ANNUAL REPORT
COMPREHENSIVE RESEARCH ON RICE
January 1, 2023 – December 31, 2023

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

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COOPERATORS:

None

LEVEL OF 2023 FUNDING: $30,800

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

The overall goal of the RB-3 project is to identify and develop novel rice germplasm for incorporation into breeding programs serving the California rice industry. We are mainly interested in traits that improve the value of the rice crop or reduce production costs. To achieve this objective, chemical agents that cause changes in DNA have been used to generate populations of rice plants with randomly altered traits. These mutant populations are subsequently screened to identify individual lines that show promising new characteristics such as improved grain quality or resistance to herbicides useful for rice production. Identification of useful mutants is achieved either directly by looking for specific traits of interest or indirectly by looking for changes in the DNA (i.e., genes) known to be responsible for an important trait. For the RB-3 project, the approaches that are taken and the traits that are targeted are typically complementary to the Rice Experiment Station's efforts to avoid redundancy. Reduced uptake and localization of metalloids (e.g., arsenic, silicon) in rice plants and resistance to selected herbicides have been a focus of the project. Grain quality and climate resiliency traits have become more relevant in recent years.

In 2023, the main goals of the RB-3 project were to continue development and trait evaluations of mutant lines from three populations (Nipponbare, Kitaake, and Sabine) and to screen remnant mutant populations for herbicide resistance. Trait evaluations were to be performed under field conditions to identify altered agronomic traits (e.g., height, heading date, vigor, tiller number, panicle morphology, and yield) and by remote sensing using a multispectral camera mounted on a small, unmanned aircraft system or drone. Seeds from the mutant lines were to be collected to serve as a source for further evaluations (e.g., grain quality, stress tolerance) and for depositing in USDA-ARS rice gene banks for long-term storage and public distribution.

Specific objectives included:

1) **Mutant population development and phenotyping by remote sensing:** Development and phenotyping of three mutant populations derived from the varieties Nipponbare, Kitaake, and Sabine will be conducted. Five hundred lines from each population will be grown in the field and evaluated for agronomic performance traits (e.g., height, tillering, heading date, panicle morphology) using standard methods. Drone-based phenotyping using multispectral imaging will be conducted over the course of the season to provide additional data for evaluating traits and establishing methods to facilitate more efficient, accurate, and detailed phenotyping in future years.
2) **Screening remnant mutant populations for herbicide resistance:** Over the past 15-20 years, numerous mutant populations have been generated while establishing genetic resources for forward and reverse genetic screens. To extract value from these remnant populations before they are no longer viable, mutant lines will be screened with selected herbicide(s) to look for resistance that may be incorporated into breeding lines.

SUMMARY OF 2023 RESEARCH (MAJOR ACCOMPLISHMENTS) BY OBJECTIVES:

1) **Mutant population development and phenotyping by remote sensing:** In 2022, a new field site for the RB-3 Project was established at the UC Davis Plant Sciences Row Crop Facility (see 2022 RB-3 Annual Report). This year we continued developing this site and determining best practices for land preparation, irrigation, weed control, planting, and harvesting. Approximately 5 acres were planted including 4 acres used for dry beans in 2022 (north field) and two smaller fields totaling about 1 acre (southeast and southwest fields) used by the RB-3 project in 2022 (Fig. 1).

Fig. 1. RB-3 Project field site for 2023. A) North field: mutant population generation advance and seed increase, variety and germplasm seed production; B) Southeast field: Kitaake mutant trial and germplasm seed increase; C) Southwest field: clethodim screening of remnant mutant populations

Our goal in 2023 was to conduct population development (i.e., generation advance) and seed increase of three mutant populations. The mutant populations were planted in the north field which consisted of eighty-four 5 ft. wide x 450 ft. long beds irrigated primarily with a single line subsurface drip per bed (Table 1).

Table 1. Rice mutant populations grown in north field

Population	Description	Generation	No. Mutant Lines Planted	Planting method, date
Nipponbare	Reverse genetics (DNA screening) population	M3 (seeds)	536	Direct dry-seeded, May 3
Kitaake	Forward genetics (trait screening) population	M9 (seeds)	484	Paperpot seedling transplant, June 5, 6
Sabine	Reverse and forward genetics population	M4 and M5 (seeds)	1072 (66 M5, 1006 M4)	Direct dry-seeded, May 3

Nipponbare and Sabine mutant seeds were dry-seeded in 6 ft. rows with 3 ft. within and 5 ft. between row spacing using manual planters (3 ft. long) borrowed from the Rice Experiment Station. Approximately 80-120 seeds were sown per row and covered by raking. Beds were leveled (flat not raised) to decrease the distance from the drip to the rice roots. Direct seeding of the north field was conducted from April 26-30 followed by application of pendimethalin (Prowl®) and watering by sprinklers starting on May 3. Emergence of seedlings observed May 15 with weed control by manual weeding and application of propanil (Stam®) on May 31. For the Kitaake mutant population, seeds were sown in Paperpots (6 inch spacing, except for one tray of 4 inch spacing planted in error). For each line, 15 seeds (7 ft. row) were planted and separated by 5 spacer plants (mung beans, which were weeded out after transplants were established). Each entry row was separated by within row spacing of 3ft. and between row spacing of 5 ft. Kitaake mutant seedlings (4-5 leaf stage) were transplanted from Paperpots into freshly tilled soil in the north field on June 5 and 6. To facilitate statistical comparisons of the mutants, an augmented randomized complete block design (RCBD) was employed for each population due to the large number of mutant lines, which could not be replicated (Table 2). For each population, a small set of checks were replicated in each block of the design. Space in the north field not planted with the mutant populations was dry-seeded with varieties (Caloro, Colusa, Sabine, Yamadanishiki, Sabine, 29Lu1, M-103, S-102, Nipponbare, and Kitaake) and some Kitaake mutant-derived germplasm lines. Seeds were

sown with push planters in 3 to 5 x 430-450 ft. rows (1 to 1.5 ft. between rowing spacing) per bed with about 1 ft. spacing between beds.

In addition to the Kitaake mutants in the north field, a second smaller Kitaake mutant trial was planted in the southeast field. In this field, the augmented RCBD consisted of three blocks with each block having 68 entries consisting of 65 Kitaake M8 mutant lines and 3 checks (two wild type Kitaake and one Purple Marker). Seeds were sown in Paperpots with 4-inch spacing. For each entry, three 3 ft. rows of Paperpot seedlings (10 seedlings per row) were transplanted centered on a 5 ft. flat bed with 1.5 ft. spacing between the rows. Within the beds, the 3 x 3ft. plots for each entry were separated by 2 ft. alleys (achieved by seeding 5 spacer sunflower seeds, which were weeded out after transplants were established, between the 10 entry seeds per row) with about 5 ft. spacing between plots (between beds). Kitaake seedlings were transplanted in the southeast field on July 10 about 1 month after seeding. In addition to generation advance/seed increase and phenotypic evaluation, the purpose of this trial was to test the planting format (3 x 3 ft. plots) using the Paperpot transplanting system and to investigate a later planting date for Kitaake and Kitaake mutants given the early flowering/short duration of Kitaake.

Table 2. Augmented RCBD employed for mutant population experiments

Population	No. Blocks (replicates)	No. mutant lines / checks per block	Check lines
Nipponbare	4	134 / 10	Nipponbare, Caloro, Colusa, Kitaake, M-103, 29Lu1, Purple Marker, NM-5448, NE-334, 6044-M*
Kitaake	4	121 / 5	Kitaake, Purple Marker, S-102, M-103, KDS-1830C**
Sabine	8	134 / 10	Sabine, L-202, Kitaake, Caloro, Colusa, Purple Marker, SAB 6-1A, SAB 7-17A, SAB 11-39A, SAB-1558***

*NM-5448, NE-334, and 6044-M are Nipponbare grain mutant lines
**S-102 did not germinate, KDS-1830C is a Kitaake grain mutant line
***SAB lines are cuticle wax deficient mutant lines derived from Sabine

Remote sensing of the north field by drone-mounted multispectral camera (Micasense Red Edge-M) was initiated on June 14 and biweekly flights were conducted to about mid-tillering stage when weekly flights were performed until flowering of Sabine in early September (Fig. 2). The southeast field was included in flights are transplanting of the Kitaake mutants in July. A total of 28 flights were conducted and spectral data were collected for five wavelengths: blue (475 nm), green (560 nm), red (668 nm), red edge

(717 nm), NIR (840 nm) with bandwiths of 20, 20, 10, 10, and 40 nm, respectively. Imaging was performed at an altitude of 30-meters and 1,000 images (5 gigabytes of data) were captured for each 4-acre flight. Due to limited resources, trait data was only collected for height, row width (as a proxy for tillering/tiller angle/biomass/growth rate), and leaf chlorophyll (SPAD-502 meter readings) from the checks in each block of the Sabine and Kitaake mutant population experiments. In addition, visual mutant phenotypes observed in the field were recorded (e.g., plant stature, leaf coloration, tiller angle).

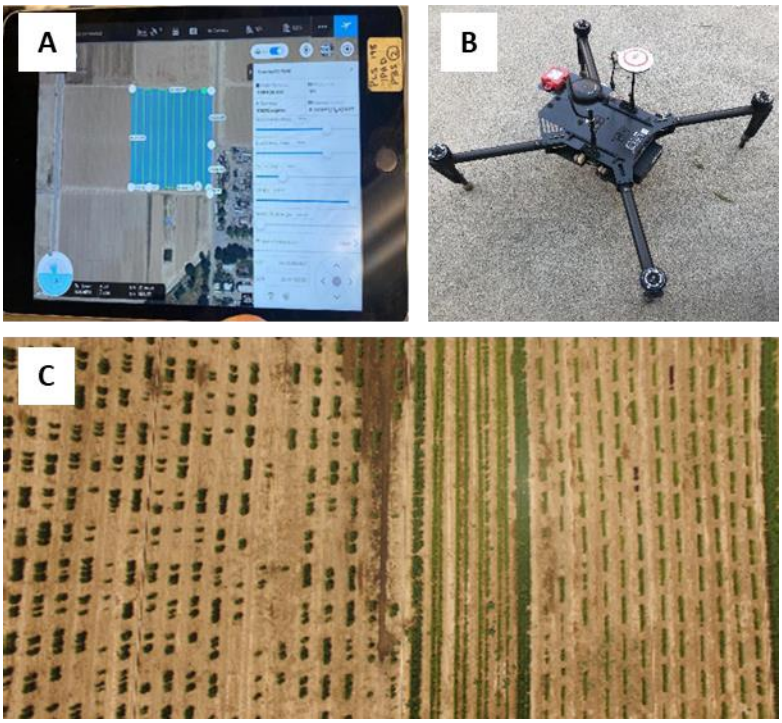

Fig. 2. Remote sensing of mutant populations grown in an augmented randomized complete block design. A) flight controller showing plotted flight path over 4-acre north field; B) drone with multispectral camera for remote sensing as well as video capabilities; C) Section of north field showing red/green/blue (RGB) image of Sabine (left) and Kitaake (far left) mutants in single row plantings. Kitaake mutants transplanted and Sabine mutants direct dry-seeded with some entries not germinating or showing sparse germination (i.e., few plants in those rows). Border and germplasm plantings separating mutant populations, just right of center of image.

No germination was observed for any of the 536 Nipponbare M2 mutant lines (M3 seeds), indicating that this population is no longer viable. Evaluation of additional lines from this population in the greenhouse produced similar results. The viability issue may be due to storage conditions and the age of the seeds (≥ 15 years). A subset of Nipponbare M3 seeds is currently housed at the Dale Bumpers National Rice Research Center and may be tested to determine if some of the Nipponbare mutant population (about 30-35%) may be recovered through these seeds. Since the Nipponbare population

failed to germinate, the checks in those blocks were not phenotyped for height, row width, or leaf chlorophyll.

For the Sabine mutant population, 1072 lines were planted including 66 M4 lines (M5 seeds) previously advanced based on visible seed/grain phenotypes and 1006 M3 lines (M4 seeds). Of the 66 M4 lines, 5 did not germinate/survive, 6 lines were sterile/late maturing under field conditions, 14 were very poor fertility (only a few panicles were harvested from these rows) and the entire row of 41 lines were harvested to provide seeds for further analysis (Fig. 3). Out of a total of 1006 M3 lines planted, 850 lines were harvested. As expected, several of these earlier generation lines exhibited segregating visual phenotypes (e.g., plant stature) and two samples were harvested from 38 lines and three samples were harvested from 1 line. A total of 156 lines were not harvested including 121 lines that did not produce any surviving plants (mostly did not germinate) and 35 lines that were sterile or too late maturing. Single panicles will be threshed and kept as the seed source for generation advance. Bulk seeds will be collected for possible use in trait evaluations (e.g., physico-chemical grain analyses) or screening (e.g., herbicide and other stress tolerance).

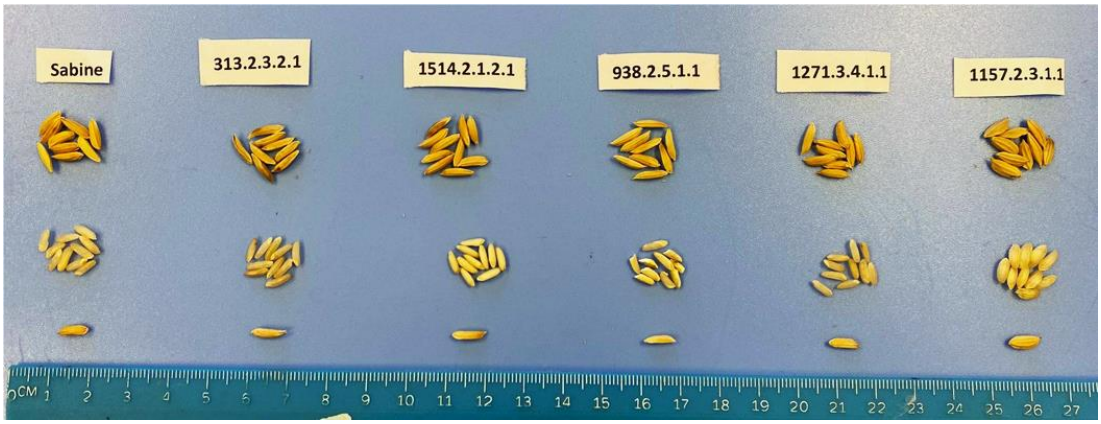

Fig. 3. Seeds from Sabine M5 plants grown in the north field. Sabine wild type grain on the far left. Grains of the mutant lines exhibit variation in shape and grain quality. For example, line 1514 appears to be a waxy/glutinous grain type, line 938 is chalky, and line 1157 exhibits a bold (larger) grain type compared to wild type.

Of the 484 Kitaake M8 mutant lines planted in the north field, 319 were harvested and are in the process of being threshed to produce seeds for trait evaluation and possible distribution to the USDA-ARS rice gene banks in Aberdeen, ID and Stuttgart, AR. Of the remaining lines, one line had no plants, and the others were not harvested due to low fertility/sterility under field conditions. None of the 195 Kitaake M8 mutant lines planted in the southeast field were harvested due to poor growth, suggesting that the late transplanting (both age of seedlings and date of transplanting into the field) and the field conditions during transplanting were negative factors. Adjustments will be made for future plantings.

Analysis of the trait data from the checks in the Sabine and Kitaake mutant populations is underway and will be compared with the remote sensing data obtain from the drone-mounted multispectral camera (Fig. 4). The image files from the drone flights will be processed over the winter to create the orthomosaic images needed to facilitate application of the multispectral data for applications such as normalized difference vegetative difference indices (NDVI). NDVI are a measure of greenness and vegetation density. These measurements will be compared to the trait data collected on the ground from the checks. Similarly, plant height measurements will be compared with digital elevation models derived from remote sensing data.

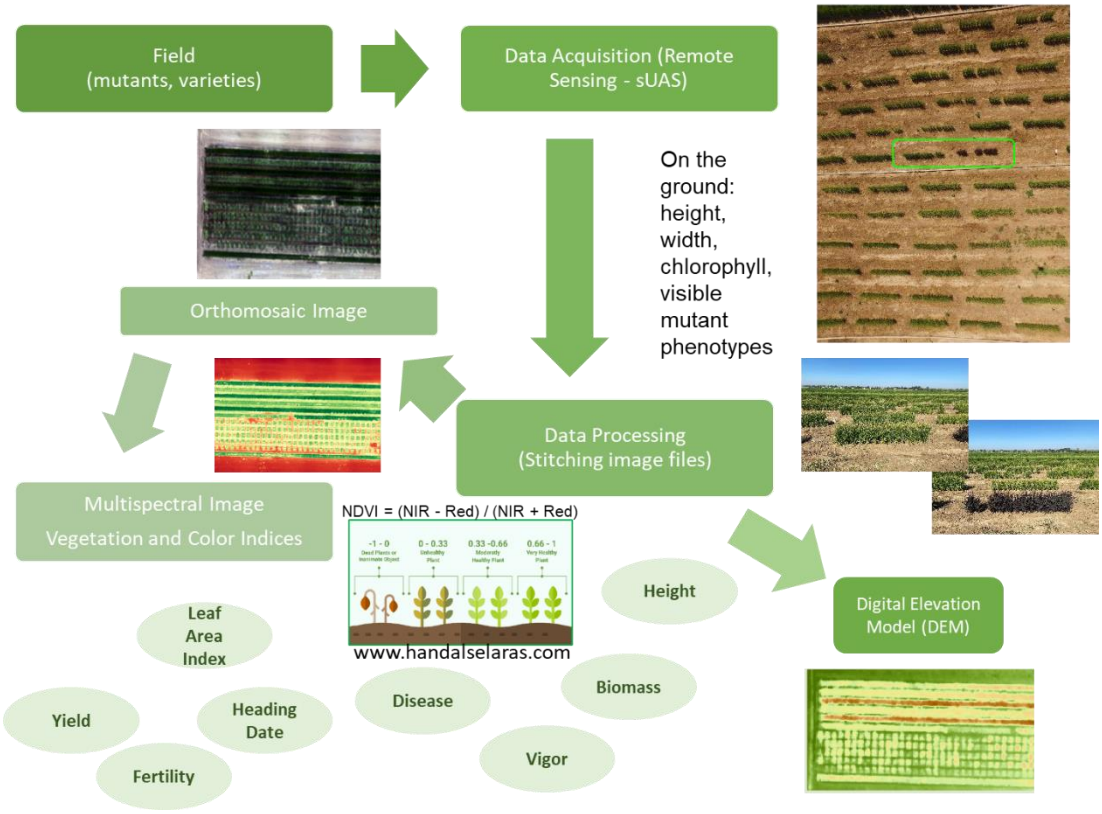

Fig. 4. Flow diagram of remote sensing-based phenotyping. Data acquisition phase completed. Data processing to generate orthomosaic images and digital elevation models for multispectral data applications and plant height measurements are being conducted over the winter.

2) **Screening remnant mutant populations for herbicide resistance:** In 2023, we continued clethodim herbicide screening of remnant mutant lines derived from several rice varieties (Table 3). In addition, the 52 survivors (putative tolerance mutants) from the 2022 clethodim screen (see 2022 RB-3 Annual Report) were re-tested this year. Seeds were dry-seeded using a push planter (bulk mutant seeds) or by hand sowing of threshed seeds (individual mutant lines or bulks of lines) or single panicles in 1 or 2 ft. rows in the southwest field, which consisted of 12 raised beds 5 ft. wide x 440 ft. long with about 1 ft. alleys between beds. Each bed was planted with 3-row tiers with spacing between

rows of about 1.5 ft. and 1-2 ft. alleys between tiers. Bulk mutant seeds were planted in 50 ft. "borders" on the north and south ends of the field with three 50 ft. rows in each bed. Weed management at pre- and post-emergence was similar to the north field (i.e., Prowl® and Stam®). At the 2-4 leaf stage (2-3 weeks after seeding), seedlings were sprayed with clethodim (1X label rate of Cleanse™ with 1% ammonium sulfate and 1% crop oil) followed 2 weeks later with a second application of Cleanse™ and ammonium sulfate at the same rate along with Loyant® and Grandstand® (16 oz. per acre with Surf90 at 1 qt./100 gal.) to control post-emergent broadleaves. Survivors were removed from the field about 1 month after the second clethodim application and transplanted in the greenhouse for seed production.

Table 3. Mutant populations screened for clethodim tolerance in 2023

Population	Variety	Mutagen	No. Lines	No. surviving plants / No. of entries (rows)*
Nipponbare TILLING	Nipponbare M2	Chemical	1590	26 / 13
S-102	S-102	Chemical	2002	23 / 18
SSA	S-102	Chemical	1304	59 / 53
SAM	S-102	Chemical	1128	30 / 16
S20	S-102	Gamma rays	1297	32 / 22
S25	S-102	Gamma rays	859	23 / 20
S30	S-102	Gamma rays	1438	25 / 8
M	M-204	TBD	559	13 / 7
M bulk	M-204	TBD	unknown	51 / 51
T3	Terso	TBD	850	58 / 30
T4	Terso	TBD	254	0 / 0
T3 Bulk	Terso	TBD	unknown	75 / 75
T4 Bulk	Terso	TBD	unknown	3 /3
B/M/T	Terso	TBD	999	16 / 7
K9	Kitaake M9	Chemical	218	0 / 0
2022 Clethodim	Sabine	Chemical and Gamma rays	52	43 / 19

*When the number of surviving plants is greater than the number of entries (rows) there is a possibility that some survivors are related (i.e., siblings) as they were found growing in the same row under the same entry number. TBD: to be determined; unknown: seeds were from a bulk source.

A total of 12,498 mutant lines (not including the 2022 clethodim survivors) and three bulk seed sources were planted in the southwest field and screened with clethodim (Cleanse™) applied twice at 1X label rate compared to twice at 0.75X label rate in the

2022 screen. From these lines and bulk seed sources, a total of 434 surviving plants were identified and transferred from the field to the greenhouse for seed production and testing of progeny in 2024. Several lines (row plantings) produced multiple (2 or more) survivors. Those may represent siblings as the chance that two or more different mutant lines with clethodim tolerance would be planted in the same row would be extremely unlikely. If confirmed, the presence of related survivors (siblings) would be consistent with genetic tolerance or resistance derived from mutations.

Of the 52 putative tolerance mutants and 3 control (Sabine wild type) lines transferred from the 2022 clethodim screen into the greenhouse last winter, 36 lines did not survive re-testing this year including all the control lines. None of the remaining 19 lines exhibited survival of all their seedlings, which suggests partial tolerance or a more complex genetic basis for tolerance than a single gene mutation. Our observation seems consistent with results of clethodim screening by Dr. R. Unan, an international collaborator of Dr. Kassim Al-Khatib. Results of the re-test of 2022 clethodim survivors (i.e., test of their progeny) for clethodim tolerance are shown in Table 4.

Table 4. Putative clethodim tolerant lines originally identified in 2022

Lines	Variety	Mutagen	No. surviving plants from 2023 test
8.B5.P43.R1.3	Sabine	1 mM sodium azide	4
8.B5.P41.R3.1	Sabine	1 mM sodium azide	2
8.B5.P40.R2.1	Sabine	1 mM sodium azide	1
8.B5.P36.R1.2	Sabine	1 mM sodium azide	2
8.B5.P36.R1.1	Sabine	1 mM sodium azide	1
8.B5.P21.R1.1	Sabine	1 mM sodium azide	2
8.B3.P99.R3.2	Sabine	150 Gy	1
8.B2.P93.R3.1	Sabine	170 Gy	5
8.B2.P93.R1.2	Sabine	170 Gy	1
8.B2.P93.R1.1	Sabine	170 Gy	5
8.B3.P95.R2.1	Sabine	170 Gy	3
8.B3.P26.R1.2	Sabine	170 Gy	1
8.B3.P26.R1.1	Sabine	170 Gy	2
8.B2.P93.R1.3	Sabine	170 Gy	1
8.B2.P87.R1.1	Sabine	190 Gy	1
8.B2.P80.R3.1	Sabine	190 Gy	4
8.B3.P50.R1.2	Sabine	190 Gy	4
8.B3.P51.R2.1	Sabine	190 Gy	1
8.B3.P57.R1.2	Sabine	190 Gy	2

Line 8.B5.P43.R1.3 (darker gray shading) was one of the first survivors spotted in the 2022 clethodim field. Lines in lighter gray shading are from plants identified in the same bed (B), plot (P), row (R), suggesting they may be sibling lines. 8 = bed number in 2023 southwest field (all 2022 re-tested lines planted in same bed). Gy = grays (unit of gamma irradiation).

PUBLICATIONS OR REPORTS:

None to report.

CONCISE GENERAL SUMMARY OF CURRENT YEAR'S RESULTS:

During 2023, we continued developing a field site for the RB-3 project. The site, located in the UC Davis Plant Sciences Row Crops Field Facility in Davis, is needed for mutant population development and evaluation as well as field-based genetic studies of specific mutants and mutant-derived germplasm. Mutants from three populations (Nipponbare, Sabine, and Kitaake) were planted this year. The Nipponbare mutants did not germinate, but the Sabine and Kitaake mutants were grown to maturity and image and multispectral data were collected over the course of the growing season using drone-based remote sensing. A small set of trait data was collected on the ground from replicated check lines for statistical comparison with the remote sensing data collected from the check lines and the non-replicated mutant lines. Data processing of the image files acquired by remote sensing and the downstream applications using those images and the spectral data will be performed during the winter. Screening for clethodim tolerant mutants continued this year. In addition to testing the progeny of 52 potential tolerant mutants identified in 2022, over 12,500 mutant lines were screened this year. A total of 19 out of the 52 lines from 2022 produced at least one surviving plant from this year's screen, which was performed using a higher rate of clethodim. For the new screen, 434 survivors were identified and transferred to the greenhouse to produce seeds that will be used for re-testing in 2024. Through additional trial and error from this year's experiments, "best" practices for land preparation, planting, irrigation, weed management, and harvesting have been developed and will be employed next season.