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GETTY

PLANT GENETICS

Branching out for crop improvement

> a detailed understanding of genetic interactions facilitates rational crop improvement

Inflorescence architecture is the arrangement of flowers and their underlying stem branching patterns, and it has important effects on the yield of the fruits or grains from agricultural plants. A new study dissects key genetic underpinnings of tomato inflorescence branching, which not only provides insight into how genes that control branching have conferred benefits and problems during historical plant domestication and breeding, but also demonstrates that genetically informed alterations to inflorescence branching can increase yield.

To search for genes controlling inflorescence architecture, Soyk, Lemmon and colleagues screened a collection of 4,193 accessions of wild and domesticated tomato plants to identify mutant lines that have an increased extent of branching relative to the unbranched arrangement of wild and current commercially grown lines. Previous work from some of the authors had identified mutations in the *COMPOUND INFLORESCENCE* gene as a cause of highly branched phenotypes, for which the branching is so extensive that the plants cannot sustain development of all flowers, hence decreasing fruit yield. In the current study, the authors focused on three weakly branching lines from the collection (and a fourth line previously derived from X-ray mutagenesis). All four lines also had 'jointless' pedicels (the short stems leading to the tomato fruits), which is an agriculturally desirable trait that prevents excessive fruit detachment and facilitates mechanical harvesting.

The team then carried out a detailed molecular characterization of the mutant lines: RNA sequencing (RNA-seq) was used to identify the developmental stages affected and the particular gene expression alterations; and mapping-by-sequencing was used to map the location of causal genes based on which mutant alleles co-segregate with phenotypically mutant progeny. Overall, the team found that the branching phenotype was caused by concurrent homozygous mutations in two different SEP4 family members of MADS box transcription factors, termed *JOINTLESS 2* (*J2*) and *ENHANCER OF JOINTLESS 2* (*EJ2*). Evidence that these are indeed the causal genes came from the identification of mutations in both of these genes in all four weakly branching accessions, disrupted expression of these genes in the RNA-seq data, and expected phenotypes from CRISPR-based mutation of these genes.

Further functional studies and genetic analyses of diverse accessions revealed an intriguing interplay between these genes. As SEP4 family members, *J2* and *EJ2* are largely functionally redundant, as mutations in both genes are required for the branching phenotype. However, there are functional differences between the two genes: mutant *EJ2* was selected during early plant domestication and is associated with a larger calyx to support larger fruit, and mutant *J2* was more recently selectively bred to confer the intended jointless phenotype. In standard genetic backgrounds of tomato, combination of these two desirable mutations displays negative epistasis (genetic interactions causing reduced fitness), as the resultant inflorescence branching is excessive and reduces fruit yield. This negative epistasis has been overcome in most commercial lines by subsequent selective breeding to introduce suppressor mutations (one of which the authors mapped) that result in unbranched plants despite the presence of mutations in both *J2* and *EJ2*.

The authors reasoned that selection of unbranched phenotypes in current commercial lines missed an opportunity to take advantage of mild branching for improved yield. Given the dependence of branching on SEP4 family function, the authors used natural and engineered alleles of *J2*, *EJ2* and a third SEP4 family member (*LIN*) and combined them in different heterozygous and homozygous configurations to create hybrid lines with a range of SEP4 gene dosages and branching complexities. Importantly, this rational fine-tuning of branching was tested in a greenhouse cultivation trial of commercial plants, which demonstrated that weak branching increases yield.

Overall, this study highlights how a detailed understanding of genetic interactions facilitates rational crop improvement, and the principles are potentially applicable to various traits and species. Finally, although the studied mutations disrupt protein-coding sequences, it will be interesting to explore whether other types of mutations (such as edited regulatory sequences) will allow further fine-tuning of these yield-associated traits.

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ORIGINAL ARTICLE Soyk, S., Lemmon, Z. H. *et al.* Bypassing negative epistasis on yield in tomato imposed by a domestication gene. *Cell* http://dx.doi.org/10.1016/j.cell.2017.04.032 (2017)
FURTHER READING Meyer, R. S. & Purugganan, M. D. Evolution of crop species: genetics of domestication and diversification. *Nat. Rev. Genet.* **14**, 840–852 (2013)