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PROSPECTS OF BIOTECHNOLOGY IN PLANTATION CROPS

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INRODUCTION

The advent of new technologies in the field of molecular biology has opened up a new vista of possibilities, so far unknown, to manipulate the genetics of crops for their improvements. The last decade has registered rapid progress in the field of plant biotechnology, and the list of crop plants which have been subjected to these new techniques is ever increasing. In general, woody tree crops are the most difficult for such manipulations and much more efforts are needed to cross certain technical barriers now slowing down the progress of work on these crops. Besides the classical tissue culture work, very little has been done in the field of molecular biology and genetic engineering in plantation crops. This paper, therefore, is confined to review briefly the progress made in other crops and to speculate the potential fields of activities which can be extended to different plantation crops. The review describes briefly some of the concepts used for transfer of genes and the problems associated with these methods. The literature cited is only indicative and by no means complete.

Once our knowledge of gene action is improved and the techniques employed in various aspects of biotechnology are refined, such studies at molecular level can be extended to more crops including plantation crops. Some of the areas where research can be initiated for plantation crops are listed below.

Micropropagation

Micropropagation techniques have been successfully employed in numerous plants eg. Lilium (Robb, 1957); tulips (Wright and Alderson, 1980); gladiolus (Hussey, 1977); gerbera (Murashige *et al.*, 1974); pineapple (Mathews and Rangan, 1979); sugar beet (Hussey and Hepher, 1978); apple (Jones *et al.*, 1977; Lane, 1978) etc., to mention a few. A wide range of tree species have also been propagated by this *in vitro* technique eg. plum (Garland and Stolz, 1981); pear (Lane, 1979); teak (Gupta *et al.*, 1980) date palm (Tisserat, 1979) and ornamental and forest trees such as fagus, quercus and ulnus (Chalupa, 1979) etc. *In vitro* studies have also been reported in some plantation crops like coconut (Guzman *et al.*, 1978; Pennitier and Buffard Morel, 1982; Iyer *et al.*, 1983); oil palm (Smith and Thomas, 1973; Jones, 1974); coffee (Sondahl and Sharp, 1977a, 1977b; Nsumbu, 1979; Dublin, 1980); cacao (Esan, 1982); tea (Phukan and Mithra, 1984) and rubber (Satchithananthavale, 1974; Sinha *et al.*, 1985.)

The technique micropropagation involves the *in vitro* sterile multiplication of plants on a precisely defined growth medium, incorporating specific growth regulators known to elicit specific growth

responses in plant tissues. This process can be used to maximise the elite individuals rapidly.

The general procedures used for *in vitro* propagation are:

(i) Selection of suitable explants.
(ii) Proliferation of shoots on multiplication medium.
(iii) The transfer of shoots to a rooting medium if required and planting out of rooted plants.

Advantages of Micropropagation

(1) Micropropagation can be profitably utilised for maintenance and mass-scale multiplication of desired genotypes.
(2) Propagation throughout the year.
(3) Lesser space requirement.
(4) Possibilities of rejuvenation from mature tissues.
(5) Disease indexing.
(6) *In vitro* selection of plants.

Somatic embryogenesis

Krikorian and Kann (1979) regarded mass somatic embryogenesis from cell suspension cultures as the ultimate goal in plant propagation and plant improvement. Steward (1958) was the pioneer worker in successfully achieving embryogenesis in carrot. Somatic embryoid production has been reported in numerous other crops eg. cotton (Price and Smith, 1979); date palm (Tisserat and De Mason, 1980); grapes (Srinivasan and Mullins, 1980); pearl millet (Vasil and Vasil, 1981, 1982); rice (Sriwardhana and Nabors, 1983); mango (Litz *et al.*, 1982; Litz, 1984a); Eugenias (Litz, 1984b) and the list goes on. Little progress has been made on this topic in plantation crops with the exception of coffee (Lanand, 1981; Sondahl *et al.*, 1984).

Applications

Difficulties do exist in transferring the somatic embryoids to the field, once efficient mass somatic embryogenesis has been obtained. Techniques involving trapping the developing embryos together with necessary nutrients in plastic strips or pellets can be used for bringing the naked embryos to the field. Refinement of fluid-drilling technique also would help in the transfer of somatic embryos/developing plantlets and their trouble-free establishment in the field.

Protoplast culture techniques and somatic cell hybridization

Protoplasts can be isolated from a wide range of species and can be induced to fuse by a variety of different fusogens to produce heterokaryons (Power and Davery, 1979). A pre-requisite for any use of protoplasts in crop improvement is the ability to regenerate plants. Success has already been achieved in several crops such as tobacco (Takabe *et al.*, 1971); rapeseed (Thomas *et al.*, 1976); cassava (Shahin and Shepard, 1980); potato (Shepard and Totten, 1977; Thomas, 1981) to name a few. Somatic hybrid plants have been produced between species that are difficult or impossible to hybridise conventionally, eg. *Lycopersicon esculentum* and Solanum tuberosum (Melchers *et al.*, 1978); *Datura innoxia* and *Atropa belladonna* (Krumbiegel and Shieder, 1979) and *Petunia parodii* and *P. parviflora* (Power *et al.*, 1980). No successful attempt is known to have been made in protoplast-fusion in any of the important plantation

crops. The most difficult part of the procedure is to induce regeneration of plants.

Applications

(1) Production of hybrids between sexually incompatible species;
(2) Production of heterozygous lines within a species which is normally vegetatively propagated;
(3) Transfer of limited parts of the genomic elements, particularly cytoplasmic organelles; and
(4) Production of somaclonal variants.

Somaclonal variation

Somaclonal variation occurs in plants regenerated from cultured tissues or cells and has been observed for morphological, physiological, bio-chemical and genetic traits (Larkin and Scowcroft, 1981). Such useful variants have been detailed in various crops like sugar cane for high sucrose content as well as disease resistance (Heinz *et al.*, 1977; Krishnamurthy, 1982); potato for its growth habit, tuber colour and uniformity, date of maturity and resistance to diseases (Shepard *et al.*, 1980; Thomas *et al.*, 1982); tiller number and seed protein in rice (Sun *et al.*, 1983); wheat for height, grain colour, tiller number and yield (Larkin *et al.*, 1984; Ahloowalia and Sherington, 1985).

Gene transfer in higher plants

Genes have been introduced into plant cells using donor materials in a variety of forms. Purified DNA is also capable of transforming recipient cells but the frequency of transformation is almost three orders of magnitude lower than that obtained using somatic cell hybridization (Cocking, 1983). Gene transfer can be done through DNA/RNA vector systems, through Ti plasmids etc. In order to develop a new organism through genetic engineering and/or gene transfer, various factors such as DNA structure, protein purification etc. have to be studied in detail. Genetic engineering is a novel technique used for changing the plants genetically-techniques that do not rely on pollination; instead involve genetic manipulations at cellular and molecular levels. This technique promises to be a powerful adjunct in modern plant breeding. But excellence in knowledge and techniques is a primary requirement to do anything worthwhile in this field.

DNA/RNA vector systems

Plant viruses received adequate attention from molecular biologists, due to their potential use as vectors for gene transfer in higher plants. The RNA genomes of some plant viruses are suitable for using as vectors for gene transfer.

Gene Transfer in higher plants through Ti-Plasmid

Crown gall is a neoplastic disease caused by the soil bacterium *Agrobacterium tumefaciens* in many dicotyledonous plants. Van Larabeke *et al.* (1974) reported that the tumour inducing capacity of the bacterium resides in large extra-chromosomal plasmids known as Ti plasmids (the T-DNA is that moiety of the DNA of the plasmid which integrates into the host cell DNA). Because the disease involves gene transfer from a bacterium to plant cells and subsequent expression of characteristics, crown gall has great potential as vector for genetic

manipulation of agricultural crops. Under natural conditions, *A. tumefaciens* cells in the soil enter the plant tissues through wounds and attack themselves to specific sites on the cell walls. A circle of DNA in the pathogen known as Ti plasmid then mobilises the transfer of a piece of DNA into the plant cell where it becomes attached to the plant's nuclear DNA (Van Larabeke *et al.*, 1974). The subsequent expression of this implanted DNA, the T-DNA, results in proliferation of tumour and produces opines, which in turn serve as food sources for *A. tumefaciens.*

Ti plasmids are useful in (1) manipulation of genes by gene transfer techniques and (2) stable introduction of foreign genes into plant cells without affecting the morphogenetic potential of the cells. This is an important pre-requisite for successful genetic engineering of plants.

Recombinant DNA Technology

It is known that recombinant DNA technology plays a pivotal role in the molecular analysis of genome organisation and its function. The application of recombinant DNA techniques to plant chromosomes has been extensively reviewed by Flavell (1980) and Bedbrook and Kolodner (1979). The main objective of recombinant DNA technology, in addition to the above mentioned analytical investigations, is the genetic modification of organisms. This includes the transfer of foreign or modified genes into eukaryotic or prokaryotic cells.

Isozymes in Plant Breeding

Isozymes are multiple molecular forms of an enzyme derived from a tissue of an organism. One of the most useful aspects of isozymes is that they are sometimes linked to important economic traits. Linkages between isozyme markers and important economic traits are being exploited in tomatoes, since the lack of effect of isozymes on appearance and performance renders them superior to the customary marker genes used for this purpose. Linkages with isozyme loci also assist in breeding programmes dealing with quantitative characteristics such as earliness or yield (Rick, 1982).

Plantation crops can be improved by some of the techniques stated earlier. However, the potential fields for immediate improvements where investigations can be intensified are given below:

Rubber:

1. Micropropagation is useful in developing elite clonal materials with its own root system to avoid stock-scion interaction or to evolve ideal stock clones.

2. Cell selection methods/somatic embryogenesis techniques can be tried to evolve clones resistant to drought or cold.

Cardamom:

As cardamom is propagated by seed (the crops is cross-fertilized) micropropagation techniques can be used for rapid multiplication to generate high yielding clonal materials.

Coconut:

Rapid multiplication of desired clones with special emphasis on disease resistant ones.

Cashew:

Rapid clonal propagation to obtain high yielding cultivars by meristem tissue culture techniques. More scion material can be generated by the above technique for use in grafting and to evolve rooted plantlets which are tolerant to pests and pathogens.

Coffee:

Evolving cultivars resistant to coffee rust disease by selection and regeneration of resistant cell lines using dual cultures of fungi and host callus tissue.

Oil palm:

Evolving elite conal progenies which give higher yield than seedling populations by either somatic embryogenesis, micropropagation techniques, etc.

Tea:

To obtain tea cultivars with leaves of better quality either by micropropagating the elite individuals or by producing new ones exploiting somaclonal variations.

In view of the current biotechnological achievements in other crops, similar techniques can be utilised in plantation crops. Several long term programmes can be envisaged to meet this objective. Some opportunities for using molecular plant genetic engineering in plantation crops can be categorised as follows (Qualset, 1982).

(1) Transfer of genes from one species to another that would not be possible with non-molecular methods.
(2) Transfer of genes at a single step, rather than through repeated crosses or back crosses.
(3) Transfer of only the target gene, without undesirable genes linked or otherwise associated with it.
(4) Transfer of genes rapidly in species with long generation times.
(5) Conservation of plant genes in cloned DNA gene banks.
(6) Assessment of genetic variation and genetic relationship among species by molecular methods.
(7) To capitalise on 'spinoff' technology for use in conventional gene transfer systems – for instance, tissue, cell or protoplast culture methods.

Requirements

The research work in the field of modern biotechnology requires sophisticated laboratories, high level of expertise and easy availability of necessary biochemicals. As long as constraints exist in acquisition of necessary equipment that will work trouble-free, availability of wide-range of ultrapure chemicals and trained man-power, the progress in this field of biotechnology is bound to be slow. Some suggestions are given to provide a conducive atmosphere for plant biotechnological research work:

(a) A central agency should be entrusted with import of required equipment, chemicals, etc. in bulk based on national requirements, so that the user agencies can purchase these from them on rupee payment without having to undergo the procedural delay in importing vital items for research.

(b) The facilities for training and conducting refresher courses for the scientists working in this field should be strengthened. Biotechnology should be

taught as a separate and independent discipline and its graduate and post-graduate programmes should be offered at leading Universities.

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