Better plant edits by enhancing DNA repair

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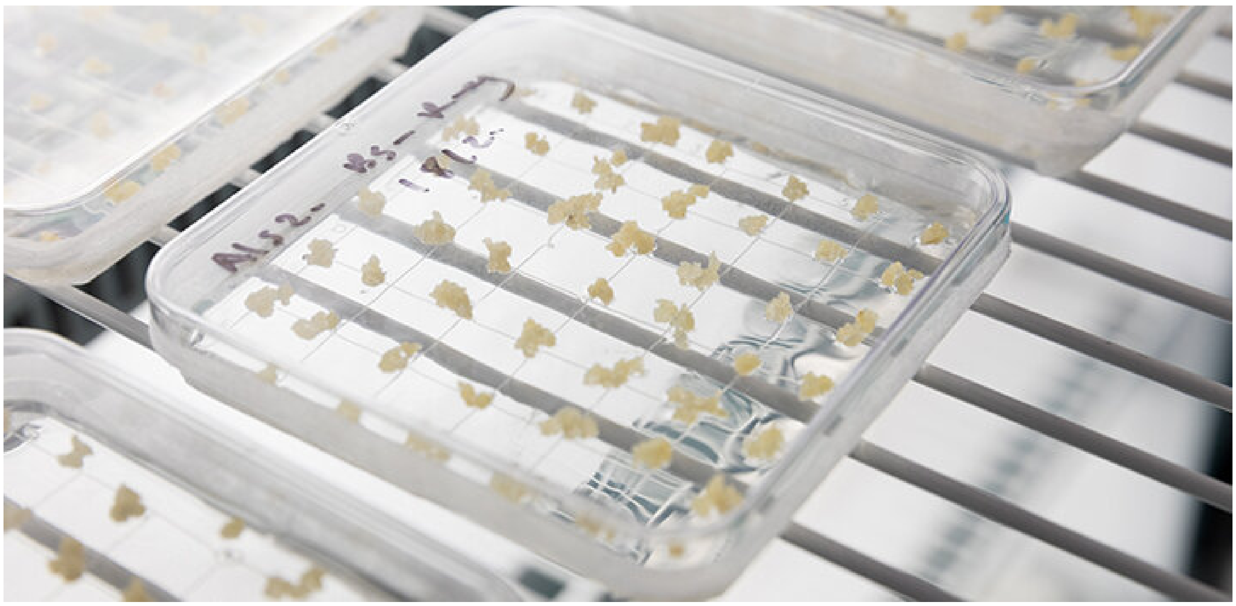


Genome editing involves cutting DNA at very specific locations and utilizing cells' natural repair pathways to modify genes. Credit: 2020 KAUST

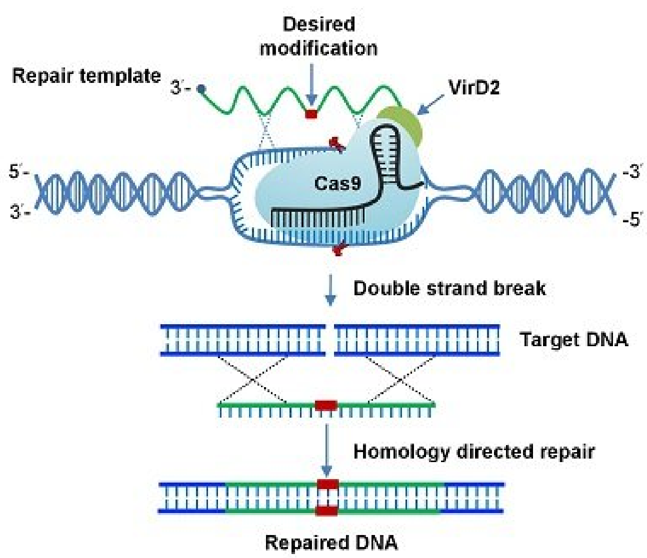
A new genome editing system enhances the efficiency of an error-free DNA repair pathway, which could help improve agronomic traits in multiple crops.

Genome editing involves cutting DNA at very specific locations and utilizing cells' natural repair pathways to modify genes. Plant cells contain two repair pathways: nonhomologous end joining (NHEJ) and homology-directed repair (HDR).

"In plants, NHEJ is the dominant repair pathway at most cell stages," says KAUST postdoc Khalid Sedeek. "But it is prone to errors. For precise genetic modifications we need more control than provided by NHEJ. HDR is error-free but is inefficient in plants. We designed a genome editing system to enhance HDR efficiency."

The system uses Cas9 as its DNA scissor. Cas9 is fused to VirD2 a protein that comes from *Agrobacterium tumefaciens*, a soil bacterium that causes disease in plants by inserting part of its own DNA into the plant genome. VirD2 leads the bacterial DNA into the plant nucleus.

"We thought we could use VirD2 to bind to a wide range of repair templates to enhance the rate of HDR in plants by bringing the template close to the DNA break," says KAUST research scientist, Zahir Ali.



Precise engineering of plants can be achieved through binding of the VirD2 protein and the Cas9 endonuclease. Credit: Reproduced with permission under a Creative Commons License CC BY from reference 1, 2020, Springer Nature

A guide RNA directs Cas9 to a specific location on the plant genome that is targeted for editing. Cas9 cuts the DNA strand, and then VirD2 holds the repair template close to the cut—the cell's natural HDR repair pathway uses it to conduct the repair with the corresponding genetic modifications encoded in the template.

"Our data showed that the binding of the repair template to the Cas9-VirD2 system improved the rate of HDR up to 5.5 fold in plant cells compared to Cas9 alone," says Ali.

The team tested their system in rice cells to show that the edits were precise and heritable. It successfully modified the gene that codes for the enzyme acetolactate synthase, a change that can help rice crops survive when herbicides are sprayed to kill surrounding weeds.

The system could also successfully attach a protein tag to the histone deacetylase gene in rice, which codes for an enzyme involved in plant development and reaction to stresses.

Finally, the system also successfully edited the gene that codes for the enzyme carotenoid cleavage dioxygenase 7. Precise editing of this gene results in dwarf rice plants with many grain-bearing branches.

"Our tests validated the use of this system for precise genome engineering in plants for the purpose of crop improvement," says KAUST molecular geneticist, Magdy Mahfouz, who led the study. "We expect our Cas9-VirD2 system could be adopted across most animal and plant species."

The researchers are testing their system for simultaneous edits in several genes and for multiple edits in a single gene. They are also testing it for improving various agronomic traits in multiple crops.

More information: Zahir Ali et al, Fusion of the Cas9 endonuclease and the VirD2 relaxase facilitates homology-directed repair for precise genome engineering in rice, *Communications Biology* (2020). DOI: 10.1038/s42003-020-0768-9

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