THE TECHNIQUE OF PLANT TISSUE CULTURE

The technique of in vitro cultivation of plant cells or organs is primarily devoted to solve 2 basic problems -

(i) First, to keep the plant cells and organs free from microbes, i.e bacteria and fungi.

(ii) Second, to ensure the desired development in the cells and organs by providing suitable nutrient media and other environmental conditions.

The technique of tissue, cell and organ culture is described as follows -

(1) SURFACE STERILIZATION, →

(i) The plant part excised for the in vitro cultivation is known as explant.

(ii) Any plant part may be used as an explant like roots, leaves or specific cell type

such as pollen or endospore.

(iii) The explant ~~may~~ must be surface sterilized to eliminate bacteria and fungi present on their surface.

(iv) This is commonly achieved by treating them with 1-2 % soln of sodium or calcium hypochlorite or with 0.1% soln of mercuric chloride.

(v) The explant is then rinsed several times with sterilized distilled water to remove the disinfectant.

(vi) obviously, this and the subsequent handling of the explants or cultured cells and organs has to be done under aseptic conditions.

(2) NUTRIENT MEDIUM →

Any nutrient preparation in which plant cells and organs are cultured is known

as nutrient medium, culture medium or simply medium.

Media for plant cell cultures can be classified as containing 6 types of components —

(i) MACRO-NUTRIENTS. → Elements usually termed required in large amount of N, Ca, Mg, K, P, S these are termed as macro-elements or macronutrients.

(ii) MICRONUTRIENTS. → Elements usually required in lesser amount than macro-nutrients are termed as micronutrients. These includes Zn, Co, Cu, Mn etc. ($CuSO_4 \cdot 5H_2O$, $ZnSO_4 \cdot 7H_2O$, $MnSO_4 \cdot H_2O$)

(iii) VITAMINS. → organic supplements such as vitamins are used. These include Nicotenic acid, Thiamine HCl, m-Inositol etc.

(IV) PLANT GROWTH REGULATORS. →

(a) Growth regulators such as auxins and / or kinins are.

commonly used.

(b) 2, 4-D is the most commonly used auxins, NAA and IAA are also used.

(c) Similarly, kinetin and benzyl aminopurine (BAP) are the most commonly used kinins.

(3) ~~ENVIRONMENTAL CONDITIONS~~ →

(v) Iron source (FeSO4 with other compounds that allows slow release into the medium.

(vi) Carbon source usually sucrose.

(3) ENVIRONMENTAL CONDITIONS →

The organ and cell cultures are maintained under a controlled environment, particularly in terms of -

(a) Temperature - The temperature may vary from 18-25°C depending upon species and the purpose of culture.

(b) Light → Light is not essential

for cell and tissue culture, but it is often beneficial for plantlet regeneration and for embryo and meristem cultures.

(4) SUBCULTURING →

After a period of time, it may be necessary to transfer organs and tissues to fresh media. This is known as sub-culturing.

In general, callus cultures are subcultured every 4-6 weeks, while suspension cultures need to be subcultured every 3-14 days.

(5) PLANT REGENERATION AND TRANSFER TO SOIL

The ultimate objective of the application of *in vitro* techniques to crop improvements is to obtain full plants and to transfer them successfully to soil.