Vol.2 Issue-9, May 2022 (e-ISSN: 2582-8223)

# Conversion of Photosynthetic Pathway in Rice from C3 to C4

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**ARTICLE ID: 11**

**Summary**

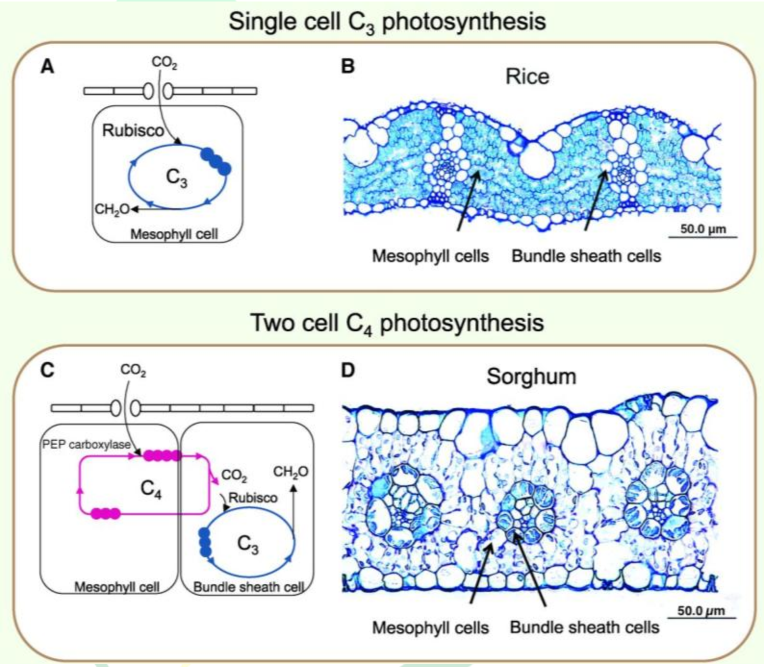
Increasing world population, degradation of natural resources and human made climate change are the important challenges to reach the world food grain demand, especially rice which is the staple food for half of the world's population. Conversion of C3 photosynthetic pathway in rice to C4 pathway can be a breakthrough to cope up with the grain demand along with improved efficiency of resource utilization in situations of changing climatic conditions.

**Introduction**

As the world population is expected to reach 9 billion by 2050 with its rapid growth. Rice, which is the staple food of over half of the world's population needs to increase the yield by at least 60% (FAO 2009). Escalating population with increase of rice consuming population at the rate of 1.098% per annum, degradation of natural resource base by diversion of large areas of farmland to industrialization and human made climate change adversely affect world food grain production and makes it an important challenge to cope up with the rice demand. The unpredictable nature of the natural climatic vagaries may lower the rice yield far more than expected if climate change continues at the same rate. The one percent increase in yield per annum by current traditional breeding programmes is not sufficient and scientists are aiming high to change the future for this crucial grain. In order to increase crop yield and simultaneously improving the efficiency of resource utilization,a sustainable agriculture calls for a second Green Revolution where crops are developed with higher productivity and better efficiency in resource use.

**Conversion of C3 to C4 Rice**

Rice uses the C3 photosynthetic pathway, that uses Calvin Benson cycle characterized by the use of ribulose biphosphate carboxylase /oxygenase (RUBISCO) which in hot dry environments is much less efficient than the C4 pathway in plants like maize and sorghum that uses Hatch-Slack cycle by phosphoenolpyruvate carboxylase (PEPC) making them more efficient than C3 plants in terms of photosynthesis and resource usage, particularly in hot dry environments where the potential for productivity is high.Thus, scientists consider introduction of metabolic pathway of C4 photosynthesis into rice that could theoretically increase productivity by 50% as a possible way of increasing the productivity in rice.




Picture source: Von Caemmerer*et al*., 2012

Based on the study of the evolution of C4 from C3 species and the associated changes, IRRI researchers has listed different modifications to establish a functional C4 photosynthetic pathway in rice such as the increasing number and size of chloroplasts in bundle sheath cells, enhancing the activity of calvin cycle in bundle sheath cells, increasing the vein density in leaf by reducing vein spacing, reducing photorespiration in mesophyll cells and by engineering of C4 pathway into rice.

**Increasing number of chloroplasts and enhancing the activity of calvin cycle in bundle sheath cells**

More than 90% of the total chloroplasts in rice are located in mesophyll cells (MCs) within the leaf and entire process of photosynthesis takes place in MC while bundle sheath cells (BSC) perform other activities, unlike that of C4 plants where chloroplasts and the process of photosynthesis are compartmentalized into MC and bundle sheath cells (BSC). In order to incorporate C4 pathway into rice, it is required to introduce more photosynthetic chloroplasts in the BSCs than rice has now. Over expression of genetic elements such as Golden2-like (GLK) genes involved in chloroplast development by help of C4 gene promoters with help in increasing the number and size of chloroplasts in BSCs of rice.C4 photosynthesis has biochemical $CO_2$ pumping mechanism thatincrease the net $CO_2$ assimilation in BSCs due to enhanced $CO_2$ concentration around Rubisco by sequestering it in BSCs, thus leading to highly efficient photosynthesis. Thus, to develop C4 rice, activity of Rubisco has to be reduced in MCs and increased in BSCs in order to confine Calvin cycle to the BSCs of rice, like in a C4 system.

**Increasing the vein density in leaf by reducing vein spacing**

In C3 plants as photosynthesis takes place in the MCs, large number of these MCs pushes consecutive veins far from each other making veins distant from each other and causes an overall reduction in vein density. But, for an efficient functioning of C4 pathway in rice, a close contact of MCs and BSCs with a ratio of 1:1 is highly desired as in the case of C4plants where it involves wreath-likecellular arrangement called Kranz Anatomy in which vein is concentrically surrounded by the BS cells which in turn are also enclosed in a layer of mesophyll cellsalso enclosed in a layer of mesophyll cells.

**Reducing photorespiration in mesophyll cells**

In C3 plants atcurrent atmospheric $CO_2$ concentrations(ca. 400 ppm) Rubisco (enzyme for carbon fixation) also catalyses a reaction between $O_2$ and RuBP, resulting in formation of one of 2- phosphoglycolate in addition to 3-PGA. In order to convert this 2-phosphoglycolate back to 3-PGA an extra amount of energy will be used in the process of conversion called as photorespiration. Whereas in C4 plants, MCs prevent the contact between Rubisco in BSCs and $O_2$ in the intercellular spaces spatially, thus preventing loss of energy through photorespiration and making $CO_2$ compensation point to nearly zero and constantly high carboxylation efficiency (CE) without responding to the changes in $O_2$ concentrations.

**Engineering C4 pathway into rice**

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Engineering of C4 rice can be a feasible solution to increase rice yield by a higher percentage. It starts with the discovery of novel genes related to kranz type anatomy followed by its engineering in to rice. Currently the C4 rice consortium members are involved with discovery and usage of these novel genes and transporters aiming to form C4 rice. With advent of efficient transformation technologies, it ispossible to study the overexpression of the C4 pathway related genes from different C4 plants in developing C4 rice. Another important efficient, precise and versatile tool is the CRISPR-Cas method, which is useful for C3-to-C4 engineering by increasing or decreasing gene expression by replacement of nuclease domain in Cas9 with transcription activation domain or repressor domain and thereby avoiding the issues associated with transgenes.

**Conclusions**

Besides a conversion of photosynthetic pathway from C3 to C4, climate changes necessitate investigations on other related pathways i.e., light reactions, synthetic processes such as biomass, water use efficiency, nutrition and hormone metabolisms.

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