



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

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This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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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

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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.

Keywords: *Ornamental crops; Genetic improvement; chemical mutagenesis; heavy ion beam irradiation; alkylating agents.*

1. 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 (Rout and Jain 2005). 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, graymold illness, or yellow or true red flower colors (Kushikawa et al. 2001). 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 (Datta 1997). 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 (Jain and Maluszynski 2004). 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 (Jain 1997 & Jain et al 1998). 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* (Kushikawa et al, 2001), *Gerbera jamesonii* (Elomaa et al, 1993),

Eustoma grandiflorum and *Osteospermum mumecklonis* (Mercuri et. 2001). 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.

2. 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-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 (Gautam et al 1992 Girija and Dhanavel 2009), relatively low cost, and high availability (Lu et al 2002, Montesano et al 1979 & Talebi et al 2012). 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 (Greene et al. 2003), which give rise to single nucleotide polymorphisms (SNPs). Furthermore, compared to physical mutagens, EMS results in fewer deletions (Koornneef et al. 1982). 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 vary 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 (Roychowdhury and Tah. 2011). 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 (LD₅₀)] (Berenschot 2008 and Hohmann 2005). Apart from seeds, nodal segments and ray florets of *Chrysanthemum* have also been subjected to in vitro treatments (Padmadevi & Jawaharlal 2011).

3. 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. (Kashikar and Khalatkar 1981) noted variations in bloom color between the white-flowering *Petunia x hybrida* M₁ and M₂ generations. The M₁ generation included a variety of violet hues, whereas the M₂ generation included hues ranging from pink to a light blueish magenta. *Antirrhinum majus* M₁ mutants with aberrant leaf shape and dwarfism were discovered. (Heffron et al. 2006) 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*.

4. 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) (Datta and Chakrabarty. 2005). 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. (Hossain 2006) 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 colour changed as a result of EMS therapy. For instance, when (Latado et al. 2004), 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 (Singh et al. 2013). There have also been reports of decreased pollen productivity and plant height (Contreras and Shearer 2020).

Table 1. Ethyl methane sulfonate (EMS) treatment conditions. The genus, mutagen, EMS concentration, treatment duration, median lethal dose (LD₅₀) when provided, treated material, and reference are shown for each study (Melsen et al,2021)

Genus	Mutagen	Treatment concn	Treatment duration	LD ₅₀	Material	Reference
<i>Agave</i>	EMS	0.25%to0.50%	4h	—	Bulbs	(Singh et al. 2013)
<i>Antirrhinum</i>	EMS	0.10%to1.00%	8–12h	—	Seeds	(Heffron et al. 2006)
<i>Begonia</i>	EMS	Unknown	Unknown	—	Leaves	(Chen et al. 2014)
<i>Bougainvillea</i>	EMS	0.80%to1.00%	6h	—	Cuttings	(Anitha et al. 2017)
<i>Buddleja</i>	EMS	1.40%	4h	—	Seeds	(Ghosh and Ganga 2019)
<i>Chrysanthemum</i>	EMS	0.025%to0.050%	5h	—	Leaf sections	(Hossain et al. 2006)
	EMS	0.02%to0.04%	Unknown	—	Cuttings	(Kapadiya et al. 2016)
	EMS	0.51%to1.03%	1h45min	0.82%	Floral pedicels	(Latado et al. 2004)
	EMS	0.10%to0.30%	1h	—	Ray florets	(Padmadev and Jawaharlal 2011)
<i>Dianthus</i>	EMS	0.10%to0.70%	6h	—	Seeds	(Roychowdhury, and Tah 2011)
<i>Gerbera</i>	EMS	0.10%to1.00%	10min	0.65%	Shoots	(Ghani 2014)
<i>Gladiolus</i>	EMS	0.20%to1.20%	Unknown	—	Cormbuds	(Gong et al 20010)
<i>Hydrangea</i>	EMS	0.50%to5.00%	3h	—	Seeds	(Greer and Rinehart. 2009)
<i>Impatiens</i>	EMS	0.32%to1.08%	24h	—	Seeds	(Weigle and Butler 1983)
<i>Jasminum</i>	EMS	0.06%to0.62%	1–6h	0.53%,0.55%	Cuttings	(Ghosh and Ganga 2019)
	EMS	0.25%to0.4%	1h	—	Cuttings	(Ghosh 2019)
<i>Ornithogalum</i>	EMS	0.20%to1.00%	24h	0.15%,0.52%	Seeds	(Contreras and Friddle 2015)
<i>Petunia</i>	EMS	0.10%to0.30%	18h	—	Seeds	(Kashikar and Khalatkar 1981)
<i>Portulaca</i>	EMS	1.20%to40.00%	4h	—	Seeds	(Bennani et al. 2021)
<i>Ribes</i>	EMS	0.20%to1.20%	24–48h	—	Seeds	(Contreras and Friddle 2015)
<i>Rosa</i>	EMS	0.50%to3.00%	2–12h	—	Apical and axillary meristems	(Senapati and Rout 2008)
	EMS	0.08%to5.00%	1–24h	—	Stem cuttings with buds	(Smilansky et al. 1986)
<i>Weigela</i>	EMS	0.50%	1h30min	—	Shoot internodes	(Duron 1992)

5. 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 *Buddle jadvavidii* seeds (Kapadiya et al. 2016). *Weigela* and *Ribes sanguineum* both had altered leaf morphology, which in *Ribes*' instance produced the cultivar Oregon Snowflake (Contreras and Friddle. 2015 & Duron 1992). After EMS treatments of cuttings, (Smilansky et al. 1986) 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 thorn lessness (Anitha et al. 2017). (Ghosh and Ganga 2019) 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 (Harten 1998). EMS has the benefit of having a relatively high mutagenesis efficiency, which reduces the proportion of undesired mutations to all mutations (Kaul and Bhan 1977). 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.

6. X-RAYS AND GAMMA RAYS

Following the discovery of the mutagenic effects of ionizing radiation by (Muller 1927) and (Stadler 1928), 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 (Kodym and Afza. 2003), (Maple and Møller 2007) & (Oladosu et al. 2016).

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 (Bala and Singh. 2013) & (Datta et al. 2014). 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 (Puchooa et al. 2005). 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 LD₅₀, should be determined for a specific subject before starting with irradiation on a large scale (Gladstones and Francis. 1965) & (Webb et al. 2005).

7. 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. (Venkatachalam and Jayabalan 1997) 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 (Doorenbos and Karper

1975) 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* (Berenschot et al. 2008).

8. 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. (Preil et al. 1983) selected two Chrysanthemum mutants that were tolerant to low temperatures. (Lee et al. 2010) 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 (Ghani and Sharma 2019). In *Dianthus caryophyllus*, X-ray irradiation restored male fertility (Sagawa and Mehliquist 1959); 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 (Shukla et al. 2018), characteristics like vase life can also be enhanced.

9. WOODY TREES AND SHRUBS

Gamma or X-rays have been used to treat a wide variety of woody species (Table 2). (Sparrow et al. 19968) calculated the gamma radiation lethal doses (LD₅₀) for 28 species of woody plants and estimated the LD₅₀ for an additional 190 species

by using the inter phase 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 (Ari et al. 2015), who showed that complicated traits like fragrance can also be improved by inducing mutations. Dwarfism was found by (Kukimura et al. 1967) in *Cryptomeria* and in *Jasminum* (Ghosh et al. 2019). Shorter internodes resulting in lower plant height were also observed in *Populus* and *Rosa* (Baig et al. 2012). 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 (Smilansky et al. 1968).

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 (Oladosu et al. 2016) & (Predieri and Virgilio 2007). However, it appears that their mutagenesis efficiency is not very high (Gautam et al. 1992) & (Wani 2009).

10. 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. (Arase et al. 2011, Kondo et al. 2009 & Matsumura et al. 2010). Neutron irradiation has also been used as a mutagen, but it has had very limited use in ornamentals (Bolon et al. 201, Broertjes 1976, Datta 2012). The mechanism of gamma and X-ray irradiation-induced mutations is somewhat

Table 2. Gamma and X-ray treatment conditions, genus, mutagen, treatment dose, median lethal dose (LD₅₀), treated material, and reference are shown for each study

Genus	Mutagen	Dose(krad) ^z	LD ₅₀ (krad)	Material	Reference
<i>Acer</i>	Gammarays	0.1–5	—	Cuttings	(Smithand Noyszewski 2018)
	Gammarays	50–225	—	Seeds	
<i>Agave</i>	Gammarays	1–40	—	Bulbs	(Navabi 2016)
<i>Begonia Berberis</i>	X-rays	1.5–2.5	—	Leaves	(Doorenbos and Karper 1975)
<i>Bougainvillea</i>	Gammarays Gammarays	0.1–5	—	Cuttings	(Smith and Noyszewski2018)
	Gammarays Gammarays	50–225	—	Seeds	(Smithand Noyszewski 2018)
<i>Chrysanthemum</i>		0.5–1	—	Cuttings	(Anitha et al. 2017)
		0.5–2	—	Cuttings	(Swaroop et al. 2015)
	Gammarays X-rays	1–4	—	Cuttings	(Dowrick and Bayoumi 1966)
	Gammarays Gammarays	0.44–1.75	—	Cuttings	(Dowrick and Bayoumi 1966)
		0.5–1	—	Ray florets	(Singh et al. 2013)
		3–10	—	Plantlets	(Kapadiya et al. 2016)
<i>Cryptomeria</i>	GammaraysX-rays	1–5	—	Cuttings	(Lee et al. 2010)
		2.5	—	Callus	(Preil et al. 1983)
<i>Dahlia</i>	Gammarays	1–3	—	Cuttings	(Sundar et al. 2017)
<i>Dianthus</i>	GammaraysX-rays	5.26–10.5	—	Cuttings	(Buiatti and Ragazzini 1965)
	X-raysGammarays	2–50	—	Nodes	(Cassells et al. 1983)
		4–13	—	Leaf segments	(Okamura et al. 2003)
		3–10	—	Leaf segments	(Okamura et al. 2003)
<i>Gerbera</i>	X-rays Gammarays	2.2–4.4	—	Plants	(Sagawa and Mehlquist 1959)
	Gammarays	2–8	—	Petal explantsShoots	(Sagawa and Mehlquist 1959)
		0.15–3	0.65		(Ghani et al. 2014)
<i>Gladiolus</i>	Gammarays	0.15–1	0.6	Shoots	(Ghani et al. 2014)
	Gammarays Gammarays	1.5–5.5	—	Corms	(Sathyanarayana et al. 2019)
	Gammarays	1.5–6	—	Corms	(Shukla et al. 2018)
		1.75–10.5	—	Corms	(Tirkey and Singh 2019)
<i>Iris</i>	X-rays	0.5–1.1	—	Bulbs	(Hekstra And Broertjes. 1968)
<i>Jasminum</i>	Gammarays	1–2.5	—	Cuttings	(Ghosh et al. 2019)
<i>Lonicera</i>	Gammarays	1–6	2.1,3.5	Microcuttings	(Cambecedes et al. 1992)
<i>Ornithogalum</i>	Gammarays	20–40	—	Seeds	(Biswas and Biswas 2006)
<i>Pelargonium</i>	Gammarays	1.5	—	Leaves	(Grunewalt 1983)

Genus	Mutagen	Dose(krad)^z	LD₅₀(krad)	Material	Reference
<i>Petunia</i>	Gammarays	2–10	10	Seeds	(Berenschot et al. 2008)
<i>Plectranthus</i>	Gammarays	1.5–6	3.762–6.52	Cuttings	(Aisyah et al. 2015)
<i>Populus</i>	Gammarays	1–30	—	Plantlets	(Nishiguchi et al. 2012)
<i>Rosa</i>	Gammarays	1–6	—	Shoot tips	(Aamir et al. 2016)
	Gammarays	1–12	3.3–5.4	Shoot tips	(Baig et al. 2012)
<i>Rudbeckia</i>	X-rays	0.5–8	4	Stem cuttings with bud	(Bala and Singh 2013)
		2.5–6	—	Micro shoots	(Walther, F. A. Sauer 1986)
<i>Saintpaulia</i>	Gammarays	1–10	5.6	Leaf cuttings	(Wongpiyasatid et al. 2007)
	X-rays	0.5–10	5.69	Leaf explants	(Zhou et al. 2007)

similar to that of neutron and ion irradiation. DNA is broken into double strands when ions and neutrons clash with it, leading to deletions (Shikazono et al. 2005). 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 (Magori et al. 2010). 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 (Ryuto et al. 2006). 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.

11. 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 (Ogawa et al. 2014) 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 (Miyazaki et al. 2006), 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 (Miyazaki et al. 2002 and Kanaya et al. 2008).

12. 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* (Sugiyama et al. 2008). Additionally, a mutant *Chrysanthemum* that flowered early and at low temperatures was discovered. (Sakamoto et al. 2016).

13. 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. (Hayashi et al. 2019) 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. (Hekstra and Broertjes 1968) 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.

14. STABILITY OF RESULTING MUTANTS

Chimeras are frequently the outcome, regardless of the mutagen of choice or the organ(s) treated.

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	
<i>Acer</i>	Neutrons	2–14krad	—	Seeds	(Hekstra et al. 1968)	
<i>Achimenes</i>	Neutrons	0.75–2krad	—	Leaves	(Broertjes 1976)	
<i>Berberis</i>	Neutrons	2–14krad	—	Seeds	(Hekstra and Broertjes. 1968)	
<i>Chrysanthemum</i>	Ion beam	1krad, unknown, 23keV/mm	—	Scions	(Hisamura et al. 2016)	
	beam Ion beam	0.1–0.8krad, 220MeV, 122keV/mm	—	Ray florets and leaf explants	(Matsumura et al. 2010)	
	Ion beam	0.1–0.5krad, 446MeV/u, 93keV/mm	—	Leaf segments ray florets	(Sparrow et al. 1968)	
	Ion beam	0.3– 0.6krad, unknown, 22.6keV/mm	—	Cuttings Stem segments	(Sakamoto et al. 2016)	
			0.1–2krad, 135MeV/u, 23keV/mm	—		(Suzuki et al. 2005)
		Ion beam Ion beam Ion beam	0.2– 0.5krad, unknown, 23/62/280keV/ mm	—	Cuttings Leaf blades Stemsegmnt	(Tanokashira et al. 2014) (Tanokashira et al. 2014)
<i>Cyclamen</i>	Ion beam	mm0.01–0.3 krad, unknown,	—	Petioles	(Wakita et al. 2008)	
	Ion beam	23/310 keV/mm0.3–0.6 krad, 135 MeV/u, 22.5 keV/mm	—	Petioles	(Ishizaka et al. 2012) (Kondo et al. 2009)	
<i>Dianthus</i>	Ion beam	0–5 krad, 220/320 MeV, unknown	—	Leaf sent	(Kondo et al. 2009)	
	Ion beam	0.7–2 krad, 320 MeV, 76 keV/mm	—	Petals	(Kondo et al. 2009)	
<i>Saintpaulia</i>	Ion beam	0.5–8 krad, 960 MeV, unknown	—	Leaf	(Zhou et al. 2006)	

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 (Marcotrigiano 1997). 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, (Hoskins and Contreras. 2019) 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.

15. 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 (Van Harten 1998). 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 (Oladosu et al. 2016 & Predieri et al. 2007). 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 (Shikazono et al. 2005 & Greene et al. 2003). Physical mutagens, on the other hand, typically result in deletions that produce mutants with lost functions (Oladosu et al. 2016). 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 (Gautam et al. 1992). 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.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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