

Biotechnology in crop improvement

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There are, broadly, three benefits to agriculture and crop-improvement programmes from use of biotechnology. The first of these, the reduction of the duration of breeding programmes, makes use of tissue culture. Plant tissue culture also allows creation of new genetic variation, from which useful new varieties can be obtained. New methods of hybridization can be combined with cell and tissue culture for selection of plants with desired traits. The third and most promising benefit is from use of recombinant-DNA techniques. Transgenic plants, engineered for resistance to diseases, pests and herbicides, and for better nutritional quality, have already been produced in the laboratory.

Even though India has proved the predictions of the prophets of doom wrong, her balance of food in relation to the growing population has been a very fragile one. In the future it will be even harder to meet the requirements—because of the shrinking land resource, the setback to sustainability posed by deteriorating environment, and the gradual decline in farm size caused by the realities of the Indian social and rural structure. The work on major food crops, which contributed to the productivity of agriculture, has begun to show stagnation. These challenges require special efforts so that more can be produced from a smaller cultivated area with the attendant economics of production. Successive increases in production will have to be achieved in progressively shorter intervals of time. It is these compulsions that have driven scientists and policymakers alike to give serious thought to innovations that can provide an impetus to agricultural production efforts. Biotechnology has emerged as a very serious candidate instrument for achieving major production advances.

Crop improvement is a designer's activity: picking up useful characteristics dispersed in numerous individuals and putting them together to produce a variety containing as many desirable attributes as possible. In doing this a plant breeder performs apparently opposite activities. He first expands genetic variability through recombination and segregation of genes and by creating variation through such processes as mutation and tissue culture. He then makes selections to narrow down the generated variability by selecting a few desirable recombinants, and rejecting the vast majority that do not meet the requirements. More efficient methods of generating variability, specially of the desirable type, and methodologies that lend efficiency to the selection procedures are of great value to the plant breeder. This is precisely why biotechnology is an attractive option.

Application of biotechnological tools in crop-improvement programmes can be effective in three different, complementary ways: speeding up the processes of conventional breeding, creating genetic variability through tissue culture, and evolving novel genotypes through recombinant-DNA (r-DNA) technology.

Speeding up plant breeding

Anther culture

Conventional plant breeding is based on attempting crosses between desirable parents followed by selection of promising recombinants in subsequent segregating generations. The fixation of the genotype to produce lines breeding true to type requires repeated cycles of selection. This procedure is time-consuming and takes anywhere from 6 to 8 years. A way out of this problem is to produce plants from the pollens of F_1 hybrids and double the chromosome number of the haploids so produced to obtain a fertile diploid plant. This technique, called anther or pollen culture, has been used effectively to produce several commercial varieties of rice (*Oryza sativa*), and wheat (*Triticum aestivum*) in China. Attempts to use the anther-culture technique in interspecific and intergeneric hybrids in rice are under way at the Central Rice Research Institute (CRRI) in Cuttack and the Indian Agricultural Research Institute (IARI) in New Delhi.

A technique to induce flowering in tissue-cultured bamboo has been developed at the University of Delhi and the National Chemical Laboratory (NCL) in Pune. This is expected to facilitate recombination breeding in bamboo. The technique may enable breeders to produce seed for the propagation of bamboos, which is currently done only by vegetative propagation. Under normal conditions most bamboo species flower once in a multiple of 12 years, which limits the application of

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conventional hybridization techniques for bamboo improvement.

Molecular markers for selection

Selection and fixation of desirable genotypes occupy a central place in a conventional breeding programme. In recent years a number of techniques involving molecular markers have made genotypic selection quite reliable and highly reproducible.

- (i) A 'sap spot' method for selecting virus-free and resistant plants has been used very effectively. This method employs cDNA probes for the given viruses and detects the presence of viral DNA with complementarity to the cDNA in the cell sap of the plant.
- (ii) Repeated DNA sequences, tagged with the heterochromatic segments of rye (*Secale cereale*) chromosomes, have been used for selecting wheat-rye chromosome combinations that are devoid of rye heterochromatin in triticale. Rye heterochromatin is known to produce shrivelled seeds. The selection that lacks rye heterochromatin possesses well-filled grains besides agronomically important traits of wheat and rye.
- (iii) Biochemical markers showing linkages with traits not so easy to score have been used for identification of the latter. Such markers have been termed 'reporter loci'. In wheat a number of phenotypic traits have been

mapped to the short arm of chromosome 1B by their linkage association with *Glia-B* and *Glu-B-1* genes, which code for gliadin and glutenin protein respectively.

A very interesting case of use of biochemical markers is in selection for nematode resistance in tomato (*Lycopersicon esculentum*). An enzyme species detectable by its characteristic electrophoretic mobility has been used as a marker for selecting nematode-resistant genotypes. Since nematode infection is soil-borne, selection for resistance is very difficult through conventional means.

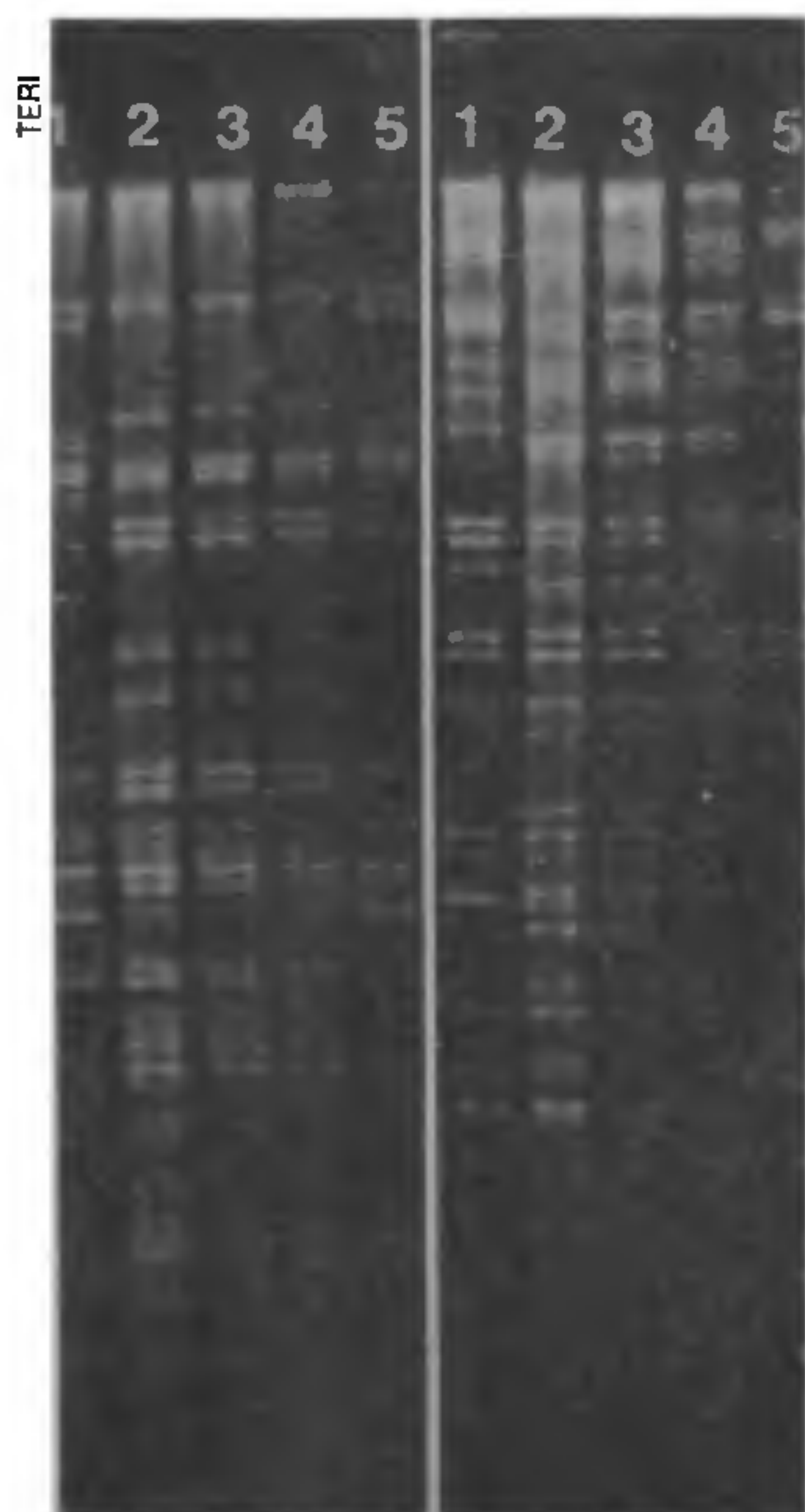
(iv) Tagging of economic traits with restriction-fragment-length-polymorphism (RFLP) markers has opened up an entirely new approach for efficient selection of desired gene combinations from a breeding population. This technique is primarily based on the thesis that restriction enzymes, which can cut DNA at specific sites, can distinguish between plant strains even if they differ by a single base in the restriction-enzyme site. Thus a changed pattern of DNA fragments is produced by cleavage with a restriction enzyme when a target site is present in the genome of one individual but is absent in the other owing to the loss of recognition by the enzyme due to the mutation. This polymorphism at the level of DNA has been used successfully for tagging the economically important traits, governed by either single or many genes. The technique has been quite helpful in selection of desirable genotypes in early generations from a segregating population and is being used extensively in breeding improved varieties of a number of crop plants across the world. For instance, in tomato, strains have been selected that carry high solid content, a character controlled by many genes. In India, RFLP studies have been initiated and are being used for molecular-mapping and character-tagging studies in mustard (*Brassica* spp), chickpea (*Cicer arietinum*) and rice at the Biotechnology Centre, IARI, New Delhi, NCL, Pune; and the International Centre for Genetic Engineering and Biotechnology (ICGEB), New Delhi.

Tissue-culture methods

It is now realized that the natural genetic variability in most of the crop plants has been mostly used by intensive breeding efforts. Future plant-breeding options will depend on the generation of usable new genetic variability within a species and on mastering the ability to mobilize genes of interest from unrelated species, genera and even taxa. We give below a few of the biotechnological approaches being followed for evolving new genotypes.

Creation of usable genetic variability

Plants regenerated from a tissue-culture cycle involving a dedifferentiated (callus) phase are known to show



Molecular markers. Restriction profiles of chloroplast DNA of (1) *Brassica juncea* Pusa Bold (2) CMS *B. juncea* Pusa Bold, (3) *B. taurinensis* (4) CMS *B. napus* BO15, (5) normal *B. napus* BO15 showing that cytoplasmic male sterility (CMS) is derived from *B. taurinensis*.

variation in a wide range of characteristics. This variation is called somaclonal variation. Since this variation arises through relatively mild conditions of *in vitro* growth, the genetic background, including adaptability characteristics, is not drastically altered. Processing of variability so generated for commercially releasable material is relatively simple. In India, somaclonal variation in sugarcane (*Saccharum officinarum*) has been processed into lines by the Sugarcane Breeding Institute in Coimbatore. These lines are at an advanced stage of testing before commercial release. At the Biotechnology Centre of IARI, New Delhi, a large number of somaclones in Indian mustard (*Brassica juncea*), which differ in plant characters of economic importance, have been isolated and are under evaluation for their use in the breeding programme.

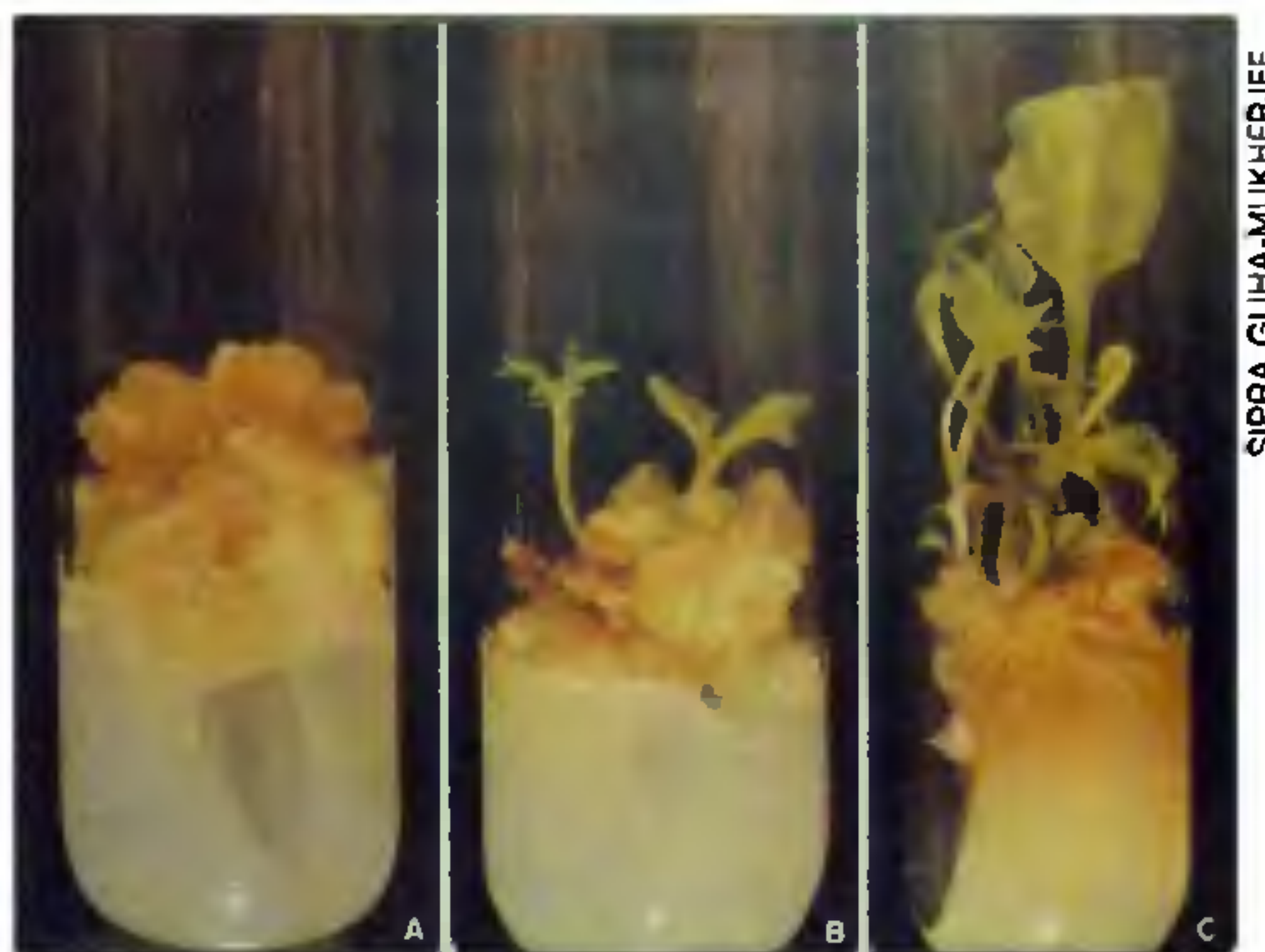
Wide hybridization

Wild and weedy relatives of crop plants are a storehouse of a number of desirable economic traits, but this gene pool generally remains unused because of sexual barriers, variation in chromosome number, lack of chromosome pairing and consequent sterility, etc. To overcome these barriers, two approaches have been employed: embryo rescue and somatic hybridization.

Embryo rescue. In those cross-combinations whose hybrid embryos are formed but fail to develop further, the embryos are rescued from collapsing by allowing them to grow on an artificial medium. Such embryo rescue allows the realization of a combination of genes of two species. At the Biotechnology Centre, IARI, the embryo-rescue technique has been employed successfully for harnessing genes imparting seed shatter resistance from *Brassica juncea* (Indian mustard) to *Brassica napus* (rape seed).

Somatic hybridization. Somatic hybrids can be produced by fusing protoplasts (cells whose cell wall has been digested enzymatically) of the two donor parents. Protoplast fusion and regeneration of hybrid plants have been achieved at the Biotechnology Centre, IARI, at both interspecific and intergeneric levels. Hybrid plants have been obtained by fusing protoplasts of Indian mustard (*Brassica juncea*) with those of *Brassica spenescence*. Intergeneric fusion has been achieved between protoplasts of Indian mustard and *Moricandia arvensis*. The purpose of producing these hybrids is to transfer biotic- and abiotic-stress-resistant genes from distant relatives into Indian mustard.

Somatic hybridization can also be used to produce cytoplasmic hybrids (cybrids) that would combine organelle genes of two distantly related parents. For example, triazine (a herbicide) resistance is chloroplast-



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Tissue culture offers many advantages.

borne while male-sterility genes reside in mitochondria. When protoplasts from a triazine-resistant plant are fused with protoplasts of a male-sterile line and selection is made for male-sterile cytoplasm, triazine-resistant and triazine-sensitive lines are recovered. Somatic hybrids thus generate combinations of mitochondrial and chloroplast genomes, which is not possible through sexual means.

In vitro selection

Selection for disease resistance. *In vitro* selection has a distinct advantage over other selection systems since it allows significant saving of space, time and money. For plant diseases that cause damage through toxins, cell selection for toxin resistance in cultures and regeneration of plants from descendants of the selected cell lines can give disease-resistant genotypes. For example, disease-resistant crop plants have been produced through *in vitro* selections in potato (*Solanum tuberosum*) against *Phytophthora infestans* (late blight of potato); in maize (*Zea mays*) against *Helminthosporium maydis*; in tobacco (*Nicotiana tabacum*) against *Pseudomonas tabaci*. At the Biotechnology Centre, IARI, plants resistant to toxin produced by *Alternaria brassicae*, a blight-causing organism, have been isolated.

Selection for tolerance of abiotic stresses. *In vitro* identification can be used for selection against abiotic stresses such as drought, and excessive salinity or minerals. Addition of polyethylene glycol (PEG) or dextran to the culture medium simulates drought conditions. PEG-induced water-stress-tolerant lines have been produced successfully in tomato. Likewise *in vitro* selections for tolerance of aluminium toxicity have been effected in tomato, soybean (*Glycine max*), rice, barley (*Hordeum vulgare*) and wheat. Screening of *in vitro*-produced somatic embryos at the Biotechnology Centre of IARI has

yielded *Brassica* regenerants tolerant to NaCl concentration of up to 0.26 M. The selected salt tolerance has been shown to be a stable genetic character.

Micropropagation of elite genotypes

Many situations in commercial horticulture, in the business of ornamentals and foliage-plant species, and also in the case of field crops demand that numerous copies of the parental elite genotype be made. Here micropropagation through tissue culture has vast potential and has already been adopted in India for commercial multiplication of plantation and horticultural crops. Hindustan Lever, Bombay, has already marketed micropropagated cardamom. Scientists of the Bhabha Atomic Research Centre (BARC) in Bombay are at an advanced stage of field testing of micropropagated oilpalm (*Elaeis guineensis*). There are a number of fruit plants like grape (*Vitis vinifera*), papaya (*Carica papaya*), etc. which depend on sexual propagation. Some of these plants are dioecious, i.e. male and female flowers are borne on different plants. From the commercial point of view it is desirable to produce more female plants, which are the ones that bear fruits. Techniques have been standardized at the Biotechnology Centre, IARI, for multiplying only female plants of papaya. These plants are in the initial stages of field testing.

Recently a tremendous innovation has been introduced into methods of mass multiplication of plants by tissue culture. Scientists at the University of Florida in the US have developed a kind of fermentor in which extensive multiplication can be achieved in an economically viable manner.

Recombinant-DNA technology

Advances in molecular biology have opened up possibilities of identifying and isolating any gene of an organism, irrespective of its phylogenetic status, and mobilizing and expressing it in a different organism of one's choice. In fact the entire living world has become one gene pool and there is no theoretical limit to the possibilities of creating desired gene assemblies.

Engineering plants for resistance to diseases

Spectacular success has been achieved with regard to viral diseases following use of r-DNA technology. A major achievement has been the transfer and expression of coat-protein genes of tobacco mosaic virus (TMV) and alfalfa mosaic virus (AIMV) in tobacco, resulting in protection against or delay of disease development in the transgenic plants. The rationale of introducing coat-protein genes to impart resistance against the virus is

that the multiplication of infecting viral RNA is somehow checked by the coat protein synthesized in the plant cells. The system, sometimes offering cross-protection, has been shown to work for several different viruses and promises to be a precise new way to reduce crop loss due to viral diseases.

Engineering plants for resistance to pests

The most common way of insect control has been widespread use of chemical insecticides. Though effective, chemical insecticides (a) proved to be an environmental hazard and (b) forced development of resistant strains of insects. In nature, only limited genetic variability is available for insect resistance, and therefore the conventional breeding programmes have been only partially successful.

However, there are genes in bacteria such as *Bacillus thuringiensis* that encode insecticidal proteins. *B. thuringiensis* strains specifically toxic to dipteran, lepidopteran and coleopteran insects have been identified and the insecticidal-protein gene cloned. Using Ti plasmid vectors of *Agrobacterium tumefaciens*, the gene encoding the insecticidal protein has been transferred to tobacco, tomato, potato, rice, corn and other crop plants. Such transgenic plants incorporate resistance to specific insects that feed on these crops. In the case of some crops, transgenic plants are at an advanced stage of field testing in the US before their commercial release.

Another approach of producing insect-resistant transgenic plants is to introduce into plants genes that inhibit enzymes responsible for breakdown of protein (protease inhibitors). Transgenic plants with cowpea (*Vigna sinensis*) protease-inhibitor gene have demonstrated resistance to tobacco budworm, corn earworm, armyworm, and hornworm of tomato and tobacco.

Engineering plants for resistance to herbicides

Herbicides are used to control weeds known to deplete soil nutrients. Application of a broad-range herbicide requires that crop plants have resistance to that herbicide so that it could selectively eliminate only the weeds. Several laboratories around the world have engineered crop plants that harbour resistance to known potent herbicides. Glyphosate, commonly known as 'round up', is a non-specific herbicide and kills green plants by inhibiting EPSP (5-enolpyruvylshikimate-3-phosphate) synthase, an enzyme involved in the biosynthesis of aromatic amino acids. Crop plants with resistance to this herbicide have been engineered by either using a mutant EPSP synthase gene from *Salmonella typhimurium* with very low affinity for glyphosate or overproducing a normal EPSP synthase such that the system can withstand EPSP

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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