Resistance from relatives

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Crops are made resistant to pathogens such as wheat stem rust, Asian soybean rust and potato late blight by methods to access the pool of resistance genes present in related plants.

Plant crop yields are often decimated by diseases. One method of disease control is to breed plant resistance (R) genes that are effective against specific microbial pathogens into crops. However, concerns have been raised over the time and cost of resistance breeding, the durability of resistance and the potential for pathogen evolution to outpace our ability to identify and deploy R genes[1,2]. In this issue, three studies[3–5] report improved approaches to identify, clone and use plant R genes from relatives of elite varieties. Collectively, these findings broaden the available pool of R genes that can be used to secure more crops against disease.

In the mid-1990's, R genes were cloned and shown to function upon transformation into distantly related species[1]. The encoded R proteins are usually present inside plant cells, where they detect pathogen effector proteins secreted into the plant cell during infection and activate effector-triggered immunity. Dozens of R genes, each conferring resistance to strains of fungal, oomycete, viral, bacterial, nematode or insect pathogens, have been cloned from diverse plant species. Indeed, there are a few hundred R genes in every plant[1]. Most of these R genes encode nucleotide binding, leucine-rich repeat (NLR) proteins (also known as NB–LRR proteins), which form a core part of the plant innate immune system. NLR homologs also function as intracellular receptors in mammalian innate immune systems. Sequences of conserved motifs in NLR proteins can be used to identify candidate R genes[1].

Breeding R genes into crop varieties takes time and can lead to yield penalties caused by the introduction of deleterious alleles (unlinked or linked to the R gene) when crossing with a plant that carries the desired R gene but is otherwise not optimal for agricultural use. Molecular marker technologies enable well-funded plant breeders (a minority) to cross in R genes from exotic plants and then efficiently recover elite genotypes, but this requires multiple crosses and generations. More importantly, despite the abundance of R genes present in the plant kingdom, classical plant breeding can only use R genes from plants that are sexually compatible with a particular crop. To produce durable disease resistance, one option is to engineer plant varieties with multiple 'stacked' R genes effective against a pathogen, but this requires a large set of available R genes. The trio of studies in this issue[3–5] report methods that expand the R gene pool (**Fig. 1**).

Steuernagel *et al.*[4] use MutRenSeq, a three-step method based on R gene enrichment sequencing (RenSeq), to clone two stem rust resistance genes from hexaploid bread wheat. One of the genes cloned is *Sr22*, a known gene from wheat relatives that confers resistance to strains of the Ug99 group of wheat stem rust pathogens. Classical breeding of *Sr22* into commercial wheat lines has been hampered by linkage to genes that cause poor agronomic performance. The authors used chemical mutagenesis to generate susceptible progeny from a resistant donor plant, then used small regions of homology to known R genes to capture[6] a fraction of transcripts enriched for candidate R genes from both susceptible and resistant lines. Comparative sequencing of cDNA libraries identified the R gene mutations likely to have converted the resistant parent to disease susceptibility. Because mutagenesis resulted in dozens of mutations in the genomic NLR-encoding R gene candidates, availability of six independent putative *Sr22* mutant lines was important to enable bioinformatic identification of *Sr22*, which was mutated in five of six independent lines. Subsequent isolation of an *Sr22* promoter

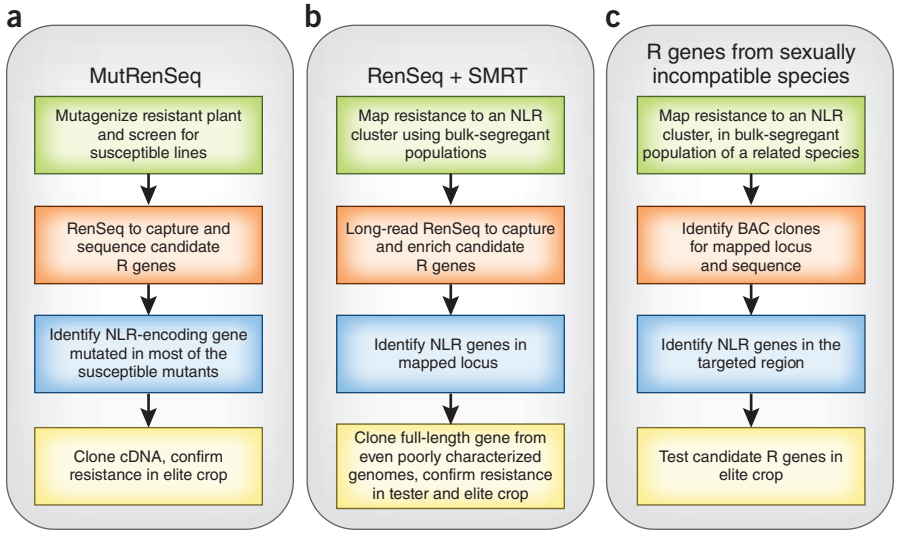

Figure 1 Strategies to clone R genes for elite crops. (**a**) The MutRenSeq method for cloning R genes from the host plant line or a related non-elite line. (**b**) RenSeq combined with single molecule, real-time (SMRT) sequencing for cloning R genes from the host plant line or a related non-elite line. (**c**) Traditional map-based cloning is used, but is focused on cloning R-gene homologs from less closely related plants.

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and full-length cDNA allowed transformation of the gene into a cultivated spring wheat variety, where it conferred resistance to stem rust.

Witek *et al.*[5] pursued a similar approach to clone resistance genes specific for potato late blight, a major potato disease that is currently managed by multiple pesticide applications during the growing season. They also used RenSeq to capture a library of gene sequences with weak homology to known R genes[6] but combined the method with long sequence reads using Pacific Biosciences technologies. Long reads and ~20× coverage with high-accuracy short reads enabled *de novo* assembly of the NLR repertoire from a previously unsequenced, highly divergent wild relative of cultivated potato. Rather than using mutagenesis, Witek *et al.*[5] relied on initial bulk-segregant genetic mapping of the resistance locus followed by fine mapping to distinguish between three linked R gene clusters. Identification of the cognate R gene was completed by expressing full-length clones of candidate R genes in *Nicotiana benthamiana* leaves and identifying the gene that conferred protection to *Phytophthora infestans* challenge. A transgenic potato line harboring the resultant R gene was resistant to *P. infestans* infection.

The third paper, by Kawashima *et al.*[3], accesses R genes from a donor species that cannot be crossed with the target crop. All commercial soybean cultivars are susceptible to Asian soybean rust, caused by *Phakopsora pachyrhizi*, which is prevalent in South America. Fungicide use in this region has subtantially increased owing to the incidence of Asian soybean rust. The authors take advantage of the observation that some accessions of pigeonpea are fully resistant to Asian soybean rust. One pigeonpea accession was found to be resistant to all of the 80 diverse soybean rust isolates that were tested. The relevant R gene from this accession was cloned by genetic mapping of closely linked markers, isolation of genomic BAC clones carrying the candidate gene region and shotgun sequencing to identify the four NLR-encoding genes on the BACs. One of these candidate genes, *CcRpp1*, produced rust-resistant genetically modified soybeans.

The methods described in these papers expedite R gene isolation, but caveats remain. For example, some R gene products 'guard' a second host protein[1,2], and for R genes from less-related plant species, both the R gene and the gene encoding the guarded protein may need to be engineered into a susceptible plant. Also, in a mutagenesis-based screen such as that adopted by Steurnagel *et al.*[4], some susceptible plants may arise from mutations in genes other than R genes, and susceptible R gene mutants may be difficult to detect if more than one R gene in the mutagenized parent recognizes the pathogen used for mutant screening. Screens for R genes may be most successful when resistance elicitation can be attributed to a single pathogen effector; such efforts require more initial research but are already well underway for many plant pathogens[7]. Use of isolated effectors may be especially preferable if it leads to identification of R genes that target those effectors that are most common in, and least readily jettisoned by, the pathogen population[7].

Most researchers agree that to increase the durability of R gene efficacy it is important to release only plants that carry at least two stacked R genes that are effective against the same pathogen strains[1,2,8]. Individual pathogen isolates must harbor two rare mutations in virulence proteins to successfully reproduce on a plant that harbors two stacked R genes. Indeed, Kawashima *et al.*[3] stress that soybeans expressing *CcRpp1* should be released to growers only as part of a multicomponent resistance package, as rusts that overcome *CcRpp1* resistance might otherwise arise within a few growing seasons. R genes pyramided as a single multigene cluster offer the further advantage of expediting plant breeding by generating co-inheritance at a single locus.

Taken together, these papers[3–5] present methods to substantially expand the pool of available R genes. The next step will require wider acceptance of technologies that move R genes from one plant to another to combat plant disease. In addition, current rules often require expensive 'event-by-event' retesting and recertification each time a previously approved transgene is transformed into a different plant[9,10]. A more rigorous science-based system for risk assessment would be likely to streamline the reuse of R genes in different plants. Unfortunately, engineering of improved R gene repertoires, with concomitant benefits in reduced pesticide application, may currently be feasible only in high-profit crops.

COMPETING FINANCIAL INTERESTS
The author declares no competing financial interests.

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Comparing CRISPR and RNAi-based screening technologies

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Two studies provide an experimental side-by-side comparison of genetic screening methods.

High-throughput loss-of-function screens are well-established approaches for characterizing genotype to phenotype relationships. Currently, both RNA interference (RNAi) and CRISPR-based screening approaches are used, but so far no systematic side-by-side comparison of their relative merits has been performed. In this issue, studies by Evers *et al.*[1] and Morgens *et al.*[2] perform viability screens in cell lines to assess how reliably and effectively the two methods identify essential genes. Both studies attest to the high sensitivity of CRISPR-based knockout screens, but differ in their assessment of the relative performance of RNAi screens.

RNAi screens in either arrayed or pooled format have been employed to study many different biological questions, ranging from basic biological processes such as signaling or cell morphology to identification of drug targets for human diseases for many years now[3]. However, the technology has several limitations. For instance, many RNAi reagents result in inefficient knockdown of the target gene, leading to false-negative results. In addition, the prevalence of off-target effects, where additional genes are unintentionally perturbed, leads to false-positive results. Such issues have led to poor reproducibility between screens[4] and considerable effort has gone into developing modified

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