MISSISSIPPI SOYBEAN PROMOTION BOARD

Development of molecular diagnostic method for the diamide resistance in soybean looper
Project # 32-2022

2022-2023 MSPB Annual Report

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Background and Objectives

The soybean looper (*Chrysodeixis includens*) is one of the most serious pests in soybeans, migrating from southern latitudes up through Mississippi and consuming massive amounts of foliage in soybean. Since the introduction of the diamide insecticides such as chlorantraniliprole, the soybean looper has been well managed without any resistance issues. Diamides belong to a class of insecticides that target ryanodine receptors (RyR), the intracellular calcium channels that play an important role in muscle and nerve functions. However, soybean loopers have a long history of resistance to other insecticides such as pyrethroids, carbamates, and organophosphates. Therefore, diamide resistance in soybean looper is expected to develop, and growers should be prepared to manage loopers without diamides in the future. Resistance to diamides has already been reported in some lepidopteran pests, including diamondback moth, tomato leaf miner, rice stem borer and beet armyworm from different regions in the world. Although diamide resistance hasn't been reported in soybean loopers, it seems that reduced diamide efficacy has been observed in the southeastern USA, probably those migrating from Puerto Rico. Therefore, it is important to regularly monitor populations in Mississippi. We have monitored soybean looper populations for resistance to diamides for the last 2 years. Although it is more directly reflecting the field situation, the bioassay method takes time and efforts to confirm the resistance status. However, if there are any molecular markers to distinguish the resistant from the susceptible strain, it would be faster and easier to detect resistance, because it does not require many insects for testing, but only a few individuals. Therefore, the molecular diagnostic method will provide a proactive detection tool for resistance and help farmers choose an insecticide wisely without suffering from low efficacy due to resistance. We screened two DNA sequence sites in RyR gene using more than 250 individual samples from the Puerto Rico, Mississippi, and other southern states' populations. Multiple bioassays were performed for their susceptibility/resistance to chlorantraniliprole. The project is a continuation to complete the following objectives. (1) Establish and maintain a resistant strain of soybean looper from Puerto Rico, (2) Compare target site genes of susceptible and resistant populations, (3) Conduct standard diamide bioassays for soybean looper populations collected in Mississippi, (4) Identify detoxification genes that differentially expressed between the two strains, and (5) Monitor resistance in field populations with a molecular diagnosis kit.

Report of Progress/Activity

Objective 1: Establish and maintain a resistant strain of soybean looper from Puerto Rico

A total of 456 larvae were collected from a soybean field in Puerto Rico. The field population (called PR strain) is assumed to be resistant to chlorantraniliprole. They have been maintained on artificial diet in the rearing facility. When tested with different concentrations from 2 to 200 ppm, the F2 generation required about 5 times the concentration to kill 50% of the population as required for the susceptible colonies. At the F5 generation, the PR strain is approximately 10 times more tolerant to the insecticide than the lab strain. It is, however, regarded as a mild resistance, probably reflecting the current field situation. At the F6 generation, however, the chlorantraniliprole resistance was lost by unknown reason. The LC50 for this assay was 0.039, which is only half as much as the susceptible lab colony (0.078). It was unexpected that the resistance suddenly disappeared after persisting for 5 generations. The loss of resistance was confirmed at the F7 generation as well. Not only to rescue any individuals harboring the resistant allele,

but also to figure out the sudden loss of the resistance, a single-pair mating system was set up to trace any genetic alleles. Among the initial 50 single-pair mates, 29 families produced fertile offspring. The larvae from each family were screened by an LC50 dose of chlorantraniliprole and, interestingly enough, two families showed descent resistance to the insecticide (>50% survivorship), whereas the larvae from the other families showed no resistance (less than 5% survivorship). However, due to the low number of adults finally obtained, the two families were unfortunately collapsed at the end. Nevertheless, it suggests that there is a genetic factor responsible for the diamide resistance in the PR strain. We have requested a new population from Puerto Rico for the continuous genetic screening in the upcoming year.

Objective 2: Compare target site genes of susceptible and resistant populations

Since the diamides insecticides target RyR, we compared the lab strain and the PR strain by sequencing the RyR gene. The two amino acid sites (I4790M and G4946E) known to be associated with the resistance in other lepidopteran insects showed no mutation in the PR strain, while other sites showed alternative splicing events. In total, four alternative splicing sites were detected. These splicing events do not change the reading frame, resulting in a mature protein with only 4-6 aa changes (AS1, 3, and 4). In the case of AS2, three different alterations were detected, spanning a longer stretch of 29 aa, probably giving more impact to the protein structure than the other three AS (Fig. 1). However, it remains unknown whether any of these AS sites are associated with resistance. Further analysis is required to understand the structural variations and their contribution to the insecticide resistance. In addition, we measured the expression levels of RyR gene in different tissues and different stages using qRT-PCR. As a result, RyR gene was highly expressed in abdominal integument where muscles are attached. It was abundantly expressed throughout the larval stage except the last instar. Based on the genome sequence, it was revealed that the soybean looper RyR contains 114 exons, which is one of the largest number of exons found in insect. The RyR sequences will be further analyzed in the upcoming year.

Objective 3: Conduct standard diamide bioassays for soybean looper populations collected in Mississippi

We have maintained a composite colony created from several field populations collected during 2021-2022. They have been challenged with moderate concentrations of chlorantraniliprole every generation to try to create a resistant strain. While there was no change between F3 and F6, it appears that some resistance has developed since then, based on survival rates at the selection concentration. The composite had been maintained for F11 generations so far. However, the colony did not show a normal growth after treating 1.2 ppm of commercial grade chlorantraniliprole (Prevathon). Even though the survivors were reared on the regular diet to prevent from collapsing, the colony did not show any better performance. Thereby, we decided the composite colony to be terminated. Instead, other field populations will be further collected and tested in the upcoming season. During this off-season, the resistance screening was focused on the PR and lab strains. Overall in 2022, no incidence of diamide resistance was detected in the soybean looper populations collected in Mississippi. The field collection and screening will resume in the 2023 season.

Objective 4: Identify detoxification genes that differentially expressed between the two strains

Since the current resistance status is mild, the resistance seems to be more likely associated with the differential expressions of detoxification genes than target genes. The detoxification enzymes usually get induced upon exposure to sublethal doses of insecticides. In order to identify detoxification genes associated with diamide resistance, third instar larvae were treated with 0.1 ppm chlorantraniliprole for 48 hr and the larvae were collected to isolate total RNA for transcriptome analysis. As a result, the RNA-Seq data have been finally produced. It ended up with 51,510 contigs in total, where a majority of contigs (71%) were expressed in both strains, while 21% were expressed only in the PR strain and 8% were only in the lab strain (Fig. 2A). It means that 2.5 times more genes were expressed in the PR strain subjected to a sublethal dose of chlorantraniliprole, compared to the lab strain subjected to the same treatment. It suggests that more molecular machineries be activated in the resistant strain. Among the genes expressed in both strains, 2,801 genes were up-regulated, while 959 genes were down-regulated in the PR strain

compared to the lab strain (Fig. 2B), demonstrating more genes were highly expressed in the PR strain, rather than being down-regulated. It also suggests the resistance might be benefited by overexpression of detoxification genes. Differentially expressed genes (DEGs) will be further analyzed in upcoming year.

Objective 5: Monitor resistance in field populations with a molecular diagnosis kit
No results available at last year.

Impacts and Benefits to Mississippi Soybean Producers

Since the soybean looper is a migratory pest species to Mississippi, it is critical to monitor whether the pest is resistant to pesticides or not. An increasing incidence of resistance cases reported in South America warns Mississippians to keep an eye on the development of resistance in the migratory pest to the relatively new insecticide (diamide). To develop a diagnostic method, it is crucial to characterize the genetic and molecular basis of the resistance. Current results will support to develop a novel diagnostic method to help growers to decide their management strategy more reasonably in a wide area of Mississippi.

End Products–Completed or Forthcoming

Presentations at conferences

Isbilir, S., Catchot, B., Musser, F. R., Ahn, S.-J., 2022. Molecular cloning and expression profiling of ryanodine receptor, a target of diamide insecticides, in soybean looper, *Chrysodeixis includens*. Mississippi Academy of Sciences - Summer Science and Engineering Symposium 2022, June 8, Mississippi State, MS.

Isbilir, S., Catchot, B., Musser, F. R., Ahn, S.-J., 2022. Molecular cloning, mutation frequency analysis, and expression profiling of insect ryanodine receptor in soybean looper, *Chrysodeixis includens*. Fall 2022 Graduate Research Symposium, October 22, Mississippi State, MS. (Oral winner, 3rd place)

Isbilir, S., Catchot, B., Musser, F. R, Ahn, S.-J. 2022. Molecular screening and expression profiling of ryanodine receptor in soybean looper, *Chrysodeixis includens*. 2022 ESA, ESC, and ESBC Joint Annual Meeting, November 13-16, Vancouver, Canada.

Isbilir, S., Catchot, B., Musser, F. R, Ahn, S.-J. 2022. Mutation frequency analysis and expression profiling of insect ryanodine receptor in soybean looper, *Chrysodeixis includens*. The 87th Annual Mississippi Academy of Sciences Meeting, February 23-24, Biloxi, MS.

Isbilir, S., Catchot, L., Musser, F. R, Ahn, S.-J. 2023. Mutation frequency analysis and expression profiling of insect ryanodine receptor in soybean looper, *Chrysodeixis includens*. 2023 Southeastern Branch Meeting of Entomological Society of America, March 12-15, Little Rock, AR.

Publications

Isbilir, S., Catchot, B., Musser, F. R., Ahn, S.-J. Molecular cloning and expression pattern of a ryanodine receptor in soybean looper moth, *Chrysodeixis includens*. *(anticipated to submit in April 2023)*

Extension/Outreach Magazine (where our project was featured)

Beeson, V., Grado, L. 2022. Under Pressure: Fight insects from crop emergence to storage. MAFES Discovers Winter Issue, pp.28-33. (https://www.mafes.msstate.edu/discovers/article.asp?id=260)

Graphics/Tables

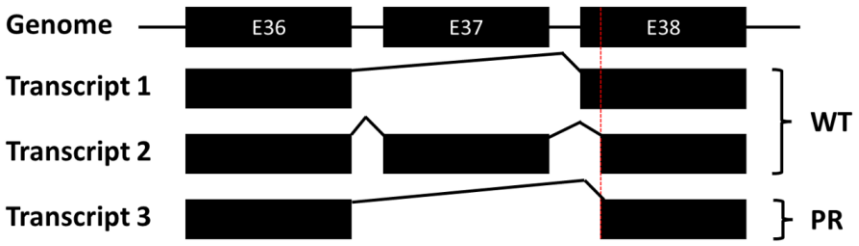

Figure 1. Alternative splicing variants of a fragment of RyR gene in soybean looper. The locus spanning exons E37-E38 shows variable transcripts which might be a candidate of resistance-associated markers.

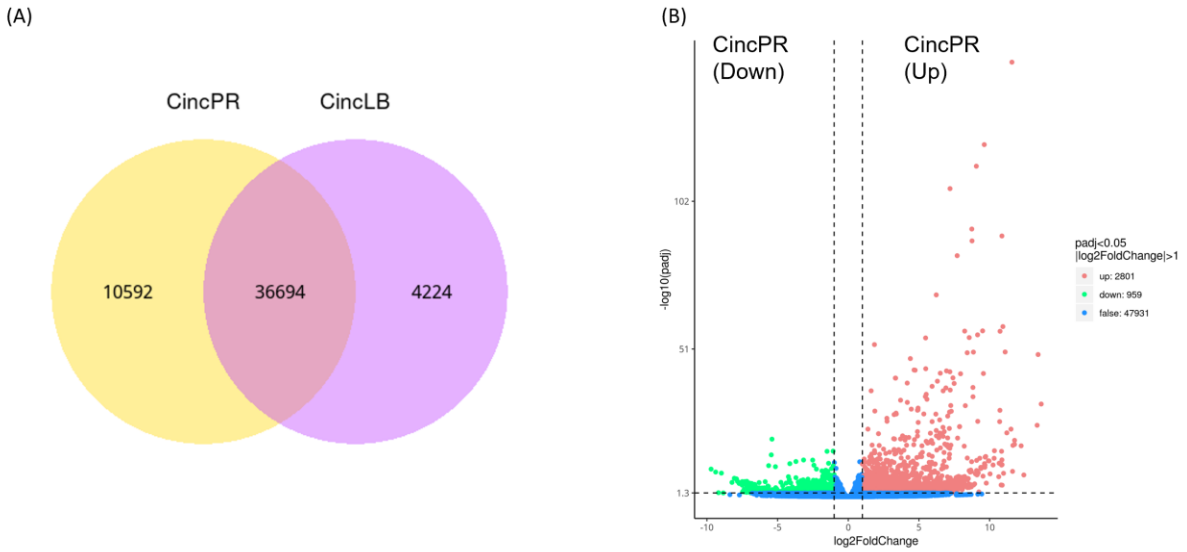

Figure 2. Analysis of differentially-expressed genes between the PR (CincPR) and lab (CincLB) strains. (A) The number of genes expressed in different strains (either alone or both). (B) Volcano plot of differentially-expressed genes with up (2,801 contigs) and down (959 contigs) regulated genes in the PR strain compared to the lab strain.