Mutation Breeding in Ornamentals

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Abstract. **The promising possibilities of mutation breeding in ornamental plants have led to a great interest in effective mutagenic treatment protocols for various species. This review discusses mutagenic treatments of a large number of ornamental genera, the advantages and disadvantages of various techniques, and the possibilities of improving the associated protocols. A number of nontargeted mutagenesis methods are available, ranging from chemical treatment with alkylating agents to irradiation with X-rays, gamma rays, and neutron or heavy ion beams at various doses. These are all relatively inexpensive and have been proven to be effective mutagens in a large number of diverse species. Genetic engineering, however, remains mostly impractical for many ornamental breeding operations because of the high cost and lack of knowledge necessary to successfully transform and regenerate ornamental crops. Of the available nontargeted mutagens, irradiation with gamma rays is still the most popular. It provides high consistency compared with chemical mutagens, albeit at a seemingly lower mutagenic efficiency. Changes in the radiation dose rate may increase the efficiency, although chronic irradiation over a longer period causes fewer deleterious mutations than the commonly used acute irradiation protocols. Heavy ion beam irradiation may also provide highly consistent mutation induction at higher efficiencies because of the high particle energy associated with these treatments. There are also opportunities to improve chemical mutagenesis. Although the required knowledge of specific gene functions in many ornamentals is still lacking, combination mutagenesis with ethyl methanesulfontate with genetic screening in a process known as TILLING (Targeting Induced Local Lesions IN Genomes) may lead to a powerful mutation breeding tool in the future. Mutation breeding is still very useful, and many opportunities are available to improve the existing methods.**

Naturally occurring changes in the DNA of organisms have been a primary source of genetic diversity that, through the processes of natural selection and genetic drift, have led to the evolution and abundance of different plant species that we know today (Huxley, 1942). These changes, called mutations, have also resulted in variations within many plant species. Mutations have been the source of improvements in many ornamental species such as japanese morning glory (*Ipomaea nil*; Miyake and Imai, 1926) and petunia (*Petunia ×hybrida*; Sink, 1973), which is the focus of this review. However, agronomic crops have benefitted from natural variation, with the IR8 "miracle rice" variety as one of the greatest examples (Chandler, 1992; Hargrove and Cabanilla, 1979; International Rice Research Institute, 1967; Li et al., 2020; Peng et al., 2010; Spielmeyer et al., 2002).

Numerous other examples illustrate that naturally occurring mutations have been an important source of genetic diversity and novel phenotypes for both ornamental and agronomic crops. The rate at which these mutations occur is quite low for most plants, however. Estimates range from 5.0×10^{-9} to 3.0×10^{-8} per site per year (Ossowski et al., 2010; Schultz et al., 1999; Wolfe et al., 1987). This low natural mutation rate and the fact that only a small portion of mutations is assumed to be observable, let alone beneficial, indicate that the chances of identifying a spontaneous mutant with a valuable, novel phenotype are very low (Elena et al., 1996; Peck, 1994; Schultz and Lynch, 1997; Sniegowski and Gerrish, 2010). Alternatively, plant material can be treated with mutagens. These treatments increase the random mutation rate, resulting in the faster accumulation of useful mutations at relatively low costs for breeding programs (Van Harten, 1998).

Such treatments were first discovered by Muller in 1927. He discovered that irradiating *Drosophila melanogaster* with X-rays caused a massive increase in the mutation rate by ≈15,000% over base levels (Muller, 1927). At almost the same time, Stadler independently showed that X-rays and gamma radiation had similar effects on barley and maize (Stadler, 1928a, 1928b). The value of these physical mutagens to plant breeding was quickly recognized (Gates, 1930). Approximately two decades later, the first chemical mutagens were discovered in the United Kingdom and Union of Soviet Socialist Republics (Auerbach and Robson, 1946; Rapoport, 1946). Since then, many other mutagens have been discovered, and the effectiveness of these physical and chemical mutagens has been proven by countless experiments (Arunyanart and Soontronyatara, 2002; Loveless, 1958; Tanaka et al., 2010; Wang et al., 1988). Furthermore, more than 3300 mutant plant varieties have been registered in the Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA) Mutant Variety Database, ranging from cereals and oilseed crops to orchids and woody perennials, indicating the widespread use of mutagenesis in modern plant breeding (International Atomic Energy Agency, 2021).

Besides nontargeted mutagens such as X-rays and gamma rays that merely increase the random mutation rate, tools that allow direct modification of plant DNA plant by genetic engineering are available. The first use of such a tool was in 1983, when Barton et al. (1983) successfully modified and regenerated tobacco using *Agrobacterium tumefaciens* as a vector to introduce exogenous DNA. Subsequently, a number of other tools were developed, among which are zinc finger nucleases, TALENs, and CRISPR-Cas9 (Khurshid et al., 2018; Shukla et al., 2009; Zhang et al., 2013). Although these methods allow efficient and directed modification of plant DNA, their use has been very limited in ornamental plant breeding for a few reasons. First, there is comparatively little knowledge of procedures for the transformation and regeneration of many ornamental species, and many that have been studied have proved exceedingly challenging. Second, genetic engineering is economically infeasible for the majority of ornamental species because of the high regulatory and development costs (Backes, 2013; Schum, 2003; Vining et al., 2012). Finally, there is little available sequence information available for ornamentals, which is required for targeted mutation or gene editing.

Because genetic engineering is largely impractical, ornamental plant breeding in particular has benefitted from the use of random mutagenesis. It is often difficult to hybridize existing cultivars with other germplasm; therefore, other ways of introducing genetic variation are needed (Van Tuyl and Lim, 2003). Additionally, vegetative propagation is common in ornamentals, which means that mutants with novel phenotypes are relatively easily maintained.

Although very useful, the application of random mutagenesis in the breeding of ornamental plants is not as straightforward as it often seems. Several factors should be considered when choosing from the available mutagens. Some require sources of radiation, whereas a place to work safely with chemicals is the only requirement for others.

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Cost and the plant material to be treated are also factors that should be considered. Besides deciding which mutagen to use, choosing the optimal treatment conditions and organ (e.g., seed, vegetative meristems, pollen) is critical to success. The large variety of ornamental species and the many genotypes within these species could all respond differently to the same treatment conditions because of varying (radio)sensitivity to mutagens (Jiang et al., 2014). Therefore, data from previous studies can be helpful when choosing the optimal mutagen and treatment conditions for new species or cultivars.

Although mutagenesis in agronomic crops has been comprehensively reviewed, there has been less focus on the mutation breeding of ornamentals (Daskalov, 1986; Goyal and Khan, 2010; Khan and Tyagi, 2013; Roebbelen, 1990; Wani et al., 2013). The goals of this review were to provide an overview of the most commonly used mutagens for a large variety of ornamental genera, to discuss the advantages and disadvantages of each mutagen, to provide some examples of resulting mutations, and to discuss possible future research opportunities that could help to improve mutation breeding methods. English and Dutch publications found by searching Google Scholar, ScienceDirect, and SpringerLink were used, with no restrictions regarding the date of publication.

This review was limited to random mutagenesis. CRISPR-CAS9 and other types of genetic engineering are not included because of their limited use in ornamental breeding. The current techniques used for ethyl methanesulfonate (EMS) and other alkylating agents, X-rays, gamma rays, fast neutron irradiation, and heavy ion irradiation and their experiences in ornamental annuals/biennials and herbaceous and woody perennials are reviewed and discussed.

EMS and Other Alkylating Agents

The widespread use of alkylating agents such as EMS in ornamental plant breeding has been observed since their discovery in 1946. Although many chemicals such as *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, *N*-ethyl-*N*-nitrosourea, and dimethylnitrosamine can also be used, EMS is by far the most common because of its mutagenic efficiency (ratio of mutations to deleterious effects (Gautam et al., 1992; Girija and Dhanavel, 2009; Yamaguchi et al., 2009), relatively low cost, and high availability (Gichner and Velemínský, 1967; Montesano et al., 1979; Schendel and Michaeli, 1984; Talebi et al., 2012).

The mechanism by which EMS modifies DNA is based on the alkylation of guanine, resulting in G:C to A:T substitutions. This causes point mutations that are randomly distributed throughout the entire genome (Greene et al., 2003), resulting in single nucleotide polymorphisms (SNPs). Furthermore, EMS causes fewer deletions than physical mutagens (Koornneef et al., 1982). Therefore, chemical mutagens such as EMS are useful for inducing missense or nonsense mutations, resulting in change-of-function or occasionally loss-of-function mutants (Shikazono et al., 2005).

Seeds are typically treated with a buffer solution containing EMS. The treatment concentrations and durations vary widely among taxa and even within the same species because different genotypes may respond differently to treatment. Deleterious effects can be caused by EMS, such as lethality, sterility, and a lower ability to regenerate plants from tissues such as floral pedicels (Latado et al., 2004; Roychowdhury and Tah, 2011). Reduced fertility resulting from the treatment of some weedy or invasive species is desirable and at least as important as other phenotypic changes. Pilot experiments to determine the optimal treatment conditions, usually a combination of concentration and duration resulting in 50% survival [(median lethal dose (LD_{50})], are always advised (Berenschot et al., 2008; Hohmann et al., 2005; Kim et al., 2006; Napoli and Ruehle 1996). In addition to seeds, in vitro treatments have been applied to nodal segments and ray florets in chrysanthemum (Padmadevi and Jawaharlal, 2011).

Annuals and Biennials

EMS has been used for several annual and biennial ornamental genera (Table 1). Seeds were treated in every case except for *Begonia*, concentrations have ranged from 0.10% to 1.20%, with an outlier at 40%, and treatment durations are commonly between 4 and 24 h. Kashikar and Khalatkar (1981) observed flower color changes in the M1 and M2 generations of white flowering *Petunia* ×*hybrida*. Several different shades of violet were found in the M1 generation, and colors ranging from pink to pale blueish magenta were found in the M2 generation. M1 mutants displaying dwarfism and abnormal leaf morphology were found in *Antirrhinum majus* (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 by Chen et al. (2014) who obtained *Begonia* ×*hiemalis* mutants that were resistant to stem rot caused by *Rhizoctonia solani*.

Herbaceous Perennials

A few nonwoody perennial ornamental species have also been treated with EMS, with the economically important genus *Chrysanthemum* being the most common (Table 1) (Datta and Chakrabarty, 2005). Several *Chrysanthemum* tissues were used, with concentrations ranging from 0.02% to 1.03% and durations ranging from 1 to 5 h. Other genera were treated with concentrations and durations ranging from 0.10% to 1.25% at 10 min to 24 h (Table 1). Different tissues, ranging from flower pedicels and leaf segments to seeds and bulbs, were used during these studies. Treatment durations and concentrations ranged from 10 min to 24 h and 0.02% to 1.25%, respectively. Hossain et al. (2006a) found a salt-tolerant *Chrysanthemum morifolium* mutant that did not suffer in terms of flower size and number when grown in high-salinity conditions. EMS treatment also led to changes in flower color in chrysanthemum. Latado et al. (2004), for example, treated the dark pink cultivar Ingrid and found mutants with white, yellow, and bronze flowers. EMS also led to an increase in the tepal number from six to eight in *Agave amica* (Singh et al., 2013). Reductions in plant height and pollen fertility have also been observed (Contreras and Shearer, 2020; Kapadiya et al., 2016; Padmadevi and Jawaharlal, 2011; Tirkey and Singh, 2019).

Woody Trees and Shrubs

EMS also has been used for woody ornamentals (Table 1). Seeds and other tissues such as cuttings and meristems were used. The treatments consisted of EMS solutions ranging from 0.05% to 5% and durations ranging from 1 h to 48 h. As with the annual, biennial, and herbaceous perennials, many traits were affected. Treatment of *Buddleja davidii* seeds led to the cultivar Summer Skies, which shows stable variegation along the edges of leaves (Smith and Brand, 2012). Leaf morphology was affected in *Weigela* and *Ribes sanguineum*, which, in the case of the latter, led to the cultivar Oregon Snowflake (Contreras and Friddle, 2015; Duron, 1992). Smilansky et al. (1986) observed a decrease in rose petal number and flower size after EMS treatments of cuttings. Flower color mutants that had lower cyanidin and pelargonidin concentrations were also found. Variations observed in *Bougainvillea spectabilis* included dwarfism, thornlessness, leaf shape, and variegation (Anitha et al., 2017). Ghosh et al. (2019) also observed dwarfism in *Jasminum grandiflorum*.

EMS is clearly capable of inducing mutations in a large range of species, thus affecting many traits. There is no need for expensive technical equipment, and the procedure is relatively straightforward, thereby making the use of EMS an attractive choice, especially for smaller-scale breeding programs. One disadvantage of using EMS or other chemical mutagens is their inability to penetrate deeply into plant tissues and seeds with thick coats, possibly leading to inconsistent treatments (Van Harten, 1998).

An advantage of EMS is that the mutagenic efficiency is quite high, which means that the number of undesirable mutations is low relative to the total number of mutations (Gautam et al., 1992; Girija and Dhanavel, 2009; Kaul and Bhan, 1977; Wani, 2009). However, the efficiency does typically change with dose and decreases on either side of the optimum. This further emphasizes the importance of determining the optimal dose before treating plants on a large scale.

The efficiency of mutation breeding using EMS may be increased further by using the reverse genetics tool known as targeting-induced local lesions in genomes (TILLING). TILLING combines mutagenesis, commonly using EMS to generate single base substitutions

Table 1. Ethyl methanesulfontate (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. EMS concentrations ranged from 0.02% to 5%, with one exceptionally high concentration at 40%. Treatment durations ranged from 10 min to 48 h. In most cases, seeds were treated in many cases; however, other materials such as cuttings have been used.

Genus	Mutagen	Treatment concn	Treatment duration	LD_{50}	Material	Reference
Agave	EMS	0.25% to 0.50%	4 h	—	Bulbs	Singh et al., 2013
Antirrhinum	EMS	0.10% to 1.00%	8–12 h	—	Seeds	Heffron et al., 2006
Begonia	EMS	Unknown	Unknown	—	Leaves	Chen et al., 2014
Bougainvillea	EMS	0.80% to 1.00%	6 h	—	Cuttings	Anitha et al., 2017
Buddleja	EMS	1.40%	4 h	—	Seeds	Smith and Brand, 2012
Chrysanthemum	EMS	0.025% to 0.050%	5 h	—	Leaf sections	Hossain et al., 2006a
	EMS	0.02% to 0.04%	Unknown	—	Cuttings	Kapadiya et al., 2016
	EMS	0.51% to 1.03%	1 h 45 min	0.82%	Floral pedicels	Latado et al., 2004
	EMS	0.10% to 0.30%	1 h	—	Ray florets	Padmadevi and Jawaharlal, 2011
Dianthus	EMS	0.10% to 0.70%	6 h	—	Seeds	Roychowdhury and Tah, 2011
Gerbera	EMS	0.10% to 1.00%	10 min	0.65%	Shoots	Ghani et al., 2014
Gladiolus	EMS	0.20% to 1.20%	Unknown	—	Corm buds	Gong et al., 2010
	EMS	0.25% to 1.25%	Unknown	—	Corms	Tirkey and Singh, 2019
Hydrangea	EMS	0.50% to 5.00%	3 h	—	Seeds	Greer and Rinehart, 2009
Impatiens	EMS	0.32% to 1.08%	24 h	—	Seeds	Weigle and Butler, 1983
Ipomoea	EMS	0.10% to 0.30%	6–18 h	—	Seeds	Bhate, 2001
Jasminum	EMS	0.06% to 0.62%	1–6 h	0.53%, 0.55%	Cuttings	Ghosh and Ganga, 2019
	EMS	0.25% to 0.4%	1 h	—	Cuttings	Ghosh et al., 2019
Ornithogalum	EMS	0.20% to 1.00%	24 h	0.15%, 0.52%	Seeds	Contreras and Shearer, 2020
Petunia	EMS	0.10% to 0.30%	6–24 h	—	Seeds	Jiang et al., 2014
	EMS	0.10% to 0.30%	18 h	—	Seeds	Kashikar and Khalatkar, 1981
Portulaca	EMS	1.20% to 40.00%	4 h	—	Seeds	Bennani and Rossi-Hassani, 2001
Ribes	EMS	0.20% to 1.20%	24–48 h	—	Seeds	Contreras and Friddle, 2015
Rosa	EMS	0.50% to 3.00%	2–12 h	—	Apical and axillary meristems	Senapati and Rout, 2008
	EMS	0.08% to 5.00%	1–24 h	—	Stem cuttings with buds	Smilansky et al., 1986
Saintpaulia	EMS	0.20% to 0.60%	30 min–4 h	—	Leaf sections	Fang and Traore, 2011
Sarcococca	EMS	0.20% to 1.20%	24–48 h	0.66%, 0.54%	Seeds	Hoskins and Contreras, 2019
Weigela	EMS	0.50%	1 h 30 min	—	Shoot internodes	Duron, 1992

observed as SNPs, with a screening tool to detect mutations in target genes in young plants (McCallum et al., 2000). Several tools are available for detecting the SNPs, including denaturing high-performance liquid chromatography, high-resolution melting, and next-generation sequencing (McCallum et al., 2000; Taheri et al., 2017).

TILLING is capable of increasing the efficiency further by allowing the selection of mutants before traits such as flower color or morphology become visible. This could be especially valuable in the case of woody ornamentals, which require several years to reach maturity (Wilde et al., 2012). TILLING also allows for a much larger initial mutant population because the majority of mutants can be discarded after genetic screening. Another advantage is that mutants obtained using TILLING are not considered genetically modified organisms, thus exempting them from expensive regulation and bans (Backes, 2013; Kurowska et al., 2011). One drawback of TILLING is that knowledge of the target genes to be screened is required but is still lacking for most ornamentals. Rinehart et al. (2018) listed a number of possible target genes for *Hydrangea macrophylla* that were selected based on homology to *Arabidopsis* genes, but the function of these genes has not been confirmed. Nonetheless, EMS mutagenesis as a part of TILLING might become a very powerful tool for ornamental breeding in the future as sequencing costs continue to decrease. However, as the regulatory landscape changes, site-directed mutagenesis or gene editing may be more efficient.

X-rays and Gamma Rays

Many mutant varieties have been created using X-rays and gamma rays after Muller (1927) and Stadler (1928a, 1928b) discovered the mutagenic effects of ionizing radiation. Gamma radiation in particular has been popular; approximately half of all mutant varieties registered in the FAO/IAEA Mutant Variety Database were created using gamma rays. X-rays only account for ≈17% of the registered varieties, and chemical mutagenesis has been used for slightly more than 10% (International Atomic Energy Agency, 2021).

X-ray and gamma ray mutagenesis are based on the direct and indirect interactions of highly energetic electromagnetic radiation with DNA. These interactions usually result in deletions and other chromosomal aberrations by breaking the DNA, with mostly loss-of-function mutants as a result (Kodym and Afza, 2003; Maple and Møller, 2007; Oladosu et al., 2016).

For gamma irradiation, plant tissue or seeds are typically treated in gamma chambers or rooms for acute irradiation and gamma fields for chronic irradiation (Bala and Singh, 2013; Datta, 2014; Girija and Dhanavel, 2009). Chronic irradiation, however, is rarely used. The most common gamma source is cobalt-60, but others, such as cesium-137, are also effective (Puchooa, 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 LD_{50}, should be determined for a specific subject before starting with irradiation on a large scale (Bala and Singh, 2013; Bhat et al., 2007; Douglas, 1986; Girija and Dhanavel, 2009; Gladstones and Francis, 1965; Puchooa, 2005; Walther and Sauer, 1986a; Webb et al., 2005).

Annuals and Biennials

Gamma and X-rays have been used to study mutagenesis in a few annual and biennial ornamental genera, with *Petunia* being the most common (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. Berenschot et al. (2008) 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

Table 2. Gamma and X-ray treatment conditions. The genus, mutagen, treatment dose, median lethal dose (LD_{50}), treated material, and reference are shown for each study. The treatment dose is shown in kilorads (krad). Doses originally reported in grays or roentgens were converted using the factors 0.1 and 0.877, respectively. Doses ranged from 0.22 krad to 20 krad for X-rays, with one outlier at 50 krad. For gamma rays, the doses ranged from 0.1 to 60 krad, with outliers at 140 krad, 225 krad, and 320 krad. Cuttings and seeds were often treated, but other materials such as leaf segments, bulbs, and callus were also used.

Genus	Mutagen	Dose (krad)[z]	LD_{50} (krad)	Material	Reference
Acer	Gamma rays	0.1–5	—	Cuttings	Smith and Noyszewski, 2018
	Gamma rays	50–225	—	Seeds	Smith and Noyszewski, 2018
Achimenes	X-rays	2–4	—	Leaves	Broertjes, 1976
Agave	Gamma rays	1–40	—	Bulbs	Navabi et al., 2016
Antirrhinum	Gamma rays	10–320	—	Seeds	Gupta and Matsuo, 1969
	Gamma rays	0.5–60	—	Plants	Sekiguchi et al., 1971
Begonia	X-rays	1.5–2.5	—	Leaves	Doorenbos and Karper, 1975
	X-rays	0.5–3	—	Leaves	Roest et al., 1981
Berberis	Gamma rays	0.1–5	—	Cuttings	Smith and Noyszewski, 2018
	Gamma rays	50–225	—	Seeds	Smith and Noyszewski, 2018
Bougainvillea	Gamma rays	0.5–1	—	Cuttings	Anitha et al., 2017
	Gamma rays	0.5–2	—	Cuttings	Swaroop et al., 2015
Calendula	Gamma rays	2.5–7.5	—	Seeds	Tiwari and Kumar, 2011
Chrysanthemum	Gamma rays	1–4	—	Cuttings	Dowrick and El Bayoumi, 1966
	X-rays	0.44–1.75	—	Cuttings	Dowrick and El Bayoumi, 1966
	Gamma rays	0.5–1	—	Ray florets	Hossain et al., 2006b
	Gamma rays	3–10	—	Plantlets	Kang et al., 2013
	Gamma rays	0.5–2.5	—	Cuttings	Kapadiya et al., 2014
	Gamma rays	1–2	—	Cuttings	Kumari et al., 2013
	Gamma rays	1–5	—	Cuttings	Lee et al., 2010
	Gamma rays	2.5–15	—	Plants	Nagatomi and Degi, 2009
	Gamma rays	0.5–1.5	—	Unknown	Padmadevi and Jawaharlal, 2011
	X-rays	2.5	—	Callus	Preil et al., 1983
	X-rays	0.5–2	—	Cuttings	Tanokashira et al., 2014
	Gamma rays	1.5	—	Cuttings	Zalewska et al., 2011
Cryptomeria	Gamma rays	0.51–3.51	—	Plants	Kukimura et al., 1976
Dahlia	Gamma rays	1–3	—	Cuttings	Sundar et al., 2017
Datura	X-rays	1.3–2.6	—	Protoplasts	Krumbiegel, 1979
Dianthus	Gamma rays	5.26–10.5	—	Cuttings	Buiatti and Ragazzini, 1965
	X-rays	2–50	—	Nodes	Cassells et al., 1993
	X-rays	4–13	—	Leaf segments	Okamura et al., 2003
	Gamma rays	3–10	—	Leaf segments	Okamura et al., 2003
	Gamma rays	2–8	2—4	Shoots	Paramesh and Chowdhury, 2005
	X-rays	2.2–4.4	—	Plants	Sagawa and Mehlquist, 1959
	Gamma rays	2–8	—	Petal explants	Simard et al., 1992
Gerbera	Gamma rays	0.15–3	0.65	Shoots	Ghani and Sharma, 2019
	Gamma rays	0.15–1	0.6	Shoots	Ghani et al., 2014
	Gamma rays	2	—	Shoots	Laneri et al., 1990
	X-rays	0.5–5	—	Microshoots	Walther and Sauer, 1990
Gladiolus	Gamma rays	1.5–5.5	—	Corms	Sathyanarayana et al., 2019
	Gamma rays	1.5–6	—	Corms	Shukla et al., 2018
	Gamma rays	1.75–10.5	—	Corms	Tirkey and Singh, 2019
	Gamma rays	5–15	—	Corms	Tiwari et al., 2010
Hebe	Gamma rays	1.5–6	3.3, 3.7, 4.8, 5.6	Nodal explants	Gallone et al., 2012
Iris	X-rays	0.5–1.1	—	Bulbs	Hekstra and Broertjes, 1968
Jasminum	Gamma rays	1–2.5	—	Cuttings	Ghosh et al., 2019
Lonicera	Gamma rays	1–6	2.1, 3.5	Microcuttings	Cambecèdes et al., 1992
Ornithogalum	Gamma rays	20–40	—	Seeds	Biswas and Biswas, 2006
Pelargonium	Gamma rays	1.5	—	Leaves	Grunewalt, 1983
Petunia	Gamma rays	2–10	10	Seeds	Berenschot et al., 2008
	Gamma rays	5–10	—	Leaf disks	Donà et al., 2013
	Gamma rays	0.87–5.26	—	Seeds	Kashikar and Khalatkar, 1981
	X-rays	0.22–2.6	—	Protoplasts	Krumbiegel, 1979
	X-rays	0.28–2.2	—	Flower buds	Moore and Haskins, 1935
Pinus	Gamma rays	0.18–3.5	—	Plants	Donini, 1967
Plectranthus	Gamma rays	1.5–6	3.762–6.52	Cuttings	Aisyah et al., 2015
Populus	Gamma rays	1–30	—	Plantlets	Nishiguchi et al., 2012
Portulaca	Gamma rays	2–6	—	Unknown	Raghuvanshi and Singh, 1979
	Gamma rays	1–4	—	Stem cuttings	Wongpiyasatid and Hormchan, 2000
Pseudotsuga	Gamma rays	0.5–10	—	Seeds	El-Lakany and Sziklai, 1970
Rosa	Gamma rays	1–6	—	Shoot tips	Aamir et al., 2016
	Gamma rays	1–12	3.3–5.4	Shoot tips	Baig et al., 2012
	Gamma rays	0.5–8	4	Stem cuttings with bud	Bala and Singh, 2013
	Gamma rays	5–20	—	Seeds	Giovanni et al., 2015
	Gamma rays	0.88–11.4	—	Stem cuttings with buds	Smilansky et al., 1986
	X-rays	2.5–6	—	Microshoots	Walther and Sauer, 1986a, 1986b
Rudbeckia	Gamma rays	0.5–4	—	Callus	Oates et al., 2013
Saintpaulia	Gamma rays	1–10	5.6	Leaf cuttings	Wongpiyasatid et al., 2007
	X-rays	0.5–10	5.69	Leaf explants	Zhou et al., 2006

(Continued on next page)

Table 2. (*Continued*)

Genus	Mutagen	Dose (krad)[z]	LD_{50} (krad)	Material	Reference
Sophora	Gamma rays	20–140	—	Seeds	Wang et al., 2017
Tagetes	Gamma rays	10–40	>40	Seeds	Singh et al., 2009
Tsuga	Gamma rays	0.5–10	—	Seeds	El-Lakany and Sziklai, 1970
Vitex	Gamma rays	1–40	4.13	Seeds	Ari et al., 2015
Zinnia	Gamma rays	7.5–12.5	—	Seeds	Pallavi et al., 2017
	X-rays	20	—	Seeds	Swarup and Raghava, 1974
	Gamma rays	2.5–12.5	—	Seeds	Venkatachalam and Jayabalan, 1997

[z]Reported in krad. Conversions to other units are 10 krad = 1 Gy and 114 R = 1 Gy.

varying leaf colors. Fertility, characterized as the number of seed capsules produced after manual pollination, was reduced in *Petunia* ×*hybrida* (Berenschot et al., 2008).

Herbaceous Perennials

X-rays and gamma rays have been widely used for several herbaceous perennials, with *Chrysanthemum* being the most commonly treated genus by far (Table 2). The X-ray treatments have consisted of doses ranging from 0.44 to 13 krad, with one exceptionally high dose at 50 krad. For gamma irradiation, the doses ranged from 0.15 to 15 krad, with two outliers at 40 krad. Many different tissues were used during these studies. For *Chrysanthemum* alone, the tissues ranged from cuttings and whole plants to individual cells and ray florets. For other genera, corms, leaf cuttings, and bulbs were used. Surprisingly, seeds were not used in the reviewed studies. Regarding annuals and biennials, many morphological traits were affected. 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). X-ray irradiation restored male fertility in *Dianthus caryophyllus* (Sagawa and Mehlquist, 1959); however, Biswas and Biswas (2006) were able to induce partial sterility in *Ornithogalum virens*. Oates et al. (2013) noticed that treating *Rudbeckia subtomentosa* callus with relatively low levels of gamma radiation resulted in high survival and a good number of mutations, including reduced height and good flower form. A large variety of changes in flower color as well as flower morphology were found in most of the other genera, and traits like vase life can also be improved, as shown by Shukla et al. (2018).

Woody Trees and Shrubs

Many diverse woody genera have been treated with gamma or X-rays (Table 2). Sparrow et al., (1968) determined the gamma radiation LD_{50} doses for 28 woody plant species and predicted the LD_{50} for another 190 species based on their interphase chromosome volumes, although the authors concede there exists wide variations because of timing and other factors involved with their method. They did not describe any visible phenotypes resulting from mutations, but the LD_{50} values provide starting points when trying to determine the optimal dose for any 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. (1976) 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; Nishiguchi 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., 1986).

Although gamma and X-ray irradiation have been very effective mutagens in a large number of plant species, they require more expensive equipment such as gamma sources and X-ray machines. They provide good penetration of tissues and seeds, making it possible to treat material with a higher consistency. They also provide ways to treat fragile tissue, such as pollen grains, that could be damaged by soaking them in chemicals (Oladosu et al., 2016; Predieri and Di Virgilio, 2007; Schum, 2003). Their mutagenic efficiency, however, seems to be relatively low (Gautam et al., 1992; Girija and Dhanavel, 2009; Kaul and Bhan, 1977; Wani, 2009).

There may be a way to increase the efficiency, however, by optimizing not only the total absorbed dose but also the dose rate. Often, only the total absorbed dose is described in treatments and relatively little attention is focused on the dose rate and/or duration of radiation treatment. Acute irradiation (high dose rate/short duration) has been favored over chronic irradiation (low dose rate/long duration) for a long time, but the efficiency of both methods has been debated (Kodym and Afza, 2003). Nagatomi and Degi (2009) showed that chronic gamma irradiation more effectively induced flower color mutants in *Chrysanthemum*, and Broertjes (1972) showed a similar effect of chronic X-ray irradiation in *Saintpaulia*. Numerous plant species have been successfully treated with chronic irradiation (Datta, 2012; Nagatomi, 1993; Nagatomi et al., 2000; Richter and Singleton, 1995). Therefore, experimentation with different dose rates and durations may lead to more efficient irradiation protocols for many ornamental species.

Neutrons and Heavy Ions

Neutron and heavy ion irradiation are being used as alternatives to gamma or X-ray irradiation. Heavy ion irradiation, mostly using carbon ions, has been used in recent years to induce mutations in several plant species (Arase et al., 2011; Kondo et al., 2009; Matsumura et al., 2010; Okamura et al., 2003; Reyes-Borja et al., 2007; Takahashi et al., 2005). Neutron irradiation has also been used as a mutagen, but it has had very limited use in ornamentals (Bolon et al., 2011; Broertjes, 1976; Datta, 2012; Love and Constantin, 1966; Smith and Noyszewski, 2018; Wang et al., 2015).

The mechanism by which neutron and ion irradiation induce mutations is somewhat similar to the way gamma and X-ray irradiation induce mutations. Neutrons and ions collide with DNA, thus causing double-strand breaks and resulting in deletions (Shikazono et al., 2005). As a result, irradiation with neutrons or ions mainly produces loss-of-function mutants.

The ions used for the irradiation of plant tissues are usually accelerated by a cyclotron and sent downrange to the sample (Magori et al., 2010). In addition to choosing the total dose, the energy of the particles themselves can be adjusted by choosing ions of a specific element. Although carbon is often used, irradiation using heavier ions such as argon or iron is also possible. 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., 2008). The particle energy is measured in either mega-electronvolts (MeV) for the total particle energy, or mega-electronvolts per nucleon (MeV/u). The amount of energy that the ions ultimately deposit in the plant tissue is described by the linear energy transfer (LET) in keV/μm. Doses typically range from 0.01 to 14 krad at LETs ranging from 22.5 to 310 keV/μm. As with all mutagens, determining the optimal dose before large-scale irradiation is important.

For neutron irradiation, samples are usually placed near nuclear reactors suitable for neutron irradiation studies (e.g., the UC Davis McClellan Nuclear Research Center

and the decommissioned Biological Agricultural Reactor Netherlands) (Bogaardt et al., 1965; Kodym and Afza, 2003; Smith and Noyszewski, 2018). The reactors must be designed in such a way that gamma ray contamination can be mostly filtered out. Doses typically range from 0.75 to 14 krad. Various types of neutrons, usually thermal or fast neutrons, can be used (Burdick, 1956). These neutrons differ in their kinetic energy levels; thermal neutrons have energies less than 1 eV, whereas fast neutrons have energies more than 1 MeV (Carron, 2006). Attention should be focused on the fact that materials treated with fast neutrons remain radioactive after treatment; therefore, the materials must be maintained until they are safe to handle (Van Harten, 1998). Again, the optimal dose for specific subjects should be determined before large-scale irradiation treatments.

Annuals and Biennials

Only a few annual or biennial genera have been treated with ion beams, and neutron beams have not been used at all (Table 3). The heavy ion doses used during these studies have ranged from 0.1 to 8 krad at LETs from 22.5 to 76 keV/μm. The tissues used for ion beam irradiation vary. Shoot cultures and apical meristems have been used, but cuttings with nodes and leaves are suitable irradiation targets as well. Flower color is often affected. Ogawa et al. (2014) found *Limonium* mutants showing lighter, darker, or more reddish shades of purple compared with the original purple flowers. Miyazaki et al. (2006) found similar *Torenia* mutants showing pale or dark pink flowers compared with blue in 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 (Kanaya et al., 2008; Miyazaki et al., 2002).

Herbaceous Perennials

A much larger number of herbaceous perennial genera have been treated with ion beam and neutron irradiation, with *Chrysanthemum* again being the most common (Table 3). Treatment conditions included ion doses ranging from 0.01 to 10 krad at LETs ranging from 22.5 to 310 keV/μm. Only *Achimenes* was treated with neutrons at doses ranging from 0.75 to 2 krad. Tissues such as ray florets, leaf segments, callus, petioles, and buds were used as targets. Seeds were used in one case, too. Basic morphological traits such as plant size (dwarfism), leaf shape, flower color, flower size, and flower shape were affected in the majority of mutants. Sugiyama et al. (2008a) found sterile *Cyclamen* mutants. A *Chrysanthemum* mutant that flowered early and under low temperatures was also identified (Sakamoto et al., 2016).

Woody Trees and Shrubs

Relatively few woody genera were treated with neutron or ion beam irradiation (Table 3). Ion beam doses ranging from 0.5 to 14 krad were used. LETs were mostly not reported, except for two cases in which the LET was ≈23 keV/μm. Neutron irradiation doses ranged from 2 to 14 krad. Seeds were most commonly used as irradiation targets, but scions, stem cuttings, and buds have been used. Among the affected traits were flower color in *Prunus* and *Rosa* and plant size (dwarfism) in *Spiraea* and *Hydrangea*. Hayashi et al. (2019) found that a *Prunus* mutant in 2007, which flowered twice during a single year, did not require a cold period for flowering and

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). Ion particle energy in either mega-electronvolts per nucleon (MeV/u) or total energy in MeV is shown. The particle linear energy transfer (LET) in kilo-electronvolts per micrometer (keV/μm) is also shown for ion beam treatments. Doses ranged from 0.75 krad to 14 krad for neutron irradiation treatments. For ion beam treatments, the doses ranged from 0.01 krad to 14 krad at LETs ranging from 22.5 to 310 keV/μm. Treated materials included leaves, cuttings, ray florets, callus, and many others.

Genus	Mutagen	Dose	LD_{50}	Material	Reference
Acer	Neutrons	2–14 krad	—	Seeds	Smith and Noyszewski, 2018
Achimenes	Neutrons	0.75–2 krad	—	Leaves	Broertjes, 1976
Berberis	Neutrons	2–14 krad	—	Seeds	Smith and Noyszewski, 2018
Chrysanthemum	Ion beam	1 krad, unknown, 23 keV/μm	—	Scions	Hisamura et al., 2016
	Ion beam	0.1–0.8 krad, 220 MeV, 122 keV/μm	—	Ray florets and leaf explants	Matsumura et al., 2010
	Ion beam	0.1–0.5 krad, 446 MeV/u, 93 keV/μm	—	Leaf segments and ray florets	Okamura et al., 2015
	Ion beam	0.3–0.6 krad, unknown, 22.6 keV/μm	—	Cuttings	Sakamoto et al., 2016
	Ion beam	0.1–2 krad, 135 MeV/u, 23 keV/μm	—	Stem segments	Suzuki et al., 2005
	Ion beam	0.2–0.5 krad, unknown, 23/62/280 keV/μm	—	Cuttings	Tanokashira et al., 2014
	Ion beam	0.01–0.3 krad, unknown, 23/310 keV/μm	—	Leaf blades	Tanokashira et al., 2016
	Ion beam	0.3–0.6 krad, 135 MeV/u, 22.5 keV/μm	—	Stem segments	Wakita et al., 2008
Cyclamen	Ion beam	0–5 krad, 220/320 MeV, unknown	—	Petioles	Ishizaka et al., 2012
	Ion beam	0.05–1.6 krad, 320 MeV, unknown	—	Petioles	Kondo et al., 2009
	Ion beam	1–8 krad, 1.62 GeV, 23 keV/μm	—	Callus, somatic embryos, plantlets, tubers	Sugiyama et al., 2008a
Dahlia	Ion beam	0.5–10 krad, unknown, unknown	—	Shoots	Hamatani et al., 2001
Dianthus	Ion beam	0.5–3 krad, 220 MeV, unknown	—	Leaf segments	Okamura et al., 2003
	Ion beam	0.7–2 krad, 320 MeV, 76 keV/μm	—	Petals	Okamura et al., 2013
	Ion beam	2–8 krad, 135 MeV/u, 23 keV/μm	—	Stem segments	Sugiyama et al., 2008b
Hydrangea	Ion beam	1–5 krad, 100 MeV, unknown	—	Seeds	Kudo et al., 1998
Limonium	Ion beam	0.5–3 krad, unknown, 23 keV/μm	—	Shoot cultures	Ogawa et al., 2014
Pelargonium	Ion beam	1–4 krad, 80 Mev/u, 34 keV/μm	—	Buds	Yu et al., 2016
Petunia	Ion beam	0–3 krad, 320 MeV, 76 keV/μm	—	Shoot apical meristem	Hase et al., 2010
	Ion beam	0.1–2 krad, 135 MeV/u, unknown	—	Nodal cuttings	Miyazaki et al., 2002
Populus	Ion beam	0–3 krad, unknown, unknown	—	Explants	Biswas et al., 2013
Prunus	Ion beam	0.5–2 krad, 135 MeV/u, 23 keV/μm	—	Scions	Hayashi et al., 2019
	Ion beam	1–2 krad, 135 MeV/u, 22.6 keV/μm	—	Scions	Ishii et al., 2009
Rosa	Ion beam	1–5 krad, 135 MeV/u, unknown	—	Buds	Hara et al., 2003
	Ion beam	0.5–10 krad, 100/220 MeV, unknown	20, 5–7.5 krad	Stem cuttings with bud	Yamaguchi et al., 2003
Saintpaulia	Ion beam	0.5–8 krad, 960 MeV, unknown	2.52, 2.32 krad	Leaf explants	Zhou et al., 2006
Sandersonia	Ion beam	2–10 krad, unknown, unknown	—	Seeds	Horita et al., 2002
Spiraea	Ion beam	0.5–14 krad, 220 MeV, unknown	—	Seeds	Iizuka et al., 2001
Torenia	Ion beam	0.5–5 krad, unknown, unknown	—	Leaves and internodes	Miyazaki et al., 2006
	Ion beam	0.5–8 krad, 1.62/2.70 GeV, 22.5/61.5 keV/μm	—	Leaf disks	Sasaki et al., 2008
Tricyrtis	Ion beam	0.5–5 krad, 135 MeV/u, 23 keV/μm	—	Callus	Nakano et al., 2010
Verbena	Ion beam	0.1–1 krad, 1.89 GeV, 31 keV/μm	—	Nodes	Kanaya et al., 2008

LD_{50} = median lethal dose.

produced three-times the number of flowers of the original variety if the mutant was exposed to cold winter temperatures. This mutant was named 'Nishina Otome' and commercially released in 2010. Smith and Noyszewski (2018) found several *Acer* mutants that did not flower and, thus, produced no seeds. They also found a *Berberis* mutant that did flower; nonetheless, it produced nonviable seeds. These mutants are still being evaluated but could provide solutions for mitigating the invasiveness of non-native *Acer* and *Berberis* species in North America.

Although neutron and ion beams have not yet been used as much as gamma rays, they are clearly suitable as mutagens in many ornamental plants. They offer the same high penetration of plant tissue and seeds as other physical mutagens, but they do so at seemingly higher efficiency. The reason for this high level of mutagenic efficiency seems to be the high energy levels associated with neutrons and ions. Whereas gamma rays and X-rays consist of photons, neutron and ion beams consist of particles. The amount of concentrated energy that is deposited in plant tissue by these photons or particles is quantified by the LET. The LET values of accelerated ions are much higher than those of gamma rays and X-rays. Whereas the LETs of ions typically range from 22 to 651 keV/μm, depending on the type of ion and its kinetic energy, the LETs of gamma and X-rays are only 0.2 and 2 keV/μm, respectively (Kazama et al., 2008, 2011; Ryuto et al., 2008). Therefore, the biological effects of ion beams are much stronger. Although gamma and X-ray irradiation mainly cause small deletions, ion beams cause much larger deletions (Hirano et al., 2015; Morita et al., 2009). Neutrons are also classified as high LET radiation and cause deletions ranging from a small number of basepairs to multiple megabases (Li et al., 2001; Men et al., 2002). As a result, neutron and ion irradiation can provide a good mutation rate at lower doses (Shikazono et al., 2005). These properties also seem to increase the mutagenic efficiency, resulting in fewer deleterious mutations (Yamaguchi et al., 2009). Therefore, moving from gamma irradiation to heavy ion or neutron irradiation as the preferred physical mutagen may lead to more efficient mutagenesis.

Stability of Resulting Mutants

Regardless of the choice of mutagen and organ(s) treated, chimeras are a common result. Chimeras are often unstable and revert to the wild type. A common example is leaf variegation, in which a portion of a histogenic layer (mericlinal), a portion of multiple histogenic layers (sectorial), or all of a histogenic layer (periclinal) is mutated. Such chimeras and their use in horticulture have been extensively treated elsewhere (Marcotrigiano, 1997). For resulting mutations to be useful, the breeder must understand how to stabilize the trait of interest to allow it to remain true-to-type during serial propagation in the case of asexually propagated crops, or it must be expressed through the LII (germ layer) histogenic layer. Often, successive phytomeres will variably express variegation and allow propagation of stems above which the trait appears stabilized. Hoskins and Contreras (2019) reported an example using *Sarcococca confuse*, in which an unstable "blotchy" variegation was allowed to continue growing and ultimately stabilized to a uniform chartreuse leaf type that remained stable after clonal propagation and also yielded true-to-type seed, although the latter is attributable to apomixis as much as an LII histogenic layer containing the trait. Even if the trait appears stable, there does exist the long-term prospect for reversion as evidence from the numerous cultivars that exhibit regular reversion in the ornamental trade. 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.

Conclusions

Several factors should be considered when choosing mutagen and treatment conditions. Although chemical mutagens are relatively inexpensive and require little technical equipment, they are regarded as having inferior ability to penetrate deeply into plant tissue or thick seeds (Van Harten, 1998). However, physical mutagens provide consistent treatments but require access to radiation sources, such as X-ray machines, gamma sources, particle accelerators, or nuclear reactors. Other advantages of physical mutagens include easy post-treatment handling of plant tissue or seeds, the ability to treat pollen grains and other fragile materials, and the lack of toxic and carcinogenic waste (Oladosu et al., 2016; Predieri and Di Virgilio, 2007; Schum, 2003). Another reason why EMS or other chemicals may be preferred in some cases is the fact that they mostly cause single base substitutions, possibly resulting in a series of phenotypically distinct change-of-function mutants for a particular trait (Greene et al., 2003; Shikazono et al., 2005). In contrast, physical mutagens usually cause deletions resulting in loss-of-function mutants (Kodym and Afza, 2003; Koornneef et al., 1982; Maple and Møller, 2007; Oladosu et al., 2016). Previously reported experiences with a large number of ornamental genera are valuable when starting mutation breeding for a new species or cultivar. Initial doses can be based on those experiences; thereafter, the treatment conditions may be fine-tuned.

In addition to the discussed advantages and disadvantages, different mutagens have different mutagenic efficiencies. Although gamma radiation has been the most common mutagen to date by far, multiple studies have shown that the efficiency of gamma rays is lower than that of EMS (Gautam et al., 1992; Girija and Dhanavel, 2009; Kaul and Bhan, 1977; Wani, 2009). There are options to improve efficiency, however. For gamma and, possibly, X-ray irradiation, the efficiency may be increased by irradiating plant tissue or seeds at lower dose rates for a longer amount of time. Neutron and heavy ion irradiation are also viable options, providing the same high penetration as traditional physical mutagens at higher efficiencies. Chemical mutagenesis may be improved by combining the use of EMS with genetic screening in a process known as TILLING.

There are many options when it comes to effective mutagens for ornamental plant breeding, and the value of random mutagenesis is underlined by the large number of entries in the mutant variety database. Although genetic engineering is becoming more accessible, it is often too expensive for use in ornamental breeding because of regulatory and development costs. Furthermore, knowledge of the procedures for modification and regeneration of ornamentals is often lacking. As a result, random mutagenesis will remain an important source of genetic diversity for the foreseeable future, with many opportunities to improve existing methods.

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