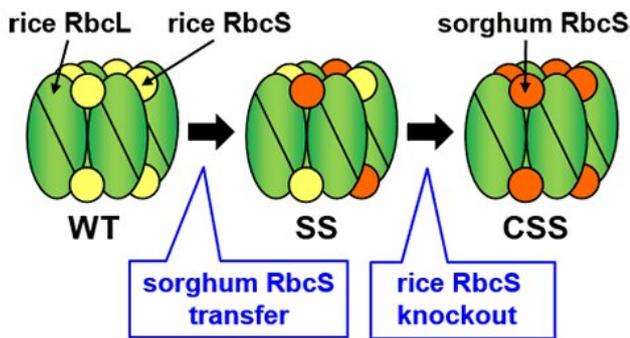


Successful improvement of the catalytic activity of photosynthetic carbon dioxide fixing enzyme Rubisco

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Strategy for improving catalytic activity in rice Rubisco: WT (Wild Type, unmodified rice), SS (rice with sorghum RbcS), CSS (rice with sorghum RbcS transferred/rice RbcS knocked out). Credit: Kobe University

A research group consisting of Associate Professor Fukayama Hiroshi (Kobe University, Graduate School of Agricultural Science) and Professor Matsumura Hiroyoshi (Ritsumeikan University) et al. have succeeded in greatly increasing the catalytic activity of Rubisco, the enzyme which fixes carbon from CO₂ in plant photosynthesis. The research team also hypothesized the mechanism which determines the catalytic activity of Rubisco, based on structural analysis of the proteins. In the future, it is hoped that increasing the photosynthetic ability of agricultural crops will lead to increased yields. These results were published in the international scientific journal *Molecular Plant* on August 31.

Growth speed in [plants](#) is mainly determined by photosynthetic ability. Thus improving photosynthesis in agricultural crops can increase their yield. In photosynthesis, Rubisco is an enzyme that acts as the initial catalyst for the reaction which turns CO₂ into organic carbon.

However, Rubisco has two major drawbacks which limit photosynthesis: its catalytic activity is very low, and it can be inhibited by O₂ (ie. Rubisco can mistakenly fix to O₂ molecules instead of CO₂ molecules, creating a toxic compound that needs to be recycled by the plant).

Rubisco's catalytic activity varies depending on the type of plant. Most major crops, such as rice, wheat and soybean are C₃ plants that use regular photosynthesis. C₄ plants, such as corn and sugarcane, on the other hand, have acquired a mechanism to concentrate CO₂ (the C₄ photosynthetic pathway).

The catalytic rate is low in C₃ plants, whereas in C₄ plants it tends to be high. Rubisco with high catalytic activity tends to be inhibited easily by oxygen, therefore it cannot function effectively in [atmospheric conditions](#) where there is a low concentration of CO₂ if the plant doesn't have a CO₂-concentrating mechanism. However, as the amount of atmospheric CO₂ is continuing to increase, it is believed that if C₃ plants had the same highly active type of Rubisco as C₄ plants then this could be utilized to improve photosynthetic ability.

Rubisco is made up of two types of protein- large subunits (RbcL) and small subunits (RbcS). The sequence of the [amino acids](#) in RbcS varies greatly between species. This team has been focusing on conducting research into RbcS. They genetically modified rice (a C₃ plant) by transferring RbcS from the C₄ plant sorghum, successfully increasing the catalytic rate of rice Rubisco 1.5 times. This rice with sorghum RbcS inserted (SS line), produced a chimera form of Rubisco from both sorghum RbcS and rice RbcS. Next, the rice RbcS gene was knocked out in the sorghum RbcS incorporated rice plants using CRISPR/Cas9 gene editing.

In this CSS line (sorghum RbcS transferred/rice RbcS knocked out), the rice RbcS was completely replaced by sorghum RbcS, producing hybrid Rubisco. This approximately doubled the catalytic rate to that which is equivalent to C₄ plants. Although many researchers have been able to improve Rubisco's catalytic characteristics, there have been no examples of such a large increase being achieved. Furthermore, CSS line plants demonstrated a higher photosynthetic rate than unmodified (wild type) rice under high CO₂ conditions, even though the amount of Rubisco in their leaves was over 30% less.

Subsequently, the researchers conducted X-ray crystallography in order to illuminate the mechanism by which sorghum RbcS increases Rubisco's catalytic activity. RbcL is present in Rubisco's catalytic site. Near this catalytic site, there is a structure called RbcS ?C. The 102 amino acid found in ?C is isoleucine in rice and leucine in sorghum. Leucine has smaller molecules than isoleucine. Therefore, it is thought that in [sorghum](#) RbcS the gaps between amino acid molecules become bigger, making the reaction site more pliable and thus increasing catalytic activity. Although further research is necessary to prove this, it is believed to be a previously unproposed ground-breaking theory for Rubisco research.

The CSS line produced in this study demonstrated high photosynthetic ability, however crop yield was not improved. Hopefully, it will be possible to vastly improve plant growth and productivity through appropriate control of Rubisco levels.

The current research used the C₃ plant [rice](#), however it is vital to consider the applications of this methodology and investigate whether or not the same strategy can be used to increase Rubisco's catalytic activity in other major crops, such as wheat, soybean and potato.

It is thought that the 102 amino acid is an important determinant of the catalytic [activity](#). Further research is being carried out to investigate this; for example by replacing only the amino acid at the 102 site with another amino acid and producing Rubisco.

More information: Hiroyoshi Matsumura et al, Hybrid Rubisco with Complete Replacement of Rice Rubisco Small Subunits by Sorghum Counterparts Confers C₄-Plant-like High Catalytic Activity, *Molecular Plant* (2020). [DOI: 10.1016/j.molp.2020.08.012](https://doi.org/10.1016/j.molp.2020.08.012)

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