ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2019 – December 31, 2019

PROJECT TITLE: Application of Forward and Reverse Genetics to Rice Improvement

PROJECT LEADER: Thomas H. Tai, Research Geneticist, USDA-ARS Plant Sciences, UCD

PRINCIPAL UC INVESTIGATORS:

Thomas H. Tai, Research Geneticist, USDA-ARS, Plant Sciences, UCD

COOPERATORS:

None

LEVEL OF 2019 FUNDING: $22,491 (includes $5,292 residual from 2018)

OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH OBJECTIVES:

The overall objective of the RB-3 project is to identify and develop novel rice germplasm for incorporation into breeding programs serving the California rice industry. Traits that improve the value of the rice crop or reduce production costs are of primary interest. To achieve this objective, rice mutant populations have been developed using traditional chemical mutagenesis of rice seeds. The resulting populations (M2 generation and beyond) are subjected to genetic screening to isolate lines exhibiting promising new traits. Identification of useful mutants is achieved directly by screening mutant populations for traits of interest (i.e. forward genetics) or indirectly by identifying changes in the DNA sequence of genes that may result in the expression of these traits (i.e. reverse genetics). Novel traits (i.e. mutant phenotypes) that are relevant to the RB-3 project's objective mainly involve grain quality and agronomic performance including tolerance to stresses experienced in the California rice growing environment and production system. Recent specific targets include reduced uptake and/or localization of arsenic in rice grains and resistance or tolerance of rice plants to selected herbicides.

In 2019, the primary focus of the RB-3 project was the continued characterization of rice mutants identified through reverse genetic screens of genes encoding silicon/arsenic transporters (i.e. *Lsi1*, *Lsi2*, and *OsABCC1*). Work on this project was conducted in the USDA-ARS rice genetics lab, UC Davis greenhouses and field nursery, and the Rice Experiment Station. Unfortunately, research efforts this year were greatly disrupted by the 35-day federal government shutdown and staff vacancies (i.e. postdoctoral research associate, biological science technician). A temporary assistant, supported in part by residual funds from the 2018 RB-3 Project, was hired to help prepare straw and brown rice samples for submission to the UC Davis Analytical Lab for processing (i.e. grinding) and elemental analysis in order to address the primary objective of

2019. The disruptions resulted in specific objectives not being completed as detailed in summary.

Specific 2019 objectives included:

1) **Complete evaluation of arsenic and silicon content of mutant lines grown in the field in 2018:** In 2018, several mutants carrying fixed (homozygous) mutations in genes involved in silicon and arsenic uptake/accumulation in rice were grown in the UC Davis field facility and the Rice Experiment Station. Samples from these lines will be processed and submitted to the UC Davis Analytical Lab for evaluation of silicon and arsenic content.
2) **Develop double mutants to examine the effect on arsenic and silicon uptake and accumulation:** Previously in 2016, crosses were performed between some of the *lsi* (low silicon) mutants obtained from the TILLING screen and the resulting progeny were planted and verified by DNA sequencing in 2017. In 2018, mutants harboring fixed mutations in both genes will be identified and evaluated. Additional crosses between mutants identified by TILLING in 2016 and 2017 will be made as warranted by results of evaluation of the silicon and arsenic content analysis and germanium assays.
3) **Evaluate remaining arsenic/silicon uptake and accumulation mutants:** In 2019, *lsi1* and *Osabcc1* mutants not previously evaluated will be characterized with regard to silicon and arsenic content and/or germanium tolerance.
4) **Complete evaluation of herbicide tolerance of rice mutants identified by TILLING:** Mutants identified by TILLING to harbor mutations in the genes encoding protoporphyrinogen oxidase, 4-hydrophenyl pyruvate dioxygenase, acetyl Co-A carboxylase, and glutamine synthetase will be evaluated for their response to selected herbicides.

SUMMARY OF 2019 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1) **Complete evaluation of arsenic and silicon content of mutant lines grown in the field in 2018:** In 2018, the same set of mutant lines grown in the UC Davis greenhouses and field facility in 2017 were grown in the UC Davis field facility and the Rice Experiment Station. The mutant lines harbored fixed mutations in the *Lsi1* (3 lines: NME-1746, NM-3403, and NM-3380), *Lsi2* (9 lines: NM-2902, NM-5988, NME-2308, NM-3483, NM-3036, NME-2143, NM-5759, NME-2244, and NME-2249), and *OsABCC1* (1 line: NM-4903). Two wild-type lines (Nipponbare parent: WT-2 and WT-7) and one wild-type sibling line (*Lsi2*: NM-3036WT, sibling line to NM-3036 which is an *lsi2* mutant) were also included in the field tests as in 2017. Plants were initially started in the greenhouse and transplanted in the field using a randomized completed block design in both locations (UC Davis, 4 replications; RES, 3 replications). Four plants per line were transplanted with 30 cm x 30 cm spacing. Plants were harvested by hand in October/November 2018. In 2019, straw and brown rice samples were prepared and submitted to the UC Davis Analytical Lab, which processed (i.e. ground samples to powder) and perform elemental (total Si and As) content analysis.

Results of the total Si (straw only) and total As (brown rice only) from the field-grown samples are shown in Table 1. Note: For the mutant lines, designations include "NM" for Nipponbare Mutant. Lines with the "E" designation were generated by ethyl methanesulfonate mutagenesis. Lines without the "E" designation were generated by sodium azide plus methyl nitrosourea mutagenesis.

Table 1 Total silicon and arsenic content analysis of mutants and Nipponbare wild type grown in the UC Davis rice field facility and RES triangle bay in 2018

Line	Mutant allele	Total Si (straw)[a] % - Davis	Total Si (straw)[b] % - RES	Total As (grain)[a] ppm - Davis	Total As (grain)[b] ppm - RES
WT-2	wild type	6.40 ± 0.175	6.50 ± 0.238	0.07 ± 0.01	0.12 ± 0.01
WT-7	wild type	6.48 ± 0.214	6.31 ± 0.131	0.07 ± 0.01	0.12 ± 0.01
NM-E1746	*lsi1*	0.13 ± 0.022**	0.22 ± 0.080**	0.10 ± 0.01**	0.18 ± 0.00§*
NM-3403	*lsi1*	3.69 ± 0.244**	3.48 ± 0.234**	0.14 ± 0.01**	0.17 ± 0.01**
NM-3380	*lsi1*	6.72 ± 0.230	6.35 ± 0.458	0.08 ± 0.01	0.14 ± 0.01*
NM-2902	*lsi2*	6.90 ± 0.180	6.82 ± 0.243	0.09 ± 0.01**	0.14 ± 0.02
NM-5988	*lsi2*	6.02 ± 0.162*	5.67 ± 0.229*	0.07 ± 0.01	0.14 ± 0.02
NM-E2308	*lsi2*	6.57 ± 0.164	6.20 ± 0.284	0.06 ± 0.01	0.12 ± 0.01
NM-3483	*lsi2*	6.11 ± 0.391	6.15 ± 0.325	0.07 ± 0.01	0.13 ± 0.02
NM-3036WT	*Lsi2*	6.26 ± 0.314	6.43 ± 0.370	0.08 ± 0.01	0.14 ± 0.01*
NM-3036	*lsi2*	6.49 ± 0.374	6.65 ± 0.188	0.09 ± 0.01*	0.14 ± 0.01**
NM-E2143	*lsi2*	6.29 ± 0.104	6.56 ± 0.193	0.06 ± 0.01§	0.12 ± 0.02
NM-5759	*lsi2*	6.43 ± 0.330	6.67 ± 0.535	0.07 ± 0.01	0.14 ± 0.01*
NM-E2244	*lsi2*	6.66 ± 0.113	6.77 ± 0.275	0.09 ± 0.01*	0.15 ± 0.01**
NM-E2249	*lsi2*	6.15 ± 0.057	5.90 ± 0.219*	0.10 ± 0.01**	0.15 ± 0.01*
NM-4903	*Osabcc1*	7.29 ± 0.175**	6.36 ± 0.163	0.05 ± 0.01§**	0.12 ± 0.01

[a] Total Si reported as a percentage of dry matter. Values are the means and standard deviations of four replications (UC Davis) and three replications (RES) with each replication for both locations consisting of four plants each except where noted

[b] Total As reported as parts per million (ppm). Values are the means and standard deviations for samples as described for the total Si analysis

*, ** means significant difference between the mean values at $p < 0.05$ and $p < 0.01$ by *t*-test between WT-2 (wild type) and the other lines within a location, respectively

§ NM-E2143 values based on two samples; NM-4903 values based on highest estimate as three of four samples were below detect limit of 0.05 and assigned values of 0.049; NM-E1746 value based on one sample

Comparison of the straw total Si content of the wild type (WT-2 and WT-7) controls from UC Davis and RES locations revealed no significant differences ($p<0.01$. t-test). Analysis of variance (ANOVA) of straw total Si of the two wild-type controls from both locations also indicated no statistically significant differences at the $p<0.05$ level [$F(3,10) = 0.619$, $p = 0.61$). In contrast, while the mean values for brown rice total As content for WT-2 and WT-7 at each location were identical (0.07 ± 0.01 ppm at UC Davis; 0.12 ± 0.01 ppm at RES), comparison between the locations indicated a significant difference ($p<0.01$, one-way ANOVA).

Consistent with last year's straw total Si analysis, the *lsi1* mutants NME-1746 and NM-3403 were significantly less than the wild type Nipponbare controls and the other mutant lines including the third *lsi1* mutant NM-3380. Unlike the previous year's results, no mutant lines with the exception of NM-4903 exhibited significantly higher straw total Si than the wild-type controls (WT-2 and WT-7). In the case of NM-4903, significantly greater total Si was only observed in the UC Davis location. In addition, two lines, NM-5988 and NM-E2249 (both are *lsi2* mutants), exhibited reduced total Si in one (NM-E2249, RES) or both (NM-5988) locations. Two points of note are that the wild-type controls in this study appeared to have higher straw total Si than the controls from the 2017 test while the total Si content of the mutants appeared to be similar between years although further data analysis is needed to confirm these observations. In this year's analysis, straw total As was not determined due to time and resource constraints and the fact that straw total Si and brown rice total As are more significant criteria for selecting mutants for additional studies (e.g., genetic crossing, evaluation of other related traits such as stress tolerance).

As noted earlier, the brown rice total As of the wild-type controls varied significantly between the two locations (Table 1). Overall in comparison to the 2017 test values, mean content values for the UC Davis location appeared to be slightly lower and those from the RES location appeared slightly higher. Statistical analysis of the total As within each location indicated significantly higher brown rice total As in the NM-E1746 and NM-3403 (*lsi1*) mutants compared to wild type (WT-2 and WT-7). This is inconsistent with the reduction in total Si content (i.e. that both Si and As uptake would be affected in the same manner) and suggests as was noted last year, that mutations may be able to separate out multiple functions encoded by one gene, in this case *Lsi1*. These total As results are in partial agreement with the analysis of the 2017 test (UC Davis) where NM-3403 showed increased total As but no significant increase was observed in NM-E1746. Significant differences in brown rice total As content of the *lsi2* and *Osabbc1* mutants were fairly consistent comparing the 2017 and 2018 UC Davis location results. One exception was NM-3036 which was not different from wild type in 2017 but did show significantly greater total As in 2018. In all cases, except NM-4903, the differences observed involved increases in grain total As which is not desirable. Total As content of brown rice from the RES location plants identified more lines exhibiting significant differences from wild type but in each case the differences were increases. Unlike the 2017 and 2018 UC Davis tests, NM-4903 from the RES location had the same content as the wild type suggesting that environmental factors are at play. To investigate this

further, it will be important to develop selected mutant lines by backcrossing to remove background mutations and by crossing to combine mutations in the *Lsi1*, *Lsi2* and *OsABCC1* genes. Based on the results of this second year of analysis, the lines prioritized for further characterization are NM-4903, NM-5988, NM-E1746, NM-3403, and NM-E2143.

In addition to completing a second year of elemental analysis of these mutants, five lines from this project were selected (WT-7, NM-E1746, NM-3380, NM-3403, and NM-E2244) along with three lines from another mutant project in my program focused on characterizing mutants with reduced/altered cuticle wax (Sabine B5, SAB-11-39A, and SAB-1558.1) for inclusion in the 2019 RES stem rot nursery. The rationale for this experiment was to examine whether silicon or leaf wax plays a role in tolerance or susceptibility to stem rot disease. Based on the 2018 elemental analysis of 2017 grown lines, two mutants with significantly lower and higher straw total Si were evaluated along with the wild type control (WT-7, i.e. Nipponbare). In addition, two mutants with altered cuticle wax (wax less and altered wax crystals) were included along with their corresponding wild type (Sabine B5). Planting, inoculation, and scoring were performed by Dr. Teresa De Leon and the results of this preliminary evaluation are presented in Table 2.

Table 2. Preliminary evaluation of selected rice mutants and their wild type progenitors in the RES stem rot nursery

	WT-7	NM-E1746	NM-3380	NM-3403	NM-E2244	Sabine B5	11-39A	1558.1	87Y550	M-206
Si[a]	wild type	very low	higher*	low	higher*	wild type	wild type	wild type	wild type	wild type
wax[b]	wild type	wild type	wild type	wild type	wild type	wild type	wax less	altered	wild type	wild type
R1	1	3	5	4	1	1	1	1	1	4
R2	3	3	2	1	1	3	5	1	2	3
R3	5	5	4	4	1	5	4	1	2	5
Rating[c]	3	3.67	3.67	3	1	3	3.33	1	1.67	4

[a] Straw total silicon based on 2018 elemental analysis of 2017 UC Davis location; non-Nipponbare mutants assumed to be wild type for Si
[*] Based on this year's analysis these lines did not significantly higher total Si
[b] Wax phenotype based on previous studies conducted in this lab; non-Sabine mutants assumed to be wild type for wax trait
[c] Average stem rot ratings (1-5 with 1 = most tolerant to 5 = most susceptible) from three replications

Visual ratings of the lines suggest that two mutants, NM-E2244 and SAB-1558.1, may be highly tolerant to stem rot. Both silicon and cuticle (leaf surface) wax have been implicated in providing protection to terrestrial plants against a wide variety of environmental stresses. These preliminary results suggest that there may be some involvement of silicon and cuticle wax in stem rot disease and indicate that further screening is needed to clarify their importance.

2) **Develop double mutants to examine the effect on arsenic and silicon uptake and accumulation:** In 2016, crosses were performed between the *lsi1* mutant NM-E1746 and the *lsi2* mutants NME-2308 and NM-2902. These were confirmed in 2017 by molecular marker analysis of the F_1. The resulting F_2 progeny were evaluated with DNA markers in 2018 but no lines were found to be homozygous for both mutations although some lines were fixed at one locus and heterozygous at the other or heterozygous at both loci. In 2019, mutants harboring fixed mutations in both genes were to be identified and evaluated but due to time and resource constraints as noted earlier, this objective was not addressed. Results from objective 1 suggest that other mutants should be prioritized including NM-4903 which is the only line to have shown a reduction in grain total As albeit in the UC Davis location only.

3) **Evaluate remaining arsenic/silicon uptake and accumulation mutants:** In 2019, *lsi1* and *Osabcc1* mutants not previously evaluated were to be characterized with regard to silicon and arsenic content and/or germanium tolerance. Due to disruptions noted earlier, this objective remains unaddressed.

4) **Complete evaluation of herbicide tolerance of rice mutants identified by TILLING:** Mutants identified by TILLING to harbor mutations in the genes encoding protoporphyrinogen oxidase, 4-hydrophenyl pyruvate dioxygenase, acetyl Co-A carboxylase, and glutamine synthetase were to be evaluated for their response to selected herbicides. As with specific objectives 2 and 3, delays due to the federal government shutdown and inability to fill staff vacancies resulted in minimal progress on this objective. Towards accomplishing this work, the project obtained access to a herbicide spray cabinet and the assistance of Seth Watkins (UC Davis, Dr. Brad Hanson's group). Through correspondences with Dr. Kassim Al-Khatib, initial efforts have been focused on PPO inhibitors and one test application of the herbicide Shark EW (2 and 4 oz. per acre rate) was conducted to evaluate the response of wild type Nipponbare (progenitor of the TILLING mutants), Kitaake, and Sabine (both of which are wild type progenitors of mutant populations developed in the USDA-ARS rice genetics lab). Furthermore, one cuticle wax mutant derived from Kitaake (KDS-2249D), which has been previously published by my lab, was included to examine whether the wax less trait expressed by this line affects its response to herbicide treatment.

   All the varieties tested showed necrotic lesions at both treatment rates and the amount of damage was consistent with the dose (more visible lesions at higher dose). Based on casual observations, the temperate *japonica* Nipponbare and Kitaake lines were the most tolerant to the Shark herbicide followed by the southern U.S. variety Sabine. The most extreme damage was observed in the KDS-2249D wax less mutants, which was consistent with the reduced protective barrier on the surface of their leaves (Fig. 1). Evaluation of the PPO gene TILLING mutants will be conducted with Shark in early 2020. In addition to Shark (carfentrazone), dose response testing of other PPO inhibitors (initially Chateau – flumioxazin) will be conducted based on recommendations by Dr. Al-Khatib and these herbicides will also be used to evaluate the mutants.

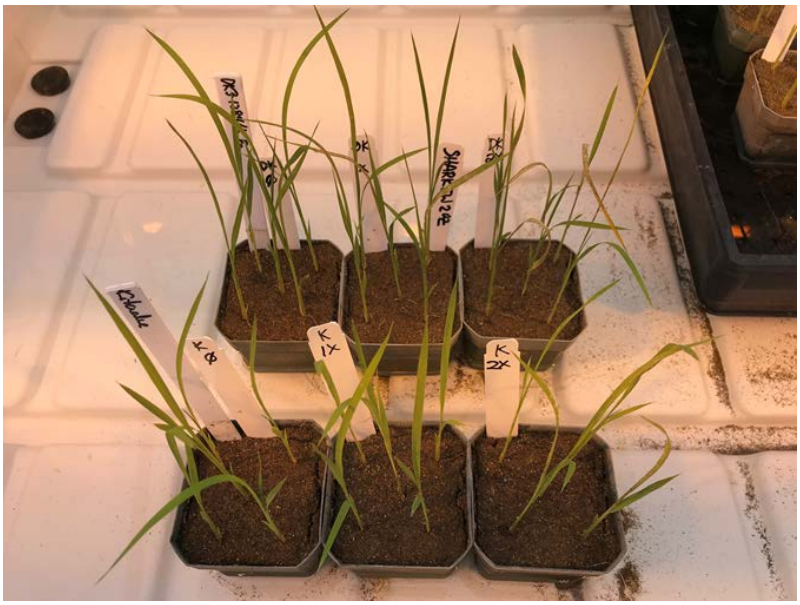

Fig. 1. Response of Kitaake (wild type) and KDS-2249D mutant line to PPO inhibitor carfentrazone (Shark EW) at 2 oz. (1X) and 4 oz. per acre rate. Seedlings were sprayed at the 3-4 leaf stage and photographed 6 days after spraying.

PUBLICATIONS OR REPORTS:

Kim, HJ and Tai, TH (2019) Identification of novel mutations in genes involved in silicon and arsenic uptake and accumulation in rice. Euphytica 215:72

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 2019, a second year of total arsenic and silicon content analysis of field-grown mutants carrying mutations in the *Lsi1* and *Lsi2* metalloid transport genes and the arsenic sequestration transporter gene *OsABCC1* was completed. Results of the content analysis from the UC Davis and RES field locations were generally consistent for straw total Si but differed from the previous analysis of UC Davis field-grown lines with regard to lines exhibiting higher total Si. It was noted that wild type controls appeared to have higher total Si in this second year (i.e. 2018 planting). Mutant line NM-4903 continued to exhibit high total Si in straw and reduced total As in grain in the UC Davis location, but these effects were not observed in the RES. This difference warrants further investigation. The two lines exhibiting the greatest reduction in total Si from previous analysis exhibited a consistent reduction in this second year of analysis. Overall, several lines have been identified for further studies and preliminary evaluation of traits that may also be influenced by reduced/increased Si (e.g. stem rot tolerance). Conditions for evaluating specific mutants and mutant populations for herbicide tolerance are being established and will facilitate completion of that objective in 2020.