

Procedures for all new students, postdocs, visitors and staff in McCouch lab

Obtaining seed for Experiments in the McCouch Rice Lab

NOTE: OUR GREENHOUSES OPERATE UNDER SPECIAL CONDITIONS CAREFULLY CONTROLLED BY THE FEDERAL GOVERNMENT. Because we import seeds from “wild” species that are considered “noxious weeds” by the USDA APHIS Quarantine service, we must meet many special rules while using our greenhouses. Based on these strict regulations, we treat all rice species other than *O. sativa* (seeds and plants) as wild. In other words, we treat any rice plant that has seeds with a red pericarp and/or seed coat as a “noxious weed” in our program.

Because of strict USDA/APHIS conditions for our Greenhouses, you **MUST** obey certain rules:

Seed already in our Rice Lab Seed Storage Area

- a. Talk to Dr. Susan McCouch about your experiment and the seeds you should use from our seed stock. Sandy maintains our RA and PA seed stock books with data on seed in our collection. Request seeds by RA or PA number from those databases.
- b. Request the seed you need to plant from Gen. Gen will check the availability of seed stock in our project database and give you an aliquot of seed.
- a. NOTE: You must be given the seed by Gen so we can keep track of the stock envelopes and how much we have left of each accession.

Ordering Seed from Sources outside the McCouch Rice Lab

1. From inside the USA

- a. To import seed from **INSIDE** the USA, restrictions apply for both *Oryza sativa* and noxious weed seed. Noxious weed seed is extremely restricted. You **MUST** obtain permission from Susan McCouch for all such shipments of seed, whether *Oryza sativa* or noxious weed (*O. rufipogon*, red pericarp, etc.). Once she gives you permission, you must inform Lois and Sandy of the details of the shipment. It should be sent to Dr. Susan McCouch, not to you. A Materials Transfer Agreement (MTA) may be associated with the seed you need from other institutions/individuals. You **MUST** present this information to Dr. McCouch before ordering. A seed accession list in excel file format will be required.
- b. This seed is logged into our Rice Accession (RA) database by Sandy before planting. Each accession you import will be assigned an RA# and will be entered into the database with all relevant accompanying information.

- c. The seeds will be put in envelopes, labeled with computer generated label (s) prepared by Sandy and stored in Gen's office (which remains locked at all times). The packaging in which the seed arrives must be autoclaved then discarded. You must be given the seed by Gen (or Sandy or Susan). You will only be given the amount of seed you need to plant and original envelopes may not be removed from the office. All seeds are to be kept in our locked facilities. You may not take seeds elsewhere to measure etc. You must do measurements in our locked facilities.

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2. From outside the USA

- a. To import seed (wild or not) from OUTSIDE the USA, you must have Susan McCouch's permission first. She will supply the appropriate USDA APHIS permit (see Lois after speaking with Susan). The seed will automatically be sent from the supplier through the US Customs quarantine service, using the permit(s). A Materials Transfer Agreement (MTA) may be associated with the seed you need from other institutions/individuals. You MUST present this information to Dr. McCouch before ordering. A seed accession list in excel file format will be required. See Lois for format.
- b. NOTE: Imported seed from outside the USA must be sent through a registered carrier accompanied by the proper permit. **NO hand-carrying or insertion in baggage.** If this is not done, US Customs will destroy it. No exceptions. Shipping is arranged by Lois.
- c. When foreign seed ordered from outside the USA arrives, it is sent to USDA/APHIS Customs Quarantine first and then directly to Dr. McCouch by express mail.
- d. It is logged into our Rice Accession (RA) database by Sandy before distribution/planting. Each accession we import is assigned an RA# and will be entered into the database with all relevant accompanying information.
- e. The seeds will be put in envelopes, labeled with a computer generated label prepared by Sandy and stored in Gen's office (which remains locked at all times). The packaging in which the seed arrives must be autoclaved then discarded. You must be given the seed by Gen (or Sandy or Susan). You will only be given the amount of seed you need to plant and original envelopes may not be removed from the office. All seeds are to be kept in our locked facilities. You may not take seeds elsewhere to measure etc. You must do measurements in our locked facilities.

Step-by-Step Instructions for Growing Seed in the Greenhouses/Growth Chambers

1. Have a tour by trained lab tech to show you several key things in the greenhouse complex, including the required reading on our Greenhouse Procedures. Attend Worker Protection Standard (WPS) training course. See <http://oeh.cals.cornell.edu/wpstrain.html> for dates and times. Please attend within 5 days of first working in the greenhouse.
2. NOTE: **All of our greenhouses are to be locked and the doors must be closed at all times.** There should be NO seeds on the floor at anytime. When you exit, you must inspect your clothing and hair in the mirrors near the doors. Use the sticky mats at the doors to be ensure you have no seed on your shoes. The greenhouses must be kept clean and tidy. Please clean up after yourself.
3. To request space in Guterman greenhouse or in any growth chamber, get permission from Dr. McCouch, then contact Gen Onishi fo13@cornell.edu to ask what is available.
4. Print or neatly write your name and the name of your experiment on a piece of paper and place it in the plastic envelope on the front of the bench you occupy, so anyone coming in can tell what experiment is proceeding in which tank.
5. After requesting seed you need to plant from Gen (see above under "Obtaining seed", Gen will check the availability of seed stock in our project database and give you an aliquot of seed.
6. All seed planted must be treated BEFORE you plant. **Surface sterilize your seeds with Clorox prior to planting (see Protocol below). This must be done EVERY TIME you plant a seed our greenhouses – NO EXCEPTIONS.**

Protocol to Clorox-treat seeds before planting

Place seeds in a flask of 20% Clorox and stir at room temp for 10 minutes. Drain and rinse in dH₂O for same amount of time with stirring. Drain thru a screen and blot dry.

7. Germinate seeds on wet paper towels in plastic Petri dishes. Space seeds evenly over the Petri dish, no more than 20 seeds per dish. Incubate under near ultra violet light or florescent light with a light/dark cycle of 12 hours, at a temperature of 20 +/- 2 o C. The germinating seeds should be examined periodically the next 8 days 8 days. After 8 days, choose healthy seedlings to plant in soil. Any diseased seeds and seedlings are to be destroyed by autoclaving.

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8. Obtain the necessary pots for your experiment from Gen. Set them up, fill with soil (sign out how much soil you use) and water them in. Place the pots in the assigned space
9. **All plants must be labeled with an RA number.** You may also use another designation *in addition* to the RA#. Plant seeds.
10. ***Pot labeling, soil preparation and planting is the responsibility of the researcher doing the experiment.*** *If you need assistance, please consult with Susan. Gen may not always be available to assist you but usually help can be found if you request it in a timely fashion..*
11. The plants will be watered and fertilized using standard protocols (see “rice fertilizer regime” below) unless you specify otherwise. Any special treatments you require (ie: shade cloths, crossing) will be the researcher’s responsibility unless Susan okays assistance. Pesticides will be applied only when necessary. The greenhouses will be posted during the restricted re-entry period. An e-mail alerting people always precedes pesticide application so you can plan your work accordingly. If the pesticide sign is on the door, you MAY NOT enter for any reason.
12. When your experiment is over, please alert Jean Koski(jas54@cornell.edu) and John Jantz (jj4@cornell.edu) (or Andy Leed (arl6@cornell.edu))that you will be collecting material that requires autoclaving. Let him know 1-2 days before you will discard your plants so he can schedule use of the autoclave. Put plant material in the autoclave bags provided in the greenhouse, tie the bags closed by knotting them securely, and leave them in the designated spot in the greenhouse. Please do not overfill the bags so they are too heavy to be lifted by an average person.
13. Let Gen (fo13@cornell.edu) and Jean Koski (jas54@cornell.edu) know that you will have pots to be cleaned the day before you take down your experiment. Clay pots will be autoclaved and deepots must be soaked and rinsed. Gen or other greenhouse staff will autoclave and/or soak your pots and put them away.
14. When your plants have been discarded, remove your planting request form from the envelope on the bench you occupy and discard.
15. **Report any maintenance, temperature/lighting or pest issues** to Jean Koski (jas54@cornell.edu) or John Jantz (jj4@cornell.edu) (Andy Leeds (arl6@cornell.edu)) **and** Gen (e-mail or in person) **as soon as you notice them** so things don’t get out of control.

Rice Fertilizer Regime

We make two types of Fertilizer stock solutions and dilute 1:100 with water just before use:

- A) Peters Water-Soluble Fertilizer* N.P.K -15.16.17 15 lbs with 5 gallons water
- B) Peters Water-Soluble Fertilizer N.P.K -15.16.17 15 lbs plus 5 lbs Sprint 330** (IRON) with 5 gallons water.

*Peters Water-Soluble Fertilizer : by SCOTTS

**Sprint 330 (Iron. Fe) 10% -10% Chelated Iron: by BECKER UNDERWOOD, Inc

We fertilize with 1:100 dilution of Solution B at 3-5 days after germination and again with Solution B one week later.

From 2 weeks of age until booting (flowering), alternate with 1:100 dilution of Solution A and B every 7-10 days.

Once plants have flowered, stop using Solution B. Apply only Solution A every 2 weeks (10-14 days) until harvest.

*This regime is subject to change as deemed necessary by the attending greenhouse crew and Susan McCouch.