Using Engineered Nucleases for Crop Improvement: Optimization of CRISPR/Cas9 System for Use in Glycine max

With the development of engineered nuclease systems such as zinc finger nucleases (ZFN) (Bibikova, Beumer, Trautman, & Carroll, 2003), TAL effector nucleases (TALENS) (Christian et al., 2012) and homing endonucleases HE (Stoddard, 2005), there has been an increased focus on the development of new or more efficient ways to carry out targeted mutagenesis. The newest of these technologies is CRISPR/Cas9.

CRISPR/Cas9 uses a guide RNA (gRNA) instead of the typical DNA binding domain for recognition of target sequence for mutagenesis (Jinek et al., 2012). The gRNA guides the Cas9 endonuclease to a 20bp recognition site in which the Cas9 causes a double stranded break. The end product results in a mutated sequence that arises from repair via the non-homologous end joining pathway. One of the reasons that CRISPR/Cas9 has become an increasing focus for targeted mutagenesis is the ability to target multiple DNA sequences in one construct (Cong et al., 2013). ZFN and TALENS are currently limited to one target per transgenic construct, with CRISPR/Cas9, the Cas9 protein can be guided by multiple gRNA in the same construct to target multiple sites (multiplexing). Unfortunately, many crop plants are minimally transformable, and the regeneration of transgenic material into fertile plants presents an additional challenge (Puchta & Fauser, 2013). Agrobacterium mediated soybean transformation can take over a year to generate seeds from a transgenic event (Paz et al., 2004) stressing the importance of obtaining multi-allelic mutations, especially in multiplexing systems.

Here we use a combination of a streamlined CRISPR Cas/9 design website, Glycine max (soybean) codon optimized Cas9 protein, variety of constitutive promoters, and single verses dual nuclear localization signal to determine the most efficient CRISPR/Cas9 system for use in Glycine max. Three different versions of the Glycine max codon optimized construct targeting five separate genes were created and will be compared side by side to test the frequency of bi-allelic mutations and average size of mutations through agrobacterium mediated soy transformation. The five target genes range from oil composition traits to a suppressor of recombination that can potentially be used for crop improvement in downstream applications.


