Germination Procedures for Wild *Oryza* Species and Hard-to-Grow *Oryza* Germplasm
Provided by USDA ARS Genetic Stock – *Oryza* (GSOR) Collection

NEEDED SUPPLIES:

Dehulled rice seeds
Sodium hypochlorite (household bleach) and water
50 ml glass beakers (1 per cultivar)
40 ml porcelain filtering crucibles (1 per cultivar)
Prepared tissue culture boxes*, aka magenta boxes with agar gel and orchid medium (1 per cultivar)
Timer, alcohol and flame for sterilizing metal forceps, lab wipes, permanent marker, gloves, fume hood

1. Carefully dehull seeds. You do not want to dislodge the germ from the seed. NOTE: If your seed is limited, you may want to only dehull a few seeds at one time since any contaminant could cause a fungal growth and you may lose seeds. However, you may plate up to 20 seeds per culture box.

2. Prepare a 70% solution of sodium hypochlorite (household bleach). You will need approximately 50 ml per cultivar.

3. For each cultivar you will need a porcelain filtering crucible placed inside a 50 ml glass beaker.

WORKING UNDER FUME HOOD,
4. Arrange beakers on top of a layer of wipes. Place dehulled seeds into crucibles (1 cultivar per container) and cover with bleach solution. Soak for 5-7 minutes. NOTE: In some cases the soaking time may need to be less than 5 minutes, especially if they are wild species and are newly harvested.

5. During soak time, mark each magenta box with cultivar identifier and date.

6. At end of soak time, sterilize metal forceps with alcohol and flame. Remove first crucible and set onto wipe to drain. (You may rinse with auto-claved water, however different labs see rinsing as optional since it may reintroduce contaminants.) Use forceps to pick up one seed and place into culture medium. The seed should be vertically suspended in the medium so there is room beneath the seed for root growth and medium above the seed to keep it moist. Place remaining seeds into magenta box. Place lid onto box. Repeat this step for each cultivar.

TRANSFER TO GROWTH CHAMBER,
7. Place magenta boxes into growth chamber set at ~30ºC and 12-hours of daylight. Seeds should germinate within 2-3 days. Seedlings may remain in culture boxes until leaves touch lid or medium begins to dry up (7-10 days). NOTE: If fungus occurs, you may need to discard and begin again.

TRANSFER SEEDLINGS TO GREENHOUSE TRAYS,
8. Fill seedling trays with sterilized soil and allow water to soak into soil from tray flat. Using forceps transfer one seedling to labeled tray cell. Maintain moisture, allowing plants to grow to 5-leaf stage, at which time you may transplant seedling to larger pot, up to 3 plants per pot.

*Suggested sources for magenta box supplies: Sigma-Aldrich Magenta Vessel with cover #C0542; 100 ml Vessel (jar) #B8630 with Magenta B-caps #B8648; 10.5g Gum Agar #A1296; 4.4 g of M6899 from Sigma; 20g Sucrose (S-5390 from Sigma). Special notes on preparing agar: you will also need 1800ml or larger beaker, stirring magnet, stirrer and hot plate, and 1000 ml distilled water. Follow manufacturer's instructions to prepare medium. Magenta boxes will need to be autoclaved and cooled prior to use.
If you have any questions regarding this protocol, please call the GSOR at 870-672-9300 or send an email to gsor@ars-grin.gov. You may also see photos which describe this process at the GSOR website www.ars.usda.gov/spa/dbnrrc/gsor.
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