19

Functional Analysis of *LHCB1* in *Arabidopsis* Growth, Development and Photosynthetic Capacity

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1. Introduction

The light-harvesting chlorophyll a/b-binding proteins of photosystem II (LHCII) are the major components of the photosynthetic machinery in plants which contain more than 60% of plant chlorophyll (Peter and Thornber 1991). LHCII has four related roles in plant photosynthesis i.e. collecting and transferring excitation energy to the reaction centers of photosystem II (PS II) and photosystem I (PS I) to promote photosynthetic electron transport (Ruban et al., 1999, Van Amerongen and Dekker, 2003), organization of the plant photosynthetic system by maintaining the tight appression of thylakoid membranes in chloroplast grana (Allen and Forsberg, 2001), distribution of excitation energy between PS II and PSI by reversible phosphorylation at its N-terminal side (Allen and Forsberg, 2001, Kargul and Barber 2008), and protection of photosynthetic system from excess energy under light saturated conditions (Horton et al 1996 and 2008).

The LHCII proteins can be grouped into six subfamilies (LHCB1-6) which are encoded by *LHC* gene family (Jansson 1999). CP29, CP26 and CP24 are the minor proteins that are encoded by *LHCB4, LHCB5* and *LHCB6* genes, respectively. LHCB1, LHCB2 and LHCB3 are the major pigment-binding proteins which are encoded by *LHCB1, LHCB2* and *LHCB3* genes, respectively (Ruban et al 1999, Lucinski and Jackowski 2006). LHCB1, LHCB2 and LHCB3 polypeptides each with about 232 amino acid residues are similar in sequence, structure and function (Standfuss and Ku¨hlbrandt 2004). LHCB1-3 precursors are synthesized in cytoplasm and following transport into chloroplasts inserted into thylakoid membranes (Li et al., 2000). LHCB1 and LHCB2 are the most abundant proteins in the light harvesting antenna complex (Ruban et al., 1999). The N-terminal domain in both LHCB1 and LHCB2 lies on the stromal side where it is involved with adhesion of granal membranes and photo-regulated by reversible phosphorylation of its threonine residues (Boekema et al 1999, Anderson 2000).

The composition and structure of LHCII complex is regulated by different factors. For example light intensity can change the amount of light-harvesting complex components (Anderson et al., 1986, Bailey et al., 2001). Meanwhile it has been reported that the expression of *LHCB1* can be down regulated by accumulation of sugars such as glucose, sucrose and trehalose (Vinti et al., 2005, Aghdasi et al., 2009).

The antisense suppression of *Arabidopsis LHCB1* also leads to *LHCB2* suppression. These plants have reduced state transitions and capacity for feedback de-excitation important to adapt to changes in light intensity (Anderson et al., 2003). Over expression of *LHCB1-2* from pea in tobacco plants led to increased grana stacking and photosynthetic capacity at low irradiance. The transgenic plants also displayed increased cell volume, larger leaves, increased biomass and increased seed weight, and greater leaf number per plant at flowering, when grown under low irradiance levels (Labata et al., 2004).
So far, the function and importance of *LHCB1* alone in *Arabidopsis* growth, development and photosynthetic capacity have not been understood very well. In the current study, we screened Leclere and Bartel collection to identify mutants in *LHCB1* (Leclere and Bartel 2001). This led to the identification of one mutant in At1g29920 gene, *lhcb1,* with pale green phenotype. Characterization of the *lhcb1* mutant was achieved through its comparison with the wild type (WT) plants when both grown under normal and low irradiances. Furthermore, the over-expression of *Arabidopsis LHCB1* was carried out to confirm the function of the encoded protein in growth and development.

2. Methods and materials

2.1 Plant materials and growth conditions:

The *Arabidopsis thaliana* wild type (WT) plants ecotype Columbia-0 (COL-0), transgenic lines and *lhcb1* mutant seeds were planted in compost and watered twice per week. Plants were grown in controlled growth chamber under normal (150 µmol photon m^{-2} s^{-1}) and low (70 µmol photon m^{-2} s^{-1}) irradiances and a 25 ^{o}C day/ 20 ^{o}C night temperature regime.

2.2 Screening for *lhcb1* mutant

The collection of *Arabidopsis* 35S-cDNA lines described by LeClere and Bartel (2001) was used in this study. Seeds from 331 pools from this collection were screened. They were surface sterilized by the gas method sterilization (Clough and Bent, 1998). Sterilized seeds were plated on ½ Murashige and Skoog (MS) medium solified with 0.8 % agar (Murashige and Skoog, 1962). Seeds were stratified in darkness at 4 ^{o}C for 2 days, before transferring to growth chamber at 25 ^{o}C. A pale green mutant was characterized from this collection. The mutant plants were transferred to soil to generate second seed generation (S2). Seeds from S2 generation were grown on medium with 12.5 mg/L PPT (Phosphinotrice). Growth on PPT, allows the segregation of the T-DNA insertion carrying the CaMV promoter driven cDNA expression cassette. After 14 days, seedlings were screened for segregation of T-DNA inserted on 12.5 mg/L PPT. Seedlings resistant to PPT were transferred to soil along with WT plants. Upon flowering of the plants, crosses were carried out with the WT plants. The individual silliques were collected in one bag after ripening. To do seed re-screening, they were sown separately from each silique on ½ MS medium supplemented by 12.5 mg/L PPT.

2.3 DNA extraction and PCR analysis

Three small leaves were frozen in liquid nitrogen, pulverized with glass beads for 2 minutes at 2800 rpm in a Dismembranator (Braun, Germany), and then DNA was extracted using the Pure Gene DNA isolation kit (Amersham Pharmaciabiotec, England) according to the manufacturer's protocols. To determine the presence of the 35S cDNA fragments

in the pale green *lhcb1* plants, PCR was performed with primers 35S-F (CGCACAATCCCACTATCCTTCGCAAG) Nos-R (GATAATCATCGCAAGACCGGAACAGG) primers. A mixture of *Taq* and PFU enzymes at unit ratio of 50:2 was used. After denaturation for 2 minutes at 94 ºC, DNA was amplified with 35 cycles (30 sec 94 ºC, 30 sec 56 ºC and 2 min 72 ºC). PCR was completed with a final step at 72 ºC for 5 minutes. An aliquot from the PCR product was run on agarose gel and the remaining was cleaned using DNA purification kit (Amersham Biosciences, England).

2.4 RNA extraction and cDNA synthesis

Total RNA was extracted from 10 days old *Arabidopsis* plants. Whole plant material was snap frozen in liquid nitrogen, pulverized with glass beads for 2 minutes at 2800 rpm in a dismembrator (Braun, Melsungen, Germany). Total RNA was isolated with the RNeasy plant mini kit (QIAGEN USA, Valencia, CA). RNA concentration and purity were determined by measuring the absorbance at 260 nm. To remove any possible contamination by genomic DNA, 10 ng of RNA was treated with 2 U of DNAse I (DNA-free, Ambion, Austin, USA). The absence of DNA was analyzed by performing a PCR reaction (40 cycles, similar to the real–time PCR program) on the DNaseI-treated RNA using *Taq* DNA polymerase. Reverse transcriptase PCR (RT-PCR) experiments were performed using 1 ng of total extracted RNA and used for first-strand cDNA synthesis with 60 U of M-MLV reverse transcriptase (Promega, Madison, WI), 0.5 µg of odT16v (custom oligo from Invitrogen, Carlsbad, CA) and 0.5 µg of random hexamer (Invitrogen, USA). PCR was performed with forward and reverse primers (5´-ctcaacaatggctctctcct-3´ and 5´- aacccaagaactgaaaatccaa-3´). Amplification conditions were performed as initial DNA denaturation at 94ºC for 2 minutes followed by 35 cycles of 1 minute denaturation at 94ºC , 30 second annealing at 56 ºC and 2 minutes of extension at 72 ºC with a final extension time at 72ºC for 10 minutes. An aliquot of the PCR product was run on an agarose gel (1%) and the remaining PCR product was cleaned using a DNA purification kit (Amersham Biosciences, England).

2.5 Cloning cDNA fragments into pGEM-T Easy vector

The resulting cDNA fragments from the previous steps were ligated into the pGEM-T Easy vector. For this purpose, cDNA was concentrated to 3 µl (25 ng) and was then added to 5 µl of 2X ligation buffer, 1 µl of T4 Ligase and 1 µl of pGEM-T easy vector. The ligation mixture was incubated over night at room temperature. An aliquot (100 µl) from the competent *E. coli* were taken from the -80 ºC freezer and thawed on ice for 20 min. The over- night ligation mixture was added to the cells and the mixture was left on the ice for 20 minutes. Heat shock was applied for 50 sec at 42 ºC, followed by a 5 min cooling period on ice. One ml of lysogeny broth (LB) medium was added and cells were incubated at 37 ºC for 1 h. The LB plates contained 50 µg/ml of Ampicillin for selection. Isopropyl-β-D-1-hiogalactopyranoside (IPTG) and XGalactopyranoside (X-Gal) were added for screening of blue and white colonies. To check colonies containing the plasmid with the ligated fragments, restriction enzyme analysis was performed. Plasmids were isolated from 5 colonies using a plasmid miniprep kit (Sigma, USA). In the digestion mixture, 2 µl of plasmid, 1 µl of 10 X buffer, 6 µl of milli-Q water and 1 µl of *Eco*R1 were used. Samples were digested at 37 ºC for 1.5 h. The obtained fragments were analyzed by agarose gel electrophoresis.

2.6 Sequence analysis

DNA sequencing was carried out at the sequencing facility in Wageningen University. Sequences obtained from analysis with forward and reverse primers (T7: 5´ atttaggtgacactatag 3´ and SP6: 5´ taatacgactcactataggg 3´) were aligned and the PCR fragment structures reconstructed by BLAST (Basic Local Alignement Search Tool) searches in TAIR (http://www.arabidopsis.org/Blast/).

2.7 cDNA over-expression constructs and re-transformation into Col-0

Full length cDNA were isolated and purified from the pGEM-T easy vector clones and over-expressed in wild type (WT) *Arabidopsis* plants. The CaMV 35S expression cassette was isolated by digestion with *Eco*RV from the pUC-18 vector. The cassette was filled with Klenow and dNTP and subsequently ligated into pBin19 (*Hin*dIII/*Eco*RI) to yield pBin-35S. Purified fragments were cloned into the pBin-35S expression cassette, resulting in pBin35S/cDNA/NOS. The plasmids containing *LHCB1* gene was digested with *Xba*I and *Xmn*I restriction enzymes. The construct was introduced by electroporation into *Agrobacterium tumefaciens* containing the pGV2260 plasmid. The resulting bacteria were used to transform *Arabidopsis* by floral dip method (Clough and Bent, 1998). Transgenic seedlings were selected on half MS media containing 50 mg/L of Kanamycin. Transgenic seedlings were grown in soil medium under either normal or low irradiances for further phenotypic characterization.

2.8 Expression analysis of the *LHCB1* gene

Quantitative-PCR (Q-PCR) analysis was performed to determine the expression level of *LHCB1* gene. Total RNA was extracted from *Arabidopsis* seedlings as described above. Following treatment of RNA with DNAase I, cDNA was synthesized using the M-MLV reverse transcriptase system (Promega, Madison, WI). Q-PCR was carried out by ABI-prism 7700 Sequence Detection System (PEApplied Biosystems, Foster City, CA). For each reaction, 12.5 µl of green PCR Master Mix (Applied Biosystems, UK) and 2.5 µl of gene-specific primers were used. Each experiment was repeated 3 times. Relative gene expression was based on the comparative Ct method (User Bulletin No. 2: ABI PRISM 7700 sequence detection system, 1997) using *AtACTIN2* as the calibrator reference (5´-ATGTCTCTTACAATTTCCCG-3´ and 5´- CAACAGAGAGAAGATGACT- 3´). The Q-PCR data were normalized against *AtACTIN2*.

2.9 Pigment content and Florescence measurements

Chlorophylls a, b and total chlorophyll were determined spectrophotometrically as described by Jeffery and Humphery (1975). In brief, 100 mg of fresh rosette leaves from 3-week-old *Arabidopsis* plants were grounded in liquid nitrogen and extracted with 80% (v/v) acetone. Absorbance was then measured at 647, 652 and 664 nm. The concentrations of chlorophylls a, b and total chlorophyll were then calculated.

Anthocyanin content was determined using the protocol of Mita *et al*. (1967). Frozen and homogenized leaves (20 mg) were extracted for 1 day at 4 °C in 1 ml of 1% (v/v) hydrochloric acid in methanol. The mixture was centrifuged at 23,000 ×*g* for 15 minutes and the absorbance of the supernatant was measured at 530 and 657 nm. Relative anthocyanin concentrations were calculated using the formula [*A*530-(1/4 × *A*657)]. The relative anthocyanin content was defined as the product of relative anthocyanin concentration and the extract volume. One anthocyanin unit equals to one absorbance unit [*A*530-(1/4 × *A*657)] in 1 ml of the extraction solution.

Chlorophyll *a* florescence was measured with OPTI-Sciences OS-30 fluorometer (Walz, Effeltrich, Germany). The *Arabidopsis* plants were adapted in the dark 15 minutes before measurement. F_0 (the initial fluorescence level of PSII reaction center) was measured in the presence of a 10 µmol photons m^{-2} s^{-1} measuring beam. The maximum fluorescence level in the dark adapted state (F_m) was determined by using a 0.8 s saturating irradiance pulse. The fluorescence parameter F_v/F_m was calculated using the DualPAM software

2.10 Chloroplast isolation and determination of Hill reaction rate

The rate of Hill reaction in the chloroplast preparations of WT, *lhcb1* and *LHCB1* over-expressed plants was measured according to Trebst (1972). Leaves (0.25 g) were homogenized in a cold mortar in a buffer consisting of 20 mM Tris-HCl (pH 7.5), 0.3 M sucrose, 10 mM EDTA and 5 mM MgCl2 and chloroplast were isolated. The rate of Hill reaction in the illuminated chloroplast preparations was determined spectrophotometrically by recording the decrease in absorbance at 600 nm due to Dichlorophenol indo phenol (DCPIP) reduction. The rate of Hill reaction was expressed as the changes in absorbance per milligram chlorophyll per minute (ΔOD.min $^{-1}$. mg chl $^{-1}$).

2.11 Carbohydrate and protein determination

The soluble and insoluble sugars were determined spectrophotometrically by the phenol-sulfuric acid method (Kuchert, 1985). The leaf soluble and total proteins were determined according to methods of Bradford (1976) and Markwell (1988), respectively.

2.12 SDS-PAGE analysis of chloroplast proteins

The reducing SDS-PAGE of the chloroplast protein samples was carried out according to Fling and Gregerson (1986). For the SDS-PAGE analysis of the chloroplast proteins, chloroplasts were isolated from leaves (1 g) as described above and finally suspended in 100 µl of the homogenization buffer. Then 5.0 ml n-Hexan: 2-propanol (3:2; V/V) was added and after a thorough mixing, it was centrifuged at 4000 *g* for 15 min. The lipid-pigment containing upper phase was discarded and the remaining pellet was re-extracted with another 5.0 ml of n-Hexane: 2-propanol mixture. The protein precipitate obtained after doing the second centrifugation, was washed with 5.0 ml acetone (80% V/V) and dried under a stream of nitrogen gas. The dried protein precipitates were dissolved in electrophoresis sample and following quantification by the Markwell (1988) method, aliquots corresponding to 50 µg protein were resolved on a 15% SDS-containing Acrylamide gel.

2.13 Statistical analyses

Data from all experiments were processed by statistical SAS package (version 9). The reported values were means of three replicates. Means were compared for significance using the Duncan's test.

3. Results

3.1 Isolation of the *lhcb1* mutant

After screening the LeClere and Bartel mutant seed collection, a pale green mutant was identified. The pale coloration was uniformly displayed by all leaves throughout the whole life of the mutant. The selected mutant was fully fertile (Fig. 1A).

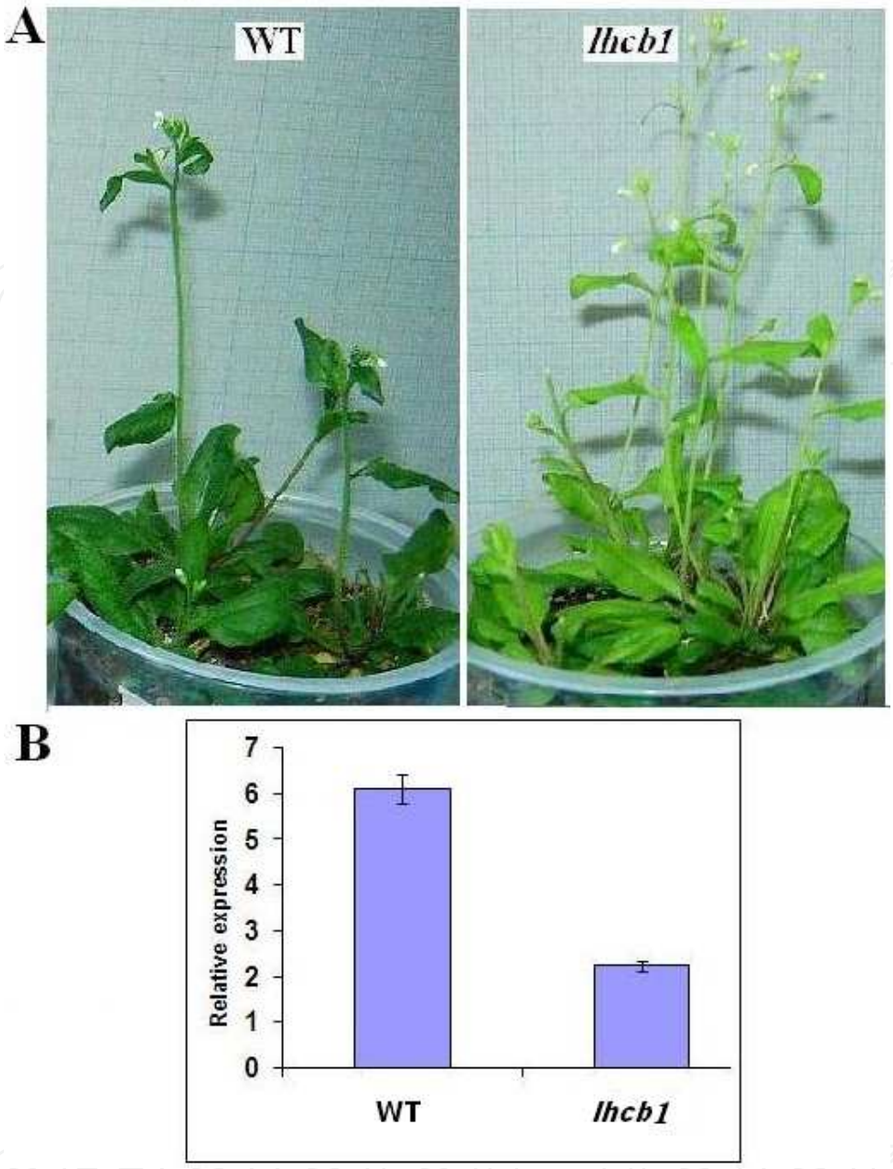

Fig. 1. Characterization of the *Arabidopsis lhcb1* mutant. A: Phenotype of the *lhcb1* mutant, as compared to the wild type (WT) Columbia-0 ecotype. B: Q-PCR analysis of *LHCB1* expression level in WT and *lhcb1*mutant plants.

To ensure the presence of T-DNA insertions in the selected line, a secondary screening was carried out on seeds from the selected pale green plants of the primary screen. Segregation analysis on PPT (phosphinotrice) showed that the line was homozygous for the T-DNA insertion. Cosegregation of resistance to PPT (flanked to T-DNA) and the pale green phenotype confirmed that the phenotype of the mutant has cosegregated with T-DNA insertion (Data not shown). To find out if the selected line contains cDNA fragments, we performed PCR using a forward primer on the CaMV35S promoter and a reverse primer on the nopaline synthase poly-adenylation sequence (LeClere and Bartel 2002). PCR reactions in the selected line yielded one fragment only. Sequence analysis of the PCR product

indicated that cDNA fragment was full length, with ATG and TGA, in sense orientation and it encodes LHCB1 (At1g29920).

To find out whether the pale green phenotype is dominant or recessive, backcrossing between the selected line and WT plants was carried out. Analysis of segregation of resistance to PPT revealed that PPT resistance segregates as a single locus.

The level of *LHCB1* expression was determined in seedlings from both WT and the selected mutant. The expression level of *LHCB1* in the selected mutant was significantly lower than that of the WT plant. This indicates that in the selected line, the pale green phenotype is due to co-suppression of *LHCB1* expression (Fig.1B). This mutant hereafter named *lhcb1* mutant.

3.2 Transformation of cDNA construct into WT plants

Transformation of the WT plants (Colombia-0 ecotype) with full length cDNA of *LHCB1* yielded 20 independent lines with resistance to the selection marker. Two independent transgenic lines were selected for mRNA level analysis (TR-1 and TR-2). The transcript levels of *LHCB1* in TR-1 and TR-2 lines were increased by approximately 53% and 47% respectively, compared to WT plants (Fig. 2).

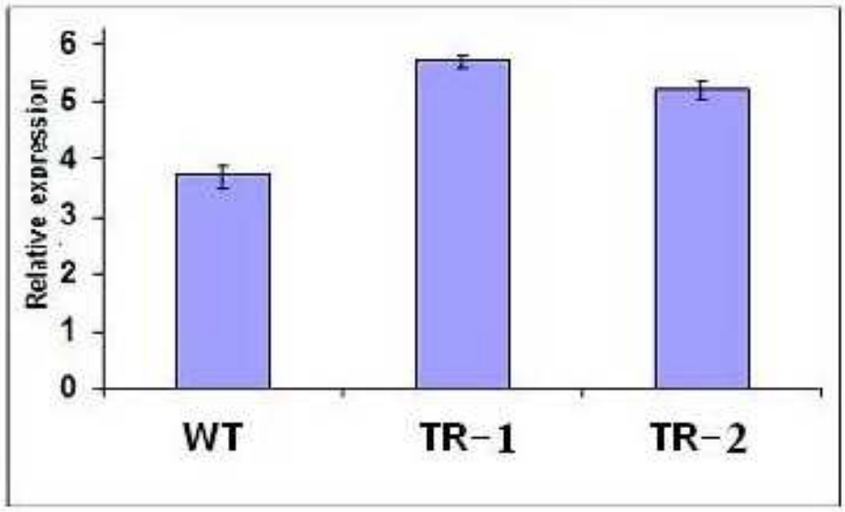

Fig. 2. *LHCB1* expression level in Transformed (TR) lines, as compared to WT plants.

3.3 Characterization of *lhcb1* mutant and WT plants

The *lhcb1* mutant plants displayed differences to the WT plants in growth, morphology, leaf area, dry and fresh weight when grown under normal and low irradiances. The *lhcb1* mutants showed pale green phenotype with smaller leaf area (Fig.1 and 3A). Dry and fresh weights were significantly lower in *lhcb1* mutant than that of the WT plants (Table 1). There was no significant difference in height between *lhcb1* mutant and WT plants under normal irradiances (Fig.1, Table 1). Plants grown under low irradiances were taller than those grown under normal irradiances. Under normal light conditions, the height of 4-week old *lhcb1* and WT plants were 13.66±3.16 and 15±1 cm respectively, while under low irradiances the height of *lhcb1* and WT plants were approximately 2 times more (Fig. 3B and Table 1). Relative to the WT plants, the *lhcb1* mutants were indifferent with respect to flowering time and fertility. The WT and the *lhcb1* mutant plants approached to flowering stage after 38 and

36 days, respectively under normal irradiances, however, this stage was shortened to 27 and 25 days, respectively under low irradiances. Carbohydrate analyses showed that both soluble and insoluble contents were similar in both *lhcb1* mutant and WT plants (Table 1).

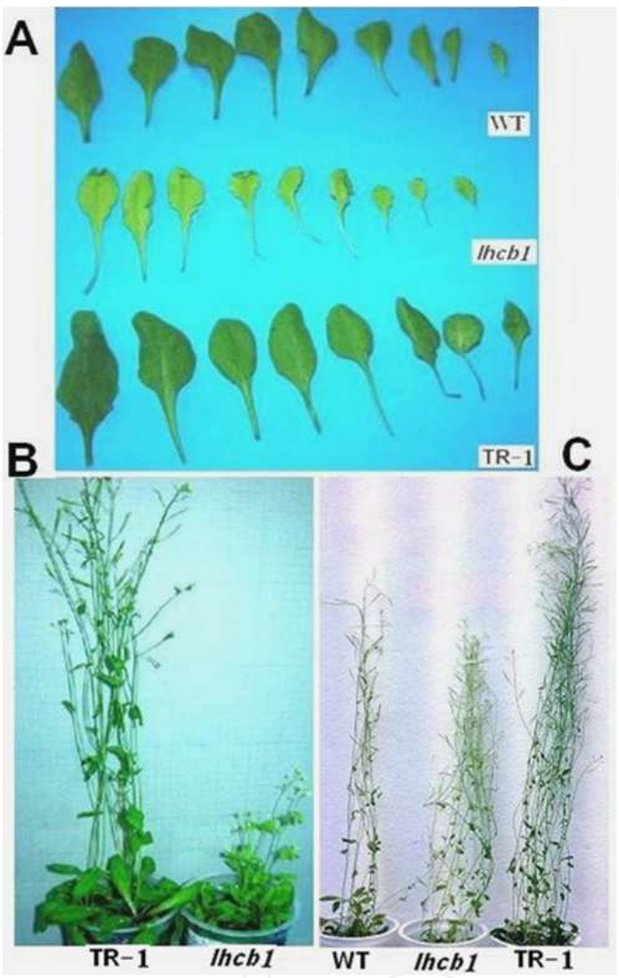

Fig. 3. Morphological characteristics of *lhcb1* mutant, transformed (TR-1) and WT plants grown under normal and low irradiance conditions. A: leaf morphology of plants grown under normal irradiance conditions, B: TR-1 and WT plants grown under normal irradiance conditions, C: WT, TR-1 and *lhcb1*mutant plants grown under low irradiance conditions.

3.4 Reduced chlorophyll contents and photosynthesis capacity in the *lhcb1* mutant

As the *lhcb1* mutant plants were clearly pale green in color when compared to WT ones, the chlorophyll contents of them were compared following growth under normal and low irradiances. Under normal light conditions total chlorophyll content of 3-week-old WT leaves was 2.09±0.1 mg/g fresh weight, while total chlorophyll content in the *lhcb1* mutant was 1.02±0.9 mg/g fresh weight (52% less). Meanwhile under low irradiances, total chlorophyll content of 3-week-old WT leaves was 2.65±0.8 mg/g, but that of the *lhcb1* mutant was 1.38±0.15 mg/g fresh weight which is about 48% of the amount found in WT plants. There was an increase in the ratio of Chl *a/b* ratio in the *lhcb1* mutants compared to WT plants grown

under any light conditions. The ratio of Chl *a/b* increased from 2.55 in the WT plants to 3.39 in the mutant plants under normal light conditions. Under low irradiances the figure rated to 1.81 in the WT plants which increased to 3.97 in the *lhcb1* mutant plants (Table 1).

Irradiance	Plant	height (cm)	number of days to flowering	dry weight (g)	fresh weight (g)	soluble sugar (µg/g)	insoluble sugar (µg/g)
Normal	WT	15.00±1	38	0.087±0.01	0.70±0.08	549.7±65.5	103.1±5.3
	lhcb1	13.66±3.16	36	0.035±0.01	0.50±0.15	576.9±557.1	107.3±38.9
	TR-1	31.33±2.08	25	0.14±0.04	1.10±0.26	700.2±690.1	115.4±83.1
Low	WT	31.22±3.44	27	0.09±0.01	0.91±0.12	3194±105.5	259.9±105
	lhcb1	26.66±2.45	25	0.04±0.01	0.47±0.65	2993±193	260.8±96.7
	TR-1	35.42±4.34	25	0.16±0.02	1.53±0.19	3625±169.7	3900±328.8

Table 1. Data on biomass, leaf total carbohydrate and total protein contents of *Arabidopsis* wild type (WT), *lhcb1* mutant and transformed (TR) plants grown under normal and low irradiance conditions.

LHCII functions as an auxiliary antenna for PSII. PSII and LHCII are close to each other in the stacked granal thylakoids. In this survey, PSII activity was analyzed by measuring the F_v/F_m value that is an indicator of the intrinsic efficiency of PSII. There was not any significant difference in fluorescence parameter (F_v/F_m) between WT and *lhcb1* mutant plants grown under normal and low irradiances. The F_v/F_m value was o.86±0.01 in WT and 0.84±0.03 in *lhcb1* mutant plants grown under normal irradiances (Table 1). These results revealed that PSII efficiency was not affected by the mutation in *LHCB1*.

We further examined the water oxidation capacity of the photosynthetic machinery of both WT and *lhcb1* mutant plants under normal irradiance condition. Measured as the rate of Hill reaction, the water oxidation capacity was significantly decreased in the *lhcb1* mutants compared to WT plants (Fig. 4).

3.5 The chloroplast protein composition of the *lhcb1* mutant and WT plants

Leaf materials from *lhcb1* mutant and WT plants were analyzed for total protein measurement and chloroplast protein composition. There was no significant difference in the total protein amount between *lhcb1* mutant and WT plants (Fig. 5A). Furthermore, chloroplasts were isolated from both the mutant and WT plants and their polypeptide compositions were analyzed by SDS-PAGE. The protein band patterns of both WT and *lhcb1* mutant chloroplasts were essentially similar. However, one protein band with a molecular mass of about 25 kDa was absent in the chloroplasts protein preparations of *lhcb1* mutant plants (Fig. 5B)

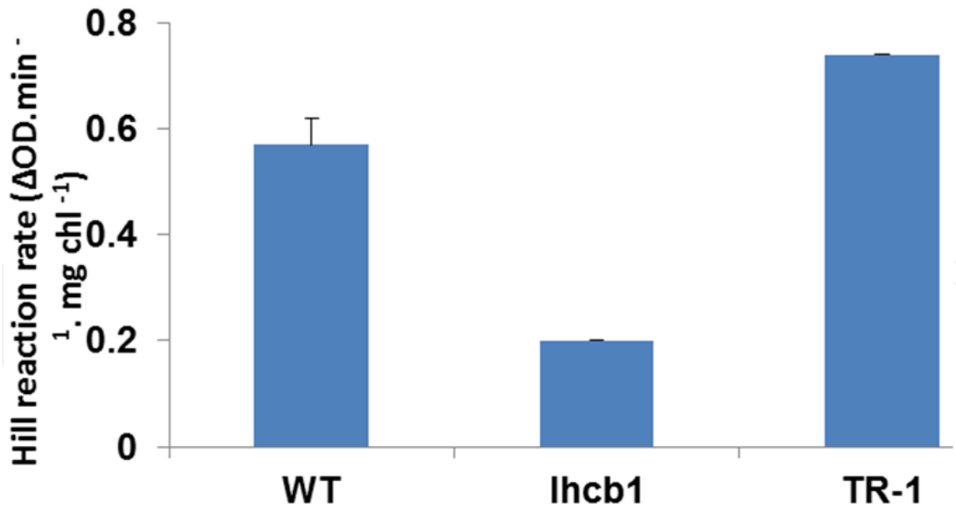

Fig. 4. The water oxidation capacity of WT, *lhcb1* mutant and TR-1 plants grown under normal irradiance conditions as measured by the rate of Hill reaction

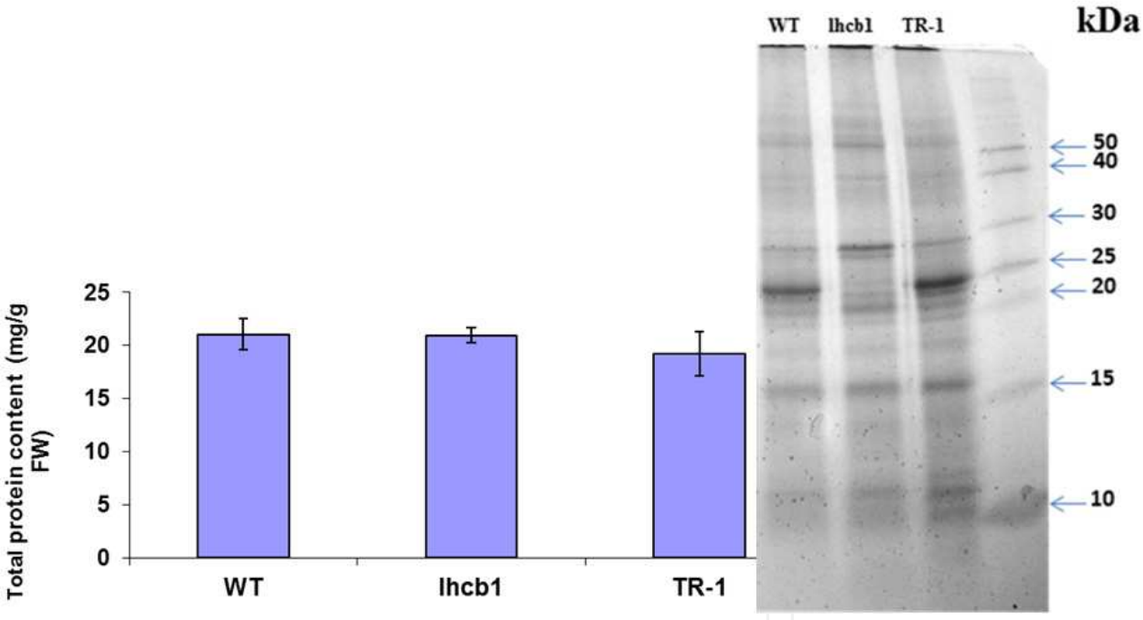

Fig. 5. Total protein content (A) and SDS-PAGE profile of WT, *lhcb1* mutant and transformed (TR) plants (B).

3.6 Transgenic plants display differences in vegetative morphology and growth under normal irradiances

Plants transformed with the full length cDNA of *LHCB1* displayed differences to the WT plants in growth, morphology, height, leaf area, dry and fresh weights, soluble and insoluble sugars when grown under normal irradiances (Fig. 3A, B and Table 1). Under normal irradiance conditions, transgenic plants (TR-1) had larger leaves than that of WT plants (Fig. 3A). Transgenic plants were generally taller (31.33±2.08 cm compared to 15±1 cm

in WT), with increased dry weight (73% more than WT) and fresh weight (75% more than WT). TR-1 plants approached the flowering stage after 25 days under normal light conditions, while in the WT plants this figure extended to 38 days. The average of seed number per silique and the weight of 1000 seeds increased by over-expression of *LHCB1* (41.7±4.4 in TR-1 versus 32.8±2.84 in WT). The leaves of transgenic plants contained a significantly higher soluble and insoluble sugar contents compared to WT plants. Thus, the soluble and insoluble sugars were respectively 700.2±690.01 µg/g FW and 115.4±83.1 µg/g in transgenic plants whereas these figures were 549.7±65.5 µg/g and 103.1±53.1 µg/g in WT plants (Table 1).

Further analysis revealed that there were not any significant differences in Chl *a*, Chl *b*, ratio of Chl *a:b* and total Chl content among WT and Transgenic plants. Meanwhile the efficiency of PSII i.e. F_v/F_m value in transgenic plants was close to that in the WT plants (Table 2). But water oxidation capacity i.e. the rate of Hill reaction was significantly higher in transgenic plants compared to WT ones (Fig 4).

irradiance	Plant line	Chlorophyll content (mg/g FW)				F_v/F_m
		chl *a*	chl *b*	total chl	chl *a/b*	
Normal	WT	1.48± 0.11	0.58±0.08	2.09±0.19	2.55	0.86±0.01
	lhcb1	0.78±0.14	0.23±0.05	1.02±0.19	3.39	0.84±0.03
	TR-1	1.31±0.05	0.53±0.05	1.84±0.10	2.47	0.83±0.05
Low	WT	1.70± 0.01	0.94±0.09	2.65±0.08	1.81	0.84±0.03
	lhcb1	1.10±0.11	0.27±0.04	1.38±0.15	3.97	0.86±0.02
	TR-1	1.52±0.07	0.58±0.05	2.11±0.12	2.59	0.87±0.04

Table 2. Chlorophyll content (chl), Chlorophyll ratio (chl *a/b*) and photosynthetic parameter of *Arabidopsis* wild type (WT), *lhcb1* mutant and transformed (TR) plants grown under normal and low irradiance conditions.

There was not any significant difference in total protein content between transgenic and WT plants. Analysis of the chloroplastic proteins showed that most of the bands were similar in both transgenic and WT plants. However, a polypeptide with a molecular mass of about 25 kDa displayed relatively thicker band intensity in chloroplast protein preparations of the transgenic plants (Fig. 5B).

3.7 Characterization of transgenic plants grown under low irradiances

Further characterization of transgenic plants was achieved after planting them under low light conditions. The height of transgenic plants was close to that in WT ones. It was interesting that *lhcb1* mutants had also the same height as both WT and transgenic plants under low irridiances (Fig. 3C). There was not any significant difference in height between

TR-1 and WT plants (35.42±4.34 cm versus 31.22±3.44 cm). Flowering occurred after 25 and 27 days respectively, in transgenic and WT plants. Dry and fresh weights, soluble and insoluble sugar contents were obviously higher in transgenic plants than in WT ones (Table 1). There was an increase in total Chl content in transgenic and WT plants, compared to those grown under normal irradiances. Total chlorophyll in WT plants was fairly greater than in transgenic plants under low irradiances (2.65±0.8 mg/g FW versus 2.11±0.2 for transgenic plant). There was not any significant difference in the efficiency of PSII (F_v/F_m value) between transgenic and WT plants.

4. Discussion

A novel system designed to co-suppress or over-express cDNA in *Arabidopsis* was developed by LeClere and Bartel (2001). They constructed a binary vector containing a novel *Arabidopsis* cDNA library driven by the CaMV35S promoter. T-DNA in this vector contains a bar-gene cassette for PPT selection of the transgenic plants and a cassette with a randomly cloned cDNA inserted between CaM35S promoter and nopaline synthase (NOS) polyadenylation (polyA) sequences. This method has the advantage that the inserted cDNA can be amplified using PCR with primers in the promoter and polyA sequences (LeClere and Bartel 2001). The cDNA insertion could be responsible for the observed phenotype if both the phenotype and cDNA co-segregate as a dominant trait. Definitive confirmation for the correlation between cDNA and the phenotype in plants exhibiting dominant trait, could be obtained following the transformation of cDNA expression cassette into WT plants. Screening of 331 pools of T4 seeds from this collection displayed one pale green mutant. Sequence analysis of the amplified gene identified *LHCB1* cDNA fragment in this mutant. It was full length, with ATG and TAG, and in sense orientation. Q-PCR data revealed that this construct co-suppressed the endogenous *LHCB1* transcript.

Studies carried out so far to reveal the functional significance of LHCII protein-chlorophyll complexes in phenotypic alterations of plants, have suffered from segregating the specific role played by each individual polypeptides constituting the complex. Thus either the impacts of over-expression of *LHCB1-2* (Labate et al., 2004) or antisense cosupression of *LHCB1-2* (Andersson et al., 2003) has been described. However, in the present study some functional significance of LHCB1 protein was investigated by producing homozygous *lhcb1* mutants (which retained *LHCB2*) and *LHCB1* over-expressed plants. Furthermore, the *LHCB1* suppression / over-expression was accompanied with the corresponding decrease / increase of a polypeptide with a molecular mass of about 25 kDa on the SDS-PAGE gel which is very close to the reported mass range of this protein (Huber et al., 2001, Zolla et al., 2003). These clearly indicate that the genetic manipulations carried out on *Arabidopsis* are translated also at the protein level.

Silencing of *LHCB1* in *Arabidopsis* significantly reduced their chlorophyll content with respect to WT plants. It was evidenced by their pale green coloration and resulted in the increased ratio of Chl *a/b*. As the *lhcb1* mutants displayed significant reduction of biomass and leaf area with respect to WT plants, it can be said that loss of *LHCB1* has greatly compromised the efficiency of carbon assimilation. Reduced chlorophyll content associated with biomass decline has also been reported for *lhcb1-2* antisense plants (Andersson et al., 2003). These might partly be attributed to the significant reduction of water oxidation capacity of the mutant versus WT plants (Fig. 4). As for the *lhcb1-2* antisense plants

(Andersson et al., 2003), no significant alteration in the quantum efficiency of PSII occurred for the *lhcb1* mutants (Table 2). Regarding that the *lhcb1* mutants displayed increased ratio of Chl *a/b* very probably due to LHCB1 loss, it is expected that they observe reduced non-photochemical quenching and feedback de-excitation (Andersson et al., 2003) with respect to WT plants. This possibly makes them more susceptible to photoinhibitory conditions which ultimately reduce their fitness. Although far from higher plants, a mutant of *Chlamydomonas reinhardtii* which lacks a major polypeptide of LHCII, also suffers from nonphotochemical quenching and thus is prone to photoinhibition (Elrad et al., 2002). Considering that LHCB1 is a major target protein for phosphorylation / de-phosphorylation required for state transition (Lunde et al., 2000), its loss in *lhcb1* mutants might decreases the capacity for state transition, a feature which has been reported for *lhcb1-2* antisense plants (Andresson et al., 2003).

Many phenotypic characteristics of transgenic *Arabidopsis* TR-1 versus the WT plants under normal irradiances were similar to those reported for transgenic tobacco plants overexpressing pea *LHCB1-2* (Labate et al., 2004). Thus the *Arabidopsis* TR-1 plants exhibited taller stature, greater biomass, increased carbohydrate contents and larger seed size as compared to WT plants. Similar to transgenic tobacco plants, there were no differences in Chlorophyll content, Chl *a/b* ratio and the quantum efficiency of PSII with respect to WT plants. However, in contrast to the tobacco transgenic plants, the flowering time of *Arabidopsis* TR-1 plants was shortened compared to WT plants. This might be due to photoperiodic behavioral differences of the two species. The increased carbohydrate content of *Arabidopsis* TR-1 plants and their overall greater biomass might represent more efficient carbon assimilation. The non-photochemical quenching of the *Arabidopsis* TR-1 is expected to be greater than WT plants, a feature which has been reported for tobacco *LHCB1-2* transgenes (Labate et al., 2004). On the other hand, the lack of *LHCB1-2* in *Arabidopsis* mutants is associated with reduced nonphotochemical quenching (Anderson et al., 2003). Taking into account that the water oxidation capacity of *Arabidopsis* TR-1 plants (and thus their potential for NADPH generation) is also greater than WT plants, the idea of more efficient photosynthesis is justified. The increase in photosynthetic carbon assimilation efficiency of TR-1 plants might also be explained by the increase in chloroplast number and improved granal stacking (Labate et al., 2004). The increased seed number per silique of TR-1 plants might have been resulted from their photosynthetic superiority with respect to WT plants.

Under low irradiances, no significant differences occurred in stature of TR-1 plants and WT ones. The differences in flowering time between them were also abolished. Apparently under these conditions differences in water oxidation capacity of WT and *LHCB1* over-expressed plants does not play anymore role in carbon assimilation competence of the transgenic plants. Further insight on the functional significance of LHCB1 can be obtained by studying the physiological responses of *lhcb1* mutant and TR-1 plants under conditions which are known to limit photosynthesis to a great extent. Thus studies focusing on the behavior of these plants under various environmental stresses might be conductive in elucidating the specific role of various LHCII proteins on plant fitness.

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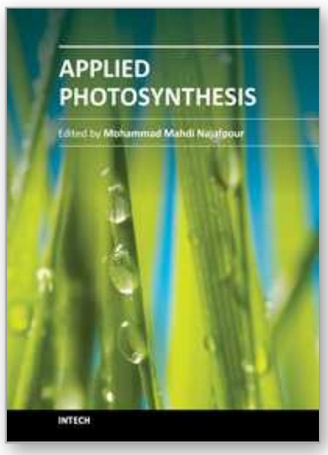

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Photosynthesis is one of the most important reactions on Earth, and it is a scientific field that is intrinsically interdisciplinary, with many research groups examining it. This book is aimed at providing applied aspects of photosynthesis. Different research groups have collected their valuable results from the study of this interesting process. In this book, there are two sections: Fundamental and Applied aspects. All sections have been written by experts in their fields. The book chapters present different and new subjects, from photosynthetic inhibitors, to interaction between flowering initiation and photosynthesis.

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