Technical report of the progresses on the MSSB project

(Quarter 1, June 12, 2024)

TITLE: Spray application of double stranded RNA (dsRNA) for simultaneous management of multiple soybean fungal and insect diseases

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The objectives of this proposed study in the third year are to: 1) Continue the effort to fine-tune the conditions to increase the efficacy of dsRNA in disease suppression; 2) Examine the potential of mixing different dsRNA to enhance their effectiveness in reducing disease symptoms under greenhouse conditions; and 3) Perform small scale field studies to determine the effectiveness of these dsRNAs in simultaneous management of CLB, FLS, and PSS through foliar applications.

In this quarter, we have mainly been focusing on exploring ways to enhance the effectiveness of applied dsRNA on disease reduction (objective 1) and on examining the potential of nano-particles in enhancing dsRNA stability on leaf surface (objective 2).

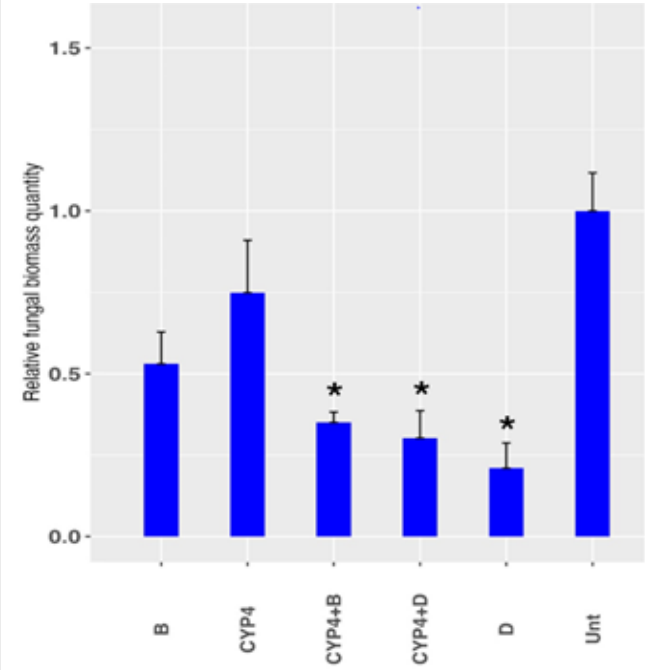

Figure 1. Evaluating the effect of different adjuvants on enhancing the dsRNA effectiveness in reducing soybean fungal diseases. Here adjuvant B was very effective in enhancing the CYP4 dsRNA uptake and in reducing soybean rust fungal infection.

For objective 1, we have been testing several new adjuvants that were recently reported to enhance dsRNA uptake to determine their potential in enhancing dsRNA delivery. The study was conducted in the greenhouse and the adjuvant B was found to enhance the effectiveness of CYP4 dsRNA in reducing soybean rust pathogen growth in infected leaf samples after dsRNA treatment based on real time PCR analysis of the collected the soybean leaf samples. We observed clear reduction in fungal growth in the presence of adjuvant in addition to dsRNA (Figure 1).

For objective 2, we have established a procedure in synthesizing Fe/Mg nano particles, coated our dsRNA on these nano materials and applied them onto soybean plants before exposing them to direct sunlight for various durations to determine how long the dsRNA can remain effective in suppressing fungal disease under natural conditions. We want to see whether there is a clear difference in soybean CLB disease symptoms and pathogen growth among soybean plants that had been sprayed with Fe/Mg nanoparticles coated with dsRNAs and exposed to direct sunlight for 0, 4, 7 and 10 days before being inoculated with *Cercospora sojina* (Figure 2). We have quantified the pathogen growth two weeks after inoculation and found that dsRNA alone can reduce the pathogen growth by about 70% compared to controls (Figure 2). However, dsRNA plus nanparticles did not seem to enhance the dsRNA effectiveness or offer some protection in comparison to dsRNA alone (Figure 2). We will repeat this study to make sure.

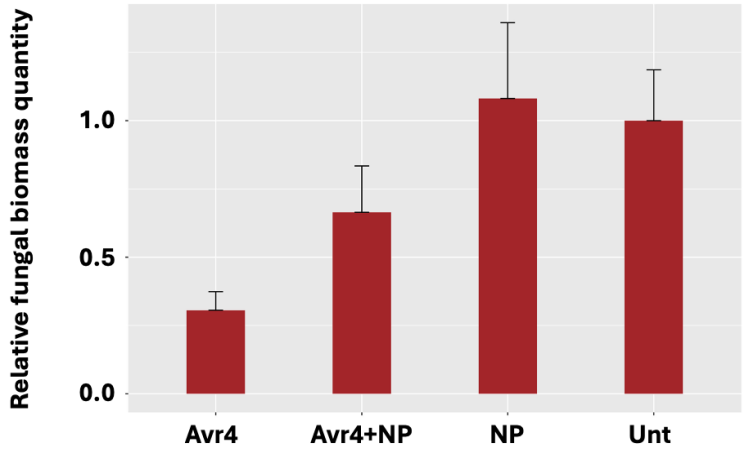

Figure 2. Differences in relative fungal growth among soybean plants that had been sprayed with dsRNA alone (Avr4) or with dsRNA coated with nanoparticles (Avr4+NP) compared to two controls (nanoparticles alone, NP and untreated plants, Unt) and exposed to direct sunlight for 10 days before spray inoculated with *C. sojina*. The leaf samples were collected two weeks after fungal inoculation and analyzed using real time PCR.

For objective 3, a field study has been planned. The first batch of 32 rows of soybean (Syngenta NK43-Y9XFS) was planted on May 22, 2024 (Figure 3). These plants will be treated with dsRNAs with or without adjuvants or nanoparticles to determine the potential of dsRNA in managing soybean fungal diseases. There will be two more plantings so we can repeat the field study two more times.

Figure 3. Soybean plants to be used for field studies listed in Objective 3 were planted at Ben Hur research station on May 22, 2024. The photo was taken on June 3, 2024.