suppression by glyphosate. Genetically engineered tobacco, tomato, petunia and rape plants, resistant to glyphosate, atrazine, etc. are currently under advanced stages of evaluation prior to release.

Engineering plants for nutritional quality

From the nutritional point of view, plant proteins *per se* suffer from amino-acid imbalances. In situations as prevailing in India, where the major source of dietary protein is of plant origin, distortions of amino-acid balance reduce the nutritional efficiency of protein. By genetic engineering, it may be possible to correct the imbalance of amino-acid profiles in seed proteins. Storage-protein genes are expressed during specifically limited periods of seed development and are, therefore, relatively easy to identify and isolate. Transgenic plants, with the gene for the zein fraction, which improves the lysine content of the storage protein in maize, have been produced. It has been demonstrated that the gene has the normal level of expression in a stage-specific manner in the seed.

Lathyrus sativus, popularly known as kesari dal, grown widely in central and eastern India, contains an aflatoxin and thus, quality-wise, is not suitable for human consumption. The aflatoxin is a neurotoxin that causes a paralysis of limbs known as lathyrism. Engineering plants devoid of the neurotoxin can be achieved by (a) mutating the gene for toxin synthesis so that the toxin is either not produced or produced in low amounts or (b) developing transgenic plants harbouring the antisense gene so that the gene product is not formed. The latter strategy is expected to work via complementary base-pairing between the antisense mRNA and the coding, sense mRNA, which will prevent translation of the latter into the gene product.

Conclusion

In the preceding overview we have tried to give a broad coverage of the areas where biotechnological methods have started yielding or are expected to provide unique results in plant-improvement programmes. Basically, biotechnological approaches involve cellular intervention using tissue-culture methods and recombinant-DNA technology. In the Indian context, while the first approach has advanced fairly well and is at the take-off stage, the latter approach is still in its infancy. It may therefore be prudent to use the tissue-culture approach in solving relevant problems for the present and build up facilities and trained manpower for absorption and application of recombinant-DNA technology in solving the more intractable problems. It must always be kept in mind that the initial product of plant-biotechnological approaches in general is the generation of novel genotypes. These must be processed into commercial viabilities through conventional breeding procedures. Therefore mechanisms should be developed to ensure that integration of biotechnology with conventional plant breeding is not opportunistic but is deliberate.

Plant biotechnology in India—the role of plant tissue culture

A. F. Mascarenhas

Plant tissue culture, besides offering many advantages for research in plant developmental biology, biochemistry and molecular biology, and the promise of improved crop plants, has opened up vast commercial possibilities. Micropropagation of ornamental plants and plantation crops is already a big business. The Department of Biotechnology supports many laboratory and commercial projects in plant tissue culture. There is much scope for use of modern disease-diagnostic methods, and cell-manipulation and recombinant-DNA techniques.

In the past three decades plant cell and tissue culture has emerged as a major tool in the study of an increasing number of applied and fundamental problems in the plant sciences. It has now become an important integral constituent of 'plant biotechnology' research and is actively pursued by scientists in universities, research institutes, laboratories and private companies.

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India is reported to have one of the largest groups of tissue-culture scientists in the world. The objectives in most cases are development of improved plants for agriculture, horticulture and forestry using now well-recognized techniques. Tissue-culture approaches are also being expanded to carry out investigations in cell and developmental biology, biochemistry, physiology, genetics and molecular biology that are intended to provide insight into the fundamental mechanisms of plant growth and differentiation.

In this article I examine efforts being made in India to commercialize plant tissue culture and the present status of research in this direction in the country. This article has been prepared purely from discussions with scientists and from published information, which may not be exhaustive. It is possible that work in some laboratories may have been inadvertently omitted. To avoid repetition the data have been tabulated.

High-quality ornamental plants are easily multiplied by tissue culture.

Micropropagation

One of the main applied areas in which plant biotechnology has begun to manifest its potential in India is micropropagation using tissue culture. Micropropagation of plants is an invaluable aid in the production of elite clones and is playing a key role in the development of high-quality plantation, foliage and horticultural crops. There is widespread interest in this industry, with several private companies looking into the possibilities of investing in it. The spurt in activity in commercializing tissue culture has arisen only in the past seven years, since A. V. Thomas and Co., Cochin (AVT), set up their first small-scale production laboratory at Manalaroo in Kerala for cloning selected cardamom plants from their own farm collections. This was based on a small-scale laboratory technology developed and released to the company by the National Chemical Laboratory (NCL), Pune. This technology was later scaled up by the company, so that the process could guarantee a cost- and quality-effective and an efficient delivery system to make the method more production-oriented. Simultaneously AVT expanded its activities in a modern, well-equipped laboratory in Cochin, with an annual turnover capacity of around eight million plants. This unit was provided with all the facilities and amenities for maintaining a very hygienic atmosphere, comparable with the best laboratories in the world. One of the priorities, in both laboratory and greenhouse, was the use of indigenously available materials. This high-risk, capital-intensive pioneering venture, at a time when commercial tissue culture was still a new concept in the country, was commendable, and was born out of the courage and conviction held by the management that plant biotechnology can play a major role in India. These pioneering efforts in biotechnology research by AVT have now resulted in the development of Ernakulam as the first 'biotechnology district' in India, and have also given new direction to several other entrepreneurs who have entered the field. Table 1 lists the different companies that have already entered the biotechnology fray or have plans for the future.

Bangalore-based Indo American Hybrid Seeds (IAHS), a pioneer in hybrid flower and vegetable seeds, has imported a modern tissue-culture laboratory and hi-tech greenhouses with a capacity of 10 million plants per annum. A tissue-culture bank is also being maintained for preservation of breeding lines of vegetables such as tomato, cabbage, onion, chilly, etc. The company proposes to introduce ready-to-use diagnostic kits and also to take up disease indexing and screening for resistance against major viral, fungal and bacterial plant diseases, mainly for vegetable crops.

Some companies have smaller targets and produce plants mainly to meet domestic requirements. Some research organizations carry out work to standardize procedures on a laboratory scale (Table 2); these technologies are then released for exploitation. Table 2 also lists laboratories where other applications of tissue culture are pursued and are at the field-evaluation stage.

Role of the Department of Biotechnology

The Department of Biotechnology (DBT), New Delhi, is playing a special role in promoting research and development in plant tissue culture. Its programmes cover a wide range of important crop plants and trees (DBT Annual Report 1989). Under a project on production of biomass (fuel, fodder, timber and commercial/industrial woods) using tissue-culture technology, 14 nationally important tree species have been

Table 1. Status of tissue-culture technology in India—Commercial scale production

Laboratory/Company	Plant species	Remarks
Bio Tissue Culture Lab, Hyderabad	Pomegranate*, banana etc	—
Harrison's Malayalam Ltd Bangalore (RPG Group)	Vegetable crops (application including micropropagation)	Biotechnology laboratory for micropropagation and other aspects of tissue culture with foreign collaboration
Hindustan Lever Bombay	Cardamom, sugarcane	For domestic market and trials
Indo American Hybrid Seeds, Bangalore	Banana foliage and flowering plants cardamom etc	For domestic and export market
Nath Seeds Aurangabad	Ornamentals etc	Foreign collaboration, for domestic and export market
Padumjee Pulp and Paper Mills Ltd Pune	—	A commercial laboratory is under consideration
Reliance Co India Pune	—	A commercial laboratory is under consideration
A V Thomas and Co, Cochin	Banana foliage and flowering plants cardamom* orchids	For domestic and export market, a laboratory to conduct research on different aspects of biotechnology being set up
Tree Tech Corporation, Haryana	Tree species	Foreign collaboration
Unicorn Biotech Hyderabad	Cardamom*, ornamentals banana	With foreign collaboration

*Process released through NCL

Table 2. Status of tissue-culture technology in India—Semi-commercial or laboratory-scale, other tissue-culture applications

Laboratory/Company	Plant species	Remarks
Botany Department, Delhi University Delhi	Flowering plants endangered plants Bamboo	Sponsored by Department of Environment (DOE) Sponsored by Department of Biotechnology (DBT) For evaluation trials
Biotechnology Centre, IARI New Delhi	Mustard Rice	Somaclonal variation for yield From haploids, undergoing trials also research on genetic engineering
Bhabha Atomic Research Centre, Bombay	Oil palm, sandalwood Medicinal plants	Oil palm micropropagation, sponsored by DBT For producing secondary products
Cardamom Research Institute Kerala	Cardamom*	For domestic market
Central Institute of Medicinal and Aromatic Plants Lucknow	Medicinal plants Endangered species	Somaclone of lemon grass isolated Sponsored by DOE
Deejay Hatcheries, Bangalore	Banana, cardamom	For domestic market
Excel India Ltd, Bombay	Orchids	For domestic market
EID Parry & Co Madras	Sugarcane	—
Grasim Forest Research Institute Harihar	Eucalyptus spp*, bamboo*	For conducting trials
National Botanical Research Institute Lucknow	*Dioscorea compositae*, *Populus*, *Citrus* sp	—
National Chemical Laboratory Pune	Cardamom, turmeric pomegranate, ginger sugarcane Turmeric Cotton papaya Custard apple Teak eucalyptus, bamboo Salvadora	Micropropagation for production of somaclones High curcumin For sexual hybrids by embryo rescue Haploids Pilot-plant facility Sponsored by DBT
National Bureau of Plant Genetic Resources New Delhi	Economically important crops like timber crops bulbous crops spices fruit crops and also medicinal and aromatic plants and endangered plant species	Activity at National Facility for Plant Tissue Culture Repository sponsored by DBT
North-Eastern Hill University Shillong	Pitcher plant	—
P P Pocha and Sons Pune	Ferns, gerberas	For domestic market
Rastriya Chemicals and Fertilizers Bombay	Orchids sugarcane	For domestic market
Regional Research Laboratory Jammu	Medicinal plants	For isolation of secondary metabolites
Rubber Research Institute, Kerala	Rubber	For their plantations
Southern Petrochemical Industries Corporation Madras	Coffee	—
Sugarcane Research Institute Coimbatore	Sugarcane	Somaclones released
Tata Tea Ltd Kerala	*Eucalyptus globulus**, tea	For their plantations
Tata Energy Research Institute New Delhi	*Brassica* Forest trees	Embryo rescue, transformation Pilot-plant facility, sponsored by DBT

*Process released through NCL

Plants of *Bambusa arundinacea* raised from *in vitro*-grown seeds.

identified, and seven R&D projects in selected forest tree species are in progress. The setting up of pilot-plant units at NCL, Pune, and Tata Energy Research Institute (TERI), New Delhi, which will serve as an intermediate step in the transfer of tissue-culture technology from the laboratory to the field, is an important component of this project.

A project to compare the performance of high-yielding tissue-culture clonal cardamom plantlets with seedlings in a 100-ha area has also been funded by DBT. Under this project AVT multiplies buds from elite selections with high yield potential, identified by the Indian Cardamom Research Institute. As a result of higher anticipated yields expected from this approach, already indicated from trials conducted by AVT, it is possible that India, which had lost its share in the world cardamom market to Guatemala on account of low productivity and inconsistent supply, may regain its earlier commanding position. A National Facility for Plant Tissue Culture Repository (NFPTCR) at the National Bureau for Plant Genetic Resources (NBPGR) in New Delhi is using tissue culture to conserve different accessions of economically important crops like tuber and bulbous crops, spices, fruit crops and also medicinal aromatic plants and endangered plant species. DBT programmes also cover research and development in other plants and trees, some of which are listed in Tables 1 and 2.

Other applications

Conventional breeding methodologies are being used to develop improved cultivars from the existing varieties. There is also, however, an urgent need to develop cultivars with greater stability in growth-limiting environments like salinity, drought and inadequate fertility; disease and pest resistance; etc. This may be accomplished by modification of conventional breeding procedures and selection under limiting and non-limiting conditions. Biotechnological methods should be applied in those situations that cannot be successfully combated by conventional breeding methodologies. Table 2 gives examples of laboratories and research institutions where research on the different tissue-culture technologies is being carried out on a variety of important crop plants. Using somaclonal- and gametoclonal-variation technology new cultivars have been obtained in sugarcane (Sugarcane Research Institute, SRI), lemon grass (Central Institute of Medicinal and Aromatic Plants, CIMAP), rice (Indian Agricultural Research Institute, IARI), *Brassica* spp. (IARI, TERI), turmeric (NCL), custard apple (NCL), etc. In some of these plants it is necessary to determine whether the variation introduced has a genetic basis and can be transmitted through seed in a predictable manner. This would require longer periods and evaluation over a few generations.

In some laboratories, like Bhabha Atomic Research Centre (BARC), Regional Research Laboratory Jammu and University of Jaipur, systems are also being developed for enhancement of secondary-product synthesis. Application of recombinant-DNA technologies to obtain transformed plants (TERI, IARI, NCL) is another area with vast potential. This, however, is a long-range application and it may still be many years before stable transformed plants are obtained. With the diverse germplasm India is fortunate in having, the technique of embryo rescue gains importance in isolation of hybrids by sexual hybridization not possible by conventional methods. Protoplast fusion followed by hybrid selection and differentiation, and the use of DNA vectors would accelerate the pace of plant breeding and also help in generating useful variability. These are long-term goals, although, with innovation, the push towards the objectives can be hastened.

Importance for India

Commercial tissue culture is expanding rapidly in India, with several companies entering the arena. It is difficult to assess the success achieved by these concerns since most of the tissue-culture laboratories are supported by their existing parent organizations. Many of the companies have business links with foreign companies who transfer complete technologies to the Indian counterparts, who in turn produce the plants and export them to the parent companies. This procedure assures a steady export market for the companies' products, and at present appears to be an area that India should fully exploit. In addition, with the vast scientific pool of tissue-culture scientists in the country, we can also undertake research to standardize tissue-culture processes for which we are paying exorbitantly to foreign companies. We would then be in a vantage position to export our technologies, thereby reversing the general trend. To achieve this we would have to carry out research on some of the grey areas that still exist, like infection of cultures, maintenance of quality, and packaging and transportation systems. Each of these problems is important. For instance the last mentioned must be carefully considered since the products are highly perishable. Contamination of cultures, on the other hand, can threaten the very survival of the industry. Product price is another important criterion to consider in making the marketed product acceptable. This means that, between the development of a laboratory-scale process and its utilization for commercial-scale production, intense research and development is essential to make the process economically viable.

New methods of automation and production of synthetic seeds can be considered for production of cost-effective propagules in the future. The big role of micropropagation in India is mainly in the case of crops and trees where the quality of the product, rotation cycle and yield are of relevance. This would include fruit and vegetable crops, ornamentals, forest trees, and horticultural, medicinal and plantation crops. Improvements in greenhouse technology provide the advantage of exploiting year-round production, unaffected by seasonal effects. Other biotechnological approaches like genetic engineering, somaclonal variation, etc. should be exploited to generate a new range of superior genotypes.

We are on the threshold of a biotechnology revolution. India can exploit the situation with its vast pool of both talented manpower in tissue culture and diverse genetic resources of plants growing in a variety of climatic and soil conditions. The initiative and bold stand taken by DBT in funding advanced research in key areas and the entry of commercial companies could help India be a leader in this field.

Biological nitrogen fixation in the context of Indian agriculture

H. K. Das

Plants obtain their nitrogen as inorganic nitrates from the soil or from added fertilizer, and synthesize organic nitrogen compounds. Leguminous plants, however, can form associations with nitrogen-fixing bacteria (Rhizobium) and obtain organic nitrogen directly. The advantages of inoculation of Rhizobium for pulse crops, which are leguminous plants, are obvious. Success in increasing yield depends on proper selection of Rhizobium strains and large-scale production of culture. Recombinant-DNA technology helps in strain improvement, increase of efficiency of nitrogen fixation by the bacteria, and introduction of other traits into Rhizobium.

Biological nitrogen fixation is the reduction of atmospheric nitrogen to ammonia by a metabolic process. The process of biological nitrogen fixation is carried out by many species of microorganisms, mostly unicellular ones. These microorganisms can be classified broadly into two main groups, the free-living and the symbiotic (Figure 1). The former can fix nitrogen by themselves; they do not require the help of any other microorganism or a plant. The symbiotic microorganisms require association with plants; they fix nitrogen when they are in close contact with plants, and the two together create a situation where nitrogen is fixed. Of the symbiotic microorganisms, the most important by far are those belonging to the genus *Rhizobium*. These microorganisms form associations with leguminous plants, and also a few non-leguminous ones. They are very specific with regard to the kind of plant they associate with. For example, *Rhizobium meliloti* will only associate with plants like lucerne (alfalfa, *Medicago sativa*) or fenugreek (methi, *Trigonella foenum-graecum*) but never with French bean (*Phaseolus vulgaris*) or soybean (*Glycine max*). Table 1 lists examples of such specificity. All *Rhizobium* species are unicellular. They form nodular structures in the roots of leguminous plants and it is in these nodules that nitrogen is fixed. In agricultural practice, seeds are coated with the appropriate *Rhizobium* culture before

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