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Acclimation of mesophyll and bundle sheath chloroplasts of maize to different irradiances during growth

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Abstract

The regulation by light of the photosynthetic apparatus, and composition of light-harvesting complexes in mesophyll and bundle sheath chloroplasts was investigated in maize. Leaf chlorophyll content, level of plastoquinone, PSI and PSII activities and Lhc polypeptide compositions were determined in plants grown under high, moderate and low irradiances. Photochemical efficiency of PSII, photochemical fluorescence quenching and non-photochemical fluorescence quenching over a range of actinic irradiances were also determined, using chlorophyll *a* fluorescence analysis. Acclimation of plants to different light conditions caused marked changes in light-harvesting complexes, LHCI and LHCII, and antenna complexes were also reorganized in these types of chloroplasts. The level of LHCII increased in plants grown in low light, even in agranal bundle sheath chloroplasts where the amount of PSII was strongly reduced. Irradiance also affected LHCI complex and the number of structural polypeptides, in this complex, generally decreased in chloroplasts from plants grown under lower light. Surprisingly moderate and low irradiances during growth do not affect the light reaction and fluorescence parameters of plants but generated differences in composition of light-harvesting complexes in chloroplasts. On the other hand, the changes in photosynthetic apparatus in plants acclimated to high light, resulted in a higher efficiency of photosynthesis. Based on these observations we propose that light acclimation to high light in maize is tightly coordinated adjustment of light reaction components/activity in both mesophyll and bundle sheath chloroplasts. Acclimation is concerned with balancing light utilization and level of the content of LHC complexes differently in both types of chloroplasts.

Keywords: Bundle sheath; Electron transport; Light acclimation; Light-harvesting complexes; Maize; Mesophyll; Photosystem

1. Introduction

Plants experience and adjust to wide daily and seasonal fluctuations in environmental conditions such as light and temperature. Changes in the environment have a particular impact on the photosynthetic apparatus, which is also a major site of damage under stress conditions. Plants have evolved many mechanisms of responding to varying growth conditions. These mechanisms operate at different levels of complexity. Responses on whole plant or individual leaf level take effect over period of weeks or months [1,2] whereas adjustments on molecular level are likely to occur within seconds to hours [3].

Irradiance affects many factors on individual leaf-level. Increase in the leaf thickness, and in its, photosynthetic capacity (increase in chloroplast numbers) are characteristic responses in plants grown under high light conditions [4,5]. Structural responses are also evident on chloroplast-level. The chloroplasts of shade or low-light grown plants have larger and more numerous granal stacks and their thylakoids are less non-appressed when compared to those in chloroplasts of sun or high-light plants [6,7].

Acclimation to different light conditions is also manifested by changes in organization and/or level of protein complexes in thylakoid membranes and by different contents of stromal

Abbreviations: BS, bundle sheath; Chl, chlorophyll; DCPIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; HL, ML and LL, high-, moderate- and low-light; LHCI and LHCII, light-harvesting complexes of photosystem I, and II; M, mesophyll; MV, methyl viologen; PPFD, photosynthetic photon flux density; PQ, plastoquinone; PSI and PSII, photosystem I and II; PVDF, polyvinylidine difluoride; SDS PAGE, polyacrylamide gel electrophoresis in presence of SDS; TMPD, tetramethyl-p-phenylenediamine

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components involved in carbon fixation. Growth irradiance modulates the composition of light-harvesting antennas of PSI and PSII [7–10]; growth under low light promotes large PSI and PSII antenna size whereas growth under high light generates a small photosynthetic unit [7,10,11]. It has been observed for several plant species [4,8,11–13] that an increase in antenna size is reflected in a decrease of Chl *a/b* ratio. Reorganization of photosystems and their associated antennas in response to high light is associated with an increase in level of PSII [11,14,15]. An increase in PSI level was also observed, but this change occurred only under very low irradiance with light intensities below 100 μmol m^{-2} s^{-1} [15]. In addition, an increase in the amount of cytochrome b_6f complex [16] and Rubisco [17] was also reported in plants grown under high light. Changes in Rubisco content appear to have adaptative significance in increasing both the capacity for and efficiency of photosynthesis [18].

Photosynthesis in maize, C_4 plant, takes place in two distinct cell types, mesophyll (M) and bundle sheath (BS) cells. Chloroplasts in mesophyll tissue are structurally similar to those in C_3 plants, but chloroplasts in BS tissue are agranal. PS II complex is expressed in a tissue-specific manner in maize [19], where chloroplasts from BS cells are devoid of PSII activity [20,21] and contain lowered amount of PSII proteins [22]. Moreover, in BS cell chloroplasts the content of LHCII polypeptides is significantly lower than that found in chloroplasts from M cells [23]. PSI is the main photosynthetic complex present in BS chloroplasts of maize. It is commonly accepted that agranal chloroplasts of BS cells of maize are not influenced by light intensity and their structure are similar in the leaves of plants grown in the full tropical sunlight or in low light greenhouses conditions [24].

In this study the effect of different irradiance conditions during growth on photosystem contents and activities, and level of light-harvesting polypeptides was compared in mesophyll and bundle sheath chloroplasts of maize. The results demonstrate that the differences in responses to light intensity between mesophyll and bundle sheath chloroplasts are related to significant changes in polypeptide composition of LHCII and LHCI complexes. Although PSI dominates in bundle sheath chloroplasts, low irradiance causes an increase in level of LHCII and decrease in level of LHCI polypeptides.

2. Materials and methods

2.1. Plant material and growth conditions

Maize (*Zea mays*) plants were grown in vermiculite in a growth chamber with a 14 h photoperiod and a day/night regime at 24/21 °C. Photosynthetic photon flux densities were 1000 μmol m^{-2} s^{-1} (HL, high light), 350 μmol m^{-2} s^{-1} (ML, moderate light) or 50 μmol m^{-2} s^{-1} (LL, low light). Plants were fertilized with Knop's solution. Leaves were harvested from 2- to 4-week-old plants.

2.2. Pigment and PQ determination

The photosynthetic pigment contents (Chl *a*, Chl *b*, and total carotenoids) in whole leaves were estimated spectrophotometrically using a UV-160A spectrophotometer (Shimadzu) from pigment extracts in 80% acetone with a small amount of $CaCO_3$, according to Lichtenthaler and Wellburn [25]. PQ pool was estimated according to Wanke et al. [26] in extracts from whole leaves. Analysis was performed by reverse-phase HPLC, the absorbance at 210 nm was monitored using UV-detector.

2.3. In vivo measurements of chlorophyll a fluorescence

Chl *a* fluorescence was measured at room temperature with an FSM 1 fluorometer (Hansatech) run by a Modfluor software provided by the manufacturer. The fluorometer was connected to a leaf-clip holder through a fiberoptic cable. Leaves adapted for 30 min to darkness were used in these assays with the actinic radiation of 60–1100 μmol m^{-2} s^{-1} and the saturation radiation of 4500 μmol m^{-2} s^{-1}. Chlorophyll fluorescence quenching coefficients: qP (photochemical) and NPQ (non-photochemical) and quantum efficiency of PSII electron transport (Φ_{PSII}) were measured at steady-state photosynthesis according to the procedure of Genty at al. [27].

2.4. Chloroplast isolation

The chloroplasts from mesophyll (M) and bundle sheath (BS) cells were isolated using mechanical method described by Romanowska et al. [22].

Chlorophyll concentration was quantified after extraction with 80% acetone as described by Arnon [28]. Chloroplasts were used immediately or stored frozen at −80 °C.

2.5. Determination of PSII and PSI activity

Activity of PS II in M and BS cell chloroplasts (10 μg Chl/ml) was monitored by measuring the rate by which the chloroplasts reduce DCPIP. This was measured, with H_2O as electron donor by a decrease in absorbance at 590 nm in a medium containing: 330 mM sorbitol, 40 mM HEPES/KOH pH 7.6, 1 mM KH_2PO_4, 5 mM NaCl, 5 mM $MgCl_2$, 5 mM NH_4Cl and 0.1 mM DCPIP. The light used for DCPIP reduction was 1800 μmol m^{-2} s^{-1}. The absorbance readings were taken at 30-s intervals during the 2-min assays.

Activity of PSI in M and BS chloroplasts was estimated polarographically with a Clark-type oxygen electrode (TriOximatic EO200, WTW, G.M.B.H., Weilham, Germany). After 3 min adaptation of isolated chloroplasts to darkness, PSI activity was measured at 25 °C in the reaction mixture (2 ml) contained: 330 mM sorbitol, 40 mM Tricine pH 7.6, 2 mM EDTA and 7 mM $MgCl_2$. Activity was measured as oxygen uptake in a reaction using TMPD (0.2 mM) reduced with sodium ascorbate (3 mM) as electron donor and MV (0.1 mM) as electron acceptor. DCMU (15 μM) and NaN_3 (5 mM) were used to inhibit PSII and catalase activities, respectively. For electron transport measurements chloroplast suspension contained 40 μg Chl/ml was illuminated at 1800 μmol m^{-2} s^{-1}.

2.6. SDS-PAGE and protein immunodetection

For immunodetection proteins were separated in 15% gels by Laemmli-type SDS-PAGE [29] 1.5–3 μg of Chl (it depended on used antibody) were loaded on each lane. Following electrophoresis polypeptides were transferred to PVDF-membrane (Millipore, Bradford, MA, USA) as described by Towbin et al. [30]. Membranes were probed with rabbit antibodies specific to the Chl *a/b*-binding light harvesting polypeptides and to PsbD and PsaD proteins from PSII and PSI reaction centers. Secondary anti-rabbit antibodies conjugated to alkaline phosphatase were used to visualize immunoreactive proteins. GeneTools software (SynGene) was used for quantitative analysis of protein bands on the membranes.

3. Results

3.1. Pigment content and composition, and PQ level in the maize leaves

Growth in different light conditions induced changes in the amount of photosynthetic pigments and in their composition

(Table 1). Acclimation of maize to HL resulted in 45% reduction (about 1600 μg g^{-1} fresh weight) of Chl $a+b$ content in comparison with ML and LL grown plants, which had similar Chl level (about 2900 μg g^{-1} fresh weight). Although there was a small increase (15%) in carotenoids content in ML grown plants, this change was not statistically significant. The Chl *a/b* ratio was found to be higher in HL and ML plants (~4.0) than in LL grown plants (~3.6). As there was only small change in carotenoid levels and significant variations in the amount of Chl among plants, HL grown plants had the Chl/Car ratio 40%–50% lower than those calculated for plants grown in the lower irradiance.

Different irradiance also affected PQ pool, which in plants acclimated to HL increased two fold compared with ML and LL grown plants, where the PQ pool remained unchanged (Table 1).

3.2. Chlorophyll a fluorescence at room temperature

Analysis of Chl *a* fluorescence is routinely used for monitoring changes in photosynthetic function. Fluorescence induction measurements (Table 1) showed a lower Fv/Fm ratio in HL grown plants (0.76), which indicates slightly decreased photosynthetic efficiency. Maximal photosynthetic efficiency (Fv/Fm ~0.8) was observed in LL and ML grown plants.

Results of analysis of PSII function for plants grown under HL, ML and LL conditions are summarized in Fig. 1. At any given actinic light the value of Φ_{PSII} was similar in ML and LL grown plants but was approximately 10% greater than that in HL grown plants. The qP values did not change at low PPFD range (40–350 μmol m^{-2} s^{-1}) and started to decrease with PPFD increasing above 350 μmol m^{-2} s^{-1}. For ML and LL grown plants the qP values were similar for all light intensities during measurements. HL grown plants had lower qP values at PPFD of 40–600 μmol m^{-2} s^{-1} however at PPFD above 800 μmol photons m^{-2} s^{-1}, the qP values were identical for all investigated plants. HL and ML grown plants exhibited similar values of the non-photochemical quenching (NPQ) over a wide irradiance range in comparison with those for LL plants, which exhibited a reduction by 30–45% in NPQ values over actinic light of 240 μmol photons m^{-2} s^{-1}.

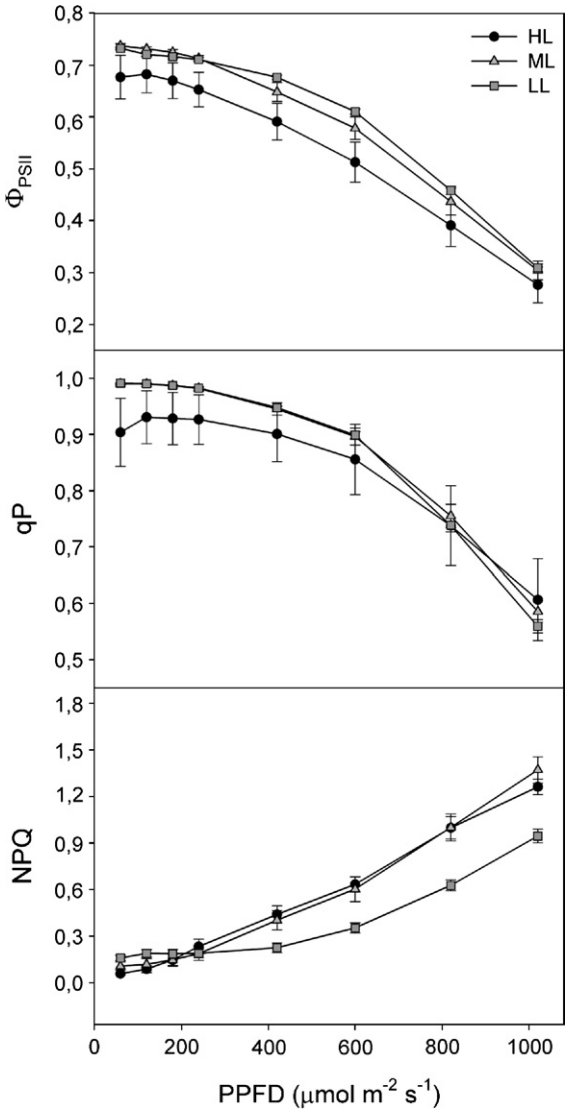

Fig. 1. Relationship between photosynthetic photon flux density and the PSII photochemical efficiency (Φ_{PSII}), photochemical fluorescence quenching (qP) and nonphotochemical fluorescence quenching (NPQ) for the leaves of maize plants grown at high light (HL), moderate light (ML) and low light (LL). Data are means±SE ($n \geq 5$).

Table 1
The pigment content, Chl *a/b* ratio, total chlorophyll/carotenoid ratio (Chl/Car), plastoquinon content and photochemical efficiency of the PSII (Fv/Fm) for maize leaves

	HL	ML	LL
Chl $a+b$ (μg g^{-1} fresh weight)	$1639\pm130^{b*}$	$2949\pm59^{a*}$	$2974\pm148^{a\#}$
Carotenoids (μg g^{-1} fresh weight)	474 ± 24	547 ± 17	$459\pm20^{b\#}$
Chl *a/b*	4.06 ± 0.05	4.00 ± 0.06	$3.64\pm0.05^{a*,b*}$
Chl/Car	$3.43\pm0.15^{b*}$	$5.40\pm0.10^{a*}$	$6.46\pm0.06^{a*,b*}$
PQ (μg g^{-1} fresh weight)	$142\pm10^{b*}$	$73\pm9^{a*}$	$79\pm10^{a\#}$
Fv/Fm	$0.76\pm0.011^{b\#}$	$0.79\pm0.004^{a\#}$	$0.79\pm0.002^{a\#}$

Plants were grown at high light (HL), moderate light (ML) and low light (LL). Data are means±SE ($n \geq 6$), "a" indicates statistically significant difference compared to samples from HL, "b" indicates statistically significant difference compared to samples from ML (*$P<0.001$, #$P<0.05$).

3.3. PSII and PSI activities

PSII and PSI activities were measured in mesophyll (M) and bundle sheath (BS) chloroplasts of plants grown under HL, ML and LL conditions. PSII in BS chloroplasts was 80–85% less active than in mesophyll chloroplasts (Table 2). Some previous reports demonstrated, that PSII was not at all active in BS chloroplast of maize [21,31,32]. But it was shown recently [22] that the activity of PSII could be detected and it strongly depended on methods of isolation of BS chloroplasts.

The PSI activity was approximately two fold higher in BS chloroplasts compared to mesophyll chloroplasts for all light intensities.

Table 2
PSII and PSI electron transfer activity in mesophyll (M) and bundle sheath (BS) chloroplasts of maize

	PSII activity (μmol DCPIP mg^{-1} Chl h^{-1})		PSI activity (μmol O_2 mg^{-1} Chl h^{-1})	
	M	BS	M	BS
HL	246±12	36±3	663±44	1286±85
ML	108±3	22±2	464±14	810±96
LL	109±2	19±1	434±21	729±21

Plants were grown at high light (HL), moderate light (ML) and low light (LL). Data are means±SE ($n \geq 3$).

There was observed influence of light intensity during growth on activity of both photosystems. Surprisingly these activities were similar in ML and LL grown plants in both types of chloroplasts but they were much more higher in HL grown plants. The PSII activity in HL plants was 2.3- and 1.6-fold higher in M and BS chloroplasts, respectively, in comparison with that in ML and LL grown plants. Similarly the PSI activity in both types of chloroplasts of HL grown plants showed a 1.5-fold increase when compared with chloroplasts from plants grown under other light conditions.

3.4. Light-harvesting polypeptides composition

Polypeptide composition of all Chl *a/b*-binding light harvesting complexes in mesophyll (M) and bundle sheath (BS) chloroplasts of maize plants grown at three investigated light conditions—HL, ML and LL was analyzed by immunodetection with specific antibodies. The immunodetection of reaction center (RC) polypeptides – PsbD and PsaD – was also conducted to relate the amount of the individual LHC proteins to the level of PSII and PSI centers. Because PsaD is extrinsic PSI protein [33] it can be lost during isolation and its level does not have to reflect the number of PSI centers. We probed the samples with a mixture PsaA and PsaB antibodies. Results obtained for these three proteins were similar (data not shown).

There were clear differences in the Chl *a/b*-binding light harvesting complex proteins among M and BS chloroplast. Distinct differences in detection referred to PSII core protein and Lhcb proteins (Fig. 2), especially proteins from the minor light harvesting antenna—Lhcb4, Lhcb5, Lhcb6 and Lhcb3 protein from the major LHCII complex. The levels of these proteins were strongly reduced in BS chloroplasts as compared to those in M chloroplasts. The amounts of detectable Lhcb1 and Lhcb2 proteins were also lower in BS chloroplasts but these differences were not as obvious. The opposite relationship was observed for proteins connected with PSI (Fig. 4). Levels of both the PSI core and antenna polypeptides in BS chloroplasts were elevated but the level of Lhca1 was similar in BS and M chloroplasts. This observation is typical to maize chloroplasts, because maize BS chloroplasts are agranal or exhibit rudimentary grana [31] and possess strongly reduced amount of PSII [22,34,35].

Antibody against Lhca2 recognized at least three polypeptides. We have some evidence that this antibody cross-reacts with Lhcb proteins and the lowest band refers to Lhca2 protein. This was apparent when isolated grana (BBY particles) from M chloroplasts were probed with this antibody, only two upper bands were detected strongly whereas the lower band was detected very weakly (data not shown).

The composition of LHCII polypeptides appears strongly irradiance-dependent, with a visible trend of, decreasing content of these proteins at high irradiance in both types of chloroplasts (Fig. 2). This is even more apparent in BS chloroplasts especially for polypeptides: Lhcb1, Lhcb4 and Lhcb6. Light intensity affected also the level of PsbD protein. In contrast to Lhcb proteins the content of PSII RC protein raised with increased irradiance. However, in BS chloroplasts where the greatest amount of PsbD was detected in chloroplasts from ML grown plants.

The LHCI composition also changed in plants grown under different light conditions. The level of PSI RC polypeptide was the lowest in BS chloroplasts from HL grown plants and only slightly differed in LL and ML grown plants (Fig. 4). Content of Lhca1 and Lhca4 proteins decreased in M and BS chloroplasts whereas the amount of Lhca3 increased in chloroplasts from BS cells as the light intensity decreased. In contrast there were not irradiance-dependent changes in levels of Lhca2 in both types of chloroplasts and Lhca3 in mesophyll chloroplasts.

Because different light conditions caused changes not only among antenna proteins but also in the amount of photosystem complexes, it was important to estimate the ratio of Lhc polypeptides per PSII and PSI reaction center proteins. We calculated Lhc/RC ratio for each tested samples and all results were normalized to Lhc/RC ratio obtained for M chloroplasts from ML grown plants (this value was defined as 1) (Figs. 3 and

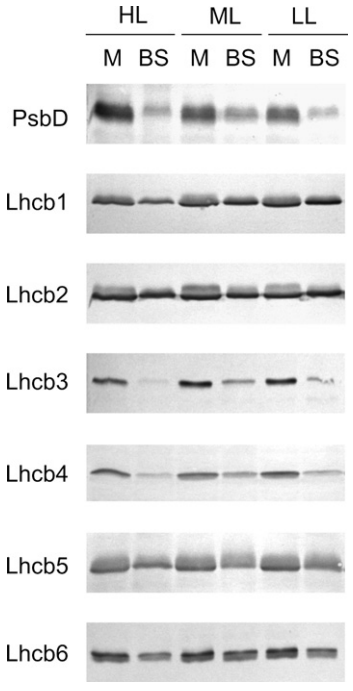

Fig. 2. Immunodetection analysis of PsbD and Lhcb polypeptides in mesophyll (M) and bundle sheath (BS) chloroplasts isolated from the leaves of high light (HL), moderate light (ML) and low light (LL) grown maize plants. Sample loading was done on an equal chlorophyll basis.

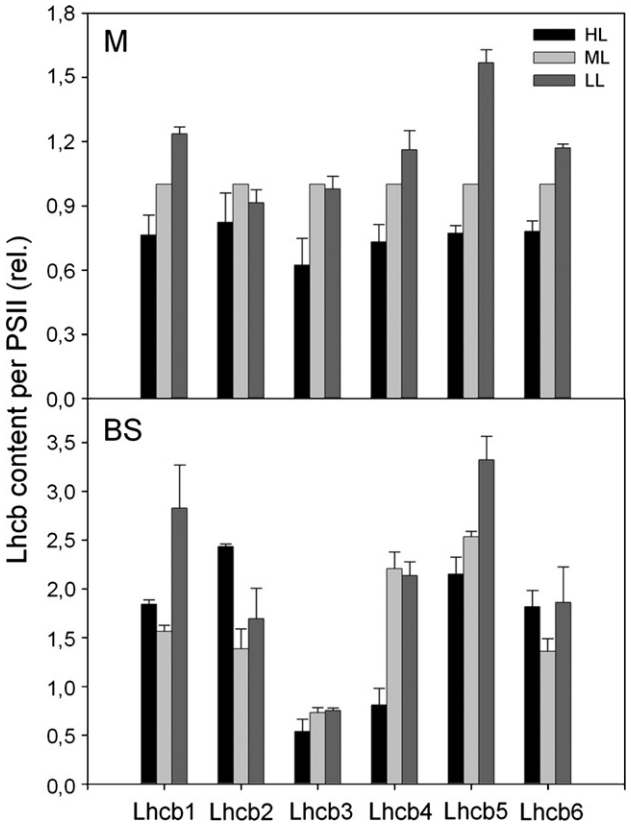

Fig. 3. Effect of different irradiance (high light—HL, moderate light—ML, low light—LL) on PSII antenna composition in mesophyll (M) and bundle sheath (BS) chloroplasts from maize. The relative abundances of Lhcb polypeptides, as determined on immunoblot by quantitative analysis (GeneTools SynGene), were normalized to quantifications of signals from PsbD. Lhcb/PsbD ratio in mesophyll chloroplasts from ML growing plants was defined as 1.

5). It has been known that LHCII/RC ratio in BS chloroplasts of maize is higher than in granal chloroplasts [34]. This result was also observed for all Lhcb polypeptides with exception of Lhcb3 for which Lhcb3/PsbD ratio was almost the same for both types of chloroplast in plants grown under HL and slightly diminished for BS chloroplasts as compared to mesophyll chloroplasts in ML and LL grown plants.

In M chloroplasts levels of Lhcb polypeptides per reaction center were reduced as growth irradiance increased, however the ratios for Lhcb2 and Lhcb3 did not changed in ML and LL conditions. For BS chloroplasts the picture was much more complicated. Like in M chloroplasts, there were not observed differences in Lhcb/PSII ratio in ML and LL conditions but in this case for Lhcb3 and Lhcb4 polypeptides. Strong relationship between the irradiance during growth and the levels of Lhcb1, Lhcb4 and Lhcb5 proteins was also observed. Levels of these proteins per PSII were reduced in HL plants and increased in both, ML and LL grown plants.

The response of LHCI proteins to growth irradiance differs from that observed for LHCII (Fig. 5). The content of Lhca1 and Lhca4 proteins per PSI reaction center decreased as growth light lowered and this phenomenon was particularly apparent in BS chloroplasts. In this case the greatest differences were between HL and ML conditions rather than between ML and LL ones. The same changes in Lhca2 and Lhca3 levels were observed relative to PSI. For BS chloroplasts this ratios were the same in ML and LL but increased in HL condition. In M chloroplasts the Lhca3/PSI ratio was similar in plants grown under ML and LL conditions but it decreased under HL. There was not evident influence of light on Lhca2/PSI ratio for mesophyll chloroplasts.

4. Discussion

C_4 plants developed method to concentrating CO_2 in bundle sheath cells which served to abolish O_2 inhibition of Rubisco oxygenase activity [36]. It is the reason that this type of photosynthesis is so efficient and it is also thought that it cannot be saturated by light. It is also thought that ultrastructure of their chloroplasts is not affected by light intensity [24].

In this study we have investigated photosynthetic acclimation of maize plants in response to varying light intensity during growth. We analyzed how different irradiance levels affected composition of light-harvesting complexes in M and BS chloroplasts and how these changes influenced activities of photosynthetic apparatus.

It has been known that irradiance affects size and composition of light-harvesting complexes [7,10,11,14,15]. We examined composition of LHCII and LHCI in both types of chloroplasts of maize grown at different light conditions. Different irradiance level influence total chlorophyll content in plant leaves [7,37] but Chl *a*/*b* ratios indicate changes in composition of photosynthetic complexes [13,15,37]. A decrease in this ratio was observed in LL leaves (Table 1), indicating that more light-harvesting complexes were present in chloroplasts of these plants because Chl *b* is mainly bound to LHCI and LHCII antennas. Surprisingly, despite decline in chlorophyll content in HL leaves there were no changes in Chl *a*/*b* ratio (about 4.0) between HL and ML leaves.

It is notable that level of Lhcb polypeptides was high in bundle sheath chloroplasts compared with that of D2 proteins (Fig. 3). It has been shown that LHCII polypeptides are present in BS chloroplasts of maize and that their precursor can be incorporated into pigment light-harvesting complex [23]. According to Bassi et al. [34] the ratio of LHCII to D1 in BS chloroplasts was approximately eight times higher than in granal chloroplasts. Results in this study are in agreement with previous observations. In BS chloroplasts, the higher Lhcb/D2 ratio was observed for all Lhcb polypeptides with the exception of Lhcb3 and Lhcb4 proteins. Lhcb3/D2 ratio in plants from different irradiance levels and Lhcb4/D2 ratio in HL grown plants was similar in both M and BS chloroplasts.

Comparison of amounts of LHCII proteins demonstrated that in HL grown plants there was generally lover level of Lhcb polypeptides per D2 proteins and this level increased at lower irradiance. The amount of some polypeptides, like Lhcb1, Lhcb4, Lhcb5 increased in LL plants more than other polypeptides, indicating changes in composition of PSII. These changes were apparent in both M and BS chloroplasts and were more prominent in BS chloroplasts. We show for the first time that at low irradiance the level of LHCII increased also

in BS chloroplasts although they are agranal with reduced amount and electron transferring activity of PSII [22,34,35]. Previous studies by Caffarri et al. [38] demonstrated that in maize multiple *Lhcb1* gene products are accumulate and genetic complexity of *Lhcb1–3* genes, including several members with high homology might be a part of basic mechanism for acclimation to environmental conditions of the photosynthetic apparatus.

Although maize was grown under HL irradiance the levels of Lhcb polypeptides were still high (Figs. 2 and 3) and observed increase in level of these proteins at lower light conditions were not as drastic as those described for *Arabidopsis* [15]. Spatial division of C_4 photosynthesis into two types of cells results in the increase in photosynthetic efficiency [36]. This is why maize can acclimate to high light conditions with only slightly decline in level of Lhcb polypeptides. High light grown plants exhibited a decreased efficiency of PSII (Fv/Fm—0.76) (Table 1). It has been known that photoinactivation of this complex can depend on antenna size [13] but, it appears, that in maize high growth irradiance (1000 μmol m^{-2} s^{-1}) does not result in visible photoinhibitory damage.

Light-dependent changes in levels of Lhca polypeptides appear to be more complex. As observed previously [15,38], contrary to PSII, the amount of PSI increase with decreasing irradiance. At lower light conditions the levels of Lhca polypeptides lowered (Lhca1 and Lhca4), did not change for Lhca2 or increased for Lhca3 (Fig. 4). The levels of Lhca1 and Lhca4 changed in a similar manner possibly because they form heterodimers whereas Lhca2 and Lhca3 exist as either separate homodimers or heterodimers [39–41]. Ben-Shem et al. [41] also suggested that Lhca1–Lhca4 dimer could act as an anchor for facilitating the binding of other LHCI monomers and dimers at varying stoichiometries, depending on environmental conditions. The differences in Lhca3/PSI ratios were observed in M and BS chloroplasts mainly due to greater changes in PSI content in BS chloroplasts. As noticed for LHCII, changes in level of Lhca and other PSI reaction centre polypeptides are also more pronounced in BS chloroplasts.

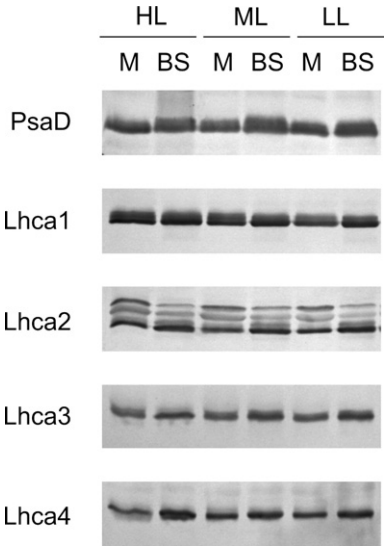

Fig. 4. Immunodetection analysis of PsaD and Lhca polypeptides in mesophyll (M) and bundle sheath (BS) chloroplasts isolated from the leaves of high light (HL), moderate light (ML) and low light (LL) grown maize plants. Sample loading was done on an equal chlorophyll basis.

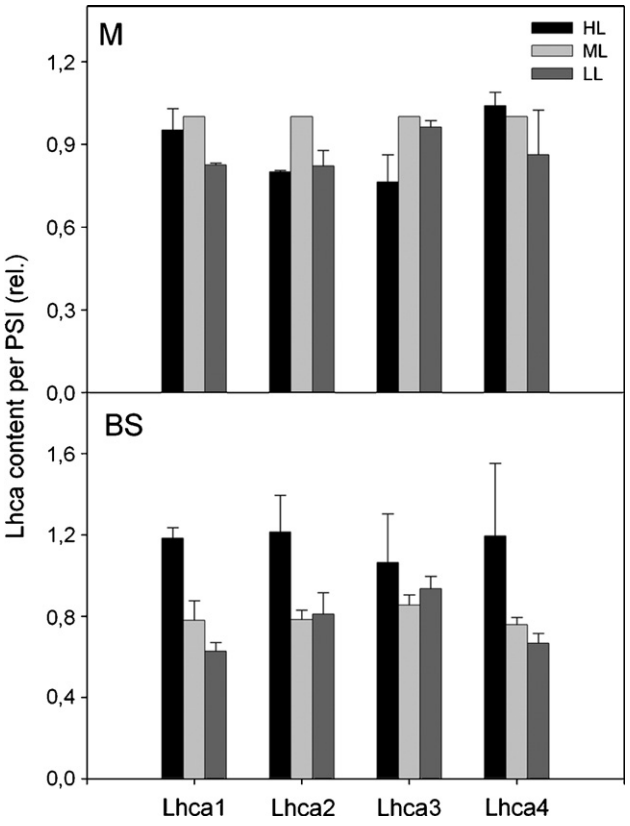

Fig. 5. Effect of different irradiance (high light—HL, moderate light—ML, low light—LL) on PSI antenna composition in mesophyll (M) and bundle sheath (BS) chloroplasts from maize. The relative abundances of Lhca polypeptides, as determined on immunoblot by quantitative analysis (GeneTools SynGene), were normalized to quantifications of signals from PsaD. Lhca/PsaD ratio in mesophyll chloroplasts from ML growing plants was defined as 1.

Nishiro et al. [42] and Evans and Voglemann [43] demonstrated that there was differential acclimation for individual cells or chloroplasts according to their position within a spinach leaf. The anatomy of maize leaf differs from that of spinach with chloroplasts from BS cells being more homogenous than the mesophyll ones mainly due to their predominant location around vascular bundles. This, in it self, may be a reason why the effect of different irradiance is more pronounced in BS chloroplasts.

We also investigated how observed changes in light-harvesting complex composition influenced functioning of photosynthetic apparatus. Measurement of Φ_{PSII} and qP (Fig. 1) showed that there were no differences in light response of quantum yield and oxidized state of PSII between plants grown under low and moderate light conditions (Fig. 1). These parameters were lower above 350 μmol m^{-2} s^{-1} of actinic light and this decline proceeded in the same way for these plants. The Φ_{PSII} and qP values also diminished for high light grown plants but these values registered above 800 μmol m^{-2} s^{-1} were similar to values for low and moderate light grown plants. The decrease for higher actinic light was not so drastic as that

recorded even in high light acclimated *Arabidopsis* [44]. There was not observed, more rapid reduction in Φ_{PSII} and qP characteristic for low light acclimated C_3 plants compared to these acclimated to high light [44–46]. Moreover, plants grown at high and moderate light showed the same pattern for NPQ in all actinic light intensities but low light grown maize had lowest NPQs at higher irradiances. It has been known that NPQ depends on a lot of processes and is not directly related to the xanthophyll cycle but it also depends on LHCII aggregation [47], electron transport capacity [45] and level of PsbS protein [48]. The PSI and PSII activities were similar in LL and ML grown plants (Table 2) hence electron transport capacity did not have an effect on NPQ. A decrease of NPQ in low light plants can be attributed PsbS level because expression of *PSBS* gene increases in light-dependent manner [44,49].

Light response curves suggest that although maize plants grown at high irradiance had lower PSII efficiency in M chloroplasts, very high activity of PSI in BS chloroplasts can compensate this effect and thus high rate of photosynthesis at high irradiance. This response of maize represents a strategy that is optimal in high light conditions to protect photosynthetic apparatus against photodamage. It allows C_4 plants to attain full competence of photosynthesis. Plants grown at low and moderate light condition maintained efficient photosynthesis over the broad range of irradiance and their electron transport capacity becomes saturated at the same irradiance. Moreover maximal electron transfer activity of PSII and PSI in mesophyll and bundle sheath chloroplast remained at the same level in plants grown in ML and LL (Table 2). HL grown plants had increased photosystem activities (2.2- and 1.5-fold for PSII and PSI, respectively) in both types of chloroplasts. An increase in PSII activity can partly depend on protein level of reaction center because there was higher amount of D2 polypeptide in chloroplasts of HL plants (Fig. 2). On the other hand, PSI activity appears to depend on other factors. Meierhoff and Westhoff [50] showed that although bundle sheath chloroplasts of maize contained amount of PSI reaction center polypeptide identical or slightly higher to that in M chloroplasts, PSI activity was approximately 2-fold higher in the BS chloroplasts. We showed that level of reaction center protein of PSI increased when irradiance decreased (Fig. 4), especially in BS chloroplasts, because in M chloroplast this level was almost the same, but activity of this complex declined (Table 2).

Apparent lack of differences in light response of Φ_{PSII} and qP, PSI and PSII activities and in the level of PQ pool (Table 1) between LL and ML plants indicates that although the plants were grown under different irradiance (50 and 350 μmol m^{-2} s^{-1}) these light conditions have similar effect on light reactions and also on CO_2 uptake (11 and 13 μmol CO_2 m^{-2} s^{-1} for LL and ML grown plants, respectively). Despite the fact that acclimation to low light did not affect functioning of photosynthetic apparatus, as compared to plants from moderate irradiance, it caused the increase in LHCII complexes in both types of chloroplasts. Irradiance in the range 50 – 350 μmol m^{-2} s^{-1} does not change photosynthesis whereas maize grown at HL has higher photosynthesis (38 μmol CO_2 m^{-2} s^{-1}) due to higher activities of photosystems, higher level of PQ pool (Table 1) and changes in antenna complexes as compared to ML and LL plants (Figs. 3 and 5).

In conclusion our data reveal that although acclimation of maize to ML and LL caused changes in LHCI and LHCII complexes it does not influence light reactions of these plants and the rate of photosynthesis. On the other hand, the changes in photosynthetic apparatus in HL grown plants resulted in higher photochemical activity and increased rate of photosynthesis. It appears that acclimation to LL, ML and HL in maize plants involves two strategies: first in the range 50–350 μmol m^{-2} s^{-1} and second at high light irradiance. Under the lower irradiance acclimation processes are limited. We suggest that agranal maize BS chloroplasts might be also the place where acclimation processes to light irradiance are realized. Further experiments are needed.

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