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Mutagenesis: Investigating the process and processing the outcome for crop improvement

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The discoveries during the late 1920s that the genetic material is amenable to changes excited geneticists who saw new opportunities for both basic and practical applications. During the 1950s, induced mutagenesis was widely pursued in the US, Europe, Japan and China. In India, Swaminathan and his team at the Indian Agricultural Research Institute, New Delhi initiated a major programme on mutagenesis in crop plants. These studies were broadly aimed at understanding the process of mutation, testing the efficacy of various mutagens, identifying optimum dose and the best method of treatment for different crop species; isolation of mutants of basic and applied value; elucidating the biological effects of radiation-treated media, seeds and vegetative propagules on the organisms consuming them.

Keywords: Mutagenesis, crop improvement, mutations, mutants.

SWAMINATHAN is an inspiring leader who is always open to new ideas. His enthusiasm for science was contagious and his team was always bubbling with activities. Above all, there was a deep sense of commitment to the good of society through the pursuit of science. I was fortunate to be associated with this group. In this article I shall attempt to capture some of the major accomplishments of the 'era of mutation breeding' at the Indian Agricultural Research Institute (IARI). The work at IARI had a cascading effect because many of Swaminathan's students went on to expand these activities in other places in India and abroad. At the end, I shall discuss the relevance of mutation breeding in the current context of biotechnology.

During mid-1950s and 1960s, when DNA had been well established as the genetic material and the Watson and Crick's double helix model of DNA received recognition as basic organization of hereditary material, there was great interest in elucidating the mechanisms underlying induced genetic mutations, especially by chemical mutagens. We in India also shared this excitement and attempted to find answers to specific questions. In particular, it was recognized that there are several steps between the initiation of a lesion in DNA by a mutagen and the ultimate expression of the change in the form of an altered phenotype. We felt that understanding of these steps would be critical for mutation breeding work, especially for increasing the frequency and range of mutations, and for lowering lethal effects of mutagens. Hence a number of test systems such as bacteria, plants and flies were employed as models.

Initial studies on induced mutations were mainly directed to finding optimum combination of mutagen and dose to elicit the best response. Both physical and chemical mutagens were tested in various crop species such as wheat, barley, rice, tobacco, corn, Brassica, fruit crops and vegetables. These studies helped to initiate large-scale mutation breeding experiments for various practical applications. The work resulting from these studies is summarized.

Effect of ploidy on mutation

Unlike animals, crop plants are represented by a variety of ploidy levels including 2*x*, 3*x*, 4*x*, 6*x*, aneuploids and higher order polyploids. Furthermore, both auto- and allopolyploidy are encountered among crop species. It was generally believed that polyploids with their duplicated sets of genes would be less sensitive to mutagenic treatment in terms of realized mutants than their diploid counterparts. Therefore, systematic studies were conducted with wheat, cotton, barley and rice.

In wheat, among the various physical mutagens examined, thermal neutrons were found to be the most effective followed by ^{35}S, ^{32}P and X-rays[1]. The diploid *Triticum monococcum* was found to be more sensitive to mutagenic treatment than polyploids. Surprisingly, the tetraploid *T. dicoccum* was relatively more resistant to fast and thermal neutrons , and X-rays than the hexaploid bread wheat, *T. aestivum*[2,3]. Greater sensitivity of hexaploids to fast neutrons was attributed to higher vulnerability of bread wheat chromosomes to breakage. A study on the effect of fast neutrons in root tip cells of *T. monococcum* showed that breaks occurred more frequently in the neighborhood of the supernumerary constriction of one of the satellite chromosomes (Sat II). Preponderance of such breakage in only one of the two homologues further implied some degree of microscopic differences with reference to localized neutron sensitivity sufficient to distinguish them. This appears to be the first report of its kind that provides cytological evidence for the existence of non-random sensitivity of chromosome segments to fast neutron action[2].

Experiments with chemical mutagens were at variance with those of physical mutagens. Among the chemical mutagens, alkylating agents, especially EMS was demonstrated to be the most potent. The mutagenic response was more or less linear with the dose and polyploids were more

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tolerant than diploids. An interesting observation was that genes near the centromere were more prone to mutagenic treatment than those located farther away. Further, chlorophyll mutants were frequent in EMS treatment but were rare in treatments with physical mutagens[4]. At that time this result was attributed to differences in the chemical composition of the chromosomes near the centromere, making them more sensitive to chemical mutagens. While it may indeed be the case, other explanations are possible. For example, genes near the centromere are less likely to be involved in recombination and hence mutations in those genes are less likely to be eliminated through selection.

Results of mutation experiments with diploid (*Gossypium arboreum*) and tetraploid (*G. hirsutum*) cotton were totally different from those of wheat. Cotton was found to be relatively resistant to mutagenic treatments and very few mutants could be recovered in M_2 and M_3 generations. It was observed that diplontic selection was highly operative in cotton which ensured that only cells with normal complement of chromosomes were functional to produce gametes. Furthermore, the histogenic aspects of inflorescence development were also found to be important in uncovering the mutants. In wheat, 2–3 shoot primordia are already present at the time of mutagen treatment of seeds and each ear is derived from one initial cell. On the other hand, in cotton, a single shoot primordium would be subjected to mutagenic treatment, which upon growth and differentiation would give rise to whole plant and flowers. Thus the chance of diplontic selection was greater in cotton than in wheat[5].

Studies with diploid and autotetraploid barley showed a steep decline in chlorophyll mutations with increasing ploidy, thus conforming to the expectation that gene duplications provide cushion against harmful effect of mutation. Following treatment, no mutants were recovered in the M_1 or M_2 generations of autotetraploids. However, in the M_3, both lethal and viable mutants were observed[6] thus confirming that mutation treatment was effective in tetraploids but uncovering of the mutants needed at least two generations of meiosis involving chromosome segregation and recombination.

In *Secale*, differences were observed among species for X-ray sensitivity. *S. anatolicum* was found to be the most sensitive. *S. cereale*, *S. silvestris* and *S. vavilovii* suffered similar degree of initial damage as evident from chromosome aberrations. However, *S. cereale* was able to overcome this initial damage, thereby showing much less adverse effect on seedling growth[7]. None of the nuclear factors such as nuclear volume, DNA content or heterochromatin content at early prophase was related to radiosensitivity.

Radiophosphorus treatment in maize produced cells with multiple nucleolar buds caused by deletion or inactivation of nucleolar organizer. Haploids were observed frequently in irradiated populations of wheat. A systematic study of mutagen dose and treatment time revealed that treatment of inflorescences prior to anthesis was effective in inducing haploids. Further, ^{32}P and ^{35}S were potent mutagens for inducing haploids[8].

Effect of combined treatment of mutagens

Since various physical and chemical mutagens are known to act in different ways to cause DNA lesions, combined effects of mutagens were investigated. In wheat, combined treatment with UV and X-rays showed dose-dependent effects. UV pretreatment of seeds reduced the frequency of mutations at low doses of X-rays (11–16 kr) but increased it at high doses of 22–30 kr[9,10]. In barley, treatment with S-2 aminoethylisothiouronium bromide hydrobromide (AET) was tested as both pre- and post-treatment with X-rays. Frequency of chromosome aberrations and chlorophyll mutations registered a significant drop when AET treatment was followed by X-ray irradiation. On the other hand, post X-ray treatment of AET caused a slight drop in chromosome aberrations[11]. Similarly, combined treatment of two chemical mutagens, ethyl methane sulfonate (EMS) and hydroxyl amine (HA), was investigated in wheat. Data of chlorophyll and viable mutations indicated that EMS is a potent mutagen in *T. dicoccum* but HA is a weak mutagen. But when HA was administered after EMS treatment, there was a significant drop in mutation frequency indicating that HA may be involved in mutational repair process[12]. Studies with *Drosophila* showed that formaldehyde, which is not mutagenic in female flies, could enhance mutation frequency when administered following X-ray treatment. This suggested that formaldehyde might be blocking some DNA repair process[13].

The importance of genetic constitution on sensitivity to mutation was investigated in *Escherichia coli*. In strain WP-2 of *E. coli* harbouring tryptophan auxotrophy (try^-), an induced adenine auxotrophy rendered the try completely stable against reversion[14]. A number of other mutants were isolated that showed differences in their vulnerability to mutagen treatment. Detailed analysis of these mutants showed that they contained lesions in their mutator loci. Both general and specific mutator loci were identified. For example, *mut4* enhanced mutations at the *nal* locus whereas *mut3* was a general mutator that influenced mutations at several loci. *mutU2* and *mutU3* were found to be UV sensitive mutators[15,16].

Induced mutations in phylogenetic studies

It was widely believed that spontaneous mutations have played a major role in speciation. The discovery of mutagens provided the opportunity to test this hypothesis. Swaminathan's team examined wheat and rice to see the contribution of major mutations to species differentiation. Based on the assumption that if major mutations were responsible for speciation, it should be possible to induce and isolate mutants with characteristic features of other species, a large number of ear mutants were induced and studied in wheat and rice.

Ear mutants were isolated in hexaploid wheats following physical and chemical mutagenesis. Mutants of phylogenetic

significance included *speltoid*, *vavilovoid*, *sphaerococcoid* and *compactoid* mutants in *Triticum aestivum* and *aestivum*-like mutants in *T. sphaerococcum* and *T. compactum*[17]. A dwarf, branched ear mutant resembling *T. turgidum* was isolated in mutagen-treated population of *T. aestivum*[18]. Deletions involving genes controlling ear branching and plant height were believed to have given rise to *turgidum*-like mutant. Occurrence of mutants with flower anomalies in *T. vavilovii* and such segregants in the cross between *T. vavilovii* and *T. vulgare* suggested that probably *T. vavilovii* lacks the basic Q-1 locus of *spelta* and hence the sterility and other flower anomalies find phenotypic expression. In the light of the above observation it was suggested that *T. vavilovii* could have arisen in a complete *speltoid-vulgare* as happens in mutagen-treated material[19,20]. Study of mutation data revealed that *T. sphaerococcum* could have arisen through a deletion in chromosome 3D of *T. aestivum* as suggested by earlier workers. However, *aestivum*-like mutants were observed frequently in the progenies of irradiated *T. sphaerococcum*. Further, sphaeroccoid mutants which did not involve chromosome 3D of wheat were isolated in *T. aestivum* which pointed to S-locus independent origin of *sphaerococcoid* trait. Hence it appears that at least two independent loci in *vulgare* are capable of generating the *sphaerococcum* syndrome of characters[21].

The relative roles of genetic and chromosomal mechanisms on phylogeny and racial differentiation of rice (*Oryza sativa*) were studied extensively through mutational, biochemical, biometric and cytological approaches. From the behaviour of mutagen-treated material in advanced generations and the response of the treated material to recurrent irradiations it was found that rice behaves more like a diploid[22]. Critical studies of the mutants suggested that the *indica* and *japonica* varieties do not involve a systematic mutation but have probably proceeded through a series of independent mutations affecting grain and plant characters. The relative response of the three subspecies of the cultivated rice *Oryza sativa*, namely, *indica*, *japonica* and *javanica* to a wide array of mutagens was determined. Judged by the indices of survival and growth inhibition, *indica* varieties appeared to be relatively more resistant to both physical and chemical mutagens as compared to *javanica* and *japonica* groups. It was found that neutrons were the most effective radiation source while nitrosomethylurea (NMU) was the most effective among chemical mutagens[23].

Radiomimetic effects

Irradiation of food and food products was known to extend their shelf life. However, consequences of irradiation on nutritive quality and possible mutagenic effects on organisms consuming them were not well understood. At IARI, studies were conducted to assess the radiomimetic effects caused by irradiated media. Irradiation of potato tubers was found to suppress sprouting thereby, reducing spoilage during storage[24]. Barley root meristems cultured on X- or gamma-irradiated potato mash or fruit juices showed chromosome aberrations[25,26]. Similarly, in *Drosophila melanogaster*, frequency of sex-linked lethals was increased in progenies of flies raised on irradiated food[33]. Experiments on the effect of irradiation of culture medium on germination and growth of pollen of *Tropeolum majus* showed that as the dose increased, the deleterious effect on pollen germination increased. The pollen culture studies on irradiated media that had been stored for different periods (0–74 h) after exposure to 1–3 kilorads of gamma rays showed that the cytotoxic effect could diminish, remain unchanged or be enhanced with storage duration depending upon the initial dose of radiation[28]. Gamma-irradiated culture medium was found to cause lethality and induce mutations in *E. coli*[29]. Further analysis of the effect of irradiation of various components of the culture medium revealed that irradiation of salt solution generated compounds causing lethality[30]. Likewise, irradiated sucrose solution was found to be cytotoxic. However, the cytotoxic effect was time dependent[31]. Similarly, it was established that feeding on irradiated medium or irradiated DNA produced dominant lethals but did not induce any visible mutations in *Drosophila*[32,33]. These studies clearly established that an array of qualitatively and functionally different radiomimetic principles might be produced at different doses and time after irradiation.

Mutants of applied value

Isolation of mutants of agronomic and economic significance was a major goal of mutation breeding exercise at IARI. Mexican wheat strains that ushered the Green Revolution in India had red hard grains which were not preferred by the Indian farmers and consumers. Mutation breeding was therefore applied to alter grain colour of wheat. A light coloured grain mutant 'Sharbati Sonora' was obtained from 'Sonora 64' while a similar light grain colour mutant 'Pusa Lerma' was derived from another popular line 'Lerma Rojo 64A'. 'Pusa Lerma' with its high resistance to stem rust and semi-hard white grains was released for cultivation in peninsular India[34]. 'Sharbati Sonora' was early besides having amber grains and hence was found to be suitable for late planting. Amber-grained mutants were also induced by gamma rays in a high yielding dwarf variety Tonari 71 of *T. aestivum*[35]. These mutants were late by about six days, and also displayed colourless auricles and waxy ears and leaves. Similarly, a mutant displaying resistance against race 40 of stem rust was isolated following X-irradiation in the popular wheat variety Pb C 591 (ref. 36). Multiple resistance to rusts was induced in *T. aestivum* varieties Lalbahadur and Kharchia Local with nitrosomethyl urea treatment. These mutants displayed resistance to several races of leaf rust at seedling stage. Besides, adult plant resistance was also found in these mutants for yellow rust, black rust and brown rust[37]. Some of these mutants displayed yield advantage, which was proved to be due to their ability to ward off rust infection.

In cotton, a jassid-resistant line was isolated through mutation breeding of the highly susceptible variety 'Mescilla Acala'[38]. In rice several dwarf mutants were induced following chemical and physical mutagenesis[39]. In tobacco Patel and Swaminathan[40] recorded significant improvement in nicotine content in mutants induced through X-rays and ^{32}P.

Mutation breeding in India

Besides IARI, mutation breeding work is being pursued at several universities and research institutes, notably, at Bhabha Atomic Research Centre (BARC) Mumbai, Tamil Nadu Agricultural University (TNAU), Coimbatore, National Botanical Research Institute (NBRI), Lucknow. Bhatia[41] while reviewing the economic impact of mutant varieties in India, had put the number of mutant varieties developed at 205 up to 1990. By 2004 this list has grown to 313 (ref. 42). The mutant varieties released include cereals, grain legumes, oil seeds fibre crops, vegetables and ornamentals (Tables 1 and 2). The success story of mutation breeding in ornamentals and horticultural crops in India is particularly impressive. In chrysanthemum alone, 46 mutants are commercially released. Considering the fact that the mutations are generally deleterious, the number of mutant cultivars released in major crops is impressive. It is relevant to emphasize here that in all cases (except ornamentals) the mutant varieties have emerged as superior to other entries in all India coordinated trials before being approved for commercial release.

Mutant varieties like K-84, IIT-48, IIT-60, Sattari and Keshari of rice[43], Aruna of castor, TG-1, TG-17, TAG-24 and Co-2 of groundnut, Pusa 408 (Ajay), Pusa-413 (Atul), Pusa-417 (Girnar) of chickpea, Co-4, Pant Moong-2, MUM-2, TARM-1 of mung bean, Co-4, TAU-1, TPU-4 of black gram, Maru Moth-1 of moth bean are among the important varieties of economic significance released in India[42,44]. Authentic information on area occupied by these varieties is unfortunately not available. However, TAU-1 variety of black gram developed by BARC, Mumbai has become the most popular variety in Maharashtra occupying about 5 lakh hectare (equivalent of 95% of the area under this crop in the state). Similarly, some varieties such as Co-4, Pant Mung-2 and TAP-7 of mung bean released in the early 1980s are still being widely grown which points to their better performance in farmers' fields. The variety TARM-1 resistant to powdery mildew and YMV diseases is the first of its kind to be released for rabi/rice fallow cultivation. The mutagen used and the main trait(s) improved in the released mutant varieties are listed in Table 3. From the table it is evident that gamma rays are the most preferred agent and plant type and higher yields are the traits most commonly manipulated through mutation breeding.

Table 1. Number of released mutant varieties in different crop species in India

Latin name	Common name	No var.	Latin name	Common name	No var
Ablemoschus esculentus L. Moench	Okra	1	*Matricario cammomilla*	German chamomile	1
Arachis hypogaea L.	Groundnut	16	*Mentha spicata*	Spearmint	1
Bougainvillea spectabilis Wild	Bougainvillea	10	*Momordica charantia* L.	Bitter gourd	1
Brassica juncea L.	Indian Mustard	6	*Morus alba* L.	Mulbery	1
Cajanus cajan Milsp.	Pigeon pea	5	*Nicotiana tabacum* L.	Tobacco	1
Capsicum annum L.	Chilli	1	*Oryza sativa* L.	Rice	41
Carica papaya L.	Papaya	1	*Papaver somniferum* L.	Opium poppy	1
Chrysanthemum sp.	Chrysanthemum	46	*Pennisetum typhoides* L.	Pearl millet	5
Cicer arietinum L.	Chickpea	7	*Phaseolus vulgaris* L.	French bean	1
Corchorus capsularies L.	White jute	2	*Pisum sativum* L.	Pea	1
Corchorus olitorius L.	Tosa jute	3	*Plantago ovata* L.	Isabgol	1
Curcuma domestica Val.	Turmeric	2	*Polyanthus tuberosa* L.	Polyanthus	2
Cymbopogon winterianus Jowitt.	Citronella	6	*Portulaca grandiflora* L.	Portulaca	11
Cyamopsis tetragonoloba L.	Cluster bean	1	*Ricinus communis* L.	Castor	4
Dahlia sp.	Dahlia	11	*Rosa* sp.	Rose	15
Dolichos lablab L.	Hyacinh bean	2	*Saccharum officinarum* L.	Sugarcane	9
Eleusine coracana L.	Finger millet	4	*Sesamum indicum* L.	Sesame	3
Gladiolus L.	Gladiolus	2	*Solanum khasianum* Clarke	Khasianum	1
Glycine max L.	Soybean	4	*Solanum melongena* L.	Brinjal	1
Gossypium arborium L.	Desi cotton	1	*Sorghum bicolor* L.	Sorghum	3
Gossypium hirsutum L.	American cotton	8	*Setaria italica* L.	Foxtail millet	1
Hibiscus sinensis L.	Hibiscus	2	*Trifolium alexandrium* L.	Snake gourd	1
Hordeum vulgare L.	Barley	13	*Trifolium alexandrium* L.	Egyptian clover	1
Hyocyamus niger	Indian henbane	3	*Triticum aestivum* L.	Wheat	4
Lantana depressa L.	Wild sage	3	*Vigna aconitifolia* Jacq. Marechal	Moth bean	5
Lens culinaris Medik	Lentil	3	*Vigna mungo* L. Hepper	Blackgram	7
Luffa acutangula Roxb.	Ridged gourd	1	*Vigna radiata* L. Wil	Mungbean	12
Lycopersicon esculentum M.	Tomato	4	*Vigna unguiculata* Walp.	Cowpea	7

Table 2. Mutant varieties of different crops released for cultivation in India

Crop	No. of varieties released	Specific crop and no. of varieties
Cereals	69	Rice (39), barley (13), pearl millet (5), finger millet (4), foxtail millet (1), wheat (4), sorghum (3)
Pulses	53	Mungbean (14), blackgram (7), chickpea (7), cowpea (7), mothbean (5), pigeonpea (5), lentil (3), lablab bean (2), cluster bean (1), common bean (1), pea (1)
Oilseeds	33	Groundnut (16), mustard (6), castor bean (4), sesame (3), soybean (4)
Fibre crops	14	American cotton (8), tossa jute (3), white jute (2), desi cotton (1)
Vegetables	12	Tomato (4), turmeric (2), bitter gourd (1), brinjal (1), green pepper (1), okra (1) ridge gourd (1), snake gourd (1)
Cash crops	10	Sugarcane (9), tobacco (1)
Medicinal crops	16	Citronella (8), German chamomile (1), Indian henbane (2), isabgol (1), Khasianum (1), opium poppy (2), Spearmint (1)
Fruit trees	2	Mulberry (1), papaya (1)
Forage crops	1	Egyptian clover (1)
Ornamentals	103	Chrysanthemum (46), rose (16), dahlia (11), portulaca (11), bougainvillea (10), wild sage (3), gladiolus (2), *Hibiscus* sp. (2), tuberose (2)

Table 3. Mutagens used and trait improved in mutant cultivars released in India

Mutagen	No. of mutants	Main attribute	No. of occurrence
Gamma rays	169	High yield	86
X-rays	26	Early maturity	65
Neutrons	7	Disease resistance	57
Ethyl methane sulphonate	15	Quality characters	39
Dimethyl sulphate (DMS)	4	Grain quality	67
Ethylene imine (EI)	2	Abiotic stress resistance	65
Sodium azide (NaN_3)	2	Improved plant type	181
Other mutagens	29	Other	9
Cross bred	47		
Natural mutants	12		

Induced mutations and biotechnology

Although traditional mutation breeding has lost its preeminent position, induced mutations continue to be in great demand for various biotechnological applications. The methods of mutation induction and analyses of mutants have witnessed great changes in recent years. For a brief period during the 1980s and 90s, when it was discovered that *in vitro* culture of plant cells gives rise to genetic variation[45], somaclonal variation was widely tested as an alternative to traditional mutation breeding. Variations were found in plants regenerated through tissue culture for almost all traits. We were successful in developing an improved variety of *Brassica juncea* 'Pusa Jai Kisan' through this approach[46]. Nevertheless, it is now concluded that somaclonal variation does not offer special advantage over existing methods of inducing genetic variation.

Induced mutations are playing a major role in basic studies especially for the elucidation of biochemical and plant developmental pathways. For example, identification of key genes involved in floral organ development, which ultimately led to the construction of ABC model of flower development, was made possible through the isolation and molecular characterization of floral mutants of *Arabidopsis* and *Antirrhinum*[47]. The genome sequencing projects have given a new fillip to mutagenesis. With the availability of complete genome sequences of major organisms including plants like *Arabidopsis thaliana* and *Oryza sativa*, scientists are now trying to find biological functions of various putative gene sequences. While computational approaches have been found useful in gene annotations, the true biological meaning can be established only by empirical experiments. Induced mutations are expected to play a key role in such functional genomic analyses.

Today scientists are interested in locating and identifying the exact genetic change at the nucleotide level. Traditional forward genetic approach 'from phenotype to genotype to gene' is very cumbersome and hence conventional mutagenesis is not considered ideal for molecular analysis. The availability of plant transformation techniques has provided novel ways to create and screen specific mutations. Mutations can now be induced through transposon or T-DNA insertion via transformation[48]. Mutant lines (i.e. lines carrying foreign DNA insertions into host chromosomes) can be isolated based on the presence of foreign DNA sequence. Thus one can avoid screening based on an altered phenotype. Further, using the sequence information of the transposon or T-DNA, the mutated gene can be easily tagged and cloned through inverse

PCR or other techniques. Reverse genetic approaches (i.e. proceeding from gene sequence information to phenotype) have also become available in recent years. For example, antisense suppression or silencing of genes is now feasible through transgenic approach. Thus any test sequence can now be employed for gene silencing via antisense or RNAi approach to elucidate its biological function[49]. However, transformation is still not very routine and easy in many plant species. Hence traditional forward genetic approach is still being followed.

One of the chief advantages of traditional mutagenesis is that it can give rise to many different mutant alleles with different degree of trait modification. This variation in expression is very useful in many basic studies, such as identification of amino acid residues critical for enzyme activity. In contrast, transposon or T-DNA mutagenesis generally leads to loss of function through gene disruption. Therefore, conventional mutagenesis is still favoured for basic studies. New molecular approaches have greatly simplified forward genetic approach with conventionally derived mutants. Saturated molecular maps are now being constructed in most crop plants. Using such maps, the mutant locus is first delimited using molecular markers. In the next step, the gene is cloned through positional cloning or chromosome walking[50].

Site-directed-mutagenesis leading to specific nucleotide modification within a gene sequence is now feasible *in vitro*. Similar efforts at introducing specific changes *in vivo* have proved successful in mouse[51] and fruitfly[52]. In plants, gene replacement experiments through homologous recombination with introduced DNA sequences have met with limited success[53]. Therefore, conventional mutagenesis will continue to be important in molecular-genetic studies of plants.

In summary, mutation breeding experiments begun in the mid-1950s at IARI have paid rich dividends. There is considerable expertise available today in our country for undertaking mutation breeding work. In view of the significance of conventionally induced mutants in functional genomics, there is great opportunity ahead for us in the era of genomics.

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