environments. In these plants, the structure–composition–function relationship of thylakoids seems to have reached a higher level of complexity than in angiosperms. Among the most challenging topics is understanding the significance of the differentiation of the thylakoid system into a lamellar and a granal region, what their molecular determinants might be, and what the consequences are for the capture and utilisation of light energy, including those of a photonic nature. Further complexity emerges at the level of the supramolecular organisation of thylakoid complexes, particularly regarding the management of LHCII in interaction with both photosystems, with several aspects remaining to be clarified, for example, the role of the phosphorylation of specific subunits, especially LHCB6. Alongside the regulation of light capture, there are also significant gaps in our understanding of electron transport control in this ancient lineage of vascular plants.

***Selaginella* thriving (slowly) through photosynthesis in the shade**

Shade-adapted *Selaginella* species exhibit photosynthetic traits that mostly align with known differences between shade and sun plants (Anderson *et al.* 2012, Lichtenthaler *et al.* 2013, Zivcak *et al.* 2014a, Huang *et al.* 2015, Bielczynski *et al.* 2016, Schumann *et al.* 2017, Mathur *et al.* 2018, Tan *et al.* 2020). In the understory, living in the shade of other plants means being reached by low or very low irradiance, down to only a few $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$; moreover, the preferential absorption of blue (400–500 nm) and red (~660 nm) light from the upper canopy causes a relative enrichment in green (500–600 nm) and far-red (~725 nm) wavelengths. While under sunlight, the red:far red ratio is generally >1.2, in the deep shade it can fall to <0.1 (Martinez-Garcia and Rodriguez-Concepcion 2023). Like other shade plants, the primary need for *Selaginella* is to maximize its light-harvesting capacity to drive photosynthesis and allow a positive balance of carbon assimilation with the few available photons. The light-compensation point (LCP) across *Selaginella* species was reported to be 1–10 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$, without clear trends related to the degree of canopy openness (Campany *et al.* 2019). However, LCP in *S. martensii* increased from 6 in the shade to 12 $\mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ when the plant was cultivated under full sunlight (Ferroni *et al.* 2021a). The downside of heavy investment in light harvesting in shade-adapted plants is their reduced carboxylation capacity, which is particularly evident in *Selaginella*. Saturated net photosynthesis (P_N) in *Selaginella* species is 1.8–5.8 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ (Campany *et al.* 2019, Carriqui *et al.* 2019, Ferroni *et al.* 2021a, Anderson 2025). Therefore, P_N in *Selaginella* is lower than in C_3 angiosperms, but falls in the range of ca. 2–10 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ reported for ferns (Tosens *et al.* 2016). For comparison, in angiosperms, P_N can reach 35 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$. *S. martensii* can long-term modulate its capacity for CO_2 fixation with a level

of plasticity comparable with that of angiosperms (Poorter *et al.* 2019), but such plasticity is expressed at intrinsically low P_N [from 1.5 under deep shade to 3.4 $\mu\text{mol}(\text{CO}_2) \text{m}^{-2} \text{s}^{-1}$ at full sunlight; Ferroni *et al.* 2021a].

As is common in pteridophytes, marked gas diffusional limitations hinder the photosynthetic assimilation in *Selaginella* (Carriqui *et al.* 2019); however, at least in *S. martensii*, there is space for enhancing stomatal and mesophyll conductance under a high-light regime, at the same time making it very clear that biochemical limitations play a major role (Ferroni *et al.* 2021a). The Calvin–Benson–Bassham cycle is a poor electron sink in *Selaginella*, which makes sense ecologically and evolutionarily. Low contents of Rubisco and ATP synthase, together with a limited capacity of photosynthetic electron transport, characterise the shade-adapted photosynthetic machinery (Anderson *et al.* 2012, Mathur *et al.* 2018). Moreover, *Selaginella* species exemplify well the conservative strategy typical of shade-tolerant plants, in which low carboxylation rates are accompanied by a tendency to slow down leaf turnover (Martinez-Garcia and Rodriguez-Concepcion 2023). However, the low carbon fixation capacity of *Selaginella* also points to the heyday of lycophytes, when CO_2 concentrations were much higher than they are today and therefore did not limit their photosynthesis.

Morphological adaptation of *Selaginella* leaves to life in the shade

In the majority of *Selaginella* species, the thin microphyll comprises three cell layers: an upper epidermis composed of large conical cells, a spongy mesophyll containing sparse, irregularly shaped cells, and a lower epidermis consisting of flat cells (Fig. 1C). All three leaf tissues contain chloroplasts, but photosynthesis is almost completely assured by the upper epidermal cells (Sheue *et al.* 2007, 2015; Colpo *et al.* 2023). While all euphyllophytes and bryophytes, except hornworts, have many small, lens-shaped chloroplasts in their photosynthetic cells, highlighting the evolutionary success of polyplastidy to flexible chloroplast placement inside the cell (Pyke 2009), a much higher variability occurs in *Selaginella*. The comprehensive survey across 76 *Selaginella* species carried out by Liu *et al.* (2020) identified five categories of plastids in microphylls: monoplastidy in epidermal conical cells, monoplastidy in mesophyll conical cells, oligoplastidy, multiplastidy, and vestigial plastidy. The first is the most frequent and characterises the subgenus *Stachygynandrum*, which includes 80% of *Selaginella* species. The giant chloroplast lies at the bottom of the epidermal cell, occurring as cup-shaped, bilobed, or disk-shaped variants, and is considered a special adaptation to a shade environment (Sheue *et al.* 2007, 2015; Liu *et al.* 2020).

The upper epidermal cell in shade-adapted *Selaginella* appears as an outstanding system to enhance photosynthesis by shaping the light environment inside the cell (Fig. 1D). The upper tangential cell wall is convex and, together with the vacuole lying just below, has a lens effect to direct

the light to the giant chloroplast beneath it (Liu *et al.* 2020). In some species, the lens effect is enhanced by papillose or conical silica bodies on the tangential cell wall (Shih *et al.* 2022). The conical shape of the cell itself funnels light toward the chloroplast through a light-pipe effect (Liu *et al.* 2020). However, light can also reach the chloroplast by alternative paths. The oblique lower cell walls, surrounded by intercellular spaces, promote multiple light reflections inside the cell thanks to an inner refractive index of $n_{\text{cell}} \sim 1.4$, higher than that of the air $n_{\text{air}} = 1$ (Liu *et al.* 2020). Conceivably, a light ray will pass through the chloroplast multiple times, which increases the probability for photons to be absorbed by the antenna system embedded in the thylakoid membranes.

Overlooked during the first observations of giant chloroplast ultrastructure in *Selaginella* (Jagels 1970a), monoplastidy is frequently accompanied by a pronounced dimorphism of the thylakoid system between an upper, highly ordered lamellar zone and a lower, grana-rich region (Sheue *et al.* 2007, Ferroni *et al.* 2016, Ghaffar *et al.* 2018). Each upper lamella consists of 2–4 thylakoids closely stacked along their full length and running parallel to the cell vacuole outline; each lamella is separated by the neighbouring one by a stroma layer (Figs. 1E, 2A). The lower region is instead populated by grana stacks connected by stroma thylakoids (Fig. 1F). In *S. erythropus*, Chiou-Rong Sheue and colleagues introduced the term “bizonoplast” to refer to this plastid type, which is unique to Selaginellaceae (Sheue *et al.* 2007). The optical properties of the epidermal cell will cause light coming from above to cross at first the upper lamellar region and then filter to the granal region, but the latter will also receive light from other directions because of multiple reflections. The predominant deposition of starch granules at the interface between the upper lamellar region and the lower grana region may also have a role in establishing the light paths inside the chloroplast.

The upper lamellar thylakoid region of the *Selaginella* bizonoplast as a photonic structure

The ultrastructure of the bizonoplast is related to the singular optical properties of the thylakoid system. As shown in *S. erythropus*, the highly periodic thylakoid arrangement of the upper lamellar region gives rise to blue iridescence, *i.e.*, reflectance of light at around 450 nm (Masters *et al.* 2018) (Fig. 1B). The photonic multilayer structure of the upper lamellar region depends on a repeating period $\Lambda \sim 130\text{--}160$ nm, formed by the thylakoid lamella and the stroma layer ($\sim 60\text{--}90$ nm) separating each lamella from the subsequent one (Masters *et al.* 2018). The number of repeats varies depending on species in a range of 10–25 lamellae (Sheue *et al.* 2007, Ferroni *et al.* 2016, Liu *et al.* 2020). It seems counterintuitive that a deep-shade adapted plant may increase the reflectance of blue light, which corresponds to a major Chl absorption. However, ecological and physiological evidence is clear in associating photonic chloroplast architectures exclusively with shade-adapted plants native to the forest understorey. Besides *Selaginella*, they characterise particularly many

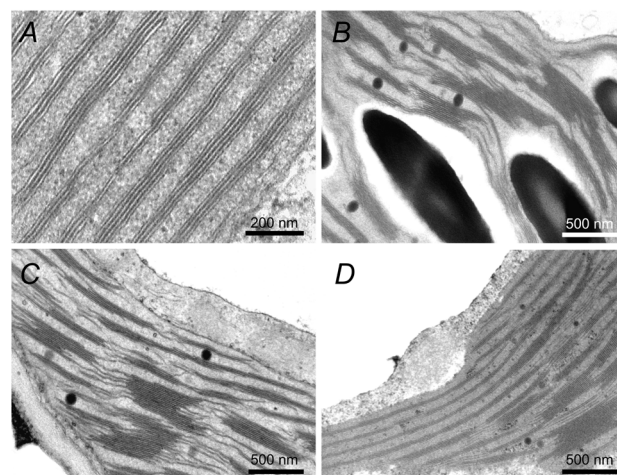


Fig. 2. The upper lamellar region of the thylakoid system in *Selaginella martensii*. (A) During the morning, in this chloroplast region, the thylakoids are appressed in groups of 2–4 in the lamella with a regular stroma spacing. (B) At the end of the night, the lamellar region is not present. (C) After the night, exposure of plants to white light [LED source, $100 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] for one hour leads to an initial induction of the lamellar region differentiation, visible as long appressed thylakoids near the chloroplast envelope facing the upper cell vacuole. (D) After the night, exposure to far red light [LED source at 730 nm, $20 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] for one hour causes a more distinct differentiation of the lamellar region.

Begoniaceae, where the lamelloplasts (also referred to as iridoplasts) hosted in the adaxial epidermal cells of leaves contain periodic structural arrangements of the thylakoids (Gould and Lee 1996, Jacobs *et al.* 2016, Pao *et al.* 2018). According to Jacobs *et al.* (2016), periodically organised thylakoid lamellae in iridoplasts of *Begonia* species enhance photosynthetic efficiency by optimising absorption of green light. This inference, which relates to the “slow light” effect in photonic thylakoid structures, was confirmed in *Begonia* and *S. erythropus* by a numerical study by Castillo *et al.* (2021), who estimated $\sim 40\%$ absorption enhancement factor in the green region peaking at 520–530 nm (Castillo *et al.* 2021). Based on Chl fluorescence imaging, Jacobs *et al.* (2016) reported that the F_v/F_m of *Begonia* iridoplasts was 10–15% higher than in mesophyll chloroplasts. However, their average measurements of F_v/F_m were in the range of 0.40–0.65, which is markedly lower than the optimal ~ 0.83 value expected upon application of a multiple-turnover saturation pulse to a dark-acclimated sample (Björkman and Demmig 1987, Stirbet and Govindjee 2012, Konyukhov *et al.* 2026). Suboptimal F_v/F_m values suggest difficulties in the reliable evaluation of PSII activity when using cross-sections or peels.

Relevance of the ordered lamellar thylakoid organisation to shade adaptation further emerges from developmental evidence. In the developing bizonoplast of *S. erythropus*, the upper lamellar region is formed under low light [$20\text{--}30 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ at midday], but not under high light [$\sim 500 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ at midday] (Sheue *et al.* 2015). Moreover, the bipartite

structure of the thylakoid system was preserved even after four days of darkness (Ghaffar *et al.* 2018). However, in contrast, in *S. martensii*, a well-structured lamellar region is preserved when the plant is cultivated under high light [$\sim 1,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$ at midday; Ferroni *et al.* 2016]. The repeating period was kept at $\Lambda \sim 122 \text{ nm}$, which is compatible with the requirements for iridescence (Masters *et al.* 2018), although in *S. martensii* the phenomenon is less evident than in other *Selaginella* species. Interestingly, in their comparative survey, Liu *et al.* (2020) could not observe the lamellar thylakoid region in *S. martensii*. In fact, it was subsequently found that in *S. martensii* the thylakoid arrangement in the bizonoplast changes according to a diurnal cycle, which is associated with the chloroplast relocation inside the cell. During the afternoon, the lamellar region mostly disappears, up to being completely absent at the end of the night (Colpo *et al.* 2023, 2024) (Fig. 2B). After the night, in *S. martensii*, the lamellar thylakoid arrangement can be induced by exposing the plants to the light, but especially to far red (Ferroni, unpublished) (Fig. 2C,D). Therefore, in *S. martensii*, the thylakoid structure of the mature bizonoplast remains flexible and responsive to environmental cues well beyond its initial development, undergoing reversible ultrastructural remodelling. The contrasting behaviour of *S. martensii* and *S. erythropus* suggests that the degree of structural plasticity of the bizonoplast can differ considerably depending on species. In *S. martensii*, the persistence of the upper thylakoid lamellae under high light suggests that this region can have a functional relevance not limited to enhancing the light harvesting in shaded environments. As it was suggested by Castillo *et al.* (2021), the stratified organisation of the bizonoplast could allow the control of light absorption to also suppress it when it is in excess and, thus, to avoid photodamage.

Distinctive characteristics of the granal architecture in *Selaginella*

The lower part of the bizonoplast (or in general the giant chloroplast) is filled with abundant grana generally not showing any preferential spatial orientation in the stroma (Jagels 1970a, Sheue *et al.* 2007, 2015; Ferroni *et al.* 2016, Ghaffar *et al.* 2018, Liu *et al.* 2020, Colpo *et al.* 2023, 2024) (Fig. 1F). Although not often emphasised in the literature, irregular orientation of grana is typical for shade-adapted plants in that it can enhance interception of light coming from different directions (Anderson *et al.* 1988). In *Selaginella*, this inference is consistent with the multiplicity of intracellular light paths allowed inside the upper epidermal cell (Liu *et al.* 2020). At a first glance, the granal organisation appears similar to that known in sciaphilous angiosperms, and so was indeed described (Jagels 1970a, Sheue *et al.* 2007, Ferroni *et al.* 2016). However, different to extremely sciaphilous angiosperms, where the stacks can be made by several tens of appressed thylakoids (e.g., Anderson *et al.* 1973, Chow *et al.* 1988, Demmig-Adams *et al.* 2015), giant grana with more than 25 thylakoids are infrequent in *Selaginella* chloroplasts

(Colpo *et al.* 2023). In *S. erythropus* and *S. martensii*, the number of thylakoids per granum is very variable but on average 15–20 (Sheue *et al.* 2007, Ghaffar *et al.* 2018, Colpo *et al.* 2023). Interestingly, the disks that form a granum are much larger in diameter – typically between 640 and 730 nm (Sheue *et al.* 2007, Ghaffar *et al.* 2018, Colpo *et al.* 2023) – than those found in angiosperm chloroplasts, which almost always have diameters below 500 nm (Höhner *et al.* 2020, Colpo *et al.* 2023). In *Selaginella*, adaptation of the granum size to the shade seems to have combined the enhancement of the vertical dimension of the stack (addition of layers), as occurs in angiosperms, with the widening of its horizontal dimension (disk diameter). This suggests that the 500-nm threshold for lateral development of grana is not universally applicable to all vascular plants and, in particular, cannot be generalised to lycophytes.

An additional feature of the grana organisation in *Selaginella* is the strong irregularity of the thylakoid stacks, quantified comparatively with euphyllphytes by Colpo *et al.* (2023). In angiosperms, electron tomography depicts grana as quasi-cylindrical entities built by precisely aligned thylakoid disks of the same diameter (Daum *et al.* 2010, Austin and Staehelin 2011, Bussi *et al.* 2019). Despite the widespread consensus that the grana are regular (Krysiak *et al.* 2026), some scientists argued that granum regularity would be, in fact, the exception, while a certain degree of irregularity is the structural norm (Mustárdy and Garab 2003, Mustárdy *et al.* 2008, Kowalewska *et al.* 2016). Accordingly, based on the most recent cryo-electron tomographic reconstructions of spinach grana, assisted by AI-based computational approaches, Wietrzynski *et al.* (2025) describe the stacks as “irregular cylinders with wavy edges”, in which “sometimes thylakoid layers change length and extend laterally beyond the stack, breaking the grana's vertical order”. Granum irregularity is a seldom considered morphometric trait, whose quantification has been proposed by Łucja Kowalewska's group as a source of functional information on the thylakoid system (Mazur *et al.* 2021, Bukat *et al.* 2025, Wójtowicz *et al.* 2025). Granum irregularity is evaluated as the deviation from the hypothetical cylindrical geometry of the stack and includes two components: the heterogeneity of disk diameters within a granum (Granum Lateral Irregularity) and the shifting of the disk on the lateral plane (Granum Cross-Sectional Irregularity) (Mazur *et al.* 2021). A high level of granum irregularity primarily means increasing the thylakoid fraction located at the interface between appressed and stroma-exposed membranes, *i.e.*, the grana margins, which are considered the most dynamic thylakoid domain because of potential mixing and interaction of PSI, PSII, and LHCII (Suorsa *et al.* 2015, Trotta *et al.* 2019, Rantala *et al.* 2020). Nonetheless, it is worth mentioning that the tomographic reconstructions by Wietrzynski *et al.* (2025) instead indicate a strict lateral heterogeneity as was formulated by Andersson and Anderson (1980), with a sharp segregation of PSI and PSII into non-appressed and appressed regions. In this simple two-domain model, there would be no space for a distinct grana margin domain in which PSI and PSII mix and interact. However,

the dynamism of the grana margins is deemed central to the photosynthetic acclimation in *Selaginella*. In grana margins, PSII–LHCI and PSII benefit mutually from free LHCII trimers, leading to the assembly of PSII–LHCII–PSI–LHCI megacomplexes or possibly higher-order clusters of these (Rantala *et al.* 2020). As will be discussed later, the proximity of the two photosystems is requisite for energy spillover to occur from PSII to PSI, and this process seems particularly intense in *S. martensii* and is related to photoprotection (Ferroni *et al.* 2018). Although this has not yet been demonstrated experimentally, it is highly probable that there is a link between the degree of granum irregularity and the extent of PSI–PSII interaction, because the former provides the ideal structural basis for the latter to occur. Consequently, just as the abundance of PSII–LHCII–PSI–LHCI megacomplexes is regulated, the irregularity of a granum could also be modulated in response to light conditions, as suggested by recent studies with angiosperms (Bukat *et al.* 2025, Falcioni *et al.* 2026).

The functional importance of granum irregularity is further supported by evidence that grana ensembles formed by misaligned stacks probably impact the nanophotonic geometric effects of the thylakoid system. Models of light–granum interaction by Capretti *et al.* (2019) show how the multi-layer granum structure gives rise to a balance between light absorption and reflection, facilitating a homogeneous distribution of light within the granum. A certain degree of “biological disorder” caused by the lateral misalignment of granum disks is still compatible with effective light absorption, but some hot spots of light intensity occur right at the disk edges of the irregular granum (Capretti *et al.* 2019), where LHCII balances excitation of PSI and PSII (Rantala *et al.* 2020). The fact that *Selaginella* tends to assemble the thylakoid disks quite “freely” in the granum most probably allows the plant to couple efficiently the light-harvesting advantage of organising large grana with the flexibility offered by increasing the thylakoid fraction located at grana margins (Krysiak *et al.* 2026). The functional implications associated with granum irregularity certainly warrant further investigation, and *Selaginella* could serve as a model for testing hypotheses.

Shade-adapted composition of the thylakoid membrane in *Selaginella*

In seven *Selaginella* species, after dimethylformamide extraction and spectrophotometry, Campany *et al.* (2019) report Chl content values corresponding to Chl *a/b* molar ratios within 1.5 and 2.2. Likewise, in HPLC determinations of acetonic extracts by Ghaffar *et al.* (2018), the amount of Chl *b* appears to be *ca.* half that of Chl *a* in *S. erythropus*. In methanolic or acetonic extracts of *S. martensii*, the spectrophotometric determination of Chl *a/b* ratio was reported to be ~2.0 or ~2.6, respectively (Ferroni *et al.* 2016, Colpo *et al.* 2023). Despite different species and methods, a low Chl *a/b* ratio undoubtedly indicates the shade character of *Selaginella* thylakoids (Lichtenthaler and Babani 2004). Although the molecular thylakoid organisation is known in some detail only for *S. martensii*,

knowledge can be conceivably valid also for other shade-adapted *Selaginella* species. Particularly, a high relative content of Chl *a/b*-containing LHCII compared to reaction centres is a well-known shade-adapted trait (Lichtenthaler and Babani 2004, Mathur *et al.* 2018). In *S. martensii*, the relative amount of LHCII is also almost invariant upon long-term acclimation to very contrasting light regimes (Ferroni *et al.* 2016, Colpo *et al.* 2022). In this respect, *Selaginella* is similar to bryophytes, in which the Chl *a/b* values are broadly dispersed around a median ratio of ~2.0 (liverworts) or ~2.3 (mosses), without clear trends related to the light regime (Marschall and Proctor 2004). Minute or inconsistent variations in the Chl *a/b* ratio are also confirmed in laboratory studies with mosses grown under different light conditions (Rincón 1993, Gerotto *et al.* 2011). Therefore, invariance of the LHCII bulk antenna appears to be an ancestral character shared by bryophytes and lycophytes.

In *S. martensii*, the main components of the photosynthetic electron transport chain are consistent with expectations for sciaphilous species (Lichtenthaler and Babani 2004, Anderson *et al.* 2012, Mathur *et al.* 2018). A high amount of LHCII obviously means relatively few photosystem reaction centres and, correspondingly, less cytb₆f, ATP synthase, and mobile electron carriers (plastoquinone, plastocyanin, and ferredoxin) than in sun-adapted plants (Mathur *et al.* 2018). In addition, the high protein crowding due to extensive LHCII in the appressed membranes hinders the mobility of plastoquinone (Ferroni *et al.* 2016), and the narrow thylakoid lumen limits the diffusion of plastocyanin (Colpo *et al.* 2023, 2024; Ferroni *et al.* 2026, see Höhner *et al.* 2020 particularly). Likewise, the very wide diameter of grana disks in *Selaginella* does not help plastocyanin mobility (Colpo *et al.* 2023). The same carrier mobility limitations may exist in the upper lamellar thylakoids of the bizonoplast, for which, however, no information is available on photosynthetic membrane composition. In *S. erythropus*, Ghaffar *et al.* (2018) suggested that the long appressed thylakoid lamellae could be interpreted as very extended grana with strongly prevailing PSI over PSII. This inference was based on imaging PAM of a microphyll section showing weaker fluorescence and lower F_v/F_m than in the grana region. Nonetheless, a specific enrichment in PSI would be contrary to the common understanding of photosynthesis adaptation to deep shade.

S. martensii contains only small amounts of PSI, as evident from electrophoresis of thylakoid proteins and very small I–P amplitude in fast Chl *a* fluorescence transient (Ferroni *et al.* 2016, Colpo *et al.* 2024). Quantitatively, electron paramagnetic resonance analysis indicates a PSI:PSII ratio of *ca.* 1:3 (Colpo *et al.* 2023). This is an expected consequence of the low red:far red ratio in the understorey. Far-red light excites preferentially PSI and, consequently, the long-term regulation of the thylakoid membrane rebalances the PSI/PSII stoichiometry in favour of PSII (Walters and Horton 1994, Walters 2005, Pantaleoni *et al.* 2009). Accommodation of the ~3.5 nm stroma protrusion of PSI in the stromal gap of the grana core is hard, if not impossible, and consequently PSI

locates in stroma-exposed thylakoid domains together with the ATP synthase (Daum *et al.* 2010, Koochak *et al.* 2019). Therefore, a consequence of low PSI/PSII ratio in association with high amounts of LHCII is a high degree of thylakoid appression, which is a very typical ultrastructural trait of sciaphilous plants (Lichtenthaler *et al.* 1981, Anderson *et al.* 1988). In *S. martensii*, the appression regards ~70% of the thylakoid membranes, which is a high fraction, though not as extreme as one would expect based on the relative amounts of PSI, PSII, and LHCII (Colpo *et al.* 2023). As will be discussed, however, the distribution of LHCII populations in different thylakoid domains explains this seeming inconsistency. Notably, if *S. martensii* is exposed to full sunlight, the almost equivalent amount of red and far-red light leads to the adjustment of the PSI/PSII ratio, with PSI relative amount increasing by 2–3 times (Ferroni *et al.* 2016).

Shade-adapted composition of the thylakoid membrane makes a plant potentially vulnerable to photodamage under high irradiance (Mathur *et al.* 2018). The risk will be inversely proportional to the plant's ability to exploit photoprotective mechanisms. Nonphotochemical quenching of Chl *a* fluorescence (NPQ) quantifies the harmless dissipation of excess absorbed energy as heat and is the first line of defence against high light (Demmig-Adams *et al.* 2012, Ruban 2016). In general, upon an abrupt and intense increase in irradiance, deep-shade species are not able to develop as much NPQ as sun-adapted species and will suffer from an excessive electron accumulation in the photosynthetic membrane (Demmig-Adams and Adams 2006, Shuang *et al.* 2022). There is a considerable variability among *Selaginella* species with respect to their capacity to induce NPQ. For example, *S. erythropus* induces $\text{NPQ} < 1$ under high light [$\sim 1,000 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$] and is particularly sensitive to even moderate light (Sheue *et al.* 2015, Ghaffar *et al.* 2018). Under similar conditions, $\text{NPQ} \sim 2$ is inducible in *S. repanda* (Sheue *et al.* 2015) and $\text{NPQ} \sim 3\text{--}4$ in *S. martensii* (Ferroni *et al.* 2014). Diversity in photoprotection capacity confirms early reports by Richard Jagels (Jagels 1970a,b) on the large variability of *Selaginella* species susceptibility to photobleaching when grown under moderate light [800 ft-c, roughly corresponding to $150 \mu\text{mol}(\text{photon}) \text{m}^{-2} \text{s}^{-1}$]. Actually, *S. martensii* can cope successfully with much higher irradiance (Ferroni *et al.* 2014, 2021a).

In *Selaginella*, the extensive LHCII lake works as flexible antenna system for both photosystems

As is easily predictable, in *S. martensii* the majority of LHCII resides in the grana core, where the Chl *a/b* ratio ~ 2.5 is completely similar to values reported for the model angiosperm *Arabidopsis thaliana* (Danielsson *et al.* 2004, Rantala *et al.* 2017, Koochak *et al.* 2019, Colpo *et al.* 2023). Some LHCII trimers are engaged in the organisation of PSII–LHCII supercomplexes, which are not very abundant in *S. martensii* compared to angiosperms (Ferroni *et al.* 2016, Colpo *et al.* 2022). Another part of LHCII, corresponding to “free trimers” or “extra trimers”,

forms the lake in which the PSII units are embedded and with which PSII can establish loose interactions (Wientjes *et al.* 2013). What makes the difference with respect to LHCII distribution in *S. martensii* thylakoids is the non-appressed domains. In angiosperms, only a small amount of LHCII is found in such regions, giving rise to Chl *a/b* ratio of ~ 4.5 or higher (Rantala *et al.* 2017, Koochak *et al.* 2019, Trotta *et al.* 2019). In *S. martensii*, the ratio is as low as 3.2, indicating that free LHCII is found in unusually high amounts in the stroma-exposed domains (Colpo *et al.* 2023). Based on large-pore blue-native electrophoresis of the digitonin-soluble thylakoid fraction, the stroma-exposed *S. martensii* membranes host an abundant and diverse population of PSII–LHCII–PSI–LHCI megacomplexes, along with free LHCII trimers (Ferroni *et al.* 2016) (Fig. 3).

In the classical “state transitions” model, a specific fraction of LHCII (mobile LHCII) shuttles between PSI and PSII based on the phosphorylation levels decided by the red:far red ratio (Allen 2003, Lemeille and Rochaix 2010). More recently, Eva-Mari Aro's group introduced a more comprehensive model, which depicts the grana margins as a lake of LHCII connecting PSI and PSII through labile, but effective interactions in a kind of supramolecular continuity, which is favourable to achieving a balanced excitation of photosystems under any light condition (Grieco *et al.* 2015, Rantala *et al.* 2020, Tikkanen and Aro 2026). LHCII phosphorylation would facilitate energy transfer to PSI specifically under low–moderate light intensity (Grieco *et al.* 2015). Moreover, the model emphasises the intertwined regulation through LHCII phosphorylation and LHCII-mediated NPQ, which would benefit not only PSII, but also the PSI population included in the quenched LHCII lake (Tikkanen and Aro 2026). The coordinated use of the two types of regulation, though still unclear in some respects (*e.g.*, the physiological significance of LHCII dephosphorylation in the dark), allows a plant to achieve an even excitation of PSI and PSII to support the photosynthetic electron flow (Mekala *et al.* 2015). The model is useful to describe how *S. martensii* manages light harvesting, excitation energy distribution between PSI and PSII, as well as photoprotection under high light. However, *S. martensii* exposed to increasing light intensity does not maintain an even excitation distribution between PSI and PSII as the angiosperms do, but, contrastingly, enhances PSI excitation (Ferroni *et al.* 2014, 2018, 2026). At the same time, already under relatively low irradiance, PSI is predominantly donor-side limited, that is, PSI is kept oxidised (P_{700}^{+} ; Ferroni *et al.* 2026).

Oxidised PSI acts as a thermal quencher of the excitation reaching the photosystem and, therefore, represents a safe state for the preservation of PSI integrity (Shimakawa and Miyake 2018a). It is conceivable that, under excess light, the LHCII lake embedding PSI and PSII does not simply work as a shared excitation quencher through the “high energy” q_E component of NPQ but also promotes an extensive direct use of oxidised PSI as a quencher by means of photoprotective energy transfer (spillover) from PSII to PSI. This latter would be reflected

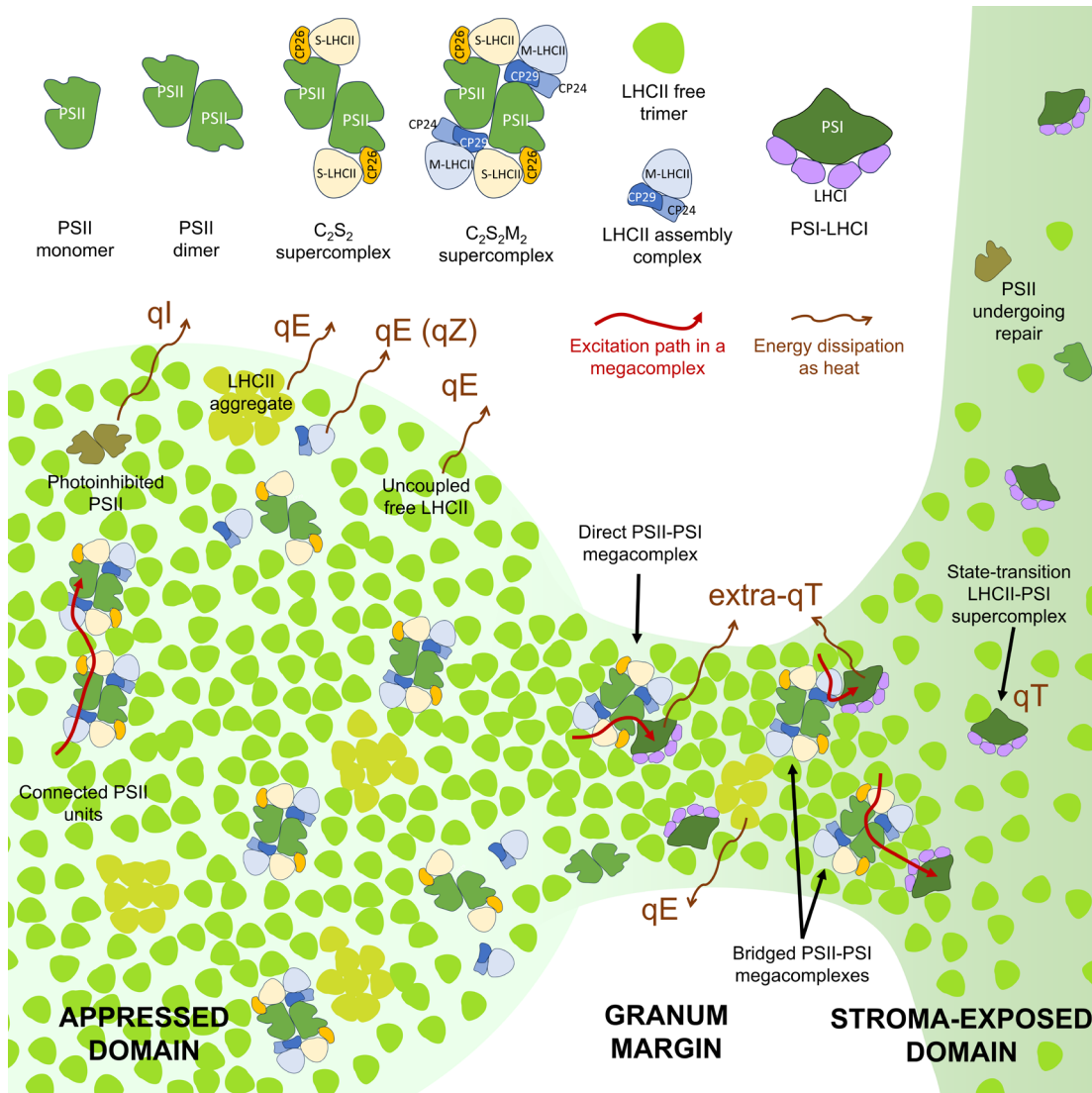


Fig. 3. Schematic model of PSI, PSII, and LHCII distribution in different domains of *Selaginella martensii* thylakoid membrane, as related to NPQ components. PSII is schematized in the form of core monomer, core dimer, C_2S_2 , and $C_2S_2M_2$ supercomplexes; additionally, LHCII free trimer, LHCII assembly complex corresponding to CP29/CP24/M-trimer subcomplex, and PSI-LHCI are shown. In the appressed domains, the lake formed by free LHCII trimers embeds the PSII supercomplexes. In the light-acclimated state, free LHCII trimers or their aggregates are the main sites for q_E . A population of PSII supercomplexes is shown while disassembling to undergo the repair cycle (see also PSII monomer in the stroma-exposed domain). The LHCII assembly complex released from the PSII supercomplex might be another q_E site and/or be involved in zeaxanthin-dependent de-excitation, q_Z . The photoinhibitory NPQ component q_I is due to inactive PSII cores. The presumably long distance between PSII units within the LHCII lake is probably the cause for low PSII connectivity in this species, which may further help the membrane photoprotection. The LHCII lake extends from the appressed domains to the grana margins and beyond, to the stroma thylakoid membrane. In the margin domain, not only PSI can be surrounded by LHCII and benefit from q_E , but LHCII is also included in PSII-LHCII-PSI-LHCI megacomplexes. In the direct type, energy spillover easily occurs from PSII core to PSI core without mediation by LHCII; in the bridged type(s), it is mediated by LHCII. Energy spillover is deemed responsible for the NPQ component called extra- q_T in *Selaginella*, which is different from the state-transition quenching q_T caused by the assembly of a PSI-LHCI-LHCII supercomplex.

in a component of NPQ termed “extra- q_T ”, different from the ordinary state-transition quenching q_T operating at low light (Ferroni *et al.* 2018). Accounting for ~10% of NPQ under high light, extra- q_T is quantitatively minor compared to q_E , but this estimate may not represent the actual importance of energy spillover in *S. martensii*. Studies by Ichiro Terashima's group highlight energy spillover

as important for photoprotection in shade-adapted plants, such as *Alocasia odora* (Araceae). Under fluctuations in light intensity and quality, as can occur in the understory, energy spilt over from PSII to PSI decreases the inflow of electrons from PSII into the chain and increases their outflow from PSI, contributing to the formation of P_{700}^+ . Therefore, upon exposure to light flecks, energy spillover

would ensure instantaneous relief against membrane overreduction in sciaphilous species, probably more relevant than NPQ, which is instead induced slowly and to generally low levels (Terashima *et al.* 2021, 2025; Kono *et al.* 2024). Accordingly, in *S. martensii*, changes in PSI/PSII emission ratio at 77K and Dual-PAM evaluations of energy distribution between PSI and PSII suggest that ~70% of excitation energy is driven to PSI at 200 $\mu\text{mol}(\text{photon})\text{ m}^{-2}\text{ s}^{-1}$ (Ferroni *et al.* 2018, 2026). Considering that, at such irradiance, more than 50% of PSI is donor-side limited (Ferroni *et al.* 2026), the use of PSI as a quencher could be important in *S. martensii*. However, different from the general case of shade-adapted plants, NPQ in *S. martensii* is readily and intensely inducible, even when the plant is acclimated for months to extreme shade (Ferroni *et al.* 2014, 2016, 2026; Colpo *et al.* 2022).

The PSII–LHCII–PSI–LHCI megacomplexes resolved in *S. martensii* are conceivably a suitable supramolecular environment to permit photoprotective energy spillover (Grieco *et al.* 2015, Suorsa *et al.* 2015, Yokono *et al.* 2015, 2019, 2024; Rantala *et al.* 2017, Kim *et al.* 2023) (Fig. 3). Accordingly, in *S. martensii*, the accumulation of PSII–LHCII–PSI–LHCI megacomplexes was emphasised when the plant was challenged by high light (Ferroni *et al.* 2016). Two main organisational models of PSII–LHCII–PSI–LHCI megacomplexes have been proposed in angiosperms, either core-mediated, in which PSI and PSII are located side by side and surrounded by LHCII, or the antenna-mediated, where LHCII is interposed between PSI and PSII (Ifuku 2023). In the core-mediated PSI–PSII megacomplex, also called “direct-type”, fast energy transfer occurs from PSII to PSI (~20 ps) and is enhanced under conditions of lumen acidification, supporting a main role in photoprotective energy spillover from PSII to PSI even when PSII is in the open state (Yokono *et al.* 2024). Conversely, the LHCII-mediated PSI–PSII megacomplex(es), also called “bridged type”, would be involved in energy sharing between the two photosystems, for example, fine-tuning LEF (and CEF) (Rantala *et al.* 2017). With both types of megacomplexes, the proximity of PSI and PSII within the LHCII lake characterises the grana margins and weakens the lateral heterogeneity of thylakoid composition, providing a compelling explanation for the high granum irregularity in *Selaginella* (Ferroni *et al.* 2016, Colpo *et al.* 2023). Furthermore, the fact that a very large proportion of LHCII is involved in PSI–PSII interactions can also explain why the degree of thylakoid appression is high in *Selaginella* but does not reach the extreme values of up to 85% reported in shade-tolerant angiosperms (Lichtenthaler *et al.* 1981, Anderson *et al.* 1988, Pancaldi *et al.* 1998). However, comparison of appression values can be biased by different morphometric methods; in this respect, recent determinations in the shade angiosperm *Alocasia odora* gave only ~60% appression ratio (Terashima *et al.* 2025).

Beside mediating the PSI–PSII interaction, the free LHCII has been proposed to “correct” the PSII antenna size thanks to PSII connectivity in grana cores (Haferkamp *et al.* 2010, Stirbet 2013, Zivcak *et al.* 2014a) and to be a main site for q_E (Horton *et al.* 2005, Holzwarth *et al.*

2009, Johnson *et al.* 2011, Nicol *et al.* 2019, Shukla *et al.* 2020) (Fig. 3). Long-term acclimation of *S. martensii* from deep-shade to high-light led to a 30% decrease in PSII functional antenna size, which was consistent with the decreasing relative amount of PSII–LHCII supercomplexes (Ferroni *et al.* 2016, 2026; Colpo *et al.* 2022). PSII connectivity remained almost invariable and low, suggesting that the distance between PSII centres determined by the LHCII lake is unfavourable to exciton migration (Ferroni *et al.* 2026). Interestingly, a low PSII connectivity could provide an additional means to avoid PSII photodamage, as proposed by Marek Živčák *et al.* (Zivcak *et al.* 2014a) for shade-acclimated barley.

A relevant role is certainly played by the free LHCII trimers, or their labile aggregates, in ensuring an effective photoprotection as q_E sites (Horton *et al.* 2005) (Fig. 3). In *S. martensii* exposed to intense light, q_E is responsible for ~85% of NPQ (Ferroni *et al.* 2018, 2021b). Interestingly, the rapid and intense induction of NPQ occurs in *S. martensii* plants grown under any light regime, which can be in line with invariable amounts of LHCII and PSBS protein (Ferroni *et al.* 2016). High NPQ in plants acclimated to various light regimes does not always lead to equally high PSII photoprotection (see Zuo 2025). This is particularly evident in *S. martensii*. Using the photochemical quenching in the dark (q_{pd}) method elaborated in Alexander Ruban's laboratory (Ruban and Murchie 2012, Ware *et al.* 2015), in *S. martensii*, the photoprotective effectiveness of NPQ against PSII photoinhibition increases consistently with the growth light intensity and is associated with a steady increment of the estimated fraction of PSII-uncoupled LHCII from plants growing under deep shade to high light (Colpo *et al.* 2022). At present, it is unknown whether the uncoupled LHCII conferring photoprotection in *S. martensii* corresponds to the q_E site(s) or is equivalent to the LHCII uncoupled from PSII to funnel excitation instead to PSI, or both. Likewise, it is difficult to make conclusions about the relative importance of NPQ or energy spillover in downregulating the electron inflow into the photosynthetic electron chain. In this respect, in *S. martensii* plants grown under extreme shade, a significant part of NPQ is due to its q_I component (Ferroni *et al.* 2016, Colpo *et al.* 2022), suggesting that the use of photoinhibited PSII as a quencher of excess absorbed energy can be important in *S. martensii*. Under stress conditions, such as chilling or fluctuating light, in angiosperms, the suppression of PSII activity diminishes the electron inflow into the electron transport chain and, consequently, can help protect PSI integrity (Takeuchi *et al.* 2025). Therefore, a sustained PSII photoinhibition was proposed to have a photoprotective function in deep-shade acclimated *S. martensii* because it is equivalent to a physiological increment in the PSI/PSII ratio (Colpo *et al.* 2022).

Involvement of LHCB6 phosphorylation in light energy management in *Selaginella*

A probable effector for the PSI–PSII interaction in *S. martensii* is the monomeric PSII antenna CP24.

Light-dependent phosphorylation of the major LHCII and PSII core in *S. martensii* confirmed previously described patterns in angiosperms and ferns, explained by the coordinated action of two kinases (Pursiheimo *et al.* 1998, Rintamäki *et al.* 2000, Bonardi *et al.* 2005, Ferroni *et al.* 2014). At low light, reduced plastoquinone activates both the LHCII kinase (STN7-like) and the PSII core kinase (STN8-like), leading to phosphorylation of trimeric LHCII, D2, and CP43 (not D1, whose phosphorylation is evolutionarily more recent; Pursiheimo *et al.* 1998). At saturating irradiance, STN7-like kinase, but not STN8-like, is inhibited by the overreduction of thioredoxins and, consequently, the trimeric LHCII is dephosphorylated while the PSII core is not (Rintamäki *et al.* 2000, Mekala *et al.* 2015). Unexpectedly, *S. martensii*, but also the lycopod *Lycopodium squarrosum* (syn. *Huperzia squarrosa*), phosphorylates also LHCB6, which forms CP24, following a light dependence like that of the PSII core – *i.e.*, without inhibition under high light (Ferroni *et al.* 2014, 2018). In the evolutionary history of plants, LHCB6 appeared, along with LHCB3, upon land colonization and indeed it is present in all land plants, except some gymnosperms (Kozioł *et al.* 2007, Kouřil *et al.* 2016, Cai *et al.* 2026). CP24 participates in the assembly of the LHCII–PSII supercomplexes $C_2S_2M_2$, formed by a PSII core dimer linked to two strongly bound S-LHCII trimers *via* CP26 and two moderately bound M-trimers *via* CP29; CP24 contacts the M trimer (Su *et al.* 2017) (Fig. 3). CP24 has been associated to the efficiency of electron transport and to the generation of q_E , possibly owing to the detachment of a CP29/CP24/M-trimer subcomplex under high light (Kovács *et al.* 2006, de Bianchi *et al.* 2008, Betterle *et al.* 2009, Passarini *et al.* 2009, Ferguson *et al.* 2025). In angiosperms, while LHCB4 (CP29) and LHCB5 (CP26) can undergo phosphorylation in response to some environmental stresses (Bergantino *et al.* 1995, Fristedt *et al.* 2012), LHCB6 does not participate in the regulation of the thylakoid membrane by dynamic phosphorylation (Longoni and Goldschmidt-Clermont 2021). In *S. martensii*, LHCB6 is distributed among the CP29/CP24/M-trimer and the high-molecular-mass complexes, but interestingly, it is also associated with PSI (Ferroni *et al.* 2014). Strong correlations between LHCB6 phosphorylation, PSI fluorescence emission and extra- q_T suggest that, once phosphorylated, LHCB6 could facilitate energy spillover from PSII to PSI within the PSII–LHCII–PSI–LHCI megacomplexes under conditions of over-reducing stroma (Ferroni *et al.* 2014, 2018, 2021b) (Fig. 3).

A few years after the discovery of phosphorylated LHCB6 in lycophytes, a similar pattern of dynamic LHCB6 phosphorylation was reported in *Physcomitrium patens* (syn. *Physcomitrella patens*) by Caterina Gerotto and coworkers. The molecular tools available for this model moss offered a mechanistic framework with respect to the involvement of kinases, particularly STN8 (Gerotto *et al.* 2019). However, STN7 also plays a role in defining the phosphorylation level of LHCB6 and another, still unknown kinase, has been hypothesized to explain the basal phosphorylation level in darkness (Gerotto *et al.* 2022).

However, in *P. patens* the role of phosphorylated LHCB6 remains elusive and, different to *S. martensii*, no direct link with the use of PSI as a quencher was found (Gerotto *et al.* 2022). Therefore, it seems that the appearance of LHCB6 in land plants has endowed them with an element of functional flexibility exploited in partially divergent ways by nonvascular plants (bryophytes), lycophytes, and euphyllophytes.

Beyond LHCII and excitation distribution: unknowns in efficient PSI donor- and acceptor-side regulation in *Selaginella*

Analysis of electron transport in *S. martensii* shows a perfectly tuned system in which high levels of PSI oxidation allow excellent photoprotection despite the shade-adapted thylakoid composition. As discussed in the previous sections, a fundamental role is certainly played by the antenna system. Dissipation of excess excitation by NPQ, including all of its components, before it reaches the PSII reaction centre, is one option. However, other important actors are involved in controlling the electron flow to avoid PSI overreduction. Different processes operate at the donor or acceptor side of PSI to prevent too many electrons from reaching PSI, and ensure that electrons are efficiently consumed downstream of it (Shimakawa and Miyake 2018a). In angiosperms, the primary donor side control of the electron flow is due to the activity of $cytb_6f$, which represents a bottleneck in the electron transport chain, being less abundant than PSII and PSI. The electron flow through $cytb_6f$ is downregulated by thylakoid lumen acidification in a process known as the “photosynthetic control” (Foyer *et al.* 1990). Lumen acidification, which depends on LEF and is strongly enhanced by CEF around PSI (Munekage *et al.* 2002), induces both NPQ and photosynthetic control. In this way, plants achieve a well-balanced LEF, testified by the quasi-linear relationship between plastoquinone reduction and PSI oxidation (Zivcak *et al.* 2014b, 2015; Brestic *et al.* 2015, Kono and Terashima 2016, Shimakawa and Miyake 2018b). However, in *S. martensii*, the fraction of oxidised PSI markedly exceeds that of reduced plastoquinone (Ferroni *et al.* 2026). The $cytb_6f$ bottleneck seems to be very tight in *S. martensii*. The $cytb_6f$ -to-photosystems stoichiometry is not known in *S. martensii*, but in blue-native/SDS 2D gels of thylakoid proteins, the spots corresponding to $cytb_6f$ subunits are minute (Ferroni *et al.* 2014, 2016). Using tobacco plants with variable levels of $cytb_6f$, Masaru Kono *et al.* showed that reduced levels of $cytb_6f$ markedly increase PSI stability under fluctuating light because of the hindered electron flow towards PSI, consequently promoting a safe PSI oxidation state (Kono *et al.* 2025). Therefore, in *S. martensii*, one could argue that NPQ, CEF, and hindered plastocyanin mobility work in addition to a low relative amount of $cytb_6f$ that is favourable *per se* to an effective PSI protection. Nevertheless, the extreme shortage of electrons at the donor side of PSI also depends on the removal of electrons from the chain. While the photosynthetic control at $cytb_6f$ is considered universal in oxygenic

photoautotrophs, the systems operating at the acceptor side of PSI have undergone evolutionary divergence in different major clades of plants (Shimakawa and Miyake 2018a). As already mentioned, in *S. martensii*, the carbon fixation is not a very strong electron sink, which consumes less than half the electrons derived from PSII photochemistry. Photorespiration removes less than 20% of the electrons (Ferroni *et al.* 2021a; for an evolutionary perspective on photorespiration as an electron sink, see Hanawa *et al.* 2017). The remaining 30–40% is diverted to electron sinks alternative to photosynthesis and photorespiration, which eventually re-reduce O₂ to H₂O (Ferroni *et al.* 2021a).

Photosynthetic electrons can be recycled from PSI to O₂ via the Mehler-peroxidase pathway (Asada 2000) or the coordinated action by NAD(P)H-dehydrogenase-like complex (NDH) and Plastid Terminal Oxidase (PTOX) (Rumeau *et al.* 2007, Messant *et al.* 2024). NDH works at the crossroad between PTOX and electron recycling in CEF, in alternative to and not completely overlapping with the PGR5/PGRL1 pathway (Shimakawa and Miyake 2018a). Almost nothing is known about the molecular details of these pathways in *S. martensii*, except an extreme upregulation of NDH in high-light-grown plants, which finds a physiological counterpart in enhanced CEF (Ferroni *et al.* 2016, 2026). A third way, which was lost in angiosperms, recycles electrons by means of flavodiiron proteins (FLVs), which provide plants with a very effective safety valve to cope especially with light fluctuations (Allahverdiyeva *et al.* 2015, Ilík *et al.* 2017). Knowledge about FLVs' structure, function, and relevance has been rapidly growing in bryophytes using the liverwort *Marchantia polymorpha* as a model (Shimakawa *et al.* 2017, 2021) but especially *Physcomitrium patens*, for which many photosynthetic mutants are available (e.g., Gerotto *et al.* 2016, Traverso *et al.* 2025). The main idea conveyed by research is that, in non-angiosperms, FLVs and CEF work in coordination: the former allows a plant to respond effectively to abrupt changes in irradiance, the latter is more important under steady-state acclimation to high light (Storti *et al.* 2019, Tan *et al.* 2025). Although in *Selaginella* and lycophytes in general, nothing is known about FLVs except their existence, it is conceivable that FLVs could be a major alternative electron sink, perhaps providing plants with a flexibility like that of bryophytes in coordination with CEF (Gerotto *et al.* 2016). Mehler-peroxidase, NDH-PTOX, and FLVs pathways are collectively called “pseudocyclic electron flows” to emphasise that they sustain ATP synthesis without generating NADPH, as CEF also does. The relative importance of CEF and pseudocyclic routes, as well as the reasons why the angiosperm ancestor lost FLVs, is a debated matter that is outside the scope of this review (see Alboresi *et al.* 2019).

Concluding remarks

The journey across shade-adapted *Selaginella* photosynthesis started with the question of whether these vascular plants, belonging to the diminutive group of lycophytes, could offer a window into the photosynthesis

of ancient vascular plants. Different elements of their photosynthetic apparatus can recall ancestral conditions: low carbon fixation capacity accompanied by constrained CO₂ diffusion from air to chloroplast, a large and almost non-resizable LHCII amount, and maybe also their relatively loose granal organisation, which is somewhat like that sometimes reported in bryophytes. Nonetheless, *Selaginella* also expresses unique adaptations to thrive in deep shade, not only emphasising light-harvesting capacity, but also enabling the plant to cope effectively with light fluctuations or, at least in some species, with a high-light regime. Highly specialised traits illustrate how, in ancient vascular plants having survived up to today, photosynthesis has followed an evolutionary trajectory distinct and parallel to those better characterised in euphyllophytes, mostly angiosperms, or in non-vascular land plants, mostly mosses. Nevertheless, a deeper understanding of the photosynthetic apparatus in *Selaginella* may also help clarify the significance of processes, structures or proteins that in angiosperms are still somewhat elusive (e.g., energy spillover, PSII connectivity), understudied (e.g., granum irregularity, photonic effects), used differently (e.g., LHCB6), or have even been completely lost (e.g., FLVs).

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