The Iowa Soybean Research Center (ISRC) works to leverage university and soybean checkoff funds with industry support to foster innovative soybean research at Iowa State University. The center solicits research ideas from farmer advisors and from industry representatives. Also, ISU researchers propose cutting-edge soybean research topics. And “Think Tank” events are organized by the center to facilitate farmers, industry representatives, and ISU researchers to brainstorm together about research needs.

For more information on the Iowa Soybean Research Center, go to iowasoybeancenter.org.
Project Title: Virus-mediated gene editing in soybean

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Summary
We are interested in expanding on our previous experience of developing viruses as vectors to silence and express genes in soybean to now deliver gene editing reagents. CRISPR/Cas9-based technologies are being used to modify crop plant genomes, but the application of CRISPR/Cas9 still requires the step of plant transformation to introduce the CRISPR guide RNAs and the Cas9 protein. If these gene editing reagents could be introduced into plants using recombinant plant viruses, the labor-intensive transformation process could be circumvented, and also, would not require integration of transgenes into the soybean genome. A project on this topic would involve testing viral vectors for ability to deliver guide RNAs and site-specific nucleases (Cas9), and approaches for enabling these reagents to be expressed in appropriate cell types that give rise to heritable gene edits.

Progress
The first part of the project was to build resources for the virus-enabled gene editing experiments, and that part was completed. We have been testing guide RNA delivery in soybean plants that express the Cas9 protein. Most recently, we have explored the possibility of using different viruses for guide RNA delivery.

A soybean virus was engineered to carry three different CRISPR guide RNAs targeting different sites within a soybean gene encoding the enzyme phytoene desaturase (Pds). These virus clones were shown to be infectious, and the infected leaves were stored to be used as inoculum for downstream experiments. The presence of the guide RNA sequences was confirmed by sequencing the viruses in the systemically infected soybean leaves. The virus clones carrying the Pds guide RNA, which we call guides 2, 3, and 4, was used to inoculate plants from five different Cas9 transgenic events. The plants were observed for phenotypes. The inoculated plants developed virus symptoms, but none of them developed a distinct photobleaching phenotype that would be expected if editing had occurred in the Pds gene. DNA and RNA were extracted from the Cas9 plants infected with virus carrying the guide sequences. DNA analyses were used to identify plants that carried Cas9. DNA analyses were also performed to determine if any edits at the target site in Pds occurred. We did not observe any edits. RNA analyses showed that the virus carrying the guide was present. Based on these results, we concluded that editing did not occur with any of the guides, and therefore, the lack of editing is probably not due to the design of the guides. Rather, we think that the original virus we selected cannot be used to deliver guide RNAs in the configuration that was tested.

We have also explored the possibility of delivering guide RNAs using a different type of virus. Preliminary studies in the model plant Nicotiana benthamiana were successful in demonstrating that guide RNA delivery is feasible. We are now in the process of transferring the knowledge to test if the same guide RNA strategy is feasible in a similar virus that infects soybean. Even though this project has ended, we will continue trying different approaches to deliver gene editing reagents using viruses in soybean. In addition, there were some new advances that were published during the course of this work that will be incorporated into future studies.