No Lhcb1 or Lhcb2 isoforms alone has a significant effect on state transitions

Siri Bäck

Bachelor thesis in plant molecular biology, 2012-03-31, 15 hp

Supervisor: Stefan Jansson
Assistant supervisor: Małgorzata Pietrzykowska
Umeå Plant Science Centre,
Department of Plant Physiology
Umeå University
SE-901 87 Umeå
SWEDEN
Abstract

Photosynthesis is essential not only for photosynthesizing organisms themselves, but also for most organisms on our planet. Even though light is necessary to drive photosynthesis, excess light may cause severe damage. Long term acclimation, where protein content in antenna complexes change, may take days or weeks, but the short term acclimation process called state transitions operate with a timescale of minutes. State transitions balance excitation energy between photosystem I and photosystem II through the mobile trimeric LHCII (light-harvesting complex of PSII), consisting of the proteins Lhcb1, Lhcb2 and Lhcb3. Lhcb1 and Lhcb2 are very similar to each other and are encoded by 5 and 3 genes respectively.

The aim of this study was to investigate whether any of the Lhcb1 and Lhcb2 isoforms have more importance to the state transition than the other. By examining different knock-out mutants via biophysical approaches, this question is addressed.

Obtained results indicate that no significant differences in protein content and no differences in state transition and non-photochemical quenching could be seen. Neither was the protein content in the plants affected by these knock out mutations.

Abbreviations:

Chl: Chlorophyll
HL: High light
LHC: Light harvesting chlorophyll a/b-binding proteins
LL: Low light
NL: Normal light
OEC: Oxygen evolving complex
Pph1: Protein phosphatase 1
PSI/II: Photosystem I/photosystem II
PQ: Plastoquinone
Stn7: State transition kinase 7.
**Introduction**

Photosynthesis is the most important process for sustaining the life on Earth, since it is the only biological solution of catching the energy provided from the sun. Photosynthesis can be carried out by many eukaryotic species, plants and in several algal clades, but also by many prokaryotes, among them cyanobacteria, that all contain pigments that are able to absorb the light energy from sun and convert them to useful substances.

Photosynthesis in plants takes place inside specialized structures called chloroplasts. Similarly to mitochondria they are semi-autonomous organelles being able to self-replicate and produce some of the proteins they contain due to the presence of DNA and ribosomes. Chloroplasts are separated from cytoplasm by a system of double membranes, called the envelopes. The envelope serves as a barrier, and the dark reaction of photosynthesis takes place in the chloroplast matrix, called the stroma. Chloroplasts also contain a highly organized system of thylakoid membranes that are organized into cylinder-like structures called grana. Grana are connected with one another by a system of lamellae, called the stroma lamellae. It is within the thylakoid membranes the light dependent reactions of photosynthesis take place (Whitmarsh et al, 1999).

The light reaction of the photosynthesis occurs in the thylakoid membranes, and involves in photosystem I (PSI) and photosystem II (PSII). Light energy is mainly captured by the chlorophyll molecules associated to light harvesting chlorophyll a/b-binding proteins (LHCI and LHCII), which are main protein-pigment antenna complexes of higher plants. LHCI and LHCII are attached to PSI and PSII respectively. The light energy collected by the antennae proteins is transferred to the reaction centers of the photosystems where the photons are used to split the water by the oxygen evolving complex (OEC) of PSII. As a result molecular oxygen and hydrogen ions (protons) are produced. The oxygen is a side product being used by the other organisms in respiration, while the proton release at the inside of the thylakoid creates a proton-motive force that drives synthesis of ATP. Water is a primary electron donor that and the electrons are going through a chain of reactions, ultimately leading to formation of reducing power in form of NADPH. ATP and NADPH are used to fix carbon dioxide the dark reaction of photosynthesis; the Calvin-Benson cycle (Tikkanen et al, 2006).
When photons captured by the antennae complexes, PSII is the first to be excited in the linear electron transport. The electrons derived from water passes through plastoquinine (PQ), the cytochrome b6/f complex and plastocyanin (PS) to PSI where they are further excited to reduce NADP\(^+\). Even though the PSII and PSI systems in this way are connected, they are separated spatially in the thylakoids. While PSI is mainly located in the non-appressed stroma lamellae, most PSII centers are found in the appressed grana stacks. In order to acquire optimal performance and avoid damage due to excess light, the distribution between the systems must be balanced (Haldrup et al, 2001). Cyclic electron transport around PSI also takes place, in parallel to the linear transport. Cyclic electron flow produces only ATP without the reduction of NADP\(^+\).

The light harvesting chlorophyll a/b-binding (LHC) proteins serve as antennae for PSI and PSII. The antenna complexes of PSII are subdivided into minor and major antenna. The minor antenna is composed of three monomeric proteins CP24 (Lhcb6), CP26 (Lhcb5) and CP29 (Lhcb4). The major antenna is connected to the PSII trough the minor one and is composed of Lhcb1, Lhcb2 and Lhcb3 polypeptides, which together form the major LHCCI complexes of PSII (Tikkanen et al 2006). Lhcb1 and Lhcb2 share very high sequence similarity, and most likely also physiological properties. Lhcb1 has been reported to have a somewhat higher chl\(a/b\) ratio (1.49 compared to 1.36 for Lhcb2) and also a higher cartenoid/chl ratio (Caffarri et al 2004). Both Lhcb1 and Lhcb2 are encoded by multiple genes (5 and 3, respectively), and some of these genes are closely clustered in tandem repeats on the chromosomes (Figure 1).

![Figure 1. The distribution of genes encoding Lhcb proteins on Arabidopsis thaliana chromosomes. Lhcb1 is encoded by five genes while Lhcb2 by 3 genes, which occur closely clustered in tandem repeats.](image)
Lhcb1 and Lhcb2 are involved in a process called state transition, which allows short term light acclimation via balancing of energy distribution between PSII and PSI. This is in contrast to long time acclimation, which involves changes in protein levels that might take days or weeks. Adaptation through state transitions can be achieved within minutes (Tikkanen et al 2006).

State transition is used for adaptation both to changes in the light intensity and spectral quality. In “steady state”, PSII and PSI is assumed to get the same amount of light energy to feed linear electron transfer from PSII to PSI, with only little contribution of cyclic electron transport around PSI. The electron transport is balanced between the systems and most LHCII is associated with PSII (state 1). If unbalance occurs in a system – for example if the spectral quality of the light change (PSII cannot utilize wavelengths above 680 nm), or if light intensity change which will change the relative proportion of linear vs. cyclic electron flow - the relative excitation pressure of PSI and PSII must change. This could, for example, lead to accumulation of electrons within PSII. To balance the energy distribution between the photosystems, LHCII trimers are detached from PSII and instead attached to PSI (state 2). The excess of excitation in PSII leads to the reduction of the plastoquinone pool, that activates state transition kinase 7 (Stn7) (Bellaﬁore et al, 2005), which in turn phosphorylates LHCII. This phosphorylation causes the LHCII dissociation from PSII (Figure 2). The process is reversed by the de-phosphorylation, which is speciﬁcally driven by a protein phosphatase 1 (Pph1) (Shapiguzov et al. 2010), or by spontaneous de-phosphorylation of LHCII. Experimentally, state transitions can be induced by exposing plants to light sources with different proportion of photons with wavelengths over 680 nm. Both Lhcb1 and Lhcb2 polypeptides are phosphorylated by Stn7, while Lhcb3 polypeptides lack the phosphorylation site and are therefore not substrates for Stn7.
Figure 2. Changes ongoing in the thylakoids membranes during the state transitions. (a) State 1. PSII and PSI get the same amount of light energy and electron transport is balanced. LHCII is mainly bound to PSII. (b) PSI cannot keep up with the light harvesting of PSII, and electrons accumulate. This leads to (c) state 2. LHCII trimers are phosphorylated and dissociate from PSII and attach to PSI. Picture adapted from Haldrup et al, 2001.

The energy absorbed by the chloroplast can have three different fates. It can enter the photosynthesis via mechanisms described above, but the energy can also be dissipated as heat, or be re-emitted as light-chlorophyll fluorescence. These three processes compete, and a decrease in photosynthesis will give an increase in fluorescence and heat dissipation, and vice versa. Thus, by measuring fluorescence, conclusions about variation in photosynthesis and heat dissipation can be drawn.
Figure 3. A schematic picture of state transitions. When PSII is overexcited, it leads to changes in the reduction of the plastoquinone pool (PQ). This activates state transition kinase 7 (Stn7), which phosphorylates a mobile pool of LHCII trimers. These LHCII trimers dissociate from PSII and associates to PSI. This process can be reversed by protein phosphatase 1 (Pph1).

This study aims to investigate whether any of the Lhcb1 or Lhcb2 isoform(s) are more important for the process of state transition than the other, and whether they have any other functions than state transitions and light harvesting. To accomplish this, eight different Arabidopsis thaliana lines with individual knockouts of Lhcb1 or Lhcb2 genes are compared with wild type Arabidopsis as control. Differences in state transitions and non-photochemical quenching (NPQ) among the knockout plants are investigated using room temperature fluorescence measurements. We compare plants grown under three light conditions: normal (NL), high (HL) and low (LL). We also investigate the changes in Lhcb1 and Lhcb2 proteins amount using thylakoid membrane protein-isolation and western blotting for detection of the proteins levels for Lhcb1 and Lhcb2 knockout plants, as well as their phosphorylation levels.

Our hypothesis is that the different knock-out will have only little effect on the measured parameters. The presence of many copies of Lhcb1 and Lhcb2 genes may indicate their great importance but also functional redundancy, and hence it is likely that one of the other Lhcb1 and/or Lhcb2 will be able to cover the loss of expression of another copy, but this is what we set out to test.
Material & Methods

Plant material and growth conditions

Eight different homozygous knock out lines of *Arabidopsis thaliana* were grown under normal light (NL: 250 µmol m$^{-2}$ s$^{-1}$) (Figure 4), high light (HL: 600 µmol m$^{-2}$ s$^{-1}$) and low light (LL: 40 µmol m$^{-2}$ s$^{-1}$) conditions under 8 hours light (23°C)/16 hours dark (18°C) photoperiod. The humidity was maintained at 75%. Wild type *Arabidopsis thaliana* ecotype Columbia (Col-0) was used as a control. The lines were:

Knockout Lhcb1:

- *koLhcb 1.1* N662639 (SALK_059893C)
- *koLhcb1.2* N597368 (SALK_097368)
- *koLhcb1.3* N846666 (SAIL_1261_C06)
- *koLhcb 1.4* N549351 (SALK_049351)
- *koLhcb 1.4* N592828 (SALK_092828)

Knockout Lhcb2:

- *koLhcb 2.1* N657479 (SALK_005774C)
- *koLhcb 2.2* N651088 (SALK_151088)
- *koLhcb2.2* N665162 (SALK_005614C)

*Figure 4.* The different knock outs after growth under normal light. Upper row from left to right: WT, *koLhcb1.1* N662639, *koLhcb1.2* N597368, *koLhcb1.3* N846666 and *koLhcb1.4* N549351. Lower row from left to right: *koLhcb1.4* N592828, *koLhcb2.1* N657479, *koLhcb2.2* N651088 and *koLhcb2.2* N665162. The fifth plant is not included in this study.
**Induction of state transitions**

Before the actual light treatment a complete (de)phosphorylation has to be achieved to ensure a ground state where 100% of proteins are phosphorylated (phosphorylation conditions) or phosphorylated (de-phosphorylation conditions).

**State I → state II transition:**

Red light of 15% intensity was on all the time, far-red light (100%) was on only for 60 min to make all LHCII dephosphorylated and was then turned off to induce phosphorylation. Samples were taken at 4 different time points: 0 s, 30 s, 10 min and 60 min after far red light was switched off. The samples were snapped frozen in liquid nitrogen and stored at –80°C before thylakoids were isolated.

**State II → state I transition:**

Plants were treated for 105 min with a constant 15% red light, and 100% far-red light that was turned on after 40 minutes to induce phosphorylation, left on for 60 minutes and then turned off. Samples were taken at 0 s, 30 s, 10 min and 60 min after far red light was switched on. All samples were frozen in liquid nitrogen and stored at –80°C until thylakoids were isolated.

**Room temperature fluorescence measurements**

Fluorescence measurements were performed on intact leaves by WALTZ’s Dual PAM100, after approximately 60 minutes dark adaptation. Two different programs were used, the first for NPQ measurements and the second for measuring the state transitions.

Non-photochemical quenching program served for observation of NPQ curves as well as qL, qP and qN. The values for NPQ were calculated from \((F_{m} – F_{m}^\prime) / F_{m}^\prime\) equation.

State transition program served for PSII quantum efficiency determination by comparison of the ratios of variable fluorescence and maximum fluorescence \((F_v/F_m)\). Also the difference in maximal fluorescence between PSI and PSII was calculated by \(F_{m1}/F_{m2}\), as well as the PSII cross-section changes, that were calculated by \((F_{m1}-F_{m2})/F_{m1}\) (Figure 5.).
**Equation** | **Explanation**
--- | ---
$F_v/F_m$ | Quantum efficiency of PSII if all the PSII centers are open
$F_{m1}/F_{m2}$ | Difference in maximal fluorescence in PSI (far-red) and PSII (red) light
$q_T$ | PSII cross section changes $(F_{m1}-F_{m2})/F_{m1}$

*Figure 5.* Equations used for comparing state transition efficiency.

**Thylakoid isolation and chlorophyll determination**

Isolation was performed in a cold room on ice under green light. The samples were homogenized in buffer containing 50 mM Hepes pH 7.5, 5 mM MgCl$_2$, 330 mM sorbitol, 10 mM NaF, 0.1% BSA (fraction V) and 5 mM ascorbate. After filtration through Miracloth, the samples were spun down at 4°C, 5000 rpm for 5 minutes. The supernatant was discarded and the pellet re-suspended in buffer containing 50 mM Hepes, 5 mM MgCl$_2$ and 10 mM NaF and the samples were spun down at 4°C, 5000 rpm for 5 minutes. The supernatant was discarded and the pellet re-suspended in buffer containing 50 mM Hepes pH 7.5, 10 mM MgCl$_2$, 100 mM sorbitol and 10 mM NaF. The samples were snap frozen in liquid nitrogen and stored at – 80°C. The chlorophyll concentration was determined via Dynamico Halo B30 spectrophotometer and Dynamico UV detective special 2.1 software, according to Porra et al 1978 using 3 different wavelengths: 646.6, 663.6, and 750 nm.

**Western Blot, visualization and quantification**

The protein content was analyzed by Western blot analysis. Mini-PROTEAN gel electrophoresis system with pre-casted gels from Bio-Rad was used. Thylakoids membranes were mixed with 2x Laemmli buffer (138 mM Tris-HCl, pH 6.8, 22.2% glycerol, 4.3% SDS) with 6 M urea and 10% β-mercaptoethanol, in 1:1 ratio, denatured at 95°C for 5 min, and then centrifuged at 10,000 x g for 5 min to pellet the debris. Samples equal to 1 µg of total chlorophyll were loaded into each well. Magic Mark (Invitrogen) and All Blue (Bio-Rad) were mixed in 1:4 ratios and 5 µl of
standard was loaded. The gels were run at 40 V for around 20 min and then current was increased to 120 V until samples reached the bottom of a gel.

Membranes were washed with MQ-H₂O, stained with 2% Poncau stain to assure a complete protein transfer, and scanned. The stain was removed with Tris Buffer Saline Tween20 (TBST) buffer.

After that, membranes were blocked for 2 hours in 5 % milk powder in TBST and then incubated for at least 2.5 h with different primary antibodies diluted in TBST: anti-Lhcb1 and anti-Lhcb2 (1:5000), anti-P-Lhcb1 and anti-P-Lhcb2 (1:1000). Primary antibodies were removed by washing 3 times in TBST for 5-10 min and exchanged for anti-rabbit IgG, horseradish peroxidase linked secondary antibody from GE Healthcare.

Visualization was performed using Amersham ECL Plus™ Western Blotting Detection Reagents, following manufacturer’s instructions. Detection was performed via Image LAS-3000 reader with exposure times of 1 and 10 minutes using a standard sensitivity. Quantification was performed via FUJI FILM Multi Gauge 2.0 software.
Results

The protein content was similar in most KO lines

After separation on SDS-PAGE gel, transfer, visualization with antibodies and quantification, the protein content of each sample could be determined (Figure 6). The results of the quantification for the normal light grown plants can be seen in Figure 7. The data is normalized to the protein level in wild type plants (set to 1) and the values for the KO mutants are presented as values relative to the wild type. For most of the lines the Lhcb1 and Lhcb2 content did not differ from wild type leaves. The exception was the two koLhcb2.2 lines, where in one case (N651088) the Lhcb1 content appeared to decrease by around 50 %, and in the other case (N665162) Lhcb1 levels appeared to increase by approximately 20 %.

The greatest difference in the phosphorylation level of Lhcb1 and Lhcb2 (P-Lhcb1 and P-Lhcb2 antibodies) was measured in koLhcb2.1 N657479, where relative P-Lhcb1 and P-Lhcb2 values were lower (0.52) and higher (1.34), respectively. Also in koLhcb2.2 N651088 the phosphorylation level of Lhcb2 was app. 40 % higher.
Figure 6. Western blot visualization of thylakoid membrane protein content isolated from plants grown under normal light. Visualization was performed, from left to right in figure, with anti-Lhcb1, anti-Lhcb2, anti-P-Lhcb1 and anti-P-Lhcb2.

Figure 7. Quantification of protein content of normal light grown plants, relative to wild type (1.00).
The phosphorylation kinetics showed no differences while de-phosphorylation kinetics did.

By using the antibodies that recognizes phosphorylated forms of proteins from not phosphorylated ones, the phosphorylation and de-phosphorylation kinetics of the KO mutants were studied. Samples were collected after 0 s, 30 s, 10 min and 60 min of light treatment inducing phospho- or de-phosphorylation and after Western blotting and quantification, the results were compared.

As can be seen in Figure 9, the wild type pattern was followed by the knockout mutants with some minor exceptions. The results of the de-phosphorylation kinetics are somewhat more scattered than the phosphorylation kinetics.

Considering phosphorylation kinetics of Lhcb1, the values for the knockout mutants correspond well to the wild type Lhcb1 phosphorylation kinetics. After 60 min of red light treatment \textit{koLhcb1.1} N66239 has the highest phosphorylation level, while \textit{koLhcb1.4} N592828 gave the lowest result.

Lhcb2 phosphorylation showed similar pattern, but here \textit{koLhcb1.3} N846666 showed the highest phosphorylation level, while \textit{koLhcb1.2} N597368 showed the lowest.

De-phosphorylation kinetics of both Lhcb1 and Lhcb2 proteins showed that \textit{koLhcb1.1} N66239 in both cases starts with more phosphorylation comparing to the wild type. Moreover \textit{koLhcb2.1} N657479 starts with lowest level of phosphorylation.
Figure 8. Western blot analysis of samples collected after 0 s, 30 s, 10 min and 60 min of phosphorylating (red) or dephosphorylating (far red) light. Membranes were blotted with P-Lhcb1 and P-Lhcb2 antibodies.
Figure 9. Phospho- and de-phosphorylation kinetics of plants grown under normal light. Quantification was done after western blotting. (a) Lhcb1 and (b) Lhcb2 phosphorylation kinetics. (c) Lhcb1 and (d) Lhcb2 de-phosphorylation kinetics.

Differences in capacity to perform state transition were small.

The data obtained from room temperature fluorescence measurements of the different KO lines were used to calculate Fv/Fm, qT and Fm1/Fm2 parameters (Figure 5.). Fv/Fm, the quantum efficiency of PSII if all the PSII reaction centers were open, gave results that were very similar between KO lines and wild type (around 0.84). The small differences that could be seen were all non-significant (data not shown).

Both qT and Fm1/Fm2 parameters – both relating to the capacity to perform state transitions - were calculated. First, qT values of the KO lines differed sometimes from to the wild type.
Grown under HL, some KO lines showed higher and some and lower values comparing to the wild type (0.039) (Figure 10). The highest value was obtained for *koLhcb1.4* N549351 (0.056) and the lowest for *koLhcb2.1* N657479 (0.019). As expected, plants grown under NL exhibited higher values comparing to the HL conditions. The highest value was recorded for the *koLhcb1.3* N846666 (0.070) and *koLhcb1.1* N662639 (0.069) lines, and the lowest for *koLhcb2.2* N665162 (0.039). When grown under LL, plants had even higher values for the cross section changes. Wild type plants had 0.072 and the values for the KO lines ranged from 0.109 for *koLhcb2.1* N657479 to 0.077 for *koLhcb1.3* N846666.
When instead measuring Fm1/Fm2, that detect differences in fluorescence between PSII and PSI and thus gives the information about the extent of the state transitions, it was also no line that exhibited consistent differences to wild type plants across the different light conditions (Figure 10.).

Knockout lines values for Fm1/Fm2 are both higher and lower than wild type values under HL and NL, but surprisingly all of the values for the mutant lines are the same or slightly higher than the wild type under LL. Overall, Fm1/Fm2 data nicely complement obtained qT data (Figure 10).

Under the HL wild type had a Fm1/Fm2 value 1.041, while the highest value (1.060) is observed for \textit{koLhcb1.4} N549351 and the lowest (1.020) for \textit{koLhcb2.1} N657479. Wild type plants grown under normal light had values of 1.050. Here \textit{koLhcb1.1} N662639 and \textit{koLhcb1.3} N846666 were the highest with values of 1.074 and 1.067 respectively, and \textit{koLhcb2.2} N665162 had the lowest value (1.041). The Fm1/Fm2 results from the plants grown under low light were higher than for the other light conditions, ranging from 1.078 for wild type to 1.122 in \textit{koLhcb2.1} N657479.

**The non-photochemical quenching differed the most between the knock out lines grown under the high light conditions.**

Finally, plants were evaluated for their capacity to perform non-photochemical quenching. For the plants grown under normal light, the values were very similar with very little variation (Figure 11.). Wild type’s maximum NPQ is 2.361, and the maximum values for the knock-out plants ranged from 2.5025 for \textit{koLhcb2.2} N651088 to 2.277 for \textit{koLhcb2.1} N846666.

Under the high light conditions the non-photochemical values were, not surprisingly, higher and there was also more variation between the lines (Figure 11). Wild type reaches a maximum at 2.394 and the some KO mutants had both lower and higher values. The highest maximum was 2.677 for \textit{koLhcb1.2} N597368, and the lowest maximum was 2.072 for \textit{koLhcb2.2} N665162.

The NPQ values obtained for the KO lines grown under the low light were also similar to the wild type values (Figure 11). Wild type’s maximum is reached at 1.945, while the highest value for a KO line was 1.905 for \textit{(koLhcb2.1} N657479) and the lowest 1.721 for \textit{koLhcb1.2} N597368.
Figure 11. Non-photochemical quenching in wild type and KO mutants grown under the (A) high light, (B) normal light and (C) low light conditions.
Discussion

Lack of expression of one Lhcb1 or Lhcb2 gene did not affect the protein content.

The Lhcb1 and Lhcb2 proteins levels show very little variation in the different KO mutants. The differences observed can most likely explained by somewhat inaccurate quantification method. This is typically addressed by doing multiple biological and/or technical replicates, but time did not allow that in this case. When quantifying protein content without enough technical or biological replicates, differences smaller than 30 % from the wild type are likely to be insignificant. The fact that two mutants alleles supposedly eliminating expression of the same gene were among those that differed most in protein content. Therefore, we believe that neither of the KO lines had a different protein content than wild type plants. The results from this study indicate therefore that different Lhcb1 and Lhcb2 isoforms do not alone play a crucial role for the composition of the photosynthetic antenna. Whenever a single isoform of Lhcb1 and/or Lhcb2 is missing, they are replaced by the overexpression of the other isoforms. In order to further investigate the specific roles of Lhcb1 and 2, more extensive studies, involving e.g. production of double knock-out lines, might be tried.

The phosphorylation and de-phosphorylation kinetics showed small differences.

When we measured the amount of phosphorylated proteins, the observed differences were somewhat greater, e.g. as in the case of phosphorylated Lhcb2 in the koLhcb2.1 N6579479 and koLhcb2.2 N651088. Here, an additional inaccuracy is introduced, differences in plant handling during the inductions of state transitions. It is common that the individual plants within the same lines can grow with a different peace and that affect the phosphorylation status of the plants. Also different lines present a different growth rate, even if the eventually reach the same size. These indicate an intrinsic natural variation among the plant species.

The phosphorylation and de-phosphorylation kinetics showed that the KO mutants followed the pattern of the wild type with some deviations. When looking at the graphs, it is tempting to draw conclusions about the kinetics, but one has to bear in mind that these experiments also need to be replicated in order to draw conclusions about differences in phosphorylation kinetics. The Lhcb1
and Lhcb2 proteins are rapidly phospho- and de-phosphorylated as a consequence of differences in the oxidation state of PQ, and the possibilities for differences in plant handling or sampling are significant. Even though care was taken to make the experiments as similar as possible we cannot expect the treatment to be equal to all lines. Again, time did not allow for replications so to confirm the obtained results more work is required.

**The differences in state transition were small.**

Concerning the fluorescence measurements, the first information from the collected values was that the quantum efficiency of PSII if all the reaction centers of PSII were open (Fv/Fm), was not affected by the knock out mutation. The values for the KO mutants were very similar to the wild type values, indicating that a plant lacking one isoform of Lhcb1 or 2, was not affected in terms of photosystem II efficiency. This was expected giver the fact that loss of one isoform was compensated by an increased expression of the other isoforms

These fluorescence measurements were performed after dark adaptation in four biological replicates for each line, nevertheless plants might have not been representative enough, thus affecting the measurements. Subtle changes in the plants’ treatment will affect the room temperature fluorescence measurements, thus the results from such measurements should be compared for instance to the phosphorylation kinetics of every plant. Even the changes recorded could probably not be trusted unless supported by the complementing data which was not the case here, as they seem to negate one another rather than confirm.

Furthermore, looking at Fm1/Fm2 (Figure 10) no great differences compared to the wild type could be observed. Since no line show a consistent pattern in all three light conditions, it is difficult to draw any conclusions and further investigation might be required.
The non-photochemical quenching differed the most among the knock out lines grown under the high light conditions.

As can be seen in Figure 11, none of the knock out mutants gave any significant difference in non-photochemical quenching when grown under both normal and low light. The biggest variation in the NPQ curves could be seen within the lines grown under high light.

Non-photochemical quenching was measured in order to draw conclusions about the fate of the energy that is reaching the specific plant. When no differences could be seen, the implication is that the different KO mutations of Lhcb1 and Lhcb2 do not affect the energy distribution between the plants’ photosystems. Both Lhcb1 and Lhcb2 isoforms might be able to replace one another if one allelic copy is not expressed.

**No Lhcb1 or Lhcb2 isoforms alone has a significant effect on state transitions**

The final conclusion to be drawn when considering the results of this study is that the single isoforms of Lhcb1 and Lhcb2 have little or no effect on state transitions. Knock-out of single Lhcb1 or Lhcb2 isoforms give no significant effect on proteins content, or on phosphorylation and de-phosphorylation kinetics, state transition and NPQ. The differences that could be seen are explained by natural variation between plants and/or by technical errors.

This may sound like a boring and uninteresting result, but in the light of the other activities in the lab, the results are relevant and important. Plants where expression of Lhcb1 or of Lhcb2 by means of artificial microRNA (amiRNA) have been generated, and show strong phenotypes in terms of both state transitions and NPQ. Therefore, the data presented in this thesis work is an important confirmation that these effects are not due to repression of expression of a single gene copy, but that only when all five (in the case of Lhcb1) or three (in the case of Lhcb2) genes are turned off, a phenotype is observed and therefore, the gene copies compensate fully for each other.
Acknowledgements

I would like to thank Stefan Jansson, my supervisor at UPSC, for patience and support. Also warm thanks to Malgorzata Pietrzykowska for help with all practical work, as well as discussions, explanations and encouragement.

References


