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CRISPR-edited plants by grafting

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Edited, transgene-free plants are produced without tissue culture by RNA migration from rootstocks to grafts.

Plant engineering and breeding are entering an era of rapid progress thanks to the sophistication of new tools for genomics research and genetic modification[1]. Foremost among these tools is CRISPR genome editing, which enables precise genetic changes to improve crop phenotypes. Yet several aspects of this technology would benefit from optimization. New work in *Nature Biotechnology* by Yang et al.[2] tackles the lengthy time frames required to produce transgene-free edited plants. The authors introduce a CRISPR delivery method that uses rootstock grafting to transfer mobile CRISPR RNAs from a transgenic donor plant to a compatible wild-type recipient plant, enabling the creation of homozygous edited *Arabidopsis thaliana* plants in just one generation of breeding.

The most common methods today for delivering the CRISPR enzyme and guide RNA into plants rely on plasmids and either *Agrobacterium*-mediated transformation or biolistic particle bombardment. These methods require time-consuming and complicated plant tissue culture and regeneration processes, as well as extensive crossing to remove the CRISPR transgene cassette from the edited plant. Removing the CRISPR components is often necessary as their prolonged expression could cause undesirable off-target effects and raise regulatory concerns in countries around the world.

A more direct approach for producing transgene-free genome-edited plants is to deliver CRISPR components in the form of mRNA or ribonucleoprotein complexes. The generation of genome-edited plantlets using mRNA or ribonucleoprotein biolistic delivery has been demonstrated in wheat[3,4] and maize[5]. PEG-mediated transformation followed by protoplast regeneration has also yielded transgene-free genome-edited lettuce, potato and other plants[6,7]. Although these methods avoid the need to remove CRISPR DNA from edited plants, they still require lengthy tissue culture, and it remains difficult to apply them to the many species that resist genetic transformation and regeneration, including leguminous plants (such as soybean) and most fruit trees.

A handful of studies have succeeded in dispensing with lengthy plant tissue culture. Edited plantlets have been generated by delivering a combination of genome-editing reagents and developmental regulators into *Nicotiana benthamiana* somatic cells, but the generated mutants were still transgenic[8]. Another culture-free approach—biolistic-bombardment-mediated direct delivery of CRISPR ribonucleoprotein into shoot apical meristems—produced transgene-free genome-edited wheat[9]; unfortunately, the genetic edits in these chimeric plants were not heritable. The HI-Edit approach is also capable of generating transgene-free genome edits in wheat, maize and *Arabidopsis*, but requires a haploid inducer line[10]. Recently, plant RNA viruses were used to deliver CRISPR reagents into plant germline cells in vivo, generating transgene-free genome-edited plants without the need for tissue culture; however, this method has been severely limited by a small viral packaging capacity or an inability to transduce meristematic cells[11,12].

The new study by Yang et al. largely overcomes these difficulties. Their method uses a transgenic rootstock expressing mobile

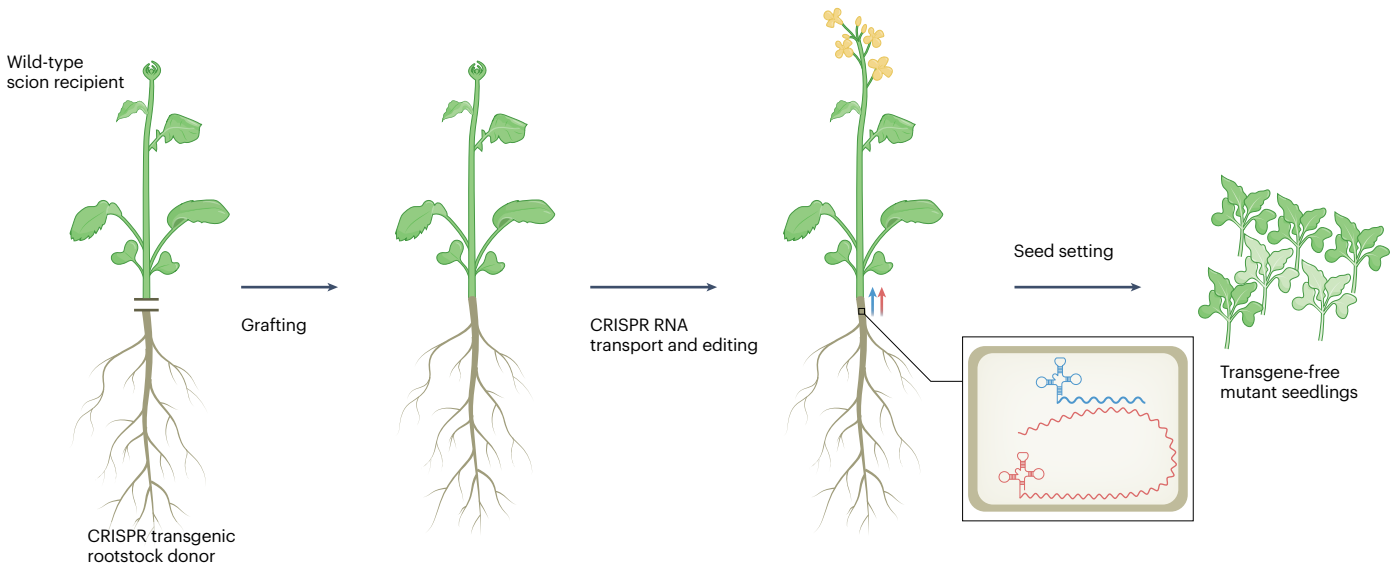

Fig. 1 | Mobile CRISPR grafting strategy for transgene-free genome editing. *Cas9* mRNA and gRNA fused to tRNA-like sequence (TLS) motifs are expressed in a transgenic rootstock line. A wild-type scion is grafted to the rootstock. The CRISPR RNAs are transported into the reproductive tissues of the grafted scion, where they perform genome editing. Transgene-free genome-edited plants are obtained in the next generation of the scion.

CRISPR RNAs, onto which is grafted a compatible wild-type plant that receives the mobile RNAs (Fig. 1). First, the authors generated stable transgenic *Arabidopsis* plants expressing *Cas9* mRNA and gRNAs fused to tRNA-like sequence (TLS) motifs that promote long-distance RNA transport. These transgenic rootstocks were then grafted with wild-type *Arabidopsis* plants. The *Cas9-TLS* and a pair of *gRNA-TLS* transcripts were detected by RT-PCR in the grafted wild-type shoots, with *Cas9-TLS* delivery frequencies ranging from 1/1,000 to 4/1,000.

Yang et al. observed the expected phenotype corresponding to an edited *nia1* gene, suggesting that both the *Cas9-TLS* and *gRNA-TLS* transcripts were successfully transported into the scion. Moreover, RT-PCR analysis and genotyping showed that the transported transcripts and genome edits were present in the siliques and flowers of the grafted plants, implying that the fusion transcripts were active in the reproductive tissues. Offspring from the grafted wild-type *Arabidopsis* plants revealed homozygous *nia1* mutants at frequencies of 1.17–1.41 per 1,000 seeds, indicating that this tissue-culture- and transgene-free delivery method could generate heritable edits in *Arabidopsis*.

Next, Yang et al. tested the method in *Brassica rapa* by grafting wild-type *B. rapa* scions across species onto *Cas9-TLS* and *gRNA-TLS* transgenic *Arabidopsis* rootstocks. The RNAs were transported to the siliques, flowers, stem and leaves of the *B. rapa* scions. Importantly, 4 out of 6 siliques and 4 out of 6 flowers of the grafted scions showed targeted genome editing as confirmed by Sanger sequencing. These findings suggest that the method is generally applicable across crop species that are graft-compatible with *Arabidopsis*.

The challenges of plant genetic transformation, tissue culture and transgene segregation represent major hurdles in efforts to accelerate CRISPR genome editing for crop breeding and basic plant research. Compared to conventional methods, the method of Yang et al. has two outstanding advantages. First, this transformation approach bypasses the need for tissue-culture-based regeneration, which is advantageous for species that are refractory to regeneration. Second, the method avoids the need for crossing and selfing because transgene-free genome-edited plants are obtained in one generation. These advances are of considerable significance for plants with long juvenile periods, such as certain fruit trees.

At the same time, the method has some limitations. A major obstacle is the need for a separate transgenic stock line for each new genetic target. One potential solution might be to engineer the rootstock using genetically movement-defective viral replicons. The approach of Yang et al. should be straightforward for recipient plants that are graft-compatible with *Arabidopsis*, such as tomato and *B. rapa*, but not for species that have no established grafting protocol, such as most monocotyledonous crop species like rice and wheat. Compared to other tissue-culture- and transgene-free genome-editing methods, the frequency of edited seeds in *Arabidopsis* is low. And the overall efficiency of heritable genome editing in other grafted species is unknown. Future research should explore the capabilities of the method and various approaches for expanding its utility.

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Competing interests

The authors declare no competing interests.