Report on “"Manipulating a major gene governing seed reserves as a means to maintain yield and oil while increasing protein, March 2020.

At the beginning of the project in October 2019 we stated the following milestones and KPIs. A brief progress report is given for each milestone and KPI. In addition, we have found several interesting research leads that were not anticipated when the initial proposal was written. Two key insights are

1. A finding that the transgenic RNAi construct is also affecting protein, but has pleiotropic effects on maturity and seed mineral content, where the original allele does not.
2. A discovery that the low protein gene found in high-yielding conventional soybean varieties may revert (at a very low frequency) back to the high protein gene. Thus, we may be able to identify revertant alleles within existing elite lines to facilitate the creation of high protein varieties.

In addition to these novel findings, which both require further research before they can be exploited in breeding and agronomy, our project to engineer protein levels using RNAi transgenics has proceeded according to plan and all milestones and KPIs are on or ahead of schedule.

Milestones:

1. Field trial completed for one growing season using RNAi transgenics

This field trial was completed at the transgenic field facility in Nebraska and we are still analyzing the data. Preliminary indications are that at least one transgenic event shows increased protein and free amino acid levels, and that oil content does not appear to be affected. However the results are preliminary and may not be statistically significant. The interpretation of yield data has been complicated by the fact that the RNAi transgenic constructs seem to affect maturity date, with the transgenic plants maturing later than the control, untransformed line. This is unexpected as the original pro/oil allele derived from PI468916 does not seem to affect maturity.

1. The high protein allele for Glyma.20G085100 downregulated and the effect on protein and oil tested in both a greenhouse and field and for yield in the field.

We have shown downregulation of the Glyma.20G085100 gene and have good data from the greenhouse and preliminary data from the field. The effect on protein appears to vary between different transgenic events, as expected. So far we are seeing a 1-2% increase in protein content in the best lines, which is also seen under field conditions, but is not yet statistically significant with the current year’s data. Interestingly we are also seeing relatively large differences in leaf mineral content in some transgenic events. Further work and additional years of field data will be needed to verify the significance of these results.

1. CRISPR guide construct designed, created and tested in transgenic soybean roots

The CRISPR guide has been designed and has been synthesized and placed in a plasmid vector. The construct has not yet been tested. Restrictions on laboratory work may slow the testing of this vector in the coming months.

We aim to complete the following Key Performance Indicators (KPIs) by the end of Year 1:

1. Data available for multiple RNAi transgenic events for yield trials in the field

We have extensive data for two RNAi events that is currently being analysed.

1. The impact of down regulating the high protein allele tested

We have demonstrated down-regulation of the allele in transgenic plants that are in the trials.

1. At least one plant transformation plasmid completed containing a guide RNA sequence targeted to the Glyma.20G085100 gene.

Although we have the guide RNA sequence it is not yet ready for plant transformation. Laboratory facilities at the University of Illinois are currently largely closed. We anticipate this will be completed by the end of the project, assuming that University laboratories are allowed to reopen.