**Objectives:**

1. Maintain an efficient soybean genetic transformation facility.  
Goal 1: Provide transformation capacity to leveraged projects funded through the National Science Foundation, United States Department of Agriculture, and the United Soybean Board.  
2. Use targeted mutagenesis and gene editing to assess gene functions and enhance traits of interest for Minnesota soybean growers.  
Goal 1: Test functions of genes hypothesized to influence seed composition and IDC tolerance using targeted mutagenesis.  
Goal 2: Generate novel architecture mutants by mutagenizing candidate genes for Lps1, CNGC20, and UGT.  
Goal 3: Enhance beta-carotene levels in the seed through transgenic approaches.

**Achievements:**

This report covers progress from 5/1/2018 to 4/30/2019.  We have continued efforts to develop targeted mutations in soybean using the CRISPR/Cas9 transgenic system.  Progress has been made on each of the following genes:

1. CNGC20 (Glyma.16g218000): Loss of function mutation may generate short petiole phenotype; Status: Two non-transgenic individuals with mutations have been identified. Both lines were evaluated in the growth chamber and showed significant architectural changes. We have also performed whole-genome sequencing of these lines to ensure that they are carrying the desired mutations and do not carry any transgenic DNA; this analysis confirmed that these plants are not transgenic. We petitioned the USDA to get these plants confirmed as non-regulated. These plants will be planted in the 2019 field season and evaluated to see how they performed compared to the control Bert line. We have also recovered two additional lines with mutations for this gene, and they will be evaluated in the growth chamber in the near future.  
2. UGT (Glyma.17g166500): Loss of function mutation may generate a reduced branch angle phenotype; Status: We have developed two different plant lineages with independent mutations. However, our preliminary growth chamber evaluations did not show significant architectural changes.   
3. P450 (Glyma13g04670): Loss of function mutation can test role in IDC tolerance; Status: CRISPR/Cas9 has been successfully transformed into whole plants and plants with mutations have been identified. T1 plants will need to be genotyped to identify individuals that inherit the mutations and do not inherit the CRISPR transgene. Seed will be bulked from these plants and evaluated for tolerance/susceptibility to IDC.  
4. Lps1 (Glyma16g33430): Loss of function mutation may generate short petiole phenotype; Status: Non-transgenic individuals with mutations have been identified. We sequenced these plants and confirmed that they are not transgenic. However, the types of alleles generated in this experiment have some small randomly inserted DNA, which may make it difficult to get APHIS approval for field testing as a non-regulated article.

In addition to the above projects, the transformation facility has supported CRISPR mutation efforts for additional targets, using funding leveraged from the National Science Foundation and the United Soybean Board. The project has also contributed to collaborative efforts with other research teams. A list of disseminated information from these projects is listed below.

**Challenges:**

No major challenges in this reporting period.

**Publication(s)/Symposium:**

In this reporting period, this project contributed work towards one published paper: 1) Liu J, Gunapati S, Mihelich NT, Stec AO, Michno JM, Stupar RM. (2019) Genome Editing in Soybean with CRISPR/Cas9. Methods Mol Biol. 1917:217-234. In this reporting period, this project contributed work towards four oral presentations: 1) Liu J, Gunapati S, Mihelich NT, Stec AO, Michno J, Stupar RM. CRISPR/Cas9-Mediated Mutagenesis in Soybean: from Single to Multiplexing CRISPR Targets. The Next Revolution: Genome Engineering 2018, Minneapolis, MN, July 2018. (~200 people attended) 2) Stupar RM, Michno J, Virdi KS, Liu J, Gunapati S, Xiong Y, Curtin SJ, Stec AO, Wang X. Cloudy with a chance of mutations: Gene editing and functional analyses in soybean. The 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean. Athens, GA, Aug 2018. (~100 people attended) 3) Stupar RM. Cloudy with a chance of mutations: Gene editing and functional analyses in soybean. Plant and Animal Genome Conference, Development and Application of Genome Engineering and Transgenic Technology to the Agriculture workshop, San Diego, CA, Jan 2019. (~250 people attended). 4) Stupar RM. Soybean breeding with genome-editing technology. 2019 Soybean Breeders Workshop, Saint Louis, MO, Feb 2019. (~200 people attended). In this reporting period, this project contributed work towards seven poster presentations: 1) Virdi KS, Campbell BW, Stec AO, Xiong Y, Stupar RM, Muehlbauer GJ. Validation of candidate genes controlling petiole development and sucrose content using CRISPR/Cas9 in soybean. The Next Revolution: Genome Engineering 2018, Minneapolis, MN, July 2018. 2) Patil GB, Michno JM, Liu J, Baxter I, Myers C, Stupar RM. CRISPR/Cas9 mutagenesis of ionomics associated genes in soybean, identified through GWAS and co-expression analysis. Loomis & CBC Symposium, Ames, IA, May 2018. 3) Liu J, Gunapati S, Mihelich NT, Stec AO, Michno J, Stupar RM. Application of CRISPR/Cas9 Technology to Target Single and Multiple Genes in Soybean. Loomis & CBC Symposium, Ames, IA, May 2018. 4) Gunapati S, Liu J, Stec AO, Michno J, Stupar RM. Advances in multiplex CRISPR/Cas9 mutagenesis in soybean. The 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean. Athens, GA, Aug 2018. 5) Toth K, Kim DW, Nguyen THN, Cho SH, Nguyen CT, Hartanto C, Stupar R, Stacey G. A protein involved in plant immunity and symbiosis. The 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean. Athens, GA, Aug 2018. 6) Patil GB, Deshmukh RK, Sonah H, Gunapati S, Belzile F, Stupar RM, Belanger R. Functional characterization of a rare silicon transport allele in soybean using a CRISPR/Cas9 multiplexing approach. The 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean. Athens, GA, Aug 2018. 7) Hoyle JW, Campbell BW, Stupar RM, Parrott W. An allelic series of CRISPR-derived mutations reveals CPR5 role in soybean trichome development. The 17th Biennial Conference on the Molecular and Cellular Biology of the Soybean. Athens, GA, Aug 2018.

**Tech Transfer:**

There was no technology transfer in this reporting period.