FEBS Letters 584 (2010) 199–202

FEBS Letters

journal homepage: www.FEBSLetters.org

Succinate dehydrogenase in *Arabidopsis thaliana* is regulated by light via phytochrome A

Vasily N. Popov [a,*], Alexander T. Eprintsev [a], Dmitry N. Fedorin [a], Abir U. Igamberdiev [b]

[a] Department of Biochemistry and Cell Physiology, Voronezh State University, 1 Universitetskaya pl., Voronezh 394006, Russia
[b] Department of Biology, Memorial University of Newfoundland, St. John's, NL, Canada A1B 3X9

ARTICLE INFO

Article history:
Received 15 October 2009
Accepted 13 November 2009
Available online 20 November 2009

Edited by Vladimir Skulachev

Keywords:
Succinate dehydrogenase
Red light
Far-red light
Phytochrome
Respiration
PhyA and PhyB mutants
Arabidopsis thaliana

ABSTRACT

The effect of light on succinate dehydrogenase (SDH) activity and mRNA content was studied in *Arabidopsis thaliana* plants. The transition from darkness to light caused a short transient increase in the SDH activity followed by a decrease to a half of the original activity. The white or red light were found to be down-regulating factors for the mRNA content of the *sdh1-2* and *sdh2-3* genes and SDH catalytic activity both in *A. thaliana* wild-type plants and in the mutant deficient in the phytochrome B gene, but not in the mutant deficient in the phytochrome A gene, while the far-red light of 730 nm reversed the red light effect. It is concluded that phytochrome A participates in the regulation of mitochondrial respiration through effect on SDH expression.

1. Introduction

The interrelationship between photosynthesis, photorespiration, and mitochondrial respiration is an important cross-talk of metabolic regulation in green tissues of plants. A common observation is that CO_2 evolution from the mitochondrial respiration is decreased under light conditions when the photorespiratory CO_2 release becomes predominant [1]. The oxidation of photorespiratory glycine actively involves non-coupled pathways of the mitochondrial electron transport such as the alternative NADH and NADPH dehydrogenases and the alternative oxidase [2]. Expression of these oxidoreductases is under control of light via the phytochrome system [3,4].

Due to the increase of the mitochondrial electron transport chain (ETC) capacity through the involvement of non-coupled pathways of electron transport, the mitochondrial glycine metabolism does not block completely the oxidation of other NADH-related substrates. However, the flux through the tricarboxylic acid cycle (TCA) cycle is reduced significantly. The known mechanisms of such reduction include the inhibition of the pyruvate dehydrogenase complex by photorespiratory ammonia [5] and the reversion of reaction catalyzed by NADP-dependent isocitrate dehydrogenase with the simultaneous inhibition of NAD-dependent isocitrate dehydrogenase by NADPH [6]. It is known also that the important point for regulation of the TCA cycle is its direct association with the ETC at the level of complex II. While in the conditions of ATP accumulation and of the increase of transmembrane potential in the light, a higher engagement of non-coupled respiration pathways can result in the increased capacity of the mitochondrial ETC, succinate can only be oxidized by succinate dehydrogenase (SDH) which acts, at the same time, as the TCA cycle enzyme and as a component in the complex II of the ETC.

SDH (EC 1.3.99.1) is located in the inner membrane of mitochondria and its activity is connected with the operation of the ETC, where it functions as a succinate:ubiquinone reductase (SUR or complex II; EC 1.3.5.1). The complex II of the plant mitochondrial ETC contains four peptides common to all eukaryotes, FAD being tightly bound to the largest subunit SDHA and three Fe/S clusters associated with the subunit SDHB, and four plant-specific subunits [7]. The activity of SDH in plant mitochondria is regulated by light through the changes of membrane potential, ATP, oxaloacetate, and CO_2 concentrations [8] but no evidence has been obtained yet that the complex II could be regulated by light directly. The complex II is not associated in the respiratory supercomplex containing complexes I and III [9], so its expression

Abbreviations: DCPIP, dichlorophenolindophenol; ETC, electron transport chain; TCA cycle, tricarboxylic acid cycle; SDH, succinate dehydrogenase; SUR, succinate:ubiquinone reductase

* Corresponding author. Fax: +7 473 2 208755.
E-mail address: pvn@bio.vsu.ru (V.N. Popov).

0014-5793/$36.00
doi:10.1016/j.febslet.2009.11.057

can be regulated separately from the supercomplex that is expressed more constitutively, and may resemble the independent regulation of non-coupled NADH and NADPH dehydrogenases [3] and the alternative oxidase [4].

The influence of light can be mediated by the photoreversible phytochrome system reacting through red (660 nm) and far-red (730 nm) light and by cryptochromes through blue light of low intensities. Phytochromes are located in plant chloroplasts, mitochondria, cytosol and, upon red light irradiation, are transported into the nucleus or chloroplasts [10]. Phytochrome-mediated reactions can be based either on gene activation or membrane modification. The aim of the present study is to show that light directly influences the expression of SDH in leaves of *Arabidopsis thaliana* via the phytochrome system. We obtained evidence that phytochrome A is involved in the inhibition of SDH expression and this can be considered as an important mechanism for regulation of the mitochondrial respiration in the light.

2. Materials and methods

2.1. Materials

Green leaves of 18–20-days old *A. thaliana* L. plants were used. The seeds were germinated in soil at 20–22 °C and the plants were transferred to the 12-h light/12-h dark cycle. In the experiments with long-term illumination, all plant samples were harvested between 11 and 12 h of the illumination period. During the experiments, plants were exposed to different intensities of light (darkness – 0 μmol quanta m^{-2} s^{-1}, intermediate – 150 μmol quanta m^{-2} s^{-1} and high – 300 μmol quanta m^{-2} s^{-1}). The light filters (LOMO, Russia) with a maximum transparence at 660 and 730 nm, respectively, were used for red and far-red irradiation. The light intensity under these conditions was 0.2 μmol quanta m^{-2} s^{-1} for 15 min. The actual ratios of red and far-red irradiances supplied to the plants in these experiments were ~20. The influence of red and far-red light on the SDH activity was investigated for three lines of *A. thaliana* plants: the wild-type (WT CoL-0), the mutant of the phytochrome A gene (PhyA-201), and the mutant of the phytochrome B gene (PhyB-1) obtained from the Max-Planck-Institute of Molecular Plant Physiology (Golm, Germany).

2.2. Isolation of mitochondria and detection of SDH activity

Mitochondria were isolated using a Percoll self-generated gradient [11]. Five grams of leaves were homogenized in a multipress MP50 (Braun) for 1 min. The homogenization medium contained 20 mM HEPES (pH 7.2), 350 mM mannitol, 1 mM EDTA, 2 mM cysteine, 0.7% PVP, and 0.5 mg ml^{-1} BSA. Rates of respiration were measured with an oxygen electrode (Yellow Springs Instruments Co. Inc., USA) at 25 °C in 1 ml of the incubation medium, containing 20 mM HEPES (pH 7.2), 350 mM mannitol, 1 mM potassium phosphate, 5 mM $MgCl_2$ and approximately 0.5 mg mitochondrial protein. As a substrate for respiration, 10 mM succinate was supplied along with 0.1 mM ATP to ensure complete activation of SDH. The SDH activity was determined by a decrease of absorption at 600 nm, caused by reduction of the artificial electron acceptor dichlorophenolindophenol (DCPIP) [12]. The reaction medium contained 30 mM potassium mono/diphosphate (pH 7.8), 1 mM NaN_3, 10 mM succinate, 0.1 mM phenazine metasulphate, and 0.008 mM DCPIP. An aliquot of the resuspended mitochondrial pellet (corresponding to 0.2 mg protein) was used for determination of enzyme activity. One unit of SDH activity was defined as the amount of enzyme which reduces 1 μmol DCPIP in 1 min at 25 °C. Total protein concentration was determined using the protein assay reagent A (Pierce, Rockford, IL, USA).

2.3. Preparation of RNA and determination of mRNA content by real-time PCR analysis

RNA was isolated by the guanidine isothiocyanate method [13]. RNA separation was conducted in 1% agarose gel [14]. Reverse transcription was catalyzed by the revertase M-MulV (Fermentas, Lithuania) using oligo-dT primers. Three micrograms of RNA were used for cDNA synthesis. The cDNA was solubilized in 10 mM Tris–HCl, pH 8.0, containing 0.1 mM EDTA, and used for the RT-PCR detection.

PCR primers for *sdh1-2* were designed using the program Primer3 (Biomatters Ltd., New Zealand) and specific sequences, obtained from BLAST (NCBI, USA): forward – 5′-CAAACGGGTCACTTCCAACT-3′; reverse – 5′-CCAAAACTGTCCCACGTCTT-3′. The primers for the *sdh2* gene family were prepared according to [15].

Real-time PCR was conducted on the RT-PCR machine Chromo4 (MJ Research, Bio-Rad, USA) using SYBR Green. The PCR cycle, after the initial denaturation at 95 °C for 5 min, consisted of 20 s denaturation at 95 °C, 20 s of primers annealing at 57 °C, 15 s of elongation at 72 °C, 15 s for detection at 72 °C. The cDNA from *A. thaliana* leaves from plants incubated in normal light was used as the basic line. The fluorescence of the elongation factor 1 (EF-α1) gene product during RT-PCR was used to normalize the mRNA content in the samples. All fluorescence measurements were repeated at least 5 times and analyzed by the Bio-Rad software.

3. Results

3.1. SDH activity depends on white light intensity

The activity of SDH from green *A. thaliana* leaves kept in darkness for 24 h (control conditions) was at the level of 0.14 μmol min^{-1} g^{-1} FW. The transfer of plants from darkness to moderate white light caused a 1.3-fold increase of the SDH activity during first 30 min (Fig. 1A). Then, within the next 2 h, a sharp reduction of enzyme activity to 50% of the original activity was observed. Subsequently, the enzyme activity remained constant for 72 h of illumination at the level of ~0.07 μmol min^{-1} g^{-1} FW. A similar time pattern was also observed for the rate of succinate oxidation by mitochondria isolated in darkness and in the course of illumination (data not shown). The twofold increase of light intensity resulted in further suppression of SDH activity (Fig. 1A) from a half to one third of the original activity in darkness resulting in the value of ~0.04 μmol min^{-1} g^{-1}.

3.2. Red light participates in the regulation of SDH activity

Irradiation of *A. thaliana* plants after 24 h darkness by low-energy red light (660 nm) for 10 min resulted in a gradual decrease of SDH activity during the consequent dark period. Irradiation by the far-red light (730 nm) resulted, on contrary, in notable increase of the SDH activity, the same result was observed when the 10 min irradiation by far-red light was applied after 10 min irradiation by red light (Fig. 1B).

The influence of low-energy red and far-red-light, as mediated by phytochrome, on SDH in three lines of *A. thaliana* was carried out to study a possible participation of phytochrome in the regulation of SDH in green leaves. As shown in Fig. 2A, in wild-type plants of *A. thaliana*, the dependence of SDH activity on the treatment by light of different wavelengths revealed a twofold decrease after irradiation by red light (660 nm) following 24 h in darkness as compared to the control (non-irradiated) level. A similar change (a 4.8-fold decrease) was observed in the *A. thaliana* mutant PhyB-1. At the same time, in the line PhyA-201, red light did not cause any change in the enzyme activity. A reduction of SDH activity

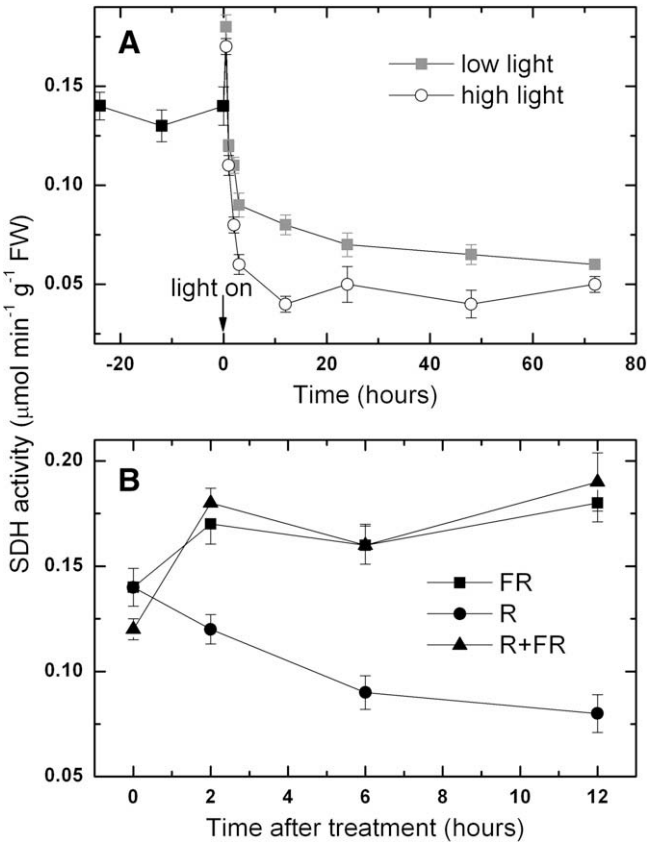

Fig. 1. Succinate dehydrogenase activity in green leaves of *A. thaliana*. (A) The activity profile after dark-light transition to the intermediate intensity white light (150 μmol quanta m^{-2} s^{-1}) and to a high intensity white light (300 μmol quanta m^{-2} s^{-1}). (B) The activity following the low-energy irradiation after 24 h darkness by the red light (660 nm) for 10 min (R), by the far-red light (730 nm) for 10 min (FR) or by 10 min far-red light applied after 10 min irradiation by red light (R + FR).

in *A. thaliana* WT Col-0 and in the mutant line PhyB-1, and no change in the enzyme activity in the mutant line PhyA-201 allows the conclusion that phytochrome A takes part in the light regulation of SDH. The influence of far-red light in all three *A. thaliana* lines was opposite to the red light effect. Upon irradiation by far-red light, the SDH activity did not change in the wild-type plants and in the mutant of the phytochrome A gene. In the mutant line PhyB the activity increased by 2.7 times. The consecutive treatment with red and far-red light caused a similar effect as far-red light (Fig. 2A). This proved that far-red light is an antagonist of red light in SDH activation and, thus, phytochrome is involved in the enzyme modulation.

3.3. Illumination controls mRNA content for the subunits A and B of SDH

We have analyzed the mRNA levels for genes *sdh1-2* and *sdh2-3* in leaves of wild-type and phytochrome mutants in response to different light treatments (Fig. 2B and C). The pattern of SDH activity (Fig. 2A) was consistent in general with the pattern of expression of the genes *sdh2-3* (Fig. 2C) and to a lesser extent of *sdh1-2* (Fig. 2B). The mRNA content for both genes was lower in the PhyA mutant in far-red light (applied solely or after red light) while in the PhyB mutant the expression of *sdh2-3* was similar to the expression level in the wild-type. For *sdh1-2*, the transcript level was higher in PhyB (not reaching the level of the wild-type) than in the PhyA mutant. Illumination by white light reduced mRNA content for both genes in all studied plants while the red-light

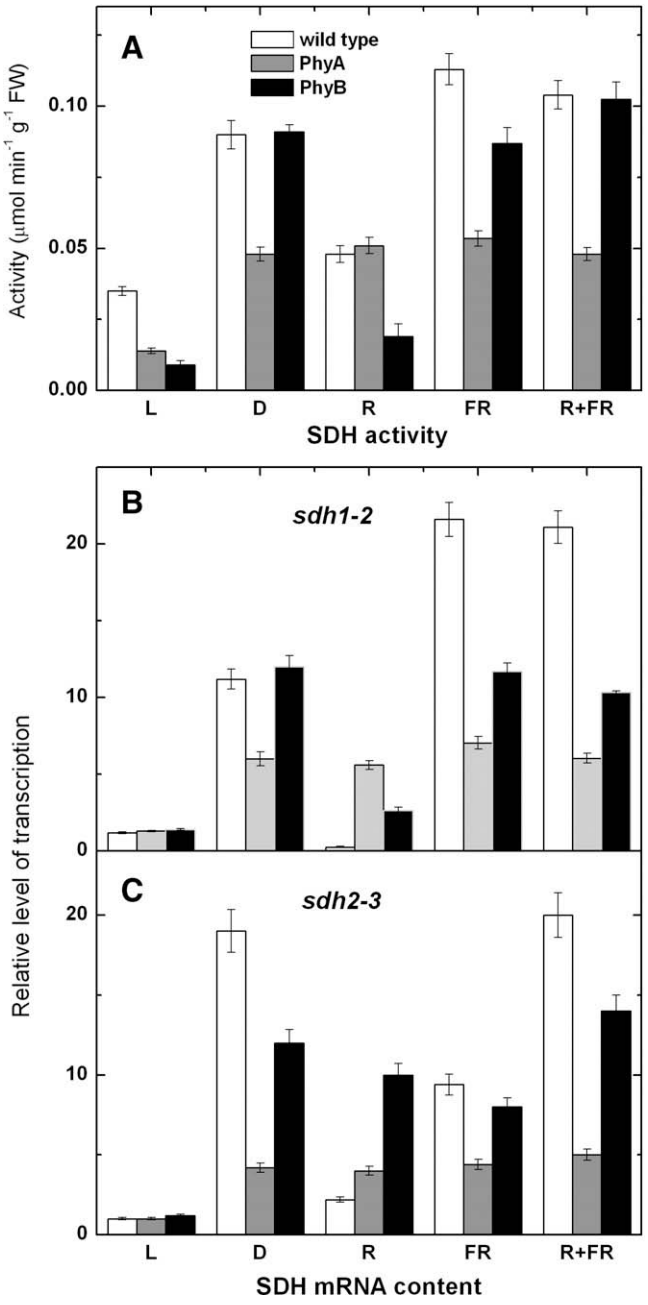

Fig. 2. Succinate dehydrogenase activity (A) and recalculation of mRNA concentration measured by the real-time amplification for the genes *sdh1-2* (B) and *sdh2-3* (C) in green leaves of wild-type and mutants PhyA-201 and PhyB-1 of *A. thaliana* after red (R) and far-red (FR) light treatments. Abbreviations: L – 12 h light treatment (150 μmol quanta m^{-2} s^{-1}); D – 24 h darkness treatment; R – 10 min red light (660 nm) treatment in the middle of darkness period (12 + 12 h); FR – 10 min far-red light (730 nm) treatment in the middle of darkness; R + FR – 10 min red light (660 nm) followed by 10 min far-red light (730 nm) in the middle of darkness. The level of expression in the wild-type plant incubated in the light was set as 1.

sensitivity was observed only for the wild-type plants and PhyB mutants. PhyA mutants did not exhibit marked changes in the expression of SDH genes under red or far-red low-intensive light.

4. Discussion

The degree of the TCA cycle inhibition in the light depends on light intensity, nitrate supply, concentrations of CO_2 and O_2 [16] and on species tested and usually is in the range of 15–55% [17]

although much stronger inhibition was suggested [16]. In the recent work [18] Tcherkez et al., by using ^{13}C labeling, showed that the TCA cycle in the light does not work like a proper cycle but rather, operates in both the reverse and forward directions to produce fumarate and glutamate, respectively, with a very negligible flux through SDH. This could be related to the light regulation of SDH observed in this study. Previously it was shown that several genes for enzymes of the TCA cycle including citrate synthase, aconitase, and NAD-malate dehydrogenase are under control of red and far-red light [19] and phytochrome A dominates transduction of red signals during greening [20], however no information about SDH had been obtained until we received a preliminary evidence that the SDH gene expression can be light-dependent [21]. The present study provides evidence of the involvement of a phytochrome mechanism in the regulation of SDH through its effect on the expression of genes encoding SDHA and SDHB subunits.

The genes encoding SDHA and SDHB are expressed differentially in ontogenesis [15,22–24]. According to our data, the gene *sdh2-3*, in coordination with the *sdh1-2* encoding the SDHA subunit, contributes to the synthesis of complex II and to the operation of mitochondrial electron transport in the light. The comparison of the pattern of SDH activity changes with corresponding expression of the genes *sdh1-2* and *sdh2-3* shows that in fact the observed levels of activity correspond to the expression of genes encoding the flavoprotein and Fe/S subunits of SDH (Fig. 2). A closer pattern is observed in the case of the iron–sulfur subunit (the gene *sdh2-3*).

The influence of red and far-red light on SDH testifies the participation of the phytochrome system in a modulation of the complex II component of the mitochondrial ETC. This view is further supported by the absence of red light effects in plants with a mutation in the phytochrome B gene. We have already excluded a possibility of light interaction with a flavin or another chromophoric group bound to SUR by showing that light had no effect on the activity of the isolated protein complex from maize [25]. The degree of inhibition of SDH expression and a corresponding decrease in its activity are comparable to the observed rates of inhibition of the TCA cycle in the light [1] representing an additional regulatory mechanism to previously described inhibition of the pyruvate dehydrogenase complex by photorespiratory ammonia and isocitrate dehydrogenase by changes in redox poise. This effect occurs via phytochrome A and is opposite to the up-regulation of rotenone-insensitive NAD(P)H dehydrogenases and the alternative oxidase.

Acknowledgments

We would like to kindly acknowledge Professor Claus Schnarrenberger (Free University of Berlin, Germany) for participation in planning the experiments, Professor Leszek A. Kleczkowski (Umeå University, Sweden) for discussion, Federal Targeted Program "Kadry" (Russia), Russian Fund for Basic Research (RFBR) and National Science and Engineering Research Council of Canada (NSERC) for partial financial support.

References

[1] Atkin, O.K., Evans, J.R. and Siebke, K. (1998) Relationship between the inhibition of leaf respiration by light and enhancement of leaf dark respiration following light treatment. Aust. J. Plant Physiol. 25, 437–443.

[2] Igamberdiev, A.U., Bykova, N.V. and Gardeström, P. (1997) Involvement of cyanide-resistant and rotenone-insensitive pathways of mitochondrial electron transport during oxidation of glycine in higher plants. FEBS Lett. 412, 265–269.

[3] Escobar, M.A., Franklin, K.A., Svensson, A.S., Salter, M.G., Whitelam, G.C. and Rasmusson, A.G. (2004) Light regulation of the Arabidopsis respiratory chain. Multiple discrete photoreceptor responses contribute to induction of type II NAD(P)H dehydrogenase genes. Plant Physiol. 136, 2710–2721.

[4] Ribas-Carbo, M., Giles, L., Flexas, J., Briggs, W. and Berry, J.A. (2008) Phytochrome-driven changes in respiratory electron transport partitioning in soybean (*Glycine max* L.) cotyledons. Plant Biol. 10, 281–287.

[5] Gemel, J. and Randall, D.D. (1992) Light regulation of leaf mitochondrial pyruvate dehydrogenase complex: role of photorespiratory carbon metabolism. Plant Physiol. 100, 908–914.

[6] Igamberdiev, A.U. and Gardeström, P. (2003) Regulation of NAD- and NADP-dependent isocitrate dehydrogenases by reduction levels of pyridine nucleotides in mitochondria and cytosol of pea leaves. Biochim. Biophys. Acta 1606, 117–125.

[7] Millar, A.H., Eubel, H., Jänsch, L., Kruft, V., Heazlewood, J.L. and Braun, H.P. (2004) Mitochondrial cytochrome *c* oxidase and succinate dehydrogenase complexes contain plant specific subunits. Plant Mol. Biol. 56, 77–90.

[8] Affourtit, C., Krab, K. and Moore, A.L. (2001) Control of plant mitochondrial respiration. Biochim. Biophys. Acta 1504, 58–69.

[9] Dudkina, N.V., Eubel, H., Keegstra, W., Boekema, E.J. and Braun, H.P. (2005) Structure of a mitochondrial supercomplex formed by respiratory-chain complexes I and III. Proc. Natl. Acad. Sci. USA 102, 3225–3229.

[10] Rockwell, N.C., Su, Y.S. and Lagarias, J.C. (2006) Phytochrome structure and signaling mechanisms. Annu. Rev. Plant Biol. 57, 837–858.

[11] Wagner, A.M. (1995) A role for active oxygen species as second messengers in the induction of alternative oxidase gene expression in Petunia hybrid cells. FEBS Lett. 368, 339–342.

[12] Cooper, T.G. and Beevers, H.J. (1969) Mitochondria and glyoxysomes from castor bean endosperm. Enzyme constituents and catalytic capacity. J. Biol. Chem. 244, 3507–3513.

[13] Chomczynski, P. and Sacchi, N. (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction. Anal. Biochem. 162, 156–159.

[14] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) Molecular Cloning. A Laboratory Manual, second ed, Cold Spring Harbor Laboratory Press, New York.

[15] Elorza, A., Roschzttardtz, H., Gómez, I., Mouras, A., Holuigue, L., Araya, A. and Jordana, X. (2006) A nuclear gene for the iron–sulfur subunit of mitochondrial complex II is specifically expressed during Arabidopsis seed development and germination. Plant Cell Physiol. 47, 14–21.

[16] Tcherkez, G., Bligny, R., Gout, E., Mahe, A., Hodges, M. and Cornic, G. (2008) Respiratory metabolism of illuminated leaves depends on CO_2 and O_2 conditions. Proc. Natl. Acad. Sci. USA 105, 797–802.

[17] Pärnik, T. and Keerberg, O. (1995) Decarboxylation of primary and end products of photosynthesis at different oxygen concentrations. J. Exp. Bot. 46, 1439–1447.

[18] Tcherkez, G., Mahé, A., Gauthier, P., Mauve, C., Gout, E., Bligny, R., Cornic, G. and Hodges, M. (2009) In folio respiratory fluxomics revealed by ^{13}C-isotopic labeling and H/D isotope effects highlight the non-cyclic nature of the tricarboxylic acid "cycle" in illuminated leaves. Plant Physiol. 151, 620–630.

[19] Thum, K.E., Shin, M.J., Palenchar, P.M., Kouranov, A. and Coruzzi, G.M. (2004) Genome-wide investigation of light and carbon signaling interactions in Arabidopsis. Genome Biol. 5, R10.

[20] Tepperman, J.M., Hwang, Y.S. and Quail, P.H. (2006) PhyA dominates in transduction of red-light signals to rapidly responding genes at the initiation of Arabidopsis seedling de-etiolation. Plant J. 48, 728–742.

[21] Popov, V.N., Fedorin, D.N. and Eprintsev, A.T. (2007) Light regulation of succinate dehydrogenase expression in *Arabidopsis thaliana* leaves. Russian J. Plant Physiol. 54, 360–365.

[22] Figueroa, P., León, G., Elorza, A., Holuigue, L. and Jordana, X. (2001) Three different genes encode the iron–sulfur subunit of succinate dehydrogenase in *Arabidopsis thaliana*. Plant Mol. Biol. 46, 241–250.

[23] Figueroa, P., Léon, G., Elorza, A., Holuigue, L., Araya, A. and Jordana, X. (2002) The four subunits of mitochondrial respiratory complex II are encoded by multiple nuclear genes and targeted to mitochondria in *Arabidopsis thaliana*. Plant Mol. Biol. 50, 725–734.

[24] Elorza, A., León, G., Gómez, I., Mouras, A., Holuigue, L., Araya, A. and Jordana, X. (2004) Nuclear SDH2-1 and SDH2-2 genes, encoding the iron–sulfur subunit of mitochondrial complex II in Arabidopsis, have distinct cell-specific expression patterns and promoter activities. Plant Physiol. 136, 4072–4087.

[25] Popov, V.N., Eprintsev, A.T., Fedorin, D.N. and Leonova, Yu.A. (2005) Light influence on succinate dehydrogenase activity in maize leaves. J. Stress Physiol. Biochem. 1, 30–36.