M-171

Gene excision using zinc finger nucleases (EXZACT) in soybean

*Thomas Hoffman**, Soybean Trait Manager, Dow AgroSciences, Indiana, USA
Dayakar Pareddy, Dow AgroSciences, Indiana, USA
Michael Ainley, Dow AgroSciences, Indiana, USA
Geny Anthony, Dow AgroSciences, Indiana, USA

The ability to delete selectable marker or other genes is valuable for commercial development of desirable multi-trait stacked products. Co-transformation strategies, and recombination systems such as *cre-lox* and *flp-frt* have been used successfully for removal of marker genes. Also, engineered nucleases resulting in double stranded breaks, e.g., homing endonucleases and zinc finger nucleases (ZFNs) were employed for gene excision in tobacco. Here, we describe gene excision in soybean using ZFNs by crossing a target event with an “excisor” event expressing high fidelity ZFNs. Specifically, stable “excisor” transgenic events were produced using three different eZFN constructs (e.g., AtUbi10/eZFN4/AtUbi10; AtUbi3/eZFN4/AtUbi3; CsVMV/eZFN4/AtuORF23). T1 events containing eZFN4 were characterized for heritability, segregation, copy number, presence of the intact gene, gene location and ZFN expression. Based on T1 characterization, six eZFN4 excisor events (two per construct) were selected and crossed with a target event consisting of AtUbi10/YFP/AtuORF23::non-functional *hpt* flanked by eZFN4 sites. Twelve cross combinations (6 excisor lines x 2 reciprocal crosses) were made in total and 1,843 F1 progeny were analyzed by qPCR. Complete (non-chimeric) gene excision at a frequency of up to 1.23% was observed with the AtUbi10/eZFN4/AtUbi10 construct in the F1 generation. Further, excision was shown to be heritable in the F2 generation. The high frequency of complete excision observed in these experiments indicates that EXZACT™ is an efficient system for gene deletion for research and commercial applications in soybean.