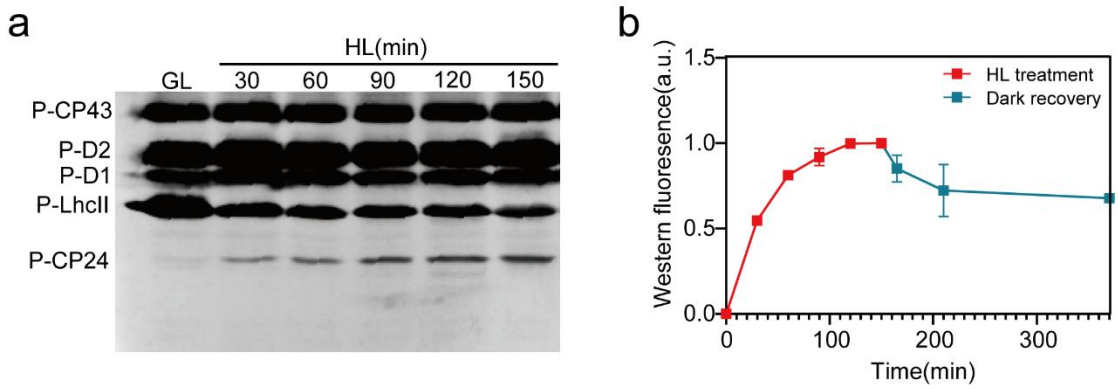


Figure S1: Phosphorylation kinetics of phosphorylated CP24

a Immunoblot of isolated thylakoid assayed with anti-P-Thr antibody. Before thylakoid isolation, leaves have been treated with HL (1,000 µmol photons m^{-2} s^{-1}) for different periods. All lanes were loaded with 3 µg Chl. The immunoblot depicts phosphorylation levels of key proteins including CP43, D2, D1, LhcII, and CP24 at different HL exposure times. **b** Densitometric analysis of immunoblot in (**a**). Symbols and error bars represent the mean ± SD calculated from three independent biological replicates. Western fluorescence intensity is normalized relative to the initial value at time zero.

Figure S2: Multiple sequence alignment of STN7 from *B. incana* and *A. thaliana*

Protein sequences were analyzed with the ESPript 3.0 online software. Chloroplast transport peptides determined using ChloroP1.1 online software are marked with black lines below the amino acid sequence.

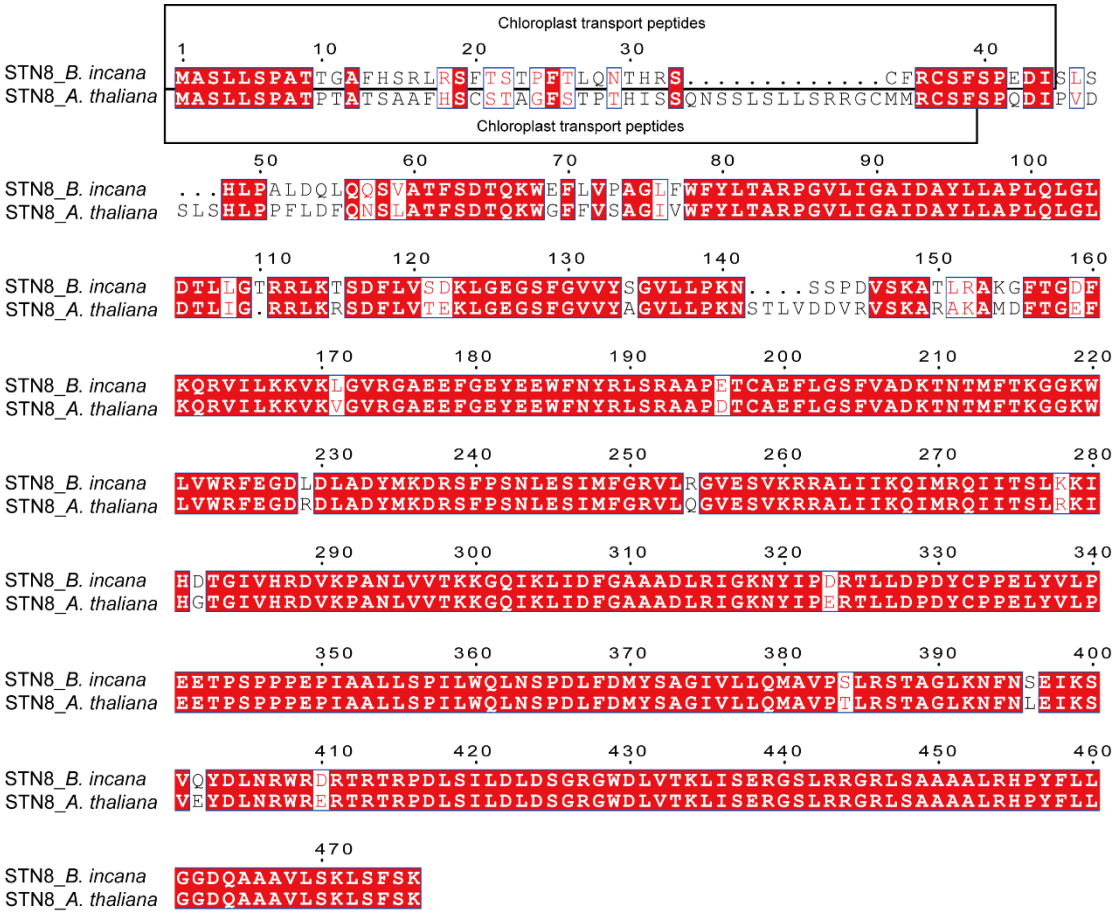


Figure S3: Multiple sequence alignment of STN8 from *B. incana* and *A. thaliana*

Protein sequences were analyzed with the ESPript 3.0 online software. Chloroplast transport peptides determined using ChloroP1.1 online software are marked with black lines below the amino acid sequence.

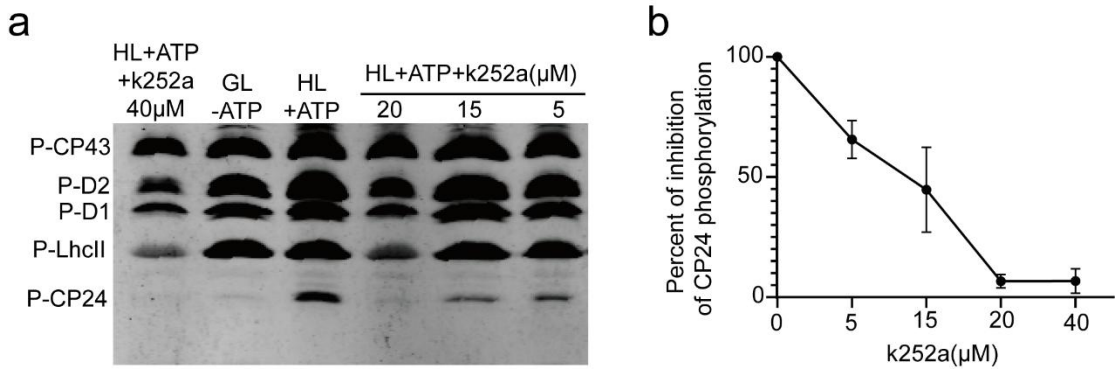
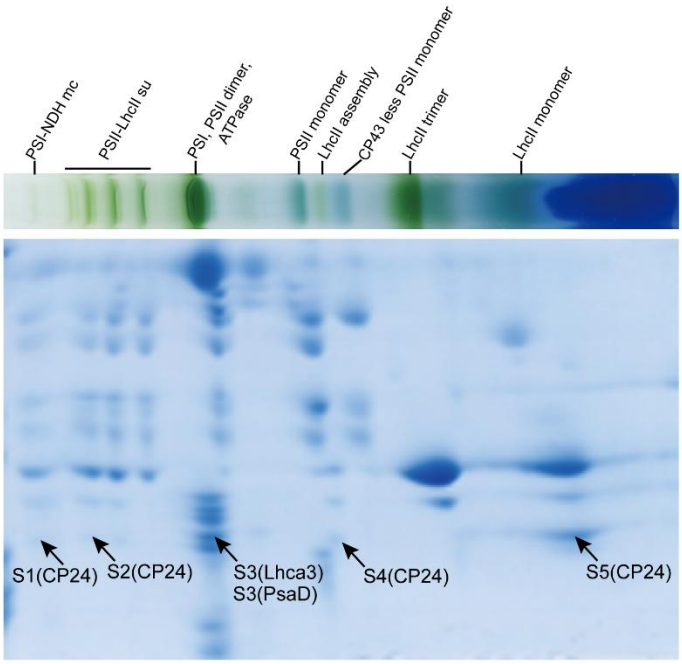


Figure S4: Inhibition of CP24 phosphorylation with varying concentrations of k252a

a Immunoblot analysis of chloroplasts exposed to growth light (GL, 100 μmol photons m^{-2} s^{-1}) in the absence of ATP, and chloroplasts exposed to high light (HL, 1,000 μmol photons m^{-2} s^{-1}) for 30 minutes in the presence of ATP, or in the presence of ATP and varying concentrations of k252a. The anti-P-Thr antibody was used to detect phosphorylated proteins. All lanes were loaded with 3 μg Chl. **b** Extent of CP24 phosphorylation inhibition by k252a. Symbols and error bars represent the mean ± SD calculated from two independent biological replicates.

a



b

Spot no.	Precursor ion mass(m/z)	Unique sequence	Protein identity	GN number reference organism	Score/E-value
S1	1917.7431	TAENFANYTGDQGYPGGR	CP24	BnaC08g38660D (Brassica napus)	149/2.6e-013
S2	1064.6318 1917.9461	FFDPLGLAGK TAENFANYTGDQGYPGGR	CP24	BnaC05g12070D (Brassica napus)	70/7e-005 161/4.8e-014
S3	1650.6882 1810.7488 1938.8240 2936.3508	EQIFEMPTGGAAIMR AQVEEFYVITWNSPK KAQVEEFYVITWNSPK EAPVGFTPPQLDPNTPSPIFAGSTGGLLR	PsaD	GA_TR2488_c0_g1_i1 _g.8116 (Noccaea caerulescens)	118/7.4e-010 90/4.2e-007 138/7.6e-012 61/0.00027
S3	2225.9983 2635.0990	WADIIKPGSVNTDPIFPNNK LTGTDVGYPGGLWFDPLGWGSGSPAK	Lhca3	BnaC04g21740D (Brassica napus)	112/2.6e-009 157/6e-014
S4	1917.8054	TAENFANYTGDQGYPGGR	CP24	BnaC08g38660D (Brassica napus)	161/3.1e-014
S5	1064.5744 1917.8470	FFDPLGLAGK TAENFANYTGDQGYPGGR	CP24	BnaC05g12070D (Brassica napus)	65/0.00031 173/2.8e-015

Figure S5: Analysis of proteins corresponding to the position of phosphorylated CP24 in *B. incana* thylakoids by MALDI-TOF-TOF MS/MS following lp-BN/SDS-PAGE

a The thylakoid membrane extracted from GL leaves (100 µmol photons m^{-2} s^{-1}) were treated with β-DM and then complexes were separated by lp-BN/SDS-PAGE. **b** The proteins corresponding to the position of molecular weight of P-CP24 (indicated by the arrow in **a**) in coomassie blue stain gel were identified by MALDI-TOF-TOF.

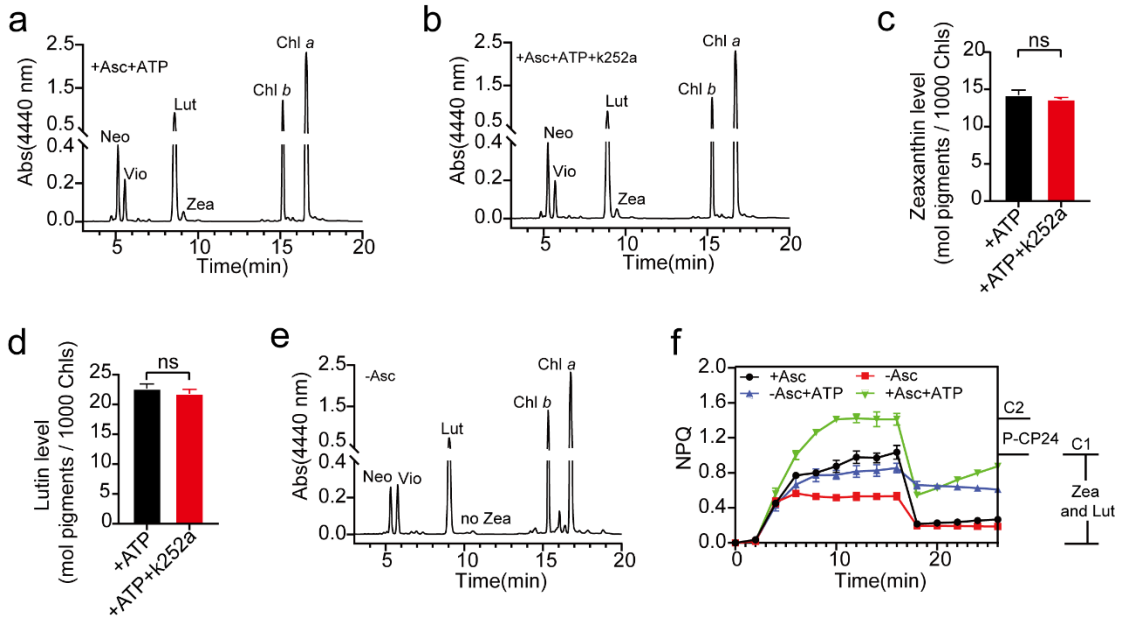


Figure S6: HPLC analysis of total pigments extracted from chloroplasts

a Pigments were extracted from functional chloroplasts exposed to HL (1,000 µmol photons m^{-2} s^{-1}) for 30 min in the absence of k252a, followed by quantification via high-performance liquid chromatography (HPLC). **b** Pigment were extracted from functional chloroplasts treated with HL (1,000 µmol photons m^{-2} s^{-1}) for 30 min in the presence of k252a, followed by quantification via HPLC. **c** Zeaxanthin levels were quantified by HPLC, as shown in (**a**) and (**b**). **d** Lutein levels were quantified by HPLC, as shown in (**a**) and (**b**). The production levels of zeaxanthin and lutein were compared under conditions with or without the inhibitor k252a using unpaired two-tailed t-tests. Statistical significance was defined as ns ($p > 0.05$), not significant. **e** Pigment were extracted from functional chloroplasts treated with HL of 1,000 µmol photons m^{-2} s^{-1} for 30 min in the absence of Asc, followed by quantification via HPLC. **f** The contribution decomposition of NPQ induced by actinic light of 1000 µmol photons m^{-2} s^{-1}. NPQ of the functional chloroplast in the presence of Asc and absence of ATP (+ Asc). NPQ of the functional chloroplast in the presence of Asc and ATP (+ Asc + ATP). NPQ of the functional chloroplast in the absence of Asc and ATP (- Asc). NPQ of the functional chloroplast in the presence of ATP and absence of Asc (- Asc + ATP). The pigment data are normalized to 1000 Chl *a* + *b* molecules. Symbols and error bars represent the mean ± SD calculated from three independent biological replicates.

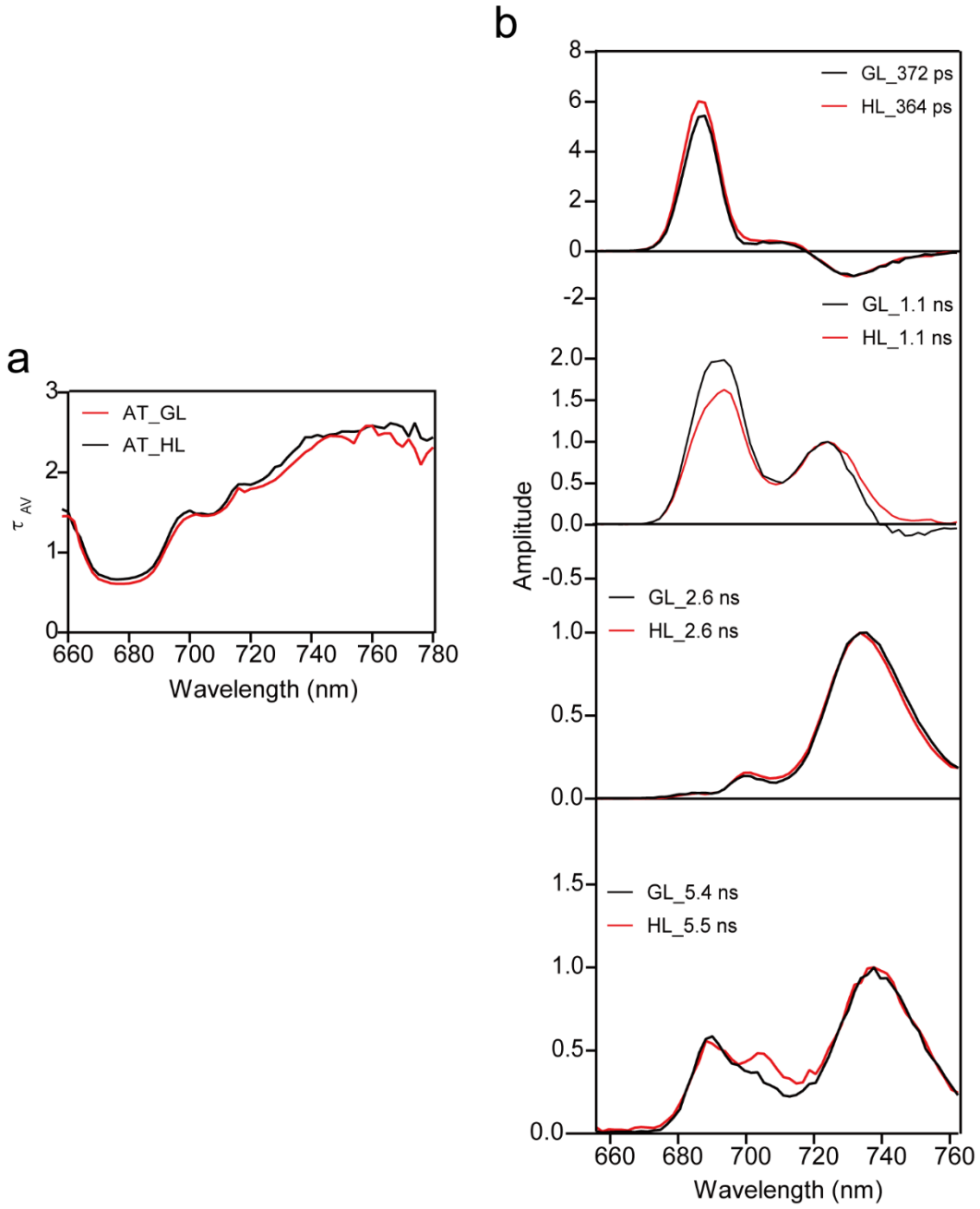


Figure S7: HL does not promote energy dissipation at around 695 nm in *A. thaliana*

a Average fluorescence lifetime (τAV) were measured in thylakoid membranes isolated from *A. thaliana* under GL (50 µmol photons m^{-2} s^{-1}) and HL (1000 µmol photons m^{-2} s^{-1}) conditions. **b** Time-resolved fluorescence decay-associated spectra (DAS) analysis was performed on thylakoid membranes isolated from *A. thaliana* grown under GL (50 µmol photons m^{-2} s^{-1}) and HL (1000 µmol photons m^{-2} s^{-1}) conditions. DAS components were normalized to the maximum fluorescence intensity of the corresponding PSI-associated lifetime components. Samples were excited at 480 nm with a chlorophyll concentration of 2 µg mL^{-1}.

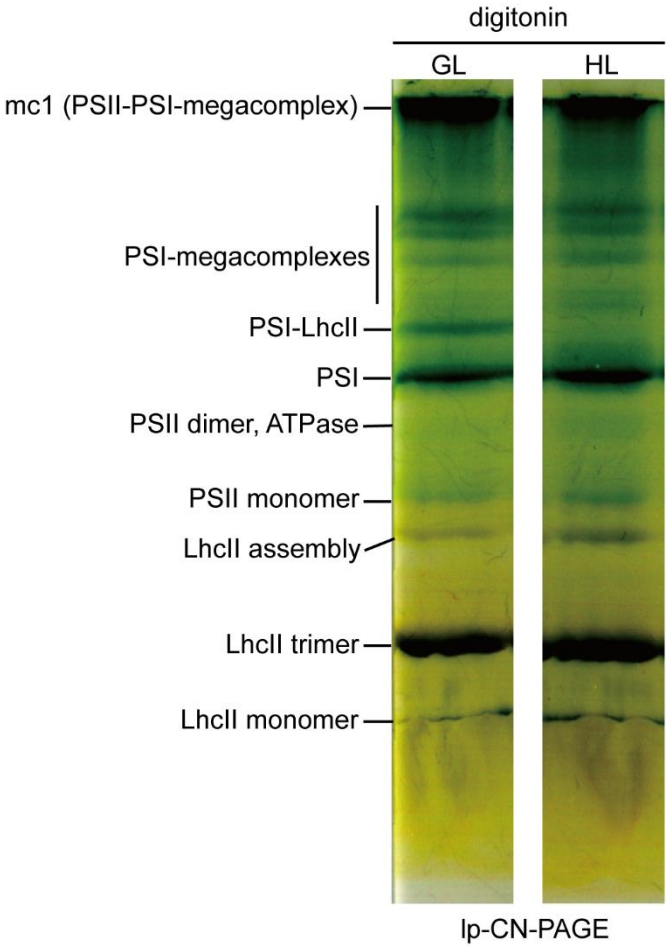


Figure S8: LpCN-PAGE profiles of thylakoids from *B. incana*

Thylakoids were isolated from *B. incana* leaves exposed to either GL (50 µmol photons m^{-2} s^{-1}) or HL (1000 µmol photons m^{-2} s^{-1}) for 2 hours. Isolated thylakoids were solubilized with 1% (v/v) digitonin (final concentration) and subjected to lpCN-PAGE. For each lane, 10 µg of Chl was loaded.

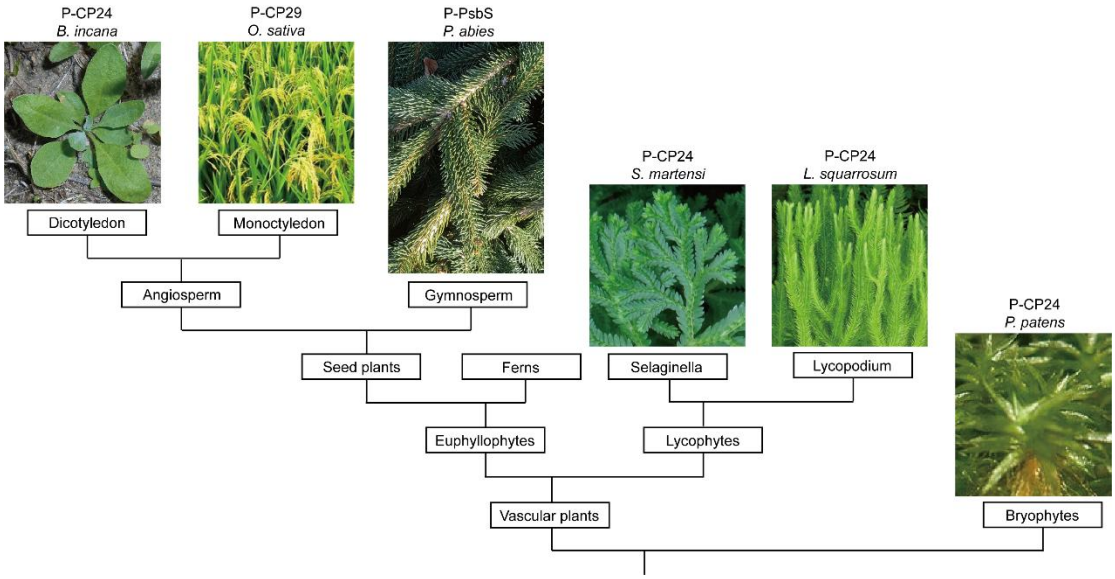


Figure S9: Evolutionary reduction of terrestrial plant phylogeny reveals lineage-specific phosphoprotein signatures.

The latter group comprises non-vascular plants, specifically bryophytes (including mosses and liverworts), and vascular plants. Among mosses, *P. patens* exhibits extensive phosphorylation of CP24, though its functional significance remains unclear. Within vascular plants, the earliest phylogenetic divergence separates lycophytes from euphyllophytes. The lycophyte lineage includes two major clades: *Selaginella* (spikemosses) and *Lycopodium* (club mosses), which exhibit substantial CP24 phosphorylation. Euphyllophytes consist of ferns and their allies, along with all seed plants. Seed plants are further classified into angiosperms and gymnosperms. Angiosperms are divided into dicotyledons and monocotyledons. The dicotyledonous plant *B. incana* studied in this work represents the first seed plant reported to exhibit substantial CP24 phosphorylation. In contrast, substantial CP29 phosphorylation has been documented in the monocotyledon *O. sativa*. In gymnosperms, substantial phosphorylation of PsbS has been detected in the Norwegian spruce (*P. abies*).