**ISA Contract Research Final Report**

**Project Title:** RNA-based approaches for resistance to nematode and fungal pathogens of soybean

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**Brief Statement of Objectives (max 250 words):**

Soybean yield always is threatened by plant diseases caused by a wide spectrum of pathogens, including fungi and nematodes. As diverse as these pathogens are, they all have two fundamental commonalities: 1) The pathogens possess genes that are expressed during and required for infection on soybean plants; and 2) These pathogens cause gene expression responses in soybean plants that are necessary for disease symptoms to develop. Therefore, interfering with these two mechanisms will have negative effects on the developing disease and, thus, can be exploited as a novel control mechanism.

The goal of this continuing research is to translate the latest research in RNA silencing into tools that stabilize and increase soybean yields by generating soybean lines that are resistant to pathogens.

The specific objectives of this continuing project are to:

• Use powerful microRNA gene regulation to interfere with soybean cyst nematode infections; and

• Use the RNA silencing approach to control soybean fungal diseases.

**Brief Statement of Expected Deliverables (max 250 words):**

Completion of the proposed project will enable us to develop and validate novel targets needed to disrupt the life cycles of soybean pathogens. Exploitation of these targets is a declared goal of public and private researchers for the last two decades. While we and others have made significant progress in understanding soybean-pathogen interactions, there are not yet any commercial biotechnological products to control soybean pathogens. Our approach is state-of-the-art, and has been reduced to practice in principle. We anticipate the following deliverables:

* Successful completion of the work proposed here will generate powerful tools to combat soybean’s most important pathogen problems that are estimated to cause billions of dollars in annual losses in the US.
* We anticipate identifying mechanisms that render the soybean plant significantly less susceptible to soybean pathogens while maintaining the ability to provide stable yields.
* Our work will serve as a proof of principle that will open a promising new area of soybean research with the goal of managing a wide spectrum of pathogen problems.
* For the first time, such novel resistance mechanisms could be effective against all biotypes or isolates of a given pathogen, thereby preventing a loss of resistance efficacy in the future.
* The transgenic solutions pursued in this proposal can directly be integrated in existing soybean germplasms, thus ensuring availability in agronomically relevant cultivars.
* This work will attract interest and funding from industry, enabling product development for the agricultural practice.
* We further anticipate that our work will lead to significant funding from federal sources.

**Final Project Results:**

**Use powerful microRNA gene regulation to interfere with soybean cyst nematode infections**

This grant has helped us immensely to advance our knowledge about the important role played by the microRNA396 family from soybean during plant nematode molecular interaction. Previously, using Arabidopsis-Sugar beet cyst nematode pathosystem, we had discovered that the microRNA396 functions as a master-regulator controlling expression of host of genes. Modulation of this microRNA clearly affected nematode’s ability to infect its host. We wanted to build on this foundation and expand our knowledge about this gene family and its role during plant-nematode interaction using soybean-soybean cyst nematode (SCN) pathosystem.

The microRNA396 family targets a group of important growth-regulating transcription factors (GRFs) and we have identified the GRFs involved in the cyst nematode infection of Arabidopsis. We worked aggressively to transfer this knowledge to the soybean-SCN system. For this purpose, we developed various strategies to identify and characterize the miRNA396 gene family as well as their potential targets in soybean. In soybean (*Glycine max*), we established the gene families encoding microRNA396 (named gma-miR396) and their GRF targets (named GmGRFs). We discovered that there are 11 different genes encoding the gma-miR396 family, each family member is considered as an isomiR, and there are 25 genes that make up the GmGRF family. We have confirmed that seven of the gma-miR396 isomiRs are present in soybean roots, as most of the GmGRFs. In soybean cyst nematode (SCN)-infected soybean roots, we found that all of the root-expressed GmGRFs are also significantly up-regulated as feeding site (syncytium) becomes established. Furthermore, a subset of GmGRFs were found to be up-regulated significantly higher than the rest of the gene family at this time-point. These most highly-expressed GmGRFs were then selected and included in a complete time-course gene expression analysis with gma-miR396 isomiRs. We discovered that at the time-point corresponding to syncytium formation, almost all gma-miR396 isomiRs are significantly down-regulated, while the GmGRFs are significantly up-regulated. Furthermore, at time-points corresponding to syncytium maintenance (i.e. after the formation phase), almost all gma-miR396 isomiRs are significantly up-regulated, while almost all of the selected GmGRFs are significantly down-regulated. We then tested to see if the small suite of GmGRFs is post-transcriptionally regulated by gma-miR396 at the time-point where GmGRFs are significantly down-regulated and gma-miR396 significantly up-regulated (i.e. during syncytium maintenance). As a result, two of the GmGRFs were found to be regulated by gma-miR396 at this time-point, one of which is among the most highly expressed GmGRFs in soybean roots. We also determined that at least some of the gma-miR396 isomiR promoters are activated in the syncytium during the maintenance phase, and thus it is likely that the results obtained from our expression analyses represent regulation that occurs in syncytia. Finally, we have established that in soybean hairy roots placing the gma-miR396 isomiRs under the control of the *GmUBI* promoter for constitutive expression results in significantly decreased susceptibility to SCN. We have submitted a manuscript to a reputed international journal describing our findings.

In addition, we also have stable transgenic soybean lines that express all seven gma-miR396 isomiRs in our possession specifically in syncytia. These transgenic soybean lines may be substantially less susceptible to SCN and developmentally normal since we are manipulating the gma-miR396-*GmGRF* regulatory module specifically in synctyia during SCN infection. We are in the process of screening these lines to assess altered nematode susceptibility. Overall this grant has been used to study the role of this novel microRNA gene family during nematode infection in soybean and our findings have laid a foundation for developing cultivars with novel and robust nematode resistance.

**Use the RNA silencing approach to control soybean fungal diseases.**

In this project, we have assessed fungal genes as potential targets for RNA-silencing based approaches to prevent fungal colonization of soybean plants. We have previously cloned and confirmed the sequence of 82 soybean rust (SBR) candidate effectors. We devised a strategy to insert a ~300 base pair fragment of each of these 82 candidate effectors into a Bean pod mottle virus vector (BPMV) and completed making the constructs. We also conducted assays to determine if any of the 82 cloned candidate effectors had the ability to suppress plant immunity. We reasoned that effectors with such an activity would be good targets for RNA silencing, because they are likely necessary for fungal colonization of soybean. Based on immune suppression assays, we are focused a few candidate effectors to learn more about the mechanism by which they suppress the soybean immune system. One effector, that we named *Pp*EC23, strongly suppressed plant immune responses consistently in various assays. We think that this protein may help to disable the soybean immune system to promote fungal colonization. The PpEC23 protein strongly interacts with a soybean protein *Gm*SPL12L, which belongs to a large protein family of transcriptional factors controlling many developmental processes. We think that GmSPL12L is a plant target of PpEC23. We have used BPMV-VIGS technology to silence *Gm*SPL12L in soybean. The *Gm*SPL12L-silencing in soybean resulted into smaller leaves and shorter stature compared to the controls, suggesting its potential involvement in developmental process. When these silenced plants were challenged with downy mildew fungus, fewer lesions were observed, confirming that *Gm*SPL12L is involved in controlling the soybean immune system. Tests that we conducted with collaborators to determine if silencing of *Pp*EC23 from the fungus or *Gm*SPL12L alters plant susceptibility to soybean rust were inconclusive to this point.