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| **Reporting Period** | Final Report  (October 1st, 2011 – September 30th, 2013) |
| **Proposal** | [2296 Genes for Insect Resistance in Soybean (Year 1 of 2)](http://moss.unitedsoybean.org/Lists/Proposals/DispForm.aspx?ID=2737&RootFolder=*) |
| **Committee** | Production |
| **Target Area** | Supply |
| **Project Start Date** | 10/1/2011 |
| **Project End Date** | 9/30/2012 |
| **Project Number** | 2296 |
| **Project Status** | **Project Summary:**  **Strategy 1:**  Test the function of Glyma07g14530 by engineering the candidate gene, and testing the resulting transgenic plants for insect resistance **(Y1)**.  Modified to fine-mapping QTL-H, an insect resistance locus from PI229358 that confers resistance to both leaf-chewing insects and kudzu bug, the most recent introduced soybean pest **(Y2)**.  **Accomplishments for Strategy 1:**  a)      Transformation of Jack with Glyma07g14530Resistant and Jack-M with Glyma07g14530Susceptible (**Figure 1**); Generated 15 – T1 Jack and 10 – T1 Jack-M **(Y1)**.  b)      Transformation of Jack and Jack-M with RNAi silencing construct for Glyma07g14530 (**Figure 1**);  Somatic tissue transformed and events under selection/regeneration **(Y1)**.  c)      Fine-mapping QTL-H **(Y2)**  i.        An F2 mapping population was developed:  Benning QTL-M x Benning QTL-MH F1 cross made Summer 2013, F2 will be available for mapping Summer 2014  ii.      Molecular marker development:  15 SNPs identified between Benning and PI229358 (resistant parent) for QTL-H (5.2 Mb pericentromeric region on Ch12) using 50k Infinium Soy SNP Chip (**Figure 2**); 50k data courtesy of Perry Cregan.  iii.    Sequencing PI229358 for polymorphisms to narrow the list of candidate genes:  Illumina HiSeq data received October 30th, 2013; In future the sequence data will be assembled and compared to Williams 82 sequence to identify polymorphisms that will narrow the list of 242 genes found in the QTL-H region to identify candidate genes.  **Strategy 2:** Determine if the meal produced from insect-resistant soybean is just as safe and wholesome as conventional soybean.  **Accomplishments for Strategy 2:**  a)      Seed increase and soybean meal processed for experiment:  Seed from Benning, Benning QTL-M, and Benning QTL-MGH was increased, processed, and analyzed **(Y1)**.  b)      Chicken feeding experiment:  Broiler chicken hatchlings were fed meal diets containing either Benning, Benning QTL-M, or Benning QTL-MGH and weights were taken at 7, 14, and 21 days **(Y2)**.  There was no difference in weights between chickens fed Benning (insect susceptible) and those fed Benning QTL-M or Benning QTL-MGH (insect resistant) soybean meal (**Figure 3)**.  **Strategy 3:**  Elimination of yield drag associated to QTL-G.  **Accomplishments for Strategy 3:**  a)      An F2 mapping population was developed:  An initial oversight was made when the original F2 population was developed in that QTL-G does not have an effect unless QTL-M is present.  Therefore a cross was made between Benning QTL-M and Benning QTL-MG during summer 2013, and F2 mapping population will be ready for screening summer 2014.  b)      Molecular marker development:  20 SNPs identified between Benning and PI229358 (resistant parent) for QTL-G (0.6 Mb region at the end of Ch18) using 50k Infinium Soy SNP Chip (**Figure 4**); 50k data courtesy of Perry Cregan.  c)      Sequencing PI229358 for polymorphisms to narrow the list of candidate genes:  Illumina HiSeq data received October 30th, 2013; In future reads will be mapped to Williams 82 sequence to identify polymorphisms that will narrow the list of 72 genes found in the QTL-G region to identify candidate genes.  **Strategy 4:**  Identification of genetic resistance to the kudzu bug.  **Accomplishments for Strategy 4:**  a)      Field tests with Benning insect resistant NILs with combinations of QTLs M, G, H and E:  i.        Summer 2012:  A wash-bucket sampling technique was used to count adults and nymphs in Athens and Midville, GA fields.  Results indicate that lines containing QTL-H host fewer kudzu bug adults (**Figure 5**), and the combination of QTL-M + QTL-H have fewer nymphs (**Figure 6**).  Yield taken on Midville, Ga field, where a heavy kudzu bug infestation was observed, shows lines with QTL-H yield 20% more than the susceptible check Benning (**Figure 7**).  ii.      Summer 2013:  Benning NILs were planted in a field cage in hill plots with 15 replications.  The plants were infested with approximately 180 adults per hill at R1 stage.  Benning QTL-MGH had 9% fewer adults than the susceptible check Benning (**Figure 8**).  Plants will be harvested for yield and stem lesions will be rated at the end of the season.  b)      Field test with soybean aphid and whitefly resistant PIs:  i.        Summer 2012:  Seed was increased for 2013 field experiment.  ii.      Summer 2013:  New stem lesion rating scale used to identify lines with lowest season long feeding by kudzu bug.  Six lines were identified as resistant to the kudzu bug for having fewer adults, nymphs, and/or egg masses in addition to low stem lesion rating (**Table 1**).  c)      Kudzu bug colony:  a.       Fall 2011:  Colony started in a 4 ft x 4 ft A-frame cage with potted soybean; Insects collected from kudzu in Athens, GA layed two rounds of egg masses before the population began to decline due to infestation by thrips and whiteflies.  b.      Fall 2012 to present:  Colony established in same size cage as 2011 on susceptible Benning potted plants.  Insects collected from UGA Plant Sciences Farm from soybean fields were reared for two rounds of egg laying.  The colony was moved to a larger cage (4 ft x 4 ft x 10 ft) where it was maintained on potted soybean and potted kudzu until May 2013.  At that time funding from GACC for Soybean was initiated to fund the maintenance of the kudzu bug colony.  A larger walk-in cage was built (10 ft x 7 ft x 20 ft) to allow space for new plants to be rotated in and to allow access to plants for insect observation.  The colony is still active and reproducing.  **Additional Non-USB Funding:**  **USDA-NIFA Plant Biology - Award Number**: **2012-67013-19456;  $388,884 (3 year total)**  Characterization Of A Gene For Resistance To Defoliating Insects In Soybean  The results obtained from USB 2296 **(Y1)** were critical to identify a candidate gene for QTL-M; also during this year, we started the cloning of the plasmids to test the QTL-M gene. With the results from **Y1**, we collected enough preliminary data to write the NIFA proposal.      **GACC for Soybean – January 1st – December 31st, 2013;   $15,000**  Soybean Genes for Resistance to the Kudzu Bug  Preliminary work to establish a kudzu bug colony from USB 2296 (**Y1**) was critical as a proof of concept and helped identify more additional workers were needed to sustain a large colony of the kudzu bug.  With this information, we procured funds for prebaccalaureate students to help maintain the larger colony.  This grant funded travel to China for a graduate student to visit scientists at Nanjing Agricultural University (Drs. Junyi Gai and Guangnang Xing) who have studied kudzu bug resistance in China. |
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| **Attachments** | |  | | --- | | [USB 2296 Final Report Figures.docx](http://moss.unitedsoybean.org/Lists/ProjectStatusReports/Attachments/7899/USB%202296%20Final%20Report%20Figures.docx) | |

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