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Chloroplast biogenesis — Correlation between structure and function ☆

Łucja Rudowska [a], Katarzyna Gieczewska [a,*], Radosław Mazur [b], Maciej Garstka [b], Agnieszka Mostowska [a]

[a] Department of Plant Anatomy and Cytology, Institute of Experimental Plant Biology and Biotechnology, Faculty of Biology, University of Warsaw, Miecznikowa 1, PL-02-096 Warsaw, Poland
[b] Department of Metabolic Regulation, Institute of Biochemistry, Faculty of Biology, University of Warsaw, Miecznikowa 1, PL-02-096 Warsaw, Poland

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ABSTRACT

Chloroplast biogenesis is a multistage process leading to fully differentiated and functionally mature plastids. Complex analysis of chloroplast biogenesis was performed on the structural and functional level of its organization during the photoperiodic plant growth after initial growth of seedlings in the darkness. We correlated, at the same time intervals, the structure of etioplasts transforming into mature chloroplasts with the changes in the photosynthetic protein levels (selected core and antenna proteins of PSI and PSII) and with the function of the photosynthetic apparatus in two plant species: bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L). We selected these plant species since we demonstrated previously that the mature chloroplasts differ in the thylakoid organization. We showed that the protein biosynthesis as well as photosynthetic complexes formation proceeds gradually in both plants in spite of periods of darkness. We found that both steady structural differentiation of the bean chloroplast and reformation of prolamellar bodies in pea were accompanied by a gradual increase of the photochemical activity in both species. This article is part of a Special Issue entitled: Photosynthesis Research for Sustainability: from Natural to Artificial.

1. Introduction

Chloroplast biogenesis is a multistage process leading to fully differentiated and functionally mature plastids. Chloroplasts develop from proplastids, small organelles in leaf primordia and possess small vesicles containing no photosynthetic complexes. On light, during the natural photomorphogenesis, proplastids develop the thylakoid network and the photosynthetic capacity.

During the natural scotomorphogenesis the initial seedling growth occurs beneath the soil surface, during germination in the dark, proplastids differentiate into etioplasts with paracrystalline prolamellar bodies (PLBs) – and prothylakoids (PT) – perforated lamellae which emerge from PLB. Etiolation is a natural process during initial plant ontogenesis which might happen during the normal plant development [1]. It is known that PLB contains protochlorophyllide (Pchlide), a precursor of chlorophyll bound to protochlorophyllide oxidoreductase (POR), NADPH, lipids, usually two carotenoids, lutein and violaxanthin and a few proteins [2 and literature therein]. The function of these two different membrane systems (PLB and PT in darkness and their role on light) is not fully understood [3]. According to Solymosi et al. [1,4,5] PLBs with maximum packing of membranes should play a crucial role in the chloroplast differentiation. In PLBs, POR accumulates in a highly stable complex with Pchlide and NADPH. This complex is responsible for a regular, paracrystalline structure [6] which protects the chlorophyll synthase against proteolysis [3,7].

Upon illumination young etiolated seedlings "switch" to photomorphogenesis and etioplasts differentiate into chloroplasts, i.e. paracrystalline tubular membrane structures of PLBs and flat porous membranes of PTs transform into the grana and stroma thylakoids [8 and literature therein]. Structural transformation is accompanied with Pchlide conversion to Chl and in addition the synthesis of proteins involved in photosynthesis, especially thylakoid proteins, gradually increases upon illumination [9–11].

The development from the precursors to mature photosynthetically active chloroplasts requires very coordinated processes in different cell compartments. During differentiation of chloroplast the following events take place: light perception and subsequent expression of nuclear and plastid genes, biosynthesis of lipids and pigments, import of appropriate pigment-binding proteins such as chlorophyll *a*/*b*-binding proteins into developing chloroplasts, insertion of these proteins into the thylakoid membranes, and protein assembly into functional complexes [12,13]. The thylakoid membranes are arranged into a 3D network

Abbreviations: CP, chlorophyll-protein; Fo, initial fluorescence; Fm, maximal fluorescence intensity; Fm′, maximal fluorescence intensity during treatment; Fv, variable fluorescence; LHCI/LHCII, light harvesting complex associated with PSI and PSII, respectively; P700, photosystem I reaction center; Pchlide, protochlorophyllide; PLB, prolamellar body; Pm, maximal P700 absorbance change; POR, protochlorophyllide oxidoreductase; qN, coefficient of non-photochemical quenching; PSI/PSII, photosystem I and II, respectively; PT, prothylakoids; TEM, transmission electron microscopy; Y(I)/Y(II), quantum yield of PSI and PSII, respectively

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* Corresponding author. Tel.: +48 22 5542109; fax: +48 22 5542022.
E-mail address: kat.gieczewska@biol.uw.edu.pl (K. Gieczewska).

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with cylindrical grana linked to each other by the stroma lamellae [8 and literature therein]. The chloroplast structure is also determined by the arrangement of the photosynthetic complexes and their protein composition within the thylakoid membranes [14]. The structure and arrangement of appressed and non-appressed thylakoids within chloroplasts depend on different features e.g. the lateral separation of LHCII and PSII from PSI and the ATP synthase. They are also characteristic for the plant species although they can change under different environmental conditions [14–17].

In spite of the fact that the crucial stages of chloroplast differentiation were already described in several plant species, all data till the present were scattered: in some papers the ultrastructural differentiation of chloroplasts during the greening was described e.g. [18–20] while in other papers the proteomic analysis and the functional analysis were done [3,6,10,11]. In this paper we correlated at the same time intervals in given developmental stages: the selected protein appearance, emergence of photochemical activity and the ultrastructure of developing chloroplasts.

Still the correlation between the structural and proteomic changes in time and the role of particular proteins in stabilization and transformation of PLBs and PT into the characteristic arrangements of thylakoid membranes remains unknown. All events that occur during the etioplast membrane transformation into the mature thylakoid chloroplast membranes require detailed structural, proteomic and biochemical analysis.

The aim of this paper was to perform complex analysis of chloroplast biogenesis on the structural and functional level of its organization during the photoperiodic plant growth. For the first time parallely with ultrastructural analysis of developing chloroplasts protein appearance and emerging of photochemical activity were performed at the same time intervals. We correlate, at the same time intervals, the structure of etioplasts transforming into mature chloroplasts with changes in selected photosynthetic protein levels and with the function of photosynthetic apparatus in two plant species: bean (*Phaseolus vulgaris* L.) and pea (*Pisum sativum* L). We show which stage of the structural differentiation and appearance of which proteins are related to full functionality of thylakoid membranes of these two species. By full functionality of thylakoid membranes we mean a developmental stage when the ratio Fv/Fm and a whole characteristic of both photosystem activities are comparable with those in fully developed plants. We selected these plant species since we demonstrated previously that the mature chloroplasts differ in the thylakoid membrane stacking and organization. We showed on 3D computer models (constructed from stacks of optical slices from a confocal laser scanning microscope of the whole pea and bean chloroplasts) a different arrangement of the appressed and non-appressed membranes. Pea chloroplasts contain large, separated from each other appressed domains while in bean chloroplasts less distinguished appressed regions are present [14,16,17]. By using various microscopic and spectroscopic methods we recently showed that the stacking process is a consequence of the supercomplexes arrangement in pea and bean. The size differences between PSII and PSI complexes and also the quantity and/or qualitative composition of microdomains are important factors in stacking process [14]. Since the mature chloroplasts of bean and pea differ in their arrangement and stacking, we expect that the biogenesis of chloroplasts of these species should also differ [14,16,17].

2. Materials and methods

2.1. Plant material and growth conditions

Pea and bean plants (*P. sativum* L. cv. Demon, *P. vulgaris* L. cv. Eureka from PlantiCo Zielonki, Babice Stare, Poland) were grown in 3 L perlite-containing pots in darkness for 8 days at 18 °C in a climate room with controlled temperature and humidity. After 8 days the growing conditions were changed into a controlled environment at 21/18 °C (day/night) at the photosynthetic active radiation of 40 μmol photons m^{-2} s^{-1} during a 16-hour photoperiod and relative humidity of 60–70%. Plants were fertilized with full Knop's nutrient solution. Samples were collected after 8 days of etiolation and at selected times during 3 days of the experiment as shown in Fig. 1. All manipulations were carried out in the darkness with dim green light, considered as safe and photomorphogenetically inactive [18,21].

2.2. Transmission electron microscopy (TEM)

Samples for TEM were prepared by cutting pieces of about 3 mm^2 area from the middle part of the leaves. The material was fixed in 2.5% (w/v) glutaraldehyde in 5 mM cacodylate buffer (pH 7.4) for 2 h, washed in the buffer and placed in a 2% (w/v) OsO_4 at 4 °C in 50 mM cacodylate buffer (pH 7.4) for about 12 h. The specimens, dehydrated in a graded acetone series, were embedded in a low viscosity epoxy resin and cut on a Leica UCT ultramicrotome. Sections stained with uranyl acetate were examined with a JEM 1400 electron microscope (Jeol, Japan). We chose 8 plastid cross sections from crucial developmental stages. The length of granal thylakoids as well as the vertical distance from one pair of the stacked thylakoids to the next pair was measured in quadrilateral area of 1 μm^2 using Digital Micrographs software (Gatan Inc, USA).

2.3. Protein extraction and Western-blot analysis

Leaf samples were frozen in liquid nitrogen, grind to a powder and transferred to 50 mM Tris–HCl (pH 8.0) extraction buffer containing 10% (v/v) glycerol, 2% (w/v) SDS, 25 mM EDTA, 1 mM PMSF. After freezing and subsequent sonication the samples were centrifuged at 10,000 *g* to remove unbroken cells. Protein content was estimated according to the Lowry method (BioRad, USA).

Extract samples were calibrated to equal amount of protein and loaded into gel wells. After separation by the standard SDS-PAGE electrophoresis specific polypeptides were detected on the PVDF membrane by using antisera containing polyclonal antibodies (Agrisera, Sweden) against selected photosynthetic proteins, followed by anti-rabbit horseradish peroxidase conjugate (BioRad, USA) and ECL Detection System (Perkin Elmer, USA).

2.4. Low temperature (77 K) fluorescence measurements

Low temperature (77 K) fluorescence emission spectra were recorded using modified Shimadzu RF-5301PC spectrofluorimeter where excitation and emission beams were led by optical fibers. Leaf samples (20 mm^2) were placed in a metal cuvette and submerged in liquid nitrogen. Excitation wavelength was set at 420 nm,

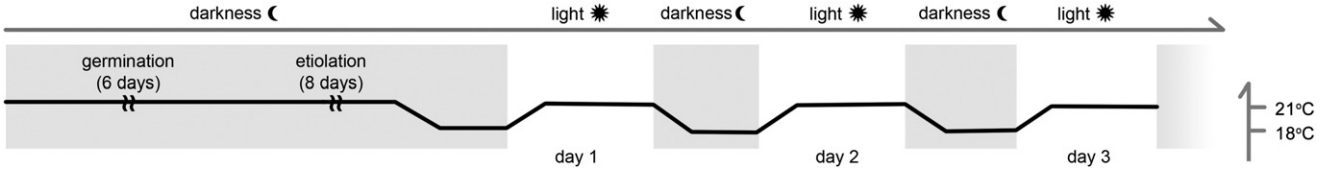

Fig. 1. Scheme of the experiment.

excitation and emission slits at 5 nm and scans were taken in the range of 600 to 800 nm. Each spectrum was recorded twice, averaged, background corrected and the obtained curve was shifted to 0 at points 640 and 780 nm.

2.5. Modulated chlorophyll a fluorescence measurements

All measurements were carried out using a pulse-amplitude modulation fluorometer — a Dual-PAM-100 instrument (Heinz Walz GmbH, Effeltrich, Germany). Experiments were carried out by using the automated Induction and Light Curve routine provided by the DualPam software, with repetitive application of saturation pulses for assessment of fluorescence and P700 parameters, from which the quantum yields of PSI (Y(I)) and PSII (Y(II)) were derived by the software (based on [22]). After Fo, Fm (300 ms saturation pulse with 10,000 μmol photons m^{-2} s^{-1}) and Pm determination the actinic light was on and saturation pulses of identical light intensity (40 μmol photons m^{-2} s^{-1}, with 1000 ms time between actinic light on and saturation pulse and with 20 s time between two consecutive saturation pulses) were given. After dark-adapted induction curve, the Light Curve was obtained.

The effective PSII quantum yield – Y(II) – is calculated according to Genty et. al. [23] by the formula: $Y(II) = (Fm' - F)/Fm'$ and measured after dark adaptation when the effective PSII quantum yield is maximal. The value of Y(II) varies between 0 and 1.

The photochemical quantum yield of PSI – Y(I) – is sustained by the fraction of overall P700 that in a given state is reduced and is not limited by the acceptor side. The Y(I) value is calculated from complementary PSI quantum yields of: (i) non-photochemical energy dissipation (Y(ND)) as a measure of a donor side limitation, which is enhanced by trans-thylakoid proton gradient and damage at the level of PSII, and (ii) non-photochemical quantum yield of PSI (Y(NA)), which represents an inability to PSI oxidation due to lack of acceptors ($Y(I) = 1 - Y(ND) - Y(NA)$). The limitation of the acceptor side is enhanced by dark adaptation and damage at the level of CO_2 fixation [24].

3. Results

3.1. Ultrastructure of plastid inner membrane transformation during chloroplast biogenesis

After 8 days of etiolation directly before light was switched on the etioplasts of both bean (Fig. 2A) and pea (Fig. 2E) had a regular paracrystalline structure of PLB and a few PT emerging from PLB. Details show a very regular structure of PLBs in both species. After 2 h of light the paracrystalline structure was transformed into an irregular one, still many PT were observed in the stroma (Fig. 2B in bean and F in pea). After 8 h of light the PLB transformation was completed in bean plastids (Fig. 2C) but not in pea plastids (Fig. 2G). Smaller PLBs together with stacked membranes formed in their proximity (Fig. 2G, details) were present in pea while no remnants of PLB were observed in bean (Fig. 2C). The first stacked membranes were observed after 8 h of light both in bean (Fig. 2C) and in pea (Fig. 2G) plastids. At this stage the average length of stacked thylakoids per 1 μm^2 of plastid section was similar in bean (312 ± 22 nm) and in pea (318 ± 19 nm) and at the same time vertical distance from one pair of membranes to the next pair of stacked thylakoids was estimated to 27 ± 2 nm and 25 ± 1 nm respectively. Longer stacked thylakoids forming grana per 1 μm^2 of plastid section were observed (Fig. 2D in bean; 597 ± 74 nm and H in pea; 368 ± 25 nm) after 16 h of light before the light was switched off. In pea plastids, groups of plastoglobules matched places where PLBs existed (Fig. 2H details). After the second night of the experiment but still before the light was switched on, numerous, but slightly shorter than before grana in plastids were seen (279 ± 38 nm per 1 μm^2 of plastid section in bean and respectively 358 ± 47 nm in plastid section in pea) (Fig. 3A and D). At this stage also the vertical distance from one pair of grana membranes to the next pair increased to 30 ± 1 nm in bean and 28 ± 1 nm in pea which means that the thylakoid membranes in grana were less appressed than before. Some small, regular PLBs could be observed but only in pea plastids (Fig. 3D). After the third night of the experiment no reconstruction of PLB occurred both in bean and pea (Fig. 3B and E). Fully developed structure of grana in both examined species was visible after 3 h of illumination (Fig. 3C and F). Average length of grana thylakoids was similar in bean (512 ± 24 nm per 1 μm^2 of plastid section) and in pea (511 ± 17 nm per 1 μm^2 of plastid section) and the vertical distance between stacked membranes was 20 ± 1 nm both in bean and pea. The ultrastructure visible in these samples was similar to the one observed in mature chloroplast of pea and bean except from smaller grana and smaller chloroplast dimensions as compared to those of mature plant mesophyll [14].

3.2. Western-blot analysis of protein extracts from leaves of developing plants

The first proteins found in the bean leaf extracts were Lhcb2 antenna proteins — antenna of PSII complex, which gave a weak band after 6 h of illumination (Fig. 4A). Later, after 12 h of light, Lhcb1 was found and after the next 2 h D2 – a reaction center protein – was registered. CP43 – a core protein – appeared as late as on the second day of the experiment, followed by Lhcb4 and finally a weak band from Lhcb3 was registered (Fig. 4A).

After 12 h of illumination, Lhca1 – the first antenna protein related with PSI complex – appeared in the extract of bean leaves. After 14 h, PsaA – a core protein of PSI – was present and Lhca2 and Lhca3 appeared respectively after 4 and 8 h of illumination on the second day of the experiment (Fig. 4A).

In short, in bean the PSII antenna proteins appeared on blots before the PSII reaction center proteins, however the PSI core proteins appeared at about the same time as the antenna proteins of PSI.

As opposed to bean, the first recognized protein in pea was from the PSI complex (Fig. 4B). It was a PsaA core protein found in etiolated pea plants. At the same time a very weak band was recognized as Lhca2 and after the next 2 h another band appeared from Lhca3. After 8 h of illumination a comparable significant signal came from Lhca1 (Fig. 4B).

Lhcb2 – an antenna protein connected with PSII as well as D2 – a PSII reaction center protein appeared after 8 h of illumination. Subsequently a weak band appeared after 12 h of light from CP43 — a core protein, and also from Lhcb1 and Lhcb4, and finally from Lhcb3 during the next day of the experiment (Fig. 4B).

In short, in pea the appearance of core proteins of PSII was accompanied by the appearance of antenna proteins. In the case of PSI the most significant was the appearance of the PSI core protein although the antenna proteins appeared soon after.

3.3. Low temperature (77 K) fluorescence of leaves of developing plant leaves

The typical 77 K fluorescence emission spectrum of thylakoids is composed of two major bands. The first one is composed of two minor bands centered at around 683 and 692 nm (Fig. 5B, D) which correspond to emission from both LHCII loosely and strongly bound to PSII and from the PSII core, respectively [25,26]. The second large band between 720 and 750 nm is related to both PSI and LHCI antenna complexes [25].

In etiolated bean and pea seedlings no trace of chlorophyll–protein complexes was found, only a single band at 655 nm was present (Fig. 5A, C, black solid lines) corresponding to the NADPH–POR–Pchilide complex in PLB [3,7,8 and literature therein, 27]. In the presence of light

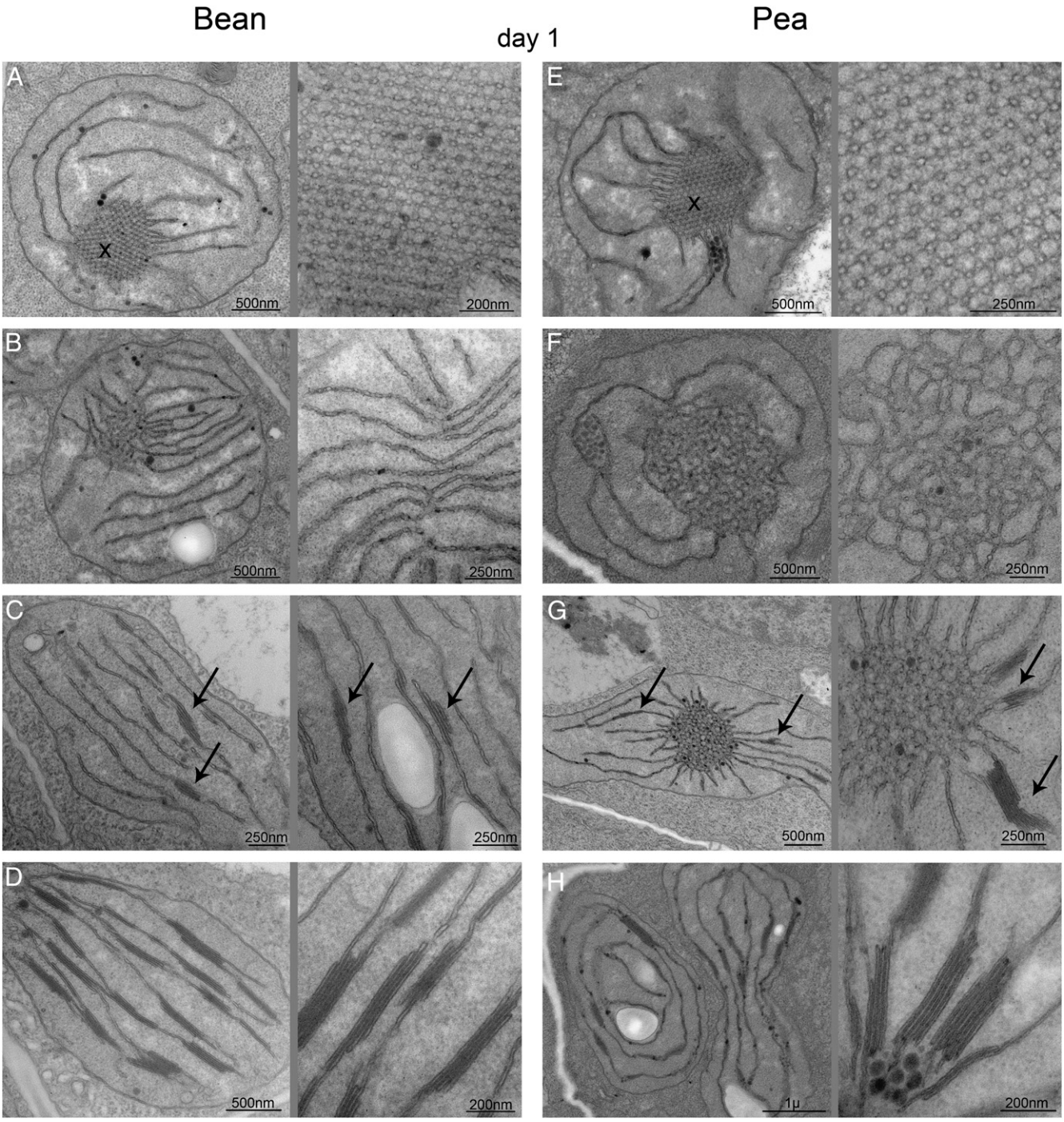

Fig. 2. Electron micrographs showing changes in the mesophyll plastids during the development of bean (left column) and pea (right column) seedlings in the subsequent hours of the illumination during the first day; left side — whole plastid, right side — details of inner membrane structure. A and E — samples collected after 8 days of etiolation just before the light was switched on (x — paracrystalline PLB); B and F — samples collected after 2 h of light; C and G — samples collected after 8 h of light (arrow — first stacked membranes formed); D and H — samples collected after 16 h of light.

Pchlide was reduced and the 655 nm band was red-shifted (Fig. 5A, C, black dashed lines).

In bean two bands were observed after 4 h of illumination: the first, with a flat top corresponding to both loosely and strongly LHCII and PSII core complexes and the second smaller related to PSI and LHCI complexes. Four hours later (8 h of illumination) these bands were clearly visible and the 680–700 nm band start to divide into two minor ones corresponding to LHCII loosely and strongly associated with PSII (683 nm) and the PSII core (692 nm).

In pea this process was delayed. After 4 h of light there was only one band in LHCII region with a small shoulder around 690 nm but after 8 h of light the three bands can be seen. At the end of light period (after16 h) in both examined species all characteristic bands are present (Fig. 5A, C, blue solid lines) in the 77 K fluorescence spectra. During the second and third days of light-growth there were no qualitative changes in bean and pea fluorescence spectra (Fig. 5B, D); only differences in relative intensities between bands were observed, especially in samples taken from darkness.

In fully developed pea thylakoids (Fig. 5D, blue solid lines) the ratio of the LHCII (loosely and strongly bound to PSII band) to the PSII core band was larger than in bean (Fig. 5B). On the other hand, the ratios of the PSI band to the PSII core and to the LHCII band

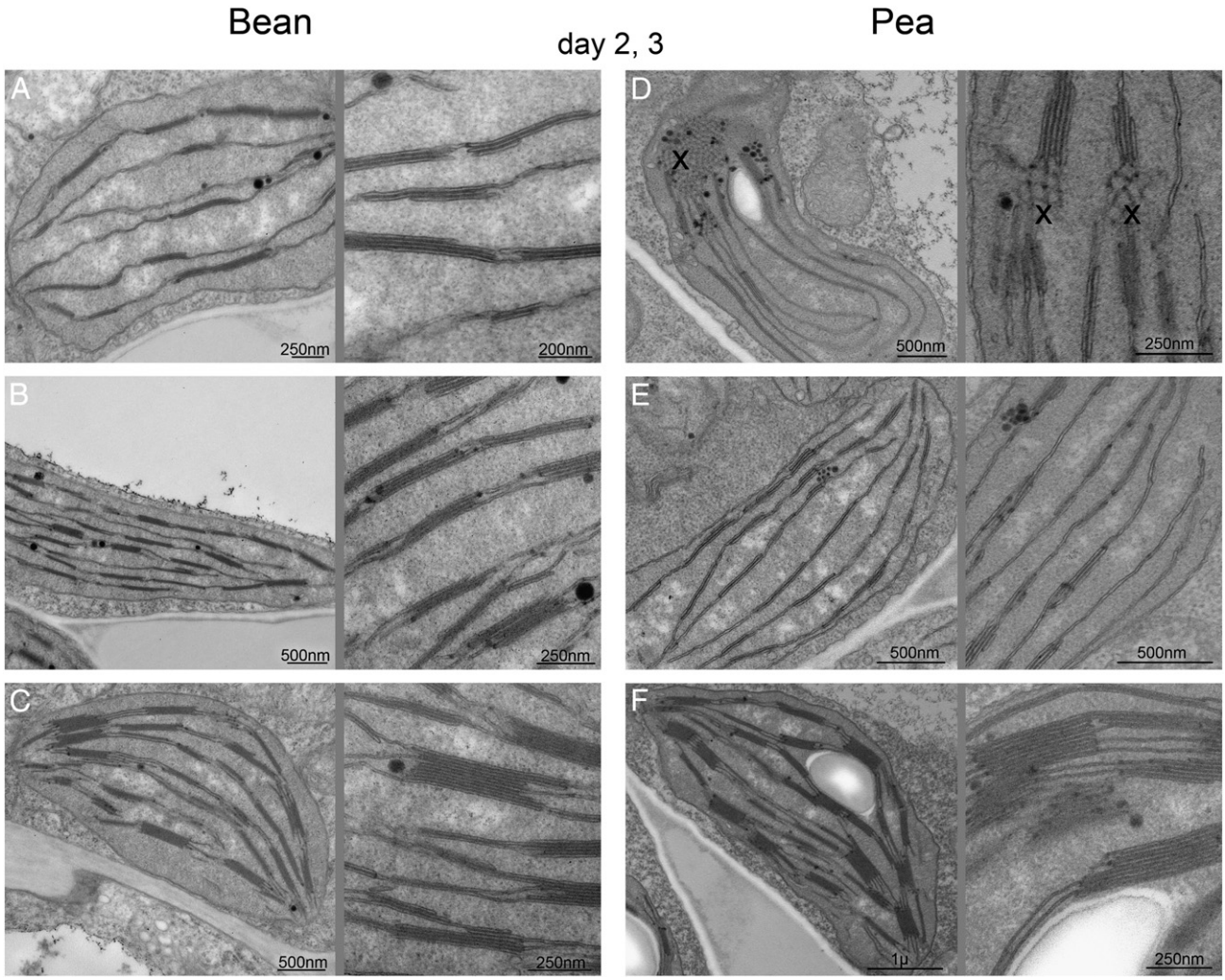

Fig. 3. Electron micrographs showing changes in the mesophyll plastids during the development of bean (left column) and pea (right column) during the second and third days of growth; left side — whole plastid, right side — details of the inner membrane structure. A and D — samples collected after the second night of experiment (x — paracrystalline PLB); B and E — samples collected after the third night of the experiment; C and F — samples collected after the third night and 3 h of light.

were higher in bean thylakoids. These observations are in agreement with the previous observation concerning the diversity in CP complexes arrangement and the whole chloroplast structure in bean an pea species [14,16,17].

3.4. Chlorophyll a fluorescence of leaves of developing plant leaves

A typical dark-adapted induction curve was composed of two initial maxima corresponding to Fm and Pm determination pulses (Fig. 6). The Fm levels induced by the repetitive saturation pulses first declined with the fluorescence signal and then rose before reaching the steady state. Lowering of Fm′ with respect to Fm reflected non-photochemical quenching (qN) induced by a large trans-thylakoidal pH change, which relaxed upon onset of the energy consumption, when the CO_2 fixation started after light-activation of the Calvin–Benson cycle [28,29].

As anticipated in etiolated bean and pea seedlings there was no photochemical activity (Fig. 6A, C black solid line). Any occurrence of fluorescence can originate only from the Pchlide species (Fig. 5A, C, black solid line) bound with the prolamellar body membranes [30]. The induction curves were unaltered in both plants after 1 h of illumination (Fig. 6A, C, black dashed line), despite changes in the Fv/Fm ratio (sudden drop after 1 h of illumination to 0.215 and 0.58 in bean and pea respectively), which can be related to the Pchlide reduction (Fig. 5A, C, black dashed line). After 4 h of light (Fig. 6A, C red solid lines) and further in time some differences between two species can be found: the course of bean induction curves became quasi-normal and – at 16 h of light (Fig. 6A, blue solid line) – normal, with proper induction points and subsequent regeneration after each saturation pulse. In the case of pea this happened little by little and only after about 8 h of light on the next day of the experiment (Fig. 6D, black dashed line).

Simultaneously with the presence of the main CP complexes shown by 77 K fluorescence (Fig. 5) and proteins (Fig. 4) during the second and third days, some differences were observed in photosynthetic activities between the two plant species (Fig. 6B, D). In pea the first symptoms of PSI activity (Y(I)) started after 8 h of light, which was consistent with the protein appearance (Fig. 4B), however the photochemical activity was very low (0.101) and varied with each of the saturation pulses. In the case of bean the PSI activity (Y(I)) started after the next 2 h rose to 0.354 and increased gradually. The value of the effective quantum yield of PSII was higher in bean than in pea (e.g. after 8 h of light on the second day of the experiment it is 0.720 in bean and 0.430 in pea), in the case of bean the maximal quantum yield of PSII — the Fv/Fm-value reaches almost the control level of a fully developed plant (0.79 confront to 0.815). The maximal Fv/Fm-value was reached in pea at the end of the experiment — third day after 3 h of illumination (0.78 confront to 0.83 of fully developed plant).

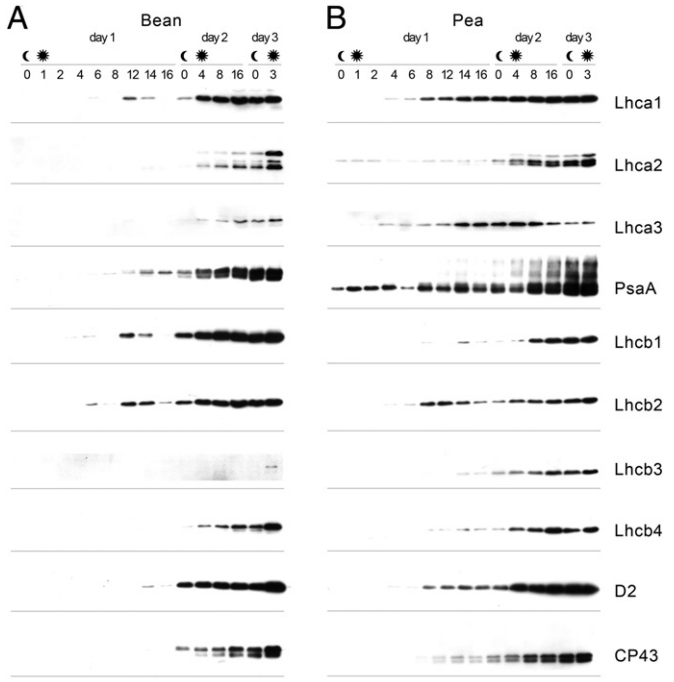

Fig. 4. Western-blot analysis of the leaf protein extracts showing the appearance of photosynthetic proteins (selected core and antenna proteins of PSI and PSII) in subsequent days and hours of growth. Results are representative for 3 independent experiments and only complete sample sets containing equal amount of protein coming from whole experiments were used. Left column — bean, right — pea.

4. Discussion

For the first time we correlated, at the same time intervals, the structure of etioplasts transforming into the mature chloroplasts with changes in the photosynthetic protein levels and with the function of photosynthetic apparatus.

Our experimental model of the differentiation of chloroplasts from etioplasts during light–dark cycles imitates the situation of the natural growth of young seedlings shortly after germination. Transformation of etioplasts into chloroplasts proceeded "smoothly" and faster during the first two days of illumination in bean. In the case of pea the reformation of PLBs during the dark period of cycle took place, although chloroplasts of both species were similarly and completely differentiated after 3 days of the experiment. The PLB reformation in darkness after their light-induced dispersal was described in other species [7,27,31] showing that the reformed PLBs were always much smaller than those initially formed. The reformed PLBs exhibited a regular structure and were localized between newly formed stacked membranes [27].

Although traces of some photosynthetic proteins were found after etiolation (Fig. 4B) no chlorophyll–protein complexes were recognized by the 77 K fluorescence. It means that proteins that have been found were not arranged in CP complexes. The only single band at 655 nm (Fig. 5A, C) corresponding to NADPH–POR–Pchlide complex was found both in bean and pea as shown also by [7,27]. The presence of the PsaA protein traces may possibly be caused by the activation of unknown green light photoreceptors [32]; all manipulations in the darkness were performed in dim green light as described in the Materials and methods section. Different examined

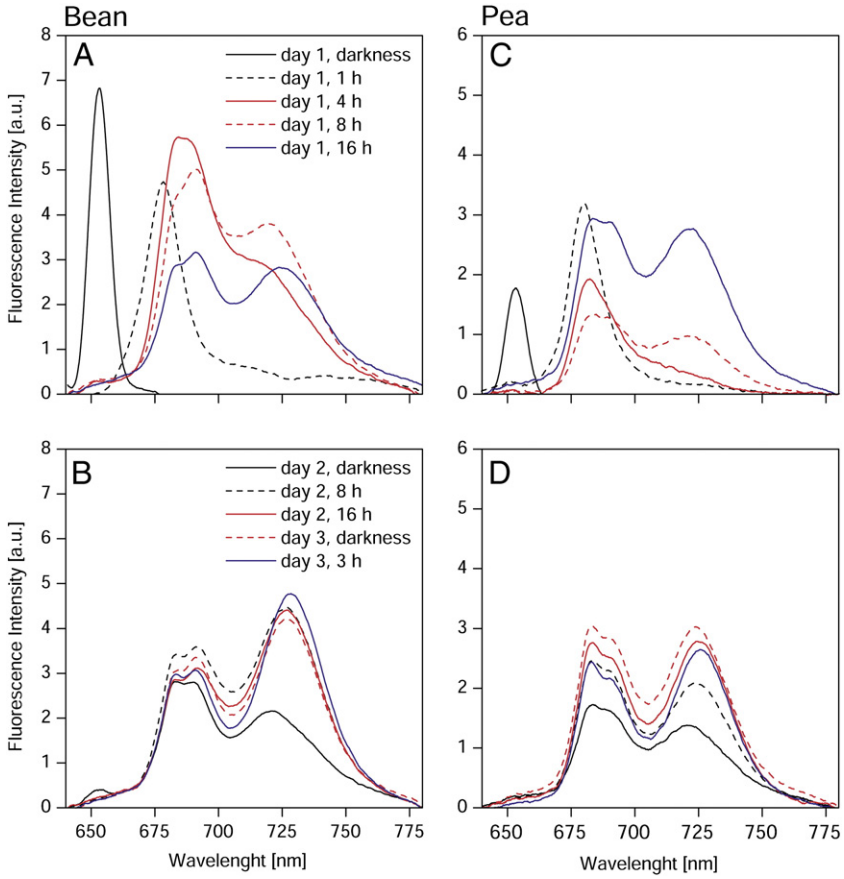

Fig. 5. Low temperature (77 K) fluorescence emission spectra (ex 420 nm) of leaves of etiolated bean (A, B) and pea (C, D) seedlings during exposure to light. The spectra are representative for 3 independent experiments.

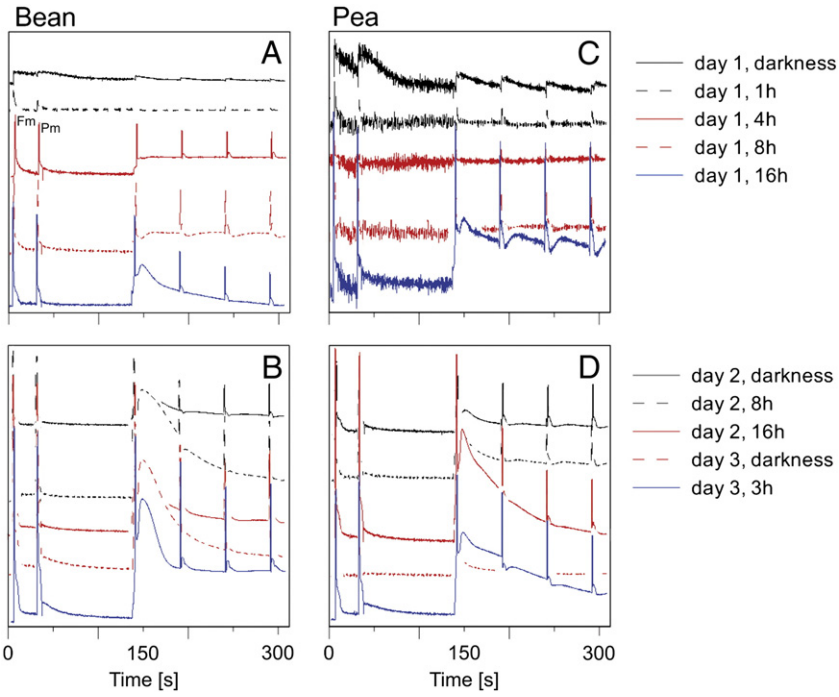

Fig. 6. Dark-adapted induction curves of leaves of etiolated bean (A, B) and pea (C, D) seedlings during exposure to light. Induction curves are representative for 3 independent experiments. Induction curves were separated from one another for a clearer view.

photosynthetic proteins related with PSI and PSII appeared in different time periods, in bean and pea leaves (Fig. 4A, B). The first proteins found in bean later become components of PSII what corresponds to the first bands related to LHCII loosely and strongly associated with PSII and PSII core observed by 77 K fluorescence. They were more clearly visible after 8 h of illumination as LHCII and as PSII core, (Fig. 5A, C). It means that either the first CP complexes revealed by 77 K fluorescence could be formed even when the protein level was very low or that the fluorescence method was more sensitive than the Western-blot.

Similarly to the structural differentiation the process of protein complex formation was delayed in pea compared with bean. However both apoprotein level (Western-blot) as well as the CP complex level (77 K fluorescence) grew gradually during the light–dark cycle. Both shapes of low temperature fluorescence and the induction curves did not change substantially after 8 h of darkness compared with 6 h of light during the first day of the experiment. Furthermore after the second and third night of the experiment qualitatively similar but quantitatively clearly visible differences in proportion between bands in bean and pea fluorescence spectra (Fig. 5B, D) were recognized.

The first stacked membranes appeared in both species already after 8 h of light what corresponds to the presence PSII-related bands registered by 77 K fluorescence. It means that formation of stacked membranes with already functioning CP complexes does not require the presence of all examined photosynthetic proteins at the level comparable with the one observed in fully developed leaves.

Maximal quantum yield of PSII comparable to mature leaves was reached earlier in bean than in pea, second day after 8 h of illumination the ratio Fv/Fm was 0.79 in bean and only 0.536 in pea. Similarly in the case of bean qN was 0.035 and 0.268 in pea. Higher photochemical activity in bean correlated with earlier thylakoid system development. Finally, after 3 days of the experiment the activities of photosynthetic complexes and grana development as well as photochemical activities were similar in both species and corresponded to a fully developed photosynthetic apparatus. It means that Fv/Fm comparable to mature leaves requires the presence of the proper CP complexes as well as completely formed grana.

Our results demonstrated that the differences in the structural development of pea and bean thylakoid system did not influence significantly the photochemical activity. A "smooth" structural transformation of etioplasts to chloroplasts in bean corresponded to a steady growth of the photochemical activity. In the case of pea the dark period in the photoperiodic growth caused reformation of PLBs but did not cause long term disturbances in the photochemical activity.

It should be taken into account that the biogenesis of the thylakoid membrane system is a complex and highly regulated process mediated by both nucleus- and chloroplast-encoded factors. The HEMERA gene, which is localized both in nucleus and chloroplast and is required for the adaptation of young seedlings transferred to light is an example of a coordinated action of nuclear and chloroplast light-regulated gene expression [32]. Another factor, ALB3 protein seems to play an important role in the thylakoid membrane biogenesis in the vascular plants and is required for the integration of the LHC proteins into the thylakoid membranes. Coordination between pigment synthesis and insertion into the thylakoid membranes has to be very precise [33]. The arrangement of supercomplexes in the thylakoid membranes is influenced and modified by changing environmental conditions; this is an advantage of the photosynthetic apparatus adaptation but on the other hand it makes its study complicated [14].

We found differences in biogenesis of pea and bean chloroplasts (species known to have different arrangements of thylakoid membranes in mature plants) such as (i) reformation of PLBs under dark period was observed in pea but not in bean despite the presence of core and antennae proteins, (ii) formation of stacked membranes in bean occurred more gradually than in pea, (iii) the order of appearance of antennae and core proteins differed in both plants, (iv) the first CP complexes revealed by 77 K fluorescence were formed even when the protein level was very low, (v) an increase of photosynthetic activity and formation of stacked membranes was delayed in pea as compared with bean. In our opinion this complex analysis provided new information about

chloroplast biogenesis on the structural and functional level of its organization during the photoperiodic plant growth.

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