SCHNABLE LAB
Plant Genomics

2035C Roy J. Carver Co-Laboratory
Plant Sciences Institute
Iowa State University
Ames, Iowa 50011-3650
515 294-7209
FAX 515 294-5256

PROTOCOL TO STERILIZE AND CHARCHOAL-GERMINATE MAIZE SEEDS

(Last revised August 2010)

This protocol is used for germinating moldy seeds that would otherwise fail to germinate under normal growing conditions. This protocol is also useful for germinating shrunken kernels.

This protocol was adapted by Dr. Marna Yandeau-Nelson of the Schnable Laboratory (Iowa State University) from various sources. Please contact Dr. Patrick Schnable (schnable@iastate.edu) regarding questions or corrections.

Reagents:
Bleach (Clorox)
Germination paper, available from Anchor Paper, St. Paul, MN, Catalog # SD3815L
10% Captan Fungicide (Arvesta)
Petri-dishes or 24-well or 48-well plates
De-colorizing charcoal (Acros Organics) available from Fisher, Catalog # AC17153-0010

Materials:
Autoclaved forceps and spoon (for scooping charcoal)

*Prior to germination, autoclave forceps, spoon and water. Cut germination paper to fit petri dishes or plate wells (three layers of paper per well). Autoclave the cut germination paper.

I. **Sterilization**

1. Place up to 10 kernels in a 14-ml tube. Cover with pure bleach and invert 3-4 times; let sit for 1 minute.
2. Rinse seeds with sterile water repeatedly until bleach odor disappears (8-10 times)

II. **Charcoal-Germination**

1. Layer three pieces of germination paper in each plate. Apply a few drops of fungicide to each plate. Add sterile water to moisten paper if necessary. Make sure that the paper is only moist and there are no pools of water.
2. Using sterile forceps, place kernels on plate with about an inch between each.
3. Using a sterile spoon, sprinkle charcoal on top of each kernel so that it is completely covered.
4. Germinate seeds in the covered plates in a 28C incubator.
5. After the first day, the paper is often dry. Apply a small amount of sterile water (250-500ul) with a pipette. It is best to add the water to the edge of the germination paper taking care not moisten the charcoal. **It is critical to keep the charcoal dry. If charcoal becomes wet, the seeds typically do not germinate.
6. If the charcoal gets wet or fungus is starting to grow, transfer kernel to a fresh plate with germination paper and fungicide and cover with charcoal (steps 1-4). It might be necessary to transfer the kernels 2-3 times during the germination process. Germination can take 3-7 days. Germination can be hastened a bit if a container of water is placed inside incubator.
7. After the kernel germinates (coleoptile and roots are visible) the seedling can be transferred to soil.