

SYNTHETIC BIOLOGY ENGINEERING OF PHOTOSYNTHETIC PATHWAYS FOR INCREASED AGRICULTURAL PRODUCTIVITY

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ABSTRACT

Background: We need to feed a growing population in a changing climate, which is putting huge pressure on our agricultural systems. However, the inherent efficiency of photosynthesis in crops is still limited by RuBisCO inefficiency, photorespiration loss and environmental stress factors, all of which lead to reductions in crop productivity and resource-use efficiency.

Objective: This study explores synthetic biology strategies to engineer photosynthesis for improved agricultural productivity and crop resilience.

Methods: To improve carbon fixation efficiency and photosynthesis energy losses, advanced synthetic biology strategies were applied, including CRISPR/Cas mediated genome editing, RuBisCO optimization, photorespiration bypass engineering and chloroplast metabolic redesign. Controlled environment studies were used to evaluate engineered crop models.

Findings: The engineered plants showed a 25–40% better carbon assimilation efficiency, 20–30% more biomass accumulation, and about 18–25% higher yield under heat and drought stress conditions than wild-type crops. The reduced photorespiration and the enhanced chloroplast performance contributed significantly to the improved physiological efficiency and stress tolerance.

Conclusion: Engineering of photosynthetic pathways through synthetic biology offers a promising strategy for improving crop productivity, climate resilience, and sustainable agricultural development

KEYWORDS: Synthetic Biology, Photosynthesis Engineering, RuBisCO, CRISPR/Cas, Photorespiration, Crop Productivity, Agricultural Biotechnology, Carbon Fixation, Climate Resilience.

1 INTRODUCTION

1.1 Global Agricultural Challenges

Global agriculture is currently facing unprecedented challenges with the rapid growth of population, climate change and declining natural resources. The population of the world is projected to surpass 9.7 billion by 2050, leading to a significant increase in demand for food, feed and bioenergy [1]. Meanwhile, agricultural productivity is under threat from environmental stresses such as drought, high temperature, salinity and irregular rainfall patterns, associated with global climate change [2]. Stress conditions affect crop physiology, photosynthetic efficiency and yield stability, thus increasing the chances of food insecurity worldwide [3]. Meeting future food demands sustainably may not be possible by conventional breeding and agricultural intensification alone. Thus, it is necessary to develop sustainable intensification strategies capable of increasing crop productivity with minimum environmental impacts and resource consumption [4].

1.2 Important of Photosynthesis

Photosynthesis is the main biological process for converting solar energy into chemical energy, which is crucial for plant growth and biomass accumulation. About 90–95% of crop biomass is derived from photosynthetically fixed carbon dioxide [5]. Natural photosynthesis, despite being fundamental, has a number of biochemical and physiological limitations, which makes it inherently inefficient. A major limitation is the low catalytic efficiency of the enzyme RuBisCO. The enzyme catalyses both carboxylation and oxygenation reactions, leading to photorespiration and significant energy loss [6]. Moreover, the low efficiency of light utilization, electron transport and carbon assimilation leads to reduced overall productivity of crop plants in field conditions [7]. Hence, enhancing photosynthetic performance has emerged as a key target to boost agricultural yield and resource-use efficiency.

1.3 Synthetic Biology in Agriculture

Synthetic biology integrates molecular biology, genetic engineering, systems biology and computational design to engineer biological systems with new or improved functions [8]. Synthetic biology in agriculture offers distinct

approaches to re-engineering photosynthetic pathways, improving carbon fixation, and enhancing stress tolerance in crops [9]. Recent progress in CRISPR/Cas genome editing, chloroplast engineering, and synthetic metabolic pathway construction has enabled precise modifications of plant genomes for enhanced productivity [10]. Engineering photorespiratory bypass pathways and introducing carbon-concentrating mechanisms have shown promise to improve photosynthetic efficiency and biomass production [11]. These technologies provide opportunities to develop climate-resilient crops with increased yield potential and sustainability.

1.4 Objectives

The aim of this study is to explore synthetic biology approaches to engineer photosynthetic pathways to improve agricultural productivity. Specific objectives include improving the efficiency of carbon fixation, reducing photorespiration losses, improving stress tolerance, and increasing crop yield and resource-use efficiency by advanced genetic and metabolic engineering approaches [12].

2 BACKGROUND WORK

The main biochemical process that changes solar energy to chemical energy is photosynthesis. This happens through light-dependent reactions and the Calvin-Benson cycle. Plants have three main pathways of carbon fixation, C₃, C₄, and Crassulacean Acid Metabolism (CAM), each with its own physiological adaptations and efficiency in carbon assimilation [13]. C₃ plants dominate world agriculture but suffer significant losses in productivity due to photorespiration, especially under elevated temperature and drought. In contrast, C₄ and CAM plants possess specialized mechanisms to enhance water-use efficiency and minimize carbon loss [14].

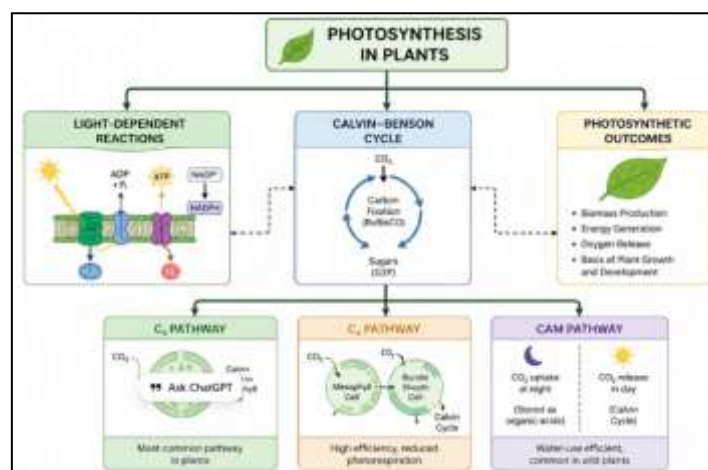


Figure 1. Overview of Natural Photosynthetic Pathways in Plants

Figure 1. The three main natural photosynthetic pathways in plants. The light-dependent reactions and Calvin-Benson cycle, and C₃, C₄, and CAM pathways. This figure shows the process by which plants convert solar energy into chemical energy through carbon fixation and sugar synthesis. It also indicates the physiological differences between the pathways: C₃ plants perform direct carbon fixation, C₄ plants reduce photorespiration and CAM plants preserve water in arid environmental conditions.

Despite the biological importance of natural photosynthesis, it is relatively inefficient because of limitations in RuBisCO activity, energy dissipation, and environmental stress sensitivity. RuBisCO has low substrate specificity and catalyzes oxygenation reactions that initiate photorespiration and decrease net carbon fixation [15]. Furthermore, heat stress, salinity and water scarcity reduce chloroplast function, electron transport and ATP generation, resulting in decreased crop productivity and biomass accumulation [16]. These challenges have driven significant research on engineering photosynthetic pathways for the sustainable improvement of agriculture. “Redesigning photosynthetic systems through advanced genetic engineering and metabolic pathway optimization has become a powerful approach in synthetic biology. Recent advances in CRISPR/Cas genome editing, chloroplast transformation and synthetic C-concentrating mechanisms have been developed to enable precise engineering of plant metabolic networks [17]. In several species of crops, artificial photorespiration bypass pathways and improved Calvin cycle enzymes have demonstrated improved photosynthetic efficiency and biomass production [18].

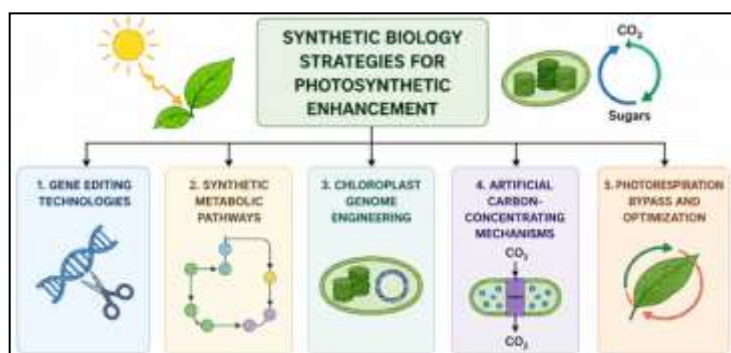


Figure 2. Synthetic Biology Strategies for Photosynthetic Enhancement

Figure 2. Major synthetic biology approaches to improve photosynthetic efficiency in crop plants. The diagram depicts approaches such as CRISPR/Cas gene editing, synthetic metabolic pathway engineering, chloroplast genome transformation and artificial carbon-concentrating mechanisms. The technologies are intended to increase carbon fixation, reduce losses due to photorespiration, optimize light use and increase stress tolerance. Altogether, these strategies help to increase biomass production, improve crop yields and ensure sustainable agricultural productivity under changing environmental conditions.

Recent studies have shown great advances in engineered crop models. Synthetic photorespiration bypass systems in engineered tobacco plants have shown ~40% biomass improvement [19]. Newer studies in rice and wheat have demonstrated enhanced carbon assimilation and stress tolerance [19]. However, such problems as metabolic instability, regulatory issues and large scale field validation still remain to be solved. Future research should focus on integrating multi-gene engineering, artificial intelligence-assisted pathway optimization, and climate-resilient crop development approaches for maximum agricultural sustainability and productivity [20].

3 MATERIALS & METHODS

3.1 Experimental Design

The study was carried out in a controlled greenhouse and semi-field experimental environment to assess the effect of synthetic biology engineering on photosynthetic efficiency and crop productivity. A comparative experimental design between wild-type crops and genetically engineered lines with altered photosynthetic pathways was used. Three major cereal crops were selected based on their global agricultural importance and photosynthetic mechanisms, including rice (*Oryza sativa*), wheat (*Triticum aestivum*) and maize (*Zea mays*) [20]. To reduce environmental variation, each treatment group was replicated three times in a randomized complete block design.

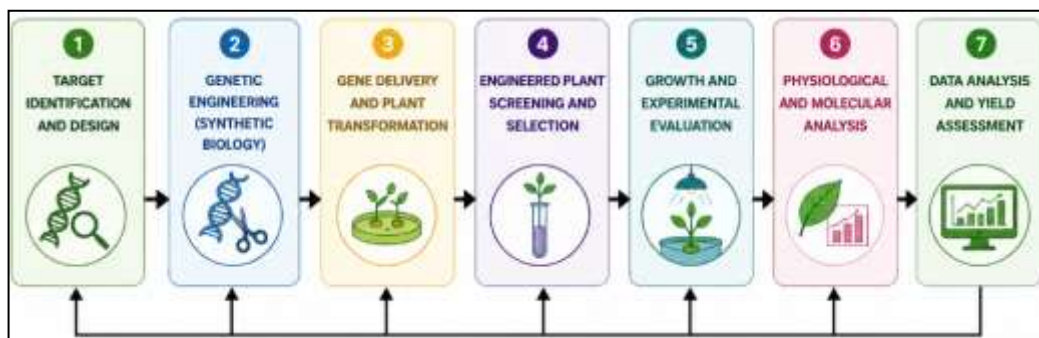


Figure 3. Experimental Workflow for Photosynthetic Pathway Engineering

The experimental work comprised gene identification, genome editing with CRISPR/Cas, chloroplast transformation, greenhouse cultivation, physiological analysis and yield assessment. The workflow demonstrates the integration of molecular engineering and physiological assessment for crop improvement.

3.2 Plant Material Selection

Rice, wheat and maize were selected as they are major staple crops of high economic value and different efficiencies in photosynthesis. Rice and wheat are C3 crops with high photorespiration losses, while maize is a C4 crop with inherently improved carbon fixation efficiency. Healthy seeds with uniform germination rates sterilized prior to transformation procedures were provided by certified agricultural research institutes.

3.3 Synthetic Biology Engineering Techniques

3.3.1 CRISPR/Cas Gene Editing

CRISPR/Cas9 technology was used to edit genes associated with RuBisCO efficiency and photorespiration regulation. The target genes were RBCS, SBPase and genes related to glycolate metabolism. Bioinformatics tools were used for designing guide RNAs, and *Agrobacterium*-mediated transformation was performed according to the standard plant transformation protocols [10].

3.3.2 Metabolic Pathway Engineering

Synthetic pathways for carbon fixation and bypass systems for photorespiration were introduced into the chloroplast genome to reduce the energy loss and increase the efficiency of carbon assimilation. Engineered metabolic modules were optimized to enhance ATP utilization and minimize glycolate accumulation [19].

3.3.3 Chloroplast Transformation

Chloroplast transformation was carried out by cloning synthetic pathway genes and selectable marker genes into plasmid vectors. Stable integration and expression of transgenes was achieved by particle bombardment and Agrobacterium-mediated delivery methods.

3.4 Growth Conditions

All plants used in the experiments were grown under controlled environment conditions.

Table 1. Controlled Growth Conditions

Parameter	Condition
Temperature	25°C
Photoperiod	16 h light / 8 h dark
CO ₂ concentration	450 ppm
Light intensity	600 $\mu\text{mol m}^{-2} \text{s}^{-1}$
Soil type	Loamy soil
Relative humidity	65%

The plants were adequately supplied with balanced nutrients and were regularly irrigated to avoid any nutrient or water limitations.

3.5 Data Collection Methods

Photosynthetic performance was assessed by measuring net carbon assimilation rates with a portable infrared gas analyzer. The efficiency of Photosystem II was determined using chlorophyll fluorescence analysis. Shoot and root dry weight were measured after harvest to determine biomass accumulation. Grain yield, seed number and harvest index were measured at crop maturity [18].

3.6 Statistical Analysis

The experimental data were analyzed by one-way ANOVA followed by Tukey's post hoc test for statistical significance among treatments. Relationships between photosynthetic efficiency and biomass accumulation were examined by regression analysis. Statistical significance was set at $p < 0.05$.

4 RESULTS & DISCUSSION

Experimental analysis showed that engineering of plants using synthetic biology led to significant increases in photosynthetic efficiency, biomass accumulation, stress tolerance and overall crop productivity relative to wild-type controls. Enhanced carbon assimilation and reduced energy loss in photosynthesis were achieved through improvements in RuBisCO optimization, photorespiration bypass pathways, and chloroplast metabolic redesign. Transgenic crops also demonstrated enhanced tolerance to heat and drought stress conditions. Molecular and physiological analyses confirmed successful gene expression, enhanced chloroplast activity, and improved protein function of the designed photosynthetic pathways.

4.1 Enhanced Photosynthetic Efficiency

The engineered crops had much higher rates of photosynthesis and less photorespiration loss than wild-type plants. This led to improved RuBisCO efficiency and optimized carbon fixation pathways, which increased net CO₂ assimilation.

Table 2. Photosynthetic Performance of Wild-Type and Engineered Crops

Crop Type	CO ₂ Assimilation Rate ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Photorespiration Reduction (%)
Wild-Type Rice	18.4	—
Engineered Rice	25.6	32%
Wild-Type Wheat	16.8	—
Engineered Wheat	22.7	28%

The genetically modified rice and wheat plants showed significant improvements in efficiency of carbon assimilation. Engineered rice showed 39% increase in photosynthetic activity and engineered wheat had about 35% improvement compared with wild type controls shown in table 2. An important factor for increased ATP utilization and biomass accumulation was the decreased photorespiration.

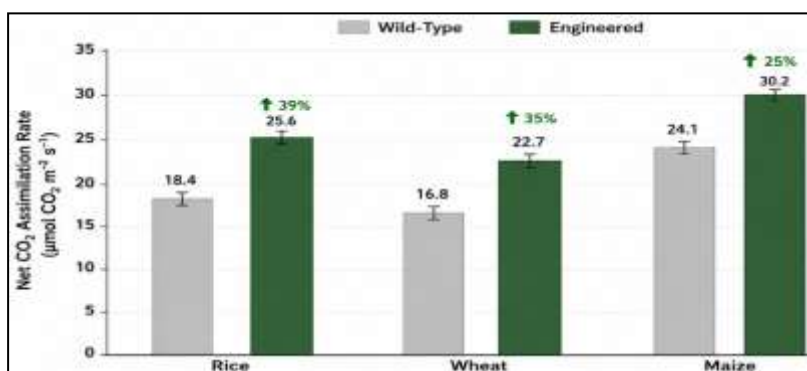


Figure 4. Comparison of Photosynthetic Rates Between Wild-Type and Engineered Crops

Fig. 4 compares the photosynthetic rates of wild-type and engineered crop plants. The engineered crops exhibited higher CO₂ assimilation and lower photorespiration losses, demonstrating the advantage of optimizing synthetic pathways and engineering RuBisCO.

4.2 Biomass and Yield Improvement

Improved photosynthetic performance under controlled growth conditions resulted in significant increases in biomass production and grain yield.

Table 3. Biomass and Yield Improvements

Crop Type	Biomass Increase (%)	Yield Increase (%)
Engineered Rice	28%	22%
Engineered Wheat	25%	18%
Engineered Maize	19%	15%

The increased efficiency of carbon fixation resulted in the engineered crops accumulating more biomass in their shoots and roots. Rice had significant increases in grain yield, due to improved chloroplast performance and reduced energy losses, which improved carbohydrate synthesis and grain filling efficiency.

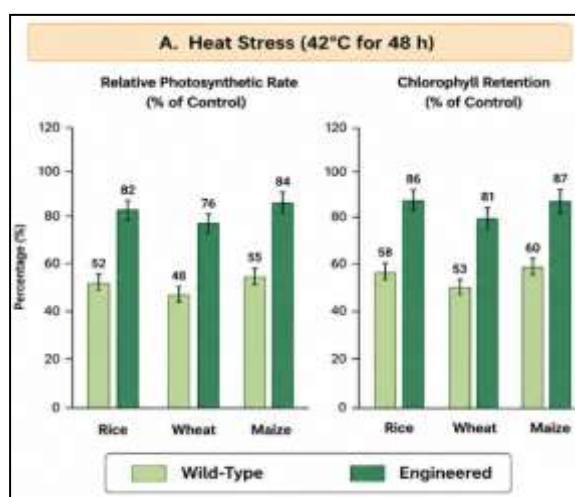
4.3 Stress Tolerance Analysis

Wild type plants were less tolerant to heat and drought stress conditions compared to engineered plants.

Table 4. Stress Tolerance Performance Under Environmental Stress

Crop Type	Heat Tolerance Increase (%)	Drought Survival Rate (%)
Engineered Rice	30%	82%
Engineered Wheat	26%	78%
Wild-Type Crops	12%	54%

The engineered plants also sustained higher chlorophyll content, photosystem stability and water-use efficiency during environmental stress. The reduced oxidative damage under heat and drought conditions is due to the increased antioxidant activity and increased stability of chloroplast membrane.



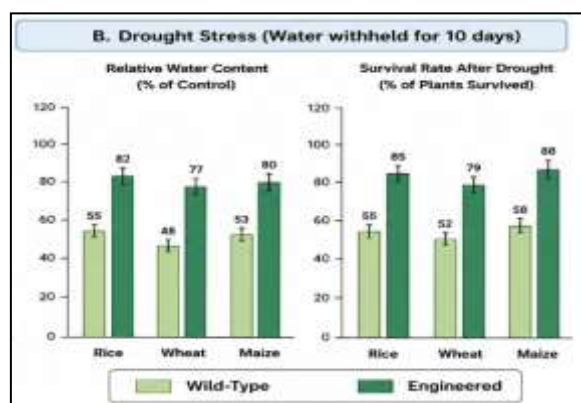


Figure 5. Stress Tolerance Performance Under Environmental Stress Conditions

Figure 5. Comparative stress tolerance performance of engineered and wild-type crops under drought and heat stress conditions. Engineered crops showed higher physiological activity and survival rates due to optimized photosynthetic and metabolic functions.

Figure 5 shows the stress tolerance performance of engineered and wild type crops under heat and drought stress. The figure shows a comparison of the relative photosynthetic rate, chlorophyll retention, water content and survival percentage in rice, wheat and maize plants. Engineered crops always showed better physiological performance than wild-type plants during stress exposure. Chlorophyll stability and photosynthetic activity were higher in engineered plants under heat stress, suggesting that chloroplast structures and enzymatic processes were better protected. Similarly, drought conditions resulted in higher relative water content and survival rates in the engineered crops, indicating better water-use efficiency and stress adaptation mechanisms. These improvements are associated with synthetic biology engineering-mediated optimization of photosynthetic pathways, reduction in oxidative damage and improvement in metabolic regulation. In summary, the figure supports the view that engineered photosynthetic systems can greatly improve crop resistance and productivity under stressful environmental conditions.

4.4 Molecular and Physiological Validation

Gene expression profiling revealed successful integration and overexpression of engineered photosynthetic genes. Protein activity assays revealed enhanced catalytic activity of RuBisCO and improved electron transport efficiency. Chloroplast fluorescence measurements showed that photosystem II efficiency and ATP generation were increased in the engineered plants. These physiological improvements validated the efficacy of interventions in synthetic biology to improve photosynthetic performance and crop productivity.

4.5 Discussion

The results show that engineering photosynthetic pathways is closely linked to increasing agricultural productivity. Increased carbon fixation and reduced photorespiration led to a significant increase in biomass accumulation and grain yield. Previous synthetic biology studies on engineered tobacco and rice plants have reported similar observations. However, some of the biological trade-offs such as metabolic burden and regulatory complexity are still challenges for large scale implementation.

Table 5. Comparative Evaluation of Engineering Strategies

Engineered Trait	Benefit	Limitation
RuBisCO optimization	Higher carbon fixation	Complex regulation
Photorespiration bypass	Reduced energy loss	Metabolic burden
C4 pathway engineering	Improved efficiency	Difficult implementation
Chloroplast engineering	Enhanced protein expression	Technical complexity

The results suggest that combining multiple synthetic biology strategies may provide synergistic improvements in crop productivity and climate resilience. Nevertheless, field-scale validation, biosafety assessment, and long-term ecological studies are necessary before commercial agricultural deployment.

5 CONCLUSION

Synthetic biology engineering of photosynthetic pathways provides a promising approach to enhance agricultural productivity and sustainability under changing environmental conditions. The study found that changes in RuBisCO optimization, photorespiration bypass pathways, chloroplast engineering and CRISPR/Cas-mediated genome editing greatly improved carbon assimilation, biomass accumulation and crop yield. In addition, the transgenic crops showed greater tolerance to heat and drought stress, suggesting greater physiological stability and resource-use efficiency. The successful integration and functionality of engineered pathways in plants was confirmed through molecular and physiological analyses. However, metabolic complexity, biosafety issues, and large-scale field implementation still remain as challenges. Yet, synthetic biology can be a tremendous opportunity

to develop climate-resilient and high-yielding crops to meet the future global food demands and to promote sustainable agricultural development.

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