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Short communication

Temperature-dependent chlorophyll accumulation and photosystem II assembly during etioplast to chloroplast transition in sunflower cotyledons

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Abstract – Despite numerous data dealing with the biogenesis of photosynthetic membranes connected with specific functional alterations in higher plants this is still an insufficiently understood topic and is one of the most promising areas of research in plant biochemistry. The main goal of our study was to detect the impact of different temperatures on chlorophyll biosynthesis and the maximum quantum yield of PSII (F_v/F_m). Therefore, we investigated the greening processes in etiolated sunflower cotyledons (*Helianthus annuus* L.) grown at different temperatures (10, 20 and 30 °C) during 24 h. The dynamics of chlorophyll *a* and *b* (Chl *a* and Chl *b*) accumulation as well as photosystem II (PSII) efficiency were observed. We also evaluated combined effects of different temperatures (20 and 30 °C) and short-term application of increased irradiation (800 μmol m^{-2} s^{-1}) on effective quantum yield of PSII ($\Delta F/F'_m$) and non photochemical quenching (NPQ) in cotyledons with fully developed PSII. Our results showed reduced chlorophyll accumulation and the arrest of PSII assembly at 10 °C in comparison with 20 and 30 °C. Further, the increased irradiance induced equal down regulation of effective quantum yield of PSII at 20 and 30 °C, with significantly higher capability of heat dissipation at 30 °C.

Keywords: chlorophyll, chloroplast biogenesis, photosynthesis, photosystem II, thylakoid membranes

Introduction

Thylakoid membranes in chloroplasts of higher plants are arranged in a highly flexible way so as to conduct efficient photosynthesis (Tikkanen and Aro 2014). They comprise several multiprotein complexes responsible for key photochemical processes such as light harvesting, trapping of excitons and electron transport to its final acceptor ($NADP^+$). A lot of studies have been undertaken with the aim of elucidating the *de novo* development of thylakoid membranes with special emphasis on the precise order of multiprotein complex assembly as well as on their interplay with photosynthetic pigments and their precursors (Rudowska et al. 2012). Still, some key processes have remained insufficiently understood and thus became much-discussed topics in scientific debates (Pudelski et al. 2009). However, it is well known that the key step in the transition of etioplast to chloroplast is regulated by catalytic activity of protochlorophyllide oxidoreductase A (POR A). The enzyme was found in etioplast prolamellar bodies and was shown to catalyze the reduction of protochlorophyllide to chlorophyllide in angiosperm plants upon exposure to light (Reinbothe et al. 2010). One of the key regulatory components of the thylakoid electron-transport chain is chloroplast water-plastoquinone oxidoreductase, known as photosystem II (PSII), which is divided into three functionally distinct parts: antennae, reaction centre (RC) and oxygen evolving complex (OEC) (Barber et al. 1997). The efficiency of PSII can be quantified thanks to the fluorescence feature of chlorophyll molecules. The methodology enables determination of the maximum quantum yield of PSII (F_v/F_m) reflecting the light-harvesting efficiency of PSII and is widely used to evaluate its general functionality (Schreiber et al. 1995).

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The aim of our study was to investigate the dynamics of chlorophyll *a* and *b* accumulation as well as PSII efficiency during greening process in etiolated sunflower cotyledons exposed to the different temperatures (10, 20, 30 °C). Since it is generally well known that low temperatures inhibit enzymatic activity, we hypothesized that the lowest temperature would result in slower accumulation of chlorophylls in developing chloroplasts. However, we aimed to place particular emphasis on the way this would be reflecteed in the F_v/F_m and Chl *a*/*b* ratio, the important parameter that influences PSII stability (Sakuraba et al. 2010). Also, we endeavored to evaluate the effects of greening temperature and short-term application of increased irradiation on the effective quantum yield of PSII ($\Delta F/F'_m$) and its capacity for non photochemical quenching (NPQ) as very important protective process against photoinhibition.

Materials and methods

Sunflower (*Helianthus annuus* L.) seedlings were germinated in the darkness at room temperature for 7 days. Etiolated cotyledons were then removed from the dark-grown plants and subjected to greening by exposure to 100 µmol m^{-2} s^{-1} of white light (Osram Powerstar HQI BT 400 W/D E40), for 3, 6, 12 and 24 h. Three separate experiments were done by changing the temperature during greening process: 10, 20 and 30 °C. Chlorophylls *a* and *b* were quantified spectrophotometrically, and concentrations were calculated according to Lichtenthaler (1987). *In vivo* chlorophyll *a* fluorescence measurement (Mini-PAM, Waltz) was used to quantify several parameters of photosystem II (PSII) efficiency: the maximum quantum yield of photosystem II (F_v/F_m), the effective quantum yield of photosystem II ($\Delta F/F'_m$) and non-photochemical quenching (NPQ) at 800 µmol m^{-2} s^{-1} (Schreiber et al. 1995). A Student t-test as well as factorial analysis of variance followed by least significance difference (LSD) test was performed to analyze statistical significance (Statistica 7.1.).

Results and discussion

Chloroplast biogenesis, usually seen as greening (Fig. 1), is characterized by coordinated biosynthesis of photosynthetic pigments and other molecules essential for the assembly of a fully functional photosynthetic apparatus. Since functional chloroplasts contain up to 4 thousand different proteins, most of them encoded by nuclear genes, it is likely that different environmental signals (e.g. light or temperature) would have a great influence on signaling during chloroplast biogenesis (Fey et al. 2005). In order to quantify green color appearance after a certain time of greening, changes in Chl *a* and *b* concentrations were measured. Our results (Tab. 1) revealed a permanent increase of Chl *a* during 24 h of greening at temperatures of 20 and 30 °C, as well as a constant increase of Chl *b* at 30 °C. Also, delay in Chl *a* accumulation was observed at 10 °C, as well as in Chl *b* accumulation at 10 and 20 °C during the first 6 h of greening. Further, Chl *b* accumulation at 10 and 20 °C was slowed down after 12 h of greening. Generally, inferior chlorophyll biosynthesis was revealed in greening cotyledons at lower temperatures, especially at 10 °C. This was also evident from the value of Chl *a*/*b* ratio after 24 h of greening, which was lower in cotyledons that were greening at 10 °C in comparison to these that were greening at 20 and 30 °C (Tab. 1). Tewari and Tripathy (1998) revealed 90% inhibition of Chl biosynthesis during greening of etiolated cucumber seedlings at 7 °C caused by the inhibition of enzymes involved in protoporphyrin IX biosynthesis, which directly decreased the biosynthesis of protochlorophyllide. So, the lower chlorophyll concentrations in such cases would be the result of diminished biosynthesis. Further, low temperatures diminish CO_2 fixation and thus increase the chance for over-reduction of the electron-transport chain and consequently for photo-damage of PSII due to inhibition of the repair cycle of D1 protein, the essential protein for optimal PSII functioning (Giardi et al. 1997).

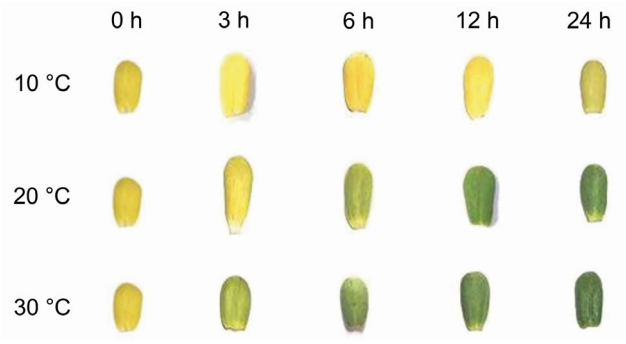

Fig. 1. Greening of etiolated sunflower cotyledons (0 h) exposed to different temperatures (10 °C, 20 °C, 30 °C) and the light intensity of 100 µmol m^{-2} s^{-1} for 24 h. Color changes were recorded after 3, 6, 12 and 24 h.

Tab. 1. Changes in concentrations of chlorophyll *a* (Chl *a*), chlorophyll *b* (Chl *b*) and chlorophyll *a/b* ratio (Chl *a/b*) in sunflower cotyledons, measured after 3, 6, 12 and 24 h of the greening period, at 10 °C, 20 °C and 30 °C. Data represent arithmetic means±standard deviations of ten replications. Factorial analysis of variance followed by least significance difference test was done separately for each temperature. Different lowercase letters indicate a significant difference at $P<0.05$. FW – fresh weight.

Greening temperature	Light exposure	Chl *a* (mg g^{-1} FW)	Chl *b* (mg g^{-1} FW)	Chl *a*/*b*
10 °C	3 h	0.034±0.012^a	0.047±0.022^a	0.828±0.372^a
	6 h	0.027±0.007^a	0.039±0.015^a	0.747±0.214^a
	12 h	0.053±0.013^b	0.080±0.024^b	0.691±0.172^a
	24 h	0.078±0.013^c	0.084±0.025^b	0.958±0.172^a
20 °C	3 h	0.065±0.010^a	0.057±0.019^a	1.311±0.586^a
	6 h	0.122±0.033^b	0.064±0.022^a	2.018±0.526^b
	12 h	0.253±0.048^c	0.097±0.034^b	2.747±0.654^c
	24 h	0.336±0.079^d	0.113±0.035^b	3.048±0.374^c
30 °C	3 h	0.082±0.011^a	0.066±0.024^a	1.352±0.383^a
	6 h	0.172±0.037^b	0.097±0.042^b	1.916±0.493^b
	12 h	0.372±0.084^c	0.125±0.024^c	2.967±0.264^c
	24 h	0.440±0.046^d	0.166±0.025^d	2.707±0.473^c

Changes in maximum quantum yield of PSII (F_v/F_m) are shown in Fig. 2. Continuous increase of F_v/F_m was observed during first 12 h of greening at 10 °C (Fig. 2A). However, the upcoming arrest of PSII assembly that was observed after 24 h of greening at 10 °C (Fig. 2) was most likely due to the inability of thylakoid membranes to accumulate *de novo* synthesized chloroplast encoded proteins (such as D1) relatively to nuclear encoded ones (Allen and Ort 2001). This would cause improper chlorophyll-protein complex assembly as well as the formation of fully competent grana (Rudowska et al. 2012) required for Fv/Fm values usually observed in mature leaves. Such a scenario consequently led to the irreversible photoinhibition that was revealed (Fig. 2A). Values of the F_v/F_m in fully competent and healthy photosynthetic leaves are usually reported to be about 0.83 (Schreiber et al. 1995). Bolhar-Nordenkampf et al. (1989) reported the F_v/F_m of 0.75 as the lowest boundary value of PSII functionality. Judging from our results (Fig. 2B, C), cotyledons that were greening at 20 and 30 °C developed fully functional PSII. Muraja Ljubičić et al. (1998) reported parallel increase of light-harvesting proteins of PSII (LHCII) with chlorophyll accumulation during greening of potato. At the temperature of 30 °C PSII functionality was achieved after as little as 6 h of greening (F_v/F_m=0.77), while at the temperature of 20 °C it needed a longer period (24 h for F_v/F_m to be 0.76). Increased accumulation of Chl *a* and *b*, as well as increased Chl *a*/*b* ratio in these cotyledons compared to those grown at 10 °C was required for the assembly of functional PSII.

Since the measurement of F_v/F_m revealed functional PSII in cotyledons subjected to greening at 20 and 30 °C, additional characterization of PSII efficiency was performed. After 24 h of greening cotyledons were exposed to short-term high irradiation (800 μmol m^{-2} s^{-1}) and the effective quantum yield of PSII ($\Delta F/F'_m$) as well as non-photochemical quenching (NPQ) were measured (Fig. 3). Although there were no differences in $\Delta F/F'_m$ upon exposure to high irradiation between cotyledons that were greening at 20 and 30 °C, cotyledons that were greening at 30 °C revealed a better capacity for NPQ than cotyledons that were greening at 20 °C (Fig. 3). NPQ represents the measure for dissipation of the excess excitation energy as heat relative to dark adapted state (Maxwell and Johnson 2000). Precise mechanisms of NPQ are under constant debate and new evidence is being continuously provided (Jurić et al. 2013). Basically, sufficient amounts of xanthophyll cycle carotenoids and structural rearrangement of PSII antennae are essential for efficient NPQ. Bilger and Björkman (1991) showed up-regulation of the rate of violaxanthin de-epoxidation by temperature increase. It seems that higher temperature during greening process (30 °C) of etiolated sunflower cotyledons favored zeaxanthin formation, thereby enabling more efficient heat dissipation by NPQ.

It can be concluded that low temperature (10 °C) decreased biosynthesis of Chl *a* and Chl *b* in comparison to two higher investigated temperatures, which was directly linked with impaired assembly of PSII after a prolonged period of greening (24 h) at 10 °C. Lebkuecher et al. (1999) reported that assembly of PSII during the transition from etioplast to chloroplast in sunflower cotyledons at 25 °C was accompanied by small initial LHCII complexes and reaction centers (RCs) that are deficient in Q_A to Q_B electron transport. Further greening in their experiment (up to 12 h) enabled conversion of Q_B-nonreducing to Q_B-reducing RCs and efficient electron transport. Hence, it is most likely that the arrest of PSII assembly after 24 h of greening at 10 °C that we have observed was due to over-reduction damage in the PSII reaction center. Also, fully developed PSII at tem-

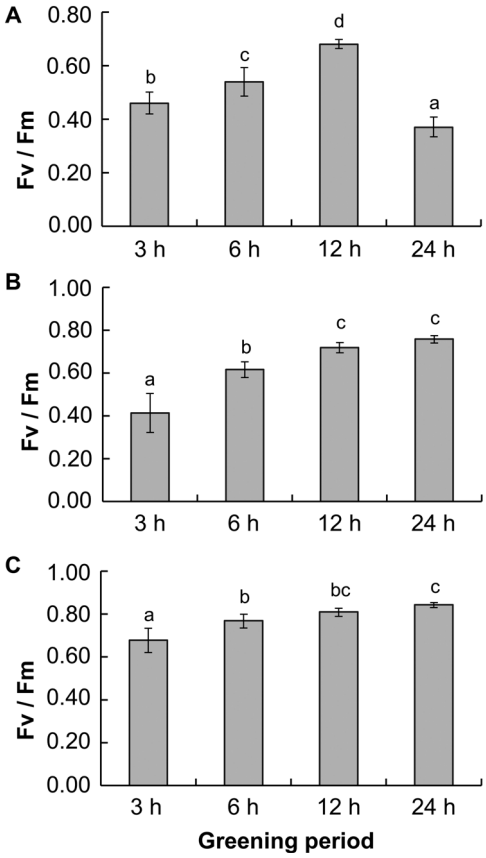

Fig. 2. Changes in maximum quantum yield of PSII (F_v/F_m) in sunflower cotyledons, measured after 3, 6, 12 and 24 h of the greening period, at 10 °C (A), 20 °C (B) and 30 °C (C). Data represent arithmetic means±standard deviations of five replications. Differences between presented values were evaluated using least significance difference test. Different lowercase letters indicate a significant difference at P<0.05.

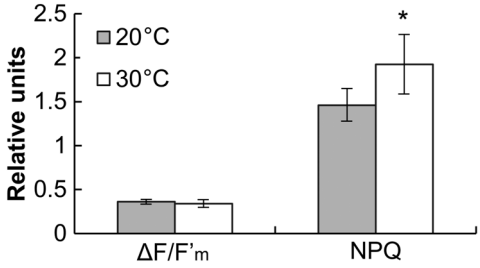

Fig. 3. The effect of short-term high irradiation (800 μmol m^{-2} s^{-1}) on effective quantum yield of PSII ($\Delta F/F'_m$) and non-photochemical quenching (NPQ) of sunflower cotyledons, after 24 h of greening at different temperatures (20 °C and 30 °C). Data represent arithmetic means±standard deviations of five replications. Differences between presented values were evaluated using Student's t-test. Asterisk (*) indicate a significant difference at P<0.05.

peratures of 20 and 30 °C was further challenged by short-term exposure to increased irradiance (800 μmol m^{-2} s^{-1}). Although the equal down regulation of PSII efficiency was revealed regardless of the greening temperature, a more efficient capability of heat dissipation was achieved by greening at 30 °C. This might be attributed to higher efficiency of the xanthophyll cycle as well as to the different structures and connectivity of LHCII proteins (Bilger and Björkman 1991, Tikkanen and Aro 2012) and requires further investigation.

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