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Plant Genetics — a new weapon against hunger

by Harsh Wardhan

THE WORLD today is characterised by economic disruptions, energy, water and food crises, diminishing agricultural land and forests and increasing environmental pollution. Substantial diversion of limited resources towards stockpiling of destructive weapons has left millions in developing countries virtually without the basic essentials of life. The population explosion in the last decade has led to pessimistic predictions for the human race because of the growing imbalance between food production and population growth. Despite an increase in total world food production, population growth has outstripped hopes of winning the battle against man's oldest foe — hunger. The 1974 World Food Conference in Rome resolved that "by 1984, no child or woman or man should go to bed hungry and no human being's physical or mental potential should be stunted by malnutrition". In fact more people are going to bed hungry today than in 1974.

Agriculture is still the basis of economy in life in less industrialised nations. The transition from food gathering to food growing paved the way to crop and animal husbandry which constitute the cornerstones of modern agriculture. Crop improvement based on plant molecular biology will be a critical scientific goal in coming years. Biotechnology, once feared as likely to unleash 'monsters' that would destroy man, has been acclaimed as the most fascinating discovery of the last decade. It not only provides a ray of hope for those suffering from 'inborn errors of metabolism' by gene therapy but will enable man to develop novel food plants to feed millions.

New crop varieties are very quickly attacked by evolved pests, necessitating further genetic development work.

The 'sunbean' experiments with sunflower plants containing soyabean genes, which code for the major fraction of a storage protein (legumin), exemplify efforts towards the creation of novel food plants by man. Although much remains to be discovered and advancement in plant molecular biology has not been as rapid as in molecular biology of microorganisms, the information on plant gene structure, organisation and expression (the process of making the specific product of a gene for which genetic information is coded in that gene) has begun to accumulate. By using plant tissue culture techniques (growing complete plants from plant cells on specific growth media) improvement of some species has already been achieved. It is speculated that crop improvement by genetic engineering would be of positive value with regard to environmental safety.

Is the new technology going to replace conventional plant breeding?

Crop improvement methods in the past have resulted in new problems which are only now coming to the fore. Extensive utilisation of specific plant varieties has led to the gradual loss of many plant genes from the world gene pool, thereby narrowing the basis of genetic variability. Plant genetic engineering, i.e. the manipulation of plant genes, opens up possibilities for extending new genetic information across diverse plant species, which are often limited in breeding programmes. However, it should not be considered as a technology that will replace plant breeding. Rather it should be used along with existing methods in an integrated manner, each being used when it will be most effective and economical. Although the ultimate value of genetic engineering will vary from species to species, large savings in terms of time and quantity produced can be foreseen.

Plant biotechnology is a promising science. However, a number of technical problems need to be overcome before a successfully engineered plant is introduced as a crop plant. The plant genome (entire genetic material of a plant cell) is extremely complex compared to the relatively simple genome of a microorganism. Plant biologists, therefore, need to identify plant genes and explain further their structure, organisation and expression.

A model genetic engineering project consists of the following general steps:

Step one: The most important task for a genetic engineer is the selection of a plant gene whose introduction in other plants will be of positive agricultural value. Plant genes of prime interest are genes which code the storage proteins (legume proteins for example), genes responsible for resistance to pests and pathogens, genes involved in nitrogen fixation and those which induce greater tolerance to environmental stresses.

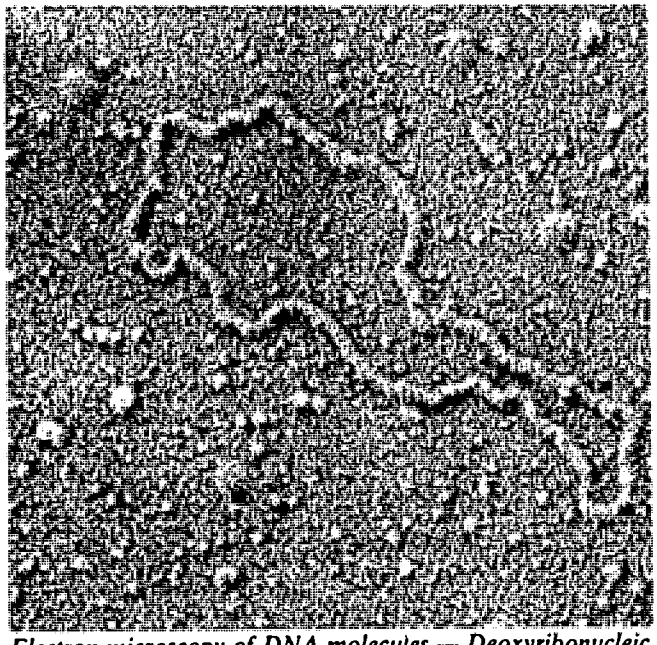
Step two: Identification and isolation of the desired gene. Techniques are currently available to select a specific gene from the plant genome. Bacteriophages (viruses which infect bacteria) have proved to be reliable tools to make "gene banks or libraries" of plant genomes (DNA from a cell is fragmented by suitable restriction enzymes (see below), and each fragment is attached to a bacteriophage and finally introduced into a bacterium for amplification, i.e. to make many copies of the fragments). The library can be screened to identify a particular gene. Already a library for the bean plant genome has been constructed and a gene which codes a storage protein identified. Restriction enzymes (bacterial enzymes which cut DNA at specific sites) are of seminal importance in identification and isolation of the desired genes.

Step three: The isolated gene needs to be transferred to the recipient plant. This can be achieved by three different methods. The desired gene is joined to a suitable vector or carrier (vehicle) to transfer a desired gene into the recipient system. Currently different vectors are being investigated for their potential to introduce the chosen 'foreign genes' into crop plants. *Cauliflower mosaic virus* is one such vector. However, it has a limited host-range and large genes cannot be accommodated in it. The most important vector is a large plasmid (extrachromosomal, autonomously duplicating, circular DNA molecules predominantly found in bacteria) called Ti plasmid (tumor inducing plasmid). This is found in a soil bacterium, *Agrobacterium* sp. which causes tumors in certain higher plants. During infection by this bacterium, a part of the Ti plasmid is transferred and integrated into the host genome. Molecular biologists have inserted some genes into the transferable portion of the Ti plasmid. A gene coding for a legume storage protein was inserted the same way, however, on subsequent infection of a sunflower plant but no protein product could be obtained from the recipient. This calls for a detailed study of factors involved in plant gene expression. Recent successful research in the expression of a gene which codes for an antibiotic by Mary-Dell Chilton and co-workers at Washington University, U.S.A. is a promising indication that similar results may be found in other cases.

Another species of *Agrobacterium* which causes an extensive proliferation of roots on the host plant has been shown to possess a root inducing Ri plasmid. This vector is being investigated as a possible alternative to the Ti plasmid, since it is easier to raise healthy plants from root tissue obtained after infection by this bacterium. Unfortunately, this *Agrobacterium* does not seem to infect cereals.

Apart from integration into the host plant genome, the stability of the "foreign gene" and its product in a new environment is an important factor.

Step four: Next, complete plants must be regenerated from transformed cells or tissue. Tissue culture plays a vital role at this stage. Plants are grown on specific growth media and subsequently transplanted. The

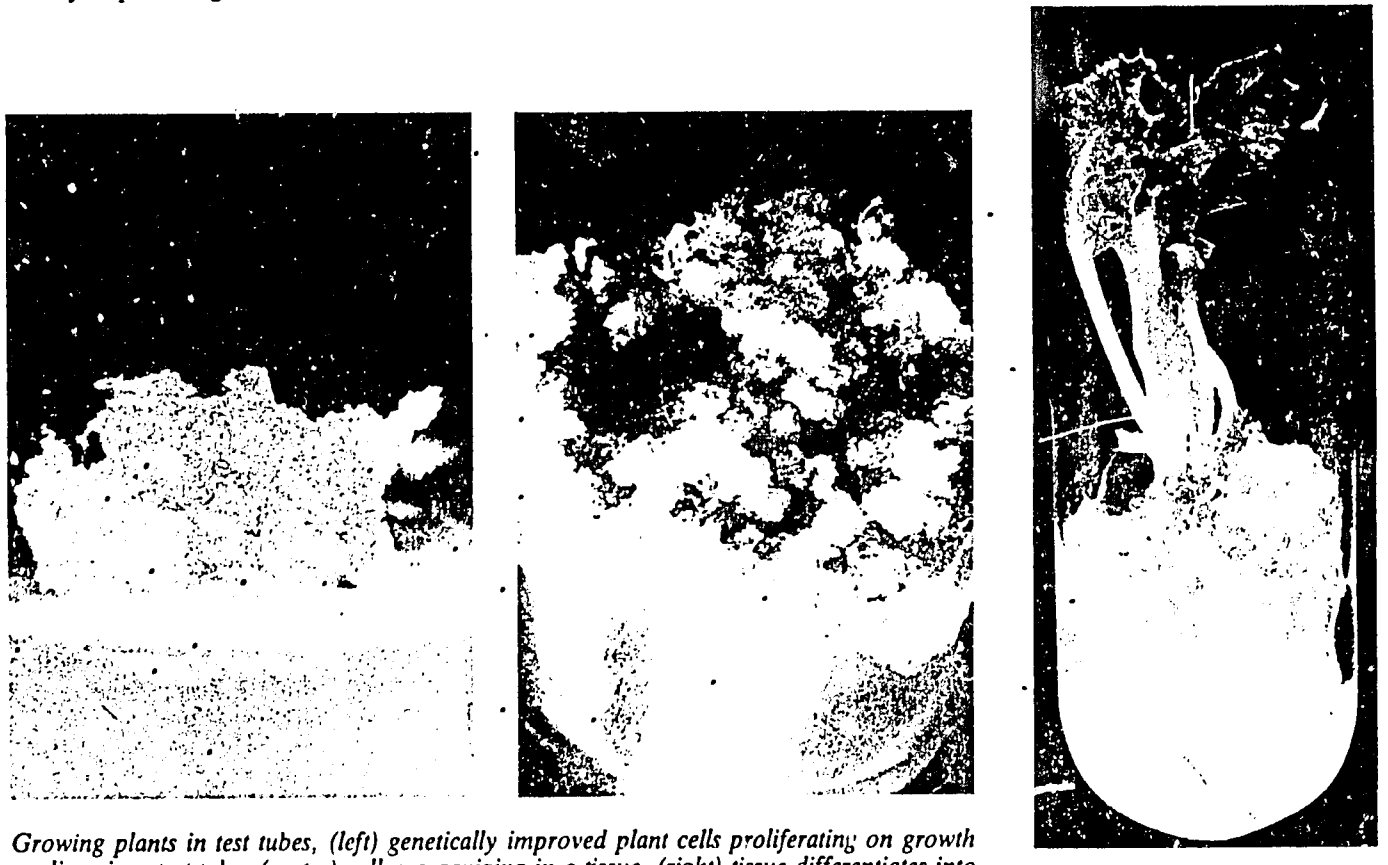


Electron microscopy of DNA molecules — Deoxyribonucleic acid (DNA) consists of long thin molecules comprised of two helically interwoven, mutually complementary single strands. The two component strands of a duplex DNA molecule can be separated by physical or chemical treatments, and will rapidly and accurately reassociate when conditions are restored to normal.

desired change or improvement by the 'foreign gene' is evaluated. Poor response in some plants to regeneration via tissue culture calls for a better understanding of physiological processes and differentiation of plant cells.

While improvements are being sought for each of these four steps, it is worthwhile considering some problems involved in the successful manipulation of agriculturally important genes.

problems make it a difficult task. Nonetheless, some success can be foreseen. The key enzyme involved in nitrogen fixation is highly sensitive to oxygen. Various approaches are being sought to protect this enzyme from oxygen. About 17 genes are involved in nitrogen fixation in the bacterium *Klebsiella,* and a similar number is expected in other nitrogen-fixing bacteria. Therefore, manipulation of these 17 genes make the system extremely complex.



Growing plants in test tubes, (left) genetically improved plant cells proliferating on growth medium in a test-tube, (centre) callus organizing in a tissue, (right) tissue differentiates into leaves, stem and roots.

The nutritive value of proteins for human nutrition can hardly be overemphasised. Legumes are rich in proteins whereas other crops, cereals for example, lack adequate protein content and some important amino acids. The storage proteins under investigation are controlled by many genes. Engineering a single gene for increased levels of a particular amino acid will have little effect on seed protein composition unless the gene is highly amplified. Stability of a foreign protein, as mentioned earlier, in a different plant will be a crucial factor. Moreover, the expression of genes for storage proteins is highly tissue specific. Introduction of an unrelated protein might upset normal seed development. Yet, due to their obvious value, the transfer of genes for the storage proteins will be an interesting exercise.

The necessity for crop plants, especially cereals, to fix their own nitrogen, thereby avoiding application of costly fertilizers is well understood. Although a number of research groups are working on this, certain inherent

In symbiotic nitrogen fixation (a mutually beneficial association between some nitrogen-fixing bacteria and roots of legumes), effective fixation depends upon genetic information in both the bacterium and host plant. Since it is easier to manipulate the bacteria than the host plant, their contribution to enhanced nitrogen fixation seems more probable.

Much energy is wasted during nitrogen fixation, through the loss of hydrogen gas released from root nodules of legume plants. Some strains of the symbiotic bacterium *Rhizobium* have genes for hydrogen uptake (hup genes) and thus are more energy efficient. Transfer of these genes to other *Rhizobium* spp. by conjugation (bacterial mating) will be an advantage.

Free-living nitrogen-fixing bacteria such as *Azospirillum* can be encouraged to associate with plant roots, thereby transfering some fixed nitrogen to plants. As more is learnt about bacterial gene expression in higher

plants, the transfer of bacterial nitrogen-fixing gene complexes could become a reality.

Resistance to pests and pathogens

Annual losses of crop yield due to pests and pathogens are enormous. Plant molecular biologists can play a significant role in saving crops. When attempts are made to solve pest and pathogen problems by gene manipulation, scientists encounter the following problems:

1. Resistance to existing pests and pathogens is overridden by new forms of the pests and pathogens which probably evolve from the original population by recombination or mutation (change in genes).
2. Suitable genes conferring disease resistance are often not available in a plant species or they cannot be incorporated because of fertility barriers.

Though genetic engineering opens up possibilities of such gene transfers, the mechanisms of resistance need to be characterised completely. Some plants produce chemical substances which mimic insect hormones, thereby upsetting insect maturation. Transfer of genetic mechanisms responsible for the synthesis of these chemical substances can provide resistance to insect attacks. Genes which code for certain inhibitors of proteases (enzymes which breakdown proteins) produced by some insects to degrade the host plant have been identified in tomato plants using gene manipulation methods. Transfer of these genes to other plants is being considered.

Genes for greater tolerance to environmental stresses

Crop plants continue to be subjected to a wide range of environmental stresses, particularly as global weather is becoming highly unpredictable. Surprisingly weeds exhibit dramatic resistance to environmental extremes. Although they are incompatible for breeding with cultivated crops, transfer of genes for tolerance by genetic engineering will be tempting. Our efforts need to be directed to understand the physiological, biochemical and genetic bases of plant response to environment.

Genetic engineering and forestry

Apart from its value in crop improvement, knowledge of plant molecular biology will contribute towards modern forestry programmes. Forestry by the next century could well be based on improvement of tree species by genetic engineering and tissue culture, optimisation of nutrient treatment and control of non-crop vegetation. Already improvement of some forest trees such as pine and spruce has been undertaken using tissue culture methods.

Some of the systems discussed above have been the centres of attraction for most plant biologists mainly because of their tremendous economic potential. In addition, advances in plant biotechnology will contribute to health care (production of new pharmaceuticals), floriculture and generation of superior fuelwood species. Enormous genetic variability is generated in plant cells proliferating in cultures, where agriculturally important genes can be looked for. Already production of "Catharanthine" — a potential anti-cancer agent and "Vincristine" — an antileukemic compound is underway — thanks to tissue culture technology.

Plant biotechnology and environmental protection

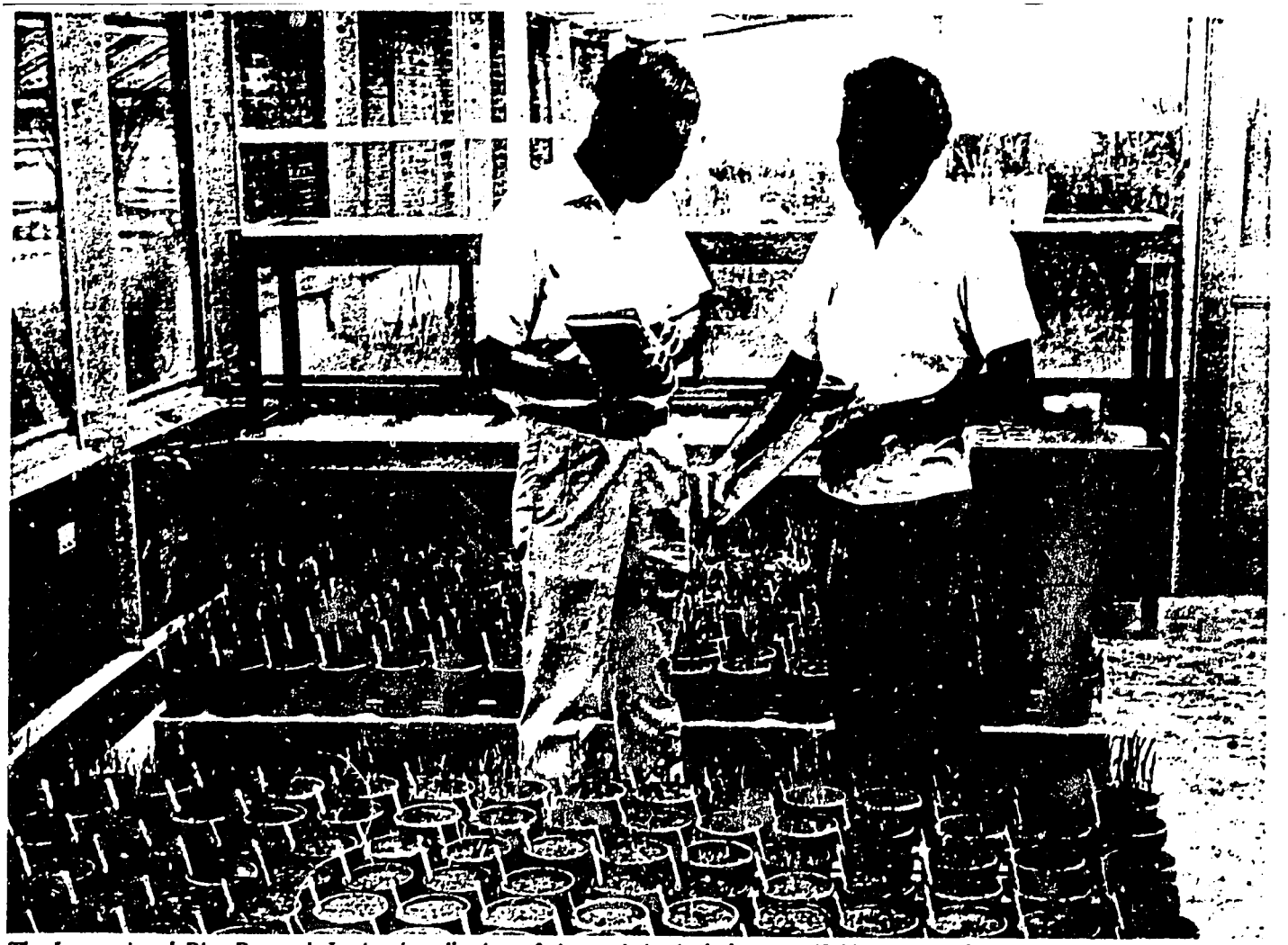
Indiscriminate use of pesticides which ushered in industrialised agriculture was said to solve all pest problems forever. Now it is evident that this optimism is partly responsible for the severe 'backlash' that resulted from saturation of the entire food chain with these 'poisons' to the point where we must phase out using many of them. The insidious effect of DDT and other chlorinated hydrocarbons on nervous systems and sex-hormone metabolism of vertebrates is well documented.

Some respite from increasing environmental pollution can be expected when applications of 'bioengineering' come into use. Genetically engineered crops with genes to thwart off pests and pathogens will dispense with spraying of pesticides and herbicides — agents of environmental pollution. Transfer of genetic mechanisms for synthesis of insect repelling chemical substances to plants is being looked at with a similar objective. Development of forest tree species with resistance to 'enemies' should also lessen damage to the environment.

Carbon-dioxide concentration in the atmosphere is increasing due to over-dependence on fossil fuel. Adverse global implications (increase in atmospheric temperature, for example) of this development have drawn the attention of environmentalists. Plant varieties with extremely efficient photosynthetic systems to achieve higher CO_2 fixation could be a partial answer to the problem.

Pollution of aquatic ecosystems with agricultural run-off (from nitrogenous and phosphate fertilisers and other agrochemicals resulting in 'algal blooms' thus choking water bodies) pose a serious challenge to ecologists. The problem of sewage disposal goes on almost unabated although city administrations are spending massive amounts of money to solve it. A lot of research work is being done on plant species fixing their own nitrogen. Plant biologists will be able to develop varieties requiring almost no addition of fertilisers thus partially restricting algal blooms — a menace to sound aquatic ecosystems. Development of modern technology utilising engineered bacteria to break down sewage into useful products (methane for example) is already at an advanced stage. Bacterial strains capable of cleaning-up oil spills have already been obtained.

Ruthless deforestation throughout the world has resulted in increasingly degraded or bare lands being rapidly colonised by weeds. These areas would gradually



The International Rice Research Institute's collection of rice varieties includes over 63,000 Asian cultivars, 2,575 African varieties, 1,100 wild rices and 680 varieties maintained to test genetic traits. Over the next five years, a further 30,000 to 40,000 varieties will be added to the collection.

turn into 'weed-parks' threatening existence of useful plant species if not restored. Transfer of genes which induce tolerance to environmental stress will enable foresters to restore such lands with plant species having better endurance to environmental extremes.

In a nutshell, plant bioengineering will not only be time and space effective, but also would cause less or almost no further damage to a fast deteriorating milieu. However, scientists will have to consider all other aspects of environmental safety before new ventures are executed.

Relevance of plant biotechnology to third world agriculture

The majority of the world population lives in developing countries which makes increasing dependence on agriculture inevitable. The countries in the tropics and subtropics face far more serious pest and pathogen problems since there are crops and vegetation throughout the year which can serve as alternate hosts to many 'plant enemies'. Also, climatic conditions promote continuous multiplication of pests and pathogens without interruption, unlike in temperate countries where severe winter is a restraining factor. Protein malnutrition is also prevalent, leading to various deficiency diseases particularly among children.

Development of crops with resistance to pests and pathogens by gene manipulation will be significant for these countries. Plants with higher protein content and rare amino acids will provide a protein rich diet which is still beyond the reach of millions in the developing world.

Third world countries will therefore have to devote considerable research and attention to absorb this fast developing technology in a phased manneraccording to their national priorities. The recent proposal to set up the International Centre for Genetic Engineering in a developing country highlights the relevance of this technology to third world agriculture and overall technological development. However, as stated earlier, crop engineering should be geared to provide additional books to conventional plant breeding and increasing food production.

The foundations of plant breeding were laid by Darwin and Mendel years ago and the knowledge of plant genertics has increased manifold since then. Bioengineering has certainly spurred man's desire to utilise new technology for food production. However, one must realise that some of the limitations of plant biology are simply too overpowering. Therefore, a lot more research is needed on all aspects of plant biology before reliable practical applications of this wonderful technology can be ready for the green revolution and a possibly safer environment for coming generations. □

Suggestions for further reading

Chilton, M.D. (1983). A vector for introducing new genes into plants. *Scientific American* June 1983, vol. 248, No. 6. *Science* — Vol. 219, 11 Feb. 1983 — A special issue devoted to Biotechnology.