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| Project Number:  | 1920-162-0115-A |
| Project Title:  | Increasing soybean oil yield through targeted gene silencing and overexpression |
| Organization:  | University of Missouri |
| Principal Investigator Name: | Jay Thelen |
| Project Status - What key activities were undertaken and what were the key accomplishments during the life of this project? Please use this field to clearly and concisely report on project progress. The information included should reflect quantifiable results (expand upon the KPIs) that can be used to evaluate and measure project success. Technical reports, no longer than 4 pages, may be included in this section.  |
| **Key Activities/Accomplishments**The story of our work over the past project year has been one of hard-earned progress in the face of significant external challenge. We began the year having just received, from the ISU Plant Transformation Facility, 25 BADC RNAi and 35 Ps α-CT overexpression (OE) soybean transformants containing our second-generation seed-specific BADC RNAi and Ps α-CT OE cassettes. Of 20 soybean transformants that were tested, 19 contained full-length inserts based on PCR and Sanger sequencing. Unfortunately, a two-spotted spider mite and *Cercospora kikuchii* leaf blight outbreak (verified by the UNL Plant and Pest Diagnostic Clinic and in-house via PCR screening) introduced by our new greenhouse neighbor then caused the loss of the majority of our young transgenics over the course of a single week, in spite of diligent attempts at intervention.As a result, we immediately arranged for the production of additional transgenic events from the Iowa State Plant Transformation Facility, while also awaiting the arrival of transformants from Dr. Tom Clemente’s group at UNL. Seed-bearing BADC RNAi and α-CT OE transformants were received from Dr. Clemente’s group early this summer, comprising 19 and 16 unique transgenic events, respectively, and including T1 progeny from 5 of the α-CT OE events. Over the summer, we screened progeny from all of the received events, and are currently growing a large population of transgenic T1 plants for oil content measurements. T1 seed from additional ISU transformants will be picked up next week.While waiting for the production of the new batches of transformants, we propagated surviving transgenic lines to the T2 generation while carefully genotyping all transformed progeny for the presence of full-length inserts through 1) glufosinate leaf painting, 2) PCR with gene-specific primers, 3) Sanger sequencing of genomic inserts. Through these efforts, and the fortuitous and generous supply of T1 plants by Dr. Clemente, we are now ready to screen oil content in T2 seeds of approximately 65 plants derived from 8 independent α-CT over-expression events (alongside appropriate controls). In order to increase the speed of the seed oil screening process, we will be heading up to Corteva in Johnston, Iowa, this coming Friday to conduct oil measurements using their high-throughput seed phenotyping pipeline. This pipeline will allow for expedited measurements and much larger sample sizes than would have been possible by manually measuring seed FAMEs via GC/MS as originally proposed. While the screening of BADC RNAi lines is delayed compared with that of α-CT OE lines (due to more severe plant losses suffered by the former during the greenhouse pest outbreak and a lack of incoming BADC T1 plants), we are nevertheless also making decent progress on catching up to our proposed timeline for BADC RNAi transformants. If Corteva’s high-throughput seed phenotyping pipeline functions well for oil measurements with our seeds, we hope that we may use it again in the future to shave further time off of our seed screening efforts.Upon completion of the initial oil content screening, we will sow T2 seeds en masse and begin selecting for homozygous transformants based on segregation analysis. We are currently optimizing an immunoblotting protocol to assess the strength of BADC repression and α-CT overexpression in our transformant lines (storage protein and lipid content has posed some immunoblotting challenges), with the further goal of narrowing down the T2/T3 generation to the 5-10 most effective homozygous BADC RNAi and α-CT OE lines. With the opening of the University of Missouri’s new East Campus Plant Growth Facility, we have been assigned substantially more and higher quality greenhouse space than we had previously been assigned and will proportionally accelerate our rate of plant screening. |
| Did this project meet the intended Key Performance Indicators (KPIs)? List each KPI and describe progress made (or not made) toward addressing it, including metrics where appropriate.  |
| **KPIs:**1. At least three independent transgenic soybean lines with a 70-100% reduction in total BADC gene expression, as compared to non-transgenic reference lines, shared with at least five public researchers.
2. At least three independent transgenic soybean lines with at least 50% higher α-CT expression, as compared to non-transgenic reference lines, shared with at least five public researchers.
3. A significant and reproducible increase in soy seed oil content of at least 5% dry weight in at least three independent transgenic events as compared to non-transgenic reference lines.

**Progress towards each KPI:**1. While verified BADC RNAi lines have been produced, due to the greenhouse pest debacle described above, we are still ascertaining the degree of BADC silencing in these lines (which requires collection of developing seeds). We anticipate that this will be completed in the near future.
2. As with BADC RNAi lines, we are still assessing the degree of α-CT over-expression in verified α-CT OE lines, but we anticipate that this will also be completed in the near future.
3. We will be conducting initial high-throughput seed oil phenotyping on T2 α-CT OE seeds this coming week and look forward to evaluating the resultant seed oil content data.
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| Expected Outputs/Deliverables - List each deliverable identified in the project, indicate whether or not it was supplied and if not supplied, please provide an explanation as to why. |
|  **Expected Outputs/Deliverables:**1. Transgenic soybean lines with significantly (t-test, one-way ANOVA) higher levels of seed oil and no significant change in protein content compared with non-transgenic reference lines.
2. Transgenic lines containing a seed-specific BADC RNAi silencing cassette.
3. Transgenic lines containing a seed-specific overexpression cassette containing a truncated form of α-CT.

**Results on Deliverables:**1. In progress. This Friday, we are conducting initial seed oil content measurements on T2 seeds from approximately 65 plants, derived from 8 α-CT OE events, and will be able to assess the success of this deliverable shortly.
2. Complete. We are currently growing progeny from 20 independent lines transformed with a seed-specific BADC RNAi silencing cassette.
3. Complete. We are currently growing progeny from 19 independent lines transformed with a seed-specific overexpression cassette containing a truncated form of α-CT.
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| Describe any unforeseen events or circumstances that may have affected project timeline, costs, or deliverables (if applicable.) |
| As discussed under Key Activities, due to a greenhouse pest outbreak over which we had no control, we suffered the loss of the majority of our transgenic plants shortly after the start of the project year. However, through our diligent efforts and the invaluable work of our colleagues Drs. Tom Clemente at UNL and Diane Luth at ISU, we were able to recover from these losses and make up much (though not all) of the delay caused by these setbacks. |
| What, if any, follow-up steps are required to capture benefits for all US soybean farmers?Describe in a few sentences how the results of this project will be or should be used. |
| If soybean oil levels are successfully increased without adversely affecting protein content, as has been observed in comparable *Arabidopsis* and *Camelina* transformants, this benefit would be available to US soybean farmers as soon as our licensing partners are able to distribute the technology. |
| **List any relevant performance metrics not captured in KPI’s.** |
| In addition to our plant work, we have also been conducting biochemical and computational research to discover additional targets for soybean oil content modulation. We have identified several promising novel soybean transcriptional regulators that may be involved in the regulation of soybean fatty acid biosynthesis. We have also developed plans to engineer two additional soybean seed oil/yield targets, which have been included as pilot projects in our USB renewal proposal. |