## 1. INORGANIC NUTRIENTS

A number of inorganic nutrients are required for normal growth of the plants. Besides, carbon, hydrogen and oxygen, other 12 elements essential for plant growth include nitrogen, calcium, potassium, phosphorus, sulphur, magnesium, iron, manganese, copper, boron, zinc and molybdenum. Out of these, former six elements are macronutrients as these are required in comparatively larger quantities (concentrations more than 0.05 mmol $l^{-1}$), while the latter six are classed as micronutrients as these are required in smaller quantities (less than 0.05 mmol $l^{-1}$). In White's medium iron is added in the form of Ferric sulphate ($Fe(SO_4)_3$) but in most of the other tissue culture media iron is supplied in the form of Fe. EDTA. Nitrogen is furnished in the medium in the forms of nitrate (oxidised) and ammonia (reduced).

Deficiencies of the macroelements N,P,S,K, Mg and Ca are more easily manifested in tissue culture when cells are cultured in liquid rather than on agar media since impurities present in the agar are considerable. Of all the mineral nutrients, the form of nitrogen is responsible for the most pronounced effects on growth and differentiation of cultured tissues.

## 2. ORGANIC NUTRIENTS

Cultured plants accomplish better growth when medium is supplemented with organic nutrients such as amino acids and vitamins. The most commonly used vitamin is thiamine (vitamin $B_1$). Other vitamins which improve the growth of cultured plants include nicotinic acid, calcium pantothenate and pyridoxine. Besides these, biotin, folic acid and aminobenzoic acid are added in various concentrations in some of the media.

As a carbon source, sucrose is the most preferred carbohydrate. Glucose, fructose, maltose, galactose, mannose and lactose are the other favourable sugars.

In order to promote growth of callus, some complex semisynthetic substances are added in the medium. These include yeast extract (YE), coconut milk (CM), casein hydrolysate (CH), tomato juice (TJ) and malt extract (ME).

## 3. GROWTH HORMONES

In addition to inorganic nutrients, vitamins and the carbon source, basal medium is supplemented with growth hormones such as auxins, cytokinins and gibberellins.

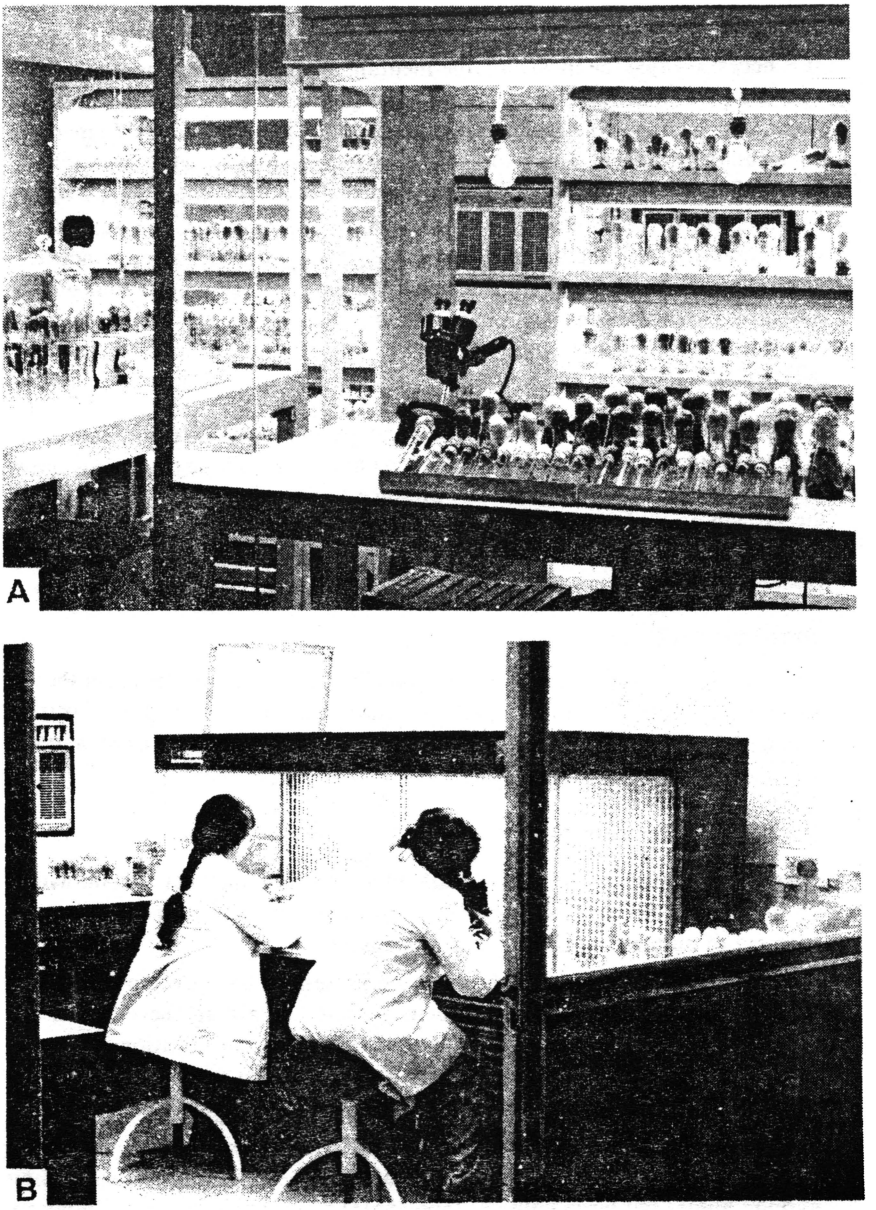


**Fig.2. A.** A view of one of the culture rooms of the Tissue Culture Laboratory, National Botanical Research Institute, Lucknow, **B.** Inoculation work being performed in a laminar flow cabinet.

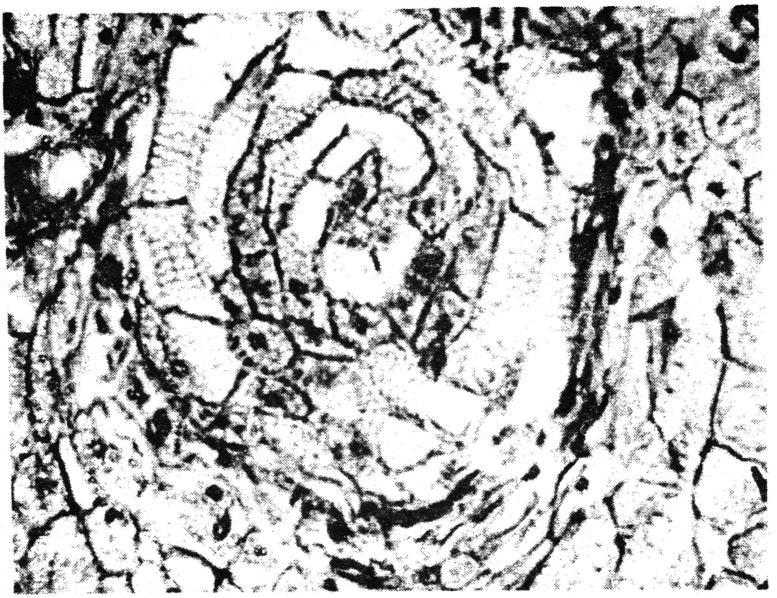

**Fig.3.1.** Cross-section of somatic callus of *Rauvolfia serpentina* showing differentiation of tracheary elements as concentric nests amongst the parenchymatous cells of callus.

### 4. Physical Factors

The effect of light on vascular tissue differentiation varies between cultured tissues. In carrot phloem slices, light induces cytodifferentiation via the synthesis of cytokinin. Light stimulates wound vessel differentiation in *Coleus.* In *Sinapis* tracheary element formation in the hypocotyle is controlled by phytochrome.

Temperature also affects cytodifferentiation. In Jerusalem artichoke callus cultures grown at a temperature below 17°C did not show vascular strands but an increase in temperature within the range of 17-31°C promotes vascular differentiation.

pH of the medium greatly affects cytodifferentiation. In *Zinnia* the optimum pH was found to be 5.0. pH below and above 5, results in the decrease of tracheary elements differentiation.

## CYTODIFFERENTIATION AND CELL CYCLE

Fosket (1968) for the first time suggested a close relationship between tracheary element differentiation and the cell cycle. He observed that in cultured *Coleus* stem segments inhibitors of DNA synthesis (mitomycin C, fluorodeoxyuridine) and inhibitor of mitosis (colchicine) prevented cytodifferentiation. In such cases cell division preceded the appearance of xylary elements. This led Fosket to conclude that cell division is essential for the induction of tracheary element differentiation.

The idea that cell division is not always a prerequisite for tracheary element differentiation was putforth by Torrey (1975). He observed that in cell cultures of *Centaurea cyanus* some of the single parenchymatous cells directly gave rise to tracheary element without cell division. The work of Torrey got support from the findings of Kohlenbach and Schmidt (1975) and Fukuda and Kommine (1980) who reported that mature mesophyll cells of *Zinnia elegans* can differentiate to tracheary elements without undergoing cell division.

It has been reported that DNA replication is not necessary for cytodifferentiation but some studies have suggested the involvement of minor DNA synthesis in differentiation. The regulation of gene expression has been studied using an inhibitor 5-Bromodeoxyuridine. Tracheary element differentiation from parenchyma cell is prevented by this inhibitor in pea and *Zinnia* but cell division is not affected. This further indicates that replication of whole genome and synthesis of specific DNA sequences are essential for cytodifferentiation. In cultured cells with the increase in endopolyploidy, differentiation ability of cells may decline.

In cultures of immature embroys of *Panicum,* the scutellum produced a compact callus that produced several cup-shaped structures which subsequently differentiated into embryoids. These embryoids germinated when auxin concentration was either reduced in the medium or omitted completely. On the other hand, callus obtained from mature embryos gave rise to shoot buds followed by differentiation and advetitious buds. For induction of embryogenic callus, the developmental stage of the inflorescences where floral primordia have just formed showed best response.

## NUTRITIONAL REQUIREMENT

A nutrient medium is needed for the initiation and maintenance of callus cultures. Usually basal medium which consists of a mixture of mineral salts, vitamins, sucrose and phytohormones is used for initiating the callus. The basal medium is supplemented with coconut milk, yeast and malt extracts for additional nutritional requirement of the callus. In forage grasses, supplementation of MS (Murashige and Skoog, 1962) medium with only 2,4-D (2,4 Dichiorophenoxy-acetic-acid) is sufficient to induce callus formations. The continued proliferation and growth of the callus in subsequent subcultures need additional growth hormones or reduced level of 2, 4-D.

For callus induction and proliferation requirement of different growth hormones varies viz., addition of 2, 4-D is essential for callus induction and proliferation. It is not required at the time of organogenesis and plantlet formation. Usually, a high concentration of auxin and low concentration of kinetin promote callus induction and proliferation.

Tissues derived from the green photosynthetic organs may grow in cultures without addition of exogenously supplied sugar. However, for continued growth of callus, presence of exogenously supplied sugar becomes indispensable. Israel and Steward (1966) reported that callus cell in cultures are deficient in some enzymes and cofactors which are essential for synthesis of chlorophyll or proper functioning of chloroplast.

## VARIATION IN CALLUS CULTURES

The genetic stability of callus cultures is one of the most important factors that determines the successful utilization of cell cultures for clonal propagation. It has been observed that spontaneous changes occur in the genetic makeup of the prolonged callus cultures. These spontaneous changes may include cytoplasmic structures such as mitochondria and plastids or chromosomes. The cytoplasmic changes may occur either through assymetric cell division or mutation. The chromosomal changes may involve increase in chromosome number, chromosomal breakage, gain, loss or reunion. In callus