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Harnessing C4 Genes to Supercharge Photosynthesis in C3 Plants

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Introduction

Photosynthesis is the fundamental process driving plant productivity and sustaining life on Earth. Among terrestrial plants, the majority—including major crops such as rice, wheat, and soybean—follow the C3 photosynthetic pathway, where carbon dioxide (CO_2) is directly fixed by the enzyme ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco). However, Rubisco is inherently inefficient due to its oxygenase activity, which leads to photorespiration and substantial loss of assimilated carbon, particularly under high temperature, drought, and light stress conditions. In contrast, C4 plants such as maize, sorghum, and sugarcane have evolved a sophisticated CO_2-concentrating mechanism that minimizes photorespiration by compartmentalizing photosynthetic reactions between mesophyll (MC) and bundle sheath cells (BSCs). This adaptation, coupled with the presence of specialized enzymes like phosphoenolpyruvate carboxylase (PEPC), pyruvate phosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME), confers superior photosynthetic efficiency and enhanced water and nitrogen-use efficiency.

The advent of recombinant DNA technology has enabled researchers to explore the molecular transfer of C4 photosynthetic traits into C3 plants. By introducing genes encoding key C4 enzymes and regulatory elements, scientists aim to reconstruct partial or full C4-like pathways in C3 crops, with the ultimate goal of improving photosynthetic performance and yield potential under stress-prone environments. This approach not only advances our understanding of the biochemical and anatomical foundations of C4 photosynthesis but also provides valuable insights into its evolutionary origin and potential for crop improvement.

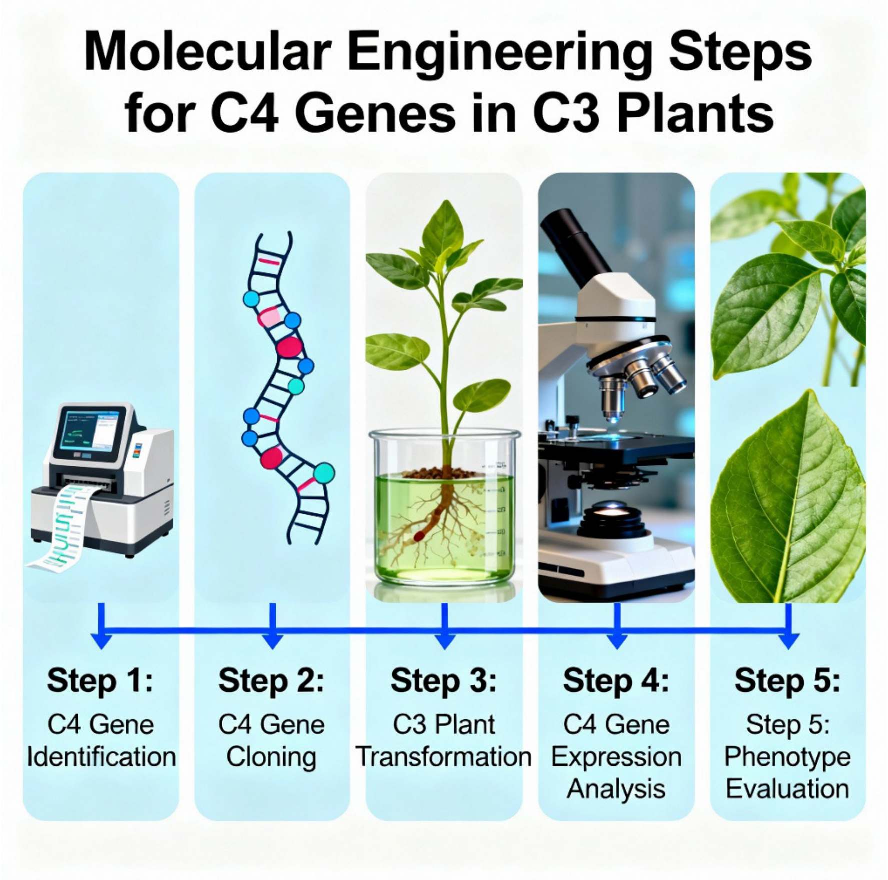
C3 and C4 Photosynthesis: Mechanisms, Efficiency, and Evolutionary Adaptation

The majority of terrestrial plants, including many important crops such as rice, wheat, soybean, and potato, are classified as C_3 plants that assimilate atmospheric CO_2 directly through the C_3 photosynthetic pathway. C_4 plants such as maize and sugarcane evolved from C_3 plants, acquiring the C_4 photosynthetic pathway to achieve high photosynthetic performance and high water- and nitrogen-use efficiencies. The recent application of recombinant DNA technology has made considerable progress in the molecular engineering of C_4 photosynthesis over the past several years. It has deepened our understanding of the mechanism of C_4 photosynthesis and provided valuable information as to the evolution of the C_4 photosynthetic genes. It also has enabled us to express enzymes involved in the C_4 pathway at high levels and in desired locations in the leaves of C_3 plants for engineering of primary carbon metabolism.

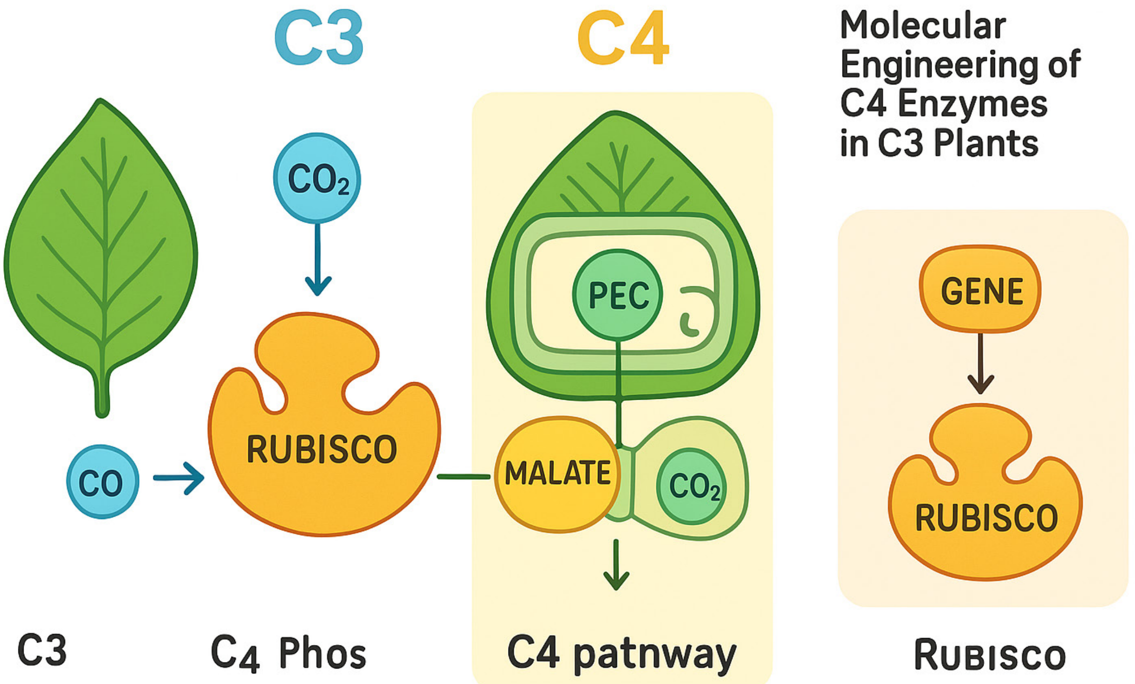
The enzyme of primary CO_2 fixation in this pathway, ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco), reacts not only with CO_2 but also with O_2, leading to photorespiration, which essentially wastes assimilated carbon. Under current atmospheric conditions, potential photosynthesis in C_3 plants is suppressed by oxygen by as much as 40%. The extent of suppression further increases under stress conditions such as drought, high light, and high temperature, through a decline of the CO_2 concentration inside leaves due to closure of stomata. C$_4$ plants such as maize, sorghum, and sugarcane have evolved a novel biochemical mechanism to overcome photorespiration. In addition to the C$_3$ pathway, they use the C$_4$photosynthetic cycle to elevate the CO$_2$ concentration at the site of Rubisco and thus suppress its oxygenase activity. This mechanism enables C$_4$ plants to achieve elevated photosynthetic capacity particularly at higher temperatures, of up to twice as high as that of C3 plants, in addition to higher water and nitrogen-use efficiencies. Leaves of C4 plants have two types of photosynthetic cells, the mesophyll cell (MC) and bundle sheath cell (BSC). While all the photosynthetic enzymes are confined in MCs in C3 plants, they are localized in MCs and/or BSCs in C4 plants. In addition, C4 plants show extensive venation, with a ring of BSCs surrounding each vein and an outer ring of MCs surrounding the bundle sheath. This unique leaf structure, known as Kranz anatomy, and the cell-specific compartmentalization of enzymes are essential for operation of the C4 pathway

Molecular engineering of C$_4$ enzymes in C$_3$ plants

Attempts have been made to transfer C4 traits to C3 plants by conventional hybridization between C$_3$ and C$_4$ plants. However, this approach was available only in several plant genera such as *Panicum, Moricandia, Brassica, Atriplex*, and *Flaveria*. Moreover, most C$_3$-C$_4$ hybrids showed infertility due to abnormal chromosome pairing and/or genetic barriers. Recent developments in plant genetic engineering have enabled us to introduce the desired genes encoding C$_4$ enzymes into C$_3$ plants. In the past several years a variety of "C4 transgenic" C3 plants have been produced .



Enzymes Located in the Mesophyll Cells of C$_4$ Plants :The first attempt of thiskind used a chimeric gene construct containing a cDNA of the maize C4-specific PEPC (*Ppc* cDNA) fused to the 50- and 30-flanking sequences of the chlorophyll *a/b* binding protein gene (*Cab*) from *Nicotiana plumbaginifolia*. The introduction of this chimeric gene into tobacco increased the PEPC activity in the leaves to 2.2-fold that of non transformants, but the levels of transcripts and protein in these transformants were far below those in maize. Similarly, the expression of a cDNA for the C4 enzyme under the control of strong promoters such as *Cab, rbcS*, and *Cauliflower mosaic virus* 35S promoters led to only two- to fivefold increases in the activity of PEPC, PPDK and NADP-MDH . In these transformants, the level of the enzyme protein was low and only detectable by immunoblotting. The expression of bacterial *Ppc* genes from either *Escherichia coli* or *Corynebacterium glutamicum* under the control of the 35S promoter increased the enzyme activity of transgenic potato leaves but the extent of increase was less than several fold, a value almost comparable to that obtained with the higher plant *Ppc* cDNA under the control of the 35S promoter. To raise the expression level of the C4 enzymes, sequences that have enhancer-like effects were included in the introduced gene. Gehlen examined the effects of the 50-untranslated region (UTR) of the chalcone synthase gene from parsley, and found that the expression of the *Ppc* gene from *C. glutamicum* under the control of the 35S promoter was enhanced by its presence. The highest expression level, however, was still only fivefold that of nontransformants in terms of PEPC activity. Thus, conventional strategies to express foreign genes in transgenic plants did not dramatically increase the activity of C4 enzymes in the leaves of C3 plants.



Enzymes Located in the Bundle Sheath Cells of C4 Plants:

Unlike the C4 enzymes located in MCs of C4 plants, those located in BSCs can be expressed at high levels in MCs of C3 plants by the introduction of a chimeric gene containing the full-length cDNA for the C4 enzyme fused to the *Cab* promoter, which directs mesophyll-specific expression in C3 plants. The expression of the maize C4-specific NADP-ME cDNA under the control of the rice *Cab* promoter increased the activity of NADP-ME in rice leaves to 30- or 70-fold that of non-transformants . The level of the NADP-ME protein was also increased to several percent of total leaf soluble protein. Such high-level expression was unique to the cDNA for the C4-specific NADP-ME, and expression of the cDNA for the C3-specific isoform increased the activity only several fold . The *Flaveria* C4-specific *Me* gene has a regulatory sequence in the 30UTR that enhances its expression , and this sequence increases expression of a reporter gene when combined with heterologous promoters in the leaves of both C4 *Flaveria* and tobacco (S Ali&WC Taylor, unpublished information). The maize C4-specific *ME* gene may therefore contain such an enhancer element for high-level expression whereas theC3-specific gene does not. The expression of a cDNAof theC4-specific PEP-CK of *Urochloa panicoides* under the control of the maize C4-specific *Ppc* or *Pdk* promoter was also effective in increasing the activity of PEP-CK in MCs of rice leaves. Recently, expression of the intact gene for C4 enzymes located in BSCs of C4 plants in C3 plants has also been addressed. When the intact gene for the mitochondrial AspAT of *Panicum miliaceum*, which is located in BSCs, was introduced into rice, high AspAT activity was detected in vascular tissues and BSCs of transgenic rice plants (M Nomura &MMatsuoka, unpublished observations).

Physiological Impacts of Overproduction of C4 Enzymes in C3 Plants

PEPC: At present, there are four independent reports of transgenic C3 plants that overproduce PEPC in the MC cytosol two independent reports of transgenic tobacco plants expressing the maize C4-specific PEPC gene transgenic potato expressing a bacterial PEPC gene from *C. glutamicum* , and transgenic rice expressing the maize C4-specific PEPC gene . In the former three cases, PEPC activities in the leaves of 2- to 5-fold greater than wild-type levels were reported, whereas in the latter, activities up to 110-fold greater than wild-type were observed.

Effects of over expression of PEPC on photosynthesis are controversial. At temperatures optimal for plant growth, practically no difference in the rate of CO2 assimilation and the CO2 compensation point (0) were observed in transgenic tobacco expressing the maize PEPC gene. Activities of PEPC were only about twofold higher than in wild-type plants. In transgenic rice plants expressing the maize PEPC gene, the rate of CO2 assimilation was also not altered significantly, but the O2 inhibition of net CO2 assimilation was mitigated with increasing activity of PEPC. The major increase in PEPC activity may lead to depletion of Pi in the cytosol, through a stimulation of glycolysis that would suppresses sucrose synthesis. These collective reactions consume one Pi molecule and release four Pi molecules, respectively. Changes in the photosynthetic characteristics at optimal temperatures have been reported only in transgenic potato expressing the bacterial PEPC . In this case, it was reported that the CO2 compensation point independent of respiration (0¤), measured according to Brooks & Farquhar , decreased by about 16% in the transformants with fivefold activity, as compared with wild-type plants. Significant changes in the photosynthetic characteristics in PEPC transformants have thus far been observed mostly at supra optimal temperatures.

PPDK There are four reports of transgenic C3 plants that express PPDK in the MC chloroplast transgenic *Arabidopsis* , potato , rice expressing the maize C4-specific PPDK gene, and transgenic tobacco expressing a PPDK gene from the CAM plant *Mesembryanthemum crystallinum* . In all cases, no changes in photosynthetic characteristics were observed in these transformants, even in the transgenic rice with PPDK activity 40-fold higher than wildtype levels. A modest increase in the ±13C value was reported in the transgenic potato but this difference was marginal in significance. Some changes in the level and composition of free amino acids were also reported in the transgenic tobacco . In general, the overall PPDK reaction is freely reversible, depending on concentrations of substrates, activators, and inactivators. This is probably the case in MCs of C3 plants, in which the activity of inorganic pyrophosphatase and adenylate kinase is low and could be the reason why the overexpression of PPDK does not result in significant effects on carbon metabolism in C3 leaves. The expression of chloroplast-targeted PPDK increased the number of seeds per seed capsule and the weight of each seed capsule by about 40% and 20%, respectively, in transgenic tobacco, with about a 1.5-fold increase in activity of PPDK in the leaves. These effects were not observed in transgenic tobacco plants that express PPDK in the cytosol . The mechanism of the increase in seed yield by PPDK is obscure at present. One possibility is that over expression of PPDK in the chloroplast enhances photosynthesis in organs surrounding seeds. In seed pods of C3 dicots and spikelets of C3 monocots, enzyme activities associated with the C4 pathway are high and C4-like photosynthesis is operative, contributing significantly to grain filling . Thus, it is possible that over expression of PPDK in the chloroplast enhances C4-like photosynthesis in organs such as hulls and ears to raise the yield of seeds and grains.

NADP-ME: There are four reports of transgenicC3 plants that express NADP-ME

in the MC chloroplast two sets of transgenic rice plants expressing the maize C4-specific isoform , transgenic rice expressing the rice C3-specific isoform , and transgenic potato expressing the C3-specfic isoform of *Flaveria pringlei* . The transformants expressing the C3-specific isoform with activities up to several fold higher than wild-type levels did not show any detectable differences in their growth and photosynthesis, whereas those over expressing the maize C4-specific isoform showed serious stunting and leaf photo bleaching, due to increased photoinhibition of photosynthesis under natural light conditions . It is proposed that the maize C4 NADP-ME in the chloroplasts acts to increase the NADPH/NADP ratio and to suppress photorespiration, rendering photosynthesis more susceptible to photoinhibition. Such detrimental effects of the maize enzyme might imply significant flexibility of carbon metabolism in MCs of C3 plants, especially in terms of transport of metabolites between the cytosol and the chloroplast stroma.

PEP-CK : There is only one report of transgenic rice plants that express the C4-

specific PEP-CK of *U. panicoides* in theMC chloroplast. Although this enzyme is located in the BSC cytosol of *U. panicoides*, the introduced construct was designed so that the enzyme was targeted to the MC chloroplasts in transgenic rice leaves. The expression of chloroplast-targeted PEP-CK showed significant alterations of carbon metabolism in rice leaves . In experiments of transgenic rice with PEP-CK activity comparable to that in *U. panicoides*, about 20% of the radioactivity was incorporated in the C4 compounds, malate, OAA, and aspartate. Feeding of 14C-labeled malate also increased the incorporation of the radioactivity into sucrose.

Reference

Lin, M.-T., Occhialini, A., Andralojc, P. J., Parry, M. A. J., & Hanson, M. R. (2020). A Partial C4 Photosynthetic Biochemical Pathway in Rice. **Frontiers in Plant Science***, 11, 564463. https://doi.org/10.3389/fpls.2020.564463*

Ermakova, M., Danila, F. R., Furbank, R. T., & von Caemmerer, S. (2021). Installation of C4 photosynthetic pathway enzymes in rice using a single construct. **Plant Biotechnology Journal***, 19(8), 1442–1456. https://doi.org/10.1111/pbi.13487*

Cui, X., Xu, Y., Wang, J., & Wang, J. (2021). Challenges and Approaches to Crop Improvement Through C4 Photosynthesis Engineering. **Frontiers in Plant Science***, 12, 715391. https://doi.org/10.3389/fpls.2021.715391*