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Re-engineering of carbon fixation in plants – challenges for plant biotechnology to improve yields in a high-CO_2 world

Christoph Peterhansel and Sascha Offermann

Source and sink strength control plant carbon gain and yield. Source strength was recently engineered by modifying the large subunit of Rubisco, replacing the small subunit, and creating improved thermostable Rubisco activases. This technological breakthrough makes Rubisco engineering feasible at last. Enhancement of leaf transitory starch synthesis or induction of artificial sinks in leaves increased biomass and yield. Importantly, such approaches also had a positive feedback on source strength. In addition, novel targets for the improvement of carbon gain in crops have been identified that are especially relevant in the light of climate change.

Address
Gottfried Wilhelm Leibniz Universitaet Hannover, Institute of Botany, Herrenhaeuser Strasse 2, 30419 Hannover, Germany

Corresponding author: Peterhansel, Christoph (cp@botanik.uni-hannover.de)

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Introduction

Agronomical yield is basically a gain in carbohydrates such as starch in grains or tubers, sugars in sugarcane or sugar beet, oils in canola, or lignocellulose in biofuel crops. There is a strong demand for yield enhancements in view of the need to feed an expected world population of over 9 billion people by the year 2050 [1], the increasing use of crops for biofuel production, and the expected arable land shortage associated with climate change [2]. In theory, improving production of carbohydrates is limited by two factors: Strength of the source and strength of the sink. The source of carbon gain is photosynthesis, whereas multiple transitory and permanent sinks exist in photosynthetic and harvested organs (see Figure 1). Whereas biomass increase of photosynthetic organs concomitantly increases source strength, the harvested yield is mostly determined by the amount of heterotrophic tissue.

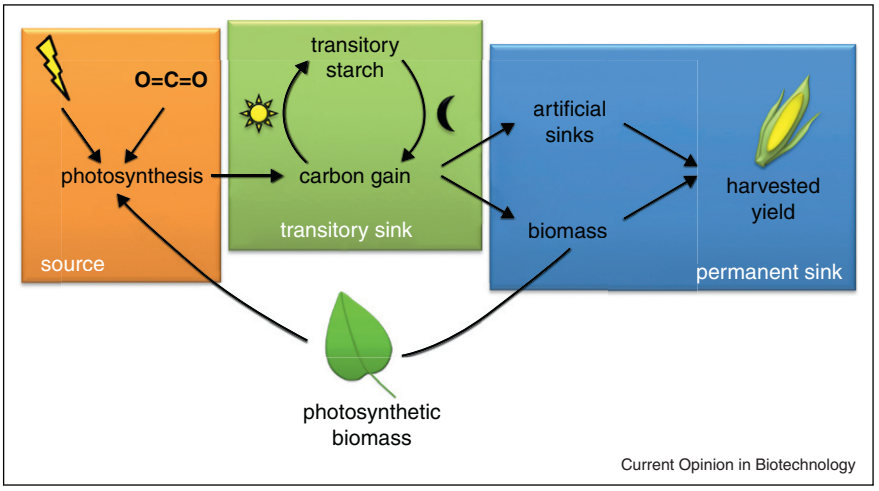
The results obtained from numerous Free-Air-CO_2 enrichment studies, where future elevated atmospheric CO_2 levels are simulated under agricultural production conditions, imply that there is still room for improvement of carbon gain in existing crops [3,4]. The increase in source strength caused by higher CO_2 availability under these conditions enhanced harvested yields indicating that source strength still limits productivity. On the contrary, this increase was much less than predicted from theory that suggests that more sink strength is required to fully exploit carbon provided by the stronger source [5].

Here, we review recent research where significant progress has been made to improve source or sink capacity with special relevance to expected future growth conditions. In addition, we discuss novel targets and future areas that might help to further optimize plant yield. Recent exciting research on the transfer of C_4 features to C_3 plants will be covered elsewhere in this issue.

Rubisco engineering becomes feasible

Ribulose-1,5-biphosphate carboxylase/oxygenase (Rubisco) is the most abundant protein on earth catalyzing the vast majority of primary carbon fixation. Despite its importance, the enzyme is an extremely slow catalyst with few turnovers per second (reviewed in [6]). Moreover, Rubisco shows appreciable affinity to O_2 as an alternative substrate that is, unfortunately, much more abundant in today's atmosphere as opposed to CO_2. O_2 fixation results in the formation of 2-phosphoglycolate that has to be recycled in photorespiration and this is associated with loss of energy as well as previously fixed carbon and nitrogen [7]. Catalytic rates and substrate specificity for CO_2 relative to O_2 show inverse correlation for most Rubiscos [8–10]. As Rubisco engineering would directly result in increases in primary productivity without any evident trade-offs, it is probably the holy grail of green biotechnology. For example, a crop prepared for the rising atmospheric CO_2 levels would benefit from a Rubisco enzyme with higher catalytic rates even if this negatively impacts on substrate specificity for CO_2 relative to O_2. However, gene replacement is hampered by the complex arrangement of genes encoding Rubisco subunits. The functional enzyme in plants is composed of eight molecules of a plastome-encoded large subunit (LSU) and a nuclear-encoded small subunit (SSU) with the latter being encoded by multiple genes [11]. In addition, the amino acids that control specific enzymatic properties were unidentified so far. Recent evolutionary analysis of Rubisco enzymes from the genus Flaveria that differ in catalytic rates and specificity for CO_2 [12] revealed that changes in only two residues of the LSU

Figure 1



Overview of source-sink relationships in carbon metabolism. Carbon dioxide (O═C═O) is fixed in source tissues into carbohydrates with the use of light energy and subsequently partitioned into transitory sinks (transitory starch) or converted to biomass. Artificial sinks provide additional means to increase sink strength and therefore have a potentially positive effect on source capacity. The source strength is further amplified through investment in photosynthetic biomass.

can explain most of the differences in catalytic properties [13]. Importantly, this has been experimentally validated in transplastomic tobacco plants by testing mutated LSUs from Flaveria with single amino acid substitutions in the LSU (Figure 2). Using this test system, the authors were able to pinpoint a switch to fast catalysis (at the expense of substrate affinity) to substitution of amino acid 309 from Met to Ile. This catalysis switch is seemingly independent of the SSU as all tested Flaveria LSUs functionally assembled with tobacco SSUs [14••]. Independent of these results, Rubisco's enzymatic properties were also engineered by overexpression of the SSU from Sorghum in rice [15•]. In this system, partial replacement of the rice SSU with the Sorghum SSU shifted enzymatic properties in the direction of the Sorghum enzyme, that is, higher catalytic rates and lower substrate affinity (Figure 2). The results indicate that exchange of small or large subunits, respectively, as well as the mutation of single amino acids in endogenous Rubiscos are suitable approaches to manipulate Rubisco enzymatics.

Besides adapting catalytic rates of Rubisco to the predicted increases in atmospheric CO_2, temperature stability of photosynthetic enzymes is another important target when preparing temperate crops species for the expected rise in global temperatures that is associated with climate change. Rubisco itself is stable and active even at high temperatures, although specificity for CO_2 declines resulting in increased photorespiration. This problem has been covered in recent reviews (e.g. [16]) and will not be discussed here. However, Rubisco activase (RCA), an essential factor for the activation of Rubisco, is unstable at elevated temperatures [17]. This property was first engineered by using a gene shuffling approach to generate a more thermostable RCA resulting in improved photosynthesis, higher biomass and better yield under moderately increased temperatures [18]. These findings were recently extended with a recombinant RCA consisting of a Rubisco activation domain from the more thermostable tobacco RCA fused to the Arabidopsis Rubisco recognition domain ([19•], Figure 2). *In vitro* analysis showed that this chimeric RCA had the positive characteristics of both, the greater heat stability of the tobacco RCA coupled with high Rubisco activation capacity of the Arabidopsis RCA. Engineering of RCA therefore seems to be a valuable add-on to approaches aiming at improving Rubisco.

Sink strength and biomass production

Any increase in carbon gain has to be translated into more biomass and yield to be of biotechnological relevance. As specified in the introduction, a positive correlation between photosynthetic rates and yield in crops exists, but actual gain in yields is lower than theoretically calculated. In most plants, major sinks for leaf photosynthesis are production of biomass or starch within the leaf or export of photosynthate to heterotrophic sink organs such as seeds or roots where it is often again stored as starch. It has been shown several times that enhancement of starch synthesis in sink organs is sufficient to increase seed or tuber yield (e.g. [20,21]). However, new data also indicate that a stronger accumulation of leaf starch used in plants to sustain metabolic functions during the night positively impacts both source strength and sink yield. In Arabidopsis, knockout mutations in starch synthase III (SS III) counter-intuitively resulted in synthesis of more transient starch, which points to a regulatory function of this isoform [22]. In a more recent approach, SS IV was