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| Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report. |
| Project Number: | 2022-47 |
| Project Title:  | Exploitation of weed species extracts as an effective and environmental friendly strategy to control insects and deer in soybean |
| Organization:  | Mississippi State University |
| Project Lead Name: | Te Ming (Paul) Tseng |
| Report Period: | September 15, 2023 |
| Progress Summary (in non-proprietary lay language suitable to be shared publicly): |
| In our research, the primary goal is to identify and develop a natural deer repellent that can be used in soybean crops. This repellent aims to protect soybean plants from damage caused by both deer and insects, ultimately safeguarding soybean yields and promoting more sustainable agriculture. First, we used a special technique called mass spectrometry to look at the chemicals inside the plant. We found that one part of the plant, called Fraction D, has the highest amount of certain compounds called anthraquinones. These compounds can help keep deer away from the plants. Fraction D is especially rich in a specific anthraquinone called oranti-obtusin. This discovery could be useful for finding ways to protect crops from herbivores.Next, we started looking at the plant's DNA to find out which genes are responsible for making these helpful compounds. We started with 10 genetic markers, but we plan to look at more in the future. So far, we've taken DNA from the leaves of the plants and used a special method called polymerase chain reaction to make copies of the genes we're interested in. However, we haven't done the full analysis to see how these genes are linked to the compounds that keep herbivores away. These genetic markers are essential for our upcoming experiments in the greenhouse, where we'll try to grow soybean plants with the same herbivore-repelling trait. |
| **Detailed Progress Status**: |
| The objectives proposed were (1) conduct chromatography and mass spectrometry analysis to identify target anti-herbivore compounds in weeds, and (2) conduct quantitative trait loci analysis to identify molecular markers associated with anti-herbivory compounds in weeds.1. **Conduct chromatography and mass spectrometry analysis to identify target anti-herbivore compounds in weeds**: The sicklepod plant fractions were collected and subjected to mass spectrometry analysis to determine the percentage composition of the four target anthraquinone compounds. The analysis was performed on six different fractions labeled as A, B, C, D, E, and F. The results of the analysis are presented in the table below, showing the percentage composition of each of the four anthraquinone compounds in the different sicklepod plant fractions:

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| **Sicklepod plant fractions collected using mass spectroscopy** | **Antrhaquinone compounds (deer repellent property) from sicklepod plant fractions (%)** |
| **Oranti-obtusin** | **Emodin** | **Chrysophanol** | **Physcion** | **Total** |
| A | 0 | 0 | 0 | 0 | 0 |
| B | 0 | 0 | 0 | 0 | 0 |
| C | 0 | 0 | 0 | 0 | 0 |
| D | 13.81 | 0.6 | 1.02 | 0.22 | 15.65 |
| E | 0.38 | 0.11 | 0.43 | 0.24 | 1.16 |
| F | 0.34 | 0.08 | 0.35 | 0.35 | 1.12 |

Fraction D shows the highest total anthraquinone content among all fractions, with a total of 15.65%. This fraction is particularly rich in Oranti-obtusin (13.81%), which is the dominant anthraquinone compound in this fraction. Emodin, Chrysophanol, and Physcion are also present but in smaller quantities. Fractions E and F also contain some anthraquinone compounds, but their overall percentages are much lower compared to Fraction D. These fractions may still contribute to the allelopathic properties of sicklepod, albeit to a lesser extent. Fractions A, B, and C do not show detectable levels of any of the target anthraquinone compounds. It's possible that these fractions contain other compounds or are not significant contributors to the deer-repellent properties of sicklepod.The analysis of sicklepod plant fractions has revealed the presence of anthraquinone compounds, with Fraction D having the highest content of these compounds. Oranti-obtusin is the predominant anthraquinone in Fraction D, indicating its potential role in the allelopathic and deer-repellent properties of sicklepod. Further research can explore the practical applications of these findings in agriculture and weed management. These results provide valuable insights into the chemical composition of sicklepod, contributing to our understanding of its allelopathic effects and potential uses in agriculture.1. **Conduct quantitative trait loci analysis to identify molecular markers associated with anti-herbivory compounds in weeds**: Presently, we have initiated the screening of a subset comprising 10 simple sequence repeat (SSR) markers, with intentions to broaden this screening effort in forthcoming months. DNA isolation from leaves followed, and polymerase chain reaction was used to facilitate amplification of genetic regions corresponding to the target anti-herbivore compound(s) as identified within objective 3. It's important to note that the statistical analysis of these markers and, consequently, the linkage analysis to anti-herbivory traits, have not been conducted at this stage. These highly promising markers will continue to serve as pivotal components in subsequent greenhouse-based breeding experiments, aimed at the meticulous selection of soybean lines characterized by the coveted anti-herbivory trait(s).
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