INTRODUCTION

I N T R O D U C T I O N

Basically three processes, growth, cellular differentiation and morphogenesis, which occur concomitantly, can be viewed as constituting the development of an organism. Growth is permanent, irreversible increase in size and dry matter during the course of whole plant development. Cellular differentiation is the transformation of identical cells, arising from a common progenitor, into diverse cell types with different structural, physiological and biochemical specializations as a result of differential manifestation of genetic control. Morphogenesis refers to the phenomenon of the inception and development of form and structure in plants. It is the resultant effect of all the physiological events associated with growth and cellular differentiation which are regarded as 'antecedent'. It is a process of higher magnitude than events occurring in single cells, wherein inter-tissue relationships and interactions among cells can not be ignored, as they constitute profound influence on the collective fates of individual cells in their growth and development (Wardlaw, 1968).

In biology the regulation of growth and development has, by far, remained the most mysterious and important problem. What requires demonstration is, how a particular physiological

process, or a system of biochemical reactions, can be closely and directly related to the ultimate visible reality i.e. the manifestation of form. This is, of course, a very rigorous requirement, demanding many and varied experimental approaches. Consequently tools and techniques of functional and analytical biochemistry, cytology and molecular biology are brought to bear (Wardlaw, 1968; Wareing, 1971).

Since all the information needed to specify an organism is contained in its DNA and if DNA is replicated in each cell of an organism, it follows that one should be able to reproduce any organism from any one of its enucleated cells. This doctrine of 'totipotency' has been vindicated in quite a few plant species (Price, 1970). Fundamentally, all morphogenesis begins with a single cell, be it a spore, a zygote or a generative cell. It takes cognizance of genetic factors, present in a cell, to be evoked in a characteristic sequence of early and embryonic development, the inception, development and final conformation of organs, the differentiation of tissues, and the harmonious development and integrated wholeness, or unity of the individual. Regulation of physiological and biochemical activities, from the inception lead to differentiation of characteristic organs and tissues, with distinctive functions. Whether the biochemical investigations are carried out of cell free systems or studies are made of morphological features of

whole plant, the objectives essentially remain the same : to understand how one cell gives rise to hundreds of thousands of specialized cells, partitioned into inter-dependent, interacting tissues and organs of a living entity (Wardlaw, 1968).

Differentiation in the intact plant is regarded as a complex phenomenon. Can this complexity be dissected in cultured tissues permitting interpretation and understanding at various levels of organization from free cells to callus to organs? Inspite of the fact, that in last few years considerable information has been accumulating regarding factors which induce and control differentiation, the sum total of the accumulated knowledge is still fragmentary. In an attempt to understand these better, more and more use has been made of cultured tissues. The value of the technique of tissue culture is that cell and tissue systems can be subjected to direct experimental control, to some extent free from the complex interactions occurring in the intact plant (Bornmann, 1974). Recent advances in plant tissue culture technique have opened up avenues for investigating genic and epigenic control of metabolic behaviour of cultured cells, tissue and organ explants during their growth and morphogenesis (Street, 1974).

The years 1902 - 1934 were most distressing and

discouraging in the history of plant tissue culture. During this period all attempts to induce isolated cells into generating tissue masses by cell division failed. Kotte's (1922 a, b) and Robbins' (1922 a, b) success with achieving limited growth of root explants of Pisum, Zea and Gossypium was, however, a bright spot during this period. Robbins and Maneval (1923, 1924) reported the beneficial effect on growth of illumination of root tips from a wide range of species. Gautheret (1935) also reported the growth of root explants in culture. The growth was initially vigorous but strictly limited in duration. These studies opened up the technique which, particularly in the hands of Philip White, led to the establishment of continuously growing root cultures. White (1934) by substituting dextrose with sucrose, succeeded in repeatedly subculturing excised tomato roots without diminution of growth rate.

Contemporary with these papers came the report by Nobecourt (1938 a, b) that explants of cambium of potato and carrot could be grown in culture and made to proliferate. Gautheret (1939) reported formation of a mass of "undifferentiated" tissue from carrot explants capable of repeated subculture. About same time White (1939) reported continuous culture of a similar undifferentiated callus derived from procambial tissue of young stems of hybrid Nicotiana

(N. glauca X N. lougsdroffii). These callus cultures of Gautheret and White were the fore-runners of similar cultures derived from a multitude of plants, both dicotyledonous and monocotyledonous.

Yet another technical advancement in the field of plant tissue culture came in 1954, when Muir, Hildebrandt and Riker reported the growth of liquid cultures containing single cells and small cell clumps of Nicotiana tabacum and Tagetes erecta.

In 1954, Muir et al. isolated single cells from their suspension cultures and placed these singly on the upper surface of squares of filter paper whose lower surface was in contact with an actively growing "nurse" callus. A high proportion of the single cells underwent divisions and gave rise to small callus masses. Jones, Hildebrandt, Ricker and Wu in 1960, examined the growth of separated cells of hybrid tobacco (N. tabacum X N. glutinosa) in hanging drop culture. Using fresh medium they observed cell divisions only when a high number of cells were present in the drop. Bergmann (1960) using suspension cultures of Nicotiana tabacum and Phaseolus vulgaris, obtained filtrates containing a high proportion of single cells. These he spread over the surface of a thin layer of culture medium solidified with agar. He observed about 20% of these plated cells underwent repeated divisions and gave rise to single cell clones.

In recent years the enzymatic isolation and culture of

plant protoplasts has caused tremendous impact on plant tissue culture technology. It has opened up new 'vistas' with its far-reaching implications in tackling fundamental and pragmatic problems in plant biology e.g. physiology, pathology, cytology, genetics, virology etc. The field was thrown open by Cocking (1960) when he isolated plant protoplasts enzymatically. Since then protoplasts have been isolated from almost every plant organ. To cite a few examples protoplasts were isolated from : roots (Cocking, 1960; Kameya and Uchimiya, 1972), leaves (Takebe et al., 1968; Power and Cocking, 1970; Potrykus and Durand, 1972; Kohlenbach and Bohnke, 1975; Kuchko and Butenko, 1977; Bajaj et al., 1978), coleoptile (Hall and Cocking, 1974), fruit tissues (Gregory and Cocking, 1965), pollen mother cells and pollen tetrads (Bhojwani and Cocking, 1972; Bajaj and Cocking, 1973; Bajaj, 1974), pollen grains (Bajaj, 1975), callus cultures (Eriksson and Jonasson, 1969; Butenko and Ivantsov, 1973; Gosch et al., 1975 a, b). Cell wall regeneration was studied in protoplasts in culture (Pojnar et al., 1967; Takebe and Otsuki, 1973; Bajaj et al., 1975 a). Protoplast culture has become a very powerful tool for genetic engineering. Protoplasts have been shown to take up viruses (Cocking, 1966; Aoki and Takebe, 1969; Takebe and Otsuki, 1974), bacterial protoplasts (Davey et al., 1973), DNA (Ohyama et al., 1972), Chloroplasts (Potrykus, 1973; Bounett and Eriksson, 1974) and nuclei

(Potrykus and Hoffmann, 1973). In quite a few species embryogenesis or whole plant regeneration has been achieved. Some notable examples are : Atropa belladonna (Gosch et al., 1975b; Bajaj et al., 1978), Daucus carota (Gosch et al., 1975a), Nicotiana tabacum (Nagata and Takebe, 1971; Takebe et al., 1971; Bajaj, 1972; Gleba et al., 1974; Bajaj et al., 1978), Petunia (Binding, 1974; Hayward and Power, 1975), Pisum sativum (Constabel et al., 1973).

In plant tissue culture serendipity has had its share. In 1964, when Guha and Maheshwari cultured anthers of Datura innoxia they observed the formation of embryos from dehisced anthers. Guha and Maheshwari (1966, 1967) traced the origin of embryos to pollen grains and found the plants to be haploids. Bourgin and Nitsch (1967) obtained complete haploid plants of Nicotiana tabacum from anthers in culture. Nitsch (1974a, 1974b, 1975, 1977) demonstrated androgenesis in isolated pollen. The culture of isolated pollen has been thoroughly exploited (Reinert et al., 1975; Bajaj et al., 1976). Haploid plants or tissues have been obtained from a large number of plants. Some notable ones are : Arabidopsis thaliana (Gresshoff and Doy, 1972), Asparagus officinalis (Raquin, 1973), Atropa belladonna (Zenkteler, 1971; Rashid and Street, 1973; Bajaj et al., 1978), Capsicum annum (George and Narayanaswamy, 1973), Hordeum vulgare (Clapham,

1973), Nicotiana tabacum (Nakata and Tanaka, 1968; Nitsch, 1969; Nitsch et al., 1969; Nitsch and Nitsch, 1969; Bajaj et al., 1976., 1978), Oryza sativa (Niizeki and Oono, 1971), Solanum tuberosum (Dunwell and Sunderland, 1973), Triticum aestivum (Picard, 1973; Bajaj, 1976), Zea mays (Murakami et al., 1972), Lycopersicum esculentum (Debergh and Nitsch, 1973).

Many attempts had been made earlier than 1964, to culture microspores and stimulate them into producing embryoids or haploid tissues. The first haploid tissue was obtained from pollen grains of Ginkgo biloba by Tulecke (1953, 1957). Later on haploid callus was also obtained from Taxus baccata (Tulecke, 1959; Zenkteler and Guzowska, 1970), Pinus resinosa (Bonga and Fowler, 1970), Ephedra foliata (Konar, 1963), Thuja orientalis (Rao and Mehta, 1969), Torreya nucifera (Tulecke and Sehgal, 1963), Tradescantia reflexa (Yamada et al., 1963), Oryza sativa (Oono, 1975), Datura innoxia (Sharma and Chowdhury, 1977), Lycopersicon esculentum (Sharp et al., 1972), Nicotiana tabacum (Bajaj, 1972), Triticum aestivum (Bajaj, 1977).

The need for successful culture of organs, tissues, cells and protoplasts resulted in continued efforts and rapid development of culture media, which normally contain mineral salts of which the most essential are nitrogen and carbon sources, and growth factors. From the very beginning it became apparent that the nutritional requirements become increasingly

complex from organ to tissue to cell and then to protoplast culture.

Nitrates are the most commonly used nitrogen sources. The incorporation of inorganic and organic nitrogenous compounds in the nutrient medium have marked influence on growth and development of the tissues. Cultured cells and tissues preferentially use nitrates, and their utilization is one of the fundamental phenomena for growth and development of all life forms. It has been established that cultured plant cells grow preferentially on either ammonium nitrate or calcium and potassium nitrates. Wheat cells grew best in a medium containing both calcium and potassium nitrates (Gamborg, 1970); whereas soybean cells and carrot cell suspensions grew with ammonium nitrate as sole nitrogen source (Gamborg and Shyluk, 1970; Dougall and Varma, 1978). For many tissues the most suitable inorganic nitrogen source is a balanced supply of ammonium salts with calcium or potassium nitrate (Steward et al., 1958; Filner, 1965; Bhatt et al., 1973). However, the standard formulation of a mixture of NH_4^+ and NO_3^- may lead to less than maximal growth in plant cell cultures if the tissue can not cope with the level of NH_4^+ provided. Part of this problem stems from the fact that at a constant NH_4^+ level, the terminal pH increased in response to increased NO_3^- concentration. At any fixed NO_3^- concentration, the terminal pH decreased in response to

increased concentration of NH_4^+. Thus the utilization of NO_3^- and NH_4^+ lead to changes in medium pH which are in opposite direction. Martin and Rose (1976) working with Ipomoea suspension cultures have shown that the rate of NH_4^+ utilization increased with increasing pH, whereas the rate of NO_3^- utilization increased with decreasing pH. This suggests an explination for the wide range of medium pH observed during growth of plant cell cultures on media containing NO_3^- and NH_4^+ as nitrogen sources (Rose and Martin, 1975). Gamborg and Shyluk (1970) demonstrated the utilization of NH_4^+ as the sole nitrogen source by soybean cells, provided a Kreb's cycle dicarboxylic acid was present. Similar results were presented by Behrend and Mateles (1975, 1976) working with tobacco. Dougall and Verma (1978) could grow carrot suspension cultures in presence of NH_4^+ as a sole nitrogen source in absence of any Kreb's cycle acid provided the pH of the medium is controlled by continuous titration. However, in most cases the substitution of nitrates or ammonium salts has not been very successful (Burkholder and Nickell, 1949; Heller, 1953, 1954; Chokshi, 1975). The ability of casein hydrolysate, either acid or enzyme hydrolysed, to support the growth of tissues has been examined by various workers (Steward and Shantz, 1955; Nitsch and Nitsch, 1957; Staba, 1962; Steinhart, 1961; Hildebrandt, 1963; Vasil and Hildebrandt, 1966; Subbaiah, 1974).

Carbohydrate nutrition has been in focus ever since the inception of plant cell culture. Intact plants meet their energy requirements autotrophically via photosynthetic fixation of carbon. In cultured plant tissues the normal functions of chloroplasts are frequently partially or totally blocked, hence the necessity for incorporation of carbohydrates into the nutrient medium. Carbohydrate requirements of callus cultures have been worked out by a number of workers (White, 1934; Gautheret, 1945; Hildebrandt and Ricket, 1949, 1953; Arya et al., 1962; Bajaj, 1971; Fadia and Mehta, 1973; Subbaiah et al., 1974; Maretzki et al., 1974; Nash and Boll, 1975; Hendre et al., 1975; Hunt and Loomis, 1976; Verma and Dougall, 1977). These studies revealed that most tissues grow best when supplied with sucrose, dextrose or laevulose. These studies further elucidated the deliterious influence of irradiated sugars (Bajaj, 1971a, 1971b), on the growth and development of tissue cultures. Filter sterilized fructose in some cases could support growth as well as sucrose and in autoclaved form proved to be deliterious for growth (Nash and Boll, 1975).

Besides nitrogen and carbon sources, vitamins, particularly thiamine (vitamin B_1) and pyridoxine (vitamin B_6) have been shown to be stimulatory to the growth of cultured plant tissues. These vitamins are involved as prosthetic groups or co-enzymes of enzyme systems vital to all living cells. Thiamine in the

form of thiamine pyrophosphate or co-carboxylase is an essential co-factor for pyruvate and α-ketoglutarate decarboxylase and of a transketolase concerned in the formation of sedoheptulose from ribulose phosphate (Racker et al., 1953). Pyridoxine is the precursor of pyridoxal phosphate the prosthetic group of the transaminases (Lichstein et al., 1945).

Tissue culture studies have made their contribution towards understanding of micronutrient requirements for growth and development. Iron deficiency has been shown to cause cessation of cell division (Brown and Possingham, 1957), impede terminal oxidation to an extent (James and Boulter, 1955), results in enhanced levels of free amino acids and of DNA but in reduced RNA content, blocks mitosis and protein synthesis (Abbott, 1963).

Other micronutrients found essential for growth were Boron (Odhnoff, 1957; Whittington, 1959), Iodine (White, 1938; Fowden, 1959), Molybdenum (Hannay et al., 1959) and Manganese.

Manganese (Mn^{++}) was found necessary for the growth of cultured tomato roots by Hanney and Street (1954) and Hanney, Fletcher and Street (1959). Abbott (1953) demonstrated disruption of vascular differentiation and cell expansion due to Mn^{++} deficiency. It also results in enhanced free amino acid level and depressed RNA content of the cells. One possible

action of manganese is that it is involved in the linkage of RNA to proteins which is essential for the development of the protein synthesizing centres of the cell; for Lyttleton (1960) has shown that Mn^{++} deficiency prevents the development of ribonucleoprotein particles in wheat embryos. Many enzymes like dehydrogenases, decarboxylases, kinases, oxidases, peroxidases and polyphenol oxidase require manganese (Nason and McElroy, 1963). Its deficiency increases the respiratory rate (Ruck and Bolas, 1954; Subba Rao and Lal, 1955), whereas its high level depresses the rate of respiration (Subba Rao and Lal, 1955). The role of Mn^{++} as a co-factor of the IAA Oxidase was first reported by Furuya and Galston (1961). Low and high Mn^{++} levels have been shown to induce higher rate of IAA destruction (Morgan et al., 1966; Taylor et al., 1968). These observations prompted the examination of the effects of Mn^{++} ions on growth and differentiation of cultured Nicotiana tissues.

Growth of plants and their isolated tissues and cells is dependent upon the availability of growth hormones (Audus, 1972), as these substances are known to play important role in cell metabolism, cell division, growth, differentiation and cell hypertrophy (Butenko, 1968; Street et al., 1968; Crocomo et al., 1976). Of these growth substances, auxins have been proved to be essential supplement for establishing

successful culture of many plant tissues (Morel, 1948; Gautheret, 1959). Most media used for successful culture of plant tissues contain either of the following auxins - Indole-3-acetic acid (IAA), Naphthaleneacetic acid (NAA) or 2,4-dichlorophenoxyacetic acid (2,4-D).

Cytokinins are reported to have an additive effect on growth of tissues (Miller, 1965, 1967; Fox, 1969). Incorporation of kinetin or zeatin into the culture medium led to substantial enhancement in growth of a number of callus and suspension cultures (Steward et al., 1969; Lalchandani, 1970; Subbaiah et al., 1974). This effect of cytokinins is mainly due to their effects on cell division, protein and nucleic acid metabolism (Skoog et al., 1967; Skoog and Leonard, 1968; Letham, 1968; Kovoor and Klambt, 1968; Letham and Williams, 1969; Fletcher, . . 1969; Piesco and Alvarez, 1972; Vajranabhaiah and Mehta, 1976, 1977).

Gibberellic acid (GA_3), yet another growth hormone which falls in the category of Gibberellins, is known for its multiferous effects on growth and metabolism of plants and their isolated tissues and cells. In common with other growth substances, GA_3 produces a variety of seemingly unrelated physiological responses (Cleland, 1969). Nickell and Tulecke (1959) reported inhibitory effect of GA_3 on the growth of a number of plant species. Both promotory and inhibitory effects

caused by GA_3 are either due to its influence on auxin metabolism (Kogl and Elma, 1960) or possibly it acts at the gene level to cause de-repression and/or repression of specific genes that would, through the production of new enzymes bring forth changes in growth and differentiation together with metabolism of active compounds involved therein (Varner et al., 1965; Nitsch, 1968).

Many of the naturally occurring phenolic compounds are known to regulate growth and metabolism of plant cells, tissues and organs. Monophenols, in general are stimulatory to growth, whereas polyphenols antagonise it (Runeckles and Conn, 1974). The known relations of phenolic substances to oxidations in plants have been implicated in the control of the IAA stability in situ (Andreae, 1952; Ray, 1958; Henderson and Nitsch, 1962; Zenk and Muller, 1963; Tomaszewski and Thimann, 1966; Hanson et al., 1967; Andreae and Collet, 1968; Shah, 1977).

The process of differentiation is common to all organisms, and may be expressed in several ways. A zygote, undergoing differentiation exhibited by various outogenic stages of embryogenesis, eventually develops into a young plantlet with root and shoot. The development of root and shoot, fundamentally unlike in structure, function and the method of growth, is the most familiar example of differentiation. One

striking feature in plant cells is that a cell which is differentiated can dedifferentiate and produce new cells that may, in turn, redifferentiate into various types of specialized cells, tissues and organs. The process of differentiation operates from the very beginning, i.e. from the single celled zygote, and undoubtedly the process of differentiation operates under genetic control. The genetic constitution cannot operate normally other than in a certain set of environment, both internal as well as external. Obviously, any change in such environment would affect the process of differentiation and the final morphological expression. In multicellular systems, such as higher plants, the cells interact in a complex fashion by the exchange of metabolites, environment of substrates from source to sink, and release of specific products. By excising the organs, tissues and cells, and studying their behaviour in isolation under various sets of environment, it would be possible to gain insight into the process of differentiation (White, 1967; Johri, 1971).

Though the tissues grown in vitro are not exactly similar to that of intact plant as they differ in a number of features (Tulecke, 1964; Street, 1969). In vitro cultured cells, tissues and organs, however, can be differentiated by subjecting them to direct experimental control.

Since the classical demonstration by Skoog and Miller(1957)

of controlled organogenesis in tobacco pith callus by manipulating auxin and kinetin balance, much knowledge has accumulated in the induction of organs from plant tissues and organs cultured in vitro (Reinert, 1959; Street, 1966; Torrey, 1966; Halperin, 1969; Murashige, 1974, 1977). The basic regulatory mechanism suggested for organ initiation usually involves balance between auxin-kinetin ratio and their interactions with other growth factors. Higher ratios of auxin to kinetin are suggested to favour root differentiation, whereas higher kinetin to auxin ratios result in shoot differentiation. Unfortunately, this scheme cannot be vindicated over a wide range of plant species and attempts to induce organ formation through treatments with auxins, cytokinins or other growth substances have often been unsuccessful. However, the present study with Nicotiana tissues in culture has bearing upon several questions regarding hormonal effect on morphogenic responses.

Like other growth hormones, gibberellic acid (GA_3), also exhibits either inhibitory or promotary effect on organogenesis. Hypothesis concerning inhibitory effects are often centred on inhibition of root initial development or subsequent organ development (Brain et al., 1964; Heide, 1969). This inhibition of organogenesis may be related to altered starch metabolism at the site of incipient organ primordia (Thorpe and Murashige, 1968, 1970). GA_3, has also been demonstrated

to induce inhibition of bud formation in Begonia (Schraudolf and Reinert, 1959), tobacco (Murashige, 1964) and Plumbago (Nitsch and Nitsch, 1967). GA_3 is further known to block the action of the cytokinin, 6-Benzylaminopurine (6-BAP), and auxin (NAA) which are ordinarily potent stimulators of buds and roots respectively (Heide, 1969).

GA_3 also exhibits promotion of root formation at high concentrations in Trianthema (Ravishankar and Mehta, 1978). Root regeneration was reported from leaf cuttings of Lycopersicon treated with exogenous GA_3 (Coleman and Greyson, 1976).

Phenolic compounds are known for their effect on growth and organogenesis in cultured plant tissues. Their influence is mediated through hormonal action on growth and organogenesis (Lee and Skoog, 1965a; Basu, 1970). This effect is postulated to be due to definite relationships of the specific structure of phenolic compounds to their activity regulating organ formation.

Sucrose, glucose or fructose is quite often used as a source of carbon and energy for plant cells grown in culture. Depending on the species from which the culture was established and the clone, other carbohydrates may support growth equally well (Maretzki et al., 1974). The effect of different carbohydrates and their levels on both growth and differentiation has, however, been elucidated only in a few cases. Wright and

Northcote (1972) concluded that for sycamore callus, any sugar which allowed good growth also allowed root formation. They also demonstrated the presence of sucrose, glucose and fructose in root-forming calli irrespective of the added carbon source. Minocha and Halperin (1974) demonstrated differential effects of maltose and sucrose on tracheid differentiation. Maroti and Levi (1977) working with meristem cultures of Dianthus caryophyllus, reported that keeping the hormonal level constant, the change in sucrose level in the medium would decide the formation of either unorganized callus or organized shoots and roots. Tran Thanh Van (1977) utilizing thin cellular layers of Nicotiana and Begonia has clearly demonstrated the importance of various sugars and their levels in the medium in controlling morphogenetic responses like the induction of flower, vegetative buds and roots. Kochba et al. (1978) working with embryogenic and non-embryogenic cell lines of Citrus have demonstrated the influence of various sugars on embryogenesis and growth. They have also demonstrated differential embryogenic responses towards increasing sugar concentration. Consequently in the present study, the influence of not only auxins, kinetin, GA_3 and phenolic acid but also of different sucrose levels on growth and organogenesis of cultured Nicotiana tissues was examined.

Gautheret (1959, 1965) observed that the hormonal

requirements for shoot and root formation varied with the plant species and even with different tissues of the same species. White (1967) reported that under no circumstances can one achieve everytime the same morphogenetic response at will. In the present investigation experiments were carried out to examine these aspects.

Somatic embryogenesis generally depends upon the hormonal balance in the culture medium. It has been demonstrated that an embryogenic medium must contain NH_4^+ ions (Halperin and Wetherell, 1964; Reinert, 1968; Reinert and Tazawa, 1969) and high auxin level (Halperin and Wetherell, 1964) or the correct auxin/cytokinin ratio (BuiDang Ha, 1974; Harada, 1973). Embryos thus obtained are transferred to hormone free medium for further development. Only a few cases are reported where embryogenesis occurs in absence of exogenous hormones (Halperin, 1967; Gamborg, Constabel and Miller, 1970).

Since no specific organ forming substance has as yet been isolated and not all cultured tissues produce roots or buds in response to auxin/cytokinin treatments, Mehta (1975) suggested that other growth factors, as yet unidentified, might also be involved in hormonal interactions regulating organogenesis.

Growth hormones are known to influence enzyme activities in cultured plant tissues and excised plant parts (Morel and Damatriades, 1955; Vajranabhaiah, 1969; Davies, 1972;Innerarity

et al., 1972; Subbaiah, 1974). Of particular interest is IAA Oxidation by the auxin destroying enzyme IAA Oxidase, which converts native auxin to physiologically inactive product, methylene oxindole, which is further converted non-enzymatically to methyl Oxindole (Teng and Bonner, 1947; Goldacre, 1949; Pilet and Gasper, 1968; Moyed and Tuli, 1968). This enzyme system consists of a flavoprotein coupled through hydrogen peroxidase to peroxidase, the latter causing inactivation or destruction of IAA (Goldacre, 1951; Galston et al., 1953; Stutz, 1957; Ray, 1958). The activity of the enzyme system appears to be limited by the phenolic cofactors (Andreae, 1952; Zenk and Muller, 1963; Van Overbeek, 1966).

Peroxidase enzyme activity is affected by growth hormones (Lavee and Galston, 1968a; Stuber and Levings, 1969; Ritzert and Turin, 1970) and has been related with growth, differentiation and lignification (Freudenberg et al., 1958; Galston and Davies, 1969; Obst and Harkin, 1973; Wolter and Gordon, 1975; Nash and Davies, 1975; Van Hoof and Gaspar, 1976; Cachita-Cosma et al., 1976; Gaspar et al., 1977; Gibson and Liu, 1978; Thorpe and Gaspar, 1978). It is also reported to bear relevance with the process of aging (Lavee and Galston, 1968b; Gordon, 1971) and disease resistance (Uritani and Stabmann, 1961; Yu and Hampton, 1964). It is reported to oxidize a number of phenolic compounds (Higuchi, 1957; Siegel, 1956; Neish, 1964; Srivastava and Van Huystee, 1977a, b; Gibson and Liu, 1978).

The role of carbohydrate metabolism in the initiation of organized structures from tissue cultures was emphasized by Thorpe and Murashige (1968). They observed heavy accumulation of starch in the areas which were potential meristemoids. The physiological significance of starch accumulation prior to organ initiation, was suggested to reflect the high energy requirement of the organogenetic process (Ross, Thorpe and Costerton, 1973). Since the degradation of starch would yield high amount of Glucos-1-phosphate, which during subsequent conversions through glycolysis and Kreb's cycle would yield ATP. Organogenesis, especially shoot formation, has been shown to be concomitant with high rate of respiration (Ross and Thorpe, 1973), high enzyme activities of the EMP and Pentose phosphate pathways (Thorpe and Laishley, 1973), high activity of starch synthesizing enzymes and also high activity of starch degrading enzymes (Thorpe, 1977). With these backgrounds, we chose the study of malate dehydrogenase (MDH) which would give the overall picture of respiratory rate and ATP generation. Furthermore, since most of the energy in aerobic systems is generated by the TCA cycle, and MDH is the terminal enzyme in the pathway producing Oxalacetate (OAA) which enters the cycle again by reacting with Acetyl-CoA , the rate of OAA turn over can act as a very reliable pointer towards energy requirements for growth and differentiation. Moreover, OAA in higher amounts becomes rate limiting, because it causes inhibition of MDH,

hence the OAA turn over and MDH activity will again be determined by the rate of reaction of OAA with Acetyl-CoA.

Since the synthesis of many secondary products is associated either with specialized differentiated cell types or with organized tissue systems, it is perhaps not surprising to study their biosynthesis during organogenesis. Biosynthetic studies have shown that Shikimic acid derivatives like phenylalanine and cinnamic acids (cinnamic acid, p-coumaric acid, caffeic acid and ferulic acid), to be good precursors for lignin biosynthesis (Hasegawa et al., 1960; Gamborg, 1967). Auxins, cytokinins and sucrose have been found to influence degree of lignification and xylem differentiation in callus cultures of Daucus carota, Phaseolus vulgaris, Syringa vulgaris and Nicotiana tabacum (Koblitz, 1962; Wetmore and Rier, 1963; Bergmann, 1964; Jeffs and Northcote, 1967; Fadia and Mehta, 1973; Mehta, 1966). Besides other factors the activity of phenylalanine ammonialyase (PAL), a key enzyme in the phenylpropanoid pathway, is the influencing factor in the biosynthesis of cinnamic acids. Indeed a correlation between lignification, xylem differentiation and PAL activity has been observed in Coleus, Glycine max, Helianthus tuberosus and Phaseolus vulgaris (Rubery and Fosket, 1969; Haddon and Northcote, 1975; Minocha and Halperin, 1976). With these observations in mind, we undertook the study of PAL

activity and accumulation of phenolics during growth and differentiation in callus cultures of Nicotiana tabacum.

"Gene controlled protein synthesis" (Jacob and Monod, 1961) is an all or none response. In this case a gene is either expressed or repressed by itself. However, the proposed scheme does not fit in with diverse "cell types" where differential gene activity is encountered. How and to what extent does gene action regulate development and differentiation of higher organs, is yet to be elucidated.

A possible way of resolving such a problem is to examine natural "built in markers" for biochemical, genetic and developmental studies. It is now an established fact that most enzymes exist in multiple molecular forms (isoenzymes). It is further acknowledged that a large amount of variation detected in isoenzyme studies is due to genetic basis, thus rendering isoenzymes as useful "markers" in a number of biological investigations (Jacobs, 1974a, b; Scandalios and Sorenson, 1977). Since peroxidase is attributed vital roles in regulation of cell growth and differentiation (Siegel, 1956), it is quite plausiable to study the development of isoperoxidase patterns.

Multiple molecular forms of plant peroxidase have been established and it may vary in content with the species, tissues and even the application of growth hormones (Shannon, 1968). The effect of growth substances is of particular interest

as peroxidase catalyses IAA Oxidation, thus suggesting the importance of this enzyme in plant growth regulation. Correlation between peroxidase activity and morphology are not uncommon. Close correspondence between increased enzyme activity, including that of peroxidase, and a remarkable influence of a gene on morphogenesis has been studied by Mathan and Cole (1964) in tomato.

Plant physiological studies as enumerated above have general biological significance, in the sense that genetic information resides intact in all the living cells derived from zygote during development. The mechanism by which the isolated cells and tissues sense changes in their immediate environment and posses "Switching Network" that permits all or none - response is the essence of the problem of growth and differentiation.

All these aspects formed the background for the present study elucidating physiology of growth and differentiation. For the present study callus cultures were initiated from Nicotiana tabacum L. var. Anand-2, which is one of the promising hybrids evolved by the Bidi Tobacco Research Station of the Gujarat Agriculture University at Anand, Gujarat. Diploid callus cultures were evolved from the floral buds of the hybrid plant and haploid callus was derived from the shoot explants of haploid tobacco plantlets raised through anther

culture. It has been amply demonstrated that the callus cultures are mossaic chromosome-wise and that the ploidy level increases with subcultures mainly as a result of endomitosis (Mitra and Steward, 1961; Shamida and Tabata, 1967; D'Amato, 1975; Reinert and Bajaj, 1977). To avoid heterogenity, experiments were, therefore, conducted with tissue subcultured for 2 to 4 culture transfers. During this period it was observed that upto 70% of the floral bud callus cells remained diploid and upto 60% of the cells of haploid callus tissues remained haploid. Freshly initiated callus tissues were used for subsequent studies. Possible role of enzymes - Peroxidase, IAA Oxidase, MDH, PAL and peroxidase isoenzymes, and phenolic content in the regulation of unorganized and organized growth forms in diploid and haploid callus were investigated. The results obtained are incorporated in this thesis under the following broad headings :

A. Initiation of Diploid and Haploid callus cultures of Nicotiana tabacum L. var. Anand-2.

B-I. Growth and accumulation of phenolic compounds in Diploid callus cultures of Nicotiana tabacum L. var. Anand-2.

B-II. Growth and accumulation of phenolic compounds in Haploid callus cultures of Nicotiana tabacum L. var. Anand-2.

C-I. Organogenesis in Diploid callus cultures of Nicotiana tabacum L. var. Anand-2.

C-II. Organogenesis in Haploid callus cultures of Nicotiana tabacum L. var. Anand-2.

D. Physiological studies with Peroxidase, IAA Oxidase, MDH, PAL, Peroxidase isoenzymes and Phenolics during growth of Diploid and Haploid callus tissues of N. tabacum L.

E. Physiological studies with Peroxidase, IAA Oxidase, MDH, PAL, Peroxidase isoenzymes and phenolics in Diploid and Haploid callus tissues of N. tabacum cultured on root differentiating medium.

F. Physiological studies with Peroxidase, IAA Oxidase, MDH, PAL, Peroxidase isoenzymes and phenolics in Diploid and Haploid callus tissues of N. tabacum cultured on shoot differentiating medium.

*****