**Engineering C4 Photosynthesis Pathways into C3 Crops to Improve Nutrient Use Efficiency: A Review**

**Abstract**

Photosynthesis is the cornerstone of plant productivity and, by extension, global food security. C4 photosynthesis, characterized by its biochemical and anatomical adaptations, offers superior nutrient and water use efficiency compared to the more common C3 pathway. With the growing demand for food due to population growth and climate change, engineering C4 photosynthetic traits into C3 crops has emerged as a promising strategy to enhance crop yields and resource use efficiency. This paper provides a comprehensive review of the molecular, biochemical, and anatomical differences between C3 and C4 photosynthesis, followed by an exploration of recent advances in synthetic biology and genetic engineering aimed at introducing C4 pathways into C3 crops. In C3 plants, the carbon fixation process is inefficient under high temperatures and low CO2 concentrations due to photorespiration. On the other hand, C4 plants employ a two-cell system to concentrate CO2 around RuBisCO, minimizing photorespiration. In mesophyll cells, phosphoenolpyruvate carboxylase (PEPC) fixes CO2 into oxaloacetate (OAA), which is then converted into malate or aspartate. These compounds are transported to bundle-sheath cells, where CO2 is released for the Calvin cycle. Several studies have successfully introduced C4 genes into rice, resulting in improved photosynthetic efficiency and nitrogen use efficiency. Moreover, the introduction of C4 enzymes into soybean has led to modest improvements in water use efficiency, particularly under drought conditions. International initiatives, such as the C4 Rice Project, highlight the importance of collaborative efforts in advancing this field. A meta-analysis of recent case studies highlights the progress, challenges, and future prospects of this transformative approach. Looking ahead, interdisciplinary research combining plant physiology, genetic engineering, and computational modeling will be key to unlocking the full potential of C4 engineering in C3 crops.

***Keywords:*** C4 photosynthesis, C3 crops, nutrient use efficiency (NUE), metabolic engineering, climate resilience, food security.

**1. Introduction**

Global food demand is projected to increase by 50% by 2050, necessitating significant improvements in agricultural productivity (Long et al., 2015; Leakey et al., 2019). However, conventional breeding approaches are reaching their limits, and climate change exacerbates the challenges by increasing the frequency of droughts, heat stress, and nutrient limitations (Cui, 2021). Climate change risks are magnified by increasing water withdrawals for households and industry to 2050, especially for irrigated agriculture that accounts for about 70% of total water withdrawals and supplies up to 40% of the global human-consumed calories (Kompas et al., 2024). Photosynthesis, the process by which plants convert sunlight into chemical energy, is a key target for improving crop productivity (Leegood, 2013; Covshoff & Hibberd, 2012). This complex and highly regulated process not only sustains plant life but also forms the basis of the food chain, underpinning global agricultural systems (Anbarasan & Ramesh, 2022). While most crops, including rice, wheat, and soybeans, use the C3 photosynthetic pathway, C4 plants such as maize and sugarcane exhibit higher photosynthetic efficiency, particularly under conditions of high temperature, light intensity, and limited water or nitrogen availability (Sage & Zhu, 2011; Ruan et al., 2012). These plants have efficient carbon-concentrating mechanisms and higher water-use efficiency, presenting a promising solution to mitigate the impacts of these human-induced stresses (Kumar et al., 2024). C4 photosynthesis is characteristic of these crops, and it initiates with the formation of a four-carbon molecule (oxaloacetate, OAA) by the enzyme phosphoenolpyruvate carboxylase (PEPC), which is then converted and releases CO2 for carbon fixation by RuBisCO (Wang, 2024).

Engineering C4 traits into C3 crops has the potential to revolutionize agriculture by improving nutrient use efficiency (NUE), water use efficiency (WUE), and overall yield (Von Caemmerer et al., 2012; Way et al., 2014). This paper reviews the biological basis of C4 photosynthesis, the genetic and biochemical tools available for engineering C4 pathways, and recent case studies that demonstrate progress and challenges in this field. A meta-analysis of recent studies is conducted to evaluate the feasibility and impact of this approach.

**1.1 Global Food Security and Agricultural Challenges**

The world’s population is projected to exceed 9.7 billion by 2050, necessitating a 50–70% increase in food production to meet growing demand. However, agricultural expansion is constrained by limited arable land, water scarcity, and environmental degradation, making yield improvement in existing crops essential. Current agricultural systems heavily rely on C3 crops—such as rice, wheat, and soybeans—which dominate the global food supply but suffer from inefficient photosynthesis under high temperatures and light intensities.

C3 plants, which include most staple crops, utilize the Calvin-Benson cycle for carbon fixation. A key limitation is the dual function of RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase), which not only fixes CO₂ but also catalyzes photorespiration—a wasteful process that occurs when RuBisCO reacts with O₂ instead of CO₂. Photorespiration can reduce photosynthetic efficiency by 20–50%, particularly under high temperatures and drought conditions.

Additionally, C3 crops exhibit lower nitrogen use efficiency (NUE) because RuBisCO constitutes up to 30% of leaf nitrogen yet operates at suboptimal catalytic rates. This inefficiency necessitates higher fertilizer inputs, contributing to environmental pollution through nitrogen runoff and greenhouse gas emissions.

Rising global temperatures and erratic rainfall patterns further exacerbate the inefficiencies of C3 crops. Heat stress accelerates photorespiration, while water scarcity limits stomatal conductance, reducing CO₂ uptake. Moreover, phosphorus (P) deficiency in soils—a critical issue in developing nations—restricts ATP production, further impairing photosynthesis (Lynch, 2019).

Given these challenges, improving photosynthetic efficiency in C3 crops is a promising strategy to enhance yields, reduce fertilizer dependency, and improve climate resilience.

**1.2. The Superiority of C4 Photosynthesis**

**Biochemical and Anatomical Adaptations**

Unlike C3 plants, C4 plants (e.g., maize, sugarcane, sorghum) have evolved a CO₂-concentrating mechanism (CCM) that minimizes photorespiration and enhances carbon fixation. The C4 pathway involves:

1. **Initial CO₂ fixation by PEP carboxylase (PEPC)** in mesophyll cells, forming a 4-carbon compound (oxaloacetate/malate).
2. **Transport of C4 acids to bundle sheath cells**, where CO₂ is released for RuBisCO, creating a high CO₂ environment that suppresses oxygenation.
3. **Recovery of the CO₂ acceptor PEP** via pyruvate orthophosphate dikinase (PPDK).

This spatial separation of carbon fixation and the Calvin cycle is facilitated by Kranz anatomy, where mesophyll and bundle sheath cells form concentric layers to optimize metabolite transport.

**Advantages of C4 Metabolism**

C4 plants exhibit:

* **Higher photosynthetic efficiency** (up to 50% greater than C3 under high light/temperature).
* **Reduced photorespiration**, leading to better water and nitrogen use efficiency.
* **Lower nitrogen requirements** (C4 plants need ~50% less nitrogen per unit of carbon fixed).
* **Enhanced drought tolerance** due to reduced stomatal conductance.

These traits make C4 plants highly productive in tropical and semi-arid regions, where many C3 crops struggle.

**Why Engineer C4 Traits into C3 Crops?**

Introducing C4 photosynthesis into C3 crops could:

* Boost yields by 30–50% under stress conditions.
* Reduce fertilizer demand, mitigating environmental pollution.
* Improve climate resilience, particularly in heat- and drought-prone regions.

**2. C3 vs. C4 Photosynthesis: Key Differences**

**2.1 Biochemical Pathways**

* **C3 Photosynthesis**: In C3 plants, carbon fixation occurs via the Calvin-Benson cycle in mesophyll cells, where ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes the reaction between CO2 and ribulose-1,5-bisphosphate (RuBP). This process is inefficient under high temperatures and low CO2 concentrations due to photorespiration (Leegood, 2002).
* **C4 Photosynthesis**: C4 plants employ a two-cell system to concentrate CO2 around RuBisCO, minimizing photorespiration. In mesophyll cells, phosphoenolpyruvate carboxylase (PEPC) fixes CO2 into oxaloacetate (OAA), which is then converted into malate or aspartate. These compounds are transported to bundle-sheath cells, where CO2 is released for the Calvin cycle (Hibberd & Covshoff, 2010).

**2.2 Anatomical Adaptations**

C4 plants exhibit Kranz anatomy, characterized by the spatial separation of mesophyll and bundle-sheath cells. This structural adaptation facilitates the efficient transfer of metabolites and CO2 concentration (Matsuoka et al., 2001).

**2.3 Nutrient and Water Use Efficiency**

C4 plants exhibit higher nitrogen use efficiency (NUE) because RuBisCO operates at near-saturating CO2 levels, reducing the need for large amounts of the enzyme (Sage & Zhu, 2011). Additionally, C4 plants have higher water use efficiency (WUE) due to their ability to maintain photosynthesis at lower stomatal conductance (Long et al., 2015).

Nitrogen is a critical nutrient for plant growth, primarily utilized in the synthesis of proteins, chlorophyll, and key enzymes like RuBisCO. In C3 plants, RuBisCO constitutes up to 30% of leaf nitrogen content, yet its inefficiency in carbon fixation—particularly due to photorespiration—results in significant nitrogen wastage. In contrast, C4 plants have evolved a biochemical pump that concentrates CO2 around RuBisCO, suppressing photorespiration and allowing the enzyme to operate near its maximum catalytic efficiency. As a result, C4 plants require substantially less RuBisCO to achieve the same photosynthetic rate, reducing their nitrogen demand by 20–50% compared to C3 plants. This enhanced nitrogen use efficiency (NUE) means C4 crops like maize and sorghum can maintain high productivity even in nitrogen-limited soils, reducing dependency on synthetic fertilizers. Engineering C4 traits into C3 crops could thus lower agricultural nitrogen inputs, mitigating environmental pollution from fertilizer runoff while maintaining or even improving yields.

Water scarcity is a growing constraint in global agriculture, making water use efficiency (WUE) a critical trait for crop resilience. C3 plants lose large amounts of water through transpiration because their stomata must remain open longer to acquire sufficient CO2, especially under high light and temperature conditions where photorespiration increases. In contrast, the CO2-concentrating mechanism (CCM) of C4 plants allows them to achieve higher photosynthetic rates at lower stomatal conductance, reducing water loss per unit of carbon fixed. Studies show that C4 species typically exhibit 2–3 times greater WUE than C3 plants, making them better adapted to arid and semi-arid environments. Introducing C4-like traits into C3 crops could, therefore, enhance drought tolerance, allowing cultivation in water-limited regions without compromising productivity. For example, C4-engineered rice could significantly reduce irrigation demands in paddy systems, which currently account for ~30% of global freshwater withdrawals.

Beyond nitrogen and water, C4 plants often demonstrate improved phosphorus use efficiency (PUE). Phosphorus is vital for ATP synthesis and metabolic regulation, but its availability in soils is frequently limited. C4 species like sugarcane and miscanthus exhibit efficient phosphorus remobilization, recycling P more effectively during senescence compared to C3 plants. This trait is linked to their faster growth rates and higher metabolic activity in bundle sheath cells. Additionally, C4 plants tend to have better potassium utilization, which is crucial for stomatal regulation and enzyme activation. By transferring these nutrient-efficient mechanisms to C3 crops, engineered plants could thrive in low-fertility soils, reducing reliance on phosphate fertilizers and addressing sustainability challenges in regions with degraded or nutrient-poor farmland.

The combined improvements in NUE, WUE, and PUE from C4 engineering could synergistically enhance crop resilience to climate change. For instance, C4 traits would allow C3 crops to maintain productivity under elevated CO2 levels, where traditional C3 plants may face diminishing returns due to photosynthetic saturation. Furthermore, the reduced stomatal conductance of C4-like plants would lower susceptibility to heat stress and soil salinity, both of which are exacerbated by global warming. Field trials with C4-like transgenic rice have already demonstrated modest improvements in drought tolerance and nitrogen retention, suggesting that full C4 integration could unlock even greater benefits.

While the potential benefits are substantial, engineering C4-level efficiency into C3 crops faces hurdles. The Kranz anatomy of C4 plants is complex, requiring precise spatial coordination between mesophyll and bundle sheath cells—a feature absent in C3 species. Even if key enzymes like PEPC and PPDK are successfully introduced, improper localization or insufficient activity may limit gains in NUE and WUE. Additionally, trade-offs may arise; for example, reducing stomatal density to improve WUE could impair cooling capacity under extreme heat. Future research must address these challenges through advanced gene-editing tools (e.g., CRISPR for tissue-specific expression) and high-throughput phenotyping to optimize trait combinations for real-world conditions.

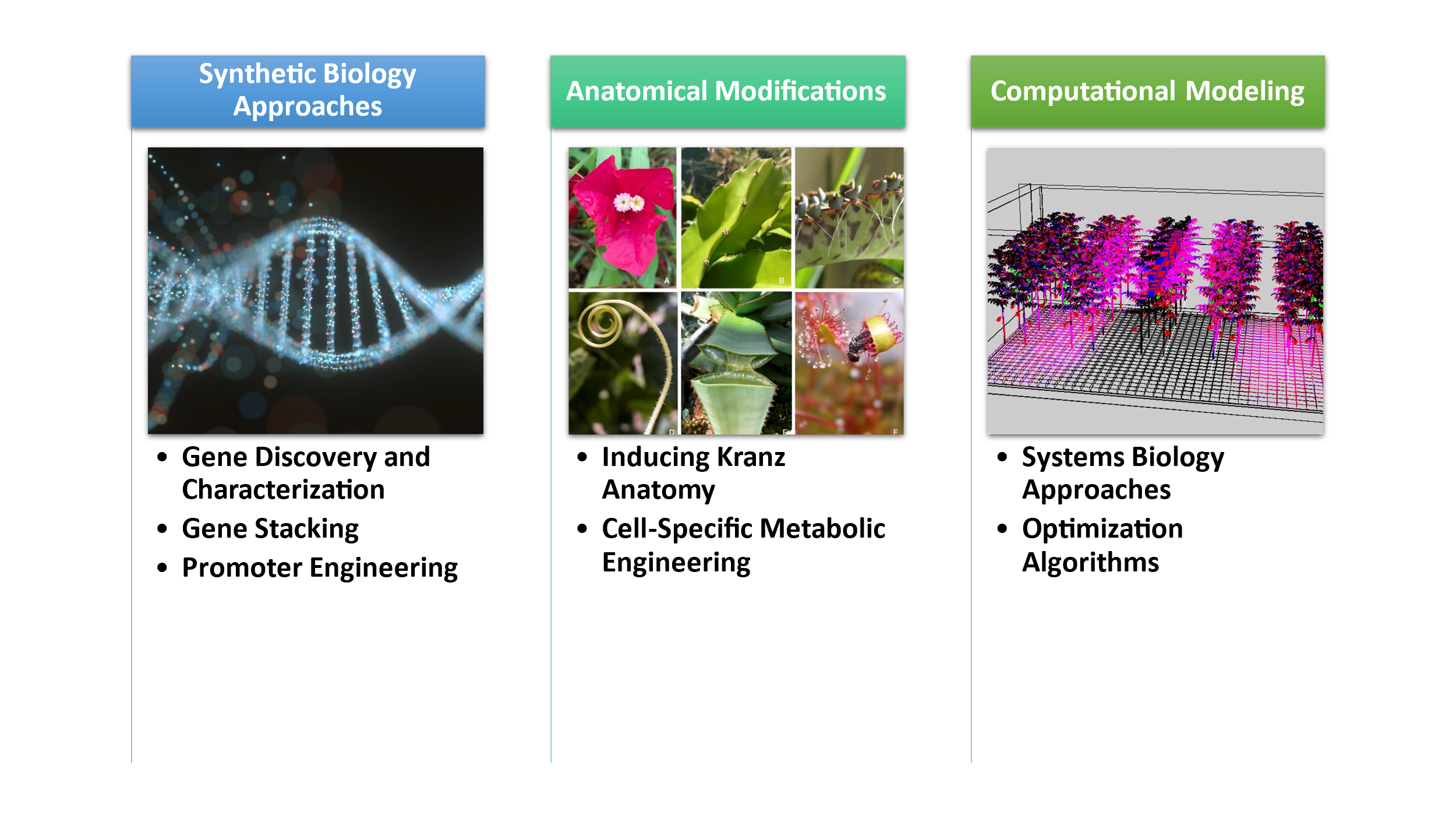
**3. Strategies for Engineering C4 Pathways into C3 Crops (fig. 1)**

**3.1 Synthetic Biology Approaches**

* **Gene Discovery and Characterization**: Identifying and characterizing key C4 genes, such as PEPC, pyruvate orthophosphate dikinase (PPDK), and NADP-malic enzyme (NADP-ME), is critical for engineering C4 traits (Joshi & Pandey, 2018).
* **Gene Stacking**: Combining multiple C4 genes into C3 plants to reconstruct the C4 pathway (Zhang et al., 2024).
* **Promoter Engineering**: Using tissue-specific promoters to ensure the spatial expression of C4 genes in mesophyll and bundle-sheath cells (Ermakova et al., 2021).

**3.2 Anatomical Modifications**

* **Inducing Kranz Anatomy**: Engineering C3 crops to develop Kranz-like anatomy through the manipulation of developmental genes (Murchie et al., 2009).
* **Cell-Specific Metabolic Engineering**: Targeting metabolic pathways to specific cell types to mimic the C4 system (Hanson et al., 2016).



**Fig. 1: Strategies for Engineering C4 Pathways into C3 Crops**

**3.3 Computational Modeling**

* **Systems Biology Approaches**: Using computational models to predict the metabolic and physiological impacts of introducing C4 traits into C3 plants (Zhu et al., 2010).
* **Optimization Algorithms**: Designing optimal gene combinations and expression levels for maximum efficiency.

**4. Meta-Analysis of Recent Case Studies**

**4.1 Methodology**

A systematic review of recent studies was conducted to evaluate the progress in engineering C4 pathways into C3 crops. Studies were selected based on their focus on genetic engineering, synthetic biology, or metabolic engineering approaches. Data on photosynthetic efficiency, nutrient use efficiency, and yield were extracted and analyzed.

**4.2 Key Findings**

* **Rice (Oryza sativa)**: Several studies have successfully introduced C4 genes into rice, resulting in improved photosynthetic efficiency and nitrogen use efficiency. For example, overexpression of maize PEPC in rice increased photosynthetic rates by 20–30% under high-light conditions (Zhang et al., 2024).
* **Wheat (Triticum aestivum)**: Efforts to engineer Kranz anatomy in wheat have shown promise, with transgenic lines exhibiting enhanced CO2 concentration and reduced photorespiration.
* **Soybean (Glycine max)**: The introduction of C4 enzymes into soybean has led to modest improvements in water use efficiency, particularly under drought conditions (Ermakova et al., 2021).

**4.3 Challenges and Limitations**

* **Complexity of C4 Pathway**: The C4 pathway involves multiple genes and intricate regulatory networks, making it challenging to fully reconstruct in C3 plants (Hibberd & Covshoff, 2010).
* **Trade-offs**: Some studies reported trade-offs between improved photosynthetic efficiency and growth, possibly due to metabolic imbalances (South et al., 2019).
* **Regulatory Hurdles**: The commercialization of genetically engineered crops faces regulatory and public acceptance challenges (Sharwood, 2017).

**Critical analysis and conclusion**

The engineering of C4 photosynthetic pathways into C3 crops represents a groundbreaking approach to addressing some of the most pressing challenges in modern agriculture, including food security, resource limitation, and climate change resilience. C4 plants, with their superior photosynthetic efficiency, water use efficiency (WUE), and nitrogen use efficiency (NUE), offer a blueprint for enhancing the productivity of staple C3 crops such as rice, wheat, and soybeans. By introducing C4 traits—either partially or fully—into these crops, scientists aim to reduce the inefficiencies of C3 photosynthesis, particularly photorespiration, while improving carbon fixation and nutrient utilization. This could lead to higher yields with lower inputs of water and fertilizers, making agriculture more sustainable and economically viable, especially in regions facing resource constraints.

Despite the promising potential, significant biological and technical challenges remain. The complexity of C4 photosynthesis, which involves not only biochemical modifications but also anatomical adaptations like Kranz anatomy, makes full engineering a formidable task. Current efforts have successfully introduced individual C4 enzymes into C3 plants, resulting in partial improvements in photosynthetic efficiency. However, achieving a fully functional C4 system in C3 crops requires coordinated expression of multiple genes, proper cellular compartmentalization, and regulatory control—all of which necessitate further research. Advances in synthetic biology, CRISPR-based gene editing, and systems biology are accelerating progress, but large-scale field trials will be essential to validate the stability and performance of engineered crops under real-world conditions.

Beyond the scientific hurdles, the widespread adoption of C4-engineered crops will depend on socioeconomic, regulatory, and ethical considerations. Public perception of genetically modified (GM) crops, intellectual property rights, and equitable access to technology are critical factors that will influence implementation. Policymakers, scientists, and agricultural stakeholders must collaborate to ensure that these innovations benefit smallholder farmers and contribute to global food security without exacerbating socioeconomic disparities. Additionally, environmental risk assessments will be necessary to evaluate potential ecological impacts, such as gene flow to wild relatives or unintended effects on ecosystems.

Looking ahead, interdisciplinary research combining plant physiology, genetic engineering, and computational modeling will be key to unlocking the full potential of C4 engineering in C3 crops. High-throughput phenotyping, multi-omics approaches, and machine learning can help identify the optimal gene combinations and regulatory networks needed for efficient C4-like photosynthesis. Furthermore, exploring alternative strategies—such as single-cell C4 systems or synthetic carbon-concentrating mechanisms—could provide simpler, more scalable solutions.

In conclusion, while the road to fully functional C4-engineered C3 crops is long and complex, the potential rewards justify continued investment in this transformative technology. Success could revolutionize agriculture by significantly boosting crop yields, reducing dependency on synthetic fertilizers, and enhancing resilience to climate stressors. As research progresses, a balanced approach that integrates scientific innovation with ethical responsibility and equitable deployment will be crucial to realizing the promise of C4 photosynthesis in securing a sustainable food future.

**Future Prospects**

**5.1 Advanced Gene Editing Tools**

The advent of CRISPR-Cas9 and other gene-editing technologies offers new opportunities for precise manipulation of C4 genes and regulatory elements in C3 crops (Joshi & Pandey, 2018).

**5.2 Integration with Other Traits**

Combining C4 engineering with other agronomic traits, such as drought tolerance and pest resistance, could further enhance crop performance (Long et al., 2015).

**5.3 Global Collaboration**

International initiatives, such as the C4 Rice Project, highlight the importance of collaborative efforts in advancing this field (Murchie et al., 2009).

**Disclaimer (Artificial intelligence)**

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

Option 2:

Author(s) hereby declare that generative AI technologies such as Large Language Models, etc. have been used during the writing or editing of manuscripts. This explanation will include the name, version, model, and source of the generative AI technology and as well as all input prompts provided to the generative AI technology

Details of the AI usage are given below:

1.

2.

3.

**References**

1. Hanson, M. R., Lin, M. T., Carmo‐Silva, A. E., & Parry, M. A. (2016). Towards engineering carboxysomes into C3 plants. *The Plant Journal*, *87*(1), 38-50.
2. Ermakova, M., Arrivault, S., Giuliani, R., Danila, F., Alonso‐Cantabrana, H., Vlad, D., ... & Furbank, R. T. (2021). Installation of C4 photosynthetic pathway enzymes in rice using a single construct. *Plant biotechnology journal*, *19*(3), 575-588.
3. Hibberd, J. M., & Covshoff, S. (2010). The regulation of gene expression required for C4 photosynthesis. *Annual review of plant biology*, *61*(1), 181-207.
4. Joshi, A. K., & Pandey, S. P. (2018). Genetic factors affecting photosynthesis. In *Handbook of photosynthesis* (pp. 539-568). CRC Press.
5. Leegood, R. C. (2002). C4 photosynthesis: principles of CO2 concentration and prospects for its introduction into C3 plants. *Journal of experimental botany*, *53*(369), 581-590.
6. Long, S. P., Marshall-Colon, A., & Zhu, X. G. (2015). Meeting the global food demand of the future by engineering crop photosynthesis and yield potential. Cell, 161(1), 56-66.
7. Matsuoka, M., Furbank, R. T., Fukayama, H., & Miyao, M. (2001). Molecular engineering of C4 photosynthesis. *Annual review of plant biology*, *52*(1), 297-314.
8. Sage, R. F., & Zhu, X. G. (2011). Exploiting the engine of C4 photosynthesis. *Journal of experimental botany*, *62*(9), 2989-3000.
9. Sharwood, R. E. (2017). Engineering chloroplasts to improve Rubisco catalysis: prospects for translating improvements into food and fiber crops. *New Phytologist*, *213*(2), 494-510.
10. Murchie, E. H., Pinto, M., & Horton, P. (2009). Agriculture and the new challenges for photosynthesis research. *New Phytologist*, *181*(3).
11. South, P. F., Cavanagh, A. P., Liu, H. W., & Ort, D. R. (2019). Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. *Science*, *363*(6422), eaat9077.
12. Von Caemmerer, S., Quick, W. P., & Furbank, R. T. (2012). The development of C4 rice: current progress and future challenges. *science*, *336*(6089), 1671-1672.
13. Zhang, D., Xu, F., Wang, F., Le, L., & Pu, L. (2024). Synthetic biology and artificial intelligence in crop improvement. *Plant Communications*.
14. Zhu, X. G., Long, S. P., & Ort, D. R. (2010). Improving photosynthetic efficiency for greater yield. *Annual review of plant biology*, *61*(1), 235-261.
15. Cui, H. (2021). Challenges and approaches to crop improvement through C3-to-C4 engineering. *Frontiers in plant science*, *12*, 715391.
16. Leegood, R. C. (2013). Strategies for engineering C4 photosynthesis. *Journal of plant physiology*, *170*(4), 378-388.
17. Ruan, C. J., Shao, H. B., & Teixeira da Silva, J. A. (2012). A critical review on the improvement of photosynthetic carbon assimilation in C3 plants using genetic engineering. *Critical reviews in biotechnology*, *32*(1), 1-21.
18. Way, D. A., Katul, G. G., Manzoni, S., & Vico, G. (2014). Increasing water use efficiency along the C3 to C4 evolutionary pathway: a stomatal optimization perspective. *Journal of Experimental Botany*, *65*(13), 3683-3693.
19. Leakey, A. D., Ferguson, J. N., Pignon, C. P., Wu, A., Jin, Z., Hammer, G. L., & Lobell, D. B. (2019). Water use efficiency as a constraint and target for improving the resilience and productivity of C3 and C4 crops. *Annual review of plant biology*, *70*(1), 781-808.
20. Covshoff, S., & Hibberd, J. M. (2012). Integrating C4 photosynthesis into C3 crops to increase yield potential. *Current opinion in biotechnology*, *23*(2), 209-214.
21. Kompas, T., Che, T. N., & Grafton, R. Q. (2024). Global impacts of heat and water stress on food production and severe food insecurity. *Scientific Reports*, *14*(1), 14398.
22. Anbarasan, S., & Ramesh, S. (2022). Photosynthesis eficiency: Advances and challenges in improving crop yield. *Plant Science Archives*, *19*, 21.
23. Wang, Y. (2024). Improving photosynthetic efficiency in fluctuating light to enhance yield of C3 and C4 crops. *Crop and Environment*, *3* (4), 184-193.
24. Kumar, S. A. H., Thomas, U. C., Pillai, P. S., Stephen, R., Rajasree, G., & Aparna, B. (2024). Resilience of C4 Crops to Climate Vagaries. *International Journal of Environment and Climate Change*, *14*(12), 846–866.