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Brief Communication

Carbon-positive photorespiratory bypass via the tartronyl-coenzyme A pathway enhances carbon fixation efficiency and yield in rice

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Photorespiration leads to the loss of over 30% of photosynthetic assimilates in C_3 plants, decreasing crop productivity substantially (Ort *et al*., 2015; Walker *et al*., 2016; Yin and Struik, 2015). To mitigate this loss, several alternative pathways have been proposed and applied in model plants and some crops to bypass photorespiration (Chen *et al*., 2025; Jin *et al*., 2023; Smith *et al*., 2023, 2025). However, these approaches still face many challenges in practical applications, including the complexity of metabolic networks, optimization of energy consumption and uncertainties in molecular regulatory mechanisms (Smith *et al*., 2023, 2025). In particular, most photorespiratory bypasses only decrease carbon losses but fail to be carbon-positive, that is, increase net carbon gain through additional CO_2 fixation.

Synthetic biology offers new solutions for modifying photorespiratory pathways through "design metabolism" strategies (Wurtzel *et al*., 2019). One such strategy is the tartronyl-coenzyme A (TaCo) pathway. The TaCo pathway consists of three enzymes: glycolyl-CoA synthase (GCS), glycolyl-CoA carboxylase (GCCM5) and tartronyl-CoA reductase (TCR), which together convert glycolate into glycerate (Scheffen *et al*., 2021). The pathway starts with the conversion of glycolate to glycolyl-CoA by GCS, followed by its carboxylation to tartronyl-CoA by GCCM5 and reduction to glycerate by TCR (Figure 1a). This pathway not only reduces ATP and reducing equivalents consumption by 20% and 30%, respectively, but also introduces additional CO_2 through glycolyl-CoA carboxylation, resulting in a carbon-positive photorespiratory bypass (Scheffen *et al*., 2021; Smith *et al*., 2025). The TaCo pathway has shown promising results *in vitro*, but its application in plants has not been tested. This study aims to introduce the TaCo pathway into rice and evaluate its effectiveness in improving crop productivity.

Given that the substrate glycolate and the product glycerate of the TaCo pathway both exist naturally in chloroplasts, introducing this pathway into chloroplasts as a photorespiratory bypass represents a strategic advantage. The integration of the TaCo pathway into chloroplast metabolism ensures efficient utilization of intermediates, minimizing metabolic bottlenecks and maximizing carbon gain. To introduce key enzymes in the TaCo pathway into rice chloroplasts, we optimized the codons of GCS, GCCM5 and TCR to enhance their expression in rice (Data S1). Subsequently, a highly efficient chloroplast-targeting peptide, RC2, was fused to the N-terminus of each enzyme and the green fluorescent protein (GFP) was attached to the C-terminus. Transient expression assays in rice protoplasts confirmed the successful expression of all three enzymes and their co-localization with chloroplast fluorescence, demonstrating the feasibility of introducing the TaCo pathway into rice chloroplasts as a photorespiratory bypass (Figure 1b).

To create stable genetically modified rice plants with TaCo bypass, we constructed *TaCo-pYL1300H* by the Cre/loxP multi-gene expression system, incorporating the genes encoding GCS, GCCM5 and TCR. Each enzyme was fused at the N-terminus with RC2 and at the C-terminus with specific tags: 3 × FLAG for GCS, 3 × HA for GCCM5 and Myc for TCR. Each cassette was driven by different promoters and terminators (Figure S1). Both the empty vector (*pYL1300H*) and *TaCo-pYL1300H* were introduced into rice (*Oryza sativa* L. cv. Nipponbare) via *Agrobacterium*-mediated transformation. At least 20 independent homozygous lines were generated for each construct, including empty vector-transformed Nipponbare as the wild-type (WT) control (Figure 1c). Finally, RT–PCR and immunoblot assay confirmed the successful expression of the three enzymes in T_3 progeny of the TaCo plants (Figure 1d,e).

Analysis of key endogenous compounds involved in photorespiratory metabolism showed significantly elevated levels of glycolate and glyoxylate, while glycine, serine and glycerate were remarkably decreased in TaCo plants compared to WT (Figure S2a–e). These findings suggested that the TaCo pathway

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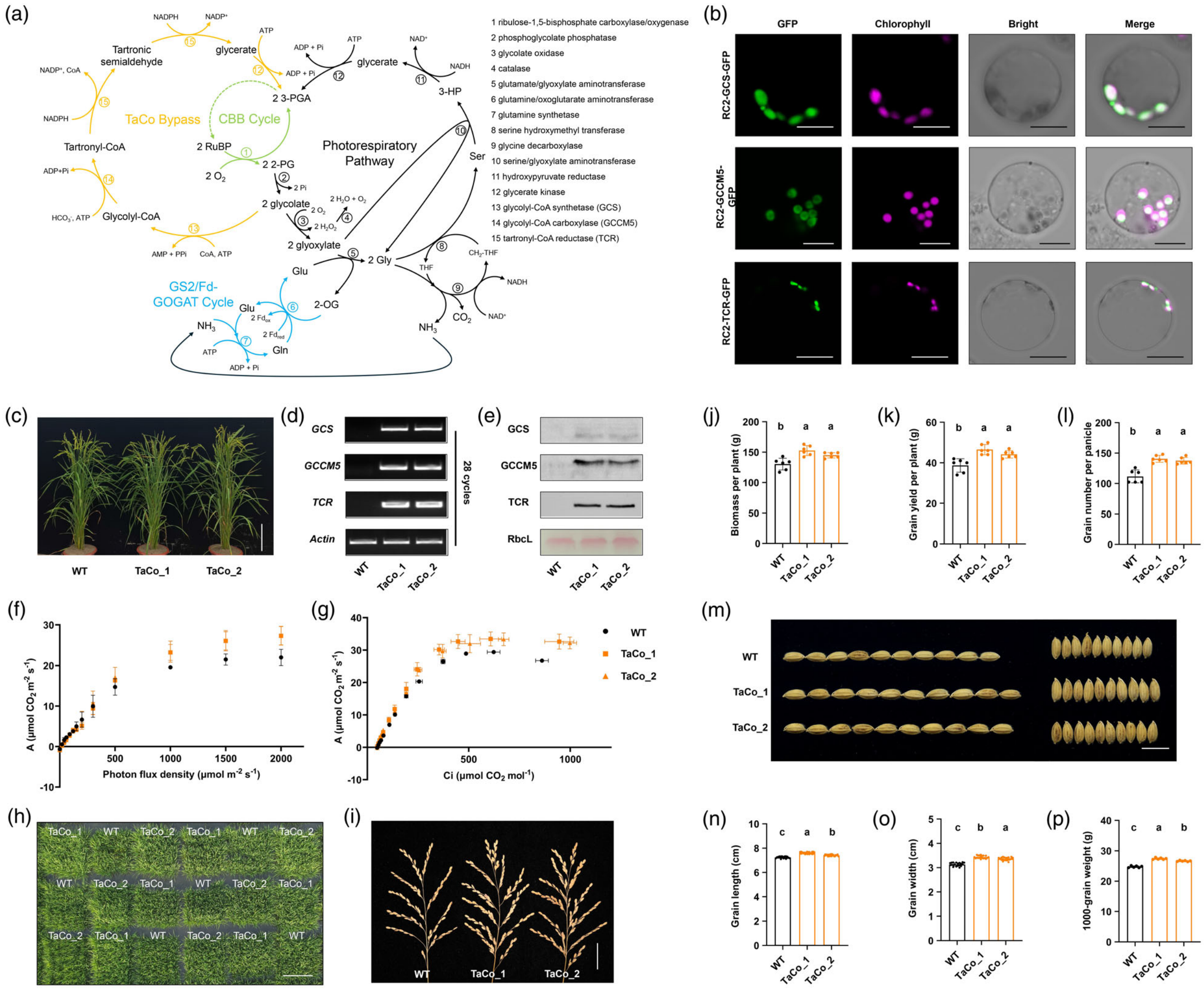

Figure 1 The TaCo pathway as a photorespiratory bypass in plant chloroplasts (Details provided in Appendix S1).

altered typical photorespiratory metabolite contents, effectively bypassing the native photorespiratory pathway. Previous studies have shown that the TaCo pathway integrates into photorespiratory metabolism, enhances CO_2 fixation efficiency and significantly boosts the production of metabolites such as malate (Scheffen *et al.*, 2021). Our analysis of malate content in WT and TaCo plants revealed that TaCo plants exhibited approximately 65% higher malate levels than WT (Figure S2f). This result indicated that the TaCo bypass could effectively promote malate synthesis through a series of carboxylation reactions, further reinforcing its carbon-positive role.

Next, we carried out gas exchange analysis to evaluate light and CO_2 response curves in both TaCo and WT plants. At photon flux densities (PFDs) exceeding 500 μmol·m^{-2}·s^{-1}, significant differences in net CO_2 assimilation rate (A) were observed between WT and TaCo plants (Figure 1f), although day respiration (R_d) and CO_2 assimilation quantum efficiency (Φ_{CO2}) under low-light levels remained unaffected in the TaCo plants (Table S1). TaCo plants demonstrated a 17.7%–18.3% increase in $A_{sat1000}$ over WT plants when adapted to a PFD of 1000 μmol·m^{-2}·s^{-1} (Table S1), with this difference expanding to 24.1%–24.5% at a PFD of 2000 μmol·m^{-2}·s^{-1} (Data S2), indicating that the bypass plants adapt superior to WT plants in high light intensity environments. Additionally, CO_2 response curves revealed that TaCo plants had higher CO_2 assimilation rates than WT plants across various CO_2 conditions, particularly when ambient CO_2 concentrations (C_a) were above 400 μmol·mol^{-1} (Figure 1g). Furthermore, TaCo plants exhibited significantly enhanced CO_2 carboxylation efficiency (CE) at low CO_2 levels ($C_a < 100$ μmol·mol^{-1}) and a lower C_i-based CO_2 compensation point in the absence of day respiration (C_i*) compared to WT rice plants (Table S1). These findings indicated that the observed increase in net photosynthetic efficiency was not due to diminished photorespiration, but rather additional carboxylation reactions introduced by the TaCo pathway.

The agronomic traits of field-grown WT and TaCo plants were evaluated in a randomized complete block design with six biological replicates for each plant type (Figure 1h). The results showed no significant differences in plant height (Figure 1c; Figure S3a). TaCo plants exhibited increases in biomass and grain yield per plant by 11.1%–17.2% and 14.2%–20.2%, respectively (Figure 1j,k), even though the effective tiller number is reduced by 7.6%–8.4%, compared to WT plants (Figure S3b). Further assessments of the panicles revealed no significant differences in panicle length between WT and TaCo plants (Figure S3c). Nonetheless, TaCo plants showed a higher number of primary and secondary branches, resulting in a 23.1%–26.0% increase in grain number per panicle compared to WT plants (Figure S3c,d; Figure 1l). These findings suggest that the increased yield in TaCo plants can be primarily attributed to their higher grain number per panicle. In addition, TaCo plants exhibited substantial improvements in grain size, including increased grain length and grain width compared to WT plants (Figure 1m–o). Specifically, 1000-grain weight increased 7.6%–10.7% in TaCo plants compared to WT plants (Figure 1p). However, the seed setting rate was not affected in TaCo plants (Figure S3f). These findings suggested that biomass and yield increase in TaCo plants could be mainly associated with the enhancements in grain number per panicle and grain size.

Transcriptome analysis revealed upregulated genes significantly enriched in the starch and sucrose metabolism pathway (Figure S4a), promoting carbohydrate partitioning and accumulation during grain filling, leading to increased grain size and weight. Conversely, downregulated genes were enriched in the zeatin biosynthesis pathway, particularly cytokinin-degrading enzymes like CKX, indicating inhibited cytokinin degradation in TaCo plants (Figure S4b). Metabolomics confirmed significantly higher levels of N^6-isopentenyladenine (iP), a cytokinin involved in panicle differentiation and grain size (Figure S5), aligning with suppressed cytokinin degradation genes. These findings suggest that improved carbon allocation and cytokinin retention contribute to the superior agronomic traits observed in TaCo plants. Primary metabolomic analyses also revealed significant changes in energy metabolism as evidenced by increases in ATP, citric acid, isocitric acid, fumaric acid, 2-OG and cis-aconitic acid. This result further validates the high malate content, which plays a central role in enhancing carbon fixation and ATP production. These metabolic shifts also contributed to enhanced amino acid synthesis, including tryptophan, methionine, tyrosine, lysine and valine. Notably, sugar metabolism changes revealed higher sucrose content and lower glucose content in TaCo plants (Figure S5; Data S3).

The TaCo pathway offers a synthetic, carbon-positive alternative to traditional photorespiration in C_3 plants, effectively reducing glyoxylate and H_2O_2 toxicity while enhancing CO_2 fixation efficiency. By converting glycolate to glycerate within chloroplasts, it minimizes ROS generation and energy loss, improving photosynthesis and yield, particularly under high light and elevated CO_2 levels. TaCo plants show increased biomass, grain yield and grain size due to enhanced carbon allocation and cytokinin retention. In summary, this study proposes a novel strategy to enhance photosynthetic efficiency and yield in C_3 crops, which is crucial for global food security. However, potential trade-offs may include increased nutrient demands and possible metabolic imbalances under stress. Future research should focus on evaluating TaCo performance under conditions such as drought, heat, salinity and nutrient limitation to ensure stability and scalability.

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Conflict of interest

The authors declare no conflicts of interest.

Author contributions

G.C., K.J. and J.W. conceived the main experiments. Y.L. performed the HPLC/MS measurements. X.T. performed the transcriptome and metabolome analyses. L.Z. and S.W. performed the transformation experiments. J.Y. performed the gas exchange measurements. G.C. and K.J. contributed to the data analysis. X.C., J.S. and X.S. contributed to the overall discussion of the data. G.C., Z.Z. and T.L. designed and wrote the manuscript.

Data availability statement

The data underlying this article are available in the article and in its online supplementary material.

References

Chen, G., Li, Y., Jin, K., Gao, J., Wu, S., Cui, X., Mao, C. *et al*. (2025) Synthetic photorespiratory bypass improves rice productivity by enhancing photosynthesis and nitrogen uptake. *Plant Cell*, **37**, koaf015.

Jin, K., Chen, G., Yang, Y., Zhang, Z. and Lu, T. (2023) Strategies for manipulating Rubisco and creating photorespiratory bypass to boost C_3 photosynthesis: prospects on modern crop improvement. *Plant Cell Environ.* **46**, 363–378.

Ort, D.R., Merchant, S.S., Alric, J., Barkan, A., Blankenship, R.E., Bock, R., Croce, R. *et al*. (2015) Redesigning photosynthesis to sustainably meet global food and bioenergy demand. *Proc. Natl. Acad. Sci. USA*, **112**, 8529–8536.

Scheffen, M., Marchal, D.G., Beneyton, T., Schuller, S.K., Klose, M., Diehl, C., Lehmann, J. *et al*. (2021) A new-to-nature carboxylation module to improve natural and synthetic CO_2 fixation. *Nat. Catal.* **4**, 105–115.

Smith, E.N., van Aalst, M., Tosens, T., Niinemets, Ü., Stich, B., Morosinotto, T., Alboresi, A. *et al*. (2023) Improving photosynthetic efficiency toward food security: strategies, advances, and perspectives. *Mol. Plant*, **16**, 1547–1563.

Smith, E.N., van Aalst, M., Weber, A.P.M., Ebenhöh, O. and Heinemann, M. (2025) Alternatives to photorespiration: a system-level analysis reveals mechanisms of enhanced plant productivity. *Sci. Adv.* **11**, eadt9287.

Walker, B.J., VanLoocke, A., Bernacchi, C.J. and Ort, D.R. (2016) The costs of photorespiration to food production now and in the future. *Annu. Rev. Plant Biol.* **67**, 107–129.

Wurtzel, E.T., Vickers, C.E., Hanson, A.D., Millar, A.H., Cooper, M., Voss-Fels, K.P., Nikel, P.I. *et al*. (2019) Revolutionizing agriculture with synthetic biology. *Nat. Plants*, **5**, 1207–1210.

Yin, X. and Struik, P.C. (2015) Constraints to the potential efficiency of converting solar radiation into phytoenergy in annual crops: from leaf biochemistry to canopy physiology and crop ecology. *J. Exp. Bot.* **66**, 6535–6549.

Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Figure S1–S5.
Table S1–S2.
Data S1–S4.
Appendix S1.