

CROP BREEDING

Variation engineering

Cell 171, 470–480 (2017)



Credit: Daniel Rodríguez-Leal, Cold Spring Harbor Laboratory

Agronomically desirable, natural mutations have been the main driver for crop breeding since the rise of agriculture. However, the paucity of these favourable alleles in plant gene pools restricts breeding efficiency. To address this problem, Daniel Rodríguez-Leal, from the Cold Spring Harbor Laboratory, and colleagues report an approach allowing rapid generation of abundant cis-regulatory alleles in tomato that confer a continuum of phenotypic variation for three yield-related traits. This approach is based on CRISPR–Cas9 genome editing and a smart genetic scheme.

Natural regulatory variations of two genes, *WUSCHEL* (*WUS*) and *CLAVATA* (*CLV3*), caused an increase in seed compartments (locules) during tomato domestication. By targeting the cis-regulatory element of the *WUS* gene, using CRISPR–Cas9, the researchers

obtained edited plants with increased locule numbers, mimicking the effect of the domestication allele.

Similarly, CRISPR–Cas9 mutagenesis of the *CLV3* promoter generated multiple novel mutations with various effects on floral organ number. To expand the number of novel alleles, the researchers designed a crossing scheme where the promoter-edited transgenic plants, containing a single-copy Cas9 transgene, were outcrossed to wild-type plants. The trans-generationally inherited Cas9 then induced diverse new mutations in the wild-type *CLV3* promoter in hundreds of F₁ individuals, resulting in a continuum of locule number variation in the F₁ population. Intriguingly, these novel alleles can be immediately fixed in a transgene-free background in 1/16 of the F₂ offspring.

Neither the level of sequence conservativeness around the mutant

alleles nor the alleles' effects on the expression of *CLV3* and *WUS* could properly predict the phenotypic effects, suggesting no linear relationship between the mutation's molecular and phenotypic effects.

A similar experimental scheme was applied to engineer new alleles in the promoters of another two genes that control tomato inflorescence and shoot architectures, respectively. In both cases, a continuum of phenotypic modification ranging from weak to strong was obtained, indicating the potential of this approach in engineering variations for diverse agronomic traits.

Jun Lyu

Published online: 6 November 2017
<https://doi.org/10.1038/s41477-017-0059-8>