Rice Germplasm and Genetic Stocks *Oryza* Best Management Practices and Operating Procedures Handbook, Version 6

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Table of Contents

Section 1 ABOUT

1.1 Introduction

Rice is primarily a staple food crop for more than 50% of the world's population. The *Oryza* genus has about 24 identified species which include *sativa* and *glaberrima*, the two rice species that are cultivated today. The National Small Grains Collection (NSGC) in Aberdeen, Idaho, houses approximately 19,000 rice accessions. The collection includes 12 of the 24 *Oryza* species, with *O. sativa* and *O. glaberrima* accounting for 98.9% and 0.7% of the collection, respectively. The Genetic Stocks *Oryza* (GSOR), housed at Dale Bumpers National Rice Research Center in Stuttgart, Arkansas, has about 38, 000 accessions. This collection is

mostly made up of biparental populations, association mapping panels, mutant populations, and chromosome segment substitution lines. These two germplasm collections are a treasure chest of genetic diversity that can be scoured to identify genes associated with disease resistance, improved yields, and nutrition, and various grain quality traits. It has become extremely difficult to acquire new varieties or germplasm from other countries, thus it is essential that we preserve and protect the rice germplasm that we have!

1.2 Objectives

The USDA Rice Germplasm and Genetic Stocks *Oryza* Best Management Practices and Operating Procedures was developed with the following objectives:

- To rejuvenate and characterize rice (*Oryza*) accessions under field and greenhouse conditions effectively and efficiently.
- To expand and refine the phenotypic descriptors used to characterize rice (*Oryza*) accessions for better consistency and efficiency.
- To ensure that accessions are true-to-type and preserve identities.
- To reduce redundancy (duplicates) of rice (*Oryza*) genetics through phenotypic and genotypic characterization.
- To provide rice breeders and researchers with improved data to better identify accessions that can fit into their programs.

The methods and protocols in this document are based on field and greenhouse experience, observations, and trial and error. These experiences have helped us to improve rejuvenation and characterization of the rice germplasm.

1.3 Versioning

This is **version 6.0** of the *Rice Germplasm and Genetic Stocks Oryza Best Management Practices and Operating Procedures Handbook* published in November 2023.

1.4 Editors

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The editors would like to thank Mrs. Lorie Bernhardt (Genetic Stocks *Oryza* staff and computer assistant, USDA-ARS, Dale Bumpers National Rice Research Center; Protocol Development, Retired) for providing ideas, insight, and the time and effort she devoted to this handbook.

Please contact trevis.huggins@usda.gov with any questions, comments, remarks, or ideas.

Keywords

- rice
- *Oryza*
- germplasm
- phenotype
- genotype
- trait
- characterization
- evaluation
- NSGC
- GSOR
- Redundant by name
- USDA-ARS
- NPGS
- DB NRRC

Section 2 GERMPLASM RESTRICTIONS

2.1 Publicly Available Germplasm

Publicly available germplasm can be shared outside of USDA-ARS. The germplasm is publicly available if it is present in GRIN-Global, has an accession number and is not restricted by a patent or PVP status. GRIN-Global can be searched at the following website: https://npgsweb.ars-grin.gov/gringlobal/search.aspx. There are no restrictions for distributing this germplasm. However, *O.rufipogon* is a noxious weed within the U.S. and recipients must present a noxious weed permit from Animal and Plant Health Inspection Service (APHIS) to receive this species. If the requestor resides outside the U.S., export regulations must be followed.

2.2 Patent or Plant Variety Protection (PVP) Restriction

Some accessions in GRIN-Global are restricted for distribution by a patent or PVP status. This is usually material from universities or private rice entities. In most cases, the original donor is the distribution source, and the requestor is referred to them. There have been

instances where the original donor has given written permission for the USDA to distribute the restricted material. This written documentation is kept in perpetuity.

2.3 Standard Material Transfer Agreement (SMTA) Restriction

A standard material transfer agreement (SMTA) restricts and determines how materials included in the SMTA is distributed. The original SMTA will specify terms of third-party distribution. Usually, a secondary SMTA is drawn up, reviewed by Office of Technology Transfer (OTT), and executed through recipient's general counsel. Signed copies are filed with OTT and the recipient. Distribution is often reported to the original donor.

2.4 California Rice Certification Act Restriction

The California Rice Commission implemented a protocol to prevent unwanted pests and transgenes from impacting rice production fields in California. This restriction is known as the California Rice Certification Act. Requestors in California who wish to receive rice seeds must receive written permission to import seeds into California. The requestor will need to submit the written permission to the curators at NSGC or GSOR to receive the germplasm. The University of California system is exempt from this act.

2.5 Experimental Status Restriction

Germplasm under development by ARS for later release is considered experimental. If a requestor wishes to use the germplasm, an MTA is prepared and reviewed by OTT for the requestor. The MTA document will specify the terms of use between USDA-ARS and recipients.

Section 3 SEED PRODUCTION AND REJUVENATION

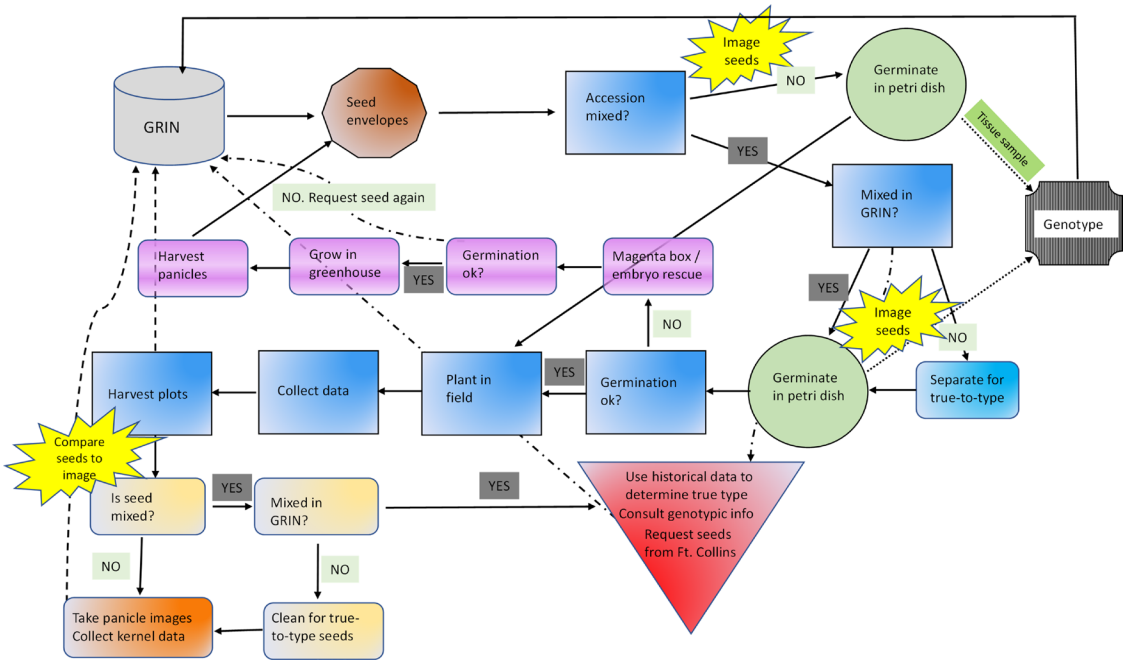


Figure 1: Flowchart illustrating the rejuvenation process of rice accessions.

3.1 NSGC and GSOR Low Inventory Seed Preparation for Field

3.1.1 Seed Audit

Accessions for rejuvenation are audited to ensure true-to-type seeds are planted. The seed from the envelopes are compared to data on ARS GRIN-Global (including images), data collected by the International Rice Research Institute (IRRI) and data collected by Ted Johnson (TJ) between 1984-1986 to verify identity.

1. Pour the entire contents of each individual envelope into a tray or on a flat hard surface, like a table.
2. Inspect the hull color and grain size of the seeds for differences.
3. Remove (dehull) the hull of 5-8 kernels to verify or identify the bran color.
4. Access the characterization and passport data in ARS GRIN-Global website (https://npgsweb.ars-grin.gov/gringlobal/search) for the current accession.
5. If a GPNO or IRGC number is listed, record it on the envelope.
6. Locate the information associated with the GPNO number in "Ted Johnson files" and the information associated with the IRGC number on the IRRI GRIN-Global website (https://gringlobal.irri.org/gringlobal/search).
7. Compare the grain type, hull color, bran color, apiculus color and awn type from each data source to the sample. Record the information on each individual envelope.
8. If only ARS GRIN-Global data is available for the seed sample and it matches the seed, it is earmarked for field rejuvenation. If the sample is mixed with the true-to-type and off-type kernels, remove the off-types, then earmark for field rejuvenation.

However, if the seed sample does not match ARS GRIN-Global, the accession is earmarked for Ft. Collins. (If there is uncertainty or confusion, discuss with supervisor)

9. If data is available from ARS GRIN, IRRI GRIN and Ted Johnson and the sample matches the information, it is earmarked for field rejuvenation. If the sample has mixed kernels, remove off-type based on the data and earmark for field rejuvenation. However, if there are discrepancies between the two or three sets of available data, the accession is earmarked for Ft. Collins. (If there is uncertainty or confusion, discuss with supervisor)
10. If an image or images for an accession on ARS GRIN does not match observation data or sample, take note of the incorrect image. (Note the image year and type of image)
11. If the kernels in a sample have red or purple bran it is earmarked for the red rice area for rejuvenation.
12. Pour the seeds onto a tray, place the seed envelope next to it and capture an image for each accession using a seed imaging system (Figure 2 and 3).
13. **ONLY *O. sativa* and *O. glaberrima* are allowed to be rejuvenated in the field. ALL other *Oryza* species will be rejuvenated in the greenhouse.**

Figure 2: Imaging system at DB NRRC.

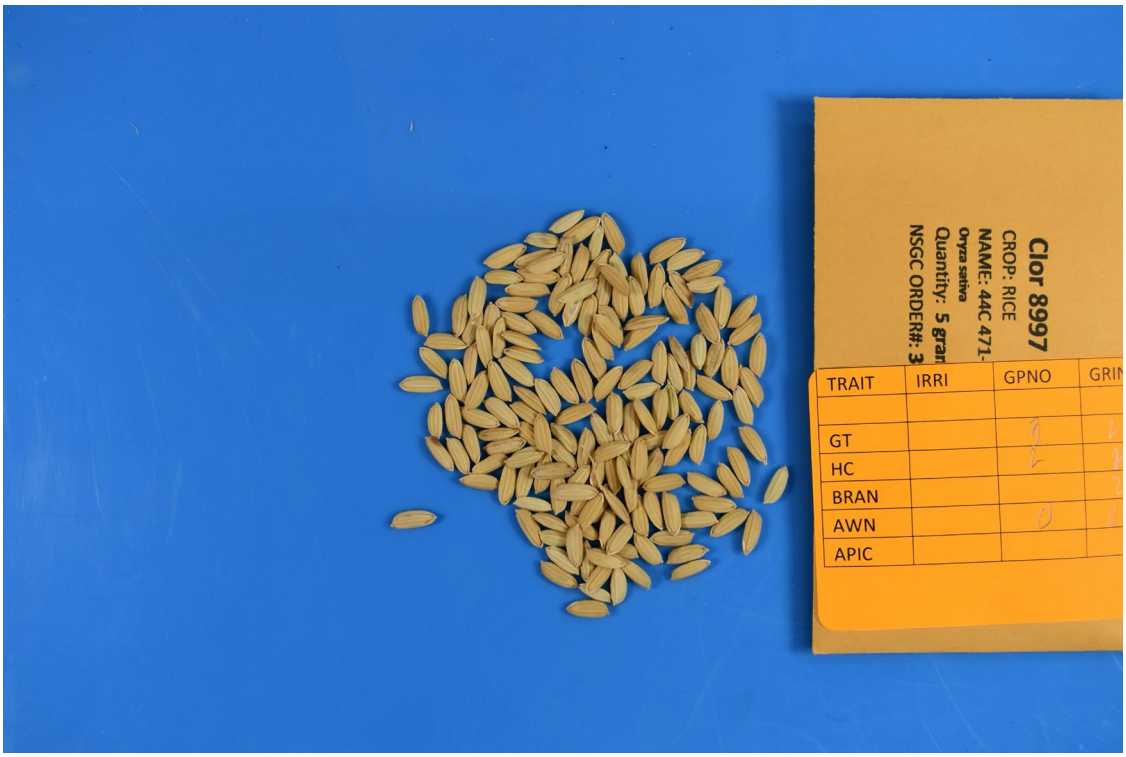


Figure 3: Image of seed and envelope as received from NSGC.

3.1.2 Seed Germination

1. Accessions earmarked for field rejuvenation are assessed for seed viability.
2. Print envelope labels having the accession information. The labels should include experiment name (LIR+YEAR, e.g., LIR2022), accession number, and accession name.
3. Collect new and unused petri dishes and filter paper.
4. Place filter paper in the deeper half of the petri dishes.
5. Place printed labels on the bottom of the petri dishes.
6. Place 10-15 kernels of the accession in the matching petri dish.
7. Place the petri dishes on benches in the greenhouse and moisten the filter paper in each petri dish.
8. Check petri dishes daily to ensure that they remain moist and free of fungi.
9. If fungus is observed in a petri dish, remove individual kernels using sterile tweezers/forceps and discard.
10. Wash tweezers/forceps in 10% bleach and distilled water after removing contaminated kernels from each petri dish.
11. Alternatively, fill germination trays with dry potting soil, add plot tags or labels and insert the seeds into the soil. Water the trays after sowing seeds and check soil moisture daily.
12. Score the petri dishes or the germination trays for seed germination. Accessions with good germination are earmarked for field rejuvenation and leaf samples are collected.

13. Accessions with poor germination are earmarked for greenhouse to acquire enough viable seeds for field planting.

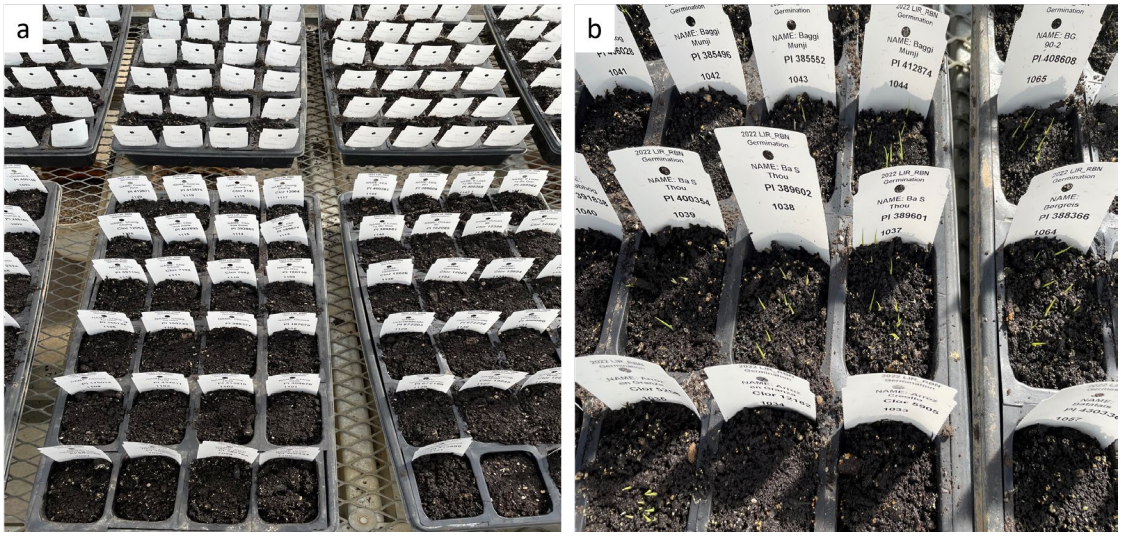


Figure 4: Germination trays with soil and label (a), and germinated seeds (b).

3.1.3 Collect Leaf Samples

Leaf tissue for DNA extraction is collected from NSGC accessions that had a good germination score. If an accession does not have a good germination score, no leaf tissue is collected. This procedure is done after germination data is collected from the petri dish or seed trays. Leaves can be collected from petri dishes, germination trays, or the field.

1. Prior to collecting leaf samples, prepare small clear zip lock bags that include an identification tag using sterile techniques (gloves and 70% alcohol).
2. Alternatively, collect coin envelopes and place printed labels with accession information on them.
3. The leaf sampling procedure can be done in the greenhouse, germplasm lab or field.
4. If collecting leaves in the greenhouse or lab, clean the area where leaf samples will be collected with 70% alcohol (gloves required).
5. Place large paper towels on the just cleaned surface and spray with 70% alcohol.
6. Collect a box of small lint-less wipes, a pair of small scissors and tweezers.
7. With gloves on, clean the scissors and tweezers with a single lint-less wipes sprayed with 70% alcohol.
8. Place the scissors and tweezers on the large paper towels.
9. While wearing gloves, collect one petri dish that has been recorded to have good germination and place on the large paper towels.
10. With tweezers and scissors, collect leaf samples from 5 or more seedlings (2 inches) and place them in the appropriate sample collection bags.
11. Close the sample bag and place it on ice to keep it cold.
12. Clean the scissors and tweezers with a small Kim wipe sprayed with 70 % alcohol, being careful to wipe each blade of the scissors.

13. The scissors and tweezers are cleaned after each sample has been collected.
14. Place the petri dish from the collected sample in the trash.
15. If collecting leaves from the field, collect an ice cooler and fill with ice.
16. Put on gloves before beginning to collect leaves.
17. Collect five young leaves from five different plants and place them in the prepared bag.
18. Place the bags with the leaf tissue in the cooler.
19. If using coin envelopes to collect leaf tissue, place the coin envelope with the leaf tissue in a Ziplock bag then place in cooler. **Do not place coin envelope directly on ice.**
20. After leaf tissue collection is complete, store the samples in a freezer at -20 ^{0}C.

3.1.4 Fill Seed Trays

1. Only accessions that pass the germination test are prepared for field planting.
2. Place the accession envelopes into seed boxes in ascending PI number.
3. The seeds are treated with an insecticide to ensure germination when planted.
4. Obtain the field map and seed packing list and lay them out on a bench or tabletop.
5. Arrange the seed trays according to the field map and field bays.
6. Place tape on the bench next to each set of trays. Label these with the bay number, tray number and pass number (e.g., Bay1 - Tray1 - Pass2)
7. Place the purple marker and check seeds into the seed trays according to the field map. Use a funnel to pour the seeds into each cell. **NOTE: Accessions are loaded in cells by ascending days to heading with accessions that have shorter days to heading planted at the front of the bay.**
8. Place the treated seeds of each accession into the seed trays according to the field map and packing list. **NOTE: It is especially important to pay close attention to the plot number and the accession number associated with it. Always double check before placing seeds in cell.**
9. After placing the seeds of an accession in a cell, check off the accession on the packing list and return the seed envelope to the seed envelope box.
10. When the complete set of seed trays are filled, place the tray cover on each set of trays for a field pass. The tray covers are labelled with the bay, tray, and pass numbers.
11. Tape the two corners of the cover on one side.
12. Stack the completed trays in a seed tray box and place them on the bench.
13. After all seed trays are completed deliver them to the field planting staff.

3.2 NSGC and GSOR Low Inventory Seed Preparation for Greenhouse

3.2.1 Seed Audit

Accessions for rejuvenation will be audited to ensure true-to-type seeds are planted. Seeds from the envelopes are compared to data on ARS GRIN-Global (including images), data collected by the International Rice Research Institute (IRRI) and data collected by Ted

Johnson (TJ) between 1984-1986. This procedure follows the steps above in **3.1.1**. Accessions with poor germination are planted in the greenhouse to generate enough viable seeds for field planting the next season. **ALL *Oryza* species other than *sativa* and *glaberrima* must be rejuvenated only in the greenhouse.**

3.2.2 Planting

In the greenhouse a 50/50 mix of field soil and potting soil is used to grow accessions. The field soil is steam sterilized to eliminate bacteria, fungi, and insects.

1. Collect field soil using buckets or other transport medium and place in the greenhouse in large tubs.
2. Collect bags of potting soil and transport them to the greenhouse.
3. Add a five-gallon bucket full of sterilized field soil to the tub.
4. Add one bag of potting soil on top of the field soil. **NOTE: One bag of potting soil is approximately equivalent to two five-gallon buckets of field soil.**
5. Add another five-gallon bucket full of sterilized field soil to the tub.
6. Use shovels or hand spades to mix the two soils into a homogenous mixture. **NOTE: Other methods can be used to mix the two soils.**
7. After the soil is homogenized, fill prepared pots to about one inch from the top.
8. Place filled pots in black rectangular tubes or other larger containers.
9. Place labels in filled pots.
10. Place ten seeds of each accession in the appropriate pot.
11. **NOTE: It is essential that the PI or CIor number on the seed envelope matches the PI or CIor number on the pot tag.**
12. Fill the tubes with water after planting and allow the soil to soak up the water.
13. Record the number of germinated seedlings after two weeks.
14. Thin the number of germinated seedlings to three per pot.
15. If a pot does not have any germinated seedlings, germinate the seeds using seed media **(see appendix 8.4)**. Transplant two-week-old seedlings to the appropriate pots in the greenhouse.
16. Record the number of seedlings that were planted and the number that survived two weeks after transplanting.
17. If any seedlings do not survive, germinate in seed media again.

3.2.3 Collect Leaf Samples

Leaf tissue for DNA extraction is collected from NSGC accessions that have germinated in pots.

1. Prior to collecting leaf samples, prepare small clear zip lock bags that include an identification tag using sterile techniques (gloves and 70% alcohol)
2. Use a lab cart as a work area to collect leaf tissue. Clean the cart with 70% alcohol (gloves required).
3. Place large paper towels on the just cleaned surface and spray with 70% alcohol.

4. Collect a box of small lint-less wipes, a pair of small scissors and tweezers.
5. While wearing gloves, Clean the scissors and tweezers with a single lint-less wipes sprayed with 70% alcohol.
6. Collect leaves from about 6-week-old plants using scissors to cut young tissue.
7. Clean the scissors after each accession.
8. Place the bags or coin envelopes in a Ziplock bags and keep on ice until they can be placed in a freezer at -20 ^{0}C.

3.3 Redundant By Name (RBN) Seed Preparation for Field

3.3.1 Seed Audit

Accessions in the collection that have the same name, but different accessions numbers are considered redundant by name (RBN). For example, three accessions with the name Blue Rose, each with a different accessions number. The goal is to determine if the RBN accessions are the same phenotypically and genotypically. If they are the same phenotypically and genotypically, one accession remains active for distribution and the others are archived. If they differ, then they all remain active for distribution. Accessions for rejuvenation are audited to ensure true-to-type seeds are planted. The seed from the envelopes are compared to data on ARS GRIN-Global (including images), data collected by the International Rice Research Institute (IRRI) and data collected by Ted Johnson (TJ) between 1984-1986 to verify identity. This procedure follows the steps above in **`3.1.1`**. Accessions with poor germination are planted in the greenhouse to generate enough viable seeds for field planting the next season. **ALL *Oryza* species other than *sativa* and *glaberrima* must be rejuvenated only in the greenhouse**. If one of the RBN accessions has poor germination or true-to-type is unverifiable, then the set of accessions is not planted in the field that season. The accessions will be held for the next field season to acquire seeds for all the accessions with that name. If one of the accessions in the group has red or purple bran, all the accessions are planted in the red rice growing area.

3.3.2 Seed Germination

Seed germination follows the same procedure outlined in **`3.1.2`** above. The labels for the RBN should include experiment name (RBN+YEAR, e.g., RBN2022), accession number, and accession name. Accessions with poor germination are earmarked for greenhouse to acquire enough viable seeds for field planting. The complete set of RBN accessions must have good germination to collect leaf tissue and be planted in the field.

3.3.3 Collect Leaf Samples

Leaf tissue for DNA extraction is collected from RBN accessions that had a good germination score. If an accession does not have a good germination score, no leaf tissue is collected. This procedure is done after germination data is collected from the petri dish or seed trays. The leaves can be collected from petri dishes or germination trays or field. This follows the same procedure outlined in **`3.1.3`** above.

3.3.4 Fill Seed Trays

1. ONLY non-colored bran accessions are placed in seed trays for direct planting. Red and purple bran accessions are germinated in the greenhouse and then transplanted to the red rice growing area.
2. Accessions with good germination are prepared for field planting.
3. Place the accession envelopes into seed boxes in alphabetical order.
4. The seeds are treated with an insecticide to ensure germination when planted.
5. Obtain the field map and seed packing list and lay them out on the bench.
6. Arrange the seed trays according to the field map and field bays.
7. Place tape on the bench next to each set of trays. Label these with the bay number, tray number and pass number (e.g., Bay1 - Tray1 - Pass2)
8. Place the purple marker and check seeds into the seed trays according to the field map. Use a paper funnel to pour the seeds into each cell. **NOTE: Accessions are loaded in cells by name in alphabetical order and are planted to the front of the bay. The accessions with the same name are planted next to each other**.
9. Place the treated accessions into the seed trays according to alphabetical order, the field map and packing list. **NOTE: It is especially important to pay close attention to the plot number and the accession number associated with it. Always double check before placing seeds in cell**.
10. After placing the seeds of an accession in a cell, check off the accession on the packing list and return the seed envelope to the seed envelope box.
11. When the complete set of seed trays have been filled, place the tray cover on each set of trays for each field pass. The tray covers are labelled with the bay, tray, and pass numbers.
12. Tape the two corners of the cover on one side.
13. Stack the completed trays in a seed tray box and place them on the bench.

3.4 Redundant By Name (RBN) Seed Preparation for Greenhouse

3.4.1 Seed Audit

Accessions for rejuvenation will be audited to ensure true-to-type seeds are planted. The seed from the envelopes is compared to data on GRIN-Global (including images), data collected by the International Rice Research Institute (IRRI) and data collected by Ted Johnson (TJ) between 1984-1986. This procedure follows the steps above in **4.1.1**. Accessions with poor germination are planted in the greenhouse to generate enough viable seeds for field planting the next season. **ALL *Oryza* species other than *sativa* and *glaberrima* must be rejuvenated only in the greenhouse**.

- If one of the RBN accessions has germination issues or true-to-type is unverifiable, then the set of accessions are not planted in the field that season.
- The accessions will be grown in the greenhouse to acquire viable seeds for all the accessions with that name.

3.4.2 Planting

In the greenhouse a 50/50 mix of field soil and potting soil is used to grow accessions. The field soil is steam sterilized to eliminate bacteria, fungi, and insects. This follows the same procedure outlined in **3.2.2** above.

- Accessions with the same name will be planted in pots next to each other.
- **NOTE: It is essential that the PI or CIor number on the seed envelope matches the PI or CIor number on the pot tag. It is also essential that the accession name matches**.

Section 4 PLANT CARE

4.1 Plants in Field

To ensure maximum seed production plants require proper care to reduce effects of disease and insects. Disease and insects should be promptly identified and reported to supervisor so that an appropriate treatment can be administered. **ALWAYS be on the lookout for snakes in the water around plots!**

- Observe plots for off types as plants begin to boot. If off types are identified, remove by cutting at the root and discarding in the trash receptacle.
- If a plot is observed to have a mixture, document the plot number and the type of mixture (e.g., mixed awns, mixed apiculus color). Verify the correct phenotype of the mixture by consulting ARS-GRIN, IRRI GRIN or Ted Johnson field books. When the correct type is identified, cut the plants at the roots to remove the incorrect plants from the plot.
- Plants are observed at least every other day for disease, insects, and any pertinent observations.
- Look for bird damage as plots begin to mature. If bird damage is observed in a plot, cover the plot with poly bags to prevent further seed loss.
- Observe plants for lodging as they progress to maturity. If lodging is observed in a plot, use bamboo stakes and string to secure the plot to prevent it from falling into the water.

4.2 Plants in Greenhouse

To ensure maximum seed production plants require proper care to reduce effects of disease and insects. Disease and insects should be promptly identified and reported to supervisor so that an appropriate treatment can be administered. Proper nutrition is also essential for healthy and productive plants.

- Two-week-old seedlings are fertilized with a solution of an all-purpose 20:20:20 and iron. This is done for transplanted and direct seeded plants. The method for making the solution can be found in **appendix 8.7**.
- Add 50 cm of fertilizer solution to each pot.

- Add about one-half teaspoon of a slow release fertilizer (17:5:11) to each pot two weeks after the fertilization solution.
- Another 50 cm of the fertilizer solution is added to each pot at the booting stage.
- Monitor plants for insects and disease throughout growth.
- Monitor plants for yellow leaves.

Section 5 DATA COLLECTION

5.1 Field

5.1.1 NSGC and GSOR Low Inventory Rejuvenation

The breeding team prepares the field for planting and plants the accessions in the field. The breeding team also manages the irrigation, pesticide, and herbicide application. We are responsible for weed management in our fields.

1. One week after the field is permanently flooded, plot stakes and tags are added to the plots according to the field map.
2. Three to four weeks after the permanent flood, record plot stand data and any pertinent (vegetative) observations accordingly.
3. Visit the field at least twice per week and observe the plots for heading and record any anomalies. **NOTE: A plot is considered to have headed when 50% of the plot has panicles**.
4. At the first sight of heading plants, walk the field daily and record heading and flowering data. When a plot has headed, mark it with red paint.
5. At flowering record awn type, awn color, leaf pubescence, presence of long outer glumes, apiculus color, leaf blade color, and any pertinent (flowering) observations. When a plot has flowered mark it with white paint. **NOTE: A plot has flowered when 50% of the panicles have flowers extruding from the florets**.
6. Collect images of two panicles per accession at flowering according to panicle image acquisition in **`appendix 8.1`**.

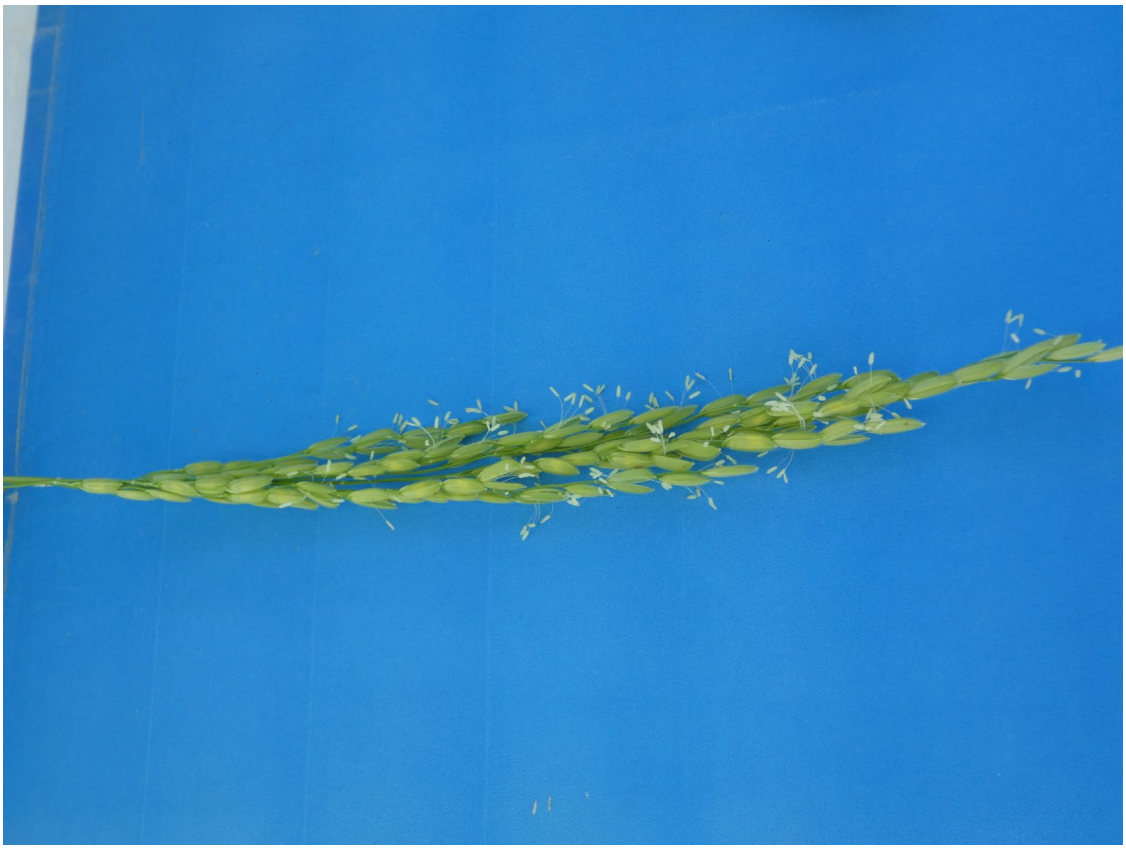


Figure 5: An image of a rice panicle at flowering.

7. At maturity, when 90% of the panicles in plot are no longer green, record maturity date, record plant type, panicle type, lodging, plant height, ratooning and any pertinent (maturity) observations. Check awn type again at this stage.
8. Harvest plants when panicles are sufficiently dry and record the date.
9. Place the harvested plots upside down on drying racks in the seed processing building.
10. Collect 3 representative panicles from each plot and place in labelled panicles envelopes and record shattering, aroma and any other pertinent data if observed.
11. After collecting 3 panicles, thresh plots and collect seed in labelled seed envelopes. **NOTE: Allow the plots to dry for about 1 week before threshing. DO NOT wait until all plots have been harvested to thresh, thresh throughout the season as enough plots accumulate**.
12. Place the collected panicles and threshed seeds in the dryer at 32 ^{0}C for 24 hours.
13. Remove the panicles and threshed seeds from the dryer and place in a freezer at -20 ^{0}C for 72 hours.
14. Remove the panicles and threshed seeds from the freezer and place in the cold room.
15. Audit the panicles and seed samples as time allows or at the end of the season by comparing them to the images of the original seed sample. If off types are identified during the audit process, remove all off types, and make a note.

16. After the audit is completed, clean the seed, and record the weight on the seed envelope. Samples with weight greater than 75 grams are packaged in boxes for shipment to NSGC. Samples weighing less than 75 grams are placed in a plastic tube to decide if they need to be regrown next field season. **DO NOT keep more than 300 grams of seed of an accession in seed envelopes**.
17. After the seed has been cleaned and packaged, image two of the panicles collected in step 10, some hulled and dehulled kernels for each accession using the imaging system (Figure 2.).

Figure 6: Images of mature panicles, dehulled and hulled grain.

18. After images of panicles are collected, hand thresh panicles and dehull approximately 100 whole kernels.
19. Use the kernels to obtain kernel length, kernel width, and kernel length/width ratio using winseedle or the seed extractor software. Add the data to the field book.

5.1.2 Redundant By Name

The RBN accessions are planted in rows next to each other for observation and comparison. Refer to **`section 5.1.1`** for information on collecting data, observations, and tasks for successful evaluation in the field.

- Record whether the RBN accessions are the same or differ phenotypically at maturity in the field book. Use the data collected at flowering and maturity.
- If an RBN accession needs seed for distribution, harvest plants when panicles are sufficiently dry and record the date.
- Collect images of panicles for each RBN set according to **`appendix 8.1`**.

Figure 7. Images of mature panicles of two sets of accessions with the same name (RBN).

5.1.3 Genetic Stocks *Oryza* Plant Images

1. Collect two images of each accession at maturity using a camera and a white height board. **Note: This is done before plants are harvested**.
2. On a sunny day, transport the white height board to the field in a truck.
3. Attach the GSOR number and accession name to the white height board.
4. Select a representative area of the plot and place the board behind the plot.
5. Capture images of the plot for the accession.
6. Remove the card with the accession information from the board.
7. Select another plot and repeat steps 3-6 above until images for all accessions have been captured.
8. Return all equipment to their original place.

Figure 8. Image of plant with mature panicles in the field.

5.2 Greenhouse

5.2.1 NSGC and GSOR Low Inventory Rejuvenation

1. Observe plants in the greenhouse daily and check for heading/flowering and record any anomalies. Place a pink flag in the pot after recording heading date. **NOTE: An accession is considered headed when two panicles emerge in most cases**.
2. Monitor the panicles daily for anther extrusion (flowering) and record the date. Place a yellow flag in the pot. **NOTE: The pink indicates that the accession has a panicle or panicles that has emerged from the leaf sheath (heading). The yellow flag indicates that the accession has panicles with extruded anthers (flowers)**.
3. At flowering record awn type, awn color, leaf pubescence, presence of long outer glumes, apiculus color and any pertinent observations.
4. Capture an image of one flowering per accession at flowering using the imaging system (Figure 2). The panicle should have anthers extruding and mostly bright yellow in color.
5. Pay close attention to any sign of lodging as panicles mature. If lodging is observed, stake plants in pot using bamboo stakes and zip ties.
6. At maturity, when 90% of the panicles in the pot are no longer green, record plant type, panicle type, lodging, plant height, ratooning, and other pertinent observations. Check awn type again at this stage.

7. Harvest panicles and record shattering, aroma, and harvest date on panicle envelope, then place them in the envelope.
8. Place the panicle envelopes in a dryer for 24 hours.
9. Remove the panicle envelopes from the dryer and place in a freezer at -20 ^{0}C for 72 hours. Place the panicles in the cold room after removing them from freezer.
10. Audit the panicles and seed samples as time allows or at the end of the season by comparing them to the images of the original seed sample. If off types are identified during the audit process, remove all off types, and make a note.
11. After the audit is completed, select two panicles for images then thresh the remaining panicles by hand or using a panicle thresher.
12. Record the weight on the cleaned seed on envelope. Samples with weight greater than 75 grams are packaged in boxes for shipment to NSGC. Samples weighing less than 75 grams are placed in a plastic tube to decide if they need to be regrown next field season.
13. Collect the two panicles from step 11, some hulled and dehulled kernels for each accession. Image them using the imaging system (Figure 2.) according to appendix 8.1.
14. Store the seed envelopes in transparent plastic box and place in the cold room. Label the boxes with the name of the experiment, the type of material, and the date placed in the cold room.
15. Hand thresh the two panicles and dehull approximately 100 whole kernels.
16. Use the kernels to obtain kernel length, kernel width, and kernel length/width ratio using winseedle or the seed extractor software. Add the data to the field book.

5.2.2 Redundant By Name (RBN)

The RBN accessions are processed following the processes outlined in **`5.2.1`** above from **`step 1-10`**. The accession is usually grown to acquire fresh seed which will then be planted the next field season.

1. After the audit is completed, select two panicles, then thresh the remaining panicles by hand or using a panicle thresher.
2. Record the weight of the cleaned seed on envelope. The seeds will be used for field planting next season.
3. Store the seed envelopes in transparent plastic box and place in the cold room. Label the boxes with the name of the experiment, the type of material, and the date placed in the cold room.

5.2.3 Genetic Stocks *Oryza* Plant Images

1. Collect two images of each accession at maturity using a camera and a white height board. **Note: This is done before plants are harvested**.
2. On a sunny day, set pots of accessions with mature grain on the ground to allow excess water to drain.
3. Set up the white height board in an area that is shaded and set up a camera on a tripod about 4 feet away from the white height board.

4. Attach the GSOR number and accession name to the white height board.
5. Place the pot on the ground right in front of the white height board.
6. Capture two images of the accession in the pot.
7. Remove the card with the accession information from the board.
8. Select another pot and repeat steps 4-6 above until images for all accessions have been captured.
9. Return all equipment to their original place.

Figure 9. Image of plant with mature panicles in the greenhouse.

Section 6 GREENHOUSE MAINTENANCE

It is essential that the greenhouse be kept clean to discourage pests, algae, and fungi. The greenhouse should remain clean and as organized as possible.

6.1 Plant Material

1. Plant material that has been removed from pots is placed in the trash bin.
2. After panicles have been harvested, the plant material is removed from pots.
3. Use a sickle to cut the plants in the pots at the base and place them in the trash in the greenhouse.
4. Sweep all plant material on floor and discard in the trash bin.

6.2 Pots and Bins

1. The used soil is discarded in the assigned area and not in the trash bin.
2. Remove plant debris from the greenhouse floor and place it in trash. Remove trash from greenhouse and take to the dumpster. Place a new trash bag in the trash bin and replace the cover.
3. After the pots are emptied, use the hose to wash each pot separately. Allow pots to dry and then store in the greenhouse.
4. Dispose of the water in black tubs that will no longer be used.
5. Remove black tubs from greenhouse and wash with soap and bleach. This is done to prevent algae and bacteria growth.
6. Allow tubs to dry upside down on empty benches in greenhouse until needed.

Section 7 RICE DISEASE

7.1 Blast

Blast disease is caused by the airborne fungus *Magnaporthe oryzae* and is the most damaging disease in rice. The fungus can infect rice and complete 2-4 life cycles throughout the entire crop season. The three leaf stage is best to begin looking for the disease. The symptoms can be seen on the leaf, stem, kernel, nodes, collars, panicle, and seed of rice. The most common symptom is diamond shaped lesions on leaves but is rare on the sheath. Rice leaves: The symptoms on leaves may vary by age, and the levels of host resistance (Figure 10a). Lesions may appear gray-green and water-soaked with a dark-green border and can expand rapidly to several centimeters in length. Older lesions often become light tan in color with necrotic borders. Symptoms of infection of the junction of the leaf and the stem sheath (collar) consist of a general area of necrosis at the union of the two tissues (Figure 10b). Panicles (Necks): The stem supports the seed head or panicle. Symptoms can be seen at the node and infection leads to neck blast (Figure 10c). Lesions look gray, and brown discolorations can also be found on the panicle branches, and spikelets.

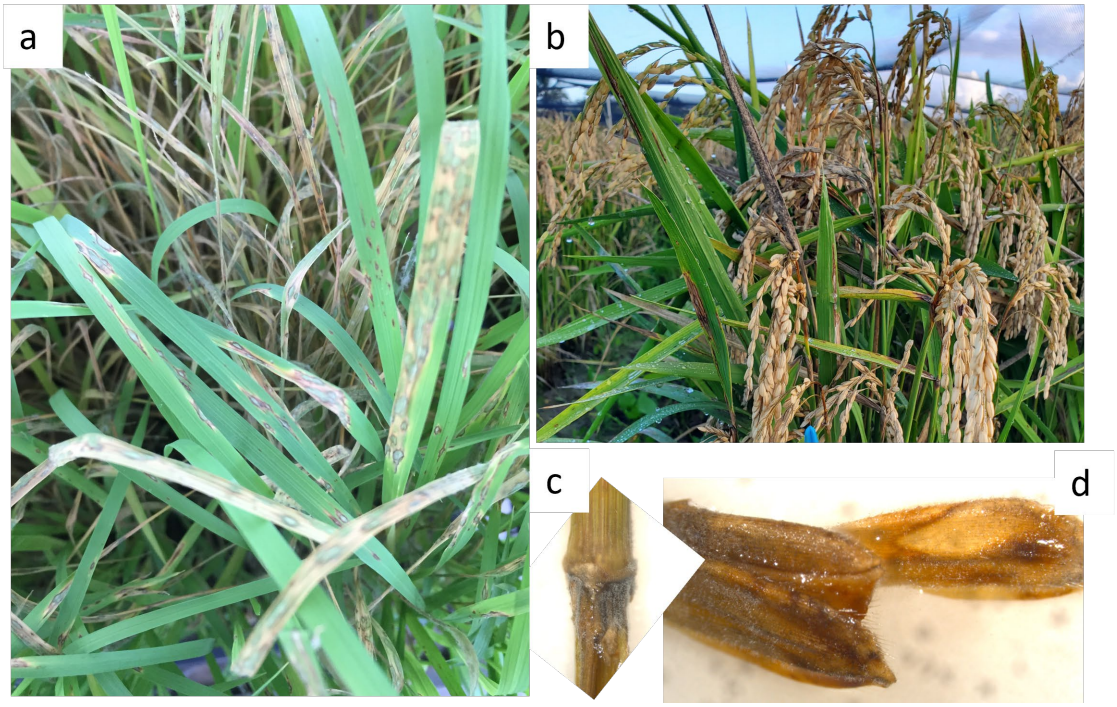


Figure 10. Rice blast disease: leaf blast (a), panicle (neck) blast (b), internode blast (c), and blast on seeds (d).

7.2 Sheath Blight

Sheath blight is caused by the soil borne fungus, *Rhizoctonia solani* and is the most damaging disease in the southern USA. The fungus infects rice in one life cycle. Begin to look for the disease after the internodes elongate to about a ½ inch. Disease symptoms are expressed as circular or oblong lesions that look green-gray, and water soaked on leaves. It can also be found on the stem and upper part of plants in highly susceptible rice varieties (Figure 11).

Figure 11. Sheath blight disease.

7.3 False Smut

Rice false smut is caused by the fungus *Ustilaginoidea virens* and damages rice grains. It is a minor disease in the southern USA. The fungus infects the floral organs of rice before heading, and smut balls can be found on spikelets. Begin looking for the disease 3-4 weeks after flowering and look for silver, orange, and greenish-black kernel. In most cases only a few neighboring smut balls can be found (Figure 12).

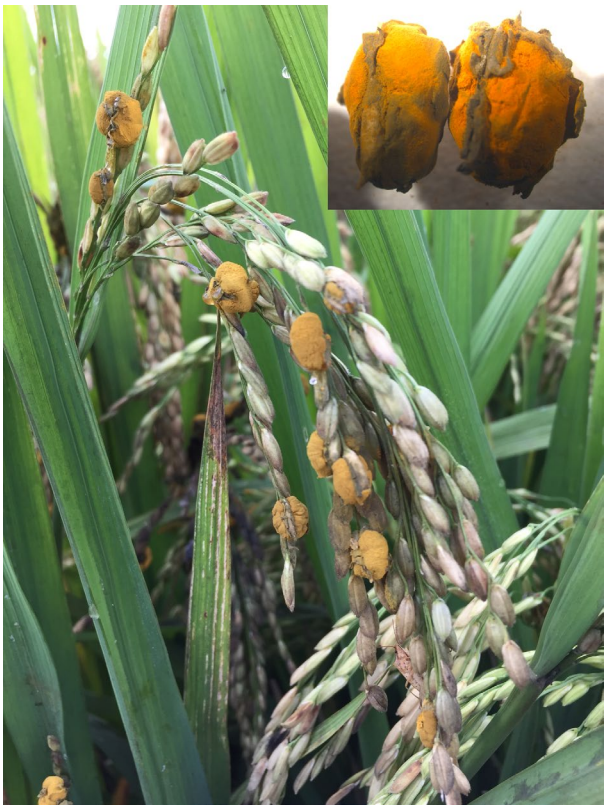


Figure 12. False smut disease.

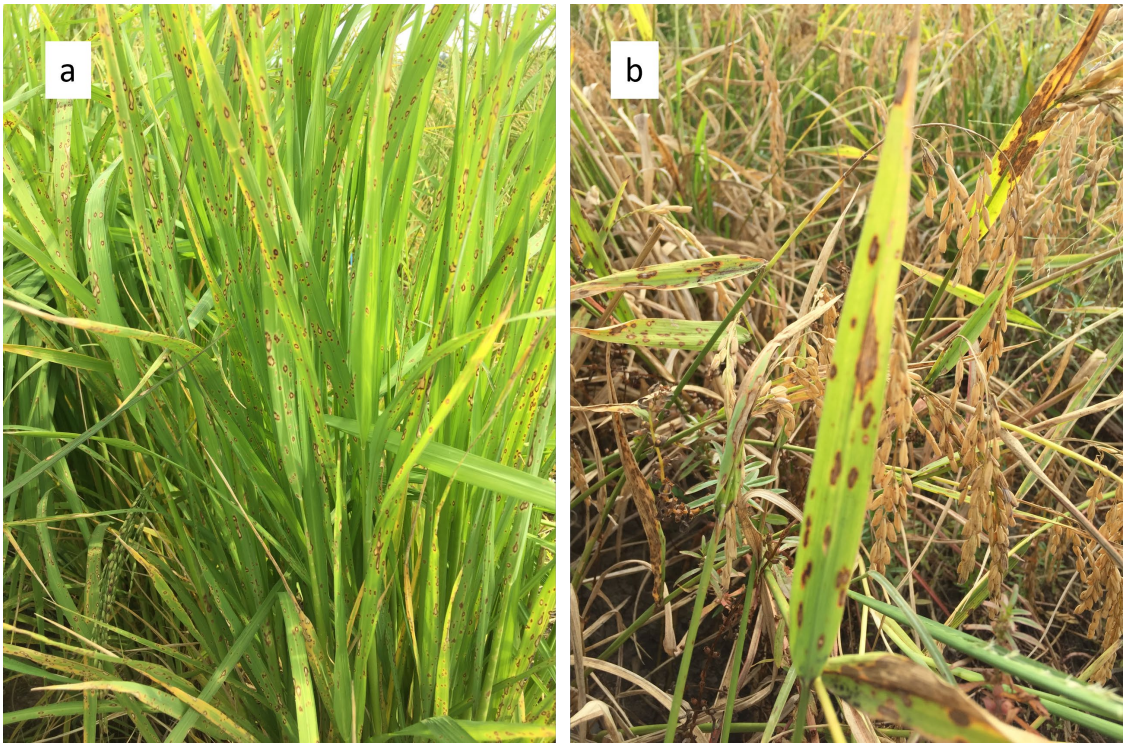
7.4 Kernel Smut

Rice kernel smut is caused by the fungus *Tilletia barclayana* and damages the rice grain. It is a minor disease in the southern USA. The fungus infects the floral organs of rice before heading and smut balls can be found on spikelets. Begin looking for black masses of spores oozing from the seam between the hulls 2-4 weeks after flowering (Figure 13).

Figure 13. Kernel smut disease.

7.5 Brown Spot

Brown spot is caused by the fungus *Cochliobolus miyabeanus*. It is one of the most prevalent rice diseases in the southern USA. The fungus infects leaves, glumes, seedlings, sheaths, stems, and grains and can occur at all rice growth stages. Look for lesions on leaves and sheath at the tillering. Symptoms are expressed as dark brown to purple small circular spots, or brown circular to oval spots with a light brown to gray center surrounded by a reddish-brown margin (Figure 14).



Figures 14. Brown spot disease at vegetative growth (a), and at maturity (b).

7.6 Narrow Brown Leaf Spot

Narrow brown leaf spot is caused by the fungus *Sphaerulina oryzina* and is a minor disease in the southern USA. The fungus can infect leaves, sheaths and panicles resulting in premature death of leaves and leaf sheaths, premature grains and lodging under severe infections. Look for light to dark brown linear lesions about 2-10 mm long and 1-1.5 mm wide at the heading stage. The elongated spots run parallel to the vein on leaves and the upper leaf sheath (Figure 15).

Figure 15. Narrow brown leaf spot at Crowley, Louisiana in 2019.

7.7 Bacterial Leaf Blight

Bacterial leaf blight is caused by *Pantoea ananatis* and is a new disease in the southern USA. The bacteria can be seed borne. Look for brown or dark brown lesion in the vein on the flag leaf at the tillering stage. It can cause panicle sterility and discoloration of seed (Figure 16).

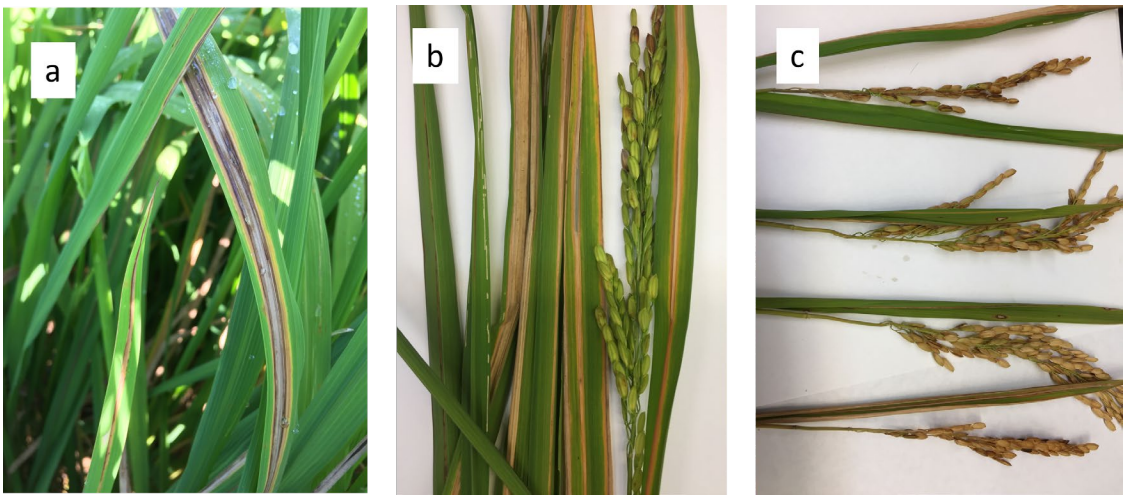


Figure 16. Leaf blast disease at vegetative growth (a), and at reproductive stage (b and c).

Section 8 APPENDIX

8.1 Panicle Image Acquisition

This procedure details the process of acquiring images for panicles for low inventory rejuvenation and redundant by name accessions grown in the field. It will also be used to capture images of mature panicles collected when panicles have matured.

The imaging system consists of a DSLR (Document Schema Renaming Language) camera attached to a portable station. The station has a flat surface (object area) that is covered with a blue background that includes a ruler on the side. Two led lights are affixed above the object area just below the camera level.

Capture image of flowering panicle

1. In the field, collect three panicles of each accession that have flowered and place them in labeled panicle envelopes.
2. Carefully transport the panicles to the lab where they will be imaged.
3. Turn on the camera and lights on the imaging station.
4. Adjust the camera and appropriate settings to capture crisp quality images.
5. Remove the panicles and place the best two panicles in the object area.
6. Take two images of each pair of panicles per accession.
7. Record the image numbers in the field book next to the accession numbers.
8. Remove the two panicles and return them to their appropriate envelopes.
9. Be sure that the object area is free of debris and particulates. Debris and particles can be removed using a soft brush or compressed air.
10. Place another panicle pair in the object area and repeat steps 7 to 9 until all accession panicles have been imaged.

Capture image of mature panicle

1. Carefully remove the panicles from envelopes and select the best two panicles.
2. Place two panicles next to each other in the object area. Place the hulled and dehulled kernels next to the panicles.
3. Capture crisp quality images of the panicles and kernels.
4. Remove the panicles and kernels from the object area and return them to envelopes.
5. Be sure that the object area is free of debris and particulates.
6. Place another pair of panicles in the object area and repeat steps 1-5 until all accession panicles have been imaged.

Capture image of RBN mature panicles

1. Carefully remove the panicles from each envelope for the RBN accessions.
2. Place the first panicle on the object area and place a label with the accession number below it.
3. Place the second panicle next to the first and place a label with the accession number below it.
4. Capture crisp quality images of the panicles.
5. Remove the panicles from the object area and return to each individual envelope.
6. Be sure that the object area is free of debris and particulates.
7. Repeat steps 1-5 until all RBN accession panicles have been imaged.

8.2 70% Alcohol Preparation

This procedure requires personal protective equipment. It is recommended that gloves, a lab coat, and safety glasses be worn during this procedure. This procedure is performed in a laminar hood or well-vented area.

Materials

- 95% Ethyl alcohol or ethyl propanol
- 100 ml measuring cylinder
- Glass bottle and cap (100 ml)
- Distilled water

Procedure:

1. The details in this procedure will produce 100 ml of 70 % ethyl alcohol.
2. Place the alcohol container in the laminar hood and turn on the vent.
3. Collect a 100 ml clean glass (Pyrex) chemical bottle and cap.
4. Place a label on the bottle with the following: 70 % alcohol, date prepared, and initials of the preparer. Place the glass bottle in the laminar hood.
5. Measure 74 ml of ethyl alcohol or propanol in a measuring cylinder. **Note: A 100-, 200-, or 500-ml measuring cylinder can be used**.
6. Pour into the glass bottle.

7. Measure 26 ml of room temperature distilled water in a measuring cylinder.
8. Pour the distilled water into the bottle containing ethyl alcohol. Stir gently.
9. Place the cap on the bottle.
10. Return ethyl alcohol container to the safety cabinet.
11. Clean up the area and wash and return all utensils/equipment.

8.3 70% Bleach Preparation

This procedure requires personal protective equipment. It is recommended that gloves, a lab coat, and safety glasses be worn during this procedure. This procedure is performed in a well-vented area.

Materials:

- Bleach
- Tween 20 (if available)
- 100 ml measuring cylinder
- Glass bottle and cap (100 ml)
- Distilled water

Procedure:

1. The details in this procedure will produce 100 ml of 70 % bleach.
2. Collect a 100 ml clean glass (Pyrex) chemical bottle and cap.
3. Place a label on the bottle with the following: 70 % bleach, date prepared, and initials of the preparer.
4. Measure 70 ml of bleach in a measuring cylinder. **Note: A 100-, 200-, or 500-ml measuring cylinder can be used**.
5. Pour into the glass bottle.
6. Measure 30 ml of room temperature distilled water in a measuring cylinder.
7. Pour the distilled water into the glass bottle and stir gently.
8. **Note: Add 2 drops of Tween 20 if available. (2 drops per 100 ml of bleach solution)**.
9. Place the cap on the glass bottle.
10. Return the bleach container to its designated area.
11. Clean up the area and wash and return all utensils/equipment.

8.4 Seed Germination Media Preparation

This procedure requires personal protective equipment. It is recommended that gloves and a lab coat be worn during this procedure. This procedure is performed in a well-vented area.

Materials:

- Agar

- Murashige and Skoog Basal salts with minimal Organics
- Sucrose (Grade 1)
- Plant Preservative Mixture (PPM)
- Magenta boxes
- 2000 ml beaker
- 1000 ml distilled water
- Stirring magnet
- Hot plate with a stirrer
- Distilled water

Procedure:

1. The measures in this procedure will produce 1000 ml of agar. They can be adjusted to make quantities of higher or lower amounts.
2. Add 900 ml of room temperature distilled water to the large beaker.
3. Place stir bar in beaker and place on the hot plate (set to high heat) and stir at medium speed.
4. Add 13g of Agar, 4.4g of Murashige and Skoog Basal salts, and 20g of sucrose to the beaker.
5. Heat and stir beaker until it come to a rapid boil then immediately lower heat to medium heat (~200 F).
6. Allow the contents to boil for 30 to 45 minutes or until the content is transparent.
7. Turn stirrer off and add distilled water to the beaker until the volume is 1000 ml.
8. Turn off heat and allow to stir for 5 minutes.
9. Add 500 ul of PPM to the contents of the beaker and stir for 5 minutes.
10. Carefully pour the contents of the beaker into magenta boxes to about a ½ inch in height.
11. Place lid on each magenta box but do not snap close, just lay the lid on top.
12. Place the magenta boxes in autoclavable containers and place them in an autoclave.
13. Choose the liquid cycle for 20 minutes on the autoclave.
14. When the cycle is complete, remove contents from the autoclave and allow magenta boxes to cool momentarily, then tightly close the lids.
15. Allow magenta boxes to cool completely then store in the refrigerator until ready to use.

8.5 Germinating Seeds in Germination Media

This procedure requires personal protective equipment. It is recommended that gloves and a lab coat be worn during this procedure. This procedure is performed in a sterile laminar flow hood or biosafety cabinet (BSC).

NOTE: The magenta boxes can be labelled after the seeds have been inserted into the agar or before placing them into the sterile laminar flow hood/BSC.

Materials:

- Magenta boxes
- Rice seed (dehulled)
- Large tweezer/forceps
- 70 % ethanol in a spray bottle
- 70 % ethanol in a large tube or small beaker
- 70 % bleach
- Bunsen burner or better, a glass bead sterilizer (no flame to set the ethanol on fire accidently)
- Large lint-less wipes
- Small beakers or disposable tubes

Procedure:

1. Turn on the UV light and fan in the laminar hood for 25 minutes to create a sterilized environment before using it. **Note: Due to the effects of UV light, DO NOT stay around the laminar hood while the light is on**.
2. While the laminar flow or BSC is being sterilized, collect the magenta boxes and seed of accessions to be germinated.
3. After 25 minutes have passed, turn off the UV light in the hood.
4. Wipe down the hood surfaces with 70% ethanol.
5. Place a series of lint-less wipes along the surface of the hood (about 80% of the surface should be covered).
6. Place the crucibles in the small beakers and line them up on the lint-less wipes close to the back of the laminar hood.
7. Place the magenta boxes in the right corner of the laminar hood.
8. Place the Bunsen burner close to the back of the laminar hood to the right of the magenta boxes. Light the Bunsen burner.
9. Place 10-15 seeds of an accession in a crucible. Place the envelope in front of the crucible with the name and PI/CIor face up.
10. Repeat this process for all accessions that will be cultured.
11. After all seeds have been placed in the crucibles, add 70% bleach solution until the seeds are submerged.
12. Let the seeds soak in the 70% bleach solution for 10 minutes.
13. After 10 minutes, remove the crucibles from the beakers and place them on the lint-less wipes behind each beaker.
14. Place a magenta box in front of you and remove the lid and place face down on the lint-less wipes.
15. Dip the tweezers/forceps in the tube with 70 % ethanol, swirl, remove, place over the flame of the Bunsen burner for 10 seconds then remove and allow to cool.
16. Take the first crucible and gently scrape all the seeds into the magenta box with the tweezers/forceps.
17. Return the crucible to its' location.
18. Take the magenta box in one hand and gently tilt towards you.

19. Use the tweezers/forceps to grab each seed and gently insert them about 2 cm below agar surface. **Note: It is best to place the seed horizontally in the agar**.
20. After the seeds have been inserted into the agar, replace the lid.
21. Place the tweezers/forceps in the small beaker or large tube containing 70 % ethanol.
22. Place a label on the magenta box and set it to the left side of the laminar hood. **Note: This step is only necessary if the magenta boxes were not labelled before the process started**.
23. Repeat this process for the remaining accessions.
24. Put out the flame of the Bunsen burner.
25. Place all magenta boxes in an incubator at 250 C.
26. Remove the crucibles, beakers, tweezers/forceps, small beaker/large tube, seed envelopes, and Bunsen burner from the laminar hood.
27. Use lint-less wipes to wipe down the laminar hood with 70 % ethanol.
28. Turn off the light and fan and close the sash of the laminar hood.
29. Wash all equipment and utensils used and return to their locations after drying.

8.6 Embryo Rescue Germination

This procedure can be performed on a lab bench or any hard surface.

Materials:

- Razorblade
- Petri dish or plexiglass sheet
- Small tweezers

Procedure:

1. Collect the seeds to be used for embryo rescue.
2. Dehull the individual seeds using a sandpaper block or by hand. **Note: DO NOT USE mechanical seed dehullers**.
3. Place the dehulled seeds in the petri dish or on the plexiglass.
4. Orient the seed so that you can identify the end that has the embryo.
5. Cut the seed in half by pressing forcefully on the seed using the razor blade.
6. Collect the seed half with the embryo and place it in a small seed envelope.
7. Repeat until all seeds have been cut and embryos collected.
8. To germinate the embryo(s), follow the procedure in **`appendix 8.5`**.
9. **NOTE: The embryos are soaked/washed in 70% bleach for 7 minutes to avoid damaging them**.

8.7 20:20:20 Fertilizer Solution

1. Measure 350 grams of the 20:20:20 (N:P:K) in an appropriate container.
2. Collect a five-liter bucket and add approximately four liters of water.
3. Add the 20:20:20 crystals to the bucket.

4. Add one teaspoon of iron to the bucket and stir until mixed.
5. Add the remaining one liter of water to the bucket and stir gently to mix the solution.
6. Poor 50 cm of fertilizer solution in each pot.
7. Add another 50 cm of the fertilizer solution to each pot at the booting stage.