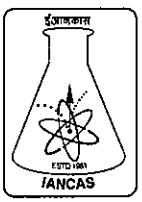

INDIAN ASSOCIATION OF NUCLEAR CHEMISTS
AND ALLIED SCIENTISTS

Nuclear Agriculture and Biotechnology

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Editorial

Agriculture in India has undergone a radical change since independence. Thanks to the 'Green revolution' of the Seventies, India is self-sufficient in food production. Development of high yielding varieties of seeds, improved fertilizer management, modern pest control techniques, better irrigation facilities, mechanized farming etc. contributed to this great achievement. Today over 25% of the GDP of India comes from agriculture.

The development of nuclear energy and the availability of radioisotopes in large quantities added a new dimension to the research in agriculture. Radiation and isotopes find application in several areas of agricultural research. Radiation is used for 'induced mutation' for the development of high yielding varieties of seeds and for controlling pest by the 'sterile insect technique'. Radiotracer techniques find applications in studying soil fertility, plant nutrition and persistence of pesticides in food. The Bhabha Atomic Research Centre has been carrying out pioneering research in all the above fields and these frontline R&D activities have largely benefited the farmers. Radiolabelled fertilizers, insecticides and isotopes supplied by DAE are used by several agricultural universities to study the uptake of fertilizers, micronutrients and for the studies on persistence of pesticides in food items.

The present issue of the IANCAS Bulletin is devoted to "Nuclear Agriculture and Biotechnology" This issue is guest edited by Dr. P.S. Rao, Formerly, Head, Nuclear Agriculture and Biotechnology Division. Dr. Rao has done an excellent job in identifying appropriate authors, relevant topics and editing the articles. I am thankful to Dr. Rao and the authors who contributed to this issue.

IANCAS has been striving hard to improve our Bulletin by having quality articles. I am glad to inform the members that we are getting very good response from our valued readers. I am thankful to all those who have expressed their comments on the Bulletins. Your views will help us to better focus the future issues.

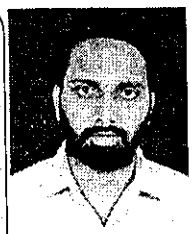
M.R.A. Pillai

CONTENTS

Agricultural Biotechnology : Integrated Approaches to Crop Improvement

Dr. P.S. Rao recently retired as Head, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, He joined BARC in December 1966 and subsequently obtained his Doctorate in Botany from Delhi University in 1967. As a Visiting Scientist, he had conducted advance research in plant morophogenesis during 1971-73 at the CNRS labs in Gif-sur-Yvette, France and in protoplast culture during 1981-82 at the Max-Planck Institut, Koln, Germany. He is a Fellow of the National Academy of Sciences of India and National Academy of Agricultural Sciences. In BARC, he has led the research group of Plant Biotechnology which had made significant contributions in the areas of technology development for micropropagation of high-value crops, protoplast culture, synthetic seeds, and production of bioactive compounds in plant cell cultures. Successful in vitro protocols have been developed for tissue culture production of banana plants and technology transfer has been made to the user agencies. Dr. Rao has guided several students for their M.Sc., M.Tech. and Ph.D. degrees and has published 171 research publications in national and international journals/books. Dr. Rao is a member of Advisory Board of several National Research Centres, Universities and Government Bodies such as Department of Biotechnology (DBT), Department of Science and Technology (DST). He has been a member of the Indian Delegation that went to USA to visit different research centres in Biotechnology. He is a member of several professional societies in India and abroad, and is serving on the Editorial Board of three scientific journals.

Dr. P. Suprasanna joined BARC in 1991 after graduating from Osmania University, Hyderabad. He has obtained his Ph.D. degre in Genetics from Osmania University and presently he is engaged in plant biotechnological research of rice and banana. His contributions have been in the areas of plant cell and tissue culture, somatic embryogenesis and synthetic seeds. Dr. Suprasanna has to his credit 40 research publications in journals and books published by national and international publishers. His interests are in using plant cell cultures for understanding mechanism of differentiation as well as for genetic manipulation.



Introduction

Over the centuries, human beings have been cultivating and improving crop plants to cater to their needs of food, feed and health. Of the 250,000 plant species that exist on this earth, only 150 are being used by modern agriculure and only 20 species are responsible for 90% calories in the diet of modern population [1]. Genetic selection, manipulation via conventional breeding and hybridization have contributed greatly to developing and evolving superior crop varieties. Increase in world food production is largely due to the green revolution which introduced high yielding varieties of rice, wheat and other grains with improved characters. In order to meet the requirements of increasing population growth in the next 40 years, the food production must double and the scenario of present food situation is far from satisfactory since productivity is affected by aberrant weather and pest epidemics. Both these tasks will require new and appropriate technologies. Integrated into these new

technologies, agricultural research must focus on addressing the problems related to improving crop productivity.

Biotechnology encompasses an array of biological and related techniques for the management and manipulation of biological systems. In the past two decades, application of biotechnology has focussed on different facets of life mainly agriculture and health. Rapid developments in *in-vitro* cell and tissue culture and recombinant DNA technology have interested plant biologists to employ them for plant improvement.

The central dogma to the use of *in-vitro* techniques is "totipotency" which means that isolated plant cells have the ability to divide and grow into complete plants. Plant cell and tissue culture has therefore emerged as a tool for plant scientists for a number of basic and applied research problems of relevance to plant improvement [2]. This has now become an important part of plant biotechnology research being pursued in Universities, Research Centres, Laboratories and Private Companies all over the world. Todate several plant species representing annuals and perenneials, herbaceous and woody species, monocots and dicots, self and cross pollinated groups that are vegetatively propagated and apomictic forms have been cultured *in-vitro* and regenerated into complete plants. A number of *in-vitro* techniques are available (Table 1) and these offer a great potential in the genetic improvement of crops for the creation of novel plant types.

Table 1. Major Reesarch Tools of Plant Biotechnology

TECHNIQUE	APPLICATION
In-vitro culture	Propagation of difficult-to-breed species
Meristem /shoot tip culture	Mass clonal multiplication of elite varieties
Embryo culture	Production of interspecific & intergeneric hybrids
Anther culture	Production of haploids for breeding purpose
Somaclonal variation	Generation of novel genetic variability
In-vitro selection	Production of novel, useful mutants
Somatic hybridization	Production of hybrids between sexually incompatible species
Genetic transformation	Introduction of foreign genes from diverse species
Synthetic seeds	Delivery system for tissue cultured plants
Cryopreservation	Preservation of valuable plant germplasm

Rapid Propagation of Superior Genotypes

In-vitro methods have been extensively used for propagation of crop species and the procedures have become very useful for commercial exploitation [2]. The first successful application of tissue culture was the *in-vitro* multiplication of orchids. Now, several orchids, ornamentals and horticultural crops are regularly multiplied through this technique. This essentially involves removal of a bit of tissue or few cells from leaf, stem, root etc., from a healthy donor plant and grow each of them into whole plants on an appropriate nutrient medium in a culture vessel under controlled conditions of light, temperature and humidity. Plants developed under these conditions are transferred to green house for acclimatization and thereafter used as normal plants. To date, we can speed up the production rate of the average plant by approximately 10,000 times and a large number of productive plants can be multiplied routinely. Meristem/shoot tip culture also has application for the production of pathogen-free plants and in potato, dahlia, strawberry, carnation, chrysanthemum, orchids and other plants, virus-free plants have successfully been produced.

Micropropagation of high value crops is a multi-billion dollar industry, practised in several small and large nurseries and commercial labs all over the World [3,4]. About 300 companies exist in this tissue culture business all over the world. In India, more than 50 companies have been established

and some have already started plant production, on a commercial scale. The urgent need for micropropagation has originated from the increased demand for flowers and indoor foliage plants. For example, the world trade in flowers is to the tune of US $ 32-35 billion (over Rs. 100,000 crores) and this trade is growing at the rate of 15% annually in value terms while the global demand is growing at the rate of 17%. The trade is expected to go over US $ 50 billions by the turn of this century. Such tremendous potential has encouraged several companies to diversify into plant biotechnology by setting up tissue culture laboratories to produce millions of plantlets per year [5]. High value ornamentals and foliage plants such as *Chrysanthemum, lillies, Gerbera, Gladioli, Rose, Carnation, Orchids, Anthuriums, Spathyphyllum, Diffenbachia, Cordyline, Ficus, Syngonium* which are in great demand are routinely grown on a commercial basis using tissue culture techniques. Protocols for propagation through tissue culture have also been developed for fruit crops such as banana, pineapple, papaya, grapes, apple and strawberry.

Agroforestry resources are dwindling every year and there is an urgent need to regenerate forests and in this regard, tissue culture has great potential. Technology for mass production of some commercially important forest trees such as teak, poplars, eucalyptus, bamboo and Himalayan pines is available. More than 15 lakhs plants of Eucalyptus species, teak, bamboo species, Anogeissuss and poplars were developed at the two Tissue Culture Pilot Plant Facilities set up by the Department of Biotechnology, Government of India, at the Tata Energy Research Institute, New Delhi and National Chemical Laboratory, Pune, and have been field planted in multi-locational trails and the data with respect to their growth performance is being ananlyzed. A highly reproducible technique for regeneration of sandalwood plants through tissue cultures from an elite mother plants has been developed at BARC [6]. Regenerated sandalwood plants have been field tested on a limited scale at Trombay.

In plantation and spice crops, tissue culture techniques have been gainfully employed to increase productivity. Successful examples include banana, cardamom and black pepper. Protocols for micropropagation of black pepper have been standardized both at Kerala Agricultural University and Calicut University. Other spice crops under investigation at Kerala Agricultural University and Indian Institute for Spices Research, Calicut, include clove, nutmeg, cinnamon and vanilla. At BARC, protocols for micropropagation have been standardized for banana, cardamom, mulberry, ginger and turmeric. In case of banana, thirty varieties including commercially valuable cultivars have been multiplied through micropropagation techniques and the tissue culture derived plants have been planted in the farmers fields [4]. Field performance of these plants has been encouraging with vigorous growth, early maturity and increase in yield. The technology has been transferred to Maharashtra State Agicultural Universities, Maharashtra State Seeds Corporation and Gujarat State Fertilisers Corporation, and Government of Pondicherry.

Cardamom, the 'queen' of spices has considerable export earnings, and is propagated vegetatively as well as through seeds. This crop is highly cross pollinated and the seed progenies often show variations and hence vegetative propagation through tiller multiplication is carried out. However, the rate of multiplication is slow and only 3 tillers are produced per plant per year. Tissue culture multiplication through shoot tip culture has been successfully employed for the propagation of elite clones. This has enhanced the cardamom productivity. In a field demonstration study on tissue cultured derived cardamom in 102 ha. conducted by the Spices Board and the Department of Biotechnology, the mean yield recorded was 260 kg/ha. as against 210 kg/ha. of the control. Thus tissue cultured clones showed an increase of over 30% over open-pollinated seedlings.

In ginger and turmeric which are important spice crops, protocols have been developed for micropropagation from vegetative buds of germinating rhizomes of commercial cultivars (6 cultivars of ginger, 5 cultivars of turmeric). Tissue cultured plants of ginger and turmeric have been established in the field and in turmeric, the performance was better over the mother plants.

Mulberry plays a vital role in the sericulture industry since the foliage constitutes the chief food

for the mulberry silkworm. It is conventionally propagated by stem cuttings and root grafts, however, there is often a low or no rooting efficiency in many cultivars. Tissue culture techniques offer a new approach for enhancing mulberry biomass production. A protocol for rapid multiplication of mulberry using axillary bud / leaves on a suitably formulated medium has been developed at BARC [7]. Mulberry plants obtained *in-vitro* have been successfully field planted at the Experimental Field Station, Trombay and at the Central Sericultural Research and Training Institute, Mysore.

Palms are normally propagated through seeds except date palm which is propagated through seeds as well as vegetatively by off shoots [8]. Seedling derived plants often show considerable variation which affects the total yield. Hence, the production of high yielding clones from selected palms through tissue culture is advantageous. Date palm has been successfully multiplied through somatic embryogenesis or axillary bud development and some of the commercial tissue culture labs have already exploited this technique. Tissue culture of oil palm is commercially important and a protocol for multiplication is now available in many labs. Results from early field trails are encouraging with more uniform growth than seedling derived plants. It is estimated that higher yields of the order of 30% and improved oil quality would be obtained by cloning palms possessing the desired characters.

Generation of wide / distant hybrids

Hybridization between wild relatives and cultivated plants has been the primary objective of plant breeders since wild species of crop plants are known to possess several useful genes for disease resistance, insect/pest tolerance, male sterility, quality and stress tolerance. Conventionally crosses have been attempted among cultivated and wild species, but in several cases, crossability barriers hinder the transfer of useful genes. Embryo culture is used to rescue such hybrid embryos that would normally abort or would not undergo progressive development following hybridization [9]. This technique involves isolation and growth of immature embryos on a nutrient medium, allowing it to develop into a viable plant. Additionally, embryo culture also helps to shorten the breeding cycle by overcoming the seed dormancy.

In general, either embryo culture or rescue of hybrid embryos is used in inter-generic and inter-specific hybridization [9]. In *Trifolium, Lotus, Ornithopus, Oryza, Hordeum, Gossypium, Brassica and Lycopersicon* species, hybrids have been developed using embryo culture. Triticale, a hybrid of Triticum and Secale sp. is a classical example of man made cereal produced through embryo rescue. In CIMMYT, Mexico, crosses between maize X sorghum, maize X *Tripsacum,* wheat X barley have been made using embryo culture. In IRRI, Philippines, genes for insect resistance have been transferred from wild rice to cultivated rices. In India, International Crop Research Institute for Semi-Arid Tropics (ICRISAT) based at Hyderabad, has a programme on using this technique for gene introgression in groundnut from its wild species. Embryo culture is one of the earliest forms of *in-vitro* culture applied to practical problems and has proven of great value to breeders.

Another technique that can be useful not only in wide crosses but also in crosses with distant plant species is **Protoplast fusion** [9, 10]. Protoplasts are naked cells without cell wall, the cell wall of which is digested by using certain cell wall degrading enzymes. Isolation and culture of protoplasts opened the avenues for genetic introgression. Successful protoplast culture and regeneration of plants has been reported in more than 320 plant species representing 146 genera and 49 families which includes cereals such as rice, maize, wheat, pearl millet, sugar cane, sorghum and barley, and woody plants like citrus, coffee, sandalwood, *Pinus, Populus, Eucalyptus*, and several other important plant species [11]. For the fusion of plant protoplasts, two methods viz., electrofusion (mediated by high electric pulses) and chemical fusion (mediated by polyethylene glycol) are generally used. Protoplasts have several uses : 1) they can be fused to produce somatic hybrids or novel plants between otherwise sexually incompatible plant species, 2) production of cytoplasmic hybrids (cybrids) for mitochondrial genetic variability and 3) for the uptake of alien DNA for generating transgenic plants. The first somatic hybrid was produced between two tobacco species, *Nicotiana glauca* and *Nicotiana langsdorffii.* Successful examples include Arabidobrassica (*Arabidopsis thaliana* + *Brassica compestris*), pomatoes and topatoes (potato + tomato) and

Erucobrassica (*Brassica napus* + *Eruca sativa*). Somatic hybrids of sexually incompatible species of Citrus, Brassica and Prunus have also been produced. The second application of protoplast fusion is the production of cybrids in which nuclear genome of one parent and the organellar genome of another parent are combined.

Protoplast fusion enables the transfer of cytoplasmic male sterility (CMS) between elite breeding lines which helps in the development of hybrid varieties. Successful examples include rice, *Petunia, Brassica, Nicotiana*, where CMS trait has been transferred to elite lines.

Obtaining Haploid Plants

Haploids with one set of chromosomes, can be produced by culturing anthers or ovaries or haploids plant explants. Of the various *in-vitro* techniques, anther culture has proved to be the most efficeint for the production of haploids [9]. Since the discovery of haploidy in *Datura* by Guha & Maheshwari in 1964, haploid plants have been produced experimentally in about 200 plant species. Successful examples include rice, brassica, wheat, corn, tomato, poplar, rye, triticale, potato, grapes and barley [3]. Cultured anthers produce callus and /or embryoids that are regenerated into plants and chromosome number of these can then be doubled through colchicine treatment to produce doubled haploids or dihaploids. Haploid material enables the selection of mutants at cellular level, in a short time. Anther culture can be applied to F1 hybrids between two inbreeding lines with complementary characters of agronomic value to fix homozygosity in a single generation, thus one can avoid several generations of inbreeding or selfing. It has been employed to develop improved varieties of wheat, rice and tobacco. In China, more than 100 rice varieties have been developed and are being grown in 100,000 ha. and superior varietes of wheat are being grown in 650,000 ha. Improved strains of Brassica, rye and potato have also been developed in Germany and Canada.

Generation of Genetic Variation and Selection

Plants regenerated from *in-vitro* culture may not always be true to type and can have genetic alterations. Somaclonal variation refers to this variation that arises in cell and tissue cultures, and is generally associated with culture procedures and explant types [12]. As callus or cell suspension cultures pass through several cycles of subcultures and sometimes as long term cultures, considerable genetic rearrangements at the chromsome and gene level occur, which accumulate and sometimes this variation is passed on to next generations. Compared to naturally occuring mutation rates of 1 in 100,000 to 1000,000 for a given locus, 15-20% variation can be expected to occur in tissue cultures. In several plant species variation has been documented for agronomic traits such as disease resistance, plant height, yield, maturity, quality and plant type [13].

Potentially useful agronomic variants have been obtained in sugarcane, tomato, celery, rice and sorghum. In sugarcane, resistance to fiji virus, eye spot and downy mildew has been achieved and cultivar 'Oono' has been developed. In tomato, promising cultivars, DNAP-9 and DNAP-17 have been developed which had high solid content and Fusarium resistance. In celery, a *Fusarium oxysporium*-resistant somaclone UC-T3 was recovered from the plants regenerated from cell suspension cultures. More stable skin colour was observed in a somaclone of sweet potato which has resulted in the development of cultivar, Scarlet. In banana, cavendish dwarf-somaclone resistant to *Fusarium wilt* has been achieved. In ornamental plants like *Pelargonium, Eustoma, Haemerocallis and Paulownia*, heritable variation has been observed.

Somaclonal variation offers to produce rapid genetic variation for crops with narrow or no genetic base and the resultant somaclones can be incorporated into plant breeding programmes aimed at crop improvement.

Analogous to microbial cells, totipotent plant cells provide an opportunity for *in-vitro* selection of useful mutations at cellular level. It is possible to select cell lines tolerant or resistant to selective agents such as pathogenic agents, aminoacid analogue, salts, herbicides etc. *In-vitro* approaches include the use of callus, cell suspensions and protoplast cultures [14,15]. Selection can either be in a single step or a multi-step, in which case, selective agent is added into the medium so that only variant cells will survive under the selection pressure and

grow. Repeated selections in the presence of the selective agent in further stages till the regeneration of plants, ensures that only tolerant plants are regenerated. Mutagenesis using physical and chemical mutagens is adopted in tissue cultures so as to enhance the recovery of the selected variants [16]. Using patho-toxins or fungal culture filterates, disease resistance has been observed in cell lines and regenerated plants in potato, maize, rice, wheat, barley, tobacco, alfalfa, sugarcane, peach and oats. Aminoacid analogues like 5-methyl tryptophan or s-aminoethyl cysteine or ethionine are used for selection for cell lines with enhanced aminoacid levels which can be further utilized for developing nutritionally superior crops. Salt tolerance has been induced in rice, tobacco, oats, by subjecting cell lines to increased levels of sodium chloride in the medium. Likewise, tolerance to herbicides, heavy metals and abiotic stresses like cold, drought and high temperature can be achieved. Mutant selection in plant cell and tissue cultures is exciting and offers considerable scope for generating genetic variability.

Somatic Embryogenesis and Synthetic Seeds

Somatic embryogenesis is the development of embryos from somatic tissues as well as from situations which do not involve directly gametes, haploid cells or gametophytes [10,17]. It is a process by which somatic cells develop into embryos which ultimately give rise to whole plants. This phenomenon has been reported in more than 150 plant species. Somatic embryogenesis can be induced from a variety of plant tissues such as germinating seedlings, shoot meristems, young inflorescences, nucellus, leaf, anther, immature embryos, floral parts, root etc. Somatic embryos proceed through similar developmental stages like that of seed embryos, acquire root and shoot meristem connected by a common vascular system and develop into a plant. Somatic embryogenesis has applications in mass propagation and cloning of genetically transformed plants [17].

The fact that somatic embryos can be produced in large numbers in tissue cultures of many plants has led to the concept of utilizing the embryos for encapsulation in an appropriate matrix to prepare synthetic seeds [18, 19, 20]. Early successful reports in this regard have been in case of carrot, celery and alfalfa. In addition to somatic embryos, vegetative propagules such as axillary buds, shoot tips, corm-lets etc., have also been encapsulated to produce synthetic seeds. The main thrust idea is to prepare a simple inexpensive delivery unit of *in-vitro* propagated plants. Although the technique is recent, it offers many useful advantages on a commercial scale. The resultant plant population from the synthetic seed will be uniform. The direct delivery of somatic embryos will save many subcultures to obtain plantlets from regenerated embryos. The encapsulated material could be packed with pesticides, fertilizers, nitrogen fixing bacteria and even microscopic parasite destroying worms [19].

At BARC laboratories, research on synthetic seeds has been conducted in several economically important plants, such as sandalwood, mulberry, banana, cardamom, finger millet and rice. In the case of sandalwood, which is one of the commercially valuable forest trees of India, young shoots from mature trees are induced to produce a callus on an appropriate nutrient medium which differentiates large number of somatic embryos. Synthetic seeds were prepared by encapsulating such somatic embryos in a matrix consisting of 3% sodium alginate as well as in composite gel. Encapsulated single embryos germinated to form sandalwood plants with roots and shoots [21]. In case of mulberry, axillary buds were encapsulated in alginate and agar to produce individual beads. The beads could be stored at 4° C for 45 days without loss of viability and developed into complete plantlets on a simplified nutrient medium. In case of mulberry, only 30-40% cuttings survive the time period between pruning, transportation and final transplantation whereas the encapsulated buds could be easily packed in bottles and transported thus limiting space and ensure the viability and survival rate [22].

New and effective means of propagating bananas would be advantageous over the conventional use of sucker material, for germplasm maintenance, exchange and transportation. In our laboratory, shoot tips excised from the multiple shoot cultures of banana cv. Basrai (Cavendish dwarf) were encapsulated in 3% sodium alginate solution. Encapsulated shoot tips were placed on different media or substrates for germination. In case

of cardamom, shoot tips of var. Malabar isolated from multiple shoot cultures were encapsulated in 3% sodium alginate with different gel matrices. Maximum conversion of the encapsulated shoot tips into plantlets occured on White's medium and the plantlets were grown successfully in soil. The encapsulated buds or shoot tips present as inexpensive, easier and safer material for germplasm exchange and transporation [23].

Synthetic seed research is now being carried out in many laboratories, but the principle limitation for commercialisation is somatic embryogeny or high quality *in-vitro* propagules. Embryo quality has been a significant issue. Because somatic embryos are still not as robust and vigorous as zygotic embryos; it has not been possible on a routine basis to produce a large number of synthetic seeds which can be planted like true seeds with their requisite performance in the green house and field. There is also a need for developing new synthetic seed coatings to give higher coversion rates under soil conditions. Synthetic seed technology in years to come would certainly find its application in Forestry, Agriculture and Horticulture.

Plant Genetic Engineering

The process of plant genetic engineering or biotechnology accomplishes the same net result as that of plant breeding : the transfer of useful genes from one species to another for improvement purposes. The past decade has seen a rapid progress in molecular biology based on the advances made in identifying, isolating and transferring desired genes from one species to another for improving certain traits [9]. Such genetically manipulated crops are referred to as 'transgenic plants' and todate, transgenic plants are reported in more than 70 plant species such as rice, wheat, maize, oats, potato, cotton, tomato, Brassica, and soybean. Foreign DNA can be introduced into plant cells by vector mediated transfer or direct transfer both of which essentially involve precise tissue culture methods [20]. *Agrobacterium tumefaciens* is the gram negative soil bacterium which upon infection of plant tissues, forms tumours and in the process, transfers part of its genetic material (transfer DNA or T-DNA). Based on this natural phenomenon, strategies have been developed to insert novel gene constructs in T DNA region for the transfer of genetic material [24,25].

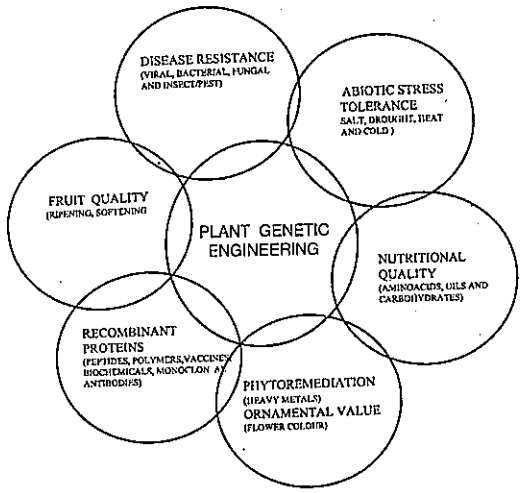


Fig. 1 Potential areas of application of plant genetic engineering.

Agronomically important genes for biotic and abiotic stresses, quality attributes have been the major focus of this research (Fig. 1). Several gene constructs, in this regard, have been made available that can be used in gene transfer : insect resistance (Bt, Cpt II), herbicide resistance (aroA, bar, bxn), virus resistance (coat protein genes, TMV, CMV, PVX), fungal resistance (chitinase), male sterility (ribonuclease-barnase), delayed fruit ripening (polygalactouronase) and cold tolerance (glycerol-3-phosphate acyltransferase).

Controlling insect pests is an integral part of the agricultural management as crop damages cause heavy economic losses. The inordinate use of insecticides/pesticides in pest management raises serious concerns for the environment. An alternative strategy is biocontrol : engineering the plant's genetic architecture by introducing novel genes like insecticidal crystal protein genes from *Bacillus thuringiensis* and protease inhibitor genes [26]. The insecticidal proteins (ICPs) interact with the receptors of the insect's midgut resulting in the structural deformities of the midgut membrane, which ultimately lead to larval death. Success in this direction has already been obtained in tomato, tobacco, cotton, alfalfa, rice and maize.

Significant progress has been achieved in protecting crop to viral diseases by developing viral

disease resistance [27]. Coat protein genes of tobacco mosaic virus (TMV), Alfalfa mosaic virus (AMV) have been transferred to tobacco and tomato resulting in protection or delay of disease development. Fungal disease resistant plants have been developed by incorporating resistance genes like chitinase, phytoalexin genes etc. Production of hybrid varieties with enhanced agronomic performance is often the goal of plant breeding programmes. Breeders require efficient pollination system and in several crops like oilseed rape and corn, hand pollination is still tedious and time consuming. A ribonuclease inhibitor was used to create plants which when crossed with male sterile plants produce hybrid plants in which fertility is totally restored. By this method one can replace the expensive practice of manual and mechanical pollination [27]. Modification of the composition and level of starch or seed oil is also possible by introducing new enzyme activities or by reducing the level of existing enzymes. In oilseed rape, fattyacid composition has been changed and in potato, starch synthesis has been enhanced through these approaches [28].

One of the major constraints for yield of many crops is premature fruit ripening and almost 50% of the fruits are thought to be lost due to spoilage. Transgenic approaches have led to the development of fruit crops with enhanced shelf life, delayed spoilage and better processing qualities [28]. Inhibition of the ripening enzyme (polygalactouronase) by introducing an antisense gene construct in tomato led to extended shelf life of fruits and *Flavr-savr* tomato has become the first genetically engineered product to reach the market place in the United States of America.

Transgenic Plants as Bioreactors

There is a growing interest all over the world in using transgenic plants as "bioreactors" for highly productive chemicals both for the pharmaceutical and industrial sectors (Table 2). A gene from bacteria *(Alkaligens eutrophs)* encodes an enzyme producing polyhydroxybutyrate polyesters in transgenic plants which resembles plastic in properties except that they are biodegradable. Biologically active peptides, proteins have many applications as vacines, immuno modulators, growth factors, hormones, blood proteins and enzymes. Monoclonal antibodies have been produced in leaves of transgenic tobacco plants and human albumins have been produced in potatoes [27]. Potential recombinant vaccines for viral and non-viral organisms have been produced in transgenic plants of tobacco and potato [29]. Research efforts are underway at the Boyce Thomson Institute, Ithaca, USA, to produce such vaccine producing genes in edible plant tissues like banana which will go long way in alleviating health problems in developing and under-developed countries [30].

Table 2. Production of High – Value Products in Crops

Products	Significance
Proteins	Plants can be genetically altered to synthesize novel proteins such as antibodies, recombinant proteins, and also antigens which act as vaccines.
Oils	Identifying and cloning key genes for production of oils or waxes with industrial use, with the intention of using them in crop plants via genetic transformation. Products are erucic acid, petro-selenic acid, lauric acid, gamma-linolenic acid, jojoba wax and epoxy fatty acids.
Carbohy-drates	Naturally occuring corn genes such as waxy, amylose-Extender, dull and shrunken-1 can be used to modify native maize starch. Genetic transformation may allow the synthesis of starch with novel desired properties.
Speciality Chemicals	Biodegradable plastics

(Modified from Duvick DN, Biotech & Develop. Monitor June 1996)

Bioactive Compounds from Plant Tissue Cultures

Plants and plant-based products provide a valuable source of medicines, flavours, fragrances and various pharmaceutical and industrial compounds. Nearly 25% of the drugs produced are of plant origin, In recent years, exploitation of medicinal flora from their natural habitat for obtaining desired product has resulted in their depletion and has consequently caused

environmental concerns. This has led to searching for alternate methods and tissue culture approach has found its place because of the following advantages:

a. Plant cell cultures provide a continuous and reliable source of natural product year round without the destruction of the entire plant,

b. Enables easy purification of the compound in the absence of significant amount of pigments,

c. Higher quantities of desired compounds can be obtained through cell line selection and/or addition of precursors into the production medium.

Thus cloning of medicial plants to increase biomass production and production of bioactive compounds through cell cultures has assumed significance [31].

Some of the important products under devlopment include Taxol and Camptothecin (anti-cancer), Castanospermine and Hypericin (anti-AIDS), Artimisinin (anti-malarial) and Forskolin (cardiotonic). Besides these, there are a host of other products which include vincristine, vinblastine, ajmalcine, diosogenin and berberine. Japanese scientists working at Mitsui Petrochemicals have demonstrated the production of shikonin, an anthraquinone used as a dyeing agent. This is the first biotech product derived at the cost of US$ 4,500 / kg in a 200 lt bioreactor. Large scale bioreactors of the capacity of 75,000 lt. have also been used for the cultivation of *Echinacea purpurea* and *Rauwolfia serpentina* cell cultures.

At BARC, tissue and / or cell cultures for the production of bioactive compounds have been established in *Catharanthus roseus* for ajmalicine, *Rauwolfia serpentina* for ajmaline, *Castanospermum australe* for tetra hydroxy indolizidine and *Nothapodytes foetida* for camptothecin. Also bioreactors have been employed for the large scale cultivation of plant cells of *Artemisia annua L.* for the production of terpenoids [32]. BARC has also signed a Memorandum of Understanding with Kabra Drugs Ltd, Indore for the transfer of know-how to produce high value compounds through the large scale cultivation of medicinal plant cell suspensions in bioreactors.

Another aspect of interest of using plant cell cultures is in the area of biotransformation; for example, hydroxylation of beta-methyl digitoxin to beta-methyl digoxin, and digitoxin to deacetylanatoside C with cells derived from *Digitalis lanata*. Immobilization of plant cell cultures is a technique by which cells are entrapped in a gel of calcium alginate, polyvinyl alcohol resin or other gelling polymers. Compared to cell suspension cultures, in immobolized cultures, there is no loss of cells while exchanging the media. These immobilized cells can then be used for the repeated production of useful compounds [33].

The significance of hairy root cultures for the production of chemicals of pharmaceutical value is gaining considerable importance. A range of plant species have been transformed with a soil bacterium, *Agrobacterium rhizogens* to establish hairy root cultures. Compounds like tropane alkaloids, flavonoids, ginsenosides and quinones have been produced. Large scale cultivation of hairy roots is the next step to economize the technique for the production of high value compounds [34].

Conclusions

Plant cell tissue and organ culture is an integral part of plant biotechnology and has become an important tool of research in agriculture, medicine and industry. In India, there exists considerable trained manpower in this area. There is also a rich reservoir of plant genetic resources which can be exploited. Several economically important crops have attracted much attention in terms of research and product development, both by government and private sector. For the industry, viable production and processing systems can be developed which are cost effective and have global relevance. Plant biotechnology is sure to play a prominent role in the development of sustainable agriculture and in conjunction with conventional breeding techniques, biotechnological tools can be supplementive in offering rapid solutions for many agricultural probelms.

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Use of Radiations in Crop Improvement

Dr. D.C. Joshua graduated from Kerala University in 1960 securing first rank. He was awarded the AEET (Atomic Energy Establishment Trombay) Fellowship for his post graduate studies. After obtaining M.Sc. Degree from the same University he joined the Biology Division of the then AEET in 1963. His early work was on seed radiobiology particularly on the effects of fast and thermal neutrons on seeds. His cytogenetic studies include pachytene analysis of rice and jute and genetics of 60 induced mutant characters in jute. He has developed a high fibre yielding jute variety. He has isolated photoperiod insensitive mutants in jute and in the green manure plant, Sesbania rostrata. Dr. Joshua obtained the Ph.D. degree from Gujarat University and was elected Fellow of Maharashtra Academy of Sciences. He has published 85 research papers.

Dr. G.S. Murty joined BARC as Scientific Officer SC1 in 1971 after post graduation in Botany from Andhra University, Waltair. From 1977, he started working in Mutation Breeding Section. His research interests are in oilseed crops, sesame and groundnut. He obtained Ph.D. degree from Gujarat University in 1982. Besides leading groundnut group and popularising the Tromby Groundnut varieties and conducting field demonstrations at Kota, Narora and Kakrapar, he is responsible for planning, production and sale of breeder seed of Trombay Groundnut varieties to various seed multiplying agencies. He is also heading the Field Facilities Section at Gamma Field and Gauribidanur.

Plant breeders and farmers depend on the genetic variability available in the different crop plants for their crop improvement programme. The available variability in some of the cultivated plants like rice is very large while in some crop plants like jute it is very low. The variability available in nature is the result of spontaneous mutations. Mutations can be induced artificially to enhance the variability many fold. Although many different kinds of mutagenic agents are known to induce genetic variability in plants, radiations such as gamma rays and neutrons have been found to be very efficient. Among the radiations, gamma rays are the most widely used as they are relatively easily accessible and induce high frequency of mutations. Crop improvement programme makes use of the induced spectrum of variability either by using the desirable mutants directly or by employing them in cross breeding to combine the desirable traits. The mutagenic effects of ionising radiations have been known for the past seventy years and the use of radiations in crop improvement is now fifty years old in India [1]. However, it was the emphasis given to the peaceful uses of atomic energy after the second world war that gave further impetus to the work on crop improvement by the use of radiations.

Radiation Induced Variability

Ionising radiations increase the frequency of mutations and widen the spectrum of variability. Increased variability has been reported for yield and yield contributing factors [2-5] resistance or tolerance to biotic and abiotic stresses [5] and for quality parameters [6-11].

Yield Trials, Release And Notification Of New Varieties

Mutants or the derivatives of mutant x mutant or mutant x variety crosses having the desired characters are evaluated for yield and other agronomic traits in the Research Station for 2 - 3 years. Those performing well are included in National or University yield evaluation trials. After completion of the different mandatory trials, if found superior, they are released by the Varietal Release Committee and notified as new varieties for commercial cultivation.

Table 1. Number of officially released mutant varities in different seed propagated crops in the world

Crops	Mutant Varities
Cereals	821
Legumes	211
Oil Crops	61
Industrial Crops	61
Vegetables	54
Others	80

This data is based on the publication of Maluszynski et al. 1995

This plant breeding approach known as mutation breeding has been most successful in self pollinated crops and in vegetatively propagated ornamentals. The IAEA data base of 1995 has 1790 accessions with induced mutations in their background (Table 1). Subsequently, Mutation Breeding Newsletter (1996) reported an addition of 65 varieties taking the total to 1855 which have been released by over 50 countries. India has so far released about 150 varieties (Table 2) as direct mutants or mutant x mutant or mutant x variety cross derivatives [4]. Of these, 77 were developed by using radiations. In ornamental plants 81 varieties were released till 1990 [2].

Mutation Breeding and Crop Improvement In B.A.R.C.

The early work at B.A.R.C. in the sixties was mainly concerned with the radiobiological studies which led to the standardisation of radiation doses for the different crop plants. These studies dealt with the basic aspects of seed radiobiology viz; RBE of different radiations and the role of modifying factors on radiation effects. The effects of neutrons, both fast and thermal, formed a major part of these studies and contributed significantly to basic seed radiobiology. In the second generation after mutagenisation, mutation frequency, spectrum, mutagenic effectiveness, and mutagenic efficiency of different radiations were studied. The concerted and systematic efforts resulted in increasing the frequency and widening the spectrum of mutations in several crop plants of economic importance. These mutants were studied for their morphological, cytogenetic, physiological, anatomical and biochemical characterisation [11-18]. Studies on the genetics of mutants showed both simple and complex type of inheritance [13,18]. Mutation breeding work using radiations resulted in the release and notification of 22 new varieties in different crops. This paper deals with mutant varieties developed in rice, groundnut, mustard and jute.

Table 2. Number of officially released mutant cultivars in different seed propagated crops in India

Crops	Mutant Varities
Cereals	43
Pulses	38
Oilseeds	23
Fibre crops	13
Millets	10
Sugar crops	9
Fodder crops	1
Vegetables	9
Others.	4

This data is based on the reference of Kharakwal, 1996

Direct Use of Induced Mutants

Mutation breeding is a useful plant breeding approach when a single specific trait is to be altered in an otherwise elite cultivar. The groundnut variety TG-1 is one such example. The most important requirement for export is the large seed size, which is usually selected and picked (hand picked selection or HPS) from the general harvest. With the recent introduction of varieties with large seeds, time, money and energy can be saved considerably. The first large seed variety to be developed using radiations was TG-1 (Trombay Groundnut-1). This is an X-ray induced mutant of the variety Spanish improved ; and was released by the Central Varietal Release Committee in 1973. This is a typical example of direct use of induced mutant. TG - 3 is another X-ray induced mutant of groundnut having higher pod yield due to increased number of tertiary branches. This variety was released for cultivation in Kerala in 1987 [5].

The mustard variety TM-2 (Trombay Mustard-2) released for cultivation in Assam was obtained by gamma ray treatment of the variety RL-9. TM-2 is characterised by appressed pods and high grain yield.

Table 3. Agronomical features of notified Trombay Groundnut (TG) varieites

Variety	Yield (kg/ha)	HKW (g)	Oil%	Important features	Released for	Year of Notification
TG-1	1700	65	48	Large pods, seeds suitable for export, seed dormancy	All India	1973
TG-3	1547	49	49	More no. of tertiary branches	Kerala	1987
TG-17	1401	61	49	Reduced plant height, less no. branches, dark green foliage, more gynophores	Maharashtra	1985
Somnath (TGS-1)	1926	65	51	Large pods, seeds suitable for export, early, tolerant to drought and stem rot	Gujarat	1989
TAG-24	2493	40	51	Semi-dwarf plant with high HI, tolerant to diseases, pests and drought	Maharashtra, West Bengal	1992
TG-22	1677	58	49	Tolerant to diseases, seed dormancy 50-60 days	Bihar	1992
TKG-19A	2260	61	46	Large pods, seeds suitable for export, seed dormancy 30 days	Maharashtra	1996
TG-26	2425	38	49	High HI, smooth pod venation, tolerant to diseases, seed dormancy 20 days.	Maharashtra, Gujarat, M.P.	1996

Mutants in Cross Breeding

The majority of the varieties developed using radiations are those resulting from crosses involving induced mutants. Often, the desired induced mutant character is transferred to an elite cultivar by mutant to cultivar cross. The rice variety Hari, which is a derivative of a cross between a radiation induced mutant and a high yielding cultivar, is a typical example. The variety SR 26 B is characterised by the desirable long slender grains and tolerance to soil salinity. But, the plants being tall lodge at maturity resulting in considerable yield loss. A mutant with reduced height with stiff and non- lodging stem was induced with neutron radiation. This mutant, TR -5 has about 300 grains per panicle but the grain size is very small. TR-5 was crossed to IR-8, a high yielding short variety to combine the desirable agronomic traits. One of the selections of this cross having non-lodging stem, high grain yield and the desired grain quality was selected and was released as Hari in Andhra Pradesh after the required trials.

Seven of the nine groundnut varieties released for cultivation are the result of cross breeding involving radiation induced mutants. Cross breeding programme identifies the genotypes with the desirable agronomic traits and uses them in crosses with the mutants to combine the different desirable characters. Although TG-1 has export quality seeds, it is late, has low harvest index and low shelling percent. Therefore, TG-1 was crossed with TG-17 a high yielding mutant variety to transfer the large seed character to TG-17. A selection from this cross had the desired seed size and agronomic traits like seed dormancy. This selection was yield tested in collaboration with Konkan Krishi Vidyapeeth, Dapoli and was officially released as variety TKG-19A for cultivation in the Konkan region. The spanish bunch type, semi-dwarf, early flowering variety TAG-24 was developed after several inter-mutant crosses involving eight parents. This plant has better partitioning ability of photosynthate leading to high harvest index of 50-55% as against the normal harvest index of 30-35%. In addition to high yield due to high harvest index, it is early maturing and tolerant to bud necrosis disease. TG-26 is another selection from a cross involving induced mutants. Like TAG-24, this variety has high harvest index, high yield and an additional useful trait viz.

fresh seed dormancy. Both these varieties have better water use efficiency [19]. TAG-24 and TG-26 has performed well in the trials over a wide range of agroclimatic zones in the country. The superior performance in the yield trials and the fact that they have been identified for diverse agroclimatic zones indicate their wider adaptability [20]. The other groundnut varieties developed with induced mutants in their background are given in Table 3.

Indian mustard is black in colour; and the first yellow seed mutant TM-1 was induced with the radioactive isotope ^{32}P. This mutant was employed in the crossing programme to combine the yellow seed trait and high yield. One of the selections from the cross TM1 x Varuna performed well in the trials in Assam and was released for cultivation in 1987. The high fibre yielding jute variety TKJ-40 released for cultivation in Orissa is also the result of using induced mutants in cross breeding.

Induction of Photoperiod Insensitive Mutants

New and highly productive multiple cropping patterns are essential requirements to improve agricultural production. This demands change in the traditional sowing time. Such changes in the sowing time without affecting the yields are possible only when we have varieties which are insensitive to photoperiod and temperature. In the bast fibre crop, jute and in the green manure plant, *Sesbania rostrata*, unlike in grain yielding crops, aerial phytomass is the economically important yield. Both these plants are typical short-day plants, and the basic vegetative phase during which they remain insensitive to the inductive photoperiod is very short. Consequently, they flower early when sown during the months of short-days resulting in uneconomically low phytomass yield. Varieties with long basic vegetative phase ie; those which are insensitive to photoperiod are the desirable agronomic types in these plants. Mutation breeding experiments using radiations have resulted in the isolation of such types in jute and *Sesbania rostrata*. The jute mutant, TCJ-5 has a basic vegetative phase of 120 days and the *Sesbania* mutant TSR-1 has a basic vegetative phase of 60 days unlike 30 days and 20 days in the respective parents [20,21].

Conclusion

The achievements in the field of crop improvement both at B.A.R.C. and in Agricultural Research Centres in India and abroad has clearly shown that mutation breeding has come of age as a powerful plant breeding tool and that ionising radiations are the most widely used mutagens for crop improvement. Since crop improvement is a continuous process, mutation breeding will remain as a plant breeding method complementing the other approaches; and radiations will be a handy and efficient tool to induce mutations.

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Grain Legume Improvement Programme at Bhabha Atomic Research Centre, Trombay

Dr. S.E. Pawar joined BARC in 1968. Presently he is a Pulses Group leader involved in the improvement of grain legumes especially mungbean, urdbean, soyabean, pigeonpea and cowpea. He is responsible for the development and release of nine varieties of pulse crops from BARC. He is the fellow of National Academy of Agricultural Sciences, National Academy of Sciences, India and Maharashtra Academy of Agricultural Sciences.

Dr. K.S. Reddy joined BARC in 1986. He is involved in mungbean breeding and has developed TARM-1, TARM-2 and TARM-18 varieties resistant to powdery mildew disease.

Shri T.G. Krishna graduated from the 16th batch of training school in 1972. He is a molecular biologist involved in studies on legume storage proteins and DNA finger printing of crop plants.

Dr. R.N. Pandey joined BARC in 1968. He is associated with pigeonpea and cowpea improvement programmes

Shri J.G. Manjaya joined BARC in 1994. He is involved in soybean and blackgram improvement programmes. He is also involved in developing cytoplasmic male sterility system in pigeonpea.

Introduction

Grain legume constitutes one of the main sources of dietary protein in India. The production of pulses has increased marginally from 11 to 14 million tonnes during the last three decades. This has affected the per capita availability of pulses. The expected requirement of pulses by 2000 AD will be around 24 million tonnes. The classical breeding methods of improvement could not so far make any dent in increasing the pulse production in the country. The large number of alleles for higher productivity have been lost during the evolution and later during domestication of these crops. The genetic variability generated by induced mutation approaches can certainly help to recover alleles for higher productivity, plant type and disease resistance which will make significant contributions for increasing the pulse production. Pigeonpea, mungbean and blackgram occupy an area of 7.8 million hectares, with a production of about 3.3 million tonnes of grain. Similarly soybean is the third major oilseed crop in the country and about 0.4 million hectares is coming under cultivation every year. Crop improvement programme was initiated at Nuclear Agriculture and Biotechnology Division of Bhabha Atomic Research Centre during early 70's in three important legume crops namely pigeonpea, mungbean and blackgram for induction of mutation for yield, disease and pest resistance and seed storage

Table 1. Released and notified mungbean and blackgram varieties developed at BARC

Crop	Variety	Year of release	Area of adaptation	Characteristic feature
Blackgram	TAU-1	1985 1996	Maharashtra Karnataka	27% higher yield with large seed
	TAU-2	1991	Maharashtra	15% higher yield over TAU-1
	TPU-4	1992	Madhy Pradesh, Gujarat Maharashtra	22% higher yield over PU-30
	TU94-2a	1997	South zone	35% higher yield over PU30
Mungbean	TAP-7	1983 1988	Maharashtra Karnataka	23% higher yield over Kopergaon Moderately resistant to powdery mildew
	TARM-2	1992	Maharashtra	85% higher yield over TAP-7. Resistant to powdery mildew, Suitable for *rabi* cultivation
	TARM-18	1996	Maharashtra	33% higher yield over TAP-7. Resistant to powdery mildew, Suitable for *kharif* cultivation
	TARM-1	1996	Central and Peninsular India	45% higher yield over PS-16. Resistant to powdery mildew and yellow mosaic virus suitable for *rabi* cultivation.
Pigeon pea	T. Vishaka 1 (TT 6)	1983	Central and Peninsular India	Early (135 days) with large seed
	TAT 10	1985	Maharashtra	Extra early (110-115 days) Medium large seed suitable for double cropping

[a]Identidfied for release by All India Pulse Improvement Project (ICAR)

proteins [1-4]. Since 1990-91, work on soybean improvement was initiated using induced mutations and conventional breeding methods.

Crop Improvement

Large number of mutations affecting plant types, maturity, leaf, pod and seed characters have been isolated and used in genetic analyses and crop improvement programme. It had been the experience from our programme that when induced mutations were used in hybridisations, the success was much more for isolating the high yielding recombinants than isolating direct high yielding mutations. Ten high yielding varieties, four of mungbean, four of blackgram and two of pigeon pea have been released from this research centre for commercial cultivation (Table 1).

Disease and pest resistance

Diseases and pests are major constraints in realising high yield potential in these crops. Among the diseases, work on powdery mildew, yellow mosaic virus, *Fusarium* wilt disease and store grain pest bruchid has been carried out at this Centre.

Mungbean

Powdery Mildew

A simple reliable method (excised leaf technique) for assessing the disease reaction has been developed [5]. Eighty germplasm lines were tested using this technique for disease reaction. All except seven RUM (Raipur Utera Mung) lines were found to be susceptible. RUM accessions were small, black seeded, late maturing, insensitive to

photoperiod and highly susceptible to dry root rot disease. A numerical rating scale of 0-5, based on the latent period of the pathogen and the leaf area infected scored visually was used for screening the disease reaction. Inheritance studies have indicated that resistance to powdery mildew disease in mungbean is governed by two dominant genes designated as *Pm1* and *Pm2*. When both are present resistant reaction is observed (R0). R1 and R2 reaction are observed when *Pm1* or *Pm2* are present individually [6].

Two selections TARM-1 and TARM-2, derived from RUM-5 X TPM-1 cross were released for commercial cultivation for South and central zones and Maharashtra respectively for *rabi* cultivation [7]. TARM-1 has been identified as a donor parent for resistance to powdery mildew disease by All India Pulse Improvement Project. Another selection TARM-18 resistant to powdery mildew disease was released for *kharif* cultivation in Maharashtra. Further selections having multiple disease resistance with large shiny seed are in the advanced stages of testing in national and state trials.

Blackgram

Powdery Mildew

Resistance sources giving differential reaction to powdery mildew isolate from Trombay have been identified. Studies on genetic behaviour and their utilization for developing high yielding blackgram varieties are in progress.

Yellow Mosaic Virus

Radiation induced early maturing mutant of exotic accession EC168200 from AVRDC, Taiwan has been identified as a resistant donor parent for yellow mosaic virus disease by All India Pulse Improvement Project (ICAR). TAU-5 has been extensively used in hybridization programmes for developing multiple disease resistant varieties for *kharif* and *rabi* cultivation. One of the selections TU-94-2 from TPU 3 X TAU-5 cross has given on an average 19-37% higher yield over checks in the All India Coordinated trials in the south zone [8].

Bruchid

The wild progenitor of blackgram *V. mungo* var. *sylvestris* collected from Trombay Hills, (Mumbai) was found to be resistant against infection by *Callosobruchus maculatus*. Inheritance studies have indicated that the two dominant duplicate genes are controlling the resistance [9]. Work on incorporating these genes in high yielding background is in progress.

Pigeonpea

Wilt

Wilt caused by *Fusarium udum* Butler is a major disease of pigeonpea. Breeding of resistant varieties to wilt will contribute to improve the yield potential of this crop. An improved laboratory screening technique for wilt resistance has been developed [10]. This technique has been successfully used for identifying the resistant genes, and studying the inheritance of *Fusarium* wilt disease. The segregation in the F_2 and F_3 generations indicated dominant monogenic inheritance of wilt resistance [11]. The mechanism of resistance was studied by post inoculation fungal growth and changes in the tissue of the wilt susceptible and resistant cultivars. Rapid spread of fungus, clogging and browning of vessels were observed in susceptible cultivars [12]. Large number of selections in the maturity group of 120 -150 days have been developed using resistant donor parents in the hybridisation programme. These are being evaluated in state and national yield trials. One of the selections TAT-93-47 has given 35% higher yield in the multilocation trials over TT-6 in Maharashtra.

Bruchid Resistance

Pigeonpea accessions and *Cajanus* species (wild) were evaluated for resistance to infection by a seed storage pest *Callosobruchus maculatus* [13]. None of the pigeonpea accessions were resistant but resistance was observed in three species of *Cajanus* namely *C. paltycarpus*, *C. scarabaeoides* and *C. sericeus*.

Crop Physiology

Spraying of mungbean crop with 1.5% urea solution one week before flowering and during the

period of pod development delayed leaf senescence and increased yield in three mungbean cultivars. ^{14}C from ^{14}C urea and ^{15}N from ^{15}N urea were traced in pods and seeds. The results support our contention based on theoretical and experimental estimation that seed yield in mungbean is limited by nitrogen supply [14].

The duration of effective filling period was similar in three mungbean cultivars differing in seed size. However, the rate of seed growth was significantly different. This indicated that the seed growth rate was directly related to seed size [15].

The amount and activity of RuBP-Carboxylase in high yielding cultivar ML-5 was higher in comparison to the low yielding selection VM-69 in leaves at identical stages of development [16].

Three anthocyanin pigments were found in *V. radiata* accessions with black seed coat while *V. mungo* had only one of them [17].

Seed Proteins

Mungbean

The fifteen mungbean genotypes studied could be grouped into four legumin variants named type I to IV in the order of increasing electrophoretic mobility on a cellulose acetate membrane. However, no easily discernible variation was observed for the slower moving vicilin protein. Genetic analysis and inheritance pattern indicated that the holoprotein as well as the subunits are inherited as simple Mendelian traits [18].

Blackgram

SDS gel electrophoresis of vicilin from 86 accessions of *V. mungo* was examined. There was considerable homology between *V .mungo* and *V. radiata*. Four major polypeptides of 69, 59, 56 and 55 kilodaltons (kd) were observed in the *V. mungo* accessions except UM-196 (radiation induced mutant) which showed an additional polypeptide of 50 kd. The protein pattern of F_2 seed analysis of a cross between NO-55 and UM-196 showed a codominant inheritance of the 50 kd polypeptide [19]. From the data obtained it was inferred that *V. mungo* and *V. radiata* might have evolved from two distinct forms of *V. sublobata* namely var. *sylvestris* and var. *sublobata* respectively. Vicilin was purified and characterised from eight *Vigna* species [20]. The data showed considerable homology amongst the *Vigna* species studied.

Pigeonpea

Pigeonpea seed contains mainly gloublins [21] and consist of three fractions named α, β and γ-globulins. Since γ-proteins is rich in sulphur amino acids [22], antibodies were raised against purified γ-protein from cultivar T-21. Using rocket immunoelectrophoresis, the variability in the amount of γ-protein was investigated. Among 48 accessions studied five showed increased amount of γ-protein compared to cultivar T-21 [18].

Vicilin from pigeonpea seeds has been purified and characterised [23]. It has a molecular weight (MW) of about 180,000 and consists of two types of subunits of MW 72,000 and 57,000 that are not linked by disulphide bonds.

Esterase isozymes were studied in pigeon pea and six *Atylosia* species [24]. Of the accessions of *Atylosia* only *A. cajanifolia* shares the esterase isozyme of *C. Cajan*.

Two protease inhibitors from pigeon pea seeds have been purified and characterised [25]. One of the inhibitors CTCI inhibits both bovine trypsin and chymotrypsin while the other CTI inhibits only trypsin. The MWs were 15,000 (CTCI) and 10,500 (CTI). The inhibitors are stable to heat and pH. The inhibitors are very specific towards mammalian serine proteases.

Soybean

The soybean improvement programme was initiated at this centre for developing bacterial leaf pustule disease resistant genotypes using induced mutations and conventional breeding methods. Soybean cultivars were screened for disease reaction using excised leaf technique [5]. The susceptible cultivars showing early and delayed symptoms were grown on plant to row basis and multiplied. Based on disease appearance, true breeding lines for early and delayed appearance of symptoms have been developed [26]. The resistant source P-4-2 [27] was used in the hybridisation with the commercial cultivars. Inheritance studies have indicated that

resistance to bacterial leaf pustule disease in soybean is governed by duplicate recessive genes (unpublished). Advanced selections in the station trials exhibited 10-20% higher yield over the best checks and showed resistant and moderately resistant reactions to bacterial leaf pustule disease in laboratory conditions.

Seed Production

Seed production programme was organised in collaboration with Agricultural Universities in Maharashtra, Orissa, Karnataka and State seed Farms Corporations, New Delhi and Maharashtra State Seed Corporation, Akola. Among the released varieties, black gram variety TAU-1 has become the most popular variety in Maharashtra. It is estimated to occupy an area of about 500,000 hectares (over 95% of the total area under this crop in Maharashtra). The Maharashtra State Seed Corporation has distributed since 1990-91 about 1,75,800 quintals of certified seed of TAU-1. This has helped to increase the area under black gram by 24% and production by 29% per hectare. The total seed production of black gram has increased in Maharashtra by 59% since 1990-91 due to the spread of TAU-1. Powdery mildew resistant variety TARM-1 suitable for *rabi* cultivation is becoming popular in Orissa state under rice fallow cultivation.

There is a need in the country for development of varieties with multiple disease resistance and suitable for different cropping systems with good agronomic characters. This will help in increasing the production and bringing more area under these crops. Taking all these into consideration the research programme is being pursued at this Centre using induced mutations and conventional breeding methods for developing multiple disease resistant varieties with high yield potential.

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Isotope Applications in Soil Fertility and Plant Nutrition

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During the last 50 years since independence Indian agriculture has undergone a radical transformation. The consumption of fertilizer nutrients has increased from a mere 0.13 million tonnes in 1955-56 to about 14.3 million tonnes in 1996-97[1]. This was possible because of introduction of high yielding varieties, improved fertilizer management, better irrigation facilities and plant protection measures which culminated into the 'green revolution' in the seventies. Currently, fertilizers are estimated to account for 40 per cent of the total food grain production in India [2]. Though the fertilizer production has been rising the cost has also increased due to increase in prices of imported crude oil, rock phosphates and other raw materials required for fertilizer production. The recent changes in the economic policies of the the Government has also brought into focus the economic use of fertilizers. Since the fertilizers are the sources of nutrients to plants, the inorganic nutrition of plants is essentially related to uptake of nutrient elements from the soil-plant system in agriculture.

The Isotopic Tracer Technique is based on the principle that since isotopes have same chemical and physical (except mass difference) properties they can be gainfully employed to follow the behaviour and pathway of inorganic ions, compounds and organic molecules in a particular physical,chemical or biological system. Various fertilizers and agrochemicals labelled with a radioisotope are tailor made by Board of Radiation and Isotope Technology (BRIT) of Department of Atomic Energy and are available for use. The system, test object such as the element or compound is termed as 'TRACEE' and the substance such as an isotope that is mixed, fixed or attached to the tracee in order to find its path, translocation or identify its location is termed as 'TRACER'. Isotopes having the potential usefulness in soil fertility, mineral plant nutrition and allied investigations are listed in Table 1.

The suitable choice of the radioisotope in soil fertility and plant nutrition studies depends on the objectives and duration of the experiments, half life, energy characteristics, facilities available such as counting equipment, radioactive label and its stability etc. In the case of Potassium due to limited availability and excessive cost, ^{40}K is unsuitable and hence its chemical analogue ^{86}Ru is normally employed for studies on potassium [3]. It is evident from Table 1 that no suitable radioisotope is available for plant uptake studies in the case of Nitrogen, Copper, Magnesium and Boron. Under these circumstances stable isotopes are used as tracers. In the case of nitrogen, the longest lived radioisotope, ^{13}N, has a half life of only 10 minutes and the use of a ^{15}N- enriched nitrogen with ^{15}N atom percent in excess of 0.365 (its natural abundance) permits its use as a tracer for nitrogen in a manner analogous to the use of specific activity concept described below. Stable isotope analysis is carried out either by Isotope Ratio Mass Spectrometry or Optical Emission Spectrometry. Literature on stable isotopes in plant nutrition, soil fertility and environmental studies has been reviewed extensively [4]. The Chemical Engineering Division of BARC has successfully developed ^{15}N enriched nitrogen and its compounds on a pilot plant scale based on chemical exchange principle and have

Table 1. Principle tracer isotopes in soil fertility and plant nutrition studies

Element	Isotope	Emission	Half-life / or (% abundance)	Measurement	Use
Macronutrients					
Hydrogen	^{3}H	Beta	12.3 y	Liquid scintillation	Water movement, metabolism
Carbon	^{14}C	Beta	5720 y	Liquid scintillation	Photosynthesis organic matter, C balance
Oxygen	^{18}O	Stable	0.204%	Mass spectrometry	Photosynthesis, Respiration hydrology
Nitrogen	^{15}N	Stable	0.366%	Mass spectrometry Emission spectrometry	Fertilizers, BNF, N balance
Phosphorus	^{32}P	Beta	14.3 d	GM counter, Liquid scintillation	Fertilizers, root distribution, rock phosphate
Potassium	^{40}K ^{41}K	Beta Stable	1.3×10^{9} y 6.77%	Liquid scintillation Mass spectrometry	Fertilizers, K balance (Rare)
Calcium	^{45}Ca	Beta	165 d	Liquid scintillation	Ion uptake, soil exchangeable Ca
Magnesium	^{28}Mg ^{26}Mg	Beta, Gamma Stable	21.3 h 11.29%	Liquid scintillation Mass spectrometry	Movement in plants, environmental pollution
Sulphur	^{35}S	Beta	87 d	Liquid scintillation	Soil availability, S cycle, Uptake
Micronutrients					
Iron	^{55}Fe ^{59}Fe	Beta Gamma, Beta	2.6 y 45.6 d	Liquid scintillation Gamma spectrometer	Spil erosion, foliar nutrition, soil availability, plant movement
Manganese	^{54}Mn	Gamma, Beta	314 d	Gamma spectrometer	Foliar nutrition, soil availability
Copper	^{64}Cu ^{65}Cu	Gamma, Beta Stable	12.8 h 30.9%	Gamma spectrometer Mass spectrometry	Soil & plant movement, complexing in soil solution
Zinc	^{65}Zn	Gamma, Beta	245 d	Gamma spectrometer	Fertilizer, soil & plant movement complexing in soil solution
Molybdenum	^{99}Mo	Gamma, Beta	66.7 h	Gamma spectrometer	Plant movement
Other elements					
Sodium	^{22}Na	Gamma, Beta	2.6 y	Gamma spectrometer	Salt tolerance, cell permeability
Chlorine	^{36}Cl	Beta	3.08×10^{5} y	GM, Liquid scintillation	Salt tolerance, solute movement in soils
Cobalt	^{58}Co ^{60}Co	Gamma, Beta Gamma	71 d 5.3 y	GM Counter Gamma spectrometer	Enzymatic studies, vitamin metabolism Plant animal food chain
Selenium	^{75}Se	Gamma	120 d	Gamma spectrometer	Animal nutrition, food chain
Chromium	^{51}Cr	Gamma	27.8 d	Gamma spectrometer	Soil and plant movement

Note : h = hours; d = days; y = years

transferred this technology to Rashtriya Chemicals and Fertilizers Ltd. (RCF) who have commissioned a ^{15}N isotope enrichment commercial plant. The products available at 5,10 and 20% atom excess of ^{15}N are ammonium sulphate, ammonium chloride, ammonium nitrate and urea.

Principle of Isotope Dilution

In isotopic tracer methodology the principle of isotopic dilution attains utmost importance due to its wider applications. For a given amount of isotopic tracer, specific activity (enrichment level in case of a stable isotope) at any time is inversely proportional to the total exchangeable mass of tracee mixed uniformly with the tracer at that time. This principle is useful when quantitative separations are either not possible or too tedious to measure the exchangeable mass 'in vivo' for the systems under study [5]. Thus,

$$S = s\left[\frac{a_i}{a_f} - 1\right]$$

Where,
S = g or moles of the test substance (tracee)
s = amount of test substance associated with added tracer (carrier)
a_i= Specific activity before equilibrium and
a_f= Specific activity after equilibrium

This principle has been applied to the measurement of the amount of inorganic mineral nutrient in soil that is available in different forms to plants. The 'L' (labile) value concept is based upon the assumption that labelled nutrient added to a soil is isotopically diluted with a clearly definable fraction of soil nutrient called labile soil nutrient and provides a reliable reference procedure for the measurement of the total quantity of available nutrient if carrier free radioisotope is employed. The 'L' value utilizes the plant to provide an integrated sample of the soil solution over the period of time the plant is grown.

The 'E' (exchangeable) value method is a laboratory measurement of the soil nutrient in which the soil solution is sampled at a specific time. Its successful application depends upon isotopic exchange between those components potentially available to plants and the isotope added to equilibrating solution.

The 'A' (available) value provides a measure of available nutrient in units of a fertilizer standard and serves as an index for either a fertilizer nutrient or a soil nutrient. 'A' values depend not only on the amount of available soil nutrient and fertilizer placement but also on the plant root distribution and the form of fertilizer. As a result the 'A' value concept has wider applications for laboratory, growth chamber, greenhouse and field experiments [5]. The implications of 'L', 'E' and 'A' values are discussed further in detail by Fried and Mistry [6].

The well planned and suitably designed experiments on soil-plant relationships of the fertilizer element under investigations with isotopic tracer techniques are able to supply valuable information derived from computation of the data on the several parameters such as percent nutrient derived from the fertilizer, from the soil, total nutrient yield, fertilizer nutrient yield, percent fertilizer nutrient utilization and transformation of the fertilizer nutrient in soil. The isotope aided investigations conducted at Nuclear Agriculture & Biotechnology Division of BARC together with collaborative projects as well as the work done at other Indian Institutes are briefly summarized below to illustrate their wider practical applications to deal with different problems.

Evaluation of Applied Fertilizer Nutrients

Studies on the nutrient requirement during the active growth phase of sugarcane crop showed that during 6th and 10th week, the range of phosphorus uptake from ^{32}P-labelled single superphosphate was between 31 to 43 and 20 to 34 percent, respectively [7]. The importance of water soluble content of phosphorus in the fertilizer was shown with ^{32}P labelled fertilizers with varying water solubility[8]. Further, a multilocational research project on ^{32}P-aided studies on ammonium nitrate phosphate (ANP) of varying water solubility showed that fertilizers with higher water soluble content of P were more efficient on acidic red soils (ultisols), acidic coastal alluvial soils (Alfisols) and highly calcarious soils (calcifluvents) [9]. Based on the findings of this project RCF improved upon the water soluble content of P to 60 percent in their ANP products.

Basic studies at BARC [10,11] on the major reaction products of ortho and polyphosphate fertilizers formed in Indian soils having a wide range of pH showed nearly equal efficiency as sources of phosphorus to plants. Further, ^{32}P-aided studies coordinated by BARC [8] with several agricultural universities have established that ammonium polyphosphate (APP) a fertilizer new to Indian agriculture providing more nutrients (N+P) per unit weight of the fertilizer is equal or superior to the orthophosphate (DAP- available in the country at present) in diverse soil-crop regimes. These findings led RCF to initially manufacture APP on a pilot scale for field trials. Further, greenhouse and field studies conducted by ICAR projects have shown that APP produced by RCF was equal or superior to DAP as a source of phosphorus and a carrier of micronutrients. Fertilizer control order for the production of APP by RCF is now awaited. Studies on APP blended with $^{65}ZnSO_4.7H_2O$ as a carrier for zinc indicated that the fertilizer-use efficiency of Zn-APP was significantly higher than that of Zn-DAP for the major crops [12] in vertisol and was equally effective in ultisol [13].

Since the natural sources such as rock phosphates,atmospheric nitrogen, sewage sludges cannot be isotopically labelled, the contribution of this unlabelled source is measured by adding a labelled common third source to the soil plant system with the indirect method [14]. ^{15}N-aided greenhouse experiments conducted at BARC for maize and rice crops using 'A' value indirect technique indicated that for both upland and lowland rice, neem- extract coated urea was a better source of N compared to other coated ureas [15]. Likewise, employing ^{32}P-labelled triple superphosphate as a common source, studies on the fertilizer-use efficiency of four types of rock phosphates (RPs) on laterite (ultisol) and alluvial (entisol) soils showed that Syrian RPs were superior to Indian Mussoorie RP and sulphur amendment enhanced the 'A' value of ultisol [16].

Soil Chemical Reactions

Application of ^{32}P labelled fertilizers to contrasting Indian soil types revealed immobility of phosphate ions in soil under conditions where diffusion was the predominant mode of ion transport or under conditions of continuous replemishment of moisture inducing hydrolytic reactions [17].

Role of Organic Matter & Complexes

Tracer studies help in studies on the decomposition of organic matter leading to formation of organic complexes attached by coordinate valency to, or precipitated on soil surfaces by adsorbed nutrient cations [18]. The complexing action of polysaccharide fraction of compost on strontium-89 was shown to result into its lower availability for adsorption in soil for plant uptake [19].

Nutrient Supply Mechanism to Plant Roots

Isotopic tracers used in the investigations on the transfer of nutrient ions from the medium into plant root cells help in proposition of different mechanisms of ion absorption and transport. Thus, employing Rubidium-86, Kannan[20] demonstrated plasmalemma as the seat of dual mechanism of ion absorption in *Chlorella pyrenoidosa.*

Root Activity Patterns

Isotope techniques offer a quick and reliable means for determining the distribution pattern of active roots by placing the radioactive compounds in the soil at various positions to be tested for root activity [21]. The amount of radioactivity taken up by the plant is used as a measure of the intensity of root activity. While studying the root distribution of rain-fed groundnut in alfisol, a positive correlation between P-uptake, yield and root distribution was observed and 66 percent of active roots near the surface were primarily involved in higher yield [22].

Biological Nitrogen Fixation

The versatility of isotopic tracer technique in nutrient availability research is utilized in measuring by indirect method the contribution of nitrogen fixed by the leguminous crop to the crop over the entire growing season. Thus, the grain yield of rice growth with Sesbania green manure was equal to that obtained with 60 Kg N/ha in urea units and the retention of applied ^{15}N in soil was 66 percent in full dose of N as Sesbania [23]. Further Sesbania rostrata mutant TSR-1 in addition to being short day insensitive is shown to be tolerant to moderate salinity levels [24] with ^{22}Na-aided studies.

Role of synthetic chelates

Chelates are known to render insoluble cations soluble and in turn they become available to plants. Multidentate synthetic chelating agents such as EDTA are used extensively to supply micronutrients to plants in agriculture [25]. Although the micronutrients are known to be completely immobile in soil, leaching with 10^{-2} M EDTA solution induced rapid mobility of ^{59}Fe, ^{58}Co, ^{54}Mn and ^{65}Zn in ultisol and vertisol [26]. This is of particular importance in view of the greater uptake of these micronutrients in crop plants from ultisol under flooded conditions than from vertisol. The differential behaviour of ^{51}Cr-EDTA complex between nutrient culture and soil medium was also clearly shown in bean and corn plants [27]. The role of EDTA, EDDHA and DTPA synthetic chelates in inducing mobility of different heavy metals and other radionuclides in three major soil groups showed high degree of chelate specificity for individual ions depending upon the soil type [28].

In view of the present scenario of sustainable agriculture, use of organic manures like vegetable compost of rural origin, city compost, municipal sewage sludges is being encouraged to avoid the likely pollution through the repeated and higher doses of chemical fertilizers. The Isotope Division of BARC has set up a Sludge Hygienisation Research Irradiator (SHRI) which produces irradiated sewage sludge free of pathogens for use in agriculture. The Coordinated Research Project with International Atomic Energy Agency to evaluate the fertilizer use efficiency of irradiated and non-irradiated (normal) sewage sludges using ^{15}N tracer through repeated application with microplot field experiments for different crops is now in progress.

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Crop Protection Through Integrated Pest Management

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Dr. T. K. Dongre joined the 17th batch of BARC training school in 1973 after Masters degree in Agricultural Entomology. He obtained his Ph.D. degree in 1987. He has made significant contributions in Integrated Pest Management of agriculturally important insect pests. Dr. Dongre is a specialist in the field of Insect Plant Interactions and he has many accomplishments in various components of IPM viz. biological control, radiation entomology, biopesticides and development of insect resistant varieties. He is actively associated with various professional bodies of Entomology.

Unabated growth of the human population is an important factor on which the destiny of our future generation is based. According to the UN population fund, the global population (5.8 billion) may reach 11 billion by the end of next century. The major question that confront us is how can we develop agriculture that would be sufficiently productive to feed this enormous population without damaging the environment and without employing pest control technology that itself is dangerous to man. Eco-friendly methods of pest management seems to be the only solution for this problem and presently scientists are looking at Integrated Pest Management (IPM) as an ecofriendly alternative method for managing different insect pests.

The term Integrated Pest Control was first proposed by Bartllet [1] in California where the concept of IPM has been introduced. According to Food and Agriculture Organisation (1968) [2] " IPM is a crop protection system that in context of associated environment and population dynamics of the pest organism, utilises all available techniques and methods in as compatible a manner as possible and reduce the pest population to level below those causing economic injury". Sound IPM requires mission oriented basic research on pest species; research and development of control tactics and system; and the integration of the systems for managing individual pest or pest complexes. Wherever possible IPM relies heavily on ecofriendly control methods like the use of resistant crop varieties, cultural methods, biological control like parasites, predators and pathogens, autocidal control methods like sterile insect techniques, use of pheromones and botanical insecticides.

The importance of IPM was realised very early at BARC and R & D activities in the area of pest management were concentrated on bio-originated pest control methods which would have low environmental cost. The chosen areas for this research were Sterile Insect Technique (SIT), development of biopesticides, biological control of insect pests with emphasis on insect parasites, use of sex pheromones for monitoring and control of insect pests of agricultural importance, studies on insect plant interactions and development of transgenic cotton resistant to bollworm complex.

Sterile Insect Technique (SIT)

In the concept of SIT, continuous field release of mass produced radiation sterilised insects would limit the reproductive ability of natural population and can bring down the insect population to a manageable level or even can erradicate completely. This method has been demonstrated to work successfully with several major insect populations in the world for control, elimination and quarantine. The screw worm fly was eliminated completely from the US and other parts of the world. Various species of fruit flies have been eliminated from islands in the Pacific ocean [3]. This method has also been successful in quarantine operations. For example the quarantine operation using pesticide against the Mexican fruit fly along US- Mexican border has been replaced by release of sterile males to prevent flies entering in to California. At BARC attempts have been made to demonstrate the feasibility of this technique to control insect pests. The insect pests chosen for this studies are red palm weevil, *Rhynchophorus ferrugineus* Oliv which is serious pest of various palms, potato tuber moth, *Phthorimaea oprculella* Zeller which infest potatoes in field as well as in storage and spotted boll worm, *Earias vittella F.* a serious pest of cotton.

SIT includes mass rearing of target insect, inducing sexual sterility in adults without affecting mating vigour and competitiveness, release of such sterile adults in overwhelming number in natural population and assessing the effectiveness. An efficient and economic mass rearing method for red palm weevil was developed based on sugar cane and artificial diet [4]. The mass rearing technique for potato tuber moth was standardised by using whole potatoes and paraffin wax coated slices. The potato slice method gave yield of adults 1.6 times more than the whole potato method [5]. Spotted bollworm of cotton was reared on natural okra fruits or artificial diet developed in our laboratory [6].

Detailed radiological studies on selected insect species were carried out in our laboratory by using cobalt-60 source. The optimum sterilising dose for red palm weevil, potato tuber moth and spotted bollworm was found to be 15 Gy, 450 Gy and 300 Gy respectively. Modifying factors like dose fractionation, temperature, diurnal rhythm and gaseous environment were tried for obtaining competitive sterile adults [7]. The ratio of ten or more sterilised males to one natural male was found to be effective ratio for suppressing progeny productions. Attempt has also been made to test the F1 sterility approach to reduce radiation damage and increase competitiveness of sterile males.

Pilot scale field trial was conducted at Kayankulam, Kerala to demonstrate the feasibility of SIT to manage the red palm weevil. The trial was carried out in collaboration with Central Plantation Crops Research Institute (CPCRI) over an area of 320 hectares [8]. The results are quite encouraging and warrants area wide management of red palm weevil. Similarly feasibility of SIT in controlling multiplication of potato tuber moth in storage was assessed in collaboration with Central Potato Research Institute (CPRI) at their regional research centre (Rajgurunagar) near Pune, Maharashra.

Development of Biopesticides

Like human beings, insects are also subjected to infections by entire array of micro-organisms like bacteria, virus, protozoa, fungi and nematodes. Effective and efficient biopesticides can be developed from above entomopathogens which may form an important component of IPM. During the past few years quite a good number of entomopathogens were isolated and tested for their pathogenecity to specific host or even to various other host species. Efforts in this direction at BARC have yielded promising results and success has been obtained in isolating, identifying and proving the toxicity of pathogens like *Bacillus thuringiensis*, *Bacillus sphericus*, Granulises Viruses (GV) and Nuclear Polyhydrosis Viruses (NPV). We have isolated 7 indigenous strains of *Bacillus* (ISPC 1 to 7), GV of potato tuber moth and NPV from *Amsacta moori, Helicoverpa armigera and Spodoptera litura.* Out of seven isolates of *Bacillus*, ISPC 1 to 4 and 7 are *B. thuringiensis var kenyae* isolated from different insect species. ISPC 4 is multicrystalliferous strain containing more than one crystal. ISPC7 is effective only against lepidoptrean pests where as ISPC 1,2 and 3 are effective against lepidopteran as well as dipteran pests. ISPC 5 and 6 are *Bacillus sphericus* strains isolated from mosquitoes which are quite effective against many mosquito species tested.

From all the above isolates, ISPC 1 was found to be the most promising isolate. This spore bearing crystalliferous *Bacillus* was isolated from the diseased larvae of *Ephestia cautella* and pure isolate clones were maintained on nutrient agar medium. This organism was found to be gram +ve, aerobic, rod shaped spore bearing *Bacillus* with bipyramidal crystal (endotoxin) in sporangium. Biochemical, morphological, serological and eastrease pattern studies confimed that this organism belongs to *B. thuringiensis var kenyae* subspecies and biotype 4a 4c. Various specific tests proved that the organism did not produce fly factor or exotoxin in growth medium. Detailed toxicity and bioefficacy of this isolate was studied in laboratory as well as in field conditions. In a comparative toxicity study when this isolate along with 11 other known *B. t.* isolates obtained from Dr. Clayton Beegle, USA were tested against *Spodoptera litura,* it was found that this isolate was more effective than other isolates tested. The toxicity of this isolate was further studied against more than 16 economically important insect pests including most serious pest like *Helicoverpa armigera* [9]. As this isolate showed great potential, detailed investigations were carried out for development of bopesticide based on this isolate. Dust and wettable powder formulations were developed and these formulations were tested under field conditions in collaboration with ICAR and agricultural universities under All India Co-ordinated Research Projects. Results of these trials were quite encouraging showing effectiveness against various insect pests. Besides, adaptive cum demonstration trials were also conducted at different locations near Pune in Maharashtra for protection of stored potatoes in country type storage known as Arni. A dust formulation at the rate of 1.0 gm/ kg potatoes offered a good protection against potato tuber moth over a period of three months.

Considering the potential of this isolate, attempts were made to mass produce this organism, using nutrient broth medium. In laboratory conditions we obtained the yield of 0.9 gm material per litter medium. This yield was further increased to 2 g per litter by using 1200 litter capacity fermentor. Recently BARC has entered in to MOU with Hindusthan Antibiotics Ltd. (HAL) Pune, for upscaling the mass production technology. This was followed by transfer of this technology to HAL for commercialisation. HAL has successfully achieved pilot scale optimisation and thus biopesticide based on indigenous isolate *Bt var kenyae* strain under the trade name of BIOKEN is soon going to be available to farmers of our country.

Pheromones in Monitoring and Control of Insect Pests

In broader sense pheromones are substances produced by insects and received by other member of same species which affect their growth and behaviour. Pheromones can be aggregating, alarm or sex pheromones. Sex pheromones have an excellent potential in IPM programme. These are chemicals used by insects for communication between opposite sexes. Sex pheromones can be used for monitoring to detect the initial infestation leading to timely application of pest control measures or they can be used for insect control through disruption of mating in feral population by distribution of additional pheromone sources in the field to confuse males from locating natural females.

BARC has an ongoing programme on indigenous development of various aspects of pheromone technology and the utilisation of pheromones in the control of insect species of economic importance. Simple and efficient trap design for potato tuber moth has been developed at BARC and these traps were evaluated for monitoring potato tuber moth infestation in field. The efficiency of traps was evaluated on farmers field near Pune and also at research centre of CPRI, Rajgurunagar. The field trial was also conducted to evaluate pheromone mediated mating disruption of same above insect. Similarly efficient trap design has been developed for bollworms of cotton and field trial has been carried out in collaboration with Central Institute of Cotton Research, Nagpur for management of cotton bollworms. In this trial using BARC developed synthetic pheromone and trap, pink bollworm activity was monitored year around. The data over three years has shown that there was positive correlation between trap catch value and ETL of larval activity. Mating disruption trials were also conducted successfully against pink boll worm using indigenous pheromone and laboratory prepared pheromone dispenser [10].

For optimum utilisation of pheromones in insect control, knowledge of pheromone related

behavior and biology of target species is important. From this point of view, source of pheromone production was identified in potato tuber moth, spotted bollworm and spiny boll worm of cotton. Influence of biotic and abiotic factors that affect pheromone production by females and pheromone response by males of these species were studied by using olfactometers and electroantennogram technique [11].

Insect Plant Interaction

Association of plant and insect is a dynamic one and may favour either the insect or plant. Plant may encourage insect visit for its own benefit such as for pollination or discourage visit through various defence strategies which they acquired during the course of co-evolution. Studies on the interaction of the insects with their host and non host plants can reveal plethora of information which can be utilised for the development of optimally designed defence of plants against insect attack. In our laboratory, attempts have been made to understand these interactions in economically important insect pests like spotted bollworm, *E. vittella* and bruchids, *Callosobruchus* species.

Detailed studies with *E. vittella* and their host plants revealed that there was vast difference in suitability of different host plants for their development and reproduction. This diversity in suitability was due to variation in the efficiency of conversion of ingested and digested food. Studies on the relative preference to these host plants revealed that preference for oviposition did not coincide with preference by the larvae for feeding and the relative preference exhibited for different host plants for oviposition could not be altered through induction. The failure of the larvae of *E. vittella* to establish on fruits of less preferred host plant, *Hibiscus sabdariffa* was due to the presence of solvent ether extractable compounds with antifeedant activity in calyx of this plant. In case of another less preferred host plant, *Thespesia populnea,* failure of larvae to establish on whole fruits was probably due to the presence of compounds with antibiosis activity in fruit pericarps. Of the various non-host plants evaluated, solvent ether extract of *Xanthium strumarium* exhibited strong antifeedant and oviposition deterrent activities against *E. vittella [12].* The oil extracted from *Blumea eriantha* leaves showed feeding and oviposition deterrent properties. Besides this oil also affected the reproductive behaviour of adult moth, in that when moths were exposed to oil vapours, mating activity particularly of males was drastically reduced [13].

Through the studies on interaction of bruchids, (*Callosobruchus maculatus* F.) with their host plants, four bruchid resistant sources were identified. Three sources belong to wild progenitor of pigeonpea (*Cajanus cajan)* and one belongs to *Vigna* species. The resistance of *Cajanus platicarpa* appears to be due to hard seed coat where as resistance of *Cajanus scarabaeoides* seemed to be due to antibiosis. Hard seed coat as well as antibiosis compounds might be involved in the resistance of *Cajuns sericeus* [14].

In *Vigna* species the resistance was evident in wild progenitor of black gram, *Vigna mungo var. silvesris.* The mechanisms of this resistance was found to be larval antibiosis expressed as reduced survival, longer developmental period and reduced body weight [15]. These resistant sources were included in our plant-breeding programme to develop bruchid resistant high yielding varieties of different pulses. *Cajanus* resistant sources were crossed with pigeonpea variety, TT5 and *Vigna* resistant source was crossed with high yielding variety of black gram, TAU1.

Development of Transgenic Crop Varieties

The development of transgenic plants with built-in mechanism for insect resistance will be of immense value to manage the insect pests through integrated pest management. Being ecofriendly, it is one of the safer insect control methods and it is expected that genetically engineered plants that carry their own built in control will overcome much of the problems of insect pest management. The usefulness of *B.t.* based transgenic crops has been recognised long back and successful applications have already been reported.

BARC has an ongoing programme on development of trangenic plants incorporating endotoxin gene from *B.t.* Initially toxin cloned constructs of E*scherichia coli* were tested against *H. armigera, S. litura* and *Bombyx mori.* Further efficiency of transgenic tobacco plants has also been

evaluated using *Heliothis* and *Spodoptera* larvae in laboratory as well as in green house conditions. Based on the expertise developed in our group, we were identified by DBT to participate in Nation-wide net work project on "Development of transgenic cotton varieties resistant to boll worm attack. Insect species selected for this work were *Pectinophora gossypiella, E. vittella, E. insulana, H. armigera, and S. litura.* Nucleus culture of above insect species were obtained from various agricultural universities and rearing of these species were standardised. Bioassay techniques to test the toxicity of Bt strains and constructs containing Bt gene were standardized by either using natural host plant or artificial diet. Various constructs containing Bt toxin gene received from NBRI, Lucknow were tested for their toxicity against all above insect species selected.

Biological Control of Insect Pests with Emphasis on Insect Parasites

In nature a balance is known to exist between plant feeding insects and their natural enemies. This balance can be shifted through manipulating the population of parasites and predators to control the insect pests of economical importance. These parasites are self-perpetuating and capable of responding to the fluctuation in population density of the pest they attack. In order to provide host stage constantly in field, Knipling has suggested the use of sterile host females along with release of egg parasites [16]. In our laboratory, a combined approach for the control of potato tuber moth by use of egg parasite, *Trichogramma chiloni* and radiation sterilised females of potato tuber moth was evaluated. We have demonstrated that *T. chiloni* could be reared on eggs laid by radiation sterilised females of potato tuber moth without any adverse effect on its development and reproduction performance [17]. We further observed that in field studies the population build up of potato tuber moth could be reduced through release of *T. chloni* and parasite sustenance could be augmented through release of radiation sterilised potato tuber moth females.

Parasite release has great potential to control the insect pests infesting stored commodities. However, in the past there were very few attempts to test their applications on a practical scale. In our laboratory a pteromide larval ectoparasite of bruchid, *Callosobruchus* species was intercepted and it was identified as *Dinarmus vagabundus* (Timberlake). Detailed investigations were carried out to find out the potential of using this parasite for arresting the multiplication of bruchids in storage. Only a single release of parasites was found to be sufficient to control the bruchids if grains were infested with single stage of host larvae, however, more than two releases were needed when grains were infested with different stages of host insect. In the searching ability studies it was found that female parasites could penetrate through the columns of healthy grains in horizontal as well as in vertical directions and could locate the infested seeds from healthy ones. Thus this parasite has shown good promise for the control of bruchids which attacks different pulse grains in field as well as in storage. Presently large-scale storage trials are in progress so as to popularise this parasite at the farmers' level.

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Environmental management of HCH and DDT residues in soils using nuclear techniques

Dr. N.B.K. Murthy joined BARC in 1964. He has been working on the fate and persistence of pesticides in soils and plants using nuclear techniques since 1970. He spent one year as a Post- doctoral fellow at United States Department of Agriculture, Belstville, USA specializing in anaerobic metabolism of persistent organochlorine pesticides. He has set up soil ecosystems in the Nuclear Agriculture and Biotechnology Division to study the metabolism of ^{14}C - labelled pesticides in soils under a continuous flow-through system to obtain complete ^{14}C - mass balance. He has been associated with many IAEA-CRP projects on the behaviour of pesticides in oil crop plants and during the refining procedure of vegetable oils. He is currently heading Plant Pathology and Pesticide Residues Section. He has over 60 publications in National and International journals. As a faculty member, he has imparted training to many agricultural scientists on the use of nuclear and allied techniques in pesticide residue research.

Dr. (Mrs.) Jharna Mitra obtained her D. Phil. degree in Microbiology from Calcutta University and joined Bhabha Atomic Research Centre in 1970. She undertook an International Training Course in Radiation Microbiology conducted by IAEA, Vienna, at BARC in 1969 and worked as a Post Doctoral Research Associate at Pennsylvania State University, USA in the year 1979-1980. She had conducted extensive research on metabolism of DDT in soils and plants which has led to the development of a protocol for decontamination of pesticides from soil. She has to her credit about 50 research papers and reviews in National and International Journals and Symposium proceedings. She has taught postgraduate students and has delivered popular scientific talks from All India Radio. Her current interest includes pesticide bioremediation by genetically engineered microorganisms.

The use of pesticides has become an integral part of modern agricultural system. The term pesticide include insecticides, fungicides, herbicides, nematicides and rodenticides. Until late 1950s, pesticides were generally considered to pose little if any long - term risks for health and environment. However, the rapid increase in use of organic pesticides during 1940s and 1950s aroused public concern about their safety as a result of publication of a book "Silent Spring" by Rachel Carson highlighting the harmful and long term side effects of pesticides [1]. The intensive use of pesticides especially chlorinated organics has resulted in serious environmental problems because they are either recalcitrant or biodegraded slowly. This resulted in contamination of soils and environment. The use of chlorinated pesticides has been restricted or banned in many developed countries by replacement with less - persistent and biodegradable pesticides. Even then, the presence of these obnoxious organochlorine pesticide residues are felt in all spheres of environment. Hence there is a need to study behaviour and impact of these pesticide residues in soil and its biological properties to evolve better pesticide management strategies.

Pesticide Type, Pattern and Usage in India

Till the year 1949, the plant protection was meager in India so also food grain production. The small scale introduction of DDT for malaria control just after the last world war may be said to be the start of modern synthetic pesticides in our country. This was followed by the use of BHC (now called as HCH) for locust control. With the introduction of grow more food campaign and National Malaria Control, and setting up of indigenous production, the pesticide usage has increased from 1952 tremendously. A look at the trend in pesticide

consumption would show that their consumption has risen from 434 MT technical grade in 1954 to about 80000 MT in 1996. But the pesticide consumption patterns are completely different in India in comparison to other countries. The consumption of pesticides in developed countries like Japan, USA and European countries are 12000, 2500 and 3000 grams per hectare, whereas it is only 570 grams per hectare in India [2].

Pest control in India is still based on insecticides. Insecticides constitute 80% of the total pesticides used in our country followed by fungicides (11%) and herbicides (8%) [3]. The demand pattern of insecticide consumption was 60700 MT for 1995-96 of which organochlorine insecticides form the bulk of the production. Among the insecticides, DDT and HCH were consumed in larger amounts till 1995 - 96. Currently due to a ban on the use of HCH and DDT for crop protection, dependence on the safer and less persistent organophosphorous and carbamate insecticides is increasing. Pesticides are used mainly on cotton (52 - 55%), followed by rice (17 - 18%) and vegetables (8%) [2]. These are used mainly in states of Andhra Pradesh, Karnataka, Gujarat, Punjab and Maharashtra. Bulk (80 - 85%) of the pesticides are manufactured locally and rest are imported. This is the general scenario of pesticide status in India.

Pesticide Residues in Environment

Pesticides, applied directly as fumigants, seed dresser and soil drencher, or indirectly as spray and dust ultimately reach the soil. It is estimated that only about 1% of the applied pesticides find their way to targets, remaining 99% of the pesticide comes to soil and is of environmental concern. Soil harbours a variety of microflora and microfauna which can transform pesticide residues either to a less or more toxic compounds. At the same time, pesticide residues can affect non - target soil organisms and their activities which are responsible for the maintenance of soil fertility. Pesticides can undergo various abiotic and biotic reactions like adsorption in soil - desorption, photodecomposition, volatilization, microbial transformation, soil bound residue formation and leaching into ground water. Factors like temperature and water regime, chemical and physical properties of soils also influence the behaviour of pesticides. The fate of a pesticide whether it is less or more persistent is governed by these factors. As stated earlier, organochlorine insecticides were used extensively in Indian agriculture till 1995 - 1996. HCH and DDT form the bulk of pesticide consumption. It has been reported that the persistence in soil varied from 1 - 20 years for DDT and 1 - 14 years for HCH [4]. In a study where DDT residues were added to soils and analyzed after 23 years, it was seen that 10 to 28% of added DDT residues were still remaining in the soil [5]. These studies are mostly related to temperate climatic conditions.

It is well known that organochlorine pesticides degrade faster and their persistence is less in tropical climate as existing in India [6]. Nevertheless, the extensive and indiscriminate use of pesticides in agriculture and public health has resulted in contamination of various abiotic and biotic matrices including air, water, soil, sediments, fish, human breast milk and food stuffs. Residue analysis has shown the predominance of HCH and DDT residues in these environmental components; sometimes exceeding the maximum tolerance limits [7-10]. Contamination of these environmental matrices is of great concern as the biomagnification and bioaccumulation of HCH and DDT in the upper strata of biosphere poses a potential threat on human life. Though HCH and DDT have been banned for use since 1997, still these residues continue to reside in environment for some more time due to their persistence unless some suitable remedial steps have been adapted to clean the environment. Information on the effect of HCH and DDT on non - target organisms, and the fate and persistence of these compounds in the soil environment is needed to develop appropriate strategies for amelioration of soils. Considerable research work has been carried out in BARC for the last two decades on the effects and fate of HCH and DDT in soils employing nuclear techniques with ^{14}C - labelled compounds. Some of the salient observations are presented here.

Work done at BARC

The effect of HCH and DDT on Non - Target Organisms

The technical grade of HCH used for crop protection is a mixture of 8 isomers predominantly containing alpha - (60-70%), beta - (5-12%), gamma

-(10-12%) and delta isomers (3-4%). Gamma - isomer also known as lindane has the highest pesticidal property. Beta isomer of HCH is considered to be the most persistent one amongst all. The effect of technical HCH and beta - HCH on soil enzymes (dehydrogenase and phosphatases) was studied to assess the impact on general microbial activity. Technical HCH and beta - HCH at various concentrations had no inhibitory effect on these soil enzymes. The mineralization of ^{14}C - labelled rice straw in a clay soil was affected by HCH applied at normal field rate [11]. However, HCH at 10 - times field rate inhibited $^{14}CO_2$ evolution from rice straw temporarily. Phytotoxic effects of HCH were noticed in sandy loam soil only but not in a clay soil indicating the importance of soil type [12].

The technical grade of DDT contains a mixture of *p,p'* - DDT(67 - 85%, *o,p'* - DDT(8 -21%) and traces of other metabolites (*p,p'* -DDD, 3 -7%; *p,p'* - DDE, 1.96% and *o,o'* - DDT, 0.1%). Studies on the interaction of DDT with soil microbial activities had shown that the parent compound and its major degradation products viz. DDE and DDD inhibited soil respiration and different soil enzymes [13]. Soil fungal and actinomycetes populations were also affected by DDD. Effect of DDT on the germination and growth of different group of crop plants was studied. It was seen that oil seed crops (groundnut, mustard and soyabean) were prone to inhibition of germination and subsequent plant growth by DDT [14], where as cereal, pulse and fiber crops were not affected. Studies with ^{14}C -labelled DDT showed that uptake of DDT by seeds was directly proportional to the seed size. No direct relationship between DDT uptake by the seeds and its subsequent translocation to the growing regions or the degree of growth inhibition was observed. However, oil content of seed per se directly affect the susceptibility or tolerance of a plant to DDT. DDT was further found to inhibit cation uptake in plants irrespective of plant types. However, in groundnut, the levels of Ca++ and K+ were inhibited considerably to affect their normal concentrations usually present in plants. The inhibitory effect on crop plants was observed even several years after DDT application in the field though total DDT residues were ranging between 0.29 - 1.25 ppm in the fields during the experimental periods [15]. Significant reduction in the yield of oil seed crops chilies and tomatoes was observed with DDT.

Fate and Persistence of HCH and DDT in Soils

The persistence of HCH isomers was studied in different water regimes and soil types. Beta - HCH was most persistent followed by delta-, alpha- and gamma isomers. HCH isomers were degraded faster in flooded soils in comparison to soils at 60 and 80% water holding capacities. HCH isomers were less persistent in black clay soil than in red sandy loam soil. Sequential moisture regimes of alternate flooded and unflooded conditions showed that HCH isomers were less persistent in unflooded soils followed by flooding compared to soil maintained under solely unflooded conditions. Rice is grown in flooded soils and rice fields are subjected to various organic and inorganic amendments to increase the soil fertility. The effects of these amendments were studied on the degradation of HCH isomers in soils. Rice straw decreased the persistence of all isomers of HCH under flooded condition. Interestingly the Dt-50 values of beta - HCH, the most persistent isomer was brought down to a great extent with rice straw treatment. Cotton seed , and neem seed cakes alone and in combination with urea in soil decreased the persistence; cotton seed cake being the most effective. Rice fields are ploughed with green leaves of plants like *Gliricidia* sp., *Sesbania* sp., *and Crotalaria* sp. to increase the organic nitrogen content of the soil. Green manuring with leaves of *Gliricidia sepium* decreased the persistence of HCH isomers even at elevated concentrations in soils [16]. In order to understand the mechanisms involved with enhanced degradation of HCH isomers in flooded soil with amendments, soil ecosystem under a continuous flow - through system with ^{14}C - gamma HCH was used. ^{14}C - mass balance of gamma - HCH had shown that the pesticide was dechlorinated to benzene which was subsequently mineralized to CO_2 in flooded soil [17]. Green manuring increased further the formation of benzene and CO_2 from gamma - HCH. Similar results were observed with ^{14}C - mass balance of beta - HCH in a flooded soil with green manure.

Studies on the persistence of DDT in soil revealed faster degradation in flooded soil compared to unflooded soil. Further enhancement in DDT degradation was noticed during hot summer months,

in organic and alkaline soils. Using ^{14}C - labelled DDT under laboratory conditions, it was observed that in continuously flooded soil DDT degrades faster to DDD and other degradation products compared to the intermittently flooded soil [13]. Organic matter amendments like rice straw and green leaf manure enhanced DDT degradation in flooded (anaerobic) soil extensively. Rice straw was found to enhance DDT degradation in different soil types [18]. Green leaves of leguminous plant, *Gliricidia sepia* (at the rate of 11230 kg per hectare) was found to be more effective in reducing the persistence of DDT in different soil types [19]. Tracer techniques with ^{14}C- labelled DDT had revealed the enhanced formation of DDD in flooded soils with rice straw and green manure when compared with flooded soil thereby indicating a selective pathway of reductive dechlorination of DDT to DDD. Inorganic amendments like $CaCO_3$ and MgO was found to convert DDT to DDE to a considerable extent in aerobic (unflooded) soils [20]. Long term studies of inorganic amendments in unflooded soils showed 49.5 and 64.5% DDE formation after two years from DDT treated and MgO amended red sandy and black clay soils respectively [13].

Bioremediation of Polluted Soils

The soil is the ultimate repository of pesticides applied for agriculture and public health purposes. The immediate concern therefore is to reduce or eliminate pesticide burden of contaminated soils so as to minimize the entry of pesticide residues into the food web through crop growth. The treatments currently used to remove or destroy contaminants include physical, chemical and biological techniques. The use of bioremediation techniques in soil have some significant advantages over others [21] The primary technique that has been used in bioremediation to enhance natural detoxification of contaminated environment is stimulation of activity of indigenous microorganisms by addition of nutrients, regulation of redox conditions, optimization of pH conditions etc. Work done at BARC using radiotracer methodology with ^{14}C - labelled pesticides had shown that HCH and DDT polluted soils even at very high concentrations could be decontaminated with rice straw and green manure amendments in flooded soil [15-18]. Rice straw and green manuring are routinely practiced in rice fields to increase the soil fertility. The increased microbial activity in amended soils has brought about the bioremediation of HCH and DDT pollution in soils through reductive dechlorination [15,17,18].

Other approaches that are still in early stages for bioremediation include, (1)- inoculation of the contaminated sites with microorganisms of specific biotransformation abilities, (2)- application of immobilized enzymes and (3)- use of plants to take up or adsorb or transform pollutants. The method to remediate through increased activity of native soil microorganisms by nutrients is best suited for land farming and cultivable fields but not the sites contaminated with recalcitrant pesticides either due to accidental spill or dumping of excessive or unused or banned chemicals. These need in situ remediation procedure through inoculation with isolated organisms having specific and enhanced biodegradable ability. Many bacterial strains such as *Arthobacter*, *Flavobacterium*, *Pseudomonas* etc which are capable of degrading pesticides have been isolated and used for bioremediation. Soil fungi seem to be more competent compared with bacteria as they can send their mycelium into the soil crevices in search of these chemicals, while bacteria can not reach such places. Successful detoxification of PCB - and PAH contaminated soils have been achieved with fungi [22,23]. Studies in this area of using fungal culture for bioremediation have been initiated recently at BARC. A fungus, *Fusarium solani* capable of degrading DDT was isolated and genetically improved further through parasexual hybridization. These hybrid strains could degrade DDT faster in liquid cultures [J.Mitra, unpublished]. Efforts are being made to study the degradation potential of *Fusarium solani* hybrids in soils. It is likely that by using such microorganisms and other cultural practices, pesticide load in the soil can be diminished to a greater extent..

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Species Variation in Phyto-Extraction of Uranium

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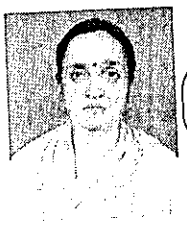

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Phytoremediation is an emerging field where plants are used to remediate heavy metals from contaminated soil and water [1, 2, 3, 4]. Among the plant families, species belonging to Cruciferae (mustard, *Thlaspi*), Chenopodiaceae (spinach, *Chenopodium*) and Compositae (Sunflower) have genetic potential to extract heavy metals from soil or water, and accumulate them in plant parts [5]. Usually species which can accumulate heavy metals at a concentration of 0.1 to 1% of their dry weight are considered as candidates for phytoremediation [6]. In addition to the genetic traits for hyperaccumulation, following characters are desirable : (a) high accumulation rate even at low environmental concentration of the contaminant (b) ability to accumulate high levels of contaminants (c) ability to accumulate several metals (d) faster growth (e) high biomass production and (f) resistance to disease and pests [7]. In general phytoremediation of heavy metals can be classified into three areas :-

1. **Phytoextraction** - in which metal-accumulating plants are used to transport and concentrate metals from the contaminated soils into harvestable parts of roots and shoots;
2. **Rhizofiltration** - in which plant roots absorb, precipitate and concentrate heavy metals from contaminated effluents;
3. **Phytostabilization** - in which heavy metal resistant plants are used to reduce the mobility of heavy metals, thereby reducing the risk of environmental pollution by leaching into the ground water or by air-borne spread.

Heavy metal tolerance of plants has been studied in depth and is the best documented example of evolution [8]. Most of the evidence produced so far indicates that only plant species embodied with the genetic variation for their tolerance in their population are able to develop tolerant populations; *de novo* mutation for a tolerant genotype induced by

Table 1. Uptake of Uranium by *Brassica* (*B. juncea*, var. Pusa Bold) callus.

Treatment	Fresh wt. (g)	Dry wt. (g)	U content (µg/g.dw)	BCF
Control	1.11	0.068	1.3	-
25 µM U	1.10	0.068	22.0	3.7
50 µM U	0.58	0.043	31.5	2.7
100 µM U	0.08	0.033	17.2	0.72

Brassica callus was grown in MS medium (pH 5.5) containing 0, 25, 50 and 100 µM Uranyl acetate (0, 5.95, 11.9 and 23.8 µg U/ml) for 20 days. Six replications were made for each treatment (standard error for Fr. wt. and Dry wt. < 6%). U content was determined from bulked dry tissue of 6 replications.

the heavy metal application has not been documented.

For a plant to resist the toxic effects of heavy metals they must either limit their uptake (avoidance), detoxify the metals which have gained entry into the cells or develop heavy metal resistant metabolism [9]. The detoxification can occur in a number of ways depending on the metal, either through chelation, compartmentalisation into vacuoles or precipitation. Phytoremediation takes advantage of the ability of certain cellular components, including proteins to bind metals. There are many metal binding proteins and peptides, e.g. metallothioneins, metalloenzymes, metal-activated enzymes and various metal storage, carrier and channel proteins. Phytochelatins are enzymatically synthesised low-molecular weight peptides of γ-glu-cys-polymers with high affinity for transition metals. They are wide spread in yeasts, lower and higher plants [10,11]. Genetics of metal tolerance has been studied in many plants. The tolerance is caused by one or two major genes [12]. The degree of tolerance in a population is controlled by additional minor genes [13]. The trait in some species is constitutive [14, 15] and in some species show co-tolerance towards many heavy metals [16].

Work at Nuclear Agriculture and Biotechnology Division

The objective of our research is focused mainly on the understanding of the physiology of heavy metal uptake by plants especially on the question why plant should take up toxic metals in default pathway of ion uptake, genetics of tolerance and possibility of inducing mutation to improve metal remediation. We are also interested to identify proteins/peptides that can bind metals such as Pb, Cd, Cr, Cs, U and Hg.

We have initiated work on uranium uptake by different species of plants and callus of some plants,

Table 2. Uptake of Uranium by *Chenopodium* (*C. amaranticolor*) callus.

Treatment	Growth rate (mg f. wt./d)		U content µg/g.d.wt. 30 d old	Bioconcentration factor (BCF)
	15 d old Mean+S.D; n=6	30 d old		
Control	177 ± 30	89 ± 21	1.9	-
10 µM	-	70 ± 23	315.0	132.0
25 µM	99 ± 30	-	-	-
50 µM	82 ± 53	67 ± 27	75.0	6.3
75 µM	92 ± 35	-	-	-
100 µM	129 ± 33	73 ± 19	180	7.6

Large variation in growth was recorded in medium containing Uranium

Table 3. Uptake and tissue distribution of Uranium in *B. juncea* seedlings.

Treatment	No. seedlings	Dry wt. Shoots (g)	Dry wt. Roots (g)	U content µg/gd.wt		BCF	
				Root	Shoot	Root	Shoot
Control	280	0.54	0.13	1	2	-	-
U-treated	350	0.42	0.15	6000	360	504.6	30.3

Seeds were germinated and grown in presence of light on nylon mesh fitted on the top of a beaker containing 400 ml of nutrient solution or nutrient solution + uranyl nitrate (11.89 µg U/ml) for 21 days. pH of the solution was adjusted to 5.5 and solution was replaced every alternate day. Constant aeration was made during the growth. Seedlings were washed thoroughly in dist. water and separated into roots and shoots. Computation of growth and uptake data shows that 26.3% dry matter was partitioned into roots and 73.7% into shoots and only 14.4% U was found in shoots while 85.6% was sequestered into roots. The experiment also deminstrated that germination and growth were not inhibited by U treatment.

Table 4. Uranium uptake and distribution in plant species

Species	Treatment	BCF			
		Whole plant	Roots	Stem	Leaves
B. juncea var. Pusa Bold	24 d seedling + 28 days treatment 8.33 µg U/ml	322.9	1526	43.4	13.4
Sunflower	20 d seedling + 7 days treatment 23.7 µg U/ml	50.6	162.6	31.4	3.9
Chenopodium amaranticolor	45 d seedling + 6 days treatment 11.89 µg U/ml	212.7	832.6	-	35.3
C. amaranticolor	64 d seedling + 8 days treatment 22.56 µg U/ml	47.6	271.7	60.7	5.8
Onion roots	Onion bulb allowed to root into medium for 7 days in U solution 4.67 µg U/ml	-	637.2	-	-

Except for onion roots, all plants were grown in nutrient solution containing uranyl nitrate (500 ml) adjusted to pH 5.5 with constant aeration and solution was replaced every day. Each experiment was done in triplicate with 6 plants in each set. SE for U content was less than 10%. Onion roots were grown in tubes containing 25 ml of solution. Roots were thoroughly washed with water and a brief wash for 15 min with 1 mM $Ca(NO_3)_2$ to remove unbound uranium in the apoplast.

grown at different concentrations of uranium and for different durations of treatment. Plants were grown in nutrient solutions containing uranium. Plant tissue was fractionated into cell wall and soluble fractions to determine the compartmentalization of uranium. Uranium was determined in the digested plant materials by Laser Fluorimeter developed at CAT, Indore (We acknowledge the help rendered to us by Shri A.G. Bhujle, Head, Laser Instrumentation Section, CAT, Indore). The composition of the nutrient solution was based on Huang and Cunningham [17] and the method for cell fractionation was followed from the procedure given by Chao and Dashek [18].

Results and Discussion

Uranium accumulation in plant tissues was expressed as BCF (Bio-Concentration Factor) which is defined as the ratio of metal concentration in plant tissue (µg/g dry wt.) to initial metal concentration in solution (µg/ml).

Table 5. Uranium distribution into cellular fractions

Species	Treatment	U content µg/g d.wt.			
		Cell wall		Soluble fraction	
		root	shoot (stem + leaves)	root	shoot
B. *juncea*	24 d seedling 28 days treatment 8.33 µg U/ml	11000	481	1375	243
C. *amaranticolor*	45 d seedling 6 days treatment 11.89 µg U/ml	3300	1321	1071	559
Onion root	4 days treatment in 4.67 µg U/ml	999	-	117	-

Uranium Uptake by Callus Tissue

Experiments were carried out to determine the toxic effect of U on callus growth and U uptake. Data are shown in Table 1 (*Brassica*) and Table 2 (*Chenopodium*). U inhibits callus growth and accumulation of U is not marked. U content of callus was determined from tissue which was not in contact with the agar medium in order to avoid U sequestered between the tissue layer and the agar medium.

Data on callus show that callus may not be good candidate for U uptake and its distribution into cell components.

Uranium Uptake by Plants and its Distribution

Brassica : Two experiments were done. In the first experiment *Brassica juncea* var. Pusa Bold seeds were germinated in presence of uranium and grown for 21 days. Data are presented in Table 3. The other experiment was done with seedlings for a brief exposure (Table 4).

Uranium uptake and distribution into plant parts and cellular components

Five plant species were used to determine U uptake and its distribution into plant parts. Plants were grown in pots and seedlings were taken out for the experiments with U treatment. Data are presented in Table 4.

U treatment did not show perceptible toxicity at lower concentration in *Chenopodium*. However, at higher concentration of U, *Chenopodium* showed necrosis of leaf at the edges and some discoloration after 6 days of treatment.

Uranium distribution into cellular fractions

U-treated plant parts were fractionated further into insoluble cell-wall and soluble cytoplasmic fractions and U content in each fraction is presented in Table 5.

About 90 % plant leaf is water. Cell wall makes up one-fourth to one-half of the dry weight of plant material. Of the one-half of dry weight which is not cell wall, roughly half is soluble cytoplasmic constituents and half is particulate material. For either fraction, roughly half is protein [19]. Our fractionation procedure was not quantitative. However, data show that U was associated with cytoplasmic constituents besides bulk of it sequestered into cell wall.

Interaction of Uranium with proteins

In order to determine the binding of U with proteins, varying concentrations of Bovine serum albumin (BSA) or gelatin (a protein devoid of sulphur amino acids) were allowed to interact with uranium (10 mg uranyl nitrate) in 100 mM Na-citrate buffer, pH 5.5 in 1 ml. The tubes containing samples were incubated at 4°C for 1 hour. Following

Table 6. Interaction of Uranium with proteins

Protein	Conc. proteins mg/ml	µg U bound	µg U bound/mg protein
BSA	50	8900	178
	25	4785	191
	12.5	1650	132
	6.25	178	28
	3.125	61	19.5
Gelatin	25	7400	296
	10	2860	286
	5	1320	264
	2.5	577.	230
	1.25	294	235
	0.625	108	173

At pH 5.5 uranium is mostly present as UO_2OH^+ and UO_2^{+2} cations [3]. Positively charged U ions might have interacted with negatively charged groups of proteins irrespective of any amino acid sequence specificity. For metallothioneins and phytochelatins metals are sequestered through metal-thiolate bonds furnished by conserved amino acid sequences involving cysteins [10]. Data show that about 56 mg of BSA can bind 10 mg U while 34 mg of gelatin would bind 10 mg U from an examination of the graph (Y-axis % U remaining soluble vs. X-axis concentration of proteins). The experiment shows that the proteins may be one of the targets of U binding in plants. However, if U-ions non-specifically bind to any proteins it encounter in plants, it would be toxic to plants as it will inactivate functional proteins. Our data show that U was detected in the cytoplasm. We believe that there must be some selective target for U sequestration. The search for U-binding proteins is therefore relevant.

incubation, pH of the reaction mixture was adjusted to 4.7 to precipitate protein and to separate unbound uranium. The precipitate was washed with acetone several times, dried and digested for U determination. Data are presented in Table 6.

Results of the experiments show that species which have never been evolved from metal contaminated sites, they have constitutive traits of U uptake and tolerance. Although U has been detected in plants, there has been no attempt to understand as to why it should be taken up by plant tissues where apparently it has no function unlike other heavy metals like Zn, Cu, Mn and Mo which is a part of functional proteins. U has been detected in crop plants like rice, wheat, maize, lentil, chickpea, green gram, spinach, carrot, raddish, brinjal, tomato, banana and others grown in soil with natural U concentration of 2.3 to 3.27 ppm. U content in these crops varies from 0.22 to 1.77 ppm [20, 21]. Our results show that most of the plant bound U was detected in roots. Precipitation on the root surface and compartmentalisation in root cells can prevent U translocation to other above-ground parts. Surface absorption of U is a combination of such physical and chemical processes as chelation, ion-exchange and chemical precipitation. As stated earlier at pH 5.5, U speciation is predominantly positively charged cations which may interact with COOH-group of polygalactouronic acid and other negatively charged binding sites within plant cells and proteins or nucleic acids. However, experiments with dead, dried plant tissues did not absorb heavy metal as compared to living plants [3] indicating uptake and sequestration of heavy metal is metabolically driven process.

Conclusion

Identification of factors governing efficient phytoremediation can provide a basis of genetic modification. The knowledge of plant physiology of absorption, sequestration, destruction and tolerance of heavy metals can be utilised to identify targets for genetic improvement of phytoremediation either by conventional breeding or molecular biology. Induced mutation for the genes involved in metal biology also may open up new variations. We are planning to use radiation induced mutation in *Brassica*, sunflower and *Chenopodium* to identify genes governing U extraction. In *Chenopodium* we have observed that it can tolerate and sequester Cd and Pb besides U. This shows that plants may have

constitutive traits of cotolerance towards many heavy metals.

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Bioremediation of Heavy Metal and Radionuclide Waste

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Biotechnology has gained considerable importance in a variety of industrial processes as well as for environmental management. Microbes have been used since ancient times in the treatment of organic waste. Historically India can take pride in contributing to the development of ancient biotechnology for environmental applications in the form of septic tanks in Mohenjodaro and Harappa regions in 3000 BC. Microbes often convert the toxic organic waste into nontoxic intermediates or catabolise it completely or can convert a waste into an industrially useful product like biogas. The rapid industrialisation has also resulted in increased disposal of heavy metal waste. This also has been true of disposal of radionuclide waste with increasing emphasis on nuclear power plants. Unlike organic toxicants which can be degraded, inorganic heavy metal species are immutable at an elemental level and persist indefinitely in the environment causing serious threat to environment, humans and animals. Removal of heavy metals from metal-bearing wastewater is usually achieved by physico-chemical processes such as precipitation, coagulation, reduction process, ion exchange, electrochemical processes and /or membrane processes. However, application of such processes is sometimes restricted because of technical or economic constraints especially when the metals are dissolved in large volumes of solutions at relatively low concentration range of around 1-100 mg/L. The search for new technologies has directed attention more recently to bioremediation using materials of microbial and plant origin. Bioremediation is being exploited as an economically alternative technology, both to reduce environmental damage from toxic metals and radionuclides and to recover those with a commercial value like the strategic metals, precious metals and nuclear fuel and radioactive elements.

Microbial Remediation

Microbial biomass has a high affinity for the actinide elements, heavy metals and also other radionuclides and form a significant component of their biogeochemical cycling thus resulting in transfer along the food-chains, ultimately to humans [1,2]. These properties however, have given rise to a considerable interest in the use of microbial biomass and derived products to remove metals and radionuclides from industrial waste. Microorganisms either live or dead can accumulate metals by several active or passive mechanisms. Many microbes specially those found in niches containing high concentrations of heavy metal have developed resistance to heavy metals through various biochemical mechanisms. Some such live organisms actually take up the metal internally

through a metabolically dependent bioaccumulation process and then store them in intracellular vacuoles. Viable cells are also known to remove heavy metals and radionuclides through enzyme dependent microprecipitation. An important example is the removal of uranium through microprecipitation on the cell walls by Citrobacter. Viable cells often contain enzymes which can also modify the metal ions like the conversion of toxic mercury ion to volatile elemental mercury. Bioaccumulation using viable cells however requires the supply of nutrients and growth factors and stringent control of environmental factors such as oxygen tension, pH and temperature in order to maintain their viability and active metabolism. Furthermore, the problem of waste stream toxicity, radiation damage on the viability of the cell etc. makes it less attractive for large scale applications. In view of this, biosorptive processes using dead biomass have received greater attention and aroused greater interest than bioaccumulation using the viable cells [3]. The dead biomass obtained by heat, chemical, or other treatments can biosorb the heavy metals or radionuclides through passive physicochemical interactions with cell wall components or cell debris and do not need a metabolic energy source. In general, the biomass shows similar biosorptive characteristics towards either heavy metals or radionuclides and have hence been discussed together in this review.

Biosorption of metal is based on several mechanisms that quantitatively and qualitatively differ according to species used, the origin of biomass, and its processing. Metal sequestration follows complex mechanisms, mainly ion exchange, chelation, adsorption by physical forces and ion entrapment in inter and intra fibrillar capillaries and spaces of the structural polysaccharide net work as a result of concentration gradient and diffusion through cell wall and membranes. The metal adsorption characteristics shown by microorganisms are partly as a result of cell surface characteristics. Microbial cell wall contains various bioplomers such as the proteins, complex carbohydrates and lipids which possess a variety of functional groups like carboxyl, amino, amide, phosphate, hydroxyl etc. which may be involved in the biosorption of heavy metals. The microbial cell walls in general have an overall negative charge which allow for cation exchange while other chemical groups allow coordination of metal cations. Biomass (unlike ion exchangers) have a variety of polarisable ligands like the nitrogen of histidine and sulphur of cysteine which have a greater binding affinity for the heavy metals than for alkali or alkaline-earth metals thus allowing them to selectively take up heavy metals from a waste stream. Removal of uranium was higher with biomass than for activated-carbon and ion-exchange resins. However it should be stressed that the presence of some functional groups does not guarantee their accessibility for sorption, perhaps due to steric, conformational, or other barriers. Studies from our laboratory as well as others have shown that such barriers can be minimised by post harvest physical or chemical treatment of the biomass. The proportions and compositions of wall macromolecular components may change with growth conditions and growth rate. This has been specifically demonstrated in the case of uranium where the sorption capacity was found to vary with growth phase and growth conditions of batch grown cells. No correlation has been found between metal ionic charge or effective nuclear charge of ions and adsorption, although larger ions appear to adsorb more strongly than ions with smaller radii [4].

There is considerable variation in heavy metal and radionuclide biosorption rate and capacity among species and even strains. Moreover, biomass uptake capacity varies with radionuclide type and environmental conditions. An understanding of the basic mechanism underlying metal/ radionuclide biosorption should aid in manipulating the process eg. through controlling the process parameters to achieve maximum radionuclide/heavy metal removal from an effluent. That knowledge could also serve as a guide in the search for potentially high sorbing materials in the natural domain. The scope of the task of prospecting for new and potentially feasible metal biosorbents from the natural biodiversity is rather large. Few species of bacteria, yeast, fungi and algae that have shown good metal uptake properties have been made public (Table 1). It must, however, be borne in mind that unlike these, new biotechnology and specially microbial biomass discoveries in the future will be considered as proprietary secrets. In view of this, efforts are being made in our laboratory to screen for novel microbes. A number of microbial strains have been isolated in

Table 1. Few typical examples of biomass used in the remediation of heavy metals.

Metal	Bisosorbent	Class	Metal mg/g, d.w.
Ag	*Streptomyces noursei*	Bacteria	38.6
Au	*Aspergillus niger*	Fungus	200
Cd.	*Saccharomyces cerevisiae*	Yeast	1
	Metal tolerant yeast isolate* (Bioaccumulation)	Yeast	7.5
Co	*Aspergillus niger*	Fungi	95
Cr(III)	*Streptomyces noursei*	Bacteria	10.6
Cr(VI)	*Rhizopus arrhizus*	Fungus	4.5
	Chlorella vuglaris	Algae	3.5
	Whey fermenting yeast*	Yeast	40
	Saccharomyces strain*	Yeast	105
	Mucilaginous seeds*	Plant	200
	Pelletised plant protein*	Plant	45
	Plant seed meal*	Plant	310
Cu	*Arthrobacter sp.*	Bacteria	148
	*Rhizopus arrhizus**	Fungi	~40
Hg	*Rhizopus arrhizus*	Fungi	54
Mn	*Bacillus subtilis*	Bacteria	44
Ni	*Candida tropicalis*	Yeast	20
Pb	*Rhizopus arrhizus*	Fungus	91
Pt	Fresh water algae	Algae	53
Zn	*Bacillus subtilis*	Bacteria	137

Note * Based on the studies carried out in our laboratory

our laboratory from heavy metal contaminated soils obtained from various industrial areas in and around Mumbai. Some of these have also been identified and characterised for their metal uptake behaviour [5]. Metal resistance of halophilic archaebacterial strains which survive at very high saline environment and hence may have potential in the treatment of heavy metal waste with high saline content have been identified.

To be successful, a biosorption process for heavy metal or radionuclide removal from waste-streams should comply with the following requirements. The microbial biomass should have a very high biosorption capacity, and biosorption should be rapid, efficient and compare favourably with conventional separation techniques. Ideally the biosorption process should be pH stable and unaffected by other waste stream constituents specially the high saline or other competing ions. Recovery of metal ions from the biosorbent by desorption should be rapid, metal selective and economic. After metal elution, the biosorbent should be reusable with no significant loss in radionuclide carrying capacity. Biosorbent should be metal selective to separate valuable radionuclides or specific valuable metals from a mixed cocktail, particularly if recovery rather than the pollution control is the process aim. However if waste treatment is the only aim biosorbents with very broad specificities would be more ideal. The economics of the process has been shown to depend on the cost of production of the biomass. This is a very important parameter for the success of this technology and has

hence led to the screening of the byproduct biomass of various fermentation industries like the antibiotic, steroid and ethanol [4]. Studies carried out in our laboratory have explored the metal uptake characteristics of easily and economically available biomass byproduct of ethanol fermentation like certain industrial yeast. Recent studies from our laboratory have also shown the possibility of using an yeast strain which can metabolise lactose to ethanol and hence can be grown on whey a zero value byproduct waste with high BOD of dairy industry to serve as a cost effective and efficient biosorbent for heavy metals. Growth of certain fungal strains on cheap carbon sources is also being explored.

The basic work on biosorption has been limited in the case of radionuclides largely to studies on single metal, particularly uranium for which biosorption seems to be a particularly potent process. Data are needed on the performance of biosorbent systems with real process solutions which often contain a complex mixture of metallic species. Information on metals other than uranium is fragmentary. A few preliminary studies carried out with free biomass however are encouraging. Cell hull of the higher fungi have been shown to have high sorption qualities for uranium, plutonium, americium, cesium and other toxic metals. Tests with real radioactive waste of Chernobyl NPP shows that such sorbents are capable of reducing 1000 times, the activity of liquid radioactive wastes. Recent studies carried out at BARC have shown the potentials of the extensively studied (for heavy metal and uranium uptake) fungi, *Rhizopus arrhizus* to biosorb various radionuclides *viz* ^{233}U, ^{239}Pu, ^{241}Am, ^{144}Ce, ^{147}Pm, $^{152+154}Eu$ and ^{95}Zr [6]. Recently a strain of *Aspergillus* spp. has been identified in our laboratory with a very good uptake capacity (423 mg U/g dry wt) and high affinity for uranium [7]. A few other examples of microbial species used specially in the removal of uranium and thorium are summarised in Table 2.

However, most of the above studies have been carried out using free biomass often in a powdered form and are of academic interest. Microbial biomass consists of very small particles with low density, poor mechanical strength and little rigidity and are amenable for putrefaction. For a practical process, however freely suspended native microbial biomass in conventional unit operations is not feasible, largely because of solid/liquid separation problems. The biomass to be used should be stable to extreme environments like pH, temperature and radiation damage and should be in a compact, accessible and recoverable form, probably as pellets or granules for efficient use in a bioreactor or biocontractor system. Immobilization of biomass in solid structures with the right size, mechanical strength, rigidity and porosity necessary for use in columns in unit operations typical of chemical engineering is an important consideration both from the process and commercial viewpoint. Immobilization can also yield beads or granules that can be stripped of metals, reactivated and reused in a manner similar to ion exchange resins or activated carbon. The properties of the beads, the biosorbent, the radionuclide ion and the desorbing ion must all be considered when planning a commercial-scale process. The benefit from recycling where the adsorbent biomass can be used many times are production of a concentrate eluate. Small volumes of concentrated nuclide solution facilitates disposal via vitrification or being put into concrete. Unlike the synthetic resins one of the major advantages of using biomass is that it is biodegradable or incinerable. Thus, the radionuclide laden biomass could be biodegraded or incinerated for reducing its effective volume for compact and safe storage. This point also has to be borne in mind while pelletising the biomass so as not to lose this important property.

A number of new techniques for the immobilization/pelletisation of biomass have been developed in our laboratory. Some of these include immobilization in synthetic acrylic polymer beads or membranes using radiation polymerisation, cross-linking in proteinic supports, entrapment in natural polysaccharide based gels as well as inorganic materials or through *in situ* cross-linking. Techniques have also been developed for obtaining biomass mats by the immobilization of fungal mycelia in highly porous synthetic as well as natural supports. Yeast and bacterial biofilms have been obtained through surface adhesion as monolayers on synthetic, inorganic and cellulosic surfaces. One of the major problem during continuous use of entrapped biomass is its putrefaction. This problem has been obviated using certain post immobilization treatments. In order to minimise the mass transfer

Table 2. Some examples of bioremediation of radionuclides

Metal	Biomass	Biomass class	Method	Uptake (mg/g) d.w.
U	*Rhizopus arrhizus*	Fungi	Adsorption to cell wall	180-220
U	*Rhizopus arrhizus (modified)**	Fungi	Adsorption to cell wall	278
U	*Acinetobacter RAG-1*	Bacteria	Binding to extracellular emulsan	800
U	*Aspergillus spp.*	Fungi	Adsorption to cell wall	423
U	*Psuedomonas aeroginosa*	Bacteria	Intracellular	150
U	*Sacchromyces cerevisiae*	Yeast	Adsorption to cell wall	150
U	*Chlorella regularis*	Algae	Adsorption to immobilized cells	159
U	*Citrobacter sp*	Bacteria	Enzymatically mediated binding to cell wall	9000
U	*Roots*	Plant	Adsorption	371
U	*Strptomyces griseofulvus*	Actinomycetes	Adsorption to cell wall	144
U	*Penicillium lilacinium*	Fungus	Adsorption to cell wall	80-90
Th	*Rhizopus arrhizus*	Fungus	Adsorption to cell wall	90-180
Th	*Sacchromyces cerevisiae*	Yeast	Adsorption to cell wall	70
Th	*Streptomyces niveus*	Bacteria	Adsorption to cell wall	34
Th	*Aspergillus niger*	Fungus	Adsorption to cell wall	138
Ra	*Penicillium chrysogenum*	Fungus	Adsorption to cell wall	5×10^4 (nCi/g)

Note * Based on the studies carried out in our laboratory

and diffusion problems as well as to increase the surface of contact of the biomass to the external liquid phase especially in gel entrapped systems, techniques have been modified to obtain such supports in open pore structures. Major emphasis has been laid on developing immobilization supports which are natural and either biodegradable or incinerable. Techniques have also been developed to obtain magnetised biomass. Magnetised biomass is gaining importance for the decontamination of soil and bottom sediments of water bodies. Such an approach has been suggested to solve the problems of Chernoboyl accident site. Most of these aspects on imobilization/pelletisation techniques developed in our laboratory have been reviewed extensively [8-10]. These techniques which have found importance in various other biotransformation studies are currently being applied for the bioremediation of heavy metals and radionuclides. The process equipment/bioreactors used for immobilized biomass would be based on solid-liquid contact and will be similar to those used in the ion exchange process or activated carbon applications. Some modifications may be required in view of the comparatively fragile nature of the biomass. More recently advantage of using air lift bioreactors has been demonstrated. Frame and annular bioreactors have been developed in our laboratory using immobilized biomass.

Phytoremediation

Plants have the unique ability to concentrate essential and non-essential elements from the environment in their tissues. Plants are one pathway for the toxic metal mobilization to the human food chain, and paradoxically they may also provide an elegant means of reducing this spread. Phytoremediation is defined as the use of green plants to remove, contain, or render harmless environmental contaminants including heavy metals and radionuclides both from liquid and solid waste [11]. Contaminated soil represents an economic liability as well as a technical challenge. Cleaning of heavy-metal-scarred industrial wastelands the old fashioned way involves physically stripping and removing the contaminated soil and starting again. This often causes more disturbances than it alleviates, and is very expensive - especially if the area concerned is large. Hence the interest in 'bioremediation' - cleaning up, the natural way. Compared to microbes, plants have certain advantages in this respect. Plants have extensive root systems that find every crevice in the soil. The total length of roots (including root hairs) of a single pot-grown rye plant is about 620 Km with a total surface area exceeding 3000 m^2. The root system of the plant can be even bigger when field grown. Plants have been redefined in engineering parlance for this purpose as a "solar driven, pumping and filtering system that has measurable loading, biodegradative and fouling capacity". Roots are "exploratory, liquid phase extractors that can find, alter and/or translocate elements and compounds against large chemical gradients". Green plants use sunlight, the most abundant source of energy to power this concentration process. Engineering modeling studies have provided research goals in terms of rooting structure, patterns water use, transpiration and metabolism. In addition to absorbing toxic chemicals, root systems stabilise the soil against erosion, preventing the kind of washout that transports soil-borne pollutants into rivers and drinking water. Capacity of plants to transpire large quantities of water helps in the prevention of downward water flux through landfill caps and containment of contaminated water down gradient of a problem site. Plants absorb both organic and inorganic contaminants from soil. Absorption, sequestration and metabolic transformations of these pollutants are possible and potentially exploitable to clean contaminated soil [11].

Several obstacles need to be over come. First, the plants have to be resistant to the poisons that are there to absorb. Evolution has thrown up plants that grow on naturally heavy-metal-rich soils, found in Southern Africa and the Western U.S. The sap of one tropical tree growing on a Nickel outcropping has been reported to have concentration of Ni in excess of 25% dry weight. However, due to their generally low growth habits and small biomass they are agronomically unsuited for phytoremediation. Nevertheless these plants are a valuable store of genetic and physiologic material and data. Transferring and extending these metal-accumulating, translocating and tolerance capacities to a plant with better agronomical characteristics may provide the ideal solution to the clean up of metal contaminated soils. Modern molecular biology may help but much exploratory biology in general is needed in this area. The plant should be able to translocate the heavy metals absorbed by the roots quantitatively only to the shoots so as to minimise the quantity of contaminated plant biomass which needs to be treated on harvest. Under these conditions the metal laden shoots can be harvested intermittantly without destroying the plant, dehydrated and incinerated to obtain low volume heavy metal/radionuclide waste contaminated biomass. Some typical examples of hyperaccumulator species used in bioremediation are shown in Table 3.

Little is known about the mechanism that allow plants to accumulate metals intracellularly or to export them to shoots. The vacuole play an important role in the storage of metal ions wherein they are thought to be chelated by organic acids like citrate or malate or by the enzymatically synthesized phytochelatins - small thiolate peptides of γ-glutamylcysteinyl-glycine with varying number of cysteinyl residues. These may be also involved in the long distance metal transport in plants. In the future, number of opportunities for enhancement of phytoremediation by plant genetic modifications either by traditional breeding, plant tissue culture or through molecular biology (transgenic plants) do exist. Some of these include altering the traits on what they absorb, sequester, destroy and tolerate as

Table 3. Some heavy metal hyper accumlator plants studied in soil phytoremediation

Plant species	Metal	Metal conc.* $g\ kg^{-1}$
Thalapi caerulenscens	Cd	1.8
Thlaspi rotundifolium	Pb	8.2
Thlaspi caerulenscens	Zn	51.6
Ipomoea alpina	Cu	12.3
Haumaniastrum robertii	Co	10.2
Macadamia neurophylla	Mn	51.8
Psychotria douarrei	Ni	47.5
Sebertia acuminata	Ni	250

Concentration on dry weight basis in harvestable materials specially shoots or sap from plants grown in containated soil.

well as alterations in root characteristics like root depth, penetration into anaerobic zones and root density. Scientists at University of Georgia, have developed transgenic plants (*Liriodendron tulipifera*) -the plants that are modified through genetic engineering techniques to include a gene, normally found in bacteria, that converts toxic mercury ions into elemental mercury. These plants could not only take up the metal but were able to convert it into elemental mercury, which they exhale into the air. There are also numerous attempts to engineer the production of the animal metallothionenes in plants.

Plants are also gaining importance in the treatment of liquid waste containing heavy metals/radionuclides. This is an emerging new technology which has been defined as rhizofiltration, makes use of plant roots to absorb, concentrate and precipitate heavy metals from polluted liquid effluents. This technique basically involves the growth of plants with high root surface area hydroponically in synthetic liquid medium and then transferring them to the aqueous solution containing the heavy metals or radionuclides, followed by harvesting of the roots, drying and incinerating. Aquatic higher plants like Water hyacinth (*Eichhornia crassipes*) and a variety of terrestrial plants, e.g. Indian mustard (*Brassica juncea*), sun flower (*Helianthus annuus*) and various grasses can be used to remove toxic metals such as Cu, Cd, Cr, Ni, Pb and Zn from aqueous solution. Terrestrial plants have been more useful in view of their ability to develop much longer, fibrous root systems covered with root hairs, which create an extremely high surface area. Unlike the phytoremediation of contaminated soil where traslocation to shoots as discussed earlier is a positive criteria, plants used for rhizofiltration should not be efficient translocators of metal from roots to shoots and stems so as to reduce contaminated plant residue. The amount of metal accumulated in roots of these plants can exceed 10% of root dry weight. At high metal concentration root-mediated precipitation of the metal from the solution in the form of insoluble metal phosphate has also been observed. Most likely this precipitation involves a release of the root exudates. Additional improvements in rhizofiltration may be achieved through better understanding the role of rhizosphere microorganisms in metal uptake and precipitation by roots. Increasing the production of phytochelatins either by biochemical or genetic means may also provide a way to improve the metal uptake capacity of the plants [11].

Ability of plants to take up radionuclides has been known for sometime. Studies of the consequence of the accident (1957) and the systematic dumping of radioactive materials in the Techa River and lake Karachay showed that plants played an important role in the radionuclides transfer in the contaminated ecosystem [12]. A systematic study carried out in this direction has demonstrated the ability of Indian mustard seedlings for the removal radionuclides such as ^{90}Sr and ^{137}Cs. Recent studies have shown that specially selected cultivars of sunflower plant proved to be more effective in uranium removal from water than Indian mustard. Rhizofiltration technology using sun flower plants has been tested in the field with U-contaminated water at concentrations of 21-874 µg/L at a former uranium processing facility in Ashtabula, Ohio. The pilot -scale rhizofiltration system provided final treatment to site source water and reduced uranium concentration to <20 µg/L before discharge to the environment [12]. The economic competitiveness of rhizofiltration is just being tested. Major success to date has been in the removal of Pb (Table 4). However this technology has time on its side since plants are one of the few renewable resources ever available to man. Studies on removal of heavy metals and U through rhizofiltration have been initiated in our laboratory using terrestrial plants and other grasses.

Table 4. Lead accumulation through rhizofiltration by some plants

Common name	Scientific name	Pb in roots (mg/g) DW
Indian mustard	*Brassica juncea*	136
Sunflower	*Helianthus annuus*	140
Colonial bent grass	*Agrostis tenuis Sibth*	169
Rough blue grass	*Poa trivialis*	100
Centipede grass	*Ermochloa ophiuroides (munro) Hack*	124
Orchard grass	*Dactylis glomerata*	60

Other biomass biosorbents

In addition to living plants a variety of agro based products have also shown potentials in the bioremediation of heavy metals. Some of these include straw, bark and saw dust. The work carried out at BARC has demonstrated the use of banana pith for the removal of radionuclide from nuclear waste [13]. Recent studies carried out in our laboratory have shown the possibility of using certain protein rich pelletised agro based biomass as well as certain seed meals for the removal of heavy metals such as Cr (VI) and Cu (Table1). Microbes especially the ones which are metal tolerant often secrete certain metal chelating polysachharides. There is a great deal of interest in using these microbial polysaccharides for the bioremediation of heavy metals in view of their high affinity for the metal. The process involves the immobilization of such microbial polysaccharides on insoluble supports. Studies from our laboratory have shown that certain mucilaginous seeds which have naturally immobilized pellicular fibrillar structure rich in metal chelating polysachharides can be used effectively in a packed bed column reactor for the removal of various heavy metals like Cr, U and Ce (Table 1). Another potential approach to solving the heavy metal contamination involves the use of metallothioneins. These are the sulphur rich proteins found in higher organism having a high affinity for various metals. The gene coding for mouse metallothioneins has been cloned in microbes and expressed thus opening up the possibilities of producing it in large amounts for bioremediation purpose. In this direction, both metallothioneins and microbial polysaccharides will play a major role in the bioremediation of heavy metals and radionuclides although time scale for realisation of this may be comparatively longer. Other novel biotechnological approaches to aqueous nuclear effluent treatment is through biomagnetic separation. It has been shown that microorganisms can be made to take up metal ions from solution and can acquire significant magnetic moments, which allow them to be captured using the technique of high gradient magnetic separation. Monoclonal antibodies specific to cell types used in a biomagnetic separation process could also be used to aid selective recovery of specific radionuclide.

Industrial aspects

Several biosorption processes are under development or have been developed, patented, and introduced for the application in removing metal contaminants from surface and ground waters [3]. The BIOCLAIM Process using pretreated and immobilized principally the bacteria of the genus Bacillus; AlgaSORB^{TM} Process a proprietary family of products that consist of several types of non-living algae and several immobilization matrixes; BIO-FIX Process biomass, including sphagnum peat moss, algae, yeast, bacteria and/or aquatic flora immobilized in synthetic polymers; and immobilized *Rhizopus arrhizus* biomass: a proprietary process involving the immobilized fungus has been evaluated for the treatment of heavy metal waste as well as in the recovery of uranium from an ore bleach solution. Economic comparisons (predictive costs) of biosorption processes with other

separation technologies have been reported. A typical proprietary 'BIOCLAIM' process (reuse 25 cycles) was found to reduce the cost per gallon of treated water by over 50% when compared with chemical precipitation process. However, the number of times the biomass can be regenerated is a key assumption in this economic analysis . Cost analysis data with various biomass preparations demonstrate that biosorptive processes are competitive with chemical treatment for liquid metal waste. Phytoremediation has been tested on site contaminated with organic waste, heavy metals and radionuclides. One patented process uses carrots to absorb lipophilic dichloro-diphenyl-trichloro-ethane. In the past three years, at least three new companies have formed to use plants to clean sites contaminated with heavy metals or organics. One of these companies - Phytotech, Inc. of Monmouth Junction, New Jersy, USA - expects to begin commercialization of a lead extraction technique very soon.

Conclusion

At present, some conventional techniques are at their limits of sensitivity in removing active radionuclides and heavy metals from waste-streams. Biosorption systems in combination with existing processes or individually offer an exciting possibility in the future for achieving even lower environmental discharge levels. Search for more efficient microbial biomass should continue. Applications of genetic engineering, plant tissue culture techniques in the future may allow modifying both the plants and microbes to achieve highly efficient metal uptake systems. Transgenic plants may play a major role. Research is necessary that addresses the fundamental as well as the applied aspects of metal uptake, particularly better understanding of biosorption mechanism, metal desorption, biosorbent regeneration and formulation of new biosorbent materials suitable for process applications. Applied biotechnology in general is a highly interdisciplinary area of research. A fruitful fusion in the future of various scientific and engineering disciplines especially biology, chemistry and chemical/ biochemical engineering, will allow biotechnology to realise its full potential in environmental protection.

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