IN BRIEF

BREEDIT: fast breeding tools to match the fast pace of climate change

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Throughout the history agriculture, humans have selected plants with the most favorable characteristics that confer higher productivity or better adaptation to new territories. Conventional breeding relies on the exploitation of genetic variation obtained by crossing varieties with desirable traits followed by the analysis of phenotypic variation among their progeny and the selection of individuals with improved characteristics. However, this is a slow process that requires multiple generations of crossing; it can take years to obtain a new variety and often leads to only modest improvements. In addition, several yield-related traits are controlled by multiple genes having redundant functions in plant development, or by many distinct genes acting in the same pathway, each having a small effect on the final phenotype (nicely reviewed by **Mickelbart et al., 2015)**, implying that several chromosomal segments must be combined to achieve considerable improvements. Modern genomics tools promise to greatly enhance the speed and precision of plant breeding (**Van Vu et al. 2022**), but improvements are still needed to fully realize their benefits (**Gaillochet et al., 2021**).

In this issue, **Christian Damian Lorenzo and colleagues** (**Lorenzo et al., 2022**) introduce BREEDIT, a new tool that speeds up plant breeding by accelerating the discovery of genetic determinants that control agronomically important traits (e.g., yield and stress tolerance) in maize. The authors set up a pipeline using CRISPR/Cas 9 to create a diverse set of multiplex gene-edited plants (i.e., with editing of multiple different genes or specific DNA loci), based on 48 candidate genes with known and conserved functions in plant growth regulation in maize, rice, and Arabidopsis.

Multiplex CRISPR/Cas9 genome editing was used to generate genetic variation in a set of target genes that have small effects on complex traits (12 guide RNAs in 4 vectors called SCRIPTs). Super-transformed plants (containing the SCRIPT and Cas9 EDITOR vectors, **Figure 1**) were genotyped using Highly multiplex (HiPlex) amplicon sequencing and crossed to generate a collection of edited plants with all possible combinations of gene knockouts. In addition to back-crosses and self-crosses, the improved crossing scheme of gene-edited plants also included intra-script crosses (i.e., complementary mutations in the same gene family generated by the same SCRIPT) and inter-script crosses (i.e., mutations in genes of different families generated by different SCRIPT). The segregating progenies were then screened for agronomically relevant traits, such as yield potential and drought tolerance, by using high throughput phenotyping. In this way, an impressive amount of genetic variability in target traits could be obtained and tested in only two generations, thus accelerating the

identification of specific combinations of gene variants associated with improved characteristics.

Given that several candidate genes used in this work act as negative regulators of plant development, their loss of function is expected to produce positive effects on plant growth. Nevertheless, multiple knockouts generated by SCRIPT1 - which targets genes involved in the catabolism of Gibberellic Acid (**Huang et al., 2015**) - caused enhanced growth of vegetative organs but abnormalities in reproductive organs that led to male sterility in transformed generations. On the other hand, multiple knockouts generated by SCRIPT2 - which targets genes involved in the metabolism of cytokinin, previously shown to be involved in plant response to drought at early developmental stages (**Rida et al., 2021**) – displayed increased growth under water-deficient conditions.

In summary, BREEDIT shows promise as an effective and fast pipeline to simultaneously modify and test multiple members of a gene family with redundant functions in plant growth.

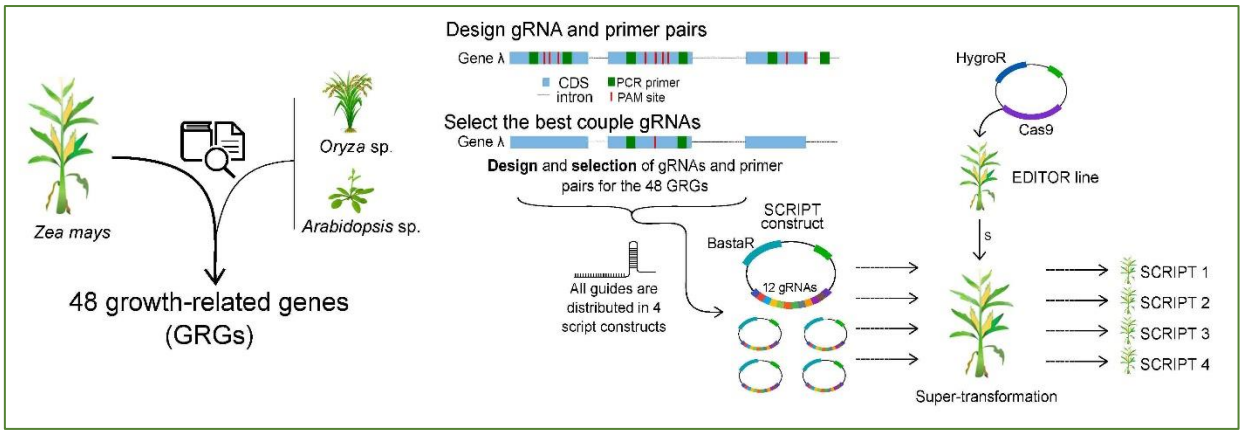

Figure 1. BREEDIT strategy to obtain multiple gene-edited maize plants
Left, selection of candidate genes involved in the regulation of plant growth based on literature search. Right, cloning of 12 guide RNAs (gRNAs) in SCRIPT vectors for multiplex gene-editing, for use in super-transformation of an EDITOR line that expresses Cas9. Adapted from Lorenzo et al. (2022), Figure 1.

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