Vol. VI. No.4 Editor : B.S. Tomar November 2007

Editorial

Ever since the first application of radioisotopes as tracer by George de Hevesy, in 1911, radioisotopes have been used in almost all spheres of life, such as, agriculture, industry, medicine, research, etc. In the field of agriculture radioisotopes are being used to increase the food production by producing improved varieties of crops, optimizing the use of fertilizers, insect control and food preservation. The increasing population and dwindling stocks of food world wide have led to exorbitantly high price of certain food items so much so that poor people can not afford to buy them.

The present thematic bulletin is aimed at bringing awareness among the scientific community about the research work that is going on and that need to be done to increase food production and improve its quality. I am grateful to Dr. S.F.D'Souza, Associate Director Biomedical Group, for accepting our request to be the guest editor of this bulletin. Thanks are due to all the contributors for their valuable contributions to this bulletin. I hope this bulletin will inspire our young researchers particularly in the universities to initiate the research based on use of radioisotopes in agriculture in their laboratories.

CONTENTS

From the Secretary's Desk

Our association has completed 25 years and auspicious was the occasion to celebrate the Silver Jubilee Function on January 2, 2008 in the company of several of its resource persons, many stalwarts of radiochemistry and retired colleagues from BARC and Universities. Momentous was the time to remember and acknowledge the yeomen services of many resource persons responsible for its growth. All the past and present President and Secretaries of IANCAS, who gave the association a wide acclaim, were felicitated by Dr.Kakodkar, Chairman, AEC and Secretary, Department of Atomic Energy, Government of India. He complimented IANCAS for its noble work through conducting of National Workshops and bringing out thematic periodic bulletins with an objective of dissemination of information on Nuclear Science and Technology.

Serious soil acidification and ecological damage are the outcome of farmer's long-term use of chemical fertilizers on a large scale. With the growing sense of the green revolution, how to get along well with Nature and develop sustainable agriculture has become the common goal of the whole world and India cannot certainly be beyond this tendency.

Conventional agriculture has made tremendous improvements in crop yield but at large costs to the environment. In response to environmental concerns, organic agriculture has become an increasingly popular option. Around the world, agriculture is moving toward natural, safe and organic biological fertilizers. Even though biological fertilizers are in a constant process of development, the pace of this development is not as fast as anticipated.

In spite of large amounts of money spent in India on Research in agriculture, the plight of the farmers, over many decades, remains as bad as ever. The link between the research laboratories and the farming society is so weak that the benefits do not reach them. Poor quality seeds, adulteration in fertilizers and pesticides and lack of proper marketing facilities have disheartened many in sustaining farming as a livelihood. The number of suicidal deaths among farmers reflects the conditions that prevail in the agriculture sector.

IANCAS has successfully organized 3 National Workshops at Universities of Nagpur, Kurukshetra and Banasthali (Jaipur).

IANCAS is gearing up to make a Directory of its members and provide a personal copy to all. This is to request all the members to provide full address along with email ID through our email ID secretary@iancas.org.

G.A. Rama Rao

Applications of Isotopes in Agriculture

Guest Editor

S.F. D'Souza

Nuclear Agriculture and Biotechnology Division
Biomedical Group
Bhabha Atomic Research Centre
Mumbai 400 085

FOCUS

Dr. K.B. Sainis

Director, Bio-Medical Group

The famous English demographer and economist Thomas Robert Malthus predicted that while the population of the world would grow geometrically, the food production would grow arithmetically. Result would be inevitable misery due to scarcity and famine. But human ingenuity reflected in scientific approaches to agriculture and allied industrial development has prolonged what Malthus considered inevitable. Discovery of pesticides, chemical fertilizers, better water management and development of new varieties of food and vegetable crops by and large kept the spectre of worldwide famine and scarcity at bay with reasonable success in most parts of the world barring perennially drought prone areas. In the 19th and 20th centuries these developments revolutionized agriculture and in India, in particular, the green revolution became a success story.

For an ever growing population of India having crossed one billion mark such success stories need to be more frequent. While the green revolution catalyzed the spurt in production of food grains, excessive use of chemical fertilizers brought in its wake growing salinity in soils, shift in cropping patterns away from food grains to cash crops like sugarcane etc. Add to it the increasing cost of agricultural production and its being proclaimed to be less than a remunerative enterprise; the cultivable area in India remains at 140 million ha. Add to that the devastation plant diseases and water scarcity may bring in. Given this state of affairs, the responsibility of providing new plant types – high yielding, disease and drought resistant, development of methods for recovery of fertility, increasing production of fruit crop and medicinal plants through in vitro micro-propagation technologies etc. needs to be shared by institutions other than National Agricultural Research Institutes and State Agriculture Universities.

In this respect the DAE have come out with flying colors having developed 35 new (Trombay) varieties of crop plants in last four decades through radiation induced mutation breeding. What's more, this effort is particularly focused on oil seeds and pulses where we are most vulnerable. For last several years we have been importing pulses and edible oil. With the cooperation of state agriculture universities the release and notification of Trombay varieties has indeed picked up a hitherto unknown momentum, a matter to be very much proud of. Lest we get branded as traditionalist in the area of crop plant research, Nuclear Agriculture & Biotechnology Division, BARC is in parallel developing the transgenic and other biotechnological approaches for both legumes as well as fruit crops. They have successfully developed micro-propagation protocols for several elite varieties of banana and pineapple and transferred them to farmers through Agriculture Universities and Krishi Vigyan Kendras. Likewise, studies being carried out on utilization of fertilizers and pesticide residue analysis as well as control of insect pests reflect an integrated approach to improvement of crop productivity.

Atomic Energy has two faces, no doubt. On relates to our defenses. The other equally importantly reflects the commitment towards generation of electricity and applications in industry, medicine and agriculture. Both the aspects of our atomic energy programme, however, inspire a source of security. In the fast changing world a nation's military and economic power will certainly be assessed in terms of its ability to harness the nuclear energy. This larger picture will become eye-catching with the shades of prosperity in food production and related agricultural enterprises facilitated by application of radiation and radioisotopes. In a distant future, the pattern of farming may change from individual's farm to cooperative or community farm or by the large industrial houses. Yet, I believe, technological developments will be at the heart of this change. Radioisotope technologies will still have their place of pride in the technological race.

I am indeed very happy that this special issue of IANCAS Newsletter focuses on the theme of Isotopes in Agriculture.

Guest Editorial

Dr. S.F. D'Souza

The DAE through its research, development and deployment activities in nuclear science and technology, has been making contributions towards enhancing agricultural production and food preservation. Bhabha Atomic Research Centre (BARC) has a broad based research programme in Food and Agriculture involving genetic improvement of crops through mutation breeding and biotechnological approaches, isotope aided soil studies on fertilizer and micronutrient uptake as well as on understanding the fate and persistence of pesticides, integrated pest management including the use of sterile insect techniques, pheromones and biopesticdes and food irradiation for food safety, shelf life extension and quarantine barriers. Use of radiation and radioisotopes in agriculture is one of the most important fields of peaceful applications of atomic energy for societal benefit.

Nuclear technology has played an important role in increasing crop productivity by developing mutants with desirable agronomic traits. Thirtyfive BARC crop varieties especially in oil seeds and pulses have been released for commercial cultivation and these have largely benefited the farmers across the nation. Various government agencies including Indian Council of Agricultural Research and State Agriculture Universities have played an important role in our agricultural developmental and deployment activities. An overview of the developments with respect to impact of radiation technology in the development of genetically improved crop plants at BARC has been presented in the first article. Basic aspects on the use of radiations in mutation breeding have been delineated in the second article. Articles 3-6 describe achievements of BARC in pulses, groundnut, soybean and mustard. Mutation breeding has also been successful in the vegetatively propagated species and these aspects have been described in the seventh article.

The experiments using fertilizers labeled with isotopes facilitate the estimation of the optimum fertilizer requirement of plants, their biological transformations, translocation, the site of utilization in the plant, time of application, and also in quantifying their losses from soil. Radioisotopes are useful in generating information on mineral plant nutrition and allied investigations. The fate of the pesticides and other agro- chemicals used in agriculture, their degradation products and their persistence in the ecosystem can be studied using radioisotopes. Radiations are useful in the control of insect pests as in the case of Sterile Insect Technique (SIT). These aspects have been discussed in the last three articles.

The uses of atomic energy in agriculture thus, are many and varied. The present issue addresses some of the topics on the potential use of radioisotopes in agriculture. I hope that the readers will find the contents interesting and informative.

I am grateful to all the authors who have contributed articles covering various aspects of nuclear agriculture. Editing this special IANCAS bulletin was an enjoyable experience and I thank IANCAS for entrusting me with this opportunity.

Radiation Technology for the Genetic Improvement of Crop Plants at BARC: An Overview

***Dr. S. F. D'Souza** joined BARC after graduating from the 15th batch of training school. He is currently the Associate Director-A, Biomedical Group and Head, Nuclear Agriculture and Biotechnology Division, BARC Mumbai. He is also Senior Professor of Homi Bhabha National Institute. Ph.D in Biochemistry and his major research interest are in the field of Enzyme and Microbial Biotechnology with special reference to immobilized biomaterials for use in bioprocessing, biosensors and bioremediation. He has authoured about 150 scientific papers in International journals/ books and has guided a number of Ph.D students. He is the recipient of the AMI- LOUIS PASTEUR AWARD for his significant contributions to the field of Microbiology and has been honoured as a Fellow of the National Academy of Science, Fellow of the Association of Food Scientists & Technologists, and Fellow of the Maharashtra Academy of Science.*

Indian agriculture in the past has witnessed events such as green revolution which changed the nation's status from a food importing nation to a self sufficient nation. In spite of industrialization, India remains an agrarian economy. The national agricultural policy now focuses on sustained production and nutritional security for the one billion plus population. Food grain production in India stands at around 212 million tons and by 2025 we may need about 340 million tons to feed the increasing population. To further increase agricultural productivity in an environmentally sustainable manner in the face of diminishing land and water resources is a highly challenging task. The approach of increasing the productivity by genetically improving the crop plants appears to be economically favourable and ecofriendly in the present scenario. Growing such genetically improved varieties along with the appropriate cultural practices can significantly boost the crop productivity.

Genetic improvement of crop plants is a continuous endeavor. Success of a crop improvement programme depends on the availability of large genetic variability, which a plant breeder can combine to generate new varieties. In nature, occurrence of natural variability in the form of spontaneous mutations is extremely low (about 10^{-6}), which can be enhanced to several fold ($\sim 10^{-3}$) by using ionizing radiations or chemical mutagens. Radiation induced genetic variability in crop plants is a valuable resource from which plant breeder can select and combine different desired characteristics to produce better crop varieties. The desirable traits which have been bred through induced mutations include higher yield, grain quality, early maturity, disease and pest resistance, improved plant type and abiotic stress resistance. Major emphasis at BARC has been on research and development in pulses and oilseed crops, however, work has also been carried out on other crops such as wheat, rice, banana and sugarcane.

In India, oilseeds and pulse crops are important food components as they are major contributors for dietary oils and proteins respectively. Productivity in the oilseeds and pulses in India remained stagnant for the past few decades. In order to generate genetic variability, mutation research in crop plants was initiated at BARC half a century back. Mutation research was concentrated mainly on the major oilseeds of the country namely, groundnut (*Arachis hypogaea*), mustard (*Brassica juncea*), soybean (*Glycine max*), sunflower (*Helianthus annuus*), sesame (*Sesamum indicum*) and among pulses, pigeonpea (*Cajanus cajan*), mungbean (*Vigna radiata*), blackgram (*Vigna mungo*) and cowpea (*Vigna unguiculata*), besides, jute (*Corchorus capsularis*), rice (*Oryza sativa*), *Sesbania rostrata*

Dr. S. F. D'Souza, Nuclear Agriculture and Biotechnology Division, Biomedical Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: sfdsouza@barc.gov.in

and wheat (*Triticum aestivum*) using X-rays, gamma rays, fast and thermal neutrons.

The era of Trombay direct mutants having superior agronomic features began with the development of TG-1 and TG-3 in groundnut, TM-2 in mustard, TT-6 in pigeonpea and TAP-7 in mungbean in the eighties and evaluation of such mutants led to their commercial release in different states. Subsequently, irradiation along with cross breeding resulted in developing wide spectrum of genetically diverse and agronomically superior crop varieties in addition to mutants of academic interest. Several of these induced mutants are also now genetic materials for functional genomics.

Induction of modified traits and their incorporation in an ideal genotype could be achieved by a well planned and judicious use of induced mutation and hybridization techniques. Mutants or recombinants initially developed at BARC are evaluated in collaboration with the Indian Council of Agricultural Research or State Agricultural Universities in multilocation trials for various agroclimatic zones. The promising ones after multilocation testing in a given agroclimatic zone/location are released for commercial cultivation. With the effective blend of mutation and recombination breeding, 35 crop varieties developed at BARC have been released and Gazette notified by the Ministry of Agriculture, Government of India for commercial cultivation. These include 18 in oil seeds (12-groundnut, 3-mustard, 2-soybean, 1-sunflower), 15 in pulses (7-greengram (mung), 4-blackgram (urid), 3-pigeonpea (tur), 1-cowpea (chowli) and one each in rice and jute (Table 1). Based on certain novel traits, eleven mutant germplasm (six groundnut mutants, three sesame mutants and one each of Sesbania and sunflower mutants) have been registered with National Bureau of Plant Genetic Resources, New Delhi (Table 2). Radiation induced mutagenesis of *in vitro* cultures of banana and sugarcane has been undertaken to isolate clones with desirable characters such as yield, dwarf type, early maturity and stress tolerance.

Some of the Trombay varieties have been very popular among the farming community. These are grown extensively in the country and have made a good impact on our national agriculture scenario by benefiting the farmers considerably. The Trombay pulse varieties are popular in Southern and Central India based on their high yielding ability and disease resistant characters. The blackgram variety TAU-1 is the most popular variety in Maharashtra and has covered most of the area under blackgram cultivation in the state. This variety is large seeded and yields about 27% more over the existing varieties. In mungbean, major bottlenecks were the susceptibility of existing varieties for yellow mosaic virus and powdery mildew diseases. Successful incorporation for powdery mildew resistance in high yielding mutants resulted in powdery mildew disease resistant varieties TARM-1, TARM-2 and TARM-18 for the first time in India. For the variety TMB-37, yellow mosaic disease resistance was recombined with early maturity (55-60 days) and that made available an additional area for mungbean in summer cultivation. Subsequently, pyramiding for multiple disease resistance led to variety TJM-3 having resistance to powdery mildew, yellow mosaic virus and Rhizoctonia root-rot diseases. The recently released mungbean variety TM-96-2 having powdery mildew resistance has made additional area under rice fallow system available for mungbean cultivation. Mungbean varieties, TARM-1 in Orissa, TM-96-2 in Andhra Pradesh and TMB-37 in Madhya Pradesh and Uttar Pradesh, are gaining importance in rice fallow and kharif/summer seasons. A pigeonpea variety (TT-401) a high yielding, early maturing (150 days), tolerant to pod borer and pod fly damage has been recently released for commercial cultivation in Maharashtra, Madhya Pradesh, Chhattisgarh and Gujarat states. The cowpea variety TRC-77-4 (Khalleshwari) having determinate plant type and suitable for rice based cropping system is released for Chhattishgarh state.

Trombay groundnut varieties are grown throughout the country and are very popular in states like Maharashtra, West Bengal, Rajasthan, Karnataka, Andhra Pradesh, Gujarat, Orissa, Punjab, Madhya Pradesh, Uttar Pradesh and Goa. Among the groundnut varieties, TAG-24 is the most popular TG variety and is used as a national check variety in rabi/summer trials in the All India Coordinated Research Project on groundnut and is grown throughout the country. It commands a major share of the national breeder seed indent. Another variety TG-26 has an additional useful trait of fresh seed dormancy of 20 days thus preventing *in situ*

seed germination due to end season rains when the crop is ready for harvest. TG-26 is popular in Maharashtra, Gujarat and Karnataka. Improved morpho-physiological traits in these Trombay groundnut varieties enabled farmers to achieve record yields of 7000 kg/ha compared to 2000 kg/ha of national average under irrigated summer situation. Many of the farmers in major groundnut growing states have been consistently harvesting an average 5000 kg/ha and above in 100 – 110 days duration. Compared to the existing large seed varieties of 140 days maturity; the new large seed varieties, TPG-41 and TLG-45 have proved to be higher yielding (4000 – 5000 kg/ha) in farmers' fields in 120 days maturity. Another large seed variety, TKG-19A is suitable for North Eastern states in view of its tolerance to aluminum toxicity, which is often associated with acidic soils. TG -37A is the recent high yielding, early maturing (110 days) variety with wider adaptability is rapidly gaining popularity in Western and Eastern India.

Among other oilseeds, soybean varieties TAMS-38 and TAMS-98-21 released for Maharashtra state are spreading in Vidharba region . The mustard TPM-1 a yellow seed coat mutant with high yield and oil content and sunflower TAS-82 a black seed coat color mutant with high seed and oil yields were notified recently for commercial cultivation in Maharashtra.

Many of the breeding programmes in national/state breeding system have been utilizing these BARC mutant varieties as parental materials/donors and developed improved varieties like R-9251, JCG-88 and TPT-25 in groundnut. Similarly, BARC varieties were utilized as donor parents for powdery mildew and yellow mosaic virus disease resistance in mungbean and for large seed trait, earliness, high harvest index, high water use efficiency in groundnut. These crop varieties also facilitated farmers to develop i) newer cropping systems like intercropping groundnut with sweet corn, Bt cotton, sugarcane, ii) usage of polythene mulch technology in groundnut, iii) intensive groundnut farming and iv) rice fallow system in mungbean.

Mutation breeding programme is also complemented with biotechnological approaches. Some programmes at BARC in plant biotechnology are in the micropropagation of banana, pineapple, sugarcane and other economically useful plants. The technology for banana has been transferred to the Maharashtra State Seeds Corporation Ltd., Akola and Kamaraj Krishi Vigyan Kendra, Pondicherry. Cell culture/hairy root based production of bioactive compounds using bioreactor has also been developed. Studies have also been initiated for developing transgenic plants for disease resistance, for the production of edible vaccines and phytoremediation. Another major activity, that is relevant to crop improvement programmes, has been the development of molecular markers for finger printing of genotypes, varietal identification, marker assisted selection, linkage studies, phylogenetic analysis and tagging and cloning of desirable genes.

For dissemination of research efforts of BARC to the farmers, effective linkages have been established with Indian Council of Agricultural Research (ICAR), State Agricultural Departments, State Agriculture Universities, National and State Seed Corporations, NGOs, National Institutes, Krishi Vigyan Kendras, progressive farmers etc. Large scale production of nucleus/breeder seeds is undertaken at BARC farms at Trombay and Gauribidanur, Karnataka and also in collaboration with progressive farmers and Agricultural Universities. Breeder seeds are supplied to different National and State Seed Corporations for multiplication into foundation and certified seeds to reach farmers. 'Paramanu Urja Agriculture Society' has been established at Tarapur Atomic Power Station, Tarapur. This Society has an agriculture farm for demonstration to the neighbourhood agriculture products developed at BARC and also to produce quality planting material of BARC varieties.

Our experience has shown that using radiations for crop improvement has come to stay as an efficient plant breeding method complementing the conventional methods. Clearly, the nuclear technologies have benefited the farmers, traders and end-users and will continue to do so in the future.

TABLE 1. BARC, Trombay Crop varieties Released and Notified for Commercial Cultivation by Ministry of Agriculture, Govt of India

Crop	Variety	Year of Release	M: Maturity (days) Y: Yield (kg/ha) YI: Yield increase (%)	Released for	Remarks
Groundnut (*Arachis hypogaea*)	TLG-45	2007	M: 114 Y: 1506 YI: 28	Maharashtra	Large seed, Kharif season
	TG-38	2006	M: 115 Y: 2500 YI: 20	W.Bengal, Orissa, Assam & N.E. States	High yield potential in residual moisture situation Rabi/Summer
	TG-37A	2004	M: 110 Y: Kharif 1993 YI: 26-38	Rajasthan, UP, Punjab, Haryana, Gujarat, W.Bengal, Orissa, Assam & N.E. States	Fresh seed dormancy Oil 51%
	TPG-41	2004	M: 120 Y: Summer 2407 YI: 26	All India	Large seed (70g/100 seeds) Fresh seed dormancy
	TG-26	1995	M: 110-120 Y: summer 2500 YI: 23-39	Gujarat, Maharashtra, MP	Semi-dwarf, early maturity, high harvest index, high partitioning efficiency, fresh seed dormancy
	TKG-19 A	1994	M: 120-125 Y: 2000-2500 YI: 12-13	Maharashtra	Large seed, fresh seed dormancy
	TG-22	1992	M: Kharif 115-120 Y: Kharif 1677 YI: 30	Bihar	Medium-large seed, fresh seed dormancy
	TAG-24	1991	M: Kharif 100-105 Summer 112-117 Y: kharif 1300 Summer 2500 YI: Kharif 24 Summer 50	Maharashtra West Bengal Rajasthan Karnataka	Semi dwarf habit, early maturity, high harvest index, high partitioning efficiency, wider adaptability
	Somnath (TGS-1)	1989	M: 110-125 Y: Kharif 2000 YI: 23	Gujarat	Large seed, Spreading habit

TABLE 1 (Contd.)

Crop	Variety	Year of Release	M: Maturity (days) Y: Yield (kg/ha) YI: Yield increase (%)	Released for	Remarks
	TG-3	1987	M: 110 Y: 2000-2500	Kerala	More branches
	TG-17	1985	M: 115-120 Y: 1700-2000 YI: 15-20	Maharashtra	Less branches
	TG-1	1973	M: 130-135 Y: 2400-2500 YI: 15-20	Maharashtra, Gujarat	Large seed
Soybean (*Glycine max*)	TAMS 98-21	2007	M: 103 Y: 2318 YI: 20	Maharashtra	High yielding, Resistant to bacterial pustules, myrothecium leaf spot and soybean mosaic virus diseases
	TAMS-38	2005	M: 90-95 Y: 1800-2000 YI: 20	Maharashtra	Early maturing, resistant to bacterial pustule, Myrothecium leaf spot
Mustard (*Brassica juncea*)	TPM-1	2007	M: 95 Y: 1396 YI: 31	Maharashtra	Yellow seed, Tolerant to powdery mildew
	TM-2	1987	M: 90 Y: 1370 YI: 25	Assam	Appressed pod
	TM-4	1987	M: 95 Y: 1470 YI: 35	Assam	Yellow seed
Sunflower Surajmukhi (Helianthus annuus)	TAS-82	2007	M: 93 Y: 1348 YI: 13	Maharashtra	Black seed coat Tolerant to drought
Greengram Mung (Vigna radiata)	TM-96-2 (Trombay Pesara)	2007	M: 69-73 Y : 1250 YI: 10	Andhra Pradesh (rabi and summer) and rice fallows	Resistant to Powdery mildew and Corynespora leaf spot

TABLE 1 (Contd.)

Crop	Variety	Year of Release	M: Maturity (days) Y: Yield (kg/ha) YI: Yield increase (%)	Released for	Remarks
	TJM-3	2007	M: 66 Y : 950 YI: 20	Madhya Pradesh (kharif and summer)	Resistant to Powdery mildew, Yellow mosaic virus and Rhizoctonia root –rot diseases.
	TMB-37	2005	M: 64 Y: 1100 YI: 20	Eastern UP, Bihar, Jharkhand, Assam, West Bengal	Tolerant to yellow mosaic virus
	TARM-18	1995	M: 65-70 Y: 1051	Maharashtra	Resistant to powdery mildew
	TARM-1	1995	M: 80 Y: 765 YI: 45	Maharashtra, Gujarat, MP, AP, Kerala Karnataka, Tamil Nadu, Orissa	Resistant to powdery mildew
	TARM-2	1992	M: Rabi: 90 Y: 1000-1100 YI: 80	Maharashtra	Resistant to powdery mildew
	TAP-7	1983	M: 60 Y: 700-800 YI: 23	Maharashtra, Karnataka	Tolerant to powdery mildew
Blackgram Udid (*Vigna mungo*)	TU 94-2	1999	M: 70 Y: 900-1000 YI: 19-37	Andhra Pradesh, Karnataka, Kerala, Tamil Nadu	Resistant to yellow mosaic virus
	TAU-2	1992	M: 70-75 Y: 900-1000 YI: 18	Maharashtra	High yielding
	TPU-4	1992	M: 70-75 Y: 900-1000 YI: 22	Maharashtra, Madhya Pradesh	Large seed
	TAU-1	1985	M: 70 –75 Y: 800 –1000 YI: 24	Maharashtra	Large seed Most popular variety in Maharashtra

TABLE 1 (Contd.)

Crop	Variety	Year of Release	M: Maturity (days) Y: Yield (kg/ha) YI: Yield increase (%)	Released for	Remarks
Pigeonpea (*Cajanus cajan*)	TT-401	2007	M: 150 Y: 1750 YI: 27	Madhya Pradesh, Maharashtra, Gujarat, Chhattisgarh	High yielding, tolerant to pod borer and pod fly damage
	TAT-10	1985	M: 110-115 Y: 900-1000	Maharashtra	Early maturing
	TT-6	1983	M: 135-140 Y: 1200-1300 YI: 15	MP, Maharashtra, Gujarat, AP, Karnataka, Kerala	Large seed
Cowpea (*Vigna unguiculata*)	TRC-77-4 (Khalleshwari)	2007	M: 90 Y: 700	Chhattisgarh (rabi)	Suitable for rice based cropping system
Rice (*Oryza sativa*)	Hari	1988	M: 135-140 Y: 6000 YI: 20	Andhra Pradesh	Slender grain type
Jute (*Corchorus capsularis*)	TKJ-40	1983	M: 125-130 Y: 2800-3100 YI: 10-13	Orissa	High yielding

M : maturity in days; Y: yield in Kg/ha; YI: yield increase over checks

TABLE 2. Trombay Germplasm Registration

Crop	Genotype	INGR No.	Year
Sesbania	TSR-1	1014	2001
Groundnut	TG-18AM	4039	2004
	TGE-1	4040	2004
	Small leaf mutant	4041	2004
	Suppressed branch mutant	4098	2004
	Imparipinnate leaf mutant	4097	2004
	TG-18A	7032	2007
Sunflower	Fasciation mutant	4100	2005
Sesame	Stiff Stem mutant	5018	2005
	Tall seedling mutant	7029	2007
	Polypetalous corolla mutant	7030	2007

Acknowledgement

Cooperation received from Indian Council of Agricultural Research, State Agricultural Departments, State Agriculture Universities, National and State Seed Corporations, NGOs and progressive farmers is acknowledged. I also thank all my Divisional colleagues especially the breeders and the field staff for their contributions.

Radiation Induced Mutations for Crop Genetics and Improvement

***Dr. S.G. Bhagwat** did his M.Sc. and Ph.D. in Botany from University of Pune. He joined the Biology and Agriculture Division in 1976 after completing one year orientation course in the 19th batch of Biology and Radiobiology. He has carried out research on wheat processing quality and components of field photosynthesis. His current interests include development and use of molecular markers for biotic and abiotic stress tolerance, use of computer based image analysis in grain morphometry. Apart from wheat he has carried out research experiments on rice, Sesbania and Jatropha. He is currently Head of Mutation Breeding Section in the Nuclear Agriculture and Biotechnology Division.*

***Dr. Suman Sud** joined BARC as Dr. K.S. Krishnan Research Associate (KSKRA) - 8th Batch in 2004. She did her Ph.D. in field of Plant Breeding from Punjab Agricultural University, Ludhiana in 2004. Currently she is working in Mutation Breeding Section of Nuclear Agriculture and Biotechnology Division. Her major interest is to look for traits controlling high temperature tolerance in wheat using conventional and biotechnological tools.*

***Shri B. K. Das** joined 39th batch (1995-96) of BARC Training School and joined in Nuclear Agriculture & Biotechnology Division in 1996. He did his M. Sc. in Agricultural Biotechnology form Assam Agricultural University, Jorhat in 1993. He has been working in Mutation Breeding Section on Genetic improvement of quality and rust resistance in Indian wheats using conventional, biochemical and molecular techniques. Currently he is working on pyramiding of rust resistance genes and combining them with quality traits by marker assisted selection.*

Historical

Mutation is a term introduced in the late-nineteenth century to refer to large scale phenotypic change observed by Hugo de Vries during his experiments on the evening primrose (*Oenothera lamarckiana*). Now we use the term to describe change in the phenotype of an individual which is heritable and not explained by segregation and recombination of genes or as a change at the level of genetic code. The change may be large or small. A point mutation is the single substitution of one base, a deletion is loss of a sequence, and a translocation is the reshuffling of a sequence while an inversion is the inverting of a sequence, and so on. Morgan (1911) studied effects of radiations from Radium on the wing mutations in Drosophila, but his studies were not conclusive, particularly about frequency of mutations. Later, Muller (1927) conclusively showed mutations in Drosophila as a result of X-ray treatment. Stadler's work on maize provided convincing evidence for mutagenic effects of X-rays in plants. In the next ten years radiation induced mutagenesis had started providing economically important barley mutants, this was followed by production of mutants in wheat, oats, lupin, flax and mustard. The utilization of induced mutations for crop improvement is now known as mutation breeding.

Spontaneous and Induced Mutations

Plant breeding involves utilization of natural variability present in gene pool of any crop species.

Dr. S.G. Bhagwat, Dr. Smt Suman Sud and Shri B.K. Das, Nuclear Agriculture and Biotechnology Division, Biomedical Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: sbhagwat@barc.gov.in

This variability is the outcome of naturally occurring mutations. There are a large number of instances in the past where naturally occurring mutations served important role in cultivar improvement. Green revolution genes (Norin 10 genes) with reduced height effect on phenotype of wheat plant are single base pair changes (point mutations) leading to stop codon. These are the examples of natural mutations. The short stature of rice variety Dee-geo- woo-gen is due to spontaneous mutation. This was widely used in breeding programs to produce many high yielding rice varieties. The naturally occurring mutations opaque-2 [1] and floury-2 in maize are examples of natural mutations with altered endosperm and amino acid profile which results in increase in level of lysine and tryptophan as compared to normal maize. Discovery of spontaneous mutants or sports has been an important means of cultivar improvement in many fruit crops such as apples and citrus.

In nature, spontaneous mutation occur at a very low rate, usually at the rate of one in a million. The natural variability may not be available or suitable for a researcher. In such a situation inducing variability or mutations is the solution. The rate of mutation is enhanced by the use of mutagens. A variety of mutagens are available which can be broadly classified as chemical mutagens and physical mutagens.

Chemical Mutagens

There are many molecules which can interact with DNA and cause mutations. Some of these are alkylating agents such as Sulphur mustards, nitrogen mustards, epoxides, imines (ethylene imine), sulphates and sulphonates e.g. ethyl methane sulphonate (EMS), methyl methane sulphonate (MMS), diazoalkanes, nitroso compounds e.g. N'-methyl-N-nitro-N-nitroso-guadine (MNNG), acridine dyes e.g. acriflavin, proflavin, acridine orange, acridine yellow, ethidium bromide, base analogues such as 5-bromouracil, 5-chlorouracil, and other compounds such as nitrous acid, hydroxyl amine, sodium azide. EMS, MNH (N-methyl-N-nitroso urea) and ENH (N-ethyl-N-nitroso urea) are some of the commonly used mutagens in plant research [2]. The chemical mutagens largely induce point mutations. Most of these chemicals are highly toxic and /carcinogenic. Special care is therefore required while handling these mutagens. Also penetration in tissues and uniformity in treatment has to be ensured.

Physical Mutagens

The electromagnetic radiations from radioisotopes such as gamma rays and particulate radiations such as alpha and beta have energy high enough to penetrate through biological tissues. They interact with water molecules and also with biomolecules. Interaction with DNA causes breaks. Some of the breaks may be repaired while others may not be repaired or repaired in such a way that the original sequence is altered. This results in a mutation.

Gamma rays have high penetration power and can penetrate deep in biological tissues. The common sources are ^{137}Cs (half life 30 years, energy 0.66MeV) and ^{60}Co (half life 5.3 years, energies 1.33MeV, 1.17MeV). Gamma rays become automatic choice when thick tissues such as seeds and stem cuttings are to be irradiated. X-rays have similar penetration and interaction properties as the gamma rays, they differ in their origin i.e. these are artificially produced. Beta ( ) radiation from radioisotopes such as ^{32}P (half life 14.29 days, energy 1709 keV) has adequate energy to penetrate through thin layers of biological tissue. These can be used when the seed size is small or these can be incorporated to ensure close contact with tissue thus enabling the beta particles to interact with DNA. Alpha ( ) radiation is particulate in nature and has low penetration power hence not commonly used in plant mutagenesis experiment. Neutrons can be useful in plant mutagenesis experiments since they have adequate penetration power. Neutrons are classified as fast neutrons (have energy greater than 1 eV, 0.1 MeV or approximately 1 MeV, depending on the definition) or thermal neutrons (energy of about 0.025 eV) based on their energies. Neutrons can be obtained from radioisotopes or from nuclear reactors. Exposure to neutrons results in neutron activation or induced activity and hence the materials cannot be handled immediately. Use of neutrons therefore requires different procedure to be followed.

Physical mutagens except neutrons do not leave any trace in the treated material and hence do not need any special precaution and are very

convenient to use. Other advantages of physical mutagens are that they are completely random in their interaction with the genome while chemical mutagens can interact differentially with chromatin. Also, physical mutagens induce large chromosomal changes such as deletions, inversions and translocations resulting in major alterations in plant characteristics. Among the induced mutants released as varieties for cultivation about 60% were produced using physical mutagens indicating the efficiency and convenience of physical mutagens [3]. Radiations can directly interact with biomolecules causing damage to the molecules. In a metabolically active cell, there is a large proportion of water. Water molecules on interaction with high energy radiations produce free radicals through a process called radiolysis of water. The free radicals are extremely reactive. The interaction of biomolecules with free radicals results in damage to the cell and also to the genetic material. The damage to vital biomolecules results in cell death, while damage to DNA can be repaired correctly or incorrectly resulting in mutation(s). In dry seeds the proportion of water is low and hence the damage to cells is reduced. This allows use of higher doses as compared to metabolically active tissues such as bulbs, rhizomes, cuttings etc. DNA is present in the nucleus and also in the cytoplasmic organelles such as chloroplasts and mitochondria, if the former undergoes mutations these are termed nuclear mutations and in organelle DNA are termed cytoplasmic mutations.

Materials that can be used for Mutagenesis

Most often seeds are used for irradiation since seeds are hardy, easy to handle and to transport and relatively small in bulk. These characteristics make them favorite among researchers. Seeds with 12-14% moisture are used for irradiation. Pre-soaking of seeds in water for 8-12 hours and post treatment drying are important in chemical mutagenesis. Other tissues which give rise to next generation such as cuttings, bulbs, rhizomes can also be used. Chimera formation is major problem in mutation induction in vegetatively propagated crops. Also, tissue cultures are used in *in vitro* mutagenesis experiments.

LD_{50} and Appropriate Dose

The amount of mutagens (physical or chemical) to be used for mutation induction varies from species to species. Criteria such as LD_{50} (50% viability) or GR_{50} (50% growth reduction) are used to choose the dose range. LD_{50} or GR_{50} is the dose of mutagen that is lethal to 50% of treated individuals. The LD_{50} value has to be determined experimentally depending on type of material and crop species used for mutation induction. The dose to be used in case of a new material is determined by exposing to a series of doses. Since mutation induction is a random process determined by probability, use of appropriate dose ensures that the loss of viability is minimized and chances of recovering mutations are maximized. In practice, use of three doses one appropriate, second a little less and third a little more than appropriate dose are used. The quantity of seed to be irradiated should take in to account the dose being used (resulting in corresponding loss in viability) so as to ensure that at least few hundred M_1 plants are available up to maturity. As a general rule, smaller seeds have higher tolerance to radiation also lower water content makes seeds more tolerant to radiation. Before taking up radiation induced mutagenesis a search for previous reports on the same species will be rewarding, also the manual of Mutation Breeding published by I.A.E.A. Vienna, lists large number of species and doses recommended.

How to start an Experiment

Self pollinated crops are most suitable for mutation breeding. Seeds of an elite variety with a single defect to be rectified makes an ideal candidate. Seeds have to be absolutely pure or else a contaminant may be identified as a mutant. Seeds should have very high germination percentage and low moisture content to reduce damage. Seeds should be sown as early as possible after irradiation to reduce loss in viability.

The irradiated seeds give rise to M_1 generation plants, which shows physiological effects such as reduced growth. A certain fraction of the population shows mortality. Also, there is some sterility induced and hence it is recommended to cover the flowers /inflorescences with bags to avoid cross pollination. Since the M_1 generation shows changes which are of

physiological nature, collecting data on M_1 population for genetic interpretation should be avoided. The seeds harvested from M_1 generation plants are sown to raise the M_2 generation. This is the important population because single genes which undergo mutations and are recessive in nature will segregate out in this generation to exhibit the mutant phenotypes. The size of M_2 generation is therefore very important and should be large enough to provide chance for the mutant to appear. A few thousand plants would provide opportunity for mutations to appear and provide scope to choose for better plant among the mutants. Also, consideration must be given to how many seeds of each M_1 plant should be used to generate the M_2 population, if there is a constraint on how many plants can be raised.

The key to the success of a mutation breeding programme is to have a precisely defined objective and a rapid screening procedure. Since large number of samples have to be analyzed, a fast, economical and rugged method is essential to complete analyses in the available time frame.

The mutant plants once identified should be carried forward in subsequent generations to confirm the trait. This is followed by multiplication of seeds and comparative testing to judge the superiority of mutant over the parent/existing check variety. If the mutant is statistically superior in performance it can be used for commercial purpose. It is however possible that the mutant may continue to segregate for a few generations before it shows complete homogeneity. Cross pollinated species and polyploid species are more difficult material to handle in induced mutagenesis experiments.

Mutations for Crop Improvement: some examples

One of the early examples of use of high energy radiation is seen in Sears' work carried out in the fifties of the last century. He transferred rust resistance from *Aegilops umbellulata* (2n=14) to wheat (2n=42). In a complex crossing programme which was necessary due to the difference in the ploidy of the two species, a series of crosses were made at the end of which a 42 chromosome plant with an extra chromosome from *A. umbellulata* which carried the gene for rust resistance was obtained. To transfer the rust resistance gene to wheat chromosome, X-ray irradiation was employed. Plants with 43 chromosomes were irradiated with X-rays, the pollen formed was used to pollinate a 42 chromosome variety 'Chinese Spring' which was susceptible to rust. Among the next generation plants, a plant showing resistance to rust was observed. The X-ray treatment had resulted in a translocation in which a fragment of the alien chromosome was transferred to wheat chromosome and rust resistance was obtained in wheat background [4]. Some of the spectacular successes include high quality brewing barley mutant varieties in Europe such as 'Golden Promise' produced using gamma rays in 1956 and 'Diamant' which was developed using X-rays in 1965 [3]. The contribution of induced mutations in plant breeding is quite significant. The IAEA database has registered over 2500 mutant varieties developed all over the world. In India, more than 300 varieties including those of ornamental plants have been developed using induced mutations. Many mutants became important varieties, e.g. the rice variety Yuanfengzao, and Zhefu 802 in China for earliness [5,6]. Several mutated genes have been integrated into modern varieties, e.g. the two independent *sd1* mutant alleles first induced in Reimei in Japan and in Calrose 76 in the US are now integrated into many new rice varieties [7]. Most spring barley varieties contain various mutant alleles of the disease resistant gene *mlo* in Europe and Australia (http://www.crpmb.org/mlo#pifanelli). Mutations have been used to improve different types of important traits, from tolerance to abiotic stress (i.e. salinity, low temperature etc.) to disease resistances, from food and nutritional quality to market preference, and from plant structure to productivity. Induced mutation sometimes is the only way for improving a particular trait while keeping the overall background unchanged. Induced mutations are also useful to break linkage with undesirable traits. At the Bhabha Atomic Research Centre several crop varieties have been developed from direct use of mutants or by using mutants in a cross breeding programme.

Mutations for Enriching Variability

Mutations either induced or spontaneous provide the genetic variability needed by the geneticist and plant breeder. Mutations which may not be useful from the point of view of a breeder can

be useful in identifying the genetic nature of the trait and identifying the genes involved in determining the trait. Therefore, these mutants are maintained as a collection for future use. The Carlsberg Collection of flavonoid mutants comprises 724 induced barley mutants (http://grain.jouy.inra.fr/ggpages/bgn/18/c18-07.html), and the collection of *Antirrhinum majus* stocks has 300-400 mutants at the John Innes Centre (http://www.jic.bbsrc.ac.uk/staff/enrico-coen/Rosemary/mutants.html). Systematic development, characterization and collection of chemically or physically induced mutants is now an important activity since researchers have realized the potential of such mutants in functional genomic studies [8]. For example, the IR64 mutant collection in the International Rice Research Institute comprises more than 38,000 M_4 lines (http://www.iris.irri.org) [9].

Characterization of mutations/ molecular characterization of mutations, why/how?

In the regime of plant breeders' right and IPR, it is now necessary to characterize the mutants with respect to morphological, biochemical and molecular characters. The novel mutants obtained by a breeder/ institution may be used in cross breeding programme and ensuring that the mutant is documented properly will help in sorting out the issue of IPR.

For characterizing the mutants, and identification, SSR and ISSR markers and techniques such as AFLP, SNP and TILLING are used. Induced mutations combined with molecular techniques will help in understanding the structural and functional aspects of plant genomes. Induced mutations are being characterized using molecular techniques. Physical mutagens produce deletions from 17 bp up to 20cM in length as revealed by molecular analysis of induced mutants [3].

Limitations of Mutation Induction

The major limitation of mutagenesis experiment is that it is based on a phenomenon governed by probability. Since the probability is low, the researcher has to deal with large number of individuals for obtaining the desirable mutation. Also, a specific type of mutation cannot be obtained, mutations are produced at random among which a desirable mutation may appear. Need for observing large population makes it difficult to handle large size plants or long duration plants in mutation breeding experiments.

New Mutagens

Particle accelerators and electron beams can be used to induce mutations. Ions or subatomic particles can be accelerated in laboratories. These have energies to penetrate in to cells to bring about mutations. Among the new ways to induce mutations space-induced mutants 971-5, 972-4, and R955 of rice are reported to have acquired new traits such as increased yield, reduced resistance to rice blast, and semi-dwarfism compared with their on-ground controls. Studies on these mutants suggest that space induced mutations might share a common mechanism with other types of mutagens [10].

Irradiation service at NA and BTD, BARC

The Nuclear Agriculture and Biotechnology Division of Bhabha Atomic Research Centre provides irradiation service facility to users on request for research purpose. Many users from all over the country have made use of the service. The service is on payment basis. Further information can be obtained from Head, NABTD or the first author of this article.

Summary

Although mutation is a natural process, rate of spontaneous mutations is not adequate for their use in intensive breeding programmes. Mutation breeding with the use of induced mutations is a technique available to breeders in their pursuit to develop new cultivars. A mutation is a change in the structure of a gene. In most cases, this is deleterious, however, mutations can result in alteration in traits to suit the specific requirement. The chance dependence is easily offset by the simplicity of the method. The method is particularly suitable where high technologies are not accessible. A large number of mutants have been used and put in to commercial use world wide. Unlike the genetically modified crop plants, the mutation derived varieties find acceptance from the consumers. Although induced mutations have been on the scene for over 75 years, authentic information is not easily available. A systematic effort is needed to enhance the gains from

induced mutations. Induced mutations also serve the purpose of enriching variability which is the starting material for geneticists.

References

1. E.T. Mertz, L.S. Bates, and O. E. Nelson, Science, 145(1964) 279.
2. R.P. Sharma, and V.L. Chopra, In: Chopra V.L. (ed.) Plant Breeding-Theory and Practice. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, (2000). pp. 321-342.
3. E. Weck, M.Maluszynski, L. van Zantel, B. Ahloowalia, and K. Nichterlein, Proceedings of RON-BEC 100, Eds. Sood D.D., Jain H.C., Reddy A.V.R., Ramakumar K.L., Kulkarni S.G., B.A.R.C., Mumbai, India, (1996). pp489.
4. E.R. Sears, 1956. In Genetics and Plant breeding, Brookhaven Symposia in Biology No. 9, Brookhaven National Laboratory, Upton, New York, 1956.
5. B.S. Ahloowalia, M. Maluszynski, K. Nichterlein, Euphytica, 135(2004)187.
6. L. Liu, L.Van Zanten, Q.Y.Shu, and M.Maluszynski, Mutat. Breed. Rev. 14(2004) 1.
7. J.N.Rutger, R.J. Bryant, and A.K. Moldenhauer, Crop Science, 46 (2006) 2340.
8. J. Li, K.A. Lease, F.E. Tax, J.C. Walker, Proc. Natl. Acad. Sci., USA, 98(2001)5916.
9. J.L. Wu, C. Wu, C. Lei, M. Baraoidan, A. Bordeos, M.R.S. Madamba, M.Ramos-Pamplona, R. Mauleon, A. Portugal, V.J. Ulat, R. Bruskiewich, G. Wang, J.Leach, G. Khush, H. Leung, Plant Molecular Biology, 59 (2005) 85.
10. Y. Li, M. Liu, Z. Cheng, and Y. Sun, Advances in Space Research, 40 (2007) 523.

Induced Mutations for Genetic Improvement of Mungbean, Urdbean and Cowpea Pulse Crops in India

***Dr. K.S. Reddy** joined BARC in 1986 after obtaining his M.Sc. in Botany from Bhopal University and obtained his Ph.D. in Botany from University of Pune. He is working in mungbean improvement programme using induced mutations. His Ph.D. work resulted in the development of 3 high yielding and powdery mildew disease resistant varieties. Further he continued to work on mungbean yellow mosaic virus and Rhizoctonia root rot diseases. He has released 7 high yielding and disease resistant varieties in mungbean. His basic work on disease resistance resulted in developing several donors for powdery mildew, yellow mosaic virus, Rhizoctonia root rot and Cercospora leaf spot diseases. He has also developed several morphological mutants contributing towards yield.*

***Shri. P. Dhanasekar** graduated from Tamil Nadu Agricultural University and joined BARC in 2001. He is involved in the improvement of grain legumes especially cowpea and pigeonpea through induced mutations and involved in developing the recently released TT-401 pigeonpea variety. He has also developed a compact dwarf ideotype in pigeonpea and isolated various mutants in cowpea. He has contributed in developing DNA fingerprints for important cowpea mutants.*

Abstract

Among the grain legumes, pulses are the main sources of dietary protein in India. The Indian pulse production has been hovering around 15 million tonnes since several years. The stagnant production is unable to meet the increasing demand of the mounting population. Several approaches have been used to develop high yielding varieties to increase national average, among them mutation breeding has contributed significantly. Mutation by physical and chemical mutagens is a good option to increase the genetic variability and use in breeding programmes. Among the physical mutagens gamma rays has been employed widely for obtaining mutants compared to X-rays and fast neutrons, while ethyl methane sulphonate (EMS) has been the most important among chemical mutagens. In pulse crops several mutations have been induced for chlorophyll, morphological and biochemical parameters like protein content and disease resistance. The inheritance studies of induced mutations have shown most of the characters to be recessive. Mutation breeding has resulted in the development of 55 varieties in India in several pulse crops including mungbean, blackgram, chickpea, cowpea, mothbean, pigeonpea, lentil, lablab bean, cluster bean, common bean and pea. Bhabha Atomic Research Centre (BARC), Trombay has succeeded in developing 15 high yielding varieties in four pulse crops: 7 in mungbean, 4 in urdbean, 3 in pigeonpea and 1 in cowpea. The Trombay pulse varieties are very popular in several states. The urdbean variety TAU-1 is cultivated in 90% of the urdbean cultivated area in Maharashtra. Mungbean varieties TARM-1, TMB-37 and TM-96-2 are becoming popular in several states of India because of their high yield and resistance for powdery mildew and yellow mosaic virus diseases. The mutation breeding has been complementing and supplementing the conventional breeding for improving the production scenario of pulses in India.

Introduction

Globally, grain legumes are the second most important group of crops. Among legumes, pulses are important in India, as most of the dietary protein is derived from them. The pulses production is

Dr. K.S. Reddy and Shri P. Dhanasekar, Nuclear Agriculture and Biotechnology Division, Biomedical Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: ksreddy122@yahoo.co.in

around 60 million tonnes in the world of which near about 15 million tonnes have been produced in India during 2004-05. The expected requirement of pulses is around 24.4 million tonnes by 2020, but the expected production is only 22.5 million tonnes around that time. Hence, there is an urgent need to develop high yielding varieties to meet the required demand. Mutation is a good option to increase the variability and to use in the breeding programmes. Although initial studies were concentrated on use of physical and chemical mutagens on mutagenisation, later studies were directed at mutation frequency, spectrum and mutagenic effectiveness. The concentrated and systematic efforts resulted in increasing frequency and widening the spectrum of mutations in several pulse crops. Several mutants isolated for higher productivity were successfully used in breeding programmes to develop 55 mutant varieties in several pulse crops including mungbean, blackgram, chickpea, cowpea, mothbean, pigeonpea, lentil, lablab bean, cluster bean, common bean and pea crops in India. Bhabha Atomic Research Centre (BARC) has contributed in developing seven mutant varieties in mungbean, four in blackgram, three in pigeonpea and one in cowpea crops.

In this article, we review the induced mutation studies conducted in mungbean (*Vigna radiata* (L.) Wilczek), urdbean (*Vigna mungo* (L.) Hepper) and cowpea (*Vigna unguiculata* (L.) Walp) in India, with special reference to mutagenic doses, mutants obtained and their use in genetic studies and developing varieties for commercial cultivation.

Mungbean (Greengram)

In India, the area of mungbean cultivation was 3.34 million hectares with production of 1.06 million tonnes with an average yield of 317 kg/ha during 2004-2005 (1). Mungbean contains 24-26% protein which is easily digestible, without any anti-nutritional factors. Although, the area under cultivation has increased from 1.99 m ha to 3.34 m ha during 1965 to 2005, the average yield did not increase proportionately, as the maximum area of cultivation was under rainfed conditions, it is cultivated in marginal soils, less responsive to chemical fertilizers and also major diseases like powdery mildew (PM), yellow mosaic virus (YMV) and *Cercospora* leaf spot (CLS) diseases are limiting factors for high yield. To increase the production and productivity, several approaches have been used to develop 43 high yielding mungbean varieties during 1985-2006. The mutation breeding has resulted in developing 15 high yielding and disease resistant varieties during 1979-2006. BARC has contributed 7 varieties which are under popularization programme and expected to increase the average yield (2,3,4,5)

Mungbean Mutation Breeding Programme in India

Physical and Chemical Mutagens in Induction of Mutation

Studies on induced mutations in mungbean were initiated in late sixties in India. Both physical and chemical mutagens were used individually or in combinations to induce mutations. X-rays, gamma rays and ethyl methane sulphonate (EMS) have been found to be the most efficient in developing genetic variability. The LD-50 of mutagens for various parameters such as germination, seedling height and survival was studied and mutagenic doses determined by several workers. The dose levels of 40 to 50 kR (400-500Gy) were critical for inducing mutations (6,7) and EMS concentrations between 0.06 – 0.08 M and the soaking duration between 6-10 hrs was found suitable for chemical mutagenesis.

Induction of Mutations

Mutagenic treatments have induced various types of morphological, physiological and biochemical mutants in mungbean

Chlorophyll Mutations

In majority of the studies, ionizing radiations were found to give the highest frequency of albina types followed by xantha (8). The viable chlorophyll mutants obtained by mutagenic treatments include chlorina, chlorotica, viridis, alboviridis, virescens, albicans and maculata etc.

Morphological Mutations

A number of mutants with altered morphological characters like growth habit, plant stature and branching pattern; leaf characters like shape, size, colour and number; flower, pod, large seed size and long root mutants for drought tolerance

characters have been induced by mutagenic treatments in mungbean (9,10,11,12).

Protein content

Mungbean mutants with high as well as low protein content ranging from 21.5% to 27.25 % vis a vis 23.8 % in control were isolated (13,14).

Disease resistance

Bahl and Gupta (8) obtained mutants with high level of disease tolerance following the seed treatment of cv. K-851 with 0.2% EMS. All disease resistance studies need good screening techniques. A simple and reliable screening technique was developed for screening foliar diseases by Reddy et al. (15) and using the technique resistance sources were identified for powdery mildew disease in mungbean. The inheritance studies showed that powdery mildew resistance is governed by two dominant *Pm1* and *Pm2* genes (16) The varieties TARM-1, TARM-2, TARM-18 and TM-96-2 were released on the basis of their high yield and resistance to powdery mildew disease. The mutant TPM-1 one of the parent in the above varieties has been identified as YMV resistant donor. The variety Kopergaon and JL-781 were identified as resistant donors for *Rhizoctonia* root-rot disease and the inheritance studies showed that the resistance was controlled by a single dominant gene (17). A mutant TM-98-50 has been identified as a donor for *Cercospora* leaf spot disease resistance (18). The Trombay variety TMB-37 was released for its high yield and resistance to yellow mosaic virus disease. The mutant variety TJM-3 has been released for its multiple disease resistance for powdery mildew, yellow mosaic virus and *Rhizoctonia* root-rot diseases. Genetic relationship and fingerprinting of some mutants and genotypes were also studied using AP-PCR technique (19). Host response to powdery mildew disease was also studied by using histochemical studies (20). The second race (Akola race) of *Erysiphe polygoni* DC was identified by genetic analysis by Reddy (21), which is useful for identification of resistance sources to make durable resistance to powdery mildew disease in mungbean.

Mutants used in Cross Breeding

Mutants with desirable characters and high yield were either released for cultivation directly or used in hybridisation with other mutants or varieties. A multi-foliate mutant obtained from EMS treatment (22) was hybridised with a large seed mutant MG50-10A (23) to develop a large seeded multi-foliate line with shiny yellow seed coat. At BARC a mutant TPM-1 obtained from S-8 cultivar characterised with multiple branching was crossed with the mungbean accession RUM-5 having resistance to powdery mildew disease to develop resistant varieties TARM-1 and TARM-2 (24). The TARM-1 and TARM-2 have been used in crossing programme with varieties and mutants for developing TMB-7, TM-96-2 and TJM-3 varieties with resistance to powdery mildew, yellow mosaic virus and *Rhizoctonia* root-rot diseases.

Mutant varieties released from BARC

Of the seven varieties developed at BARC and released for commercial cultivation for different States, one is direct mutant and six are mutant derivatives. The salient features of the Trombay varieties are given below.

TAP-7

Developed and released for cultivation in 1983 for the states of Maharashtra and Karnataka. It is an early maturing (65 days) mutant variety with yield potential of 700-800 kg/ha in *kharif* season. It has tolerance against powdery mildew disease.

TARM-2

A medium-late maturing variety (80 days) was released for *rabi* cultivation in the year 1993 for the sate of Maharashtra. It has yield potential of 1000-1100 kg/ha and resistance to powdery mildew disease.

TARM-1

A powdery mildew resistant variety with medium maturity (80 days) was released for commercial cultivation in 1996 for central and southern regions of India comprising Maharashtra, Gujarat, Madhya Pradesh, Andhra Pradesh, Karnataka, Kerala, Tamil Nadu and Orissa. It has yield potential of 1200 kg/ha.

TABLE 1. Mungbean varieties developed through induced mutations in India

Variety	Year of release	Institution	Characteristic features for release
Dhauli (TT9E)	1979	OUA&T, Bhubaneshwar	High yielding
Co-4	1981	TNAU, Coimbatore	High yielding
Pant Moong-2	1982	GBPUA&T, Pantnagar	High yielding
TAP-7	1983	BARC, Mumbai	High yielding, large seed size
BM-4	1992	ARS, Badnapur	High yielding, tolerant to YMV, large seed size
MUM-2	1992	CCSU, Meerut	High yielding, resistant to YMV
TARM-2	1993	BARC, Mumbai & PDKV, Akola	High yielding, resistant to PM, medium maturity
LGG-407	1993	APAU, Lam	High yielding, tolerant to YMV
LGG-450	1993	APAU, Lam	High yielding, tolerant to YMV
TARM-1	1996	BARC, Mumbai & PDKV, Akola	High yielding, resistant to PM, medium maturity
TARM-18	1996	BARC, Mumbai & PDKV, Akola	High yielding, resistant to PM
OUM 11-2	2002	OUA&T, Bhubaneshwar	High yielding, moderately resistant to YMV and CLS
TMB-37	2005	BARC, Mumbai	High yielding, early, resistant to YMV, medium large seed
TM-96-2	2007	BARC, Mumbai & ANGRAU, A.P.	High yielding, medium large seed , resistant to PM
TJM-3	2007	BARC, Mumbai & JNKVV, Jabalpur	High yielding, early, medium maturity, large seed size, resistant to PM, YMV and *Rhizoctonia* root rot

TARM-18

A powdery mildew resistant variety, has yield potential of 1051 kg/ha with 65-70 days maturity. This was released in 1996 for the state of Maharashtra.

TMB-37

An extra-early maturing variety (55-60days) having resistance to yellow mosaic virus disease was released in 2005 for summer cultivation in north-east plain zone comprising eastern UP, Bihar, Jharkhand, West Bengal and Assam states. It has a yield potential of 1000-1200 kg/ha with medium bold seed size .

TM-96-2

A powdery mildew resistant variety, with yield potential up to 1200 kg/ha in rice fallows and rabi season was released for commercial cultivation in the state of Andhra Pradesh during 2007.

TJM-3

An early maturing (60-65 days), high yielding (950-1200 kg/ha) variety with resistance to powdery mildew, YMV and *Rhizoctonia* root-rot diseases

was released for cultivation in the state of Madhya Pradesh during 2007.

Impact of Mungbean Mutant Varieties Released in India

In India, fifteen mutant varieties of mungbean have been released for cultivation for different agro-climatic regions. The varieties Co-4 and Pant Mung-2, though released in early eighties are still being grown in the country. In mungbean, major bottlenecks were the susceptibility of existing varieties for YMV and PM diseases. Successful incorporation of powdery mildew resistance in high yielding mutants resulted in PM disease resistant varieties TARM-1, TARM-2 and TARM-18 for the first time in India. The variety TARM-1 suitable for *rabi* and rice fallow cultivation became very popular in Orissa State. A recently released variety TMB-37 is becoming popular in Madhya Pradesh and Uttar Pradesh. The Department of Agriculture Co-operation (DAC) has produced 19.63 quintals of breeder seed for the variety TMB-37 as compared to 31.50 quintals of most popular variety Kopergaon during 2006-07. TMB-37 is also being introduced in the Bihar, West Bengal and Assam States. Another recently released variety TM-96-2 is becoming popular in Andhra Pradesh as it is suitable for rice fallow cultivation and cultivated in an area of near about 15 thousand hectares during 2006-07. The variety TJM-3 having resistance to powdery mildew, yellow mosaic virus and *Rhizoctonia* root-rot diseases is expected to become popular in Madhya Pradesh. Fifty seven mungbean varieties are in the breeder seed production (DAC) in the country of which 6 have been developed by BARC.

Blackgram (Urdbean)

Blackgram is one of the important pulse crops and is cultivated in 3.17 million hectares with production of 1.33 million tonnes and with an average yield of 419 kg/ha in India during 2004-05 (1). Important aspects on the mutation breeding in blackgram have been reviewed here.

Physical and Chemical Mutagens for Induction of Mutants

Thakare (25) used gamma rays doses in the range of 15-75 kR (150-750 Gy) and fast neutrons in the range of 2-6 kR (20-60Gy) for urdbean variety No. 55 and obtained a very large number of mutants. Various studies on radio-sensitivity and mutagenicity in urdbean showed that a dose range of 30 - 40 kR (or 300-400 Gy), and the treatment of seeds under dry condition were useful for inducing mutations (26). Mahna *et a*l. (27) found sodium azide to be moderately effective when used in acidic solution. Routaray *et al* (28) found 0.2% and 0.4% of EMS and 0.015% of sodium azide to be most useful doses.

Mutation Studies for Different Characters

Chlorophyll Mutations

Several chlorophyll mutants like *albina*, *xantha* (non-viable) and *chlorina*, *virescens* (viable) were obtained following X-ray irradiation of urdbean variety T-9 (29). In later studies several viable and non-viable chlorophyll mutants including *chlorina, virescens, viridis, flavo-viridis, albo-viridis, chlorina-terminalis, chlorina-virescens, albo-virescens; chlorotica, aurea*, *albina* and *xantha* were observed following treatments with physical or chemical mutagens or their combinations (25).

Morphological Mutations

Several morphological mutants were developed in urdbean affecting growth habit, leaf characters, floral characters, pod characters(25) and seed characters(30).

Nodulation Studies

Pentafoliate mutants with increased weight and number of nodules were obtained following seed treatment of T-9 with 20 kR (200Gy) gamma rays (31). Mutants with increased number of nodules obtained from the same cultivar following seed treatment with hydroxyl amine or sodium azide showed high levels of total nitrogen and seed protein (27).

Protein Content Studies

Mutants with 7.2 - 12.3% increased protein content were obtained in urdbean (25). Ignacimuthu and Babu (32) obtained variations with both positive and negative directions in seed protein of variety

TABLE 2. Urdbean varieties developed through induced mutation.

Variety	Year of release	Institution	Characteristic features for release
Co-4	1978	TNAU, Coimbatore, India	High yielding , early maturing
TAU-1	1985	BARC, Mumbai & PKV, Akola	High yielding, large seed size
Manikya	1988	GBPUA&T, Pantnagar, India	High yielding, early, large seed size
TPU-4	1992	BARC, Mumbai & MPKV, Rahuri, India	High yielding, large seed
TAU-2	1993	BARC, Mumbai & PKV, Akola, India	High yielding, large seed size
Vamban-2	1997	TNAU, Vamban	High yielding, resistant to YMV
TU94-2	1997	BARC, Mumbai, India	High yielding, large seed, resistant to YMV

T-9, following treatment with 20-30 kR (200-300 Gy) gamma rays.

Disease Resistance

Mutants with vigorous growth and resistance to yellow mosaic virus disease were obtained (33). Shaikh and Majid (34) obtained a high yielding mutant having combined resistance to *cercospora* leaf spot and yellow mosaic virus diseases following treatment with gamma rays in accession No. B-10. The variety TU-94-2 was released for its high yield and resistance to yellow mosaic virus.

Genetic Studies on Induced Mutants

The inheritance of mutant traits in urdbean has been reported. In majority of the cases the mutant traits have been found to be monogenic recessive. Thakare (25), in his studies on the genetics of induced mutants of urdbean, found that out of 20 mutant x parent crosses, 19 showed monogenic recessive inheritance. In case of a brown seeded mutant x parent, he found 1:2:1 segregation for seed colour in the F_2 generation.

Mutants in Cross Breeding and Development of Varieties

The large seed mutants, UM-196 and UM-201 were used in cross breeding with the elite cultivar T-9 for developing high yielding varieties TAU-1, TAU-2 and TPU-4 (35,36). So far seven varieties have been developed through induced mutation and released for cultivation in India (Table 2). Four of these varieties are the derivatives of mutants used in cross breeding.

Impact of Urdbean Mutant Varieties in India

Mutation breeding has made significant contribution in increasing the production of urdbean in India. Four of the seven mutant varieties of urdbean released in India have been developed at the Nuclear Agriculture & Biotechnology Division of BARC, Mumbai. The variety TAU-1, developed at BARC in collaboration with Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola, has become the most popular variety in Maharashtra occupying an area of about 5 lakh hectare (over 90% of the total area under urdbean cultivation in Maharashtra).

Cowpea (Lobia)

Cowpea Cultivation in India

India alone accounts for half of the total acreage of about 1.3 million hectares under different forms of cowpea in Asia (37). Nevertheless, cowpea is considered a minor pulse crop in India, and is cultivated in the semi-arid regions of Rajasthan, Gujarat, Karnataka, Tamil Nadu and Maharashtra mostly as grain legume.

Improvement of Cowpea Through Induced Mutagenesis

Mutation Breeding Programme Using Chemical and Physical Mutagens

Narsinhani and Kumar (38) studied the response of two cowpea varieties to the treatments with EMS (0.25%) and Methyl Methane Sulphonate (MMS) (0.025%) in the M_1 generation and observed reduction in germination and seedling height only in Pusa Barsati.

Brunner (39) had found 32.5 kR (325 Gy) dose of gamma rays causing 50% reduction in shoot height and estimated 10-25 kR (100-250 Gy) gamma rays dose to be useful for inducing mutation in cowpea. John (40) however, found 30 kR(300 Gy) gamma rays to be the efficient one for cowpea varieties Co-4 and C-152 and their hybrid on the basis of sterility and lethality studies. Observations from the recently conducted experiments at BARC on an exotic cowpea, EC394763 treated with 25 kR (250 Gy) showed that the germination was adversely affected at this dose, indicating the useful doses were lower to 25 kR. Treatment of cultivar V-130 with 20 kR gamma rays led to the development of mutant variety TRC 77-4 at Trombay with dwarf compact plant type suitable for rice fallows. The various studies, however, have indicated the differential response of cowpea varieties to gamma rays treatment.

Development of Mutants for Different Characters

Chlorophyll Mutations

Narshinghani and Kumar (38) observed a large number of chlorophyll mutants in the M_2 generation of cowpea treated with 0.25% EMS and 0.025% MMS. Albino, Xantha, chlorina, striata and viridis were observed in the EMS treatment, while only albino, xantha and chlorina mutants appeared in MMS treatment. John (40) observed mutant viridis more frequently and chlorina and xantha in equal proportions followed by albino and albo-viridis following 30-40 kR gamma rays treatment of cowpea seeds of cultivars Co-4, C-152 and their hybrid.

Morphological Mutations

Mutations affecting various plant parts and characters have been observed in cowpea following mutagenic treatments. Sharma (41) had observed

TABLE 3. Cowpea varieties developed through induced mutations in India

Variety	Year of release	Institution	Characteristic features for release
V-16 (Amba)	1981	IARI, New Delhi	High yielding and tolerant to diseases
V-37 (Shreshtha)	1981	IARI, New Delhi	High yielding, luxuriant vegetative growth
V-38 (Swarna)	1981	IARI, New Delhi	High yielding, early, non-trailing, synchronous maturity
V-240	1984	IARI, New Delhi	High yielding, medium late, tolerant to diseases
Co-5 (forage cowpea)	1986	TNAU, Coimbatore	High yielding, suitable for forage
Cowpea-88 (forage cowpea)	1990	PAU, Ludhiana	Large seed size, resistant to YMV and anthracnose
Khalleswari (TRC-77-4)	2007	BARC, Trombay & IGKVV, Raipur	High yielding, dwarf compact plant type, long pod, large seed, suitable for rice fallow cultivation

very drastic mutations affecting several characters like growth habit, flower colour, pod size, spotting on pods, and colour, size and form of the seed in the M_2 generation following mutagenic treatments. Pandey and Dhanasekar (42) observed a wide range of altered characters in one of the mutants TCM 308 following recurrent gamma irradiation of dwarf mutant TCM 77-4.

Disease Resistance

An induced mutant tolerant to seed borne mosaic virus disease was indentified (43). A mutant variety V-240 developed from Pusa Phalguni following DMS (0.8%) treatment has shown tolerance to major fungal, bacterial and viral diseases (44). Mahadevu et al. (45) isolated a *Pythium* rot resistant mutant from the M_2 population of cowpea variety KBC-1 treated with gamma rays. One of the mutants TCM 148-1 isolated at Trombay was found to have multiple disease resistance against yellow mosaic virus, root rot, leaf curl and leaf blight diseases (46).

Cowpea Mutant Varieties Released in India

Cowpea varieties developed through induced mutation and released for commercial cultivation in India are given in Table 3. While varieties Co-5 and cowpea-88 were developed for the fodder, other varieties were of grain types. Mutation breeding at BARC has resulted in developing a variety TRC-77-4 (Khalleswari) in collaboration with Indira Gandhi Krishi Vishwa Vidyalaya, Raipur.

Thus mutation breeding in conjunction with conventional breeding has immense potential to increase the genetic variability in the existing germplasm, paving way for development of elite varieties suitable for different agro-climatic zones and improving the pulses production and nutritional security of our country.

References

1. B.B. Singh, Project Coordinator's Report, All India Coordinated Research Project on MULLaRP, Annual Group Meet, Kharif, 13-15 May (2007), IIPR, Kanpur, India. Pp. 12
2. S.E. Pawar, K.S. Reddy, R.G. Thakare and C. R. Bhatia. In proceedings " Indian Nuclear Society on Nucear Science and Technology in India Past, Present and Future" (1989) Pp. 106.
3. S.E. Pawar, R.G. Thakare, K.S. Reddy and C.R. Bhatia. In Proceedings "FAO\IAEA symposium on the contribution of plant mutation breeding to crop improvement". IAEA/SM311/509. IAEA Vienna. Vol; I(1990) Pp.413.
4. K.S. Reddy, S.E. Pawar , K.B. Wanjari and C.R. Bhatia. In Proceedings "International Symposium on Pulses Research" New Delhi (1994) Pp-46.
5. K.S. Reddy, S.E. Pawar, K.B. Wanjari and C.R. Bhatia. In Proceedings "Recent Advances in Mungbean Research" Indian Society of Pulses Research and Development, IIPR. Kanpur, India (1996) Pp144.
6. D.P. Singh and B.P. Pandya. Trop. Grain Leg. Bull. 9 (1977) 25.
7. C.M Sharma, M.F. Haque. Journal of Research, Birsa Agricultural University; 9 (1997) 5.
8. J. Bhal and P.K. Gupta. Mut. Breeding News. 20 (1982) 13.
9. D. P. Singh, K.R. Vaidya and D.D. Bhatt. Indian J. Agri. Sci.; 49 (1979) 835.
10. R.G. Thakare, S.E. Pawar and D.C. Joshua. Curr. Sci. 49 (1980) 945.
11. K. S. Reddy. In "BARC golden Jubilee and BARC-BRNS Life Science Symposium" December18-20 (2006) Pp.57.
12. V.J. Dhole and K.S. Reddy. In "national Symposium on Legumes for Ecological sustainability: Emerging Challenges and Opportunities" IIPR, India, November 3-5th (2007) Pp.233.
13. B.S. Dahiya, Indian J. Genet. and Plant Breed. 33 (1973) 460.
14. S.G. Bhagwat, C.R. Bhatia, T.G. Krishna, D.C. Joshua, R.K. Mitra, P. Narahari, S.E. Pawar and R.G. Thakare. In "Seed protein improvement in cereals and grain legumes", Vol. II. IAEA, Vienna, (1979) Pp. 225.
15. K.S. Reddy, S.E. Pawar and C.R. Bhatia. Proc. Ind. Acad. Sci. (Plant Sci.) 97 (1987) 365.

16. K.S. Reddy, S.E. Pawar and C.R. Bhatia. Theor. Appl. Genet. 88 (1994) 945.

17. K. S. Reddy In "National Seminar on Breeding-Impact of DNA revolution" UAS Dharward, India October 30-31st (2003) Pp.147

18. R.A. Singh, Pulses news letter, Indian Institute of Pulses research, Kanpur Vol. 18, No 2 (2007)Pp. 4.

19. S. Ajay, K.S. Reddy and N. Jawali, Ind. J. Biotech. 3 (2004) 511

20. K.S. Reddy, S.E. Pawar and C.R. Bhatia, Indian Phytopathology (2001) 54;117.

21. K.S.Reddy, Plant Breeding. 126 (2007) 603

22. B.N. Samol. S.K.Sinha and J.M. Singh.. Ind. J. Agri. Sci. 49 (1979) 776.

23. S. Dwivedi and D.P. Singh.. SABRAO Journal 17 (1985) 177.

24. K.S. Reddy and S.E. Pawar. In Proceedings "Symposium on Genetics and Biotechnology in crop improvement", Meerut, (1997) Pp. 4.

25. R.G. Thakare. 1988. Induced mutants in blackgram *(Vigna mungo* (L.) Hepper). Ph.D. Thesis, Nagpur University.

26. A. Kumar and M.N. Mishra . Adv. Plant. Sci. 17 (2004) 475.

27. S.K. Mahna, R. Garg and M. Parvateesam. 1989. Curr. Sci. 58 (1989) 584.

28. B.N. Routaray, S.K. Mishra, R.C. Mishra and S.N. Das. Current Agri. Res. 8 (1995) 113.

29. P. Seenaiah, A. Satyanarayana, Y.K. Rao, N.V. Naidu.. Indian J. Agri. Sci. 60 (1990) 337.

30. A. S. Rao and M. K. Jana. 1974. Indian J. Agri. Sci. 44 (1974) 657.

31. R.K. Singh and S.S. Raghuvanshi. Mutation Breeding Newsletter 30 (1987) 16.

32. S.Ignacimuthu and C. R. Babu, In Proceedings, "The contribution of plant mutation breeding to crop improvement". Vienna, (1990) Pp. 171.

33. H.R. Kalia. and D. Tashi,. Science and Culture 30 (1964) 47.

34. M.A. Q. Shaikh and M. A Majid,. In: Proceedings, Sym. on "Induced mutations and molecular techniques for crop improvement", Vienna, (1995) Pp. 635.

35. S.E. Pawar and J. G. Manjaya. (Abstract). 2nd "International Crop Science Congress on Crop productivity and sustainability - Shaping the Future" New Delhi, (1996) Pp.. 258.

36. S.E. Pawar, R. G. Thakare and C. R. Bhatia, In: Proc. "FAO/IAEA Workshop on Improvement of grain legume production using induced mutations" Vienna, (1988) Pp. 235.

37. B.B. Singh, O.L. Chambliss and B. Sharma. 1997. In BB Singh, DR Mohan Raj, KE Dashiell and LEN Jackal (Eds). Co-publication of International institute of tropical agriculture and Japan International Research Centre for Agricultural Sciences, IITA, Ibadan, Nigeria.

38. V.G. Narsinghani. and S. Kumar. 1976. Ind. J. Agric. Sci. 46 (1976) 61.

39. H. Brunner. Mutation Breeding Newsletter 5 (1975) 7.

40. S.A. John.. Ind. J. Genet. Plant Breed. 59 (1999) 353.

41. B. Sharma. In Proceedings "Symposium on radiation and radiomimetic substances in mutation breeding". Sept 26-29.. BARC, Bombay, (1969) Pp. 13.

42. R.N. Pandey and P. Dhanasekar. Journal of Arid Legumes Vol. 1 (2004) 1.

43. S.B. Choulwar and S.T. Borikar. Journal of Maharashtra Agricultural Universities 11 (1986) 244.

44. M.C. Kharkwal. H.K. Hain and B. Sharma. In Proc. workshop, Pullman, 1-5 July, 1986, on Improvement of grain legume production using induced mutations, IAEA, Vienna. (1988) Pp. 89.

45. P. Mahadevu, S.K. Thimmaiah and R.S. Kulkarni. In "DAE-Symposium on induced mutations and molecular techniques in improving crop productivity and quality". Oral presentation-50. Chennai, (1998).

46. D. Kumar Annual Progress Report, National Network Research Project on Arid Legumes, CAZRI, Jodhpur, India, (2003) Pp. 6.

Mutation Experiments and Recent Accomplishments in Trombay Groundnuts

Dr. Anand M. Badigannavar *did his M.Sc. (Agriculture) from University of Agricultural Sciences, Dharwad and joined BARC in 1997. He obtained his Ph.D. (Botany) from University of Mumbai, Mumbai on the project "Genetic improvement for agronomical and biochemical traits in groundnut (Arachis hypogaea L.)". His field of interest includes genetics, mutation and recombination breeding in groundnut leading to the development of improved groundnut varieties. Presently, he is involved in the pyramiding of disease resistant and salt tolerant genes with large seed trait in groundnut and deployment of Trombay groundnut varieties.*

Shri Suvendu Mondal *did his M.Sc. (Microbiology) from Indian Agricultural Research Institute, New Delhi and joined BARC in 2003 through 46th training school. He is currently pursuing Ph.D. on the project "Induction of mutations and its morphological, biochemical and molecular characterization in groundnut." His major research interest is to develop molecular markers for the biotic stresses and characterization of mutants in groundnut. Besides, he is involved in developing Trombay groundnut varieties and their dissemination.*

Introduction

The cultivated groundnut (*Arachis hypogaea* L.) in India is an important oilseed, food and feed crop grown in an area of 6.45 million hectares with a total production of 6.57 million tons. This contributes to 26.6% of world's groundnut area and 18.5% of world's groundnut production. Groundnut occupies nearly 28.3% of the cultivated area and contributes 31.7% of the production of the total oilseeds in the country. It is widely used as principal source of cooking oil, digestible protein, minerals and vitamins in many countries. About 80% of India's groundnut production is crushed for oil, 12% for using as seed, 5% for food and 2% for export. The oil is used primarily for cooking, manufacture of margarine and soaps. Seeds are consumed directly either raw or roasted, chopped in confectioneries or ground into butter. Young pods are consumed as vegetable. The high protein in the de-fatted cake is very good livestock feed. Microbial processing of the groundnut shells and the cakes for the production of the industrially important enzymes holds a good promise.

Induced Mutagenesis in Groundnut

Genetic variability is the most important requirement for success in plant breeding. In nature, mutations are the main source of variability, although the occurrence of natural mutations is less. Ionizing radiations and chemical mutagens enhance the mutation frequency. Keeping specific objectives and aiming for improving one or two traits in a well-adapted variety is the key to success in mutation breeding experiments. Mutation breeding consists of inducing the genetic variability and using the variability either directly or in recombination breeding.

In most of the groundnut mutation experiments, the objectives were to develop high yielding varieties with early maturity, high harvest index, large seed, high oil content, high shelling percentage, moderate seed dormancy, tolerance to biotic and abiotic stresses and improved seed quality traits. The radiation source used for groundnut breeding in the early years was X-rays. Later, gamma rays took a leading role in mutation breeding. The effective dose of 200-350 Gy gamma rays was close to lethal dose $(LD)_{50}$ depending on the

Dr. Anand M. Badigannavar and Shri Suvendu Mondal, Nuclear Agriculture and Biotechnology Division, Biomedical Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: anandgnut@gmail.com

factors influencing radio-sensitivity at the time of mutagen treatment.

Materials used for mutagen treatment are seeds of cultivar, mutant, selection, hybrids or advanced lines. Mutagen treated seeds are sown in the field. Mutants are isolated from second (M_2) generation onwards and their breeding behaviour is studied in the subsequent generations. Since then, pedigree breeding method is followed for agronomic evaluation. Such induced mutants are utilized directly or in recombination breeding by hybridizing mutant X mutant, mutant X cultivar, mutant derivative X mutant or mutant derivative X cultivar (Fig. 1).

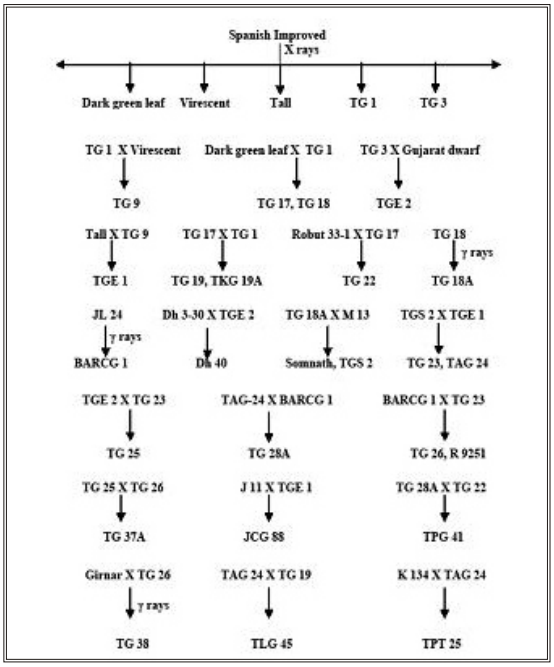

Fig. 1 Evolution of Trombay groundnut varieties

Induction of Genetic Variability in TAG 24

Popular Trombay groundnut (TG) variety, TAG 24 was irradiated with 150, 250 and 350 Gy gamma rays. In all, 71 mutants affecting various characteristics were induced with a frequency of 0.62% [1]. Out of these, one mutant was obtained spontaneously from other mutant, 28 were from 150 Gy, 36 from 250 Gy and six from 350 Gy. Plant height mutants included 16 for dwarf with 24.5% to 41.0% reduction and three for tall with 30% increase in height compared to parent. Plant height in groundnut mutants varies due to more or less internodal length by maintaining similar number of internodes in tall or dwarf mutants, respectively.

Induced mutants for leaf colour included waxy leaf, disease lesion mimic leaf, virescent leaf and golden yellow leaf mutants. Leaves of the disease lesion mimic mutant mimic the symptoms of groundnut rust disease. In virescent mutant, terminal 3-5 leaves are characterized by yellowish light green young leaflets with white coloured rachii and midribs as compared to dark green leaflets with green rachii and midrib in parent. In golden leaf mutant, newly formed leaves had golden yellow colour and remaining leaves were green. The yellowness was more intense in midribs and rachii. After 70 days of emergence, leaf colour turned towards green and whole plant appeared normal. Among the leaf size mutants, six small leaf mutants with 19% to 57% reduction and three large leaf mutants with 33% to 48% increase in leaflet area compared to parent were induced. Reduction in leaf size was common phenomenon in mutant populations.

There were ten mutants with modified leaf shape. In three mutants, instead of two apical leaflets, leaf had a combination of either i) one leaflet and a midrib, ii) two midribs, iii) one leaflet and stalked funnel, iv) two stalked funnels and v) one midrib and one stalked funnel. In long rachis mutant, rachis length had almost doubled. The leaflets of three mutants were near-circular with acute tip i.e., sub-orbicular leaflets as compared to oblong-elliptic in parent. This was evident from significant reduction in leaflet length/width ratio by maintaining similar width compared to parent. These mutants were dwarf with small leaflets. One mutant had drooping leaves facing downwards. All the leaflets of two mutants had involute (cup) leaflets.

The most common flower colour in groundnut is yellow and orange. In one mutant, flower colour was from white to light-orange as compared to orange in parent. At any given time, the mutant was having either all the flowers in white colour or a combination of white and light-orange flowers. Among the pod mutants, four mutants had slight

beak, two had slight constriction and four had deep constriction, four had slight reticulation and one had prominent reticulation compared to moderate beak, constriction and reticulation in parent. Further, pods of one mutant had parallel reticulation because of prominent reticulation along the length of pod. Two mutants had dumb-bell shaped pods when pods are positioned with beak facing down. Among the seed size mutants, four mutants were with smaller seeds with 9 to 25% reduction and ten were with larger seeds with 20.0 to 54.9% increment over parent were evolved. Increased seed size was attributed to the increased cotyledonary cell volume by retaining similar cell number within unit area. As compared to rose testa colour in parent, one mutant each with purple, chocolate and light red testa was induced and three pink testa mutants were induced.

Induction of Genetic Variability in TG 66

Rust resistant breeding line, TG 66 (TFDRG 5) was treated with gamma rays (200 Gy and 300 Gy) and Sodium azide (1mM, 2mM and 3mM for 22 hrs) singly or in combination. In total, 63 true breeding mutants for plant height, plant habit, leaf related traits, flower colour, pod traits, seed size and testa colour were isolated [2]. Leaf mutants included light green leaf, small leaf, narrow leaf, disease lesion mimic leaf, mosaic leaf, virescent leaf, twisted leaf and fused terminal leaflet mutants. There were seven dwarf mutants. Besides, ten rose seed colour mutants, 21 large seed mutants, one hard kernel mutant and two mutants for Virginia bunch (VB) habit were also identified. Probably, these VB mutants were arisen due to reversion as one of the progenitors of the TG 66 was VG 9514, which is a Virginia bunch and resistant to rust and LLS diseases [3]. Among these VB mutants, one was resistant to both the diseases and other was only to LLS. In 21 large seed mutants, seed size increased by 15 to 53% compared to parent. Of these, 18 are with red testa like parent and three are with rose testa and two are with superior pod and seed weights by 15.7 to 46.3%. All the large seed mutants retained their resistance against rust.

Inheritance of Mutant Traits

Inheritance pattern of some of the induced mutants of groundnut was studied. Dwarf mutant of TAG 24 showed incomplete dominance. Disease lesion mimic leaf, funnel leaflet, long rachis mutant traits induced from TAG 24 were due to suppressive gene action. Sub-orbicular leaflet, white to light orange flower and chocolate seed color induced from TAG 24 were governed by single recessive gene [1]. A rose seed colour mutant from TFDRG 5 was monogenic dominant to red seed color [4].

Breeding for Disease Resistance in Groundnut

About 70% of groundnut cultivation is under uncertain rainfed situation due to which the crop often suffers from biotic and abiotic stresses leading to low productivity. Among the biotic stresses, late leaf spot (LLS) and rust are economically predominant diseases, as they together can cause > 70% of yield losses. Breeding for resistance to these diseases is the most economical, cost-effective and viable option. Towards this, recombination breeding was initiated with TAG 24, TG 26 and TG 49 (high yielding TG lines), GPBD 4, B 37c, Mutant 28-2 and R 9227 (disease resistant lines from University of Agricultural Sciences (UAS), Dharwad) [5]. Subsequently, shuttle breeding for higher productivity and disease resistance was practiced between BARC and UAS. Based on the evaluation for resistance and higher yield, three lines had significantly higher pod yield (14-19%) or seed yield (18-24%) than TAG 24 with resistance to LLS and rust (disease score of 3). Three lines had resistance to LLS (score 2-3) and rust (score 2-4) and yields were at par with TAG 24 (score 8). These lines are currently in the national and state level evaluation trials.

In the subsequent disease resistant breeding programme, TAG 24 was hybridized with VG 9514, an inter-specific derivative resistant to both the diseases [3]. F_2 seeds from three F_1 plants out of five were irradiated with 200 Gy of gamma rays. At the end of F_6 and F_6M_5 generations, five recombinants (Trombay foliar disease resistant groundnut (TFDRG) 1 to 5) having rust and LLS resistance (score 1 to 3) were established [6]. VG 9514 scored 1 for LLS and rust disease while, TAG 24 scored 7. Additionally, all the selections showed either enhanced or similar tolerance level to peanut bud necrosis disease compared to their parents. All the selections recorded significantly superior pod and seed yields over VG 9514. Further, TFDRG 1 also showed superiority for pod and seed yield and

TFDRG 2 for seed yield alone as compared to TAG 24. TFDRG 5 had better shelling out turn and TFDRG 2 had higher 100-seed weight over TAG 24. Oil content of the recombinants was maintained as that of TAG 24 except TFDRG 3 where it was lower. This study emphasizes the importance of gamma rays in evolving recombinants having high yield, disease resistance and other superior agronomic traits.

Foliar disease resistant breeding line, TFDRG 5 was treated with gamma rays (200 Gy and 300 Gy) and/or sodium azide (1mM, 2mM and 3mM for 22 hrs) to improve seed size [7]. Apart from maintaining rust resistance like parent, eight mutants registered 15.7-45.6% superiority for pod and seed yield over parent and one mutant with 11.3% superiority over TAG 24. Further, nine mutants had significantly higher seed size (51-61g/100 seeds) than parent and three mutants than TAG 24 (51g). When seven of the mutants screened for peanut bud necrosis disease at Regional Research Station, Raichur, these mutants had 2.9% to 9.3% disease incidence compared to 32.7% in susceptible check, KRG 1.

In order to pyramid the resistance genes for LLS and rust into large seed source, TPG 41, TG 37A, TGM 59, TGM 62, TGM 94 and LSVT 8 were hybridized with TFDRG 5 and Mutant 28-2 [7]. Selection pressure for LLS and rust resistance and for progeny performance resulted in 19 progenies having resistance (score 1-4) as well as increased pod (14-78%) and seed (10-57%) weights by maintaining larger seed (65-128g/100 seeds).

Breeding for Large Seed size in Groundnut

In groundnut, large seeds have consumer preference and contribute considerably to direct consumption and fetch premium price in both domestic and international markets. Some of the available large seed varieties have certain inherent constraints such as longer crop duration (> 140 days), long seed dormancy (> 30 days), lower productivity and lower proportion of large seeds. Further, it is desirable to have greater uniformity in seed size to improve shelling, blanching and roasting process. It would be beneficial to farmers and traders if the varieties have higher proportion of large seeds with higher productivity, less number of days for crop maturity and quality than existing cultivars. One of the objectives of breeding programme at this institute is to develop large seed types. In this context, varieties TG 1, TKG 19A, Somnath, TPG 41 and TLG 45 developed at BARC had addressed some of these constraints [8, 9, 10, 11, 12].

Based on selection pressure for higher productivity, early maturity, bigger seed size, greater frequency of large seeds and testa colour, five recombinants, TG 53, TG 54, TG 65, TG 67 and TG 68 were selected from the cross between TG 22 and TG 40 with BAU 13. TG 53, TG 65 and TG 54 registered increased pod and seed yield (3.5-9.6%) over TG 40. TG 65 had the largest seed with 149-155g 100 seed weight (HSW) followed by TG 67 (140-149g HSW) and TG 53 (130-142 g HSW) compared to BAU 13 (117-129g HSW). Further the highest seed area and perimeter of 223.0 mm^2 and 58.3 mm was noted in TG 65, followed by TG 67 (213.3 mm^2; 57.5 mm) and TG 53 (204.5 mm^2; 57.0 mm) compared to BAU 13 (194.7 mm^2; 55.6 mm). Both seed length and width in TG 65 (22.3 mm; 12.7mm) and TG 67 (22.0 mm; 12.3 mm) were higher than BAU 13 (21.4 mm; 11.8 mm). Seed size distribution among recombinants indicated that TG 53, TG 65, TG 67 and TG 68 had the highest proportion of seeds >80g HSW than BAU 13. Among these, TG 65 and TG 67 also had significantly higher proportion of >100g HSW and >120g HSW seeds. Particularly from the farmers' point of view, higher yield with greater proportion of large seeds is crucial for getting the premium price. It is of interest to note that even with large seeds, their maturity period was around 120 days.

Breeding for Salt Tolerance in Groundnut

Salinity is one of the important abiotic stress, which significantly affects seedling, vegetative and reproductive growth, seed quality and yield of groundnut. Since more and more cultivated land is turning saline, development of salt tolerant groundnut genotype is an appropriate strategy to sustain economic yield and seed quality. In order to breed such a genotype, it is essential to develop a simple and reliable laboratory technique by which large populations can be screened. A simple screening technique was standardized by testing several groundnut varieties from 25mM to 150mM NaCl, wherein 100 mM NaCl was found ideal for

screening large mutant/segregating populations by taking the least reduction in radicle growth as a selection criterion.

A large seeded variety, TPG 41 was irradiated with 200 and 300 Gy gamma rays to induce genetic variability for salinity tolerance [13]. Around 45,775 M_3 seeds from 4,240 M_2 plants were screened with 100mM NaCl. Further, 1,790 seeds were having radicle growth similar to unirradiated distilled water control. Plant-wise M_4 seeds from 1,021 plants were screened without bulking with 100mM NaCl. Using radicle growth criterion for all seeds from each plant, 302 M_4 plants were identified as tolerant plants for radicle growth. Since all the seeds in each of the tolerant plant were with radicle growth as that of unirradiated distilled water control, these plants were genetically true breeding for their response to NaCl treatment. In order to avoid escapes in these tolerant plants, plant wise M_5 seeds were screened with NaCl and 91 true breeding M_5 plants were isolated for radicle growth tolerant to NaCl. As a consequence, only 0.2% of the M_3 seeds treated were true breeding tolerant mutants.

F_3 seeds from the crosses involving TG 37A, TPG 41, NRCG 2419 and NRCG 7548 were screened with 100mM NaCl [13]. Further from all the crosses, 795 F_3 seeds (6.1%) were found to be NaCl tolerant as they were having radicle growth similar to distilled water control and of these 614 plants were survived at harvest in the field. Plant-wise F_4 seeds from all the crosses were screened with 100mM NaCl and 266 plants (43.3%) were found to be tolerant to NaCl based on radicle growth. Following the same protocol, 246 F_5 plants were screened with NaCl and 114 true breeding F_5 plants were isolated for radicle growth tolerant to NaCl. Thus, 0.8% of the F_3 seeds treated were true breeding tolerant genotypes and will be screened at salt affected soils to ascertain their salt tolerance.

Trombay Groundnut Varieties

Mutation research in groundnut evolved several morphologically distinct mutants, which formed an initial gene pool for developing groundnut varieties. Sustained research using mutation and recombination breeding at BARC resulted in the release of 12 TG varieties for commercial cultivation in the country (Table 1). TG 1 was the first induced large seed mutant variety developed by X-ray irradiation of 'Spanish Improved' variety and released in India [8]. Another direct mutant, 'TG 3' was released for Kerala state and became popular in Orissa state [14]. Subsequently, 'TG 17' was released for Maharashtra state [15]. 'TG 17' along with 'TG 1' resulted in large seed variety 'TKG 19A' with 120 days maturity and 20 days fresh seed dormancy for Maharashtra [9]. Under the genomic backgrounds of 'Robut 33-1' and 'M 13', mutant and mutant derivatives of 'Spanish Improved' generated two varieties, 'TG 22' and 'Somnath', and were released for Bihar and Gujarat states, respectively [10, 16]. Genomic blend of five different mutants of 'Spanish Improved' was brought under the background of 'M 13' to develop 'TAG 24' [17] and 'TLG 45' [12]. TAG 24 was released for Maharashtra, Andhra Pradesh, Karnataka, Gujarat, Rajashtan and West Bengal while, TLG 45 for Maharashtra. Similarly, five mutants of 'Spanish Improved' and one mutant of 'JL 24' with 'M 13' background resulted 'TG 26' [18]. TG 26 was initially released for Gujarat, Maharashtra and Madhya Pradesh and later became popular in Karnataka. The genomic combination of TG 26 was diversified with natural mutant Gujarat dwarf to evolve 'TG 37A' [19] and with variety 'Girnar 1' to evolve 'TG 38' [20]. Similarly, 'TPG 41' was resulted from five mutants of 'Spanish Improved' and one mutant of 'JL 24' under 'M 13' and 'Robut 33-1' backgrounds [11]. Among these varieties, TG 37A, TG 38, TPG 41 and TLG 45 were recently released.

TG 37A

TG 37A is a high yielding Spanish bunch variety released for Haryana, North Rajasthan, Punjab, Uttar Pradesh States (Zone I) for rainy season (June-October) in 2004 and Southern Rajasthan and Gujarat (Zone II) for rainy season in 2006 and Orissa, West Bengal, Bihar and North Eastern states (Zone IV) for post-rainy (September-January)/summer (January-May) in 2006 (Fig. 1). This variety is an improvement over TG 26 with respect to seed size, plant biomass and wider adaptability. In the All India Coordinated Research Project on Groundnut (AICRPG) Trials for Zone I, TG 37A recorded mean pod yield of 1963 kg/ha and seed yield of 1246 kg/ha with a superiority

of 26% and 40% over the best check variety, respectively [19]. In these trials, it matured in 114 days with a shelling out turn of 64 % and 100 seed weight of 39g. In Zone II, TG 37A performed well with pod yield of 3048 kg/ha and seed yield of 2173 kg/ha with superiority of 22%. It also recorded pod yield of 3186 kg/ha and seed yield of 2231 kg/ha with superiority of 20% in Zone IV. Thus, TG 37A not only had agronomic superiority but also showed wider adaptability in these groundnut growing states [21]. It is erect, semi-dwarf with sequential flowering, medium-size leaflets, compact pod setting and smooth pod surface. Seeds are with spherical shape and rose colour, containing 48.0% oil, 23.0% protein, 19.3% carbohydrate, 4.5% sucrose and 2.8% crude fibre. Its oil contains 40.7% oleic, 39.8% linoleic and 12.3% palmitic acid. TG 37A has tolerance to collar rot and peanut bud necrosis diseases.

TG 38

TG 38 is a high yielding, Spanish bunch variety evolved by irradiating F_1 seeds of the cross Girnar 1 X TG 26 with 300 Gy gamma rays. It was released for Zone IV for post-rainy/summer in 2006 (Fig. 1). This variety is an improvement over TG 26 for seed size and shelling out turn. In the AICRPG trials for Zone IV, TG 38 recorded mean pod yield of 2768 kg/ha and seed yield of 1984 kg/ha with a superiority of 19% and 21% respectively over the best check variety [20]. It matures in 100-110 days with a shelling of 75 % and 100 seed weight of 45g. It is erect, semi-dwarf with sequential branching, medium-size dark green leaflets, compact pod setting and smooth pod surface. Seeds are more spherical with rose colour. Seed contains 48.0% oil, 22.6% protein, 20.4% carbohydrate, 5.0% sucrose and 2.7% crude fibre. TG 38 oil contains 39.6% oleic, 39.6% linoleic and 12.1% palmitic acid. It has shown tolerance to stem rot and dry root incidence under natural field conditions.

TPG 41

TPG 41 is a Spanish bunch large seed variety released for the entire country for post-rainy/summer situation (Fig. 1). It is an improvement over TKG 19A with respect to higher productivity, larger seed size and greater proportion of large seeds. In AICRPG large seed trials, TPG 41 produced mean pod yield of 2313 kg/ha and seed yield of 1586 kg/ha registering 19% and 29% superiority over the best check variety [11]. It also recorded 49% increased pod yield in 26 farm trials spread over Maharashtra. It matures in 120 days with an average 100-seed weight of 70g and 70% shelling out turn. TPG 41 is erect, semi-dwarf with sequential flowering. Seeds are cylindrical with pinkish rose colour. Seed contains 48.6% oil, 25.0% protein, 20.3% carbohydrate and 3.9% sucrose. Its oil contains 62.4% oleic and 19.3% linoleic acid. TPG 41 has a fresh seed dormancy of 25 days, which prevents in situ seed germination due to end-season rains when crop is ready for harvest.

TLG 45

TLG 45 is another Spanish bunch large seed variety released for Marathwada region of Maharashtra in 2007 (Fig. 1). In multi-location large seed varietal trial in Marathwada during rainy season, TLG 45 produced mean pod yield of 1506 kg/ha and seed yield of 1031 kg/ha registering 28% and 37% increase over the best check variety [12]. It also recorded 23% and 33% pod and seed yield superiority in inter-institutional large seeded varietal trial spread over Maharashtra. It matures in 115 days with an average 100-seed weight of 75g and 70% shelling out turn. It is erect, semi-dwarf with sequential flowering. Seeds are cylindrical with rose colour. Seed contains 49.6% oil, 26.9% protein, 54.3% oleic acid, 27.5% linoleic acid and 12.1% carbohydrate and 4.5% sucrose.

Groundnut mutant/mutant derivatives developed at this centre also contributed as a parental material in the release of varieties at the other agricultural universities in the country. Towards this, University of Agricultural Sciences, Dharwad and Raichur released 'Dh 40' from the cross Dh 3-30 X TGE 2 and 'R 9251' from the cross BARCG 1 X TG 23, respectively for Northern Karnataka. Similarly, Acharya N.G. Ranga Agricultural University, Jagatial and Tirupati released an early maturing variety 'JCG 88' from the cross J 11 X TGE 1 and drought tolerant variety 'TPT 25' from the cross K 134 X TAG 24, respectively for Andhra Pradesh state. Besides, > 300 groundnut induced mutants and breeding lines were developed and maintained at BARC.

Yield Potentials of New Trombay Groundnut Varieties

Among the released TG varieties, TAG-24 and TG-26 in normal seed class and TKG 19A in large seed class became popular among the farming community in India, due to incorporation of desirable traits like earliness, wider adaptability, large seeds and high harvest index, through planned breeding. Farmers' reach for these TG varieties is spread over major groundnut growing states in India. TAG 24 is used as national check for rabi/summer condition in AICRPG trials. In addition, TKG 19A, TG 26 and Somnath are also used as check varieties in the respective national and state varietal trials. As a first step to transfer the benefits of new varieties like TG 37A, TG 38, TPG 41 and TLG 45 to the farmers, large-scale breeder seed production was undertaken. Between 2004 and 2007, >100 tonnes of breeder seed of these varieties was produced and supplied to the State Agricultural Universities in Rajasthan, Gujarat, Madhya Pradesh, Maharashtra and Karnataka and NGOs to conduct frontline demonstrations/adaptive trial and National and State Seed Corporations for further seed multiplication. Feedback received from some of the agencies on the performance of these new varieties is reported here.

Rajasthan

Maharana Pratap University of Agriculture and Technology, Udaipur had conducted 51 frontline demonstrations with TG 37A in 27 villages and 15 with TPG 41 in eight villages along with local varieties. In these demonstrations, TG 37A registered an average pod yield of 2,111 kg/ha with 48% increase over local variety. Similarly, TPG 41 recorded an average pod yield of 2,447 kg/ha with 59% superiority. By cultivating both the varieties, farmers grossed an average net profit of Rs. 10,707/ha and Rs. 13,819/ha, respectively. Rajasthan Agricultural University, Bikaner had also conducted frontline demonstrations using TG 37A on 23 farmers' fields at Bikaner, Jaisalmer, Jaipur, Sikar and Hanumangarh districts. Farmers had harvested an average yield of 1,906 kg/ha.

Maharashtra

Earlier, Mahatma Phule Krishi Vidyapeeth, Digraj had conducted adaptive trials of TPG 41 on 26 farmers' fields in Pune, Nashik, Dhule, Jalgaon, Solapur, Raigadh, Yawatmal, Parbhani, Latur, Sangli, Kolhapur and Satara districts. TPG 41 gave an average yield of 4,551 kg/ha with 49% increase over local variety. Further, from adaptive trials of TG 37A on 15 farmers' fields in Sangli, Kolhapur and Satara Districts, farmers had harvested average pod yields of 3,622 kg/ha with 15% superiority. Krishi Vigyan Kendra (KVK), Nandurbar initially obtained an average pod yield of 2,500 kg/ha with 46% increase over local variety from adaptive trials with TG 37A on fields of seven tribal farmers during summer 2005. Further, during 2005 rainy season adaptive trials extended to 17 tribal farmers resulting an average productivity of 1,512 kg/ha with 71% superiority. During summer, 2006 an average productivity of 3,500 kg/ha with 48% superiority was reported on 19 farmers' fields. Farmers from Pusad, Yawatmal district had harvested 5,000 kg/ha pods by cultivating TPG 41 with a net profit of Rs. 50,000/ha. Farmer from Kehal village, Parbhani district has been producing an average yield of 3,500 kg/ha in Rabi and 6,000 kg/ha in summer from both TG 37A and TPG 41. Another farmer from Nool village, Kolhapur district had harvested 7,000 kg/ha pods of TG 37A.

Madhya Pradesh

Under the Indira Gandhi Gareebi Hatao Yojana, Department of Rural Development, Chhatarpur, 54 quintals of TG 37A breeder seed was supplied to cover around 40 hectares area spread over three villages. More than 100 farmers cultivated TG 37A during 2005 rainy season and produced about 700 quintals seed with an average productivity of 1,680 kg/ha. With an initial encouraging productivity levels, Nowgong Agriculture Producer Company Private Limited, Nowgong, Chhatarpur drawn year-wise plan for producing 10,000 quintals certified seed each of TG 37A and TPG 41 upto 2011. Recently, Jawaharlal Nehru Krishi Vishwavidhyalaya, Jabalpur has reported superior productivity of TG 37A on several farmers. TG 37A was also cultivated under the "Adivasi Sewashram Trust", Jhabua, during 2005 rainy season, on around four hectares area with a total production of 45 quintals and productivity of 1,260 kg/ha, which was encouraging compared to what local varieties grown by the farmers. Further to encourage spread of TG

varieties, 30 quintals of seeds were supplied during 2006.

Gujarat

Both TG 37A and TPG 41 varieties were introduced in Bhuj, Junagadh and Rajkot Districts. Looking into initial encouraging results, one farmer had sown 25 ha with TG 37A and TPG 41 and harvested more than 700 quintal pods. Of which, he sold more than 500 quintals to seed companies and some quantity to Orissa University of Agriculture and Technology and also distributed seeds to more than 100 fellow farmers at Bhachau, Nakatrana and Mandvi Talukas. Later he had harvested around 900 quintals in summer and 1300 quintals in rainy season. Farmers claimed that 70% of the groundnut area in Bhayawadar village in Rajkot district was covered by TG 37A variety. It was learnt that around 15,000 quintals of TG 37A was produced during rainy season, 2007 in this village. In Junagadh District, several farmers had cultivated both the varieties and obtained promising yields compared to local varieties. Farmers started intercropping TPG 41 with Bt cotton hybrid, which adds bonus to their profits. Already few seed companies in Junagadh and Ahmedabad started seed multiplication of these varieties in a big way to facilitate seed availability to the farmers in the state and elsewhere.

Andhra Pradesh

Farmer from Cuddappa District cultivated TG 37A and TPG 41 and obtained an average pod yields of 4,500 and 7,200 kg/ha, respectively. He sold the entire produce to the fellow farmers who in turn reported him an average yield of around 3,000-5,000 kg/ha. He also obtained 5,000kg/ha pod yield with TG 38. Another farmer from Jangalapalle village harvested 3,960 – 4,400 kg/ha pods of TG 37A in 105 days and 4,400 – 5,280 kg pods of TPG 41 in 115 days compared to 1,760 – 2640 kg in local variety.

Karnataka

University of Agricultural Sciences, Dharwad undertaken breeder seed multiplication of TPG 41 in order to facilitate the seed availability and disseminate the new variety among farming community. Under the "Seed Village Concept", 17 quintals of TPG 41 was produced with farmers' participation during 2004-05. Next year, by roping in their own seed farms and farmers at Dharwad, Nippani and Haveri, the University had multiplied 100 quintals of TPG 41. This helped to meet the seed demand by different seed agencies, corporations and farmers by maintaining active seed chain for TPG 41. Farmer from Nippani also harvested 5,500 kg of TG 37A and 5,000 kg/ha of TPG 41 in his first year cultivation.

Goa

Cultivation of groundnut has gained lot of scope in Goa particularly in rice fallows after rainy season. In this situation, groundnut can make use of residual moisture for its growth. Among the groundnut varieties, large seed varieties are preferred as they are utilized for table and confectionery purposes and fetch premium prices. The large seed variety, TPG 41 was supplied to ICAR Research Complex, Goa for conducting frontline demonstrations on farmers' fields in 2004 and 2007. Farmers were impressed with the high yields and larger seed size of TPG 41.

In order to popularize new TG varieties, Directorate of Oilseeds Development, Hyderabad has allocated 7,000 minikits (each with 20 Kg) of TG 37A and 2,700 of TPG 41 among the farmers across the country for the rainy season 2007. For TG 37A, allocation of 2,000 minikits was to Karnataka, 1,500 each to Andhra Pradesh and Chattisgarh, 1,000 each to Madhya Pradesh and Maharashtra. Similarly for TPG 41, allocation of 1,000 each was to Andhra Pradesh and Gujarat, 500 to Madhya Pradesh and 200 to Karnataka. For this purpose, National Seed Corporation Limited supplying 1,400 quintals of TG 37A and 500 quintals of TPG 41 and State Farms Corporation of India supplying 40 quintals of TPG 41.

Molecular Studies in Trombay Groundnut

Random amplified polymorphic DNA (RAPD) and Inter simple sequence repeat (ISSR) markers were used to analyze genetic diversity among 20 cultivated groundnut genotypes differing in disease reaction against rust and late leaf spot. Of the 50 RAPD primers screened, 11 exhibited polymorphism from 12.5% to 76.9% among the genotypes [22]. Genetic distance was minimum between TMV 2 and GPBD 4 and was maximum

between VG 9514 and DTG 57. TFDRG 5 and VG 9514 formed separate cluster and are resistant to LLS and rust. Besides all large seed genotypes (TG 39, TG 40 and TPG 41) formed separate sub-cluster and incidentally they are susceptible to rust and LLS.

With the initial screening disease resistant (VG 9514) and susceptible (TAG 24) genotypes with 50 ISSR primers, 21 produced consistent polymorphism [23]. Out of 154 amplicons produced, 75% were polymorphic. The 3'-anchored primers based on poly 'GA' and poly 'AG' motifs produced high average polymorphism of 75% and 77% respectively. Among these, six having 'AG' repeats generated 38% of total bands where as four having 'GA' repeats generated 15%, indicating higher frequency of 'AG'/'GA' repeats in the groundnut genome. Genetic distance was minimum between TG 40 and TPG 41 and was maximum between TFDRG 5 and SB XI based ISSR analysis. Resistant genotypes like VG 9514, DTG 27, TDFRG 5 and GBFDS 272 were clustered together. On the other hand, five susceptible genotypes clustered separately. Based on Kruskal-Wallis one-way ANOVA, UBC 810_{540} was found to be associated with both rust and LLS resistance and UBC 810_{500} with LLS resistance.

A F_2 mapping population comprising 117 individuals was developed from a cross between rust resistant parent VG 9514 and rust susceptible parent TAG 24. Rust resistance was governed by single dominant gene in this cross. Using bulk segregant analysis, primer kit J7 produced a single coupling phase marker ($J7_{1350}$) and a repulsion phase marker ($J7_{1300}$) for rust resistance [24]. Screening of entire F_2 population by primer kit J7 revealed that coupling phase marker $J7_{1350}$ was linked with rust resistance gene at a distance of 18.5 cM. On the other hand, the repulsion phase marker $J7_{1300}$ was completely linked with the rust resistance. The marker $J7_{1300}$ identified all the homozygous rust resistant genotypes in the F_2 population and was not genotype specific when tested in a group of 11 other resistant and eight susceptible groundnut genotypes. Thus, $J7_{1300}$ can be used for marker assisted selection in the rust resistance breeding programme in groundnut.

References

1. A.M. Badigannavar, Ph D Thesis, University of Mumbai, Mumbai (2007) Pp 1.
2. S. Mondal, A.M. Badigannavar, D.M. Kale, G.S.S. Murty, BARC Newslett., 285 (2007) 237.
3. P.V. Varman, Indian J. Agril. Sci. 69 (1999) 67.
4. S. Mondal, A.M. Badigannavar, D.M. Kale, G.S.S. Murty, Indian J. Genet. 67 (2007) 177.
5. A.M. Badigannavar, D.M. Kale, S. Mondal, M.V.C. Gowda, G.S.S. Murty, 4th International Food Legumes Research Conference, (2005) 345.
6. A.M. Badigannavar, D.M. Kale, S. Mondal, G.S.S. Murty, Mut. Breed. Newslett. Rev., 1 (2005) 11.
7. A. M. Badigannavar, S. Mondal, Progress Rep. 3nd RCM of the CRP "Pyramiding of Mutated Genes Contributing to Crop Quality and Resistance to Stress Affecting Quality", (2007) 1.
8. S.H. Patil, Nuclear India 13 (1975) 7.
9. C. Mouli, D.M. Kale, S.H. Patil, Groundnut News 2 (1990) 3.
10. C. Mouli, D.M. Kale, S.H. Patil, Groundnut News 1 (1989) 5.
11. D.M. Kale, G.S.S. Murty, A.M. Badigannavar, International Arachis Newslett., 24 (2004) 21.
12. D.M. Kale, G.S.S. Murty, A.M. Badigannavar, V.G. Makane, V.N. Toprope, S.P. Shirshikar, N.P. Jangawad, J. SAT Agril. Res. (2008) In press.
13. A.M. Badigannavar, S. Mondal, BARC Newslett., 285 (2007) 226
14. S.H. Patil, R.G. Thakare, DAE Symp. " Radiations and Radiomimetic substances in mutation breeding", (1970) 375.
15. S.H. Patil, Nuclear India, 16 (1977) 3.
16. G.S.S. Murty, A.M. Badigannavar, S. Mondal, D.M. Kale, "Groundnut Research in India" (Eds. Basu M.S. and Singh N.B.), NRCG, Junagadh, India (2004) 57.

TABLE 1. Trombay groundnut varieties released for commercial cultivation across the country

S. No	Variety	Year of release	Pedigree	Released for	Special features	Refer-ence
1	TG 1	1973	X-ray mutant of Spanish Improved	Maharashtra	High yield, large seed	[8]
2	TG 3	1985	X-ray mutant of Spanish Improved	Kerala	High yield	[14]
3	TG 17	1985	Dark green leaf mutant X TG 1	Maharashtra	Less number of branches.	[15]
4	Somnat h (TGS 1)	1991	TG 18A X M 13	Gujarat	Large seed, Semi-runner type	[10]
5	TKG 19A	1993	TG 17 TG 1	Maharashtra	Large seed size, Fresh seed dormancy	[9]
6	TG 22	1992	Robut 33-1 X TG 17.	Bihar	Medium seed size, Fresh seed dormancy	[16]
7	TAG 24	1992	TGS 2 X TGE 1	Maharashtra, Orissa, Karnataka, Rajasthan, West Bengal	Semi-dwarf, earliness, High yield, high partitioning %	[17]
8	TG 26	1996	BARCG 1 X TG 23	Gujarat, North Maharashtra, Madhya Pradesh	High partitioning%, Fresh seed dormancy, smooth pods	[18]
9	TG 37A	2004	TG 25 X TG 26	Haryana, Rajasthan, Punjab, Uttar Pradesh, Gujarat, Orissa, West Bengal, Assam, North Eastern states	High yield, wider adaptability, collar rot tolerance	[19]
10	TG 38	2006	Gamma ray mutant from the cross Girnar-1 X TG 26	Orissa, West Bengal, Assam, North Eastern states	High shelling %, stem rot tolerance, more 3-seeded pods,	[20]
11	TPG 41	2004	TG 28A X TG 22	All India	Large seed, Medium maturity, High oleic acid.	[11]
12	TLG 45	2007	TG 19 X TAG 24	Maharashtra	Large seed, Medium maturity	[12]

17. S.H. Patil, D.M. Kale, S.N. Deshmukh, G.R. Fulzele, B.G. Weginwar, J. Oilseeds Res. 12 (1995) 254.
18. D.M. Kale, C. Mouli, G.S.S. Murty, M.V.P. Rao, Mut. Breed. Newslett. 43 (1997) 25.
19. D.M. Kale, G.S.S. Murty, A.M. Badigannavar, International Arachis Newslett., 24 (2004) 19.
20. D.M. Kale, G.S.S. Murty, A.M. Badigannavar, International Arachis Newslett., 27 (2007) 16.
21. A.M. Badigannavar, D.M. Kale, S. Mondal. G.S.S. Murty, J. Oilseeds Res. 24 (2007) 16.
22. S. Mondal, S. Ghosh, A.M. Badigannavar, International Arachis Newslett., 25 (2005) 27.
23. S. Mondal, S.R. Sutar, A.M. Badigannavar, G.S.S. Murty, DAE-BRNS LSS - 2006, (2006) 93.
24. S. Mondal, A.M. Badigannavar, G.S.S. Murty G.S.S., Euphytica 159 (2007) 233.

Genetic Improvement of Soybean through Induced Mutations

Dr. J. G. Manjaya *joined BARC in 1994. His specialization is in the field of plant breeding and genetics. He has been actively involved in blackgram and soybean improvement programmes using induced mutations and conventional breeding approaches. He has also worked extensively on development of cytoplasmic male sterility system in pigeonpea. He obtained his PhD degree from Mumbai University in 2003 for his work on "Studies on genetic improvement for productivity and quality of soybean". He is responsible for the development of yellow mosaic virus resistant blackgram variety TU 94-2 and multiple disease and pest resistant soybean varieties TAMS -38 and TAMS 98-21 for commercial cultivation. Dr. Manjaya is life member of Indian Society of Genetics and Plant Breeding, Indian Society of Oilseed Research, Soybean Research and Indian Science Congress Association, Kolkata and authored more than 30 scientific publications. Currently he is working on development of soybean varieties resistant to biotic and abiotic stresses with improved oil and protein quality.*

Abstract

In India, soybean has emerged as one of the major oilseeds crop and has revolutionized rural economy and lifted the socio-economic status of farmers. It is mainly grown as a kharif crop and it occupies an area of about 8.0 million hectares with and production of over 7.5 million tonnes. As a result of high protein and fat soybean has a multifold use and can be utilized domestically for meeting the acute protein deficiency in our country. The average yield of soybean in India is 1 tonne per hectare as compared to a world average of 2.2 tonnes per hectare. Narrow genetic base of cultivated varieties in soybean is one of the reasons for low productivity. Improvement in yield is normally attained through exploitation of the genetically diverse genotypes in breeding programmes. Mutations, spontaneous or induced, are an important source for inducing genetic variability. Efficient mutant production systems, through either physical or chemical mutagenesis, have been well established in soybean. A vast amount of genetic variability, of both quantitative and qualitative traits, has been generated through experimental mutagenesis in the past 50 years. At BARC, genetic variability is generated in soybean through induced mutations. Mutants for morphological traits, high oil, low Trypsin inhibitor content, altered protein profile, high harvest index and high yield have been identified and characterized. Two soybean varieties TAMS-38 and TAMS 98-21 has been developed and released for commercial cultivation. In this review article, the success story of induced mutations in soybean in the world and at BARC will be discussed.

Introduction

Soybean (Glycine max L. Merr) is the most important grain legume in the world in terms of production and international trade. It is an economically important leguminous crop for oil, feed, and soy food products and has occupied a coveted place among the oilseed crops being cultivated all over the world. Soybean is ranked number one in world oil production and is widely cultivated in the United States, Brazil, Argentina, China and India. In India, it contributes about 13 per cent to the domestic edible oil pool [1] and the country earns substantial foreign exchange to the tune of Rs. 33,000 million through export of soy meal.

Dr. J. G. Manjaya, Nuclear Agriculture and Biotechnology Division, Biomedical Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: manjaya@barc.gov.in

The history of soybean can be traced back to China where as early as 5000 years ago, Chinese farmers grew soybean. It is considered to be a native of China and has spread to many developing and developed countries of the world. Soybean was introduced in India around 1882 and has been grown since long in Kumaon and Garhwal region where it is known as Bhatt, Bhattawar, Bhatmas and similar type in Madhya Pradesh called Kalitur.

Soybean seed contains 18-23% oil and 38-40% protein. As a result of high protein and fat soybean has a multifold use. It is mainly grown for seeds, which are used for fresh, fermented and dried food products. A large quantity of seed is crushed to extract oil for food and industrial purpose. The oil is converted to margarine, mayonnaise, shortening, salad oils and salad dressing. The soybean meal remaining after oil extraction is used primarily as a source of high protein for animal and poultry feeds. It is also being used in the production of fermented foods like soy sauce, miso, natto, tempeh and sufu and non-fermented foods like soymilk and tofu. The soybean protein is also used in the form of concentrates, isolates and textured protein for human consumption. Thus, the role of soybean as a protein rich food crop and oil crop is well known and can be utilized domestically for meeting the acute protein deficiency in our country. Despite its rich nutritional profile, use of soybean in food has been limited because soybean proteins are often associated with compounds, which could be considered toxic or harmful to the animal body. These are called the anti-nutritional factors. Some of these anti-nutritional factors can be destroyed by heat (protease inhibitors, lectins, goitrogens, antivitamins) while others (saponins, tannins, estrogens, flatulence factors, lysinoalanine, allergens, phytate) cannot be destroyed. Hence, development of cultivars with low or null anti-nutritional factors will help to improve nutritional quality of soybean for domestic use and export.

Soybean is commercially grown in 35 countries around the world. The major soybean producing countries are United States, Brazil, Argentina, China and India. United States accounts for about 50 percent of the total world production. Soybean provides over half the world supply of vegetable protein and about one third of the oil. In India soybean is mainly grown as a kharif crop under rainfed condition and it occupies an area of about 8.0 million hectares with production of over 7.5 million tonnes. The major soybean growing states in India are Madhya Pradesh, Maharashtra, Rajasthan, Karnataka and Rajasthan. Madhya Pradesh, known as "Soybean State" occupies 65 percent of the total acreage under soybean cultivation.

The average yield of soybean in India is 1 tonne per hectare as compared to a world average of 2.2 tonnes per hectare. The major constraints for low productivity of soybean are poor seed viability, non-availability of early maturing, photoperiod insensitive high yielding cultivars carrying resistance to biotic and abiotic stresses. Narrow genetic base of cultivated varieties in soybean is of global concern. The degree of genetic variability available for selection can play an important role in overcoming yield barriers. Improvement in yield is normally attained through exploitation of the genetically diverse genotypes in breeding programmes. Mutations, spontaneous or induced, are an important source for inducing genetic variability. Improvement in either single or few economic traits and quality characters can be achieved with the help of induced mutations within the shortest possible time. Induced mutations have been widely accepted as a supplementary approach in the crop improvement programme, thus speeding up the breeding programme considerably. Mutation breeding has played a significant role in the development of many crop varieties [2]. In India, five soybean varieties (Birsa soy 1, VLS-1, NRC-2, NRC-12 and TAMS 98-21 have been developed using induced mutations.

In this paper the role of mutation breeding in soybean improvement has been reviewed. Soybean improvement work carried out at BARC has been highlighted.

Mutation Breeding Studies in Soybean

Mutation breeding in crop plants has its significance in selection of desirable genotypes and directly using them either as cultivars or as parents in recombination breeding programme. Efficient mutant production systems, through either physical or chemical mutagenesis, have been well established

TABLE 1.Mutagens and doses commonly used for soybean mutagenesis

Mutagen	Material	Practiced/recommended Doses
Fast neutron	Dried seeds	10-45 / 15-25 Gy
X rays	Dried seeds	40-200 / 100-200 Gy
rays	Dried seeds Seedling (chronic)	80-400 / 200-300 Gy 15-50 Gy/ —
Ethyl methane sulphonate (EMS)	Pre-soaked seeds	0.025-0.05 M, 8h / —
Diethyl sulphate (dES)	Pre-soaked seeds	0.15 %, 1.5-2.5h / —
N-methyl-N-nitroso urethane(NMU)	Pre-soaked seeds	0. 5 .0 mM, 5h / —
Sodium azide (NaN_3)	Pre-soaked seeds	3.0 .0 mM, 2h / —
Ethyleneimine (EI)	Pre-soaked seeds	0.04% 5h/—

in soybean. A vast amount of genetic variability, of both quantitative and qualitative traits, has been generated through experimental mutagenesis in the past 50 years.

Mutagenesis

Various physical and chemical mutagens had been tested for soybean mutagenesis. The commonly studied parameters for the mutagen of interest include its dosage effect, optimum doses, mutation frequency and spectrum. The decision of using a particularly mutagen is not always based on its effectiveness, but on their availability, the convenience for treatment and post-treatment management. Studies on induced mutation in soybean were first carried out by neutrons by Humphrey in 1951[3]. Various physical and chemical mutagens had been tested for soybean mutagenesis during the past 50 years. The recommended optimum doses could vary significantly from one study to another since mutagenic effect can be influenced by genetic susceptibility and its physiologic status. Several mutagens have been tried and proven to be capable of inducing mutations in soybean. Gamma rays have so far been the most widely used mutagen; more than 90% of the mutant varieties were developed from mutants induced through gamma irradiation (http://www-mvd.iaea.org). Combined treatment of physical and chemical mutagens are also been reported to have synergetic effects. Viable mutants have been isolated from 250 Gy + UV treatment [4]. Two soybean varieties were exposed to the beam from a helium–neon laser in combination with 0.025% or 0.05% ethyleneimine (EI) and greater genetic variation was observed [5]. Combined treatments of fast neutrons with NMU showed synergistic effects in soybean [6]. Treatment of 100 Gy gamma rays plus 0.4 M EMS was the most effective in inducing genetic variability [7]. The different mutagen used, the doses commonly practiced in soybean mutagenesis are summarized in Table 1.

Mutational Enhancement of Genetic Diversity

Morphological Mutants

Mutagenic treatment have induced a number of mutants with altered morphological characters like growth habit, plant stature, leaf characters, leaf shape, size and colour, flower colour, pod and seed characters. Mutants induced in soybean by mutagenic treatment are listed in Table 2.

Mutations for Quantitative Traits and Qualitative Traits

Agronomic Traits

Induced genetic variability of quantitative traits, i.e. maturity, plant height, and yield components (pods per plant, seed per pod), were observed in many experiments. A number of mutants

TABLE 2. Induced mutants for morphological characters

Character	Characteristics
Chlorophyll deficiency	Albina, xantha, chlorina and virescent
Growth habits	Tall, dwarf, bushy and viny plant type, semi determinate
Root Character	Non fluorescent
Leaf characters	Two opposite trifoliate leaves per node; small leaf & crinkled leaf
Floral Characters	White flower, purple flower ,sterility
Seed colour	Yellow and green, brown and white, light brown coloured hilum

were generated with altered performance of quantitative traits around the world. However, most mutant varieties released for commercial production involved improved performance of quantitative traits, i.e. maturity, plant height, pods per plant, seed per pod and high yield.

High Oil and Protein

Mutation techniques had also been successfully used to develop a range of novel characteristics in soybean, from changing the content of seed oil and protein, reducing anti-nutritional factors, eliminating allergenic components, resistance to insect pests and diseases and tolerance to herbicides. Soybean oil contains 11% palmitic, 3% stearic, 22% oleic, 56% linoleic and 8 % linolenic acid. The content of linolenic acid is relatively high in commercial soybean and is responsible for oxidative instability of cooking oil. Mutation techniques are regarded as the most effective breeding approach to modify fatty acid composition in soybean [8]. A large number of mutants with altered fatty acid composition have been isolated in soybean.

Altered Protein Profile

Two types of proteins are found in legumes, water-soluble protein fraction known as albumins and saline soluble protein fraction known as globulins. Soybean protein is mainly of globulin type. Two types of globulins are found in soybean termed as glycinin and conglycinin. -Conglycinin (7S globulin) and glycinin (11S globulin) are the major components of storage protein in soybean. The 7S globulin is low in sulphur-containing amino acids, and thus exhibits poor nutritional and food processing properties than the 11S globulin. Furthermore, the -subunit of -conglycinin is a major allergenic protein of soybean. Therefore, the reduction of -Conglycinin level is deemed useful. Mutants which lacked both and subunits of -conglycinin and which lacked the ' subunit but had low level of and subunits were isolated through gamma rays treatment [9]. A mutant soybean line with very low level of -conglycinin was observed following seed irradiation with 200 Gy gamma rays. Mutants lines lacking and '-subunits of -conglycinin and A_3 subunit of the glycinin were observed following seed irradiation with 250 Gy gamma rays [10].

Lipoxygenase lacking Soybean

Soybean has a rancid or grassy beany flavour and is a major antinutritional factor which restricts the acceptance of full fat soy protein-based foods. The enzyme lipoxygenases catalyse the oxidation of polyunsaturated fatty acids to produce hydroperoxides which is broken down by other enzymes to form off-flavours. Soybean seeds have three lipoxygenases named L-1, L-2 and L-3, respectively. From the progenies of irradiated F_2 seeds of the cross between two lines lacking lipoxygenase enzymes L-1L-3 and L-2L-3 with 150 Gy gamma rays, a new mutant variety was released for commercial cultivation [11].

Low phytic Acid

Phytic acid is a major storage form of phosphorus in soybean seed. However, organic

phosphorus in phytic acid is indigestible in humans and non-ruminant animals, which affects nutrition and causes phosphorus pollution of ground water from animal wastes. Development of low phytic acid mutant soybean lines would help to over come the problem cause by phytic acid. Low phytic acid content mutants were identified and characterized [12-14]

Supernodulating and Herbicide Resistant Mutants

Soybean plants have the ability to fix atmospheric nitrogen (N) thereby can restore soil fertility. Mutants that had significantly high nodulating ability (supernodulating or hypernodulating) were generated through either EMS mutagenesis [15] or through fast neutron irradiation [16]. Resistance to herbicides is an important character in modern soybean production, mutants with a high degree of resistance to both post- and pre-emergence of various sulfonylurea or chlorsulfuron herbicides were isolated [17]. A supernodulating variety Sakukei 4 with high yield is developed in soybean [18-19].

Genetic Studies on Induced Mutants

Studies on the inheritance of mutant traits carried out by various workers in soybean are summarised in Table 3.

Released Varieties

According the FAO/IAEA Mutant Variety Database (http://www-mvd.iaea.org) more than 100 new soybean varieties were developed worldwide using mutation technique. Among those varieties, more than 90% were developed using gamma rays treatment. Most of the mutant varieties were selected for high yielding, high harvest index, early maturity, quality.

Induced Mutants and Functional genomic Studies

Soybean functional genomic studies are far behind Arabidopsis and rice since soybean genome is not fully sequenced. Induced mutants will constitute an important genetic resource for annotating the function of many genes. Once the soybean genome sequence is completed mapping of mutated genes will become easier and common practice similar to rice. With the rapid development of molecular and genomic tools, the screening of induced mutations in a mutated population could be undertaken at the DNA Level, using TILLING (Target-Induced-Local-Lesions-IN-Genomes) [20]. All these molecular and genomic tools will undoubtedly increase the efficiency and effectiveness of the use of induced mutations for soybean improvement.

Work carried out at BARC

Induced Mutation Studies

Mutants for Morphological Characters

Soybean genotypes JS 80-21 and VLS-2 were irradiated with 250 Gy gamma rays for improving yield and biochemical characters. A large number of mutants affecting the morphological characters were identified and characterized. The total morphological mutants observed in the genotype JS 80-21 were 55 with mutation frequency of 1.34 percent and VLS-2 were 277 with a mutation frequency of 3.68 percent. In the M_2 generation, chlorophyll and viable mutants affecting morphological characters were identified from the two cultivars JS 80-21 and VLS-2. Two pink flower mutants were observed in the cultivar JS 80-21 having light pink flower colour. Two crinkle leaf mutants were also observed in the cultivar JS 80-21. In the cultivar VLS-2 nearly 80 different types of dwarf mutants were isolated and the frequency (1.07%) was high as compared to JS 80-21. Good pod bearing mutants were observed in both the cultivars. One of the mutant exhibited pleiotropic effect having alteration in more than one character. The mutant was dwarf with small leaves, more branches and dark green foliage with late maturity.

Mutants for High Oil and Low trypsin Inhibitor (TI) Content

Oil content of seed samples was determined by solvent extraction using Soxlet apparatus [Soxtec system – HT (1043)]. Screening of 7480 M_2 plants of the cultivar VLS-2 for high oil content and low TI content resulted in the identification of high oil content mutant M-387 (22.7 %) as compared to parent VLS-2 (19.7 %). Trypsin inhibitors are one of the most important antinutritional factors in soybean. They decrease the digestibility of protein

TABLE 3. Inheritance of mutant traits in soybean

Mutation affecting plant parts/characters	Inheritance
Morphological mutants	
Dwarf mutant Short petiole Sterile Necrotic root mutants Root fluorescence	Single recessive gene Single recessive gene (sp) Single recessive gene (ms5) Single recessive gene Single recessive gene
Biochemical mutants **Proteins**	
-subunit deficiency of -conglycinins	Single recessive allele
and -subunits deficiency of conglycinin	Single recessive gene
Fatty acids	
Reduced palmitic acid	Recessive fap2(J10) fapx(KK7), Different alleles at two independent loci (fapx), Recessive alleles, Recessive non allelic, sop1 (J3) & sop2(J10), Four independent loci,
High stearic acid	Recessive alleles at single locus
High Oleic acid	Single gene,Ola(M11) & Ol(M23), Two alleles at single locus with partial dominant effect
Low linolenic acid	Recessive fan Xa, Additive gene effect Fanafanb, Single recessive nonallelic fanX(KL-8) & fanX(M5), Recessive allele FanXa(M24)
High linolenic acid	Linr (major single gene)
Root nodulations	
Hypernodulating	Recessive allele, Single recessive rj7
Super nodulations	Single recessive allele

and cause pancreatic hypertrophy. A modified rapid and reliable method was used for screening trypsin inhibitor content in soybean seeds. Trypsin inhibitor activity was estimated in the seeds of 7480 M_2 plants by micro titration plate technique. Three mutants namely M-213 (13.7 TIU (trypsin inhibitor unit)/mg seed meal), M-104 (15.4 TIU/mg seed meal) and M-291 (15.9 TIU/mg seed meal) showed lower levels of TI content as compared to the parent VLS-2 (21.8 TIU/mg seed meal). One of the mutants M-225 (28.5 TIU/mg seed meal) exhibited higher trypsin inhibitor content.

Protein Profile

The protein profile studies showed variability in the SDS-PAGE profiles of the VLS-2 mutants. Four mutants M-291, M-17, M-231 and M-468 were characterized by the absence of A_3 subunit of glycinin. Two mutants M-291 and M-468 also showed low levels of ', subunits of the 7S protein

and low levels of A's subunit of 11S protein. Mutants M-17 and M-231 were also characterized by the absence of ' and subunits of -conglycinin and increased levels of subunits of -conglycinin. Mutants M-231, M-17, M-291 and M-468 demonstrated absence of A-4 subunit of 11S protein [10].

Genetic Diversity of Mutants

Multivariate analysis has been found to be a potent biometrical tool in quantifying the degree of divergence in the germplasm. Mutants of JS 80-21 and VLS-2 were analyzed for studying diversity. Three cluster groups were formed on the basis of cluster analysis among the mutants of JS 80-21. Mutant M-59 grouped in Cluster III showed maximum dissimilarity of 26% from the parent JS 80-21. Six major clusters were observed in the mutants of VLS-2. Only one mutant M-17 was grouped in cluster VI and showed maximum dissimilarity value of 24% from the parent. This indicates that mutants M-59 and M-17 are genetically diverse and may give rise to high heterotic response when used in hybridization programme.

Breeding for Disease Resistance

Bacterial Pustule Disease

Bacterial Pustule disease caused by *Xanthomonas campestris* pv. glycines is one of the major bacterial diseases of soybean prevalent in the world. Yield losses due to the bacterial pustule disease in soybean are reported to be 15% in United States, 30 to 95% in Ukraine and 40% in Thailand. In India considerable damage to the soybean crop by bacterial pustule is reported. The studies were carried out at BARC with the objective to develop rapid and reliable in vitro screening method, inheritance of resistance and development of resistant varieties for the bacterial pustule disease.

Screening Technique

An improved laboratory technique of screening for bacterial pustule resistance was developed. Rooting and survival studies indicated that soybean leaves excised from 25 day old plants could be maintained at 22^0C to 28^0C for about 30 days in enamel trays filled with water. Inoculation of excised leaves with 5ìl bacterial suspension of 10^7 to 10^9 colony-forming units (cfu/ml) and incubation at 27 ± 1^0C with 12 h/day illumination of 4136 lux/m^2 showed initiation of chlorotic symptoms 48 hours after inoculation (HAI) and clearly visible symptoms were observed at 72 hours after inoculation in the susceptible genotypes [21].

Identification of Resistant Sources

Screening of 55 soybean genotypes for bacterial pustule resistance showed that only one genotype P-4-2 did not develop symptom up to 120 HAI and was identified as resistant genetic stock. All the other genotypes showed initiation of chlorotic lesions 48 hours after inoculation and clearly visible symptoms were observed at 72 hours after inoculation and were identified as susceptible.

Inheritance Studies

Reciprocal crosses were made between susceptible genotype Monetta, PK 472, JS 80–21 and resistant stock P-4-2. Inheritance of the resistance was studied using excised leaf technique. The F_1 plants showed susceptible reaction 96 hours after inoculation indicating that susceptibility was dominant over resistance. The F_2 population segregated into 15 susceptible to 1 resistant indicating that duplicate recessive genes control resistance [22]. In the F_3 generation, the genotypic segregation among the progenies showed excellent fit to the expected ratio of 7 (susceptible true breeding) : 4 (segregating into 15 susceptible to 1 resistant) : 4 (segregating into 3 susceptible to 1 resistant) : 1(homozygous resistant).

Development and release of Soybean Varieties

Two soybean varieties TAMS-38 and TAMS 98-21 have been released for commercial cultivation. The salient features of both the varieties are given in Table 4. Both the varieties are becoming popular among the farmers in Vidarbha region of Maharashtra. TAMS-38 was cultivated in more than 1 lakh hectares during 2005-2006. The collaborative partner Dr. Punjabrao Deshmukh Krishi Vidyapeeth, Akola produced and distributed more than 165 Q of breeder seed of TAMS-38 during 2005-2006.

TABLE 4. Trombay soybean varieties released and notified for commercial cultivation

Name	Year of Release	M: Maturity (days) Y: Yield (kg/ha) YI:Yield increase (%)	Released for	Remarks
TAMS-38	2005	M: 95 Y: 1800-2200 YI: 20	Maharashtra	Early maturing, resistant to bacterial pustule, Myrothecium leaf spot
TAMS 98-21	2007	M: 103 Y: 2200 -2600 YI: 21	Maharashtra	Resistant to bacterial pustules, myrothecium leaf spot and soybean mosaic virus and moderately resistant to rust and other leaf spot disease

References

1. B.U. Dupare, S. S. Vinayagam, S. D. Billore and O.P. Joshi, Soy Res 3 (2005) 58.
2. K. Nichterlein, H. Bohlman, S. Niclen and M. Maluszynski, Proc. Of DAE – BRNS-2000, Pg2.
3. H. W. Johnson and R. L. Bernard, 1963. Soybean Genetics and breeding. In book: The soybean, Edited by A. G. Norman Academic Press. pp 61 – 65.
4. P. S Bhatnagar, S. P. Tiwari, and J. S. Sandhu, Mutation Breeding Newsletter 339 (1989) 15.
5. S. Plesnik, Acta Facultatis Rerum Naturalium Universitatis Comeniawae, Genetica et Biologia Molecularis 24 (1993) 105.
6. T. Sodkiewcz, and W. Sodkiewcz, Bodenkultur 50 (1999) 191.
7. D. D. Ahire, R. J.Thengane, J. G., Manjaya, M. George and S. V. Bhide, Soybean Research 3 (2005) 1.
8. Y. Takagi, A. B. M. Hossain,, T. Yanagata and S. Kusaba, Japan J. Breed. 39 (1989) 403.
9. I. Nakamura, H. Odonaka and N. Kaizuma, National Institute of Genetics. Japan (1989) 115.
10. J. G. Manjaya, K. N. Suseelan, T. Gopalakrishna, S. E Pawar and V. A. Bapat, Food Chemistry 100 (2007):1324-1327.
11. M. Hajika, K. Igita, and Y. Nakazana, Jpn. Agric. Res. Q 29 (1995) 73.
12. S. A. Sebastian, P. S. Kerr, R. W. Pearlstein and W. D. Hitz, In JK Drackley, ed, Soy in Animal Nutrition. Federation of Animal Science Societies, Savoy, IL, (2000) Pg 56.
13. J. Wilcox, G. Premachandra, K. Yound and V. Raboy, Crop Sci 40 (2000) 1601.
14. F. J. Yuan, H. J. Zhao, X. L. Ren, S. L. Zhu, X. J. Fu, Q. Y. Shu,. 2007 (In press)
15. B. J. CarolL, D. L. Mcneil and P. M. Gresshoff, Proc. Natt. Acad. Sci. USA. 82 (1985) 4162.
16. A. E. Men, T. S. Laniya, I. R. Searle, I. Iturbe-Ormaetxe, I. Gresshoff., Q. Y. Jaing, B. J. Caroll and P. M. Gresshoff, Genome Lett. 3 (2002) 147.
17. S. A. Sebastian, G. M.Fader, J. F. Ulrich, D. R. Forney, and R. S. Chaleff, Crop Sci. 29 (1989) 1403.
18. M. Takahashi, J. Arihara, N. Nakayama, M. Kokubun, S. Shimada, K. Takahashi and M. Hajika, Bull. Natl. Inst. Crop Sci.. 4 (2003) 17.
19. M. Takahashi, J. Arihara, N. Nakayama and M. Kokubun, Plant Prod.Sci. 6 (2003) 112.
20. C. M. McCallum, L. Comai, E. A. Greene and S. Henikoff, Plant Physiol. 123 (2000) 439.
21. J.G. Manjaya, Changing Global Vegetable Oils Scenario: Issues and Challenges before India , Directorate of Oilseeds Research, Hyderabad. (2007) Pg 71.
22. J.G. Manjaya, and S. E. Pawar, Euphytica 106 (1999) 205.

Genetic Improvement of Rapeseed-Mustard Through Induced Mutations

Dr. S. J. Jambhulkar *SO 'F' obtained M.Sc., Ph.D. Degrees from Indian Agricultural research Institute (IARI, New Delhi) in 'Genetics' and joined BARC in 1991. His area of specialization is mutation breeding for genetic improvement of oilseed crops namely sunflower and Indian mustard. He has developed two high yielding varieties namely TAS 82 in sunflower and TPM1 in mustard. He has been actively involved in crop improvement programme of various Agricultural universities*

Shri A.S. Shitre *is B.Sc.(Agri.) from Dr. P.D.K.V., Akola and joined BARC in 2001. Since then he is assisting the research activities on sunflower and mustard.*

Introduction

Brassica species are second most important oilseed crops in India after groundnut. It's cultivated area is 6mha with the productivity in the range of 900-1000kg/ha. To fulfill ever-increasing demand of oil, seed yield and oil yield needs to be improved. Crop improvement is a key route to ensuring continued benefits arising from food and plant products. Wide range of genetic, biochemical and metabolic variation needs to be generated for effective crop improvement. Mutation breeding is one of the approaches to enhance the spectrum of variability for characters of agronomic and economic significance, which is a prerequisite in crop improvement programme. It has been successfully employed to enhance the production and productivity of crop plants [1,2,3]. Mutagenesis has also been successfully employed in genetic improvement of qualitative and quantitative traits in oleiferous *Brassica* species [4,1]. Overview on the modifications in morphological, biochemical and yield attributes through mutagenesis, and their direct and indirect use to develop high yielding varieties have been discussed [5]

Morphological Characters

Morphological characters play an important role as phenotypic markers to identify and maintain the purity of genotype as well as in linkage mapping. Desirable changes in a specific character without affecting the rest of the genome could be induced and utilised in basic studies and for the development of high yielding varieties.

Dwarfing genes could also be useful in *Brassica* species to increase seed yield by reducing lodging and increasing harvest index. Dwarf mutants compared to their parents have been isolated in rapeseed-mustard using physical and chemical mutagens. The exploitation of male sterility for the production of hybrids is one of the approaches to break the yield barrier in crop plants. Male sterile mutants in *B. juncea* have been isolated using chemical mutagens.

Siliqua of rapeseed-mustard stands approximately 50^0 angle to the raceme branch. Reduction of angle would cut down the vigorous growth of aphids. In *B. juncea*, X-ray induced appressed pod mutants were isolated from variety Rai5 and RL 9. Tri and tetra-locular siliqua and

Dr. S. J. Jambhulkar and Shri A.S. Shitre, Nuclear Agriculture and Biotechnology Division, Biomedical Group, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085

non-shattering mutations were isolated from variety Pusa Jaikisan using chemical mutagens.

In general, seeds of Brassica species are brown/black in colour. Yellow seeded rapeseed-mustard are more desirable than brown/black seed because it has thinner seed coat, higher oil content, high protein and lower fibre content than brown seeded varieties within same genetic background. It has improved nutritive value of the meal after oil extraction. Yellow seeded genotypes are available in *B. rapa, B. juncea*, and *B. carinata*. No natural or induced mutations have been reported in *B. napus*. Till late 1960s all *B. juncea* genotypes available in the germplasm collection had brown or black seed coat. Induced mutations to isolate yellow seed coat was initiated at BARC, Mumbai, India. Using ^{35}S radioisotope, two yellow seed coat mutants (YSM1 and YSM2) were isolated from blackish brown seed variety Rai5. New yellow seeded mutant was isolated from the same variety Rai5 using ^{32}P radioisotope [6]and named as Trombay Mustard 1 (TM1). Using this mutant in cross breeding programme, improved high yielding genotypes were developed .The yellow seed coat mutants and their derivatives were extensively used in cross breeding programme throughout India and large number of high yielding bold seeded genotypes has been developed [7] In recent years, we have also isolated yellow seed coat mutant from most popular variety 'Varuna' using gamma rays (data unpublished). These mutants are being used in cross breeding to develop high yielding varieties [8].

Yield Contributing Characters

Various yield components are directly or indirectly contribute to seed and oil yields. Induced mutations have been successfully employed to isolate mutants for desirable economic characters such as plant height, number of siliquae per plant, number of seeds per siliqua, seed weight, seed yield and oil content. The most important yield contributing characters in rapeseed-mustard are numbers of primary and secondary branches, siliquae per plant, siliquae on main fruiting axis, number of seeds per siliqua and seed weight. Mutants with more branches and siliquae per plant have been reported in rapeseed-mustard.

Reduction in time to flowering could well be at the expense of yield potential. However, mutation for early flowering in the agronomically superior lines could be useful in the various cropping patterns. Early flowering mutants were obtained in *B. napus* and in *B. juncea*.

Modification in Fatty Acid Composition

Edible oil is an important component of the human diet and provides energy. In vegetable oils, the major part of the fatty acids is represented by oleic acid (C18:1), linoleic acid (C18:2) and linolenic acid (C18:3) along with other fatty acids like palmitic and stearic acids. Traditional rapeseed-mustard oil contains high proportion (~50%) of erucic acid (C22:1) and thus it is an exception to other vegetable oils. Reduction of erucic acid to zero level was found to be nutritionally desirable. Mutation breeding has been successful in tailoring oil crops for desirable fatty acid composition [4] because oil crop plants tolerate wide range of fluctuation in fatty acid composition without loosing viability and single mutation can result into desirable oil composition. Liho is the first zero erucic acid mutant in *B. napus*, which opened the era of mutant assisted quality improvement in oilseed crops. [9] Suggested that erucic acid free oil should also occur in *B. campestris* as it is one of the parents of *B.napus* and reported zero erucic acid natural mutant/variability in *B. campestris*. Two zero erucic acid natural mutants/variability were found in Chinese accession of *B. juncea* and termed them as zem1 and zem2. High level of erucic acid also has industrial applications, however, mutation for high erucic acid has not yet reported.

Linolenic acid is easily oxidized and oil cannot be stored for long period. However, no variety or species of cruciferae was found free from linolenic acid. In view of this, efforts in Germany on mutation breeding programme resulted in reduced linolenic acid mutant in *B. napus*. High oleic acid in oil is considered as nutritionally desirable for human health. High oleic acid mutant in *B.napus* has been reported. This initial success of mutant isolation has laid foundation stone for improvement of oil quality in Brassica crops.

Varietal Developmet

Utilization of induced mutations in crop improvement programme has resulted in the development of 31 high yielding varieties in rapeseed–mustard crops. Among them, 12 are in *B. juncea*, 14 in *B.napus*, 2 in B.rapa, and 3 in white mustard. Fifteen varieties have been developed using gamma rays and 4 by X-rays. Remaining varieties were developed using chemical mutagen and mutants used in hybridization. In *B. juncea*, a gamma ray induced mutant RLM-198 with 25% higher seed yield, moderately resistant to aphids and leaf miners, higher oil content and 5-6 days earlier maturity. Another mutant RLM 214 with 22% more seed yield with high oil content and shattering resistant from parent RL-18 using X-rays. Mutations for increased seed yield resulting into the development high yielding varieties have also been reported . Backcrossing of M-11, a low linolenic acid mutant with 'Regent' resulted into the development of high yielding low linolenic acid variety 'Stellar' and Apollow . Mutation breeding efforts at BARC has resulted into the development of 3high yielding varieties namely, TM2, TM4 and TPM1. Among them TM2 and TPM 1 are direct mutants whreas TM4 is derivative of mutant used in breeding programme. TM4 and TPM1 are yellow seed coat varieties.

References

1. C.R. Bhatia, K. Nichterlein and M. Maluszynski, Mutation Breeding Review 11 (1999) 1-36.
2. V.L. Chopra, Curr. Sci., 89: (2005) 353-359.
3. M. Maluszynski, B. S. Ahloowalia and B. Sigurbjornsson, Euphytica, 85 (1995) 303-315.
4. G. Robbelen, Mutation Breeding Review 6 (1990) 1- 43.
5. S. J. Jambhulkar, Botanical Research (Rapeseed Breeding) (2007) 418-428.
6. G. G. Nayar, F. Hook and Thomas, Sci. and Cult., 34 (1968) 421-422.
7. V. Abraham and C. R. Bhatia, Plant Breed. 97 (1986) 86-88.
8. S. J. Jambhulkar, A.S. Shitre, and B. Proceedings of Conference on Resource Development and Marketing Issues in Rapeseed-Mustard, March 28-29, Jaipur, India, (2005) 27-28.
9. R. K. Downey, Can J. Plant Sci., 44 (1964) 295.

Radiation Induced Mutagenesis of Plant Cell and Tissue Cultures

Dr. P. Suprasanna *joined BARC in 1991 after obtaining Ph.D. degree in Genetics from Osmania University, Hyderabad. He is working in the Nuclear Agriculture and Biotechnology Division and is engaged in plant biotechnological research on banana and sugarcane. His interests are in using plant tissue and cell cultures for plant genetic manipulation. Under "DBT Overseas Associateship" he worked as host professor at the Katholic University of Leuven, Belgium in 2000-2001. Dr. Suprasanna has more than 100 research publications to his credit and is a member of the editorial board and referee of scientific journals. He has guided several students for their M.Sc. and Ph.D. in Biotechnology and Agricultural Biotechnology. Dr. Suprasanna is serving as the Member, Research advisory committee, UGC-SAP, University of Pune, and as a Visiting Faculty Member at the Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola (since 2006). His current research activities include cellular and molecular aspects of abiotic stress tolerance.*

Dr. T.R. Ganapathi *joined BARC in 1991, did his Ph.D. degree in Botany from Karnatak University, Dharwad and is working in the Nuclear Agriculture and Biotechnology Division. He has established micropropagation protocols in banana and the technology has been transferred to user agencies. Dr. Ganapathi was awarded "DBT Overseas Associateship" for his post-doctoral research on genetic transformation in banana during 1997-99, and has worked at the Boyce Thompson Institute for Plant Research Inc., Cornell University, Ithaca, NY, USA. He was awarded INS medal in 2004 for his contribution to the banana biotechnological research. Currently, he is working on genetic engineering of crop plants for the incorporation of useful traits.*

Dr. Vishvas M. Kulkarni*, basically a plant breeder, holds the Ph.D. (Biotechnology) degree of Mysore University. After spending a few years at the "Plant Molecular Biology Group, NCL, Pune" and "All India Coordinated Pearl Millet Improvement Project, Pune", he joined BARC as a Scientific Officer in 1993. Since then he has been working on genetic manipulation of Banana and Pearl Millet using tissue culture, genetic transformation and related molecular biological techniques. He was awarded the Biotechnology Overseas Associateship Award (2002-03), of the Department of Biotechnology, Government of India, and worked at the Coastal Plain Experiment Station (Tifton), University of Georgia, USA, during 2004-05. His work on mutagenesis in vitro using Gamma irradiation has resulted in the isolation of banana mutants. To his credit have been several research papers published in National-International Journals / Symposia / Workshops etc.*

Dr. P. Suprasanna, Dr. T.R. Ganapathi and Dr. V.M. Kulkarni, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: prasanna@barc.gov.in

Introduction

Since the dawn of agriculture, humans have been cultivating and manipulating crop plants to enhance their quality and yield. Genetic selection and manipulation via conventional breeding, mutation induction and hybridization have contributed greatly in developing superior crop varieties. Despite such noteworthy accomplishment in enhancing agricultural productivity of crop plants, the ever-changing situation in world population, land resources, farming methods and climate have imposed a continuous pressure on renovating and consolidating existing technologies. Such a task will necessitate new and appropriate technologies and integrated into these new technologies, agricultural research must focus on addressing the problems of controlling plant development and yield, improving the tolerance of plants to abiotic stresses (salinity, drought, and extreme temperatures), improving pest control and enhancing food quality related to improving crop productivity [1].

Induced mutations have played a seminal role in the improvement of plants and in some crop plants, have made a significant impact on increasing plant productivity. So far, more than 2500 mutant varieties involving cereals, oilseeds, pulses, vegetables, fruits, fibers and ornamental plants have been officially released (http://www-infocris.iaea.org/MVD/). Although this is a remarkable success, certain limitations do exist, such as less-effective screening techniques, unrealistically large but necessary size of mutated population for screening and time required for development of mutant lines. In view of these, effective and alternative techniques need to be employed in conjunction with conventional plant breeding strategies. Plant cell and tissue culture techniques fill this gap and offer as supplementive tools. Successful utilization of in vitro techniques for propagation, maintenance and manipulation of plant germplasm has been possible for a great number of plant species [2]. The ability to culture and manipulate a large number of totipotent cells provides a greater opportunity for in vitro selection of useful mutations at cellular level [3]. An overview of the in vitro studies on mutagenesis and mutant selection clearly shows that it is possible to induce mutations and select cell lines with desirable attributes: resistance to biotic stress for example disease resistance and tolerance to abiotic stress viz. aluminum tolerance, salt, drought and frost tolerance. Mutation induction can be empowered by in vitro techniques as many examples related to different plant species showed that the combination of in vitro culture and mutagenesis is relatively inexpensive, simple and efficient. Both induced mutations and somaclonal variation have generated a wide range of genetically stable useful variants or mutants [4]. In this article, we present an overview of different facets of radiation-induced mutagenesis of in vitro plant cell and tissue cultures and case studies on banana and sugarcane.

The Mutagens and their Effects

Both physical and chemical mutagens are used for enhancing and generating variability by inducing mutations. The most widely employed chemical mutagens in vitro are EMS (ethyl methane sulphonate), MNNG (1-methyl-3- nitro- 1-nitrosoguanidine) and some nitrosoureas, while the physical mutagens are gamma rays, X-rays and UV radiation (Table 1). In the case of chemical mutagens, several precautions have to be followed during the handling and washing process in addition to the problems of penetration of the chemical in the plant tissue, dosimetry and distribution of mutagens in the target tissue. Use of appropriate carriers or agents that can improve penetration of chemical mutagen, like dimethyl sulphoxide (DMSO) can

TABLE 1. Mutagens and doses employed in plant tissue cultures

Mutagen	Dose	Dose rate / Duration
- rays	10-80 Gy	5-20 Gy/min
X- rays	20-100 Gy	1-10 Gy/min
UV	2500-2900 nm	0.1-0.7 J/cm2.min
EMS	0.1-1.0%	2-6 hrs
MNNG	5-20mg/l	0.5-4 hrs
MNH	0.1-1.0 mM	6-24 hrs
ENH	0.1-1.0mM	6-24hrs

increase the effectiveness. Physical mutagens may be preferred because they are less hazardous and the user has no contact with them during the mutagenesis. Ultra-violet light (250-290 nm) has limited penetration ability and can be used for single cell or pollen systems while ionizing radiations (X-rays and gamma rays) penetrate deep into the tissue. On the other hand, particle radiation (fast and thermal neutrons) dissipates a large number of ions along the track. An important characteristic of ion beams is that they can make localized irradiation to the target organisms. Ion beam is expected to increase the mutation frequency and a wide spectrum, since it has a high linear energy transfer (LET). The combination of ion beam irradiation and tissue culture has been found to be beneficial for high frequency of mutation induction [5].

Considerations a Priori Mutagenesis

Considerations of target crop, culture system, and trait to be improved need to be given due attention [6] besides choice of starting material, organ type and ways to handle chimerism. The heterozygous genetic constitution of the material would be very interesting since most mutations go from dominant towards recessive, and for the recovery of dominant mutations, homozygous recessive starting material can be useful. Higher ploidy generally leads to higher radiosensitivity but this effect can be compensated by the genetic redundancy of having more than two copies of the genome. Mutations occurring in apical or axillary shoot meristems will result in a lineage of daughter cells and mutated cells and then large mutated sectors. A mutant plant complete for a specific trait could result from a single mutated cell. In situations where mutations occur outside the shoot meristem, the cells have to be stimulated to develop further shoots in order to encourage the mutation to be maintained. For example, mutagenized callus can be multiplied and stimulated to produce shoots and whole plants can be regenerated for evaluating the mutant [7]. In chrysanthemum, gamma irradiated ray florets were cultured for direct regeneration through shoot buds and the technique proved useful to avoid unstable chimeras by forcing the regeneration of buds from single cells [8]. A mutagenic event in a single cell of multicellular tissue entity can result in a chimera and handling the chimeric situation for eventual segregation is often complicated but not impossible.

In the post-irradiation phase, several factors can influence recovery of mutations. These include metabolic activity (e.g. oxygen and temperature), specific metabolic pathway such as protein synthesis, and repair of DNA damage and physical modification of dose rate and dose fractionation [9]. In addition, radiolysis products produced in the culture medium can have influence on the subsequent growth of both irradiated and unirradiated cells. Cells irradiated with ionizing radiation in the absence of oxygen generally exhibit a $2/3^{rd}$ reduction in damage or lethality. This oxygen effect may be due to the formation of peroxide and their subsequent secondary damaging interactions with cellular constituents. Oxygen can be eliminated by equilibrating cells with stream of nitrogen gas before and during irradiation, thus allowing the effect of oxygen on radiation induced lethality or on some of the molecular aspects of radiation damage. Low temperature incubation following irradiation can suppress repair of chromosomal damage. Radiation can produce chemical changes in culture media in addition to the direct effects produced in the irradiated cells. These indirect effects on the growth and differentiation of cultured plant cells have usually been observed only after massive doses of ionizing radiations to the sugar component of the media.

Most of the in vitro mutagenesis studies have employed mutagen treatment prior to selection for achieving higher frequency of mutant cell lines. It is often necessary to decide on LD50 by giving a series of doses and comparing the survival of the propagated cultures to that of control. In vitro cultures often require lower doses of irradiation (around 20Gy) than seeds and this dose has been the optimal LD_{50} dose for a majority of horticultural crops (Table 2). Thus mutagenic treatment is often included in the selection experiments, as the chances of isolating variants are usually enhanced by 10-12 times compared with untreated cells. Optimal conditions for mutagenesis for each selection scheme, crop plant and culture system may vary and for this reason, mutagenic treatments have to be carefully designed.

TABLE 2. Some examples of radiosensitivity of tissue culture of fruit crops

Species	Mutagen	Explants	LD50 (Gy)
Apple	rays	Leaves	10-20
Banana	rays	Shoot tips	20-30
Grapevine	rays	Shoots	20-40
Japanese plum	rays	Shoots	30
Kiwi fruit	rays	Leaves	40-60
Pear	X-rays	Shoots	30
Pear	rays	Leaves	20-30
Prunus	X – rays	Shoots	22 -29
Strawberry	rays	Shoot clumps	50

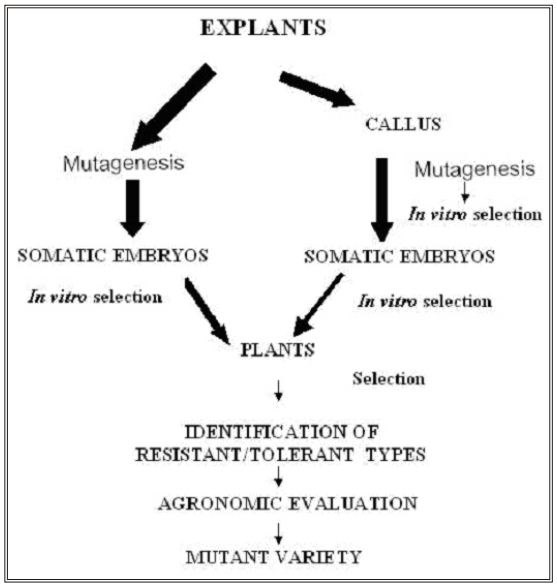

Fig. 1 Schemes for the induction and selection of mutations using in vitro cultures

Different in vitro Culture Systems

One of the prime considerations of using in vitro cultures for mutagenesis is based upon the fact that large populations of cells can be treated and screened before being regenerated into complete plants. Furthermore, a number of subcultures can be performed in a short time for chimera separation and to increase the mutagenized population for selection. Either the explants are mutagenized before culture and then cultured in vitro, or the in vitro culture systems (multiple shoot cultures / callus / cell suspensions / protoplasts) can be mutagenized followed by their culture (Fig.1). Among the different in vitro methods, somatic embryogenesis is the most useful tool for mutagenesis as somatic embryos usually originate from single cells. Consequently, non-chimeric mutants (homo-histonts) can be isolated from the irradiated explants through callus proliferation. The possibilities are much higher for obtaining such desired mutants if cultures can be induced into secondary embryogenesis or repetitive embryogenesis. In vitro subcultures are usually carried through M1 (irradiated explants) for 4-6 cycles. The major factors that can influence during the regeneration process are mutagen treatment per se, the gene affected or trait selected and expressed during the selection and the in vitro culture passage. Optimized mutation – selection conditions combined with an early regeneration of selected variants can reduce the time required for regeneration. Since somatic embryogenesis has been reported for a number of plant species, these approaches should become feasible.

Protoplast cultures offer as an ideal system for mutagenesis and selection [10]. Protoplasts can be prepared from cell suspensions, callus and also directly from whole plant tissues. Compared to shoot meristems, callus and cell suspensions, protoplasts are in G1 phase and have better chances of yielding non-chimeric mutant lines. Haploid protoplasts are certainly the choicest material for mutagenesis as non-chimeric recessive mutants can be recovered. The use of protoplast system for mutagenesis including different aspects like mutagen treatment, time of application, and calculation of mutation frequency and selection of mutants have been described in detail by Negrutiu et al. [10].

Haploid callus cultures derived from microspores / ovules are also the choicest targets for

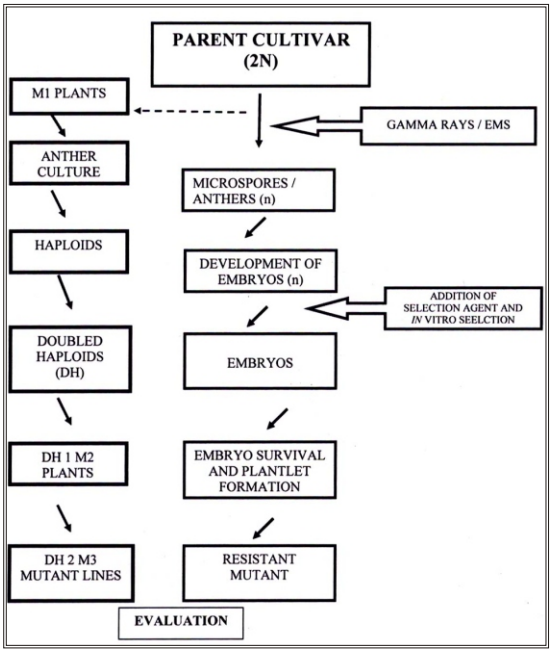

Fig. 2 Mutagenesis and selection using haploid culture system

mutagenesis. Haploid cell and protoplast cultures have advantages in studies on mutant selection in vitro, since mutations particularly recessive in nature can easily be detected in the subsequent generations without any interference. Also breeding lines can be developed in the immediate generation from any segregating population, thereby reducing the breeding cycle of new varieties. Mutagen treatment can be given at different stages: at the parent cultivar stage so that M1 plants are used for culturing microspores or anthers for subsequent selection and doubled haploid mutant or M1 plants are developed from which haploid explants are cultured for obtention of doubled haploid mutant lines (Fig. 2). Using the microspore culture combined with mutagenesis, selection for tolerance to herbicides (chlorosulphuron, imidazolinone), resistance to blackleg, high oleic acid and low linoleic acid and low level of saturated fatty acids has been successfully accomplished through haploid embryos followed by haploid plants and doubled haploids [11,12].

In addition to such approaches of in vitro mutagenesis, tissue culture induced variation ("somaclonal variation") may offer additional source of variation [13]. Such a variation can be most effective if it is successfully associated with cellular level selection and handling of large populations for screening. Chromosomal variation with numbers (polyploidy, aneuploidy) and structural changes (deletions, translocations and inversions) have been observed in somaclonal variants and also in X-ray treated plants of potato. In tomato, 13 different single gene mutations were reported among the 230 somaclonal regenerants [14], and such mutations normally are obtained among plants irradiated with gamma rays. Although the exact mechanism of somaclonal variation is not known, it is possible that the process of somaclonal variation itself could result from a transposon or mutagenic phenomena and that additional mutagenic exposure may enhance likelihood of getting novel variations.

Selection of Mutants at Cellular Level

During in vitro selection, two types of selections viz.. single step selection and multi-step selection are practiced. Generally, an inhibitor or an antimetabolite is added into the culture medium at a level that will either kill or inhibit the growth of the control or wild type cells. The mutagenized cells on the other hand will be able to grow in the presence of the inhibitor. The concentration of the inhibitor depends on the sensitivity of the cells/tissues used in the experiment. It is often desirable to establish a dose-response curve with respect to growth inhibition. In single step selection, the inhibitor is added into the culture medium, at least 2-3 times the level of maximum inhibitory concentration (MIC) and cultures are maintained for several subculture regimes. Examples include selection for herbicide tolerance [15], aminoacid enrichment through lysine + threonine resistance [16] and disease resistance [17,18]. In a multi-step selection method, a sub-lethal concentration (less than MIC) is added into the medium for cultures to grow and in the subsequent subcultures, a gradual increase in inhibitor level is maintained. With this method, it has been suggested that mutant trait selected will often be more stable and more expressive, since the variant cells are in constant exposure to the increasing levels of the inhibitor [19-22].

TABLE 3. Traits that are selectable in cultured plant cells

	Trait	Selection Criteria
AGRONOMIC TRAITS	Disease resistance	Culture filtrate / patho-toxin
	Herbicide tolerance	Herbicide
	Salt tolerance	Salt (Na Cl), Sea Water
	Metal tolerance	Metals
	Flooding tolerance	Anaerobic conditions
	Cold tolerance	Cold temperature
	Drought	Polyethylene glycol or High osmoticum
	Enhanced amino acid Accumulation	Amino acid analogues or Amino acids
OTHER TRAITS	Nitrate reductase	Chlorate
	Cytoplasmic mutants	Antibiotics
	Developmental mutants	Mutagenesis
	Hormonal mutants	Hormones
	Temperature variants	High temperature
	Auxotrophs	BudR

Traits Selectable in vitro

In any selection scheme, it is advantageous that the trait of interest be selectable at the cellular level and also express in the regenerated plants. Not all the traits can be selected at cellular level, for example, yield, seed color or plant height, which are mostly under polygenic control [23]. On the other hand, some traits of agronomic importance and some with a fundamental interest can be selected using selection agents in plant cell cultures (Table 3). Disease resistance, stress tolerance particularly for salt and drought, enriched nutritional quality and herbicide tolerance are some of the traits selected and, mutants have been induced and recovered (Table 4).

Work conducted at BARC

Radiation-induced Mutagenesis in Banana

Banana belonging to family Musaceae is an important fruit crop and a staple food crop in some parts of the world. Banana cultivation and production is threatened by biotic (diseases and pests) and abiotic stresses. This warrants the development of improved clones resistant / tolerant to different biotic and abiotic stresses. However, genetic improvement of cultivated bananas is difficult due to triploidy and sterility. In this regard, prospects of using biotechnological approaches including in vitro mutagenesis are high and relevant [24]. The components of a successful in vitro mutagenesis programme involve the establishment of efficient in vitro regeneration procedure, optimization of mutagenic treatments of in vitro cultures (radio-sensitivity to ^{60}Co gamma rays), and finally, the screening of mutagen treated populations for desired variations (Fig. 3).

Radiosensitivity of in vitro multiple shoot cultures of six banana cultivars (Musa species) belonging to different genomic groups (AAA, AAB, ABB and BB) to gamma rays has been studied [25]. Lower doses (10, 20 Gy) enhanced shoot

TABLE 4. Some examples of in vitro selection for desirable traits

Plants	Traits
Herbicide tolerance	
Wheat	Atrazine, picloram
Tobacco	Atrazine, Amitrole, Picloram, Chlorosulfuron
Brassica	Atrazine, Phenmedipham
Maize, Barley	Glyphosate
Sugarbeet	Chlorosulfuron
Disease resistance	
Tobacco	*Alternaria alternata*
Potato	*Fusarium solani, F. oxysporum, Phytopthora infestans, Alternaria solani, potato leafroll luteovirus*
Strawberry	*Phytopthora cactorum, Rhizoctonia fragariae, Botrytis cinera*
Barley	*Fusarium sp.*
Apple	*Phytopthora cactorum*
Triticale	*Fusarium head blight*
Wheat	*Helminthosporium sativum, Septoria nodorum*
Alfalfa	*F. oxysporum f. sp. medicaginis*
Sugarcane	*Eyespot disease*
Peach	*Xanthomonas compestris*
Mango	*Colletotrichum gloeosporiodes*
Banana	*Fusarium oxysporum f. sp. cubense, fusaric acid*
Abiotic stresses	
Rice, maize, wheat	Aluminium tolerance
Alfalfa	Salt tolerance
Rice, maize, wheat	Salt tolerance
Potato	Salt tolerance
Brassica	Salt tolerance
Tomato, tobacco	Salt tolerance
Other traits	
Arabidopsis	Valine resistance
Sugarcane	Ametryn

multiplication ratio, which however decreased with increased dose. Lethal dose 50% was 40 Gy. Multiplication seldom occurred beyond 50 Gy and doses exceeding 70 Gy were 100% lethal. More than 6,300 plantlets have been produced. In the early stages of field growth, a few chlorophyll and morphological variants have been noticed.

Higher radio-sensitivity was observed in cultivars with hybrid genome (Rasthali, AAB and Karibale Monthan, ABB) compared to cultivars with single genome (Lal Kela, AAA and Wild, BB), which were less sensitive. High multiplication ratio in either AAA or BB genomes and lower in hybrid (AAB and ABB) genomes indicated that efficiency of chimera separation could be dictated by proliferation rate of genotype. The low multiplication ratios were possibly due to development of apical dominance in several cultures, which strongly resisted production of multiples. It is suggested that certainly not the higher but lower in vitro multiplication ratios may affect efficient separation of chimeric regions. Hence, multiple shoot cultures with at least moderately high multiplication ratios (3.0 or more) should be

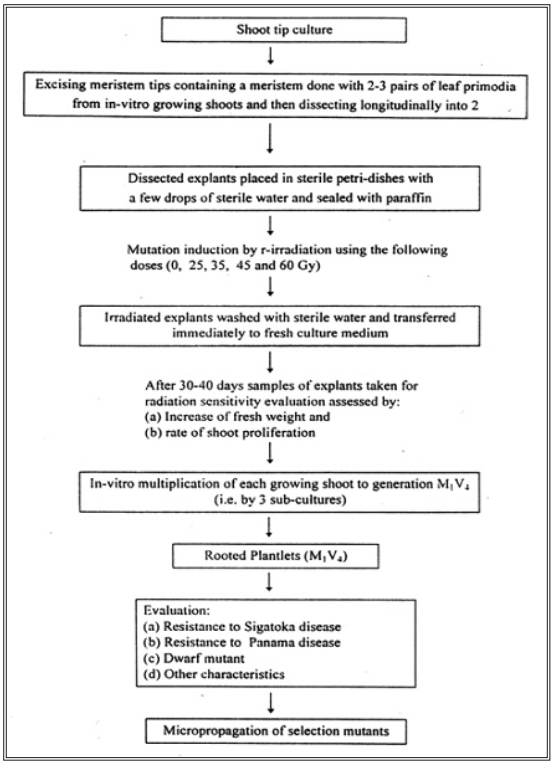

Fig. 3 Schematic diagram of the strategies involving shoot tip culture and mutagenesis in banana [FNCA, 30]

established, or otherwise subculturing of mutagenized populations may be continued up to M1V6 or M1V7 [26]. Thus, radio-sensitivity is a complex function of several factors such as physiological status of plant tissue, method of post-irradiation handling (in vitro or in vivo), degree of differentiation of tissue, stage and rate of growth (dividing or non-dividing), irradiation dose and dose rate, and also genetic architecture of species [25].

In another study, multiple shoot cultures of three banana cultivars (Basari, Chakkarakela and Rasthali) belonging to AAA and AAB genomic groups were gamma irradiated. AAB cultivars appeared to be more sensitive as compared to AAA cultivars. The multiple shoots were also subjected to recurrent mutagenesis at an interval of 15 days. The % survival was found to decrease after each irradiation treatment, whereas multiplication ratio was found to be enhanced after third irradiation in Basrai. Individual shoots derived after different irradiation experiments were rooted and the plantlets were hardened in the green house. Among these, few showed morphological variations such as thick shiny dark green colored leaves, ovate leaves and a dwarf with a rosette of leaves in AAB cultivar, Rasthali [27].

Multiple shoot cultures of an elite variety of Indian banana var. Giant Cavendish (Musa spp. AAA group) were irradiated with gamma rays (5, 10 and 30 Gy) and the resulting plant population was field evaluated for their agronomical performance. A few variant plants for agronomic traits including dwarf stature and early flowering were selected and are being evaluated in further generations at NPCIL, Kaiga. In case of 30 Gy irradiated plants although dwarf stature was noted, the yield was comparable to control plants. The selected variants were also characterized using RAPD markers and a SCAR marker. The GC rich primers exhibited DNA amplification in the variants with a total of 717 bands, of which 44% were polymorphic. The dwarf types were also corroborated using the SCAR marker specific to dwarf off-types. The results are indicative that the gamma irradiation can be employed for recovering agronomic variants in Cavendish bananas.

Effect of gamma irradiation on callus/cell cultures in banana is advantageous over in vitro shoot cultures and in vivo system (sucker) because it offers: (i) high propagation rate facilitating proper chimera separation, (ii) generation of a large plant populations for screening, (iii) possibility of exposing in-vitro cultures to higher irradiation doses (than in vivo explants) and thereby expecting high frequency of mutations, (iv) reduction in time and space requirement, (v) optional facility of in vitro selection and (vi) better chances of selecting dominant or desired mutations. The embryogenic cell suspension cultures are probably the best choice for in vitro mutation induction, as their use overcomes the problem of chimerism. Since there is no need to dissociate chimeras after mutagenic treatment this will fasten the in vitro mutagenesis. Such approaches will be useful for designing strategies for in-vitro mutagenesis in banana improvement.

Radiation-induced Mutagenesis in Sugarcane

Sugarcane (Saccharum spp. hybrids) is a highly polyploidy plant (2n = 36–170) grown in different parts of the world from the tropics to subtropics, and accounts for around 60% of the world's sugar. Sugarcane is vegetatively propagated through setts and work is being carried out on establishing in vitro cultures for the purposes of somatic cell genetics through culture-induced mutations and other biotechnological approaches [28]. In our laboratory, embryogenic callus cultures were gamma irradiated at different doses and observations on % survival was taken in terms of white proliferating clumps [22]. Percent survival showed decreasing trend with increasing irradiation dose. The highest survival was observed in the control cultures (85.7%) while the lowest survival was noted in 50 Gy irradiated cultures. Cultures exposed to 20 Gy dose exhibited almost 50 % survival response and this was taken as the LD50 dose for sugarcane embryogenic cultures. Lower doses (10 and 20 Gy) and control exhibited higher plant regeneration than the higher doses which had low regeneration response beyond 20 Gy dose. In the initial culture cycle, the 30 Gy and 40 Gy irradiated cultures did not show any sign of regeneration, but in further subcultures, these cultures showed signs of regeneration.

In case of sugarcane, irradiated callus cultures have also been subjected to in vitro selection. Irradiated callus on salt selection media showed decreased survival and growth with increase in salt concentration. Regeneration was observed in case of 10 Gy irradiated cultures only on 0.5 % NaCl selection medium. The selections with the higher concentrations resulted in callus browning. The cultures with 20 Gy irradiation showed regeneration on 0.25 and 0.5 % NaCl in the selection medium and higher levels (0.75% and higher) exhibited more browning. Culture survival was noted on 1.25 % NaCl selection medium, in case of 30 Gy irradiated cultures. The 40 Gy irradiated cultures did not exhibit regeneration upon transfer to NaCl selection media. The 50 Gy irradiated cultures produced shoots upon exposure to 0.75 % salt and further increase of NaCl (1.25 %) affected survival and callus growth. The irradiated and selected plants have been field planted at Dr. Punjabrao Deshmukh Krishi Vidyapeeth (PDKV), Akola.

Radiation induced mutagenesis and in vitro techniques have also been used in the selection for salt tolerance. Embryogenic calli were subjected to gamma irradiation at different doses (0, 10, 20, 30, 40 and 50 Gy) and exposed to different levels of NaCl (42.8, 85.6, 128.3, 171.1, 213.9, 256.7, 299.5 and 342.2 mM). Cell viability decreased and higher levels of osmolytes like free proline and glycine betaine were found in stressed calli as compared to control. Electrolyte leakage was 2.8 times more under salt stress and leached out Na^+ and K^+ was much more than that of retained in tissue in both adapted and unadapted callus cultures. The results are indicative that physiological and biochemical attributes are critical in alleviating salt stress effects and in improving salt tolerance in sugarcane.

In vitro techniques can be employed in various steps of a mutation breeding programme and offer a number of advantages. The meristematic cells or tissues and mitotically active cells can be propagated under tissue culture conditions to prepare sufficient amount of material for mutagenic treatments. Although single cells in a cell culture can be used for this purpose, it is possible to use an organized tissue, if single cells from this can be induced to develop into buds or somatic embryos. Intrasomatic competition (also referred to as diplontic selection) discriminating mutagen affected cells and potentially causing a loss of their cell progenies may be controlled by modifying in vitro conditions (medium composition or some other factors) resulting in a better competitiveness of mutant cells [7]. In sugarcane, high-dose gamma-irradiated embryogenic callus cultures were subjected to partial desiccation for 4-6 hrs to stimulate and enhance somatic embryo differentiation and regeneration response [29]. In subsequent studies, this method has been extended successfully to other cultivars, thus suggesting that partial desiccation treatment can offer as a simple and novel approach in stimulating regeneration response of higher dose gamma- irradiated cultures.

Conclusion and Future Prospects

In vitro mutagenesis and selection can be useful in increasing genetic variability in crop plants.

A complete operating strategy should involve in vitro mutagenesis and selection, ex vitro confirmation of tolerance, precise field evaluation of existence, stability, and usefulness. Several methods have been developed for selecting somaclonal variants, while some were developed for in vitro evaluation of known cultivars. All these can be useful only if they are reasonably correlated with field responses. Both mutation breeding and molecular breeding have a common objective of modification of an existing cultivar for a single trait. Desirable traits to be improved include resistance to pests and diseases, tolerance to salinity, drought and cold stresses, improved fruit quality, and alteration of plant form and architecture. Despite the research potential and extensive in vitro study, the strategy of combining mutation induction and in vitro techniques remain to be exploited to their benefit and use in breeding programmes. As describe here, it emerges that different approaches (mutagenesis, somaclonal variation, selection) can yield novel genetic variants with the desired traits. Nevertheless, studies were limited to the lab scale with not much of extension into breeding or development of mutant cultivars. Somaclonal variation may prove to be the preferable source, when dependable, early selection methods for the trait of interest are available. Therefore, induced mutation, which has set pace with a wider interest among plant breeders, could still become the right tool for biotechnologists, given the current need of simple and affordable approaches for plant improvement.

References

1. P. Suprasanna and S.F. D'Souza, In: National Seminar on "Steps towards sustainable agriculture". Indian Soc. Environ. Sci. & Tech., (2007) pp 25.
2. P. Suprasanna and V.A. Bapat, In: National Seminar on Applications of biotechnology in agriculture and food processing sectors in Konkan. Indian Soc. Environ. Sci. & Tech., (2005) pp 26.
3. P. Suprasanna and P.S. Rao, In: Advances in Plant Physiology, Vol. 1. Ed. Hemantaranjan, A. Scientific Publishers, Jodhpur, India. (1997) pp 103.
4. M. Maluszynski, I. Szarejko, J. Maluszynska, Encyclopedia of Applied Plant Sciences 1–3 (2004) 186.
5. K. Ishii, Y. Yamada, Y. Hase, N. Shikazono and A. Tanaka, RAPD analysis of mutants obtained by ion beam irradiation to hinoki cypress shoot primordial. Nucl. Instr. Methods Physics Res. B 206(2003) 570.
6. S. Predieri, Plant Cell, Tissue and Organ Culture 64(2001) 185.
7. A.M. Van Harten (1998) Mutation Breeding: Theory and Practical Applications. Cambridge Univ. Press, Cambridge
8. S.K. Datta, P. Mishra, A..K.A. Mandal. Curr Sci 88(2005) 155.
9. G.P. Howland and R.W. Hart, In: Applied and fundamental aspects of plant cell, tissue and organ culture. Ed. Reinert J, Bajaj YPS. Springer Verlag. Berlin, Newyork., (1977) pp 731.
10. I. Negrutiu , M. Jacobs and M.Caboche, Theor. Appl. Genet. 67: (1984) 289.
11. I. Szarejko, M. Maluszynski, K. Polok and A. Kilian, In; Plant mutation breeding for crop improvement. Vol 2. IAEA, Vienna (1991) pp 355-378.
12. M. Maluszynski, B.S. Ahloowalia, B. Sigurbjoornsson, Euphytica 85(1995) 303.
13. B.S. Ahloowalia, In: Somaclonal variation and induced mutations in crop improvement. Ed. Jain SM Brar DS, Kluwer Acad. Publ. Dordrecht. (1998) pp 293-309; S.M. Jain, Plant Cell Tissue and Organ Culture 82(2005).113.
14. D.A. Evans and W.R. Shar, Science 221(1983) 949.
15. R.S. Chaleff and R.E. Parsons, Proc. Natl. Acad. Sci. (USA), 75 (1978) 5104.
16. K.A. Hibberd and C.E. Green, Proc. Natl. Acad. Sci. (USA), 79 (1982) 559.
17. B.G. Gengenbach and C.E. Green, Crop Sci. 15 (1975) 645.
18. H.W. Rines and H.H. Luke, Theor Appl Genet 71 (1985) 16.

19. O.K. Miller and K.W. Hughes, In vitro 16 (1980) 1085.

20. T.J. McCoy, Plant Cell. Rep. 6 (1987) 417.

21. S. Miao, D.R. Duncan and J. Widholm, Plant Cell Tissue Organ Cult. 14 (1983) 3.

22. V.Y. Patade, P. Suprasanna, U.G. Kulkarni and V.A. Bapat, BARC Newsletter 273(2006) 244.

23. J.M. Widholm, Iowa State Jour. Res. 62(4) (1985) 587.

24. V.A. Bapat, T.R. Ganapathi, V.M. Kulkarni, G.B. Sunil Kumar, L. Srinivas and P. Suprasanna, In: Banana: technological advancements. Ed. Singh HP, Uma S, AIPUB, NRCB, Trichy, India. (2007) pp.160.

25. V.M. Kulkarni, T.R.Ganapathi, P. Suprasanna, V.A. Bapat and P.S. Rao, Jour. Nucl. Agr. Biol. 26 (3) (1997) 168.

26. V.M. Kulkarni, T.R. Ganapathi, P. Suprasanna and V.A. Bapat, In: Protocols for Micropropagation of Woody trees and fruits. Jain SM, Haggman H, (Ed.), Springer, Netherlands. (2007) pp. 543–559.

27. P.J. Mishra., T.R. Ganapathi, P. Suprasanna and V.A. Bapat, Int. Jour. Fruit Sciences 7(2007)

28. P. Suprasanna, V.Y. Patade and V.A. Bapat, In: Advances in Plant Biotechnology. GP. Rao, Yipeng Zhao, Volodymyr V Radchuk, S Bhatnagar, (Ed.) Science Publishers, USA. 2007. Pp 313.

29. P. Suprasanna, C. Rupali, N.S. Desai and V.A. Bapat, Plant Cell Tiss Org Cult. 92 (2008) 101.

30. FNCA (2004) Mutation Breeding Manual (Ed.) F S. Medina, Etsuo Amano and Sigemitsu Tano, Forum for Nuclear Cooperation in Asia, Philippines.

Role of Isotopes in Soil-Plant, Water and Fertility Research

Dr. V. Ramachandran *joined B.A.R.C. in 1976 after graduating from University of Madras. He has obtained his M.Sc. and Ph.D. degrees in Chemistry from University of Mumbai. He has to his credit 56 research publications in National/International journals, IAEA reports, book chapters and symposia proceedings. His research interests are on 'toxic' heavy metal pollution of agricultural soils-plant system through application of inorganic/organic fertilizers like processed fertilizers, rock phosphates, sewage sludges, city composts, etc. and macro-/micronutrient research on different soil types of India utilizing different agro-industrial wastes with the help of isotopic techniques.*

Shri Manoj Shrivastava *has done M.Sc. (Ag.) from G.B. Pant University of Agriculture and Technology, Pantnagar, Uttrakhand. He has worked as Research Associate at Central Soil Salinity Research Institute, Karnal, and as Soil Scientist at Nahar Group of Industries (OWM group), Ludhiana, prior to joining Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre as Scientific Officer (C). His area of research interest is Soil Fertility. Currently he is involved in research on phosphorus dynamics in soil and development of phosphate solubilizing bio-fertilizers using low grade Indian rock phosphates and agro-industrial wastes. He is using nuclear as well as conventional techniques for these studies.*

Dr. S. F. D'Souza *joined BARC after graduating from the 15th batch of training school. He is currently the Associate Director-A, Biomedical Group and Head, Nuclear Agriculture and Biotechnology Division, BARC Mumbai. He is also Senior Professor of Homi Bhabha National Institute (Deemed University). Ph.D in Biochemistry and his major research interest are in the field of Enzyme and Microbial Biotechnology with special reference to immobilized biomaterials for use in bioprocessing, biosensors and bioremediation. He has authoured about 150 scientific papers in International journals/ books and has guided a number of Ph.D Thesis. He is the recipient of the AMI- LOUIS PASTEUR AWARD for his significant contributions to the field of Microbiology and has been honoured as a Fellow of the National Academy of Science, Fellow of the Association of Food Scientists & Technologists, and Fellow of the Maharashtra Academy of Science*

Introduction

The ever increasing population, especially in developing countries, demands more food and emphasise the need to do more research for higher agricultural production. About 180 million hectares of gross land area is used for agriculture in our country, and the nutrients in the soil are very much depleted. To overcome the nutrient demand, huge amount of fertilizers are required. However, compare to other countries, where fertilizers (N, P and K) are used in the range of 100-900 kg ha^{-1}, in India, only 70 kg ha^{-1} is currently being used. A good crop yield simultaneously results in depletion of nutrients from the soil. A wheat crop, for example, removes about 16 kg N, 2 kg P_2O_5 and and 3.5 kg K_2O per tonne of grain, and 4.5 kg N, 0.1 kg P_2O_5 and

Dr. V. Ramachandran, Shri Manoj Shrivastava and Dr. S.F. D'Souza, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 400 085; E-mail: vrchand@barc.gov.in

17.5 kg K_2O per tonne of straw. In addition to major nutrients N, P and K, micronutrients are also removed by crops and about 47%, 5%, 3%, 12%, 35% and 7% of cultivable area in the country are deficient in Zn, Mn, Cu, Fe, B and Mo, respectively [1]. Since the nutrient requirements of crops vary considerably with the soil type, climatic conditions and plant species, precise knowledge is required on the type, amount, method and time of application of fertilizer materials best suited for specific soil-crop combinations. Use of isotope labeled fertilizer materials permits such determinations and reveals directly within weeks, information which otherwise takes long period and that too with elaborate field studies. The availability of radioisotopes for many of the plant nutrients such as ^{32}P, ^{35}S, ^{59}Fe, ^{54}Mn, ^{65}Zn and stable isotopes like ^{15}N, ^{65}Cu and ^{11}B makes it possible to study the behaviour of these nutrients in soil-plant system using isotopic techniques. The availability of radioisotopes for many of the 'toxic' heavy metal pollutants like Cr, Cd, Pb, Se, etc. makes it convenient to study their behaviour in soils and plants. Some of the important isotopes are presented in Table 1.

Monitoring and managing soil water resources are also very essential in agriculture. Bulk density, one of the important soil properties depends upon soil water content. Effective soil water management can be achieved not only on the assessment of soil water content and bulk density, but on the movement of dissolved constituents with the soil water, namely, fertilizers, pesticides and other inorganic and organic materials which undergo physical, chemical and microbiological reactions within soil profiles. Isotopes play an important role in soil water management.

Maintenance of adequate levels of organic matter in soil is another important factor in productive agriculture [2,3]. Soil organic matter plays an important role in soil fertility; it acts like a reservoir from which nutrients N, S and P can be released, following decomposition and metabolism of organic substrates by soil animals and microorganisms. Also, organic matter plays major role in the formation of aggregates, and thus in stabilizing soil structure, facilitating root growth and improving both aeration and water infiltration and retention; further, it influences biological activity in soil. It is very essential (i) to establish conditions which enable organic matter to accumulate in soils at suitable levels and (ii) to know the rates at which plant nutrients are incorporated into and released from organic matter due to biological activity. The diversity of origin, chemical nature and physical distribution of organic matter in soils are major factors in the dynamics of organic matter turnover. Here again, both stable and radioisotopes are very useful.

Soil-Plant-Water Research

Radioisotopes play a significant role in maintaining and management of soil water resources, which is of prime importance in agriculture. The development of neutron moisture gauges, which make use of radio isotopes like americium, radium and beryllium is routinely used for both research and practical investigations of soil water. Before the nuclear technology development, soil water contents were estimated by indirect methods or by laborious destructive sampling techniques that were inefficient and time-consuming. Neutron moisture gauge is very useful in research on soil-water-plant relations, irrigation and drainage, evapotranspiration and soil moisture storage in relation to agronomic alternatives. Mederski [3], Jarvis and Slatger [4] and Laing [5] have introduced beta radiation gauge for non-destructive method for measuring relative water content of plant leaves.

Both stable and radioisotopes are used as tracers in field investigations of soil water. The criterion for an ideal tracer is that it must be chemically and physically indistinguishable from the tracee and that the introduction of the tracer must not disturb the soil water system. In soil systems, tracers are often used to identify the fate of inorganic and organic soil water constituents, fertilizers and pesticides as well as crop and animal residues. Tritium, a radioactive tracer is very useful in estimating the movement of soil water or the age of water in an aquifer. It serves as an indicator of the rate at which water moves through the unsaturated zone of soil towards the water table. Smith et al.[7] measured profiles of tritium in a clay soil to depth of 4 m. On the other hand, Corey and Horton [8] used ^{18}O, a stable isotope for water movement studies in soil.

TABLE 1. Important isotopes in soil-plant, water and fertility research

Elements	Isotope	Emission	Half-life/or % abundance	Measurement	Use
Hydrogen	^{3}H	Beta	12.3 years	LS	Water movement, metabolism
Carbon	^{14}C	Beta	5720 years	LS	Photosynthesis, organic matter, C balance
Oxygen	^{18}O	Stable	0.204 %	MS	Photosynthesis, Respiration, Hydrology
Nitrogen	^{15}N	Stable	0.366 %	MS ES	Fertilizers, BNF, N balance
Phosphorus	^{32}P	Beta	14.3 days	GM counter, LS	Fertilizers, root distribution, rock phosphates
Potassium	^{40}K ^{41}K	Beta Stable	1.3 X 10^{9} years 6.77 %	LS MS	Fertilizer, K balance
Calcium	^{45}Ca	Beta	165 days	LS	Ion uptake, soil exchangeable Ca
Magnesium	^{28}Mg ^{26}Mg	Beta, Gamma Stable	21.3 hours 11.29 %	LS MS	Movement in plants, Environmental pollution
Sulphur	^{35}S	Beta	87 days	LS	Soil availability, S cycle, Uptake
Iron	^{55}Fe ^{59}Fe	Beta Gamma, Beta	2.6 years 45.6 days	LS GS	Soil erosion, foliar nutrition, soil availability
Manganese	^{54}Mn	Gamma, Beta	314 days	GS	Foliar nutrition, Soil availability
Copper	^{64}Cu ^{65}Cu	Gamma, Beta Stable	12.8 hours 30.9 %	GS MS	Soil and plant movement
Zinc	^{65}Zn	Gamma, Beta	245 days	GS	Fertilizer, soil and plant movement
Molybdenum	^{99}Mo	Gamma, Beta	66.7 hours	GS	Plant movement
Sodium	^{22}Na	Gamma, Beta	2.6 years	GS	Salt tolerance, cell permeability
Chorine	^{36}Cl	Beta	3.08 X 10^{5} years	GM, LS	Salt tolerance, solute movement in soils
Cobalt	^{58}Co ^{60}Co	Gamma, Beta Gamma	71 days 5.3 years	GM GS	Enzymatic studies, Vitamin metabolism, plant animal food chain
Selenium	^{75}Se	Gamma	120 days	GS	Animal nutrition, Food chain
Chromium	^{51}Cr	Gamma	27.8 days	GS	Soil and plant movement

Note : GS = Gamma spectrometer; LS = Liquid Scintillation; GM = Geiger Muller; MS = Mass spectrometry; ES = Emission spectrometry

Soil Organic Matter

Soil organic matter is considered a key factor in maintaining soil quality and it is very essential for long-term soil fertility [9]. It provides a reservoir of plant nutrients and improves soil structure. The fate of carbon and nutrients released during organic matter decomposition is an important determinant of the short- and long-term soil fertility. Hence, maintenance of soil organic matter must be considered as one of the main objectives of sustainable land-management of productive

agriculture. Stable isotopic tracers like ^{15}N, ^{13}C, ^{11}B, ^{34}S, ^{18}O and ^{25}Mg and radioactive tracers such as ^{3}H, ^{14}C, ^{32}P, ^{35}S and ^{86}Rb are very useful in nutrient dynamics in soil. The difference in ^{13}C between C3 and C4 plants has been used to study carbon turnover rates. The different carbon assimilation pathways in C3 and C4 plants result in different ratios of ^{12}C and ^{13}C between these two groups of plants [10]. Using ^{25}Mg, a stable isotope in their study, Jentschke et al. [11] found that ectomycorrhiza was capable of enhancing the Mg supply to Norway spruce seedlings.

The diversity of origin, chemical nature and physical distribution of organic matter in soils are major factors in the dynamics of organic matter turnover. The use of isotopes has been invaluable not only for obtaining measures of organic carbon entering a soil annually, but also for enabling assessments to be made of the rates of decomposition of specific substrates, simple and complex, under natural or controlled conditions and for periods long after the initial substrate has been metabolized. Factors influencing decomposition rates, and the release and tie-up of nutrients can be assessed. Further, isotopic measurements, coupled with soil organic matter fractionation by chemical or physical means, have demonstrated differences in turnover rates of organic matter components [12, 13, 14, 15].

Soil Fertility

In soil fertility and fertilizer studies, isotopes have found extensive application in the assessment of nitrogen and phosphorus fertility status of soil, evaluation of different nitro phosphates and polyphosphates for specific soil-crop conditions, and optimum placement of nitrogenous and phosphate fertilizers.

In the case of nitrogen, the longest-lived radioisotope, ^{13}N, has a half-life of only 10 minutes, and the use of stable isotope is consequently a necessity for tracer technique applications in agricultural research. Nitrogen-15 with ^{15}N atom % 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 in the case of a radioisotope.

Nitrogen-15 - aided greenhouse experiments conducted at Nuclear Agriculture and Biotechnology Division of BARC for maize and rice crops using 'A' value technique have indicated that for both upland and lowland rice, neem-extract coated urea was a better source of N compared to other coated ureas. Further, for rice, sulphur-coated urea and urea gypsum were superior or equal to prilled urea, whereas, for maize, prilled urea was as good as all other modified forms of urea [16].

Tracer research on phosphorus in soil and plants could be conveniently carried out using the radioisotope ^{32}P having a half-life of 14.3 days. Isotope-aided studies coordinated by NABTD, BARC with several agricultural universities/ institutions have established that ammonium polyphosphate (APP), a fertilizer new to Indian agriculture, and which provides more nutrients (N+P) per unit weight of the fertilizer with reduction in the cost of handling, transport, storage and application is equal or superior to the orthophosphate fertilizer (DAP-presently available in the country) in diverse soil-crop regimes. Greenhouse and field experiments conducted by agricultural universities and ICAR Coordinated Projects have shown that APP was superior to DAP as a source of phosphorus and a carrier of micronutrients. Studies on APP blended with $^{65}ZnSO_4 . 7 H_2O$ as a carrier of zinc indicated that the fertilizer use efficiency of Zn-APP was significantly higher than that of Zn-DAP for the major crops in vertisol and was equally effective in ultisol [17, 18]. Natural sources like rock phosphates, atmospheric nitrogen, sewage sludges can not be labeled; hence, the contribution of this unlabeled source is measured by adding a labeled common third source to the soil-plant system with the indirect method as described by Zapata [19]. Studies on the fertilizer use efficiency of rock phosphates (RPs) using ^{32}P-labeled triple super phosphates as a common source (indirect method) indicated that Syrian RPs were superior to Indian Mussoorie RP [20].

Recently, ^{32}P-aided tracer studies carried out at NABTD, BARC indicated that the agronomic efficiency of single super phosphate (SSP) and di ammonium phosphate (DAP) were comparable and are superior to nitro phosphate (NP) for cotton crop grown on a vertisol under greenhouse conditions

[21]. In another ^{32}P-aided tracer studies on Indian rock phosphates [22], it was found that Purulia rock phosphate (PRP) performed better than Mussoorie rock phosphate (MRP) in two acid soils.

Studies carried out under a BRNS-funded project of NABTD, BARC and G.B. Pant University of Agriculture & Technoloty, Pantnagar, to evaluate the efficacy of radio labeled P- and Zn-enriched bio-sludges from molasses based distillery as P and Zn sources to rice and wheat crops resulted in two new fertilizer formulations and patents have been filed for them (Patent file Nos. 757/MUM/2007 and 758/MUM/2007).

Micronutrients play an important role in crop nutrition and have a tendency to become immobilized, or fixed in soil thereby becoming unavailable to plants. Isotopes have been useful in studying the mobility of such nutrients through different soil types. At NABTD, BARC, the mobility of labeled micronutrients ^{65}Zn, ^{54}Mn and ^{59}Fe has been studied after surface application on two different soil types, namely, a black clay loam and a laterite. All the four micronutrients were found to be immobile when leached with rain water or irrigation water in both soils. However, synthetic chelating agents like EDTA significantly induced mobility of all the four micronutrients by the formation of soluble and stable micronutrient-EDTA complexes [23]. The uptake, distribution and metabolic fate of the micronutrients (Zn, Mn and Fe) in plants have been studied by D'Souza and Mistry [23] and they found that the uptake of these micronutrients was more in laterite than in black soil; further, the accumulation of Zn and Mn in aerial tissues was more than that of Fe.

The uptake and distribution studies of chromium, a heavy metal pollutant, in bean plant was studied by Ramachandran et al. [24, 25] using ^{51}Cr in nutrient culture. The results showed that $^{51}Cr^{3+}$ uptake was more than that of $^{51}CrO_4^{2-}$. Shoot uptake of both forms of Cr decreased with increasing pH and salt concentrations of culture solution. Distribution of both forms of Cr in bean shoots followed an acropetal gradient. More than 70% of the accumulated Cr was found to be associated with ionic forms.

Work on the mobility of heavy metals, Cr, Pb and Zn using their radionuclides ^{51}Cr, ^{210}Pb and ^{65}Zn in different soil types was evaluated by D'Souza et al. [26]. Dilute solutions of EDTA and DTPA were found to be effective in leaching of these heavy metal pollutants; EDDHA was found to be very effective for leaching Cr from various soil profiles. Rapid formation of stable, soluble complexes like metal-EDTA, -DTPA and –EDDHA facilitated the leaching of these pollutants from contaminated soils. These results have practical implications in terms of distribution of heavy metals in different tropical and sub-tropical soil profiles and the development of practices for leaching these pollutants below the root zone of crop plants grown on contaminated soils.

The mobility of heavy metal pollutants, namely, Se and Cd in different soil profiles was studied by Athalye and Mistry [27] and Ramachandran and D'Souza [28], respectively using radioisotopes, ^{75}Se and ^{115m}Cd. Diethylene triamine pentaacetic acid (DTPA) at $10^{-4}M$ concentration enhanced the mobility of ^{75}Se and lime ($CaCO_3$) application effectively enhanced the mobility of ^{115m}Cd in various soils of India.

Root Distribution and Soil Erosion Studies

Soil fertility research is also related to root distribution of nutrients and assessment of soil erosion and sedimentation. Intercropping involves simultaneous cultivation of more than one plant species in the same field, which can improve the use of plant-growth resources in space and time. Isotopes are very useful to elucidate root distribution and competition of nutrients in intercropped plant species [29]. Soil erosion and associated land degradation are serious problems, which affects the soil fertility and productivity. There is a need to quantify the soil erosion for the development of effective soil conservation. Radioisotopes such as ^{137}Cs, ^{210}Pb and ^{7}Be are useful in assessing soil erosion losses and sedimentation rates [30, 31].

Conclusions

The isotopes, both stable and radioactive have proved to be very useful in agricultural research. They play a major role in the advancement of soil-plant and fertility studies. Their availability and advantages of using them in agricultural research as

a tool would definitely result in increased food production.

References

1. G.U. Malewar, J. Indian Soc. Soil Sci. 53, 484-499 (2005)
2. F.E. Allison, "Soil Organic Matter and Its Role in Crop Production", Elsevier, New York (1973)
3. E.W. Russel, "Soil Conditions and Plant Growth" 10th edn., Longman, London (1973)
4. H.D. Mederski, Soil Sci. 92, 143-146 (1961)
5. P.G. Jarvis and R.O. Slatyer, Science, 153, 78-79 (1966)
6. D.P. Laing, Ph.D. Thesis, Iowa State University (1966)
7. D.B. Smith, P.L. Wearn, H.J. Richards and P.C. Rowe, "Isotope Hydrology", IAEA, Vienna, 73-87 (1970)
8. J.C. Corey and J.C. Horton, Soil Sci. Soc. Am. Proc. 32, 471-475 (1968)
9. A. Wallace, Comm.Soil Sci. Plant Anal. 25, 29-35 (1994)
10. G. Cadisch and K.E. Giller, Soil Biol. Biochem. 28, 823-825 (1996)
11. G. Jentschke, B. Brandes, A.J. Khun, W.H. Shroder, J.S. Becker and D.L. Godbold, Plant Soil 220, 243-246 (2000)
12. S.L. Jansson, In "the Use of Isotopes in Soil Organic Matter Studies", FAO/IAEA Symp. Brunswick – Volkenrode, Pergamon Press, Oxford, 283-296 (1966)
13. S.L. Jansson, In "Soil Biochemistry" Vol. II (A.D. McLaren and J.J. Skujins, eds.), Marcel Dekker, New York,pp.129-166 (1971)
14. J.M.Oades and J.N. Ladd, In "Soil Factors in Crop Production in a Semi-arid Environment", (J.S. Russel and E.L. Greacen, eds.), University of Queensland Press, Adelaide, pp.127-160 (1977)
15. J.D. Stout, K.M. Goh and T.A. Rafter, In "Soil Biochemistry" Vol. V (E.A. Pauland J.L. Ladd, eds.), Marcel-Dekker, New York, pp.1-73 (1981)
16. B.N. Vyas, Proc. Natl. Symp. Use of Nuclear and Allied Techniques in research on Soil Fertility Fertilizer Usage, Coimbatore, Dept. of Atomic Energy, 41-42 (1987)
17. V.B. Yadav and K.B. Mistry, J. Nucl. Agric. Biol. 14, 102-104 (1985)
18. T.J. D'Souza and V.B. Yadav, Proc. FAO/IAEA Seminar for Asia and the Pacific on Nuclear Related Methods in soil/Plant Aspects of Sustainable Agriculture, Colombo, Sri Lanka, 209-217 (1995)
19. F. Zapata,"Use of Nuclear Techniques in Studies of Soil and Water Relationships", G.B. Harderson (ed.), IAEA, Vienna, p.61 (1990)
20. S. Kanakri and B.M.Bhujbal, J. Nucl. Agri. Biol. 22, 155-162 (1993)
21. M. Srivastava and S.F. D'Souza, J. Radioanal. Nucl. Chem. 274, 397-401 (2007)
22. M. Srivastava, B.M. Bhujbal and S.F. D'Souza, Comm. Soil Sci. Plant Anal. 38, 461-471 (2007)
23. T.J. D'Souza and K.B. Mistry, Proc. Symp. Isotopes and Radiations in Research on Soil Plant Relationships, IAEA, Vienna, 407-425 (1979)
24. V. Ramachandran, T.J. D'Souza and K.B. Mistry, J. Nucl. Agri. Biol. 9, 126-128 (1980)
25. V. Ramachandran, T.J. D'Souza and K.B. Mistry, Experientia 37, 718-719 (1981)
26. T.J. D'Souza, B.N. Vyas, V.V. Athalye, V. Ramachandran and K.B. Mistry, Proc. Symp. Isotopes and Radiation Techniques in Soil Physics and Irrigation Studies, IAEA/FAO, Vienna, 165-178 (1983)
27. V. V. Athalye and K.B. Mistry, Proc. Ann. Con. Indian Soc. Soil Sci. 6 (1985)
28. V. Ramachandran, T.J. D'Souza, J.Nucl. Agri. Biol. 26, 133-141 (1997)
29. E.S. Jensen, Plant Soil 182, 25-38 (1996)
30. D.E. Walling, Proc. Symp. Tracer Technologies for Hydrological Systems, IAHS Publication No. 229, 163-172 (1995)
31. W.H. Blake, Appl. Radiat. Isot. 51, 599-605 (1999)

Applications of Radioisotopes in Pesticide Biodegradation Studies

***Dr. Sharad P. Kale** is engaged in research on pesticide degradation and environmental pollution for last 30 years. His major research interests are agricultural and marine pollution due to environmental chemicals, bioremediation, biological control and solid waste management. He has studied microbial degradation of several 14C-labelled pesticides. He has worked on rice fish ecosystem and marine ecosystems to study the environmental behavior of pesticides in various components. He has developed NISARGRUNA plant for solid waste management. In addition to his research work he is a popular science writer in Marathi and he conducts 1-2 days symposia on inculcating scientific spirit in society.*

Pesticide can be defined as a substance or mixture of substances, which is used for controlling the pests. Pesticides were introduced into intensive agricultural systems several decades ago. The use of systemic organic pesticides began in early 1930s after the demonstration of the insecticidal properties of alkyl thiocyanates and fungicidal properties of dithiocarbamates. The introduction of DDT marked a revolution in the pesticide field in 1942. During same period the first herbicide 2,4-D was introduced into the market. The second generation pesticides viz. the organ-phosphorous group compounds were introduced in agriculture during 1945-1955. The carbamates and ureas were developed in this period. Many of these compounds are still actively used in agriculture.

Fungicides were introduced during 1960s and 70s. Benzimidazoles, pyrimidines, triazoles and imidazoles were prominent among these fungicides. During this period the risks involved in usage of chemical pesticides started surfacing. These were apparently the result of ecotoxicological effects following intensive use of DDT and other organochlorine pesticides. The euphoria of DDT discovery was short lived and USA banned the usage of DDT in early 1970s. Many of the countries soon followed the suit and by 2001 most of the countries in the world have banned use of persistent organochlorine pesticides in Agriculture as well as in the field of public health.

The third generation pesticides were developed in the decade 1970-80. They included the pyrethroids and sulfonylureas. The important and useful aspect of these pesticides is that they can be used in low dosage rates because of their strong biological activity. In recent years sterilants, pheromones and chitin inhibitors have been introduced to control the pest problem. The biopesticides like Bacillus thuringiensis and viruses have made significant contribution in pesticide field in last decade but they have still a long way to go before making quantitative impact.

India is basically an agricultural country. Forty million hectares of cultivable land is available here for agricultural production. Sixty percent of our population lives in rural areas. Intensive and multiple cropping systems are practised wherever irrigation facilities are provided but still majority of the farmer remains dependent on rain gods for water requirements. It is estimated that about 70% of the farming is rain fed. All crops suffer from damages caused by various pests including insects, rodents, mites, nematodes, birds, wild animals and plant diseases due to infection of bacteria, viruses and fungi. All these pests cause about 25 to 30% losses in crop yields. The consequence is reduction in economic gains. Stored food grains and other market commodities are spoiled due to the constant attacks of insect pests, rodents and invasion by moulds. In agriculture, high yielding genotypes particularly hybrids and synthetics were introduced through

Dr. Sharad P. Kale, Nuclear Agriculture And Biotechnology Division, Bhabha Atomic Research Centre, Mumbai 400 085;
E-mail: sharadkale@gmail.com

"Green Revolution" in late sixties. These genotypes could be grown only under intensive use of inputs like fertilizers, pesticides, irrigation, improved seed and labour. Thus pesticides were responsible for increasing the crop production. Thereafter insist for pesticides increased by lumps and bounds.

Consumption of insecticide in agriculture has been increased more than 100% from 1971 to 1994-95. For instance, insecticide consumption in India, which was to the tune of 22013 tonnes, has increased to 51755 tonnes by 1994-95. Consumption of all of these pesticides in same duration has increased more than two times, that is from 24305 tonnes to 61357 tonnes. In recent past, change has been observed in trends of pesticides consumption. As a consequence of adoption of bio intensive Integrated Pest Management Programme in various crops the consumption of chemical pesticide (Tech. Grade) has come down from 66.36 thousand MT during 1994-95 to 43.59 thousand MT during 2001-02 with a reduction of 27.69%(Thirty Seventh Report of Standing Committee on Petroleum and Chemicals, 2002). Since then it is more or less stable at this level. Consumption pattern of pesticides in India is also very different from world. In India insecticide account for 76% of the total domestic market while herbicides & fungicides have a significantly higher share in the global market. There are wide ranges of regional variations in pesticide consumption in the country. In the year 2000-01, States of Haryana, Punjab and Uttar Pradesh by consuming more than 5,000 MT (technical grade) pesticides annually come under category I state in consumption of pesticides. States viz., Andhra Pradesh, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Rajasthan, Orissa and Tamil Nadu, which consumed between 1000 MT and 5000 MT fall in the category II states. States viz., Assam Bihar and Himachal Pradesh that consumed pesticide between 100 and 1000 MT come under category III. States viz., Arunachal Pradesh, Jammu & Kashmir, Manipur, Mizoram, Nagaland, Tripura, Delhi and Union Territory (UT) of Pondicharry consumed pesticides between 10 and 100 MT annually fall under category IV. States viz., Goa, Meghalya, Sikkim and UTs viz., Andaman & Nicobar Islands, Candigarh, Dadara & Nagar Haveli, Daman & Diu and Lakshadweep consumed less than 10 MT pesticides annually as fall in the last category in pesticide consumption (Thirty Seventh Report of Standing Committee on Petroleum and Chemicals, 2002).

The assessment of distribution and effects of pesticide residues in the environment requires a consideration of the usage of pesticides either applied in past or in active use as on today. Data on pesticide usage and the amounts of active ingredients are rarely complete and need a constant input from the research field. Pesticides along with drugs, detergents and variety of environmental chemicals have received a major attention. Part of this flurry can be attributed to Ms. Rachel Carson who published a novel called "Silent Spring" depicting the various hazards of pesticides. The continuing environmental interest in the fate of all organic substances has stimulated much new and original research on the fate of pesticides in soil, water, air and living systems.

The recognition that as much as 90% of the applied quantity of most of the pesticides remain unaccounted for, scientific and practical interest in environmental transformation products has increased sharply. It is now apparent that a highly toxic material may be converted environmentally to an innocuous one while a presumably safe pesticide can yield decomposition products, which are unexpectedly lethal to some innocent part of life. The parent compound does not necessarily remain the most significant one toxicologically and environmentally.

Radioisotopes have played an important part in agricultural research in last 40 years. Several pesticides have been extensively evaluated for their degradation in the environment using labelled compounds. The residue analysis in particular has been considerably strengthened by their use in research laboratories. Common labels used for this purpose are ^{14}C, ^{35}S (in sulfur-containing pesticides) and ^{3}H. All these isotopes are soft beta emitters and can be easily detected in biological samples by liquid scintillation counting. There are definite benefits of labelled isotopes in pesticide research.

1. They are helpful to determine the extraction efficiency and efficiency of cleanup procedures in the laboratory.

2. Because of specific labelling potentials, the path of individual atoms can be traced in biological materials.
3. The detection limit is considerably lowered making residue analysis more accurate and complete.
4. Extremely low concentrations can be recognized and determined.
5. The fate of labelled pesticide can be followed specifically in the presence of background levels of the pesticides including the one being studied.
6. Co-chromatographic radioisotope techniques with non-radioactive carriers provide for identification of labelled metabolites or degradation products.
7. Detection of soil bound components of residues is possible.

However, there are certain disadvantages of the technique. It is not possible to use the radioactive compounds on a field scale because of their hazardous nature. This is the biggest limiting factor as the research is laboratory oriented and results may not be representative of field scale. The use of lysimeter has picked up in last few years overcoming this limitation and has now been accepted by Environmental Protection Agency of US as prerequisite for pesticide registration.

The pesticides applied to the soil interact with natural biological systems. Chemical, photochemical and microbiological processes can cause the transformation of pesticides in soil. Chemical transformations in soil such as hydrolysis and oxidation are widespread phenomena, but reduction or isomerization has also been observed. Clay surfaces, metal oxides, metal ions and organic surfaces may catalyze the chemical reactions. Photochemical decomposition also can play a significant role in the pesticide degradation. The microbial breakdown of pesticides must be considered the most important cause of degradation in soil. The mineralization or complete biodegradation of an organic compound in waters and soils is almost always a consequence of microbial activity. Mineralization is a typically growth linked process.

These studies have been conducted in various ecosystems. They include simple laboratory systems like biometer flask to complex systems like field lysimeters. Many of such studies have been fruitful in predicting the possible bioaccumulation potential of either the parent molecule or the degradation products. They have been useful in determining the waiting period for consumption of pesticide treated field materials without any risk. They have been useful in tracing the path of these chemicals in ground water. The lysimeters provide an excellent tool in modelling studies. All these applications thus are of practical value and considering the precise and accurate results they yield with detection limits exceeding much lesser than the conventional techniques, they certainly are indispensable till pesticides are used. Let us study these various systems in detail to understand the finer intricacies of the technique as well as the difficulties involved.

Numerous experimental approaches have been described in the literature to study the fate of pesticides in soil. The model systems have been classified in four categories:

1. Soil perfusion systems
2. Soil biometers
3. Gas flow through systems
4. Integrated systems.

Soil perfusion systems were originally developed for studying soil microbiological processes, such as nitrification, denitrification and microbiological utilization of water insoluble substrates and were successfully applied to examine microbial degradation of pesticides. However these systems have some distinct disadvantages in that the bacterial appear to be only predominant organisms in these systems. This factor and also other conditions in the system are far away from the regular practice. Moreover, the data obtained are not representative for the actual soil environment. Biometer type systems have been frequently used to study the degradation and metabolism of ^{14}C-labelled pesticides in soils. Bartha and Pramer developed the first biometer flask.

A simple system like biometer flask can be developed in laboratory to study the mineralization of radiolabelled pesticides either in soil or any other matrix under consideration like sea sediments,

manure etc. Measured amount of soil is taken on dry weight basis in a 250 ml Erlenmeyer flask. This flask can be designed to have a side tube for holding KOH or similar trapping solution. Alternatively such trapping solution can be accommodated in the flask itself in a vial with firm base to avoid loss of experimental material by accidental tilting. When soil is used in the experiment, it is moistened to 60% o its water holding capacity (WHC) or to any desired level of WHC. This moisture level is maintained throughout the period of experimentation. Similarly moisture contents of other matrices used in the experimentation are adjusted to predetermined levels. Three to four millilitre of 1N KOH are placed in a glass vial or side arm tube of the biometer flask if available. The soils can be treated with either labelled pesticide or labelled organic matter to study the mineralization of pesticide or effect of pesticide on mineralization of organic matter. The aims of the experiments can vary but methodology is similar. Aliquots of KOH are counted in a liquid scintillation counter ((LSC) after suitable time intervals and quantitation can be done. KOH is to be replaced before it gets saturated. The duration of the experiment will vary depending upon the quantity of degradation of the labelled compound.

Flows through systems have been used extensively for pesticide transformation studies in soil. They have varied from a few flasks connected in series to the complex manifold systems. The advantages of these systems are:

1. Soil respiration experiments can be conducted under more precisely defined conditions.
2. They permit characterization of both $^{14}CO_2$ and volatile products.
3. Exposure to the atmospheric CO_2 can be prevented.
4. Total CO_2 production can be monitored and used as an indication of soil microbial activity.
5. They can be operated on either positive or negative pressure.

A continuous flow through system consists of a 250ml Erlenmeyer flask holding the pesticide treated soil/sediment connected to a series of traps for capturing $^{14}CO_2$ and other organic volatile which may possibly released as a result of degradation of the pesticide. KOH is used to trap CO_2 while ethoxyethanol is used to trap the organic volatiles. Polyurethane foam plugs are used to trap anything that may escape earlier traps. The soil in the system is extracted after a certain period of time after monitoring $^{14}CO_2$ and organic volatiles continuously over a period of time. This time interval will vary for various pesticides and could be few weeks to few months. The solvent extracts can be characterized using thin layer chromatography and autoradiographic procedures. The phosphoimagers are now available which can help in faster characterization of the ^{14}C-residues. The extracted soil or sediment matrix is subjected to combustion to convert the bound residues into $^{14}CO_2$. This is achieved with the help of Biological Material Oxidizer. This is an instrument, which can burn the organic matter completely. Thus every component of the ecosystem is analyzed thoroughly and almost complete ^{14}C-mass balance of ^{14}C-labelled pesticide can be obtained.

Metcalf introduced the model ecosystem technique for studying the degradation of the pesticides and to determine their biomagnification. The model ecosystem is developed in an all glass aquarium tank. This tank accommodates the terrestrial and aquatic habitats. Plants, phytophagus insects, decomposer invertebrates, microorganisms and predaceous fishes form the biotic components of this ecosystem. Soil, sand or sediment and water form the abiotic components. When a radiolabelled pesticide is added to such an ecosystem, its fate and degradation can easily be followed through over a period of time. The uptake of labelled pesticide can be traced in food grains like rice or wheat in such ecosystems. The biomagnification of the pesticide in various components of ecosystem can throw more light on their ability to get concentrated in various strata of food chain. A rice-fish ecosystem has been developed for studying behavior of ^{14}C-labelled agrochemicals.

The rice-fish ecosystem developed in our laboratory simulates the rice fields especially in Eastern and North-Eastern States of India like Andhra Pradesh, West Bengal and Assam. Farmers in these States have developed a technique to grow fish in floodwater in rice fields. The floodwater is available for about two months. The catfish is a

popular variety grown in these areas. Since pesticides are used on rice quite extensively it is likely that they may be taken up by these fishes and in turn may bioaccumulate. Such bioaccumulation potential of the fishes can be easily studied using tracer techniques.

The concept of lysimeter developed by German scientists has revolutionised the radioisotope research. The application of radioisotopes in natural systems is not possible because of their hazardous nature. When the experiments are conducted using radioisotopes in laboratory systems, there is always a problem in the extrapolation of the results on field scale. Many a times we find that the laboratory experimental results are not reproducible especially in case of heterogeneous matrices like soils and waters. The model ecosystems come close to field scale from climatic point of view. The soil used in such ecosystem can never be considered as representative of field sample as its physical as well as biological structure get disrupted.

In laboratory experiments it is scarcely possible to control most of the environmental and edaphic parameters in the field. The precipitation can be supplemented to a limited extent by irrigation or reduced with shelters. Light may be reduced with shading devices but environmental variables can not really be controlled in a defined way. Soil is variable. This variability includes the variations between soils, which can to some extent quantified in pedological terms and variability within fields or even smaller units. It is known that the coefficients of variation for measurements of soil properties in individual samples taken within an area of 0.1 ha are of the order of 10-40%. Similar variations for pesticide persistence in samples incubated in laboratory have been reported. The situation is further complicated because many properties notably those related to water movement do not follow a normal distribution.

Lysimeters offer an excellent tool for using radioisotopes on undisturbed soil cores and get more realistic results. Lysimeters can be useful models to study the fate of pesticides in soil/plant system a per the FAO guidelines. In the latest context with so much research input with lysimeters this is really an understatement. Lysimeters have a number of features, which give them apparent advantages over other experimental systems. Compared with laboratory techniques they have the advantage that they almost exactly reproduce the environmental conditions that occur in the corresponding field soil. They do not significantly perturb the soil either mechanically or microbiologically. In addition it is possible to grow crops and carry out cultivation in line with agricultural practices and they can be maintained for a long time which could be in terms of several years. The results integrate the processes that are normally measured separately in the laboratory. Some of the problems of field experimentation can be atleast reduced in that soil monoliths from different sources can be grouped at the same site so it is possible to explore soils from different climates. It is usually easier to install equipment to monitor environmental parameters at a lysimeter station than it is in the field, particularly when a large number of field sites is involved. There is also no restriction on using radioisotopes.

With several years research work using lysimeters to study the fate of pesticides, it has been demonstrated that it is possible to achieve following things more effectively.

To quantify overall residues in plants, soil and leachate water and bound residues in soil and plants.

To integrate degradation and leaching studies (including aged residues) and obtain a more realistic assessment that can be derived from laboratory studies.

To define detailed studies of degradation, sorption, release and availability of residues to untreated rotational crops.

To obtain information on translocation of aged residues in the topsoil and with sufficient high specific labelling, also on realistic, extremely low concentrations in the subsoil.

To use the information obtained to improve the interpretation of field ecotoxicological studies.

To validate pesticide transport and degradation models.

Their major disadvantages are listed here.

They are expensive.

The environmental parameters can be monitored quite accurately but can not be very well controlled.

The reproducibility due to field variability may not be accounted for by lysimeters.

Optimistically it is quite conceivable that a well-conducted series of lysimeter studies could answer almost all of the questions that must be answered for registration purposes with regard to the fate and mobility of agrochemicals. This also includes studies concerned with dissipation pathways and rates as well as mobility, which seem to account for most current regulatory interest in lysimeters. If such development could be achieved, lysimeter studies would be the core source of registration data supplemented from laboratory and field studies wherever necessary to resolve particular uncertainties.

One lysimeter unit is composed of an absolutely watertight square or round container embedded permanently in the ground. In this unit a square or round casing is inserted keeping the undisturbed monolith and standing in a rack open at one side with a perforated bottom having a cutting edge at the front. All this equipment is made from stainless steel (Figure 4). The lysimeter unit is placed on a concrete bed and surrounded by top soil in control area. The filled lysimeter casing stands in the container on four arms fastened to the walls at a depth of 120cm. The container is 130-140cm deep, so that there is ample space available below the lysimeter to collect the water percolated through soil monolith. This water is sampled via a suction tube inserted into a pipe, which is fixed to the higher wall of the container. The lysimeter have effective areas of 0.5m^2 (square) to 1 m^2 (square or round). Critical attention has to be given to the filling of lysimeters in order to create conditions representative of the actual field situation.

To collect an undisturbed soil monolith, the lysimeter casing is covered at the top with a thick steel plate. At the bottom, the walls of the casing have sharpened edges for cutting the soil. The casing is rammed or pressed into the soil 110cm deep by the shovel of an excavator. This technique guarantees that the soil monolith is pressed close to the walls of the casing and that there are no gaps between the walls and the soil. This is very important while studying the mobility of the pesticides. After the casing is pressed into the soil, the surrounding soil is removed and the bottom of the rack is pushed under the casing cutting the soil with sharpened edge at the front of the bottom. Then the lysimeter casing with the undisturbed soil monolith standing in the rack is lifted from the hole and transported to the lysimeter station. There it is inserted into the container, which is permanently installed in the ground. Now it is ready to use.

The special advantages of experiments with ^{14}C-labelled pesticides using the lysimeter systems are as follows.

^{14}C-Labelled pesticides can be used in conditions close to agricultural systems.

The behaviour of pesticides can be studied over several growing seasons.

Mass balances can be drawn up considering all processes of dissipation including volatilisation.

Sufficient soil is available for detailed studies of specific processes like degradation, availability, formation of non-extractable residues etc.

The water percolating through the soil monolith can be collected and investigated for ^{14}C-residues.

Uptake of pesticides by plants and internal distribution can be studied with autoradiography.

It is possible to determine the biological activities at various depths in the lysimeter. This can be achieved by taking sections at various depths.

To summarise there are several advantages of using ^{14}C-labelled pesticides. The bioaccumulation and bioconcentration potentials of these xenobiotics in food chain can be accurately predicted. The residues can be detected at very low levels due to sensitivity and accuracy of the technique. The otherwise undetectable bound residues can be easily determined using a biological material oxidiser. The TLC-autoradiographic procedures strengthened with techniques like cochromatography and HP-TLC can play very important role in residues analysis. The leaching studies using lysimeters can throw light on potential of these agrochemicals to pollute the ground water. It is possible to obtain information on heterogeneity of soil and other intricately complex soil properties like role of organic carbon, microbial activity and physical structures in determining the fate of agrochemicals. These studies can be extended with suitable

modifications in the model ecosystems to reveal the marine pollution. The nuclear techniques thus provide us enormous scope in the field of pesticides and other environmental chemical studies.

References

1. N.B.K.Murthy, D.D. Kaufman and G.F.Fries. J. Environ. Sci. Health, B 14,1, 1979.
2. N.B.K.Murthy, W.R. Lusby, J.E. Oliver and P.C. Kearney. J. Nuclear Agric. Biol., 13, 16, 1984.
3. N.B.K. Murthy and K. Raghu. J. Environ. Sci. Health, B 24, 479, 1989.
4. N. B. K. Murthy, S. P. Kale and K. Raghu. Soil Biol. Biochem., 24, 857-859, 1990.
5. J. Drego, N.B.K.Murthy and K. Raghu. ^{14}C - gamma - hexachlorocyclohexane in a flooded soil with green manuring. J. Agric. Food Chem., 38, 266, 1990.
6. N.B.K.Murthy and K. Raghu. Bull. Environ. Contam. Toxicol., 46, 374, 1991.
7. T. Puetz. In "Lysimeter studies of the fate of pesticides in the soil" Ed. F. Fuhr and R. J. Hance BCPC Monograph No. 53, 1992.
8. S. P. Kale and K. Raghu. Bull. Environ. Contam. Toxicol., 53, 298-302, 1994.
9. S. P. Kale and K. Raghu. J. Plant Nutr. Soil Sci., 159, 519-523, 1996.
10. S. P. Kale, G. Sarma, U.C. Goswami and K. Raghu. Chemosphere, 33, 449-451, 1996.
11. G. Reinken, S. P. Kale and F. Fuehr. Sciences of Soils, (Internet Journal: HTTP://www.hintze-online.com/SoS/Art2) 1, 1-28, 1996.
12. K. Raghu, S. P. Kale, N. B. K. Murthy, J. Mitra Science and Culture, 62, 177-180, 1996
13. S. P. Kale, N. B. K. Murthy and K. Raghu. Bull. Environ. Contam. Toxicol., 59, 72-75, 1997.
14. Murthy, N.B.K. and K. Raghu. J. Nucl. Agr. Biol., 26, 111, 1997.
15. Murthy, N.B.K., Kulkarni, M.G. and Raghu, K. Toxicol. Environ. Chem., 65, 241, 1998.
16. S. P. Kale, F. P. Carvalho, K. Raghu, P. D. Sherkhane, G. G. Pandit, A. M. Mohan Rao, P. K. Mukherjee, and N. B. K. Murthy. Chemosphere, 39, 969-976, 1999.
17. S. P. Kale, F. P. Carvalho, K. Raghu, P. D. Sherkhane and N. B. K. Murthy. Chemosphere, 39, 959-968, 1999.
18. S. P. Kale and N. B. K. Murthy. Pestology 23, 331-335, 1999.
19. N. B. K. Murthy, S. P. Kale and K. Raghu. J. Nuclear Agric. Biol. 29(2) 100-103, 2000.
20. S. P. Kale, N. B. K, Murthy and K. Raghu. Chemosphere 44, 893-895, 2001.
21. S. Dubey, S. P. Kale, P. S. Dubey and N. B. K. Murthy. J. Nuclear Agric. Biol., 30(1), 34-40, 2001.

Sterile Insect Technique (SIT) for the Management of Insect Pests of Economic Importance

***Dr. T. K. Dongre** joined BARC through 17th batch of training school in 1973-1974 and presently leading a Pest Management Section of Nuclear Agriculture and Biotechnology Division. He is engaged in research activities pertaining to Integrated Pest Management (IPM) with emphasis on Sterile Insect Technique (SIT) and biological insect control. He had many accomplishments as a collaborative scientist in national and international projects. The IAEA and FAO have recognized him as an expert in field of SIT and IPM. He has made outstanding contributions in area of pest management and completed various international experts' assignments successfully. He is actively associated with different professional bodies of Entomology.*

The radiation has direct application in controlling insect pests through Sterile Insect Technique (SIT). The SIT is a species-specific and it can be considered as a form of birth control in insects. It is an environmentally non-polluting method of insect control that relies on mass rearing, radio-sterilization and release of large numbers of sterile insects in field. The continuous field release of sterile insects brings down the reproductive ability of natural insect population and consequently it controls or even eradicates the targeted insect pest completely.

The idea of sterile insect technique was first conceived in the 1930s [1], and applied on a significant scale in the 1950s against the New world screwworm fly, *Cochliomyia hominivorax* (Coquerel) [2] and subsequently to a number of other insect pest species [3]. The attractive features of the SIT are its absolute specificity to the targeted insect pest, ability to integrates well with other control methods and its action is inverse-density dependent, means as field population declines, the pressure increases on the population from a constant rate of sterile insect release. This characteristic makes SIT more desirable for eradication, suppression and containment of targeted insect pest [4].

Principles of " SIT "

The principle behind the sterile insect technique is the genetic effects of radiation on insect reproduction. Low dose radiation affects the reproductive cells of the insects and makes them sterile. By taking advantage of this fact in 1930, E.F.Knipling conceived an idea of controlling the insect pests from defined area by using SIT. In this concept of pest control, continuous release of large number of mass produced sterile insects into natural population would limit the reproductive ability of native population which would result in reduction of density of field population. As the density of natural population decreased, the influence of continuous release of sterile insects would increase and finally it may lead to possible elimination of insects from that area. The effectiveness of sterile insect technique can best be understood by theoretical mathematical model prepared by Knipling (Table 1).

In this model we assume that no practical problem of immigration, both the sexes are completely sterile and equally competitive and one generation time equal to the life cycle of targeted insect. This model follows a hypothetical insect population for 5 generations. Assumption is made that in parent generation there are one million populations. If sex ratio is considered to be 1:1, then there would be 500,000 males and 500,000 females. Here sex is ignored for simplicity because the theoretical example will be the same in both the cases. The population growth rate was assumed to be five fold in each generation. Assuming that there is no environmental constraint, one million populations in parent generation would increase to 625 million in F-4 generation.

Dr. T. K. Dongre, Nuclear Agriculture and Biotechnology Division, Bhabha Atomic Research Centre, Trombay, Mumbai 4000 85;
E-mail: tkdongre@barc.gov.in

TABLE 1. Hypothetical example of SIT

Generation	Insects in field	Sterile insects released	Ratio of sterile: fertile	% Sterility	Fertile insects in field	Growth rate
P	1000000	9000000	9:1	90	100000	5x
F1	500000	9000000	18:1	94.7	26316	5x
F2	131580	9000000	68:1	98.6	1907	5x
F3	9535	9000000	944:1	99.9	10	5x
F4	50	9000000	180000:1	99.999	0	5x

In the example the trend is reversed by the release of 9 million completely sterile insects which establish a ratio of 9 sterile insects to every one wild insect. The 90% wild females assumed to mate with sterile males would result in 90% reduction in fertility. Only 10% wild population would be reproducing. In other words with sterile release only 1 lakh females would be producing and these individuals can increase their number by five fold, then the next generation (F1) would be represented by 5 lakhs individuals in place of 5 millions in case of unreleased case. The positive side of this outcome is that the population is reduced by 50% in the first generation. In F1 generation if we release another 9 million sterile insects then the wild population would now be over flooded with ratio of sterile to fertile 18:1 that is, twice as high as it was in parent generation (9:1). In further generations since the density of wild population will go on reducing, the ratio of sterile to wild will go on increasing. The theoretical out come is the development of ratio of sterile to fertile is so high that the population becomes extinct.

Requirements of " SIT "

The SIT cannot be used for all the insect pests. There are some general and specific requirements for using SIT for insect control. As SIT is sophisticated, difficult and not easily accomplished program, it should be restricted to insect pest of major economic concern where the current cost of damage and control justify large expenditure. The SIT approach of managing insect population should be in terms of area wide i.e. control of particular insect species in a given area rather than on a conventional field by field basis. Area wide insect control is possible only where government or agricultural co-operative working in different area assume responsibilities. There is need to have some naturally isolated area to avoid migration of the insects and other control measures to minimize insect number as SIT is more effective at lower density of the insect population.

Besides these general requirements there are few specific requirements, which have to be considered, when one decide to go for SIT. Before starting SIT program one needs to have detail information about population dynamics of the concerned insect. It is essential to collect information about population density, growth rates from generation to generation, survival rate of various life stages and their developmental times. The basic requirement of any insect control program is colonization and rearing of that particular insect species.

Following colonization and small scale rearing, for field studies mass rearing of that species is essential. The mass rearing operation can be described as insect factory and involves all the components and operations of large factories viz., planning, location, facilities, budgets, accounting, personnel, equipments, supplies, purchasing, waste management etc. Quality control and handling of released insects is of utmost importance for the successful implementation of SIT. Insects of poor quality cannot perform satisfactorily in natural environment. The quality of insects can be lost in the

colonization, rearing, sterilization and handling. In SIT program, release of males are always preferred over the release of both the sexes. So sexing at early stage will help to reduce insect rearing cost.

"SIT " as a Component of " IPM "

In principle, SIT can be used to control or to eradicate the targeted insect pest completely. However, presently scientists are looking at SIT as one of the component of integrated pest management program (IPM). The sterile insect release method is completely compatible with other types of insect control that might be used in IPM programs. In fact, SIT must be integrated with other measures in order to be used in the most effective way. The SIT is the most efficient and economical when pest population is already at low level. In SIT we need to release laboratory or factory reared insects in field. Thus, the number of insects that can be reared will determine the size of the area of release. If less native insects are in the release zone, then a higher release ratio will be attained or a larger release area can be covered. Any insect control measure that will reduce population densities either before or during the application of SIT program will enhance the effectiveness of both the methods. For example, use of biocontrol organisms is generally most effective at high pest densities, but lose efficiency as pest densities decrease. Thus, a sterile insect release program would be a natural adjunct to any type of biological control program. This same reasoning applies to use of insecticides to reduce high pest population and use of pheromones in confusion program.

Cultural control methods, which are generally used to reduce pest populations during their most vulnerable stages are completely compatible with the use of SIT programs because the latter programs would generally be used during the time of high reproductive potential of the target species. The area wide IPM with an SIT as one of the component has been used with great success to control several major horticultural pests following four major strategies: eradication, suppression, containment and prevention [5].

World Scenario

The successful implementation of sterile insect technique against number of key veterinary and plant insect pests has clearly demonstrated the peaceful application of nuclear technology. Over the past 35 years, the joint FAO/IAEA committee has played a critical role in supporting member states in the development and application of this effective, environment-friendly insect control method. In different parts of the world SIT was successfully used or being used for managing following insect pests of economic importance.

Screw worm Fly

The first success story of SIT was eradication of screwworm fly from North America. It is a serious problem to livestock production in southern and south western USA. This is an ideal candidate for SIT because it inflicted heavy losses in limited geographical area and its population density was comparatively less. This project started way back in 1954 and continued till 1984. The first successful trial to control this insect was conducted at an island of Curcaao (435Km^2) in 1954. In this trial more than 70,000 sterile flies were released per week for 3.5 months. This resulted in complete eradication of flies from this island.

After success of this trial, wide spread trials have been conducted for next 30 years in different parts of USA and this fly was completely eradicated from North America. In 1958 within 18 months flies were eradicated from Florida by releasing 50 million sterile flies per week. Further in 1962 Screw worm SIT program was started in south west United States and released 100 million flies per week which resulted in 95 percent success. Here problem was migration of flies from Mexico. Therefore in 1977 the joint program between USA and Mexico was started where release of 3.5 million flies per week resulted in complete eradication of flies. Since 1984 sterile fly barrier was maintained on the border of Mexico and USA.

In 1988 screw worm outbreak was reported in Libya and first time this insect was found to be established in out side of USA. An international program has been launched to eradicate this out break. For this program flies were reared in Mexico and flown to Libya. Total 1.3 million flies released over a period of 18 months which eradicated flies from Libya. Eradication is also well underway in Costa Rica , Panama, Cuba, and Jamaica. An

international effort to contain and eventually control an outbreak of the old world screwworm fly in the Gulf States, with a focus in Iraq and southern Iran, is launched by AOAD, FAO and IAEA [6].

Tsetse Fly

Tsetse fly is an important veterinary insect pest, which is being controlled through SIT. Tsetse flies have a devastating effect on humans and livestock production in 38 countries south of the Sahara covering area of 7 to 11 million square kilometers. The tsetse fly is the carrier of animal trypanosomosis that has retarded cattle production in Africa for centuries, as well as of "sleeping sickness" in humans. No healthy cattle are available in tsetse infested areas for agricultural work, thus effectively preventing economic development in many countries south of the Sahara. The SIT program started to eradicate the tsetse fly from Zanzibar and Tanzania had made a considerable progress. Recent break through in mass rearing and aerial release technology have culminated in the first area-wide and integrated use of SIT to eradicate tsetse in Zanzibar, initiated in 1994 against tsetse fly, *Glossina austeni*. Intensive monitoring over Zanzibar during the three years eradication shows no sign of tsetse fly or animals affected by trypanosomosis [7].

Fruit Flies

Due to globalization, the trade in fresh fruits and vegetables has gained special interest on worldwide basis. Fruit and vegetable industries in many developing countries are aware that their profitability depends on exporting fruits and vegetables to lucrative markets in developed countries, however in order to export fruit and vegetables, developing countries must comply with increasing stringent Sanitary and Phytosanitary Standard (SPS) measures being mandated. The fruit fly damage is major concern in fruit export. Many developed countries do not tolerate the pesticide residues as well as introduction of pests like fruit flies. The highly successful programs in Australia, Mexico, Japan, and elsewhere have already proven that the SIT can be used effectively to control various species of fruit fly. IAEA/FAO helped Chile and Mexico to eradicate the Mediterranean fruit fly using SIT. The UN agencies are currently assisting national plant protection authorities in Argentina, Guatemala and several countries in the Near East to eradicate this pest and establish pest-free zones.

The first large SIT program against medfly was initiated in Mexico in 1977, with the construction of a 500 million sterile fly mass rearing facility. The aim of the program was to prevent the spread of medfly, which had become established in Central America, into Mexico and the USA, which threatened Mexico's multi-million fruit and vegetable export trade with the USA. The program succeeded in 1982 in eradicating medfly and since then maintained a sterile fly barrier to assure the fly-free status of Mexico, USA and Guatemala.

Bactrocera and *Anastrepha* are devastating fruit fly pests of economic and quarantine importance. Great advances have been made in developing sterile insect technology for some of these species. The SIT melon fly program in southern Japan started in mid 1990s was the most successful program to eradicate this species from islands of the Okinawa. In Australia, SIT was successfully applied to eradicate the Queensland fruit fly from Western Australia, and preventive SIT release are being used in Southern Australia to protect fruit growing areas from the seasonal movement of the pest into commercial areas.

In the Philippines the sterile insect technology has been adapted to *Bactrocera philippinensis* and a pilot program has been in progress in mango producing Guimaras Island. The economic feasibility of this approach has been confirmed by a number of benefit-cost analyses. For medfly and other fruit flies, the current worldwide weekly production capacity of sterile flies has now reached several billion sterile flies. The SIT technology is well developed for some other fruit flies like Caribbean fruit fly, Oriental fruit fly, Onion fruit fly, Mexican fruit fly and Cherry fruit fly. These programs showed a good success not only for controlling flies, but also for quarantine purpose in USA, Mexico and Japan [8,9].

Pink Bollworm

The pink bollworm, *Pectinophora gossypiella*, is a serious cotton pest in many parts of the world. A program against this pest in California has since

1968 been successfully integrating aerial sterile moth releases and various cultural and other controls. Over a 24-years period there has been a continuous release of sterile pink bollworm adults during each day of the cotton-growing season. Its objective has been to prevent the migrating pest from becoming established in the San Joaquin Valley. In order to address moth migration at its origin in other cotton growing areas like Phoenix and Arizona, mass rearing facility was enlarged in 1994 and the program was extended to the Imperial Valley in 1998 to eradicate the established pest in that area [10].

Codling Moth

Codling moth, *Cydia pomonella*, is a key pest of apples and pears in many temperate regions of the world. In early 1990s the SIT program against this pest was initiated in British Columbia, Canada. With funding from the federal and provincial governments, a mass rearing facility was built with a capacity of producing 15 million moths per week. After four years of operations, the program has been successful in controlling the pest with growers essentially reporting no damage and most not having to spray against codling moth, allowing the production of organic fruits [3].

Mosquitoes

The high reproductive rate of mosquito populations will place special requirements on any use of SIT. Feasibility studies for the control of the mosquitoes *Anopheles albimanus* and *An. stephensi* using SIT were conducted in El Salvador and in India in the 1970s respectively. New technology and the concept of integrated vector management will be combined in an attempt to evaluate the use of SIT as a component of mosquito control. Two consultant reports commissioned by the IAEA have recommended several candidates of *Anopheles* species for SIT together with potential target sites for initial field trials. However, they stressed that before this stage, important technical constraints relating to several key components of SIT technology must first be removed. Recently a renewed interest in SIT for malaria vectors has led to a 5-year feasibility study to investigate all aspects of an SIT program including sexing, mass production, sterilization, and release methodology [11]. The project initially focuses on the African malaria vector *Anopheles arabiensis* Patton.

Other Insects

In addition to above insect species recently IAEA has initiated SIT program for controlling date moth and cactus moth. The date moth (*Ectomyelois ceratoniae* Zeller), also know as the carob moth, is a devastating pest of dates in Tunisia, Morocco, Algeria, Libya, Iran, Iraq, Saudi Arabia and Israel. The cactus moth (*Cactoblastic cactorum* (Berg)), a native to Argentina, was first detected in Florida in the USA in 1989. However, the accidental introduction of the cactus moth into the USA raised serious concerns for its potential spread to the Opuntia-rich areas of the western USA and Mexico . The list of insects for which SIT has been used or being developed for the management of different insect pests of economical importance is given in Table 2.

Indian Scenario

At BARC attempts have been made to demonstrate the feasibility of SIT to control insect pests of national importance. The insect pests chosen for this studies are red palm weevil, *Rhynchophorus ferrugineus* Oliv, a serious pest of coconut and date palms and potato tuber moth, *Phthorimaea oprculella* Zeller which infest potatoes in field as well as in storage.

Red palm Weevil

Attempts have been made to demonstrate the feasibility of SIT to control red palm weevil under field condition. Besides coconut this pest also attacks date palm, oil palm and many other ornamental palms. This is serious pest in South Asia and Arabian countries. In India, it is the most serious pest of coconut, whereas recently this has become very serious pest on date palm in Arabian countries. This pest believed to have crossed into Arabian countries through ornamental plants in 1985. Presently all 50 million of Arabia's date palms are under threat of extinction from this virulent pest. Present control methods have failed to manage this insect pest due to the absence of any visual symptoms of damage at early stage of infestation. By the time insect attack become discernible,

TABLE 2. Insect pests for which SIT is being used or is being developed

Insect	Countries where SIT has been used	Countries where SIT is being developed
Screwworm	Curaçao, USA, Mexico, Puerto Rico, US Virgin Islands	Guatemala, Belize, Libya
Mediterranean fruit fly	Italy*, Peru*, Mexico, USA, Israel*	Guatemala, USA (Hawaii)
Pink bollworm	USA*	USA (quarantine)
Caribbean fruit fly	USA (Florida)*	USA(Florida)fly-free zone
Melon fly	Japan*	Japan, Brazil
Oriental fruit fly	Rota, Hawaii*	
Onion fly	Netherlands*	Netherlands (control)
Mexican fruit fly	USA/Mexico*	USA/Mexico (quarantine + fly-free zone)
Cherry fruit fly	Switzerland*	
Codling moth	Canada*, USA*	Canada (control)
Gypsy moth	USA*	USA (quarantine)
Tsetse flies	United Republic of Tanzania*, Nigeria*, Nigeria, Zanzibar	
Tobacco budworm		USA*
Boll weevil	USA*	
Sheep blowfly	Australia*	Bangladesh*
Mosquitoes	El Salvador* India*	Africa*
Stable fly	St Croix, USA*	
Tobacco hornworm	St Croix, USA*	
Cattle fevertick	St Croix, USA*	St Croix. USA
Date moth		Tunesia*
Cactus moth		USA*
Red palm weevil		India*

* Experimental pilot test.

irreparable damage would have been already caused leading to the total destruction of the palm trees.

Pest Management Section of the Nuclear Agriculture and Biotechnology Division has developed sterile insect technique (SIT) for the management of red palm weevil. An efficient and economic mass rearing methods were developed by using coconut petioles, sugar cane and artificial diet [12, 13]. Detail radiological studies were carried out by using cobalt 60 source [14] and initially pilot scale field trial was conducted at Kayangulam, kerala. The trial was carried out in collaboration with Central Plantation Crop Research Institute (IPCRI). The results of this trial were quite encouraging and warrant area wide management of red palm weevil. Recently Sterile Insect Technique was tested in the field in collaboration with three Indian agricultural universities viz. Kerala Agriculture University, Thiruvanantapuram, University of Agricultural Sciences, Dharwad and Dr. B.S. Sawant Konkan Krishi Vidyapeeth, Dapoli. Initially the pest incidence was surveyed extensively along the coastal districts of three Indian states - Maharashtra, Karnataka and Kerala. Three relatively isolated hot spots one in each state were selected and insect population density was estimated by using marked sterile insects. The four years release of sterile males in selected hot spots resulted in significant reduction in wild insect population as well as trees infested with red palm weevil. As this insect has become the most dangerous pest of date palms in Gulf countries, recently IAEA and Gulf countries are taking keen interest in sterile insect technique developed by us. There are some attempts to test this technique for managing red palm weevil under Gulf conditions. Author has visited Saudi Arabia as a IAEA and FAO experts to assess the extent of damage by red palm weevil and to find out the feasibility of using SIT for the management red palm weevil in the Gulf region

Potato Tuber Moth

Potato tuber moth is the most destructive pest of potatoes, causing serious damage in field as well as in storage. In field it appears as leaf minor and later stage infest the exposed tubers, which act as nucleus culture for the multiplication of this pest in storage. Recommended cultural, chemical and biological control methods have proved to be either unsafe or inadequate to control this pest. Under storage conditions, fumigation or the dust formulation of chemical insecticides are commonly used. The toxic residues of these insecticides pose great health hazards when potatoes were stored for the human consumption. As the insect population in storage structure is a discrete entity, the use of sterile insect technique has been advocated, as a promising method for controlling this pest in storage conditions.

In our laboratory, investigations were carried out to evaluate the feasibility of using sterile insect technique for the control of potato tuber moth under storage condition. Mass rearing technique has been developed and required radiological data have been generated. The adult stage was found to be an ideal stage for the irradiation and almost complete sterility was induced when freshly emerged males and females were irradiated with the dose of 450 Gy and 300 Gy respectively. The irradiation at the dose of 450 Gy had no appreciable effect on the mating periodicity, propensity and overall competitiveness of sterile males. The 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. The multiplication of insect population was significantly suppressed by the release of sterile males in ratio of 10:1 [15]

Recent Advances in SIT

There are many limitations in SIT program which need to be addressed for their successful implementations. With recent advancement in molecular biology and biotechnology there are possibilities of removing these hurdles in near future. Modern biotechnology could potentially provide several improvements such as improving the identification of released individuals (genetic markers) and providing automated sex-separation prior to release (genetic sexing).

In many cases it is considered important to release only males, but large-scale sex-separation is problematic. This difficulty can be overcome by using engineered strains of insects carrying a dominant, repressible, lethal gene or genetic system. As a proof of this principle, scientists have constructed strains of *Drosophila melanogaster*

with the required genetic properties [16]. One method, called RIDL, involves using a dominant lethal gene associated with a female-specific promoter so that expression of the dominant lethal is switched off so long as a particular nutrient is provided to the breeding stock; however, when insects are being reared for field release, then nutrient could be removed, causing the death of all females. This would reduce costs of producing the millions of sterile males needed for control programs and reduce the likelihood of releasing females that could bite or transmit disease.

For easy evaluation purpose it is essential to have genetic marker for released insects. The fluorescent proteins GFP and DsRed [17] and their derivatives are likely to be the most suitable markers which allow easy discrimination between wild type and released insects. This is the basis of the current pink bollworm release trials. A transgenic pink bollworm strain expressing EGFP was constructed by John Peloquin at UC Riverside [18]. This strain could potentially be used to monitor the dispersal and longevity of released insects in the pink bollworm SIT program.

Genetic modification using recombinant DNA methods can now be used, almost routinely, to transform pest and beneficial insects for different purpose. However, it is essential to resolve the potential risk issues prior to releases of transgenic sterile insects in field. These risks include whether the inserted gene is stable; the traits, especially pesticide or antibiotic resistance genes be horizontally transferred to other populations or species; released insects will perform as expected with regard to their geographic distribution, host or prey specificity and other biological attributes. Risk assessments of fitness and host specificity are relatively easy to assess in the laboratory, but the potential risk assessment of horizontal gene transfer under field conditions and unintended effects on ecosystem function are much more challenging.

Even though Sterile Insect Technique has many limitations, it was found to be the most effective method against certain insect pests which are successfully controlled or being controlled in different parts of the world. Over the last 40 years, FAO and IAEA have played a vital role in supporting their Member States in the development and application of this environment-friendly pest control method. The successful implementation of Sterile Insect Technique (SIT) for controlling different insect pests all over the world, clearly demonstrated the peaceful application of nuclear technology.

References

1. E.F. Knipling, J. Econ. Entomol., 48, 459-462 (1955)
2. A.H. Baumhover, A.J. Graham, B.A. Bitter, D.E. Hopkins, W.D. New, F.H. Dudley and R.C. Bushland, J. Econ. Entomol., 48, 462-466 (1955)
3. A. Dyck , J. Hendrichs, A.S. Robinson, In Area-Wide Integrated Pest Management. Heidelberg, Germany: Springer. (2005)
4. J. Hendrichs , M..J.B Vreysen, W.R. Enkerlin, J.P. Cayol , In: Sterile Insect Technique, Principles and Practice in Area-wide Integrated Pest Management. V.A. Dyck, J. Hendrichs and A.S. Robinson (eds..). Springer, Dordrecht, The Netherlands pp. 563-600. (2005)
5. M.J.B.Vreysen, J. Gerardo-Abaya, J.P. Cayol, In Area-wide Control of Insect Pests. From Research to Field Implementation, M.J.B. Vreysen, A.S. Robinson and J. Hendrichs (eds.) Springer, Dordrecht, The Netherlands (2007).
6. Alfred H. Baumhover, Florida Entomologist 85, 666-673 (2002)
7. L.E. LaChance and W. Klassen, IAEA Yearbook 1991, IAEA, Vienna B23, (1991),
8. W. Enkerlin and, J.D. Mumford, J. Economic. Entomol., 90, 1066–1072 (1997).
9. E.S. Krafsur, J. Agric. Entomol., 15, 303-317 (1998).
10. R.T.Staten, R.W. Rosander and D. F. Keaveny, In Management of Insect Pests: Nuclear and Related Molecular and Genetic Techniques. (Proc. Symp. Vienna, 1992), IAEA Vienna, pp. 269-283. (1993).
11. M.E.H. Helinski, B. El-Sayed, B.G.J. Knols, Entomol. Berichten., 66,13-20 (2006).

12. H.D. Rananavare, K. Shantaram, M.R. Harwalkar and G.W. Rahalkar, J.Plant.crops. 3, 65-67 (1975).
13. G.W. Rahalkar, A.J. Tamhankar and K. Shantaram, J. Plant. Crops., 6, 61-64 (1978).
14. G.W. Rahalkar, M.R. Harwalkar, H.D. Rananavare, K. Shantaram, and G. Ayengar, J. Plant. Crops., 1, 141-146 (1973).
15. H.D. Rananavare, M.R. Harwalkar and G.W. Rahalkar, J. Nuclear Agric. Biol. 20, 199-295 (1991).
16. Luke Alphey, Insect Biochem. Mol. Biol., 32, 1243-1247 (2002).
17. M. Matz, A. Fradkov, Y. Labas, A. Savitsky, A. Zaraisky, M. Markelov and S. Lukyanov, Nat. Biotechnol. 17, 969–973 (1999).
18. J.J. Peloquin, S.T. Thibault, R. Staten and T.A. Miller , Insect Mol. Biol. 9, 323– 333 (2000).