Fundamentals of

Nanotechnology

Recent Advances

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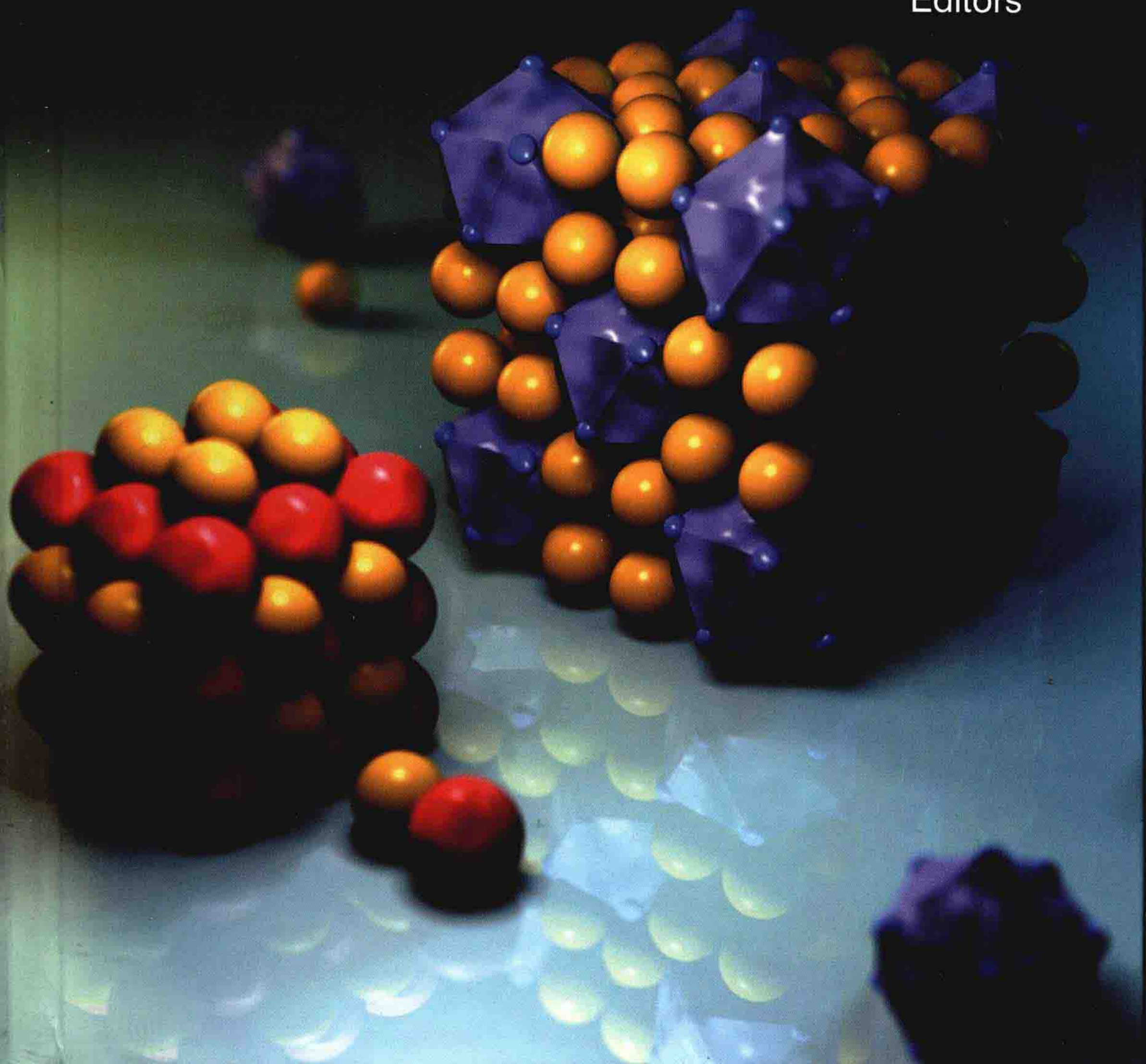

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Preface

Nanotechnology is the manipulation of matter on an atomic and molecular scale. The earliest, widespread description of nanotechnology referred to the particular technological goal of precisely manipulating atoms and molecules for fabrication of macroscale products, also now referred to as molecular nanotechnology. A more generalized description of nanotechnology was subsequently established by the National Nanotechnology Initiative, which defines nanotechnology as the manipulation of matter with at least one dimension sized from 1 to 100 nanometres. This definition reflects the fact that quantum mechanical effects are important at this quantum-realm scale, and so the definition shifted from a particular technological goal to a research category inclusive of all types of research and technologies that deal with the special properties of matter that occur below the given size threshold. It is therefore common to see the plural form "nanotechnologies" as well as "nanoscale technologies" to refer to the broad range of research and applications whose common trait is size. Because of the variety of potential applications (including industrial and military), governments have invested billions of dollars in nanotechnology research. Through its National Nanotechnology Initiative, the USA has invested 3.7 billion dollars. The European Union has invested 1.2 billion and Japan 750 million dollars.

Nanotechnology as defined by size is naturally very broad, including fields of science as diverse as surface science, organic chemistry, molecular biology, semiconductor physics, microfabrication, etc. The associated research and applications are equally diverse, ranging from extensions of conventional device physics to completely new approaches based upon molecular self-assembly, from developing new materials with dimensions on the nanoscale to direct control of matter on the atomic scale. Scientists currently debate the future implications of nanotechnology. Nanotechnology may be able to create many new materials and devices with a vast range of applications, such as in medicine, electronics, biomaterials and energy production. On the other hand, nanotechnology raises many of the same issues as any new technology, including concerns about the toxicity and environmental impact of nanomaterials, and their potential effects on global economics, as well as speculation about various doomsday scenarios. These concerns have led to a debate among advocacy groups and governments on whether special regulation of nanotechnology is warranted. Molecular nanotechnology, sometimes called molecular manufacturing, describes engineered nanosystems (nanoscale machines) operating on the molecular scale. Molecular nanotechnology is especially associated with the molecular assembler, a machine that can produce a desired structure or device atom-by-atom using the principles of mechanosyn thesis. Manufacturing in the context of productive nanosystems is not related to, and should be clearly distinguished from, the conventional technologies used to manufacture nanomaterials such as carbon nanotubes and nanoparticles. When the term "nanotechnology" was independently coined and popularized by Eric Drexler it referred to a future manufacturing technology based on molecular machine systems. The premise was that molecular scale biological analogies of traditional machine components demonstrated molecular machines were possible: by the countless examples found in biology, it is known that sophisticated, stochastically optimised biological machines can be produced. It is hoped that developments in nanotechnology will make possible their construction by some other means, perhaps using biomimetic principles. However, Drexler and other researchers have proposed that advanced nanotechnology, although perhaps initially implemented by biomimetic means, ultimately could be based on mechanical engineering principles, namely, a manufacturing technology based on the mechanical functionality of these components that would enable programmable, positional assembly to atomic specification. The physics and engineering performance of exemplar designs were analyzed in Drexler's book *Nanosystems*.

It will also be an invaluable reference source for libraries in universities and industrial institutions, government laboratories and independent research institutes working in nanotechnology and related fields.

—*Editor*

Contents

Chapter 1

Application of Nano Cell Biotechnology

Cell biotechnology or plant tissue culture is an enabling technology from which many novel tools have been developed to assist plant breeders. These tools can be used to increased the efficiency of the breeding process, to improve the accessibility of existing germplasm and to create new variation for crop improvement.

They include micropropagation, another culture, in vitro selection, embryo rescue, somaclonal variation, somatic bybridization and transformation. Of these somaclonal variation occupies a somewhat unique position, because it has both the advantages and disadvantages of tissue culture systems.

Micropropagation is another technique of great significance to agriculture which is based on in vitro culture of plant cell. Each and every cell of a plant has the potency to be regenerated into complete plant by tissue culture techniques and this property of the cell is known as totipotency. Using this technique hundreds and thousands of seedlings can be generated in a very short time, starting from a limited number of explants. This methods of multiplication has two major advantages. First is the multiplication of new elite cultivars.

For example, after its release, a new sugarcane cultivar may take 5-10 years before sufficient planting material may be generated for wide coverage in the farmers field. But using micropropagation techniques a comparable level of multicipation may be achieved within 2 years. The second advantage is that we get disease free planting material because of the use of aseptic conditions during tissue culture. There is also reports of rejuvenation and increased vigous following tissue culture. This can also help in rescue of important materials which are on the verge of extinction due to disease attack. For example, a rare variety of banana used for temple offering in Karnataka has been rescued through tissue culture techniques. Micropropogation of fruit and ornamental plants provides a useful avenue for the employment generation for rural youths at cottage level industries. In species like Daffodies and gladioli, microporpagation techniques are being used to speed up the release of new varieties. In strawberry, millions of plants can be produced from a single mother plant in one year.

Micropropagation work undertaken at our Defence Agricultural Research Laboratory. Pithograh was resulted into successful multiplication of strawberry, petunia, carnation and tomato hybrid. In case of strawberry a well adapted variety of hill vi., Jyolikit Selection was taken for micropropagation studies with objective to large scale production of disease free propagules since this fruit plant is highly susceptible to number of fungal, bacterial and viral diseases. The result showed that internodal segments are most suitable explant material for micropropagation under in Vitro condition. MS formulated with 1ppm of BAP and 0.1ppm of GA3 was found optimal protocal for maximizing shoot proliferation in explant where MS media modified with 1 ppm 1AA was most suitable for early rooting in shootlets. Peat moss was found to be most suitable potting culture for hardening of tissue culture raised plants of strawberry var.

Jyolikot Selection. Micropropagation studies in Petunia Hybrida were undertaken in order to raise the plant seedlings in mass. The result showed that optimum number of shootlets could be obtained after culturing the leaf explant of size 5mm X 2.5mm in MS media supplemented with 1ppm of BAP + 0.5 ppm of IAA with an inclubation period of 34 days. Protocol standardisation revealed that MS medium supplemented with 1 ppm of IBA was suitable for an early and healthy rooting in tissue culture raised shootlets. Hardening process was most successful with high relative humidity (90-95%), Temperature 23 + 10 C and potting mixture of sand and soil in equal proportion. Work on micropropagation of carnation was resulted into standardization of an vitro culture media for shoot and root proliferation. Protocol found most promising for shoot proliferation was MS medium supplemented with 1 ppm of BAp AND 0.1 ppm of IAA where MS medium supplemented with 1 ppm of NAA was identified to most suitable for rooting in shootlets.

Micropropagation studies were also undertaken in hybrid tomato to generate large scale seedings to fulfil the need of army personnel as well as local growers with pure and healthy planting material. Based on the observation it was found that tissue culture medium comprised of MS,0.5 ppm kinetin, 0.5 ppm IAA and 0.3 ppm GA3 was most suitable for shoot growth and MS medium supplemented with ppm of IAA or MS with 1 ppm of IBA was most suitable for early and healthy rooting in the explant material. Tissue culture regenerated plantlets showed high survival under relative humidity varied from 85-95 per cent, temperature 25+20C and potting mixture sand and oak forest humus in equal ratio.

Application of DNA Technology

Genetic engineering opens a totally new dimension for bioprospecting. The search for new genes and their application is the primary objective of the biotech industry. Gene technology now enable humans to integrate revolutionary new properties in to cultivated plants through inter-specific or inter-generic gene transfer which was not possible through classical approach of crop improvement.

Genetic engineering is the direct introduction into a plant of an isolated or modified single gene using transformation techniques. Now it is possible to transform almost all of the plants cultivated by man. Where this has not happened, it is more to do with the insignificance of the plant in agriculture or forestry rather than a reflection of a stubborn resistance to all attempts to transform it. A couple of years ago, cereals were regarded as being recalcitrant species. This has new changed and transformation has been reported as being successful for nearly all cereal species.

Transgenic Plants

Transgenic is used to describe plants which have had DNA introduced into them by means other than by the transfer of DNA from a sperm cell to an egg. Development achieved through modern biotechnology in the form of transgenics have given new options of controlling insects, diseases and weed pests, while improving the overall integrity and consequences of agricultural practices. Biotechnology combined with modern crop management techniques and the responsible use of pesticides gives the best tools available to ensure healthy, high yielding crops. However, the benefit expected from the release of genetically modified organisms into environment are quite more which are seems to pay major role in bioremediation, environmental improvement, agriculture, food industry and health care. A number of transgenic plants in various crop species covering wide range of altered characteristics have been approved for commercial cultivation.

Resistant to Biotic Stresses

Damage to crops by biotic factors like insects-pests and weeds is a major limiting factor in agriculture economic in tropical and temperate regions of the world. Despite large scale investment in the chemical control of pests, they cause great economic loss by damaging or destroying the crops. With a tremendous development in techniques to engineer crops genetically, we now have the ability to make broader use of natural insecticides. The choice of a Bt endotoxin, as the first insecticidal protein for introduction into plants, was based on the extensive knowledge gathered about this class of crystal protein since 1902 and such a strategy has been successfully achieved. The application of glyphosate kills crop plants just as effectively as it kills weeds. The usefulness of glyphosate as a weed control agent in agriculture could be enhanced if resistance to herbicide could be selectively engineered into crop plants. Transgenics have been developed by introducing the corresponding resistance genes from weeds and microbes into crop plants such as tobacco. In addition genes conferring resistance against herbicides, viz., Sulphobylurea, Imidazolinones, Phosphinothricin, Asulam, Bromoxynil and 2,4-D have been engineered and transgenic plants developed.

Abiotic Resistance

A large area of our country is under stress conditions i.e. saling, alkaline water, cold and heat stress etc. transgenics produced from introducing mtl 1D gene from E. coli in tobacco and arabidopsis showed tolerance to high leave of NaCl. Thermoc tolerance was found in Arabidopsis plants engineered with hsf gene. It has been reported that over-expression of sac B gene from Bacillus subtillis leads to high level of fructans in tobacco cells, and this is associated with increased drought tolerance whereas chemically synthesized antifreeze protein genelala 3 leads to improved freezing tolerance of tobacco plants.

Genetic engineering for improved tolerance to abiotic stresses is, therefore, the need of the hour as the existing cultivars in most cases are capable of giving much higher biomass that what we harvest today. While it is true that the response of plants to these stresses is multigenic, the recent success achieved in genetic engineering against excess salt, high and low temperature as well as less or excess water by altering individual gene is noteworthy.

Product Quality

A group of scientists have reported the ethylene synthesis in transgenic tomato plants, using anti-sense to a gene that has been identified as ACC oxidase. The enzyme convert ACC to ethylene. Ethylene production was inhibited by 97% with a signification reduction in over-ripening and shrivelling. Flavr Savr and Endless Summer in tomato, Freedom II in squash, high lauric acid reposed (Canola) and Round Up Ready soybean are some examples of the crops that already being commercially grown in developed countries.

Genetic engineering of metabolic pathways of fruits and vegetables has therefore given control over post-harvest process which not only leads to improvement of quality characteristics but also enhances processability, transportability and prologation of shelf like.

In addition, engineered plants with change fatty acid composition of edible oil, reduced antinutritional components in many food crops, production of palm oil or animal derived industrial oil into plants like Brassica are some of the good examples of novel products that can be expected from biotechnology. Significant progress has been achieved for production of biodegradable plastics derived from biomsass of genetically altered plants having gene from bacteria.

With the advancement in DNA technology and understanding the structure and function of gene and its products more and more transgenics are coming out from various research programme all over the world. However, boon of biotechnology has been confined to few developed countries and developing countries are still in vogue to harvest the benefits of modern technology. More that 48 transgenic has picked up remarkably in last couple of years.

During 1986-87, 25000 field trials were conducted with transgenics of more that 60 crops in 45 countries. Sixty per cent of these trials were in first 10 years and 40% in the last two years. Area under transgenics cultivation has also increased tremendously. During 1996, the transgenic crops covered an area of 2.8 million has which increased about 4 times during 1997 and 10 times during 1998.

Table: *Area of transgenic crops planted (m ha)*

Country	*1997*	*1998*
USA	8.1	20.5
Argentina	1.4	4.3
Canada	1.3	2.8
Australia	0.1	0.1
Mexico	<0.1	0.1
Spain	0.0	<0.1
France	0.0	<0.1
South Africa	0.0	<0.1
Total	11.0	27.8

Source: Anne Simon Maffat (1998)

A major challenge for agriculture in the 21st century in developing nations would be to speed up production process economically to fulfil the aspirations of huge populace, to achieve diversification and adding value to the primary produce so as to make agriculture enterprise farmers as well as environmental friendly. Biotechnology based advance technologies are expected to materialize many of our expectations in the coming millennium.

Viruses

To minimize the losses due to viral disease and to reduce the use of chemicals, the cross protection phenomenon of viral coat protein and movement proteins have been employed. This method has been used to develop transgenic plants which conferred significant levels of resistance in tobacco plants to TMV or to alfa mosaic virus (AMV) using the AMV coat protein gene.

In some viruses it has been found that certain sequences termed satellite RNAs act to reduce disease symptoms in infected plants and as such it is being utilized for developing transgenic with virus resistance. Antisense RNA which binds to sense RNA and artificial ribozyme molecules which cleave specific gene transcripts were found very powerful tools for the inhibition of viral disease development. Genetic engineering of antisense or ribozyme genes complementary to viral genes whose products are essential may prove one of the best way of viral disease control.

Insects

Transgenic crop plants that produce pesticidal proteins such as Bt toxin can offer the growers advantage of increased yields, numerous environmental benefits due to decreased use of conventional insecticidals. A transformed tobacco plants with the gene encoding the cowpea trypsin inhibito, in feeding experiments demonstrated that expression of the gene conferred resistance to a range of insect genera, including Heliothis, Spodoptera, Diabrotica and Tribolium the protein is not however toxic to humans.

Herbicides

The use of herbicidea has become an important feature of modern agriculture as a means of controlling weed species, however, it is economically feasible to produce specific herbicides for use with every individual crop species. A more generic approach would be to transform a range of crop plants with a gene which confers tolerance to a single non-selective herbicide.

Biotechnology for the Cotton Farmer Problems and Prospects

Cotton or white gold as it is aptly called is grown for its lint and seed which yield cotton fibre and seed oil, respectively. This crop occupies 32-33 mha of world area with a production of 20-25 metric tonnes. In India its area spans over 7.5 mha with an average yield of 290 kg/ha of lint and 870 kg/ha of seed cottom. To meet the challenges of 2000AD with a population of more that 960 million, a total production of 19 million bales is required as against the 13-14 million bales of today. This can be achieved by the use of improved crop production practices coupled with appropriate pest management tactics. In addition, generation of novel. Transgenics may help achieve the near impossible.

Genes that have been identified as potentially profitable, if engineered into acceptable cultivars can be used to generate such transgenics. Among these are genes imparting resistance to herbicides, insects, pathogens and abiotic stresses. It is also widely accepted now that a number of other qualitative characters can be improved, such as fibre strength, fineness, colour and thermal adaptability of the fibre.

Thansgenic plants have become realistic components of stress management world over. Bollworm and herbicide resistant transgenic cotton have received the approval of the Environmental Protection Agency and have been commercially released in the US for cultivation.

Considering the fact that numerous biotic and abiotic stresses limit cotton production, it is likely that future strategies might orient towards the development of a multiadversity resistant high yielding transgenic cotton variety with superior fibre qualities.