Effect of Light Intensity under Different Photoperiods on Expression Level of Carbonic Anhydrase Genes of the α- and β-Families in *Arabidopsis thaliana* Leaves

N. N. Rudenko, D. V. Vetoshkina, T. P. Fedorchuk, and B. N. Ivanov*

Institute of Basic Biological Problems, Russian Academy of Sciences,
142290 Pushchino, Moscow Region, Russia; E-mail: ivboni@rambler.ru

Abstract—Changes in expression levels of genes encoding carbonic anhydrases α-CA1, α-CA2, α-CA4, β-CA1, β-CA2, β-CA3, β-CA4, β-CA5, and β-CA6 in *Arabidopsis thaliana* leaves after light increase from 80 to 400 µmol PAR quanta·m^{-2}·s^{-1} were investigated under short day (8 h) and long day (16 h) photoperiods. The expression of two forms of the gene, *At3g01500.2* and *At3g01500.3*, encoding the most abundant carbonic anhydrase of leaves, β-CA1, situated in chloroplast stroma, was found. The content of *At3g01500.3* transcripts was higher by approximately an order of magnitude compared to the content of *At3g01500.2* transcripts. When plants were adapted to high light intensity under short day photoperiod, the expression level of both forms increased, whereas under long day photoperiod, the content of *At3g01500.3* transcripts increased, and the content of transcripts of *At3g01500.2* decreased. The expression levels of the *At3g01500.3* gene and of genes encoding chloroplast carbonic anhydrases α-CA1, α-CA4, α-CA2 and cytoplasmic carbonic anhydrase β-CA2 increased significantly in response to increase in light intensity under short day, and these of the first three genes increased under long day as well. The expression level of the gene encoding α-CA2 under long day photoperiod as well as of genes of chloroplast β-CA5 and β-CA4 from plasma membranes and mitochondrial β-CA6 under both photoperiods depended insignificantly on light intensity. Hypotheses about the roles in higher plant metabolism of the studied carbonic anhydrases are discussed considering the effects of light intensity on expression levels of the correspondent genes.

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Nineteen genes encoding carbonic anhydrases (CA) belonging to three families, α, β, and γ [1], out of six families known to date, have been found in the *Arabidopsis thaliana* genome [2]. All five carbonic anhydrases of the γ-family were found in mitochondria as part of the so-called CA domain located on the inner surface of the mitochondrial membrane [3]. The CAs of the β-family were found in different parts of the cell: β-CA1 and β-CA5 in chloroplasts, β-CA2 and β-CA3 in cytoplasm, β-CA4 in cell membrane, β-CA6 in mitochondria (nomenclature proposed by Fabre et al. is used [1]). The product of the *At4g20990* gene encoding α-CA4 was found using

proteome analysis of thylakoid membrane proteins [4]. Another CA of this family, α-CA1, was found in chloroplast stroma [5]. The expression of the gene encoding α-CA3 is detected in flowers and pods, and α-CA2 in is detected in roots, stems, and leaves of *Arabidopsis* [1]. In an article by Zhurikova et al. [6], evidence of α-CA2 functioning in the thylakoid membranes was presented.

The components of the reversible hydration reaction of carbon dioxide, $CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$, are involved in virtually all metabolic processes, and although the participation of CA in the catalysis of this reaction in several processes is assumed, it is not reliably established. For the most abundant CA in leaves, β-CA1 located in the stroma of chloroplasts, the most rational assumption about its function was its participation in the acceleration of the conversion of bicarbonate to CO_2, the substrate of ribulose-bisphosphate-carboxylase/oxygenase (Rubisco). However, in transgenic plants, where the expression of the CA gene was suppressed by 99% using antisense

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Abbreviations: CA, carbonic anhydrase; Chl, chlorophyll; HL, high light (light of high intensity); LL, low light (light of low intensity); PAR, photosynthetically active radiation; PSII, photosystem II; Rubisco, ribulose-bisphosphate-carboxylase/oxygenase.
* To whom correspondence should be addressed.

sequence, no significant changes in the rate of photosynthesis were observed [7]. It was hypothesized that another stromal CA, α-CA1, participated in the delivery of CO_2 to Rubisco [8]. Studies of the carbonic anhydrase activity of thylakoid membranes and their fragments have led to the assumption that CAs can participate in many reactions there [9], but these CAs have not been identified. Many observations indicate that CAs can play a role in the plant's system of stress protection [10, 11].

One approach to clarifying the functions of enzymes in plant metabolism is to study the dependence of their content and the expression of the genes encoding them on the intensity of light. Changes in illumination cause significant rearrangements in plants, primarily in the structure and activity of the photosynthetic apparatus: as illumination increases, the content of the components of the photosynthetic electron transport chain, ATP synthase [12], and Rubisco [13] increases, and the size of the Photosystem II (PSII) antenna decreases [14]. An important factor affecting the metabolism of plants is the duration of daylight (photoperiod). In addition to changing the active time of the photosynthetic apparatus during the day, the photoperiod is important for inducing the transition of plants to the flowering phase. This transition is accompanied by activation or inhibition of many processes in plants, including changes in gene expression [15]. In Eurasia, which is the native area for *A. thaliana*, the duration of daylight during the growing season is 14-20 h, and flowering is induced when plants stay in the light for 12 h or more during the day [16].

In this report, we show how the expression levels of nine CA genes are correlated and how they change in response to increased light intensity under "long" and "short" day conditions. The conditions of the "long" day, 16 h day/8 h night, are close to natural, and the "short" day, 8 h day/16 h night conditions, artificially created in a climate chamber, are usually used to study the long-term adaptation of plants to various external factors because it maintains *Arabidopsis* in the vegetative state for several weeks.

MATERIALS AND METHODS

Growing conditions. Wild-type *A. thaliana* plants, ecotype Columbia, were grown in heat-treated generic soil, trademark "Generous earth", in a climate chamber. The scheme of cultivation is shown in Fig. 1. Eighteen days after sowing the seeds, at the stage of 2-3 true leaves, the plants were transplanted into separate containers filled with fresh soil mixture (replanting). After 21 days of growing the plants at 450 ppm CO_2 concentration in air, 19°C temperature, and light intensity of 80 μmol PAR quanta·m^{-2}·s^{-1} (low light, LL) at 8 h day/16 h night cycle ("short" day), one fourth of the plants were left under these conditions, and one fourth was transferred to light with intensity of 400 μmol PAR quanta·m^{-2}·s^{-1} (high light, HL) while maintaining the "short" day. The remaining plants were placed under 16 h day/8 h night cycle ("long" day) while LL was preserved, and 10 days after adaptation to this photoperiod, half of these plants were left in such conditions, and the other half was transferred to HL while retaining "long" day. When studying the effect of plant illumination on the transcription of the carbonic anhydrase genes in leaves, the day of moving plants from LL to HL was considered as the beginning of the experiment under both "short" and "long" day conditions (i.e. "zero" day, see "Results and Discussion"). Measurements for the "short" day plants were carried out for 14 days, and for the "long" day plants – within 7 days after placing the plants under light of different intensity, because after a total of 17 days of growing in the "long" day the plants entered the generation phase.

Measurement of fluorescence characteristics of chlorophyll (Chl) *a* in leaves. Prior to measurements, the plants were kept in the dark for 30 min; fluorescence was measured with a Walz Mini-PAM fluorometer (Germany). The effective quantum yield of PSII under illumination:
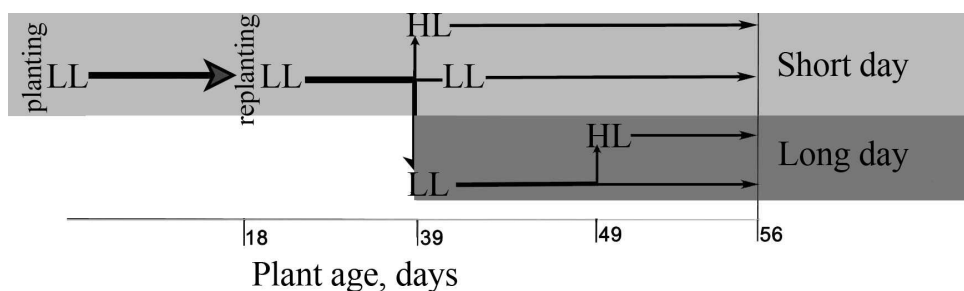
$$Y = (F'_m - F_s)/F'_m,$$

Fig. 1. Scheme of cultivation of *Arabidopsis* plants during experiments on the influence of the intensity of the illumination on the expression of CA genes under different photoperiod conditions. Conditions of "short" day (indicated by light gray): 8 h day/16 h night; conditions of "long" day (indicated in dark gray): 16 h day/8 h night. LL (low light), light intensity 80 μmol PAR quanta·m^{-2}·s^{-1}; HL (high light), light intensity 400 μmol PAR quanta·m^{-2}·s^{-1}.

and the coefficient of nonphotochemical quenching of Chl a fluorescence:

$$qN = (F_m - F'_m)/(F_m - F'_0),$$

were measured; F_m — the maximum yield of Chl a fluorescence in the dark-adapted leaves in response to a flash of saturating light, F_s — the fluorescence yield under actinic light, F'_m — the maximum value of the fluorescence yield in response to a flash of saturating light during actinic light illumination, and F'_0 — the minimum level of fluorescence after illumination. Relaxation of nonphotochemical quenching of Chl a fluorescence from the moment of switching off the light to the third minute in the dark (qN_3) was calculated as:

$$qN_3 = (F_{m3} - F'_m)/(F_m - F'_0),$$

and relaxation to the 15th min of darkness as:

$$qI = (F_m - F_{m15})/(F_m - F'_0);$$

F_{m3} and F_{m15} — the fluorescence yield values in response to saturation light flashes delivered 3 and 15 min after the light was turned off, respectively. The photosynthetic active radiation (PAR) was measured with an LI-190SA Quantum Sensor, which is sensitive in the 400-700 nm wavelength range.

Measurement of protein content of light-harvesting complex of PSII. Isolation of thylakoids and determination of the chlorophyll content in them were carried out as described in Ignatova et al. [17]. Electrophoresis under denaturing conditions was carried out using the Shagger and Von Yago method [18] in a Protean II xi Cell (Bio-Rad, USA). Protein bands were visualized with Coomassie dye. The position of proteins Lhcb3 and Lhcb6 was determined in the gel after electrophoresis under denaturing conditions [19]. Western blotting with antibodies to Lhcb1 and Lhcb2 was carried out on nitrocellulose membranes by the method of Onda et al. [20]. Densitometric analysis of protein bands was performed using the gel-documenting system "Gel Doc System" (Bio-Rad) and Gel-Pro Analyzer 3.1 software.

Conventional PCR. RNA was isolated using the Aurum total RNA Mini Kit (Bio-Rad) from leaves previously frozen in liquid nitrogen. Reverse transcription was performed using the iScript Reverse Transcription Supermix reagent kit (Bio-Rad). The resulting cDNAs and specific primers to the CA genes (Table 1), designed in such a way that at least one primer from the pair is complementary to the terminal sequences of the two exons, were used in PCR, followed by electrophoresis in 1% agarose gel with 89 mM Tris borate buffer in the presence of 20 mM EDTA and ethidium bromide as an intercalating agent. The 2-Log DNA Ladder 100-3000 bp (New England Biolabs, USA) was used as size markers.

Quantitative PCR. Quantitative PCR (qPCR) was carried out using an iQ5 Bio-Rad instrument with Evrogen reagents (Russia) in three biological and two statistical samples using leaves from three individual plants. The data were normalized to the actin 7 gene *At5g09810* and calculated as $2^{-\Delta CT}$.

RESULTS AND DISCUSSION

Characteristics of plants. *Arabidopsis* is a shade tolerant plant, thus the optimal light intensity for it is about 150 µmol PAR quanta·m^{-2}·s^{-1} [21]; therefore, we designated the light intensities of 80 and 400 µmol PAR quanta·m^{-2}·s^{-1} as LL and HL, respectively (see "Materials and Methods"). It is known that, when adapting to high light, the size of the light-harvesting complex of PSII decreases due to a decrease in the amounts of Lhcb1, Lhcb2, Lhcb3, and Lhcb6 proteins [22]. The data in Table 2 show that during 14 days in the plants of the "short" day, and during 5 days in the plants of the "long" day, there was a marked decrease in the content of these proteins in the leaves, which indicated the adequacy of the selected periods of adaptation of the photosynthetic apparatus to HL in various photoperiod conditions.

Characteristics of the photosynthetic apparatus by measuring fluorescence of Chl a in leaves of plants growing at low or high light intensity were compared at the same light intensity at the end of the periods of plant adaptation to HL, i.e. after 14 days of parallel growth at both light intensities under "short day" conditions, and after 5 days — under "long day" conditions (Table 3). The effective quantum yield of PSII by this time was lower in plants under HL than in plants under LL, and the coefficient qN, characterizing nonphotochemical quenching of Chl a fluorescence, was higher. It is significant that the value of the quantum yield achieved during this period was higher, and the value of qN is lower than these values in the first days after the transfer of plants to HL (not shown), which, according to the data of Borisova-Mubarakshina et al. [23], reflects the adaptation that has taken place. We found that the difference in the nonphotochemical quenching under light of different intensities is due to processes that can be characterized by the parameter qN_3 (Table 3), the rapidly relaxing component of the nonphotochemical quenching, which depends on protonation of the PsbS protein [24]. In the "short day" plants, increase in illumination leads to a greater increase in qN_3 than in the "long day" plants, and this indicates a difference in the effect of the intensity of light for different daytime durations.

Despite the adaptation to HL, the coefficient qI in plants under HL was higher than in plants under LL in both "long day" and "short day" conditions, which shows that photoinhibition of PSII was higher in plants under HL.

Table 1. Primers used for quantitative and conventional polymerase chain reaction. F is the "forward" and R is the "reverse" primer

Genes		Nucleotide sequences of primers
At3g01500.2 (β-ca1a)	F R	CCTCTCCGAAACTAGCTCTGTTAA CTGTCCCCCAAGATTTTAATTCTGAAA
At3g01500.3 (β-ca1b)	F R	GAAAGGGAGGCGGTGAATGTT GATGATGATGATGGATTGGTTCATACAGA
At5g14740 (β-ca2)	F R	CGAAAGGGAGGCAGTGAATGTG AACCGATGGTGATGGTGATGTGTTA
At1g23730 (β-ca3)	F R	TACAGCTCCCACTAAGACCGAGTT CTTGTTCTTCACCACTCTTTCTCTCACGA
At1g70410 (β-ca4)	F R	AAGAATAGTACTTTGTTCAATCATCTTGCC GTGTCTCTTCTGGTCAAAAGGTGG
At4g33580 (β-ca5)	F R	CCTTAATCTCCAAACTCAAGCGATC GTCGTCAAATACATCAGTGTTGTTTTGT
At1g58180 (β-ca6)	F R	AAAAATGGCGTTTACACTAGGTGGAAG CCCTGATTCCTAATTCTTGAACCATGT
At3g52720 (α-ca1)	F R	TGCTCAGACAGAAGGAGTAGTGTTTG GTTTGTGAAGTAGTATTCACGGTGTATTG
At3g52720 (α-ca2)	F R	GGCGGTTCACGATAATTCAGACACA AAAAAATTAAATAAATAGTACACTGTGGTTGTA
At4g20990 (α-ca4)	F R	TCCTCACCAAGCTACTAAATGGAATAAA TTGACGACAGTCCAAATGACGC
At1g08065 (α-ca5)	F R	TCTCCTCACGTTGGAAAGATACTTGAAG TTGTTTTAATGTCACAGTCCTCATCTC
At4g21000 (α-ca6)	F R	ATTGGCATTCACCGTCTGAGCATA TCCCTACTCCTTTTATTCCATTTAGTATCTTTG
At1g08080 (α-ca7)	F R	CAGATCGTTGGAGAAAGAATTAGAGGG TCTTGTCACGGTCCTAACCTTT
At5g56330 (α-ca8)	F R	CCAAGTCATCAGTTTATCCCTAAAAACATC CCAAGTCATCAGTTTATCCCTAAAAACATC
At5g09810 (gene, encoding actin 7)	F R	GAAGGCTGGTTTTGCTGGTGAT CCATGTCATCCCAGTTACTTACAATACC

Expression of genes encoding CAs. Although the protein content in the plant does not always directly correlate with the content of the transcripts of the corresponding genes, the approach based on measuring the level of transcription when changing the plant's living conditions is most convenient in the study of CAs, since the determination of the content of these proteins by Western blot analysis in leaves of higher plants is difficult due to the similarity of the amino acid sequences of representative proteins within one family of CAs. The construction of primers, sufficiently short DNA fragments used to measure the expression level of genes by the qPCR method, makes it possible to select specific sequences for each gene that do not occur in the genes of other CAs, and to determine the relative levels of expression of the genes of all CAs.

Primers for the *At3g01500* gene encoding β-CA1, the most abundant CA of the plant cell located in the chloroplast stroma, were selected while considering the possible alternative splicing of this gene at the 3′-end: the last two exons can be spliced during transcription with excision of the last intron (*At3g01500.2*), or the last intron can serve as the untranslated region of the exon (*At3g01500.3*) (Fig. 2). We designed primers complementary to both forms of the gene (designated by us as *β-ca1a* and *β-ca1b*, respectively), and for the first time it was shown that both forms of this gene are expressed in the leaves of *Arabidopsis* (Fig. 3, lanes *1* and *2*). In leaves of *A. thaliana* plants, all the

Table 2. Effect of light intensity on content of proteins of light-harvesting complex of PSII in leaves of *Arabidopsis* plants under conditions of "short" (8 h day/16 h night) or "long" day (16 h day/8 h night)

| Proteins | Protein content at high light intensity as percentage of its content at low light intensity | |
	"short day"	"long day"
Lhcb1	69.6 ± 2.8	83.1 ± 2.8
Lhcb2	53.5 ± 18.3	62.3 ± 1.8
Lhcb3	90.4 ± 3.5	32.5 ± 0.6
Lhcb6	31.8 ± 2.7	27.4 ± 4.0

Note: Measurements with "short day" plants were carried out after 14 days, and with "long day" plants − 5 days after transfer of plants adapted to corresponding photoperiod, from low-intensity light (80 µmol PAR quanta·m^{-2}·s^{-1}) to high-intensity light (400 µmol PAR quanta·m^{-2}·s^{-1}). Average values of five experiments are given ± standard error of the mean. Differences between values at high and low light intensities are statistically significant ($p <$ 0.01).

genes of the β-family CAs and three genes of the α-family − α-CA1, α-CA2, and α-CA4 − were expressed. The size of the PCR products with the corresponding primers was 170-200 bp (Fig. 3, lanes *1-11*). All primers reacted only with cDNA, because the size of the PCR products in each case corresponded to the calculated one, and there were no other bands in the gel. Thus, the use of these primers for qPCR made it possible to measure the content of the RNA of the corresponding genes. PCR products with primers to genes encoding α-CA3, α-CA5, α-CA6, α-CA7, and α-CA8 were not detected (not shown), which corresponds to literature data.

In the leaves of 40-55-day plants, which were at the stage of vegetation (plants of the "short day"), all the studied CA genes were actively transcribed (Figs. 4 and 5, left columns). By the 55th day, there was an increase in the content of transcripts of some genes (*β-ca3, β-ca6, α-ca2*). Expression of other genes of CAs − *β-ca2, β-ca4,* and *β-ca5* − also showed a tendency to increase. This is apparently because at the age of 39-55 days the *Arabidopsis* plants are still in the growth phase, when the mitotic index (the percentage of dividing cells from the total number of cells) in all annuals is high [25]. In the "long day" conditions, just before the plants started the formation of generative organs, approximately on the 17th-20th day of growth at this photoperiod, when leaves began to die and flower stems started to form, the contents of the transcripts of the CA genes fell practically to zero (Figs. 4 and 5, right columns), since changes in mRNA levels occur before visible changes in plants [25].

Adaptation of plants to an increased light intensity (Figs. 4 and 5) led to a change in the expression level of most CA genes, and these changes for many genes depended on the length of the photoperiod during cultivation. Obviously, in plants of both "short day" and "long day", an increase in illumination leads to an intensification of photosynthesis in the leaves, but the different duration of this process during the day can have different demands on the functional activity of the CA in high-light conditions. In addition, the "long day" conditions in *Arabidopsis* plants is the main factor initiating the transition of the *Arabidopsis* plants to the generative phase by triggering the cascade of expression of groups of flowering genes [15], including a variety of genes not directly related to the formation of flower organs. The "long day" and "short day" plants were, therefore, in different ontogenetic phases, which could affect the transcription of the CA genes, changing the dependence of this process on the light intensity, and on the duration of photosynthesis during the day. Thus, the experimental conditions used in this study provide additional information on the possible functions of the CAs.

Effects of light intensity on level of gene expression of extrachloroplastic CAs. Under the "short day" conditions, the level of expression of the gene encoding β-CA2,

Table 3. Effect of light intensity on parameters of Chl *a* fluorescence of *Arabidopsis* leaves under conditions of "short day" (8 h day/16 h night) or "long day" (16 h day/8 h night)

Day length (photoperiod)	Light intensity, µmol PAR quanta·m^{-2}·s^{-1}	Y	qN	qN3	qI
8 h	80	0.72 ± 0.01	0.23 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
	400	0.38 ± 0.01*	0.63 ± 0.01*	0.23 ± 0.03*	0.18 ± 0.02*
16 h	80	0.54 ± 0.03	0.36 ± 0.01	0.13 ± 0.01	0.03 ± 0.01
	400	0.39 ± 0.01*	0.63 ± 0.01*	0.33 ± 0.01*	0.12 ± 0.01*

Note: Measurements with "short day" plants were carried out in 14 days, and with "long day" plants − in 5 days after moving the plants adapted to the corresponding photoperiod in low light into high light. Measurements were carried out at saturating concentration of CO$_2$ (700 ppm) and intensity of light at which the plants grew. The mean ± standard error of the mean is presented. * Statistically significant differences between values under different light intensity and same day length ($p <$ 0.01).
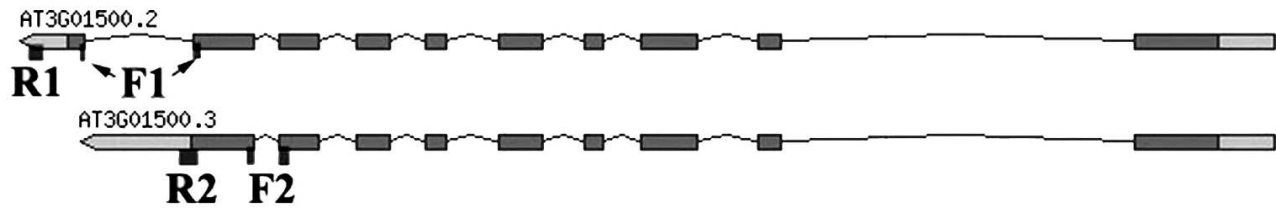
At3g01500

Fig. 2. Alternative splicing scheme of the *At3g01500* gene encoding β-CA1. Rectangles denote the gene exon parts, connected by introns. Light areas are untranslated end regions. F1 and R1, and F2 and R2 are the locations of the sections complementary to the "forward" and "reverse" primers, respectively.
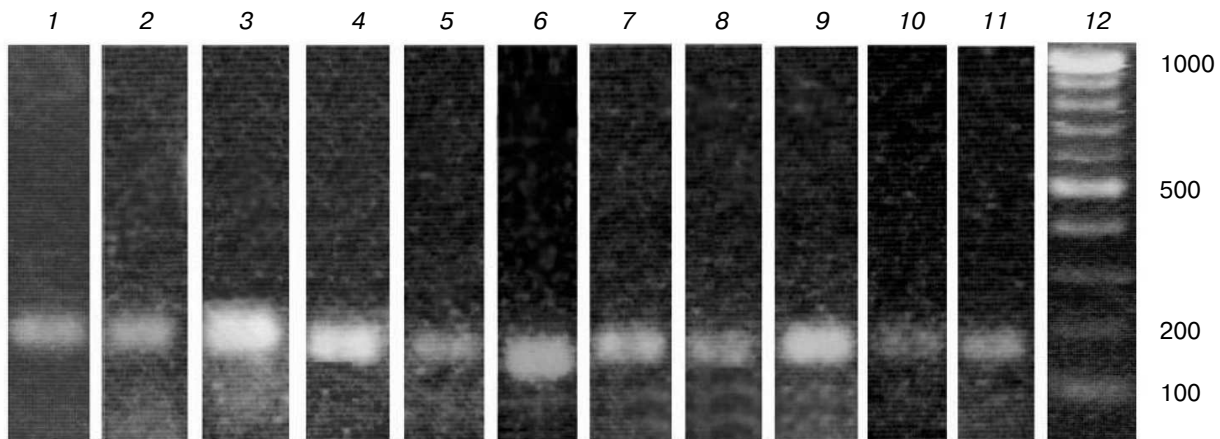

Fig. 3. Result of conventional PCR with designed for quantitative PCR primers to genes encoding: *1*) actin; *2*) β-CA1a; *3*) β-CA1b; *4*) β-CA2; *5*) β-CA3; *6*) β-CA4; *7*) β-CA5; *8*) β-CA6; *9*) α-CA1; *10*) α-CA2; *11*) α-CA4; *12*) markers of length of DNA fragments; numbers on the right are the sizes of double-stranded DNA fragments in base pairs.

the second most abundant CA in the cells of higher plants and located in the cytoplasm, following a slight decrease in the first day after transferring plants to HL in 14 days was six times higher than under LL. In the "long day" plants, the expression of this gene did not change significantly with increasing light intensity (Fig. 4). The content of the transcripts of the gene of the second cytoplasmic CA, the minor β-CA3 protein, was 3-4 orders of magnitude lower than that of *β-ca2* transcripts (Fig. 4). The level of expression of the *β-ca3* gene, in contrast to that of the *β-ca2* gene, in HL was lower than in LL in both "short day" and "long day" conditions. It was suggested that the cytoplasmic CA is involved in the regulation of pH of the cytoplasm [26] and in the influx of inorganic carbon into the cell [27]. Although the effect of high light intensity on the number of transcripts of the genes of cytoplasmic CAs is opposite, and their content in the cell is very different, it is possible that they both fulfill these functions, but under different physiological conditions. An example is the presence of two CAs in the periplasmic space of

Chlamydomonas reinhardtii cells: highly expressed CAH1, induced by low concentrations of CO_2 in the environment, and very lowly expressed CAH2 [2], induced by high concentrations of CO_2. It is believed that these CAs perform the same physiological function, facilitating the supply of inorganic carbon to the cell.

The content of transcripts of the *β-ca4* gene, which encodes plasma membrane CA, changed very little during the two weeks of cultivation under the "short day" conditions and did not depend on the intensity of light (Fig. 4, left column). In the "long day" plants, it was approximately two times lower in HL than in LL (Fig. 4, right column), which is similar to the change in the *β-ca3* gene transcript content. The function of β-CA4 was confirmed in processes taking place in stomata guard cells, where in cooperation with the β-CA1 located in the same cells it participated in regulation of the size of the stomatal pore [28]. It was suggested [29] that the function of the plasma membrane CA in the mesophyll cells of the leaf is the adjustment of the proton exchange between the cell and
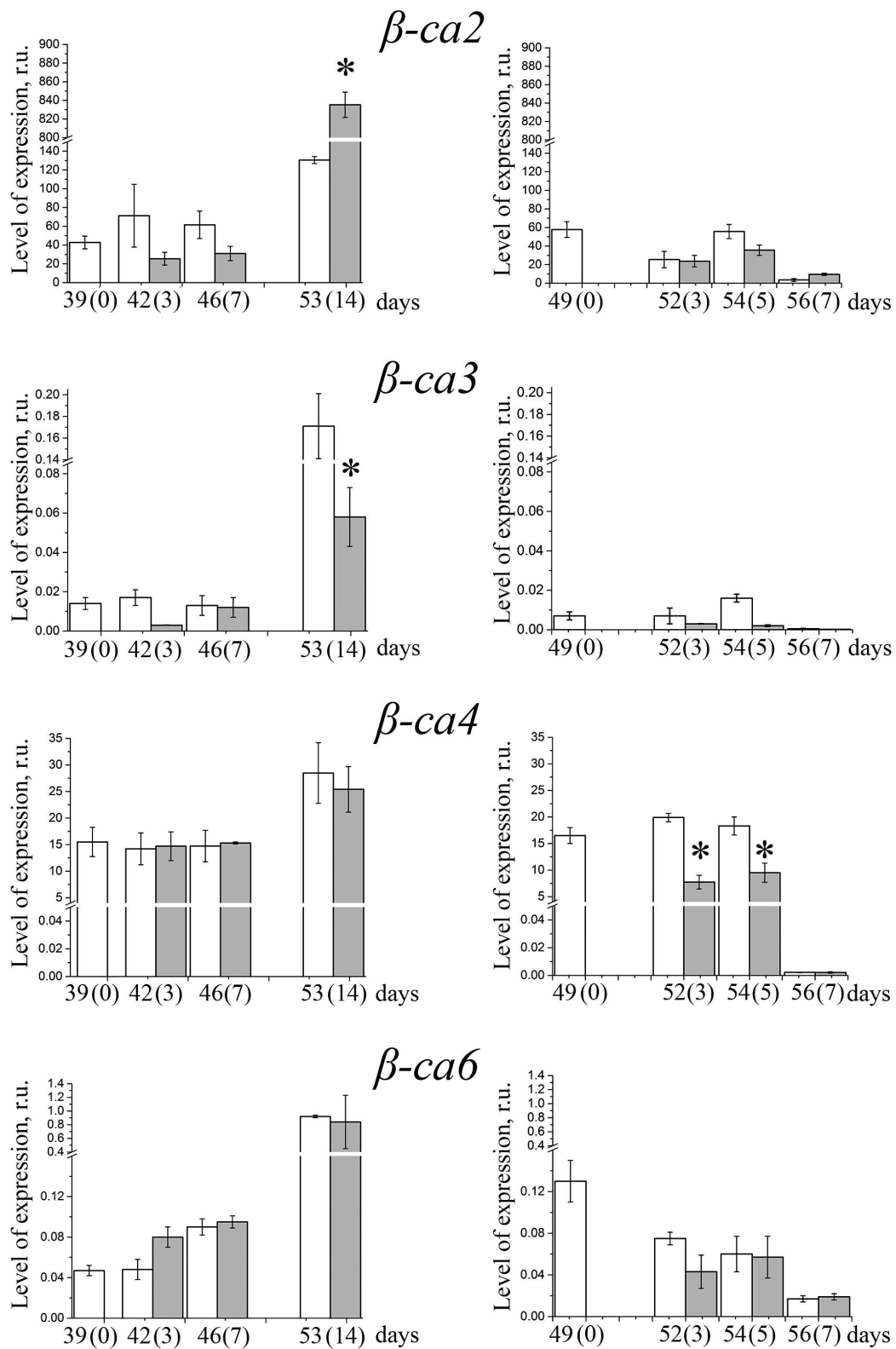
Fig. 4. Levels of expression of genes encoding extracellular carbonic anhydrases at low (80 μmol PAR quanta·m^{-2}·s^{-1}) and high (400 μmol PAR quanta·m^{-2}·s^{-1}) light intensities (white and dark bars, respectively) in *Arabidopsis* plants grown with "short day" (left column) or "long day" (right column). Numbers on the X-axis show the age of the plants and (in parentheses) the number of days after moving the plants adapted to the corresponding photoperiod from low-intensity light to high-intensity light. The data are presented as mean ± standard error of the mean. Asterisk denotes statistically significant differences between values for different illumination at the same day length, $p < 0.01$.
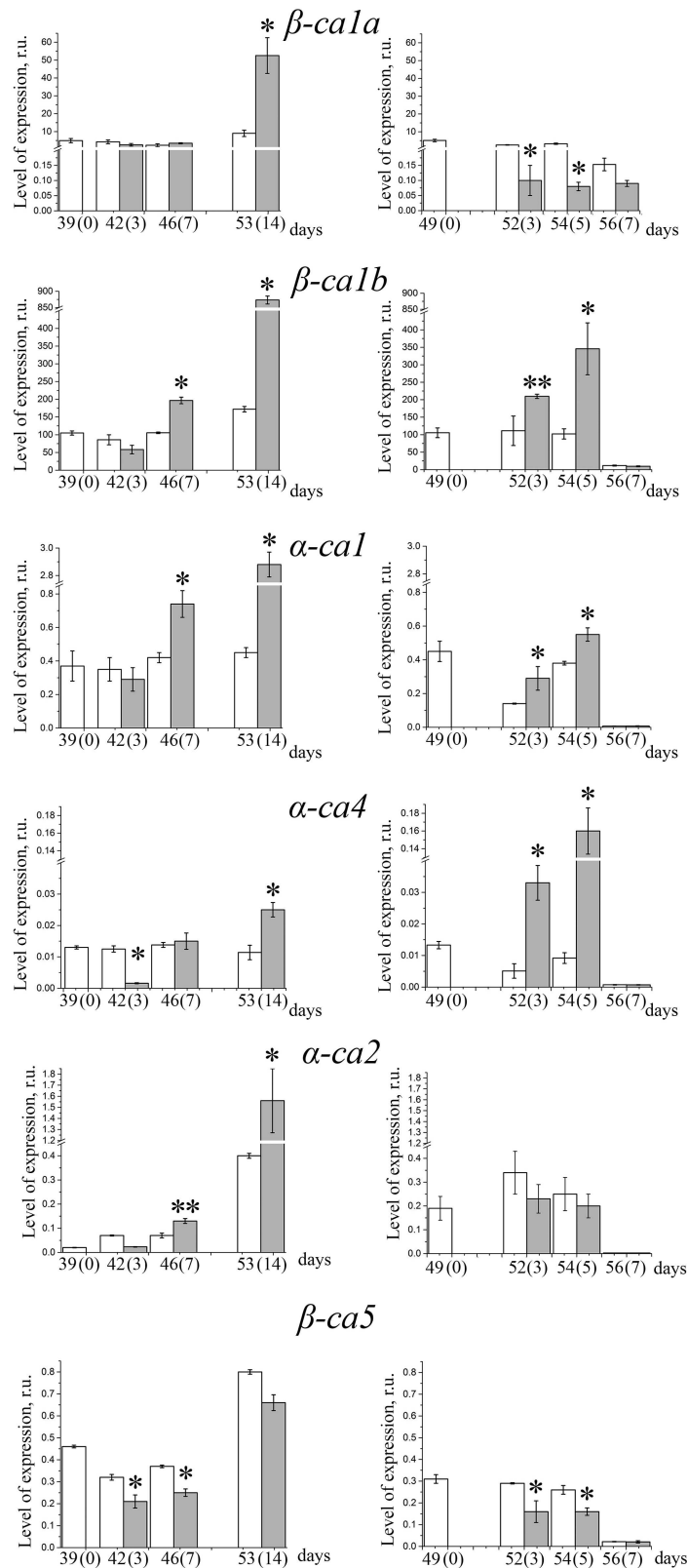
Fig. 5. Levels of expression of genes encoding chloroplast carbonic anhydrases at low (80 µmol PAR quanta·m^{-2}·s^{-1}) and high (400 µmol PAR quanta·m^{-2}·s^{-1}) light intensities (white and dark bars, respectively) in *Arabidopsis* plants grown with "short day" (left column) or "long day" (right column). Numbers on the X-axis show the age of the plants and (in parentheses) the number of days after moving the plants adapted to the corresponding photoperiod from low-intensity light to high-intensity light. Mean ± standard error of the mean is presented. Statistically significant differences between values for different illumination at the same day length; * $p < 0.01$, ** $p < 0.05$.

the environment that is provoked by the consumption of CO_2 in photosynthesis.

Only contents of transcripts of the gene of mitochondrial β-CA6 remained approximately the same under both HL and LL, and in both "short day" and "long day" conditions, changing only with the age of the plant (Fig. 4). A hypothesis about the physiological role of this CA assumes its participation in cooperation with the γ-CA domain in the active inorganic carbon transport system from mitochondria to chloroplasts in conditions when the stomata are closed and CO_2 inflow from the environment is limited [3]. In this case, the activity of β-CA6 should be induced by low concentration of carbon dioxide.

Effects of light intensity on level of expression of CA genes of chloroplasts. The adaptation of plants to increased illumination should obviously be reflected in the activation of processes taking place in chloroplasts. In "short day" plants, after two weeks of adaptation to HL the level of expression of the majority of chloroplast CAs was higher than under LL (Fig. 5). The level of *β-ca1a* transcripts in the "short day" conditions increased approximately 5-fold after 14 days under HL (Fig. 5, left column), while in the "long day" conditions, after just 3 days under HL it was 30 times lower than under LL (Fig. 5, right column). The level of transcripts of *β-ca1b* in "short day" plants under HL increased approximately 5-fold after 14 days (Fig. 5, left column), exactly like the level of expression of *β-ca1a*, but in the "long day" conditions, it showed a tendency to increase, and after 5 days it was more than three times higher than under LL (Fig. 5, right column). Light intensity had a similar effect on the expression of yet another stromal CA, α-CA1 (Fig. 5): its level of expression under high light was also higher for both "short day" and "long day" conditions, after the initial decrease in the "long day" conditions at both light intensities.

Thus, the study of the content of transcripts of stromal CA genes under "long day" conditions revealed significant differences in the expression level of these genes during the adaptation to high light intensity. The opposite effect of light intensity on the level of expression of the two forms of the gene encoding β-CA1, *β-ca1a* and *β-ca1b*, under these conditions may indicate different functions of the proteins encoded by them. The lack of the effect of the decrease in the content of β-CA1 on photosynthesis (see introduction) served as the basis for assumptions about a minor role of CA in this process [7]. However, it is likely that more than an order of magnitude difference in expression levels between the two forms of the α-CA1 gene should be considered. Considering the similar increase in the expression of *α-ca1* and *β-ca1b* genes with increasing illumination, we assume their cooperation in the supply of CO_2 to Rubisco. From the literature, the joint participation of two CAs is known to perform an important function. For example, it has been

shown that β-CA1 and β-CA4 of the *Arabidopsis* stomatal guard cells together control the gas exchange of the plant [28]. It is possible that in this case β-CA1a functions together with β-CA4. This possibility is supported by the similar effect of HL on the genes *β-ca1a* and *β-ca4* in "long day" plants (Figs. 4 and 5, right columns).

The level of expression of the thylakoid CA gene, *α-ca4*, did increase under HL in the "short day" conditions after the initial decrease, and became two times higher than under LL after 14 days (Fig. 5, left column). Under the "long day" conditions, the number of transcripts of this gene under HL became 16 times higher than under LL after five days (Fig. 5, right column). A study of α-CA4 function, using knockout plants lacking the *α-ca4* gene, showed that in its absence energy-dependent nonphotochemical quenching of Chl *a* fluorescence was significantly suppressed, and it was suggested that this CA is involved in local protonation of the PsbS protein [30]. This function of α-CA4 is consistent with a significant increase in the level of expression of its gene in plants under HL, when the need for nonphotochemical quenching protecting PSII from photoinhibition increases: in plants adapted to HL, nonphotochemical quenching of Chl *a* fluorescence was indeed significantly higher than in plants under LL (Table 3).

The gene encoding α-CA2 had a relatively low level of expression, which, under "short day" conditions, in our experiments increased with increasing plant age (Fig. 5, left column). An increase in the illumination of plants under these conditions led to an additional increase in the level of its transcription, similar to the genes discussed above. In the "long day" conditions (Fig. 5, right column), there were no significant differences in the content of transcripts of the *α-ca2* gene under both HL and LL, although there was a tendency for its decrease under HL, like that for *β-ca1a* and *β-ca5* (Fig. 5). There are no direct data on the location of α-CA2, but some properties of knockout mutants lacking *α-ca2* gene suggest that this CA is located in the thylakoid membranes: in these mutants, the level of energy-dependent nonphotochemical quenching was higher than in wild-type plants [6]. Similar trends in the level of expression of *α-ca4* and *α-ca2* genes with increasing illumination in plants under "short day" conditions and different trends in "long day" conditions can be compared with the data of Zhurikova et al. [6] about the opposite effects of α-CA4 and α-CA2 carbonic anhydrases on nonphotochemical quenching of Chl *a* fluorescence; these effects, owing to a change in the ratio of their contents, can provide the nonphotochemical quenching value that is necessary under the current environment conditions.

The level of transcription of only one gene encoding the chloroplast CA β-CA5 decreased under HL in "short day" conditions (Fig. 5, left column). Under HL in "long day" conditions, the level of transcription of this gene also decreased, which coincided only with the behavior of

the *β-ca1a* gene. The location of β-CA5 in the chloroplasts of *Arabidopsis* has not been established [1]. Previously, we found that there is a soluble CA of the β-family in the lumen of the thylakoids of *Arabidopsis* and pea [31]. Even before the discovery of this enzyme, it was suggested [32] that the soluble CA could, with the participation of the CO_2/HCO_3^- buffer, provide the facilitated diffusion of protons to the ATP synthase channel. The importance of such CA should obviously increase at low illumination, when the proton flux into the lumen is low, and they can be "lost" along the way to ATP synthase. This corresponds to the observed dependence of the expression of the *β-ca5* gene on the intensity of illumination of plants, and one can safely assume that β-CA5 is a lumenal CA. Seeds of *Arabidopsis* with knocked out *β-ca5* gene sprouted up only under increased CO_2 concentration and the plants were far smaller than wild-type plants (J. V. Moroney, personal communication), and this is the only case where knockout of only one CA affected the phenotype so negatively.

The study provides data on the expression of genes of several CAs belonging to the α- and β-families in plant leaves. It is known that the roles of many enzymes can manifest themselves to a greater or lesser extent when placing the plant in stress conditions, and such a natural factor for plants as increased illumination can induce a stress response in *Arabidopsis*, a heliophobic plant. It turned out that the expression of the genes of some of the CAs during the adaptation of *Arabidopsis* plants to increased illumination depends on the photoperiod, i.e. the period of illumination of plants during the day; and with increasing illumination, the level of expression of the genes of some CAs changed in the opposite directions for different photoperiods. The results suggest that when the same external factor (light intensity, temperature, humidity, etc.) changes, the participation of CAs in the metabolism of higher plants, which for most of them is still not completely clear, can depend radically on accompanying conditions, which must be taken into account when analyzing the functions of these enzymes.

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