

Preparation of Stock Plants

-Prior good care of stock plants may lessen the amount of contamination that is present on explants.

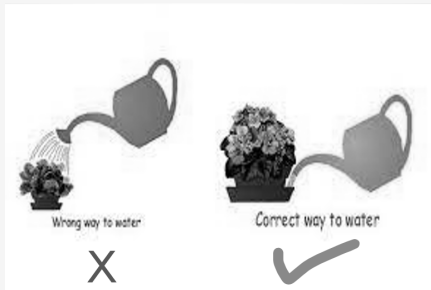
-Transfer plants to a greenhouse to reduce endemic contaminants

-Plants grown in the field are typically more "dirty" than those grown in a greenhouse or growth chamber, particularly in humid areas like Malaysia.

-Sources of contamination:

- Overhead watering increases contamination of initial explants
- Splashing soil on the plant during watering will increase initial contamination.

Correct Way to Water a Plant



Ways to have "Cleaner" Stock Plants

- Force outgrowth of axillary buds

-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 detergents and then placed under running water for 1 to 2 hours.

- Remove surface contaminants by rinsing with sterilizing solutions

- Treatment of stock plants with fungicides and/or bactericides is sometimes helpful.

Fungicides

-pesticides that kill or prevent the growth of fungi and their spores

-used to control fungi that damage plants, including rusts, mildews and blights

-used to control mold and mildew in other settings

Bactericide or Bacteriocide

-substance which kills bacteria

-it is disinfectants, antiseptics, or antibiotics

Sterilization of Explants

-Plants growing in the external environment are invariably contaminated with micro-organisms and pests

-These contaminants are mainly confined to the outer surfaces of the plant, although, some microbes and viruses can even grow within the root.

-Most of the surface microbes do not form tight associations with the plant tissue.

-They are easily removed from the explant by gentle rinsing, and the remaining are killed by surface sterilization.

-Place the explant in a 70% ethyl alcohol (ethanol) solution prior to treatment with another disinfectant solution.

-Conducting the sterilization process under vacuum results in the removal of air bubbles and provides a more efficient sterilization process.

Sterilant

- Oxidants
- Active halogens
- Heavy metal poisoning
- Powerful chemicals such as concentrated sulphuric acid may be used on seeds.

-cut surfaces should be protected

Common Explant Sterilization Agents

- Sodium Hypochlorite
- Ethanol or Isopropanol
- Calcium Hypochlorite
- Mercuric Chloride
- Hydrogen Peroxide

1. Sodium Hypochlorite

- most frequent choice for surface sterilization
- readily available and can be diluted to proper concentrations
- 5.25% sodium hypochlorite (laundry bleach) → 0.5 - 1.0% sodium hypochlorite (sterilant)
- 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.

2. Ethanol or Isopropanol

- powerful sterilizing agent but also extremely phytotoxic
- plant material is typically exposed to it for only seconds or minutes
- more tender the tissue, the more it will be damaged by alcohol
- Generally 70% ethanol is used prior to treatment with other compounds.
- 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.

3. Calcium Hypochlorite

- obtained as a powder and must be dissolved in water
- concentration that is generally used is 3.25 %
- solution must be filtered prior to use since not all of the compound dissolve into solution
- Calcium hypochlorite may be less injurious to plant tissues than sodium hypochlorite

4. Mercuric Chloride

- used only as a last resort
- concentration of 0.1-1.0% denatures proteins
- 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.

5. Hydrogen Peroxide

- concentration of 30% is used, which is 10 times stronger than that obtained from pharmacy
- useful for surface-sterilizing material while in the field

Enhance Effectiveness of Sterilization

- Surfactant/ wetting agent (e.g. Tween 20 or Triton-X) is frequently added to the sodium hypochlorite.
- Wetting agents makes the surface of plant wet and repel the air, thus making the treatment more effective.
- The solutions that the explants are in are often shaken or continuously stirred.

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

Use of Antibiotics and Fungicides in vitro

- The use of antibiotics and fungicides in vitro is not very effective in eliminating microorganisms
- These compounds are often quite phytotoxic.

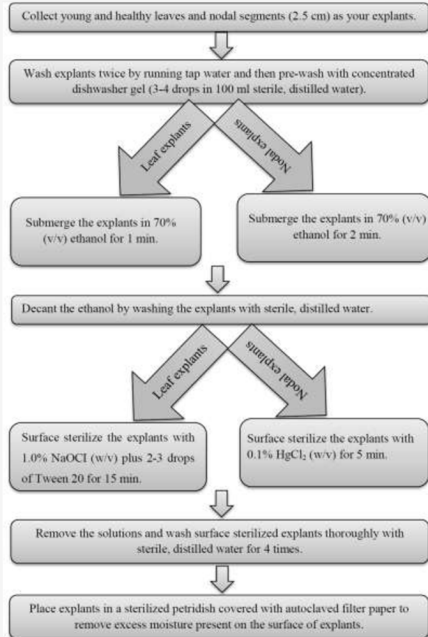
If Sterilization is a Problem

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| 1. Seeds can be used instead | -Most seeds have hard outer coat that can withstand harsh and prolong sterilization procedure |
| | -Germinate the seed in vitro to obtain axenic seedlings providing sterile meristems, leaves, roots and stem nodes for culturing |
| 2. Grow plant under controlled environment | -Enclosed environment to prevent accumulation of dust |
| | -Treatment with fungicide and to lesser extent antibiotics |
| | -Clean organs can be collected later |

If Sterilization is a Problem (cont)

*Axenic: state of a culture in which only a single species, variety, or strain of organism is present and entirely free of all other contaminating organisms

Explant Sterilization Process



Phytotoxic

-poisonous to plants

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By **woozing**
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