

## Surface-sterilizing Plant Material

### 1. Preparation of Stock Plants

Prior good care of stock plants may lessen the amount of contamination that is present on explants. Plants grown in the field are typically more “dirty” than those grown in a greenhouse or growth chamber, particularly in humid areas like Florida. Overhead watering increases contamination of initial explants. Likewise, splashing soil on the plant during watering will increase initial contamination. Treatment of stock plants with fungicides and/or bacteriocides is sometimes helpful. It is sometimes possible to harvest shoots and force buds from them in clean conditions. The forced shoots may then be free of contaminants when surface-sterilized in a normal manner. Seeds may be sterilized and germinated in vitro to provide clean material. Covering growing shoots for several days or weeks prior to harvesting tissue for culture may supply cleaner material. Explants or material from which material will be cut can be washed in soapy water and then placed under running water for 1 to 2 hours.

### 2. Sodium Hypochlorite

Sodium hypochlorite, usually purchased as laundry bleach, is the most frequent choice for surface sterilization. It is readily available and can be diluted to proper concentrations. Commercial laundry bleach is 5.25% sodium hypochlorite. It is usually diluted to 10% - 20% of the original concentration, resulting in a final concentration of 0.5 - 1.0% sodium hypchlorite. Plant material is usually immersed in this solution for 10 - 20 minutes. A balance between concentration and time must be determined empirically for each type of explant, because of phytotoxicity.

### 3. Ethanol (or Isopropyl Alcohol)

Ethanol is a powerful sterilizing agent but also extremely phytotoxic. Therefore, plant material is typically exposed to it for only seconds or minutes. The more tender the tissue, the more it will be damaged by alcohol. Tissues such as dormant buds, seeds, or unopened flower buds can be treated for longer periods of time since the tissue that will be explanted or that will develop is actually within the structure that is being surface-sterilized. Generally 70% ethanol is used prior to treatment with other compounds.

### 4. Calcium Hypochlorite

Calcium hypochlorite is used more in Europe than in the U.S. It is obtained as a powder and must be dissolved in water. The concentration that is generally used is 3.25 %. The solution must be filtered prior to use since not all of the compound goes into solution. Calcium hypochlorite may be less injurious to plant tissues than sodium hypochlorite.

### 5. Mercuric Chloride

Mercuric chloride is used only as a last resort in the U.S. It is extremely toxic to both plants and

humans and must be disposed of with care. Since mercury is so phytotoxic, it is critical that many rinses be used to remove all traces of the mineral from the plant material.

## **6. Hydrogen Peroxide**

The concentration of hydrogen peroxide used for surface sterilization of plant material is 30%, ten times stronger than that obtained in a pharmacy. Some researchers have found that hydrogen peroxide is useful for surface-sterilizing material while in the field.

## **7. Enhancing Effectiveness of Sterilization Procedure**

- Surfactant (e.g. Tween 20) is frequently added to the sodium hypochlorite.
- A mild vacuum may be used during the procedure.
- The solutions that the explants are in are often shaken or continuously stirred.

## **8. Rinsing**

After plant material is sterilized with one of the above compounds, it must be rinsed thoroughly with sterile water. Typically three to four separate rinses are done.

## **9. Use of Antibiotics and Fungicides in Vitro**

We have found that the use of antibiotics and fungicides in vitro is not very effective in eliminating microorganisms and these compounds are often quite phytotoxic.

## **10. Plant Preservative Mixture**

PPM™ is a proprietary broad-spectrum biocide, which can be used to control contamination in plant cell cultures, either during the sterilization procedure, or as a medium component. PPM™ comes in an acidic liquid solution (pH 3.8). The recommended dose is 0.5–2.0 mL of PPM™ per liter of medium. Higher doses are required to treat endogenous contamination and for *Agrobacterium*.

Its makers say that PPM™ has several advantages over antibiotics: It is effective against fungi as well as bacteria, thus it can be substituted for a cocktail of antibiotics and fungicides. PPM™ is less expensive than antibiotics, which makes it affordable for wide and routine use. The formation of resistant mutants toward PPM™ is very unlikely because it targets and inhibits multiple enzymes. Many antibiotics adversely affect plant materials. If used as recommended, PPM™ does not adversely affect in vitro seed germination, callous proliferation, or callous regeneration. Seeds and explants with endogenous contamination can be sterilized at doses of 5–20 mL/L of PPM™. This is useful when routine surface sterilization is insufficient.