Protocols & Procedures

The procedures described below are generic. Slight modifications might be needed for your specific plant species. For assistance, contact Labconsult.

PPM™ significantly simplifies the tissue culture working procedures as follows:

1. Media containing PPM™ may be dispensed outside the laminar flow hood (LFH) exposed to the ambient air. The plates should be covered soon after agar solidification. In the event that media dispensing is done by a pump, we recommend passing autoclaved hot water through the hoses prior to and after media dispensing.

2. Heat sensitive or heat stable liquid media containing PPM™ does not need to be filter sterilized or autoclaved provided that it will be stored in sterile containers and that the stock solutions are not contaminated. In rich media containing 200 mg/liter or more of amino acids or proteins, it is recommended to filter the media with the PPM™.

3. If working in the LFH the utensils (forceps or scalpels) do not need to be flamed. They should be periodically dipped in 70% alcohol. The LFH does not need to be certified and the work can be done as well outside the LFH on a clean surface for a period not exceeding 1 hour.

4. PPM™ is less effective when exposed to high density of bacteria or fungi spores found regularly on seed's coat. For in vitro germination, seeds should be conventionally surface sterilized with EPA registered bleach. Therefore, in the presence of PPM™ (in the germination medium), the seeds can be rinsed under tap water in a non-sterile strainer and left to dry preferably in the LFH. If the utensil ends have touched active bacteria, fungi culture or otherwise suspected of being contaminated, they should be sterilized by autoclave or by use of an electric heating element.

5. General Dosage levels: With the exception of endogenous contamination, the recommended dose range is 0.05%-0.2%. (For callus proliferation, organogenesis and embryogenesis, the recommended range is 0.05-0.075%.) To eliminate higher endogenous contamination densities, higher doses of PPM are needed (see paragraph 6 below).

6. Endogenous Contamination: (a) For explants: gently and routinely shake / stir 1 cm. long explants (or shorter) for 4-12 hours in 4-5% v/v PPM™ solution supplemented as above with full MS strength basal salts without pH ing and without Tween 20. Without rinsing, insert into a medium supplemented with 0.05 - 0.1% PPM™ for herbaceous plants and 0.2% PPM™ for woody plants.

Note

Paragraphs 6(b) through 10 below are intended for ornamental plants only.

(b) For tubers, bulbs and scales: shake / stir the entire tuber / bulb / scale in bleach. Rinse with water (can be done under non-sterile conditions). Slice the tuber / bulb / scale to thin slices. Shake / stir for 12-24 hours in 4 - 5% PPM™ solution supplemented with full strength basal salts without pH ing and Tween 20. Without rinsing, insert into a medium supplemented with 0.1 - 0.2% PPM™.

7. In cases where the above protocols do no yield satisfying results (especially thick explants, highly infested explants, seeds), we recommend the following:

(a) Shake / stir the explants in water (1hr for soft tissues and 2 hr for hard tissues).

(b) Shake / stir the explants in (50%) PPM™ supplemented with full strength MS basal salts (without pH ing and without Tween 20) for 5 -10 minutes.

(c) Without rinsing, insert the explants into the medium. In fungal contamination, the addition of PPM™ to the medium is optional. However, with bacterial or mixed contamination, the addition of 0.05 - 0.2% PPM™ to the medium in the first month is essential. Do not discard highly oxidized explants as approximately 50% of the explants will recover within 4 - 6 weeks.

Note

Refer to notes 2 and 3 below.

8. To eliminate Agrobacterium:

After co-cultivation, rinse the leaf discs with water. Dip (entirely) the transfected discs in a 100% PPM™ solution (supplemented with full strength basal salts) for approximately 2 minutes. Blot the discs between two sterile paper towels and place onto a medium supplemented with full-strength of the commonly used antibiotics. After 3 weeks, transfer to the medium with solely PPM at 0.05 0.075%

General Notes:

1. For the first transfer following the sterilization with PPM™, we recommend to insert the explants entirely into a semi-solid medium.

2. The 50% PPM™ solution can be reused but is not recommeded. The number of uses depends on the volume of the explants treated and the inoculum density. Keeping the 50% PPM™ solution stored at 4ºC prolongs its activity. If necessary, prepare two PPM™ solutions: one to disinfect endogenous contamination and the second, to disinfect "in-culture" contamination. The second solution should be filtered after each treatment, using 0.2 micrometer Millipore. The filtration process can be done in non-sterile atmosphere. A single filter can be used for the entire "lifespan" of the solution.

3. In cases where the treatment with 50% PPM™ is still insufficient, full strength PPM™ (100%) can be used. The treatment with 100% PPM™ is similar to the one described above for 50% PPM™, however, the exposure time should not exceed 10 minutes.

Summary

PPM™ most definitely will facilitate the work in any plant tissue laboratory and should significantly increase technician and laboratory productivity. However, conditions in each lab may vary which could have a bearing on the effectiveness of PPM™. It is advisable that staff follow the above guidelines initially and adjust parameters accordingly.

When used as recommended:

- PPM™ is effective against airborne contamination, waterborne contamination and contamination introduced from human contact.
- If used correctly, PPM™ will eliminate endogenous contamination from explants.
- At recommended doses (0.5 - 2ml/l), PPM™ does not impair in vitro seed germination, callus proliferation, callus regeneration, and axillary or adventitious buds' induction.

Safety and Handling - See MSDS

DO NOT CONCENTRATE THE MATERIAL
DISPOSAL INFORMATION:
Dispose of media containing PPM™ in the same manner in which you dispose of media without PPM™. In an emergency, contact the following numbers: 1 (202) 271-0328 or +1.800.746.8535. A toxicological assessment has been performed by a qualified toxicologist. This assessment is available upon request. For more information on PPM™, or to request test results contact: Tel: 1.202.778.8522 ex. 0, Fax: 1.202.429.9812