ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
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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 2018 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, UC Davis greenhouses and field nursery, and the Rice Experiment Station.

Research efforts continued to be disrupted by the 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. In addition, the only support staff and the UC Davis cooperator on this project unexpectedly departed for other opportunities. As a result, some specific objectives were not completed and some 2018 funding was rolled over to 2019 through a no-cost extension.

Specific 2018 objectives included:

1) **Complete evaluation of arsenic and silicon content of mutant lines grown in the field in 2017:** In 2017, 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. In 2018, samples from these lines will be processed and submitted to the UC Davis Analytical Lab for evaluation of silicon and arsenic content.
2) **Develop *lsi1*/*lsi2* double mutants:** 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 new *lsi1* and *Osabcc1* mutants:** In 2017, additional mutants in the *Lsi1* and OsABCC1 genes were identified by TILLING. These mutants will be characterized for silicon and arsenic content and/or germanium tolerance.
4) **Evaluate herbicide tolerance and development of rice mutants identified by TILLING:** Mutants identified by TILLING in 2016 and 2017, which harbor mutations in the genes encoding protoporphyrinogen oxidase, 4-hydrophenyl pyruvate dioxygenase, acetyl Co-A carboxylase, glutamine synthetase (chromosome 2), and glutamine synthetase (chromosome 10) will be evaluated for their response to selected herbicides. These mutants will also be grown for observation of developmental and agronomic traits.

SUMMARY OF 2018 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1) **Complete evaluation of arsenic and silicon content of mutant lines grown in the field in 2017:** Lines harboring 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) that were grown in the UC Davis greenhouse and field facility in 2017 were analyzed for their total As (straw and brown rice) and total Si (straw) content. The lines were initially started in the greenhouse and transplanted in the field for elemental analysis and large pots in the greenhouse for generation advance/seed increase. 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*: NM-3036WT, sibling line to NM-3036

which is an *lsi2* mutant) were also included in the field test. Results of the elemental analysis of the straw and brown rice for total silicon (straw only) and total arsenic (straw and brown rice) from the field-grown lines are shown in Table 1. Note: Mutant lines designations now include "NM" for Nipponbare Mutant. Previously mutant lines were referred to by line numbers with and "E" designation indicating the line was generated by ethyl methanesulfonate mutagenesis. Lines with 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 in 2017

Line	Mutant allele	Total Si (straw)[a] %	Total As (straw)[b] ppm	Total As (grain)[b] ppm
WT-2	wild type	5.34 ± 0.290	0.70 ± 0.071	0.09 ± 0.010
WT-7	wild type	5.81 ± 0.592*	0.66 ± 0.084	0.10 ± 0.006
NM-E1746	*lsi1*	0.20 ± 0.021**	1.47 ± 0.234**	0.10 ± 0.013
NM-3403	*lsi1*	3.26 ± 0.220**	3.37 ± 0.236**	0.16 ± 0.010**
NM-3380	*lsi1*	7.00 ± 0.256**	0.95 ± 0.079**	0.11 ± 0.013
NM-2902	*lsi2*	6.74 ± 0.121**	1.56 ± 0.179**	0.12 ± 0.000**
NM-5988	*lsi2*	5.60 ± 0.170	0.69 ± 0.075	0.10 ± 0.008
NM-E2308	*lsi2*	6.24 ± 0.116**	0.65 ± 0.111	0.09 ± 0.006
NM-3483	*lsi2*	5.76 ± 0.511*	0.87 ± 0.066*	0.11 ± 0.010
NM-3036WT	*Lsi2*	6.53 ± 0.187**	0.79 ± 0.051	0.10 ± 0.014
NM-3036	*lsi2*	6.58 ± 0.499**	0.77 ± 0.044	0.11 ± 0.006
NM-E2143	*lsi2*	5.68 ± 0.113	0.68 ± 0.053	0.09 ± 0.006
NM-5759	*lsi2*	6.62 ± 0.176**	0.74 ± 0.082	0.10 ± 0.010
NM-E2244	*lsi2*	6.88 ± 0.085**	0.85 ± 0.055	0.12 ± 0.006**
NM-E2249	*lsi2*	6.47 ± 0.230**	1.45 ± 0.091**	0.12 ± 0.005**
NM-4903	*Osabcc1*	7.27 ± 0.350**	0.51 ± 0.033*	0.08 ± 0.010**

[a] Total Si reported as a percentage of dry matter. Values are the mean of four replications (consisting of four plants each) and the standard deviation

[b] Total As reported as parts per million (ppm). Values are the mean of four replications (consisting of four plants each) and the standard deviation

*, ** means significant difference between the mean values at $P < 0.05$ and $P < 0.01$ by *t*-test between T-2 (wild type) and the other lines, respectively

Note: This table has been submitted as part of a manuscript for publication and is currently in review.

Although a significant difference was observed between the two wild type controls (WT-2 and WT-7), comparisons of each of the controls with the mutants revealed essentially the same results. The differences between the two control lines are likely to be the result of sample processing (e.g., contamination of plant samples with soil). Analysis of the *lsi1* mutants revealed that the two mutants (NME-1746 and NM-3403) which showed altered (more tolerant) response to germanium exposure were consistent with an altered (reduced) uptake of silicon whereas the third mutant, NM-3380, actually appeared have increased total silicon in its shoot tissues. The reductions in silicon in NME-1746 and NM-3403 were consistent with the germanium tolerance observed in these lines. NME-1746, which showed no symptoms of germanium-induced lesions, had significantly less silicon than NM-3403, which exhibited lesions albeit more slowly and less severe than wild type (Fig. 1). All three mutants had significantly higher total arsenic in their straw while NM-3403 also showed a significant increase in total arsenic in its brown rice relative to wild type. The increase in total arsenic in NME-1746 and NM-3403 seems inconsistent with the role of *Lsi1* in transporting all three (arsenic, germanium, silicon) elements into rice roots. If this effect can be confirmed it would suggest that the corresponding mutations alter *Lsi1* in a way that differentiates the uptake of silicon/germanium from that of arsenic.

In contrast to the *lsi1* mutants, the majority of the *lsi2* mutant lines exhibited increased total silicon in shoot tissues. This finding was consistent with the fact that differences in germanium tolerance in the *lsi2* mutants were not obvious. The exception was NM-3036 which showed a noticeably earlier appearance of (and possibly more severe) germanium-induced lesions. However, this increased sensitivity was also observed in its wild-type sibling line NM-3036WT (Fig. 1) indicating that it is not related to the *lsi2* mutation in NM-3036. While most of the *lsi2* mutant lines unexpectedly showed increased total silicon (mutations were expected to mostly result in reduced silicon), most of these lines showed no changes in total arsenic in the straw and brown rice samples. Notable exceptions are NM-2902, NME-2244, and NME-2249, which all showed significant increases in total arsenic in their brown rice samples.

Interestingly, the *Osabcc1* mutant NM-4903 was the only line to exhibit a significant reduction in total arsenic in its brown rice. Unlike *Lsi1* and *Lsi2*, which are involved in transport of arsenic from the soil into the root and from the root into the rest of the plant, respectively, *OsABCC1* encodes a transporter that is involved in sequestering arsenic in the vacuoles of phloem companion cells thus preventing accumulation in rice grains. Given this function, the identification of a mutation that would enhance this activity could provide used to produce reduced grain arsenic rice varieties.

To confirm the total arsenic and silicon content results, mutant lines (M5 generation derived from the lines grown in 2017) and wild-type controls were grown in two locations (UC Davis rice field facility and the Rice Experiment Station) using the same experimental design as in 2017 except only three replications were planted in the Rice

Experiment Station test. Plants (straw and grain) were hand-harvested and will be processed and submitted for analysis by the UC Davis Analytical Lab.

Re-evaluation of the response of the lines to germanium was performed in 2018. 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. Results were consistent with those reported in 2017. Two of the *lsi1* mutants (NME-1746 and NM-3403) exhibited tolerance with NME-1746 showing no germanium-induced lesions and NM-3403 having a delay in the onset of lesions and a reduction in their severity (Fig.1). As noted earlier, NM-3036 and its wild-type sibling line NM-3036WT exhibited earlier onset and more severe germanium-induced lesions than wild type Nipponbare.

Fig. 1. Response of rice seedlings to germanium after nine days of treatment. One week old rice seedlings were grown hydroponically in nutrient solution for one week and then transferred to 50 µM Ge. Lesions started to appear after 2-3 days of treatment. Lines (left to right): Nipponbare WT-2, NM-E1746, NM-3036WT, NM-3036, NM-3403, NM-4903, Nipponbare WT-7.

2) **Develop *lsi1*/*lsi2* double mutants:** In 2016, crosses were performed between some of the *lsi* (low silicon) mutants obtained from the TILLING screen. The resulting progeny were planted and verified by DNA sequencing in 2017. In 2019, 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. In 2016, crosses were performed between the *lsi1* mutant NME-1746 and the *lsi2* mutants NME-2308 and NM-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.

3) **Evaluate new *lsi1* and *Osabcc1* mutants:** Due to the unexpected departure of all staff (technician and UC Davis Associate Specialist) and administrative delays in re-hiring, this objective was not addressed and will be completed in 2019.

4) **Evaluate herbicide tolerance and development of rice mutants identified by TILLING:** As with specific objective 3, a lack of personnel delayed completion of this objective which will be addressed in 2019.

PUBLICATIONS OR REPORTS:

None to report at this time.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

In 2018, total arsenic and silicon content analysis of field-grown mutants carrying mutations in the silicon/arsenic/germanium transport genes *Lsi1* and *Lsi2* and the arsenic sequestration transporter gene *OsABCC1* were conducted. Results indicate that the transport/accumulation of silicon and germanium may be functionally separated from that of arsenic by induced mutagenesis of the *Lsi1* and *Lsi2* genes. Two of three *lsi1* mutations showed strong effects on reducing straw silicon content and sensitivity to germanium, which is consistent with the transport of both these elements from the external environment into the rice roots via the *Lsi1* transporter. All three *lsi1* mutants exhibited increased straw total arsenic but only one, NM-3403, may affect brown rice total arsenic (increasing content). In contrast to the *lsi1* mutants, most *lsi2* mutants showed increased total silicon but no effect on total arsenic in the straw and grain or germanium response. Notable exceptions are *lsi2* mutants NM-2902 (increased in total arsenic in straw and brown rice), NM-E2244 and NME-2249 (both increased in total arsenic in brown rice). The single *Osabbc1* mutant NM-4903 was the only mutant to appear to have reduced total arsenic in brown rice. Two caveats are that these results must be confirmed by testing materials grown in 2018 and the total arsenic analysis does not provide information on the various arsenic species, which differ in the health risk posed.