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REVIEW PAPER

Plant Biotechnology: Promises and Challenges

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ABSTRACT

Development of procedures in cell biology to regenerate plants from single cells in any desired quantity provides the prerequisite for the practical use of plant tissue culture and genetic engineering in crop improvement. Such regenerating cell cultures are used for selection of mutants and for DNA transformation experiments. DNA transfer by means of engineered *Ti* and *Ri* plasmids has become an established technique for the rapidly growing list of dicotyledonous plants. Considerable success has also been achieved in making gene transfer techniques independent of cell culture methods. These techniques have given the opportunity to create, characterise and select plant cultivars which cannot be obtained by traditional breeding methods. The exploitation of plant cell cultures for production of pharmaceuticals, natural products of commercial importance and mass propagation of high-value crops by automation, have developed into an important industry with considerable potential for future. This paper discusses the recent advances and applications of plant biotechnology in agriculture and industry and the challenges that still exist.

1. INTRODUCTION

Dramatic gains in agriculture have been made in the last few decades largely as a result of intensified use of fertilizers and pesticides and by planting improved crops developed through a variety of breeding programmes[1-3]. The huge scale of inbreeding and the narrowing of the genetic base of cultivated plants cause increasing concern about the susceptibility of crops to major disease outbreaks.

Traditional plant breeding methods rely primarily on the recombination of interesting characteristics through sexual reproduction. This has led to the development of large number of high yielding varieties in a number of crop plants. However, by conventional means it is not possible to associate genes of practical interest from two incompatible species. It is even less feasible to consider inserting genes coming from non-plant sources such as microorganisms or animals into a plant. Compatibility, stability and breeding cycle duration are clearly the main factors limiting the efficiency of traditional plant breeding. Any process fully or partially overcoming these restrictions will, therefore, represent progress in the field.

The recent advances made first in cell biology and later in molecular biology have permitted the development of a whole set of new tools generally referred to as plant biotechnology, which can overcome many of the shortcomings of conventional plant improvement methods[4]. Like its biomedical counterpart, plant biotechnology has two important but critical interacting components, those of tissue and cell culture and molecular biology[5,6]. The rapid development of these new tools in no way challenges the validity of traditional plant breeding methods. They simply serve to improve the performance of the methods or to widen their scope of application.

2. TECHNIQUES IN PLANT BIOTECHNOLOGY

The main objective of plant biotechnology is to eliminate or minimise the major limitations of traditional plant breeding. There are two types of techniques: those aimed at modification of a genotype

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(A) and those aimed at stabilising or multiplying a pre-existing genotype (B) (Fig. 1). They may be exploited either in the form of whole plant, or remaining at cellular level as cell culture in bioreactors. These techniques primarily address the need of agriculture but they may also serve industry either indirectly through improvement of agriculture raw materials, or directly through production of plant-derived substances in bioreactors.

A volume of literature already exists on biological and methodological aspects of various plant biotechnology techniques in depth[7,8] and an attempt is made here to highlight only the major applications of these techniques and the inherent challenges.

3. PRACTICAL APPLICATIONS

3.1 Clonal Propagation & Somatic Embryogenesis

Plants can be regenerated in vitro from cultured meristems (micropropagation) or by formation of somatic embryos or adventitious buds. Clonal propagation has obvious advantages for many species, especially those in which seed propagation is not practical. Micropropagation also has application for many other species in which breeding methods require extended progeny testing to identify desirable plants. In vitro micropropagation is now used in many horticultural species, like strawberry, citrus, potato, sugarcane, cassava, geranium, etc. to make them

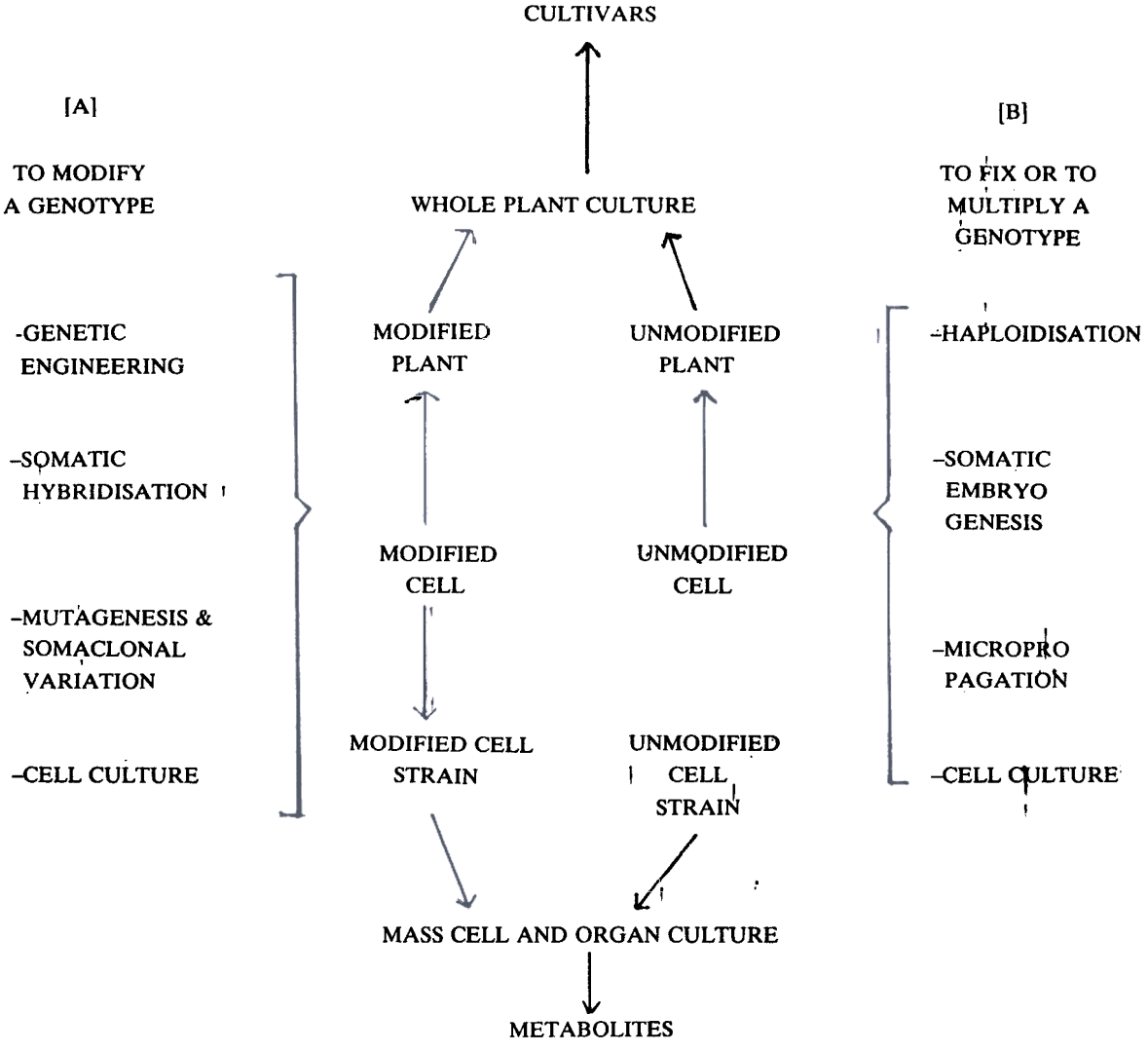

Figure 1. Techniques in plant biotechnology.

disease-free[11]. However, the current methods of micropropagation are time consuming and rely heavily on manual labour. The resulting high costs and low profitability have restricted the market to only high-value plants, such as medicinal, spices and fruit species. Therefore, automated methods of micropropagation can provide substantial reduction in the costs of production and in extending the technology to a wider variety of crop plants.

The greatest potential impact which can be made biologically on micropropagation is through the development of somatic embryogenesis, since somatic embryos are produced from callus in many species (e.g. alfalfa, bean, rapeseed, wheat, rice) in astounding numbers per gram of tissue and in theory are naturally self-singulating[12,13]. When a somatic embryogenesis system is well established, the embryos from callus act as independent, unattached biological units making them highly amenable to mechanical handling techniques. Synthetic seed production via the encapsulation of somatic embryos produced from callus offers an excellent system for automation and mechanical planting. High-value crops, such as celery, asparagus, lettuce, tomato, and brassicas are currently amenable for mass propagation by synthetic seeds. Newer procedures for encapsulating somatic embryos for synthetic seed production have been developed and are in field-testing stages[14-16].

Perhaps the most useful application of this technology can be in the reforestation of vast areas in many parts of the world. Mass propagation of buds has been widely used for multiplication of *Eucalyptus terreticornis, E. camaldulensis, E. citriodora,* and *Pinus radiata*. The multiplication of germplasm through micropropagation and tissue culture has been possible in hardwood and softwood species like sandalwood, populus, birch, douglasfir, *Thuja plicata, Acacia, Albizia lebbek, Peltophorum pterocarpum,* etc[17-19]. Integration of tissue culture methods with automation can provide reliable and efficient means to produce unlimited supply of high quality planting material for energy plantations, social forestry and biomass production.

3.2 Virus-free Plants

Another application of meristem culture is in the elimination of viruses from many plants. This is based on the observation that viruses are not present in cells of the meristem or if present are in very low numbers. Excision and culture of shoot meristem with only one or two leaf primordia, therefore, often results in the elimination of virus. Such virus-free meristems can be rapidly multiplied in culture by micropropagation. This simple procedure is being used widely in many countries to obtain virus-free potato, sugarcane, cassava, garlic, banana and many ornamental and vegetable species[20].

3.3 Germplasm Exchange

Germplasm resources are important prerequisites for initiating any meaningful plant improvement efforts. Many opportunities exist for locating novel genetic traits in wild, semidomesticated, or indigenous cultivars of important horticultural, forest or agronomic species. Plant cell and tissue culture allows the cryopreservation of shoot meristems, callus, somatic embryos and subsequent recovery and complete plant regeneration. Some of the successfully cryopreserved cultures include *Hyoscyamus muticus, Zea mays, Datura innoxia, Triticum aestivum,* and *Arachis hypogea*. Cryopreservation also facilitates large scale storage and transfer of plant materials in a disease-free condition for utilisation in crop improvement programmes worldwide[21].

3.4 Embryo Rescue

Agronomically useful hybrids between closely related species often cannot be obtained because of sexual incompatibility and abortion of embryos at various stages of development. The technique of embryo rescue which involves the excision and culture of young hybrid embryos has proven to be useful in growing such embryos to maturity and in obtaining many useful hybrid plants[22]. Genetic and field evaluation of the hybrids can be accelerated by cloning the hybrid embryos in culture to produce multiple embryos and plants from a single hybrid embryo.

3.5 Haploids

In vitro cultures of gametophytes of *Datura innoxia*[23] and *Nicotiana tabacum* opened the way to haploid production in a number of herbaceous and woody species. Cultured anthers, microspores and ovaries of more than hundred plant species have been used to recover haploid plants (plants with gametic chromosome number (n)) by stimulating the development of haploid gametophyte cells either to

form embryos directly or callus tissues which can be induced later to differentiate shoot buds. The doubling of chromosome numbers of such haploid plants readily yields homozygous diploids and fertile plants which are of much use in breeding programmes[24-27]. The few new cultivars of rice, wheat, maize, tobacco, oilseed rape and some other species that have been obtained by this method were of only limited commercial success, partly because the efforts require more coordinated and integrated approach with established breeding programmes.

3.6 In Vitro Selection

During the normal life cycle of the plant the mutant somatic cells are eliminated during sexual reproduction and are not passed on to the progeny. However, such mutant cells have an excellent opportunity to divide and multiply as do the non-mutant cells when plant tissues are placed in culture. Imposition of selection pressure in cell culture has been found to be very useful in preferential growth of mutant cells and in the establishment of mutant cell lines from which whole plants can be regenerated[28] (*Nicotiana tabacum, Zea mays, Medicago sativa, Solanum tuberosum, Lycopersicon esculentum*). Mutant selection is useful only to single gene traits such as resistance or tolerance to certain pathogenic toxins, herbicides, anti-metabolites, heavy metals and for producing mutants which overproduce useful amino acids[29].

Though many attempts have been made to select mutant cell lines and plants that are resistant to a variety of stresses (salt, aluminium, drought, frost, etc.), none of these attempts has been successful, largely because these traits are complex and multigenic in nature and no methods are known which allow simultaneous selection of desirable mutations in a number of genes. It is more likely that genes involved are active only in specific tissues or organs during specific phase of plant development, making it impossible to select for mutations in such genes during cell growth in culture.

3.7 Somaclonal Variation

Genotypic variation among regenerated plants from both somatic and gametic cell cultures (i.e., somaclonal and gametoclonal variations) has been suggested as a useful source of potentially valuable germplasm for plant breeding[30]. Variation for karyotype, isozyme characteristics and morphological variation has been observed in many instances, viz., *Apium graveolens, Dendrobium, Lactuca sativa,* tomato, potato, maize, etc[31]. In spite of many claims of the potential uses of somaclonal variation, so far there is not a single example of significantly improved new variety of any major crop species developed as a result of somaclonal variation and which is grown commercially.

3.8 Somatic Hybrids & Cybrids

Fusion of protoplasts has long been proposed as a novel and important method for the production of hybrid plants[32]. Somatic hybrids have been produced between species that are difficult or impossible to hybridise conventionally (*Lycopersicum esculentum* × *S. tuberosum, Datura innoxia* × *Atropa belladona, Arabidopsis thaliana* × *B. campestris*). Successful production of useful hybrids includes *Brassica naponigra* produced by the fusion of protoplasts of *B. napus* and *B. nigra* which is resistant to certain diseases. However, the recent work on the production of asymmetric hybrids is promising[33,34]. The greatest potential for the use of cell fusion methods will be in the production of cybrids which contain the nuclear and cytoplasmic genome of one parent and only the cytoplasmic genome of the second parent. Although complex and not clearly understood, certain agriculturally important traits are the result of interaction between the nuclear and cytoplasmic genomes. Well-studied cases include species of tobacco (*Nicotiana sp*) and combination of rapeseed (*B. napus*) nuclear genomes with cytoplasms from radish (*Raphanus sativus*). Cybrids can be of particular advantage in the transfer of cytoplasmic male sterility, an important trait in plant breeding[35].

3.9 Secondary Metabolites Production

The use of plant cell and organ culture for commercial production of natural products is one of the most challenging areas of plant biotechnology[36]. The production of pharmaceuticals by in vitro culture of plant cells, tissues and roots has been pursued for over 30 years and still the results have not met expectations. Only a handful of species are being used by industry for commercial exploitation (Table 1). The methodology used in metabolite production by plant cell cultures is very similar to that used in conventional microbial fermentation processes: first, a suitable strain must be isolated and then culture conditions must be

optimised for product formation with regard to scaling-up.

Table 1. Plant tissue cultures developed for industrial applications

Product	Species	Company	Country
Shikonin	*Lithospermum erythrorhizon*	Mitsui	Japan
Berberine	*Coptis japonica*	Mitsui	Japan
Biomass	*Panax ginseng*	Nitto Denki	Japan
Peroxidase	*Raphanus*	Toyobo	Japan
Geraniol	*Pelargonium sp.*	Kanebo	Japan
Rosmarinic acid	*Coleus blumei*	Nutterman	F.R.G.
Digoxin	*Digitalis lanata*	Boehringer Mannheim	F.R.G.

Recent advances in bioreactor design and technology coupled with study of elicitors as well as cellular immobilisation may allow expansion of this industry for production of additional useful compounds. Nevertheless, the high cost of production, coupled with limited market size of many of the products, has severely restricted the commercial application of this technology to only a limited number of compounds.

Biosynthetic potential of hairy root cultures is being explored in several laboratories. A massive increase in biomass over relatively short periods of culture and complete differentiation of root tissue warrant production of root-specific phytochemicals at substantial levels. In the near future hairy root cultures may become raw material for industrial processes in the production of pharmaceuticals or flavours or pigments[37] (Table 2).

Table 2. Examples of compounds synthesised by hairy root cultures

Class of compounds	Species	Examples
Pyrrolidine alkaloids	*Nicotiana tabacum*	Nicotine
Tropane alkaloids	*Hyoscyamus muticus*	Hyoscyamine
Piperidine alkaloids	*Securingea sp.*	Securinine
Quinoline alkaloids	*Cinchona ledgeriana*	Quinine
Steroids	*Panax ginseng*	Ginsenoside
Betalains	*Beta vulgaris*	Betanine
Polyacetylenes	*Tagetes erecta*	Thiophene
Naphthoquinones	*Lithospermum sp.*	Shikonin
Sesquiterpenes	*Hyoscyamus muticus*	Hyoscyamine
Phenolics	*Lupinus sp.*	Isoflavonoids

3.10 Environmental Clean-up by Cultured Cells

There may be a role for the use of actively growing cell cultures to remove certain toxic substances from contaminated effluent. It has already been demonstrated that suspension cultures of rose plants can remove polychlorinated biphenyls (PCBs) from solution and then chemically modify them[38]. Jackson *et al.*[39], have shown that suspension cultured cells derived from *D. innoxia* anthers could rapidly remove barium from solution forming a nontoxic, immobilised complex with a component of cell wall. *D. innoxia* cells grown in large fermentors may provide a relatively efficient and inexpensive means of removing barium from industrial effluents and cleaning up of contaminated sites. A similar process can be used for removing small toxic inorganic compounds, such as 2,4,6-TNT.

3.11 Gene Transfer & Transgenic Plants

Genetic transformation can be defined as the controlled introduction of nucleic acids into a recipient genome. It opens up the possibility of moving a specific gene from any potential donor to a desired species. Such genetically transformed plants are commonly termed as transgenic plants[40-43]. The introduction of DNA into plant cells can be achieved in several ways, e.g. the use of natural plant pathogens such as the soil bacterium *Agrobacterium tumifaciens* (*Ti* plasmid) and *A. rhizogens* (*Ri* plasmid) and viruses (which may contain either DNA or RNA), as carriers of new information to plants[44].

Agrobacterium vector system is being used extensively now for the transfer of various traits to crop plants as well as for the study of gene function in plants. Applications include transfer of genes effecting such widely diverse traits such as resistance to viruses, herbicide tolerance, altered flower colour, altering shelf-life of tomato, male sterility, cold tolerance, altered starch composition and resistance to pathogenic bacteria.

The most serious limitation of *A. tumifaciens* is its inability to transfer genes to graminaceous monocots, especially the cereals. The limitation is overcome to a large extent by the use of direct gene transfer or vectorless gene transfer techniques. Such alternative gene transfer methods include electroporation[45], sonication, UV laser, pollen transformation[46], microinjection into somatic embryos and reproductive

organs, and the highly advanced microprojectile or particle bombardment by DNA particle gun[47,48].

Microprojectile bombardment employs high velocity metal particles to deliver biologically active DNA into plant cells. The ability to deliver biologically active foreign DNA into tissues or organs, appears to provide the best method for achieving truly genotype-independent transformation in many agronomic crops bypassing *Agrobacterium tumifaciens* host specificity and tissue culture related regeneration difficulties[49,50].

Transgenic plants have been produced employing some of the above methods in a number of crop plants like potato, cotton, tomato, soybean, rice, etc. (Table 3). Transgenic plants can also be used as 'bioreactors' for obtaining virtually unlimited quantities of commercially useful proteins, biologically active peptides, blood factors, growth hormones, and antibodies[51].

Table 3. Transgenic crops that have been field-tested for the listed recombinant traits

Plant	Traits incorporated
Alfalfa	
Apple	
Corn	
Cotton	
Cucumber	
Melon	
Rape oilseed	
Papaya	
Potato	
Rice	
Soybean	
Squash	
Sunflower	
Tomato	
Walnut	

Detailed information about the yield and quality of transgenic plants and their products is not generally available, but these factors play a significant role in determining their practical use. Many of the transformed plants listed above are now undergoing extensive field and laboratory trials for regulatory purposes. Nevertheless, the concerns about the excessive use of herbicides with the availability of herbicide-resistant crops, the evolution of herbicide-resistant weeds by cross pollination with transgenic plants, the presence of bacterial and viral genes (proteins) in transgenic plants to be used for food, and the environmental consequences of the new technology, must be considered seriously, and appropriate safeguards and strategies must be developed to satisfy governmental and public concerns[52].

4. AUTOMATED PLANT TISSUE CULTURE FOR MASS PROPAGATION

The successful expression of foreign genes in model systems, such as tobacco and tomato, highlights the enormous potential of the marriage of molecular biology and plant cell culture. But biological and technical problems hamper the application of these technologies to important food crops such as cereals and legumes. Nevertheless, mass propagation by tissue culture technique – another facet of plant biotechnology – has developed into an important industry with considerable potential for the future[53].

One of the key unresolved issues in mass propagation is the high cost of producing plants from tissue culture. Recent development of the fully integrated, automatic plant tissue culture system aims at overcoming these problems and achieving that potential. This advanced biological, mechanical vitriomatic system integrates a bioreactor (fermentor) with a bioprocessor in a closed system. An automated transplanting machine transfers the propagules to soil mix in greenhouse trays[54]. *Begonia rex, Saintpaulia, Syngonium, Philodendron, Lilium,* potato, tomato, celery, asparagus, etc. are some of the species commercially produced with such automated vitriomatic system. Many of these advanced automated propagation systems can be of commercial use in developed countries to minimise the involvement of manual labour but they may not be appropriate and economically viable for developing countries.

5. CONCLUSION

The benefits of the most powerful and novel procedures of plant biotechnology will be realised only in the long run after more complete understanding of plant growth and development, and of the structure, function and expression of agronomically important genes. Some of the most difficult challenges facing

mankind during coming years and early part of 21st century will be production of food, reducing global pollution, and restoring the integrity of the environment. Plant biotechnology can play a critical and useful role in resolving each of these problems by providing better and more food, by reducing the need for pesticides, herbicides[55], fertilisers and improving the environment and slowing the greenhouse effects through massive global reforestation[56]. However, the fundamental knowledge of plant growth and development, and its molecular control, will be the key to realising the above objectives.

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