Chapter

Role of Plant Carbonic Anhydrases under Stress Conditions

Natalia N. Rudenko, Maria M. Borisova-Mubarakshina, Lyudmila K. Ignatova, Tatiana P. Fedorchuk, Elena M. Nadeeva-Zhurikova and Boris N. Ivanov

Abstract

Carbonic anhydrases (CAs) are enzymes catalyzing the reversible hydration of carbon dioxide with the generation of protons and bicarbonate. The components of the reaction are involved in almost all metabolic processes in higher plants and algae, maintaining the balance of electrolytes and pH, gluconeogenesis, lipogenesis, ethylene synthesis, and others. The CAs may take part in transmitting signals to activate cascades of protective response genes. Our findings reveal significant changes in the content of carbonic anhydrase gene transcripts in response to changes in environmental conditions. Here we discuss the functions of CAs located in the plasma membrane, chloroplast envelope, chloroplast stroma, and in thylakoids in plant protection under stress conditions, such as high illumination, low and high concentration of carbon dioxide in the environment, drought, and salinity.

Keywords: carbonic anhydrase, plants, chloroplasts, thylakoids, high illumination, carbon dioxide

1. Introduction

Carbonic anhydrases (CAs) are the group of Zn-containing enzymes that are the biological catalysts accelerating both the carbon dioxide hydration reaction and the bicarbonate dehydration reaction:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+$$

In the absence of CA, these reactions proceed relatively slowly to ensure the physiological needs of the cell. The CAs were found in cells of all living organisms: prokaryotes, fungi, plants, and animals. Notably, both binding and release of protons in this reaction are important for many biochemical processes in cellular metabolism. All metabolic processes in higher plants and algae, including the electrolyte balance and pH, gluconeogenesis, lipogenesis, ethylene synthesis, and others depend on/require the components of this reaction. In this review, we focus on the functioning of plant CAs, including algae and angiosperms with different types of CO_2 fixation: on C3 and C4 organic matter as well as under normal and stress conditions respectively.

CO_2 is the main source of carbon in higher plants and algae cells. The compounds with a common name "inorganic carbon" (Ci) were found in nature both in the form of an unhydrated or hydrated carbon dioxide molecule and in the form of a bicarbonate or carbonate ion. The content of various forms of Ci in solution is pH-dependent. CO_2 prevails at pH value lower than 6.4, HCO_3^- at pH between 6.4 and 10.3, and CO_3^{2-} at a pH of 10.3 and higher [1]. The substrate for the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), which carries out the first Ci fixation reaction in the Benson-Calvin cycle, is the CO_2 molecule. This poses difficulties for aquatic photosynthetic organisms, since at pH of 8.0–8.3 in seawater about 95% of Ci is in the form of HCO_3^-, whereas the dissolved CO_2 represents only a small part of Ci. Thus, the main stress that the aquatic photosynthesizers, namely cyanobacteria and algae, especially in the habitats with alkaline water in seas and oceans, have to face is a low CO_2 content in their environment. Therefore, these organisms have evolved the mechanisms of Ci concentrating to increase CO_2 content near Rubisco. The main role in supplying Ci and converting it from HCO_3^- to CO_2 is played by CAs located in various cellular compartments of cells in aqueous photosynthesizers [2].

The terrestrial higher plants have adapted to survival under constant stress conditions, such as high temperature, high illumination, and low soil moisture, leading to the closure of stomata. They do not suffer from CO_2 deficiency since another type of Ci concentrating mechanism, C4 type of photosynthesis, has evolved. The first reaction that ensures the concentrating of Ci in plants of C4 species is converting CO_2 to bicarbonate, which is used by phosphoenolpyruvate carboxylase in forming a four-carbon product. It has been established that the CA, which is mainly located in cytoplasm in C4 plants, plays a key role in this transformation. In C4 species, the suppression of the synthesis of these CAs leads to dramatic effects in these plants [3, 4].

In C3 plant leaves, the existence of a Ci-concentrating mechanism has not been stated. At the same time, the story is much more complicated since C3 plants have a large number of genes encoding CAs, the physiological role of which has not been established yet. It is still not entirely clear whether all these genes are expressed, since the expression of a number of CA genes is induced under certain conditions, such as low CO_2 [5] and high illumination [6] and osmotic stress [7].

In chloroplasts, performing C3 photosynthesis, at least six CAs have been discovered to date, both soluble and membrane-bound [8], and belonging to different CA families. In the review, we discuss the possible role of two soluble CAs in photosynthesis, one of them was known for a long time [9] and another one discovered rather recently [10]. Both of them are situated in the chloroplast stroma, where carbohydrates from Ci are formed. Thus, here we discuss the functional relationship of these CAs with Rubisco and their response to stress factors. Recent studies show that the CAs are important not only for photosynthesis and for a number of metabolic pathways, including the interconversion of Ci forms, but also they are necessary under certain stress conditions. In this case, changes (fluctuations) in the CA activity can orchestrate the intensity of certain metabolic processes including the rate of photosynthesis [11–13].

Recently, a functional relationship between cytoplasmic and chloroplast CAs and aquaporins has been found [14–16]. The last ones are the protein channels that facilitate the transport not only of water, but also of CO_2, and even of H_2O_2 [16], known as a signal molecule in retrograde signaling.

The enzymatic activity and the content of thylakoid CAs also change under stress conditions. CA activity in thylakoids of higher plants was detected in granal thylakoid membranes, enriched with PSII, as well as in lamellar membranes, enriched with PSI and ATPase in thylakoid lumen [17–20]. Incorporation of these CAs in plant defense systems of higher plants under changing environmental conditions is reviewed.

2. Modern classification of carbonic anhydrases

Based on the conservative nucleotide sequences in the genes encoding CAs, they could be classified into nine evolutionarily independent families (α, β, γ, δ, ζ, ε, η, θ and the recently discovered ι-CAs) [21–24]. Some researchers do not ascribe ε-CAs to a separate family, since these CAs are highly modified β-CAs [25]. Although these enzymes have completely different primary, tertiary, and quaternary structures and differ in the organization of the active center, they all are called CAs because they catalyze the same reaction using similar catalysis mechanisms.

Representatives of rare and small δ-, ζ-, θ-, and ι-CA families in eukaryotes were found in diatoms and some other unicellular microalgae, whereas α-, β-, and γ-CAs were discovered in most algae species and in all higher plants, including mosses and Lycopodium.

2.1 α-CAs

Since all CAs in human cells belong to the α-CA family, this family is the most studied and widespread. Representatives of this family were found in eubacteria [26, 27], ascomycetes [28], algae [29, 30], higher plants [5, 31], and animals [32]. In green algae *Chlamydomonas reinhardtii*, there are three α-CAs: CAH1, CAH2 [30, 33] in the periplasm, and CAH3 found on the luminal side of thylakoid membranes [34]. Eight genes encoding α-CAs were found in *Arabidopsis thaliana* genome. α-CA1 was discovered in chloroplast stroma [10], and α-CA4 was found during proteomic studies among the proteins of the thylakoid membranes [35, 36]. Evidence suggests that α-CA2 is present in thylakoid membranes [37]. α-CA3 was found in flowers and pods [5], as well as during proteomic analysis of mature pollen proteins [38, 39].

All functionally active α-CAs contain three residues of histidine, which are conservative in all active α-CAs. These histidines are the ligands of Zn atom [40, 41] located at the bottom of the conical cavity of the active center. Histidines in α-CAs' structure enhance the net positive charge of the metal ion, which is essential to achieve effective catalysis [42]. Most α-CAs are the monomers with molecular mass of about 30 kDa, although the periplasmic CA in *Chlamydomonas reinhardtii*, CAH1, is a heterotetramer with two 27-kDa subunits and two 4-kDa subunits connected by disulfide bridges [43].

2.2 β-CAs

The representatives of β-CAs were found in archaebacteria [44], cyanobacteria [45], eubacteria [46], chemoautotrophic bacteria [47], fungi [48], algae [49], higher plants with C3- and C4-type photosynthesis, dicotyledons and monocotyledons [50, 51]. In *Chlamydomonas reinhardtii* cells, there are six β-CAs located in mitochondria, chloroplast stroma, and cytoplasm [2]. The analysis of the Arabidopsis genome has shown the presence of six genes encoding β-CA; moreover, as it was found by Fabre et al. [5], all of these genes are expressed. The same group of researchers using the method of gene fusion with a green fluorescent protein gene confirmed that the two most active CAs are located in stroma and cytoplasm. These CAs were discovered by Atkins et al. in 1972 [9] in the soluble fractions of higher plant leaves of different species. Fabre et al. [5] have found these CAs in the chloroplast stroma and in the cytoplasm. They were called β-CA1 and β-CA2, correspondingly. Other β-CAs were also located in Arabidopsis cells: β-CA3, in the cytoplasm, β-CA4 in plasma membranes, β-CA5 in chloroplasts, and β-CA6 in the mitochondrial matrix.

The enzymes of this family are as effective in catalysis as α-CAs are. One histidine and two cysteine residues are zinc ligands in the active center of β-CAs [52, 53]. The active site in β-CAs' structure is a tight pocket and the only access to it from the bulk solvent is via a bottleneck between Gln, Gly, Asp, and Tyr residues. This bottleneck is too narrow for anything larger than a water molecule, meaning that to implement the catalytic cycle some rearrangement should take place [54]. The study of the structure of β-CAs revealed the presence of the so-called non-catalytic bicarbonate binding site [55], which represents a "pocket" located 8 Å from the active center zinc. A network of at least seven molecules linked together by hydrogen bonds is capable of holding a bicarbonate molecule. Studies have shown that this is not just an anion-binding site, the addition of bicarbonate leads to the reorganization in the protein molecule and in the active center optimizing its functioning [56]. The hydrogen bonds of the Trp, Arg, and Tyr residues in this site are well organized to recognize the bicarbonate ion such anions as acetate or nitrate are not able to carry out all the necessary interactions with the hydrogen bonds of these amino acid residues. Tyr residues in the active site and bicarbonate binding site structures were recently found to be the key amino acids for the reversible modification by phosphorylation and nitration in response to abiotic and biotic stresses [11, 12]. Such modifications allow for blocking the passage of substrate and inhibiting the activity of β-CAs under stress conditions.

2.3 γ-CAs

γ-CAs are present in cells of bacteria, green algae, diatoms [2], and higher plants [57]. In *C. reinhardtii,* three γ-CAs are situated in mitochondria [58]. In *A. thaliana*, the CA domain consisting of five γ-CAs was discovered as a part of mitochondrial complex I [57]. Each of these CAs is encoded by a separate gene [59]. Three CAs of the domain, γ-CA1, γ-CA2, and γ-CA3, are close in structure to the first discovered γ-CA from *Methanosarcina thermophila* (Cam). Two more CAs were called γ-CAL1 and γ-CAL2 ("gamma carbonic anhydrase like") since they had sequences less similar to those of Cam for readability [60].

γ-CAs function as trimers consisting of identical subunits [61], which contain one zinc atom per subunit; however, unlike α and β-CAs, the active center is located between the subunits. In the active center of γ-CAs, there are three histidine and one H_2O residues coordinating Zn atom, like in α-CAs, but these are histidines of two opposite subunits. The mechanism of catalysis is similar to that of α-CAs [62].

3. The participation of CAs of aquatic photosynthesizers in the CO_2-concentrating mechanism

To increase the CO_2 content near Rubisco during the evolutionary adaptation to growth conditions, some groups of plants, that is, aquatic photosynthesizers, developed CO_2 concentrating mechanisms (CCMs) in their cells. All currently known CCM pathways require at least one or, more often, several CAs.

In cyanobacteria, negatively charged HCO_3^- penetrates the cells through specialized transporters on the plasma membrane, utilizing the energy of ATP and NADPH [63]. The dissolved Ci is captured with the help of extracellular CA, supplying HCO_3^- to bicarbonate transporters and preventing leakage of Ci from the cell [45]. The other CAs situated in cyanobacterial carboxysomes accelerate the formation of CO_2 for Rubisco. The last one is also placed in carboxysomes, which serve as a barrier to CO_2 leakage.

In algae cells, Ci should cross the cell wall, plasma membrane, and chloroplast membrane to reach the place of CO_2 fixation. Like in cyanobacteria, CAs in algae cells play a crucial role in CCM, which is important under conditions of low CO_2 in water. Periplasmic CA in *C. reinhardtii*, the so-called CAH1, is the most important CA in CCM [2]. This enzyme is intensively expressed at a low concentration of CO_2 in water. If CAH1 activity is inhibited, photosynthesis in *C. reinhardtii* cells is also suppressed at high pH in media, when most Ci is in the form of bicarbonates [64]. This result suggests that CAH1 facilitates the CO_2 entry into the cell. Other CAs are also involved in the delivery of carbon dioxide into the cell, namely, CAH8 and CAH9. CAH8 appears to be localized in the plasma membrane and this protein might also facilitate the diffusion of CO_2 across the plasma membrane by facilitating the conversion of HCO_3^- to CO_2 at the cell surface [65]. Likewise, CAH9 might mediate the movement of CO_2 across the cytoplasmic region to the chloroplast. One more CA, CAH3, located in thylakoid membranes of chloroplasts in *C. reinhardtii* cells is also important for algae survival under conditions of low carbon dioxide content in water. The fact that *C. reinhardtii* cells with inhibited synthesis of CAH3 were not able to grow at a normal CO_2 concentration indicates that CAH3 is involved in photosynthesis [66, 67]. However, the expression of CAH3 is constitutive; the concentration of CO_2 had no effect on the transcription level of the gene encoding this enzyme and the role of CAH3 in CCM is still not accepted.

4. CAs in higher plants

4.1 The role of CAs in photosynthesis of C4 higher plants

The plants constantly growing under stress conditions, such as drought, salinity, or high temperature, have to keep stomata closed most of the day to reduce water loss as a result of transpiration. At the same time, to avoid starvation in the absence of CO_2 in leaves, a C4 type of photosynthesis has evolved. It includes an additional carbon conversion cycle, called the Hatch-Slack cycle. CCM exists in the form of the so-called four-carbon (C4) photosynthesis with the spatial separation of the primary carboxylation reactions and the Calvin cycle. The Hatch-Slack cycle allows Ci to be concentrated in leaf tissues, carrying out the primary fixation of carbon dioxide through the carboxylation of phosphoenolpyruvate using the enzyme phosphoenolpyruvate carboxylase (PEPC). In the cytosol of the mesophyll cells of C4 plants, there is a high amount of CA [68]. This enzyme plays a decisive role in C4 photosynthesis. CA catalyzes the first reaction of C4 pathway, increasing its rate by 10^4 times, due to providing bicarbonate to PEPC [3]. After that, four-carbon acids produced as a result of PEPC activity are decarboxylated in bundle-sheath cells. This leads to an increase in carbon dioxide concentration around Rubisco, which is located in the chloroplasts of the bundle-sheath cells in C4 plants [69]. Interestingly, the Genomic Southern analysis by Tetu et al. in 2007 [70] revealed the presence of two forms of CA from the β-family in the cytoplasm in the cells of *Flaveria bidentis* leaves. The only one of them, the abundant CA, located in the cytoplasm, plays the described role in C4 photosynthesis. The other CA, which is also a cytosolic CA isoform, is not directly involved in C4 photosynthesis. This CA was suggested to be the housekeeping form of the enzyme supplying bicarbonate for such anaplerotic processes, as replenishment of tricarboxylic acid cycle intermediates, carbon for amino acid biosynthesis, seed maturation, and pH balance [71, 72]. The transformation of the gene encoding the abundant cytosolic CA in *Flaveria bidentis* cells with an antisense construct confirmed the important role this enzyme plays in the C4 photosynthetic pathway. Some of the primary transformants had impaired CO_2

assimilation rates and required a high level of CO_2 for growth. In the mutants with the CA activity, less than 10% of the WT CO_2 assimilation rate was very low and these transformants grew poorly at ambient CO_2 in the atmosphere. Reduced CA activity also increased the partial pressure of carbon dioxide required to saturate the assimilation rate of CO_2 [4].

4.2 The role of CAs in photosynthesis of C3 higher plants and in the plant defense systems under stress conditions

4.2.1 CA in the plasma membrane and envelope chloroplast membrane in C3 plants

The mechanisms of CO_2 penetration inside leaf cells have been studied for decades. Chemically, CO_2 is a lipophilic compound and it should easily diffuse through membranes [73, 74]. However, biological membranes appear to have a low CO_2 permeability [75] due to a high level of protein and sterol content [74, 76]. Recently, using plasma membrane vesicles of pea leaves it has been demonstrated that plasma membrane aquaporins could facilitate CO_2 transport [77]. The same data were earlier presented for tobacco, Arabidopsis, fava bean, and others [78–80]. The plant aquaporins are the plasma membrane intrinsic proteins (PIPs). They are mainly represented by two groups: PIP1 and PIP2, which possess some structural differences in the N and C terminal end and are subdivided into several subgroups [81, 82]. Each aquaporin has different transport properties for H_2O, CO_2, and solutes; however, the functional interaction between PIP1 and PIP2 was proposed to occur with the emphasis on their coupling under stress conditions [82]. Using the inhibitor analysis, the presence of CA located in the plasma membrane of photosynthetic pea leaf cells was proved [83] and the importance of the CA functioning in the plasma membrane for Ci transport into higher plant leaves was confirmed [84]. Later, the presence of β-CA4 in the plasma membrane was shown [5].

Wang et al. in 2016 [14] identified β-CA4 as an interactor with aquaporin PIP2;1 in Arabidopsis. This connection allows CO_2 permeability across the plasma membrane to be facilitated. Further, the decrease in the CA concentration was shown to lead to a lower CO_2 permeability in plasma membrane vesicles [77]. Therefore, it can be proposed that the functioning of plasma membrane aquaporins depends on the CA activity under both normal and stress conditions. The roles of aquaporins and CA in plant defense responses against biotic and abiotic stress factors are broadly discussed [85]. Aquaporins should interact with CAs at the membrane-liquid phase interfaces, and a term that describes their combined functioning as "cooporin" was proposed [86]. The extent of water and CO_2 permeability via aquaporins of the plasma membrane is consistent with the expression levels of both PIPs and CA when exposed to environmental challenges. Experimental evidence suggests that aquaporins and CA are mutually involved in the regulation of CO_2 in stomatal and mesophyll conductance in leaves of higher plants [15] that is crucial for both processes: acclimation to stress and recovery after stress. A number of studies conducted using genetic transformation methods showed that changes in the content of aquaporins per unit area of leaves lead to the corresponding changes in mesophyll conductance [87–89].

In tobacco plants, aquaporin NtAQP1 has been identified as a membrane CO_2 pore not only in the plasma membrane but also in the inner chloroplast envelope membranes [79]. Therefore, it was proposed that aquaporins may be involved in CO_2 transfer in both the plasma membrane and in the chloroplast envelope [75]. Along with PIP, various forms of tonoplast intrinsic proteins (TIPs) and other intrinsic proteins were described in the Arabidopsis envelope fraction [90]. We have

previously provided data showing the presence of the CA activity, associated with the isolated chloroplast envelope [16]. CA activity was also detected in chloroplast envelope membranes of *Chlamydomonas reinhardtii* and shown to be induced under conditions of low inorganic carbon concentrations [91]. Perez-Martin et al. [15] have studied the relationship between the functioning of envelope aquaporins and CA of the β-family (Olea_CA) in leaves of *Olea europaea*, which is a drought-tolerant plant. By studying the change in the expression intensity of genes encoding these proteins in the leaves of olive plants grown under conditions of sufficient moisture, drought, and during the recovery period after drought, the authors made the conclusion that these proteins can function together by supplying carbon dioxide to the stroma chloroplast. In this case, the authors presume that Olea_CA takes part in the joint functioning with envelope aquaporins, proceeding from the assumption that this CA corresponds to β-CA1. Moreover, they also demonstrated that the nucleotide sequence of the Olea_CA gene corresponds to encoding the other CA in *A. thaliana*, β-CA5. This CA was discovered by Fabre et al. in 2007 [5] in Arabidopsis chloroplasts, but the exact location of this enzyme in the chloroplast is still unknown. What is important, β-CA5 gene is the only CA encoding gene, the knockout of which leads to death when growing under ambient CO_2. The seeds of these mutants could germinate only at high CO_2 concentrations, although they were much worse developed as compared to WT plants (J. Moroney, personal communication). Based on the above, it seems more likely that not the stromal β-CA1 but β-CA5 (or one of the isoforms of β-CA5) located in the chloroplast envelope membrane may function mutually with aquaporins.

CA protects the cells from H_2O_2-induced apoptosis [92]. It is known that among various chloroplast signals H_2O_2 plays a major role in various signaling pathways under stress conditions [93, 94]. An essential factor to implement the retrograde signal (the signal from the organelle to the nucleus) is the ability of H_2O_2 to diffuse over long distances from the place of formation to the place of signaling. Earlier, we demonstrated that H_2O_2 that was produced inside chloroplasts diffused from a chloroplast to cytoplasm through chloroplast envelope membranes and the amount of H_2O_2 outside the chloroplasts increased under the conditions of ascorbate peroxidase inhibition [95]. Using acetazolamide (AZA), which is known as CA inhibitor, as a non-specific aquaporin inhibitor, we have shown that aquaporins facilitate the diffusion of H_2O_2 molecules through the chloroplast membrane [16]. AZA was established to be an efficient inhibitor of aquaporins through interacting with the guanidyl group of Arg, backbone carbonyl of Gly, carboxyl of Asp, Ser, His, Ile and Asn of aquaporins [96–98]. Taking into consideration that AZA also inhibits the activity of CA, we cannot exclude that inhibition of H_2O_2 diffusion through the envelope membrane in the presence of AZA was the only result of blocking of the aquaporins. This inhibition could also result from inhibiting the envelope CA, especially if this CA is attached to aquaporins in the envelope. If this is the case, the inhibitory effect of AZA on the H_2O_2 diffusion could be a consequence of AZA binding to envelope CA, leading to the conformational changes of CA with subsequent conformational changes of aquaporin proteins and therefore blocking the envelope aquaporins. Thus, the data in [16] can represent the evidence of the joint functioning of the envelope CA with aquaporins in diffusing hydrogen peroxide through the chloroplast envelope. Considering the presence of CA of β-family in the chloroplast envelope (see above) and the facilitation of not only CO_2, but also H_2O_2 diffusion through the envelope by the functioning of envelope aquaporins, several propositions on the mechanisms of the CA incorporation in signaling under stress conditions can be made. One of them is that CA is involved in the cascades of mitogen-activated protein kinases (MAPKs), which are serine-threonine kinases mediating intracellular signaling through changes in the redox state of their

cysteine residues. Since cysteine residues are the main target of hydrogen peroxide in MAPK cascades [99], and two cysteine residues are located in the β-CAs active center, we can assume that H_2O_2 changes the redox status of cysteine residues of β-CAs, resulting in incorporation of CA in the global MAPK signaling network in plant cells.

It was quantified that the amount of water in chloroplast stroma and thylakoid lumen was much lower than it is enough to perform photosynthetic water oxidation per day. Therefore, the existence of aquaporins in the thylakoid membrane was also suggested [100]. This question is still under debate, some researches have detected some forms of TIPs in the thylakoid membranes of *Arabidopsis thaliana* [101, 102]. However, there is still little direct experimental evidence demonstrating the existence of aquaporins in thylakoid membranes and this question needs to be clarified in the future.

4.2.2 CA in mitochondria and the possible role of CA in chloroplast-mitochondria communication for activation of the CO_2-concentrating-like mechanism

C3 higher plants are believed to lack any mechanisms of CO_2 concentrating. The carbon dioxide content in a liquid phase in leaves is not very high, about 11 μM at the current concentration of CO_2 in the atmosphere. Under the illumination pH value of the chloroplast stroma, where Rubisco is located in higher plant cells, increases up to 7.7. The bicarbonate content there reaches 230 μM at 25°C. This effect can be considered as inorganic carbon concentrating.

For higher plants, especially when they grow under stress conditions, such as high light or high temperature, Zabaleta et al. [103] supposed the existence of CMM as in plants in such conditions stomata are closed leading to the decrease of Ci content in chloroplasts. At the same time, a large amount of CO_2 is released in mitochondria due to the reactions of both the tricarboxylic acid cycle and photorespiration. The authors supposed that CO_2 from mitochondria can be used by chloroplasts in the Calvin cycle. Possibly, CO_2 may transfer into chloroplast by diffusion, harnessing some mechanisms of an active HCO_3^- transport would be more efficient. In the genome of *A. thaliana*, five genes encoding γ-CA, which form part of the mitochondrial complex I, were found [57, 104]. The CAs of the three subunits of the complex have a fully active center (γ-CA1, γ-CA2, and γ-CA3), and the CAs of two other subunits, in which some conservative amino acid residues forming the active center are absent, are called CA-like proteins named γ-CA4 and γ-CA5. The domain consisting of five γ-CAs attached to mitochondrial complex I probably plays a role in the conversion of CO_2 to HCO_3^- and/or even in a transfer of HCO_3^- from mitochondria to cytosol. Possibly, one more CA, β-CA6, located in the mitochondrial matrix, can also participate in the formation of HCO_3^-. The last one can be subsequently transferred from mitochondria to chloroplasts by a putative bicarbonate carrier in the chloroplast membrane or C4-like pathway; however, the mechanism of such transfer is yet unknown [103].

Interestingly, in double mutants without γ-CA4 and γ-CA5, light-dependent activation of the key enzyme for the synthesis of anthocyanins, chalcone synthase, was observed [105]. The authors suggested that both these CAs play an important role in the growth and development of *A. thaliana* in conditions of high illumination.

4.2.3 Carbonic anhydrases in chloroplasts of C3 plants

The most known and the most studied CA in chloroplasts of C3 higher plants is the stromal CA, which belongs to β-family. This CA was named β-CA1 by Fabre

et al. in 2007 [5] in the study of CAs of β-family in Arabidopsis leaves. Later, this name started to be used to designate the corresponding stromal CAs in leaves of other plants [12, 15]. This enzyme is the second after Rubisco in terms of the amount of protein in the cell (0.5–2% of the total) [106]. The rate of the spontaneous interconversion of Ci forms is low, which implies the role of CA in accelerating the supply of CO_2 to carboxylation centers; however, direct data on the role of the enzyme in this process are lacking.

In some studies, no association of CA activity with photosynthesis was found. Growing plants under conditions of zinc deficiency showed that the rate of photosynthesis in these plants remained almost unchanged with a sharp decrease in CA activity [107]. In transgenic plants of *Nicotiana tabacum* containing 10% or less soluble β-CA activity compared to WT plants, there were no significant differences in Rubisco activity, chlorophyll content, stomatal conductivity, dry weight per unit leaf area, and in the ratio of the partial pressure of intracellular and external CO_2 in comparison with WT plants [108]. However, in these mutants, the carbon isotopic composition of the leaf dry matter was changed.

Another group of researchers has found that plants compensated for a decrease in CA activity by increasing the permeability of stomata, which, however, has led to a higher rate of water loss [109]. Plant growth under conditions of nitrogen deficiency in the soil led to a significant decrease in the activity of soluble CA in leaves of sugar beet plants, and upon restoration of nitrogen nutrition, a gradual reverse increase in CA activity by 80% of the initial values has been observed (Novichkova, personal comunication). Importantly, many researchers have discovered the convincing evidence of the functional relationship of Rubisco and stromal β-CA. In *Phaseolus vulgaris* plants grown under the increased CO_2 content in the air, a significant decrease in the activities of both CA and Rubisco has been observed [110]. The activity of these enzymes and the content of transcripts of the genes encoding them were reduced in *Pisum sativum* plants grown at 1000 ppm CO_2, compared with plants grown at atmospheric carbon dioxide concentration; the transfer of pea plants grown at a carbon dioxide concentration of 1000 ppm to the conditions of normal CO_2 content in the atmosphere led to a rapid increase in the expression level of CA and Rubisco genes, following which the activity of the corresponding enzymes in the leaves increased [50]. Immunocytolocalization experiments indicated that CA is a neighbor of Rubisco in the stroma of pea leaves' chloroplast [111]. One CA in the stroma of chloroplasts was later discovered to be associated with Rubisco on the outside of the thylakoid membrane [112]. In the early stages of the adaptation of sugar beet plants to a high concentration of carbon dioxide, of 700 ppm, a decrease in both the activity of the soluble and of the membrane-bound CAs was observed. However, the activity of Rubisco was the same in plants grown in conditions of high CO_2 concentration and in the ambient carbon dioxide content [113].

In the study of the drought tolerance mechanisms in *Brassica napus* [12], β-CA1 was identified using mass-spectrometry analysis as a protein interacting with isoforms of the large Rubisco subunit in several protein spots obtained by 2D electrophoresis of the proteins of the rapeseed plant leaves. The content of β-CA1 in these spots was higher in the samples from plants after drought treatment in comparison to those from the control plants. RT-PCR analysis of the expression level of the gene encoding β-CA1 has shown a similar trend at the transcriptional and translational levels meaning that both the expression level and the protein level increased under drought stress in leaves of *Brassica napus* plants.

Nevertheless, the activity of CA in leaves of plants exposed to water deficit was lower than in the control plants grown under normal conditions [12] due to the phosphorylation of several amino acid residues in the active site and

substrate-binding site of β-CA1. The same mechanism of inhibition of CA activity was observed by studying the action of high-temperature stress in the leaves of *Helianthus annum* [11]. The key suppression mechanism of this activity was the nitration of tyrosine residues in the structure of β-CA. Nitration, as well as phosphorylation of Tyr, blocks the passage of substrate to active site cavity.

It is not entirely clear why the content of CA protein increases with the simultaneous suppression of CA activity. It can be assumed that plants apply this mechanism to prepare for normal moisture conditions. The answer to this question is very important to determine the physiological role of β-CA1 stroma in higher plant metabolism. Although the participation of β-CA1 in photosynthesis is not considered to be proved, the described facts support this suggestion. The fact that it turned out to be one of the main differentially abundant proteins in response to drought testifies to the assumption that β-CA1 supplies CO_2 for Rubisco activity by catalyzing the reversible reaction of bicarbonate to carbon dioxide and thus regulating the rate of photosynthesis under stress condition. Water deficit is known to cause stomatal closure with a reduction in plant photosynthetic efficiency and inhibition of Rubisco activity [114]. These responses often result in a change in photosynthetic and energy metabolism-associated protein accumulation. The inhibition of the activity of β-CA1 under stress by phosphorylation and nitration [11, 12] may play an important role in regulating the photosynthetic process in response to stress.

Herewith Yu et al. [7] showed that in rice seedlings the expression of the gene encoding CA of β-family (OsCA1) as well as the total CA activity were upregulated by osmotic stress, in particular, by salt stress. Moreover, the same group showed that *Arabidopsis thaliana* mutant plants over-expressing OsCA1 had a greater salt tolerance. These data imply that the OsCA1 has an important role in the response of plants to environmental stress conditions. In the rice genome among salt tolerance genes, the *LOC_Os09g28910* gene encoding CA has been recently identified [115]. This gene, as well as the gene encoding OsCA1, had the chloroplast precursor sequence, which implies the location of both CAs in the chloroplasts in rice.

The experimental data presented, however, show that the role of β-CA1 in photosynthesis, as well as the participation of this enzyme in regulating the rate of photosynthetic processes under stress conditions, is still controversial. This contradiction can be explained by the suggestion that two stromal CAs, β-CA1 and α-CA1, participate in CO_2 supply to Rubisco. The latter was detected in the chloroplast stroma in the study of the pathway of newly synthesized proteins into the chloroplast through the endo-membrane system of the Golgi apparatus [10]. The data on the decrease in photosynthetic activity, as well as the ability to accumulate starch in plants with a knockout of the gene encoding α-CA1 [116], may indicate the role of α-CA1 in photosynthesis and the possibility that this CA is involved in CO_2 supply to Rubisco. Using primers designed for possible alternative splicing of the template RNA of the *At3g01500* gene encoding β-CA1, two isoforms were found to be present in the leaves of Arabidopsis [6]. An increase in plant illumination led to a change in the content of transcripts of most genes encoding CA of chloroplasts, and a significant difference was observed in the expression intensity of α-CA1 genes and two forms of β-CA1. The opposite effect of increasing the light intensity on the content of transcripts of two RNA forms of a gene encoding β-CA1, an increase in the content of transcripts of one form and a decrease in the other, can indicate different functions of the proteins encoded by them. An increase in the expression of α-CA1 and one of the forms of RNA of the *β-ca1* gene with increased illumination suggests their cooperation in the CO_2 supply to Rubisco [6]. In general, it can be assumed, on the basis of the available data, that plant CAs, as a rule, jointly control one or another metabolic process.

Studies using double mutants showed that the reduced synthesis of several CAs had an effect on photosynthesis. Plants with knocked-out genes encoding β-CA1 and β-CA4, which are located in the stroma and plasma membrane, respectively, showed a higher stomata conductivity compared to WT plants [117]. Plants are known to activate anion channels in response to environmental signals such as drought, high levels of carbon dioxide, and bacterial invasion. Recently, two new gene families encoding major groups of anion channels have been identified. SLAC/SLAH channels are the representatives of this group characterized by slow voltage-dependent activation (S-type) [118]. Xue et al. in 2011 [119] and Tian et al. in 2015 [120] showed that β-CA4 and β-CA1 take part in the regulation of gas exchange between the atmosphere and leaves by opening/closing the stomatal aperture. Intracellular bicarbonate generated by β-CA4 and β-CA1 acts as a second messenger and activates S-type anion channels in guard cells.

Evidence suggests that β-CA1 may be important not only for photosynthetic processes but, for example, for the synthesis of ethylene during plant germination, a process that is also dependent on CO_2. The seeds of the *A. thaliana* mutant with knocked-out gene encoding β-CA1 showed a significant decrease in germination on sterile artificial media at ambient CO_2 concentration in air [121]. The germination ability of these mutants was restored to that of WT when grown at a high CO_2 content (1500 μl/L) or after adding sucrose to the medium [122].

To understand the role of β-CA1 in plants, we should take into account that this CA located in the stroma of the chloroplast not only exhibits CA activity but can also bind salicylic acid (SA) [123]. As it is known, SA plays a role in signaling cascades that activate the synthesis of the proteins involved in protecting plants from oxidative stress at both the transcription and translation levels. The intensity of stromal CAs' gene expression was shown to respond to signals associated with activation of biotic stress protection systems: infection with the late blight of potato plants [124], treatment of tomatoes with mycotoxin fusicoccin [125] and treatment of Arabidopsis plants with methyl jasmonates [126]. *Nicotiana benthamiana* mutant plants with knocked-out gene encoding chloroplast soluble CA showed high susceptibility to late blight [124]. Medina-Puche et al. [127] showed that in *A. thaliana* plants inoculated with the phytopathogenic bacterium *Pseudomonas syringae*, the expression level of *β-ca1, β-ca2*, and *β-ca4* genes was repressed, with the induction of the expression of the gene encoding the other CA, β-CA6, located in the mitochondrial matrix.

There is also a hypothesis that the stromal β-CA can ensure protection against the stress through the biosynthesis of fatty acids. The lower content of stromal β-CA was shown [128] to suppress the fatty acid synthesis leading to a lower expression of genes regulated by jasmonic acid, another signaling molecule that triggers an alternative gene cascade of a protective response. Omega-6 fatty acids are the intermediate compounds of biosynthesis of fatty acids that are synthesized in the stroma of chloroplasts.

4.2.4 CAs in lamellar thylakoid membranes

In the early 2000s, the information on the presence of more than one carrier of CA activity in the thylakoid membranes began to appear [17, 129]. The CA activity was identified in preparations of lamellar thylakoid membranes enriched with PSI and ATP synthase complexes isolated from pea and Arabidopsis plants [18, 20]. The activity of this CA differed in properties from the CA activity of granular thylakoid membranes enriched with PSII complexes. The CAs in lamellar and granal thylakoid membranes were different in their effect on the activity of inhibitors and detergents, their molecular masses were different. The CAs' activity of lamellar

thylakoid membranes was inhibited equally by both ethoxyzolamide, which is able to penetrate into membranes and acetazolamide, poorly penetrating into lipid membranes. These data show that this CA is situated at the stromal surface of the thylakoid membrane, where it is accessible to both inhibitors. Recently [130, 131] the stimulating effect of adding bicarbonate on the rate of phosphorylation in isolated thylakoids that is known since the 60th of the last century was explained by the presence of CAs in lamellar thylakoid membranes. We have shown that mafenide, a water-soluble inhibitor of CAs, suppressed the phosphorylation stimulation by HCO_3^- without inhibiting the rate of electron transport and had no effect on phosphorylation rate in the absence of bicarbonate [132].

We suggested a hypothesis of the mechanism of this CA's involvement in the increase of photophosphorylation in thylakoid membranes. In the reaction of bicarbonate dehydration catalyzed by membrane-bound CAs, CO_2 molecules are formed near or even in the surface layer of the thylakoid membrane. With an increase in their local concentration, a stream of CO_2 in the thylakoid lumen is possible. In the lumen, CO_2 is hydrated by the luminal CAs (see below) with proton release. As a result, an increase in proton concentration in lumen may cause an intensification in ATP production, that is, acceleration of photophosphorylation. Since the uncoupling effect of ammonium salts on ATP synthesis in thylakoids resulted mainly from proton bonding in the thylakoid lumen by NH_3 molecules penetrating there, the facts that the addition of bicarbonate reduced the uncoupling effect of these salts [132, 133] is in line with the proposed hypothesis.

Thus, CAs located in lamellar thylakoid membranes, that is, directed immediately to the stromal phase, may operate as one of the suppliers of CO_2 for Rubisco, and in the case of low effectiveness of CO_2 fixation under some stressful conditions can be involved in the extra production of ATP that is especially important under such conditions.

4.2.5 CA in granal thylakoid membranes

In 2004, α-CA4 was detected among the proteins of thylakoid membranes [35]. We have shown that the fresh weight of the plant leaves with knocked-out *α-ca4* gene was 10% higher, the starch and H_2O_2 content was significantly higher, and the rate of CO_2 assimilation in leaves was lower in comparison to WT plants. The effective quantum yield of photosynthetic electron transport at saturating light intensity and CO_2 concentration was higher in mutants than in the WT, while nonphotochemical chlorophyll fluorescence quenching (NPQ) was lower [13, 37, 134, 135]. The content of transcripts of the *At4g20990* gene encoding α-CA4 was two times higher under high (400 μmol quanta m^{-2} s^{-1}) than under low illumination (80 μmol quanta m^{-2} s^{-1}) when grown under "short-day" (8 hours day/16 hours night) and 16 times higher under "long-day" conditions (16 hours day/8 hours night) [6]. Thus, the above-described effects of the *At4g20990* gene knockout are consistent with a significant increase in the expression level of this gene in plants under high illumination; these are the conditions where the activation of NPQ, which protects PSII from photoinhibition, is important. Since the energy-dependent quenching (the part of the NPQ that is associated with the accumulation of protons in the thylakoid lumen) is the main process, which had the effect on changes in NPQ, we suggested that α-CA4 is involved in the protonation of either PsbS protein or violaxantin deepoxidase. The role of α-CA4 in NPQ in PSII antenna was confirmed by the fact that the knockout of the *At4g20990* gene affected both the protein content of the light-harvesting complex PSII and the expression level of the genes encoding these proteins [135]. The content of the major PSII antenna proteins, Lhcb1, and Lhcb2 was lower in α-CA4 knockouts than in the WT plants. We have also found that

the mutant plants grown under high illumination compensated for the absence of α-CA4 and for reduced NPQ by the increase in the contents of both PsbS protein and the violaxanthin cycle components, the latter accompanied by an increase in violaxanthin deepoxidase activity [13]. In addition, *α-ca4* gene was found among the genes that change the expression level under osmotic stress [136]. The content of *α-ca4* gene transcripts became an order of magnitude higher in conditions of drought stress (Rudenko et al., in preparation).

Our research has provided evidence that one more CA, α-CA2, is present in higher plant thylakoids. The knockout of *α-ca2* gene has led to the opposite changes in the properties of plants compared with those resulting from the knockout of *α-ca4* gene. Fresh leaf weight, chlorophyll a/chlorophyll b ratio, and starch and H_2O_2 content in leaves of α-CA2 mutants were lower than in the WT plants, while CO_2 assimilation rate was higher [37, 134, 135]. In α-CA2 knockouts, the effective quantum yield of photosynthetic electron transport was lower than in WT, while NPQ was higher, also due to the energy-dependent component. The set of physiological effects that occur when the α-CA2 and α-CA4 genes are turned off suggests that not only α-CA4, but also α-CA2, is present in chloroplasts, participating in the functioning of the opposite "regulatory pathways" that respond to changes in external conditions. This phenomenon of regulation of metabolic states, when there are two enzymes that oppositely affect particular conditions, for example, kinase and phosphatase on protein phosphorylation, is well known. The detection of this phenomenon in the regulation of NPQ is very important. It shows that the change in NPQ involved in the protection of the photosynthetic apparatus from photoinhibition is under operational control, which allows the fast increase in the thermal dissipation of solar energy with its excess. In the opposite situation, in conditions of the low illumination, the above-described mechanism could reduce this dissipation when it could decrease the useful incoming energy, which is necessary for photosynthesis.

Since the regulation of the energy-dependent component of NPQ taking place with the participation of α-CA4 and α-CA2 is based on a change in proton concentration in the lumen, these CAs are also able to regulate the redox state of plastoquinone pool by the regulation of plastohydroquinone oxidation rate by cytochrome complex in the photosynthetic electron transport chain. According to a number of studies [137–139], it regulates the adaptive response of plants to environmental conditions' changes. In addition, the concentration of protons is important for the functional activity of enzymes such as thioredoxins and kinases, whose activities are dependent on the state of sulfhydryl groups.

4.2.6 CA in the thylakoid lumen

The CA activity in thylakoid membranes of higher plants was discovered in the early 1980s. A number of properties distinguish the CA activity of thylakoid membranes from the activity of soluble stromal CA. In particular, the dehydration activity of thylakoid CA depends on pH with a maximum at 6.8–7.0, and the activity of soluble CA does not depend on pH [140]. Antibodies against soluble CA from spinach have also shown a strong cross-reaction with soluble CA from pea chloroplasts, but not with thylakoids exhibiting similar CA activity [141]. In our studies, a soluble CA that belongs to β-family located in the thylakoid lumen has been discovered [19, 142], and it was suggested that this is β-CA5, previously detected in chloroplasts from Arabidopsis [5]. The exact position of β-CA5 in Arabidopsis chloroplasts is unknown, but it cannot be excluded that there are two forms of this enzyme with one located in the chloroplast envelope (see above) and the other situated in the thylakoid lumen.

The expression level of the *At4g33580* gene encoding β-CA5 is 2–3 orders lower than that of, for example, the *At3g01500* gene encoding stromal β-CA1 in plants grown at atmospheric CO_2 concentration in low-light conditions [135]. The transcription intensity of the gene encoding β-CA5 was higher when illumination decreased both in short-day and long-day conditions. CA in thylakoid lumen may enable more free diffusion of protons to ATP-synthase channel together with CO_2/HCO_3^- buffer, and the value of such diffusion should decrease at low light intensity when proton inflow into the lumen is low and they may be "lost" on the way to the ATP-synthase.

5. Conclusion

The role of CAs in mechanisms of stress tolerance has been studied in plants possessing CCM, that is, in algae and C4 higher plants. The lack of understanding of the functions of individual CAs in higher plants with C3 type of photosynthesis may be explained by an involvement of more than one of CAs in one biochemical pathway. The effect of the absence of CAs in mutants is more obvious under various adverse conditions, which are comfortable for plants. These are the conditions when CO_2 supply and fixation are not limited to the general plant metabolism under optimal light intensity, temperature, mineral nutrition, etc. Moreover, the effect of the absence of CAs in mutants is more obvious in various adverse conditions. That means that the functioning of CAs in plants is the most important under stress.

Funding information

The preparation of the manuscript was supported by the Russian Science Foundation (project no. 17-14-01371) and by the Ministry of Science and Higher Education of the Russian Federation, State Scientific Program, theme no. AAAA-A17-117030110135-1.

Author details

Natalia N. Rudenko*, Maria M. Borisova-Mubarakshina, Lyudmila K. Ignatova, Tatiana P. Fedorchuk, Elena M. Nadeeva-Zhurikova and Boris N. Ivanov
Institute of Basic Biological Problems, Federal Research Center–Pushchino Scientific Center for Biological Research of the Russian Academy of Sciences, Pushchino, Russia

*Address all correspondence to: nataliacherry413@gmail.com

References

[1] Manahan S. Environmental Chemistry. Boston: Willard Grant Press; 1984

[2] Moroney J, Ma Y, Frey W, Fusilier K, Pham T, Simms T, et al. The carbonic anhydrase isoforms of *Chlamydomonas reinhardtii*: Intracellular location, expression, and physiological roles. Photosynthesis Research. 2011;**109**:133-149. DOI: 10.1371/journal.pone.0001426

[3] Hatch M, Burnell J. Carbonic anhydrase activity in leaves and its role in the first step of C4 photosynthesis. Plant Physiology. 1990;**93**:825-828. DOI: 10.1104/pp.93.2.825

[4] von Caemmerer S, Quinn V, Hancock N, Price G, Furbank T, Ludwig M. Carbonic anhydrase and C4 photosynthesis: A transgenic analysis. Plant, Cell and Environment. 2004;**27**:697-703. DOI: 10.1111/j.1365-3040.2003.01157.x

[5] Fabre N, Reiter I, Becuwe-Linka N, Genty B, Rumeau D. Characterization and expression analysis of genes encoding alpha and beta carbonic anhydrases in Arabidopsis. Plant, Cell & Environment. 2007;**30**:617-629. DOI: 10.1111/j.1365-3040.2007.01651.x

[6] Rudenko N, Vetoshkina D, Fedorchuk T, Ivanov B. Effect of light intensity under different photoperiods on expression level of carbonic anhydrase genes of the α- and β-families in *Arabidopsis thaliana* leaves. Biochemistry (Moscow). 2017;**82**(9):1025-1035. DOI: 10.1134/S000629791709005X

[7] Yu S, Zhang X, Guan Q, Takano T, Liu S. Expression of a carbonic anhydrase gene is induced by environmental stresses in Rice (*Oryza sativa* L.). Biotechnology Letters. 2007;**29**:89-94. DOI: 10.1007/s10529-006-9199-z

[8] Rudenko N, Ignatova L, Fedorchuk T, Ivanov B. Carbonic anhydrases in photosynthetic cells of higher plants. Biochemistry (Moscow). 2015;**80**(6):674-687. DOI: 10.1134/S0006297915060048

[9] Atkins C, Patterson B, Graham D. Plant carbonic anhydrases. II. Preparation and some properties of monocotyledon and dicotyledon enzyme types. Plant Physiology. 1972;**50**:218-223. DOI: 10.1104/pp.50.2.218

[10] Villarejo A, Buren S, Larsson S, Dejardin A, Monne M, Rudhe C, et al. Evidence for a protein transported through the secretory pathway en route to the higher plant chloroplast. Nature Cell Biology. 2005;**7**:1224-1231. DOI: 10.1038/ncb1330

[11] Chaki M, Carreras A, Lypez-Jaramillo J, Begara-Morales J, Sάnchez-Calvo B, Valderrama R, et al. Tyrosine nitration provokes inhibition of sunflower carbonic anhydrase (β-CA) activity under high temperature stress. Nitric Oxide. 2013;**29**:30-33. DOI: 10.1111/j.1399-3054.1992

[12] Wang L, Jin X, Li Q, Wang X, Li Z, Wu X. Comparative proteomics reveals that phosphorylation of β carbonic anhydrase 1 might be important for adaptation to drought stress in *Brassica napus*. Scientific Reports. 2016;**6**:39024. DOI: 10.1038/srep39024

[13] Rudenko N, Fedorchuk T, Terentyev V, Dymova O, Naydov I, Golovko T, et al. The role of carbonic anhydrase α-CA4 in the adaptive reactions of photosynthetic apparatus. The study with α-CA4 knockout plants. Protoplasma. 2020;**257**:489-499. DOI: 10.1007/s00709-019-01456-1

[14] Wang C, Hu H, Qin X, et al. Reconstitution of CO_2 regulation of

SLAC1 anion channel and function of CO_2-permeable PIP2;1 aquaporin as carbonic anhydrase4 interactor. The Plant Cell. 2016;**28**(2):568-582. DOI: 10.1105/tpc.15.00637

[15] Perez-Martin A, Michelazzo C, Torres-Ruiz J, Flexas J, Fernández J, Sebastiani L, et al. Regulation of photosynthesis and stomatal and mesophyll conductance under water stress and recovery in olive trees: Correlation with gene expression of carbonic anhydrase and aquaporins. Journal of Experimental Botany. 2014;**65**:3143-3156. DOI: 10.1093/jxb/eru160

[16] Borisova (Mubarakshina) M, Kozuleva M, Rudenko N, Naydov I, Klenina I, Ivanov B. Photosynthetic electron flow to oxygen and diffusion of hydrogen peroxide through the chloroplast envelope via aquaporins. BBA – Bioenegetics. 2012;**1817**:1314-1321. DOI: 10.1016/j.bbabio.2012.02.036

[17] Lu Y, Stemler A. Extrinsic photosystem II carbonic anhydrase in maize mesophyll chloroplasts. Plant Physiology. 2002;**128**:643-649. DOI: 10.1104/pp.010643

[18] Ignatova L, Rudenko N, Khristin M, Ivanov B. Heterogeneous origin of carbonic anhydrase activity of thylakoid membranes. Biochemistry (Moscow). 2006;**71**:525-532. DOI: 10.1134/S0006297906050099

[19] Rudenko N, Ignatova L, Ivanov B. Multiple sources of carbonic anhydrase activity in pea thylakoids: Soluble and membrane-bound forms. Photosynthesis Research. 2007;**91**(1):81-89. DOI: 10.1007/s11120-007-9148-2

[20] Ignatova L, Rudenko N, Mudrik V, Fedorchuk T, Ivanov B. Carbonic anhydrase activity in *Arabidopsis thaliana* thylakoid membrane and fragments enriched with PSI or PSII. Photosynthesis Research. 2011;**110**:89-98. DOI: 10.1007/s11120-011-9699-0

[21] Hewett-Emmett D, Tashian R. Functional diversity, conservation, and convergence in the evolution of the α, β, and γ-carbonic anhydrase gene families. Molecular Phylogenetics and Evolution. 1996;**65**:50-77

[22] Liljas A, Laurberg M. A wheel invented three times. The molecular structures of the three carbonic anhydrases. EMBO Reports. 2000;**1**(1):16-17. DOI: 10.1006/mpev.1996.0006

[23] De Simone G, Di Fiore A, Capasso C, Supuran C. The zinc coordination pattern in the η-carbonic anhydrase from plasmodium falciparum is different from all other carbonic anhydrase genetic families. Bioorganic & Medicinal Chemistry Letters. 2015;**25**:1385-1389. DOI: 10.1016/j.bmcl.2015.02.046

[24] Jensen E, Clement R, Kosta A, Maberly S, Gontero B. A new widespread subclass of carbonic anhydrase in marine phytoplankton. The ISME Journal. 2019;**13**:2094-2106. DOI: 10.1038/s41396-019-0426-8

[25] DiMario R, Clayton H, Mukherjee A, Ludwig M, Moroney J. Plant carbonic anhydrases: Structures, locations, evolution, and physiological roles. Molecular Plant. 2017;**10**:30-46. DOI: 10.1016/j.molp.2016.09.001

[26] Soltes-Rak E, Mulligan M, Coleman J. Identification and characterization of a gene encoding a vertebrate-type carbonic anhydrase in cyanobacteria. Journal of Bacteriology. 1997;**179**:769-774. DOI: 10.1128/jb.179.3.769-774

[27] Elleby B, Chirica L, Tu C, Zeppezauer M, Lindskog S. Characterization of carbonic anhydrase from *Neisseria gonorrhoeae*.

European Journal of Biochemistry. 2001;**268**:1613-1619. DOI: 10.1046/j.1432-1327.2001.02031.x

[28] Elleuche S, Poggeler S. Evolution of carbonic anhydrases in fungi. Current Genetics. 2009;**55**:211-222. DOI: 10.1007/s00294-009-0238-x

[29] Fukuzawa H, Fujiwara S, Yamamoto Y, Dionisto-Sese M, Miyachi S. cDNA cloning, sequence, and expression of carbonic anhydrase in *Chlamydomonas reinhardtii*: Regulation by environmental CO_2 concentration. PNAS. 1990;**87**: 4383-4387. DOI: 10.1073/pnas.87.11.4383

[30] Fujiwara S, Fukuzawa H, Tachiki A, Miyachi S. Structure and differential expression of two genes encoding carbonic anhydrase in *Chlamydomonas reinhardtii*. PNAS. 1990;**87**:9779-9783. DOI: 10.1073/pnas.87.24.9779

[31] Tuskan G, Difazio S, Jansson S, et al. The genome of black cottonwood, *Populus trichocarpa* (Torr., Gray). Science. 2006;**313**(5793):1596-1604. DOI: 10.1126/science.1128691

[32] Meldrum N, Roughton F. Carbonic anhydrase: Its preparation and properties. Nature. 1933;**80**:113-142. DOI: 10.1113/jphysiol.1933.sp003077

[33] Rawat M, Moroney J. Partial characterization of a new isoenzyme of carbonic anhydrase isolated from *Chlamydomonas reinhardtii*. The Journal of Biological Chemistry. 1991;**266**:9719-9723. DOI: 10.1371/journal.pone.0079909

[34] Karlsson J, Hiltonen T, Husic H, Ramazanov Z, Samuelsson G. Intracellular carbonic anhydrase of *Chlamydomonas reinhardtii*. Plant Physiology. 1995;**109**:533-539. DOI: 10.1007/s11120-011-9635-3

[35] Friso G, Giacomelli L, Ytterberg A, Peltier J, Rudella A, Sun Q, et al. In-depth analysis of the thylakoid membrane proteome of *Arabidopsis thaliana* chloroplasts: New proteins, new functions, and a plastid proteome database. The Plant Cell. 2004;**16**: 478-499. DOI: 10.1105/tpc.017814

[36] Sun Q, Zybailov B, Majeran W, Friso G, Olinares P, van Wijk K. PPDB, the plant proteomics database at Cornell. Nucleic Acids Research. 2009;**37**:969-974. DOI: 10.1093/nar/gkn654

[37] Zhurikova E, Ignatova L, Rudenko N, Mudrik V, Vetoshkina D, Ivanov B. The participation of two carbonic anhydrases of alpha family in photosynthetic reactions in Arabidopsis thaliana. Biochemistry (Moscow). 2016;**81**(10):1182-1187. DOI: 10.1134/S0006297916100151

[38] Holmes-Davis R, Tanaka C, Vensel W, Hurkman W, McCormick S. Proteome mapping of mature pollen of *Arabidopsis thaliana*. Proteomics. 2005;**5**:4864-4884. DOI: 10.1002/pmic.200402011

[39] Noir S, Brautigam A, Colby T, Schmidt J, Panstruga R. A reference map of the *Arabidopsis thaliana* mature pollen proteome. Biochemical and Biophysical Research Communications. 2005;**337**:1257-1266. DOI: 10.1016/j.bbrc.2005.09.185

[40] Eriksson A, Jones T, Liljas A. Refined structure of human carbonic anhydrase II at 2.0 angstrom resolution. Proteins: Structure, Function, and Genetics. 1988;**4**:274-282. DOI: 10.1002/prot.340160104

[41] Christianson D, Cox J. Catalysis by metal-activated hydroxide in zinc and manganese metalloenzymes. Annual Review of Biochemistry. 1999;**68**:33-57. DOI: 10.1146/annurev.biochem.68.1.33

[42] Christianson D, Fierke C. Carbonic anhydrase: Evolution of the zinc binding site by nature and by design. Accounts

of Chemical Research. 1996;**29**:331-339. DOI: 10.1021/ar9501232

[43] Kamo T, Shimogawara K, Fukuzawa H, Muto S, Miyachi S. Subunit constitution of carbonic anhydrase from *Chlamydomonas reinhardtii*. European Journal of Biochemistry. 1990;**192**: 557-562. DOI: 10.1111/j.1432-1033.1990.tb19261.x

[44] Smith K, Ferry J. A plant-type (beta-class) carbonic anhydrase in the thermophilic metanoarchaeon Methanobacterium thermoautotrophicum. Journal of Bacteriology. 1999;**181**:6247-6253

[45] So A, Espie G. Cloning, characterization and expression of carbonic anhydrase from the cyanobacterium Synechocystis PCC6803. Plant Molecular Biology. 1998;**37**:205-215. DOI: 10.1023/A:1005959200390

[46] Smith K, Jakubzick C, Whittam T, Ferry J. Carbonic anhydrase is an ancient enzyme widespread in prokaryotes. PNAS. 1999;**96**(26):15184-15189. DOI: 10.1073/pnas.96.26.15184

[47] Sawaya M, Cannon G, Heinhorst S, Tanaka S, Williams E, Yeates T, et al. The structure of beta-carbonic anhydrase from the carboxysomal shell reveals a distinct subclass with one active site for the price of two. The Journal of Biological Chemistry. 2006;**281**:7546-7555. DOI: 10.1074/jbc

[48] Gotz R, Gnann A, Zimmermann F. Deletion of the carbonic anhydrase-like gene NCE103 of the yeast Saccharomyces cerevisiae causes an oxygen-sensitive growth defect. Yeast. 1999;**15**:855-864. DOI: 10.1002/(SICI)1097-0061(199907)15:10A<855::AID-YEA425>3.0.CO;2-C

[49] Eriksson M, Karlsson J, Ramazanov Z, Gardestrom P, Samuelsson G. Discovery of an algal mitochondrial carbonic anhydrase. Molecular cloning and characterization of a low-CO_2-induced polypeptide in *Chlamydomonas reinhardtii*. PNAS. 1996;**93**(21):12031-12034. DOI: 10.1073/pnas.93.21.12031

[50] Majeau N, Coleman J. Effect of CO_2 concentration on carbonic anhydrase and ribulose-1,5-bisphosphate carboxylase/oxygenase expression in pea. Plant Physiology. 1996;**112**(2):569-574

[51] Fett J, Coleman J. Characterization and expression of two cDNAs encoding carbonic anhydrase in Arabidopsis thaliana. Journal of Plant Physiology. 1994;**105**:707-713. DOI: 10.1104/pp.112.2.569

[52] Provart N, Majeau N, Coleman J. Characterization of pea chloroplastic carbonic anhydrase. Expression in Escherichia coli and site-directed mutagenesis. Plant Molecular Biology. 1993;**22**:937-943. DOI: 10.1007/BF00028967

[53] Rowlett R, Chance M, Wirt M, Sidelinger D, Royal J, Woodroffe M, et al. Kinetic and structural characterization of spinach carbonic-anhydrase. Biochemistry. 1994;**33**:13967-13976. DOI: 10.1021/bi00251a003

[54] Kimber M, Pai E. The active site architecture of Pisum sativum β-carbonic anhydrase is a mirror image of that of α-carbonic anhydrases. The EMBO Journal. 2000;**19**:1407-1418. DOI: 10.1093/emboj/19.7.1407

[55] Cronk J, Rowlett R, Zhang K, Tu C, Endrizzi J, Lee J, et al. Identification of a novel noncatalytic bicarbonate binding site in eubacterial beta-carbonic anhydrase. Biochemistry. 2006;**45**:4351-4361. DOI: 10.1021/bi052272q

[56] Rowlett R. Structure and catalic mechanism of the β-carbonic anhydrase. BBA. 1804;**2010**:362-373. DOI: 10.1007/978-94-007-7359-2_4

[57] Parisi G, Perales M, Fornasari M, Gonztalez-Schain A, Gomez-Casati D, Zimmermann S, et al. Gamma carbonic anhydrases in plant mitochondria. Plant Molecular Biology. 2004;**55**:193-207

[58] Cardol P, Vanrobaeys F, Devreese B, Van Beeumen J, Matagne R, Remacle C. Higher plant-like subunit composition of mitochondrial complex I from *Chlamydomonas reinhardtii*: 31 conserved components among eukaryotes. BBA. 1658;**2004**:212-224. DOI: 10.1007/s11103-004-0149-7

[59] Sunderhaus S, Dudkina N, Jansch L, Klodmann J, Heinemeyer J, Perales M, et al. Carbonic anhydrase subunits form a matrix-exposed domain attached to the membrane arm of mitochondrial complex I in plants. The Journal of Biological Chemistry. 2006;**281**(10):6482-6488. DOI: 10.1074/jbc

[60] Perales M, Eubel H, Heinemeyer J, Colaneri A, Zabaleta E, Braun H. Disruption of a nuclear gene encoding a mitochondrial gamma carbonic anhydrase reduces complex I and supercomplex I + III2 levels and alters mitochondrial physiology in Arabidopsis. Journal of Molecular Biology. 2005;**350**:263-277. DOI: 10.1016/j.jmb.2005.04.062

[61] Kisker C, Schindelin H, Alber B, Ferry J, Rees D. A left-handed beta-helix revealed by the crystal structure of a carbonic anhydrase from the archaeon *Methanosarcina thermophila*. The EMBO Journal. 1996;**15**:2323-2330. DOI: 10.1002/j.1460-2075.1996.tb00588.x

[62] Smith K, Ferry J. Prokaryotic carbonic anhydrases. FEMS Microbiology Reviews. 2000;**24**:335-366. DOI: 10.1111/j.1574-6976.2000.tb00546.x

[63] Price G. Inorganic carbon transporters of the cyanobacterial CO_2 concentrating mechanism. Photosynthesis Research. 2011;**109**:33-45. DOI: 10.1007/s11120-010-9608-y

[64] Moroney J, Husic H, Tolbert N. Effect of carbonic anhydrase inhibitors on inorganic carbon accumulation by *Chlamydomonas reinhardtii*. Plant Physiology. 1985;**79**:177-183. DOI: 10.1104/pp.79.1.177

[65] Ynalvez R, Xiao Y, Ward A, Cunnusamy K, Moroney J. Identification and characterization of two closely related beta carbonic anhydrases from *Chlamydomonas reinhardtii*. Physiologia Plantarum. 2008;**133**:15-26. DOI: 10.1111/j.1399-3054.2007.01043.x

[66] Spalding M, Spreitzer R, Ogren W. Carbonic anhydrase deficient mutant of *Chlamydomonas reinhardii* requires elevated carbon-dioxide concentration for photoautotrophic growth. Plant Physiology. 1983;**73**:268-272. DOI: 10.1104/pp.73.2.268

[67] Moroney J, Tolbert N, Sears B. Complementation analysis of the inorganic carbon concentrating mechanism of *Chlamydomonas reinhardtii*. Molecular & General Genetics. 1986;**204**:199-203. DOI: 10.1007/BF00425498

[68] Ku M, Kano-Murakami Y, Matsouka M. Evolution and expression of C4 photosynthesis genes. Plant Physiology. 1996;**111**:949-957. DOI: 10.1104/pp.111.4.949

[69] von Caemmerer S, Furbank R. The C4 pathway: An efficient CO_2 pump. Photosynthesis Research. 2003;**77**:191-207. DOI: 10.1016/B978-0-12-675408-7.50012-4

[70] Tetu S, Tanz S, Vella N, Burnell J, Ludwig M. The *Flaveria bidentis* β-carbonic anhydrase gene family encodes cytosolic and chloroplastic isoforms demonstrating distinct organ-specific expression patterns. Plant Physiology.

2007;**144**(3):1316-1327. DOI: 10.1104/pp.107.098152

[71] Raven J, Newman J. Requirement for carbonic anhydrase activity. Plant, Cell & Environment. 1994;**17**:123-130. DOI: 10.1111/j.1365-3040.1994.tb00275.x

[72] Chollet R, Vidal J, O'Leary M. Phosphoenolpyruvate carboxylase: A ubiquitous, highly regulated enzyme in plants. Annual Review of Plant Physiology and Plant Molecular Biology. 1996;**47**:273-298. DOI: 10.1146/annurev.arplant.47.1.273

[73] Missner A, Pohl P. 110 years of the Meyer–Overton rule: Predicting membrane permeability of gases and other small compounds. ChemPhysChem. 2009;**10**:1405-1414. DOI: 10.1002/cphc.200900270

[74] Boron W, Endeward V, Gros G, Musa-Aziz R, Pohl P. Intrinsic CO_2 permeability of cell membranes and potential biological relevance of CO_2 channels. ChemPhysChem. 2011;**12**:1017-1019. DOI: 10.1002/cphc.201100034

[75] Uehlein N, Otto B, Hanson D, Fischer M, McDowell N, Kaldenhoff R. Function of *Nicotiana tabacum* aquaporins as chloroplast gas pores challenges the concept of membrane CO_2 permeability. The Plant Cell. 2008;**20**:648-665. DOI: 10.1105/tpc.107.054023

[76] Endeward V, Al-Samir S, Itel F, Gros G. How does carbon dioxide permeate cell membranes? A discussion of concepts, results and methods. Frontiers in Physiology. 2014;**4**:21. DOI: 10.3389/fphys.2013.00382

[77] Zhao M, Tan HT, Scharwies J, Levin K, Evans JR, Tyerman S. Association between water and carbon dioxide transport in leaf plasma membranes: Assessing the role of aquaporins. Plant, Cell & Environment. 2017;**40**(6):789-801. DOI: 10.1111/pce.12830

[78] Terashima I, Ono K. Effects of $HgCl_2$ on CO_2 dependence of leaf photosynthesis: Evidence indicating involvement of aquaporins in CO_2 diffusion across the plasma membrane. Plant & Cell Physiology. 2002;**43**:70-78. DOI: 10.1093/pcp/pcf001

[79] Uehlein N, Lovisolo C, Siefritz F, Kaldenhoff R. The tobacco aquaporin NtAQP1 is a membrane CO_2 pore with physiological functions. Nature. 2003;**425**:734-737. DOI: 10.1038/nature02027

[80] Uehlein N, Sperling H, Heckwolf M, Kaldenhoff R. The Arabidopsis aquaporin PIP1;2 rules cellular CO_2 uptake. Plant, Cell & Environment. 2012;**35**:1077-1083. DOI: 10.1111/j.1365-3040.2011.02473.x

[81] Soto G, Alleva K, Amodeo G, Muschietti J, Ayub N. New insight into the evolution of aquaporins from flowering plants and vertebrates: Orthologous identification and functional transfer is possible. Gene. 2012;**503**:165-176. DOI: 10.1016/j.gene.2012.04.021

[82] Yaneff A, Sigaut L, Marquez M, Alleva K, Pietrasanta L, Amodeo G. Heteromerization of PIP aquaporins affects their intrinsic permeability. PNAS 2014;111(1):231-236. DOI: 10.1073/pnas.1316537111.

[83] Ignatova L, Romanova A. Participation of carbonic anhydrase in inhibition of photosynthesis in pea protoplasts by CO_2 excess. Russian Journal of Plant Physiology. 1992;**39**:82-88

[84] Ignatova L, Moskvin O, Ivanov B, Romanova A. The effect of CO_2 uptake by pea protoplasts on O_2 evolution rate and parameters of chlorophyll fluorescence quenching. Journal of

Plant Biochemistry & Physiology. 1993;**31**:295-301

[85] Afzal Z, Howton T, Sun Y, Mukhtar M. The roles of aquaporins in plant stress responses. Journal of Developmental Biology. 2016;**4**(1):9. DOI: 10.3390/jdb4010009

[86] Terashima I, Hanba Y, Tazoe Y, Vyas P, Yano S. Irradiance and phenotype: Comparative eco-development of sun and shade leaves in relation to photosynthetic CO_2 diffusion. Journal of Experimental Botany. 2006;**57**:343-354. DOI: 10.1093/jxb/erj014

[87] Hanba Y, Shibasaka M, Hayashi Y, Hayakawa T, Kasamo K, Terashima I, et al. Overexpression of the barley aquaporin HvPIP2;1 increases internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants. Plant & Cell Physiology. 2004;**45**:521-529. DOI: 10.1093/pcp/pch070

[88] Flexas J, Ribas-Carbo M, Hanson D, Bota J, Otto B, Cifre J, et al. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. The Plant Journal. 2006;**48**:427-439. DOI: 10.1111/j.1365-313X.2006.02879.x

[89] Katsuhara M, Hanba YT. Barley plasma membrane intrinsic proteins (PIP aquaporins) as water and CO_2 transporters. Pflügers Archiv. 2008;**456**:687-691. DOI: 10.1007/s00424-007-0434-9

[90] Simm S, Papasotiriou D, Ibrahim M, Leisegang M, Müller B, Schorge T, et al. Defining the core proteome of the chloroplast envelope membranes. Frontiers in Plant Science. 2013;**4**:11. DOI: 10.3389/fpls.2013.00011

[91] Villarejo A, Rolland N, Martínez F, Sültemeyer D. A new chloroplast envelope carbonic anhydrase activity is induced during acclimation to low inorganic carbon concentrations in *Chlamydomonas reinhardtii*. Planta. 2001;**213**:286-295. DOI: 10.1007/s004250000508

[92] Raisanen S, Lehenkari P, Tasanen M, Rahkila P, Harkonen P, Vaananen H. Carbonic anhydrase III protects cells from hydrogen peroxide-induced apoptosis. The FASEB Journal. 1999;**13**:513-522. DOI: 10.1096/fasebj.13.3.513

[93] Gadjev I, Vanderauwera S, Gechev T, Laloi C, Minkov I, Shulaev V, et al. Transcriptomic footprints disclose specificity of reactive oxygen species signaling in Arabidopsis. Plant Physiology. 2006;**141**:436-445. DOI: 10.1104/pp.106.078717

[94] Ivanov B, Kozuleva M, Mubarakshina M. Oxygen metabolism in chloroplast. In: Babulya P, editor. Cell Metabolism - Cell Homeostasis and Stress Response. Rijeka: InTechOpen; 2012. pp. 39-73. DOI: 10.1134/S1990747814060026

[95] Mubarakshina MM, Ivanov BN, Naydov IA, Hillier W, Badger MR, Krieger-Liszkay A. Production and diffusion of chloroplastic H_2O_2 and its implication to signalling. Journal of Experimental Botany. 2010;**61**:3577-3587. DOI: 10.1093/jxb/erq171

[96] Gao J, Wang X, Chang Y, Zhang J, Song Q, Yu H, et al. Acetazolamide inhibits osmotic water permeability by interaction with aquaporin-1. Analytical Biochemistry. 2006;**350**:165-170. DOI: 10.1016/j.ab.2006.01.003

[97] Haddoub R, Rutzler M, Robin A, Flitsch S. Design, synthesis and assaying of potential aquaporin inhibitors. In: Beitz E, editor. Handbook of Experimental Pharmacology: Aquaporins. Berlin: Springer; 2009. pp. 385-402. DOI: 10.1007/978-3-540-79885-9_19

[98] Huber V, Tsujita M, Yamazaki M, Sakimura K, Nakada T. Identification of arylsulfonamides as aquaporin 4 inhibitors. Bioorganic & Medicinal Chemistry Letters. 2007;**17**:1270-1273. DOI: 10.1016/j.bmcl.2006.12.010

[99] Kim J, Yoon H, Kwon K, Lee S, Rhee S. Identification of proteins containing cysteine residues that are sensitive to oxidation by hydrogen peroxide at neutral pH. Analytical Biochemistry. 2000;**283**(2):214-221. DOI: 10.1006/abio.2000.4623

[100] Beebo A, Mathai J, Schoefs B, Cornelia S. Assessment of the requirement for aquaporins in the thylakoid membrane of plant chloroplasts to sustain photosynthetic water oxidation. FEBS Letters. 2013;**587**: 2083-2089. DOI: 10.1016/j.febslet.2013.05.046

[101] Zybailov B, Rutschow H, Friso G, et al. Sorting signals, N-terminal modifications and abundance of the chloroplast proteome. PLoS One. 2008;**3**(4):e1994. DOI: 10.1371/journal.pone.0001994

[102] Gao L, Lu Z, Ding L, Guo J, Wang M, Ling N, et al. Role of aquaporins in determining carbon and nitrogen status in higher plants. International Journal of Molecular Sciences. 2018;**19**(1):35-47. DOI: 10.3390/ijms19010035

[103] Zabaleta E, Hans V, Braun P. A basal carbon concentrating mechanism in plants? Plant Science. 2012;**187**:97-104. DOI: 10.1016/j.plantsci.2012.02.001

[104] Millar A, Sweetlove L, Giege P, Leaver C. Analysis of the Arabidopsis mitochondrial proteome. Plant Physiology. 2001;**127**:1711-1727. DOI: 10.1104/pp.010387

[105] Wang Q, Fristedt R, Yu X, Chen Z, Liu H, Lee Y, et al. The γ-carbonic anhydrase subcomplex of mitochondrial complex I is essential for development and important for photomorphogenesis of Arabidopsis. Plant Physiology. 2012;**160**(3):1373-1383. DOI: 10.1104/pp.112.204339

[106] Badger M, Price G. The role of carbonic anhydrase in photosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology. 1994;**45**:369-392. DOI: 10.1146/annurev.pp.45.060194.002101

[107] Edwards G, Mohamed A. Reduction of carbonic anhydrase activity in zinc deficient leaves of *Phaseolus vulgaris* L. Crop Science. 1973;**13**:351-354. DOI: 10.2135/cropsci1973.0011183X001300030018x

[108] Price G, von Caemmerer S, Evans J, Yu J, Lloyd J, Oja V, et al. Specific reduction of chloroplast carbonic anhydrase activity by anti-sense RNA in transgenic tobacco plants has a minor effect on photosynthetic CO_2 assimilation. Planta. 1994;**193**:331-340. DOI: 10.1007/BF00201810

[109] Majeau N, Arnoldo M, Coleman J. Modification of carbonic anhydrase activity by antisense and over-expression constructs in transgenic tobacco. Plant Molecular Biology. 1994;**25**:377-385. DOI: 10.1007/BF00043867

[110] Porter M, Grodzinski B. Acclimation to high CO_2 in bean carbonic anhydrase and ribulose bisphosphate carboxylase. Plant Physiology. 1984;**74**:413-416. DOI: 10.1104/pp.74.2.413

[111] Anderson L, Carol A. Enzyme co-localization with rubisco in pea leaf chloroplasts. Photosynthesis Research. 2004;**82**:49-58. DOI: 10.1023/B:PRES.0000040443.92346.37

[112] Lazova G, Stemler A. A 160 kDa protein with carbonic anhydrase activity is compexed with rubisco on the outer

surface of thylakoids. Cell Biology International. 2008;**32**:646-653

[113] Ignatova L, Novichkova N, Mudrik V, Lyubimov V, Ivanov B, Romanova A. Growth, photosynthesis, and metabolism of sugar beet at an early stage of exposure to elevated CO_2. Russian Journal of Plant Physiology. 2005;**52**(2):158-164

[114] Alam M, Nahar K, Hasanuzzaman M, Fujita M. Exogenous jasmonic acid modulates the physiology, antioxidant defense and glyoxalase systems in imparting drought stress tolerance in different Brassica species. Plant Biotechnology Reports. 2014;**8**:279-293

[115] Liu C, Chen K, Zhao X, Wang X, Shen C, Zhu Y, et al. Identification of genes for salt tolerance and yield-related traits in rice plants grown hydroponically and under saline field conditions by genome-wide association study. Rice. 2019;**12**(1):88-100. DOI: 10.1186/s12284-019-0349-z

[116] Buren S. Targeting and function of CAH1-Characterisation of a novel protein pathway to the plant cell chloroplast [thesis]. Umea: Umea University; 2010

[117] Hu H, Boisson-Dernier A, Israelsson-Nordstrom M, Bohmer M, Xue S, Ries A, et al. Carbonic anhydrases are upstream regulators of CO_2-controlled stomatal movements in guard cells. Nature Cell Biology. 2010;**12**:87-93. DOI: 10.1038/ncb2009

[118] Roelfsema MRG, Hedrich R, Geiger D. Anion channels: Master switches of stress responses. Trends in Plant Science. 2012;**17**(4):221-229. DOI: 10.1016/j.tplants.2012.01.009

[119] Xue S, Hu H, Ries A, Merilo E, Kollist H, Schroeder J. Central functions of bicarbonate in S-type anion channel activation and OST1 protein kinase in CO_2 signal transduction in guard cell. The EMBO Journal. 2011;**30**(8): 1645-1658. DOI: 10.1038/emboj.2011.68

[120] Tian W, Hou C, Ren Z, Pan Y, Jia J, Zhang H, et al. A molecular pathway for CO_2 response in Arabidopsis guard cells. Nature Communications. 2015;**6**:6057. DOI: 10.1038/ncomms7057

[121] Kende H. Ethylene biosynthesis. Plant Molecular Biology. 1993;**44**: 283-307. DOI: 10.1146/annurev.pp.44.060193.001435

[122] Ferreira F, Guo C, Coleman J. Reduction of plastid-localized carbonic anhydrase activity results in reduced Arabidopsis seedling survivorship. Plant Physiology. 2008;**147**:585-594. DOI: 10.1104/pp.108.118661

[123] Slaymaker D, Navarre D, Clark D, del Pozo O, Martin G, Klessig D. The tobacco salicylic acid binding protein 3 (SABP3) is the chloroplast carbonic anhydrase which exhibits antioxidant activity and plays a role in the hypersensitive defense response. PNAS. 2002;**99**(10):11640-11645. DOI: 10.1073/pnas.182427699

[124] Restrepo S, Myers K, del Pozo O, Martin G, Hart A, Buell C, et al. Gene profiling of a compatible interaction between *Phytophthora infestans* and *Solanum tuberosum* suggests a role for carbonic anhydrase. Molecular Plant Microbe Interactions Journal. 2005;**18**(9):913-922. DOI: 10.1094/MPMI-18-0913

[125] Frick U, Schaller A. cDNA microarray analysis of fusicoccin-induced changes in gene expression in tomato plants. Planta. 2002;**216**:83-94. DOI: 10.1007/s00425-002-0887-1

[126] Schenk P, Kazan K, Wilson I, Anderson J, Richmond T, Somerville S, et al. Coordinated plant defense responses in Arabidopsis revealed by microarray analysis. PNAS. 2000;**97**:11655-11660. DOI: 10.1073/pnas.97.21.11655

[127] Medina-Puche L, Castelló M, Canet J, Lamilla J, Colombo M, Tornero P. β-Carbonic anhydrases play a role in salicylic acid perception in Arabidopsis. PLoS One. 2017;**12**(7):e0181820. DOI: 10.1371/journal.pone.0181820

[128] Hoang C, Chapman K. Biochemical and molecular inhibition of plastidial carbonic anhydrase reduces the incorporation of acetate into lipids in cotton embryos and tobacco cell suspensions and leaves. Plant Physiology. 2002;**128**:1417-1427. DOI: 10.1104/pp.010879

[129] Pronina N, Allakhverdiev S, Kupriyanova E, Klyachko-Gurvich G, Klimov V. Carbonic anhydrase in subchloroplast particles of pea plants. Russian Journal of Plant Physiology. 2002;**49**(3):303-310. DOI: 10.1023/A:1015589215862

[130] Punnett T, Iyer R. The enhancement of photophosphorylation and the hill reaction by carbon dioxide. The Journal of Biological Chemistry. 1964;**239**:2335-2339. DOI: 10.1104/pp.40.6.1074

[131] Cohen W, Jagendorf T. Inhibition of energy-linked reactions in chloroplasts by polygalacturonate. Archives of Biochemistry and Biophysics. 1972;**150**:235-243. DOI: 10.1016/0003-9861(72)90031-8

[132] Fedorchuk T, Opanasenko V, Rudenko N, Ivanov B. Bicarbonate-induced stimulation of photophosphorylation in isolated thylakoids: Effects of carbonic anhydrase inhibitors. Biological Membranes. 2018;**35**:34-41. DOI: 10.7868/S0233475518010048

[133] Cohen W, MacPeek W. A proposed mechanism for the stimulatory effect of bicarbonate ions on ATP synthesis in isolated chloroplasts. Plant Physiology. 1980;**66**(2):242-245. DOI: 10.1104/pp.66.2.242

[134] Zhurikova E, Ignatova L, Semenova G, Rudenko N, Mudrik V, Ivanov B. Effect of knockout of α-carbonic anhydrase 4 gene on photosynthetic characteristics and starch accumulation in leaves of *Arabidopsis thaliana*. Russian Journal of Plant Physiology. 2015;**62**:564-569. DOI: 10.1134/S1021443715040214

[135] Rudenko N, Fedorchuk T, Vetoshkina D, Zhurikova E, Ignatova L, Ivanov B. Influence of knockout of At4g20990 gene encoding α-CA4 on photosystem II light-harvesting antenna in plants grown under different light intensities and day lengths. Protoplasma. 2018;**255**(1):69-78. DOI: 10.1007/s00709-017-1133-9

[136] Li Y, Zhu Y, Shu Y, Meng F, Lu Y, Bai X, et al. Genome-wild identification of osmotic stress response in *Arabidopsis thaliana*. Genomics. 2008;**92**:488-493. DOI: 10.1016/j.ygeno.2008.08.011

[137] Allen J. Plastoquinone redox control of chloroplast thylakoid protein phosphorylation and distribution of excitation energy between photosystems: Discovery, background, implications. Photosynthesis Research. 2002;**73**:139-148. DOI: 10.1007/1-4020-3324-9_17

[138] Maciejewska U, Polkowska-Kowalczyk L, Swiezewska E, Szkopinskae A. Plastoquinone: possible involvement in plant disease resistance. Acta Biochimica Polonica. 2002;**49**:775-780. DOI: 024903775

[139] Frigerio S, Campoli C, Zorzan S, Fantoni L, Crosatti C, Drepper F, et al. Photosynthetic antenna size in higher plants is controlled by the plastoquinone redox state at the post-transcriptional rather than transcriptional level. The Journal of Biological Chemistry.

2007;**282**:29457-29469. DOI: 10.1074/jbc.M705132200

[140] Ignatova L, Moskvin O, Romanova A, Ivanov B. Carbonic anhydrases in the C3-plant leaf cell. Australian Journal of Plant Physiology. 1998;**25**:673-677. DOI: 10.1071/PP97137

[141] Moskvin O, Shutova T, Khristin M, Ignatova L, Villarejo A, Samuelsson G, et al. Carbonic anhydrase activities in pea thylakoids. Photosynthesis Research. 2004;**79**:93-100. DOI: 10.1023/B:PRES.0000011925.93313.db

[142] Fedorchuk T, Rudenko N, Ignatova L, Ivanov B. The presence of soluble carbonic anhydrase in the thylakoid lumen of chloroplasts from Arabidopsis leaves. Journal of Plant Physiology. 2014;**171**(11):903-906. DOI: 10.1016/j.jplph.2014.02.009