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General discussion.
Written instructions for these procedures, including a general supply list, are available in downloadable form [here](#). Adobe Acrobat reader is required. Special thanks to Biological Science Technicians Tiffany Sookasern and Quynh Ho Grunden for technical assistance and comments in preparing the photos and compiling these instructions.



Magenta box is an autoclavable polycarbonate/polypropylene box used often in plant tissue culture. An alternative to the plastic box is a glass baby food jar with a polypropylene cap. Our technicians use either container, but use the term 'magenta box' to describe both in their protocols.



Supplies needed:
For each rice cultivar you will need a prepared magenta box, a strainable porcelain crucible, a 50ml glass beaker; and 70% bleach solution, timer, metal forceps and alcohol for flame sterilization, sterile working area such as chemical vent hood.



Rice seeds should be dehusked by hand instead of mechanical means in order to preserve the embryo. Click [here](#) to see tools that our technicians use to quickly dehusk seeds for this procedure.

Please let us know if these instructions were helpful to you by sending an email to gsor@ars-grin.gov.

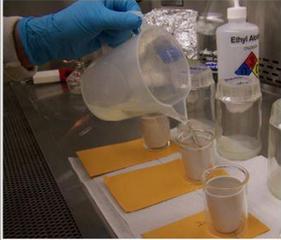
Please continue to next pages for additional photos and instructions.

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1. Prior to setting up work area, you should mark cap of each magenta box with unique information using a permanent marker.

2. Place dehusked rice seeds into crucible that has been placed inside 50ml beaker. Beaker is then placed in front of corresponding magenta box.



3. Fill beaker with 70% bleach solution to cover rice seeds. Begin 7-minute soak. NOTE: Please see full print-out of procedures to learn about using a different amount of soaking time.

Printable instructions for these procedures are available [here](#).

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4. Following timed soak, use flame-sterilized forceps to lift crucible out of bleach solution and rinse with auto-claved water. Set crucible on paper towel to drain.

5. Using flamed forceps, place treated seed into crucible out of bleach solution and rinse with auto-claved water. Set crucible on paper towel to drain.



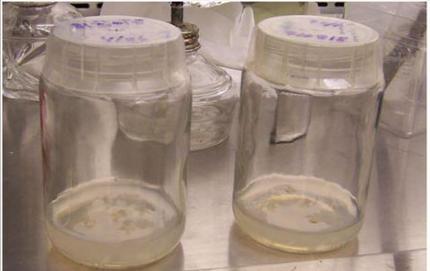

6. The seeds should be suspended equally within the medium to allow for ample room for root growth beneath the seed and coverage by medium over the seed.

7. This magenta box is complete with 5 seeds evenly spaced within the medium area.

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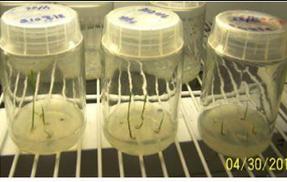


8. Numbered caps have been replaced onto the completed magenta boxes.



9. Completed magenta boxes have been placed in a growth chamber which is set at 30 degrees C and programmed for 12 hours of light per day. Do not open magenta boxes during germination process. You should observe radicle growth within 2-3 days of seed treatment. If a fungus begins in the magenta box, you may have to discard and begin again.

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The coleoptile is easily visible 3 days after seeds were placed in magenta boxes.

The seedlings in the jars on the left and in the middle are 4 days old, the seedlings in the jar on the right are 3 days old.




This picture shows the seedling growth 7 days later.

This photo shows that although a fungus has begun to grow, the seedlings are thriving. However, in some cases contamination does inhibit or prevent successful germination of the seed.

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Germination procedures - page 6 Transplanting seedlings




Forceps are used to gently remove seedlings from magenta box and plant into moistened soil in seedling tray. The tray will be moved to the greenhouse to allow the seedlings to continue growing until such time the plants can be moved to the field plot. NOTE: During the first 24 hours in the greenhouse, you might want to place the tray in the shade, or loosely cover to protect the seedlings from direct sunlight.

Above are the transplanted seedlings 4 days after being moved to the greenhouse.



Download written instructions, including a supply list [here](#). (Adobe Acrobat required for PDF document.)
Download screenshots of all these pages [here](#). (Adobe Acrobat required for PDF document.)
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After about two weeks in the greenhouse, seedlings are then transplanted by hand in the field.

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