Trends in
Plant Science

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Spotlight

Foxtail Millet: A New Model for C4 Plants

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Arabidopsis and rice are major models for C3 plants, but we still lack a model for C4 plants. Recently, Yang and coworkers developed foxtail millet as a C4 plant model; with the rapid development of clustered regularly interspaced short palindromic repeats (CRISPR)/Cas technology, this will open a new era for plant functional studies and crop improvement.

The Scientific Community Needs More Model Plants

A good plant model not only provides a tool and resource for plant gene functional studies but also speeds up crop improvement and simplifies agronomic practices. In the past 20 years, first arabidopsis (*Arabidopsis thaliana*) and then rice (*Oryza sativa*) have become plant genetic models that have not only enabled the elucidation of many biological pathways but have also facilitated basic genetic and genomic functional studies in plants, leading to fundamental discoveries and technological advances [1]. However, both arabidopsis and rice are C3 plants, and therefore cannot be used for studying C4 plant-associated traits, such as highly efficient CO_2 fixation through the Hatch–Slack pathway. Although C4 plants make up only a small percentage (~3%) of flowering plant species, they account for ~25% of terrestrial primary productivity and ~30% of global agricultural grain production [2]. This is primarily due to the fact that photosynthesis in C4 plants is highly efficient for fixing and converting CO_2 from the air into carbohydrates; C4 plants also generally show higher nitrogen and water usage efficiencies [3]. However, almost all C4 plants have relatively large stature and a long life cycle, and are also refractory to plant tissue culture and regeneration. This limits the study of plant traits associated with C4 plant species, and therefore identifying a suitable model for studying C4 plants is long overdue. Developing a C4 plant model will accelerate the elucidation of the molecular mechanisms controlling CO_2 fixation via the Hatch–Slack pathway, and may allow C4 pathway genes to be introduced into C3 plants to obtain new crops with higher biomass yield and lower nitrogen and water requirements to meet the growing global demands for bioenergy and food production [4].

Foxtail Millet Is Becoming a New Model Plant

The annual grass Foxtail millet (*Setaria italica*) is one of the most ancient, domesticated crops in the world, with cultivation starting >10 000 years ago [5]. It is also an important staple food that is widely cultivated in semi-arid regions in Asia, Eurasia, and Africa; foxtail millet is also thought to be an important crop to ensure food and nutritional security in the face of a quickly growing world population, particularly during abnormal situations such as the current coronavirus disease 2019 (COVID-19) pandemic [5,6]. Compared with other plants, especially crops, foxtail millet has many elite traits, including tolerance to abiotic stresses (e.g., drought and salinity), ease of cultivation with low fertilizer requirement, and the ability to grow on infertile land. More importantly, foxtail millet is a C4 plant species that can covert CO_2 into carbohydrate with a higher photosynthetic efficiency than C3 plants. Foxtail millet has a small diploid genome of ~490 Mb and only nine pairs of chromosomes. Thus, the simple genome of foxtail millet is easier to investigate and modify for both fundamental and applied research. Since it was first fully sequenced in 2012, foxtail millet has attracted increasing attention from the scientific and industrial communities [7]. A recent study by Yang and colleagues has now reaffirmed that foxtail millet could become an arabidopsis-like model [3] for studying fundamental and applied aspects of plant genetics and agronomy.

A large mutant population (~20 000), generated chemically by ethyl methanesulfonate (EMS)-mutagenesis of an elite foxtail millet cultivar Jingu21, allowed Yang and coworkers to successfully screen for and identify a mutation called *xiaomi* [3]. The *xiaomi* mutant shows traits that make it a good candidate for a plant model species similar to arabidopsis. First, the *xiaomi* mutant has a short life cycle that was reduced compared with the wild type from about 130 days to 70 days, and its flowering time was reduced from 82 days to 39 days. This short life cycle makes this mutant more accessible for gene functional studies on plant growth and development. Although the new mutant line is much smaller, it produces more seeds (up to 12.8% more) than the wild type. These new features of small size, a short life cycle, and high seed production make *xiaomi* culturable in the laboratory and even in a standard growth chamber with multiple generations per year. These are perfect features for a plant model in which to study different plant traits. In addition, the authors also sequenced the mutation and developed a multiomic searchable database with whole-genome information and a transcriptome atlas at different developmental stages. All these genetic and genomic data, including noncoding RNA expression profiles [8], are an important resource for a model plant species. More importantly, Yang and colleagues also developed a highly efficient *Agrobacterium*-mediated genetic transformation system for foxtail millet *xiaomi* and obtained an average transformation rate of 23.28% with a marker gene *NPTII*, which is comparable with the transformation rates in the current model species, arabidopsis, and rice. All these data suggest that foxtail millet *xiaomi*

is a suitable plant model for C4 plants. This model will accelerate the study of traits unique to C4 plants such as CO_2 fixation via the Hatch–Slack pathway.

In the past couple of years foxtail millet has already been employed to study some important traits. Using foxtail millet as a model, Zhao and coworkers demonstrated that DROOPY LEAF1 (DPY1), a leucine-rich repeat (LRR) receptor-like kinase, controls leaf architecture, including leaf droopiness, by orchestrating early brassinosteriod signaling [9]. Overexpression of DPY1 significantly enhanced the number of upright leaves, and led to thicker stems and bigger panicles [9]. DPY1 is also evolutionarily conserved across the plant kingdom [9]. This suggests that DPY1 has potential for studying plant architecture and improving crop yield.

CRISPR/Cas9 Speeds Up the Development of New Model Plants

The new plant model foxtail millet was generated by chemical EMS-induced mutagenesis. *Xiaomi* is a point mutation in the phytochrome C (*PHYC*) gene encoding a light receptor that is essential for photoperiodic flowering [3]. Generating and screening genetic mutations is a common method for creating new germplasm. In the past this has mainly been achieved by chemical (e.g., EMS) and/or physical (e.g., X- or γ-ray) mutagenesis. However, these methods are time-, cost-, and labor-intensive, and they require the generation of a large mutant population followed by screening for a suitable mutation. The recently developed CRISPR/Cas9 genome-editing technology, including base editing and prime editing, provides a powerful and robust tool for creating desired genetic mutations. CRISPR/Cas9 is a precision method for editing a target gene for various purposes, including silencing, overexpressing, regulating, and monitoring individual genes. CRISPR/Cas9 has been widely used to create diverse animal models for studying human genetic

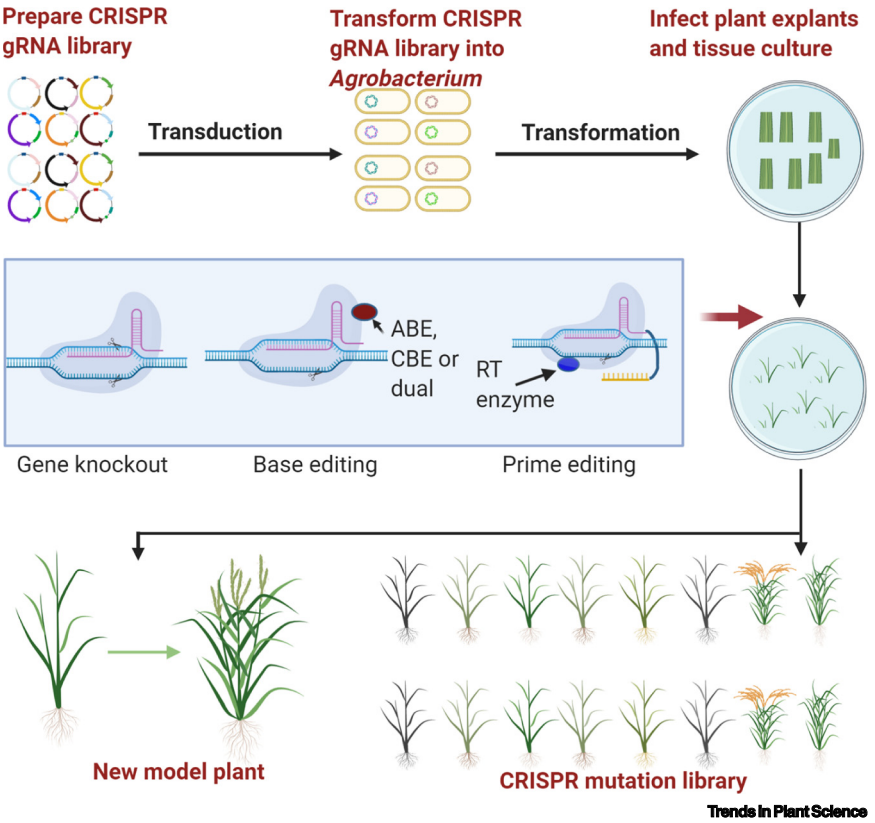

Figure 1. Creating a New Plant Model and a Mutation Library Using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 Genome-Editing Tools. CRISPR/Cas-based genome editing has revolutionized research and applications in almost all biological and biomedical fields, including plant gene function studies and crop improvement. By targeting a specific gene, it is possible to create a plant that can be used as a model system for investigating specific traits, such as the Hatch–Slack pathway in C4 plants. Through the construction of a CRISPR/Cas guide RNA (gRNA) library, it is also possible to target all potential genes for precision knockout and to produce a mutant population as an important resource for creating a new model plant. During gRNA library preparation, different gRNAs can be inserted into the same tumor-inducing (Ti) vector or different Ti vectors, then transformed into *Agrobacterium* for genome-wide editing of plant genes. Several CRISPR/Cas-based genome-editing tools, including the newly developed prime editor, can be employed to generate genetic mutation libraries and model mutation lines with a specific trait in plant species. The commonly used CRISPR/Cas technology first generates a double-strand break (DSB); DNA repair mechanisms that repair the DSB can cause indels (insertion or deletion of nucleotides), primarily via the nonhomologous end-joining (NHEJ) pathway. In some cases the DSB may be also repaired by the homology-directed repair (HDR) pathway in the presence of a homologous DNA template. Because Cas enzymes have independent binding and cutting activities, and loss of cutting activity does not affect binding, scientists have created nickase Cas (nCas) and catalytically dead Cas (dCas). nCas generates a single-strand DNA break, whereas dCas does not cleave the DNA sequence, but both bind tightly to the target DNA sequence. Importantly, other functional enzymes/molecules can be fused to Cas, nCas, or dCas enzymes and offer CRISPR/Cas new functions, including base editing and prime editing. Base editing coverts an individual base to another for introducing a point mutation. Prime editing is a new application of the CRISPR/Cas technology that employs a reverse transcriptase fused to nCas and a prime editing guide RNA (pegRNA) instead of a gRNA; when nCas cuts a DNA strand it serves as a template for pegRNA and generates a new strand DNA. Thus, prime editing can introduce new DNA sequence, including point mutations, without a DNA template. Prime editing is becoming a game-changer for modifying plant genomes, including all 12 types of base-to-base conversions [12]. CRISPR/Cas-mediated genome editing will facilitate the study of gene function and precision breeding in plants. This figure was created using BioRender (BioRender.com). Abbreviations: ABE, adenine base editor; CBE, cytosine base editor; RT, reverse transcriptase.

diseases [10]. Although great progress has been made in plants for gene functional studies and crop improvement, there has been no report on creating genetic models by using CRISPR/Cas9-mediated genome editing, but this is likely to change soon. The CRISPR/Cas9 technology is now a valid alternative strategy for developing designer plant models for specific purposes (Figure 1).

Concluding Remarks and Future Perspectives

A short life cycle and small plant size, in addition to whole-genome sequencing data and an efficient transformation method, make the foxtail millet *xiaomi* mutant a suitable model for studying plant traits at the molecular, cellular, biochemical, and physiological levels; *xiaomi* may also become a model to study agronomic practices as well as nutrient and stress physiology. However, before *xiaomi* can become a genetic model for studying plant traits, different genetic mutations should be created and/or developed. The well-developed transformation method for foxtail millet [3] and new CRISPR/Cas9 genome-editing tools [11] provide a solid foundation for *xiaomi* to quickly become a new plant model, particularly for C4 plants. By designing CRISPR/Cas9 guide (g)RNA libraries, we can quickly obtain genetic mutations in foxtail millet that are suitable for studying different traits. Arabidopsis and rice are current plant models, not only because of their short life cycles, small size, and highly efficient transformation procedures but also because large mutant libraries are available. These mutant collections have been generated by the *Agrobacterium* T-DNA method or chemical mutagenesis for different research purposes, by scientists for decades. The novel CRISPR/Cas9 technology now allows the creation of large mutant libraries much faster than ever before in the new model plant species, foxtail millet, to address questions of plant evolution, development, and response to environmental factors, particularly for traits unique to C_4 plants (Figure 1).

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Spotlight

GRF-GIF Chimeras Boost Plant Regeneration

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Low regeneration efficiency hampers plant transformation. Two independent studies demonstrate that GROWTH-REGULATING FACTORs (GRFs) alone or in chimeras with GRF-INTERACTING FACTOR (GIF) dramatically boost regeneration from tissue cultures from a broad range of species. GRF-GIF chimeras could be a game-changer in plant transformation and gene editing.

In response to hormones such as auxins and cytokinins, most plant tissues can dedifferentiate *in vitro* and form an undifferentiated mass called a callus, which consists of dividing pluripotent stem cells. Entire plants can be regenerated from such explants provided proper conditions are applied to stimulate organogenesis or somatic embryogenesis. However, despite considerable progress in recent years, the regeneration efficiency in most plants remains low, which limits genome editing applications and transformation for crop improvement [1]. Expression of developmental regulators, such as *BABY BOOM* (*BBM*) and *WUSCHEL* (*WUS*), is viewed as a promising strategy to boost plant regeneration [2–4]. Unfortunately, these developmental regulators have negative pleiotropic effects when constitutively expressed and have to be excised from engineered plants. This restricts their application and calls for new methods that facilitate regeneration.