

LAB EXERCISE Seed Culture

Objectives

1. To teach the students the proper procedure in sterilizing seeds for tissue culture.
2. To show the students different protocols used in sterilizing different explants.

Materials and Method

Each group followed the protocol assigned and a lab report was prepared based on the format provided.

Protocol: Sterilization of seeds

1. Seeds washed under running water for 10 minutes to clean off debris and dirt.
2. The following was prepared while waiting for the seeds to be washed:
 - a. 20% commercial bleach solution: 40ml of commercial bleach added to 160ml of sterile distilled water in a sterile beaker.
 - b. 70% alcohol: 70ml absolute alcohol added to 30ml sterile distilled water in a sterile beaker.
3. After 10 minutes of washing, seeds were submerged in 20% commercial bleach solution + 2 drops of surfactant Tween 20 for 20 minutes with constant agitation.
4. Seeds were washed three times with sterile distilled water.
5. Solution was discarded and seeds were swab with 70% alcohol for 1 min.
6. The whole seeds was plated out on agar medium with different concentrations of hormones:
 - a. 4mg/l NAA
 - b. 4mg/l BAP
7. The petri dish was sealed with parafilm and incubated under 16 hours photoperiod.

Data Observation and Preparation of Report

Culture was observed at every interval of 5 days up to 30 days. Report on the observation was prepared and the result of this laboratory exercise was discussed.

Data and Results

Medium with 5mg/l NAA

Changes/Date	2609	0110	0610	1110	1610	2110
% Contamination	-	-	-	-	-	-
% Color change	-	stem is white in colour	stem is brownish in colour	brown colour spot on stem		
Morphological change	root formation	root formation	root growth increase	normal root grown		

Discussion

1. Seeds should be washed under running water for 10 minutes to clean off debris and dirt. Washing for shorter time is inadequate while too long will suffocate the seeds and may cause other, undesired mechanical injuries.
2. Typical commercial bleach solution used is Clorox, which contains approximately 5.25% of sodium hypochlorite as active ingredient. 20% Clorox = 1.05% active ingredient.
3. Seeds were submerged in 20% commercial bleach solution + 2 drops of surfactant Tween 20 for 20 minutes to allow more and better penetration. Using higher concentration may shorten the time but only kills superficial undesired element or penetrates exterior area only.
4. Seeds were washed three times with sterile distilled water (step 4) to minimize Clorox presence and activity.
5. Temperature when culture was incubated under 16hr photoperiod is around 24-27°C.
6. NAA (1-Naphthaleneacetic acid) is a white powder, with the molecular formula $C_{12}H_{10}O_2$. NAA is a plant hormone in the auxin family and is an ingredient in many commercial plant rooting horticultural products; it is a rooting agent and used for the vegetative propagation of plants from stem and leaf cutting.
7. BAP (6-Benzylaminopurine; Benzyl adenine) is the first generation synthetic cytokinin, which elicits plant growth and development responses setting blossoms and stimulating fruit richness by stimulating cell division.
8. Parafilm is stretchable, moldable, waterproof, odorless, thermoplastic, semitransparent and self-adhering sealer. It is also used to further seal a lidded container against moisture for long-term storage – makes it perfect to use in tissue culture laboratory.

Conclusion

Proper procedure in sterilizing seeds for tissue culture and different protocols used in sterilizing different explants was studied and get familiar with.