regularly interspaced, short palindromic repeats (CRISPR)-associated protein (Cas)9 reagents will likely appear in the coming months. The expectation is that new varieties created through targeted mutagenesis will be no different, from a regulatory perspective, than varieties created using chemical mutagens or ionizing radiation, approaches that do not trigger regulation.

Regulatory policy has important consequences for the application of targeted mutagenesis in agriculture owing to the costs of assembling the data packages needed to address safety requirements, which often exceed tens of millions of dollars per transgenic product. The absence of regulation would open the door to using SSN technology in a wide range of crop plants and horticultural species. The polyploid crops that might be engineered using SSNs include potato (tetraploid, 0.84 Gb), canola (tetraploid, 1.2 Gb) and sugarcane (polyploid and aneuploid, 10 Gb), as well as other hexaploid cereals like oat (13 Gb) and triticale (19.4 Gb). Many valuable traits could be created simply by knocking out genes through SSN-induced nonhomologous end joining, from disease and drought resistance to reduced immunotoxicity (e.g., reduction in the content of immunoreactive gluten for celiac patients) and increased nutritional quality (e.g., through the accumulation of healthier proteins or fatty acids).

Even greater opportunities are afforded by targeted gene replacement, in which homologous recombination is used to incorporate specific, templated DNA sequences into genomes. This approach is more difficult than gene knockouts in that both the SSNs and a repair template must be delivered to cells. New delivery vehicles, such as DNA replicons, overcome some of these hurdles and increase the frequency of homologous recombination[10]. However, additional technological advances are needed if the potential of gene replacement for crop improvement is to be fully realized. With an increasing number of laboratories using SSNs, such advances are surely forthcoming.

COMPETING FINANCIAL INTERESTS
The authors declare competing financial interests: details are available in the online version of the paper.

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New plant species through grafting

Grafting, the process by which adjacent tissues fuse and establish vascular continuity, has been used for centuries to improve plant architecture, productivity and tolerance of environmental stresses. Now, a team led by Ralph Bock of the Max Planck Institute of Molecular Plant Physiology in Potsdam, Germany, reports that entire nuclear genomes can be transferred across graft junctions to generate a new polyploid species that produces fertile progeny[1].

Many modern crops, including wheat, cotton, canola, Arabica coffee, leek, oat and peanut, are allopolyploids—species that contain two or more chromosome sets derived from different species—which were created through sexual hybridization. However, allopolyploidization is a relatively rare phenomenon. "Strong barriers prevent sexual hybridization between most species," explains Luca Comai of the University of California, Davis. "This demonstration that allopolyploids and chromosome addition lines (the approach should also enable the transfer of single chromosomes) can arise by an asexual mechanism might open significant new opportunities for crop improvement."

The most striking outcome of the study arose from grafting stems of transgenic, hygromycin-resistant cigarette tobacco (*Nicotiana tabacum*, an herbaceous species with 48 chromosomes) to stems of transgenic, kanamycin-resistant tree tobacco (*Nicotiana glauca*, a woody species with 24 chromosomes). After fusion of the tissues from the different species had occurred, the graft site was excised and cultured on regenerative media that contained both kanamycin and hygromycin. Analysis of 45 doubly resistant plants derived from 12 grafted plants indicated not only that they had 72 chromosomes, which is the sum of the chromosome numbers of their parents, but also that the new species—tentatively named *Nicotiana tabauca*—outgrows its progenitor species while having many other traits that are intermediate between those of its parents.

Gloria Moore of the University of Florida in Gainesville points to the potential of the approach for breeding new citrus varieties. "Many citrus types display nucellar embryony, which is a process by which somatic cells of the nucellus (the tissue in the ovule but outside the embryo sac) produce multiple embryos that are genetically identical to the maternal parent plant. Nucellar embryony poses a major impediment to the production of citrus hybrids because male cells make no contribution to the formation of nucellar embryos. Self- and cross-incompatibilities are also common among citrus species," she explains.

Notwithstanding the potential of the grafting technique, both Moore and Comai point out that the use of selectable markers in the proof-of-concept study leaves open the question of whether the frequency with which hybrids or polyploid plants arise is sufficient to identify their nontransgenic variants by marker scoring alone. "Considering the ease with which tobacco plantlets can be regenerated from callus, rates of shoot regeneration might be limiting for certain crops," cautions Comai. "Nonetheless, grafting is much simpler and more widely applicable than protoplast fusion, which has been the only approach to circumvent obstacles to sexual hybridization in efforts to generate improved plant cultivars," he adds.

Peter Hare, Senior Editor, Nature Biotechnology

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