

## **STANDARD AXENIC CULTURE PROCEDURE**

- 1 package of MS6899 dissolved in 1000mls of dH<sub>2</sub>O
- Dispense the 1000mls of MS6899 of MS medium into 4 bottles of 250mls
- Use 1 bottle containing 250 mls of MS6899 and add 650 mls of dH<sub>2</sub>O
- Add 10 grams sucrose
- Add 7 grams agar
- Bring to a boil
- Add 100mls of coconut endosperm
- Adjust pH to 6.00 with NaOH (about 6 drops)
- Dispense ~10mls into culture tubes and autoclave 235 degrees F for 20 minutes
- Cool in slant racks
- Inoculate with surface sterilized seeds\*
- Store in dark at room temperature

### **\*SURFACE STERILIZATION AND INOCULATION OF SEEDS INTO MEDIUM**

Prepare the seed sterilizing solution: a 10% solution of common bleach with one drop of detergent. (since common bleach is about 5% sodium hypochlorite, a 1:10 dilution of bleach will give a 0.5% solution of sodium hypochlorite).

To prepare the solution, take 10 mls of bleach and add 90 mls of sterile water and add one drop of detergent as a wetting agent. Refrigerated seeds are added to this solution and shaken gently for 30 minutes. The surface sterilized seeds are poured into a conical funnel lined with a clean filter paper folded in quarters. The seeds are rinsed 3-4 times with sterile water. Seeds are removed from the filter paper with a sterile loop and placed into culture tubes and stored in the dark at room temperature.