Romanian Biotechnological Letters *Vol. 15, No.2, Supplement, 2010*

ORIGINAL PAPER

Mutagenesis in crop improvement under the climate change

Received for publication, January 29, 2009
Accepted, February 10, 2010

S. MOHAN JAIN
Department of Agricultural Sciences, University of Helsinki, PL-27, Latokartanonkaari 5, Helsinki, Finland
Email: mohan.jain@helsinki.fi

Abstract

The purpose of mutation induction is to enhance mutation rate in a short duration in developing new plant varieties. The occurrence of spontaneous mutation frequency rate is very low and difficult to use in plant breeding. Traditionally mutations are induced by physical (e.g. gamma radiation) and chemical (e.g. ethylmethane sulfonate) mutagen treatment of both seed and vegetatively propagated crops. Recently high energy ion beams have been used for mutation induction. They induce largely deletion mutants. In International Atomic Energy Agency (IAEA) mutant database, over 3000 officially released mutant varieties have been released worldwide in cereals, ornamental plants, fruits, vegetables, and oil crops. As a result, sustainable food production has been maintained. By in vitro selection, desirable mutants with useful agronomical traits, e.g. abiotic and biotic stress tolerant can be isolated in a short period of time. The genetic fidelity of the regenerated plants is highly desirable for developing new improved plant varieties and a useful as a reliable tool for feeding the ever-growing human population, genomic function especially under climate change and limited arable land.

Keywords: physical mutagen, chemical mutagen, mutation, deletion mutants, *in vitro* selection, genomic function

Introduction

Exploitation of natural and induced genetic diversity is the basic requirement of plant breeding in developing plant varieties for sustainable food production. Plant breeders are handicapped due to lack of availability or non-existence of desired genotypes. However, they have successfully recombined the desired genes from the existed available gene pool and the related plant species by sexual hybridization, and successfully develop new cultivars with desirable traits such as high yield, abiotic and biotic stress resistance. The purpose of induced mutations is to enhance the mutation frequency rate in order to select appropriate variants for plant breeding. The mutation frequency rate of spontaneous mutations is rather very low and difficult to exploit by the plant breeders. Mutations are induced by physical (e.g. gamma radiation) and chemical (e.g. ethylmethane sulfonate) mutagen treatment of both seed and vegetatively propagated crops. The mutagen treatment breaks the nuclear DNA and during the process of DNA repair mechanism, new mutations are induced randomly and heritable. The changes can occur also in cytoplasmic organelles, and also results in chromosomal or genomic mutations and that enable plant breeders to select useful mutants such as flower colour, flower shape, disease resistance, early flowering types (1,2,3) A specific advantage of mutation induction is the possibility of obtaining unselected genetic variation, improvement of vegetatively-propagated plants when one or few characters of an outstanding cultivar are to be modified.

The use of induced mutations has over the past 50 years played a major role in the development of superior crop varieties translating into a tremendous economic impact on agriculture and food production that is currently valued in billions of dollars and millions of cultivated hectares. There is no difference between artificially produced induced mutants and spontaneous mutants found in nature. As in "traditional" cross-breeding, induced mutants are passed through several generations of selfing or clonal propagation, usually through *in vitro*

techniques. This is exactly what happens in nature (through evolution) and leads to the fixation of the mutation events. All that plant breeders do is mimic nature in this regard. It should also be noted that in most cases, the induced mutants are merely "raw materials" that in order for their potentials to be realized must be integrated into established breeding schemes. Thus, mutation induction is a flexible, workable, unregulated, non-hazardous and low-cost alternative to genetically modified organisms (GMOs). The economic benefit of nuclear and non-nuclear techniques is given in Table 1, describing higher output from nuclear applications over non-nuclear techniques such as genetic engineering. For more, see the website of the International Atomic Energy Agency (IAEA) and the Food and Agriculture Organization (FAO) (http://www-mvd.iaea.org/) that covers over 3000 new crop varieties in some 170 species, including both seed and vegetative propagated crops such as rice, wheat, barley, apples, citrus, sugar cane, banana, among others.

Table 1. Economic benefits of using nuclear techniques and non-nuclear techniques, in terms of US dollars

Technology used	Crop	Traits/ Variety	Total input MD	Total output MD/MR	Period	Country
Nuclear	-	-	68.9	61,568.2	1959-2001	Japan
Nuclear	-	-		11234	1997	USA
Nuclear	-	-		804	1997	Japan
Nuclear	Cotton	NIAB-78		36744 MR	1983-2005	Pakistan
		NIAB-Karishma		17699 MR	1998-2005	
Nuclear	Rice	Niab-Irri-9		971 MR	2000-2005	Pakistan
Nuclear	Chickpea	CM-98 CM-2000		4514 MR	1998-2005	Pakistan
Nuclear	Mung bean	NM-92, 98; NIAB Mung 2006		4729	1997-2005	Pakistan
Non nuclear (GE)	papaya	Ringspot (VR)		24.0 4.7	Since 1999	USA Hawaii alone
(GE)	squash	VR		21	2005	USA
(GE)	potato	Insect resistant		Was withdrawn	1996-2000	USA
(GE)	corn	Root worm resistant		26.5 MD 410000.00	2005 2004	USA Canada (Can)
(GE)	Soybean	HT		7570;5197; 1367; 132;69; 2.2	1996-2005	USA, Argentina (Arg), Brazil, Paraguay, S. Africa (Saf),Can,
(GE)	maize	HT		771;0.2;24; 0.3	1996-2005	USA, Arg, Can., Saf
(GE)	cotton	HT		919;4.0;0.2 ; 4.1	1996-2005	USA, Arg, Saf, Australia (Aus)
(GE)	canola	HT		101;792	1996-2005	USA, Canada,
(GE)	Insect resistant (IR)			1957;159; 145;59;8;28	1996-2005	USA, Arg, Can, Saf, Philippines (Phil), Spain

	maize					
(GE)	IR cotton			1627;29;14 ; 5168;463;1 50;55	1996-2005	USA, Arg, Saf, China, India, Aus, Mexico,
(GE)	Herbicide resistant (HT) Soybean			Farm income 1183;1658	2005	Developed and developing countries
(GE)	IR maize			Farm income 364;53	2005	Developed and developing countries
GE	HT Maize			Farm income 212;0.3	2005	Developed and developing countries
GE	IR cotton			Farm income 354;1378	2005	Developed and developing countries
GE	HT cotton			Farm income 163; 3	2005	Developed and developing countries
GE	HT canola			Farm income 195; 0	2005	Developed and developing countries
GE	VR papaya, squash			Farm income 25; 0	2005	Developed and developing countries

Abbreviation: US million dollars (MD); Million Rupees (MR); Genetic engineering (GE); Virus resistant (VR); Herbicide resistant (HR)

Now new challenges such as climate change, human population growth, etc., are posing a big threat and challenge to sustain food production worldwide. Local climates and terrestrial ecosystems will change, in many cases threatening human livelihoods. The developing countries will have greater adverse impact of climate changes on food and fibre production, environmental services and rural livelihoods. They are currently faced with urgent needs for development to improve food security, reduce poverty and provide an adequate standard of living for ever growing populations. Moreover large percentages as high as 80% of the populations of poor countries depend on agriculture on their livelihoods and therefore are more vulnerable to climate changes. In addition, climate induced changes must be considered in light of other stress factors including economic globalization, urbanization and its effect on rural labor and land availability, population growth and its effect on water other resource availability, crop pests and diseases, land degradation and low soil fertility, poverty etc. Moreover, there is no further scope of expanding arable land and rather gradually losing to various human developmental activities. The rapid industrialization in newly developed economies like China and India as well as existed developed economies are mounting heavy toll on environment including atmospheric ozone layer depletion, acid rain, erratic weather conditions, and global warming. The erratic rainfall pattern may either lead to shortage of water or increase in flooding and that will ultimately have an adverse impact on shortage of food production and would increase the food price. Plant breeders will look for new innovative tools, such as genetic engineering together with traditional breeding for sustaining food production to feed the world. In this paper, we will mainly focus *in vitro* mutagenesis and their potential in plant breeding.

Impact of climatic change on agriculture

Agriculture is totally dependent on resources, weather and climate. Massive industrialization and ever-increasing human population growth are gradually placing a great strain on existing water resources, arable land, environment, germplasm resources, and sound forestry practices. There are visible signs on the negative impact on world food production and rise in food price. One of the major reasons of this problem could be the impact of climatic changes such as *gaseous pollution*, *depletion of atmospheric ozone, increase in UV-B radiation level, increased atmospheric CO_2, extreme variability of rainfall time and locations, irregular growing season lengths, intermittent dry spell, global warming, high temperatures, degradation of water and soil resources.* Also it is a formidable task for planners to overcome inherent uncertainties which are complex such as: our inability to make region specific predictions of the rate, nature and extent of climatic change-especially rainfall patterns, the threat of irreversible damage to ecosystems, a very long planning horizon, long time lags between greenhouse gas emissions and climate effects, wide regional variation in causes and effects, the global scope of the problem and the need to consider multiple greenhouse gases and aerosols. Global warming may become disastrous to agriculture production especially by appearance of new insects and pests and diseases and some existing ones may disappear. The increase in atmospheric CO_2 levels and potential global climate change can alter growth rates, distribution of weeds and insect pests, and their impact on agricultural productivity. An increase in CO_2 levels can enhance growth rate of plants that could offset the competitiveness between crop plants and weeds. It seems that cotton is highly responsive to elevated CO_2 where as wheat is much less responsive. Also, increased level of UV-B radiation to ground level as a result of a decrease in protective ozone shield of the stratosphere could lead to negative effects for life on earth: the spectral regime changes within leaves after exposure to the enhanced UV-B radiation as a consequence of altered structure, surface reflectance and pigments. The ambient ozone concentration alone or in combination with other pollutants also decrease yield of several important crops.

Strategies to overcome impact of climatic changes

Plant breeders and agronomists are under pressure to sustain food production under the climatic changes. The food prices have already gone up worldwide and both developed and developing countries are facing economic crunch due to food and fuel price rise, however, the developing countries are hardest hit with current crisis. There is no short term magic formula to solve the world food problem. Thus, identify the most appropriate cost effective techniques to sustain food production. Conventional breeding in combination with other techniques such as mutagenesis, biotechnology, genetic engineering or molecular breeding utilize local genetic resources for developing new cultivars that could handle frequent climatic changes and targeted breeding varieties may not be very much helpful.

Genetic engineering

Even though genetic engineering has a lot of potential in genetic improvement of crops, this technology may not readily be operational, especially in the developing countries due to high cost, lack of trained manpower, lack of isolated genes, consumer acceptance of genetically modified (GM) food etc. They will have to depend on the developed world or commercial companies for gene supplies and that may not come cheap. Moreover, transgenic approach is useful for a single gene trait. One of the big challenges facing genetic engineers today is the regulation of transgene expression, with the position of integration of a transgene within a genome influencing its expression. This is known as the genome position effect. The insertion of multiple random copies of a transgene in the genome can effectively abolish its expression and the

insertion of a transgene in or close to another gene can result in the production of an undesirable phenotype. Therefore, to ensure long-term stable expression of a transgene post-transformation, the insertion of a single copy of a gene into a location in the genome where expression of the transgene is not adversely affected by the surrounding genomic sequences is desirable. *Bt* cotton-insect resistant that has been the most successful crop. However, recent reports indicate that some insects have developed resistance to *Cry* proteins (responsible for insect resistance) as high as 250 fold after 36 generations; and in some others seven fold increase after 21 generations. These resistant insects can become a huge problem for sustainable crop production and may either reduce or complete loss of economic returns to the farmers. In this situation, farmers in poor countries would be adversely affected. In poor countries, most of the farmers are small land holders (even less than one hectare). For example, for them high cost of transgenic cotton seeds, lack of resources, and high inputs won't be beneficial. In India, nearly 3000 farmers committed suicide after sowing costly *Bt* cotton seeds, and they could not repay the debts whereas in China small farmers improved production by getting cheap seeds and inputs. Therefore, the Federal government has to play a major role to make sure that the seed cost is not too high, readily availability of seeds, proper guidelines to grow seeds and agronomical practices.

Mutation breeding

By mutation breeding, one of the major advantages is that mutants with multiple traits can be identified. The chances of survival of mutant varieties are much higher under rapid fluctuating climatic conditions. To my mind, the use of nuclear techniques for developing new varieties under the changing climatic conditions would be the most ideal approach before any new cost effective techniques are developed which are freely available without too many regulations. Molecular biology and transgenic research are at the experimental stages for many traits even though transgenic research has made substantial progress for single gene traits, e.g. *Bt* cotton and maize, herbicide resistant soybean; farmers have gained economically. Transgenic crops may not survive in the rapid changing climate, e.g. during erratic drought and rainfall conditions or appearance of new insect and pests or diseases as result of global warming. However, transgenic crops could be developed for certain traits e.g. nutrition (golden rice) or shelf-life (tomato). Gene pyramiding or gene stacking could be useful for the introduction of multiple novel genes into plants by straightforward iterative strategies. Plants containing several transgenes can be produced by crossing parents with different transgenes until all the required genes are present in the progeny. An alternative iterative strategy is to sequentially transform and re-transform plants with different individual transgenes by several rounds of transformation. This strategy could be particularly useful in asexual or vegetative propagated crops, such as woody plants and trees. Table 2 shows the differences between transgenic plants and mutants.

Table 2. Differences between transgenic and mutant plants

Transgenic plants	**Mutant plants**
Uses tools of molecular biology to isolate, clone and incorporate genes into plants	Mutation is a random event
It is a more precise technique than mutation	Requires large population for screening the best desired mutants
Transgene integrates into plant genome randomly	It cannot be directed to make changes at the DNA
Changes at both DNA and protein are well understood.	Mutants possibly can carry other changes in their DNA
It can add new gene/genes in plants. Gene(s) can be from any source including animals, insect, plants, bacteria	Mutagenesis can only modify the genes of an organism. Mutagen treatment fragments DNA first followed by DNA repair mechanism, and that results in mutations

It requires tissue culture protocols for transgenic plant regeneration	*In vitro* mutagenesis also requires tissue culture for plant regeneration of mutants
Consumer's lack of confidence towards transgenic food	Consumers accept food derived from mutagenesis
Transgenic plants has less probability to carry other changes in their DNA	Selection system is placed for agronomic desirable trait mutant selection
Molecular techniques are used to confirm the integration of transgene in plant	TILLING (Target induced local lesions in genomes), a new strategy for reverse genetics
Costly research. Developing countries may have problem to support it due to lack of infrastructure and finance to support research. Most of the chemicals are imported.	Cheaper. International Atomic Energy Agency (IAEA, Vienna, Austria) provides free services of gamma irradiation of experimental material
Most transgenes are not readily available to anyone interested in using them. The developing countries will be become dependent on the supply of transgenes from abroad.	Mutant variety seeds are readily available. Moreover IAEA can assist in supplying mutant seeds.
Bio-safety a big concern	No problem with bio-safety
Mostly single gene trait	Both single gene and polygenic traits
T-DNA mutagenesis or transposons leads to loss of function through gene disruption	Gives rise to many different mutant alleles with different degree of trait modification of plants
Concern of Intellectual Property Right (IPR)	Not a serious problem

Molecular markers

The recent developments in genomics have provided new tools for discovering and targeting novel alleles and genes, which can enhance efficiency of plant breeding by using molecular marker-assisted selection (MAS) (4). The use of MAS in plant breeding is relevant to all target crops: a) traits that are difficult to manage through conventional phenotypic selection-because they are expensive or time consuming to measure, or have a low penetrance or complex inheritance; b) traits whose selection depends on specific environments or developmental stages that influence the expression of the target phenotype; c) traits maintenance of recessive alleles during backcrossing or for speeding up backcrossing breeding in general; and d) pyramiding multiple monogenic traits (pest and disease resistances or quality trait) or several QTL for a single target trait with complex inheritance such as drought tolerance (5). So far, most of the funds from private sector have gone in molecular breeding for crops of greatest commercial interests such as maize, soybean, canola, cotton and sunflower. This has led to the development of holistic molecular breeding strategies for variety development aimed at generating an ideal genotype based on mosaic of favourable chromosomal segments, including simultaneous MAS for multiple traits (selection based marker information only) such as yield, biotic and abiotic stress resistance, and quality attributes, several of them polygenic in nature. As a result, very limited information is available to the public research organisations. Within next 4-5 years, commercial varieties developed through molecular breeding are expected to be released in the USA. However, many challenges remain to be resolved before MAS can routinely provide value added breeding very complex traits. There are many technical hurdles that have hindered the speed and scope of MAS applications. These include the unit cost and scalability of DNA extraction systems, the capital costs associated with high throughput genotyping equipment, the prolonged and labour-intensive methods for identifying marker-trait association (MTAs), and the absence of freely available software tailored to the needs of molecular breeding programs. Therefore, application of genomics tools is long way to go to assist plant breeding programs in the developing countries.

Induced Mutagenesis

Mutations have been induced to broaden the genetic diversity with physical and chemical mutagens for enhancing crop productivity in both seed and vegetatively propagated crops (see Tables 3, 4).

Table 3. Different methods for improving seed propagated crops

No.	Nuclear technologies used	Objective/purpose it is used	Alternative non nuclear technologies used
A.		**Abiotic stress**	
1	Laser, XR GR	*Drought (water shortage stress)	
2		Water quality	
3	GR	*Salinity	**GE,** *in vitro*
4	GR, TN	Extreme temperatures-heat, cold	CM, CB, **GE**
5	GR	Flooding/water logging	**GE**
6		Gaseous Pollution	
7.		Alleviated CO_2	
8.		Ozone layer depletion	
9	GR	Acid soils	
		Aluminum tolerance	CM, **GE**
10	GR	Soil with heavy metals (phyto-remediation)	**GE**
11		Herbicides	**GE**
12		insecticides	**GE**
13		fungicides	
B		**Biotic stress**	
1	GR, FN, XR, BR, TN	Fungal diseases	
2	GR	Bacterial diseases	
3	XR, GR	Viruses	CB, **GE**
4	GR	Insects and pests	**GE**
C.		**Plant traits**	
1	GR, TN, XR, laser	Earliness	
2	GR, Laser,	Early maturation	
	GR	Late maturation	CM
3	GR	Water logging resistant	
4.	GR, BR, TN, Laser	Plant architecture-tall, dwarf, semi-dwarf	CM, CB,
5	GR, TN, GR +CM	High yield	CB, CM, colchicine
6.	GR,	Nutrition-lysine	
7	GR	Straw quality	
8		Malting quality	CB
9	GR, XR, laser	Edible oil content	CM, CB
10	GR, FN	Plant growth-vigour	CM
12	GR	Seed retention trait	CM, CB
13	GR, FN	Seed size and quality	CM
	GR	Seed colour	CM, CB, *In vitro,*
14	BR, XR	Pod size and numbers	
15	XR	Pod morphology	
16	XR	Arachin content	
17	GR	Harvest index	
18	GR	Storage quality	
19	GR	Root length - short	
	GR	Shattering resistance	
		Uniform maturity	CM
	GR, TN	Lodging resistance	CB

XR GR,	Photosynthesis rate	CB, CM
XR	Nitrogen fixation rate	
GR, TN	Adaptability	CM
	Fibre yield	CM
GR, XR, TN. laser	Vigor	
TN, GR	Threshability	
GR	Grain quality	CB,
GR	Grain weight	
GR	Grain morphology	CB
GR	Grain size	
GR	Grain color	
GR, XR	Stiffness	CB, CM
	Alkaloid content	CB, CM
GR	Fruit ripening	
GR	Fruit color	
GR	Leaf color	
	Leaf quality	CB
GR	Leaf size	
	Amylose content	CB, CM
GR, FN	Glutinous content	
GR	Cooking quality	CM
GR	Non-branching	
GR	Capsule size	
GR	Protein content	CB, CM
TN, XR,FN	Stiffness	CB, CM

* A polygenic trait. Many genes have been identified and isolated

Methods of crop improvement

1. Cross breeding (CB)
2. Mutation assisted breeding (MAB): Gamma radiation (GR) X-rays (XR), High energy beam (HIB), Chemical mutagen (CM), Fast Neutrons (FN), Beta rays (BR), Thermal neutrons (TN)
3. Genetic engineering/transgenics/ (GE)
4. Molecular biology (MB): Molecular assisted breeding
5. *In vitro* techniques- doubled haploid, somatic embryogenesis, microprpagation

Table 4. Various methods for improving vegetative propagated crops

No.	Nuclear technologies used	Objective/purpose it is used	Alternative non nuclear technologies used	Benefit
A.		**Abiotic stresses**		
1		*Drought (water shortage stress)		
2		Water quality		
3		*Salinity		
4		Extreme temperatures		
5		Flooding		
6.		Gaseous Pollution		
7.		CO_2		
8		Ozone layer depletion		
9		Acid soils		
10		Soil with heavy metals		
11		Herbicides/insecticides	**GE**	
B		**Biotic stresses**		
1	GR, HIB, FN	Fungal diseases	**GE**	
2		Bacterial diseases		
3	GR, FN	Viruses	**GE**	
4	GR+CM	Insects and pests		
C.		**Plant Traits**		

1	GR, XR, FN	Earliness	CM, cross	
2	GR	Late maturation		
3	GR, FN	Fruit weight		
	GR, FN, XR, FN	Fruit colour and shape	CM,	
	GR	Fruit size	cross	
	GR	Fruit quality		
	GR	Juice quality		
4.	GR, XR	Plant architecture-shortness		
5	GR, FN, XR	Yield		
6.		Nutrition-		
7	GR, XR	Flower colour	*In vitro,* CM, **GE**	Blue Carnation and rose
8	GR	Shelf-life	In Vitro, **GE**	tomato
9	TN, GR	Seedless		
	GR	Seed number		
	GR	Seed color	*In vitro, CM*	
	TN, GR, XR	Fruit color		
	GR, XR	Skin color	*In vitro,*	
	XR	Fruit set		
		Fruit taste	CM	
	GR, XR	Variegated leaves	*In vitro,*	
	GR, FN, XR	Leaf characters	CM	
	GR	Leaf colour		
	XR	Flower petal		
	GR	Flower number	*In vitro*	
	GR	Flower morphology	*In vitro* CM	
		Flower fragrance	**GE**	
	GR	Seed retention		
	GR, XR	Longer harvesting period		
		Bract color	CM- colchicine	
	XR	Flower size	*In vitro,*	
	TN	Starch content		
	GR	Cooking quality		
	GR	Internode length		
	GR	Bunch size	*In vitro*	

Methods of crop improvement

1. Cross breeding (CB)
2. Mutation assisted breeding (MAB): Gamma radiation (GR) X-rays (XR), High energy beam (HIB), Chemical mutagen (CM), Fast Neutrons (FN), Beta rays (BR), Thermal neutrons (TN)
3. Genetic engineering/transgenics/ (GE)
4. Molecular biology (MB): Molecular assisted breeding
5. *In vitro* techniques- doubled haploid, somatic embryogenesis, microprpagation

Various mutants with a wide range of traits have been isolated and utilized for breeding programs, which are high yield, flower color, disease resistance, early maturity in crop, vegetables, medicinal herbs, fruit and ornamental plants (1,6). The mutant database of International Atomic Energy Agency (IAEA), Vienna, Austria indicated that since 1960, over 3000 mutant varieties had been officially released in 60 countries. The top six countries are China, India, the former USSR, The Netherlands, Japan, and USA. Rice stands first by having the largest number 700 mutant varieties followed by barley, wheat, maize, durum wheat, oat, millet, sorghum, rye and dura (2,7. A high yielding barley “naked grain” has been released Altiplano, Peru by selecting mutants from gamma irradiated seeds. This area is more than 3600 m above the sea level, and is stress-prone area characterized by short vegetation periods. This variety has improved adaptability in stress-prone areas and is well accepted by the consumers. Maluszynski et al (7) pointed out that even though most of the induced mutations

are recessive and deleterious from breeding point of view, a significant improvement in plant improvement worldwide has been achieved by induced mutations. For example, salt tolerant rice in China, high yielding cotton in Pakistan, heat tolerant wheat in India etc., varieties have been released (8). Yuandong No. 3 wheat variety was developed by IAEA in 1976 with gamma irradiation, and possesses a complete resistance to rust, powdery mildew and aphids and is also tolerant to saline, alkaline and other environmental stresses. This variety is cultivated in 200,000 hectares, an increase from 1000 hectares in 1986. In India, Behl et al (9) reported to develop heat-tolerant mutants of wheat and their yield performance was much better than the heat-sensitive types under the field conditions.

Genetic improvement of fruits is essential for increasing the productivity of fruits. The major problems with fruit breeding work is long life cycle of many fruit crops, which varies from 3-25 years or more, and the large juvenile period and has hampered fruit breeding work. The fruit production figures show banana, citrus, mango, cashew, and date palm are grown extensively.To date, genetic improvement of fruit crops has relied heavily upon vegetative propagation techniques and classical breeding. In fruit crops, mutagenesis has already been found able to introduce many useful traits (Table 5) affecting plant size, blooming time and fruit ripening, fruit colour, self-compatibility, self-thinning, and resistance to pathogens.

Table 5. Mutant traits of different fruits by radiation-induced mutations (updated table 59) .

Fruit	Mutagen and dosage	Mutant traits
Apple	GR	Early maturing, red fruit skin colour, compact tree, russet-free fruit skin, variegated leaf, dwarf
Apricot	GR	Earliness
Banana	GR	Earliness, bunch size, reduced height, Tolerance to *Fusarium oxysporu* f sp. c*ubense* (FOC) race 4, large fruit size, putative mutants resistant to Black sigatoka disease
Citrus	GR	Seedless, red colour fruit and juice, *Xanthomonas citri* disease resistant, resistant to Tristeza virus
Japanese pear	GR	Black spot disease resistance
Indian jujube	GR	Fruit morphology, earliness
Loquat	GR	Fruit size
Pineapple	GR	Spineless, drought tolerant producing unmarketable fruits
Plum	GR	Early flowering
Pomegranate	GR	Dwarf
Papaya	GR	Short, striated fruits, malformed top disease resistance, split stem
Pear	GR	Disease resistance
Peach	GR	Disease resistance, fruit size and yield
Date palm	GR	Bayoud disease resistance
Mulberry	GR	Tetraploids and cytochimeras, high rooting ability
Guava	GR	Seedless, low seeded fruits, cluster fruiting, segmented fruits, cylindrical fruit shape
Jujube	GR	Fruit shape, leaf shape and size variation
Strawberry	GR	Thick and small leaf, light leaf colour, white flesh, and long fruit; *Phytophthora cactorum* resistance

Gamma radiation- GR

Traditionally, new flower colors in ornamental plants are obtained through screening the natural occurring variation. However, traditional breeding methods have failed to produce cultivars with yellow or true red flower colors, resistance to gray mold disease, or cold resistance, *e.g.* in saintpaulia. The genetic improvement of ornamental plants for improving or

developing new varieties requires genetic variation. However, the desirable genetic variation is most often lacking and that hampers the breeding of ornamental plants. This is due to existing germplasm fails to provide the desired recombinants, and it is necessary to resort to other resources of variation. Since spontaneous mutations occur with extremely low frequency, mutation induction techniques provide tools for the rapid creation and increase in variability in crop species. The genetic variability can be induced by mutagenic agents, such as radiation and chemicals, and from which desired mutants could be selected (10, 11, see Table 6)

Table 6. Different mutated traits among officially released mutant varieties of ornamental and decorative plants (Jain and Spencer, 2006).

Number	Mutated traits	Number of mutants
1.	Flower color	417
2.	Flower morphology	31
3.	Plant architecture	25
4.	Leaf color	13
5.	Variegated leaves	9
6.	Ornamental type	9
7.	Leaf morphology	7
8.	Earliness	6
9.	Compact growth	5
10.	Dwarf	4
11.	Flower type	3
12.	Others	27

In vitro mutagenesis

The plant breeder has available several *in vitro* techniques such as micropropagation, protoplasts, embryo rescue, somatic embryogenesis etc., which increases the efficiency in obtaining variation, selection and multiplication of the desired genotypes (12). The frequency of tissue culture-derived variation or somaclonal variation is low in cereals, and that hindered the frequent use of somaclonal variation in cereal improvement (13). It is desirable to increase the genetic variability by combining mutagenesis and tissue culture for breeders to exploit in crop improvement (14). Few more benefits of *in vitro* mutagenesis are: a) mutagen treatment can be given to large cell or protoplast or somatic embryos density, b) fast multiplication of mutant plant material, c) *in vitro* selection of mutation, d) less space required for shoot multiplication under the controlled conditions. Tissue culture of periclinal chimeras often results in segregation of the component genotypes, depending on the pattern of differentiation and proliferation of the shoots formed (15)..They found new ornamental cultivars of *Saintpaulia* and *Rhododendron.* Gavazzi et al (16) compared somaclonal variation and chemically induced mutagenesis and found differences in their effects, changing the spectrum and frequency of mutants and even in some cases, the pattern of segregation of mutant character in *Lycopersicon esculentum*, and in *Brassica napus* (17). Jain (18) irradiated axillary buds with gamma rays excised from *in vitro*-grown strawberry plants; 5% plants survived the selection pressure of *Phytophthora cactorum* crude extract and these plants were also able to withstand water holding for 5-6 days. It seemed that pathogen-related (PR) proteins may be responsible for both drought and disease tolerance.. Banerjee and Kallo (19) observed high total phenol content in disease and pest resistant wild type tomato (*Lycopersicon hirsutum f glabratum*) lines as compared to susceptible cultivated tomato (*Lycopersicon esculentum*), and suggested the use of this parameter for the selection of disease and pest resistant lines in

cultivated tomato. While studying somaclonal and *in vitro* mutagen-induced variability in grapevine, Kuksova et al (20) found an increase in tetraploid plants among somatic seedlings after gamma irradiation, and also variability was seen among regenerated plants after field testing..

Mutation induction methods

Nuclear technology has benefited greatly in genetic improvement of seed and vegetative propagated crops worldwide (21,22). Both chemical and physical mutagens are used to induce mutations. Among them, gamma rays and ethyl-methane sulphonate (EMS) are widely used for mutation induction. Initially LD_{50} dose is determined, which is used as an optimal dose for mutation induction. By ignoring this step, mutagen dose can either be high or low resulting mutation frequency.

Physical mutagens

Mutation induction with radiation has been the most frequently used method to develop direct mutant varieties, accounting for about 90% of obtained varieties (64% with gamma-rays, 22% with X-rays) (21). The types of radiations available for induced mutagenesis applications are ultraviolet radiation (UV) and ionizing radiation (X-rays, gamma-rays, alpha and beta particles, protons and neutrons). Ultraviolet radiation (250-290 nm) has a moderate capacity to penetrate tissues when compared with ionizing radiation.). Ionizing radiation penetrates deeper into the tissue and can induce a great number of different types of chemical changes.. The advantages of using physical as compared to chemical mutagens are the accurate dosimetry, allowing for a sufficient reproducibility and, particularly for gamma rays, a high and uniform penetration in plant tissues (21). Gamma radiation have provided an high number of useful mutants (23) and is still showing an elevated potential for improving vegetative propagated plants. Recent report showed the recovery of a putative mutants of banana (*Musa* spp., AAA group, Cavendish subgroup), named DPM25, with improved yield and fruit size, as well as a degree of resistance to Fusarium wilt a potentially devastating disease throughout the world (24). . Figure 1 shows black Sigatoka disease putative resistant mutants were isolated from susceptible banana variety 'Grande Naine' by gamma irradiation.

Susceptible Grande Naine **Tolerant Grande Naine**

Figure 1. From a population of 4000 M_1V_4 plants derived from gamma irradiated shoot tips, 15 plants were selected for their tolerance to Juglone (5-Hydroxy-1,4-naphthoquinone), a toxic metabolite of the fungus *Mycosphaerella fijiensis* responsible for Black Sigatoka disease (25)

Ion beam technology

Heavy ion beam (HIB) is used as a new physical mutagen instead of gamma rays, X-rays and neutrons, which has been predominantly used for mutation induction in plants (26,27,28). These beams are responsible for linear energy transfer (LET) and as LET increases that induces higher biological effects such as lethality, chromosomal aberration etc., as compared to most commonly used physical mutagens. The LET can be of ^{14}N or carbon or ^{20}Ne or Fe-ions for mutation induction (29, 30). Double-strand DNA breaks induced by ion beams are less repairable as compared to caused by gamma radiation. Ion beams deposit high energy on a target densely as opposed to low LET radiation that suggests that ion beams predominantly induce complicated damage (clustered damage) including double-strand DNA breaks with damaged end groups whose reparability would be low. Therefore, it is possible that ion beams could cause lethality and mutation at higher frequency compared to low LET (27). A range of mutants have been induced in several different ornamental plants (Table 7), as well as in maize and rice (31, 32), wheat (33.. Nishihara et al (30) obtained different type of mutants including deletions, insertions and chromosomal translocations by ion-beam irradiation in *Arabidopsis thaliana*. Fe-ion irradiation tended to induce larger deletions than C-ion irradiation, and concluded that deletion size could controlled by selecting appropriate LETs. Shikazono et al (28) irradiated *Arabidopsis thaliana* by carbon ions to investigate the mutational effect of ion particles in higher plants. Frequencies of embryonic lethals and chlorophyll-deficient mutants were significantly higher after carbon-ion irradiation than after electron irradiation (11-fold and 7.8-fold per unit dose, respectively. Nagatomi et al., (34 analyzed the frequency rate of flower color mutation induced by heavy-ion beam and gamma radiation treatment in chrysanthemum. The flower color mutation frequency rate induced by carbon ions was approximately half of those induced by gamma irradiation, which showed complex and stripe types that were never obtained by gamma rays, other than single color such as white, light pink, dark pink, orange, and yellow. The most of flower color mutants induced by gamma rays were light pink and few were dark pink yellow. Thus isolation of novel mutants and the high mutation rate suggest that ion particles can be used as a valuable mutagen for plant genetics (28).

Table 7. Update on type of mutations induced with Heavy ion beams in ornamental plants (Jain 2006)

Species	Induced mutation	Reference
Chrysanthemum	Flower color Flower shape and color	Suzuki et al., 2005; Matsumura et al 2010 (29)
Verbena hybrida	Sterile	Saito et al., 2005
Pelargonium	Male sterile	Sugiyama et al., 2005
Ficus	Leaf variegation – chlorophyll mutations	Takahashi et al., 2005
Rose	Flower characteristics	Hara et al., 2003; Kitaura et al., 2000; Yamaguchi et al 2003
Petunia	Variegation in flower color	Miyazaki et al. 2002; Suzuki et al., 1999
Dhalia	Flower color	Hamatani et al., 2001
Sandersonia	Leaf variegation	Hortita et al., 2002
Carnation	Flower color and shape	Okamura et al . 2003 (58)

Low-energy (10-10^3 keV) ion beam has been used for bombarding biological organisms for broad applications on mutation breeding, gene transfer, and others (35, 36). This method has provided many advantages, such as low damage rate, higher mutation rate, and wider mutation spectrum. By low energy nitrogen ion irradiation of rice seeds, Yu et al (32) discovered rice mutations for the first time, and since than11 new lines of rice mutants with higher yield, broader disease resistance, and shorter growing period and high grain

quality were grown in China. In Thailand, low-energy ion beam induced mutations in Thai jasmine rice revealed phenotypic variation including short stature, red/purple color of leaf sheath, collar, auricles, ligules and dark brown stripes on leaf blade, dark brown seed coat and pericarp (37,38).

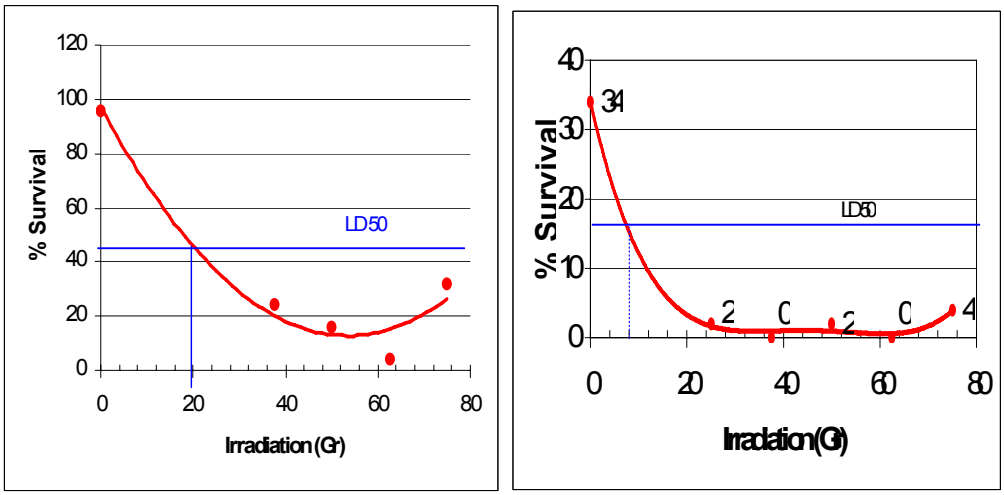
Chemical mutagens

Chemical agents can be useful since they provide high mutation rates and, mostly point mutations (21). The chemical mutagens most used for mutation induction belong to the class of alkylating agents [ethyl methanesulphonate (EMS); diethyl sulphate (dES); ethyleneimine (EI); ethyl nitroso urethane (ENU), ethyl nitroso urea (ENH), methyl nitroso urea (MNH)] and azides. The dose assessment for chemicals is determined by varying the concentration and duration of treatment, the solvent used [*e.g.* dimethyl sulfoxide (DMSO)], or the pH of the solution. Chemical mutagens (EMS, DES, sodium azide) were also used by treating banana shoot tips to produce variants for tolerance to Fusarium wilt (39). EMS has been successfully used on chrysanthemum**,** yielding a frequency of 5.2% mutants. A wide range of variations in petal color (pink-salmon, light-pink, bronze, white, yellow and salmon color) have been recorded. (10). Luan et al (40) treated sweet potato (*Ipomoea batatas* L.) callus with EMS and obtained salt tolerant lines.

Radiosensitive curve

One of the first steps in mutagenic treatment is the estimation of the most appropriate dose to apply. The unit of the absorbed dose of radiation energy is the Gy (Gray) (equivalent to 1 J kg^{-1}; equivalent to 100 rad). A precise method for the determination of the radiation dose absorbed has been proposed by Neville et al. (41). However, the common procedure in assessing the most appropriate dosage is based on radiosensitivity, which is estimated through the response of the irradiated material. Radiosensitivity varies with the species and the cultivar, with the physiological condition of plant and organs, and with the manipulation of the irradiated material before and after mutagenic treatment (Figure 2). Correlations between the physiological status of plants and their radiosensitivity are often correlated to water content of the tissue, since the most frequent primary target of ionizing radiation is the water molecule (42). The irradiation of hyperidric tissues affected can increase the frequency of induced mutations (43).

Figure 2. Left: Radiosensitive curve of guava (LD_{50} 20Gy). Right: Gamma radiation response curve for Jaboticaba (LD_{50} 8Gy).(adapted from IAEA-312.D2.RC.823-2)



Materials used for in vitro mutagen treatment

Fine embryogenic cell suspension cultures are most suitable for inducing mutations by transferring to the filter paper and plated on the agar-solidified culture medium for gamma irradiation. Irradiated cells are transferred to the fresh culture medium for cells to recover from radiation treatment. Irradiated cells are further cultured to the fresh medium for the development, maturation, and germination of mutated somatic embryos. This approach provides mutated somatic seedlings in a short period and also prevents chimeras problem which otherwise requires to multiply plants up to $M1V_4$ generation for chimera dissociation. The problem with this approach is low rate of somatic embryo germination, which is highly genotypic dependence. A combination of somatic embryogenesis and organogenesis would be a realistic approach for mutation induction and multiplication of mutant plants in large numbers (44,45). Table 5 provides a list of mutants of different fruits with wide range of traits with radiation treatment.

Shoot tips are used to induce direct shoots, preventing callus formation, in order to micropropagate plants in large numbers. This would be ideal system for mutant plant multiplication in large numbers for further evaluation. Shoot tips can also be irradiated and culture them on a culture medium containing appropriate plant growth regulators. By this approach, regenerated plants will be chimeras, and mutations will be unstable, and would require chimera dissociation by micropropagation of shoot cultures up to M1V4 generations to make sure mutated plants are stable. This approach would be suitable to follow in crops recalcitrant to somatic embryogenesis

***In vitro* selection**

The occurrence of genetic variation is very common in tissue culture-derived plants as a result of *in vitro* mutations. These mutations are not always expressed and only when few of the changes are able to express evident as phenotypic and cytological modifications in the regenerated plants from the callus tissue (46). However, the frequency of somaclonal variation rate should be high enough for the selection of agronomically desirable variants, and the selected lines should perform well under multiple environments (47). While making selections under the natural conditions, plant breeders don't have a precise tool to make the right selections for desirable traits and takes years and generations to accomplish their goals. *In vitro* selection shortens the time considerably for the selection of a desirable trait under the selection pressure without having any environmental influence, and should complement the field conditions. Duncan (47) reported that subjecting somaclonal variants to performance evaluations in the field includes additional genotype x environmental interactions..Heath-Pagliuso et al (48) selected *Fusarium oxysporum* f.sp.*apii* resistant celery, and some of the selected somaclones were superior to the parental type.

In vitro selection against salt tolerance is commonly achieved as a result of a temporary adaptation; cells are able to compartment the excessive salts into the vacuoles, and survive by adjusting the osmotic pressure, but this adaptation causes reduction in cell division and expansion (49). The mechanism of this relationship is not yet known. However, some plants become well adapted to high salts to become halophytic than others, and are unable to grow in the absence of salt selection pressure (46). Bressan et al (49) found reversible tobacco salt tolerant cell lines at 10 g/l NaCl concentration. As the salt concentration was raised to 25g/l, stable salt tolerance lines were obtained indicating high salt concentration acted as a selective as well as mutagenic agents, and caused specific modifications of the plant morphology, including unbalanced polyploidization, sterility, longer reproduction phase, and dwarfism coupled with heritable salt tolerance. Moreover, when salt stress is given osmoprotectants (e.g. glycinebetaine; (50), proline (51), and 24kDa protein, called osmotin-1

(52) accumulate. Sabry et al (50) found accumulation of glycinebetaine and sucrose in salt stressed wheat plants. Glycinebetaine is known as osmoprotectant which is accumulated in certain plant species under salt and drought conditions. Remotti (46)has listed *in vitro* selection against salt in several crop species, and still long-term salt adaptation requires more studies so that salt tolerant selected plants grow more like halophytic plants. Perhaps all plant species may not response to long-term salt adaptation and therefore, we may have to screen plant species for these studies.

Conclusion and prospects

Induced mutations are necessary to enhance rate of genetic variability since spontaneous mutation rate is very slow and that prevents breeders to exploit them in plant breeding programs. The major advantage in induced mutations is that multiple trait mutants can be isolated as compared to transgenic approach where single trait can be introduced in the crop and moreover there is a lack of acceptance of genetically modified (GM) food. A specific advantage of mutation induction is to develop a range of mutant lines, and identify trait specific genes in order to set up molecular gene database, study molecular functional genomics and develop bio-informatics for future to develop plant variety to grow the existing arable land under climate change to feed rapid human population growth. Moreover another advantage of mutagenesis is to isolate mutants with multiple traits and hopefully would be ideal to grow under climate change.

The use of mutagenesis with plant tissue culture and length of culture period especially in cereals could increase the genetic variability for exploitation by plant breeders. This approach could be utilized more frequently before genetic engineering becomes a regular and a reliable tool in plant breeding. The developing countries with high population growth can't wait before genetic engineering can reap high harvest. Thereby, a combination of tissue culture and mutagenesis could create genetic variability and that is utilized by plant breeders in plant breeding programs.

The new gene discovery with reverse and forward genetics will open the way for developing functional genomic plant breeding (53,54). The general strategy for reverse genetics is called TILLING (Targeting Induced Local Lesions in Genomes) or coming together of traditional mutagenesis with functional genomics (55) . TILLING enables the reverse selection of single point mutations by cleavage of mismatches in heteroduplex DNA with the endonucleases CEL 1. This strategy was first applied in an Arabidopsis thaliana mutant collection induced with ethyl methanesulphonate (56). EcoTILLING, a variation of this technique, represents a means to determine the extent of natural variation in selected genes in crops (55). It may be a cost effective approach for haplotyping and SNP discovery. Furthermore, DNA sequence information of crop plants facilitates the isolation of cisgenes (57), which are genes from crop plants themselves or from crossable species. The increasing number of these isolated genes, and the development of transformation protocols that do not leave marker genes behind, provide an opportunity to improve plant breeding while remaining within the gene pool of the classical breeder or mutation breeding.

Important recommendations

1. Collect detailed information about the existing available seeds of mutants, arrangements for multiplication and exchange/supply of mutant seeds and plant material.
2. Induce regularly new mutants using local germplasm for developing new mutant varieties as well as for basic research in gene discovery and functional genomics.

3. It is difficult to predict the impact of climate changes on global or regional or national agriculture and therefore new varieties must be developed and distributed regularly at the national and regional levels for sustainable crop production.
4. Develop new varieties that can be readily adapted in a short period on different locations with varying agro-climatic and growing conditions, and low available resources.
5. There is no further scope of expanding arable land worldwide. Now the attention should be given to develop plant varieties suitable to grow in marginal lands.
6. Exploit neglected and underutilized crops
7. Study root architecture and root growth of plants to withstand abiotic and biotic stress conditions.

References

1. P. CRINO, A. LAI, R.D. BONITO, P. VERONESE,. Genetic variability in tomato plants regenerated from irradiated cotyledons. *J Genet. Breed.* 48: 281-290 (1994).
2. P. DONINI, A. SONNINO, *Somaclonal Variation and Induced Mutations in Crop Improvement,* S.M. JAIN, D.S. BRAR, B.S. AHLOOWALIA, eds., Kluwer Aacdemic Publishers, Dordrecht, 1998, pp 255-291
3. S.M. JAIN, M. MALUSZYNSKI 2004.: *In vitro applications in crop improvement: Recent Progress*. A. MUJIB, M. CHO, S. PREDIERI, S. BANERJEE, eds., IBH-Oxford, India. Pp 169-202.
4. E.C.GUIMARAES, J.RUANE, B.D.SCHERF, A.SONNINO, J.D. DARGIE (eds.), *Marker-assisted selection. Current status and future perspectives in crops, livestock, forestry and fish,* FAO, Rome (2007).
5. S. D. TANKSLEY, J. C. NELSON**,** Advanced backcross QTL analysis: a method for the simultaneous discovery and transfer of valuable QTLs from unadapted germplasm into elite breeding lines. *Theor. Appl. Genet*. 92: 191-203 (1996)
6. A. MICKE, B. DONINI, M. MALUSZYNSKI, 1990. Induced mutations for crop improvement. *Mutation Breed Rev., FAO/IAEA*, Vienna, No 7, pp 1-41
7. M. MALUSZYNSKI, B.S. AHLOOWALIA, B. SIGURBJÖNSSON, Application of *in vivo* and *in vitro* mutation techniques for crop improvement. *Euphytica* 85: 303-315 (1995).
8. S.M. JAIN, *Crop improvement for stress tolerance*, R.K. BEHL, D.P. SINGH, G.P. LODHI, eds., CCSHAU, Hissar and MMB, New Delhi, India, 1998a, pp 218- 232.
9. R.K. BEHL, H. S. NAINAWATEE, K.P. SINGH, *International Crop Science 1*, D.R. BUXTON, R.SHIBLES, R.A. FORSBERG, B.L.BLAD, K.H.ASAY, G.M.PAULSEN, R.F.WILSON, EDS.,. Crop Sci. Soc. America, Madison, Wisconsin, USA, 1993, pp 349-355.
10. S.M. JAIN. M. M. SPENCER, Floriculture, Ornamental, and Plant Biotechnology J.A. Teixeira da Silvae, ed., Global Science Books, UK, . 2006, pp 589-600
11. S.M. JAIN. 2006. Mutation-assisted breeding in ornamental plant improvement. Acta Hort. 714: 85-98
12. B. S. AHLOOWALIA, *Somaclonal Variation and Induced Mutations for Crop Improvement*, S.M. JAIN, D.S. BRAR, B.S. AHLOOWALIA, eds., Kluwer Academic Publishers, Dordrecht, 1998, pp 293-309.
13. S.E. MADDOCK, *Somaclonal Variation and Crop Improvement*, J. SEMAL, ed., Martinus Nijhoff Publishers, Dordrecht, 1986, pp 127-137
14. S.M. JAIN, Induction of somaclonal variation and mutation in developing new improved cultivars. MIIT PUNE J, pp 23-31, (1998b)
15. R.D. LINEBERGER, M. POGANY, T. MALINICH, M. DRUCKENBROD, A. WARNR, *Proc. XVIIth Eucarpia symposium, Creating Genetic Variation in Ornamentals*, T. SCHIVA,A. MERCURI, eds., Sanremo, Italy, 1993, pp 83-92.
16. G. GAVAZZI,C. TONELLI, G. TODESCO, E. ARREGHINI, F. RAFFALDI, F. VECCHIO, G. BARBUZZI, M.G. BIASINI, F. SALA, Somaclonal variation versus chemically induced mutagenesis in tomato (*Lycopersicon esculentum*). *Theor. Appl. Genet.* 74: 733-738 (1987).
17. S.M. JAIN, R.J. NEWTON, Evaluation of protoclonal variation versus chemically induced mutagenesis in *Brassica napus* L. *Current Sci.* 58: 176-180 (1989).
18. S.M. JAIN, Creation of variability by mutation and tissue culture in improving plants. *Acta Hort*. 447: 69-78. (1997)
19. M.K. BANERJEE, KALLOO, Role of phenols in resistance to tomato leaf curl virus, *Fusarium* wilt and fruit borer in *Lycopersicon*. *Curr. Sci.* 58: 575-576 (1989).
20. V.B. KUKSOVA, N.M. PIVEN, Y.Y. GLEBA, Somaclonal variation and *in vitro* induced mutagenesis in grapevine. *Plant Cell Tiss. Org. Cult*. 49: 17-27. (1997)

21. S.M. JAIN. Major mutation-assisted plant breeding programs supported by FAO/ IAEA. *Plant Cell Tiss. Org. Cult.* 82: 113-123 (2005).
22. B. S. AHLOOWALIA, M. MALUSZYNSKI, K. NICHTERLIN, Global impact of mutation derived varieties. Euphytica 135: 187-204. (2004)
23. S. PREDIERI, Mutation induction and tissue culture in improving fruits. *Plant Cell Tiss Org. Cult*. 64: 185-219.(2001)
24. M.K. SMITH, S.D. HAMILL, P.W. LANGDON, J.E. GILES,V.J. DOOGAN, K.G. PEGG, Towards the development of a Cavendish banana resistant to race 4 of fusarium wilt: gamma irradiation of micropropagated Dwarf Parfitt (*Musa* spp., AAA group, Cavendish subgroup). *Australian J. Expt. Agric,* 46: 107-113 (2006)
25. S.M.JAIN, R. SWENNEN (eds.), *Banana improvement: cellular, molecular and mutagenesis approaches*. Science Publishers, New Hampshire, USA, 2004
26. H.YAMAGUCHI, S. NAGATOMI, T. MORISHITA, K.DEGI, A TANAKA, N.SHIKAZONO, Y. HASE, Mutation induced with ion-beam irradiation in rose. *Nucl. Instr. Meth. Phys. Res*. B 206:561-564 (2003
27. H.WATANABE, Significance and expectations of ion beam breeding. *Gamma field Symposia, No. 40, Institute of Breeding, NIAS, Japan*, 15-19 (2001).
28. N. SHIKAZONO, Y. YOKOTA, S. KITAMURA, C. SUZUKI, H. WATANABE, S. TANO, A. TANAKA, Mutation rate and novel tt mutants of Arabidopsis thaliana induced by carbon ions. *Genetics* 163: 1449–1455 (2003)
29. A. MATSUMURA, T. NOMITZU, N. FURUKANI, K. HAYASHI, Y. MINAMIYAMA, Y. HASE, Ray florets color and shape mutants induced by $^{12}C^{5+}$ ion beam irradiation in chrysanthemum**.** *Scien. Hort.* 123: 558-561(2010)
30. K. NISHIHARA, Y. KAZAMA, T.HIRANO, S. OHBU, T. MATSUYAMA, S. KAWANO, T. ABE, LET-dependent effects of ionizing radiation on deletion mutants in Arabidopsis thaliana. Abs. book of Intern Conf. on Molecular Aspects of Plant Development, Feb 23-26, Vienna, Austria, p72
31. 31. M. MEI, H. DENG, Y.LU, C.ZHUANG, Z.LUI, Q. QIU, Y.QIU, T.C.YANG, Mutagenic effects of heavy ion tradiation in plants. *Adv. Space Res*. 14: 363-372. (1994).
32. 32. Z.YU, J.DENG, J. HE, Y.HUO,Y.WU, X. WANG, G.LIU, Mutation breeding by ion implantation. *Nucl. Instr. and Meth*. B25726: 705-708 (1991).
33. 33. T. MORISHITA, H.YAMAGUCHI, K.DEGI, N. SHIKAZONO, A.TANAKA, T.ABE. Dose response and mutation induction by ion beam irradiation in buckwheat, *Nucl Instr. and Meth* B196: 565-569 (2003).
34. 34. S. NAGATOMI, A.TANAKA, A.KATO, H.WATANABE, S.TANO, Mutation induction on chrysanthemum plants regenerated from in vitro cultured explants irradiated with 12C ion beams. *JAERI-Review 96-017*:50-52 (1995)
35. 35. L.D.YU, S. SANGYUENYONGPIPAT, S. ANUNTALABHOCHAI, B. PHANCHAISRI, T. VILAITHONG, I.G. BROWN, Effects of low-energy ion beam bombardment on biological cell envelopes. *Surface Coatings Tech*. 201: 8055-8061 (2007).
36. 36. T.VILAITHONG, L.D.YU, P.APAVATJRUT, B. PHANCHAISRI, S. SANGYUENYONGPIPAT, S. ANUNTALABHOCHAI, I.G.BROWN, Heavy ion induced DNA transfer in biological cells. *Rad. Phys Chem 71:* 927-935 (2004).
37. 37. S. ANUNTALABHOCHAI, R.CHANDEJ, B. PHANCHAISRI, L.D.YU, S. PROMTHEP, S. JAMJOD, T.VILAITHONG, Mutation induction in Thai purple rice by low-energy ion beam. *Proc. 9th Asia Pacific Phys. Conf.* Hanoi, Vietnam: 1-6 (2004)
38. 38. B. PHANCHAISRI, R.CHANDET, L.D.YU, T.VILAITHONG, S. JAMJOD, S. ANUNTALABHOCHAI, Low-energy ion beam-induced mutation in Thai jasmine rice. *Surface Coatings Tech*. 201: 8024-8028 (2007).
39. 39. B. BHAGWAT, E.J.DUNCAN, Mutation breeding of banana cv. Highgate (*Musa acuminate* AAA Group) for tolerance to *Fusarium oxysporum* f. sp. *cubense* using chemical mutagens. *Sci. Hort* 73: 11-22.
40. 40. Y.S. LUAN, J. ZHANG, X. R. GAO, L.J. AN, Mutation induced by ethylmethanesulphonate (EMS), in vitro screening for salt tolerance and plant regeneration of sweet potato (*Ipomoea batatas* L.) *Plant Cell, Tiss. Org. Cult.* 88: 77-81 (2007).
41. 41. P.A.NEVILLE, N.B.NAYANA, M. TANGUAY, Mutagenic effects of acute gamma irradiation on miniature roses: target theory approach. *HortSci*. 33: 127-129. (1998)
42. 42. A.B. BRITT, DNA-damage and repair in plants. *Ann. Rev. Plant Physiol. Plant Mol. Biol.* 47: 75-100 (1996).
43. 43 A.C. CASSELS, *Somaclonal variation and induced mutations in crop improvement,* S.M.JAIN, D.S.BRAR, B.S. AHLOOWALIA, eds., Kluwer Academic Publishers, Dordrecht, 1998, pp367-378.
44. S.M. JAIN, Mutation-assisted breeding in ornamental plant improvement. *Acta Hort.* 714: 85-98 (2006)
45. S.M. JAIN, Recent advances in plant tissue culture and mutagenesis. *Acta Hort* 736:205-211. (2007)
46. P.C. REMOTTI, *Somaclonal variation and induced mutations in crop improvement*, S.M. JAIN, D.S. BRAR, B.S. AHLOOWALIA, eds.,. pp 169-201. Kluwer Academic Publishers, Dordrecht, 1998, pp 169-201

47. R.R. DUNCAN, 1997. Tissue culture-induced variation and crop improvement. *Adv. Agron.* 58: 201-240 (1997).
48. S. HEATH-PAGLIUSO, J. PULLMAN, L. RAPPAPORT, Somaclonal variation in celery: screening for resistance to *Fusarium oxysporum* f.sp.*apii*. *Theor. Appl. Genet.* 75:446-451 (1988).
49. R.A. BRESSAN, N.K. SINGH, A.K. HANDA, A.K. KONONOWICZ, P.M. HASEGAWA, *Plant Genetics*, M. FREELING, ed., New York: Liss, 1985, pp 755-769
50. S.R.S. SABRY, L.T. SMITH, G.M. SMITH, Osmoregulation in Spring wheat under drought and salinity stress. *J Genet Breed*. 49: 55-60 (1995).
51. G.R. STEWART, J.A. LEE, The role of chlorine accumulation in halophytes. *Planta* 20: 279-289 (1974).
52. R.A. BRESSAN, N. K. SINGH, A.K. HANDA, R. MOUNT, 1987. *Drought Resistance in Plants*, L. MONTI, E. PORCHEDDU, eds., Commission of the European Communities, Brussels, Belgium, 1987, pp 41-57.
53. Q.Y. SHU (Ed.), Induced plant mutations in the genomics era, FAO, Rome, (2009).
54. H.HIROCHIKA, E. GUIDERDON,G.AN, Y.HSING, M.Y.EUN, C.HAN, N.UPADHYAYA, S. RAMACHANNDRAN, Q.ZGANG, A.PEREIRA, V.SUNDARESAN, H.LEUNG, Rice mutant resources for gene discovery. *Plant Mol. Biol*. 54: 325-334. (2004).
55. B. MARTIN, M.RAMIRO, J.M. MARTINEX-ZAPATER, C. ALONSO-BLANCO, A high-density collection of EMS-induced mutations for TILLING in Landsberg erecta genetic background of *Arabidopsis*. *BMC Plant Biol*. 9: 147 (2009).
56. T. BRADLEY, J. TILL, J. COOPER, T. H. TAI, P. COLOWIT, E. A. GREENE, S. HENIKOFF, L. COMAI, Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biol.* 7**:** 19 (2007).
57. E. JACOBSEN, H. J. SCHOUTEN, Cisgenesis strongly improves introgression breeding and induced translocation breeding of plants. *Trends in Biotechnology* 25: 219-223 (2007).
58. M.OKAMURA, N.YASUNO, M.OHTSUKA, A.TANKA, N.SHIKAZAZONO, Y.HASE, Wide variety of flower-color and shape mutants regenerated from leaf cultures irradiated with ion beams. *Nucl. Instr. Meth. Res.* B206:574-578 (2003)
59. S.M. JAIN, A review of induction of mutations in fruits of tropical and subtropical regions. *Acta Hort*. 575: 295-302 (2002).