Review Article

A Comprehensive Review on Application of Conventional and Mutation Approaches in Genetic Improvement of Ornamental Crops.

Abstract: Effective mutagenic treatment techniques for different species are of tremendous interest due to the exciting potential of mutation breeding in ornamental plants. The present article addresses the mutagenesis treatments of numerous ornamental genera, the benefits and drawbacks of different methods, and the potential for enhancing the related protocols. There are several techniques for non-targeted mutagenesis, from chemical treatment with alkylating chemicals to dose-dependent exposure to X-rays, gamma rays, neutron or heavy ion beams. All of these have been shown to be efficient mutagens in a wide range of different species and are reasonably priced. However, due to the high cost and lack of understanding required to efficiently transform and regenerate attractive crops, genetic engineering is still generally impracticable for many ornamental breeding operations. The most widely used non-targeted mutagen currently in use is gamma radiation. Although it appears to have a lower mutagenic efficacy than chemical mutagens, it offers excellent consistency. Although chronic irradiation over a longer period of time induces less harmful mutations than the routinely employed acute irradiation protocols, changes in the radiation dose rate may boost the efficiency. Because of the high particle energy associated with these treatments, heavy ion beam irradiation may also offer extremely consistent mutation induction at greater efficiencies. Additionally, there are chances to enhance chemical mutagenesis. It is still highly beneficial to use mutation breeding, and there are plenty of chances to make the current techniques better.

INTRODUCTION

Since ancient times, people have grown decorative plants for all significant occasions, including the expression of emotions. The value of the world's floriculture trade has surpassed USD 50 billion and continues to rise. The area that may be grown for different flower crops is continually growing. More than 90% of the global commerce in floriculture products is with wealthy nations. Currently, tissue culture is being used to propagate over 156 ornamental genera in various commercial laboratories across the globe. [1]. The major ornamental plant producing country worldwide is the Netherlands, claiming 33% of the total global market.

Screening the naturally occurring diversity is the traditional method used to create new flower colors in ornamental plants. Unfortunately, traditional breeding techniques have not been able to create cultivars that,

for example in saintpaulia, are resistant to cold, gray mold illness, or yellow or true red flower colors. [2]. Genetic variation is necessary for the genetic improvement of ornamental plants in order to create new or improved kinds. However, the breeding of ornamental plants is hampered since the desired genetic variety is frequently absent. This is because the available germplasm is unable to produce the required recombinants, necessitating the use of alternative sources of variety. Mutation induction techniques offer a means of rapidly creating and increasing crop species variety, as spontaneous mutations happen very seldom. Mutagenic substances like radiation and chemicals can cause genetic diversity, which can then be used to select desired mutants. [3]. The nuclear DNA is broken by the mutagen therapy, and new, random, heritable mutations are produced during the DNA repair procedure. Changes in cytoplasmic organelles can also lead to chromosomal or genomic mutations, which allow plant breeders to choose beneficial mutants for traits including disease resistance, early flowering types, and flower color and form. [4]. 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.

Alternatively, somaclonal variation is another way to induce genetic variability in ornamental plants, e.g. Begonia and Saintpaulia [5&6]. Point mutations, DNA methylation, changed sequence copy number, transposable elements, genotype, explant type, culture media, age of the donor plants, single gene mutations, and chromosomal rearrangements are the modifications linked to soma clonal variation. Since gene mutations are typically common in plants grown from tissue cultures, tissue culture systems can be thought of as a means of facilitating mutations that will enhance agricultural plants. It is important to keep in mind that somaclones should not be used for breeding programs until their genetic stability has been established.

By genetic engineering, flower variants have been obtained in several ornamental plants, such as *Saintpaulia ionantha* [2], *Gerbera jamesonii* [7], *Eustoma grandiflorum* and *Osteospermum ecklonis* [8.] Florigene Company, Australia has sold transgenic carnation flowers for six years [9]. Flowers were first sold in Australia, followed by Japan and USA. They developed two carnation types – the spray, which has a branching stem with flowers from each branch, and the standard, which is a single stem with a single large flower.

EMS and Other Alkylating Agents

Since its discovery in 1946, alkylating compounds like EMS have been widely used in the breeding of ornamental plants. N-methylN0 -nitro-N-nitrosoguanidine, N-ethyl-N-nitrosourea, and dimethyl nitrosamine are only a few of the several compounds that can be utilized; however, EMS is by far the most popular due to its mutagenic efficiency ratio of mutations to harmful consequences. [10&11], relatively low cost, and high availability [12,13&14]. The process by which EMS alters DNA is predicated on guanine's alkylation, which produces G:C to A:T replacements. This results in randomly dispersed point mutations across the whole

genome [15], which give rise to single nucleotide polymorphisms (SNPs). Furthermore, compared to physical mutagens, EMS results in fewer deletions [16]. Consequently, missense or nonsense mutations can be induced by chemical mutagens like EMS, which can lead to mutants with altered or occasionally lost functions. Typically, a buffer solution containing EMS is used to treat seeds. Treatment doses and durations varies significantly between taxa and even within species, as distinct genotypes may react differently to the same intervention. EMS can have harmful effects, including sterility, mortality, and a decreased capacity to regenerate plants from tissues like floral pedicels [17]. If certain weedy or invasive species are treated, the desired outcome is reduced fertility, which is at least as significant as other phenotypic alterations. It is always advisable to conduct pilot tests to identify the ideal treatment settings, which are often a mix of concentration and duration that results in 50% survival [median lethal dose (LD_{50})] [18&19]. Apart from seeds, nodal segments and ray florets of Chrysanthemum have also been subjected to in vitro treatments. [20].

Annuals and Biennials

Several annual and biennial ornamental genera have been treated with EMS (Table 1). With the exception of Begonia, all cases included treating the seeds; concentrations have varied from 0.10% to 1.20%, with a 40% outlier; treatment times are typically between 4 and 24 hours. [21] noted variations in bloom color between the white-flowering Petunia x hybrida M_1 and M_2 generations. The M_1 generation included a variety of violet hues, whereas the M_2 generation included hues ranging from pink to a light blueish magenta. *Antirrhinum majus* M_1 mutants with aberrant leaf shape and dwarfism were discovered. [22]. In addition to changing flower color and other morphological traits, EMS has also been used to create mutants with resistance against pathogens, as shown who obtained *Begonia x hiemalis* mutants that were resistant to stem rot caused by Rhizoctonia.

Herbaceous Perennials

A small number of non-woody perennial decorative species have also been treated with EMS; the most common is the economically significant genus Chrysanthemum (Table 1) [23]. A variety of Chrysanthemum tissues were employed, with durations ranging from 1 to 5 hours and concentrations ranging from 0.02% to 1.03%. Table 1 shows the concentrations and times at which different genera were treated, which ranged from 0.10% to 1.25% for 10 min to 24 h. These experiments used a variety of tissues, including seeds, bulbs, flower pedicels, and leaf segments. The concentrations and treatment times varied from 0.02% to 1.25% and 10 minutes to 24 hours, respectively. [24] discovered a salt-tolerant mutant of *Chrysanthemum morifolium* that, when grown in high saline conditions, showed no decline in bloom size or quantity. Chrysanthemum flower color changed as a result of EMS therapy. For instance, when [25], a dark pink cultivar, was given treatment, mutants with golden, yellow, and white flowers were discovered. Agave Americana tepal count increased

from six to eight as a result of EMS [26]. There have also been reports of decreased pollen productivity and plant height. [27&28].

Woody Trees and Shrubs

Table 1 shows that EMS has also been applied to woody ornamentals. It was employed with seeds as well as other tissues including cuttings and meristems. Treatments ranged from 1 to 48 hours and used EMS solutions with concentrations between 0.05% and 5%. Several features were impacted, much like in the case of the biennial, annual, and herbaceous perennials. The cultivar Summer Skies, which exhibits consistent variegation along the margins of leaves, was created by treating *Buddleja davidii* seeds. [29]. *Weigela* and *Ribes sanguineum* both had altered leaf morphology, which in Ribes' instance produced the cultivar Oregon Snowflake [30&31]. After EMS treatments of cuttings, [32] saw a decrease in the number of rose petals and the size of the flowers. There were additional mutations in flower color with reduced cyanidin and pelargonidin concentrations. *Bougainvillea spectabilis* showed variations in leaf form, variegation, dwarfism, and thornlessness. [33]. [34] dwarfism in *Jasminum grandiflorum* has also been noted. It is evident that EMS can cause mutations in a wide variety of species, hence altering a wide range of properties. Using EMS is a desirable option because it doesn't require pricey technical equipment and is a reasonably simple process, especially for smaller-scale breeding initiatives. The inability of EMS or other chemical mutagens to deeply enter plant tissues and seeds with thick coatings is a drawback that could result in uneven treatment outcomes [35]. EMS has the benefit of having a relatively high mutagenesis efficiency, which reduces the proportion of undesired mutations to all mutations. [36]. On either side of the optimal, however, the efficiency usually fallsand varies with dose. This highlights even more how crucial it is to ascertain the correct dosage prior to applying a large-scale plant treatment.

Table 1. Ethyl methane sulfontate (EMS) treatment conditions. The genus, mutagen, EMS concentration, treatment duration, median lethal dose (LD_{50}) when provided, treated material, and reference are shown for each study.

Genus	Mutagen	Treatment concn	Treatment duration	LD_{50}	Material	Reference
Agave	EMS	0.25% to 0.50%	4 h	—	Bulbs	[26]
Antirrhinum	EMS	0.10% to 1.00%	8–12 h	—	Seeds	[22]
Begonia	EMS	Unknown	Unknown	—	Leaves	[37]
Bougainvillea	EMS	0.80% to 1.00%	6 h	—	Cuttings	[33]
Buddleja	EMS	1.40%	4 h	—	Seeds	[29]
Chrysanthemum	EMS	0.025% to 0.050%	5 h	—	Leaf sections	[24]
	EMS	0.02% to 0.04%	Unknown	—	Cuttings	[28]
	EMS	0.51% to 1.03%	1 h 45 min	0.82%	Floral pedicels	[25]
	EMS	0.10% to 0.30%	1 h	—	Ray florets	[20]
Dianthus	EMS	0.10% to 0.70%	6 h	—	Seeds	[17]
Gerbera	EMS	0.10% to 1.00%	10 min	0.65%	Shoots	[38]
Gladiolus	EMS	0.20% to 1.20%	Unknown	—	Corm buds	[39]
Hydrangea	EMS	0.50% to 5.00%	3 h	—	Seeds	[40]
Impatiens	EMS	0.32% to 1.08%	24 h	—	Seeds	[41]
Jasminum	EMS	0.06% to 0.62%	1–6 h	0.53%, 0.55%	Cuttings	[34]
	EMS	0.25% to 0.4%	1 h	—	Cuttings	[42]
Ornithogalum	EMS	0.20% to 1.00%	24 h	0.15%, 0.52%	Seeds	[30]
Petunia	EMS	0.10% to 0.30%	18 h	—	Seeds	[21]
Portulaca	EMS	1.20% to 40.00%	4 h	—	Seeds	[43]
Ribes	EMS	0.20% to 1.20%	24–48 h	—	Seeds	[30]
Rosa	EMS	0.50% to 3.00%	2–12 h	—	Apical and axillary meristems	[44]
	EMS	0.08% to 5.00%	1–24 h	—	Stem cuttings with buds	[32]
Weigela	EMS	0.50%	1 h 30 min	—	Shoot internodes	[31]

X-rays and Gamma Rays

Following the discovery of the mutagenic effects of ionizing radiation by [45] and [46&47], numerous mutant types have been produced utilizing X-rays and gamma rays. Particularly popular has been gamma radiation, which was used to develop around half of all the mutant kinds listed in the FAO/IAEA Mutant Variety Database. Only 17% of the registered types have been exposed to X-rays, and little more than 10% have been subjected to chemical mutagenesis (International Atomic Energy Agency, 2021). The foundation of both gamma and X-ray mutagenesis is the direct and indirect interaction with DNA with extremely intense electromagnetic radiation. These interactions typically break the DNA, resulting in deletions and other chromosomal abnormalities, the majority of which are loss-of-function mutants. [48,49&50].

Plant tissue or seeds are normally exposed to gamma radiation in gamma fields for long-term exposure and gamma chambers or rooms for acute exposure [51&52]. However, chronic irradiation is not often employed. Cobalt-60 is the most often used gamma source, however there are other efficient ones as well, such cesium-

137. [53]. The procedure for X-ray irradiation is similar and uses an X-ray source instead of a gamma source. Dosage is typically measured in kilorads (krad), grays (Gy), or sometimes roentgens (R). Converting among units is simple: 10 krad = 1 Gy and 114 R = 1 Gy. A wide range of absorbed radiation doses are used depending on the radiosensitivity of the treated material. Physical mutagens have the same deleterious effects as chemical mutagens. Therefore, it is recommended that the optimal dose, usually close to the LD_{50}, should be determined for a specific subject before starting with irradiation on a large scale [11, 53, 51, 54, 55, 56&57].

Annuals and Biennials

Petunia is the most popular annual and biennial ornamental genera whose mutagenesis has been studied using gamma and X-rays. (Table 2). For gamma irradiation, the total absorbed doses have ranged from 0.5 to 320 krad. A dose of 320 krad is exceptionally high, however; the median maximum dose was 12.5 krad. For X-rays, the doses have ranged from 0.22 to 20 krad. Seeds are the most commonly irradiated tissues, but others such as leaf discs and cuttings have been used. Many traits were affected. [58] identified a Petunia mutant with a higher density of trichomes and a distinct leaf shape. [59] found zinnias with novel flower colors such as yellow, magenta, and red with white spots in mutants of the cultivar Crimson Red. A Zinnia mutant showing a larger number of whorls in its flowers was also found. [60] identified *Begonia hiemalis* mutants displaying dwarfism, petaloid stamens, and varying leaf colors. Fertility, characterized as the number of seed capsules produced after manual pollination, was reduced in *Petunia hybrida* [58].

Herbaceous Perennials

Many herbaceous perennials have been treated with gamma and X-rays; Chrysanthemum is by far the most often treated genus (Table 2). The doses used in the X-ray treatments ranged from 0.44 to 13 krad, with one dose at 50 krad being extremely high. The dose range for gamma irradiation was 0.15 to 15 krad, with two exceptions at 40 krad. In these investigations, a wide variety of tissues were used. The tissues varied from cuttings and entire plants to individual cells and ray florets for Chrysanthemum alone. Bulb cuttings, corms, and leaf cuttings were used for other genera. Remarkably, none of the research under consideration used seeds. Numerous morphological characteristics of biennials and annuals were impacted. [61] selected two Chrysanthemum mutants that were tolerant to low temperatures. [62] irradiated cuttings of the Chrysanthemum cultivar Beakma and found a mutant that did not form a hollow stem when grown in high summer temperatures, leading to plants with stronger stems that are easier to handle. A *Gerbera jamesonii* mutant that was tolerant to powdery mildew was found by [63]. In *Dianthus caryophyllus*, X-ray irradiation restored male fertility [64]; in *Ornithogalum virens*, however, [65] was able to produce partial sterility. observed that applying very moderate doses of gamma radiation to the callus of *Rudbeckia subtomentosa* produced a good number of mutations, including decreased height and improved flower morphology, as well

as high survival. Most of the other genera exhibited a wide range of variations in flower form and color, and as demonstrated by [67], characteristics like vase life can also be enhanced.

Woody Trees and Shrubs

Gamma or X-rays have been used to treat a wide variety of woody species (Table 2). [68] calculated the gamma radiation lethal doses (LD_{50}) for 28 species of woody plants and estimated the LD_{50} for an additional 190 species by using the interphase chromosomal volumes of those plants. The authors acknowledge that there are significant differences in the results due to timing and other methodological issues. Although they did not list any obvious characteristics brought on by mutations, the LD50 values offer a place to start for figuring out what dose is best for each of these species. The aforementioned studies used doses ranging from 0.1 to 40 krad with outliers at 140 and 225 krad for gamma rays and 2.5 to 6 krad for X-rays. Seeds and cuttings were often the choice of tissue to treat, but whole plants and explants were also treated. Different traits were affected by the mutations. A jasmine-like fragrance was found in a Vitex agnus-castus mutant by [69], who showed that complicated traits like fragrance can also be improved by inducing mutations. Dwarfism was found by [70] in Cryptomeria and in Jasminum [71]. Shorter internodes resulting in lower plant height were also observed in Populus and Rosa [72]. Other variations in Rosa were restoration of fertility and changes in color (orange, pink, etc. compared with red in the original cultivar), possibly because of changes in cyanidin and pelargonidin content [73].

While many plant species have found great success with gamma and X-ray irradiation as mutagens, these techniques need more expensive apparatus, such as gamma sources and X-ray machines. They provide for good tissue and seed penetration, enabling the treatment of material with a higher uniformity. Additionally, they offer methods for caring for delicate tissue that could be harmed by soaking things in chemicals, including pollen grains [93&94]. However, it appears that their mutagenesis efficiency is not very high [95&96].

Table 2. Gamma and X-ray treatment conditions, genus, mutagen, treatment dose, median lethal dose (LD_{50}), treated material, and reference are shown for each study.

Genus	Mutagen	Dose (krad)[z]	LD_{50} (krad)	Material	Reference
Acer	Gamma rays	0.1–5	—	Cuttings	[74]
	Gamma rays	50–225	—	Seeds	[74]
Agave	Gamma rays	1–40	—	Bulbs	[75]
Begonia	X-rays	1.5–2.5	—	Leaves	[60]
Berberis	Gamma rays	0.1–5	—	Cuttings	[74]
	Gamma rays	50–225	—	Seeds	[74]
Bougainvillea	Gamma rays	0.5–1	—	Cuttings	[33]
	Gamma rays	0.5–2	—	Cuttings	[76]
Chrysanthemum	Gamma rays	1–4	—	Cuttings	[77]
	X-rays	0.44–1.75	—	Cuttings	[77]
	Gamma rays	0.5–1	—	Ray florets	[26]
	Gamma rays	3–10	—	Plantlets	[28]
Cryptomeria	Gamma rays	1–5	—	Cuttings	[62]
	X-rays	2.5	—	Callus	[61]
Dahlia	Gamma rays	1–3	—	Cuttings	[78]
Dianthus	Gamma rays	5.26–10.5	—	Cuttings	[79]
	X-rays	2–50	—	Nodes	[80]
	X-rays	4–13	—	Leaf segments	[81]
	Gamma rays	3–10	—	Leaf segments	[81]
Gerbera	X-rays	2.2–4.4	—	Plants	[82]
	Gamma rays	2–8	—	Petal explants	[64]
	Gamma rays	0.15–3	0.65	Shoots	[38]
	Gamma rays	0.15–1	0.6	Shoots	[38]
Gladiolus	Gamma rays	1.5–5.5	—	Corms	[83]
	Gamma rays	1.5–6	—	Corms	[67]
	Gamma rays	1.75–10.5	—	Corms	[84]
Iris	X-rays	0.5–1.1	—	Bulbs	[85]
Jasminum	Gamma rays	1–2.5	—	Cuttings	[71]
Lonicera	Gamma rays	1–6	2.1, 3.5	Microcuttings	[86]
Ornithogalum	Gamma rays	20–40	—	Seeds	[65]
Pelargonium	Gamma rays	1.5	—	Leaves	[87]
Petunia	Gamma rays	2–10	10	Seeds	[58]
Plectranthus	Gamma rays	1.5–6	3.762–6.52	Cuttings	[88]
Populus	Gamma rays	1–30	—	Plantlets	[89]
Rosa	Gamma rays	1–6	—	Shoot tips	[90]
	Gamma rays	1–12	3.3–5.4	Shoot tips	[72]
	Gamma rays	0.5–8	4	Stem cuttings with bud	[51]
Rudbeckia	X-rays	2.5–6	—	Microshoots	[56]
Saintpaulia	Gamma rays	1–10	5.6	Leaf cuttings	[91]
	X-rays	0.5–10	5.69	Leaf explants	[92]

Neutrons and Heavy Ions

Gamma or X-ray irradiation is being replaced with neutron and heavy ion radiation. Recent years have seen the use of heavy ion irradiation, primarily with carbon ions, to cause mutations in a number of plant species. [97,98, 99 & 81]. Neutron irradiation has also been used as a mutagen, but it has had very limited use in ornamentals [100, 101, 102 &103]. The mechanism of gamma and X-ray irradiation-induced mutations is somewhat similar to that of neutron and ion irradiation. DNA is broken into double strands when ions and neutrons clash with it, leading to deletions [104]. Therefore, the majority of mutants produced by neutron or ion radiation are loss-of-function mutants. Typically, a cyclotron is utilized to accelerate the ions required to irradiate plant tissues, and then the ions are transmitted downrange to the sample [105]. Selecting ions of a certain element allows one to modify the energy of the particles themselves in addition to the total dose. Even though carbon is frequently utilized, it is also feasible to irradiate employing heavier ions like iron or argon. Another way of altering the particle energy of the ions is by forcing the beam to pass through aluminum disks of a certain thickness, thus causing the particles to lose kinetic energy [106]. Mega-electron volts (MeV) or mega-electron volts per nucleon (MeV/u) are the two units used to quantify particle energy. The linear energy transfer (LET), expressed in keV/mm, characterizes the final energy that the ions deposit in the plant tissue. At LETs ranging from 22.5 to 310 keV/mm, doses usually range from 0.01 to 14 krad. Like with all mutagens, it's critical to figure out the ideal dosage prior to widespread radiation exposure.

Annuals and Biennials

Ion beams have only been applied to a small number of annual or biannual genera, while neutron beams have never been employed (Table 3). These investigations have employed heavy ion doses ranging from 0.1 to 8 krad at LETs ranging from 22.5 to 76 keV/mm. Different tissues are exposed to ion beam radiation. Although apical meristems and shoot cultures have been employed, cuttings with nodes and leaves also make good targets for radiation. Color of flowers is frequently impacted [107] discovered that, in contrast to the natural purple blooms, Limonium mutants displayed brighter, deeper, or more reddish-purple hues. Similar Torenia mutants, as reported by [108], displayed pale or dark pink flowers as opposed to the blue blooms of the wild type. They also revealed that the pink color likely resulted from the inhibition of dihydromyricetin biosynthesis, thus preventing build-up of the anthocyanidins delphinidin, petunidin, and malvidin. Other traits such as variegation in Petunia and sterility in Verbena were observed [109 &110].

Herbaceous Perennials

Ion beam and neutron irradiation have been applied to a far greater number of herbaceous perennial species, with Chrysanthemum once again being the most common (Table 3). The treatment parameters comprised of ion doses between 0.01 and 10 krad at LETs between 22.5 and 310 keV/mm. Neutrons were administered to Achimenes alone, at doses ranging from 0.75 to 2 krad. Target tissues included ray florets, leaf segments,

callus, petioles, and buds. One case also involved the usage of seeds. The majority of mutants exhibited abnormalities in basic morphological features as plant size (dwarfism), leaf form, flower color, flower size, and flower shape. discovered sterile mutations of Cyclamen [111]. Additionally, a mutant Chrysanthemum that flowered early and at low temperatures was discovered. [112].

Woody Trees and Shrubs

Only a small number of woody taxa underwent ion beam or neutron irradiation (Table 3). Doses of ion beams between 0.5 and 14 krad were employed. With the exception of two occurrences where the LET was 23 keV/mm, LETs were primarily not recorded. Doses of neutron irradiation varied between 2 and 14 krad. Although scions, stem cuttings, and buds have all been employed as irradiation targets, seeds were most frequently used. Prunus and Rosa flower color as well as dwarfism in Spiraea and Hydrangea plants were among the impacted features. [113] discovered that in 2007, a Prunus mutant that flowered twice in a single year did not require a cold period to flower. If the mutant was exposed to harsh winter temperatures, it produced three times as many flowers as the original variety. In 2010, this mutant—dubbed "Nishina Otome"—was made available for purchase. [85] discovered a number of Acer mutants that failed to blossom and generate any seeds. A Berberis mutant that did flower was also discovered, but the seeds it produced were not viable. Although these mutations are still being assessed, they may offer ways to lessen the invasiveness of non-native Berberis and Acer species in North America.

Stability of Resulting Mutants

Chimeras are frequently the outcome, regardless of the mutagen of choice or the organ(s) treated. Frequently unstable, chimeras revert to their wild form. Leaf variegation is a typical example, wherein a mutation affects all of a histo-genic layer (periclinal), a section of many histo-genic layers (sectorial), or a fraction of a histo-genic layer (mericlinal). There has been a lot of discussion on these chimeras and their application in horticulture elsewhere [114]. In the case of asexually propagated crops, the breeder must know how to stabilize the trait of interest so that it can stay true to type during serial replication; alternatively, the mutations must be expressed through the LII (germ layer) histogenic layer in order for them to be helpful. Subsequent phytomeres frequently exhibit variable variegation and permit the proliferation of stems above which the characteristic seems stabilized. In a *Sarcococca confuse* example, [115] described how an unstable “blotchy” variegation was allowed to grow until it stabilized into a uniform chartreuse leaf type. This leaf type remained stable after clonal propagation and also produced true-to-type seed, though the latter is more likely due to apomixis than to a LII histogenic layer containing the trait. The many cultivars that regularly revert in the ornamental trade provide evidence that, even in cases where the characteristic seems stable, there is a long-term potential for reversion. Seed propagation, independent of apomixis, represents a more reliable method of stabilizing the trait but does require the mutation present in the LII histogenic layer.

Table 3. Neutron and heavy ion treatment conditions. The genus, mutagen, treatment conditions, treated material, and reference are shown for each study. For the treatment conditions, the dose is shown in kilorads (krad).

Genus	Mutagen	Dose	LD_{50}	Material	Reference
Acer	Neutrons	2–14 krad	—	Seeds	[85]
Achimenes	Neutrons	0.75–2 krad	—	Leaves	[101]
Berberis	Neutrons	2–14 krad	—	Seeds	[85]
Chrysanthemum	Ion beam	1 krad, unknown, 23 keV/mm	—	Scions	[116]
	Ion beam	0.1–0.8 krad, 220 MeV, 122 keV/mm	—	Ray florets and leaf explants	[99]
	Ion beam	0.1–0.5 krad, 446 MeV/u, 93 keV/mm	—	Leaf segments and ray florets	[68]
	Ion beam	0.3–0.6 krad, unknown, 22.6 keV/mm	—	Cuttings	[112]
	Ion beam	0.1–2 krad, 135 MeV/u, 23 keV/mm	—	Stem segments	[117]
Cyclamen	Ion beam	0.2–0.5 krad, unknown, 23/62/280	—	Cuttings	119]
	Ion beam	keV/mm 0.01–0.3 krad, unknown,	—	Leaf blades	118]
	Ion beam	23/310 keV/mm 0.3–0.6 krad, 135	—	Stemsegmnt	[120]
	Ion beam	MeV/u, 22.5 keV/mm	—	Petioles	[121]
	Ion beam	0–5 krad, 220/320 MeV, unknown	—	Petioles	[98]
Dianthus	Ion beam	0.5–3 krad, 220 MeV, unknown	—	Leafsent	98
	Ion beam	0.7–2 krad, 320 MeV, 76 keV/mm	—	Petals	98
Saintpaulia	Ion beam	0.5–8 krad, 960 MeV, unknown	—	Leaf	[122]

Conclusions

When selecting the mutagen and treatment circumstances, a number of parameters need to be taken into account. Chemical mutagens are thought to have a poorer capacity to profoundly enter plant tissue or thick seeds, despite the fact that they are comparatively cheap and need less technical equipment [123]. Physical mutagens, on the other hand, offer reliable therapy but necessitate the presence of radiation sources, such as nuclear reactors, gamma or X-ray equipment, or particle accelerators. Physical mutagens also have the benefit of not producing hazardous or cancer-causing waste, making it simple to handle plant tissue or seeds after treatment, and treating fragile materials like pollen grains [124 & 125]. The fact that EMS and other chemicals primarily result in single base changes, which may produce a number of phenotypically unique change-of-function mutants for a certain feature, is another reason why they might be preferable in some circumstances [126 & 127]. Physical mutagens, on the other hand, typically result in deletions that produce mutants with lost functions [49 & 124]. When beginning mutant breeding for a new species or cultivar, already documented experiences with a multitude of ornamental genera are useful. These experiences can inform the initial dosages; the treatment parameters can then be adjusted. Apart from the above mentioned benefits and drawbacks, distinct mutagens exhibit varying degrees of mutagenicity. Despite being the most widely used mutagen to date, gamma radiation has not been as effective as extracellular magnesium sulfide (EMS), according to several research [11 & 95]. Nonetheless, there are ways to increase efficiency. The efficiency of gamma and maybe X-ray irradiation can be improved by irradiating plant tissue or seeds over extended periods of time at lower dosage rates. Other practical possibilities include heavy ion and neutron irradiation, which offer higher efficiency and the same high penetration as conventional physical mutagens. Even though genetic engineering is becoming more widely available, the expenditures associated with development and regulation make it frequently too costly for application in ornamental breeding. Moreover, there's frequently a dearth of understanding regarding the processes involved in changing and regenerating ornamentals. For the foreseeable future, random mutagenesis will therefore continue to be a significant source of genetic diversity with plenty of room for advancement in current techniques.

Reference

1. Rout, G. and Jain, S.M. 2005. Micro propagation of floricultural crops. p.310-365. In: S.J. Murch and P.K. Saxena (eds.), Journey of a single cell to a plant, Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India.
2. Kushikawa, S., Hoshino, Y. and Mii, M. 2001. Agrobacterium-mediated transformation of Saintpaulia ionantha Wendl. Plant Sci. 161:953-960.
3. Datta, S.K. 1997. Ornamental plants-role of mutation. Daya Publishing House, Delhi, India.
4. Jain, S.M. and Maluszynski, M. 2004. Induced mutations and biotechnology on improving crops. In: In vitro applications in crop improvement: Recent progress. p.169-202. In: A. Mujib, M. Cho, S. Predieri and S. Banerjee (eds.), IBH-Oxford, India.
5. Jain, S.M. 1997. Micro propagation of selected somaclones of Begonia and Saintpaulia. J. Biosci. 22:585-592.
6. Jain, S.M. and De Klerk, G.J. 1998. Soma clonal variation in breeding and propagation of ornamental crops. Plant Tissue Culture & Biotechnol. 4(2):63-75.
7. Elomaa, P., Honkanen, J., Seppanen, P., Helairutta, Y., Mehto, M., Kotilainen, M., Nevalainen, L. and Teeri, T. 1993. Agrobacterium-mediated transfer of antisense chaconne synthase DNA to Gerbera hybrida inhibits flower pigmentation. Biotech. 11:508-511.
8. Mercuri, A., Sacchetti, A., De Benedetti, L., Schiva, T. and Alberti, S. 2001. Green fluorescent flowers. Plant Sci. 161:961-968.
9. Lu, C.Y., Chandler, S.F., Mason, J.G. and Brugliera, F. 2002. Florigene flowers: From labpratory to market. p.333-336. In: I.K. Vasil (ed.), Plant biotechnology 2002 and beyond, Kluwer Academic Publishers, The Netherlands.
10. Gautam, A.S., K.C. Sood, and A.K. Richarria. 1992. Mutagenic effectiveness and efficiency of gamma-rays, ethyl methane sulphonate and their synergistic effects in black gram (*Vigna mungo* L.). Cytologia (Tokyo) 57:85–89, doi: 10.1508/cytologia.57.85
11. Girija, M. and D. Dhanavel. 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combined treatments in cowpea (Vigna unguiculata L. Walp). Glob. J. Mol. Sci. 4:68–75.
12. Gichner, T. and J. Velemınsky. 1967. The mutagenic activity of 1-alkyl-1-nitrosoureas and 1-alkyl-3- nitro-1-nitrosoguanidines. Mutat. Res. 4:207– 212, doi: 10.1016/0027-5107(67)90073-5.
13. Montesano, R., H. Bresil, and G.P. Margison. 1979. Increased excision of O6 - methylguanine from rat liver DNA after chronic administration of dimethylnitrosamine. Cancer Res. 39:1798–1802.
14. Talebi, A.B., A.B. Talebi, and B. Shahrokhifar. 2012. Ethyl methane sulphonate (EMS) induced mutagenesis in malaysian rice (cv. MR219) for lethal dose determination. Amer. J. Plant Sci. 3:1661–1665, doi: 10.4236/ajps.2012.312202.
15. Greene, E.A., C.A. Codomo, N.E. Taylor, J.G. Henikoff, B.J. Till, S.H. Reynolds, L.C. Enns, C. Burtner, J.E. Johnson, A.R. Odden, L. Comai, and S. Henikoff. 2003. Spectrum of chemically induced mutations from a large scale reverse-genetic screen in Arabidopsis. Genetics 164:731–740, doi: 10.1093/genetics/ 164.2.731.

16. Koornneef, M., L.W.M. Dellaert, and J.H. van der Veen. 1982. EMS- and radiation-induced mutation frequencies at individual loci in Arabidopsis thaliana (L.). Heynh. Mutat. Res. 93:109–123, doi: 10.1016/0027-5107(82)90129-4.
17. Roychowdhury, R. and J. Tah. 2011. Assessment of chemical mutagenic effects in mutation breeding programme for M1 generation of carnation (Dianthus caryophyllus). Res. Plant Biol. 1:23–32.
18. Berenschot, A.S., M.I. Zucchi, A. Tulmann-Neto, and V. Quecini. 2008. Mutagenesis in Petunia x hybrida Vilm. and isolation of a novel morphological mutant. Brazilian J. Plant Physiol. 20:95–103, doi: 10.1590/S1677-04202008000 200002.
19. Hohmann, U., G. Jacobs, and C. Jung. 2005. An EMS mutagenesis protocol for sugar beet and isolation of non-bolting mutants. Plant Breed. 124:317–321, doi: 10.1111/j.1439-0523.2005. 01126.
20. Padmadevi, K. and M. Jawaharlal. 2011. Induction of in vitro mutation in chrysantemum (Dendranthema grandiflora Tzvelev) ray florets (var. Ravi Kiran) using gamma rays and EMS. Floric. Ornam. Biotechnol. 5:74–77.
21. Kashikar, S.G. and A.S. Khalatkar. 1981. Breeding for flower colour in Petunia hybrida Hort. Acta Hort. 111:35–40, doi: 10.17660/ActaHortic. 1981.111.3.
22. Heffron, L., A. Blowers, and S. Korban. 2006. Chemical mutagenesis in snapdragon (Antirrhinum majus). HortScience 41:1021, doi: 10.21273/HORTSCI.41.4.1021.
23. Datta, S.K. and D. Chakrabarty. 2005. Classical mutation breeding and molecular methods for genetic improvement of ornamentals, p. 260–303. In: S.K. Datta (ed.). Role of classical mutation breeding in crop improvement. Daya Publishing House, Delhi.
24. Hossain, Z., A.K.A. Mandal, S.K. Datta, and A.K. Biswas. 2006a. Isolation of a NaCl-tolerant mutant of Chrysanthemum morifolium by gamma radiation: In vitro mutagenesis and selection by salt stress. Funct. Plant Biol. 33:91–101, doi: 10.1071/FP05149.
25. Latado, R.R., A.H. Adames, and A.T. Neto. 2004. In vitro mutation of chrysanthemum (Dendranthema grandiflora Tzvelev) with ethylmethane sulphonate (EMS) in immature floral pedicels. Plant Cell Tissue Organ Cult. 77:103–106, doi: 10.1023/B:TICU.0000016481.18358.55.
26. Singh, P.K., R. Sadhukhan, K. Roy, and H.K. Sarkar. 2013. Effect of EMS on morpho-anatomical changes in tuberose (Polianthes tuberosa L.). Floric. Ornam. Biotechnol. 7:103–105.
27. Contreras, R.N. and K. Shearer. 2020. Exposing seeds of Galtonia candicans to ethyl methane sulphonate reduced inflorescence height, lodging, and fertility. HortScience 55:621–624, doi: 10.21273/HORTSCI14775-19.
28. Kapadiya, D.B., S.L. Chawla, A.I. Patel, and D. Bhatt. 2016. Induction of variability through in vivo mutagenesis in chrysanthemum (Chrysanthemum morifolium Ramat.) var. Jaya. Indian J. Hort. 73:141–144, doi: 10.5958/ 0974-0112.2016.00035.9.
29. Smith, W.A. and M.H. Brand. 2012. ‘Summer Skies’ Buddleja davidii. HortScience 47:126– 127, doi: 10.21273/HORTSCI.47.1.126.

30. Contreras, R.N. and M.W. Friddle. 2015. 'Oregon Snowflake' flowering currant. HortScience 50: 320–321, doi: 10.21273/HORTSCI.50.2.320.
31. Duron, M. 1992. Induced mutations through EMS treatment after adventitious bud formation on shoot internodes of Weigela cv. Bristol Ruby. Acta Hort. 320:113–118, doi: 10.17660/Acta Hortic.1992.320.15.
32. Smilansky, Z., N. Umiel, and N. Zieslin. 1986. Mutagenesis in roses (cv. Mercedes). Environ. Exp. Bot. 26:279–283, doi: 10.1016/0098-8472 (86)90040-7.
33. Anitha, K., R. Surendranath, M. Jawaharlal, and M. Ganga. 2017. Mutagenic effectiveness and efficiency of gamma (g) rays and ethyl methane sulphonate on Bougainvillea spectabilis Willd. (cv. Lalbagh). Intl. J. Bio-resource Stress Mgt. 8:247–256, doi: 10.23910/IJBSM/2017.8.2.1634.
34. Ghosh, S. and M. Ganga. 2019. Determination of lethal dose for ethyl methane sulphonate induced mutagenesis in jasmine. Chem. Sci. Rev. Lett. 8:6–10.
35. Van Harten, A.M. 1998. Mutation breeding: Theory and practical applications. Cambridge University Press, Cambridg.
36. Kaul, M.L.H. and A.K. Bhan. 1977. Mutagenic effectiveness and efficiency of EMS, DES and gamma-rays in rice. Theor. Appl. Genet. 50:241–246, doi: 10.1007/BF00273758.
37. Chen, Y., Y. Zhang, S. Yuan, H. Liu, X. Zeng, and H. Zhang. 2014. Ethyl methane sulfonate induced disease resistance in Begonia x hiemalis Fotsch. Hort. Environ. Biotechnol. 55:498–505, doi: 10.1007/s13580-014-0053-2.
38. Ghani, M., S. Kumar, and M. Thakur. 2014. Physiological and biochemical responses of gerbera (Gerbera jamesonii Hook.) to physical and chemical mutagenesis. J. Hort. Sci. Biotechnol. 89:301–306, doi: 10.1080/14620316.2014.1151 3083.
39. Gong, S., H. Fu, and J. Wang. 2010. ISSR Analysis of M1 generation of Gladiolus hybridus Hort treated by EMS. J. Northeast Agr. Univ. 17:22–26.
40. Greer, S.P. and T.A. Rinehart. 2009. In vitro germination and dormancy responses of Hydrangea macrophylla and Hydrangea paniculata seeds to ethyl methane sulfonate and cold treatment. HortScience 44:764–769, doi: 10.21273/ HORTSCI.44.3.764.
41. Weigle, J.L. and J.K. Butler. 1983. Induced dwarf mutant in Impatiens platypetala. J. Hered. 74:200, doi: 10.1093/oxfordjournals.jhered.a109764.
42. Ghosh, S., M. Ganga, and K. Soorianathasundaram. 2019. Studies of mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in jasmine. Curr. J. Appl. Sci. Technol. 38:1–8, doi: 10.9734/cjast/2019/ v38i630414.
43. Bennani, F. and B.D. Rossi-Hassani. 2001. Seed mutagenesis in Portulaca grandiflora (Hook), INIS-XA–427. International Atomic Energy Agency, Vienna.
44. Senapati, S.K. and G.R. Rout. 2008. In vitro mutagenesis of rose with ethyl methane sulphonate (EMS) and early selection using RAPD Markers. Adv. Hort. Sci. 22:218–222.
45. Muller, H.J. 1927. Artificial transmutation of the gene. Science 66:84–87, doi: 10.1126/science. 66.1699.84.
46. Stadler, L.J. 1928a. Mutations in barley induced by X-rays and radium. Science 68:186–187, doi: 10.1126/science.68.1756.186.

47. Stadler, L.J. 1928b. Genetic effects of X-rays in maize. Proc. Natl. Acad. Sci. USA 14:69–75, doi: 10.1073/pnas.14.1.69.
48. Kodym, A. and R. Afza. 2003. Physical and chemical mutagenesis, p. 189–203. In: E. Grotewold (ed.). Plant functional genomics: Methods and protocols. Humana Press, Totowa, NJ, doi: 10.1385/1-59259-413-1:189.
49. Maple, J. and S.G. Møller. 2007. Mutagenesis in Arabidopsis, p. 197–206. In: E. Rosato (ed.). Circadian rhythms: Methods in molecular biology. Volume 362. Humana Press, Totowa, NJ, doi: 10.1007/978-1-59745-257-1_14.
50. Oladosu, Y., M.Y. Rarfii, N. Abdullah, G. Hussin, A. Ramli, H.A. Rahim, G. Miah, and M. Usman. 2016. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnol. Biotechnol. Equip. 30:1–16, doi: 10.1080/13102818.2015.1087333.
51. Bala, M. and K.P. Singh. 2013. In vitro mutagenesis of rose (Rosa hybrida L.) explants using gamma-radiation to induce novel flower colour mutations. J. Hort. Sci. Biotechnol. 88:462–468, doi: 10.1080/14620316.2013.11512992.
52. Datta, S.K. 2014. Induced mutagenesis: Basic knowledge for technological success, p. 97–140. In: N.B. Tomlekova, M.I. Kozgar, and M.R. Wani (eds.). Mutagenesis: Exploring genetic diversity of crops. Wageningen Academic Publishers, Wageningen, doi: 10.3920/ 978-90-8686-796-7_5.
53. Puchooa, D. 2005. In vitro mutation breeding of Anthurium by gamma radiation. Intl. J. Agr. Biol. 7:11–20.
54. Bhat, R.S., N.M. Upadhyaya, A. Chaudhury, C. Raghavan, F. Qiu, H. Wang, J. Wu, K. McNally, H. Leung, B. Till, S. Henikoff, and L. Comai. 2007. Chemical- and irradiation-induced mutants and TILLING, p. 148–180. In: N.M. Upadhyaya (ed.). Rice functional genomics. Springer, New York, NY, doi: 10.1007/0-387-48914-2_8.
55. Gladstones, J.S. and C.M. Francis. 1965. Studies on the use of mutagenic agents in plant breeding. ii. The effects of dose and seed moisture content on mutation production in Lupinus angustifolius by X-rays. Austral. J. Agr. Res. 16:301–310, doi: 10.1071/AR9650301.
56. Walther, F. and A. Sauer. 1986a. Analysis of radiosensitivity: A basic requirement for in vitro somatic mutagenesis II. Gerbera jamesonii, p. 155–159. In: International Atomic Energy Agency (ed.). Nuclear techniques and in vitro culture for plant improvement. International Atomic Energy Agency, Vienna.
57. Webb, K.J., M. Robbins, T.L. Wang, M. Parniske, and A. Marquez. 2005. Mutagenesis, p. 177–186. In: J. Marquez (ed.). Lotus japonicus handbook. Springer, Dordrecht, doi: 10.1007/ 1-4020-3735-X_18.
58. Berenschot, A.S., M.I. Zucchi, A. Tulmann-Neto, and V. Quecini. 2008. Mutagenesis in Petunia hybrida Vilm. and isolation of a novel morphological mutant. Brazilian J. Plant Physiol. 20:95–103, doi: 10.1590/S1677-04202008000200002.
59. Venkatachalam, P. and N. Jayabalan. 1997. Effect of gamma rays on some qualitative and quantitative characters in Zinnia elegans Jacq. Indian J. Genet. Plant Breed. 57:255–261.
60. Doorenbos, J. and J.J. Karper. 1975. X-ray induced mutations in Begonia hiemalis. Euphytica 24:13–19, doi: 10.1007/BF00147163

61. Preil, W., M. Engelhardt, and F. Walther. 1983. Breeding of low temperature tolerant poinsettia (Euphorbia pulcherrima) and chrysanthemum by means of mutation induction in in vitro culture. Acta Hort. 131:345–355, doi: 10.17660/ ActaHortic.1983.131.41.
62. Lee, J., Y. Chung, Y. Joung, T. Han, S. Kang, Y. Yoo, and G. Lee. 2010. Induction of mutations for stem quality in chrysanthemum (Dendranthema grandiflora) by using gamma-ray irradiation. Acta Hort. 855:177–182, doi: 10.17660/ ActaHortic.2010.855.25.
63. Ghani, M. and S.K. Sharma. 2019. Induction of powdery mildew resistance in gerbera (Gerbera jamesonii) through gamma irradiation. Physiol. Mol. Biol. Plants 25:159–166, doi: 10.1007/ s12298-018-0613-5.
64. Sagawa, Y. and G.A.L. Mehlquist. 1959. Some X-ray induced mutants in the carnation: Dianthus caryophyllus. J. Hered. 50:78–80, doi: 10.1093/ oxford journals. jhered.a106881.
65. Biswas, S.C. and A.K. Biswas. 2006. Cytogenetic characterization of induced sterility in Ornithogalum virens L. Cytologia (Tokyo) 71:119–123, doi: 10.1508/cytologia.71.119.
66. Oates, K.M., D.H. Touchell, and T.G. Ranney. 2013. Induced variation in tetraploid Rudbeckia subtomentosa 'Henry Eilers' regenerated from gamma-irradiated callus. HortScience 48:831– 834, doi: 10.21273/HORTSCI.48.7.831.
67. Shukla, A., S. Kashyap, V. Ramteke, L. Sinha, and M. Netam. 2018. Effect of gamma rays on flowering and vase life of gladiolus (Gladiolus grandiflorus L.). J. Pharmacognosy Phytochem. 7:558–561.
68. Sparrow, A.H., A.F. Rogers, and S.S. Schwemmer. 1968. Radiosensitivity studies with woody plants —I acute gamma irradiation survival data for 28 species and predictions for 190 species. Radiat. Bot. 8:149–174, doi: 10.1016/ S0033-7560(68)80071-7.
69. Ari, E., H. Djapo, N. Mutlu, E. Gurbuz, and O. Karaguzel. 2015. Creation of variation through gamma irradiation and polyploidization in Vitex agnus-castus L. Scientia Hort. 195:74–81, doi: 10.1016/j.scienta.2015.08.039.
70. Kukimura, H., F. Ikeda, H. Fujita, T. Maeta, K. Nakajima, K. Katagiri, K. Nakahira, and M. Somegou. 1976. Genetical, cytological and physiological studies on the induced mutants with special regard to effective methods for obtaining useful mutants in perennial woody plant, p. 93–137. In: International Atomic Energy Agency (ed.). Improvement of vegetatively propagated plants and tree crops through induced mutations. International Atomic Energy Agency, Vienna.
71. Ghosh, S., M. Ganga, and K. Soorianathasundaram. 2019. Studies of mutagenic effectiveness and efficiency of gamma rays and ethyl methane sulphonate in jasmine. Curr. J. Appl. Sci. Technol. 38:1–8, doi: 10.9734/cjast/2019/ v38i630414
72. Baig, M.M.Q., I.A. Hafiz, N.A. Abbasi, M. Yaseen, Z. Akram, and D.J. Donnely. 2012. Reduced-stature Rosa species through in vitro mutagenesis. Can. J. Plant. Sci. 92:1049–1055, doi: 10.4141/cjps2011-199.
73. Smilansky, Z., N. Umiel, and N. Zieslin. 1986. Mutagenesis in roses (cv. Mercedes). Environ. Exp. Bot. 26:279–283, doi: 10.1016/0098-8472 (86)90040-7.

74. Smith, A.G. and A.K. Noyszewski. 2018. Mutagenesis breeding for seedless varieties of popular landscape plants. Acta Hort. 1191:43–52, doi: 10.17660/ActaHortic.2018.1191.7.
75. Navabi, Y., M. Norouzi, M. Arab, and S.D. Daylami. 2016. Mutagenesis via exposure to gamma-rays in tuberose (Polianthes tuberosa). Electronic J. Biol. 12:168–172.
76. Swaroop, K., R. Jain, and T. Janakiram. 2015. Effect of different doses of gamma rays for induction of mutation in Bougainvillea cv Mahatma Gandhi. Indian J. Agr. Sci. 85:1245–1247.
77. Dowrick, G.J. and A. El Bayoumi. 1966. The induction of mutations in chrysanthemum using X and gamma radiation. Euphytica 15:204–210, doi: 10.1007/BF00022325.
78. Sundar, P., K. Ajit, C. Preeti, S. Ranjan, and T. Shailesh. 2017. Determination of lethal dose for gamma rays induced mutagenesis in different cultivars of dahlia. J. Hill Agr. 8:279–282, doi: 10.5958/2230-7338.2017.00055.6.
79. Buiatti, M. and R. Ragazzini. 1965. Gamma-ray induced changes in the carnation Dianthus caryophyllus L. Radiat. Bot. 5:99–105, doi: 10.1016/S0033-7560(65)80029-1.
80. Cassells, A.C., C. Walsh, and C. Periappuram. 1993. Diplontic selection as a positive factor in determining the fitness of mutants of Dianthus 'Mystere' derived from X-irradiation of nodes in in vitro culture. Euphytica 70:167–174, doi: 10.1007/BF00023756.
81. Okamura, M., N. Yasuno, M. Ohtsuka, A. Tanaka, N. Shikazono, and Y. Hase. 2003. Wide variety of flower-color and -shape mutants regenerated from leaf cultures irradiated with ion beams. Nucl. Instrum. Methods Phys. Res. B 206:574–578, doi: 10.1016/s0168-583(03)00835-8.
82. Sagawa, Y. and G.A.L. Mehlquist. 1959. Some X-ray induced mutants in the carnation: Dianthus caryophyllus. J. Hered. 50:78–80, doi: 10.1093/ oxford journals. jhered.a106881.
83. Sathyanarayana, E., G. Sharma, T. Tirkey, B.K. Das, K. Divya, and J. Kumar. 2019. Studies of gamma irradiation on vegetative and floral characters of gladiolus (Gladiolus grandiflorus L.). J. Pharmacognosy Phytochem. 8:227–230.
84. Tirkey, P. and D. Singh. 2019. Effect of induced mutagenesis on different characters of gladiolus (Gladiolus grandifloras L.). J. Pharmacognosy Phytochem. 8:650–654.
85. Hekstra, G. and C. Broertjes. 1968. Mutation breeding in bulbous Iris. Euphytica 17:345–351, doi: 10.1007/BF00056234.
86. Cambecedes, J., M. Duron, L. Decourtye, and R. Jalouzot. 1992. Methodology of in vitro gamma rays irradiations from Lonicera species; mutant description and biochemical characterization. Acta Hort. 320:119–126, doi: 10.17660/ ActaHortic.1992.320.16.
87. Grunewalt, J. 1983. In vitro mutagenesis of Saintpaulia and Pelargonium cultiva131:339–344, doi: 10.17660/ActaHortic.1983. 131.40.
88. Aisyah, S.I., Y. Marthin, and M.R.M. Damanik. 2015. Improvement of coleus performance through mutation induction using gamma ray irradiation. J. Trop. Crop Sci. 2:26–32, doi: 10.29244/jtcs.2.1.26-32.

89. Nishiguchi, M., T. Nanjo, and K. Yoshida. 2012. The effects of gamma irradiation on growth and expression of genes encoding DNA repair related proteins in lombardy poplar (Populus nigra var. italica). J. Environ. Radioact. 109:19–28, doi: 10.1016/j.jenvrad.2011.12.024.
90. Aamir, S.S., M.M.Q. Baig, T. Ahmad, A. Ghafoor, I.A. Hafiz, N.A. Abbasi, I. Ali, and M. Yaseen. 2016. Molecular and morphological characterization of rose mutants produced via in vitro mutagenesis. Philipp. Agr. Sci. 99:25–33.
91. Wongpiyasatid, A., T. Thinnok, T. Taychasinpitak, P. Jompuk, K. Chusreeaeom, and S. Lamseejan. 2007. Effects of acute gamma irradiation on adventitious plantlet regeneration and mutation from leaf cuttings of african violet (Saintpaulia ionantha). Agr. Nat. Resour. 41:633–640.
92. Zhou, L.B., W.J. Li, S. Ma, X.C. Dong, L.X. Yu, Q. Li, G.M. Zhou, and Q.X. Gao. 2006. Effects of ion beam irradiation on adventitious shoot regeneration from in vitro leaf explants of Saintpaulia ionahta. Nucl. Instrum. Methods Phys. Res. B 244:349–353, doi: 10.1016/j. nimb.2005.10.034.
93. Oladosu, Y., M.Y. Rarfii, N. Abdullah, G. Hussin, A. Ramli, H.A. Rahim, G. Miah, and M. Usman. 2016. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnol. Biotechnol. Equip. 30:1–16, doi: 10.1080/13102818.2015.1087333.
94. Predieri, S. and N. Di Virgilio. 2007. In vitro mutagenesis and mutant multiplication, p. 323–333. In: S.M. Jain and H. H€aggman (eds.). Protocols for micropropagation of woody trees and fruits. Springer, Dordrecht, doi: 10.1007/ 978-1-4020-6352-7_30
95. Gautam, A.S., K.C. Sood, and A.K. Richarria. 1992. Mutagenic effectiveness and efficiency of gamma-rays, ethyl methane sulphonate and their synergistic effects in black gram (Vigna mungo L.). Cytologia (Tokyo) 57:85–89, doi: 10.1508/cytologia.57.85.
96. Wani, A.A. 2009. Mutagenic effectiveness and efficiency of gamma rays, ethyl methane sulphonate and their combination treatments in chickpea (Cicer arietinum L.). Asian J. Plant Sci. 8:318–321, doi: 10.3923/ajps.2009.318.321.
97. Arase, S., Y. Hase, J. Abe, M. Kasai, T. Yamada, K. Kitamura, I. Narumi, A. Tanaka, and A. Kanazawa. 2011. Optimization of ion-beam irradiation for mutagenesis in soybean: Effects on plant growth and production of visibly altered mutants. Plant Biotechnol. 28:323–329, doi: 10.5511/plantbiotechnology.11.0111.
98. Kondo, E., M. Nakayama, N. Kameari, N. Tanikawa, Y. Morita, Y. Akita, Y. Hase, A. Tanaka, and H. Ishizaka. 2009. Red-purple flower due to delphinidin 3,5-diglucoside, a novel pigment for Cyclamen spp., generated by ion-beam irradiation. Plant Biotechnol. 26:565–569, doi: 10.5511/plantbiotechnology.26.565.
99. Matsumura, A., T. Nomizu, N. Furutani, K. Hayashi, Y. Minamiyama, and Y. Hase. 2010. Ray florets color and shape mutants induced by 12C51 ion beam irradiation in chrysanthemum. Scientia Hort. 123:558–561, doi: 10.1016/j. scienta.2009.11.004.
100. Bolon, Y., W.J. Haun, W.W. Xu, D. Grant, M.G. Stacey, R.T. Nelson, D.J. Gerhardt, J.A. Jeddeloh, G. Stacey, G.J. Muehlbauer, J.H. Orf, S.L. Naeve, R.M. Stupar, and C.P. Vance. 2011. Phenotypic and genomic analyses of a fast neutron

mutant population resource in soybean. Plant Physiol. 156:240–253, doi: 10.1104/pp.110.170811.
101. Broertjes, C. 1976. Mutation breeding of autotetraploid Achimenes cultivars. Euphytica 25:297–304, doi: 10.1007/BF00041560.
102. Datta, S.K. 2012. Success story of induced mutagenesis for development of new ornamental varieties. Bioremediation Biodivers. Bioavailab. 6:15–26.
103. Love, J.E. and M.J. Constantin. 1966. The induction of bud sports in Coleus blumei by fast neutrons. Proc. Amer. Soc. Hort. Sci. 88:627–630.
104. Shikazono, N., C. Suzuki, S. Kitamura, H. Watanabe, S. Tano, and A. Tanaka. 2005. Analysis of mutations induced by carbon ions in Arabidopsis thaliana. J. Expt. Bot. 56:587–596, doi: 10.1093/jxb/eri047.
105. Magori, S., A. Tanaka, and M. Kawaguchi. 2010. Physically induced mutation: Ion beam mutagenesis, p. 3–16. In: K. Meksen and G. Kahl (eds.). The handbook of plant mutation screening: Mining of natural and induced alleles. Wiley-Blackwell, Chichester, doi: 10.1002/ 9783527629398.ch1.
106. Ryuto, H., N. Fukunishi, Y. Hayashi, H. Ichida, T. Abe, M. Kase, and Y. Yano. 2008. Heavy-ion beam irradiation facility for biological samples in RIKEN. Plant Biotechnol. 25:119–122, doi: 10.5511/plantbiotechnology.25.119.
107. Ogawa, D., T. Fujioka, T. Hirano, and T. Abe. 2014. Effect of C-ion beam irradiation on survival rates and flower color mutations in statice (Limonium sinuatum Mill.). RIKEN Accelerator Prog. Rpt. 47:295.
108. Miyazaki, K., K. Suzuki, K. Iwaki, T. Kusumi, T. Abe, S. Yoshida, and H. Fukui. 2006. Flower pigment mutations induced by heavy ion beam irradiation in an interspecific hybrid of Torenia. Plant Biotechnol. 23:163–167, doi: 10.5511/plantbiotechnology.23.163.
109. Miyazaki, K., K. Suzuki, T. Abe, Y. Katsumoto, S. Yoshida, and T. Kusumi. 2002. Isolation of variegated mutants of Petunia hybrida using heavy-ion beam irradiation. RIKEN Accelerator Prog. Rpt. 35:130.
110. Kanaya, T., H. Saito, Y. Hayashi, N. Fukunishi, H. Ryuto, K. Miyazaki, T. Kusumi, T. Abe, and K. Suzuki. 2008. Heavy-ion beam-induced sterile mutants of verbena (Verbena hybrida) with an improved flowering habit. Plant Biotechnol. 25:91–96, doi: 10.5511/plantbiotechnology.25.91.
111. Sugiyama, M., H. Saito, H. Ichida, Y. Hayashi, H. Ryuto, N. Fukunishi, T. Terakawa, and T. Abe. 2008a. Biological effects of heavy-ion beam irradiation on cyclamen. Plant Biotechnol. 25:101– 104, doi: 10.5511/plantbiotechnology.25.101.
112. Sakamoto, K., Y. Takatori, R. Chiwata, T. Matsumura, K. Tsukiashi, Y. Hayashi, and T. Abe. 2016. Production of mutant line with early flowering at a low temperature in spray-type chrysanthemum cultivar induced by C-ion Beam irradiation. RIKEN Accelerator Prog. Rpt. 49:262.
113. Hayashi, Y., S. Ishii, T. Hirano, K. Ichinose, Y. Kazama, and T. Abe. 2019. New ornamental cherry cultivars induced by heavy-ion beam irradiation. Acta Hort. 1235:99–104, doi: 10.17660/ActaHortic.2019.1235.13.
114. Marcotrigiano, M. 1997. Chimeras and variegation: Patterns of deceit. Hort. Science 32:773–784, doi: 10.21273/HORTSCI.32.5.773.

115. Hoskins, T. and R.N. Contreras. 2019. Exposing seeds of Sarcococca confusa to increased concentrations and durations of ethyl methane sulphonate reduced seed germination, twinning, and plant size. HortScience 54:1902–1906, doi: 10.1111/j.1439-0523.2005.01126.
116. Hisamura, A., D. Mine, T. Takeda, T. Abe, Y. Hayashi, and T. Hirano. 2016. Breeding of summer-autumn flowering Chrysanthemum cv. Hakuryo with a little generation of malformed flower. RIKEN Accelerator Prog. Rpt. 49:24.
117. Suzuki, K., Y. Takatsu, T. Gonai, M. Nogi, K. Sakamoto, N. Fukunishi, H. Ryuto, H. Saito, T. Abe, S. Yoshida, and M. Kasumi. 2005. Flower color mutation in spray-type chrysanthemum (Dendranthema grandiflorum (Ramat.) Kitamura) induced by heavy-ion beam irradiation. RIKEN Accel. Prog. Rpt. 38:
118. Tanokashira, Y., M. Tamari, S. Nagayoshi, Y. Hayashi, T. Hirano, and T. Abe. 2016. Induction of flower color mutants by heavy-ion irradiation to leaf blades of spray-mum 'Southern Chelsea'. RIKEN Accelerator Prog. Rpt. 49:261.
119. Tanokashira, Y., S. Nagayoshi, T. Hirano, and T. Abe. 2014. Effects of heavy-ion-beam irradiation on flower-color mutation in chrysanthemum. RIKEN Accelerator Prog. Rpt. 47:29
120. Wakita, N., Y. Kazama, Y. Hayashi, H. Ryuto, N. Fukunishi, K. Yamamoto, S. Ijichi, and T. Abe. 2008. Induction of flower-color mutation by C-ion irradiation in spray-type chrysanthemum. RIKEN Accelerator Prog. Rpt. 41:230
121. Ishizaka, H., E. Kondo, and N. Kameari. 2012. Production of novel flower color mutants from the fragrant cyclamen (Cyclamen persicum x C. purpurascens) by ion-beam irradiation. Plant Biotechnol. 29:201–208, doi: 10.5511/plantbio technology.12.0116.
122. Zhou, L.B., W.J. Li, S. Ma, X.C. Dong, L.X. Yu, Q. Li, G.M. Zhou, and Q.X. Gao. 2006. Effects of ion beam irradiation on adventitious shoot regeneration from in vitro leaf explants of Saintpaulia ionahta. Nucl. Instrum. Methods Phys. Res. B 244:349–353, doi: 10.1016/j. nimb.2005.10.034.
123. Van Harten, A.M. 1998. Mutation breeding: Theory and practical applications. Cambridge University Press, Cambridge.
124. Oladosu, Y., M.Y. Rarfii, N. Abdullah, G. Hussin, A. Ramli, H.A. Rahim, G. Miah, and M. Usman. 2016. Principle and application of plant mutagenesis in crop improvement: A review. Biotechnol. Biotechnol. Equip. 30:1–16, doi: 10.1080/13102818.2015.1087333.
125. Predieri, S. and N. Di Virgilio. 2007. In vitro mutagenesis and mutant multiplication, p. 323–333. In: S.M. Jain and H. H€aggman (eds.). Protocols for micropropagation of woody trees and fruits. Springer, Dordrecht, doi: 10.1007/ 978-1-4020-6352-7_30.
126. Shikazono, N., C. Suzuki, S. Kitamura, H. Watanabe, S. Tano, and A. Tanaka. 2005. Analysis of mutations induced by carbon ions in Arabidopsis thaliana. J. Expt. Bot. 56:587–596, doi: 10.1093/jxb/eri047.
127. Greene, E.A., C.A. Codomo, N.E. Taylor, J.G. Henikoff, B.J. Till, S.H. Reynolds, L.C. Enns, C. Burtner, J.E. Johnson, A.R. Odden, L. Comai, and S. Henikoff. 2003. Spectrum of chemically induced mutations from a largescale reverse-genetic screen in Arabidopsis. Genetics 164:731–740, doi: 10.1093/genetics/ 164.2.731.