Biotechnological Approaches to Improve Carbon Fixation in Agricultural Crops

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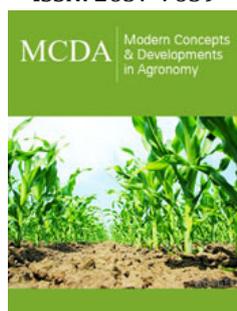
Abstract

Photosynthesis is the basis of the primary production. Conventional breeding has produced notable increases in crop productivity, although a substantial improvement in photosynthesis per se has not yet been achieved. This mini review summarizes the possibilities for biotechnological manipulation of photosynthesis and their possible application for crop improvement.

Keywords: Photosynthesis; Rubisco; Photorespiration; Stomatal conductance; Carboxysomes; Pyrenoids

Abbreviations: RUBISCO: Ribulose-1,5-Bisphosphate Carboxylase Oxygenase; NPQ: Nonphotochemical Quenching; PhAR: Photosynthetic Active Radiation

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Introduction

Currently, the knowledge of the biochemical and molecular basis on which the complex processes of CO_2 acquisition by plants are based may allow attempts to improve it by genetic modification. Since photosynthesis is the basis of plant growth and productivity, improving its efficiency may contribute to greater food security in the coming decades as the world population increases. Multiple targets have been identified that could be improved through biotechnology to increase photosynthesis of crops [1-6].

Engineering photosynthetic enzyme activity

Under intense light conditions, photosynthesis is limited by the CO_2 supply, by the working capacity of Rubisco and by the regeneration rate of ribulose 1,5-bisphosphate (the five-carbon substrate for Rubisco). Potential possibilities are to increase the amount of Rubisco molecules and to improve their affinity for the CO_2 to increase their carboxylase capacity and decrease oxygenase activity (photorespiration) [3,7,8]. However, the problem arises since carboxylase activity is not only a function of the number of Rubisco molecules, but is modulated by the concentrations of its cofactors (mainly supply of CO_2 and Mg_2^+) and by the regeneration rate of ribulose 1,5-bisphosphate, in addition to other enzymes of the Calvin cycle [3,7,9]. However, by overexpressing the small and large subunits of the Rubisco, together with an assembly chaperone protein, it was possible to increase photosynthesis and biomass in corn [10]. In tobacco, enzymes involved in glycolate metabolism have been inserted into chloroplasts to reduce photorespiration, along with reduced expression of a glycolate and glycerate transporter to minimize the flow of glycolate out of the chloroplast, increasing thus vegetative biomass in a 40% under field conditions [11]. Other actions at the enzymatic level have involved an increase in the activity of sedoheptulose 1,7-bisphosphatase, one of the key enzymes in the regulation of the Calvin-Benson cycle [12]. Thus, in transgenic wheat, the overexpression of this enzyme caused an increase in photosynthesis and grain yield [13]. It has also been suggested that the tolerance of photosynthesis at higher temperatures could be increased by improving the thermal stability of Rubisco activase, the enzyme that induces its activity under lighting conditions [14].

Introducing the C_4 cycle in C_3 crops

The possibility of introducing C_4 photosynthetic metabolism in C_3 crops such as wheat or rice, is one of the most important challenges in engineering photosynthesis [15-17]. At present, transgenic C_3 plants have been obtained that express at least one set of key enzymes of the photosynthetic C_4 pathway. However, those C_3 transgenic plants that overproduce a single C_4 enzyme show alterations in carbon metabolism [15-17]. Therefore, the correct post-translational regulation of the introduced heterologous enzymes, the adjustment of the levels of auxiliary enzymes (such as carbonic anhydrase, adenylate kinase and pyrophosphatase) and the metabolite transporters must also be addressed, as well as to achieve an effective mechanism of CO_2 concentration in the chloroplast stroma [15-17]. Alternatively, work is currently underway on the engineering of cyanobacterial carboxysomes or pyrenoid algae in C_3 plants. Carboxysomes are bacterial polyhedral micro-compartments consisting of protein layers containing Rubisco and a carbonic anhydrase, so that they act by concentrating CO_2, thus improving photosynthesis efficiency, while reducing photorespiration. Currently, it has been possible to partially assemble carboxysomes of cyanobacteria in tobacco, as well as to express genes for the enzyme Rubisco specific for cyanobacteria [18-20]. The introduction of the BicA and SbtA membrane transporters with a high affinity for bicarbonate ion of cyanobacteria in the envelope of the chloroplast has also been proposed as a mechanism for CO_2 concentration [21]. Another strategy, although less developed, is to introduce algae pyrenoids, subcellular organelles located in the plastid stroma of almost all eukaryotic microalgae with large amounts of Rubisco together with a high activity of carbonic anhydrase [22,23].

Engineering components of the photochemical system

Under excessive radiation and to avoid photooxidation of chlorophylls and subsequent damage to Photosystems, green leaves have several mechanisms that allow to dissipate excess energy that cannot be used for photosynthesis. One of these mechanisms is the so-called nonphotochemical quenching (NPQ) that protects chlorophylls from damage by heat emission, but in this process, energy is lost that is not used for photosynthesis and biomass production [24-26]. In the case of the NPQ, its activation is quite fast, but not its return to the basal level, so a faster thermal relaxation of the NPQ after a decrease in the light level, could increase the energy available for photosynthesis in instead of continuing to dissipate it in the form of heat, especially under conditions of fluctuating light, as they usually occur inside the leaf canopy. In fact, some theoretical studies of the kinetics of the process indicate that the loss in CO_2 fixation due to the slow deactivation of NPQ could be as high as 30% [27].Recently, it has been possible to accelerate the return of NPQ to its basal level in tobacco by introducing genes from *Arabidopsis thaliana* for three proteins (PsbS, VDE and ZEP) involved in the xanthophyll cycle*,* thus increasing biomass production by up to 20% in greenhouse trials and around 15% in field trials [28]. Another strategy is to extend the spectrum of photosynthetic active light absorption (PhAR), which is located in the band between 400 and 700nm wavelengths and represents approximately 50% of the energy of sunlight [29]. Few photons with wavelengths greater than 700nm are absorbed by the leaves, as they are almost entirely reflected or transmitted by leaves. However, chlorophylls *d* and *f* from cyanobacteria are capable of capturing light up to 750nm, so they could be used to extend photochemistry in terrestrial plants, thus increasing photons available for photosynthesis in sunlight by up to 19% [30].

Increased CO_2 uptake

To increase the uptake of CO_2 in C_3 plants, the classic approach has been to increase stomatal conductance [31], but this also drastically increases the rate of transpiration, since under most circumstances the outflow of water is much greater than the flow of CO_2 input [3]. Consequently, increased stomatal conductance would be detrimental to productivity in dry environments, although it has been found to correlate with higher yields under good irrigation conditions [32]. Another alternative is to modify the conductance of the mesophyll to CO_2, which depends on two anatomical attributes of the leaf, the surface of the mesophyll cells exposed to the intercellular airspaces and the thickness of their cell walls, factors that are currently difficult to modify by Genetic Engineering [33,34]. Furthermore, the permeability to CO_2 of the plasmalema and the chloroplast membranes must be also considered.

In this sense, it has been pointed out that certain aquaporins could also function as channels for CO_2 because although lipid bilayers are highly permeable to CO_2, biological membranes are very rich in proteins, which greatly reduce the area available for the diffusion of CO_2 through lipids [35,36]. In fact, the overexpression of some aquaporins such as HvPIP2;1 in rice [37], NtAQP1 in tobacco [38] and PIP1;2 in *Arabidopsis thaliana* [39] was associated with an increase in the conductance of mesophyll to CO_2. However, the physiological interpretation of these results is not straightforward, due to associated pleiotropic changes and technical challenges since, unfortunately, measuring the permeability of the membrane to CO_2 is difficult. For example, in rice, overexpression of HvPIP2;1 was accompanied by an increase in the thickness of the mesophyll cell walls [37]. However, the modification of certain aquaporins is a very interesting strategy to facilitate the diffusion of CO_2 through the plasma membrane and the envelope of the chloroplast and to collaborate in the possibility of introducing the C_4 photosynthetic pathway in C_3 agricultural crops.

Conclusion

While modern crops are highly efficient at rapidly unfolding their leaves to maximize light interception, they are not as efficient at converting absorbed light energy to carbohydrates through photosynthesis. This may be due to the fact that the proteins and enzymes implicated in the photosynthetic process evolved in a marine environment with little light and absence of oxygen, very different therefore from current agronomic and atmospheric conditions. However, as we have discussed, there are several biotechnological possibilities, although the modification of photosynthesis at the chloroplast or leaf level should be accompanied by an improvement at the level of the crop canopy.

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