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Plant Biotechnology: Importance of Plant Tissue Culture, Applications and Advantages

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Abstract:

The Science of Plant Tissue Culture means aseptic cultivation of plant protoplasts, cells, tissues, organs or complete plantlets in vitro, i.e. "in glass" in certain culture media and their incubation under controlled physical conditions, like, light, temperature and humidity. It provides the best system for experimental research where all the factors acting upon the explants are within control and can be varied selectively to pin-point the causal factor/s for a particular response. To be precise, in Plant Tissue Culture one studies In Vitro Morphogenesis to understand the process of chemical regulation of growth and differentiation – both morphological and biochemical – with the aim of unravelling the underlying causal processes and mechanisms, the least understood aspects of development. Morphogenesis reveals in succession, the genetic information in a most orderly sequence as reflected in differentiation and developmental events to give a format, which is species specific. When the cells and tissues are freed from the restrictions imposed by the organization of a whole individual, these can be induced to forget their commitments as a part of the organization and differentiate and develop as an individual entity, since these possess the complete genetic information for programming the development of a whole individual as well as different chemical pathways for synthesizing a whole array of metabolites. As such, for understanding the discipline of morphogenesis, knowledge of such other branches of Science as Morphology, Anatomy, Embryology,

Cytology, Genetics, Pathology, Physiology, Biochemistry, Molecular Biology, etc., is required.

Key words: plant biotechnology, plant tissue culture

Introduction

In Plant Tissue Culture, both fundamental investigations of science and applied aspects of industrial research can be pursued with equal intensity. The very first concept of Gottlieb Haberlandt (1902) of not only culturing plant cells *in vitro*, but also expecting vegetative cells to grow "into embryos", which developed into the science of Plant Tissue Culture, forms the basis of both fundamental and applied studies. The remarkable insight Haberlandt had and the prophecy he made can be appreciated by his statement: "Without permitting myself to pose more questions, or to prophesy too boldly, I believe, in conclusion, that it will be possible to grow, in this manner, artificial embryos from vegetative cells. In any case, the method of growing isolated plant cells in nutrient solutions could be a new experimental approach to various important problems". In fact, it has been the concept of "totipotency", which was later demonstrated by Steward with carrot cells in 1958.

A fallacy that the Plant Tissue Culture is empirical is largely based on ignorance of the fact that we have hardly understood Morphogenesis in so much so that the genetic make-up and the state of development of an individual have to decide the response that could be evoked by the morphogenetic stimuli, the suitability of which has to vary greatly in a biological system. For example, when the regeneration phenomenon is under genetic control and there is specific regeneration genes deciding the extent of ease with which a genotype can be or cannot be regenerated with particular morphogenetic stimuli, how all plant species or genotypes can behave similarly. For example, Oelck and Schieder (1983) have

demonstrated that regenerant differentiation in legume calli is under direct genetic control, implying that selection of species and cultivars can be critical and more important than the choice of culture media. It has been demonstrated by Lazar and colleagues studying with 7 addition lines, in which chromosomes from rye (*Secale cereal*) were incorporated into Chinese Spring wheat (*Triticum saestivum*) that rye chromosome 4 contains genes that control organogenesis in anther cultures and chromosomes 6 and 7 contain genes that control organogenesis in immature embryo cultures and there has been no correlation between organogenesis in another culture and that in embryo culture (Lazar et al., 1987). A particular genotype of tobacco may be induced to regenerate simply by submerging its somatic callus in liquid nutrient medium with no supplement of growth substances, i.e., perhaps by creating O_2 tension or certain others by adenine or kinetin, whereas that of other plant species may require 6-benzylaminopurine (BAP) or of still others may require isopentenyladenosine (2iP) or zatin or thidiazuron (TDZ) and so on. To make the point more explicit, Gautheret (1948) did not succeed in regenerating shoots from cambial callus tissue cultures of *Ulmus* and *Salix*, since cytokinins were not discovered by that time. Likewise, certain human beings may be treated with sulpha drugs or penicillin, while others may develop deadly allergic reactions. Similarly, some persons especially of 'O' blood group may have an enzyme of disintegrate sulphone drug, depsone (DDS), making it ineffective for *Mycobacterium leprae*, while in others it has been found very effective in controlling leprosy. Three could be many such examples, but they do not go to prove that the science of medicine is empirical.

The discovery of kinetin by Miller et al. (1955) led to the convincing first demonstration by Skoog and Miller (1957), using tobacco callus, or regulation of organ formation, shoots or roots, by changing kinetin-auxin ratio, where more kinetin than

auxin favours shoot formation, while the reverse favours root formation, which lays the foundation of study of Morphogenesis *in vitro*. Similarly, the astonishing rate of multiplication of an orchid *Cymbidium* by meristem culture demonstrated by Morel (1960) forms the basis of micro propagation and precisely clonal multiplication. It cannot be over-emphasized that right from the very beginning Plant Tissue Culture has been envisaged to solve applied problems. For example, White (1934) initiated tomato excised root culture actually to study multiplication of viruses in it and their elimination. Similarly, Morel and Martin (1952) undertook shoot meristem culture for virus elimination in *Dahlia* and later in *Cymbidium* (Morel, 1960). The former investigations by White led to the fundamental discovery that vitamins B1 (thiamine) and B6 (pyridoxine) are the root growth factors (Robbins and Bartley, 1937, 1939), while the latter investigations by Morel led to the most applied discovery of mericloning. Along an another dimension, i.e., production of useful secondary metabolites by Plant Tissue Culture, first significant studies were conducted through large-scale cultivation of cells or mainly tobacco during late 1950's to early 1960's at Pfizer Inc. by Tulecke and Nickell (1959), but it took about 20 years for its industrial utilization. Now, with the revival of interest in this branch, production of several high value compounds through cell suspension cultures using fermentation technology is being investigated, while a few have already been produced. It is now also known that in certain cases secondary metabolites are produced in higher quantities in cell cultures than by the plants, e.g., shikonin, ginsenosides, anthrquinones, akmalicine, and berberine, which are produced at the levels (% dry wit) of 20, 27, 18, 1, 10 from cell cultures as compared to their concentrations in the respective plants, i.e., 1.5 (*Lithospermum erythrorhizon*,), 4.5 (*Panax ginseng*), 0.3 (*Morinda citrifolia*), 0.3 (*Catharanthus roseus*) and 0.01 (*Thalictrum minor*).

Plant Tissue Culture is an important facet of Biotechnology and in many cases it becomes a limiting factor for the fruition of the goals of Biotechnology. A general connotation of Biotechnology is industrial utilization of biological processes. As such, the examples which first come to mind are things, like the use of yeast for fermentation or production of antibiotics, but if looked closely there are several important ramifications of this powerful branch of Biology. Broadly speaking, Biotechnology can be differentiated into aspects of Biomedicine and those of Agriculture, including Horticulture and Forestry. There are some glaring examples of useful products in the Medicinal Biotechnology, like, production of new drugs, vaccines and diagnostic procedures for detecting genetic disorders and, of course, a single example of production of human insulin from transformed bacteria cells (Johnson, 1983) (employing recombinant DNA technology in *Escherichia coli*, achieved by Eli Lilly and Co., Indiana, USA, in 1978 and its production started by 1982) can outweigh the achievements of the Agricultural Biotechnology so far as the improvement of quality of life is concerned. But with regard to human welfare and survival, the Agricultural Biotechnology is more important, particularly in view of population explosion in the third world countries. Biotechnology may further be distinguished between the area dealing with genetic modifications precisely Molecular Biology and the area where the principles of Morphogenesis are utilized, i.e., Tissue Culture. However, Tissue Culture becomes the nerve-centre of Molecular Biology as well in certain situations.

On the global scene and studying science for achieving novel things, the Molecular Biology approach is very attractive, but in the Indian context, the priorities are to be drawn keeping in view the financial resources, time period involved and the extent of dependence on import that can be permitted or sustained. The priorities of developed countries, like, USA can hardly be matched with that of a developing country like ours.

Transgenosis can render wonderful and very useful results, like those of transgenic cotton recently produced by introducing in it the insect control protein gene of *Bacillus thuringiensis* by Monsanto Company, USA, which is resistant to lepidopteran insect pests, which take a heavy toll of cotton produce in field. Likewise, if maize and some other field crops, like *Cajanus Cajan* and *Cicer arietinum* can be made resistant to such insects, their yields may tremendously be improved. And if, like the transgenic tomato with enhanced firmness and shelf life, produced by Calgene Inc., USA, by incorporating a bacterial 1-aminocyclopropane-1-carboxylate (ACC) deaminase gene, which limits the ethylene production, the mango fruit is also transformed, it will be of immense commercial value. However, upto 1991, though 395 transgenic plants have been field released, the list does not include any from India (Dale et al., 1993). But, with this impressive list, a parallel disappointing phenomenon of inactivation of transgenes following propagation and field cultivation is also at work, leading to the loss of engineered traits (Finnegan and McElory, 1994). Thus, the situation is not so rosy as once thought, but on the contrary is alarming in view of the News recentlypublished in Nature (Mccilwain, 1996): "A large question mark is hanging over the effectiveness and safety of one of the first genetically-engineered crops to be extensively planted, after cotton bollworms were found to have infested thousands of acres planted with the new breed of cotton in Texas", i.e., 'Bt cotton'. Besides, production of transgenic plants, despite spending enormous amount of money and time and employing sophisticated infrastructure, may not be possible in certain other cases because of several intricacies involved in the process. Leaving insect resistance, most of the useful traits are polygenic. The frustrating experience on this account faced in respect of nif-gene transfer to non-leguminous plants is of near past only. Techniques are not available to effect integration of many genes in the plant genome. Besides poor acceptability of

bacterial genes by higher plants, there may be several other problems, like, if multicopies of single genes are incorporated at random and at multiple sites, it will certainly adversely affect the activity of other vital genes. Furthermore, genes for a multigenic trait must be integrated at specific loci in order to have position effect. While such problems are yet to be resolved, the very basic requirement of identification of genes for specific traits and their cloning in most of the polygenic traits are not yet accomplished and may take several years before being solved. Thus, in the national context, first the concerted efforts need to be made where the problems are practicable and objectives realizable immediately or in the near future for economic growth of the country, while the strides in long-term realizable problems of Biotechnology requiring much basic research may be taken up later. The creation of blue rose, red tuberose and yellow petunias may be extremely fascinating, but commercial multiplication of ornamentals is definitely down to erth proposition, on which the economy of a commercially advanced country, like The Netherlands is based. Certainly, the second approach has to be a priority in the Indian context too, if we have to differentiate between the facts and fantasies.

Though, it is true that micropropagation is still the most commercially applicable aspect of Tissue Culture, there are some other facets too which are of immense practical value, like embryo rescue, *in vitro* pollination and intraovarian fertilization, meristem culture for production of clean stocks, androgenesis and gynogenesis for haploid production, endosperm cuylture for triploid production, induced nucellar polyembryony, somaclonal variation, somatic hybridization, synthetic seed production, germplasm preservation for conservation of phytodiversity and, of course, also production of active principles. The viewpoint expressed above may be appreciated by knowing some instances of achievements in these various aspects.

1. Micro-propagation

Multiplication of plants under micro-propagation can be effected by inducing caulogenesis, i.e., shoot bud differentiation and somatic embryogenesis in explants. Caulogenesis can be adventitious, without intervening callusing, at the cut end of an excised shoot tip as well as in the axil of nodal stem explants, or, it can be in callus tissue; the former results into direct regeneration and clonal multiplication, whereas the latter results into indirect regeneration, which may be of non-clonal nature. The basic difference between somatic embryos and shoot buds is that the former are bipolar structures and the latter unipolar. Thus, the somatic embryogenesis is advantageous since the embryoids possess a ready-made root system, while in regenerated shoots, rooting is to be induced. Here, a a point has also to be made clear that only the embryoids differentiated from somatic tissue directly without intervening callus phase or from very young zygotic embryos of homozygous plants are expected to produce clonal plants, but those differentiated from embryos, mainly from their cotyledons, of heterozygous plants cannot produce true-to-mother type plants.

1.1. Somatic Embryogenesis vs. Caulogenesis Regeneration via somatic embryogenesis or caulogenesis is generally species and/or explants specific (Chaturvedi and Mitra, 1975). For example, the vegetative explants of carrot produce somatic embryos, while those of tobacco differentiate shoot buds under the same morphogenetic stimuli. Similarly, tissues of vegetative parts of *Citrus* differentiate through caulogenesis, while those from reproductive parts via somatic embryogenesis under the influence of similar growth substances (Chaturvedi and Sharma, 1988).The aspect of somatic embryogenesis, particularly to understand the factors governing this phenomenon is of current interest, so that somatic

embryogenesis could be induced at wll in plant species which do not regenerate through this pathway (Thorpe, 1995). The role of arabinogalactan proteins in somatic embryogenesis (Kreuger and Van Holst, 1993) and that of polar transport of auxin in establishing bilateral symmetry at early stage of embryo development (Liu et al., 1993) are yet to be established. However, control of pattern of morphogenetic differentiation is of great significance and certain clues in this respect have been found out, albeit the information is far from being sufficient. A shift from caulogenesis to embryogenesis in root explants of *Solanum khasianum* is found to be due to the extent of hydration of the culture medium; perfect shoot buds are differentiated on nutrient agar, but embryoids are formed in liquid medium in th submerged state of explants, while transitory stages of shoot bud-embryoid are formed with the increasing hydration of agar gel with the same culture medium and growth hormones (Chaturvedi and Sinha 1979a; Chaturvedi and Sharma, 1990). In case of citrus, a shift from caulogenesis to embryogenesis in the stem callus tissue has been observed as a result of habituation (Chaturvedi and Mitra, 1975).

1.2. Some Examples Orchid micropropagation is the best example of Tissue Culture applications in the applied field that within a period of a decade of its initiation (Morel, 1964), it revolutionized the orchid multiplication and got stabilized as a multi-million-dollar trade in such small countries, like, Thailand and Singapore. Tremendous scope in this field exists in India, which is extremely rich in orchid flora. Besides orchids, there are a number of ornamentals valued for their flowers and foliage now being commercially multiplied throughout the world, where The Netherlands remains at the top. Tissue culture of ornamentals is more lucrative than other plants and that of foliage ornamentals still more, since their individual specimens are quite costly, even Rs. 1,000 per plant

in case of *Anthurium scherzerianum*. In our Laboratory a number of ornamentals, like, orchids (*Vanda hybrid, Dendrobium chrysotoxum, Rhynchostylis retusa*), *Chrysanthemum morifolium, Petunia hydrida, Rosa hybrid, Gladiolus, Amaryllis, Peperomia obtusifolia, Ficus elastic, Bignonia chamberlaynii, Monstera pinnatifida* and *Bougainvillea* have been multiplied by tissue culture, albeit to different extents (Chaturvedi et al., 1988, 1995). Similarly, a number of medicinal plants, namely, *Dioscorea floribunda, D. deltoidea* (the richest source of raw material for steroidal drug production), *Rauvolfia serpentine, Rosmarinus officinalis, Atropa belladonna, Digitalis purpurea* and *Chrysanthemum cinerariaefolium* have been multiplied at a very fast rate. To illustrate the point of superiority of tissue culture multiplication over the conventional method, not more than 8 plants can be obtained from a particular high-yielding individual of *D. floribunda* by tuber segments, whereas the method developed in our Laboratory can produce ca. 2.5 million cloned plants from a single axillary bud (Chaturvedi, 1975; Chaturvedi and Sinha 1979b).

Tissue culture has been advantageously used for multiplication of a number fruit plants. In our laboratory, extensive work has been done on *Citrus*, and the *in vitro*-raised plants, first for any fruit tree, have been grown in field, where they produced fruits (Chaturvedi and Sharma, 1988). Citrus production in Spain has been remarkably increased by the use of tissue culture, precisely micrografting (Navarro and Juarez, 1977). Recently, success has been achieved also in micropropagating forest trees, albeit they pose several difficulties as compared to herbaceous plants. Neverthless, some woody plants, like *Eucalyptus, Pinus radiate*, oil palm, rhododendrons, etc. are commercially multiplied. In our Laboratory, *Populus deltoids*, bamboos, *Dalbergia latifolia* and *Mitragyna parvifolia* have been successfully micropropagated. Success has been spectacular in case of *P. deltoids*,

multiplication of which has been eluding success so far in India and abroad alike. It is being mass propagated with our technology through the pilot plant facility of the Tata Energy Research Institute, New Delhi. The success in proliferating the shoots of *Shorea robusta in vitro* also deserves special mention, since this tree is most intractable-to-multiply even in nature. Multiplication of forest trees at a rapid rate for afforestation is imminent in view of the mass deforestation obtained all over the world. By a rough estimate, the forest cover has been reduced by 55 per cent over the years. In USA alone, there is a need of planting 1.5 million trees per day (Durzan, 1985). Such a high demand cannot be fulfilled without using Plant Tissue Culture.

1.3. Commercial Micropropagation In recognition of the great potential of micropropagation, it is indeed heartening that in our country also the wave of Tissue Culture Revolution is fast spreading. Commercialization of Tissue Culture in India started in the year 1987 by M/s A.V. Thomas and Company, Cochin. Its success with tissue culture of cardamom and orchids and their export was an encouragement for other firms, like, Indo-American Hybrid Seeds, Bangalore, which followed the suit to create the capacity to produce 10 million plants annually. Commercial tissue culture is taken as synonymous to micropropagation of ornamentals, which is valid for earning profits, particularly in global market, but may be erroneous if considered in the interest of the survival of mankind on the planet. In the latter context, micropropagation of plants necessary for restoring the green cover on earth should be given top priority. But, how to make it commercial is a big question? There is going to be a global demand of over 2 billion plants by the turn of the century against around 500 million plants produced presently by micropropagation and the market for it is expected to be U.S. $ 50 billion / year. There are about 100 commercial companies of the capacity for producing more than

a million plants, of which in India there are hardly 6. So far, the commercial micropropagation is confined to ornamentals, but a trend towards fruit plants has already set in. Covered area under floriculture in the world is about 20,000 ha. There are some startling figures which at one hand expose our miserable status in the world market of about U.S. $ 40 billion of hardly having 0.1% contribution, but on the other hand assure of a great scope for expanding micropropagation of ornamentals. The leading countries in Commercial Tissue Culture trade are The Netherlands, USA, Japan, Israel, France, Germany, Columbia, etc. However, per capita consumption of cut-flower and other floral products is maximum in Norway followed by Switzerland, Denmark and Austria, where it runs into U.S. $ 100-130. The cost of production / sq. ft. in Europe is around Rs. 350/- as compared to Rs. 75/- in India. Unfortunately with all the seasonal and agro-climatic and area-wise blessings obtained in India, where though about 35,000 ha land is under floriculture, its world share remains only 0.1% in contrast with The Netherlands, where the area under floriculture is only 3,600 ha, but its share is as much as 67%. The India Government has announced certain incentives and concessions for promoting export of floriculture with the target of about Rs. 100 crores in 1995-96. The main reason of India lagging behind even the small countries is that so far there is no self-reliance worth the name in respect of exploitation if indigenous technology. Whatever trade in micropropagation exists in the country is mostly based on tie-ups of Indian companies with the multinationals in The Netherlands, Israel, France, etc. How to change the scenario is again a major question, for which new strategies are to be drawn without losing any more time and ground. With the renewed interest in herbal drugs, its market in USA alone is expected to be of about US $ 47 billion per year by the turn of this century, however, the role played by Biotechnology in this area so far is negligible. This makes rapid clonal multiplication

of medicinal plants imperative, in which respect Plant Tissue Culture is indispensable. Besides, it also ensures production of high-yielding plants all the year round unaffected by seasonal or ecological barriers. The plants so produced, yield standard raw materials both in respect of quality and quantity of their active principles.

2. Embryo Rescue and *In Vitro* Pollination and Fertilization

A very practical and useful approach of immediate application is the embryo rescue, by which new hybrids even through wide crosses have been created, which otherwise are unexpected to be produced in nature. In fact, embryo culture is amongst the oldest applications of Tissue Culture when Laibach (1925) successfully created hybrid seedlings from a cross between *Linum perenne* x *L. austriacum*. A new crop, rich in protein triticale owes its existence to embryo rescue of the cross between *Triticum durum* x *Secale cereal* (Hulse and Spurgeon, 1974). Likewise, there are a number of examples where wide crosses in cotton, legumes, medicinal plants, etc., have been successfully utilized to create useful hybrids and variation. In our Laboratory, seed sterility in *Rauvolfia serpentine* due to degeneration of endosperm has been overcome by embryo culture from young seeds. In a, more or less, similar approach, the *in vitro* pollination and fertilization have resulted in intergeneric hybrids, which have been unknown in nature, involving plant species of Solanaceae, Caryophyllaceae, Cruciferae, etc. (Zenkteler, 1984).

3. Somatic Hybridization

Contrary to embryo rescue and *in vitro* fertilization, the most expensive research of somatic hybridization pursued for nearly 30 years could not yield any worthwhile parasexual hybrid,

which could not be produced in nature or by other methods. Such investigations must have consumed millions of dollars before being given up few years ago. The anomalous situation can be assessed by the fact that somatic hybridization involving *Nicotiana glauca* (2n=24) x *N. longsdorfii* (2n=18), worked by 3 groups (Carlson et al., 1972; Smith et al., 1976; Chupeau et al., 1978), has been reported to yield parasexual hybrids having variable chromosome numbers (28-183) in each case and only occasionally they showed the sum total of chromosome numbers of the two species involved. That is why, the chapter of parasexual hybridization has now been closed for creation of novel hybrids amongst the plant species or genera, which are genetically incompatible. However, its role for creating cybrids, transfer of cytoplasmic male sterility or to some extent imparting disease resistance still appears valid. To give a few examples, a parasexual hybrid, *brassica naponigra* produced from protoplast fusion of *B. napus* and *B. nigra* is resistant to infection of *Phoma lingam* and the parasexual hybrid of potato involving *Solanum tuberosum* and *S. brevidens* is resistant to several viral diseases. Similarly, glaring examples of producing disease resistant plants is in the cases of maize and tobacco. The cells were screened against resistance to methionine sulfoximide, added to the medium, which is similar in effect to the toxin produced by *Helminthosporium* in case of maize and *Pseudomonas tabaci* in case of tobacco. Such procedures are definitely simpler, realizable within a short time and far less expensive than transgenosis.

4. Shoot Meristem Culture for Pathogen Elimination

Elimination of viruses and other pathogens through shoot meristem culture is one of the most established and trusted methods. The clean stocks for more high-yielding than their diseased counterparts. The tremendous increase in *Citrus* production through *in vitro* micrografting in Spain is an event

of the near past (Navarto and Juarez, 1977). And to cite the earliest instance, the potato varieties King Edward and Arran Victory have been freed from viruses through shoot meristem culture and thus saved from extinction (Kassanis, 1957). Remarkable success has been achieved in our Laboratory in regenerating excised shoot meristems, measuring 1 mm in length, of field-grown trees of *C. aurantifolia*, for the first time, and in raising plantlets of *C. aurantifolia* and *C. sinensis* through micrografting of meristems measuring less than 1 mm in length.

5. Androgenesis and Gynogenesis for Haploid Production

Production of haploids through androgenesis or in certain cases through gynogenesis cannot be over-emphasized for their role in creation of new improved varieties. Recently, a treatise devoted to *in vitro* haploid production is being published in three volumes, of which one is already out of press (Jain et al., 1996). Its significance is immense in case of perennial crops, like, trees having long reproductive cycles, in case of outbreeding plants and that too with seedless character for making homozygous plants in respect of desired genetic characters. Production of haploids through anther culture in rubber, horse chestnut, *Populus*, and litchi, are some examples in trees besides some other woody plants, like, grape and tea. But the break-through in creating new improved varieties of rice, wheat and tobacco achieved in China is a real success story in this respect (Hu, 1978). In our Laboratory, androgenic plants of *Citrus aurantifolia* have been raised and grown in soil (Chaturvedi and Sharma, 1985). Haploids can also be produced byt eh chromosome elimination method, also called as bulbosum method. By crossing *Hordeum vulgare* with *H. bulbosum,* the haploid *H. vulgare* is produced, while the chromosome complement of *H. bulbosum* is eliminated.

Likewise, crosses between *Nicotiana tabacum* and *N. Africana, Solanum tuberosum* and *S. phurjea, Medicago sativa* and *M. falcate* and *Triticum aestivum* cv. Solamon and *Aegilops* result into haploids of the former species by chromosome elimination procedure.Although there are fewer examples of haploid production through gynogenesis than by androgenesis, these include such economic plants as *Triticum aestivum, Oryza sativa, Solanum tuberosum* and *Hevea brasiliensis* (Yang and Zhou, 1990).

6. Endosperm Culture for Triploid Production

Triploids have at least two outstanding traits compared to their diploid counterparts, viz., more vigour and seedlessness of fruit (Elliott, 1958). Triploids of many fruit crops are already in commerce because of their seedless fruits, namely, grape, banana, apple, mulberry, watermelon, etc. Triploids of a few plant species have been raised through regenerant differentiation in endosperm culture. The first triploid has been formed in *Putranjiva roxburghii* (Srivastava, 1973), while some other prominent examples are: *Prunus persica* (Shu-quiong and Jia-qu, 1980), *Pyrus malus* (Mu et al., 1977), *Citrus grandis* (Wang and Chang, 1978), *Citrus* hybrid (*C. reticulata* x *C. paradise*) (Gmitter and Ling, 1991). In our Laboratory, regeneration of shoots has been obtained from excised endosperm tissue of *Santalum album* and efforts are being made to produce triploids of *Emblica officinalis* by endosperm culture with promising results.

7. Induced Nuclellar Polyembryony

Induction of nucellar polyembryony is of immense practical value in case of a number of fruit trees where true-to-type disease-free plants are required, while cloning by other methods is far more difficult as even if somatic embryos are

differentiated, they pose difficulty in producing high frequency normal plants. And if somatic embryos are not produced, the adventitiously rooted cuttings are inferior in their performance. There are some examples in fruit trees, where nucellar polyembryony is either induced or augmented, such as, *Vitis vinifera, Malus domesticum, Eriobotrya japonica* (loquat), *Eugenia jambos, Carica papaya*, etc. (Thorpe, 1995). Enormous work has been done on nucellar polyembryony in *Citrus* in our Laboratory and abroad. Nucellars of polyembryonic species, *C. aurantifolia* and *C. sinensis* and of a monoembryonic species, *C. grandis* and of root-stocks, like, *C. karna* and *C. jambhiri* have been produced and grown in field conditions (Chaturvedi and Sharma, 1988). Success has been achieved in obtaining proliferation and development of nucellar embryos of *Mangifera indica*, both scion and root-stock varieties (Chaturvedi et al., 1994).

8. Somaclonal Variation

Somaclonal variation for creating variation through somatic cell cultures has been introduced as a big hope (Larkin and Scoweroft, 1981). There may be several causes of somaclonal variation and besides involving chromosome and nuclear DNA, it may be of epigenetic nature as well. However, when variation is caused by transposable elements or demethylation of DNA, there are great chances of reversions, but still there are hopes of crop improvement by this phenomenon (Semal and Lepoivre, 1990). In any case, to-date a number of somaclonal variants have been produced in several important crop plants, like, sugarcane, potato, tobacco, etc. In sugarcane, besides creation of new varieties in terms of yield, certain varieties resistant to diseases, like, eye spot disease (by *Helminthosporium sacchari*), Fiji disease (by a virus transmitted by aphids) and downy mildew (by *Sclerospora sacchari*) have also been produced. Similarly, in potato the variants produced both in respect of

characters of tuber as well as resistance to early blight (by *Alternaria solani*) and late blight (by *Phytophthora infestans*). Amongst the ornamentals, a new cultivar of geranium known as 'Velvet Rose' has been created from callus cultures. The new cultivar differs from the original cultivar 'Rober's Lemon Rose' in having symmetrical flowers and seedlings. In our Laboratory also, several stable variants of *Dioscorea floribunda* and 1 of *Solanum surattense* have been created and the one in *Citrus sinensis* is being evaluated.

9. Synthetic Seed Production

Synthetic seeds produced by encapsulating somatic embryos or other regenerants may prove to be of great practical value both in propagation and storage of germplasm (Redenbaugh et al., 1986; Redenbaugh, 1993). Synthetic seeds if developed in crops, like, sugarcane may revolutionize its cultivation, as the stem pieces in storage are not only difficult to manage because of their big size, but are also susceptible to several fungal diseases. In our Laboratory, the basal pieces (3-5 mm in length) of *in vitro* regenerated and proliferating tillers when encapsulated in calcium alginate have been made to regenerate multiple shoots on morphogenetic medium. In potato, microtubers, in gladioli, microcorms and in orchids, protocorms have been likewise encapsulated to produce synthetic seeds, which on storage at 4^{o}C for several months have shown normal 'germination'.

10. Germplasm Preservation

The use of Tissue Culture for germplasm preservation in conservation of phytodiversity is one of the emerging areas of paramount significance for human welfare by establishing 'Gene Banks' for posterity. Of late, there is global awakening on this score. The tissue culture strategy is essential in case of

hybrids, which must be propagated vegetatively and in those cases where either the seeds re recalcitrant or not produced or the palnt material is very limited.

Cryopreservation of plant propagules, like, shoot meristems and somatic embryos has not yielded satisfactory results commensurate to the efforts made, time spent and resources utilized. The main drawbacks of the process being low recovery of plants and loss of regeneration potential within few weeks or months of storage. There are only a few examples to cite where the plants have been stored for ca. two-and-a-half years, viz., strawberry and pea (Kartha, 1985). In our Laboratory, two approaches have been successfully examined for long-term germplasm preservation. First, by limited growth cultures of shoots of a number of economic plants, notable amongst which are: *Dioscorea floribunda, D. deltoids, Solanum khasianum, Costus speciosus, Rauvolfia serpentine, Chrysanthemum cinerariaefolium, Atropa belladonna, Rosmarinus officinalis, Solanum tuberosum, Chrysanthemum morifolium,* orchids, *Rosa hybrid, Ficus elastic, Simmondsia chinensis,* bamboos, *Glycyrrhiza glabra*, etc. In the second approach, which is novel, the plant species are preserved in terms of their root cultures, which may be induced to regenerate, after several years of their establishment, normal plants. The root-regenerated plants have been grown in soil under field conditions. The method has been successfully tested in *S. khasianum* (spiny and spineless), *S. torvum, A., belladonna, Kalankoe fedtschenkoi* and to some extent also *R. serpentine* (Chaturvedi et al., 1991). Also, a tree species, like, *P. deltoides* has been regenerated from more than 3-year-old excised root cultures and normal plantlets raised. Recently, success has been achieved in inducing somatic embryogenesis in root explants of *Elaeis guineensis*, which may prove to be a break-through for conservation of germplasm of this important tree, a major source of oil, which cannot be cloned by any other

means. Generally, the period of preservation of germplasm of these plant species ranges from 4 to 16 years, tested so far.

11. Production of Active Principles

This important commercial aspect of Tissue Culture for producing active principles or drugs from cultures of plant tissues in bio-reactors has, of late, taken strides mainly in Japan involving private companies (Morris et al., 1986). A vegetable dye shikonin used for making harmless lipsticks and other cosmetics besides being used as a natural antiseptic agent is commercially produced by Mitsui Petrochemical Industry Co. Ltd., Japan, from cultures of cells of *Lithospermum erythrorhizon*. Similarly, the elixit of life is produced from large-scale culture, in 20 kl fermenters, of roots and cells of ginseng (*Panax ginseng*) by Nitto Denko Co. Ltd., Japan. Berberine, digitoxin and codeine are some other drugs, which are near to commercial production through suspension culture of cells of *Coptis japonica*, foxglove and poppy, respectively. In USA, commercial production of vanilla flavour from cell cultures of *Vanilla fragrance* by Escagenetics and sanguinarine from cell cultures of *Papaver somniferum* by Vipon Research Laboratories, at the behest of Colgate Co., have been undertaken. Several firms and academic institutions are engaged in production of taxol, a very promising antitumour compound from cell cultures of *Taxus brevifolia* as also *T. baccata* spp. *wallichiana* (an Indian species), the prominent amongst the companies in this regard is Phyton Catalytic, USA. Similarly, Mitsui Petrochemical, Japan, is now studying commercial production of vinblastine one of the most potent anticancer compound, costing 5 million U.S. $ / kg, employing a process developed by a Canadian Company, Allelix combining the cell cultures of *Catharanthus roseus* and a chemical coupling reaction. In our Laboratory, a number of active principles, including alkaloids, steroids, a cardenolide, and an

essential oil have been produced from *in vitro*-grown plant tissues and organs (Chaturvedi, 1979; Jain et al., 1991) diosgenin and solasodine, the base materials for manufacturer of steroidal hormones, have been biosyntehsized from somatic calli of *Bioscorea deltoidea* and *Solanum khasianum*, respectively, whereas, atropine and hyocyamine from excised root cultures of *Atropa belladonna*. A correlation between organogenesis and enhanced degitoxin biosynthesis has been demonstrated in seedling callus of *Digitalis purpurea*, while essential oil, reosemary oil has been obtained from *in vitro*-proliferating shoots of *Rosmarinus officinalis*.

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