

Student 2.

Steps within Tissue Culture that Provide Conditions that are Important for Successful Propagation.

Steps that are very important in plant tissue culture are collecting the correct sample, making it sterile, correct hormone concentrations, then growing process and hardening off.

First of all, a sample has to be collected from a plant that you want to have more of. For example, if a plant is a good fruiter you would want to have a whole crop of them, you can use an asexual propagation technique like tissue culture. To do this, you take a sample off the plant, it must demonstrate totipotency, normally pieces of plant leaves or sometimes the roots are used as these parts demonstrate totipotency and the collection of these does not harm the parent plant [2].

Secondly these explants are taken and put through a strict sterilisation process involving glass that is heated under pressure, the heating under pressure is to render the glass aseptic. The sterilisation process of the explants involves them being rinsed in an alcohol or bleach solution, being swirled then rinsed after which they are really aseptic. The water must be pure to reduce contamination. The sterilisation is extremely important as any bacterial or fungal growth in the extremely nutrient rich tissue culture solution would destroy the explants, rendering it useless, the tissue culture would have to be repeated which is costly. So the explants and glassware must be totally aseptic before it placed in the tissue culture medium [2].

After it is rendered aseptic, the explants is placed in a growth medium which is rich in specific hormones (auxin hormones like IAA and NAA, these cause a proliferation of roots) and cytokinins hormones (these cause a proliferation of shoots). These hormones need to be correctly proportioned if the two hormones are in equal proportions a mass of cells form, it is called a callus [2]. The solution is also rich with inorganic salts like ammonium nitrate and organic compounds also vitamins specific to early plant growth. The solution needs to have the nutrients in correct proportions for correct plant growth, too much or too little can cause problems, such as too much nitrogen means the plant grows fast but is brittle and unhealthy. These solutions are chosen over soil for many reasons. They can be scientifically designed for the individual plant with correct hormone and nutrient concentrations also the solution can be aseptic, soil is hard to make aseptic. So these solutions give the explants the best possible chance at growing strong healthy plants [1].

After the explants have reached a stage where they have developed a good root system, 2 healthy leaves with no curling, and a certain size depending on the species of plant. For example, *Pinus radiata* must be 10 – 15 cms tall from shoot to highest part before transplantation can begin. When the explants have reached these milestones, they are removed from the tissue culture media and begin the process of hardening off. This occurs when they are taken out and put into soil in greenhouse where overtime the conditions go towards what the outside environment will be like. This is to get the plants used to the outside environmental conditions, instead if the tissue culture medium, the plants will undergo less shock when transplanted to the field so less plant will die and the process of tissue culture will be successful [1].