ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2017 – December 31, 2017

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

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OBJECTIVES AND EXPERIMENTS CONDUCTED, BY LOCATION, TO ACCOMPLISH OBJECTIVES:
The overall objective is to employ forward and reverse genetic screens to identify novel rice germplasm for incorporation into breeding programs serving the California rice industry. Primary emphasis will be placed on screening populations of rice generated by traditional mutagenesis for new traits affecting grain quality and reducing production costs. This will be achieved by directly screening plant materials for traits of interest and by identifying changes in the DNA sequence of genes that may result in the expression of these traits. Specific targets include reduced uptake and/or localization of arsenic in milled rice grains and resistance/tolerance of rice plants to selected herbicides.

The traditional approach to utilizing mutant populations is to conduct screens to identify mutant phenotypes of interest such as semidwarfism, male sterility, and herbicide tolerance. Reverse genetics is a complementary approach for exploiting mutant populations. This strategy requires prior knowledge of the genes involved in the target traits. These genes are used to screen populations to identify mutated versions that may result in the expression of novel traits. One method of reverse genetics is Targeting of Induced Local Lesions in Genomes (TILLING). The TILLING strategy is based on the detection of mutations in target gene sequences by screening DNA isolated and pooled from hundreds of mutant lines (typically ~2,000 lines total). A service to identify mutations of interest from this population is operated by the UC Davis TILLING Lab at the Genome Center. We have successfully used this service to identify mutations in genes involved in arsenic uptake/accumulation and genes that encode protein targets of various herbicides. The major objectives of our research in 2017 were 1) characterization of the rice mutants identified by the forward and reverse genetic screens, and 2) confirmation and evaluation of additional mutants identified by reverse genetic screens of genes encoding proteins

targeted by selected herbicides and genes that control arsenic uptake/accumulation. Work on this project was conducted in the USDA-ARS rice genetics lab, greenhouses, field nursery and other research facilities at UC Davis.

Research efforts were disrupted by the required relocation of the USDA-ARS rice genetics lab from the Plant and Environmental Sciences Building to a substantially smaller lab and less equipped facility (Sprocket Building, formerly the Food Science and Technology Building) in 2017. As a result, some specific objectives were not completed and some 2017 funding was rolled over to 2018.

Specific 2017 objectives included:

1) **Characterization of the uptake and accumulation of arsenic and silicon in rice mutants:** In 2016, several lines carrying mutations in genes involved in silicon and arsenic uptake in rice were identified by TILLING and confirmed. Seeds from mutants harboring homozygous (i.e. fixed) mutations in one of three genes (*Lsi1*, *Lsi2*, and *OsABCC1*) were harvested. Forward genetic screening resulted in the identification of several candidate mutants for altered uptake of silicon and arsenic. In 2017, the lines identified by the TILLING approach will be planted in the field at the UC Davis rice facility and in controlled environments and silicon and arsenic contents will be determined. The mutants identified by forward genetics will be re-tested and promising lines will be evaluated for silicon and arsenic uptake.
2) **Evaluation of the response of rice mutants to selected herbicides and characterization of their growth and development:** In 2016, several rice mutants carrying mutations in genes that encode proteins targeted by various herbicides were identified and confirmed. Mutant lines with homozygous mutations in one of six genes (alpha-2 tubulin, protoporphyrinogen oxidase, 4-hydrophenyl pyruvate dioxygenase, acetyl Co-A carboxylase, glutamine synthetase – chromosome 2, and glutamine synthetase – chromosome 10) were isolated and seeds were harvested. In 2017, these lines will be evaluated for their response to selected herbicides under controlled environments. These mutants will also be grown for observation of developmental and agronomic traits.
3) **Identification and confirmation of additional rice mutants using the TILLING approach:** The initial TILLING screen yielded few mutations (≤ 3) in five out of the nine genes targeted. An additional 2,048 mutants (M2 generation) are currently being screened for mutations in those genes (Lsi1, OsABCC1, 4-hydrophenyl pyruvate dioxygenase, acetyl Co-A carboxylase, and glutamine synthetase – chromosome 2) and two of the other high value targets (protoporphyrinogen oxidase and glutamine synthetase – chromosome 10). Any mutations identified in this screen will be confirmed and homozygous individuals will be identified and subjected to preliminary trait evaluations.

SUMMARY OF 2017 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1) **Characterization of the uptake and accumulation of arsenic and silicon in rice mutants:** This year lines harboring fixed mutations in the *Lsi1* (3 lines: E-1746, 3403, and 3380), *Lsi2* (9 lines: 2902, 5988, E-2308, 3483, 3036, E-2143, 5759, E-2244, and E-2249), and *OsABCC1* (1 line: 4903) were grown in the UC Davis field facility and greenhouse. The lines were initially started in the greenhouse and transplanted in the field and large pots in the greenhouse. A randomized completed block design with four replications was employed in the field. Five plants per line were transplanted with 1 foot spacing. Two wild-type lines (Nipponbare parent: WT-2 and WT-7) and one wild-type sibling line (*Lsi2*: 3036-2) were also included in the field test. Several lines showed reduced fertility likely due to the number of mutations (i.e. mutational load) in the lines, which have not been backcrossed to wild-type Nipponbare to remove possible deleterious mutations, or cold stress typical of the UC Davis nursery location (or a combination of the two factors). Plants (entire aboveground tissues) were harvested and are currently being prepared for submission to the UC Davis Analytical Lab for analysis of arsenic and silicon content. Preliminary evaluation of the lines was performed using the germanium assay. Seeds of each line from the greenhouse grown materials were germinated in an incubator and small seedlings were transferred to Kimura B nutrient solution and grown until the 2-3 leaf stage. Seedlings were then treated with 50 µM of germanium in Kimura B. Two of the *lsi1* mutants (E-1746 and 3403) exhibited high tolerance (few to no lesions on the plants) in contrast to the controls (Fig. 1). The location of the mutations and their effects on the protein encoded by Lsi1 are shown in Fig. 2. Lesions, which form as the results of the toxicity of germanium to rice plants, were observed on all the remaining mutants (Fig. 3). The presence of lesions in the *lsi2* and *Osabcc1* mutants is consistent with previous reports on how these genes function (i.e., these genes have a greater impact on grain arsenic uptake and accumulation).

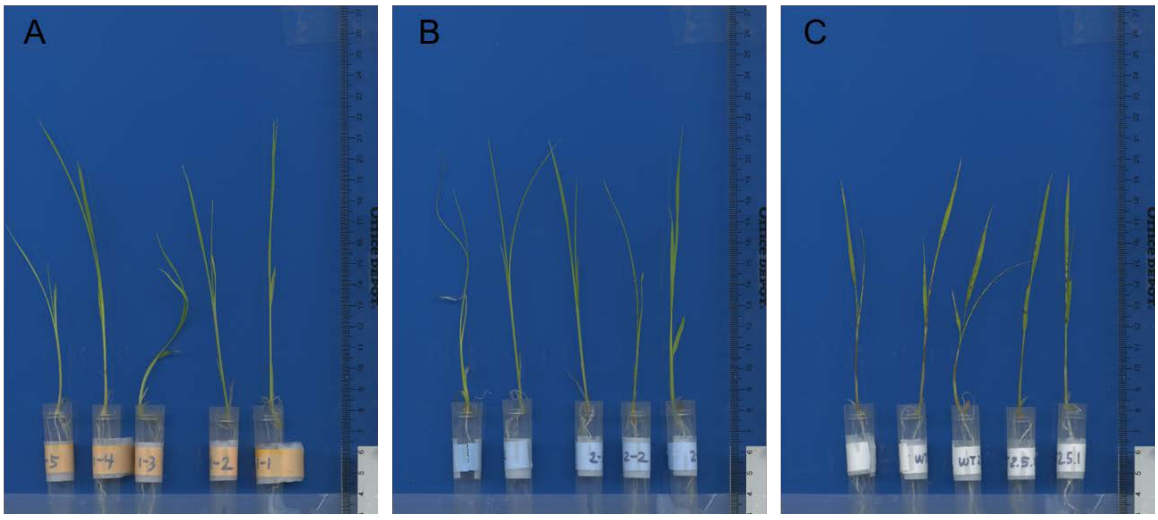

Fig. 1. Response of rice seedlings to germanium treatment. A) *lsi1* mutant E-1746, B) *lsi1* mutant 3403, C) wild-type Nipponbare WT-2. Few or no lesions developed in the lsi1 mutants compared to the numerous lesions observed in the wild type seedlings. Lack

of lesions, which are the result of germanium toxicity, are indicative of reduced uptake of germanium and most likely reductions in silicon and arsenic uptake.

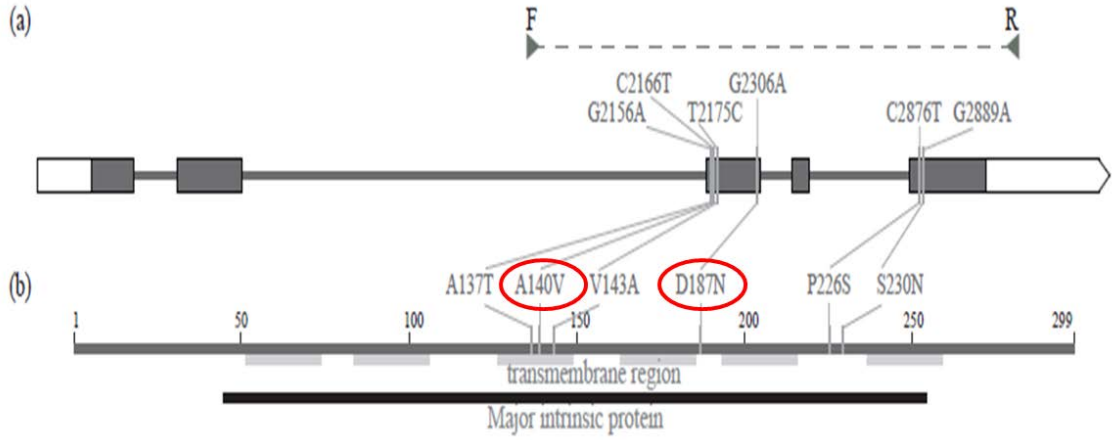

Fig. 2. The location of mutations in Lsi1 identified by TILLING and corresponding effects on the protein encoded by the gene. (a) Gene model with gray boxes indicating exons or coding sequences, the gray line representing the introns or non-coding regions, and the open boxes indicating the untranslated regions of the gene. The mutations (base changes) and their locations are indicated above the model and the dotted lines represent the region of the gene used to screen the TILLING mutant population by sequencing (F: forward, R: reverse DNA primers). (b) Protein model indicating motifs/domains and functional regions. Amino acid changes (single letter amino acid code) predicted to result from the mutations and their locations in the protein are indicated above the model. The two changes circled correspond to lines E-1746 (A140V) and 3403 (D187N).

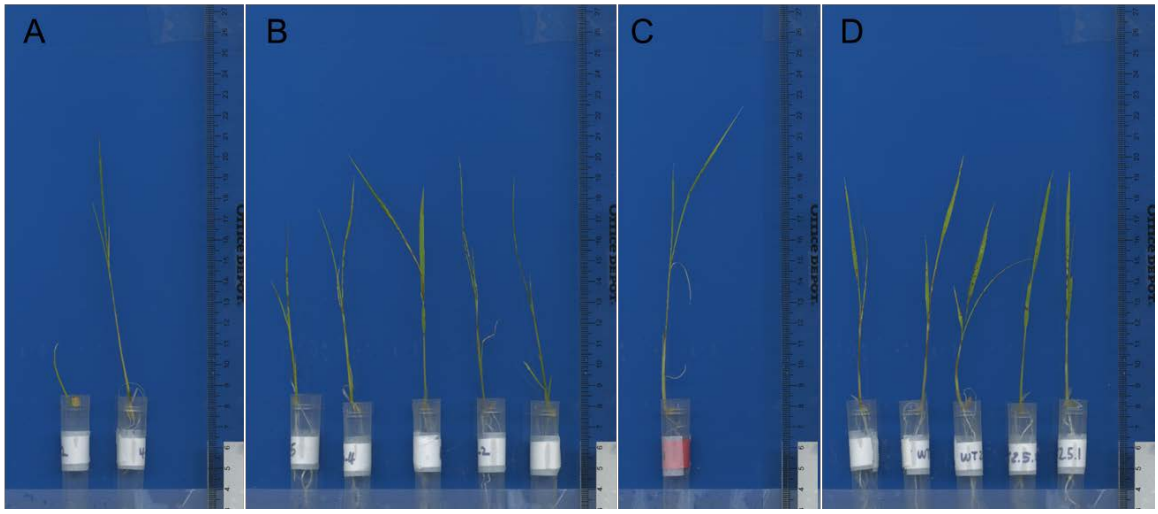

Fig. 3. Lines with mutations in *Lsi2* and *OsABCC1* developed lesions similar to wild type. A) *lsi2* mutant 2902, B) *lsi2* mutant E-2038, C) *Osabcc1* mutant 4903, D) wild-type

Nipponbare WT-2. The lesion phenotype is consistent with previous reports on the function of these genes and is not indicative of arsenic content in the rice grains.

In 2016, crosses were performed between the *lsi1* mutant E-1746 and the *lsi2* mutants E-2038 and 2902. These were confirmed this year by molecular marker analysis of the F_1. The resulting F_2 progeny will be screened with DNA markers in 2018 to identify double mutants (i.e., lines harboring fixed mutations in both *lsi1* and *lsi2*) for evaluation of the impact of having both mutations on the arsenic and silicon content.

In addition to work on the mutants identified by TILLING, the germanium assay was performed on a previously identified mutant, KDS-557B, which exhibited greater sensitivity to germanium as evidenced by more rapid and intense development of lesions. This phenotype was confirmed in comparison to the wild-type parent Kitaake and the wild-type variety Sabine (Fig. 4). KDS-557B has been crossed with Sabine (KDS-557B/Sabine) and F_2 seeds were harvested this year for genetic mapping studies.

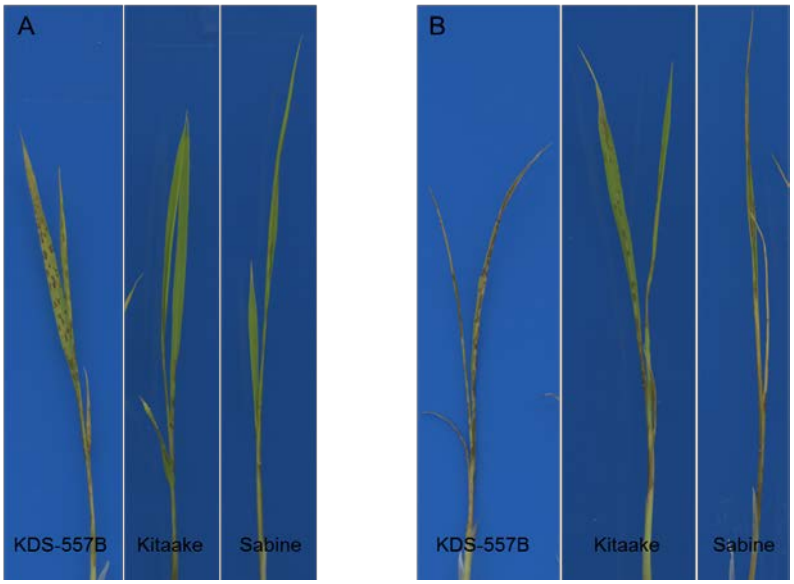

Fig. 4. Response of KDS-557B mutant and wild-type varieties Kitaake and Sabine to germanium. A) Three days post-treatment with 50 µM germanium in Kimura B solution. B) Five days post-treatment.

Further genetic and physiological analysis of the KDS-557B mutant is needed to determine if this increased sensitivity to germanium is due to altered uptake and, if so, whether this also effects uptake of silicon and arsenic. In 2016, other mutants in the Kitaake background, which showed altered responses to germanium, were reported but have not been re-tested to confirm the original assessment.

2) **Evaluation of the response of rice mutants to selected herbicides and characterization of their growth and development:** Due to disruptions to the rice

genetics program noted above, efforts on the characterization of mutants carrying mutations in herbicide target genes was limited to evaluation of the response to alpha-2 tubulin mutants to the herbicide Prowl® (active ingredient pendimethalin). Seven mutations were detected by TILLING (Fig. 5).

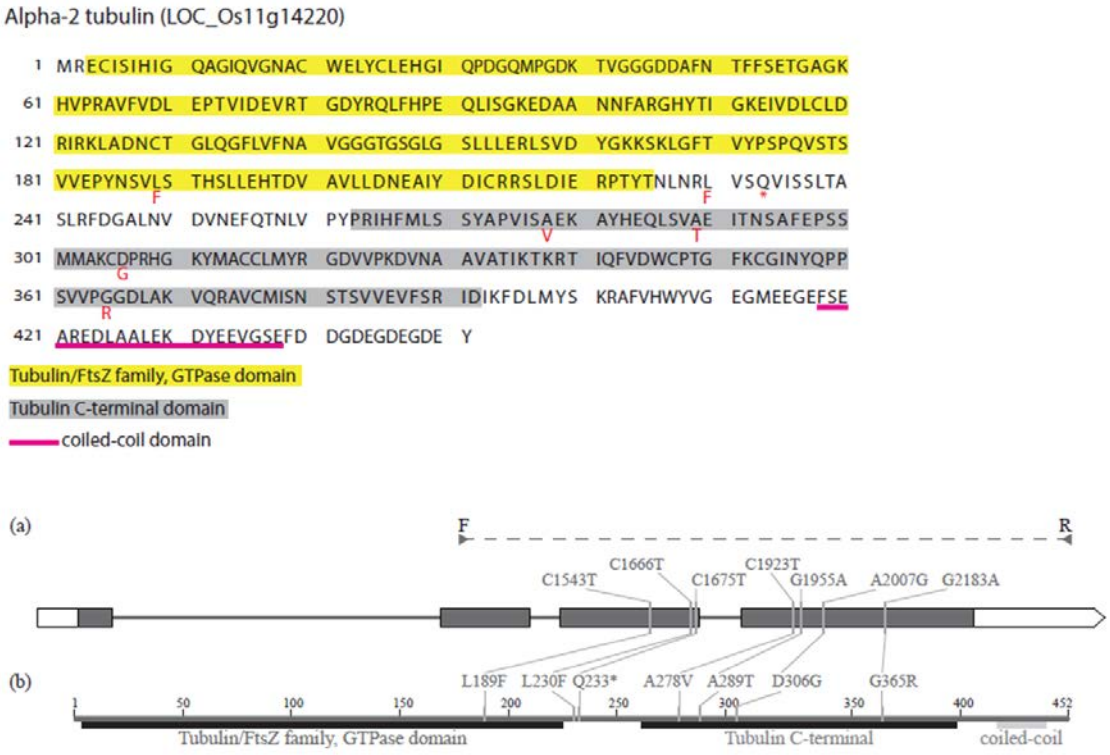

Fig. 5. Mutations in the alpha-2 tubulin gene of rice and their corresponding predicted effects of the protein. Amino acid sequence and corresponding protein domains as indicated. Single letters under the sequence indicate amino acid changes resulting from mutations. Asterisk indicates premature termination of protein. (a) Gene model with gray boxes indicating exons or coding sequences, the gray line representing the introns or non-coding regions, and the open boxes indicating the untranslated regions of the gene. The mutations (base changes) and their locations are indicated above the model and the dotted lines represent the region of the gene used to screen the TILLING mutant population by sequencing (F: forward, R: reverse DNA primers). (b) Protein model indicating motifs/domains and functional regions. Amino acid changes (single letter amino acid code) predicted to result from the mutations and their locations in the protein are indicated above the model.

In cooperation with Prof. Bo Liu, these mutants were evaluated for their sensitivity to pendimethalin (200 nM; as determined by the Liu lab). Results of this evaluation indicated that all the mutants were more sensitive to the pendimethalin than wild-type Nipponbare (Fig. 6). Characterization of the growth and development of these mutants is underway.

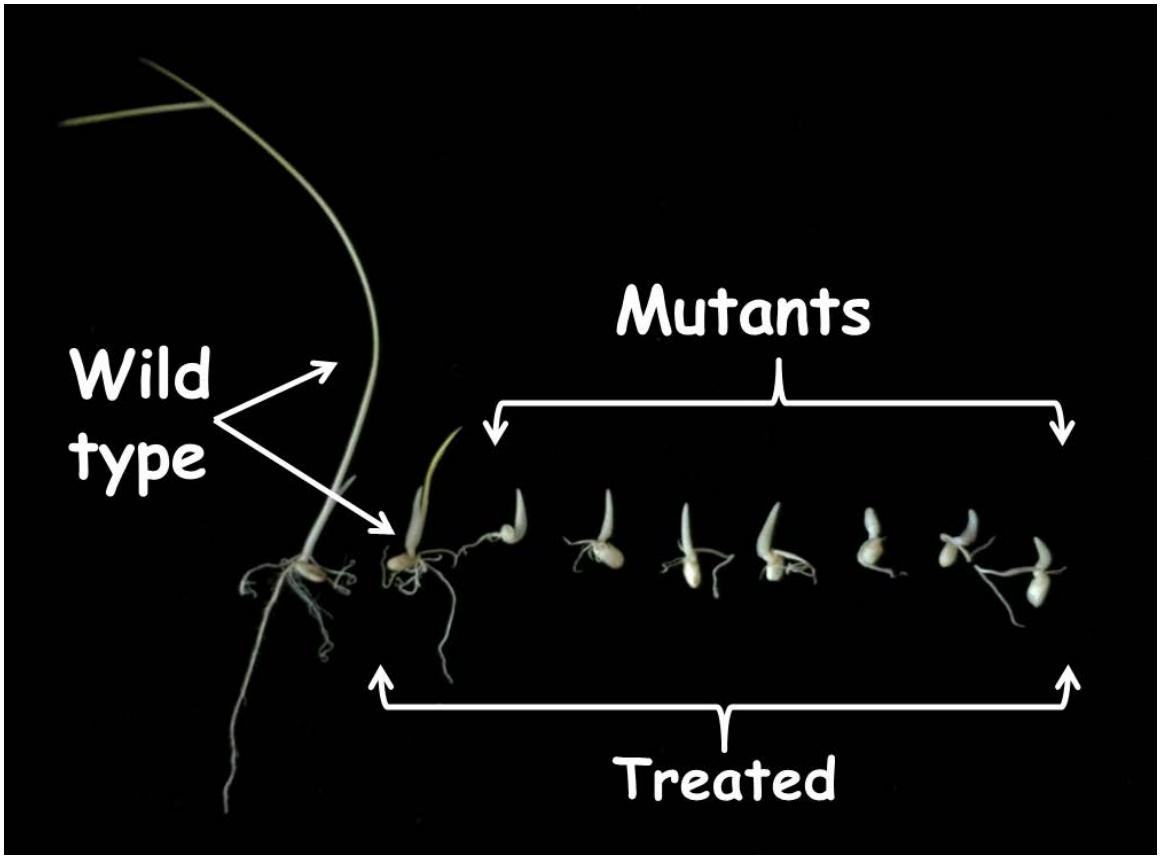

Fig. 6. Response of alpha-2 tubulin mutants identified by TILLING to pendimethalin. Mutants appear to be more sensitive than the wild-type Nipponbare.

3) **Identification and confirmation of additional rice mutants using the TILLING approach:** In 2017, TILLING of an additional 2,048 M2 mutants in the Nipponbare TILLLING mutant population was completed for seven gene targets [*Lsi1*, *OsABCC1*, 4-hydrophenyl pyruvate dioxygenase (HPPD), acetyl Co-A carboxylase (ACCase), glutamine synthetase (GS) – chromosome 2, protoporphyrinogen oxidase (PPO) and glutamine synthetase (GS) – chromosome 10]. The number of mutations detected in the 2017 screening is shown in Table 1. These mutations are in various stages of confirmation and identification of fixed mutant lines.

Table 1. Mutations detected by additional TILLING in 2017

Target gene	Locus Identifier	Additional mutations	Total mutations
ACCase	LOC_Os05g22940	1	3
HPPD	LOC_Os02g07160	2	3

PPO	LOC_Os01g18320	6	11
GS chromosome 2	LOC_Os02g50240	3	6
GS chromosome 10	LOC_Os10g31820	16	21
Lsi1	LOC_Os02g51110	3	6
OsABCC1	LOC_Os04g52900	3	4
Lsi2	LOC_Os03g01700	N/A	9
Alpha-2 tubulin	LOC_Os11g14220	N/A	7

N/A: not applicable

PUBLICATIONS OR REPORTS:

None to report at this time.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 2017, nine mutant lines carrying fixed mutations in genes involved in silicon and arsenic uptake/accumulation in rice were grown in the field to provide samples for analysis of silicon and arsenic content in 2018. Evaluation of these mutants using the germanium assay revealed that two of three mutations detected in the *Lsi1* gene appeared to confer high tolerance to germanium, which likely corresponds to reduced uptake of germanium, silicon, and arsenic. Nine lines carrying *Lsi2* mutations and one carrying an OsABCC1 mutation did not appear to qualitatively different in their response to germanium compared to wild type. Analysis of seven alpha-2 tubulin mutants indicates that all are more sensitive to pendimethalin (Prowl®) than wild type. Screening of another set of 2,048 mutants using the TILLING approach identified a number of new mutations in several of the target genes, which are being confirmed and developed for trait analysis in 2018. The TILLING screening work has concluded and future efforts will focus on mutant trait evaluation and targeted mutagenesis using a gene editing approach.