Restriction Release: Improved Maize Transformation Efficiency

Improvement of crops using traditional breeding is too slow to ensure food production able to sustain the growing human population, especially in the face of climate change (Hickey et al., 2019). Transformation methods for monocot crops depend on the availability of immature embryos and are effective only for a limited number of genotypes, oftentimes eliminating the most productive elite varieties, which are recalcitrant to transformation. Lowe et al. (2016) recently addressed the above issues by equipping a vector for DNA delivery with the embryogenesis-inducing genes *Wuschel* (*WUS*) and *Baby Boom* (*Bbm*). Expression of *WUS* and *Bbm* in the positive transformants promoted callus regeneration, thereby increasing the recovery rate and widening the range of cultivars available for transformation.

In this issue of *Plant Physiology*, Zhang et al. (2019) introduce a new system that employs *WUS* and *Bbm* for efficient delivery of CRISPR/Cas9 in maize (*Zea mays*). They constructed a ternary vector system, carrying maize *WUS2* and *Bbm* genes, two glyphosate-resistance genes (*GAT* and *CP4EPSPS*), and a Cre/*lox* module. The Cre/*lox* module is driven by a drought-inducible promoter (*Rab17*), which ensures that the cassette containing the morphogenesis regulators (*WUS2* and *Bbm*) is removed upon desiccation treatment of the developing callus. The transformation efficiency for the plants transfected with the morphogenesis regulators increased from 20% to 60% compared with the vectors without the *WUS2* and *Bbm* genes. The efficiency of gene editing with these vectors carrying a single guide RNA was similar to the traditional vector, but the increased transformation efficiency of the vector carrying morphogenic regulators resulted in an overall higher success rate.

The authors further improved *Agrobacterium tumefaciens*-mediated DNA delivery by developing a new binary vector (pGreen3), which carries the T-DNA insertion and the replication origin pRK2 (oriV; Zhang et al., 2019). The helper plasmid (pVS1-VIR2) carrying the virulence genes ensures efficient transformation and serves as a replication helper for pGreen3. The two plasmids, pVS1 and pGreen3, showed improved stability in transformed *A. tumefaciens*, further enhancing transformation efficiency.

Oftentimes, the availability of new methods is restricted by the limited availability of the resources; however, the tools developed by Zhang et al. (2019) are available in public repositories (Addgene and Molecular Cloud), and the guide RNA can be exchanged in a jiffy using a one-step Gateway reaction. The method developed by Zhang et al. (2019) illustrates that combining tissue dedifferentiation with compatible vectors can unlock a higher transformation efficiency, releasing the restrictions on the transformation of obstinate maize inbred lines like ND88, which can now be successfully transformed. This work further confirms that driving transformed tissue to a quasi-embryonic state provides an important contribution to the gains of the transformation efficiency.

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