Review

Transport and Use of Bicarbonate in Plants: Current Knowledge and Challenges Ahead

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Abstract: Bicarbonate plays a fundamental role in the cell pH status in all organisms. In autotrophs, HCO$_3^-$ may further contribute to carbon concentration mechanisms (CCM). This is especially relevant in the CO$_2$-poor habitats of cyanobacteria, aquatic microalgae, and macrophytes. Photosynthesis of terrestrial plants can also benefit from CCM as evidenced by the evolution of C$_4$ and Crassulacean Acid Metabolism (CAM). The presence of HCO$_3^-$ in all organisms leads to more questions regarding the mechanisms of uptake and membrane transport in these different biological systems. This review aims to provide an overview of the transport and metabolic processes related to HCO$_3^-$ in microalgae, macroalgae, seagrasses, and terrestrial plants. HCO$_3^-$ transport in cyanobacteria and human cells is much better documented and is included for comparison. We further comment on the metabolic roles of HCO$_3^-$ in plants by focusing on the diversity and functions of carbonic anhydrases and PEP carboxylases as well as on the signaling role of CO$_2$/HCO$_3^-$ in stomatal guard cells. Plant responses to excess soil HCO$_3^-$ is briefly addressed. In conclusion, there are still considerable gaps in our knowledge of HCO$_3^-$ uptake and transport in plants that hamper the development of breeding strategies for both more efficient CCM and better HCO$_3^-$ tolerance in crop plants.

Keywords: bicarbonate; transporter; metabolism; carbonic anhydrase; carboxylases; carbon concentration mechanisms; algae; seagrass; higher land plants; limestone soil

1. Introduction

Life on Earth is based on the photosynthetic transformation of inorganic carbon (C$_{\text{inorg}}$) and water into energy-rich organic carbon (C$_{\text{org}}$) compounds. In turn, these are oxidized by heterotrophs to obtain cellular energy, releasing again C$_{\text{inorg}}$ in the form of CO$_2$ into the atmosphere. Atmospheric CO$_2$ is the main form of C$_{\text{inorg}}$ assimilated by the terrestrial photosynthetic organisms. Dissolution of CO$_2$ in water provides carbonic acid, which dissociates into bicarbonate (HCO$_3^-$) and carbonate (CO$_3^{2-}$). Ocean water contains about 90% of C$_{\text{inorg}}$ in the form of HCO$_3^-$ . It is calculated that, at preindustrial concentrations of atmospheric CO$_2$, the seawater concentration of HCO$_3^-$ was 1757 µmol/kg. Even higher HCO$_3^-$ concentrations are currently observed due to increasing atmospheric CO$_2$, which leads to acidification of the ocean and higher solubility of carbonate under these lower pH conditions. Photosynthetic marine organisms as well as submerged freshwater plants can use this abundant HCO$_3^-$ as a source for the biosynthesis of C$_{\text{org}}$ [1–3].

Due to the weathering of limestone and dolomite, bicarbonate enters into the soil solution. The HCO$_3^-$ concentration in a solution phase should be controlled by the solubility of CaCO$_3$. Calcite is the main carbonate mineral with an ion concentration product (Ksp) at 25 °C of $10^{-8.35}$ [4].
However, considerably higher $\text{HCO}_3^-$ levels than those predicted based on carbonate solubility constants may occur in soil solutions [5]. Biological activity contributes to $\text{HCO}_3^-$ build-up in soil solutions by hydrating $\text{CO}_2$ from the atmosphere and from the respiratory activity of plant roots, microorganisms, and soil fauna. The $\text{CO}_2$ hydration process catalyzed by soil carbonic anhydrase activity provided mostly by soil cyanobacteria and microalgae [6] can be considerably higher than the uncatalyzed process [7].

Terrestrial cyanobacteria can use the $\text{HCO}_3^-$ dissolved in the surrounding aqueous film for photosynthesis [8]. In contrast, high soil $\text{HCO}_3^-$ concentrations can injure the performance of higher land plants especially of the carbonate sensitive calcifuge species. In these calcareous soils with high pH, the availability of Fe and other essential micronutrients like Zn, Mn, and Cu is usually low due to precipitation as oxides or carbonates. This leads to the so-called lime-induced chlorosis and low yield in sensitive varieties of crops. Dicots such as citrus, deciduous fruit trees, vineyard, and legumes are the most sensitive mainly because of the interference of bicarbonate with their Fe acquisition mechanisms (strategy I). Grasses are less affected. Their Fe acquisition is based on phytosiderophore production (strategy II) [9]. Nonetheless, monocots like rice, maize barley, or wheat can be affected by severe Zn deficiency on carbonate-rich soils [10]. To what extent terrestrial higher plants are able to assimilate $\text{HCO}_3^-$—either soil-derived or of respiratory origin produced by soil microorganism and plant roots—is still under debate [11] and will be discussed below.

Heterotrophs, which are animals and humans, are net producers of $\text{CO}_2$ by respiration. Bicarbonate is the main transport form of $\text{C}_{\text{inorg}}$ from cells to the lungs where it is exhaled in the form of $\text{CO}_2$. The carbonic acid/bicarbonate buffer is considered the most important system for cell pH homeostasis. Kidneys filter and reabsorb $\text{HCO}_3^-$. These processes are essential for the acid-base balance of the body [12]. Furthermore, $\text{HCO}_3^-$ transport plays an essential role in pH regulation during amelogenesis, which is the formation of enamel during tooth development [13], and in other biological calcification processes such as the development of reef cnidarians [14].

The presence of $\text{HCO}_3^-$ in all organisms opens the question of how this molecule is taken up, membrane-transported, and compartmentalized in these different biological systems. This review aims to give a comprehensive overview of the transport and metabolic processes related to $\text{HCO}_3^-$ in plants. Bicarbonate transport in cyanobacteria and human cells is much better documented and will be briefly presented for comparison.

2. Bicarbonate Transport

As an anion, $\text{HCO}_3^-$ is not freely permeable to the lipid bilayer of biological membranes. The presence of $\text{HCO}_3^-$ inside cells is either due to $\text{HCO}_3^-$ transport mediated by membrane transporter proteins or transmembrane diffusion of $\text{CO}_2$ followed by fast transformation into $\text{HCO}_3^-$ using carbonic anhydrase (CA).

$\text{CO}_2$ crosses biological membranes by diffusion either through the lipid bilayer or through pores. A subset of aquaporins and related proteins [15,16] can behave as $\text{CO}_2$ channels [17]. Models based on artificial lipid bilayers indicated that the resistance for $\text{CO}_2$ diffusion is small and mostly limited by unstirred layers. According to the authors, an estimated permeability of 3.6 cm s$^{-1}$ makes it unlikely that $\text{CO}_2$ is transported through aquaporins or other transporter proteins [18]. Contrastingly, studies on real bio-membranes provided clear evidence that membranes can offer resistance to $\text{CO}_2$ diffusion and that this resistance depends on the membrane’s protein composition [19]. There is now functional evidence that some, but not all, plant aquaporins can enhance $\text{CO}_2$ diffusion into both stomatal guard cells and mesophyll cells [20,21]. However, the relative contribution of both $\text{CO}_2$ diffusion pathways to the downhill, non-energized movement of $\text{CO}_2$ through the membranes of cyanobacteria, eukaryotic algae, and embryophytes has to be further clarified [22]. Transport of $\text{HCO}_3^-$ through aquaporins has not been shown. As a charged chemical species, the $\text{HCO}_3^-$ ion is submitted to electrochemical gradients that govern plasma membrane ion transport. Since cyanobacteria, algae, and plant cells have an inside negative membrane potential ($E_m$), the uphill $\text{HCO}_3^-$ uptake must
be energized. It takes place through transporters in cyanobacteria [23], microalgae [24,25], macroalgae [26], and seagrasses [27]. The plasma membrane downhill HCO$_3^-$ efflux takes place through anion channels. The only available evidence for an HCO$_3^-$ permeable anion channel is the R-type, which has been found in the hypocotyls of Arabidopsis thaliana with a selectivity sequence for anions. NO$_3^-$ (2.6) > SO$_4^{2-}$ (2.0) > Cl$^-$ (1.0) > HCO$_3^-$ (0.8) >> malate$^{2-}$ (0.03) [28,29]. In addition, the inner chloroplast envelope protein LCIA of Chlamydomonas reinhardtii has been proposed to be an HCO$_3^-$ permeable channel [30–32]. Furthermore, Raven et al. [33] have proposed the existence of HCO$_3^-$ permeable anion channels in the thylakoid membrane as an element of the carbon concentration mechanisms (CCM) in microalgae.

2.1. Bicarbonate Transport by Solute Carriers (SLC) in Humans and Mammals

Most bicarbonate transporters belong to the solute carriers (SLC), which is a large group of secondary active membrane transporters for relatively small molecules. The best studied bicarbonate transporters are SLC in humans due to severe diseases related to the malfunctioning of these transporters [34,35]. In humans, 430 members organized in 52 families have been identified [36]. Proteins transporting HCO$_3^-$ belong to the families SLC26 (Sulfate permease SulP) and SLC4. The phylogenetically ancient gene family SLC26 encodes for multiple anion exchangers and channels. Some are relatively ion specific, but others have a broad substrate range. Besides transporting inorganic anions like HCO$_3^-$, Cl$^-$, SO$_4^{2-}$, and I$^-$, oxalate and formate may be transported. Structural models indicate that these polypeptides have 10 to 14 membrane-spanning domains flanked by a cytoplasmic N-terminal and a cytoplasmic C-terminal bound to a STAS (sulphate transporter anti sigma factor-like) domain [37]. The gene family SLC4 contains genes that code for proteins transporting HCO$_3^-$ or the closely related CO$_3^{2-}$ along with a monovalent anion (Cl$^-$) or cation (Na$^+$) [38]. These proteins have 14 transmembrane spanning domains grouped into a 7 + 7 inverted repeat topology.

Different ways for HCO$_3^-$ transport through the membranes in mammalian and human cells can be distinguished (see Figure 1). The first includes electroneutral, Na$^+$- independent anion exchange between HCO$_3^-$ and Cl$^-$ using anion exchange transporters (AE) encoded by genes of the SLC4A family. The second includes sodium-driven Cl$^-$/HCO$_3^-$ exchanger (NDCBE) encoded by SLC4A8. This transporter is thought to exchange 1 Cl$^-$ for 2 HCO$_3^-$ and 1 Na$^+$. The next transport mechanism comprises electrogenic Na$^+/HCO_3^-$-cotransport performed by NCBT transporter proteins NBCe1 and NBCe2 encoded by SLC4A4 and SLC4A5. The fourth way is the electroneutral Na$^+$-HCO$_3^-$ cotransport or Na$^+$-driven Cl$^-$/HCO$_3^-$ exchange through the transporter protein encoded by SLC4A10 [39–41]. The fifth mechanisms involves electroneutral Cl$^-$/HCO$_3^-$ exchangers that also can exchange either I$^-$, NO$_3^-$, SCN$^-$, or formate encoded by SLC26A (Pendrin) or NO$_3^-$, OH$^-$, SO$_4^{2-}$, oxalate, and formate (SLC26A6). (Electrogenic Cl$^-$/HCO$_3^-$ exchange, with channel activity for Cl$^-$, SO$_4^{2-}$, and oxalate (SLC26A7) and Electrogenic Cl$^-$/HCO$_3^-$ exchange with Cl- channel activity, NaCl cotransport or Cl$^-$-independent HCO$_3^-$ transport (SLC26A9) are also HCO$_3^-$ transport mechanisms [35] (see Figure 1).
2.2. \( \text{C}_{\text{inorg}} \) Transporters in Cyanobacteria

Five modes of \( \text{C}_{\text{inorg}} \) transport have been described in cyanobacteria. (i) BCT1 is an inducible, high affinity \( (K_{\text{m}} \text{ for } \text{HCO}_3^- \approx 15 \text{ mM}) \) transporter located in the plasma membrane that belongs to the ATP binding cassette (ABC) transporter family [42] although transport energization by ATP consumption has not been proven [43]. BCT1 is a multi-meric complex composed by four subunits. CmpA is located in the periplasmic space and binds \( \text{HCO}_3^- \) with a very low \( K_{\text{m}} \) of 5 \( \mu \text{M} \) [44] and also binds \( \text{Ca}^{2+} \) as a cofactor [45]. CmpB is a dimer within the plasma membrane and CmpC and CmpD are extrinsic proteins that share binding sites for ATP. CmpC has an extra domain involved in the allosteric regulation of BCT1 similar to the NrtC protein of NRT1 transporter. In this later case, the domain of the NrtC protein is involved in the inhibition of transport in the presence of \( \text{NH}_4^+ \) [46]. BCT1 is found in \( \beta \)-cyanobacteria but absent in marine cyanobacteria. However, it is present in the \( \alpha \)-cyanobacteria Synechocystis WH5701, which can live in a wide range of \( \text{C}_{\text{inorg}} \) concentrations and salinities [43].

(ii) SbtA is a low \( \text{C}_{\text{inorg}} \)-inducible, high affinity \( (K_{\text{m}} \text{ for } \text{HCO}_3^- \approx 2–5 \text{ mM}) \) plasma membrane \( \text{HCO}_3^- \) transporter that uses \( \text{Na}^+ \) as a driving ion with a half saturation constant around 1 \( \mu \text{M} \) for this ion [47]. Although initially considered a single unit-type transporter, it has a bigger complex size, which suggests that, in its functional form in the plasma membrane, this transporter is a tetramer [48]. It has been suggested that SbtA is activated by a serine-threonine protein kinase [49] that also depends on \( \text{Na}^+ \) [50]. SbtA homologs seem to be present in many \( \beta \)-cyanobacteria although this has only been confirmed in Synechocystis PCC6803 [47] and Synechococcus PCC7002 [50].

(iii) BicA \( \text{HCO}_3^- \) transporters are also dependent on \( \text{Na}^+ \). Their affinity for \( \text{HCO}_3^- \) transport with a \( K_{\text{m}} \) ranging from 74 \( \mu \text{M} \) to 353 \( \mu \text{M} \) (1.7 \( \text{mM} \) for \( \text{Na}^+ \)) is lower than that of SbtA. Nonetheless, BicA is able to maintain a high flow of \( \text{C}_{\text{inorg}} \) for photosynthesis. BicA transporters are expressed at low levels under conditions of high \( \text{CO}_2 \) but they are highly inducible under low \( \text{CO}_2 \). They have been discovered in the coastal marine cyanobacterium Synechococcus PCC7002 [51] and they are present in both \( \alpha \)-cyanobacteria and \( \beta \)-cyanobacteria. BicA transporters belong to the large family of prokaryotic and eukaryotic transporters often described as sulphate, SulP family transporters. The C-terminus includes a hydrophilic STAS domain (see also Section 2.1) involved in the allosteric regulation that has also been found in \( \text{A. thaliana} \) sulfate transporters [52].
(iv) NDH-I4 is a constitutive protein complex located in the plasma membrane that accelerates CO₂ uptake. The passive entry of CO₂ is followed by the conversion (NDH-I mediated) to HCO₃⁻ [53,54]. (v) NDH-13 is a second, complex, low CO₂-inducible system involved in CO₂ uptake located in the thylakoid membrane. It works in a similar manner to NDH-I4 [53,54].

2.3. C_inorg Transport in Microalgae

*C. reinhardtii* uptake of C_inorg has been associated with the activity of an ATP-binding cassette transporter, HLA3, and the homolog of a formate-nitrite transporter LCIA that is also called NAR1.2 [30]. HLA3 is located in the plasma membrane and LCIA in the chloroplast envelope. The absence of LCIA decreases the amount of HLA3 mRNA, which indicates a regulation by the chloroplast-encoded LCIA of the expression of HL3 encoded in the nuclear genome [31]. While the HCO₃⁻ transport mechanism of HL3 seems to be clear, LCIA has been proposed to be an HCO₃⁻ channel [22,30,31]. If so, HCO₃⁻ ions would be transported through such a channel downhill and could not accumulate HCO₃⁻ over the equilibrium prediction. However, the addition of mM concentrations of HCO₃⁻ to *Xenopus laevis* oocytes expressing NAR1.2 evokes a membrane depolarization as does the addition of mM concentrations of NO₂⁻, which suggests an HCO₃⁻ transport into the chloroplastic by H⁺ symport instead of the transport through a channel. This mechanism would also be consistent with the need to overcome the electrochemical gradient for HCO₃⁻ in the stroma relative to the cytosol [55]. The ycf10 is also related to C_inorg transport. Disruption of the plastid ycf10 inhibits the C_inorg accumulation in the chloroplast. Its gene product known as the protein CemA was originally proposed as a C_inorg transporter, but its similarities with the cyanobacterial PxcA involved in Na⁺-dependent H⁺ extrusion suggest that CemA may play a similar role in the energization of the chloroplast envelope [55]. The HCO₃⁻ uphill transport through the plasma membrane and the chloroplast envelope agrees with the early observation of a vanadate sensitive C_inorg transport at both levels. A second C_inorg transporter proposed for the plasma membrane in *C. reinhardtii* is LCI1 [56,57]. The overexpression of this protein increases the affinity for C_inorg and enhances HCO₃⁻ uptake. The protein is encoded by an orphan gene [55] and does not have any known functional motif. The proteins CCP1/2 have also been proposed to take part in C_inorg uptake by the chloroplasts. They show similarities with the mitochondrial carrier proteins superfamily, but knock-outs of CCP1/2 do not show defects in photosynthesis [58]. Thus the specific role of CCP1/2 proteins in C_inorg transport has yet to be clarified.

The active uptake of HCO₃⁻ was described for natural populations of marine phytoplankton dominated by large diatoms [59]. However, the HCO₃⁻ transport mechanisms at the molecular level have been studied in the model diatom species *Phaeodactylum tricornutum* and *Thalassiosira pseudonana* [60,61]. In *P. tricornutum*, ten putative HCO₃⁻ transporters have been identified. They are similar to the unrelated SLC4 and SLC26 mammalian protein families (see Section 2.1). SLC4 has been characterized as a HCO₃⁻ transporter in the plasma membrane of *P. tricornutum* and SLC4 homologs have also been found in *T. pseudonana* [25]. Photosynthesis in the diatom species is sensitive to 4,4′-diisothiocyanatostilbene-2, 2′-disulfonic acid (DIDS), which is an inhibitor of anion exchange, and depends on the presence of Na⁺ in the medium (K₀.₅ 28 mM, saturation at 100 mM Na⁺). This suggests the existence of an HCO₃⁻ uptake mechanism based on Na⁺ symport or on a Na⁺ dependent Cl⁻ /HCO₃⁻ anti-port [25]. A different group of SLC4 transporters located in the chloroplast envelope have been proposed for transporting HCO₃⁻ to the chloroplast stroma [25,60,61]. The active transport rate of dissolved inorganic carbon through the chloroplast envelope is ten-fold that of HCO₃⁻ transport across the plasmalemma [54]. However, further investigations are required to elucidate the molecular identity of the protein and the transport mechanism in the context of the complex four-layer chloroplast envelope of diatoms [62].

In micro-algal species, genetic tools are still not available and HCO₃⁻ uptake has been revealed by physiological methods that include the photosynthetic sensitivity to inhibitors of external CA, pH buffers to dissipate electrochemical H⁺ gradients, and inhibitors of anion exchangers. Therefore,
a direct entry of HCO$_3^-$ has been proposed for the marine eustigmatophycean *Nannochloropsis gaditana* [63–65]. The absence of external CA and the sensitivity to DIDS suggest an anion exchange mechanism for HCO$_3^-$ transport. A DIDS and 4-acetamido-4′-isothiocyanato-stilbene-2, 2′-disulfonic acid (SITS) sensitive photosynthesis has been described in *Eminliania huxleyi* [66]. SITS is the putative inhibitor of the anion exchanger 1 (AE1), which works as a Cl$^-$/HCO$_3^-$ antiporter in red blood cells [67] (see Figure 1). In contrast, a DIDS/SITS insensitive HCO$_3^-$ transport has been described for *Dunaliella tertiolecta* [68].

### 2.4. C$_{inorg}$ Transport in Macroalgae

One of the first examples for the use of HCO$_3^-$ in macro-algae was described in the giant inter-nodal cells of Characeae living in alkaline media [69]. The active efflux of H$^+$ through the putative H$^+$/ATPase causes a local acidification of the apoplast in about two pH units [69]. The presence of CA activity in the acidic zones accelerates the conversion of HCO$_3^-$ to CO$_2$ that diffuses across the plasmalemma [70]. The cytosolic pH homeostasis requires the presence of alkaline areas between the acid zones, which produces the spectacular banding observed in these organisms under the light [71]. An alternative mechanism for HCO$_3^-$ use and hence for banding was given by Lucas et al. [72]. These authors, by using quasi apoplastic pH measurements in flow-through experiments, provide evidence for an H$^+$/HCO$_3^-$ symport in the acid bands in which the electrochemical proton gradient generated by the H$^+$-ATPases is secondarily used for HCO$_3^-$ transport. According to the model by Walker et al. [69], the alkaline zones are needed for compensating cytosolic pH through OH$^-$ efflux, which originated via the catalyzed cytosolic dehydration of HCO$_3^-$.

A similar model to the one proposed by Walker et al. [69] has been described for freshwater flowering plants where the acid zone is the abaxial (lower) leaf surface and the alkaline zone is the adaxial (upper) leaf surface [22,73].

The use of HCO$_3^-$ as a source of inorganic carbon for photosynthesis has been described for the majority of marine macro-algae and seagrasses [74–76]. The most common mechanism of HCO$_3^-$ use is the apoplastic conversion to CO$_2$, which is shown in *Condrus chrispus* [77], *Porphyra leucosticta* [78], a series of red macroalgae [79], and *Phyllariopsis puspurascens* [80]. More information is available in References [33,75,81,82]. Alternatively, other algal species have been described as direct HCO$_3^-$ users. Most of the evidence for a direct uptake of HCO$_3^-$ ions comes from experiments in which the inhibitors of anion exchanger, mainly DIDS and SITS, are used to inhibit HCO$_3^-$ transport and, therefore, photosynthesis [82]. Larsson and Axelson [83] examined 11 green, 5 red, and 11 brown macro algae. Photosynthesis was DIDS-sensitive only in Chaetomorpha, Monostroma, and ulvaceans (Ulva and Enteromorpha), but not in the rest of green, red, or brown algae tested. More information is available in Reference [84]. Fernández et al. [26] show a DIDS-sensitive anion exchanger as the main mechanism for HCO$_3^-$ uptake in the giant kelp *Macrocystis pyrifera*. DIDS-sensitivity has also been reported in the red algae *Eucheuma denticulatum* [85] while a residual DIDS-sensitive photosynthetic activity was found in *Gracilaria gaditana* [86]. Calculations made from photosynthetic conductance were used to suggest direct HCO$_3^-$ uptake in *Laurencia pinnatifida* [87].

### 2.5. C$_{inorg}$ Transport in Seagrasses

Seagrasses have been described as HCO$_3^-$ users [82,88–91]. Based on the lack of photosynthesis inhibition in seagrasses by DIDS and SITS, Larkum et al. [91] hold that HCO$_3^-$ influx through anion exchangers does not take place in the leaves of seagrasses. These substances inhibit AE1 that are present in algae but in angiosperms (including marine) DIDS and SITS have been described as inhibitors of anion channels [29,92] that may have a distinct role in plasma membrane anion transport [87]. As an alternative, Larkum et al. [91] suggest a proton symport as the mechanism for direct uptake of HCO$_3^-$.

Such a mechanism has been proposed for *Zostera marina* [88,93,94], *Zostera noltii* [95], *Posidonia oceanica* and *Cymodocea nodosa* [96], *Halophila stipulacea* and *Ruppia maritima* [96], *Ruppia cirrhosa* [97], and *Halophila ovalis* [98]. Based on the response of seagrasses to acetazolamide (AZ) and TRIS buffers, Beer et al. [88] suggest three mechanisms for carbon acquisition. First, an apoplastic
dehydration of HCO$_3^-$ catalyzed by CA and the subsequent diffusion of CO$_2$ across the plasmalemma. This mechanism is proposed for plants that show AZ-sensitive TRIS-insensitive photosynthesis, but the ubiquitous presence of plasmalemma H$^+$-ATPase cannot be ignored [99]. Second, the catalyzed apoplastic dehydration of HCO$_3^-$ to CO$_2$ in acid regions generated by the activity of the H$^+$-ATPases. This mechanism would be sensitive to AZ and TRIS. Third, the direct uptake of HCO$_3^-$ ions by symport with H$^+$. In this case, the electrochemical gradient for H$^+$ generated by the activity of the plasmalemma H$^+$-ATPases drives the direct HCO$_3^-$ transport. This mechanism would be AZ-insensitive and TRIS-sensitive. The two first mechanisms involve apoplastic accumulation of OH$^-$ and CO$_2$ diffusion across the plasmalemma and the third one implies accumulation of HCO$_3^-$ and likely OH$^-$ in the cytosol (see Table 1). In contrast to humans (see Figure 1) and cyanobacteria (Section 2.2), no Na$^+$-dependent HCO$_3^-$ uptake system has been reported in plants. The only example for a Na$^+$-driven ion transport system is the high affinity transporter for NO$_3^-$ and P$_i$ in the seagrass Zostera marina [100]. In that case the electrochemical gradient for Na$^+$ is maintained because of very low membrane permeability for Na$^+$ and the action of a Na$^+$/H$^+$ antiporter, which is similar to the SOS1 present in terrestrial vascular plants [101].

| C$_{inorg}$ Uptake Mechanism | AZ | TRIS | Seagrass Species | References |
|-----------------------------|----|------|------------------|-----------|
| Apoplastic dehydration of  |    |      | Zostera marina   | [93]      |
|  HCO$_3^-$ catalysed by CA  | +  | -    | Cymodocea nodosa | [96]      |
|                             |    |      | Halophila ovalis | [98]      |
|                             |    |      | Cymodocea serrulata | [98] |
|                             |    |      | Cymodocea rotundata | [98] |
|                             |    |      | Synringodium isoetifolium | [98] |
|                             |    |      | Halodule wrightii | [98]      |
|                             |    |      | Thalassia hemprichii | [98] |
|                             |    |      | Thalassodendron ciliatum | [98] |
|                             |    |      | Enhalus acoroides | [98] |
|                             |    |      | Posidonia australis | [102] |
| Apoplastic dehydration of  |    |      | Halophila stipulacea | [88] |
|  HCO$_3^-$ in acid regions  | +  | +    | Rupia maritima | [88] |
|                             |    |      | Cymodocea nodosa (?) | [96] |
|                             |    |      | Cymodocea rotundata | [98] |
|                             |    |      | Synringodium isoetifolium | [98] |
|                             |    |      | Halodule wrightii | [98] |
|                             |    |      | Thalassia hemprichii | [98] |
|                             |    |      | Thalassodendron ciliatum | [98] |
|                             |    |      | Enhalus acoroides | [98] |
Table 1. Cont.

| Plasma membrane | HCO$_3^-$/$\text{H}^+$ symport | Posidonia oceanica | Zostera marina | Halophyla stipulacea | Rupia maritima | Cymodocea nodosa | Halophila ovalis |
|-----------------|-------------------------------|--------------------|----------------|----------------------|----------------|----------------|-----------------|
|                 | +                             | [27] $^1$          | [94]           | [88]                 | [88]           | (?)            | [98]            |

+ sensitive; $^-$ insensitive; $^1$ Direct evidences for a plasma membrane HCO$_3^-$/$\text{H}^+$ symport mechanism.

The availability of the genome of Zostera marina [103] allows the in silico search for genes potentially involved in C$_{\text{inorg}}$ transport. Using the web application Phytozome (http://www.phytozome.net), which is a comparative platform for green plant genomics [104], we searched for genes with homologies with the HLA3 transporter of C. reinhardtii and SLC4 transporter of P. tricornutum. The search for homologies in the genome of Z. marina with ChHLA3 sequence results in six genes with high homology, all of them listed as ABC transporters. In contrast, the search for homologies with PhSLC4 results in five sequences of medium-to-low homologies with genes encoding boron transporters and anion exchangers. The public availability of the genome of seagrasses will be a valuable tool for the future investigation of the exact molecular identities of C$_{\text{inorg}}$ transporters, cellular location, mechanism, kinetic properties, and regulation.

2.6. C$_{\text{inorg}}$ Transport in Higher Land Plants

While HCO$_3^-$ transporters are already quite well-characterized in cyanobacteria, algae, and mammals, the information on higher vascular land plants is scarce. Seven loci of genes coding for transporters of the HCO$_3^-$ family are listed in the gene databases of the genetically well-characterized A. thaliana. The best studied protein is BOR1. This protein belongs to the solute carrier family type SLC4 and presents homology to SLC4A1, which is the band 3 transporter highly abundant in erythrocytes. As SLC4A1, BOR1 has a gate and a core domain and acts with an elevator mechanism. However, BOR1 has an inward rotated core domain providing an occluded state, which suggests that it may undergo structural transitions allowing access from either side of the membrane [105]. Bicarbonate transport by Band 3 is a unidirectional pathway out of the erythrocyte. A further substantial difference is that BOR1 is an efflux-type borate transporter responsible for root-to-shoot transport of this essential plant nutrient. BOR1 is located in the xylem parenchyma cells and loads borate into the xylem, which is then transported to shoots by the transpiration stream [106]. The other six genes code for BOR2 to BOR7 [107]. All seem to be involved in the transport of borate or boric acid rather than in HCO$_3^-$ transport.

Although no selective HCO$_3^-$ transporters or channels have so far been characterized in higher land plants, the possibility of membrane transport by specific or unspecific anion transporting proteins cannot be excluded. Several studies provide indirect support for HCO$_3^-$ uptake by plant roots. Under exposure to high HCO$_3^-$ (5 mM to 20 mM), a strong inhibition of nitrate, sulphate, and phosphate uptake by roots has been observed [108]. Such inhibition could be caused, at least in part, by competition between HCO$_3^-$ and other anions for transport mechanisms with low anion specificity. An electrophysiological approach to ion selectivity of a voltage-dependent anion channel in A. thaliana hypocotyls revealed low but reproducible HCO$_3^-$ currents. A permeability ranking of NO$_3^->\text{SO}_4^{2-} > \text{Cl}^->\text{HCO}_3^- >> \text{mal}^{2-}$ could be established [28]. More recently, such a channel with permeability for several anions has been identified as QUAC1/ALMT12, which is a channel that releases anions from guard cells [109]. In fact, anion channel currents in plants have mainly been studied in guard cells where they contribute to the mechanisms for controlling stomatal resistance.
As plants can acquire $\mathrm{CO}_3^{2-}$ by flow, either through the young root tips where the Casparian strip has still not fully formed or at sites where lateral root emergence from the pericycle disrupts this hydrophobic barrier, a substance has to first pass through the apoplastic bypass due to the symport of 2 $\mathrm{H}^+$ into CO$_2$, which may easily diffuse into the stele. Apoplastic flow, by either the root tips where the Casparian strip has still not fully developed or at sites where lateral root emergence from the pericycle disrupts this hydrophobic barrier, may be another way $\mathrm{HCO}_3^-$ enters the stele. Contribution of this apoplastic bypass is relatively small in the case of NaCl [117] or Cd [118]. We could not find specific data for HCO$_3^-$. Early investigations using $^{13}$C or $^{14}$C isotopes as markers for HCO$_3^-$ provided evidence for uptake of HCO$_3^-$ by roots and transport to the shoots [119–122]. However, the contribution of C$_{\text{inorg}}$ taken up by roots may be less than 1% taken up by leaves [123]. The $^{14}$C from labelled H$^{14}$CO$_3^-$ supplied through the roots was found to be incorporated into sugar, starch, and proteins of leaves [124]. As plants can acquire C$_{\text{inorg}}$ from different sources including atmospheric CO$_2$ and respiratory CO$_2$, the experimental design is critical. Solution pH used for supplying labelled HCO$_3^-$ to the plants deserves special attention. At pH 8, most of the labelled C$_{\text{inorg}}$ is in the form of HCO$_3^-$ but a small percentage of labelled CO$_2$ can be present and CO$_2$ diffusion into the root cells may occur, which will be followed by transformation of this CO$_2$ into labelled HCO$_3^-$ by CA. This transformation can be even more relevant considering that the pH of cell walls and xylem sap are usually in the acid range. The pH of the leaf apoplast of sunflowers remained stable around 6.4 to 6.5 even if roots were exposed to 10 mM HCO$_3^-$ [125]. However, it has to be taken into account that apoplastic alkalinization is a general response to stress in plants [126]. Enhanced Cl$^-$ supply under stress causes alkalinization of the root apoplast due to the symport of 2 $\mathrm{H}^+$ per 1 Cl$^-$ [127]. Increasing the external pH of the root bathing solution also increases pH of both A. thaliana root cell walls [128] and xylem sap [129]. This favors HCO$_3^-$ over CO$_2$ formation. Nonetheless, even under severe stress conditions, such as drought or fungal infection with a strong alkalinization effect in the apoplast, the increased pH values remain nearly neutral [130]. Therefore, in plants with their roots exposed to HCO$_3^-$, the proportion of HCO$_3^-$ over CO$_2$ during radial transport of C$_{\text{inorg}}$ from soil to the stele and within the xylem sap up to the leaves may be considerably lower than in the soil solution surrounding the plant roots. The direct use of root-derived HCO$_3^-$ by CA in chloroplasts to supply CO$_2$ for Rubisco is unlikely when taking into account the low chloroplast permeability of HCO$_3^-$ ($1 \times 10^{-8}$ m s$^{-1}$) in comparison to CO$_2$ (range from $2.3 \times 10^{-4}$ to $8 \times 10^{-4}$ m s$^{-1}$), which was recently shown by mass inlet mass spectrometry (MIMS) using $^{18}$O labelled C$_{\text{inorg}}$ [131].

3. Formation and Use of Bicarbonate in Plants

As seen in higher plants, no selective HCO$_3^-$ transporter or channel has been characterized at the molecular level. Membrane transport of HCO$_3^-$ in these organisms is still unclear. Contrastingly,
the contribution of HCO$_3^-$ to essential metabolic pathways and the total assimilation of C$_{\text{inorg}}$ in plants is reliably documented.

The CA-generated HCO$_3^-$ serves as a substrate for different carboxylases among others acetyl-CoA carboxylase (ACCase, EC.6.4.1.2) and phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31). ACCase contains a biotin carboxylase, a biotin carboxyl carrier protein, and a carboxyl transferase. It catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in the chloroplast and the cytosol [132]. Malonyl-CoA is the precursor for fatty acid formation and elongation. Moreover, it participates in the biosynthesis of ring A of flavonoids through the polycetic pathway and in the biosynthesis of malonylated aminocyclopropane-1-carboxylic acid (MACC), which is involved in the down-regulation of ethylene production in plants (see Figure 2). Other biotin-containing carboxylases operating with HCO$_3^-$ are 3-methylcrotonyl-CoA carboxylase, which is involved in the mitochondrial pathway of leucine catabolism. Geranyl-CoA carboxylase likely works in the metabolism of cyclic terpenes [133,134].

**Figure 2.** Metabolic pathways related to three major plant carboxylases using HCO$_3^-$ as substrate.

CA, carbonic anhydrase; PEPC, phosphoenolpyruvate carboxylase; ACC, acetyl-CoA carboxylase; MCC, malonyl-1-aminocyclopropane-1-carboxylic acid.

PEPC plays a major role in the carbon assimilation processes in plants. This enzyme in the presence of Mg$^{2+}$ or Mn$^{2+}$ ions catalyzes the β-carboxylation of phosphoenolpyruvate (PEP) yielding oxalacetate (OAA) and inorganic phosphate (P$_i$) in an irreversible reaction (see Figure 2).

The relative importance of the contribution of HCO$_3^-$ to the total plant C$_{\text{org}}$ as well as the assimilation mechanisms and their consequences for plant adaptation to different environmental conditions depend on the plant species and the characteristics of the habitat (see Sections 3.2 and 4). In all cases, the cooperation of the two enzymes, CA and, in higher plants, PEPC, is essential.

### 3.1. Plant Carbonic Anhydrase and Phosphoenolpyruvate Carboxylase

Carbonic anhydrases (CAs, EC 4.2.1.1) are metallo-enzymes that catalyze the reversible hydration of CO$_2$ forming HCO$_3^-$ . Zinc is the required metal at the catalytic site for CA activity. Some exceptions are several coastal diatoms with cadmium-containing CA (CDCA). The Cd$^{2+}$ at the catalytic site is fully exchangeable for Zn$^{2+}$ [135]. The natural use of Cd$^{2+}$ in this "-CA class enzyme is considered an evolutionary adaptation to low Zn$^{2+}$ availability in marine habitats [136].

CA enzymes are ubiquitous in nature (animals, plants, archaebacteria, and eubacteria) and are an example of convergent evolution. Based on sequence comparison, CA proteins are grouped into
seven distinct classes: $\alpha$, $\beta$, $\gamma$, $\delta$, $\zeta$, $\eta$, and $\theta$-CAs [137–140]. In higher land plants, only $\alpha$, $\beta$, $\gamma$ CAs are found. The $\delta$ and $\zeta$ classes are restricted to marine diatoms and $\eta$-CA so far has only been reported in Plasmodium falciparum [141]. $\theta$-CA seems more widely distributed in algae and cyanobacteria [142] and it has been reported critical for photosynthesis in the diatom Phaeodactylum tricornutum [143]. The ubiquity of the distribution of CAs implies that they play diverse and essential roles in many biological processes. They have been related to respiration and transport of $\mathrm{CO}_2/\mathrm{HCO}_3^-$ between tissues, pH and $\mathrm{CO}_2$ homeostasis, electrolyte secretion in a variety of tissues/organs, various biosynthetic reactions, and $\mathrm{CO}_2$ fixation [142,144]. In addition, CA is a plausible source of hydrogen sulphide ($\mathrm{H}_2\mathrm{S}$) within plant leaves by catalyzing the conversion of carbonyl sulphide (COS) to $\mathrm{CO}_2$ and $\mathrm{H}_2\mathrm{S}$ [145].

Higher plants contain three evolutionarily distinct CA families including $\alpha$CAs, $\beta$CAs, and $\gamma$ CAs where each family is represented by multiple isoforms in all species [142,146,147]. Alternative splicing of CA transcripts is common. Consequently, the number of functional CA isoforms in a species may exceed the number of genes [147]. CAs are expressed in numerous plant tissues and in different cellular locations. The most prevalent CAs are those in the chloroplast, cytosol, and mitochondrion. CAs have been found in the thylakoid lumen of Chlamydomonas and Phaeodactylum. They are an important component of the CCM in these species and, therefore, essential for photosynthesis and growth [143,148]. This diversity in location is paralleled in the many physiological and biochemical roles that CAs play in plants [142,147,149,150]. As in humans and animals, many of these roles are related to the CA-driven regulation of cell pH, which, in turn, can participate in multiple regulatory processes through electrical signals, changes in cytosolic $\mathrm{Ca}^{2+}$ concentrations, and plant hormones [150–152] among others.

3.1.1. Plant $\alpha$-Carbonic Anhydrases ($\alpha$CA)

Arabidopsis thaliana contain eight $\alpha$CA (At$\alpha$CA1-8) [153]. Genes for $\alpha$Ca1, $\alpha$Ca2, and $\alpha$Ca3 are expressed in green and reproductive tissue (stems, rosette leaves, caulinar leaves, and flowers). Only $\alpha$Ca2 presents root expression. While expression of $\alpha$Ca1 is independent of the level of $\mathrm{CO}_2$, the expressions of $\alpha$Ca2 and $\alpha$Ca3 are induced under conditions of low $\mathrm{CO}_2$ concentrations [149]. $\alpha$Ca1 is expressed in chloroplasts and $\alpha$Ca2 is expressed in the plasma membrane. $\alpha$-CA4 is implicated in the processes leading to energy dissipation in the PSII antenna [154]. Arabidopsis $\alpha$Ca8 is clearly a pseudogene since it encodes in-frame stop codons [147]. Tissue-specific expression has also been reported for other species. In sorghum, the $\alpha$Ca Sb5G039000 is expressed specifically in anthers while, in the legume species Medicago truncatula $\alpha$CAs Mt1g059900 and Mt1g059940, are expressed in root nodules [147]. There is increasing evidence that $\alpha$CAs can play an important role in photosynthesis [150]. Under conditions of increasing light intensity, the expression of $\alpha$Ca2 decreases while the expression of $\alpha$Ca4 increases. Knock-out mutants of these chloroplast-located $\alpha$CAs exhibit contrasting responses in comparison of the wild type. Both the quantum yield at photosystem 2 (PS2) and the electron transfer to $\mathrm{O}_2$ decreased while non-photochemical quenching (NPQ) and $\mathrm{CO}_2$ assimilation were enhanced in plants lacking $\alpha$Ca2. The opposite was observed in $\alpha$Ca4 knock-outs [155]. The authors hypothesize that these $\alpha$CAs may participate in the regulation of $\mathrm{H}^+$ flux into the PS2 protein PsbS, which regulates qE-type NPQ.

3.1.2. Plant $\beta$-Carbonic Anhydrases

$\beta$CAs are most abundant in land plants where they participate in photosynthesis [147]. Arabidopsis thaliana has six $\beta$CAs [147]. $\beta$CAs genes are highly expressed in leaf tissue. Expressed sequence tag experiments revealed that $\beta$Ca1 to $\beta$Ca6 are expressed in rosette leaves, caulinar leaves, and flowers. $\beta$Ca3 is also strongly expressed in reproductive tissue while $\beta$Ca4, $\beta$Ca5, and $\beta$Ca6 are expressed in all tissues including roots. $\beta$CAs have been found in chloroplasts, mitochondria, the cytosol, and the plasma membrane [144,149]. Targeting analysis using green fluorescent protein fusion proteins confirmed the subcellular localization of plant $\beta$CAs: $\beta$Ca1 and $\beta$Ca5 are expressed in chloroplasts...
while $\beta$CA2 and $\beta$CA3 are cytosolic. Isoforms $\beta$CA4, $\beta$CA4.1, are localized in the plasma membrane while the short form, $\beta$CA4.2, is cytosolic. $\beta$CA5 and $\beta$CA6 are localized in the chloroplast and mitochondria, respectively [149].

The role of $\beta$CAs in photosynthesis of land plants seems especially relevant in grasses with C$_4$-type photosynthesis [156] or for plants under limited C$_{\text{inorg}}$ supply (see Section 3.2). Carbonic anhydrases could be versatile. They may be involved not only in photosynthesis and responses to CO$_2$ and light but also in seed germination, morphogenesis, nodule development, and responses to abiotic stress [157,158]. The tobacco salicylic acid-binding protein 3 (SABP3) is a chloroplast $\beta$CA that exhibits antioxidant activity and plays a role in the hypersensitive defense response [159]. Furthermore, $\beta$CA1 is related to ethylene signaling responses, photosynthetic performance of cotyledons, and Arabidopsis seedling survival [160].

3.1.3. Plant $\gamma$-Carbonic Anhydrases

Plant $\gamma$CAs are codified in the nucleus but localized in mitochondria [139]. So far, no higher plant $\gamma$CA with CA activity has been identified. Nonetheless, plant proteins with the active-site residues found in $\gamma$CAs from archaeabacteria and cyanobacteria have been found. In A. thaliana, five $\gamma$CA-related genes have been reported including three $\gamma$CA genes and two genes encoding $\gamma$CA-like proteins. In contrast to $\gamma$CA proteins, the $\gamma$CA-like proteins do not have the required Zn-coordinating amino acid residues. Plant $\gamma$CA genes encode for a part of the mitochondrial Complex I (NADH-ubiquinone oxidoreductase). Complex I knock-out lines present adverse effects: non-viable seeds, high levels of mitochondrial Complexes II and IV, and the alternative oxidase. However, this is in contrast with reduced levels of photosynthetic proteins [161]. A proteomic approach has recently found enhanced $\gamma$CA root levels during the induction phase of Al-tolerance in the hyper-resistant grass Urochloa decumbens. This increase occurred along with higher adenylate kinase activity and supports a role for $\gamma$CA in the maintenance of ATP-production during the Al tolerance response [162].

3.1.4. Plant PEP Carboxylases

Phosphoenolpyruvate carboxylases (PEPC) are located in the cytosol and catalyze the $\beta$-carboxylation of PEP to oxaloacetate using HCO$_3^-$ in an irreversible process. The OAA can then be reduced through NADH or NADPH-dependent malate dehydrogenase to malate in a reversible process. PEPCs are present in bacteria, algae, and plants. The typical plant PEPC (class 1 PEPC) has four identical subunits of 107 kDa. Multiple isoforms have been identified in leaves of C$_3$, C$_4$, and CAM plants [163–165]. In Sorghum bicolor, which is a plant with C$_4$-type photosynthesis, five PEPC genes (PEPC1-5) have been identified. The plant PEPC is highly regulated. Phosphorylation through PEPC carboxykinase (PEPCK) at the N-terminal phosphorylation domain [166] and allosteric regulation by glycine and glucose-6-P enhances the activity. Inhibition is achieved by both allosteric regulation especially by malate and by ubiquitination [167]. In addition to the typical plant-type PEPC, plants also contain a bacterial-type PEPC (BPEPC) of 118 kDa [153]. The BPEPC is highly expressed in floral tissues as well as in seeds and fruits. It has recently been shown that high BPEPC occurs in tissues that accumulate high malate concentrations [168]. There is a tight interaction between PTPC and BTPC, which forms the class 2 PEP. This is an enzyme complex that, in contrast to class 1 PEPC, is mostly insensitive to allosteric inhibition by high malate concentrations [169,170]. While class 1 PEPCs are constitutively expressed in the cytosol, the BPEPC is associated with the outer mitochondrial surface. This location is in line with a central role of this enzyme in collaboration with CA in the efficient fixation of respiratory CO$_2$ and the anaplerotic supply of organic acids to the Krebs cycle [171]. This is especially relevant in developing seeds that store fatty acids such as castor bean seeds [172]. PEPC activity also plays a central role in symbiotic N$_2$ fixation in root nodules (see Figure 2) where it provides OAA for nitrogen assimilation and malate for the bacteroids [173].
3.2. Carbon Concentration Mechanisms (CCM) in Terrestrial Plants

Under certain environmental conditions, CO$_2$ may become a limiting factor for photosynthesis not only in cyanobacteria, algae, and aquatic macrophytes where CCMs have been intensively studied but also in terrestrial higher plants. High-temperature favoring photorespiration and drought imposes an increase of stomatal resistance. These are the main factors limiting CO$_2$ availability for RuBisCo in land plants [174].

Long distance transport of HCO$_3^-$ from roots to leaves usually makes only a small contribution to C$_{inorg}$ for photosynthesis (see Section 2.6). Exceptions are aquatic plants in the Lycophyta genus Isoetes and the non-stomatous land plant Stylites. They acquire all C$_{inorg}$ for photosynthesis from the soil through the roots and recycle carbon by CAM [175]. Other terrestrial plants take up most of the C$_{inorg}$ in the form of CO$_2$ through stomata of the leaves. This CO$_2$ diffuses into the chloroplast where it is assimilated by RuBisCO, which forms phosphoglycerate (PGA) as the first stable product of C$_{inorg}$ assimilation. After activation with ATP and reduction by NADPH provided by the light-driven chloroplastic electron transport, PGA forms phosphoglyceraldehyde, which is the first sugar molecule of the photosynthetic carbon metabolism. Most terrestrial plants fix CO$_2$ directly onto ribulose-bis-phosphate. In contrast to plants with this so-called C$_3$-type photosynthesis, plants with C$_4$-type photosynthesis convert CO$_2$ entering through the stomata into the mesophyll cells to HCO$_3^-$ using a cytosolic βCA. This C$_{inorg}$ in the form of HCO$_3^-$ is initially fixed by PEP carboxylase in the cytosol of the outer mesophyll cells of the leaves. In this case, OAA is a first stable organic compound. Oxalacetate is either reduced to malate or transformed by transamination to aspartate. Malate or aspartate are the molecules that transfer the newly fixed carbon to the inner bundle sheet cells of the leaves where decarboxylation provides CO$_2$, which is the substrate for Rubisco [176]. While CA activity is high in C$_3$ chloroplasts where it facilitates the availability of CO$_2$ for RuBisCo, the absence of CA activity from bundle sheet cells seems essential for the C$_4$ mechanism [177].

This CCM around RuBisCo in C$_4$ plants is considered an evolutionary adaptation to reduce the oxygenase activity of RuBisCo, which inhibits photorespiration and is especially enhanced under high temperature in tropical or subtropical areas [178]. However, C$_4$-type photosynthesis can also be induced in certain amphibious plant species such as Eleocharius vivipara [179] under conditions of leaf emergence under dry conditions. Extreme adaptation to drought is observed in many CAM plants, which capture CO$_2$ during the night when a lower temperature and a higher relative humidity in the atmosphere reduces transpiratory water loss. During the dark period, this C$_{inorg}$ is fixed in the form of HCO$_3^-$ by PEP carboxylase and stored in large vacuoles mostly in the form of malate. The CO$_2$ for fixation with RuBisCo is obtained by decarboxylation of malate during the following day-light period [180].

Limitations of CCMs in higher plants, especially of the C$_3$-type of photosynthesis, and advances in our understanding of CCMs in cyanobacteria and microalgae like C. rheinhardii have promoted genetic engineering approaches to introduce efficient CCM into crop plants for increasing yield. Different approaches include manipulation of photorespiration, C$_3$ to C$_4$ engineering, and introduction of CCMs of cyanobacteria of C. rheinharddii into C$_3$ crops. This well-known topic has recently been reviewed in detail by Mackinder [181] who identified gaps in our knowledge on bicarbonate transporter structure, functioning, and localization as important constraints that need priority attention for successful development of CCM engineered plants.

3.3. CO$_2$/Bicarbonate Signalling in Stomatal Guard Cells

Regardless the type of photosynthesis, C$_3$, C$_4$, or CAM, the CO$_2$ flux from the atmosphere into the plants is regulated by the stomatal opening and closure due to turgor changes in the stomatal guard cells. These changes are strictly controlled by multiple external and internal factors. Among those, the binomial CO$_2$/HCO$_3^-$ plays a central role (see Figure 3). High CO$_2$ promotes stomatal closure, which is brought about by the activation of efflux anion channels: SLAC1 (S-type) facilitating Cl$^-$ or NO$_3^-$ efflux and R-type (AtALMT12/QUAC1 in A. thaliana) for malate efflux (see Section 2.6).
The signal for stomatal closing in response to high CO$_2$ seems to be a combination of alkaline pH, high Ca$^{2+}$, and high HCO$_3^-$ in the cytosol [182]. The carbonic anhydrase double mutant ca1ca4 does not show any effect on stomatal conductance when CO$_2$ concentration is changed from 100 ppm to 80 ppm [183]. This points to HCO$_3^-$ being the key signal. Abscisic acid (ABA) dependent and ABA-independent mechanisms seem to operate in stomatal closure under a high amount of CO$_2$ (see Figure 3). OST1 (Open Stomata 1) is a positive regulator of the anion efflux channels. In the ABA-independent signaling pathway, a high amount of HCO$_3^-$ activates a MATE-like transporter protein (RHC1, Resistance to High CO$_2$), which acts as a positive regulator of OST 1 by inhibiting HT1 (High Leaf Temperature) known as a protein kinase that inactivates OST1 [183,184].

![Figure 3. Mechanisms of stomatal closure induced by high CO$_2$ or HCO$_3^-$ concentrations according to [183,184]. CA, carbonic anhydrase; ABA, abscisic acid; ABA receptor, PYR/RCAR, Pyrabactin Resistance (PYR) Regulator Component of ABA Receptor (RCAR); ABI1/PP2C2, Abscisic acid Insensitive Protein Phosphates C2; RHC1 Resistant to High CO$_2$, MATE-type transporter specific activated by HCO$_3^-$; HT1, High Leaf Temperature kinase; OST1, Open Stomata1 protein kinase; SLAC1, Slow Anion Channel1; QUAC1, Quick Anion Channel1.]

### 4. Plant Response to Bicarbonate-Rich Soils

It is common knowledge that limestone soils containing high carbonate/bicarbonate concentrations restrict the performance of calcifuge plant species and limit yield especially in iron-inefficient crops such as certain varieties of citrus, peach, pear, or soybeans suffering from lime-induced chlorosis [185,186]. Low pH leads to low availability of essential nutrients (especially Fe, Zn, and P) and high Ca soil concentrations are considered the main constraining factors. However, HCO$_3^-$ at concentrations occurring in the solution of limestone soils can inhibit root growth in sensitive plant species like the calcifuge grass *Deschampsia caespitosa* [187]. However, dicots like peas, beans, or sunflowers suffer more intense root growth inhibition due to CO$_2$ and/or HCO$_3^-$ than the monocots barley and oats [123]. Recently soil carbonate has been identified as a main selection factor that drives local adaptation in natural populations of *A. thaliana*, which is a calcifuge species able to colonize soils with moderate carbonate contents [188].

Currently, no HCO$_3^-$ transporter has been characterized in higher plants (see Section 2.6). Nonetheless, HCO$_3^-$-induced root growth inhibition is paralleled by enhanced root production of organic acids especially malate, succinate, and citrate [189]. This suggests that excess HCO$_3^-$ enters the root and is metabolized by CA and PEP, which yields enhanced organic acid levels. However, CO$_2$ diffusing from the soil’s atmosphere into the root can also be transformed inside the root into HCO$_3^-$ by CA. Bicarbonate can be released again into the soil rhizosphere, which contributes to the plant’s...
cation-anion balance. Especially under conditions of high nitrate uptake, enhanced HCO$_3^-$ efflux from the roots has been claimed to contribute to the characteristic alkalization of the rhizosphere when nitrate is the main N source for the plants [190,191]. In fact, in maize and a tomato, the sum of K$^+$ and NO$_3^-$ uptake and the HCO$_3^-$ efflux have been reported to be in electrical equilibrium [192]. However, no selective bicarbonate efflux transporters in plant roots have been reported and alkalization can also be a consequence of either or both OH$^-$ release or H$^+$ uptake in cotransport with nitrate [193]. Actually, root supply of low HCO$_3^-$ concentrations tended to increase rather than decrease root nitrate uptake in *Populus canescens*. Exposure to 1 mM external NaHCO$_3$ enhanced both nitrate reduction and assimilation as well as exported nitrogen to the shoots of poplar plants [194]. Higher HCO$_3^-$ concentrations cause net K$^+$ and NO$_3^-$ efflux as well as accumulation of organic acids, mainly malate, in the roots [195]. To what extent dark fixation of C$_{inorg}$ entering the roots plays a role in the carbon budget of terrestrial plants has been considered mainly in relation to lime-induced chlorosis in calcifuge plants. This type of chlorosis affects sensitive plant species when growing on carbonate-rich soil and may reflect an interference of HCO$_3^-$ in the mechanisms of Fe acquisition and transport [196]. Key processes potentially impaired by HCO$_3^-$ include the dicots’ strategy 1 such as the acidification of the rhizosphere due to the strong buffer ability of HCO$_3^-$ and the reduction of Fe(III) to Fe(II) by ferric reductase, which operates optimally at acid pH [197]. The induction of root exudation of phenolic substances is not affected and is even stimulated by HCO$_3^-$, Induction of root accumulation and exudation of coumarin-type phenolics with high affinity for Fe has been reported as a response to Fe-deficiency under high pH conditions in *A. thaliana* [198]. An *A. thaliana* population which is naturally adapted to moderate soil carbonate had higher rates of coumarin root exudation than a sensitive population [188]. Furthermore, prevention of the imbalance of organic acid concentrations caused by dark fixation of HCO$_3^-$ and shifting of C$_{org}$ into the shikimate pathway for the production of phenolic compounds has been reported as a mechanism of the extreme HCO$_3^-$ tolerance in *Parietaria difusa* [199]. In the view of the multiple implications of HCO$_3^-$ in plants’ metabolism, breeding programs for better crop yield on carbonate-rich soil would greatly benefit from the characterization at the genetic and molecular level of the of bicarbonate uptake and efflux mechanisms in higher land plants.

5. Conclusions

During the last decade, there has been significant progress of our knowledge on the mechanisms of HCO$_3^-$ transport and CCM in cyanobacteria, algae, and seagrass species due to improved genetic and molecular tools and electrophysiological approaches. In contrast, in higher land plants, no HCO$_3^-$ transporter has been characterized so far. Advanced knowledge of the metabolic use of HCO$_3^-$ in terrestrial plants has mainly been made in relation to C$_4$ and CAM metabolism including the genetic and molecular characterization of CAs and PEPC involved. However, there are still important gaps in our knowledge about the mechanisms of compartmentation and regulation especially regarding the complex interactions between light and dark fixation of C$_{inorg}$, the recycling of respiratory and photosynthetic CO$_2$, and the importance of anaplerotic supply of organic acids to the Krebs cycle. Filling these gaps is essential for progress in both genetic engineering approaches for transferring CCMs from cyanobacteria or microalgae to higher plants and breeding for bicarbonate tolerance in crops sensitive to lime-induced chlorosis.

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Abbreviations

ABA Abscisic acid
CCM Carbon concentration mechanism
CA Carbonic anhydrase
PEPC Phosphoenolpyruvate carboxylase
SLAC Slow anion channel
SLC Solute carriers
QAC Quick anion channel

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