

## 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.

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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.

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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**

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Since its discovery in 1946, alkylating compounds like EMS have been widely used in the breeding of ornamental plants. N-methyl-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

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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<sub>50</sub>)] [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<sub>1</sub> and M<sub>2</sub> generations. The M<sub>1</sub> generation included a variety of violet hues, whereas the M<sub>2</sub> generation included hues ranging from pink to a light blueish magenta. *Antirrhinum majus* M<sub>1</sub> 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

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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 falls and varies with dose. This highlights even more how crucial it is to ascertain the correct dosage prior to applying a large-scale plant treatment.

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**Table 1. Ethyl methane sulfonate (EMS) treatment conditions. The genus, mutagen, EMS concentration, treatment duration, median lethal dose (LD<sub>50</sub>) when provided, treated material, and reference are shown for each study.**

Genus	Mutagen	Treatment concn	Treatment duration	LD <sub>50</sub>	Material	Reference
<i>Agave</i>	EMS	0.25% to 0.50%	4 h	—	Bulbs	[26]
<i>Antirrhinum</i>	EMS	0.10% to 1.00%	8–12 h	—	Seeds	[22]
<i>Begonia</i>	EMS	Unknown	Unknown	—	Leaves	[37]
<i>Bougainvillea</i>	EMS	0.80% to 1.00%	6 h	—	Cuttings	[33]
<i>Buddleja</i>	EMS	1.40%	4 h	—	Seeds	[29]
<i>Chrysanthemum</i>	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]
<i>Dianthus</i>	EMS	0.10% to 0.70%	6 h	—	Seeds	[17]
<i>Gerbera</i>	EMS	0.10% to 1.00%	10 min	0.65%	Shoots	[38]
<i>Gladiolus</i>	EMS	0.20% to 1.20%	Unknown	—	Corn buds	[39]
<i>Hydrangea</i>	EMS	0.50% to 5.00%	3 h	—	Seeds	[40]
<i>Impatiens</i>	EMS	0.32% to 1.08%	24 h	—	Seeds	[41]
<i>Jasminum</i>	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]
<i>Ornithogalum</i>	EMS	0.20% to 1.00%	24 h	0.15%, 0.52%	Seeds	[30]
<i>Petunia</i>	EMS	0.10% to 0.30%	18 h	—	Seeds	[21]
<i>Portulaca</i>	EMS	1.20% to 40.00%	4 h	—	Seeds	[43]
<i>Ribes</i>	EMS	0.20% to 1.20%	24–48 h	—	Seeds	[30]
<i>Rosa</i>	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]
<i>Weigela</i>	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<sub>50</sub>, 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].

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### 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

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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  $LD_{50}$  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].

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**Table 2. Gamma and X-ray treatment conditions, genus, mutagen, treatment dose, median lethal dose (LD<sub>50</sub>), treated material, and reference are shown for each study.**

Genus	Mutagen	Dose (krad) <sup>a</sup>	LD <sub>50</sub> (krad)	Material	Reference
<i>Acer</i>	Gamma rays	0.1–5	—	Cuttings	[74]
	Gamma rays	50–225	—	Seeds	[74]
<i>Agave</i>	Gamma rays	1–40	—	Bulbs	[75]
<i>Begonia</i>	X-rays	1.5–2.5	—	Leaves	[60]
<i>Berberis</i>	Gamma rays	0.1–5	—	Cuttings	[74]
	Gamma rays	50–225	—	Seeds	[74]
<i>Bougainvillea</i>	Gamma rays	0.5–1	—	Cuttings	[33]
	Gamma rays	0.5–2	—	Cuttings	[76]
<i>Chrysanthemum</i>	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]
<i>Cryptomeria</i>	Gamma rays	1–5	—	Cuttings	[62]
	X-rays	2.5	—	Callus	[61]
<i>Dahlia</i>	Gamma rays	1–3	—	Cuttings	[78]
<i>Dianthus</i>	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]
<i>Gerbera</i>	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]
<i>Gladiolus</i>	Gamma rays	1.5–5.5	—	Corms	[83]
	Gamma rays	1.5–6	—	Corms	[67]
	Gamma rays	1.75–10.5	—	Corms	[84]
<i>Iris</i>	X-rays	0.5–1.1	—	Bulbs	[85]
<i>Jasminum</i>	Gamma rays	1–2.5	—	Cuttings	[71]
<i>Lonicera</i>	Gamma rays	1–6	2.1, 3.5	Microcuttings	[86]
<i>Ornithogalum</i>	Gamma rays	20–40	—	Seeds	[65]
<i>Pelargonium</i>	Gamma rays	1.5	—	Leaves	[87]
<i>Petunia</i>	Gamma rays	2–10	10	Seeds	[58]
<i>Plectranthus</i>	Gamma rays	1.5–6	3.762–6.52	Cuttings	[88]
<i>Populus</i>	Gamma rays	1–30	—	Plantlets	[89]
<i>Rosa</i>	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]
<i>Rudbeckia</i>	X-rays	2.5–6	—	Microshoots	[56]
<i>Saintpaulia</i>	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 <sub>50</sub>	Material	Reference
<i>Acer</i>	Neutrons	2–14 krad	—	Seeds	[85]
<i>Achimenes</i>	Neutrons	0.75–2 krad	—	Leaves	[101]
<i>Berberis</i>	Neutrons	2–14 krad	—	Seeds	[85]
<i>Chrysanthemum</i>	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]
<i>Cyclamen</i>	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	—	Stemsegment	[120]
	Ion beam	MeV/u, 22.5 keV/mm	—	Petioles	[121]
	Ion beam	0–5 krad, 220/320 MeV, unknown	—	Petioles	[98]
<i>Dianthus</i>	Ion beam	0.5–3 krad, 220 MeV, unknown	—	Leafsents	98
	Ion beam	0.7–2 krad, 320 MeV, 76 keV/mm	—	Petals	98
<i>Saintpaulia</i>	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.

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