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# PLANT TISSUE CULTURE TECHNIQUES

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*Plant cell and tissue culture in a simple fashion refers to techniques which utilize either single plant cells, groups of unorganized cells (callus) or organized tissues or organs put in culture, under controlled sterile conditions.*

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Plant cells shall divide indefinitely in appropriate culture medium and in many cases (but not with all species), can be induced to regenerate whole plants, so that thousands of clones can be produced from a single plant. One of the most important applications concerns *in vitro* plant *propagation* from apical tips or meristem culture. It is commonly used for rapid clonal propagation of superior genetic material, with an immediate effect on plant improovement. This technique allows also to produce virus-free plants and cryopreservation of germoplasm. Another very important application is in the field of genetics and plant breeding. Somatic cell genetics include: (a) induction of genetic variability (somaclonal variation and induced mutation); (b) *in vitro* selection of mutants (for toxins produced by pathogens or other stress factors like toxic ions, herbicides and salinity, for example); (c) embryo rescue (from interspecific crosses with embryo/endosperm incompatibility) for transfer of resistance genes from wild species; (d) haploid culture (from anthers or microspores) followed by chromossome duplication, for the production of homozigote lines; (e) somatic hybridization by means of protoplast fusion. More recently this techniques have been largely used in plant molecular biology studies and genetic engeneering by means of plant cell transformation with exogenous DNA, isolated from chromossomes, organelles or from cloned genes as well as from DNA constructions. Finally the utilization of batch cultures in fermentators for production of secondary metabolites like pharmaceutical or other chemical compounds, are progressess that shall be achieved in a near future for many products. Although plant tissue culture are comparatively recent techniques, its progress is very rapid; one shall expect that its utilization will help to solve many practical problems concerned to plant breeding and will enlarge the scientific basis of plant biology as a whole.

*Totipotency* – A notable property that distinguishes plant from animal cells is the ability to regenerate fertile plants. Even though not all plant cells retain their regenerating ability, this property is the basis for the success of gene transfer technology for the production of genetically modified/engineered superior plants. Many plant cells have the ability to reorganize as embryos following the path of somatic embryogenesis in a manner analogous to that observed after fertilization. Alternatively, under appropriate conditions, somatic cells can establish meristematic centers and regenerate mature plants through an organogenic pathway. This regenerating ability is not restricted only to diploid sporophytic cells, but micorspores also retain the capacity of regenerating haploid plants. In some cases, the lack of high regenerating ability from single cells in species of agricultural and commercial interest such as corn, soybean and beans has been an obstacle for the production of superior varieties by genetic engineering (Goldberg, 1988).

*Clonal multiplication* – Plant tissue culture can be used for large-scale plant propagation. Shoot tip culture is being established for many higher plant species (Murashige, 1977; Vasil & Vasil, 1980) and the number of descriptive

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protocols has been constantly increasing. Shoot tip culture is being used for the clonal propagation of ferns and of ornamental and horticultural plants, as well as trees (fruit and forest species) for several reasons, among which (according to de Fossard, 1976; Hussey, 1978) are the following: (1) rapid multiplication of hybrid cultivars of proven agricultural and commercial potential; (2) elimination of viral disease from infected stocks; (3) vegetative propagation of species of difficult propagation; (4) clone propagation throughout the year; (5) propagation of genetically uniform parent plants for hybrid on a commercial scale.

*Meristems* – Plant diseases are caused by fungi, bacteria, viruses, mycoplasms and nematoids. Efficient controls are available to fight most types of disease except those caused by viruses and by mycoplasm-like agents. Disease of viral origin are present in pratically all plants, whether they reproduce by seed or by vegetative propagation, and are responsible for serious quality and productivity losses (for reviews, see Walkey, 1978; Kartha, 1981, 1984). The process of meiosis, fertilization and seed formation functions as a filter for many types of viral disease, however, in species of vegetative propagation the pathogens are all passed on to the progeny. In the absence of chemical or physical treatments capable of eradicating viral disease from infected plants, tissue culture techniques, and meristem culture in particular, have been used to eliminate viral infection for the production of healthy plants. Since 1949 it has been known that the density of viral infestation in a plant decreases with closeness to the meristem apex (Limmaset & Cornuet, 1949). Starting in 1952, many infected species have been freed from viral disease, with very positive results in terms of quality and increased productivity, these results being simply attributed to plant health improvement. Another important application of meristem culture is the *cryopreservation* of germplasm collections. Prolonged preservation of genetic material at ultra-low temperatures such as that of liquid nitrogen (– 196 °C) is one of the most promising approaches for guaranteeing the genetic stability of plant material over long periods of time, since at this temperature the matabolic activity of cells pratically ceases. The meristems of plants are ideal organs for cryopreservation because they can withstand long periods of storage in liquid nitrogen after which they regenerate normal plants. Plant cells in culture are less ideal systems for cryopreservation mainly because of the genetic changes that occur during proliferation before or after freezing (Kartha, 1984).

*Applications for improvement* – In parallel to clonal multiplication and obtention of virus-free plants, plant tissue culture techniques have a very strong impact on the genetic improvement of plants. These new techniques, generically termed *genetic improvement by non-conventional methods*, in association with *recombinant DNA technology*, have opended possibilities for the combination of new traits in a more precise or elegant manner, permitting the selection of rare genotypes within a large population as well as the transfer of specific traits coded by a given gene of a species to an already improved variety. Within the context of non-conventional improvement techniques, particularly interesting are the methodologies described below. Plant regeneration from tissue culture either by organogenesis or by somatic embryogenesis can give origin to *somaclonal variation* which, in combination with *in vitro selection* systems, can be used to isolate variants and mutants with resistance to, or tolerance for, stress-inducing factors such as pathogen toxins, high ion concentrations in soil, herbicides and salinity for example. Somaclonal variation is also found among plants regenerated from cells and protoplasts. The use of *cell cultures in suspension* and of *protoplasts* permits the manipulation of plant cells by microbiological techniques since 1 g of leaf tissue provides approximately 1 to 2 million protoplasts from which 100,000 to 200,000 plants can be regenerated (Dodds, 1985). Isolated protoplasts are the basic prerequisite for the study of *somatic cell genetics* of higher plants, including *protoplast fusion* for the derivation of *somatic hybrids* or cybrids (asymetrical fusion products) and the transfer of organelles, chromosomes, isolated or vector-borne genes (plasmids or viruses) in order to obtain genetic transformation (Lörz et al., 1988). Isolated DNA transfer to protoplasts, cells or even intact plants can be performed directly or through vectors such as *Agrobacterium tumefasciens* or *A. rhizogenes*, among others. Certain genes can be isolated from a species and be integrated after cloning into the genome of another by *recombinant DNA technology*. A basic condition for its expression in the entire plant is that tissue culture techniques including plant regeneration from proto-

plasts or cells be fully dominated, a fact that is not yet the general rule for many species of agricultural or commercial interest such as grain producing crops in general.

Plant derivation from *haploids* by anther and microspore culture followed by the production of homozygous lines is an important methodology for selecting rare genotypes such as certain recessives since it opens the possibility of rapidly including them in cross combinations (Nitsch, 1981).

Gene transfer from wild species to closely related cultured species by *interspecific crosses* (introgression) is an important tool for recovering the variability lost during the genetic erosion produced by the domestication and selection process. Thus, important traits such as resistance to disease and pests and tolerance to environmental stress are being reintroduced into cultured species from wild species. To overcome hybrid embryo/maternal endosperm incompatibility, the *embryo rescue* technique followed by *in vitro* culture has been used with considerable success, facilitating the transfer of lost genetic variability (Fonseca, 1987).

*Bioreactors* – There is growing interest in plant cell culture as a source of secondary metabolism products. This is partly due to concern about the disappearance of natural habitats harboring wild species as well as to overcome problems of geopolitical instability in countries that supply crude plant material.

Cells in suspension could be cultured in fermenting units, thus representing a constant source of production of natural plant compounds. Considering that plants accumulate high secondary metabolite concentrations in specific cells and at well-defined stages of development, it may be assumed that, under special conditions, cells may also accumulate such high concentrations *in vitro*. Maximization of production and accumulation of secondary metabolites by cells in culture requires: (a) selection of appropriate genotypes; (b) selection of highly productive cell lines; (c) manipulation of environmental factors for increased metabolite production. Using this technique, more than 30 natural products have been synthesized in cell cultures in amounts exceeding those found in the intact plant (Balandrin et al., 1985).

An alternative method for secondary metabolite production is the use of plant cell cultures for the conversion of exogenous precursors to the desired metabolites, i.e., *biotransformation*. An example is methyldigitoxin hydroxylation to methyldigoxin by *Digitalis lanata* cells. Within a few years it became possible to select cells that carry out this reaction with 60 times higher efficiency (Barz & Ellis, 1981).

*Hairy roots* – The interest in the use of plant cell culture for the production of fine chemicals dates back to a long time ago. With rare exceptions, the commercial applications of this process have developed quite slowly mainly owing to the low and/or unstable productivity of cells in culture. New approaches have been recently tested based on the rapid growth of organized tissue cultures, the so-called *hairy roots* obtained by genetic transformation of plants by *Agrobacterium rhizogenes*. The integration of part of the plasmid of these bacteria into the plant's genome alters the auxin metabolism of transformed cells, leading to a phenotype characterized by abundant root proliferation. Hairy root cells have a great potential for the production of metabolites synthesized in dicotyledon species (Hamil et al., 1987). The use of organized tissue culture is still in the initial stage, but investigators are quite optimistic about this new technology both in terms of basic and applied research.

## CONCLUSIONS

Natural plant products have always been important sources of drugs, seasonings, fragances, oils, stains, gums, resins, rubber, insecticides and other compounds of industrial interest. Some investigators have gone as far as to attribute the advancement of the Industrial Revolution partly to the discovery and commercial exploitation of the rubber tree *Hevea brasiliensis*. Nevertheless, the full potential of the world flora in terms of chemical and biological components is still unknown today, with much still to be discovered. However, if the tendency towards destruction of forest reserves should continue at today's rhythm, investigators will have little time to study the useful components of the plant kingdom.

Collaboration between researchers who dominate advanced chemical technique (chromatography and spectroscopy) and researchers who develop plant tissue culture and genetic

manipulation techniques could lead to new ways of economically producing chemical substances of high value. These new technologies will certainly lead to an increased use of higher plants as sources of renewable materials.

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