Review

Current and possible approaches for improving photosynthetic efficiency

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ABSTRACT

One of the most important tasks laying ahead today's biotechnology is to improve crop productivity with the aim of meeting increased food and energy demands of humankind. Plant productivity depends on many genetic factors, including life cycle, harvest index, stress tolerance and photosynthetic activity. Many approaches were already tested or suggested to improve either. Limitations of photosynthesis have also been uncovered and efforts been taken to increase its efficiency. Examples include decreasing photosynthetic antennae size,

increasing the photosynthetically available light spectrum, countering oxygenase activity of Rubisco by implementing C4 photosynthesis to C3 plants and altering source to sink transport of metabolites. A natural and effective photosynthetic adaptation, the sugar alcohol metabolism got however remarkably little attention in the last years, despite being comparably efficient as C4, and can be considered easier to introduce to new species. We also propose root to shoot carbon-dioxide transport as a means to improve photosynthetic performance and drought tolerance at the same time. Different suggestions and successful examples are covered here for improving plant photosynthesis as well as novel perspectives are presented for future research.

Key words: transgenic plant, energy plant, harvest index, productivity, food security

Contents

1. Introduction

There is a huge demand on biotechnology and plant breeding these days to increase crop productivity, for many reasons. Among them, the climate change, the increase of world human population, losses of agricultural land due to urbanization, soil degradation, and the growing demand for food crops as energy-sources should be considered [1,2]. The recent climate change explains a third of global crop yield variability [3]. It is widely accepted that the climate change is largely caused by carbon-dioxide emitted by human activity [4]. Therefore carbon-neutral alternative energy sources such as plant biomass are increasingly considered. Using energy plants as energy sources has some debate due to the rising food prices. However, non-crop plants, cellulose-bioethanol and energy plants cultivated in polluted areas which are unsuitable for food production are still considered as potentional options [5]. An often over-looked fact is that intensive agriculture itself is very energy-demanding. For many crops including wheat and potato, bioethanol produced from the harvested part of the plant would contain only as much energy as its cultivation had been, having a net energy balance (NEB) of 1, reviewed by [5]. C4 crops perform better with this regard having NEB values of 1.2 (corn) or 6.7 (sugarcane). The usage as energy plant requires a high NEB and/or the improvement of plant productivity. While much has to be done to improve energy efficiency and waste management of agriculture, crop productivity also has to be increased by 70% to feed a human population growing by 34% and estimated to reach 9.1 billion till 2050 [6].

Plant productivity depends on genetic and environmental factors [7]. Among genetic factors, life cycle and longevity must be underlined. Generally, longer leaf life correlates well with higher productivity and increased drought tolerance, see the excellent review of [8]. Both traditional and molecular breeders have already been eager to profit from this effect. Stay-green mutants have been bred, while cytokine overproduction in transgenic plants also lead to

71 higher productivity and yield [9,10]. The over-expression of the Growth Regulating Factor 5
72 also delayed senescence and increased productivity of *Arabidopsis thaliana* as it co-operates
73 with cytokonins to stimulate chloroplast division [11]. Although the mechanism is more
74 complicated, and the stay-green approach may be species-specific and not applicable for
75 wheat for example [8]. Assimilate remobilization during senescence considerably increases
76 the yield of wheat, while senescence delay could result leaving much non-structural
77 carbohydrates in the straw [12]. Other important traits to be counted for productivity increase
78 are harvest index, photosynthetic efficiency and stress tolerance or more precisely, tolerance
79 to adverse environmental factors (mineral deficiency/pollution, water shortage/flooding, cold,
80 heat, pathogens, etc.) [13]. The harvest index refers to the rate of the plant biomass which can
81 be harvested [13]. In the past decades, traditional plant breeding has achieved a huge increase
82 in harvest index, mostly by dwarfing. Dwarfing also helped to reduce lodging [14]. Many data
83 show however a stagnation in yield, which indicates that the harvest index has already been
84 optimised for the most important crop plants like maize, rice and wheat [15]. Therefore, the
85 remaining options for productivity improvement are the altered life cycle, enhanced stress
86 tolerance and increased photosynthetic efficiency. These are connected as for example C4
87 plants were shown to exhibit high water and N-use efficiency [16], while sugar-alcohol
88 metabolizing plants have high osmotic stress tolerance [17]. Plants with crassulacean acid
89 metabolism excel with extreme drought tolerance. This type of photosynthesis was suggested
90 for implementation to C3 plants, improving tolerance to water deficit [18]. According to some
91 estimates, environmental stress reduces the potential yield of crop plants by as high as 70%,
92 [19]. It is of no surprise therefore, that stress tolerance has been intensively studied for
93 decades. Many of the underlying mechanisms have been understood and the gathered
94 knowledge was successfully utilised resulting in crops with enhanced stress tolerance. Three
95 main approaches emerged so far. These are over-expression of effectors like antioxidant

enzymes [20–22], over-expression of regulators like transcription factors and receptors which activate stress-inducible genes [23,24] and preparing the plant for the oncoming stress by applying external signals like salicylic acid or S-methylmethionine [25,26]. While the most effect can often be accomplished by providing external or internal signals, over-expression of effectors can also be suggested in some cases. For instance, during the work of [27] the over-expression of a dehydration-responsive element binding factor did not yield frost-tolerant tomato, since cold responsive effector genes were completely missing from this sub-tropical species.

Another approach to improve plant productivity is to make photosynthesis more efficient. Interestingly, such possibilities have only been tested in the last two decades. Land plant photosynthesis can be considered remarkably inefficient. C3 crops generally achieve light conversion efficiency of around 1-2% and C4 crops around 3-4% under normal field conditions and during active phase of the vegetation period [28]. The theoretical maximal photosynthetic efficiency at 30 ºC and 380 ppm CO_2 was calculated to be 4.6% for C3 and 6% for C4 plants, respectively [29]. On the other hand, photovoltaic solar-powered cells work up to 44.7% efficiency [30]. Based on these data, we believe there is much to improve on terrestrial plant photosynthesis. Attempts have been made to boost all major steps, including light reactions, dark reactions and source-sink carbohydrate transport. In our review we also propose possible solutions to improve each, together with summarization of earlier findings and suggestions. Emphasis is placed on fields which previously got less coverage.

2. Improving light reactions

In short, the light reactions of land plant photosynthesis consist of two photosystems (PSI and PSII) accompanied by light-harvesting antennae (LHCI and LHCII) and an electron-

120 transport chain connecting the two photosystems, reviewed by [31]. The PSII is capable of
121 splitting water, and together with PSI takes part in a linear electron transport, producing ATP
122 and NADPH. The PSI is also able to produce solely ATP in a cyclic electron transport,
123 without the PSII but in co-operation with elements of the electron transport chain [31]. Many
124 failings have been uncovered within this system. Some scholars argued for the inefficiency of
125 the photosynthetic electron transport chain. Supplement of plastocyanin with algal
126 cytochrome C6 protein has increased photosynthesis and growth of *Arabidopsis thaliana* by
127 providing an accelerated electron transport [32]. Furthermore, instability and photo-oxidative
128 damage of PSII have been reported at high light intensity, possibly due to its evolution in low-
129 light marine conditions, reviewed by [33]. To avoid this damage, an over-expressed maize
130 PSII reaction centre protein D1 in tobacco resulted in higher growth, lesser oxidative damage
131 and lesser photosynthesis inhibition during water shortage [34]. Others argue that during
132 fluctuating light conditions, a more dynamic activation and relaxation of photoprotective
133 mechanisms can also be a way of photosynthetic improvement [35].

134 In addition to these structural imperfections, many argue for the unnecessarily huge
135 size of light harvesting antennae [7,36,37]. As result of over-absorption, much of the absorbed
136 light cannot be converted to chemical energy and dissipated as heat instead, especially in
137 upper leaves during peak sunlight at the midday. Current antennae may have resulted from
138 competition in the nature, preventing other plants to capture light [36]. However, this issue is
139 of lesser importance in intensive agriculture where weeds are controlled by the farmer
140 therefore the yield may be increased by truncating antennae [36,37]. Indeed, reduced antennae
141 size of chlorophyll b-deficient soybean lines has caused 30% increases in the daily integral of
142 photosynthesis [38]. Mutant tobacco plants possessing truncated light-harvesting chlorophyll
143 antenna size (TLA) exhibited 25% higher stem and leaf biomass [39]. The loss of the
144 regulator protein HPE1 also reduced photosynthetic antennae size and led to improved

145 photosynthesis and biomass production of *Arabidopsis thaliana* mutants [40]. The
146 optimisation of plant architecture may also prevent futile over-absorption of light by the upper
147 leaves leading more light absorption for shade leaves. Brassinosteroid mutant rice plants have
148 been reported with erected leaves and an enhanced biomass production and grain yield [41].
149 According to the authors, shade of the upper leaves was minimized, and the lower leaves
150 received more light to drive higher rates of photosynthesis [41].

151 For lower leaves however, the extension of light absorption spectrum looks beneficial.
152 One inherent weakness of terrestrial plant photosynthesis is that usually only part of the
153 sunlight, the photosynthetically active radiation (PAR, 400-700 nm) can be absorbed and
154 converted to chemical energy. It is only around 48.7% of the total incident solar energy [13].
155 While the infrared (IR) light cannot be utilised, the plants may use wavelengths of UVA as
156 well [42–44]. Introduction of algal pigments like chlorophyll *d* and *f* with infrared absorption
157 maxima (696 and 705 nm, respectively) was considered to increase the absorption range,
158 especially in lower leaves which mostly receive IR light [45]. The synthesis enzyme of
159 chlorophyll *f* has been since isolated from terrestrial cyanobacteria and this pigment has been
160 successfully produced ectopically in other cyanobacteria [46]. An innovative plan was also
161 envisaged to replace the PSI of land plants with a purple bacterial photosystem having IR
162 absorption maximum [7]. The approach could replace the competition between the two
163 photosystems for photons with completion of each other's function, absorbing different parts
164 of the solar spectrum [7]. It also must be noted, that even the 400-700 nm radiation is not fully
165 utilised, green plants are unable to use the green light effectively, consisting of 4.9% of
166 sunlight and 10% PAR, respectively [29]. However, there are known photosynthetic pigments
167 in the nature with specific green light absorption, notably the proteorhodopsin proteins in
168 marine eubacteria [47]. These are about 27 kDa proteins coded by single genes and being
169 capable of proton transport across the biological membrane after capture of a green photon

(absorption peak at 520 nm). The proton can then be used for ATP generation. Proteorhodopsin has already been ectopically expressed in *Escherichia coli*, a heterotrophic bacterium and powered it with enough energy for movement in an energy-less medium [48]. While the system has some limitations compared to terrestrial plant photosynthesis (lack of antennae, less proton transport per photon campture) its introduction can still be considered because of its apparent ease and also to extend the absorbed light spectrum. Proteorhodopsin and beta-carotene 15,15'-monooxygenase (producing the chromophore retinal from beta-carotene) should be expressed in green tissues of plants [49]. Either the inner membrane of mitochondrion or the chloroplast thylakoid membrane can be considered for targeting proteorhodopsin.

3. Improving dark reactions

Carbon is fixed during the Calvin-Benson cycle using the produced reducing power and ATP from the light reactions, reviewed by [50]. Most efforts to improve photosynthetic efficiency, has been taken on this process. Woodrow and colleagues [51] found in their pioneering work, that some of the Calvin-Benson cycle enzymes (fructose-1,6-bisphosphatase, seduheptulose-1,7-bisphosphatase) were rate-limiting. Over-expression of any of these enzymes caused a 20-50% increase in the growth parameters of transgenic tobacco [52]. On the other hand, downregulation of the mitochondrial Krebs cycle enzymes like aconitase and malate-dehydrogenase also resulted an enhanced rate of photosynthesis [53,54]. Mitochondrion was suggested to play an important role in photosynthesis by providing carbon skeletons, taking part in photorespiration and stoma regulation and it was also marked as target for further photosynthetic improvement [55,56]. The most important limitation of dark reactions is however the futile oxygenase activity of the carbon-dioxide-assimilating enzyme, Rubisco [57,58]. Some scholars even proposed to build alternative,

195 Rubisco-less CO_2-fixation pathways instead [1,50,59]. Oxygen capture by Rubisco leads to
196 photorespiration that converts 2-phosphoglycolate formed in oxygenation into 3-
197 phosphoglycerate which then re-enters the Calvin cycle. The process is carried out in co-
198 operation by three organelles, the chloroplast, the mitochondrion and the peroxisome and the
199 carbon dioxide molecule is formed in the mitochondrion. A natural adaptation to counter this
200 CO_2 loss has been described by [60]. The authors have shown that chloroplasts are arranged at
201 the surface of mesophyll cells of wheat and rice, blocking the escape of CO_2 derived from
202 respiration and photorespiration. Meanwhile, Kebeish and co-workers [61] managed to build
203 an alternative photorespiratory route within the chloroplasts of *Arabidopsis thaliana*. The
204 emitted carbon dioxide could be readily refixed there, increasing photosynthetic efficiency.
205 The approach was also adapted for potato, causing 2.3-fold tuber yield [62]. It is traditionally
206 held that CO_2-specificity of Rubisco can only be improved at the expense of its speed,
207 reviewed by [13]. However, many natural Rubiscos were characterized recently having better
208 specificity and higher speed at the same time, like in *Poa palustris* and *Puccinellia maritima*
209 [63,64]. Soybean Rubisco was suggested to be replaced by these more effective monocot
210 counterparts [64]. Enzymatic properties of Rubisco are also temperature-dependent. Rubisco
211 optimisation has been proposed for future climatic conditions [2]. The specificity of Rubisco
212 decreases with rising temperature, therefore over-expression of Rubisco's chaperone, the
213 Rubisco activase could increase photosynthetic efficiency at high temperature [65,66].
214 Building cyanobacterial-like carboxysomes around Rubisco were also suggested to increase
215 local CO_2 concentration and diminish oxygenase activity of the enzyme [67].

216 An extensively studied natural adaptation to overcome Rubisco's oxygenase activity is
217 the well-known C4 photosynthesis. Full coverage of this issue is not within the scope of the
218 present review as it has been excellently reviewed elsewhere [16,68]. New results and some
219 of the most important approaches are noted here, however. Most commonly, C4

220 photosynthesis is a result of co-operation between two cell types, the mesophyll and bundle
221 sheath cells, though single-cell examples have also been reported from a few species,
222 reviewed by [69]. Bicarbonate ion is fixed by PEP-carboxylase in mesophyll cells producing
223 an oxaloacetate. Oxaloacetate is chemically labile, therefore it is either converted to malate or
224 aspartic acid which is then transported to the bundle sheath cells. Carbon dioxide is released
225 during decarboxylation and fixed by C3 photosynthesis in bundle sheath cells. The system is
226 generally considered a carbon-dioxide pump to the site of Rubisco, preventing its oxygenase
227 activity [70]. Three subtypes of C4 photosynthesis are traditionally considered based on the
228 transported intermediate (malate or aspartic acid) and the decarboxylation process, the
229 NADP-malic enzyme (NADP-ME), the NAD-malic enzyme (NAD-ME) and PEP-
230 carboxykinase (PEPCK). However, both experimental evidence and the modelling of energy
231 requirement indicate that the traditionally characterised C4 types do not exist in pure form,
232 but flexibility exist between them [71]. Some critical steps of C4 photosynthesis (e.g.
233 substrate availability of PEPC, carbonic acid anhydrase activity of mesophyll cells, transport
234 between the two cell types) have also been underlined and alterations were suggested to
235 improve this highly efficient process even further [72]. C4 plants generally do not tolerate low
236 temperature well, with the notable exception of *Miscanthus×giganteus*. Protection and
237 maintenance of photosynthetic proteins were found to be the key to the exceptional chilling
238 tolerance of that plant [73]. Huge effort has already been taken to equip the C3 plant rice with
239 C4 photosynthesis within the international C4 Rice Project [74,75]. Mutant populations of
240 *Sorgum bicolor* and *Sorghum viridis* were screened for regulator genes governing the C3-to-
241 C4 switch [74]. It became clear, that number and size of chloroplast needs to be increased in
242 rice bundle sheath while Calvin-cycle and photorespiration needs to be down-regulated in the
243 mesophyll to make rice amenable to act as as C4 plant [75]. Rice lines have also been bred for
244 the purpose with increased leaf vein density [76,77]. Functional promoters and enzyme genes

were already evaluated for the introduction of C4 biochemistry [78]. Despite this process, true C4 rice had not yet been produced, but the project is still ongoing. The key enzyme of C4 photosynthesis, the PEPC was also over-expressed in itself in many transgenic plants either constitutively or during the mesophyll. Although PEPC alone cannot carry out a full C4 cycle, interestingly many over-expressing transgenic plants were reported with unusually high drought tolerance and photosynthetic performance [79–84]. Increased sugar, amino acid content and higher level of cytoskeletal synthases, S-adenosylmethionine synthetase and N-metabolism enzymes have been observed and labelled as explanation [85,86]. Altogether, the implementation of the C4 photosynthesis to C3 plants appears to be one of the most straightforward approaches to increase crop productivity, but is still not without pitfalls. Lower cold tolerance of C4 plants and slower recovery of C4 photosynthesis after drought stress were marked as limitations of such projects, among others [87]. These effects can decrease the yield and narrow down the range of climatic condition when the actual yield could increase [87]. Forecasts also show an increased CO_2 level of 700 ppm for the year 2100, a condition where C4 photosynthesis will no longer be more efficient than C3, except at extreme high temperature [29]. These predictions indicate that more options should also be considered while improving C3 photosynthesis.

4. Source to sink transport

Although many of the mentioned studies achieved increased growth parameters by enhancing photosynthetic activity, Paul and co-workers [88] have emphasised that these approaches did not always lead to an increased yield of the harvested organ. Source-to sink transport of assimilates should also be redirected for maximal effect [88]. The point is not only that researchers should go back to the old story of increasing the harvest index once the

photosynthetic efficiency has been grown, but also that increased sink (i.e. heterotrophic tissue) demand is also able to increase photosynthetic activity in retrospection by energizing the entire transport pathway, reviewed by [89,90]. Examples for increasing sink demand include over-expression of the starch producing enzyme, the ADP-glucose pyrophosphorylase in various cereal caryopses [91], seed-specific over-expression of the potato sucrose transporter StSut1 in pea [92] and the endosperm-specific over-expression of the barley sucrose transporter HvSUT1 in wheat [93]. These efforts fostered starch and protein accumulation and enlarged the seeds in many cases. Seed-specific over-expression of the amino acid transporter (VfAAP1) in pea also increased the storage protein content [94]. Not only the increase in sink strength, but the accelerated export of assimilates from source tissue increased yield and photosynthetic efficiency. For instance, over-expression of a key enzyme for sucrose synthesis, the sucrose-phosphate synthase caused higher fruit production in tomato, possibly because of an enhanced carbohydrate export from leaves [95]. Rice mutants (originally bred for the C4 Rice Project) with increased number of veins per leaves also showed improved photosynthetic characteristics, possibly because of enhanced transport of photoassimilates [77].

Not only the direct acceleration of assimilate export or uptake, but some regulators were also proposed for altering source to sink assimilate transport. Uncoupling the apoplastic phloem-loading from the sucrose-sensing system regulating assimilate partitioning was also suggested to expand the transport and increase yields [89]. The authors underline that constitutive expression of a Suc symporter would increase the carbohydrate export from leaves leading to high photosynthetic activity [89]. It would also avert the onset of senescence associated with sugar accumulation in the leaf [89]. It was also demonstrated that increased cytokinin content raises sink strength and yield of transgenic rice, also leading to an enhanced drought tolerance [96]. Trehalose 6-phosphate has been proposed as a regulatory molecule

too, signalling sugar availability [88]. Low trehalose 6-phosphate level in sinks may act as a starvation signal up-regulating sucrose uptake [88]. Flower-specific over-expression of the catabolic enzyme trehalose phosphate phosphatase1 (TPP1) indeed enhanced yield and photosynthetic efficiency in transgenic maize [97].

5. Sugar alcohol metabolism

A natural adaptation, the sugar alcohol metabolism simultaneously achieves efficient source to sink transport and high photosynthetic activity [17,98,99]. Sugar alcohols like mannitol, xylitol and sorbitol are widely distributed in the nature, found in bacteria, fungi, animals and higher plants [99]. Sweetness, low glycemic index and high osmotic activity also made these compounds attractive food and pharmaceutical ingredients [100,101]. Sugar alcohols are present in many plants including *Arabidopsis thaliana* at a low level (0.1 – 2 μmol $gfwt^{-1}$) and have a role in osmotic and oxidative stress protection due to osmotic and ROS-scavenging activity [102]. Polyols were shown to scavenge hydroxyl radicals [103]. However, in certain species like celery, apple and some other woody members of the Rosacea family, an increased amount (up to 200 μmol $gfwt^{-1}$ in leaves) of energy-rich sugar alcohol, either mannitol or sorbitol is produced in source leaves and supplied to sinks as the main photosynthetic product [17,98,99]. In celery plants, mannitol accounts for as high as 50% of the phloem-translocated photoassimilates [98]. Sugar alcohol metabolism means more efficient carbon use, and better energy supply of sink tissues [17,104]. Two molecules of mannitol or sorbitol contains the same number of carbon atoms as a sucrose molecule, but also the reducing power of two NADHs, enough to produce 6 ATPs. In addition, both celery (a mannitol synthesizer) and apple (a sorbitol synthesizer) are C3 species, but were reported to have CO_2 fixation rates at around 40 mg $CO_2/dm^2 \times hr$, identical to C4 values varying between

318 30 and 60 mg $CO_2/dm^2 \times hr$, while C3 photosynthesis rate varies between 10 and 30
319 $CO_2/dm^2 \times hr$ [98,105,106]. CO_2 fixation of celery was the most studied and showed typical C3
320 characteristics but had a low CO_2 compensation point [106]. It is also notable, that apple and
321 pear, sorbitol synthesizers, and ash tree, a mannitol synthesizer, were reported among the tree
322 species having the highest photosynthetic activity [107]. Such remarkable photosynthetic
323 performance is quite surprising without carbon-concentrating mechanisms, but the following
324 explanation has been provided [98]. Oxygenase activity of Rubisco and the excess NADPH
325 generated in chloroplasts are common limitations of photosynthesis. The excess NADPH
326 possesses a serious risk, because without available $NADP^+$, photoreduction of the oxygen
327 molecule in photosystem I (PSI) generates superoxide radical [108]. In most plants
328 photorespiration takes part in solving both issues, but at a cost of carbon loss and futile
329 oxidation of the reducing power, i.e. the so called water-water cycle [109]. Meanwhile sink
330 tissues of the plant could have benefitted from these carbon and reducing power. Mannitol
331 and sorbitol synthesis has been proposed as a supplementary mechanism to dissipate reducing
332 power (NADPH) accumulated during the light reactions of photosynthesis [108]. Thus, the
333 role of polyol metabolism may be analogous to that of photorespiration in some degree by
334 dissipating excess photochemically produced reducing power (NADPH), thereby preventing
335 photoinhibition of CO_2 fixation [110]. Sugar alcohol synthesis also provides an additional
336 cytosolic sink for photosynthetically fixed CO_2, which may thereby contribute to the increase
337 in CO_2 fixation [17].

338 Sugar alcohol metabolism gets remarkably little attention in this decade, despite
339 leading to as efficient photosynthesis as C4 and would probably be easier to introduce to new
340 species. Unlike C4, anatomical alterations may not be necessary, only enzyme and transporter
341 genes need to be over-expressed, we believe. A possible scheme is suggested here. Efficient
342 loading of sugar alcohols to the phloem, followed by effective uptake and catabolism in sinks

343 can be considered key to the success. Rice can be considered as a potential candidate, owing
344 to its phloem loading pathway. According to most studies, this plant primarily utilizes
345 symplastic phloem loading and therefore in theory the produced sorbitol could freely move
346 from source to sink organs [111–114]. Symplastic continuity between the phloem and the
347 surrounding leaf tissues of rice was experimentally confirmed using different low molecular
348 weight dyes [113,114]. As for phloem unloading, the symplastic route is a common feature
349 for many sink types in most plants as well [115], though apoplastic phloem unloading
350 mechanism in certain plants/tissues cannot be excluded either. For example the first step of
351 unloading of the sucrose molecules in corn roots is found to be symplastic, but it is often
352 followed by sucrose hydrolysis by cell wall invertase enzymes and an active uptake of
353 monosaccharides by the root cells [116]. Assimilate-uptake in rice grains possibly also
354 involves apoplastic mechanisms [117,118]. To introduce sugar-alcohol metabolism to rice,
355 green tissue-specific expression of the apple-derived sorbitol-6-phosphate dehydrogenase
356 (S6PDH) can be used. The rice *rbcS* promoter can be considered for driving the expression to
357 green tissues [119]. S6PDH catalyzes the biochemical reaction to convert glucose-6-
358 phosphate to sorbitol-6-phosphate in the presence of NADPH [120]. The glucose-6-
359 phosphate, an intermediate of sucrose synthesis, is abundant in all plant leaves. The produced
360 sorbitol-6-phosphate is subsequently dephosphorylated by nonspecific endogenous
361 phosphatases to release sorbitol [121]. For utilisation of sorbitol, the NAD-dependent sorbitol
362 dehydrogenase (SDH) needs to be introduced in a sink tissue-specific manner. For instance,
363 promoter of the rice *osl43* gene can be considered for the purpose, being active in panicles,
364 stems, roots and dark-induced leaves [122]. The root-specific rice *catB* promoter or the wheat
365 endosperm-specific *Glu-1Bx17* HMW GS promoter fused to the first intron of the rice actin
366 gene (already tested in rice) may serve as alternatives [123,124]. SDH enzymes convert
367 sorbitol to fructose and produce NADH [125–128]. The uptake of sorbitol to rice grain and

368 root cortex may require the seed and root-specific expression of the apple MdSOT3 inward
369 sorbitol transporter as well [129]. Altogether, the over-expression of three genes coding the
370 S6PDH and the SDH enzymes and the MdSOT3 transporter may thus substantially increase
371 photosynthetic efficiency and growth parameters of transgenic rice. Other important cereals,
372 like barley, wheat and maize possess apoplastic phloem-loading pathway [130–132] and
373 therefore the introduction of efflux sorbitol transporters would be required to load sorbitol to
374 the phloem. It also has to be noted, that many transgenic plants engineered to produce extreme
375 high amounts of sorbitol, showed necrotic lesions [133,134]. It could be the result of osmotic
376 imbalance caused by sorbitol hyper accumulation [133]. It is of no surprise however, because
377 neither phloem loading process nor sorbitol catabolism was considered during these projects.
378 The engineered plants, tobacco and sugarcane are apoplastic phloem loading species [135–
379 137] and so the produced sorbitol could not leave the shoot system, where it was mostly
380 produced from the available glucose-6-phosphate and NADPH. SDH was not introduced into
381 these transgenic plants for sorbitol degradation either. Introduction of sugar alcohol
382 metabolism thus needs a complex multigene approach that simultaneously considers not only
383 the synthesis, but also the transport and catabolism of the newly produced assimilate.
384 Therefore we believe, the successive or separate introduction of the S6PDH (in green-tissue
385 specific manner) and the SDH (to sink tissues) enzymes and the MdSOT3 transporter (to sink
386 tissues) would not increase the productivity of rice. These genes should be co-transformed at
387 the same time for the highest effect.

388

389 **6. Conclusions and perspectives**

390 Various approaches have been either proposed or carried out to improve plant
391 productivity. Successful strategies dealt with aspects like harvest index, senescence, stress

392 tolerance and photosynthetic activity (see **Fig. 1**). As for photosynthetic improvement,
393 decreasing antennae size and optimising plant architecture are proven options to make light
394 reactions more efficient. The option to extend the absorbed spectrum of light towards IR has
395 already been suggested introducing algal pigments like chlorophyll *d* and *f* but has not been
396 tested yet [45]. Our suggestion is to introduce proteorhodopsin for effective utilisation of
397 green light. Potential benefits as well as costs of such approaches should also be considered
398 e.g. on photoinactivation, photoinhibition or on PSI and PSII coordination. While the
399 absorption of excess light might cause photoinhibition, ATP synthesis has no effect on the
400 photodamage [138]. It may argue for the application of proteorhodopsin which could act as an
401 ATP-pump, separate from either PSI or PSII. In theory, intrinsic dynamic mechanisms of the
402 chloroplasts [139] could react to the increased ATP production by proteorhodopsin and
403 maintain the optimal ATP:NADPH ratio for functioning of the dark reactions. It also has to be
404 kept in mind that many stress conditions lead to stomatal closure, so probably not the light
405 reactions, but carbon dioxide uptake and the dark reactions present the most important
406 limitations these cases. Various approaches have also been carried out to improve dark
407 reactions of photosynthesis. The implementation of C4 photosynthesis to C3 plants and
408 alternative photorespiration pathways within the chloroplast should be underlined. We believe
409 the dark reactions can be also improved by the introduction of sugar alcohol metabolism. This
410 metabolic route also means more efficient carbon utility and source to sink transport. Sugar
411 alcohols can also provide additional benefits like osmoprotection and ROS-scavenging
412 [17,140].

413 We also call for an even more ambitious approach including some form of carbon
414 dioxide transport from root to the shoot system within the xylem. Such plans are motivated by
415 the long-known fact that CO_2 level is generally higher in the soil compared to the air [141].
416 Even with equal levels, CO_2 uptake in the damp environment of roots would provide the

benefit of keeping water, unlike during CO_2 uptake in leaves through stomata. CO_2 transport to the shoot system would result in stomatal closure, since this effect has been observed for high intercellular CO_2 level in most plants [142]. We believe that while water deficit is one of the most prevalent global stressor, limiting plant productivity [143], the above mentioned approach could promise of a high photosynthetic activity and extreme drought tolerance at the same time. A C4 photosynthesis splitted between the root and shoot system (see **Fig. 2**) or active transport of HCO_3^- ion to the xylem using the cyanobacterial ictB transporter [144] can be considered for this purpose. However, these processes would put an extra energy demand on the root system which may be alleviated by the co-introduction of sugar alcohol metabolism, providing roots with more energy-rich metabolites to consume. Other factors to be counted are decreased traspirational cooling and altered, possibly decreased xylem-based transport of minerals. This form of root to shoot carbon dioxide transport thus could possibly supplement but not completely substitute the stomatal transpiration and carbon dioxide uptake. Further research should clarify the reliability and possibility of our scheme which has not been seen in the nature. Implementation of such a C4 split would require the coordinated expression of many enzymes and transporters which could be achieved only in large teams or in international consortia. During the implementation of a classic leaf-based two-cell C4 photosynthesis to C3 plants, anatomical alterations like suberization of bundle sheath cell walls may be necessary to prevent the re-diffusion of transported CO_2 to the nearby site of primary fixation [145]. Anatomical alterations may not be required however for the introduction of root to shoot carbon-dioxide transport as the distance of different plant organs could prevent the re-diffusion. It could be even easier to be engineered into existing C4 species. It is also notable, that almost all efforts to improve photosynthetic efficiency involve GMO technology. There is much to done to foster its acceptance [146]. Hopefully

biotechnology will come out with reliable solutions for enhancing plant productivity and thereby contributing to solve food and energy crises.

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Figure legends

Fig. 1. Elements of plant productivity (P): senescence, harvest index, photosynthesis and stress tolerance. Strong interactions are marked by arrows. These are connected as care always should be taken to maintain a high harvest index while improving any other trait. Increasing harvest index by enhanced sink strength also upregulates photosynthesis. However improving photosynthetic performance in itself does not always lead to an increased yield of the harvested organ. The photosynthetic route both affects the overall productivity and stress tolerance. C4 plants were shown to exhibit high water and N-use efficiency, while sugar-alcohol metabolizing plants have high osmotic stress tolerance. Plants with crassulacean acid metabolism excel with extreme drought tolerance. Stress tolerance is also in relation with senescence, because senescence delay could considerably increase drought tolerance. Delayed senescence and longer photosynthetically active period also increase productivity overall. The

873 senescence may also affect the harvest index as assimilate remobilization during senescence
874 considerably increases the yield of wheat.

875

876 **Fig. 2.** A proposed scheme of root to shoot carbon dioxide transport, through NAD ME or
877 PEPCK-type C4 photosynthesis split between the two organs. Our scheme (A) is intended to
878 transfer energy (through sugar alcohol metabolism) from the energy-rich leaves (B) to the
879 energy-poor roots (C) in exchange for CO_2, which is abundant in the vicinity of roots. CO_2 is
880 fixed by PEP-carboxylase in roots, yielding oxaloacetate (OAA). Oxaloacetate is
881 transaminated to form the more stable aspartic acid (Asp), which is then transported to the
882 shoot system through the xylem. Aspartic acid is converted to alanine (Ala) and CO_2 in leaves
883 through either NAD-ME or PEPCK C4 photosynthesis process. The produced CO_2 is fixed by
884 Rubisco. Alanine is transported back to the roots through the phloem. Pyruvate (Pyr) is
885 formed from deamination of alanine. Phosphoenolpyruvate (PEP) is regenerated from
886 pyruvate at the expense of 2 ATP. The approach may be supported by the implementation of
887 sugar alcohol metabolism (see the text for details). It would involve sorbitol supply of roots
888 (green) which is more energy-rich per carbon atom, compared to the common sucrose.
889 Sorbitol is degraded to fructose by sorbitol-dehydrogenase, forming fructose and NADH.
890 Reducing power of a NADH molecule is eligible to produce three ATPs.

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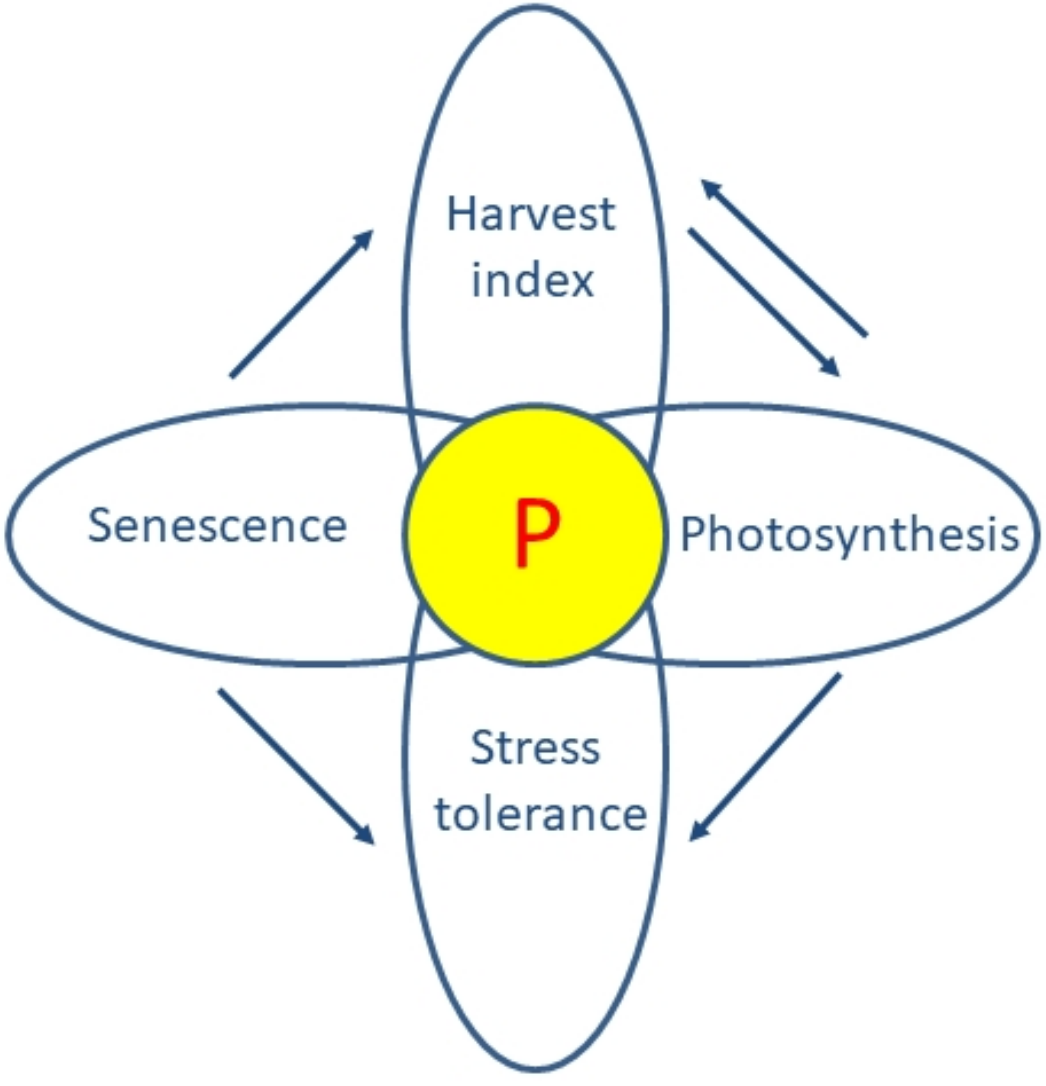
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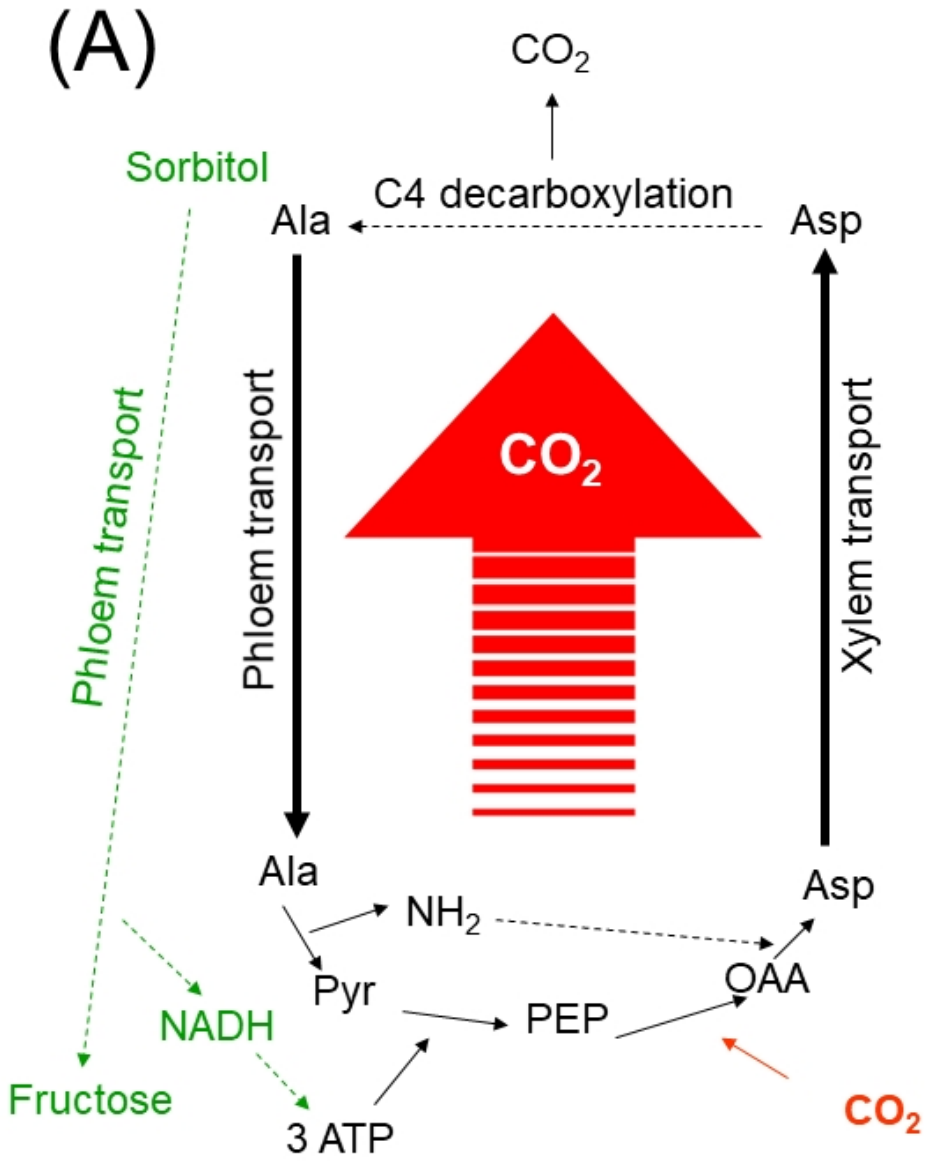
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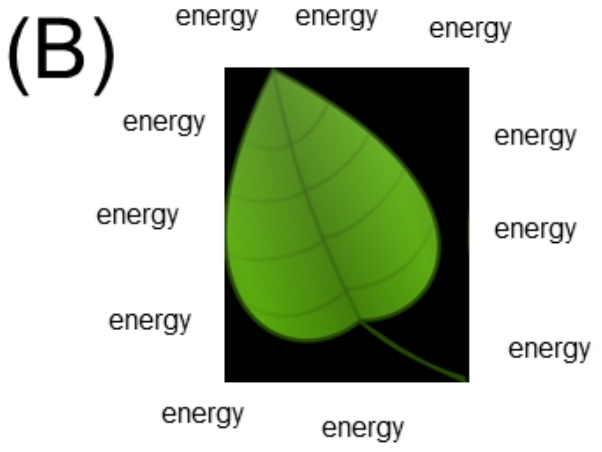
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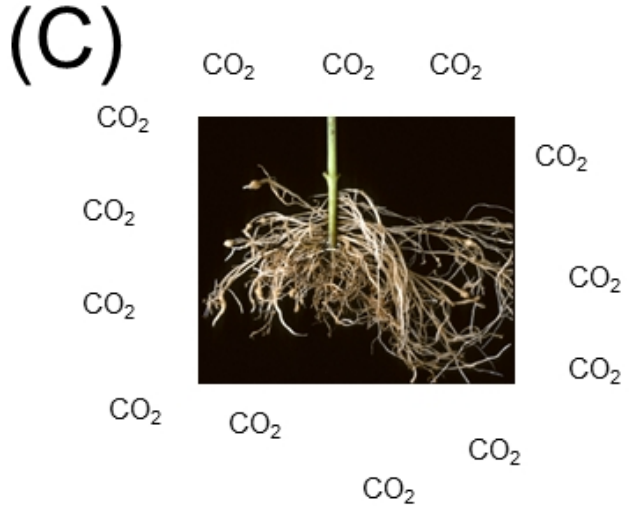
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