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Breeding Strategies in Conversion of C3 to C4 Rice

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Abstract

To boost food production for a rapidly growing global population, crop yields must significantly increase. One of the avenues being recently explored is the improvement of photosynthetic capacity by installing the C4 photosynthetic pathway into C3 crops like rice to drastically increase its yield. As the genes and the genetic makeup of C3 plants resembles with the C4 plants so it is somehow possible to convert it.A better approach to increase rice yield in the present climate scenario involves the introduction of C4 genes into the C3 rice crop via novel gene cloning and genome editing technologies via Golden Gate cloning method, CRISPR-Cas9& TALE method, Transgenic via C4 enzymes.

Introduction

There are various types of plants found on the earth. They have their own way of photosynthesis and during the biosynthetic phase CO_2 and H_2O combine to form carbohydrates. This is known as carbon fixation. Different plant follow its own pathway of carbon fixation via3 ways C3, C4 and CAM.

C3 plants such as wheat, barley, rye, cotton, rice, tobacco where carbon fixation firstly the carbon is breakdown in to 3 carbon compound (PGA) by the enzyme called ribulose-1, 5-biphosphate carboxylase oxygenase (Rubisco). Rubisco also does the conversion of RuBP (Ribulose-1, 5-biphosphate)by using $O_2$and this process is known as photorespiration. It can loss of upto 25% of previously fixed carbon(Sriraj and Gurjar,2022)[7].

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C4 plants pineapple, corn, sugar cane that convert carbon dioxide to 4-carbon sugar compounds (OAA)by PEP Case enzyme first in order to enter the C_3 or the Calvin cycle. The C_4 plants are very productive in climatic conditions that are hot and dry and produce a lot of energy. The leaves possess Kranz anatomy, and the chloroplasts of these plants are dimorphic.

The Golden Gate Cloning Method

The Golden Gate cloning method is of particular interest because it allows the stacking of multiple genes in a straightforward manner **(**Lee *et al.*, 2020)[1]. Using this method, (Ermakova *et al.*, 2021)[2] recently demonstrated that a C4 photosynthetic pathway could be installed in rice by simultaneously expressing maize photosynthetic enzymes using a single construct.

To introduce the C4 pathway into rice, more photosynthetic chloroplasts are required in the BSCs than rice has now. This could be done by over expressing the genetic elements that are necessary for the chloroplast development such as Golden2-like (GLK) genes in a cell phosphoenolpyruvate carboxykinase (PCK) promoter for BSC specific expression in rice leaves.

CRISPR-Cas9& Talen Method

(Cermak *et al.*, 2017; Zhang *et al.*, 2019 and Zhu *et al.*, 2020)[3,4,5]usedanother powerful tool that is the CRISPR-Cas method, which is not only highly efficient and precise but also very versatile in gene editing. The sequence replacement function of the CRISPR-Cas method is particularly useful for C3-to-C4 engineering C3-to-C4 engineering is a complex project requiring collaborative efforts from the community. The C4 rice project is a good example It can be envisioned that C4 rice will be made available in the near future, thus ushering in a new wave of Green Revolution.

Transgenic Rice

Sen *et al.* (2017)[6] have developed transgenic rice plants expressing threeC4 enzymes, namely *CA, PEPC* and *PPDK*. The over expression of *CA* along with*PEPC* and *PPDK* resulted in a significant increase in photosyntheticefficiency and grain yield of up to 12 % comparedto control plants. Metabolic functions of C4 enzymes werelinked to altered phenotypic functions in C3 plants indicatingthat significant changes in cell physiology occur in thetransgenic plants.

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Abbreviations

CA- Carbonic anhydrase, **PEPC**- Phosphoenol pyruvate carboxylase, **PPDK**- Pyruvate orthophosphate dikinase.

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