Genome-editing by CRISPR/Cas9 in Sorghum Through Biolistic Bombardment

Guoquan Liu (g.liu2@uq.edu.au), Karen Massel, and Ian D. Godwin

Background

Sorghum, generally considered as the 5th important crop among cereals, is used for food, feed, and energy. It is well-known as a drought tolerant, heat resistant, high biomass, C4 model crop. CRISPR/Cas9 has become a powerful genome-editing tool for improving important agronomic traits such as yield and quality and it has gained momentum worldwide for molecular biology research in recent years. In the big five cereals that are used as a food source for human, many have numerous publications, however, there are only a few reports of CRISPR research on sorghum.

Results

The CRISPR/Cas9 system has been investigated for sorghum genome-editing through biolistic bombardment in our lab. Two target genes, cinnamyl alcohol dehydrogenase (CAD) and phytoene desaturase (PDS), both of which have been successfully edited in transgenic lines. Genome-editing was achieved within the sorghum inbred line Tx430, and confirmed by sequencing of PCR products. Homozygous editing of the PDS gene generating albino plantlets (Fig. 1) was observed in the T1 generation of genome-editing line, likely due to insufficient expression of the Cas9 nuclease in the primary transgenics. In the CAD gene, both homozygous and heterozygous editing (Fig. 2) of the CAD gene were found in primary transgenic lines, and achieved an editing efficiency of 21%. In both cases, the edited sequence of the target gene was passed down to the next generations. More experiments, such as optimizing promoters for guide RNA (gRNA), multiplexing targeting the same gene, as well as codon-optimisation of the Cas9 gene are currently under investigation. Three factors were considered crucial elements to establish an efficient CRISPR/Cas9 system for genome editing in sorghum: (1) an efficient transformation system (Fig. 3), (2) the design of targeted gene sequence for gRNA, (3) effective expression of CRISPR/Cas9 components including Cas9 and gRNA.

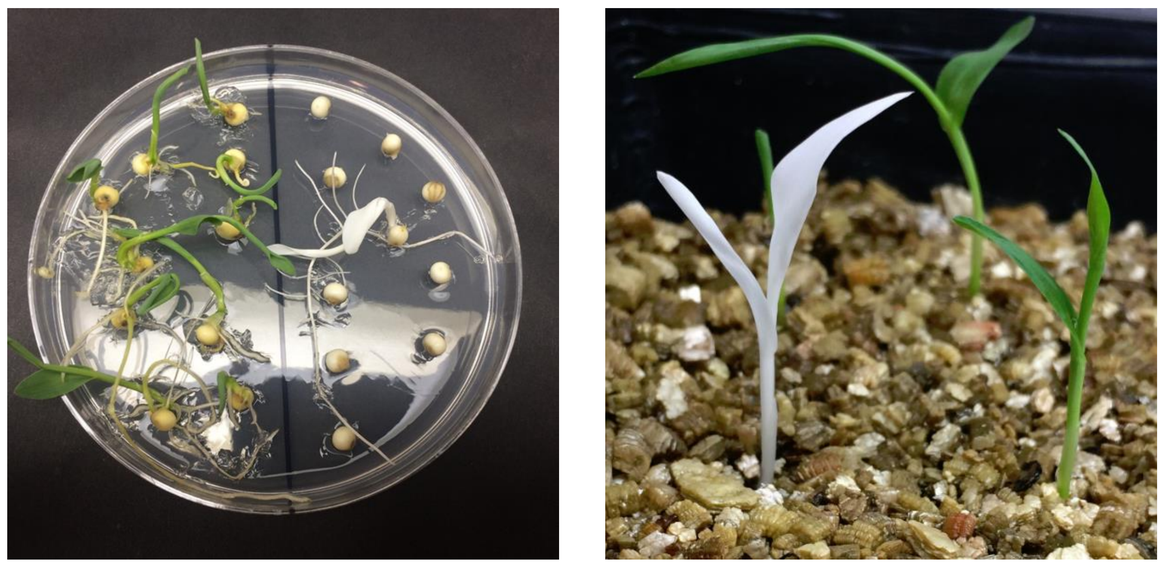

Fig. 1 Genome-editing albino plants of PDS gene

The genome editing of one nucleotide insertion:

SbRio.10G086300.1:-ATGGCTGCTGAATCAG - AGCACGGCAACTGCAATGCTTGGGCAGCG-
Wildtype Tx430: -ATGGCTGCTGAATCAG - AGCACGGCAACTGCAATGCTTGGGCAGCG-
Edited line GS411: -ATGGCTGCTGAATCAG A AGCACGGCAACTGCAATGCTTGGGCAGCG-

The genome editing of 15 nucleotide deletion:

SbRio.10G086300.1: -ATGGCTGCTGAATCAGAGCACGGCAACTGCAATGCTTGGGCAGCGA-
Wildtype Tx430: -ATGGCTGCTGAATCAGAGCACGGCAACTGCAATGCTTGGGCAGCGA-
Edited line GS415: -ATGGCTGCTGAATCAGAG----------GCTTGGGCAGCGA-

Fig. 2 Genome-editing sequence of CAD gene

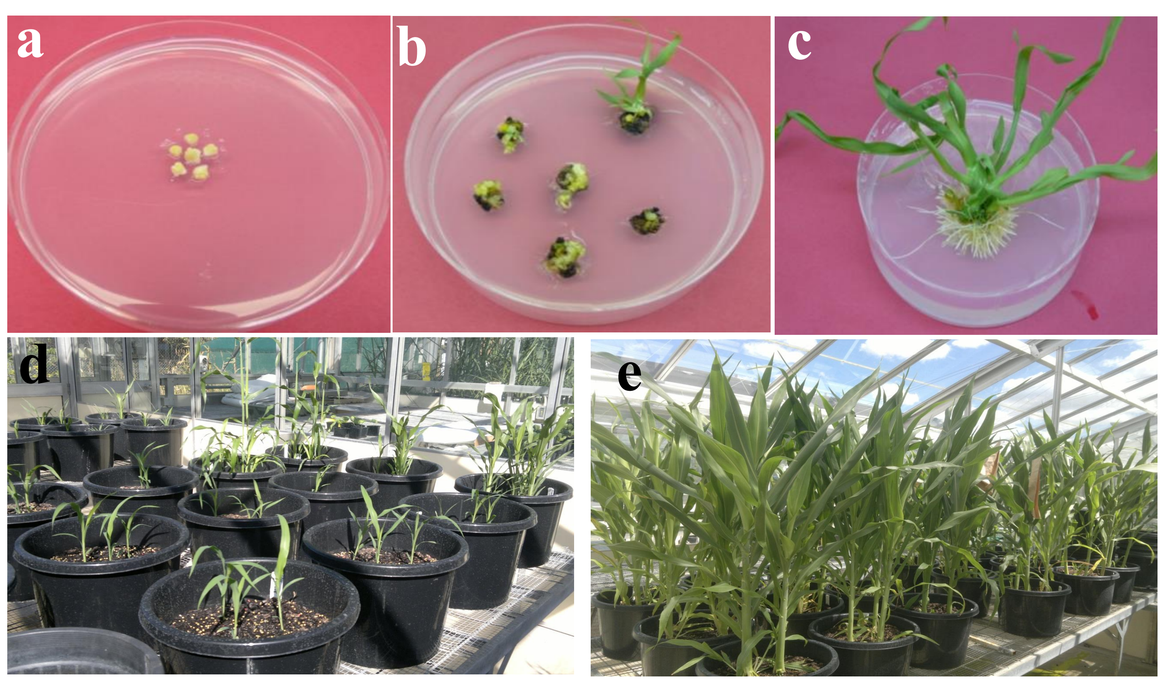

Fig. 3 An efficient sorghum transformation system

a Young embryogenic callus; *b* Transformed calli on selective regeneration medium (30 mg/L geneticin) 4 wks post-bombardment; *c* Putative transgenic plantlet on selective root induction medium for 4 wks; *d* Putative transgenic plants in glasshouse; *e* Transgenic plants before flowering in glasshouse.

References

Liu, GQ, Li, J.Q, and Godwin, I.D, Sorghum Genome Editing by CRISPR/Cas9 in Sorghum Through Biolistic Bombardment (2018). Zuo-Yu Zhao and Jeff Dahlberg (eds.): Methods and Protocols, Methods in Molecular Biology, vol. 1931

Liu GQ, Gilding EK & Godwin ID (2015) A robust tissue culture system for sorghum [Sorghum *bicolor (L.) Moench*]. S. Afr. J. Bot. 98:157-160

Liu GQ, Campbell BC, Godwin ID (2014) Sorghum Genetic Transformation by Particle Bombardment. Cereal Genomics: Methods and Protocols, Methods in Molecular Biology, 219-234.

Liu GQ, Gilding EK, Godwin ID (2013) Additive effects of three auxins and copper on sorghum in vitro root induction. In Vitro Cell Dev Biol Plant 49 (2):191-197.

Liu GQ, Godwin ID (2012) Highly efficient sorghum transformation. Plant Cell Rep 31 (6):999-1007.

The University Of Queensland AUSTRALIA | Queensland Alliance for Agriculture & Food Innovation | Queensland Government