Aim 48

To Inoculate Seeds on MS Medium

Introduction

The medium used in *in vitro* propagation is sterilized before use. The all the procedure of inoculation is carried out in aseptic conditions in laminar air flow chamber. The explants are surface sterilized to check the contaminants present on the surface of the explant and protect the living tissue inside. Aseptic manipulation are conducted in a closed transfer area filled with UV lamp and sterilized air flowing inside the culture room. UV sterilized the inner portion of chamber before performing the inculcation. In the laminar air flow chamber the HEPA filter block dust particles and microbes in the air.

Requirements

Viable seed of sunflower, culture tubes, petridish, $HgCl_2$/chlorinated water, rectified spirit, distilled forceps, burner, Teepol-20 and transfer needle etc.

Procedure of inoculation

1. The culture tubes containing M.S. medium, forceps, rectified spirit, petridish etc. (Except any living material) are put in the laminar air flow chamber and switch on the U.V. lamp for 30 min.
2. Outside the laminar air flow chamber, the sunflower seeds are washed under running tap water for about 10 minutes using liquid detergent like Teepol-20 to remove any adhering dust particles.
3. U.V. is turned off and air flow is turn on before the chambers wall is opened. Hand are sterilized with

rectified spirit before starting to work in laminar chamber.

4. The petridish/forceps/bottles etc in the cabinet and floor of cabinet is flame sterilized.
5. The seeds are brought inside the laminar air flow chamber and are transferred to the freshly prepared solution of sterilizing agent $HgCl_2$ for 6 minutes or chlorine water in a petridish/bottle, for 15-30 minutes with the help of forceps.
6. Finally, rinse the seeds in sterilized D.W. for 3-4 times and keep it ready for inoculation in a flame sterilized petriplate inside the laminar air flow.
7. The cotton plug of culture tube is opened near the flame. Forceps used for transferring the seeds is flame sterilized every time and be used after it get cooled inside the laminar air flow. The seed is then inculcated on the medium. The mouth of the tube should be always over the flame while transferring. Then, the cotton plug is put back rotating the tube on all side in front of flame.
8. Similarly, rest of seeds are also inoculated aseptically in separate culture tubes.
9. Then, put all the tubes in maintained in culture room maintained with photoperiod and temperature of 25°C for 10 days to observe the germination.

Precautions

1. Totally aseptic condition of the whole process is very much necessary. Otherwise there will be microbial contamination.
2. Hands should be rinsed off with alcohol, explants should be surface sterilized.
3. The transferring of seed to culture tube should be right in front of the flame and forcep should be flow sterilized each time.

4. Forcep/petriplates etc be cooled after flame sterilization before taking them in contact with the seeds because heat will the kill the living embryo inside the seed.