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Review

Physiological Factors Limiting Leaf Net Photosynthetic Rate in C_3 Crops like Rice and Approaches for Improving It

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Abstract: Improving leaf photosynthetic capacity is one of the most promising approaches to further boost crop yield. Clarifying factors limiting leaf photosynthetic capacity, especially in C_3 crops, is meaningful for designing strategies to improve it. Leaf net photosynthetic rate (A) is one of the parameters describing leaf photosynthetic capacity. In the present study, physiological factors limiting A in C_3 crops such as rice were discussed and different approaches for A improvement were summarized to provide theoretical guidance for increasing leaf photosynthetic capacity. A will be limited by both CO_2 availability and light intensity over periods from a few hours to several days, and by one of them over shorter intervals. Under current ambient atmospheric conditions, A of C_3 crops is mainly limited by Rubisco activity and the CO_2 concentration in chloroplasts. Leaf nitrogen content affects A by regulating Rubisco content and leaf anatomy; leaf morphological and anatomical traits limit A by impacting stomatal and mesophyll CO_2 diffusion. Further improvements of A in C_3 crops can be achieved by designing or introducing high-activity Rubisco; adjusting leaf nitrogen allocation to optimize leaf anatomy and leaf chemical composition; modifying leaf morphology and anatomy for greater CO_2 diffusion; improving the activity of proteins and enzymes associated with sugar transportation and utilization; introducing C_4 photosynthetic mechanisms and combining high photosynthetic traits by conventional breeding.

Keywords: C_3 photosynthesis; Rubisco; leaf nitrogen content; stomatal conductance; leaf hydraulics; leaf vein traits; mesophyll conductance; leaf anatomy

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Citation: Ye, M.; Wu, M.; Zhang, Y.; Wang, Z.; Zhang, H.; Zhang, Z. Physiological Factors Limiting Leaf Net Photosynthetic Rate in C_3 Crops like Rice and Approaches for Improving It. *Agronomy* **2022**, *12*, 1830. https://doi.org/10.3390/agronomy12081830

Academic Editor: Eiji Goto

Received: 15 June 2022
Accepted: 30 July 2022
Published: 2 August 2022

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1. Introduction

The leaf net photosynthetic rate (A, μmol (CO_2) m^{-2} s^{-1}) is defined as the amount of CO_2 assimilated by 1 m^2 leaf per 1 s and is calculated as follows:

$$A = V_c - 0.5V_o - R_d \quad (1)$$

where V_c is the velocity of ribulose-1,5-bisphosphate (RuBP) carboxylation; V_o is the rate of oxygenation, V_o is multiplied by 0.5 because half a mole of CO_2 is evolved for every mole of oxygenation event; R_d is the day respiration rate, which is referred to the mitochondrial respiration rate under light.

A will be limited by both CO_2 availability and light intensity over periods from a few hours to several days, and by one of them over shorter intervals [1]. The Farquhar-von Caemmerer-Berry (FvCB) model for C_3 photosynthesis assumes that carboxylation of RuBP by ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) is limited by one of three factors: (1) the activity of Rubisco; (2) the RuBP regeneration rate; and (3) the release of phosphate during the metabolism of triose phosphate to either starch or sucrose (rate of triose-phosphate utilization, TPU) [2–5]. Under low CO_2 conditions, sufficient nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) are produced

by the light reaction, and A linearly increases with the intercellular CO_2 concentration (C_i) (Figure 1). At this time point, A is mainly limited by the activity of Rubisco. As C_i increases, ATP is insufficient to regenerate adequate RuBP, thereby increasing A with C_i decreases (Figure 1). Thus, A is mainly limited by the RuBP regeneration rate. As C_i further increases, triose-phosphate in the chloroplasts is redundant and will inhibit A as a feedback mechanism [6] (Figure 1). Then, A is mainly limited by TPU.

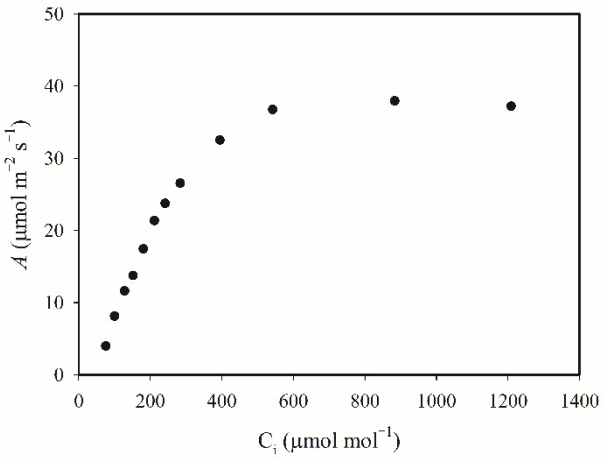

Figure 1. The A/C_i curve of rice leaf. A, leaf net photosynthetic rate; C_i, intercellular CO_2 concentration.

Under current ambient atmospheric conditions (400 μmol (CO_2) mol^{-1} (air)), the A of C_3 crops is mainly limited by Rubisco carboxylation capacity, which is related to both the content and the specific activity of Rubisco [7,8]. Rubisco is a type of bifunctional enzyme that can catalyze both carboxylation and oxygenation reactions and CO_2 competes with O_2 as a substrate for Rubisco. The carboxylation and oxygenation rate depends on the affinity of Rubisco for CO_2 and O_2 as well as the ratio of CO_2 and O_2 concentrations in chloroplasts. The low solubility of CO_2 in water at ambient temperatures means dissolved CO_2 in equilibrium with atmospheric CO_2 in the range of 10–20 μM, well below the Michaelis constant for carboxylation reaction (K_c) of C_3 Rubisco, although the Michaelis constant for oxygenation reaction (K_o) (typically higher than 300 μM) is far higher than K_c [9–11]. Therefore, the affinity of Rubisco for CO_2 and the CO_2 concentration in chloroplasts (C_c) represent the main limiting factors for A in C_3 crops under natural conditions [12–16].

2. Physiological Factors Limiting Leaf Net Photosynthetic Rate in C_3 Crops

2.1. Effects of Rubisco on Leaf Net Photosynthetic Rate

Rubisco is the most abundant enzyme in plants, accounting for approximately 40% of soluble proteins in leaves; 22–33% of leaf nitrogen (N) is allocated to Rubisco in the typical C_3 crop—rice [17,18]. Although Rubisco is redundant under high CO_2, low temperature, or low light conditions [18–22], A significantly increases with Rubisco content under normal conditions [23]. Increasing the leaf N content can remarkably increase the Rubisco content, improve the total activity of Rubisco, and finally enhance A [16,24]. However, Suzuki et al. [25] found that greater allocation of N to Rubisco in rice decreased the allocation of N to other proteins and enzymes associated with the tricarboxylic acid cycle and consequently decreased A.

As CO_2 and O_2 compete to use one site on Rubisco to process carboxylation and oxygenation, the ratio of V_c and V_o is expressed as follows [9]:

$$\frac{V_c}{V_o} = \frac{k_c}{k_o} \times \frac{K_o}{K_c} \times \frac{[CO_2]}{[O_2]} \quad (2)$$

where k_c and k_o are the turnover rate constants for carboxylation and oxygenation reactions, respectively, and the unit is mol mol^{-1} (site) s^{-1}. $[CO_2]$ and $[O_2]$ are the CO_2 and O_2

concentrations in chloroplasts, respectively, and the unit is μmol mol^{-1}. The specificity of Rubisco ($S_{c/o}$) is expressed as follows [9]:

$$S_{c/o} = \frac{k_c}{k_o} \times \frac{K_o}{K_c} = \frac{k_c K_o}{k_o K_c} \quad (3)$$

$S_{c/o}$ is greatly affected by temperature, increasing with temperatures between 15–30 °C and decreasing with temperatures above 30 °C [9,26].

$S_{c/o}$ differs greatly between species, for example, in *Rhodospirillum rubrum* it is only 12.3, in rice it is 85, but in *Griffithsia monilis* it reaches 167 under 25 °C [10]; hence, studies have attempted to introduce high-activity Rubisco to C_3 crops to improve their photosynthetic capacity. Lin et al. [27] introduced Rubisco from cyanobacteria into tobacco plants, however, the transgenic plants did not survive under normal atmospheric CO_2 concentration. Introducing high-efficiency Rubisco, for example from *Griffithsia monilis* to C_3 crops to improve the carboxylation efficiency needs more investigation.

2.2. Effects of Leaf Nitrogen Content on Leaf Net Photosynthetic Rate

Mass-based (N_{mass}) or area-based (N_{area}) leaf N content is expressed to represent leaf N content (LNC). In addition to affecting Rubisco content, LNC can affect leaf photosynthesis by impacting other factors. Taylaran et al. [28] reported that increasing N_{area} can significantly improve stomatal conductance (g_s) and finally enhance A in rice. Moreover, LNC can alter leaf anatomy. Polesskaya et al. [29] found that leaf and leaf cell walls became thick under N deficit conditions, and the cell wall under ammonium N was thicker than that under nitrate N. Li et al. [30] reported that the chloroplast size under high N supply (HN) was larger than that under low N supply (LN), and Rubisco content also increased with chloroplast size in C_3 plant rice. Xiong et al. [31] demonstrated that the cell wall under HN was thinner than that under LN, whereas the surface area of cells exposed to intercellular airspace (IAS) per leaf area (S_m) and the surface area of chloroplasts exposed to IAS per leaf area (S_c) under HN were both higher than those under LN in rice. The thin cell wall and high S_m and S_c promoted mesophyll CO_2 diffusion and improved A.

To enhance the understanding of the intrinsic relationship between A and LNC, the concept of photosynthetic nitrogen utilization efficiency (PNUE) was established. PNUE is the ratio of A to N_{area}. Improving PNUE can be achieved by improving A, decreasing N_{area}, or promoting a larger increase in A than in N_{area}. To avoid N loss and environmental pollution caused by excessive N application, scientists have proposed "N reducing" strategies, including site-specific N management (SSNM) for crops [32]. Reducing N application means that N_{area} will likely decrease, and PNUE will subsequently increase. Therefore, it is particularly important to breed cultivars exhibiting high A under low N_{area}. It was reported that in the field, N needs to be applied to improve crop yield when the N_{area} of the newest fully expanded leaf is less than 1.40 g m^{-2} [32]. Coincidentally, Ye et al. [33] found that N_{area} did not contribute to the improvement in A when N_{area} was greater than 1.40 g m^{-2}, and PNUE was positively correlated with A across 121 cultivated rice lines. In Ye et al. [33], the 121 rice lines were divided into four sections according to their leaf net photosynthetic rate, and it can be found that only when A was lower than 20 μmol m^{-2} s^{-1} the average N_{area} was lower than those of other sections (Table 1). These findings indicated that when A is higher than 20 μmol m^{-2} s^{-1}, the further improvement in A was not attributed to the increase in N_{area}, it is possible to select varieties showing both high PNUE and high A. How to regulate other limiting factors for A, such as CO_2 diffusional conductance, by adjusting N application to further improve A requires more investigations.

Table 1. The average leaf net photosynthetic rate (A) and area-based leaf nitrogen content (N_{area}) of 121 cultivated rice lines in different sections.

Section	A (μmol m^{-2} s^{-1})	N_{area} (g m^{-2})	Number of Rice Lines
$A \geq 30$	31.5 ± 0.9 a	1.67 ± 0.17 a	5
$25 \leq A < 30$	27.0 ± 1.5 b	1.57 ± 0.16 a	34
$20 \leq A < 25$	22.4 ± 1.4 c	1.53 ± 0.18 a	60
$A < 20$	18.4 ± 1.2 d	1.35 ± 0.22 b	22

The data followed by different letters are significant at $p < 0.05$ level.

2.3. Effects of Stomatal Conductance on Leaf Net Photosynthetic Rate

Diffusing from air to the carboxylation sites, CO_2 first needs to overcome the boundary resistance between air and leaf to reach the leaf surface, and then enters the stomatal pores to reach the substomatal cavity (Figure 2a). CO_2 diffusional resistance from the leaf surface to the substomatal cavity is referred to as stomatal resistance (r_s) and the reciprocal of r_s is called stomatal conductance (g_s).

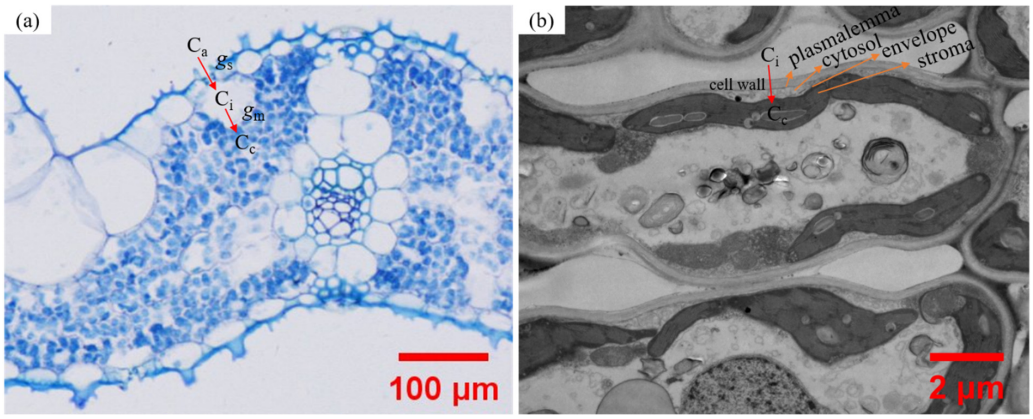

Figure 2. The diffusing pathway of CO_2 from the atmosphere to the chloroplast. (**a**) The light microscope image of rice leaf and (**b**) the transmission electron microscope image of rice leaf. C_a, atmospheric CO_2 concentration; C_i, intercellular CO_2 concentration; C_c, chloroplastic CO_2 concentration; g_s, stomatal conductance to CO_2; g_m, mesophyll conductance to CO_2.

CO_2 entering a leaf and H_2O evaporating from a leaf both need to pass through the stomatal pores. g_s is usually obtained by measuring the stomatal conductance to H_2O (g_{sw}), $g_s = g_{sw}/1.6$, as the diffusing rate of H_2O in the air is 1.6 times that of CO_2. g_s is mainly determined by stomatal size, stomatal density, stomatal distribution, and especially by stomatal aperture [34,35]. Large stomatal pores and stomatal apertures as well as high stomatal density were suggested to be of benefit for improving g_s and A in species [36–39]. As an important determinant for g_s, the stomatal aperture is greatly influenced by plant hydraulic status [40,41]. A, g_s, and transpiration rate (E) were found to be positively correlated with the plant hydraulic conductance [42].

As leaf hydraulic resistance accounts for greater than one-quarter of whole-plant hydraulic resistance [43,44], leaf hydraulic conductance (K_{leaf}), the reciprocal of leaf hydraulic resistance, determines leaf gas exchange parameters greatly [40,45,46]. In higher plants, water from the stem moves into the petiole through the xylem in different vein orders, then passes through the bundle sheath and the mesophyll tissue before evaporating into the IAS and diffusing out of the leaf from the stomatal pores [47,48]. Therefore, leaf hydraulic transport can be divided into two parts—xylem hydraulic transport and outside-xylem hydraulic transport.

As leaf hydraulic transport consists of the xylem and outside xylem hydraulic transports, K_{leaf} is mainly determined by leaf vein and mesophyll anatomical traits [48,49]. High leaf vein density, short distances between veins and stomata, large xylem conduit diameters,

high S_m, S_c, and the fraction of intercellular airspace (f_{ias}) as well as thin cell walls were reported to be beneficial for improving K_{leaf} [46,48,50].

In addition to leaf hydraulic resistance, root hydraulic resistance also significantly accounts for approximately one-third to half of the whole-plant hydraulic resistance [51,52]. Thus, K_{root}, the reciprocal of root hydraulic resistance, was also found to impact A. Else et al. [53] found that a reduction in K_{root} can decrease the leaf water potential and cause stomatal closure in *Ricinus communis* plants. Some species growing in flooded conditions, for instance, rice, form numerous aerenchyma in leaves, stems, and roots to transport O_2. To reduce the outward diffusion of O_2 in the root aerenchyma, a layer of wood components was formed on the root surface in rice plants, which greatly decreased the K_{root} [54]. In addition, the formation of aerenchyma will reduce the effective area for radial water transport, which will also decrease K_{root} [55]. Moreover, aquaporins (AQPs) are also involved in regulating K_{root} [56]. Thus, traits that affect K_{root} mainly include the root surface area, the degree of lignification, and the expression of AQPs [57–60].

2.4. Effects of Mesophyll Conductance on Leaf Net Photosynthetic Rate

After reaching the substomatal cavity, CO_2 needs to diffuse to the surroundings of the cell wall, then successively passes through the cell wall, plasmalemma, cytosol, chloroplast envelope, and stroma to get to the carboxylation sites [12] (Figure 2b). The CO_2 diffusional resistance from the substomatal cavity to the carboxylation sites is referred to as mesophyll resistance (r_m). r_m limits photosynthesis to the same magnitude as r_s under many conditions [61,62]. The reciprocal of r_m is called mesophyll conductance (g_m). A was found to be positively correlated with both g_m and g_s in numerous previous studies [63,64].

g_m is greatly determined by leaf anatomical traits [12,62]. Many anatomical properties were reported to determine g_m, including the fraction of intercellular airspace (f_{ias}), the surface area of cells exposed to intercellular airspace (IAS) per leaf area (S_m), the surface area of chloroplasts exposed to IAS per leaf area (S_c), and cell wall thickness (T_{cw}). High S_m, S_c, and f_{ias} were suggested to be of benefit for improving g_m [48,63,65–69]. Among these anatomical traits, T_{cw} is particularly important for determining g_m given that cell wall resistance accounts for about half of the total mesophyll resistance [62] and g_m was found to be negatively correlated with T_{cw} in many species [65–67,70–72].

Leaf mass per area (LMA), a composite parameter describing leaf anatomy, is defined as the leaf dry weight per leaf area. Some studies reported that LMA was negatively correlated with g_m [69,70,73], and it was suggested to be related to T_{cw} and IAS [67,74]. High LMA is usually accompanied by a thicker cell wall [67,71,74]. Ye et al. [71] found that high LMA rice genotypes showed low g_m mainly due to the relatively thick cell wall but not high S_m and S_c. However, some studies also found that g_m did not correlate with LMA [68,75–78], which may be due to different physiological and biochemical features.

Apart from leaf anatomy, leaf biochemical components (e.g., carbonic anhydrase (CA) and AQPs) were also found to be involved in regulating g_m [79,80]. CO_2 needs to dissolve in the apoplastic water of the cell wall to pass through the cell wall as HCO_3^-, and the conversion from CO_2 to HCO_3^- is catalyzed by CA located external to the cell membrane [62,81,82]. AQPs are a family of membrane-bound water channel proteins that play a major role in regulating the water transport distribution in leaves [83,84]. Recently, an increasing number of studies have found that AQPs also participate in regulating CO_2 diffusion across membranes [12,80,85]. Some AQP genes, such as *NtAQP1* and *AtPIP1;2*, were found to play roles in CO_2 diffusion across membranes [86–88].

In addition to leaf anatomy, CA, and AQPs, other factors, such as leaf chemical composition, have also been reported to affect g_m. Ye et al. [71] demonstrated that increased investment of leaf mass to the cell wall leads to low g_m in rice plants. A more recent study also reported that changes in cell wall composition, particularly in pectin content, determined leaf elasticity in both g_s and g_m [89]. The influence of cell wall composition on g_m and photosynthesis has started to attract attention in recent years. For example, Flexas et al. [90] summarized the effects of cell wall structure and composition on leaf pho-

tosynthesis and proposed the idea of improving leaf photosynthesis by manipulating the cell wall, e.g., improving the mesophyll conductance and photosynthetic rate by decreasing cell wall thickness and reducing cellulose. Improving g_m and A in C_3 crops by altering leaf anatomy and leaf chemical composition requires further investigation.

Furthermore, based on the determinants for K_{leaf} and g_m, we can know that K_{leaf} and g_m are both affected by leaf anatomical traits f_{ias}, S_m, S_c, and T_{cw}. Thus, g_m was found to be positively correlated with K_{leaf} [48]. However, although Xiong et al. [48] explained that the coordination between g_m and K_{leaf} is partly attributed to the shared diffusion pathways of CO_2 and H_2O inside leaves, the complete mechanisms remain unclear, i.e., the location of evaporating sites. Canny et al. [91] reported that cells in the spongy mesophyll and palisade cells surrounding substomatal cavities shrank more in size compared to the majority of cells in the palisade tissue during initial dehydration, indicating that H_2O mainly evaporates from the spongy mesophyll and palisade cells surrounding substomatal cavities. However, Rockwell et al. [47] reported that the H_2O dominantly evaporates from the vascular bundle sheath and its surroundings to the IAS in *Quercus rubra*. The H_2O diffusion pathway inside leaves and its coordination with g_m require further investigation.

2.5. Effects of the Rate of Sugar Utilization on Leaf Net Photosynthetic Rate

Although the A of C_3 crops is mainly limited by the activity of Rubisco and C_c under natural conditions, it is still affected by the rate of triose-phosphate utilization. The quantity of triose-phosphate transported from the chloroplasts to the cytoplasm is regulated by the Pi level in the cytoplasm. The triose-phosphate was transported out of the chloroplast by the Pi transporter on the envelope, at the same time, equal amounts of Pi are transported from the cytoplasm to the chloroplast. When the triose-phosphate forms the sucrose in the cytoplasm, Pi is released. If the transport of sucrose from the cytoplasm is restricted, or the utilization decreases, the transport of triose-phosphate from the chloroplast will be restricted, and finally, inhibit photosynthesis. During the triose-phosphate utilization process, sucrose phosphate synthase (SPS) plays an important role [92], while sucrose transporter and SWEET protein on plasmalemma and cell wall invertase play pivotal roles during the apoplast transport of sucrose [93–95].

2.6. Response of Leaf Photosynthesis to Changing Environments

Above we generally talked about the factors limiting the instantaneous CO_2 assimilation rate. However, steady-state conditions are rare in nature, and growth environments, especially irradiance, are intrinsically heterogeneous in time and space within canopies. Leaves within a canopy experience a highly variable light environment in magnitude (PAR 1–2000 μmol m^{-2} s^{-1}) and time (seconds to minutes or longer) over the course of a day due to changes in the incoming solar irradiance, cloud cover, wind, and self-shading of the upper leaves [96,97]. When shifting from low irradiance to high irradiance, the recovery of Rubisco activity and stomatal and mesophyll conductance are crucial for the recovery of leaf photosynthesis [97]. Generally, the full activation of Rubisco needs 10–30 min, which strongly restricts the recovery of photosynthesis. Recently, Zhang et al. [98] found that the increasing rate of light-induced stomatal conductance is related to stomatal size in the genus *Oryza*, as small stomata are not beneficial for the rapid activation of Rubisco. How the adaption of leaf physiological properties to changing irradiance affects leaf photosynthetic capacity needs more investigation.

Except for irradiance, other environmental factors such as temperature also change in nature, although it changes much slower and to a smaller degree. Temperature responses of photosynthesis have also attracted attention. Studies showed that the temperature response of photosynthesis is strongly determined by the temperature response of mesophyll conductance [72,99]. Regarding the underlying mechanisms of the temperature response of mesophyll conductance, classic hypotheses have been proposed. Bernacchi et al. [100] illustrated that CA and aquaporins are candidates for determining the temperature response of g_m, while Xiong et al. [31] suggested that the rapid temperature response of g_m is

related to the regulation of diffusion through biological membranes but not the response of leaf structure. Recently, Li et al. [101] reported that leaf water potential (Ψ_{leaf}) plays important role in determining the temperature response of g_m and they revealed that the decline of Ψ_{leaf} with leaf temperature is one of the reasons for the decreasing of g_m with temperature. Except for these hypotheses, other models including the two components model [102] and the optimal g_{mem} (membrane conductance to CO_2) model [103] have also been established to investigate the temperature response of g_m. Recently, Ye et al. [72] reported that the stronger response of g_m to temperature was related to the larger activation energy of membrane in rice. Factors limiting the responding rate of photosynthesis to temperature need more investigation.

3. Approaches for Improving Leaf Net Photosynthetic Rate in C_3 Crops

According to equation 1, we know that improving the carboxylation rate and decreasing the respiration rates can both increase A. However, recent studies demonstrated that depressing the photorespiration or the day respiration both affected the nitrogen metabolism in plants and reduced the quality of crop products [104–106]. In addition, under stressed conditions such as high light intensity, drought, or salt stress, the photorespiration can consume the redundant NADPH, reducing the peroxide production, thus protecting the photosynthetic apparatus [107–109]. Moreover, the glycine and serine produced during the photorespiration process also participate in plant metabolism [110] and photorespiration is necessary for plant growth and development. Modifying Rubisco to depress respiration by gene engineering is not of benefit for plant growing [111].

Whether improving the carboxylation rate or decreasing the respiration consumption, the final aim is to improve the photosynthetic capacity. Many gene engineering approaches were suggested to improve the photosynthetic capacity, for example, designing efficient Rubisco or improving the heat tolerance of the Rubisco kinase to keep the Rubisco efficient even under high temperatures [27,112–116]; improving the activities of the enzymes associated with the Calvin cycle, e.g., fructose-1,6-bisphosphatase (FBPase) and sedoheptulose 1,7-bisphosphatase (SBPase), to improve the carboxylation rate [117–122]; improving the electron transport rate on the thylakoid membranes [123–125]; introducing CO_2 concentrating mechanisms or the Kranz anatomy from C_4 plants to C_3 crops [126–129]; modifying leaf morphology and anatomy to improve the light capturing and the CO_2 diffusion [130–133]. Alternatively, based on the natural variations of photosynthetic characteristics in the plant germplasm, combining high photosynthetic traits through traditional breeding can also improve photosynthetic capacity [16].

Under natural conditions, the A of C_3 crops is usually lower than that of C_4 plants, as A of C_3 crops is usually lower than 40 μmol m^{-2} s^{-1}, while it is commonly higher than 40 μmol m^{-2} s^{-1} in C_4 plants [16,134,135]. There is no doubt that the A of C_3 crops will be greatly improved if the C_3 crops were modified into C_4 plants. The phosphoenolpyruvate carboxylase (PEPC) in C_4 plants, which catalyzes the reaction between phosphoenolpyruvate (PEP) and HCO_3^-, has a higher affinity for HCO_3^- than Rubisco for CO_2. Sheehy et al. [136] illustrated that the success of building C_4 rice can improve the rice yield by 50%. The International Rice Research Institute (IRRI) also started 'The C_4 Rice Project' from 2008 (https://c4rice.com/the-project-2/our-history/), aimed at building C_4 rice. Taniguchi et al. [137] introduced the PEPC, pyruvate, orthophosphate dikinase (PPDK), and malate dehydrogenase (MDH) from maize to rice plants, however, the gene-modified rice plants did not show an increase in A, the A of gene-modified rice plants even showed lower A than that of wild plants, due to an increasing photorespiration rate. Lacking knowledge about the genetic control of Kranz anatomy and dimorphic chloroplast formation, and detailed information about the regulation of cellular metabolism and metabolite trafficking between cellular compartments, there are still great obstacles to building C_4 rice [138]. However, although it is a big challenge, progress has still been made with C_4 rice engineering, for instance, the flux of CO_2 fixation by PEPC was increased within C_4 transgenic rice [139,140].

Moreover, although it is difficult to switch from C_3 crops to C_4 plants in a short time, there are still some conservative approaches to improve the *A* in C_3 crops, such as combining high photosynthetic traits by traditional breeding work to build plants with higher *A*. Adachi et al. [16] identified two rice lines with extremely high values of *A* among the backcrossed inbred lines derived from the indica variety Takanari, one of the most productive varieties in Japan, and the elite japonica variety Koshihikari (Koshihikari/Takanari//Takanari). Combining the advantages of small mesophyll cells and more developed lobes of mesophyll cells from Koshihikari, high K_{root}, strong nitrogen accumulation capacity, and thick mesophyll from Takanari, the *A* of the two lines reached 38.8 μmol m^{-2} s^{-1} and 37.6 μmol m^{-2} s^{-1}, respectively, significantly higher than their parents, which were 30.8 μmol m^{-2} s^{-1} and 24.4 μmol m^{-2} s^{-1}. Thus, selecting varieties with high photosynthetic traits and combining them together can further improve the photosynthetic rate in C_3 crops.

Therefore, according to the factors limiting the *A* and attempts made to improve the *A* in C_3 crops, we can summarize approaches to improving the *A* in C_3 crops: design or introduce high-activity Rubisco; adjust leaf nitrogen allocation to optimize leaf anatomy and leaf chemical composition; modify leaf morphology and anatomy for greater CO_2 diffusion; improve the activity of proteins and enzymes associated with sugar transportation and utilization; introduce C_4 photosynthetic mechanisms to C_3 crops and combine high photosynthetic traits by conventional breeding (Figure 3).

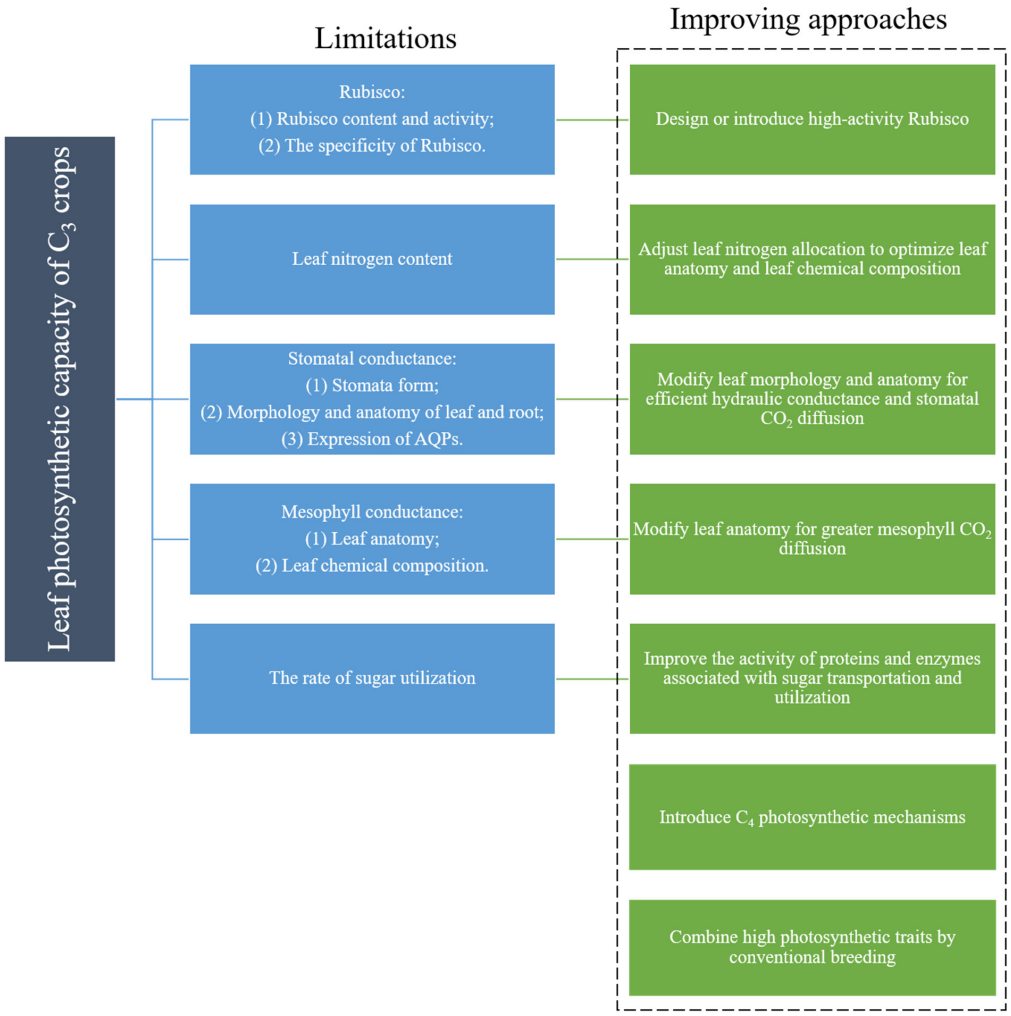

Figure 3. Physiological factors limiting leaf photosynthetic capacity in C_3 crops under natural conditions and approaches for improving it.

4. Conclusions

Under current ambient atmospheric conditions, the leaf net photosynthetic rate of C_3 crops is mainly limited by Rubisco activity and the CO_2 concentration in chloroplasts. Leaf nitrogen content affects leaf net photosynthetic rate by regulating Rubisco content and leaf anatomy; leaf morphological and anatomical traits including stomatal size, stomatal density, stomatal distribution, leaf vein density, leaf vein diameter, the fraction of intercellular airspace, the surface area of cells exposed to intercellular airspace (IAS) per leaf area, and the surface area of chloroplasts exposed to IAS per leaf area and cell wall thickness limit leaf net photosynthetic rate by impacting stomatal and mesophyll CO_2 diffusion. Further improvements in leaf net photosynthetic rate in C_3 crops can be achieved by designing or introducing high-activity Rubisco; adjusting leaf nitrogen allocation to optimize leaf anatomy and leaf chemical composition; modifying leaf morphology and anatomy for greater CO_2 diffusion; improving the activity of proteins and enzymes associated with sugar transportation and utilization; introducing C_4 photosynthetic mechanisms, combining high photosynthetic traits by conventional breeding, etc.

Author Contributions: M.Y. designed the frame of the manuscript and wrote it. M.W., Y.Z. and Z.W. participated in writing the physiological factors limiting leaf net photosynthetic rate part. H.Z. and Z.Z. commented and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the China Postdoctoral Science Foundation (2021M702769); the R&D Foundation of Jiangsu province, China (BE2022425, BK20220017) and the National Natural Science Foundation of China (31871559).

Acknowledgments: We would like to express our heartfelt appreciations to the Jiangsu Funding Program for Excellent Postdoctoral Talent.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Harley, P.C.; Loreto, F.; Di Marco, G.; Sharkey, T.D. Theoretical considerations when estimating the mesophyll conductance to CO_2 flux by analysis of the response of photosynthesis to CO_2. *Plant Physiol.* **1992**, *98*, 1429–1436. [CrossRef] [PubMed]
2. Sharkey, T.D. Photosynthesis in intact leaves of C3 crops: Physics, physiology and rate limitations. *Bot. Rev.* **1985**, *51*, 53–105. [CrossRef]
3. Farquhar, G.D. Models of integrated photosynthesis of cells and leaves. *Philos. Trans. R. Soc. B Biol. Sci.* **1989**, *323*, 357–367.
4. Sage, R.F. A model describing the regulation of ribulose-1,5-bisphosphate carboxylase, electron transport, and triose phosphate use in response to light intensity and CO_2 in C3 crops. *Plant Physiol.* **1990**, *94*, 1728–1734. [CrossRef] [PubMed]
5. Harley, P.C.; Sharkey, T.D. An improved model of C_3 photosynthesis at high CO_2: Reversed O_2 sensitivity explained by lack of glycerate reentry into the chloroplast. *Photosynth. Res.* **1991**, *28*, 169–179. [CrossRef] [PubMed]
6. Long, S.P.; Bernacchi, C.J. Gas exchange measurements, what can they tell us about the underlying limitations to photosynthesis? Procedures and sources of error. *J. Exp. Bot.* **2003**, *54*, 2393–2401. [PubMed]
7. Makino, A.; Shimada, T.; Takumi, S.; Kaneko, K.; Matsuoka, M.; Shimamoto, K.; Nakano, H.; Miyao-Tokutomi, M.; Mae, T.; Yamamoto, N. Does decrease in ribulose-1,5-bisphosphate carboxylase by antisense rbcS lead to a higher N-use efficiency of photosynthesis under conditions of saturating CO2 and light in rice plants? *Plant Physiol.* **1997**, *114*, 483–491. [CrossRef]
8. Spreitzer, R.J.; Salvucci, M.E. Rubisco: Structure, regulatory interactions, and possibilities for a better enzyme. *Annu. Rev. Plant Biol.* **2002**, *53*, 449–479. [CrossRef] [PubMed]
9. Brooks, A.; Farquhar, G.D. Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. *Planta* **1985**, *165*, 397–406. [CrossRef]
10. Tcherkez, G.G.B.; Farquhar, G.D.; Andrews, T.J. Despite slow catalysis and confused substrate specificity, all ribulose bisphosphate carboxylases may be nearly perfectly optimized. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 7246–7251. [CrossRef]
11. Iñiguez, C.; Capó-Bauçà, S.; Niinemets, Ü.; Stoll, H.; Aguiló-Nicolau, P.; Galmes, J. Evolutionary trends in RuBisCO kinetics and their co-evolution with CO_2 concentrating mechanisms. *Plant J.* **2020**, *101*, 897–918. [CrossRef] [PubMed]
12. Evans, J.R.; Kaldenhoff, R.; Genty, B.; Terashima, I. Resistances along the CO_2 diffusion pathway inside leaves. *J. Exp. Bot.* **2009**, *60*, 2235–2248. [CrossRef] [PubMed]
13. Li, Y.; Gao, Y.; Xu, X.; Shen, Q.; Guo, S. Light-saturated photosynthetic rate in high-nitrogen rice (*Oryza sativa* L.) leaves is related to chloroplastic CO_2 concentration. *J. Exp. Bot.* **2009**, *60*, 2351–2360. [CrossRef] [PubMed]
14. Yamori, W.; Nagai, T.; Makino, A. The rate-limiting step for CO_2 assimilation at different temperatures is influenced by the leaf nitrogen content in several C_3 crop species. *Plant Cell Environ.* **2011**, *34*, 764–777. [CrossRef] [PubMed]

15. Flexas, J.; Barbour, M.M.; Brendel, O.; Cabrera, H.M.; Carriquí, M.; Díaz-Espejo, A.; Douthe, C.; Dreyer, E.; Ferrio, J.P.; Gago, J.; et al. Mesophyll diffusion conductance to CO_2: An unappreciated central player in photosynthesis. *Plant Sci. Int. J. Exp. Plant Biol.* **2012**, *193–194*, 70–84. [CrossRef]
16. Adachi, S.; Nakae, T.; Uchida, M.; Soda, K.; Takai, T.; Oi, T.; Hirasawa, T. The mesophyll anatomy enhancing CO_2 diffusion is a key trait for improving rice photosynthesis. *J. Exp. Bot.* **2013**, *64*, 1061–1072. [CrossRef]
17. Makino, A.; Sakashita, H.; Hidema, J.; Mae, T.; Ojima, K.; Osmond, B. Distinctive responses of ribulose-1,5-bisphosphate carboxylase and carbonic anhydrase in wheat leaves to nitrogen nutrition and their possible relationships to CO_2-transfer resistance. *Plant Physiol.* **1992**, *100*, 1737–1743. [CrossRef]
18. Makino, A.; Nakano, H.; Mae, T. Responses of ribulose-1,5-bisphosphate carboxylase, cytochrome J and sucrose synthesis enzymes in rice leaves to leaf nitrogen and their relationships to photosynthesis. *Plant Physiol.* **1994**, *105*, 173–179. [CrossRef]
19. Makino, A. Biochemistry of C_3-photosynthesis in high CO_2. *J. Plant Res.* **1994**, *107*, 79–84. [CrossRef]
20. Sage, R.F.; Santrucek, J.; Grise, D.J. Temperature effects on the photosynthetic response of C3 crops to long-term CO_2 enrichment. *Vegetatio* **1995**, *121*, 67–77. [CrossRef]
21. Medlyn, B.E. The optimal allocation of nitrogen within the C_3 photosynthetic system at elevated CO_2. *Aust. J. Plant Physiol.* **1996**, *23*, 593–603. [CrossRef]
22. Lauerer, M.; Saftic, D.; Quick, W.P.; Labate, C.; Fichtner, K.; Schulze, E.D.; Rodermel, S.; Bogorad, L.; Stitt, M. Decreased ribulose-1,5-bisphosphate carboxylase-oxygenase in transgenic tobacco transformed with "antisense" *rbcS*. VI. Effect on photosynthesis in plants grown at different irradiance. *Planta* **1993**, *190*, 332–345. [CrossRef]
23. Makino, A. Rubisco and nitrogen relationships in rice: Leaf photosynthesis and plant growth. *J. Soil Sci. Plant Nutr.* **2003**, *49*, 319–327. [CrossRef]
24. Evans, J.R. Nitrogen and photosynthesis in the flag leaf of wheat (*Triticum aestivum* L.). *Plant Physiol.* **1983**, *72*, 297–302. [CrossRef] [PubMed]
25. Suzuki, Y.; Ohkubo, M.; Hatakeyama, H.; Ohashi, K.; Yoshizawa, R.; Kojima, S.; Makino, A. Increased Rubisco content in transgenic rice transformed with the 'sense' *rbcS* gene. *Plant Cell Physiol.* **2007**, *48*, 626–637. [CrossRef] [PubMed]
26. Jordan, D.B.; Ogren, W.L. The CO_2/O_2 specificity of ribulose 1,5-bisphosphate carboxylase/oxygenase. *Planta* **1984**, *161*, 308–313. [CrossRef]
27. Lin, M.T.; Occhialini, A.; Andralojc, P.J.; Parry, M.A.J.; Hanson, M.R. A faster Rubisco with potential to increase photosynthesis in crops. *Nature* **2014**, *513*, 547–550. [CrossRef]
28. Taylaran, R.D.; Adachi, S.; Ookawa, T.; Usuda, H.; Hirasawa, T. Hydraulic conductance as well as nitrogen accumulation plays a role in the higher rate of leaf photosynthesis of the most productive variety of rice in Japan. *J. Exp. Bot.* **2011**, *62*, 4067–4077. [CrossRef] [PubMed]
29. Polesskaya, O.G.; Kashirina, E.I.; Andreeva, S.E.; Goryaeva, O.V.; Glazunova, M.A.; Alekhina, N.D. Morphophysiological indices of the source leaf in wheat plants acclimated to conditions of nitrogen nutrition. *Russ. J. Plant Physiol.* **2001**, *48*, 716–722. [CrossRef]
30. Li, Y.; Ren, B.; Ding, L.; Shen, Q.; Peng, S.; Guo, S. Does chloroplast size influence photosynthetic nitrogen use efficiency? *PLoS ONE* **2013**, *8*, e62036. [CrossRef] [PubMed]
31. Xiong, D.; Liu, X.; Liu, L.; Douthe, C.; Li, Y.; Peng, S.; Huang, J. Rapid responses of mesophyll conductance to changes of CO_2 concentration, temperature and irradiance are affected by N supplies in rice. *Plant Cell Environ.* **2015**, *38*, 2541–2550. [CrossRef]
32. Peng, S.; Garcia, F.V.; Laza, R.C.; Sanico, A.L.; Visperas, R.M.; Cassman, K.G. Increased N-use efficiency using a chlorophyll meter on high-yielding irrigated rice. *Field Crops Res.* **1996**, *47*, 243–252. [CrossRef]
33. Ye, M.; Peng, S.; Li, Y. Intraspecific variation in photosynthetic nitrogen-use efficiency is positively related to photosynthetic rate in rice (*Oryza sativa* L.) plants. *Photosynthetica* **2019**, *57*, 311–319. [CrossRef]
34. Ocheltree, T.W.; Nippert, J.B.; Prasad, P.V.V. Changes in stomatal conductance along grass blades reflect changes in leaf structure. *Plant Cell Environ.* **2012**, *35*, 1040–1049. [CrossRef] [PubMed]
35. Ouyang, W.; Struik, P.C.; Yin, X.; Yang, J. Stomatal conductance, mesophyll conductance, and transpiration efficiency in relation to leaf anatomy in rice and wheat genotypes under drought. *J. Exp. Bot.* **2017**, *68*, 5191–5205. [CrossRef]
36. Tanaka, Y.; Sugano, S.S.; Shimada, T.; Hara-Nishimura, I. Enhancement of leaf photosynthetic capacity through increased stomatal density in *Arabidopsis*. *New Phytol.* **2013**, *198*, 757–764. [CrossRef]
37. Sarwar, A.G.; Karim, M.A.; Rana, S.M. Influence of stomatal characteristics on yield and yield attributes of rice. *J. Bangladesh Agric. Univ.* **2013**, *11*, 47–52. [CrossRef]
38. Wu, L.; Boer, H.J.; Zhang, Z.; Chen, X.; Shi, Y.; Peng, S.; Wang, F. The coordinated increase in stomatal density and vein dimensions during genetic improvement in rice. *Agron. J.* **2020**, *112*, 2791–2804. [CrossRef]
39. Li, S.; Hamani, A.K.M.; Zhang, Y.; Liang, Y.; Gao, Y.; Duan, A. Coordination of leaf hydraulic, anatomical, and economical traits in tomato seedlings acclimation to long-term drought. *BMC Plant Biol.* **2021**, *21*, 1–14. [CrossRef] [PubMed]
40. Brodribb, T.J.; Feild, T.S.; Jordan, G.J. Leaf maximum photosynthetic rate and venation are linked by hydraulics. *Plant Physiol.* **2007**, *144*, 1890–1898. [CrossRef]
41. Xiong, D.; Nadal, M. Linking water relations and hydraulics with photosynthesis. *Plant J.* **2020**, *101*, 800–815. [CrossRef]
42. Tabassum, M.A.; Ye, Y.; Yu, T.; Zhu, G.; Rizwan, M.S.; Wahid, M.A.; Li, Y. Rice (*Oryza sativa* L.) hydraulic conductivity links to leaf venation architecture under well-watered condition rather than PEG-induced water deficit. *Acta Physiol. Plant.* **2016**, *38*, 92. [CrossRef]

43. Sack, L.; Cowan, P.D.; Jaikumar, N.; Holbrook, N.M. The 'hydrology' of leaves: Co-ordination of structure and function in temperate woody species. *Plant Cell Environ.* **2003**, *26*, 1343–1356. [CrossRef]
44. Sack, L.; Holbrook, N.M. Leaf hydraulics. *Annu. Rev. Plant Biol.* **2006**, *57*, 361–381. [CrossRef]
45. Hirasawa, T.; Tsuchida, M.; Ishihara, K. Relationship between resistance to water transport and exudation rate and the effect of the resistance on the midday depression of stomatal aperture in rice plants. *Jpn. J. Crop Sci.* **1992**, *61*, 145–152. [CrossRef]
46. Ye, M.; Wu, M.; Zhang, H.; Zhang, Z.; Zhang, Z. High leaf vein density promotes leaf gas exchange by enhancing leaf hydraulic conductance in *Oryza sativa* L. plants. *Front. Plant Sci.* **2021**, *12*, 693815. [CrossRef]
47. Rockwell, F.E.; Holbrook, N.M.; Stroock, A.D. The competition between liquid and vapor transport in transpiring leaves. *Plant Physiol.* **2014**, *164*, 1741–1758. [CrossRef]
48. Xiong, D.; Flexas, J.; Yu, T.; Peng, S.; Huang, J. Leaf anatomy mediates coordination of leaf hydraulic conductance and mesophyll conductance to CO_2 in *Oryza*. *New Phytol.* **2017**, *213*, 572–583. [CrossRef]
49. Flexas, J.; Scoffoni, C.; Gago, J.; Sack, L. Leaf mesophyll conductance and leaf hydraulic conductance: An introduction to their measurement and coordination. *J. Exp. Bot.* **2013**, *64*, 3965–3981. [CrossRef] [PubMed]
50. Sack, L.; Scoffoni, C. Leaf venation: Structure, function, development, evolution, ecology and applications in the past, present and future. *New Phytol.* **2013**, *198*, 983–1000. [CrossRef]
51. Simonneau, T.; Inra, R.H. The use of tree root suckers to estimate root water potential. *Plant Cell Environ.* **1991**, *14*, 585–591. [CrossRef]
52. Javot, H.; Maurel, C. The role of aquaporins in root water uptake. *Ann. Bot.* **2002**, *90*, 301–313. [CrossRef] [PubMed]
53. Else, M.A.; Coupland, D.; Dutton, L.; Jackson, M.B. Decreased root hydraulic conductivity reduces leaf water potential, initiates stomatal closure and slows leaf expansion in flooded plants of castor oil (*Ricinus communis*) despite diminished delivery of ABA from the roots to shoots in xylem sap. *Physiol. Plant.* **2001**, *111*, 46–54. [CrossRef]
54. Miyamoto, N.; Steudle, E.; Hirasawa, T.; Lafitte, R. Hydraulic conductivity of rice roots. *J. Exp. Bot.* **2001**, *52*, 1835–1846. [CrossRef] [PubMed]
55. Yang, X.; Li, Y.; Ren, B.; Ding, L.; Gao, C.; Shen, Q.; Guo, S. Drought-induced root aerenchyma formation restrains water uptake in nitrate-supplied rice seedlings. *Plant Cell Physiol.* **2012**, *53*, 495–504. [CrossRef] [PubMed]
56. Postaire, O.; Tournaire-Roux, C.; Grondin, A.; Boursiac, Y.; Morillon, R.; Schäffner, A.R.; Maurel, C. A PIP1 aquaporin contributes to hydrostatic pressure-induced water transport in both the root and rosette of *Arabidopsis*. *Plant Physiol.* **2010**, *152*, 1418–1430. [CrossRef] [PubMed]
57. Kramer, P.J. *Water Relations of Plants*; Academic Press: New York, NY, USA, 1983.
58. Steudle, E.; Peterson, C.A. How does water get through roots? *J. Exp. Bot.* **1998**, *49*, 775–788. [CrossRef]
59. Lu, Z.; Neumann, P.M. Water stress inhibits hydraulic conductance and leaf growth in rice seedlings but not the transport of water via mercury-sensitive water channels in the root. *Plant Physiol.* **1999**, *120*, 143–152. [CrossRef]
60. Grondin, A.; Rodrigues, O.; Verdoucq, L.; Merlot, S.; Leonhardt, N.; Maurel, C. Aquaporins contribute to ABA-triggered stomatal closure through OST1-mediated phosphorylation. *Plant Cell* **2015**, *27*, 1945–1954. [CrossRef]
61. Flexas, J.; Ribas-Carbó, M.; Diaz-Espejo, A.; Galmés, J.; Medrano, H. Mesophyll conductance to CO_2: Current knowledge and future prospects. *Plant Cell Environ.* **2008**, *31*, 602–621. [CrossRef]
62. Terashima, I.; Hanba, Y.T.; Tholen, D.; Niinemets, Ü. Leaf functional anatomy in relation to photosynthesis. *Plant Physiol.* **2011**, *155*, 108–116. [CrossRef] [PubMed]
63. Giuliani, R.; Koteyeva, N.; Voznesenskaya, E.; Evans, M.A.; Cousins, A.B.; Edwards, G.E. Coordination of leaf photosynthesis, transpiration, and structural traits in rice and wild relatives (genus *Oryza*). *Plant Physiol.* **2013**, *162*, 1632–1651. [CrossRef] [PubMed]
64. Carriquí, M.; Cabrera, H.M.; Conesa, M.À.; Coopman, R.E.; Douthe, C.; Gago, J.; Gallé, A.; Galmés, J.; Ribas-Carbo, M.; Tomás, M.; et al. Diffusional limitations explain the lower photosynthetic capacity of ferns as compared with angiosperms in a common garden study. *Plant Cell Environ.* **2015**, *38*, 448–460. [CrossRef]
65. Scafaro, A.P.; von Caemmerer, S.; Evans, J.R.; Atwell, B.J. Temperature response of mesophyll conductance in cultivated and wild *Oryza* species with contrasting mesophyll cell wall thickness. *Plant Cell Environ.* **2011**, *34*, 1999–2008. [CrossRef] [PubMed]
66. Peguero-Pina, J.J.; Flexas, J.; Galmes, J.; Niinemets, U.; Sancho-Knapik, D.; Barredo, G.; Villarroya, D.; Gil-Pelegrin, E. Leaf anatomical properties in relation to differences in mesophyll conductance to CO_2 and photosynthesis in two related Mediterranean *Abies* species. *Plant Cell Environ.* **2012**, *35*, 2121–2129. [CrossRef]
67. Tosens, T.; Niinemets, Ü.; Westoby, M.; Wright, I.J. Anatomical basis of variation in mesophyll resistance in eastern Australian sclerophylls: News of a long and winding path. *J. Exp. Bot.* **2012**, *63*, 5105–5119. [CrossRef]
68. Tomás, M.; Flexas, J.; Copolovici, L.; Galmés, J.; Hallik, L.; Medrano, H.; Niinemets, Ü. Importance of leaf anatomy in determining mesophyll diffusion conductance to CO_2 across species: Quantitative limitations and scaling up by models. *J. Exp. Bot.* **2013**, *64*, 2269–2281. [CrossRef]
69. Muir, C.D.; Hangarter, R.P.; Moyle, L.C.; Davis, P.A. Morphological and anatomical determinants of mesophyll conductance in wild relatives of tomato (*Solanum* sect. *Lycopersicon*, sect. *Lycopersicoides*; Solanaceae). *Plant Cell Environ.* **2014**, *37*, 1415–1426. [CrossRef] [PubMed]
70. Hassiotou, F.; Renton, M.; Ludwig, M.; Evans, J.R.; Veneklaas, E.J. Photosynthesis at an extreme end of the leaf trait spectrum: How does it relate to high leaf dry mass per area and associated structural parameters? *J. Exp. Bot.* **2010**, *61*, 3015–3028. [CrossRef] [PubMed]

71. Ye, M.; Zhang, Z.C.; Huang, G.J.; Xiong, Z.; Peng, S.B.; Li, Y. High leaf mass per area *Oryza* genotypes invest more leaf mass to cell wall and show a low mesophyll conductance. *AoB Plants* **2020**, *12*, plaa028. [CrossRef]
72. Ye, M.; Zhang, Z.; Huang, G.; Li, Y. Leaf photosynthesis and its temperature response are different between growth stages and N supplies in rice plants. *Int. J. Mol. Sci.* **2022**, *23*, 3885. [CrossRef] [PubMed]
73. Hassiotou, F.; Ludwig, M.; Renton, M.; Veneklaas, E.J.; Evans, J.R. Influence of leaf dry mass per area, CO_2, and irradiance on mesophyll conductance in sclerophylls. *J. Exp. Bot.* **2009**, *60*, 2303–2314. [CrossRef] [PubMed]
74. Onoda, Y.; Wright, I.J.; Evans, J.R.; Hikosaka, K.; Kitajima, K.; Niinemets, Ü.; Poorter, H.; Tosens, T.; Westoby, M. Physiological and structural tradeoffs underlying the leaf economics spectrum. *New Phytol.* **2017**, *214*, 1447–1463. [CrossRef] [PubMed]
75. Hanba, Y.T.; Kogami, H.; Terashima, I. The effect of growth irradiance on leaf anatomy and photosynthesis in Acer species differing in light demand. *Plant Cell Environ.* **2002**, *25*, 1021–1030. [CrossRef]
76. Peguero-Pina, J.J.; Sisó, S.; Flexas, J.; Galmés, J.; García-Nogales, A.; Niinemets, Ü.; Gil-Pelegrín, E. Cell-level anatomical characteristics explain high mesophyll conductance and photosynthetic capacity in sclerophyllous Mediterranean oaks. *New Phytol.* **2017**, *214*, 585–596. [CrossRef]
77. Fini, A.; Loreto, F.; Tattini, M.; Giordano, C.; Ferrini, F.; Brunetti, C.; Centritto, M. Mesophyll conductance plays a central role in leaf functioning of Oleaceae species exposed to contrasting sunlight irradiance. *Physiol. Plant.* **2016**, *157*, 54–68. [CrossRef]
78. Ren, T.; Weraduwage, S.M.; Sharkey, T.D. Prospects for enhancing leaf photosynthetic capacity by manipulating mesophyll cell morphology. *J. Exp. Bot.* **2019**, *70*, 1153–1165. [CrossRef]
79. Nakhoul, N.L.; Davis, B.A.; Romero, M.F.; Boron, W.F. Effect of expressing the water channel aquaporin-1 on the CO_2 permeability of *Xenopus oocytes*. *Am. J. Physiol. Cell Physiol.* **1998**, *274*, C543–C548. [CrossRef]
80. 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. [CrossRef]
81. Fukuzawa, H.; Suzuki, E.; Komukai, Y.; Miyachi, S. A gene homologous to chloroplast carbonic anhydrase (*icfA*) is essential to photosynthetic carbon dioxide fixation by Synechococcus PCC7942. *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 4437–4441. [CrossRef]
82. Badger, M. The roles of carbonic anhydrases in photosynthetic CO_2 concentrating mechanisms. *Photosynth. Res.* **2003**, *77*, 83–94. [CrossRef] [PubMed]
83. Sade, N.; Shatil-Cohen, A.; Attia, Z.; Maurel, C.; Boursiac, Y.; Kelly, G.; Granot, D.; Yaaran, A.; Lerner, S.; Moshelion, M. The role of plasma membrane aquaporins in regulating the bundle sheath-mesophyll continuum and leaf hydraulics. *Plant Physiol.* **2014**, *166*, 1609–1620. [CrossRef] [PubMed]
84. Secchi, F.; Zwieniecki, M.A. Down-regulation of plasma intrinsic protein1 aquaporin in poplar trees is detrimental to recovery from embolism. *Plant Physiol.* **2014**, *164*, 1789–1799. [CrossRef] [PubMed]
85. Huang, X.; Wang, Z.; Huang, J.; Peng, S.; Xiong, D. Mesophyll conductance variability of rice aquaporin knockout lines at different growth stages and growing environments. *Plant J.* **2021**, *107*, 1503–1512. [CrossRef] [PubMed]
86. Heckwolf, M.; Pater, D.; Hanson, D.T.; Kaldenhoff, R. The *Arabidopsis thaliana* aquaporin AtPIP1;2 is a physiologically relevant CO_2 transport facilitator. *Plant J.* **2011**, *67*, 795–804. [CrossRef]
87. Uehlein, N.; Sperling, H.; Heckwolf, M.; Kaldenhoff, R. The *Arabidopsis* aquaporin *PIP1;2* rules cellular CO_2 uptake. *Plant Cell Environ.* **2012**, *35*, 1077–1083. [CrossRef]
88. Groszmann, M.; Osborn, H.L.; Evans, J.R. Carbon dioxide and water transport through plant aquaporins. *Plant Cell Environ.* **2017**, *40*, 938–961. [CrossRef] [PubMed]
89. Roig-Oliver, M.; Fullana-Pericàs, M.; Bota, J.; Flexas, J. Adjustments in photosynthesis and leaf water relations are related to changes in cell wall composition in Hordeum vulgare and *Triticum aestivum* subjected to water deficit stress. *Plant Sci.* **2021**, *311*, 111015. [CrossRef]
90. Flexas, J.; Clemente-Moreno, M.J.; Bota, J.; Brodribb, T.J.; Gago, J.; Mizokami, Y.; Nadal, M.; Perera-Castro, A.V.; Roig-Oliver, M.; Sugiura, D.; et al. Cell wall thickness and composition are involved in photosynthetic limitation. *J. Exp. Bot.* **2021**, *72*, 3971–3986. [CrossRef] [PubMed]
91. Canny, M.; Wong, S.C.; Huang, C.; Miller, C. Differential shrinkage of mesophyll cells in transpiring cotton leaves: Implications for static and dynamic pools of water, and for water transport pathways. *Funct. Plant Biol.* **2012**, *39*, 91–102. [CrossRef]
92. Lunn, J.E.; MacRae, E. New complexities in the synthesis of sucrose. *Curr. Opin. Plant Biol.* **2003**, *6*, 208–214. [CrossRef]
93. Sauer, N. Molecular physiology of higher plant sucrose transporters. *FEBS Lett.* **2007**, *581*, 2309–2317. [CrossRef] [PubMed]
94. Braun, D.M. SWEET! The pathway is complete. *Science* **2012**, *335*, 173–174. [PubMed]
95. Patrick, J.W.; Botha, F.C.; Birch, R.G. Metabolic engineering of sugars and simple sugar derivations in plant. *Plant Biotechnol. J.* **2013**, *11*, 142–156. [CrossRef] [PubMed]
96. Timm, H.C.; Stegemann, J.; Küppers, M. Photosynthetic induction strongly affects the light compensation point of net photosynthesis and coincidentally the apparent quantum yield. *Trees* **2002**, *16*, 47–62. [CrossRef]
97. Lawson, T.; Kramer, D.M.; Raines, C.A. Improving yield by exploiting mechanisms underlying natural variation of photosynthesis. *Curr. Opin. Biotechnol.* **2012**, *23*, 215–220. [CrossRef]
98. Zhang, Q.; Peng, S.; Li, Y. Increase rate of light-induced stomatal conductance is related to stomatal size in the genus *Oryza*. *J. Exp. Bot.* **2019**, *70*, 5259–5269. [CrossRef]
99. Yang, Y.; Zhang, Q.; Huang, G.; Peng, S.; Li, Y. Temperature responses of photosynthesis and leaf hydraulic conductance in rice and wheat. *Plant Cell Environ.* **2020**, *43*, 1437–1451. [CrossRef]

100. Bernacchi, C.J.; Portis, A.R.; Nakano, H.; von Caemmerer, S.; Long, S.P. Temperature response of mesophyll conductance. Implications for the determination of Rubisco enzyme kinetics and for limitations to photosynthesis in Vivo. *Plant Physiol.* **2002**, *130*, 1992–1998. [CrossRef]
101. Li, Y.; Song, X.; Li, S.; Salter, W.T.; Barbour, M.M. The role of leaf water potential in the temperature response of mesophyll conductance. *New Phytol.* **2020**, *225*, 1193–1205. [CrossRef]
102. von Caemmerer, S.; Evans, J.R. Temperature responses of mesophyll conductance differ greatly between species. *Plant Cell Environ.* **2015**, *38*, 629–637. [CrossRef] [PubMed]
103. Warren, C.R. The photosynthetic limitation posed by internal conductance to CO_2 movement is increased by nutrient supply. *J. Exp. Bot.* **2004**, *55*, 2313–2321. [CrossRef]
104. Myers, S.S.; Zanobetti, A.; Kloog, I.; Huybers, P.; Leakey, A.D.; Bloom, A.J.; Carlisle, E.; Dietterich, L.H.; Fitzgerald, G.; Hasegawa, T.; et al. Increasing CO_2 threatens human nutrition. *Nature* **2014**, *510*, 139–142. [CrossRef] [PubMed]
105. Asensio, J.S.; Rachmilevitch, S.; Bloom, A.J. Responses of Arabidopsis and wheat to rising CO_2 depend on nitrogen source and nighttime CO_2 levels. *Plant Physiol.* **2015**, *168*, 156–163. [CrossRef]
106. Bloom, A.J. Photorespiration and nitrate assimilation: A major intersection between plant carbon and nitrogen. *Photosynth. Res.* **2015**, *123*, 117–128. [CrossRef]
107. Lovelock, C.; Winter, K. Oxygendependent electron transport and protection from photoinhibition in leaves of tropical tree species. *Planta* **1996**, *198*, 580–587. [CrossRef]
108. Heber, U.; Walker, D. Concerning a dual function of coupled cyclic electron transport in leaves. *Plant Physiol.* **1992**, *100*, 1621–1626. [CrossRef]
109. Kozaki, A.; Takeba, G. Photorespiration protects C3 crops from photooxidation. *Nature* **1996**, *384*, 557–560. [CrossRef]
110. Zelitch, I.; Schultes, N.P.; Peterson, R.B.; Brown, P.; Brutnell, T.P. High glycolate oxidase activity is required for survival of maize in normal air. *Plant Physiol.* **2009**, *149*, 195–204. [CrossRef]
111. Whitney, S.M.; von Caemmerer, S.; Hudson, G.S.; Andrews, T.J. Directed mutation of the Rubisco large subunit of tobacco influences photorespiration and growth. *Plant Physiol.* **1999**, *121*, 579–588. [CrossRef]
112. Whitney, S.M.; Sharwood, R.E.; Orr, D.; White, S.J.; Alonso, H.; Galmés, J. Isoleucine 309 acts as a C_4 catalytic switch that increases ribulose-1,5-bisphosphate carboxylase/oxygenase (rubisco) carboxylation rate in Flaveria. *Proc. Natl. Acad. Sci. USA* **2011**, *108*, 14688–14693. [CrossRef] [PubMed]
113. Kurek, I.; Chang, T.K.; Bertain, S.M.; Madrigal, A.; Liu, L.; Lassner, M.W.; Zhu, X.G. Enhanced thermostability of *Arabidopsis* Rubisco activase improves photosynthesis and growth rates under moderate heat stress. *Plant Cell* **2007**, *19*, 3230–3241. [CrossRef] [PubMed]
114. Kumar, A.; Cishan, L.; Portis, A.R., Jr. *Arabidopsis thaliana* expressing a thermostable chimeric Rubisco activase exhibits enhanced growth and higher rates of photosynthesis at moderately high temperatures. *Photosynth. Res.* **2009**, *100*, 143–153. [CrossRef] [PubMed]
115. Yamori, W.; von Caemmerer, S. Effect of Rubisco activase deficiency on the temperature response of CO_2 assimilation rate and Rubisco activation state: Insights from transgenic tobacco with reduced amounts of Rubisco activase. *Plant Physiol.* **2009**, *151*, 2073–2082. [CrossRef]
116. Yamori, W.; Masumoto, C.; Fukayama, H.; Makino, A. Rubisco activase is a key regulator of nonsteady-state photosynthesis at any leaf temperature and, to a lesser extent, of steady-state photosynthesis at high temperature. *Plant J.* **2012**, *71*, 871–880. [CrossRef] [PubMed]
117. Miyagawa, Y.; Tamoi, M.; Shigeoka, S. Overexpression of a yanobacterial fructose-1,6-/sedoheptulose-1,7-bisphosphatase in tobacco enhances photosynthesis and growth. *Nat. Biotechnol.* **2001**, *19*, 965–969. [CrossRef] [PubMed]
118. Lefebvre, S.; Lawson, T.; Zakhleniuk, O.V.; Lloyd, J.C.; Raines, C.A.; Fryer, M. Increased sedoheptulose-1,7-bisphosphatase activity in transgenic tobacco plants stimulates photosynthesis and growth from an early stage in development. *Plant Physiol.* **2005**, *138*, 451–460. [CrossRef]
119. Rosenthal, D.; Locke, A.; Khozaei, M.; Raines, C.; Long, S.; Ort, D. Overexpressing the C_3 photosynthesis cycle enzyme sedoheptulose-1–7 bisphosphatase improves photosynthetic carbon gain and yield under fully open air CO_2 fumigation (FACE). *BMC Plant Biol.* **2011**, *11*, 123. [CrossRef]
120. Köhler, I.H.; Ruiz-Vera, U.M.; VanLoocke, A.; Thomey, M.L.; Clemente, T.; Long, S.P.; Ort, D.R.; Bernacchi, C.J. Expression of cyanobacterial FBP/SBPase in soybean prevents yield depression under future climate conditions. *J. Exp. Bot.* **2017**, *68*, 715–726. [CrossRef]
121. Zhong, M.; Wang, Y.; Hou, K.; Shu, S.; Sun, J.; Guo, S. TGase positively regulates photosynthesis via activation of Calvin cycle enzymes in tomato. *Hortic. Res.* **2019**, *6*, 92. [CrossRef]
122. Li, C.; Li, N.; Huang, R.; Chen, C.; Guo, J.; Yang, X.; Zhang, X.; Sun, C.; Deng, X.; Wang, P. A single nucleotide substitution at the 3′-end of SBPase gene involved in Calvin cycle severely affects plant growth and grain yield in rice. *BMC Plant Biol.* **2020**, *20*, 1–14. [CrossRef] [PubMed]
123. Hajirezaei, M.R.; Peisker, M.; Tschiersch, H.; Palatnik, J.F.; Valle, E.M.; Carrillo, N.; Sonnewald, U. Small changes in the activity of chloroplastic NADP+- dependent ferredoxin oxidoreductase lead to impaired plant growth and restrict photosynthetic activity of transgenic tobacco plants. *Plant J.* **2002**, *29*, 281–293. [CrossRef] [PubMed]

124. Pesaresi, P.; Scharfenberg, M.; Weigel, M.; Granlund, I.; Schroder, W.P.; Finazzi, G.; Leister, D. Mutants, overexpressors, and interactors of Arabidopsis plastocyanin isoforms: Revised roles of plastocyanin in photosynthetic electron flow and thylakoid redox state. *Mol. Plant* **2009**, *2*, 236–248. [CrossRef]
125. Takahara, K.; Kasajima, I.; Takahashi, H.; Hashida, S.N.; Itami, T.; Uchimiya, H. Metabolome and photochemical analysis of rice plants overexpressing *Arabidopsis* NAD kinase gene. *Plant Physiol.* **2010**, *152*, 1863–1873. [CrossRef] [PubMed]
126. Price, G.D.; Badger, M.R.; von Caemmerer, S. The prospect of using cyanobacterial bicarbonate transporters to improve leaf photosynthesis in C_3 crop plants. *Plant Physiol.* **2011**, *155*, 20–26. [CrossRef]
127. Price, G.D.; Pengelly, J.J.L.; Forster, B.; Du, J.; Whitney, S.M.; von Caemmerer, S.; Evans, J.R. The cyanobacterial CCM as a source of genes for improving photosynthetic CO_2 fixation in crop species. *J. Exp. Bot.* **2013**, *64*, 753–768. [CrossRef] [PubMed]
128. Covshoff, S.; Hibberd, J.M. Integrating C_4 photosynthesis into C_3 crops to increase yield potential. *Curr. Opin. Biotechnol.* **2012**, *23*, 209–214. [CrossRef]
129. von Caemmerer, S.; Quick, W.P.; Furbank, R.T. The development of C_4 rice: Current progress and future challenges. *Science* **2012**, *336*, 1671–1672. [CrossRef]
130. Tholen, D.; Boom, C.; Zhu, X.G. Opinion: Prospects for improving photosynthesis by altering leaf anatomy. *Plant Sci.* **2012**, *197*, 92–101. [CrossRef]
131. Hanba, Y.T.; Shibasaka, M.; Hayashi, Y.; Hayakawa, T.; Kasamo, K.; Terashima, I.; Katsuhara, M. Overexpression of the barley aquaporin HvPIP2; 1increases internal CO_2 conductance and CO_2 assimilation in the leaves of transgenic rice plants. *Plant Cell Physiol.* **2004**, *45*, 521–529. [CrossRef]
132. Flexas, J.; Ribas-Carbó, M.; Hanson, D.T.; Bota, J.; Otto, B.; Cifre, J.; Kaldenhoff, R. Tobacco aquaporin NtAQP1 is involved in mesophyll conductance to CO_2 in vivo. *Plant J.* **2006**, *48*, 427–439. [CrossRef] [PubMed]
133. Bailey-Serres, J.; Parker, J.E.; Ainsworth, E.A.; Oldroyd, G.E.; Schroeder, J.I. Genetic strategies for improving crop yields. *Nature* **2019**, *575*, 109–118. [CrossRef] [PubMed]
134. Leakey, A.D.; Uribelarrea, M.; Ainsworth, E.A.; Naidu, S.L.; Rogers, A.; Ort, D.R.; Long, S.P. Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. *Plant Physiol.* **2006**, *140*, 779–790. [CrossRef]
135. Markelz, R.J.; Strellner, R.S.; Leakey, A.D. Impairment of C_4 photosynthesis by drought is exacerbated by limiting nitrogen and ameliorated by elevated [CO_2] in maize. *J. Exp. Bot.* **2011**, *62*, 3235–3246. [CrossRef] [PubMed]
136. Sheehy, J.E.; Ferrer, A.B.; Mitchell, P.L.; Elmido-Mabilangen, A.; Pablico, P.; Dionora, M.J.A. How the rice crop works and why it needs a new engine. In *Charting New Pathways to C_4 Rice*; Sheehy, J.E., Mitchell, P.L., Hardy, B., Eds.; International Rice Research Institute: Los Banos, Philippines, 2007; pp. 3–26.
137. Taniguchi, Y.; Ohkawa, H.; Masumoto, C.; Fukuda, T.; Tamai, T.; Lee, K.; Miyao, M. Overproduction of C_4 photosynthetic enzymes in transgenic rice plants: An approach to introduce the C_4-like photosynthetic pathway into rice. *J. Exp. Bot.* **2008**, *59*, 1799–1809. [CrossRef] [PubMed]
138. Zhu, X.G.; Shan, L.; Wang, Y.; Quick, W.P. C_4 rice—An ideal arena for systems biology research. *J. Integr. Plant Biol.* **2010**, *52*, 762–770. [CrossRef] [PubMed]
139. Lin, H.; Arrivault, S.; Coe, R.A.; Karki, S.; Covshoff, S.; Bagunu, E.; Lunn, J.E.; Stitt, M.; Furbank, R.T.; Hibberd, J.M.; et al. A partial C_4 photosynthetic biochemical pathway in rice. *Front. Plant Sci.* **2020**, *11*, 564463. [CrossRef]
140. Ermakova, M.; Arrivault, S.; Giuliani, R.; Danila, F.; AlonsoCantabrana, H.; Vlad, D.; Ishihara, H.; Feil, R.; Guenther, M.; Borghi, G.L.; et al. Installation of C_4 photosynthetic pathway enzymes in rice using a single construct. *Plant Biotechnol. J.* **2021**, *19*, 575–588. [CrossRef] [PubMed]