Review of Literature

Any landmarking discovery is a fruit of sustained longstanding contemplation spread over a period of decade or more which is often the emergence of a geneous. However, such discoveries do not receive a general appreciation of a contemperory scientific world, not because it is mere speculative but is beyond the grasp of contemperory scientific world and hence the work remains hidden or unknown. While tracing the history of genetics for instance Gregor Mendel, the father of genetics is often refered. However, Mendel's work so precisely and logically and convincingly putforth did not receive the appreciation till a contemperory biological tenet gained this power for almost 35 years and in the year 1900 when his work was discovered by Tshremark D-Varies and Corens, the birth of the science of genetics has been resolved as year 1900. In three quarter of a century not only this scientific tenet revolutionised food grain production to meet the mouth of millions but paved the foundation for technology. Genetic engineering is a major biotechnological boom vis-a-vis the tissue culture both of which have been richly harvested today.

In keeping with the remark that we made in the beginning the great geneous who laid the foundation of tissue culture and opened new horizon of research, may not be with the intension nor with a slightest inklink that eventually it will emerge into a technology but with an urge know the unknown and to make impossible a possible by way of exploring

the possibility as to whether one can make plant tissue to grow and differentiate if necessary nutrients are provided in culture conditions. This great geneous possibly a German engineer dubbed in to a plant anatomist was Hildbrandt.The imagintion made in 1902 by him , almost the contemperory year of the discovery of Mendels work pressumed to have been based on the logic that the chlorophyll bearing tissues must be able to supply organic substances required for the growth for photosynthesis and hence they must grow. Although the results were frustating , they certainly awaken the awareness and inculcated interest in the experimental scientists. Hildebrants media contained glucose and peptone and prevented contamination . Possibly his experiments yielded success had the contemperory science provided the information about the growth hormones. All the same his experimentation did not vain at this juncture. The careful examinations of reasons for the failure laid him to perform new experiments where he cultured a small piece of potato tuber tissue and found that the cell division is occuring without exception if these discs contained vascular tissues. Based on this he immediately concluded that some sort of stimuli were elicitated through the mediation of peptone and this stimuli diffuses through the adjesent storage tissue and initiate cell division . It may be added here that the concept of stimulus in growth has origin from the eye-brow of the great naturalist of last century Charls Darvin. Possibly the -

science of reasoning of subsequent experimental scientist laid to apprehent and transform the idea in to chemical stimulus i.e. auxin.

Soon after the Haberlandt's experiment with culturing of potato tuber tissue Winkler (1908) tried to culture segments of spring beans in the same way as Haberlandt and noted cell division taking place. But even in his experiment no further proliferation of explant could be achieved. During the same year Simon (1908) tried to culture stem segments of poplar and for his pleasent surprise he noticed the development of bulky callus and dedifferentiation of buds and roots from it. Starting with Haberlandt's experiments in a short span of five to six years first successful culturing of plant in the test tube has been achieved. From this experiment of Simon a concept of polarity has emerged. It will not be inappropriate to give credit to this great scientist for establishing the base of callus culture and micropropogation. While accomplishing the success what all has been over-looked is septicity, because of which, it has not been persued and nor it attracted the attension of many scientists.

Simultaneously efforts were being made by the zoologists to culture mammelian tisue in vitro. They were trying to grow the tissues in complex nutrient media prepared out of blood plasma and embryonic fluid which subsequently were replaced by synthetic ones. Inspired by the work of zoologists Czech (1926),

Prat (1927) and many other plant scientists attempted to grow excised plant tissues directly on plant extracts which was of no avail. Number of other ingredients such as yest extract were tried. Only the negative inference which was useful that they drew is meristem tissue grows in synthetic nutrient media.

A great turn to those scattered and unsuccessful efforts of raising the plant under culture condition in vitro was given by the great scientists White in the US and Gautheret in France. The reasons behind the success of these experimental botanists are :

i) The careful choice of materials for investigation.

ii) The careful selection of nutrient media suitable for growing plant tissues.

iii) The careful examination of work of previous workers constrains in their achievement and search the reason behind their failure.

They persued the objective of growing plants in vitro with great perserverence. Therefore they are regarded as the Masters of method for cultivating excised plant tissues in vitro. The sustained work of White (1931, 1932, 1932a, 1933, 1933 a,b, 1939) has culminated in the discovery that the excised to nato roots can be grown in vitro indefinately by transferring of growing tissue periodically to the fresh nutrient media. His work from 1931 to 1954 not only laid him to master the

methodology but also to the discovery of several combinations of nutrient media required for successful culture of varieties of plant tissues.

Almost brushing the shoulder with White Gautheret (1932) unravelled his work of plant tissue culture in vitro with root tips. However, he chose to start with cambium and stem parenchyma of root and tubers quite some time. He and his colleagues basically engaged themselves in studying the nutrition of plants both inorganic and organic i.e. carbohydrate. The effects growth hormones such as auxins, vitamins and coconut milk on the growth of both normal and habituated tissues (Heller, 1953; Goris, 1954; Kulescha, 1950, 1954, Duhamet, 1955; 1957). Thus the contribution of Gautheret and co-workers has placed the technique of tissue culture on a very sound platform because they have taken into consideration the number of physiologicaly active substances and their effect on growth and dedifferentiation of tissue under culture conditions (Gautheret, 1961, 1962). Subsequently many workers tried several media supplimented with various nutrient substances. For instance Torrey and Reinert (1961), Sastri (1963) have tried the media supplimented with defferent amino acids.

While tracing the history of development of tissue culture technique one considers the period, 1930 to 1969 has been the great period of methodology, because, during this

period only the discovery of many plant growth substances has taken place and use of plant growth hormones in tissue culture pivotale role to play. A leading group of workers in USA. Wisconsin who made a landmarking discovery is the school of Prof.Skoog. The discovery of kinetin is of great significance and single solid contribution made by skoog which gave an impetous to plant tissue culture for as we know today, that Kinetin has been the backbone in tissue culture. This opened a new horizon of research with its application in tissue culture. Skoog and Miller (1957), Skoog (1955) eventually characterised the structure of this non auxinous physiologically active substance analoguous to the purine base. It attracted the attention of numerous scientists to study the effect of purines and their analogues on the process of plant growth. Working on this active substance Skoog became interested in experimentation with tobacco tissue and in the process he found that, when tobacco tissue is maintained in the medium solid in nature remained unorganised but upon immersing in the liquid medium produced buds. This observation of Skoog and Tsui (1948) made earlier led to demonstrate that adenine stimulated cell division and induced bud formation in the tobacco tissue. It also led them to spaculte a possibility of altering nucleic acid and protein metabolism. The culmination of this is the discovery of Kinetin.

Miller (1961) subsequently detected in young maize endosperm a substance similar to that of kinetin but many fold potent than kinetin. This substance is called zeatin was eventually purified by Letham and in colleboration with other workers he established its molecular structure (Letham et al, 1964). Finally he demonstrated that the cytokinin found in coconut milk is nothing but Ribosyl zeatin (Letham, 1964). This work has added a new dimension to the tissue culture that the auxin and coconut milk together could provide a powerful stimulus to the growth of plant tissue under culture condition.

Almost inspired by Gautheret's contribution James Nitsch and Collette Nitsch are the two renouned French plant physiologists who made loudable contribution to the field of plant tissue culture. James Nitsch was one of the first to cultivate excised ovaries and tissues of plant fruits (Nitsch; 1947, 1951, Nitsch and Nitsch; 1956). They carefully examined the tissue nutrition and auxin metabolism under culture conditions.

Another leading school of palnt physiologist contributed to the tissue culture in Cornel University, USA is of Prof.Steward. This school developed original methodology for growing the explant in liquid media. The famous Carrot tissue culture which teaches the alphabets of tisue culture is the contribution of Steward's school. Working on cell division,

tissue formation and organogenesis under culture conditions Steward and his Co-workers from 1949 to 1962 made very valuable contributions (Steward; 1958, 1961, 1961 a, 1962). Steward and his associates proposed the use of revolving apparatus which facilitated dissociation of tissues in to cell suspension. Thus the culture was established. Microscopic observations showed that the medium was crowdedwith isolated cells or cell clumps. Eventually Steward et al (1966) with their revolving technique noticed the differentiation of each cell in to embryo from which eventually a normal plants grew. Based on tnis experiment many researchers subsequently reported the phenomenon of somatic embryogenesis and formation of plantlets from cell culture. The limitation of cell suspension technique was that, it did not enable continuous observation of single cell differentiating into embryo.

Another group of workers in Wisconsin actively engaged in the study of nutritional aspect of tissue culture is Hildbrandt, Riker, Muir, Jones and others. In 1943 White who first suggested a mineral solution which was of Knop's and supplimented with trace elements. However, optimisation of this combination was achieved by Hildbrandt in 1946, who proposed new media containing rather high concentration of Sodium sulphate. Burkholder and Nickell (1949) working on tumorous tissue modified the solution. But this didnot make any

significant difference. Eventually Heller (1953) after extensive investigation especially with respect to carrot tissue suggested increase in the concentration of salt.

The greatest contribution in evolving ideally accomplished nutrient medium has been made by Murashige and Skoog (1962). They studied the mineral requirement of the tobacco tissues and proposed solution far more concentrated than the routine formula. This medium enabled 5-7 times more active growth of tissue than the other media and is characterised by the presence of large amount of nitrates and ammonium salts. Todays successful application of this medium facilitates comfortable differentiation of tissues in varieties of plants belonging to both monocots and dicots.

The demonstration of totipotency in plant cell culture has been made by number of workers. Tulecke (1953) working on pollen grains cultured in a medium enriched with vitamines and amino acids obtained cell colonies. Muir et al (1954) carried out experimentt on tissue culture of plant similar to that of animals. They tried to place a single cell of tobacco on a small piece of filter paper which in turn was placed on the top of callus mass. They noticed 8% of these isolated cells multiplied and formed colonies. Torrey (1957) working on root culture successfully achieved the multiplication of isolated cell placed near the active cell colony. But the interesting aspect

is his mdium was enriched with 2,4-D a powerful stimulating substance plus yeast extract. Muir *et al* subsequently demonstrated single cell culture and differentiation of embryoids from these culture was eventually made by Steward *et al* (1952).

Vasil and Hildbrandt (1965) working with interspecific hybrid of *Nicotiana* evolved a method where they employed cloning technique to culture isolated single cell and there by raised colonies of cells and successfully produced plantlets. This removed some of the irritants prevailed in the revolving technique of Steward. Lutz (1966) evntually demonstrated the totipotency of somatic cells. Later on Backs-Hismann and Reinert (1970) demonstrated the formation of somatic embryo from a single plated cell of carrot followed by formation of proembryonal mass of cells.

Great amount of active research was going on in Delhi School on organogenesis and embryology of angiospersms under the leadership of Prof. P.Maheshwari spread over a period of decade and half (1950-65). They have been pioneers in making valuable contribution of *in vitro* cultivation of young ovaries, seed buds, embryonic seed tissues. They ellucidated reproductive system of plants in vitro and studied the effect of various physiologically active substances on plant development under culture. This sustained work culminated into a symposium in 1962 on the aspect of plant tissue and organ culture. (Maheshwari, 1958, Murgai, 1959, Vasil, 1957, 1958, Maheshwari and Rangaswamy, 1963, Maheshwari and Gupta

1965, Mohan Ram, 1963, Guha and Maheshwari, 1964, 1966).

As discussed earlier, efforts to culture pollen grains were first made by Tulecke, (1953) in Ginkgo biloba from which the cell colonies were obtained. Later on it was realised that his cultured pollen grains were nothing but male prothallium consisting of variable number of cells depending upon the thallus.Almost a decade later Lamada et al.(1963) tried to culture whole anther instead of pollen grains and obtained haploid callus. Based on the cytological nature of the callus cells he concluded that this might have developed from microspore mother cells following meiosis. Soon after an exciting development took place when Guha and Maheshwari (1964) culturing the anthers of Dhatura innoxia a member of Solanaceae at a pollen grain stage observed that the anthers burst and release normal embryos and platlets. They used a medium supplimented with casein hydrolystate, Inndole acitic acid and Kinetin or coconut milk or grape juice or pulm juice. Their subsequeent work confirmed that these plants were haploids and developed from pollen grains (Guha and Maheshwari, 1966). This not only open a new vistas in plant tissue and organ culture but provided an impetus to an aorist in the new direction. Soon after Bourgin and Nitsch (1967) using Nicotiana anther demonstrated that there is a pollen embryo develops directly into haploid plants. These workers not only confirmed totipotency and answered the qurious question as to why in

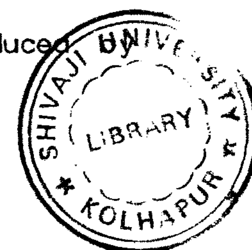
in the higher plants the haplpoid stage is confined only to the pollen grain, but totipotency inherent even in the haploid cell. It also led to establish androgenesis.

Unravelling of the possibility of haploid culture by way of anther culture is an important step towards the direction leading to the technology. It opened a vast opportunity in the field of plant breeding which has been an essential technology exploited to meet the food requirement of the steeming million. Isolation of inbreads or pure lines or isogenic line is an essnetial step in breeding programme and is of great labour and time consuming. With raising a haploid through culture and doubling its chromosome number leads to achieve isogenic line in a stroke. A technique has been soon developed and even the plants have been raised in two ways-

i) By treating the plantlets with colchicine while they are still attached to anther in culture (Kasperbauer and Collins, 1972, Berk et al, 1972,)

ii) Explant of the haploid seedling culture accomplished by the natural phenomenon of endomitosis and led to the isogenic diploid (Nitsch, 1949, Kameya and Hinata, 1970, Kochhar et al 1971).

In the latter experiment the explant of the haploid has been grown on auxin and cytokinin mdium to induce callus formation (Nitsch, 1972). While it is callusing during the growth of callus deploid homozygous cells were produced by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endomitosis where from a large number of isogenic plants could be differentiated.

However, number of different methodologies to culture excised anthers and raised haploids have been developed depending upon the individual requirements. Few of the examples are in Barley (Clapham, 1971), in tomato (Sharp, 1971a), in wheat (Ovyng et al 1973) and in Atropa (Rashid and Street, 1973). The interesting point to be emphasizing here is modifications that they have made. The medium used for this is of Murashige and Skoog (1962) and Nitsch and Nitsch (1969), of course, the latter with modifications. It has been shown that androgenesis in tobacco anther occurs if activated charcoal is incorporated into the medium (Anagnostakis, 1974). Subsequently Bajaj et al (1974) working with culture of Nicotiana tabaccum showed that the androgenic anthers under culture conditions can be varied from 41 to 91% by simply adding activated charcoal to the media. This phenomenon has often interprited as activated charcoal stimulates adrogenesis. In the mean while Fridborg and Eriksson (1975) have shown that charcoal enhances the induction of embryogenesis in cell suspension of carrot.

As already mentioned the possibility of culturing anther has opened vast opportunity in plant improvement programme. Rapid strides in this direction has been made. One of the

steps ahead in this direction is the technique developed by Bajaj and Devey (1974) in isolating protoplast from pollen grains where they have combined enzymatic and mechanical method to digest the exine. Soon the procedure for isolation and culture of protoplast from pollen mother cell and tetrads have been developed by number of workers(Bhojwani and Cocking, 1972, Bajaj and Cocking 1972, Bajaj 1974c).

With a successful culture of an anther as an organ, the technique of tissue culture branched into tissue and organ culture. Johri and Guha (1963), Nitsch (1963a) tried to culture ovaries in vitro with variable degrees of success. Although ovary culture was first attempted in 1942 by La-Rue, who obtained limited growth of ovaries acccompanied either by rooting of pedicel in case of several species. Subsequently Nitsch (1951) extended this technique for study of fruit physiology. Later on similar results have been obtained by several investigators (Rangan, 1982). Referring to the various organ culture in brief are embryo culture by Maheshwari and Rangaswamy (1963), Rangan (1982) embryo and ovule culture by Withner (1943,1959) Ovule culture by Rangaswamy (1963). These techniques and successful attempts have been shown to have various applications besides understanding the morphogenesis in better way. However, they made their own niche in appliction of tissue culture technique.

The preliminary experiments carried out by earlier workers led them to notice disappearance of secondary metabolites sucn as tannin and alkaloides, (Ball, 1950, Telle and Gautheret 1947). Although the alkaloides disappears quickly but not completely during the first stages of tissue culture, in those instance where secondary metabolites are produced by specific organs it was recognized that their synthesis was not obtained in callus which did not undergo such a organisation(Paupardin, 1971). However, when the callus produced buds and specific structure associated with secondary metabolites, terpine synthesis started again. Finally it was Benveniste et al (1964) observed that in tobacco tissue culture there is a deviation of steroid synthesis which is enriched and partly oriented towards new steroides hitherto unknown in this palnt.

The first attempt for the industrial production of secondary metabolites was made Pfizer Company in the US between 1950 and 1960 which met with failure. This led to disuade many workers for many years and hence the problem remained unresolved. Clearly the industrial production of secondary metabolites require equipment allowing suspension culture of very large masses of callus cells. Although the technology was ready to industrially produce secondary products, the economic aspects were not encourageing. From

1975 the fundamental aspect of subject were explored carefully and very intensively in order to find the way to success. (Gautneret, 1985). Although the rapid progress in this area attracted many new workers (Bohm, 1980, Staba, 1980,1982), the industrial point of view the main problem still remained for the production in vitro of substances to make cheaper than directly tapping from the plants.

It is generally considered that plant cells are totipotent i.e. all cells except for certain highly specialized one contain a full genetic information when given appropriate conditions will allow the regeneration of whole plant from a single cell. By extrapolation, then given the appropriate chemical and physiological environment, it should be possible to induce any cell to synthesize substances characteristic of parent plant. Accordingly, it has traditionally been the approach to initiate cultures from high yielding plants in the hope that this will provide a best chance of developing high yielding cultures. Although this belif is contradicted by many workers that the yield characteristic of parent material are of no consiquences (Foweer, 1986). The practical varification has been carried out by Kinnersley and Dougall (1981) in Nicotiana tabaccum where they studied Nicotine synthesis in callus cultures from isogenic lines of both high and low yielding cultivars. This clearly showed

that high yielding plants give high yielding cultures indicating that the Nicotine yield is genotypically linked. This observation is in conformity with the reports of Zenk et al (1977) and Roller (1978) both who worked independently with Catheranthus reseus.

It is an established fact that in plants secondary metabolities typically accumulate in the localized parts or Organs which is normally used as source material in normal industrial tapping of the substance. This immediately raises question whether one should use the tissue of that particular part where the metabolite is localized. But if one examines the biosynthetic path way, one realises that often the synthesis takes place in the organ or the tissue other than where its localization or accumulation occurs.

The commercial exploitation of secondary metabolities through tissue-culture required lot of basic work to be carried out and creation of the simulated condition in which the synthesis of secondary metabolites occurs in nature. Vast amount of basic work in some of the established plants from point of view of tissue culture such as Tobacco and Potato were principally carried out to initiate cell line culture. This included even the formulation of special and defined media beside selecting certain chemical substances which induce the synthesis of secondary metabolities. In this

direction the work of Gamborg et al (1976) is loudable. In the process of finding the proper formulation of media for cell line culture and secondary metabolites, what is unearthed is that, precise nature of the coconut milk is not known. For many years coconut milk was taken as almost universal component of plant tissue culture media. This material contains a variety of regulators together with other nutrients. Not only is the precise nature of various components is little understood, despite much research, but coconut milk exhibits a great variation in both qualitative and quantitative composition. This obviously makes precise growth, and hence process control difficult to achieve. Definition of growth regulator requirements for many cell lines has led to decline in the use of coconut milk (Fowler, 1986). The basal formulation of many media has come about through analysis of cell sap and tissue extracts.

Although no media have been fully optimized, some knowledge about the nutrient regime for tobacco culture have been aquired by Noguchi, et al (1977). Ulbrich and Arents (1984) optimized the culture medium for the production of Rosmarinic acid by Coleus blumei cultures. Beginning with this the technique for the production of biomass through cell line culture has been mastered by Stepan-Sarkissian and Fowlar (1984). They investigated wide range of carbohydrates and carbohydrate containing materials and have shown these as a potential source of carbon for plant cell culture. Secondly, photoautotropic growth by plant cell culture has also been

demonstrated where few of these cultures exhibited levels of biomass production equal to those observed with hetertrophic culture. These lines of research prepared a ground for large scale raising of cell line. The only thing remains to be achieved is to induce the plants to synthesize secondary metabolites inadequate quantity so as to make possible to exploite commercially. In order to achieve the goal emphasis is laid on selection or on biochemical research on regulation of secondary metabolism. An alternative approach was to understand the condition that forces the organism to produce secondary metabolites. Normally this is monitored often by abiotic factors(Swain,1977, Harborne, 1982). The secondary metabolities are produced as a protective measure against the physical and biotic environment. Frischknecht et al (1986b) showed that there is an increased level of secondary compounds under environmental stress conditions. In ecological terms it is interpreted as by a shift from effective but costly defence mechanism e.g. tannins. There are number of examples to support these findings. In vitro stress situation in Papaver somniferum resulted in release of the thebaine in culture medium (Lockwood, 1984). And in Catherantnus roseus increase in indol alkaloid formation has been shown by Giger et al (1985). Immitation of biotic stress by addition of fungal elicitors led to a sanguinarine accumulation of 2-9% (26 times that of the control). In suspension culture of Papaver somniferum (Eilert et al, 1985) several physical, chemical

and biological factors which educe secondary metabolites have been tested. It has been shown that high and alternating temperatures had no effect, where as low temperature and polyethylene glycol had negative effect on purine alkaloid formation in Coffea arabica suspension cultures. HIgh light intensity has shown to stimulate increase in alkaloid synthesis (Frischknecht and Bawmann, 1985). They also showed that the combination of both stress forms, lignt and Sodium chloride has a cumulative effect.

There is great amount of urge of tapping the secondary metabolites through in vitro culture at a commercial scale. Many compounds of medicinal value both alkaloids, flavonoids, quinones,,tannins, anthocyanins, saponins, terpenes and biologically active compounds having insecticidal property besides purines, phytohormones and glycosides have been looked for through cell line culture, voluminous information has accumulated (Vasil,1988).For the quick and rapid Screening of the literature to refers to the some of the landmarking discoveries. To site with the number of workers contributed in this direction are Huesemann and Barz (1977), Yamada and Sato (1978), Yasuda et al (1980). They have successfully isolated photoautotropnic cells. Palmer and Widholm (1975), Midiga et al (1976), Nabors et al (1980) have successfully isolated resistant cell lines. Yamada and Watanabe (1980), Matsumoto (1980), Watanabe and Yamada

(1982) isolated high vitamin producing cells. Kinnersley and Dougal (1980), Yamamoto et al (1982) could successfully isolated high pigment producing cells. Besides high alkaloid poroducing cell lines have also been developed in number of species (Zenktel, 1977, Ogino et al 1978, Yamada and Hoshimoto, 1982, Ramawat and Irya, 1979 a & b).

As one of the steps in increasing alkaloid content Eapens et al (1978) studied polyploid and tetraploid plants of Atropa belladona and have shown in them high alkaloid content than their diploids. Similarly in polyploids and tetraploids of Dhatura are also shown to have high alkaloid content than their diploids (Robinson, 1981). Muhitch and Fletcher (1985) noted that the polyamines extend the life span of mature nondividing cells. This observation has led them to investigate the effect of polyamine addition on the production of phenolics in rose suspension cultures. Addition of polyamines induced changes in the type of polyphenolics as well as their quantity in culture. However, the same was not applicable for the production of caumarines in the tobacco suspension cultures. (Okazaki, 1982 a,b).

Although the cell line culture opened an opportunity to raise biomass of cells producing secondary metabolities, added to this methodology has been a technique to isolate such cell lines and immobilize them (Yeoman,1986).

Immobilization is the newest culture technology for plant cells. As a result of immobilization cells can be encourage to grow together in a multicellular partially organised condition particularly if the cells are maintained physically stationary. The physical and chemical gradients so eastblished can provide an environment which of all the culture techniques, most closely resemble in vivo condition. Thus it is a technique which facilitates the sequential manipulation of cells derived approximately a homogenous suspension culture and permits some structural and biochemical differentiation of those cells. These advantages are the strength of immobilization techniques and they can be exploited most readily and effectively in the field of secondary metabolite production (Lindsay and Yeoman,1986). Immobilization technique, though considered recent one, but is with respect to cell culture technology. It has been in practice in microbial and animal cells (Mosbach and Mosbach 1966). They experimented with entrapment of enzymes and microorganisms in synthetic cross linked polymers. In higher organisms Kitajima and Butler (1976) microcapsulated the chloroplast particles. Similarly Kiersten and Bukae (1977) immobilized microbial cells and chloroplast in calcium alginates. Within few years time the methodology has been adopted by number of plant biochemist and cell physiologist

(Brodelins et al, 1979; Alferman et al, 1980, Lindsay and Yeoman,1983). Lindsay and Yeoman (1983) described the method for microbial and animal cell immobilization in following categories :

1 Immobilization of cells in one or in combination with cell involving the entrapment of cells in one or in combination with cell matrices such as alginates, polyacrylamides, agar or collagen or in man made plastic polymer (Hackel et al; 1975; Klein and Klüge 1981).

2 Th adsorption of cells to an inert substratum such as charged microsphere or glass bead beds (Spiea and Whiteside, 1976 a,b) DEAE saphadex.

3 Cells are adsorbed to an inert substratum such as a gel via biological macromolecules such as lectins (Carlsson et al. ,1979).

4 The fourth technique involves the covalent bonding of cells to one otherwise inert substratum such as carboxymethyl cellulose (Jack and Zagic,1977).

As already mentioned it has been long realised that the regulation of metabolism is largely under epigenetic control that is determined by the interaction between genome and endogenous and exogenous environment. The various intermidiates of secondary metabolities produced work as barriers to influence physical and chemical factors of exagenous

nature. This observation has led to artificial stress and study the effect. Zeleneva and Khavkin, (1980) showed that the ezymatic compliment of organized cells is less disrupted than the freely suspended cells. This made a valuable suggestion that immobilized cells may be better able to respond to environmental stimulii. In support of this there are evidences to show that under immobilized condtions some species retain secondary metabolities intracellularly which otherwise released to the surrounding media. For instance Solanum nigrum, Dhatura innoxia accumulated alkaloids with culture within the cell itself in that flat bed reactor or entrapped in calcium alginate (Lindsay, 1982). On the other hand the cell of Capsicum frutescens immobilized in the flat bed reactor in alginate or in polyurathene foam released capsaicin in the circulating nutrient medium which could be continuously harvested by passing the medium containing the metabolite through a column of fabricated kiedsselgur (Lindsay and Yeoman, 1986).

Immobilization of cell and protoplast of Catheranthus roseus was tried by Lambe and Rosevear (1982) and Bapat et al (1986) in alginae, agar and agarose. They found that cells immobilized in alginates, agar and agarose produce uniform cell suspension in 2-3 weeks. The protoplast so immobilized comfortably underwent division giving rise to cell

suspension which other-wise in nomral condition fail to divide or even if divided it nacrosed within the few weeks. With all the studies barring few exceptions profitable exploitation of plant cell cutlure in the production of natural compounds has not been achieved.

There are various tenets of tissue culture as a part of micropropogation and genetic preservation and conservation of germplasm. Encapsulation of the buds of growing parts, regenerated plantlets and so on have been achieved. It was first tried in Morus indica where axillary buds were encapsulated in alginate and agar to produce individual beads. The beads could be cryopreserved at 4 °C for 45 days without loss of viability. This technique opened a new possibility of generating a complete plantlet, a noval propogation system.

Rapid multiplication of recalcitrant plant such as orchid by root meristem was developed by Morel (1964). This created a great interest in the recent years in the application of tissue culture technique, as an alternate means of asexual reproduction of economically important plant (Hu and Wang, 1983). The size of propogate in culture is so minute that in vitro asexual propogation technique has refer to micropropagation. There are three possible means available for in vitro propagule multiplication :

1 Enhanced release of axillary buds, 2. production of adventitious shoots 3. Somatic embryogenesis. As already mentioned above George Morel (1964) was pioneer in developing methods of meristem culture for the elimination of viruses and shoot tip culture as a clonal multiplication tool. After his success in cloning the orchid <u>Cymbidum</u> in vitro, clonal multiplication of plants has become one of the popular techniques. One of the greatest discoveries that he made has been the two unique substances of the same chemical family <u>Octopine</u> and <u>Nepoline</u> belonging to group opines not characeristic of the plant but a bacterium in the crowngall inducing tumors. Morel (1971), suggested that the synthesis of opines depends on the process in the tumor cells of the genes coming from the bacterium.

Although the great progress has been made in micropropagation of herbacious horticultural species, the micropropagation of woody species is restricted. The constraints in woody species are induction of rooting when explants are taken from mature trees (Hu and Wang. 1983). All the same breakthrough was made while culturing the apple shoot when the medium was supplimented with a phenolic compound floroglucinol (Jones and Hatfield, 1976). This achivement stimulated the wave of micropropagation of woody species. Begining with this many forest species which are ratner shy enough to root from the cutting could successfuly regenerated and voluminous information is inflowing. One of

of the loudable achievements is of Laxmisita et al (1982) who produced plantlets from shoot tips of red sandle wood which normally took long period to regenerate. Although it is not possible to list all of them both in herbaceous and woody angiospermous plants, few worth mentioning are : Bajaj and Maeder (1974) successfulcultured adventetious buds and embryos in Anagalis arvensis Corcus (1973) raised plantlets of Arabidopsis thaliana under culture conditions. Regeneration of embryos in Asclepia curassavica was achieved under culture condition by Prabhudesai and Narayanswami (1973). Micropropagation of plantlets from the shoot buds of Codiaeum variegatum has been achieved by Chikkannaiah and Gayatri (1974). Nataraja (1971) raised seedlings of Consolida orientalis from shoot buds. Jayakar (1970) regenerated plantlets from shoot bud of Crepis capillaris. Kavathekar and Ganapthy (1973) obtained new plants of Eschscholtzia californica under culture condition. Nataraja et al (1973); Delanghe et. al (1974); Nataraja (1975) raised new plants from soot buds of Euphorbia pulcheriima Mehra and Mehra (1974) obtained regenerated plantlets from shoot buds and embryos of Mesebryomthemum floribundum and Prunus amygdalis respectively. Organogenesis in callus of Crotolaria medicagenia has been achieved by Raj Bhahsali and Arya (1981). Bajaj et al (1981 a) obtained geneticaly variable plants from anther

derived callus of Arachis hypogea and Arachius villosa. Similarly Sastri et al (1982) got improved plants of Arachis hyhpogea in culture.

Some of the successfully regenerated plants of the cereals under culture conditions are Maize, Sorghum, rice, Pennisetum and Eleusin . In the latter two they have been transfered for even cultivation (Rangan, 1973, 1974). Some of the medicinally important plants that have been cultured are Discorea Sensibarensis (Rao, 1969) Rauvolfia serpentina (Mitra and Chaturvedi, 1970) Tylophora indica (Rao and Narayanswamy, 1972). Some of the bulbous plants regenerated from various organs such as shoot buds, protocorms and roots etc. are Allium cepa (Fridborg ,1971) Gladiolus (Ziv et al 1970, Simonsen and Hildbrandt, 1971), Howarthia (Mujumdar,1970, Sabharwal and Kaul, 1972).

The most significant development in the field of plant tissue culture of this century has been the isolation and culture and fusion of protoplast. This makes the padestal for single cell hybridization and genetic engineering. The technique was first developed by Cocking (1960). Since the time a far reaching implications in the studies of plant improvement by cell modification and somatic hybridization is appriciated. (Cocking, 1972; Bajaj, 1974 a). The literature accumulated so far has revealed that the protoplast in culture can be regenerated in to an entire new plant. They can be induced to undergo intra and interspecific fusion to form somatic hybrid

and also to take foreign organlles or genetic material or DNA or the gene itself (Bajaj, 1977). This opened a new vistas in genetic modification of plants and transgenic technique or gene transfer by passing the conventional method. Many interested workers have come forward. Cocking (1976) observed the uptake of TMV by tomato fruit protoplast and studied the initial stages of infection by using electron microscope. This interesting experiments stimulated many workers to bring out the infection of protoplast of the plants with TMV. These studies have demonstrated that TMV is taken up by pinocytosis through the plasmolema and that the infection is stimulated by poly-ornithine. Such studies have given an insite to develop methods to put some useful organism or organalles such as bacterium and chlorophyll in-to the protoplast of interest. For example symbic tic nitrogen fixing baacterium Rhizobium could be introduced in legume protoplast (Davey and Cocking, 1972). Even efforts have been made to fuse these nodule protoplasts (Davey and Short, 1973) and such a investigations could result in to new symbiotic relationship. Introduction of free nitrogen fixing bacteria such as Azotobacter and blue green algae in to number of legumes has been tried. The incorporation of Nitrogen fixing gene (Nif-gene) in to nonleguminous plants was first tried by Child, (1975) Scowcroft and Gibson (1975).

Genetic transofrmation in bacterium by exogeneous DNA in the protoplast has been deeply contemplated and several techniques have evolved in the recent years. The work of Doy et al (1972, 1973 a, b) has attracted much attention. They have transformed haploid tomato and Arabidopsis callus culture

by Lambada phage. To test whether the lambada gene is functioning inside the Arabidopsis cell these transofrmed cells were grown on a medium containing galactose and lactose as the exclusive source of carbon. The act that it was able to grow provide sufficient proof that lambada phage DNA is functional. These authors called this phenomenon by new term "Transgenosis" for this type of transformations in higher plant systems. Sicne then the extensive search for different ways to put the desired gene inside the protoplast have been attempted by number of workers. Among the notable methods of gene transfer utilizing Agrobacterium tumifaciens was available. Devey (1980) and Krens et al (1980) showed that protoplast could be transformed by the DNA of bacterial-free Ti plasmids. The expression 'Direct gene transfer' appears to have been first used by Paszkowski et al (1984) to refers to uptake of DNA to plant protoplasts without the mediation of Agrobacterium or its Ti plasmid. One of the interesting aspects of Tii plasmid is that not only it can be used as vector to transfer the gene but the transformed tissue to acquire an ability to grow in medium devoid of auxins and cytokinins. This reflects that the genetic loci for auxin and cytokinin production, it is delivered in to genome of the growing cell and there by enable them to make the required hormones themselves. However the interest in direct gene transfer arose chiefly because of opportunity in surmountable

restiction of the Agrobacterium method to its dicotyledonous host because of which many important monocotyledonous crop species, notably cerels, are left out without a transformation procedure. Moreover even for species amenable to Agrobacterium infection, it could be agreed that the gene constructs required for direct gene transfer were simpler than for Agrobacterium (Larkin et al, 1990).

Most amusing work is transformed plant of Nicotiana tabaccum which is known to produce luminescence. Here the luciferous gene from the fire fly Photinus pyralis was transducted in to tobacco plant using Agrobacterium tumifaciens (Ow et al, 1986). Several methods of direct gene transfer has been developed but most commonly sued one is putting the gene in to he protoplast by electroporation where genes are knwon to express transiently (Fromm et al, 1985, Ou-Lee, 1986, Hauptmann et al,1987, Bates et al 1988). Successful electroporation can either shorter (5-200 micro seconds, high voltage 2000-1000 volts per cm) square wave pulses or longer (2-20 mili seconds, lower voltage 250-2000 volts per cm) capacitive discharge pulses. Polyethylene glycol (PEG) used gene transfer as an alternative method for electroporation is also employed (Krens et al 1982). In the recent years the microprojectile has been developed by plant virologist who used high velosity microprojectile to wound plant cells and facilitated entry of virus particles or nuclic acids (MacKenzie et al, 1966). However this is being employed to introduce gene or DNA. here DNA coated microcapsules are fired at the plant cells. This is often called biolistic or biobelistics. The particles are usually tungston, gold or platinum and the guns

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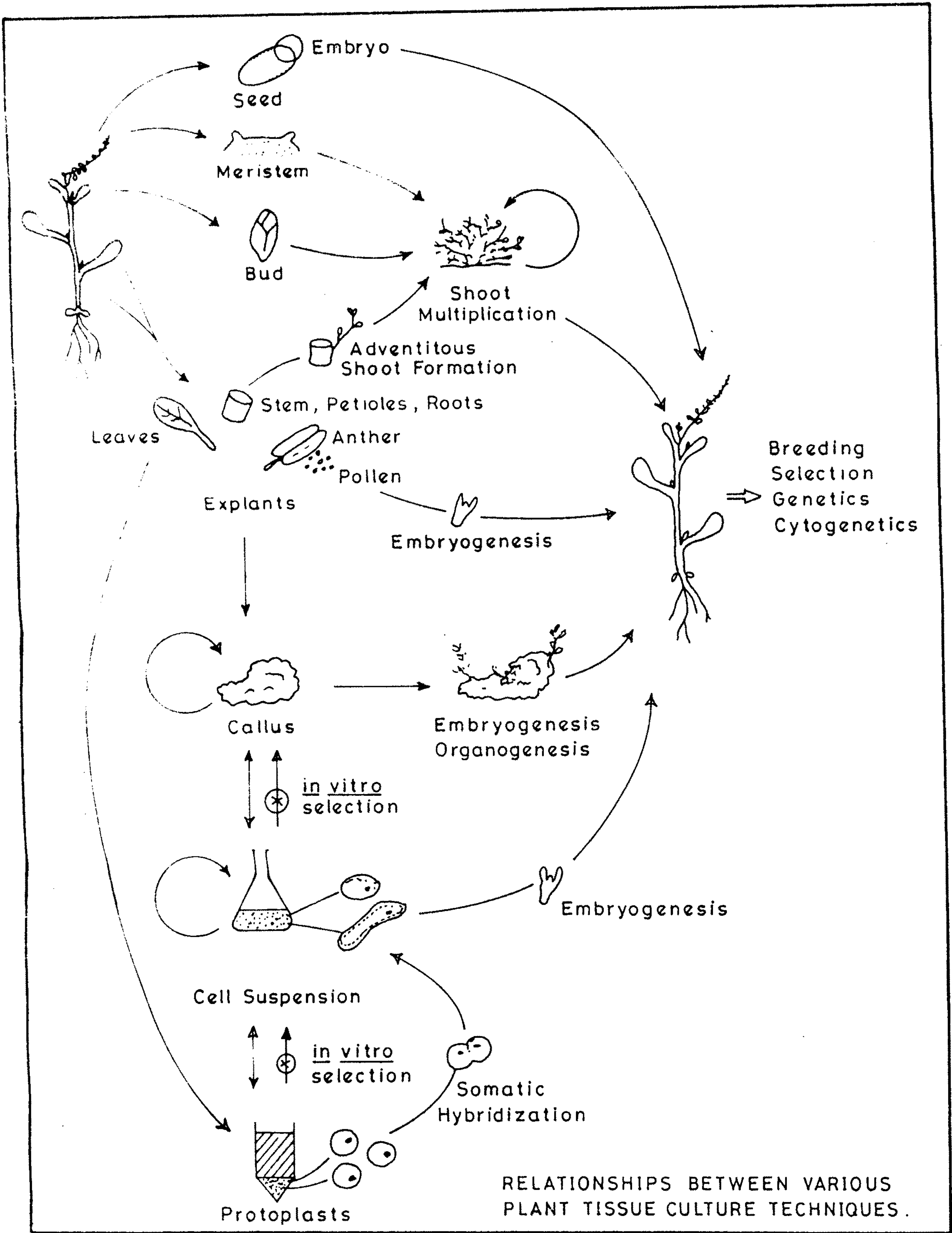
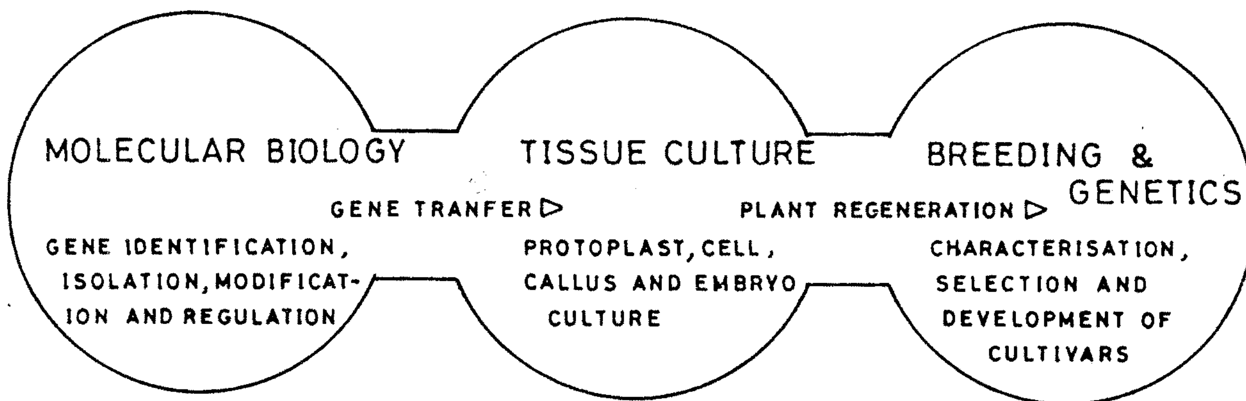
used to aceelerate DNA coated particles can involve explosive charges or high voltage discharges to explode water droplets (Klein et al, 1987; Chrisstou et al, 1987). The attrction of biolistcs is that the delivery of DNA promises to be independent of protoplast and not limited by Agrobacterium host range. The optimization of the microprojectile system for plant genetic transformation has been achieved by Birch and Frands (1991).

The chloroplast implataion is another mode of accomplishing genetic transformations. Nass (1969) for the first time tried incorporation of Spinach chloroplast in to the animal cell culture. Following this Giles and Sarafis (1971) implanted Nitella chloroplast in to chicken egg. They demonstrated amazingly enough, that these chloroplasts not only survived but were metabolically active and maintained their morphological intigrity and divided repeatedly. Subsequently many experiments were conducted in the field of organale implantation in to the protoplast. This opened possbility of improving the photosynthetic efficnecy of one crop plant by introducing the chloroplatst of other which has great effiency.



RELATIONSHIPS BETWEEN VARIOUS PLANT TISSUE CULTURE TECHNIQUES.



THE ROLE OF TISSUE CULTURE FOR THE GENETIC IMPROVEMENT OF PLANTS AND ITS INTERACTION WITH MOLECULAR BIOLOGY AND PLANT BREEDING.