Please cite this article in press as: Ali et al., CRISPR-TSKO: A Tool for Tissue-Specific Genome Editing in Plants, Trends in Plant Science (2019), https://doi.org/10.1016/j.tplants.2019.12.002

Spotlight

CRISPR-TSKO: A Tool for Tissue-Specific Genome Editing in Plants

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Functional genomics is at the core of studying the exact function of genes. However, homozygous knockouts of essential and pleiotropic effectors (almost 10% of the genome) are not always possible, thus, functions of these genes remain obscured. The tissue-specific genome editing tool (CRISPR-TSKO) recently described by *Decaestecker et al.*, can characterize these indispensable genes and has wide applications in plants.

Heritable Genome Editing in Plants

The adaptive immune system of prokaryotes, [clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR-associated proteins (CRISPR/Cas system)] CRISPR-Cas9 system, has been domesticated as a powerful genome-editing tool that has revolutionized the functional genomics of eukaryotes [1]. In the *Streptococcus pyogenes* CRISPR/Cas9 system, Cas9 endonuclease is directed by a single guide RNA to a complementary 20-nucleotide target DNA sequence where, upon the recognition of the protospacer-associated motif (NGG), the active Cas9 make DNA double-stranded breaks (DSBs) [2] and the following imprecise repair of DSBs leads to targeted mutations in DNA. The CRISPR-Cas9 system has been established successfully in the majority of model and crop species for functional knockouts and trait improvements [3,4]. Similarly, coupling of the CRISPR-Cas9 system with a homology-directed repair system paved the way for precise genome engineering in plants [5]. Moreover, CRISPR-Cas9 was applied to produce foreign DNA-free, non-genetically modified organism (GMO)-improved crops [6]. However, all of these genome-editing approaches have two main objectives, that is, to improve the rate of targeted mutagenesis and to recover mutant progenies [1]. For that, various methodologies like use of regulatory elements (enhancers, promoters, and terminators), specific cell-cycle/phases, viral replicons, and physical and chemical conditions were applied to enhance expression of CRISPR-Cas9 machinery and hence the recovery of edited plants [3,4,7]. Despite their success, it remains that more than 10% of *Arabidopsis* (*Arabidopsis thaliana*) genes, cannot be characterized with these methods. These genes are essential or have pleiotropic effects and their complete removal interferes with the cellular machinery, reproduction, or development [1]. In the absence of heritable homozygous knockouts for these essential and pleiotropic genes, their functions are assumed and their real roles remain unknown.

Tissue-Specific Gene Knockdown

In contrast to their animal counterparts, plant tissues and organs are biologically more independent. For example, complete removal or replacement of tissue or organs are common in plant biology and are practically applied in agriculture [8]. Along the same lines, genetic modifications of specific cells, tissues, and organs will have similar potential and advantages (Figure 1) that can now be realized with a recently developed CRISPR-tissue-specific knockout (TSKO) tool kit. Using the cell-specific promoters (*pSMP* for root-cap, *pTMM*, *pFAMA* for stomatal linage, *pGATA23* for lateral root progenitor cell-specific expression), CRISPR-TSKO locked the expression and activity of the genome-editing machinery in a subset of cells, leading to localized genome modification of these tissues [1]. Similarly, CRISPR-Cas9 expression was restricted to xylem cells to knockdown *HCL*, hence lowering the local lignin contents while avoiding *HCL* pleotropic effects [9]. Tissue-specific genetic modifications can provide an easy approach to elucidate the functions of essential and pleiotropic genes. Moreover, compared with other approaches used for the same purpose (i.e., conditional knockouts, tissue-specific gene silencing, and virus-induced gene silencing) the tissue-specific gene knockdown approach has clear advantages. Firstly, it can be initiated early in progenitor cells to develop mutated tissues [1] and so would be more specific and robust. Secondly, these knockdowns are DNA based and would not leak to neighboring cells, and once DNA mutations are made in the progenitor cells, cell division of modified cells would lead to the development of genetically modified tissues and organs.

Advantages and Applications of Tissue-Specific Gene Knockdown

Tissue-specific gene knockdown can characterize essential genes or genes having pleiotropic effects on multiple tissues (Figure 1A). For example, in a pilot experiment, complete removal of *PDS3* (PHYTOENE-DESATURASE3), an important protein in carotenoid, chlorophyll, and gibberellin biosynthetic pathways, by ubiquitous CRISPR-Cas9, leads to albino short-stature plants with severe growth and survival problems on soil. But local knockdown of *PDS3* in a particular subset of cells (stomatal lineage) evaded the pleotropic or lethal effects of these factors on the whole plant and allowed the characterization of such genes in particular

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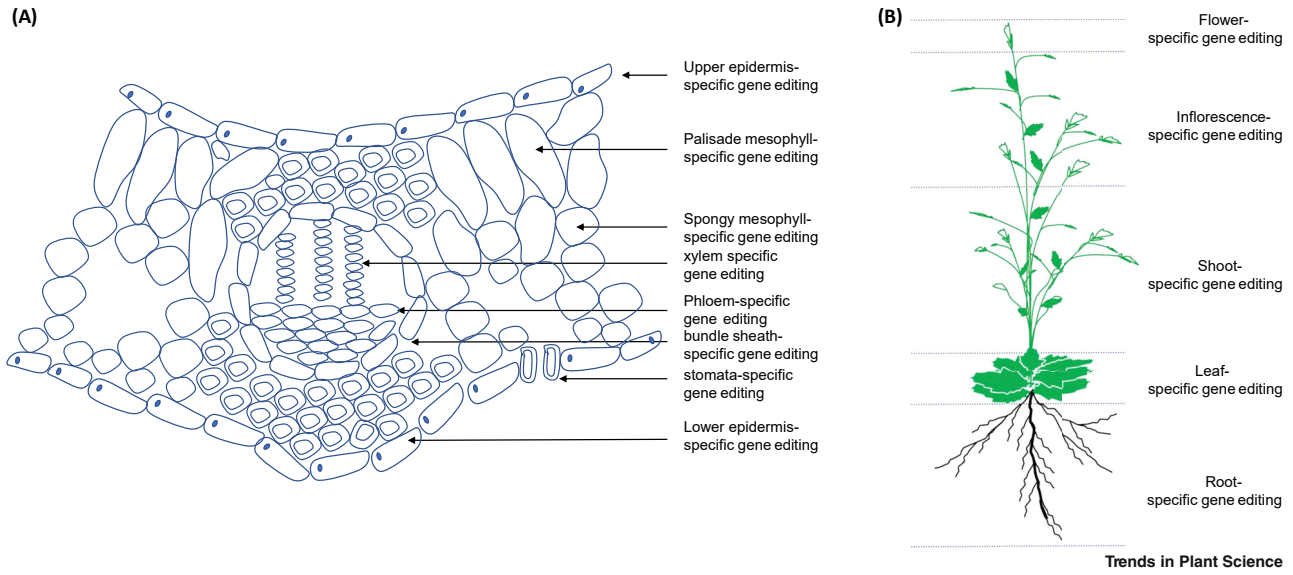

Figure 1. Cell Type-, Tissue-, and Organ-Specific Genome Editing.

(A) Schematic of different cell types and tissues of the plant leaf that could be targeted for cell- and tissue- specific genome modification. Pleiotropic effects of essential genes can be avoided by limiting gene-knockdown events to specific type of cells or tissues. This could also provide a way for characterization of the essential genes in these tissues. (B) Schematic of organ-specific genome editing. Using the clustered regularly interspaced short palindromic repeats-tissue-specific knockout (CRISPR-TSKO) approach, functional knockouts of a particular gene can be produced in one specific organ of the plant. Also, with organ-specific genome modification, plant interacting partners of pathogens can be knocked down in tissues that serve as a replication niche for viruses and other pathogens. Similarly, the genome of a specific organ can be modified, for metabolite engineering, to cope with hostile environmental conditions, to modulate the plant life cycle, or as an alternative to the grafting procedure for horticultural purposes.

tissues [1]. Similarly, members of the gene family have explicit roles in specific tissues, but the functional redundancy of the family members obscures the precise role of single members in a specific tissue [10]. Targeting the unique sequence of individual members or a subset of family members in a particular tissue/organ would allow identification of the exact spatial and temporal role of each member of the gene family (Figure 1).

Likewise, tissue-specific gene editing has the ability to alter the genome of specific cells, tissues, and organs of wild type plants. In the proof of concept experiment, CRISPR-TSKO efficiently knocked down the nuclear localization signal (NLS)-GFP only at the targeted root cap cells without targeting the same sequence in other cells or tissues [1]. This has potential applications, as it will allow a set of cells, tissues, or organs of variant allelic backgrounds for stress adoptability. Similarly, this approach can be used for tissue-specific directed genetic evolution for biotic or abiotic stress tolerance [11]. Once selected under specific stress conditions and confirmed, resistant tissues can be converted to whole plant or the resultant genetic information can be used to create resistant tissues *de novo* in plants.

Plants are sessile and depend on signaling and stress tolerance pathways to cope with hostile environmental conditions. These pathways use multiple common factors for crosstalk and knockdown of these factors have pleotropic effects on respective pathways active in roots, shoots, and leaves [10]. Tissue-specific knockdown of these common factors can provide an alternative approach for better understanding of signaling and tolerance mechanisms. Also, this system can be adopted for differential root/shoot biotic and abiotic tolerance or to modulate the perennial/annual flowering system and to engineer metabolites (i.e., to enhance aroma and alkaloids in certain tissues and to reduce gossypol in cotton and erucic acid and glucosinolate in mustard seeds).

Similarly, as an alternative to the laborious grafting practice, tissue-specific approaches can be used to modify the root, shoot, leaves, and flowering primordial cells (Figure 1A) for environmental adoptability or agricultural applications without compromising the genome of other parts and crop quality.

Another major application would be the use of this system for pathogen resistance in plants. For example, viruses systemically infect and tend to replicate in particular tissues of plants. Using the

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plant promoters (virus-inducible or specific promoters of plant tissues where viruses replicate) to express CRISPR-Cas9 machinery for local knockdown of the plant interacting partners will restrict the systemic spread and replication of viruses in plants. Plants will grow normally in the absence of viruses and the system would be induced only upon viral infection and would then also prevent virus evolution and escape, as has been shown with CRISPR-Cas9-based direct targeting of viral genome in plants [12].

Limitations of Tissue-Specific Gene Knockdown

Tissue-specific genome editing is completely promoter-dependent in expressing the CRISPR-Cas9 machinery in a subset of cells. So far, only a limited number of promoters have been characterized and the strength of the promoters will be crucial for efficient editing. Most importantly, the complete editing of the targeted tissue may be impossible and most of the promoters have leaky expression in the neighboring cells. Another major limitation is the requirement for producing transgenics, as the technique can be applied only in plant species amenable to introducing transgenes and will face similar public and legal challenges, as seen for other transgenic systems.

Acknowledgments

This work in the lab of S.M. is supported by a research grant from Higher Education Commission, Government of Pakistan.

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