

## **FSI Annual Report 2016-2017**

**Project Leader (PI):** Dr. Din-Pow Ma

**Collaborator(s):** Dr. W. Paul Williams

### **Milestones for FY 2016-17:**

1. Project title: Differential expression of *Aspergillus flavus* miRNA-like small RNAs in aflatoxin resistant and susceptible maize inbred lines
2. Milestones substantially met

The project started in January, 2016 and extended to June 30, 2017. Two maize inbred lines, Mp719 (resistant) and Va35 (susceptible), were inoculated with *A. flavus* NRRL 3357 (toxin producing) and *A. flavus* NRRL 21882 (non-toxin producing), respectively. Maize kernel samples of Mp719 and Va35 (inoculated and un-inoculated with *A. flavus*) were collected and used for small RNA extraction with the Sigma mirpremier isolation kit. The isolated small RNAs had high quality and were used for construction of miRNA libraries for Illumina RNA deep sequencing (see progress report). The project is currently in its final stage to collect sequence data and perform gene expression analysis. The milestones have been substantially met in this project.

### **Progress Report:**

Isolated high-quality Small RNAs with sizes smaller than 200 nucleotides were from two maize inbred lines, Mp719 (resistant) and Va35 (susceptible), using the Sigma mirpremier isolation kit. The isolated high-quality small RNAs from maize kernel samples inoculated and non-inoculated with the fungi *A. flavus* (toxin and non-toxin producing strains) were used to construct 24 miRNA-like (milRNA) libraries (Table 1) using the New England Biolabs (NEB) Next Multiplex Small RNA Library Prep Set. The 24 milRNA library samples were pooled based on their concentration and sent to BGI (Beijing Genomics Institute) for RNA sequencing service using the HiSeq 4000 system. The sequenced data will be analyzed by IGBB at MSU to identify differentially expressed *A. flavus* milRNAs in the maize kernels.

### **Accomplishments**

1. Had collected maize kernel samples inoculated and non-inoculated with the fungi *A. flavus* (toxin and non-toxin producing strains).
2. Had successfully isolated high quality of small RNAs from maize kernel samples and used them to construct 24 milRNA libraries.
3. Had pooled the milRNA libraries and sent to BGI for RNA sequencing.
4. Had determined the expression level of 10 milRNAs in Mp719 and Va35 lines inoculated with *A. flavus* NRRL 3357 and NRRL 21882 strains with qRT-PCR. The expression data indicated that two *A. flavus* milRNAs, Af-miR-1 (GCUGGUAUCUGCGAACGACUUGC) and Af-mir-4 (GUGGAGGAUUGGGACGGGU), had 4.8 and 1,300-fold reduced expression, respectively, in

resistant Mp719 vs susceptible Va35 when the two lines were inoculated with NRRL 3357 compared with NRRL 21882.

**Significant Activities that Support Special Target Populations:** The differentially expressed *A. flavus* miRNAs can be used as molecular markers in the development of maize lines with enhanced resistance to aflatoxin accumulation. They can be also utilized for the study of molecular mechanisms of maize resistance to *A. flavus*.

**Technology Transfer:** None

Patent Disclosure: None

**International Cooperation / Collaboration:** None

**Publications:**

Harper, A., Franks, K., and Ma, D. (2017). Differential Expression of *Aspergillus flavus* miRNAs in aflatoxin resistant maize inbred lines. *Mississippi Academy Sciences* 62 (1): 40.

**Presentations:**

Ma, D. (2016, May). Expression analysis of *Aspergillus flavus* miRNA like RNAs in aflatoxin resistant and susceptible maize inbred lines. Oral presentation at the 2016 FSI Conference, Mississippi State, MS.

Harper, A., Franks, K., and Ma, D. (2017, February). Differential Expression of *Aspergillus flavus* miRNAs in aflatoxin resistant maize inbred lines. Poster presentation at the 81<sup>st</sup> annual meeting of Mississippi Academy of Sciences, Hattiesburg, MS.

Harper, A., Franks, K., and Ma, D. (2017, March). Expression analysis of *Aspergillus flavus* MicroRNA-like RNAs in aflatoxin resistant and susceptible maize inbred lines. Poster presentation at the 15<sup>th</sup> graduate student research symposium of Mississippi State University, Mississippi State, MS.

Franks, K., Harper, A., and Ma, D. (2017, April) Identification of *Aspergillus flavus* microRNA-like RNAs differentially expressed in maize lines with different levels of resistance/susceptibility to aflatoxin accumulation. Poster presentation at undergraduate student research symposium, Mississippi State University, Mississippi State, MS.

<b>Table 1. List of 24 miRNA libraries for RNA sequencing</b>							
Sample ID	Barcode	Tag seq	Qubit con (ng/ul)	Ave frag size (bp)	Qubit con (nM)	Vol (ul) lib stock	Vol (ul) of ET buffer added
Mp719-3357-1	1	ATCACG	3.24	141	34.82	17.2	12.8
Mp719-3357-2	2	CGATGT	8.58	142	91.55	6.6	23.4
Mp719-3357-3	3	TTAGGC	7.03	141	75.54	7.9	22.1
Mp719-21882-1	4	TGACCA	6.63	140	71.75	8.4	21.6
Mp719-21882-2	5	ACAGTG	12.7	140	137.45	4.4	25.6
Mp719-21882-3	6	GCCAAT	6.21	141	66.73	9	21
Mp719-H2O-1	7	CAGATC	14.8	141	159.04	3.8	26.2
Mp719-H2O-2	8	ACTTGA	13.3	141	142.92	4.2	25.8
Mp719-H2O-3	9	GATCAG	15.7	140	169.91	3.5	26.5
Mp719-Control-1	10	TAGCTT	13.5	140	146.10	4.1	25.9
Mp719-Control-2	11	GGCTAC	16.1	141	173.01	3.5	26.5
Mp719-Control-3	12	CTTGTA	10.6	139	115.54	5.2	24.8
Va35-3357-1	13	AGTCAA	17.1	139	186.40	3.2	26.8
Va35-3357-2	14	AGTTCC	8.36	141	89.83	6.7	23.3
Va35-3357-3	15	ATGTCA	10.4	140	112.55	5.3	24.7
Va35-21882-1	16	CCGTCC	4.29	142	45.77	13.1	16.9
Va35-21882-2	17	GTAGAG	5.47	141	58.78	10.2	19.8
Va35-21882-3	18	GTCCGC	3.43	138	37.66	15.9	14.1
Va35-H2O-1	19	GTGAAA	7.03	139	76.63	7.8	22.2
Va35-H2O-2	20	GTGGCC	6.18	140	66.88	9	21
Va35-H2O-3	21	GTTTCG	3.83	139	41.75	14.4	15.6
Va35-Control-1	22	CGTACG	9.94	140	107.58	5.6	24.4
Va35-Control-2	23	GAGTGG	2.42	139	26.38	22.7	7.3
Va35-Control-3	24	GGTAGC	7.78	140	84.20	7.1	22.9