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## Review

# Mutation breeding of ornamental plants using ion beams

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Ornamental plants that have a rich variety of flower colors and shapes are highly prized in the commercial flower market, and therefore, mutant cultivars that produce different types of flowers while retaining their growth habits are in demand. Furthermore, mutation breeding is well suited for ornamental plants because many species can be easily vegetatively propagated, facilitating the production of spontaneous and induced mutants. The use of ion beams in mutation breeding has rapidly expanded since the 1990s in Japan, with the prospect that more ion beam-specific mutants will be generated. There are currently four irradiation facilities in Japan that provide ion beam irradiation for plant materials. The development of mutant cultivars using ion beams has been attempted on many ornamental plants thus far, and some species have been used to investigate the process of mutagenesis. In addition, progress is being made in clarifying the genetic mechanism for expressing important traits, which will probably result in the development of more efficient mutation breeding methods for ornamental plants. This review not only provides examples of successful mutation breeding results using ion beams, but it also describes research on mutagenesis and compares results of ion beam and gamma ray breeding using ornamental plants.

**Key Words:** ion beam, mutation breeding, ornamental plants.

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## Introduction

Mutation breeding is an important method for improving crops, with more than 3,200 mutant cultivars produced worldwide thus far (FAO/IAEA Mutant Variety Database). Ornamental plants with a rich variety of flower colors and shapes are highly prized, and the production of mutant cultivars that differ in these traits is in demand because all other growth habits are identical. Many species of ornamental plants, such as chrysanthemum (*Chrysanthemum* spp.), rose (*Rosa* spp.), and carnation (*Dianthus caryophyllus*), are vegetatively propagated, making it relatively easy to propagate mutants. Therefore, mutation breeding is well suited for breeding ornamental plants, as evidenced by the approximately 720 spontaneous and induced mutant cultivars that have already been produced.

One method for inducing mutations is using ion beam irradiation. Ion beams consist of ion particles that are accelerated by a cyclotron. The resulting high linear energy transfer (LET) radiation has greater biological effects on plants than low LET radiation, such as gamma rays and X-rays. The use of ion beams in plant mutation breeding was

first undertaken in the 1990s in Japan using the cyclotron in the Japan Atomic Energy Agency, the predecessor of the National Institutes for Quantum and Radiological Science and Technology (QST) and RIKEN. Mutation breeding using ion beams has spread quickly among researchers because of the expectation that ion beams, being different from gamma rays, will create new mutagens; this has probably renewed scientific interest in mutation breeding. Specifically, it appears that the report by Nagatomi *et al.* (1997) on the induction of the ion beam-specific flower-color mutant in chrysanthemum, based on an accumulation of research in mutation breeding using gamma rays, was particularly influential in creating great expectations on the usefulness of the “ion beam breeding” approach.

There are currently four irradiation facilities in Japan that can be used for irradiating plant materials: (1) the RIKEN RI Beam Factory at RIKEN, (2) the Takasaki Ion Accelerators for Advanced Radiation Application (TIARA) and (3) the Heavy-Ion Medical Accelerator in Chiba (HIMAC) at QST, and (4) the Wakasa Wan Energy Research Center Multi-purpose Accelerator with Synchrotron and Tandem (W-MAST) at the Wakasa Wan Energy Research Center. An ion beam irradiation facility for plant materials is also available in China [Dong *et al.* (2016) reviewed a plant mutation breeding program conducted at this facility].

The production of mutant cultivars using ion beams has been attempted on many ornamental flower species to date.

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Communicated by Ryutaro Aida

Received August 4, 2017. Accepted November 28, 2017.

First Published Online in J-STAGE on February 17, 2018.

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Moreover, research on mutagenesis has also been stimulated as a consequence of ion beam mutation research. Differences in mutational effects between ion beams and gamma rays have also been investigated, particularly in studies using ornamental plants, because flower color is one of the most important commercial traits; thus, color is a useful index of mutation effects because changes in color (in response to mutation) can be easily recognized. In addition, progress is being made in our understanding on genetic mechanisms responsible for expressing important traits in ornamental plants, such as flower color in chrysanthemum (*Chrysanthemum morifolium*; Ohmiya *et al.* 2006) and in cyclamen (*Cyclamen* spp.; Hase *et al.* 2012). Therefore, we are gaining a better understanding of mutation breeding based on these results, which will help researchers develop more efficient mutation breeding methods.

This review provides examples of successful mutation breeding results in ornamental plants using ion beams, outlines the progress of research on mutagenesis, and compares results of ion beam and gamma ray breeding approaches on ornamental plants.

### Induction of mutation and production of mutant ornamental plant cultivars using ion beams

Induction of mutation using ion beams has been attempted with many ornamental plant species produced and sold as cut flowers, potted plants, and bedding plants, including chrysanthemum (Asami *et al.* 2011, Furutani *et al.* 2008, Hisamura *et al.* 2016, Imakiire *et al.* 2006, Matsumura *et al.* 2010, Nagatomi *et al.* 1997, Okada *et al.* 2010, Okamura *et al.* 2015, Sakamoto *et al.* 2016, Shirao *et al.* 2007, Suzuki *et al.* 2005, Tamaki *et al.* 2017, Tamari *et al.* 2017, Tanokashira *et al.* 2014, 2016, Wakita *et al.* 2008), carnation (Okamura *et al.* 2003, 2012, 2013), rose (Hara *et al.* 2003, Yamaguchi *et al.* 2003), lily (*Lilium* spp.; Chiba *et al.* 2007, Chinone *et al.* 2007, Kondo *et al.* 2008), *Limonium* spp. (Chinone *et al.* 2008, Ogawa *et al.* 2014), *Gypsophila* spp. (Tsuji *et al.* 2008), *Gentiana* spp. (Tsuji *et al.* 2008), tulip (*Tulipa* spp.; Ikegawa *et al.* 2016), *Delphinium* spp. (Chinone *et al.* 2008, 2009), dahlia (*Dahlia* spp.; Hamatani *et al.* 2001, Uyama *et al.* 2013), *Dianthus* spp. (Sugiyama *et al.* 2008a), *Tricyrtis hirta* (Nakano *et al.* 2010), azalea (*Rhododendron* spp.; Kobayashi *et al.* 2009), *Asclepias* spp. (Kobayashi *et al.* 2011), hydrangea (*Hydrangea* spp.; Kodama *et al.* 2015), petunia (*Petunia* spp.; Miyazaki *et al.* 2002, Okamura *et al.* 2009), cyclamen (*Cyclamen* spp.; Ishizaka *et al.* 2012, Sugiyama *et al.* 2008b), begonia (*Begonia* spp.; Ito *et al.* 2007), *Dendrobium* spp. (Affrida *et al.* 2008), *Cymbidium* spp. (Yuki *et al.* 2007), *Osteospermum* spp. (Iizuka *et al.* 2008), salvia (*Salvia* spp.; Yamaguchi *et al.* 2017), verbena (*Verbena* spp.; Kanaya *et al.* 2008), cherry blossom (*Prunus* spp.; Ishii *et al.* 2009, 2011), and torenia (*Torenia* spp.; Miyazaki *et al.* 2006, Sasaki *et al.* 2008).

The main objective of mutation breeding (using ion beams) in ornamental plants is to obtain new flower colors

and shapes. It is also hoped that the use of ion beams will improve traits that affect the production of cut flowers, such as fewer malformed flowers (Hisamura *et al.* 2016), fewer lateral buds (Imakiire *et al.* 2006, Tamari *et al.* 2017) and the ability for early flowering at a low temperature (Sakamoto *et al.* 2016, Ueno *et al.* 2013) in white-flowered cultivars of the standard-type chrysanthemum. In addition, mutation breeding using ion beams has successfully produced two types of sterile mutants (males and females with non-functional mutations and self-incompatibility) in a *Verbena* sp. with a long inflorescence life (Kanaya *et al.* 2008) and in a mutant *Lilium* sp. with twisted anthers that produces less pollen (Kondo *et al.* 2008).

Traditionally, mutants were often produced using a combination of irradiation and *in vitro* techniques, such as regeneration from cells or re-differentiation from the callus, which allow generation of mutants via an efficient release of chimeras. However, the ion beam irradiation approach has also been used on cuttings (definite buds) in some mutation breeding programs, such as those involving cherry blossom (Ishii *et al.* 2009), salvia (Yamaguchi *et al.* 2017), rose (Hara *et al.* 2003, Yamaguchi *et al.* 2003), and chrysanthemum (Hisamura *et al.* 2016). A plant (including its shoot apical meristem) consists of three layers L1, L2, and L3 (outer to inner layers) in dicotyledonous angiosperms and two or three layers in monocotyledons (qtd. in van Harten 1998). For example, a mutated initial cell in the shoot apical meristem of the L1 layer grows a mutated sector only in the L1 layer, resulting in a mericlinal chimera. Then, a periclinal chimera mutant in the L1 layer is produced through the expansion of a mutated sector in the L1 layer by repeatedly cutting back. This method is available for plant species in which *in vitro* techniques have not yet been developed and it can eliminate the risk of introducing somaclonal variation during *in vitro* culture.

Because the epidermis (L1 layer) is the tissue responsible for flower color, usually a mutation only in the L1 layer can lead to a change in the flower color. It was thought that a flower color mutant shows the same growth as the original (non-mutated) plant because the L2 and L3 layers preserve their original genotype. Subsequently, it is believed that a combination of irradiation and cutting is adequate for producing mutant cultivars that can exhibit a variety of flower colors.

**Table 1** lists registered mutant cultivars of ornamental plants that have been induced with ion beams [More information on the main improved attribute is available in the database of cultivars registered under the Plant Variety Protection System of the Ministry of Agriculture, Forestry and Fisheries of Japan]. According to the FAO/IAEA Mutant Variety Database, mutant cultivars of ornamental plants induced with ion beams have only been developed in Japan. Because most of the main ornamental plants species are vegetatively propagated, the induced mutants can generally be used directly as the cultivar (direct use of induced mutant). While, there are also some example of indirect use of

**Table 1.** Main mutant cultivars of ornamental plants that were induced using ion beams

Scientific name	Common name	Name of cultivar	Parent cultivar	Main improved attribute of mutant cultivar	Notes	Reference
<i>Catharanthus roseus</i> (L.) G. Don	Madagascar periwinkle	Setofuku CAP	Setofuku CAMR	Altered flower color and petal shape		–
<i>Catharanthus roseus</i> (L.) G. Don	Madagascar periwinkle	Setofuku GPN	Kamihon BPN	Altered flower color		–
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Aladdin	Jimba	Few lateral buds		Imakiire <i>et al.</i> (2006)
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Imajin	Jimba	Few lateral buds		–
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Aladdin 2	Aladdin	Early flowering at a low temperature		Ueno <i>et al.</i> (2013)
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Ion-no-Kouki	Taihei	Altered flower color (Complex with light yellow and pink)	Flower color of parent cultivar 'Taihei' is pink.	Nagatomi <i>et al.</i> (2003)
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Ion-no-Mahou	Taihei	Altered flower color (Light orange on adaxial, dark yellow orange on abaxial side)	Flower color of parent cultivar 'Taihei' is pink.	Nagatomi <i>et al.</i> (2003)
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Ion-no-Koumyou	Taihei	Altered flower color (Complex with light yellow and light pink)	Flower color of parent cultivar 'Taihei' is pink.	Nagatomi <i>et al.</i> (2003)
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Ion-no-Seikou	Taihei	Altered flower color (Complex with light pink and yellowish orange)	Flower color of parent cultivar 'Taihei' is pink.	Nagatomi <i>et al.</i> (2003)
<i>Chrysanthemum</i> × <i>morifolium</i> Ramat.	Chrysanthemum	Ion-no-Hatsune	Taihei	Altered flower color (Complex with light pink and bright orange yellow)	Flower color of parent cultivar 'Taihei' is pink.	Nagatomi <i>et al.</i> (2003)
<i>Cyclamen</i> L.	Cyclamen	Tennyo-no-Mai	Uruwashi-no-Kaori	Altered flower color (salmon pink)	Flower color of parent cultivar 'Uruwashi-no-Kaori' is pink.	Ishizaka (2018)
<i>Delosperma</i> N. E. Br.	Delosperma	Reiko Pink Ring	Reiko	Altered flower color	Selfed line originated from ion-beam treated plant	–
<i>Delosperma</i> N. E. Br.	Delosperma	Reiko Rose	Reiko	Reduced plant height and flower diameter		–
<i>Dianthus caryophyllus</i> L.	Carnation	Misty Pink Vital Ion	Vital	Altered flower color (bi-colored) and petal shape	Flower color of parent cultivar 'Vital' is cherry pink.	Okamura <i>et al.</i> (2003)
<i>Dianthus caryophyllus</i> L.	Carnation	Dark Pink Vital Ion	Vital	Altered flower color (dark pink) and petal shape	Flower color of parent cultivar 'Vital' is cherry pink.	Okamura <i>et al.</i> (2003)
<i>Dianthus caryophyllus</i> L.	Carnation	Red Vital Ion	Vital	Altered flower color (red)	Flower color of parent cultivar 'Vital' is cherry pink.	Okamura <i>et al.</i> (2003)
<i>Osteospermum</i> L.	African daisy	Viento Flamingo	Mother Symphony	Altered flower color (pastel pink)	Flower color of parent cultivar 'Mother Symphony' is deep yellow.	Izuka <i>et al.</i> (2011)
<i>Osteospermum</i> L.	African daisy	Viento Labios	Viento Flamingo	Altered flower color (dark orange)	Flower color of parent cultivar 'Viento Flamingo' is pastel pink.	Okada <i>et al.</i> (2012)
<i>Pelargonium peltatum</i> (L.) L'Her.	Pelargonium	Fringe Fiorerosa	Kimura KM33	Very weak conspicuousness of zone on upper side of leaf blade, and altered shape and color of upper petal		–
<i>Prunus</i> L.	Cherry blossom	Nishina Zao	Gyoiko	Altered flower color (yellow)	Flower color of parent cultivar 'Gyoiko' is green.	Ishii <i>et al.</i> (2009)
<i>Prunus</i> L.	Cherry blossom	Nishina Otome	Keiouzakura	Perpetual		Ishii <i>et al.</i> (2011)
<i>Prunus</i> L.	Cherry blossom	Nishina Haruka	Shungetsuka	Bigger flower diameter	Crossing with two lines originated from ion-beam treated buds	–
<i>Salvia</i> L.	Salvia	TL585	Lady in Red	Plant height		–

Scientific name in **Table 1** is followed as described in database of varieties registered under the Japan's Plant Variety Protection System of MAFF.

induced mutants, such as the cherry blossom cultivar ‘Nishina Haruka’, which was produced by crossing two irradiated lines and the *Delosperma* cultivar ‘Reiko Pink Ring’ (originating from a selfed line from an ion beam-irradiated seed).

The development process for a flower-color mutant cultivar of a fragrant cyclamen is separately reported in this issue (Ishizaka 2018), whereas that for the chrysanthemum mutant cultivar ‘Aladdin 2’ is discussed below.

### Development of the chrysanthemum mutant cultivar ‘Aladdin 2’

Cut flowers of white-flowered cultivars of the standard-type chrysanthemum are extensively used at funerals or as offering flowers in Japan. Therefore, it is essential that these cultivars are highly productive so that cut flowers of the same quality of color and shape can be supplied in large quantities at a low cost.

‘Jinba’ is one of the main, standard-type, autumn-flowering chrysanthemum cultivar with white flowers. However, this cultivar produces many lateral buds, resulting in high labor costs associated with removing the buds; also, because of its late flowering at low temperature, its cultivation entails a high heating cost. Consequently, because ‘Jinba’ is an unregistered cultivar, its defects have been improved through line selection and by mutation breeding at research stations in several prefectures, including Kagoshima Prefecture (Imakiire *et al.* 2006), Fukuoka Prefecture (Ikegami *et al.* 2006), Oita Prefecture (Watanabe *et al.* 2008), and Aichi Prefecture (Asami *et al.* 2011).

‘Aladdin 2’ is the improved mutant cultivar of ‘Jinba’, which was developed in Kagoshima Prefecture. ‘Aladdin 2’ is a mutant with two targeted trait improvements: few lateral buds and early flowering at a low temperature. Originally, ‘Aladdin’ was produced by improving ‘Jinba’ so that it produced fewer lateral buds (Imakiire *et al.* 2006). ‘Aladdin 2’ was then produced by adding the characteristics of early flowering at a low temperature to ‘Aladdin’ (Ueno *et al.* 2013).

During the development of ‘Aladdin 2’, Ueno *et al.* (2013) showed that the mutant that exhibited early flowering at a low temperature was induced at a higher frequency by using ‘Aladdin’ than by using ‘Jinba’, indicating that the “re-irradiation” of the mutant cultivar ‘Aladdin’ with ion beams was the most efficient method for obtaining this characteristic.

Other studies have also shown that re-irradiation of mutants can increase the variation in certain traits. For example, the variation in flower color mutants is efficiently increased through re-irradiation of ion beam-irradiated mutants of *Osteospermum* spp. (Iizuka *et al.* 2008, Okada *et al.* 2012), cyclamen (Ishizaka 2018), and chrysanthemum (Sato *et al.* 2006). Further details about the re-irradiation of ion beam-irradiated lines, also known as “step-wise irradiation”, can be found in the study by Hase *et al.* (2012).

During the development of mutant cultivars by “re-irradiation”, Ueno *et al.* (2013) showed that the mutant ‘Imajin’, which has few lateral buds (as does ‘Aladdin’), but also has a reduced nuclear DNA content, was unsuitable as the parent plant for re-irradiation because candidates of the cultivar were not obtained from ‘Imajin’. This indicates the importance of mutant selection for re-irradiation in chrysanthemum. In chrysanthemum, the mutation frequency in a desired trait, for instance flower color, increases as the irradiation dose of ion beams and gamma rays increases (Yamaguchi *et al.* 2010). However, the mutation frequency of undesired traits in a plant also increases with increasing dosage as does the amount of reduction in its nuclear DNA content (Yamaguchi *et al.* 2010). The chromosome number in chrysanthemum is reduced by irradiation with gamma rays or X-rays (Dowrick and El-Bayoumi 1966, Ichikawa *et al.* 1970). Furthermore, the reduction in chromosome number correlates with a reduction in the diameter of inflorescences (Ichikawa *et al.* 1970) and a decrease in nuclear DNA content (Yamaguchi *et al.* 2010). Ueno *et al.* (2013) showed that the mutant ‘Imajin’, which had few lateral buds like ‘Aladdin’ but also had a reduced nuclear DNA content, was unsuitable as the parent plant for re-irradiation because mutants as candidates of the cultivar were not obtained from ‘Imajin’, highlighting the importance of mutant selection for re-irradiation in chrysanthemum. A similar result has been reported in sugarcane (Degi *et al.* 2001), wherein there is a significant correlation between the nuclear DNA content and the length and diameter of the stem. Thus, a reduced nuclear DNA content is undesirable for obtaining useful mutants of commercial cultivars, although only polyploidy plant species, such as chrysanthemum and sugarcane, may be able to survive a reduction in chromosome number usually associated with irradiation. Consequently, low-dose ion beam irradiation was used to irradiate ‘Jinba’ (Imakiire *et al.* 2006) and re-irradiate ‘Aladdin’ (Ueno *et al.* 2013) for preventing the incorporation of undesired mutations and a reduction in the nuclear DNA content.

Using the two technical points described above, ‘Aladdin’ was selected from approximately 10,000 plants that originated from ‘Jinba’ (Imakiire *et al.* 2006), whereas ‘Aladdin 2’ was selected from approximately 15,000 plants that originated from ‘Aladdin’ (Ueno *et al.* 2013).

### Research on mutagenesis using ion beams and ornamental plants

It was once believed that mutation was randomly induced. However, (using chrysanthemum) Nagatomi *et al.* (1997, 1998) showed that flower color mutants are obtained at a higher frequency when cultured petals rather than cultured leaves are irradiated with gamma rays. This result suggests that mutagenesis in genes related to flower color differ between petals and leaves. More recently, Okamura *et al.* (2015) confirmed this finding in a large-scale experiment using chrysanthemum. They found that the mutation

frequency of ion beam-irradiated petal clones is significantly higher than that of ion beam-irradiated leaf clones.

Hase *et al.* (2010) also demonstrated that flower color mutants were obtained at a higher frequency when sucrose-treated petunia seedlings (which contain a high concentration of anthocyanin) were irradiated with ion beams than when non-treated seedlings were irradiated, whereas there was no difference in the frequency of chlorophyll mutants between the two treatments. Hase *et al.* (2012) stated that this result indicated that the direction of mutation might be controlled, to some extent, by ion beam irradiation combined with a specific type of pretreatment.

Control of mutagenesis is expected to produce desired mutants at a higher frequency. It is thought that this could be achieved by changing the gene expression profile (Hase *et al.* 2012) and that a method for mutagenesis control could be established by clarifying the mechanism of gene regulation in various plant traits important for the production of crops. Moreover, an increased frequency of desired mutations obtained by controlling mutagenesis may make it possible to obtain a desired mutant even at low irradiation doses, thus reducing irradiation damage and mutation frequency of undesired traits. In many ornamental plants, it is easy to produce somaclones using *in vitro* culture techniques. Therefore, we expected that the science of mutation breeding is headed toward the development of methods that combine irradiation, *in vitro* culture, and treatments for controlling mutagenesis.

### Comparison between ion beams and gamma rays for mutation breeding using chrysanthemum

Chrysanthemum is a useful material for studying mutation breeding because the plant flowers uniformly and the flower color can be used as an index of mutation. Chrysanthemum is also an important commercial flower and has been produced with mutation breeding using gamma rays and X-rays. Thus, mutation studies performed using chrysanthemum are important from a practical standpoint. In this section, we compared the mutagenetic efficiency and width of the mutated sector produced by ion beam irradiation with that of the mutated sector produced by gamma rays using flower color mutation in chrysanthemum as an index.

**Mutagenetic efficiency:** Mutagen treatment not only induces mutation but also causes undesirable damage. Consequently, it has been suggested that the usefulness of any mutagen used in plant breeding depends not only on the mutation frequency per unit dose but also on its efficiency (Konzak *et al.* 1965), which is defined as the proportion of specific desirable mutagenic changes relative to plant damage (Konzak *et al.* 1965, Mikaelson *et al.* 1971, Nilan *et al.* 1965).

Yamaguchi *et al.* (2010) compared the mutagenetic efficiency of three types of ion beams (220 MeV carbon ions, mean LET = 107 keV/μm; 320 MeV carbon ions, mean LET = 76 keV/μm; and 100 MeV helium ions, mean

LET = 9 keV/μm) and gamma rays in chrysanthemum, using flower color as an index of mutation and the reduction in nuclear DNA content as an index of radiation damage. They found that there were differences between the three types of ion beams and gamma rays with respect to the mutation induction effect, nuclear DNA content, and the relationship between these. Specifically, the 220 MeV carbon ion beam appeared to be the most appropriate type of ion beam because it provided a high mutation frequency with low-intensity damage to the chromosomes, whereas the 100 MeV helium ion beam had a lower mutagenetic efficiency than the gamma rays. This result indicates that ion beams widely differ in their mutation efficiency, and thus their adequacy for use in mutation breeding. Ion beams of various ions and LET suitable for irradiation of plant materials are available at four facilities in Japan. Therefore, it is desirable to examine both mutation induction effects and any adverse effects that a particular ion beam might cause.

**Width of the mutated sector:** Each layer (L1, L2, and L3) contains one or a few initial cells (qtd. in van Harten 1988). When mutation is induced in one of the few initial cells containing apical meristem, shoots are formed into mericlinal chimera. To establish a mutant by irradiating the shoot apices, it is necessary to produce periclinal chimera from mericlinal chimera through the expansion of the mutated sector originated from a mutated initial cell by cutting back. The probability that reproductive organs and definite buds are formed from a mutated cell becomes greater when the width of the mutated sector is maximized, resulting in the high possibility that the mutation can be retained in its progeny.

Yamaguchi *et al.* (2009) estimated the width of a mutated sector using the segregation ratio of flower color mutants in plants that had been produced by irradiating their lateral buds with ion beams and gamma rays and then releasing them from their chimeric status by cutting them back twice. They found that the width of the mutated sector coding for flower color in the epidermis (L1 layer) was similar regardless of whether it was formed by ion beam or gamma ray irradiation. However, an analysis of the chimeric structure of flower color mutants, determined by comparing flower color expressed by the L1 layer of the mutants with that of plants that had been regenerated from their roots (L3 layer), suggested that some of the mutants obtained using ion beams were solid mutants. (i.e., both the L1 and L3 layers were derived from the same mutated cell). However, no such solid mutants were obtained using gamma rays. Thus, the expansion of the mutated sector through all layers of the shoot apex only occurs with ion beam irradiation.

It seems that these solid mutants were produced from sectorial chimeras that developed from a single mutated cell of only a few initial cells that had survived the ion beam irradiation. Yamaguchi *et al.* (2009) argued that the survival of these cells was because of two characteristics of ion beams: their ability to induce DNA damage in a limited region in the nucleus (Yang and Tobias 1979) and the relatively small number of ion particles that penetrate the cells as

estimated by Tanaka (1999). Thus, although there was no difference in the width of the mutated sector coding for flower color in the L1 layer (epidermis) following irradiation with ion beams and gamma rays, the mutated sector of a particular layer may be wider when it is formed by ion beams rather than by gamma rays (Yamaguchi *et al.* 2009).

### Concluding remarks

Many mutation cultivars created using ion beams have been produced in the past 25 years, indicating the usefulness of ion beams in mutation breeding. Differences between mutagens used in ion beams and gamma rays for mutation breeding is expected to be clarified further because currently, there are only a few published studies comparing irradiation effects of ion beams and gamma rays.

One objective of mutation breeding is to improve only one desired trait. I hope to identify the difference in ability of one trait improvement between ion beams and gamma rays because ion beams have the possibility of few affect to genetic background supposed from penetration of few ion particles to cell. For example, Tanaka *et al.* (2010) estimated that irradiation with 220 MeV carbon ions at a dose of 1 Gy resulted in four tracks being produced in a cell at TIARA in QST, whereas the same dose of gamma rays resulted in 2,000 spurs being produced in a cell. Thus, it is expected that ion beams cause less damage to chromosomes than gamma rays and X-rays, allowing them to be used to obtain mutants that only exhibit a change in the target trait with no effect on the remaining genetic background. This is a great benefit to the science of mutation breeding of ornamental plants.

To fully exploit these characteristics of ion beams, it is essential that ion beam irradiation is performed at low doses, which results in a low mutation rate. However, this problem may be solved by developing a more efficient mutant screening technique. Asami *et al.* (2010) developed an easy screening method for evaluating the non-branching characteristics of a chrysanthemum line in the juvenile plantlet and *in vitro* stages. Similarly, Hase *et al.* (2012) developed a polymerase chain reaction screening technique for evaluating irradiated deletion mutants at the early growth stage in cyclamen. Recent advances in clarifying the expression mechanism of important traits will probably assist in the development of a suitable screening technique. Furthermore, there are plans for establishing a technique for mutagenesis control in the future, building on the findings of Hase *et al.* (2010). Consequently, it is expected that there will be significant advancements in the use of ion beam breeding in the future.

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