IONIZING RADIATIONS FOR PLANT MUTAGENESIS: SUCCESS STORY AT BARC, TROMBAY

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Abstract
Ionizing radiation has immense applications in agriculture for crop improvement, crop production and crop protection. Crop improvement is reliant on wide genetic diversity of economic characters towards achieving food and nutritional security. In nature, occurrence of genetic change (mutation) in plants is evolutionarily slow and gradual process. The frequency of such mutations can be increased through ionizing radiations. The radiations cause mutations at chromosomal, gene or DNA level, which may be manifested into desirable characters in crop plants. Since fifties, Bhabha Atomic Research Centre (BARC), Mumbai has been engaged in radiation-based induced mutagenesis to develop improved varieties in cereals, food legumes and oilseeds. Earlier, experiments were undertaken to study radiosensitivity, mutation frequency and cytological aberrations upon induced mutagenesis and its effect on morphological, biochemical and physiological traits in crop plants. Subsequent induced mutagenesis generated large spectrum of mutants in different crops. By identifying promising induced mutants and their utilization in recombination breeding, BARC has developed 62 improved varieties in various crops. These mutants

and mutant derived varieties were released for commercial cultivation across the country in collaboration with State Agricultural Universities and national ICAR institutes. Several of Trombay varieties have been cultivated extensively by the farmers in most of the states and have immensely benefitted by enhancing their income.

Keywords: *Induced mutagenesis, Ionizing radiation, Mutation breeding, Cereals, Food legumes, Oilseeds*

1. Introduction

Mutation is a heritable alteration in the genetic material in an organism, which is the main driving force for crop evolution. Both spontaneous and induced mutations constitute the basis for genetic diversity in crop plants. Genetic diversity for various characters is crucial to crop improvement and enhanced food production, which in turn for ensuring national food and nutritional security. Hugo de Vries in 1901 first identified mutagenesis as a phenomenon of creating genetic variability, which he distinguished from recombination and segregation. Spontaneous mutations were the only source of novel genetic variation till twentieth century, which were used in plant selection for domestication and breeding. After the discovery of the mutagenic potential of X-rays as demonstrated by Stadler in 1928 and 1930 in maize, barley and wheat, radiation based induced mutagenesis was employed as a tool for generating novel genetic variability in plants. In 1928, Gustafsson and Nilsson-Ehle first identified valuable mutations in diploid barley by using X-rays and UV rays. The first commercial mutant variety was produced in tobacco in 1934. Bhabha Atomic Research Centre (BARC), Mumbai has been engaged in the field of crop improvement by using ionizing radiations through mutation breeding since fifties.

2. Induced plant mutagenesis

Mutagenesis is the process of bringing stable genetic changes, which may include, a) **Induced mutagenesis,** wherein mutation is induced by treating the seeds with physical or chemical mutagens; b) **Insertion mutagenesis,** wherein mutation is obtained due to DNA incorporation from transformation or transposon activation and c) **Site-directed mutagenesis** by creating a mutation at a target site by transformation followed by homologous recombination between native DNA and T-DNA. Usually induced mutants occur at random, whose genes mostly are recessive in nature, occasionally with pleiotropic effects and are influenced by genetic background and environmental effects.

3. Physical and chemical mutagens for induced mutagenesis

A mutagen is an agent that cause mutations in DNA sequence. These agents are physical, chemical and biological in nature. Each mutagen type acts differently in the genome. Physical mutagens, generally ionizing radiations, have been effectively employed for inducing mutations in crop plants. Globally, >85% mutant varieties were developed through irradiation. Ionizing radiation dislodges an electron from its orbit around the nucleus after passing through a plant tissue, thus producing an ion (ionization) and free radicals. Ionizing radiations such as X rays, gamma rays, beta particles, neutron, proton, electron and ion beams have been utilized for induced mutagenesis in crop plants. They bring changes in DNA sequence either by base substitutions (transition or transversion) or by indels (insertions or deletions). Majority of ionizing radiations are emitted from naturally decaying isotopes and can also be produced artificially in reactors and through accelerators.

Using chemical mutagens is a simple and effective method of altering a single base in the DNA sequence. The most commonly used chemical mutagens for crop improvement include Ethyl methanesulphonate (EMS), sodium azide, colchicine, nitrosoethyl urea and N-methyl N-nitrosourea. EMS is the most effective and potent mutagen causing point mutations, loss of small DNA regions and reorganization in other chromosomes, alkylation of DNA to form base pairs of guanine with thymine instead of cytosine, and conversion of GC base pair to AT. For mutation induction, factors like moisture content of irradiated tissue, mutagen type, irradiation dose, dose rate or concentration and treatment duration are crucial.

4. Mutation breeding methodology

Crop improvement is a continuous process of development of improved varieties suitable for different agro-climatic situations. Natural radiations bring new variability in different characters spontaneously at extremely low frequency (one in a million). Using radiations and/or chemical mutagens, mutation frequency can be enhanced to several folds (one in thousand). Mutation breeding includes induction, isolation and stabilization of mutants and their judicious utilization in cross breeding. In mutation breeding, the main goal is to create suitable varieties with increased seed yield and nutrients, earliness, desired seed size and dormancy, tolerance to diseases, insects, drought, salinity, heat etc.

Successful mutation breeding starts with well-defined objectives to generate new genetic variability for nuclear and/or cytoplasmic traits; to improve one or a few traits in popular and well adapted varieties; to break the tight linkages between the traits; to enhance chromosomal translocations in inter-specific crosses and to improve vegetatively propagated crops. To begin with, effective dose for a mutagen has to be standardized for the selected genotype in given crop by treating seeds with different doses of radiation. After the treatment, values on seed germination and seedling growth from different treatments are subjected to Probit analysis to derive the dose close to LD_{50} (dose that brings 50% reduction in germination) and/or GR_{50} (dose that brings 50% growth reduction). For large scale induced mutagenesis, seeds are treated with 2-3 doses of radiation around LD_{50} or GR_{50} values (M_1 generation) using gamma irradiator (**Fig. 1a**). These treated M_1 seeds are sown in the agricultural fields to raise M_1 plants. Seeds (M_2) from the M_1 plants are sown to raise M_2 generation. Fig. 2 shows field view of M_2

generation of rice and groundnut. Usually mutants are identified from M_2 generation onwards.

Fig. 1: (a) Seed irradiation with gamma rays for induced mutagenesis; (b) Crossing of crop mutants to recombine favourable alleles.

Fig. 2: Field view of M_2 generation of (a) transplanting of rice material; (b) groundnut population at Gamma field, BARC, Trombay.

Homozygosity (stable genetic nature) of the induced mutants is ascertained by studying their breeding behavior in subsequent generations. These stabilized mutants are evaluated with the existing varieties over the locations and seasons to find their superiority in yield parameters, suitability and adaptability in the trials carried out by State Agriculture Universities (SAUs) and Indian Council of Agricultural Research (ICAR). Based on the superiority of new mutant, varietal identification committee of ICAR/SAU recommends the suitable mutant for release. Further, Department of Agriculture and Farmers' Welfare, Ministry of Agriculture & Farmers Welfare, Government of India releases and notifies new mutant for commercial cultivation. Sometimes, such mutants are crossed with other mutant, variety or distant parent to combine the beneficial traits from both the parents (Recombination or cross breeding) (**Fig. 1b**).

5. Ionizing radiation based mutation breeding at BARC

5.1. Basic experiments on plant mutagenesis

Seed irradiation studies were initiated in the fifties at Atomic Energy Establishment, Trombay (AEET), to study radiosensitivity, mutation frequency, cytological aberrations upon induced mutagenesis and their effect on morphological, biochemical and physiological traits by treating seeds of over 50 varieties of rice, maize, sorghum, water melon, groundnut, cotton, foxtail millet and isabgol with X rays, neutrons and gamma rays. Considerable differences for radiosensitivity among these varieties were observed. LD_{50} values ranged from 70 Gy in brinjal to 400 Gy in mustard for X rays and 100 Gy in peas to 550 Gy in mustard for gamma rays. Stimulating effects of low doses of thermal neutrons were also observed. In 1957, thermal neutron irradiation in rice evolved a plant with awned seeds. Chlorophyll deficiency of several types, pigment development affecting various parts of rice, fasciation of stem and branch in groundnut and water melon were also noted. It was also observed in water melon that the ratio of male and female flowers was altered by pile neutrons in favour of female flowers.

Dosimetry studies were conducted for barley seeds by irradiating with fast neutrons at APSARA reactor and gamma rays in gamma cell. Based on LD_{50} values, barley seeds showed increased radiosensitivity with storage time for fast neutron, while there was no such effect with gamma rays. With a view to study the ploidy-dose level effect on the relative biological efficiencies (RBE) of the radiations, seeds of tetraploid and hexaploid wheat were irradiated with X-rays, fast neutrons and thermal neutrons. It was shown that RBE for fast neutron/X-rays was not significantly influenced by the ploidy, but was dependent on the dose level. Further in another study, RBE values for gamma rays/fast neutron ranged from 6.75 in *Phaseolus lunatus* to 33.70 in barley. RBE values had also been determined for specific mutations affecting culm height in rice. Fast neutrons were effective to induce variability for this trait and offer possibility of dwarf mutants with good fertilizer response. Recently, the pulsed electron beam treatment from linear accelerator in wheat showed slightly higher RBE compared to gamma rays. Further with accelerated proton ions from BARC-TIFR Pelletron accelerator facility, significant differences were observed in wheat seedling growth and survival parameters and later for mutation spectrum compared to gamma rays. Based on the survival and growth curves, the thermal neutron from Dhruva research reactor showed considerably high RBE in wheat compared to gamma rays.

5.2. Effective doses of mutagen for induced mutagenesis in different crops

In food legumes, the effective doses for gamma rays (pigeonpea: 100-200 Gy; groundnut, cowpea: 200-300 Gy; mungbean, urdbean, chickpea: 300-400 Gy) and for electron beam (groundnut: 150-200 Gy; cowpea: 270 Gy, chickpea: 300 Gy; urdbean: 400 Gy; mungbean: 500 Gy) have been standardized through radiosensitivity assays. Gamma rays of 300 Gy and electron beam of 250 Gy are suitable for rice and sorghum mutagenesis. In wheat, LD_{50} and LC_{50} values were 290–315 Gy and 0.90–1.35%, respectively for gamma rays and EMS under laboratory conditions, 240–290 Gy and 0.50–1.1% under field conditions. In another wheat experiment, it was found that 300 Gy and 350 Gy of gamma rays and 200 Gy and 250 Gy of electron beam were most effective in HD2967 and PBW343, respectively. The frequency of yellow rust resistant mutants was higher in electron beam than in gamma rays. Similarly, in chickpea, electron beam irradiation showed higher mutagenic effectiveness and efficiency compared to gamma rays.

5.3. Cytogenetic effects of radiation based mutagenesis

Radiations cause chromosomal aberrations in the first generation of their treatment (M_1). In an earlier experiment in rice, a single quadrivalent was observed in thermal neutron induced awned mutant. In groundnut, X-rays induced irregular association of chromosomes and their separation at anaphase, abnormal spindle development, chromosomal bridges and fragments, polyad occurrence, cytokinesis failure and developmental anomalies in pollen grains. Further studies have reported reciprocal translocations with chain and ring multivalents, inversions with fragments and bridges at anaphase I and II. In groundnut, trisomics, tetrasomics, long chromosomes with altered coiling, cytomixis and pollen mother cells with 15–18 chromosomes were reported. In X-ray-induced dwarf groundnut mutant, asynaptic chromosomes with reduced number of bivalents at diakinesis were observed.

5.4. Inheritance of induced mutant traits

In various crops, radiation induced mutants were studied for their inheritance pattern of some of the mutant traits and are given in table 1. Certain mutants exhibited unusual genetic behaviour such as preferential segregation for leaflet size and type, paternal inheritance for foliaceous stipule and hard kernel and suppressive gene action for disease mimic leaflet in groundnut.

5.5. Development of Trombay varieties through mutation breeding

With basic understandings from earlier radiation induced mutation experiments and in line with national and state crop breeding programmes, BARC had initiated and streamlined mutation breeding programmes for the improvement of cereals, oilseeds and food legumes. Over six decades of consistent efforts in induced mutagenesis had resulted in hundreds of mutants with several desirable agronomic features in these crops at BARC. Such mutants were utilized directly or made inter-mutant or mutant-variety crosses to develop 62 improved Trombay varieties. These varieties have been released and notified for cultivation by the farmers in different states during 1973-2023 in synergistic research collaboration with the ICAR and SAUs (Table 2). Of these, 19 varieties are direct mutants and rest are mutant derivatives and 17 varieties are released in the national ICAR system, while rest are released through SAUs. Some of the desirable traits in these crops are enhanced seed yield, higher nutrient content, earliness, ideal plant type, greater seed size, seed dormancy, wider adaptability and resistance to lodging, diseases and moisture stress. These characters not only advanced the crop productivity but also facilitated the development of new or alternate cropping systems, which in turn generated additional farm income.

Table1: Inheritance of induced mutant traits in different crops

Crop	Mutant trait	Inheritance of genes
Mungbean	Large seed	Incomplete dominance
	Yellow mosaic disease resistance	Single recessive gene
	Lanceolated leaf	Single recessive gene
	Cerrated leaf	Single recessive gene
	Small leaf	Single recessive gene
	Chlorina	Single recessive gene
	Yellow seed	Single recessive gene
Urdbean	Bruchid resistance	Duplicate dominant genes
Cowpea	Connate foliaceous stipules	Duplicate recessive genes
	Subsessile leaf	Single recessive gene
	Monopodial branching	Double recessive genes
	Seed coat pattern (Solid, Holstein and eyed)	Duplicate recessive epistasis (Supplementary genes)
	Determinate plant type	Single recessive gene
	Self-incompatibility	Single recessive gene
Pigeonpea	Compact-dwarf plant type	Single recessive gene
Wheat	Reduced height	Single recessive gene
	High tillering, grassy bunch and clustered panicle	Single recessive gene
Rice	Lesion mimic	Single recessive gene
Sesbania rostrata	Late flowering	Single recessive gene
Chickpea	Elongated organ and large seed size	Incomplete dominance
Groundnut	Dwarf plant height	Single recessive gene
	Gibberellin-insensitive dwarf	Single dominant gene
	Suppressed branches	Single recessive gene
	Imparipinnate leaf	Single recessive gene
	Bifurcated leaf	Triple recessive gene
	Funnel leaflet	Suppressive gene action
	Suborbicular leaflet	Single recessive gene
	Lupinus leaflet	Suppressive gene action
	Dark green leaf	Duplicate recessive gene
	Virescent, *chlorina*, variegated	Single recessive gene
	Disease lesion mimic leaf	Suppressive gene action
	Foliaceous stipules	Duplicate recessive gene
	Yellow flower	Single recessive gene
	Sequential flowering	Duplicate recessive gene
	Purple, Chocolate testa	Duplicate recessive gene
	Rose testa	Single dominant gene
	Hard seed	Single recessive gene

5.5.1. Food legumes

Food legumes (pulses) comprise an important crops that provide high quality protein supplementing cereal proteins for country's majority vegetarian population. Pulse production in India is 24.49 million tonnes (mt) during 2023-24, where chickpea contributed 11.57 mt, followed by pigeonpea with 3.38 mt and mungbean with 2.91 mt. Genetic improvement of food legumes such as, blackgram/urdbean, greengram/mungbean, cowpea, pigeonpea, chickpea and cluster bean has been undertaken by the BARC through radiation induced mutagenesis and recombination breeding. BARC has developed 24 varieties in pulse crops, which include nine in mungbean, eight in urdbean, five in pigeonpea and two in cowpea (Table 2; **Fig. 3**). Pulse varieties such as TAP-7 in mungbean, TT-6 in pigeonpea and TRC77-4, TC-901 in cowpea are direct mutants, while rest of them are mutant derivatives. In urdbean, mutants, UM-196 and UM-201 were crossed with variety T-9 to evolve three varieties, TAU-1, TAU-2 and TPU-4 for Maharashtra. Similarly in pigeonpea, cross between a fast neutron induced large seed mutant variety TT-6 and ICPL 84008 had led to development of early maturing varieties, TT-401, TJT-501 for Chhattisgarh, Gujarat, Madhya Pradesh and Maharashtra and PKV-TARA for Maharashtra. While in mungbean, an early maturing variety TMB-37 was developed by crossing Kopargaon and TARM-2 and released for Assam, Bihar, Jharkhand, Uttar Pradesh and West Bengal. Trombay pulse varieties like TU-40 in urdbean, TM-96-2, TM-2000-2 in mungbean and TRC-77-4 in cowpea are also suitable for cultivation in rice fallows. Cowpea mutant variety, TC-901 is the first variety in the country suitable for summer. Recently urdbean varieties TJU 130 and TJU 339 are released for Madhya Pradesh and TRCRU 22 for Karnataka. Mungbean variety TRCRM 147 is released for Karnataka. Most of the pulse varieties are with ideal plant type, better seed size and disease resistance.

Fig. 3: Plants of (a) mungbean variety, TRCRM-147, (b) urdbean variety, TJU 339, (c) cowpea variety, TC-901 and (d) pigeonpea variety, TJT-501

5.5.2. Oilseed crops

Indian oilseed production crossed 39.59 mt during 2023-24. Rapeseed-Mustard ranked first by contributing 13.16 mt, followed by soybean (13.05 mt) and groundnut (10.28 mt). Since sixties, BARC has been engaged in mutation breeding of Indian mustard using beta particles, X-rays, gamma rays and developed spectrum of mutations for plant height, inflorescence, flower morphology, maturity, seed colour, seed weight and oil content. Sustained breeding efforts with these mutants has evolved eight varieties, which are commercialized in different states (Table 2). In mustard, yellow seed coat mutant has more oil, more protein, thinner seed coat and lower fiber compared to brown seed coat parents. First yellow seed coat mutant in India, Trombay Mustard 1 (TM 1) was developed by BARC by treating Rai 5 variety with beta rays from Phosphorus-32 radioisotope. Subsequent recurrent selection in the same mutant has resulted in high yielding variety, TPM 1 with reduced erucic acid for Maharashtra. Earlier years, a direct X ray short mutant with appressed pods and brown seeds, TM 2 and a mutant derivative TM 4 have been released for Assam. TPM 1 and TM 4 are with yellow seed coat. Further, TM 2 was diversified using IC264133 to develop two varieties: Trombay Him Palam Mustard 1 (THPM-1) for Himachal Pradesh and Birsa Bhabha Mustard-1 (BBM-1) for Jharkhand. Similarly, TM 102 was successively recombined with other breeding lines to evolve TAM 108-1 for Maharashtra; TBM-143 (**Fig. 4a**) and TBM-204 for West Bengal.

In soybean, BARC has developed two varieties viz., TAMS 38 (**Fig. 4b**), a gamma ray mutant of JS 80-21 and TAMS 98-21, a cross derivative with superior seed yield, non-pod shattering, resistance to diseases and pests. Both the varieties were cultivated widely by the farmers in Vidarbha region of Maharashtra.

Fig. 4: Plants of (a) mustard variety, TBM-143; (b) soybean variety, TAMS 38; (c) groundnut variety, TAG 73; (d) linseed variety, TL 99

Groundnut is an important food, oilseed and feed crop in our country. At BARC, its mutation studies were started with X-ray irradiation in 1957 and with gamma rays and electron beam in subsequent years. Periodical induced mutagenesis in groundnut had generated gene pool having many divergent mutants. Succeeding breeding efforts using these mutants in recombination breeding has developed and released 16 Trombay groundnut (TG) varieties for cultivation across the country (Table 2). First BARC variety TG 1 was developed in 1973 through X-ray mutagenesis. Its large seed mutant trait contributed immensely in the succeeding TG varieties. Later, another X-ray mutant variety, TG 3 was commercialized for Kerala. Inter-mutant cross variety, TG 17 was developed for Maharashtra. Crosses involving both TG 1 and TG 17 had developed a mutant derivative, TKG 19A having large seed for Maharashtra. Further, these TG mutants and their derivatives were genetically diversified by other varieties to develop TGS-1 (Somnath) and TG 22 for Gujarat and Bihar, respectively. Recombination breeding involving these mutants and M 13 has resulted in the development of four varieties: TAG 24 and TLG 45 for Maharashtra; TG 39 for Karnataka and Rajasthan and RARST-1 (TG 47) for Andhra Pradesh. Genetic diversification of these mutants was continued by involving more parents for incorporation of newer characters, which has evolved five varieties, TG 26, TG 37A, TG 38, TPG 41 and TG 51 and are released by ICAR for different states. Recently, gamma ray mutagenesis has evolved mutant, TG 73, suitable for Maharashtra and Gujarat (**Fig. 4c**). These TG varieties were with semi-dwarf height, compact plant type, large seed, early maturity, fresh seed dormancy, desired seed and pod type, drought tolerance, high oleic acid, which make them suitable to different seasons and cropping systems.

Linseed is the winter oilseed crop and its oil contains linolenic acid (36–50 %), linoleic acid (18–24 %) and oleic acid (16–24 %). Its oil is non-edible as it develops off-flavours during storage. Varieties with minimal linolenic acid will enable linseed oil for edible purpose. BARC has developed a high yielding variety, TL 99 with 2-5% linolenic acid which was released for commercial cultivation in Assam, Bihar, Jharkhand, Nagaland, Uttar Pradesh and West Bengal. TL 99 is the first Indian variety for edible oil (**Fig. 4d**). In sunflower, gamma ray mutagenesis of zebra stripped seed coat variety, Surya has resulted in high yielding black seed coat variety, TAS 82 with 2-7 % more oil than its parent for Maharashtra.

5.5.3. Cereals

Rice is the vital Indian staple food crop with 136.7 mt production during 2023-24 and is ensuring country’s food security. Radiation induced mutation breeding in rice at BARC has led to the development and release of seven varieties (Table 2). In early seventies, a cross between fast neutron mutant TR-5 and IR-8 resulted in breeding line TR-RNR-21, which was released as Hari in 1987 for Andhra Pradesh. India is known for traditional rice landraces having unique grain quality, which has separate niche in the domestic market. Usually, these are late maturing with poor yield and lodging due to tall stature, hence their cultivation is minimal. Gamma ray induced mutagenesis was successfully employed at BARC for improvement of these landraces. Recently, concentrated breeding efforts have resulted in release of five high yielding mutant varieties with improved agronomic traits for Chhattisgarh; Trombay Chhattisgarh Dubraj Mutant-1 (TCDM-1), Vikram-TCR (**Fig. 5a**), CG Jawaphool Trombay (CGJT), Trombay Chhattisgarh Sonagathi Mutant (TCSM) and Trombay Chhattisgarh Vishnubhog Mutant (TCVM). Further, a promising mutant derivative, Trombay Karjat Rice Kolam (TKR-Kolam) has also been released for Maharashtra. These high yielding varieties are lodging resistant due to semi-dwarf stature and with enhanced milling and head rice recovery. TCDM 1, CGJT and TCVM are with aromatic grains like their parents. TKR Kolam has superfine grain and better taste like other Kolam rice varieties. Grains of Vikram-TCR are suitable for puffed rice making, while that of CGJT and TCVM for kheer making.

Sorghum is a climate resilient crop cultivated for grain, fodder and biofuel under drought prone areas with low input conditions. Its cultivation is on 4.0 mha with 4.74 mt production. Gamma ray induced mutagenesis has generated a mutant, TRJP1-5 with superior grain and fodder yield and grain quality, which has been released for Karnataka in 2023 (**Fig. 5c**). It has synchronous maturity, bold and lustrous seeds with better rheological properties (roti making), besides moderate tolerance to charcoal rot, rust and blight diseases. In another experiment, gamma ray mutant for *hurda* (consumed at green stage) type, TAKPS-5 was developed and was released for Maharashtra as Suruchi (**Fig. 5b**). It is early maturing with compact and easily threshable panicles, better *hurda* grain yield. The grains showed more spongy tissues with improved organoleptic properties.

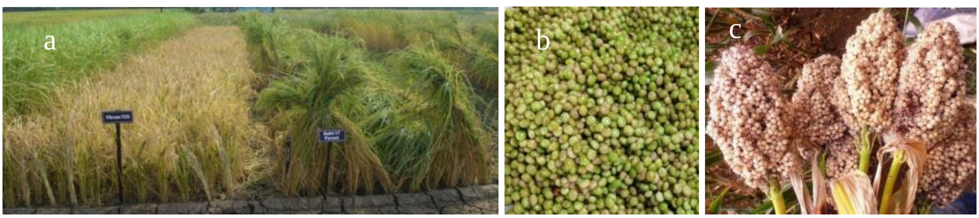

Fig. 5: (a) Field view of rice variety, Vikram TCR, (b) hurda type grains of TKPS-5, (c) panicles of TRJP 1-5 of sorghum

Trombay crops with certain novel mutant characters have been registered with National Bureau of Plant Genetic Resources, New Delhi (Table 3). Of the 20 genotypes registered, 10 mutants belong to groundnut, three to sesame, two each to sorghum and wheat, one each to sesbania, sunflower and urdbean.

5.5.4. Success with Trombay varieties

Trombay mutant varieties have contributed as parental material in respective crop breeding programmes at various state universities. Using Trombay varieties as parents, 14 groundnut and two urdbean varieties were developed in the country. Groundnut varieties include JCG 88, TPT 25, TCGS 894 for Andhra Pradesh; JL 501, GG 34, GG 35, GG 41 for Gujarat; GPBD 5 for Jharkhand, Manipur; Dh 40, R 9251, Dh 232 for Karnataka; JL 501, PDKVG 335 for Maharashtra; GG 21, RG 559-3 for Punjab; GG 21, JL 501, RG 559-3 for Rajasthan; TCGS 894 for Tamil Nadu, Telangana and RG 559-3 for Uttar Pradesh. In urdbean, TAU-1 was used to develop DU-1 for Karnataka and PDKV Blackgold for Maharashtra. Many of the Trombay varieties are being used as check varieties to test new entries in the state and national varietal evaluation trials.

Table 2: Chronological list of released Trombay varieties

Year	No of varieties	Crop	Trombay crop varieties
1973	1	Groundnut	TG 1
1983	2	Jute	TKJ-40 (Mahadev)
		Mungbean	TAP-7
1985	4	Pigeonpea	TT-6, TAT-10
		Groundnut	TG 17
		Urdbean	TAU 1
1987	1	Groundnut	TG 3
1988	1	Rice	Hari (TR-RNR-21)
1991	1	Groundnut	Somnath (TGS 1)
1992	2	Groundnut	TAG 24
		Urdbean	TPU-4
1993	3	Mustard	TM 2, TM 4
		Urdbean	TAU-2
1994	2	Mungbean	TARM-2
		Groundnut	TG 22
1996	2	Groundnut	TKG 19A, TG 26
1997	2	Mungbean	TARM-1, TARM-18
1999	1	Urdbean	TU 94-2
2004	2	Groundnut	TG 37A, TPG 41
2005	2	Mungbean	TMB 37
		Soybean	TAMS 38
2006	1	Groundnut	TG 38
2007	8	Groundnut	TLG 45
		Mungbean	TJM-3, TM 96-2
		Mustard	TPM 1
		Soybean	TAMS 98-21
		Sunflower	TAS 82
		Cowpea	TRC-77-4
		Pigeonpea	TT 401
2008	2	Groundnut	TG 51, TBG 39
2009	1	Pigeonpea	TJT-501
2010	1	Mungbean	TM-2000-2 (Pairy Mung)
2011	1	Groundnut	RARST-1 (TG 47)
2013	2	Pigeonpea	PKV-TARA
		Urdbean	TU-40
2018	1	Cowpea	TC 901
2019	2	Rice	TCDM-1
		Mustard	TBM-204
2020	2	Linseed	TL-99
		Rice	TKR Kolam
2021	8	Rice	Vikram TCR, CGJT, TCVM, TCSM
		Mustard	THPM-1, BBM-1, TAM 108-1
		Groundnut	TAG 73
2022	1	Mustard	TBM 143
2023	6	Urdbean	TRCRU-22, TJU 130, TJU 339
		Mungbean	TRCRM 147
		Sorghum	TRJP 1-5, TAKPS 5

Table 3: Trombay mutant germplasm registered with National Bureau of Plant Genetic Resources, New Delhi

Year	INGR No.	Crop	Name of Trombay germplasm	Trait
2001	1014	Sesbania	TSR-1	Photoperiod insensitivity
2004	4039	Groundnut	TG-18AM	Disease lesion mimic leaf
2004	4040	Groundnut	TGE-1	Early (95 days), foliaceous stipule, high shelling outturn (80%)
2004	4041	Groundnut	Small leaf mutant	Dwarf with small leaflet
2004	4097	Groundnut	Imparipinnate leaf mutant	Imparipinnate leaf with small leaflet
2004	4098	Groundnut	Suppressed branch mutant	Suppressed primary branches
2004	4100	Sunflower	Fasciation mutant	Fasciation and more leaves
2005	5018	Sesame	NM-58	Non-lodging due to stiff stem
2007	7029	Sesame	N-129	Tall seedling with greater initial vigour
2007	7030	Sesame	N-29	Polypetalous corolla
2007	7032	Groundnut	TG-18A	Large pod and seed
2010	10133	Urdbean	Trombay wild urid	Bruchid resistance
2011	11058	Groundnut	TGM-112	White to light orange flower
2013	13011	Groundnut	TGM-167	Gibberellin insensitive dominant dwarf
2013	13025	Groundnut	TGM-38	Sub-orbicular leaflet, erect, compact and dwarf plant type
2013	13026	Groundnut	TGM-51	Funnel leaflet, dwarf plant
2021	21201	Wheat	TAW-33	High seed hardness index
2023	23038	Sorghum	TAKPS-1	High yielding hurda with better sucrose and organoleptic properties
2023	23039	Sorghum	TAKPS-3	Semi-compact, threshable hurda with taste and flavour
2023	23081	Wheat	TAW-41	Spot blotch resistance & terminal heat tolerance

BARC has followed multi-pronged approaches to reach Indian farming community with Trombay varieties. It has undertaken large-scale seed production, participated in *Kisan Melas*, exhibitions of SAUs, conducted field demonstrations and attended the Public Awareness Programme on Peaceful Uses of Atomic Energy (**Fig. 6**). Some Trombay varieties like TAG 24, TG 37A of groundnut, TAU 1 of urdbean and TJT 501 of pigeonpea have made distinct impression and impact across the states due to their consistent better yield, wider adaptability and other desirable characteristics. Farmers have harvested record yields by cultivating these varieties.

Fig. 6: Field visit of farmers to learn about Trombay crop varieties at Gamma field, BARC

Cumulative share in national breeder seed indent for Trombay varieties by different states for 2021-2025 period is extremely encouraging. TG varieties constitute 74.5% of the Maharashtra's cumulative national indent for groundnut; followed by 73.2% of Gujarat and 62.2% of West Bengal's indent (**Fig. 7**). For urdbean, Maharashtra indents 68.7% for Trombay urdbean varieties and in the rest of the states, it ranges from 8.5% for Madhya Pradesh to 37.5% for Tamil Nadu. Similarly, for pigeonpea, Tamil Nadu indents 34.5% for Trombay pigeonpea varieties and in the rest of the states, it ranges from 5.0% for Jharkhand to 17.0% for Chhattisgarh. For recently released Trombay rice varieties, the demand is picking up by the marginal and progressive farmers in Chhattisgarh, where its share is 3.8% for the above period.

As per the demand, more than 2500 tonnes breeder seed of Trombay varieties were supplied to National Institutes; National Seed Corporation, State Seed Corporations of Andhra Pradesh, Bihar, Chhattisgarh, Gujarat, Maharashtra, Odisha, Rajasthan and West Bengal; State Agricultural Departments & Universities; Non-Governmental Organizations and farmers. Apart from this, various SAUs also dispersed hundreds of tonnes breeder seeds of BARC crop varieties. Some of the seed material was further multiplied and distributed horizontally to many farmers in the country. Most of these varieties have considerably benefited thousands of farmers, traders, exporters and other stakeholders.

5.6. Molecular characterization of mutant traits

Among the various radiations, gamma rays are the most extensively used physical mutagen in crop mutation breeding. In cowpea, large seed, small seed and cowpea aphidborne mosaic disease resistant mutants were isolated through gamma ray mutagenesis of cv. CPD103. Genomic DNAs from these mutants and parent were subjected to whole genome resequencing. The mutation rate was 1.4×10^{-7} per base pair. Gamma rays brought 88.9% of single base substitutions (SBSs) with an average transition to transversion ratio (Ti/Tv) of 3.51. A > G and T > C transitions were the major transition mutations, while all four types of transversion mutations were identified. Of the total induced variations, 11% were the indels followed by 6.3% small insertions and 4.8% small deletions. Distributed across all 11 chromosomes, only a fraction of SBSs (19.45%) and indels (20.2%) potentially altered the encoded amino acids/ peptides.

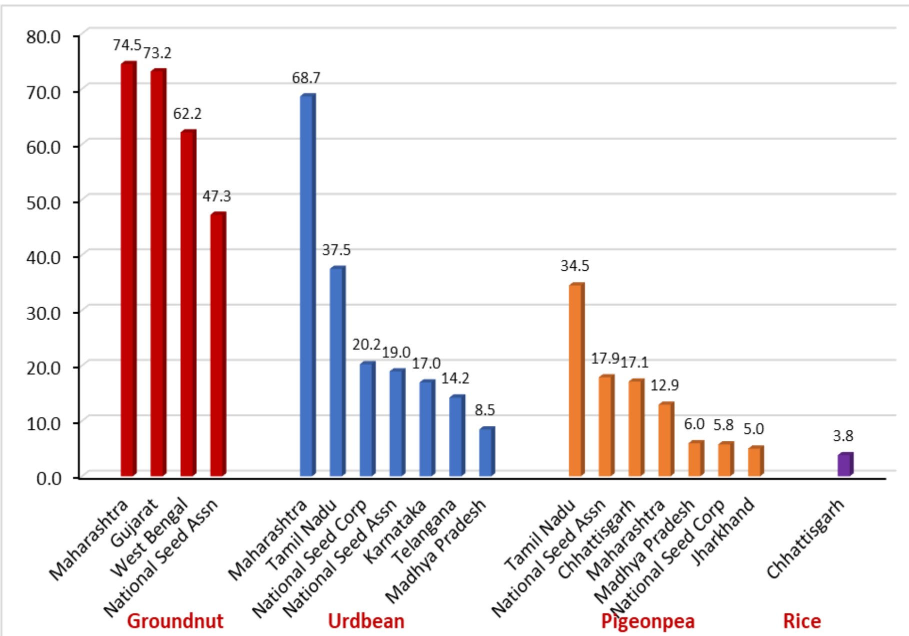

Fig. 7: Cumulative share (%) in national breeder seed indent for Trombay varieties by different states during 2021-2025 period.

Assessment of gamma ray wheat mutant with medium-hard texture found variations in either *pina* or *pinb* or both genes. This suggested that gamma rays brought mutations in loci coding puroindloines. Besides, characterization of gamma ray dwarf mutant of dicoccum wheat showed that dwarfing locus was different from the existing *Rht B1b* of tetraploid varieties. Further locus specific primer analysis of gamma ray GA_3 insensitive semi-dwarf dicoccum mutant showed absence of known wild and mutant alleles, *Rht-B1a* and *Rht-B1b* genes indicating reduced height mutant carried an altered mutation for the dwarf trait. Another gamma ray mutant TAW 41 had lower spot blotch infection and had higher normalised difference vegetation index (NDVI) and chlorophyll content (SPAD) values under terminal heat stress. Expression analysis showed that down-regulation of genes promoting senescence (TaSAG4, TaSNAC11) and up-regulation of wheat copper binding protein 1 (WCBP1) in the TAW41 exposed to spot blotch and terminal heat stress. In another high temperature stress tolerant gamma ray wheat mutant, HSP20, serine threonine kinase, calcium dependent protein kinase, ATP-binding cassette transporters, SOD, heat shock transcription factor, starch synthase, sucrose synthase and debranching enzyme were upregulated.

A chickpea mutant (*elm*) that showed increased organ and seed size was characterized and a novel, previously uncharacterized gene (*CaEl*) with a role in regulating organ size, early vigor and seedling establishment under salinity stress was identified. The gene LOC101503252, corresponding to *CaEl* is found to be deleted in the mutant resulting in its complete loss of expression. Both cell proliferation and expansion are also affected in the mutant. Transcriptomic profiling identified cell cycle, cell wall organization/biogenesis and related carbohydrate metabolism as major pathways involved in the regulation. The *in-silico* analysis showed that this single copy gene acquired a broader function in regulating cell division and/or proliferation in multiple tissue types. In high oleate groundnut mutant, sequencing of *ahFAD2A* gene detected several point mutations in the coding region. Further analysis of mutant confirmed that the G to A transition is responsible for high oleate trait.

6. Future prospects

Crop improvement is a continuous breeding process, wherein new breeding lines are to be developed in line with future needs of the farmers in various growing conditions. Categorically, a thoughtful merger of mutation and recombination breeding has a greater prospect in the genetic improvement of crop plants, which can be strengthened with genomic tools. Further, mutation breeding needs to be explored for the improvement of inbred lines of the existing, established and popular hybrids in respective crops; nutraceutically important minor millets; unexplored medicinal and aromatic crops; widely adapted land races with rich nutritional and other quality traits; vegetatively propagated crops along with *in vitro* mutagenesis. Additionally, many more crop species need to be experimented for induced mutagenesis with mutagens like electron beam, proton beam, ion beam, neutron or with novel chemical mutagens or their combination. Consequent mutant reservoir in different crops would be ideal genetic resource material for understanding structural and functional genomics of various economic traits. In future, radiation breeding could help to feed billions by promoting crop flexibility amid looming climate change, shrinking water and land resources, growing soil exhaustion and increasing insect resistance.

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