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Electron Beam Irradiation for Quarantining Cut Flowers and Its Effect on the Postharvest Quality

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UNDER THE DIRECTION OF DR. KI SUN KIM SUBMITTED TO THE FACULTY OF THE GRADUATE SCHOOL OF SEOUL NATIONAL UNIVERSITY

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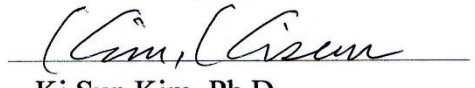
VICE-CHAIRMAN 
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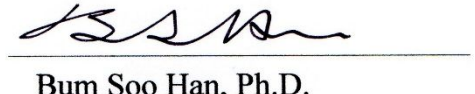
MEMBER 
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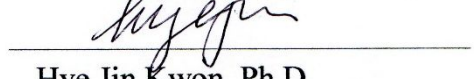
MEMBER 
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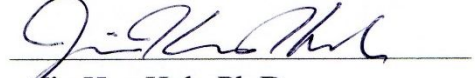
MEMBER 
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Electron Beam Irradiation for Quarantining Cut Flowers and Its Effect on the Postharvest Quality

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ABSTRACT

The main purpose of this study was to develop a reliable quarantine technology using electron beam irradiation that ensures the postharvest quality of cut flowers. Fresh weight, flower longevity, and flower bud opening of cut flowers decreased with increasing the irradiation dose. The effective irradiation doses for 10% reduction of postharvest quality (ED_{10}) was 269.7-448.4, 431.4-653.1, 144.4-306.3, 451.6, and 841.2 Gy in the roses, chrysanthemums, lilies, carnations, and eustomas, respectively. It is conceivable that tolerance of cut flowers to electron beam irradiation vary according to species and cultivars. Radiation-induced failure of flower opening may be due to insufficient osmotic pressure, discontinuation of the persistent sugar supply, DNA damage, and cell death. A trend toward increase in osmolarity was maintained up to day 6 in 'Siberia' lily flowers irradiated $\leq$ 200 Gy, while osmolarity was less increased or gradually decreased during bud development of flowers irradiated at $\geq$ 400 Gy. Total soluble

sugars of the tepal irradiated at ≤ 200 Gy gradually increased with the developmental bud stage, and then decreased between day 4 and 8. In the tepal irradiated at ≥ 400 Gy, it increased at day 2, while followed by a decline between day 2 and 6. When subjected to electron beam irradiation, 'Siberia' and 'Medusa' tepal cells showed broad CV of G0/G1 peaks, high percentage of Background Aggregates and Debris (BAD), and apoptotic peaks in a dose-dependent manner. Trypan blue staining also showed a radiation-induced cell death in tepals of 'Siberia' and 'Medusa' lilies irradiated at ≥ 400 and ≥ 100 Gy, respectively. 'Medusa' lilies were more sensitive to irradiation than 'Siberia'. Sucrose treatment enabled more 'Siberia' lily flowers to open fully. Pretreatment substances were less effective in extending flower longevity and in preventing fresh weight loss as the radiation dose increased in both 'Siberia' lily and 'Leopard' chrysanthemum flowers. In 'Leopard', pretreatment combined with preservative solution was effective in preventing radiation-induced deterioration. The effects of electron beam irradiation on control of *Botrytis cinerea*, troublesome to cut flowers during storage and shipment were examined. Electron beams inhibited spore germination and mycelial growth of *B. cinerea* with increasing irradiation doses. Conidia of *B. cinerea* were more radiation resistant than mycelia: the effective irradiation doses for 50% inhibition (ED_{50}) of spore germination and mycelial growth were 2.02 and 0.89 kGy, respectively. Electron beam irradiation was more effective in reducing mycelial growth of *B. cinerea* at 10°C than at 20°C. The results suggest that lowering the effective doses to inactivate *B. cinerea* throughout low temperature helps reduce radiation-induced damage of commodity. Therefore, electron beam irradiation can be available as a quarantine treatment without

detriment to postharvest quality of cut flowers if optimal postharvest treatment and low temperature is applied, considering that an irradiation dose of 400 Gy is established to be effective for quarantine security.

Keywords: *Botrytis cinerea,* cell death, cut flower, DNA damage, electron beam irradiation, osmolarity, postharvest quality, preservative solution, pretreatment, quarantine, sugar, temperature, tolerance

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CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL INTRODUCTION

The world floriculture industry is in a state of unrest, with drastic changes in supply and demand positions. New markets as well as new suppliers are emerging and disappearing in short span of time (van Liemt, 1999). Although the growth of international trade in floriculture is significantly affected by the recent global economic crisis, the global flower exports more than doubled from US$ 7.18 billion in 2001 to US$ 17.87 billion in 2012, an average annual growth of 9% (ITC, 2013). Flower exports in Korea have sharply increased since the 1990s as the floriculture industry has grown. Flower exports expanded 3.6 times, growing from US$ 28.89 million in 2000 to US$ 103.07 million in 2010, with cut flowers leading the exports. These cut flower exports centered on roses, chrysanthemums, and lilies, with three major export items comprising over 73% of flower exports. Rose is the most extensively cultivated cut flower grown for exporting, accounting for over 33% of total flower exports. Chrysanthemum exports have been on the rise every year, increasing from US$ 4.68 million in 2000 to US$ 13.82 million in 2010. There has been sharply increasing lily exports from US$ 4.40 million in 2000 to US$ 27.85 million in 2010, it has grown into the leading flower export recently (KATI, 2010).

Exported cut flowers should be certify conformity with the phytosanitary requirements provided by importing countries in order to prevent the introduction of new invasive pests and diseases. Actions which may be taken when an imported cut flowers does not comply with regulations and is initially refused entry include: treatment, reshipment, and destruction (FAO, 2004). Phytosanitary

treatments is an “official procedure for the killing, inactivation or removal of pests, or for rendering pests infertile or for devitalization”, including mechanical, chemical, irradiation, physical (heat, cold), and controlled atmosphere treatments (FAO, 2007).

Methyl bromide, the predominant fumigant for phytosanitary purposes, is commonly used to disinfest or disinfect cut flowers to satisfy quarantine requirements (APHIS, 2014b). Although methyl bromide is a colorless, clear, nonflammable, and extremely volatile, it easily penetrates commodities and some plant species are sensitive to its use (Van den Oever et al., 1982). Some cut flowers may show severe damage and unacceptable deterioration in quality by exposure to methyl bromide (FAO, 1984). Damage includes reduced shelf life, burning on leaves and petals, and discolored pitting of the peel (Hatton and Cubbedge, 1979). Furthermore, methyl bromide was listed as an ozone-depleting substance, and the Montreal Protocol of the United Nations Environment Programme (UNEP) recommends that methyl bromide be phased out by 2005 in developed countries and by 2015 in developing countries (UNEP, 2012). Although the Montreal Protocol explicitly exempts quarantine and pre-shipment (QPS) use of methyl bromide from regulation, the International Plant Protection Convention (IPPC) promulgated a recommendation for replacement or reduction of the use of methyl bromide as a phytosanitary measure (IPPC, 2008). Numerous chemicals have been considered as alternative fumigants to methyl bromide, however none are as fast acting as it. Typically, cut flowers must be marketed quickly before a loss in quality occurs, so rapid fumigation is essential (Fields and White, 2002). Furthermore, these alternatives have some limitations, such as phytotoxicity

(phosphine, hydrogen cyanide, carbonyl sulfide, and ethyl formate), unacceptable residues (carbon disulfide), slow activity (phosphine), and health or economic reasons (Fields and White, 2002; Hansen et al., 1991; Weller and van S. Graver, 1998).

There has been a growing interest in irradiation as a phytosanitary treatment due to its non-chemical, residue-free approach (FAO, 2003). Irradiation promises rapid, efficacious, and approved quarantine treatments for many pests while causing minimal damage to agricultural commodities at the doses used to control pests (Follett, 2009). Additionally, irradiation can be applied to the commodity after packaging and may even extend shelf life (Follett and Griffin, 2006). Ionizing radiation for phytosanitary treatment may be provided by radioactive isotopes (γ-rays from the ions Co^{60} and Cs^{137}), accelerated electrons, or x-rays (FAO, 2003). Compared with γ-rays, electron beam irradiation has been considered to be safer, more cost-effective, shorter exposure time, and superior to dose rate (Rami Reddy et al., 2006). In addition, electron beams are generated electrically, they can be turned on and off as needed (Gomes et al., 2008). On the basis of the extensive research carried out to estimate and confirm the minimum absorbed dose for a specific pest treatment, the IPPC adopted an international standard for the use of irradiation as a phytosanitary treatment (FAO, 2003). The Animal and Plant Health Inspection Service (APHIS), the United States Department of Agriculture agency also approved irradiation as a phytosanitary treatment for all imported fruits and vegetables (APHIS, 2014a). Irradiation has not yet been contained in phytosanitary treatment schedules for cut flowers because the efficacy data were insufficient.

Flowers can also be damaged by irradiation, depending on the tolerance of flowers to irradiation as well as the irradiation dose (Hayashi et al., 1998). Postharvest quality studies should be done on commodities in conjunction with pest disinfestation tests to make sure that treatments do not lead to unacceptable levels of injury to the commodity. Therefore, it is essential to determine the appropriate dose level for irradiation of cut flowers without detriment to their quality and the adequate postharvest treatment for enhancing tolerance of flowers to irradiation.

International trade of cut flowers relies heavily on low temperature and high humidity handling to retard flower senescence, which may favor fungal pathogens like *Botrytis cinerea. B. cinerea,* a widespread necrotizing fungus that infects a variety of flower crops (Hammer et al., 1988), significantly reduces the ornamental value of the cut flowers. The damage can occur during various stages of plant growth and in postharvest during storage, transport, and shipment (Elad, 1988). Increasing worldwide demand for high quality floricultural produce requires due attention by industry to postharvest disease management (Dinh and Joyce, 2007). Irradiation is one of the most promising approach. Irradiation can be used for the purpose of reducing the attack of pathogens and maintaining the postharvest quality of fresh commodities. Irradiation allows the shelf life of fruits and vegetables to be extended throughout the inactivation or delay of the development of microorganisms which cause deterioration and quality loss during postharvest (Gomes et al., 2008).

The main goal of this study was to develop a reliable quarantine technology using electron beam irradiation that ensures the postharvest quality of cut flowers.

The specific objectives were (1) to verify the tolerance of cut flowers to electron beam irradiation, (2) to examine the effect of electron beam irradiation on flower opening, (3) to determine whether radiation-induced deterioration of cut flowers can be prevented with postharvest treatments, and (4) to assess the effect of electron beam irradiation on control of *B. cinerea*.

LITERATURE REVIEW

Ionizing Radiation

Radiation is defined as energy travelling through a distance in the form of waves or particles. Ionizing radiation has enough energy to directly affect the structure of atoms of impacted materials, breaking chemical bonds on account of the electromagnetic spectrum or high-energy particles (Shu et al., 2012). The action of ionizing radiation on living organisms can be divided into direct and indirect effects. In direct effects, radiation energy is deposited directly in the targets. DNA in the chromosomes represents the most critical target for ionizing radiation. In indirect effects, energy is absorbed by the external medium, which leads to the production of diffusible intermediates that then attack the targets. When an ionizing radiation passes through a water molecule, highly reactive free radicals such as $OH^{\bullet}$, $H^{\bullet}$, and hydrated electrons are produced, which induce DNA damage indirectly (Sharma, 2004). The action of the hydroxyl radical ($OH^{\bullet}$) is the most important, these radicals formed in the hydration layer around the DNA molecule are responsible for 90% of DNA damage. Thus, in living cells, the indirect radiation damage is especially significant, resulting in a break in the phosphodiester backbone in one strand of the molecule (single-strand break) or in

both strands at the same place (double-strand break) (Aquino, 2012). Irradiated organisms such as microorganisms, insect gametes, and plant meristems are prevented from reproducing because of the inhibition of cell division (Arvanitoyannis et al., 2009). Therefore, irradiation can be used for insect disinfestation, inhibition of sprouting, alteration of ripening and senescence, control of postharvest disease, and inducing mutagenesis.

Phytosanitary Irradiation

Irradiation as a phytosanitary treatment is increasing in commercial use because it has some advantages over other treatments, such as applicability to packed commodities and broad tolerance by fresh commodities (Heather and Hallman, 2008). Because ionizing radiation penetrates commodities quickly, its treatment time short, and the required dose cannot change the commodity's temperature, most commodities can tolerate irradiation at doses that control the pest (Follett, 2009).

Radiotolerance can vary with insect taxa and normally increases as the developmental stage of the insect progresses. Thus, adults are usually the most tolerant, followed by pupae, larvae, and then eggs, being the least tolerant (Kader, 1986). It has generally been accepted that female insects are more sensitive to irradiation than males (Hallman, 2000). Unlike other treatments, irradiation does not need to kill the target pests immediately to provide quarantine security, therefore live pests may occur with the exported commodity. The objective of phytosanitary irradiation is to prevent reproduction, thus prevention of adult emergence, induction of adult sterility, or F_1 sterility should be required for a radiation treatment (Follett, 2009).

Table 1. Phytosanitary irradiation treatments approved by USDA-APHIS.

International Standards for Phytosanitary Measures (ISPM) No. 18 provides technical guidance on the specific procedures for the application of ionizing radiation as a phytosanitary treatment for regulated pests or articles (FAO, 2003). Pest-specific minimum absorbed doses have been determined in accordance with the requirements outlined in ISPM 18, several phytosanitary irradiation treatments approved by APHIS and IPPC (Table 1) (APHIS, 2014b; FAO, 2003).

Scientific name	Common name	Minimum absorbed dose (Gy)
Anastrepha ludens	Mexican fruit fly	70
Anastrepha obliqua	West Indian fruit fly	70
Anastrepha serpentina	Sapote fruit fly	100
Anastrepha suspensa	Caribbean fruit fly	70
Aspidiotus destructor	Coconut scale	150
Bactrocera cucurbitae	Melon fruit fly	150
Bactrocera dorsalis	Oriental fruit fly	150
Bactfrocera jarvisi	Jarvis fruit fly	100
Bactfrocera tryoni	Queensland fruit fly	100
Brevipalpus chilensis	Chilean false red mite	300
Ceratitis capitata	Mediterranean fruit fly	100
Conotrachelus nenuphar	Plum curculio	92
Copitarsia declora		100
Cryptophlebia ombrodelta	Litchi fruit moth	250
Cryptophlebia illepida	Koa seedworm	250
Cylas formicarius elegantulus	Sweet potato weevil	150
Cydia pomonella	Codling moth	200
Euscepes postfasciatus	West Indian sweet potato	150
Grapholita molesta	Oriental fruit moth	200
Omphisa anastomosalis	Sweet potato vine borer	150
Pseudaulacaspis pentagona	White peach scale	150
Rhagoletis pomonella	Apple maggot	60
Stermochetus frigidus	Mango pulp weevil	165
Stermochetus mangiferae	Mango seed weevil	300
Fruit flies in the Family Tephritidae not listed above		150
Plant pests of the Insecta not listed above, except Lepidoptera pupae and adults		400

Moreover, irradiation has been very amenable to generic doses, one dose serves for a group of pests and commodities although not all have been tested for efficacy (Follett, 2009; Hallman, 2012). Currently, two broad generic treatments, 150 Gy for all Tephritidae and 400 Gy for all Insecta except pupal and adult Lepidoptera, are approved for use on imports to the United States (Table 1).

Irradiation has been documented to be effective against various quarantine pests of cut flowers. Preliminary tests on armyworm, *Spodoptera litura*, suggested that the fecundity of adults developed from irradiated pupae is entirely inhibited at 150 Gy (Yun et al., 2014). Koo et al. (2012) also reported that a minimum dose of 150 Gy should be sufficient for female sterilization of American serpentine leafminer fly, *Liriomyza trifolii*. Moon et al. (2010) suggested that a radiation dose of 100 Gy applied to pupae of diamondback moth, *Plutella xylostella*, might be sufficient to sterilize adults and stop egg hatch. When *Bemisia tabaci*, *Myzus persicae*, and *Tetranychus urticae* adults were irradiated with 100 Gy, fecundity decreased and egg hatch was inhibited (Moon et al., 2010). If the efficacy of this dose can be confirmed through large-scale tests, irradiation would be an effective mitigation option for cut flower export.

Irradiation as a Postharvest Application of Fresh Fruits and Vegetables

Fresh horticultural commodities can exhibit certain life processes such as deterioration of postharvest quality during the period between harvest and consumption. Irradiation can be used to arrest these changes (Arvanitoyannis et al., 2009). When tubers and bulbs are treated with ionizing energy, remarkable morphological and histological changes in dormant buds are induced. Irradiation

processing with doses ranging from 50 to 150 Gy has been shown to inhibit sprouting of potato, ginger, sugar beet, turnip, carrot, onion, and garlic (Sharma, 2004). Doses below 150 Gy also prevent elongation and curvature of asparagus, but higher doses are detrimental to quality and storage life (Kader, 1986).

Ionizing radiation acts on cellular metabolism and, therefore, delays ripening and senescence of some fruits and vegetables by inducing a global decrease in respiratory activity, ethylene synthesis, and moisture loss (Kader, 1986; Lacroix and Ouattara, 2000). The ripening and senescence of tropical fruits including banana, mango, papaya, and guava, can be inhibited using doses between 250 and 350 Gy (Kader, 1986). Some temperature-zone fruits, such as apple, pear, and apricot, require much higher doses (> 1 kGy) for effective inhibition of ripening (Wani et al., 2007). However, irradiation can also cause biochemical and textural changes. Examples include increased surface blemishes, peel pitting, internal browning, skin discoloration, and softening (Kim et al., 2010; Moreno et al., 2006; Yu et al., 1996). Therefore, it is important to consider an approach that maintains quality, extends shelf life, and offers economic incentives for production and commercialization of this commodity.

Control of Postharvest Diseases by Irradiation

Microbial decay is one of the main factors lowering the quality of the fresh horticultural commodities. Although fungicides will probably remain a major tool to control postharvest diseases, their overuse has led to proliferation of fungicide resistance within pathogen populations. They are also becoming more unacceptable due to negative effect of fungicide residues on human health and

environment (Dinh and Joyce, 2007).

Irradiation has the potential to control or eliminate microbial pathogens. The DNA in the chromosome is the most critical target of ionizing radiation. The correlation of radiation sensitivity is roughly inversely proportional to the size of targets (Borrely et al., 1998). There is considerable variability in radiotolerance between microbial species; in general, viruses are more radiation resistant than bacterial spores, which in turn are more resistant than vegetative organisms, yeasts and molds. Moreover, resistance of microorganisms is affected not only by environmental conditions during irradiation such as water content, temperature, and nature of the suspending medium, but also by post-irradiation conditions (Aquino, 2012).

The feasibility of irradiation has recently been evaluated as a pathogen decontamination treatment of fruits and vegetables. Farkas et al. (1997) showed that γ-radiation at 1 kGy reduced loads of bacteria, improved microbiological shelf life, and extended sensory quality of pre-cut bell peppers and carrots. Prakash et al. (2000) found that cut romaine lettuce irradiated at 0.35 kGy decreased aerobic counts by 1.5 logs and the difference was maintained through 22 days of storage at 4^{o}C. Hagenmaier and Baker (1997) found that commercially prepared fresh-cut lettuce irradiated at 0.19 kGy significantly reduced microbial populations for 8 days. Zhang et al. (2006) also showed that the number of aerobic mesophilic bacteria on fresh cut lettuce irradiated with 1 kGy was reduced by 2.35 logs and also proved that 1 kGy irradiation appeared to be the best treatment for maintaining quality of fresh-cut lettuce. The added advantage in this case was that the sensory quality was maintained for a period of 8 days at 4°C.

The potential use of ionizing radiation to control postharvest diseases depends on the radiation sensitivity of pathogen relative to the ability of the fresh commodity to withstand the required radiation level with little acute damage or other detrimental effects (Gomes et al., 2008). The challenge is how to reduce the damage to produce quality while applying sufficient doses of radiation to inactivate pathogens.

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CHAPTER I

Tolerance of Cut Flowers to Electron Beam Irradiation

ABSTRACT

Effects of electron beam irradiation on the postharvest quality of cut flowers were examined. Cut flowers were irradiated with electron beam at 100, 200, 400, 600, 800, 1,000, and 2,000 Gy with a 10 MeV linear electron beam accelerator to evaluate their irradiation tolerance. Postharvest quality was determined by monitoring fresh weight loss, flower longevity, flower bud opening, flower diameter, visual quality of flowers and leaves, and chlorophyll content. Flower longevity and fresh weight of cut flowers decreased with increasing the irradiation dose. Flower bud opening was also inhibited in a dose-dependent manner. The effective irradiation doses for 10% reduction of postharvest quality (ED_{10}) was 269.7, 434.1, 448.4, 363.0, and 373.2 Gy in 'Decoration', 'Il se Bronze', 'Queen Bee', 'Revue', and 'Vivian' roses, respectively. ED_{10} values were 431.4, 653.1, and 574.1 Gy in 'Baekma', 'Baekseon', and 'Leopard' chrysanthemums, respectively. ED_{10} values were 144.4, 306.3, and 243.4 Gy in 'Medusa', 'Siberia, and 'Augusta' lilies, respectively. ED_{10} values were 451.6 and 841.2 Gy in the 'Montezuma' carnations and 'Rosina White' eustomas, respectively. Although tolerance of cut flowers to electron beam irradiation varied with species and

cultivars, chrysanthemum, carnation, and eustoma were tolerant when the dose of 400 Gy was considered as the minimum value for tolerance of the cut flowers.

Additional keywords: flower bud opening, flower longevity, irradiation tolerance, postharvest quality

INTRODUCTION

Postharvest quality studies should be done on commodities in conjunction with pest disinfestation testing to ensure that treatments do not cause unacceptable injury to the commodity. Flowers can also be damaged by irradiation, depending on the tolerance of flowers to irradiation as well as the irradiation dose (Hayashi et al., 1998). Therefore, the appropriate dose level for electron beam irradiation is needed to be determined for cut flowers without detriment to their quality.

Hayashi et al. (1998) reported that carnation, alstromeria, gladiolus, tulip, statice, stock, dendrobium, prairie gentian, oncidium, campanula, gloriosa, fern, gypsophila, freesia, lobelia, triteleia, and gerbera were tolerant to electron beams at 400-600 Gy, while chrysanthemum, rose, lily, calla, anthurium, sweet pea, and iris were intolerant. Kikiuchi (2003) also reported that the dose of 300 Gy was considered as the minimum value for tolerance of the flowers to γ- and electron beam irradiation. *Lilium speciosum*, *Alpinia purpurata*, and *Lisianthus* sp. were tolerant to both kinds of radiation. However, previous investigations have been limited by the lack of explanation about cultivar, harvest stage, conditioning methods, test room conditions, and flower longevity terminating symptoms. Few studies have been performed to prove dose-response relationship between irradiation dose and postharvest quality of cut flowers for sufficiently accurate dose estimation.

The present study was conducted to examine the effect of electron beam irradiation on the postharvest quality of cut flowers and to verify the tolerance to electron beam irradiation.

MATERIALS AND METHODS

Plant Materials

Rose. Cut rose flowers (*Rosa hybrida* 'Decoration', 'Il se Bronze', 'Queen Bee', 'Revue', and 'Vivian') were obtained from Rosepia (Jeonju, Korea). Flowers were harvested at stage I, with very tight buds showing only the outer petals clearly visible, according to the chronological stages of development by Ho and Nichols (1977).

Chrysanthemum. Two standard-type cultivars, *Dendranthema grandiflorum* 'Baekma' and 'Baekseon', were used for experiments. 'Baekma' flowers were obtained from Rosepia, whereas 'Baekseon' flowers were obtained from Yesan Chrysanthemum Export Complex (Yesan, Korea). Flowers were harvested at stage III according to the chronological stages of development suggested by Yoo and Roh (2012b). Cut 'Leopard' flowers, spray-type chrysanthemum, were obtained from Gumi Infrastructure Corporation (Gumi, Korea). Flowers were harvested at stage III, with 30-40% of flowers fully opened, according to the chronological stages of development suggested by Yoo and Roh (2012a).

Lily. Three lily cultivars, *Lilium* Oriental hybrid 'Medusa', *L.* Oriental hybrid 'Siberia', and *L. longiflorum* 'Augusta' were used for experiments. 'Medusa' lilies were obtained from Guidoon Flower Export Complex (Inje, Korea). 'Siberia' and 'Augusta' lilies were purchased from a local wholesaler in Seoul, Korea. Flowers were at the commercial harvest stage, with the largest bud showing color.

Carnation. Cut carnation flowers 'Montezuma' (*Dianthus caryophyllus* 'Montezuma') were purchased from a local wholesaler in Seoul, Korea. Flowers

were at the commercial harvest stage, paint brush stage.

Eustoma. Cut eustoma flowers 'Rosina White' (*Eustoma grandiflorum* 'Rosina White') were purchased from a local wholesaler in Seoul, Korea. Flowers were at the commercial harvest stage, with the largest bud showing color.

Electron Beam Irradiation

Electron beam irradiation was conducted in EB-Tech Co., Ltd. (Daejeon, Korea) using a high energy linear accelerator (UEL V10-10S, 10 MeV). Cut flowers were treated with doses of 100, 200, 400, 600, 800, 1,000, and 2,000 Gy. Target doses were monitored by dosimetry with a radiochromic film dosimeter (GAF3002DS, GEX Corp., Centennial, CO, USA) (ISO/ASTM51275:2004(E)).

Quality Evaluation

After irradiation, stems were recut to 30 cm for rose and eustoma flowers, to 40 cm for spray-type chrysanthemum, lily, and carnation flowers, and to 50 cm for standard-type chrysanthemum flowers. After re-cutting, stems were placed in distilled water and then kept in an air-conditioned room with a 12 h light cycle at 23 ± 1°C, a relative humidity of 60 ± 10%, and a leaf level photosynthetic photon flux density at 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool white fluorescent lamps.

Postharvest quality was determined by monitoring fresh weight loss, flower longevity, flower bud opening, flower diameter, visual quality of flowers and leaves, and chlorophyll content. Flower longevity was determined with senescence symptoms according to the general criteria. A flower was considered senesced when the rose flower showed bent-neck or wilting, more than 50% of the

chrysanthemum flowers have wilted or turned brown, the lily flower has wilted or dropped, the carnation flower has shrunk or shown brown discoloration, and more than 50% of the eustoma flowers have wilted. Flower bud opening was rated on a 5-point scale from 1 (very tight) to 5 (fully open). Flower diameter was measured at the widest point by using a digital caliper (ABS Digimatic Caliper, Mitutoyo Co., Ltd., Tsukuba, Japan). To evaluate aesthetic quality, an index of visual quality of flowers and leaves was applied. The scale ranged from 1 (very poor) to 5 (excellent) based on the amount of discoloration and necrosis. The chlorophyll content was measured using a SPAD-502 meter (Konica-Minolta, Tokyo, Japan). The experiments were replicated five times.

Determining Dose Effects

Dose effects were determined by dose response relationship between irradiation dose and postharvest quality. Postharvest quality loss was measured using parameters, such as flower longevity, flower diameter, flowering rate, visual quality of flowers and leaves, and chlorophyll content. Dose effects were estimated using the following equation: Dose effects (%) = B/A × 100, where A = parameter value of non-irradiated flowers and B = parameter value of irradiated flowers. Nonlinear regression was conducted to determine the dose effect by Sigma Plot, version 10.0 (SPSS Science, Chicago, IL, USA). The four-parameter logistic sigmoidal curves was used to determine the effective irradiation doses for 10% reduction of postharvest quality (ED_{10}) and was calculated as $y = y0 + a/(1 + (x/x0)^b)$.

Statistical Analysis

Statistical analysis was performed using SAS software, version 9.2 (SAS Inst., Cary, NC, USA). Differences among the group means were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test at 5% level.

RESULTS

Rose

In ‘Decoration’, longevity of the flowers irradiated up to 600 Gy was similar to that of non-irradiated flowers (Table I-1). Flower senescence as indicated by discoloration, wilting, and bent-neck was rapidly progressed in flowers irradiated at ≥ 800 Gy, while the flowers irradiated at 100 Gy showed a slight increased longevity. Longevity was not significantly different in the flowers irradiated up to 800 Gy in ‘Il se Bronze’, up to 600 Gy in ‘Queen Bee’ and ‘Revue’, and up to 200 Gy in ‘Vivian’ from those non-irradiated. In these cultivars, the irradiation doses ranging from 100 to 200 Gy were effective in extending flower longevity. Flower bud opening was evaluated as a potential indicator of irradiation sensitivity (Table I-1, Fig. I-1). Flower bud opening was inhibited in a dose-dependent manner, whereas no significant differences between the flowers irradiated up to 400 Gy and control were found. Flower buds irradiated at ≥ 1,000 Gy did not develop, but wilted in a closed state. The fresh weight of cut roses decreased with increasing the irradiation dose (Fig. I-2). Flowers irradiated up to 200 Gy showed a similar declining pattern in fresh weight to control. Furthermore, fresh weight of flowers irradiated at 2,000 Gy was sharply declined. ED_{10} values were 269.7, 434.1, 448.4, 363.0, and 373.2 Gy in ‘Decoration’, ‘Il se Bronze’, ‘Queen Bee’, ‘Revue’, and ‘Vivian’, respectively (Fig. I-3). Therefore, ‘Il se Bronze’ and ‘Queen Bee’ were tolerant to irradiation dose of 400 Gy, while ‘Decoration, ‘Revue’, and ‘Vivian’ were not. These observations were attributed to the irradiation sensitivity of cut roses vary according to cultivars.

Table I-1. Effects of electron beam irradiation on flower longevity and flower bud opening in five rose cultivars.

Irradiation dose (Gy)	Decoration	Il se Bronze	Queen Bee	Revue	Vivian
			Flower longevity (day)		
0	7.4 ab[z]	7.3 a	6.5 ab	9.8 a	8.0 a
100	7.6 a	7.3 a	6.5 ab	9.3 ab	8.3 a
200	7.2 abc	7.5 a	7.0 a	10.0 a	8.2 a
400	6.6 bcd	7.0 a	5.8 bc	9.8 a	6.8 b
600	6.6 bcd	6.7 ab	5.8 bc	9.2 ab	6.4 bc
800	6.4 cd	6.5 ab	5.0 c	6.8 b	5.5 cd
1000	6.4 cd	5.3 b	5.0 c	6.8 b	5.5 cd
2000	6.0 d	5.3 b	5.0 c	6.8 b	4.5 d
Significance	***	*	**	*	****
			Flower bud opening[y]		
0	4.2 a[y]	4.2 a	4.4 a	4.2 a	4.4 a
100	4.0 a	4.4 a	4.2 ab	4.0 ab	4.2 a
200	3.8 ab	4.4 a	4.2 ab	4.2 a	4.0 a
400	3.6 abc	3.8 ab	4.0 ab	3.6 abc	3.8 ab
600	3.2 bcd	3.4 bc	3.6 bc	3.2 bcd	3.2 bc
800	3.0 cd	2.8 cd	3.2 cd	2.8 cd	2.6 cd
1000	2.6 d	2.8 cd	2.8 d	2.6 d	2.4 d
2000	2.6 d	2.6 d	2.6 d	2.6 d	2.4 d
Significance	**	****	****	**	****

[z]Duncan's multiple range test within columns for each experiment, $P = 0.05$.
[y]Based on a scale of 1 to 5. 1 = very tight bud, only the outer petals are clearly visible; 2 = bud starts to open, the tips of most inner petals are visible; 3 = bud is open, the outer petals are starting to stand out; 4 = bud is open, the outer petals are fully expanded, the flower has not yet reached its maximum size; 5 = bud is fully open, all petals are fully expanded, the flower has reached its maximum size.
*,**,***,****Significant at $P = 0.05$, 0.01, 0.001, or 0.0001, respectively.

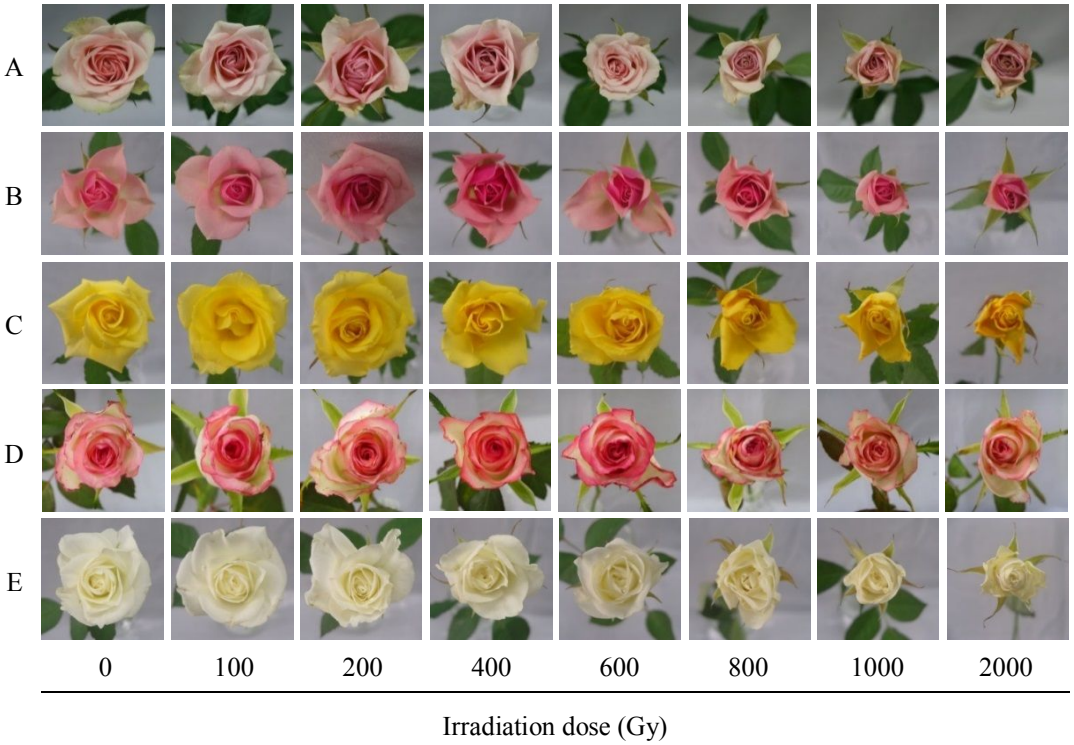

Fig. I-1. Appearance of ‘Decoration’ (A), ‘Il se Bronze’ (B), ‘Queen Bee’ (C), ‘Revue’ (D), and ‘Vivian’ (E) rose flowers at 6 days after electron beam irradiation at various doses.

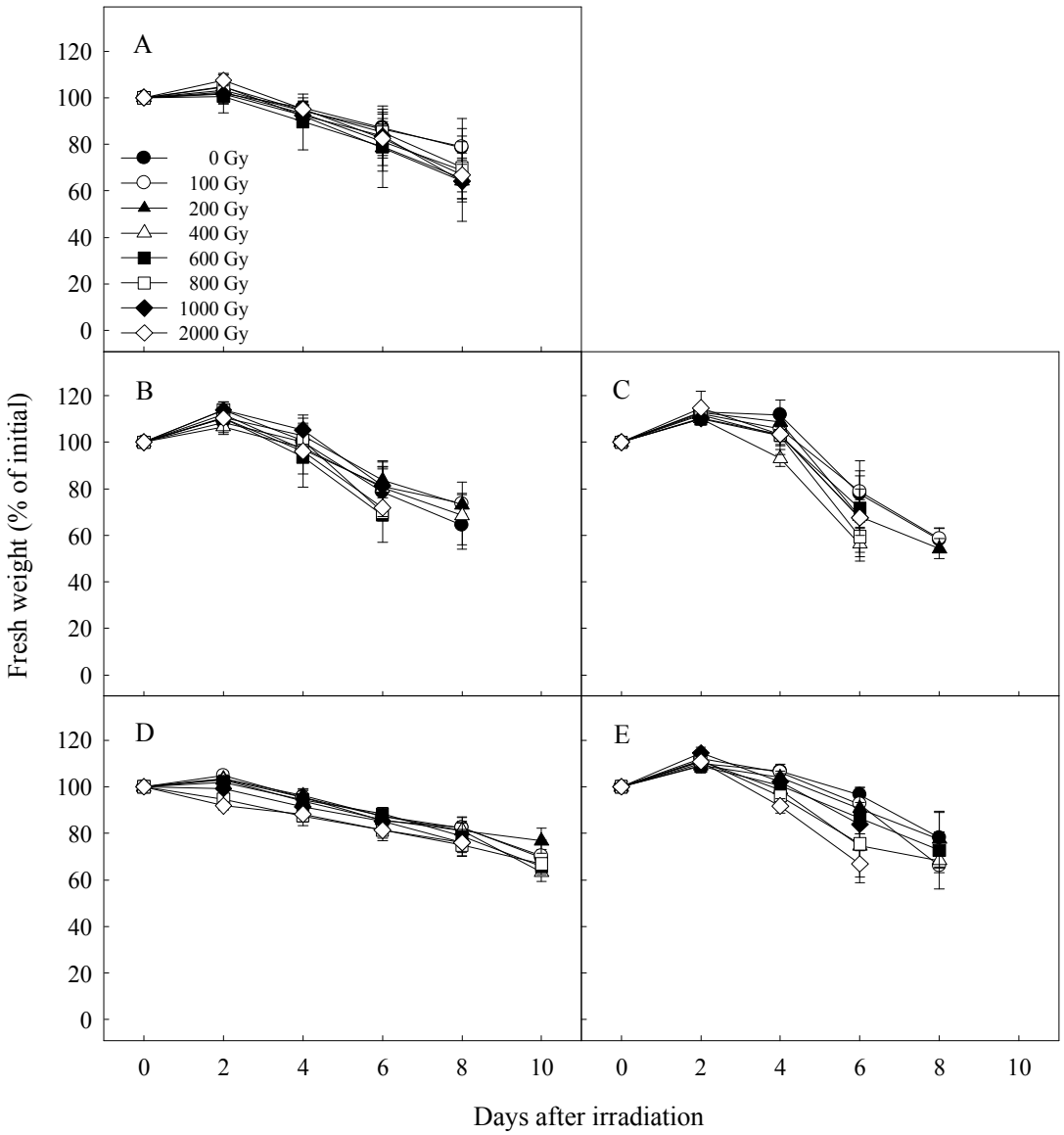

Fig. I-2. Effects of electron beam irradiation on fresh weight of 'Decoration' (A), 'Il se Bronze' (B), 'Queen Bee' (C), 'Revue' (D), and 'Vivian' (E) roses. Data are the means ± SD from five replications.

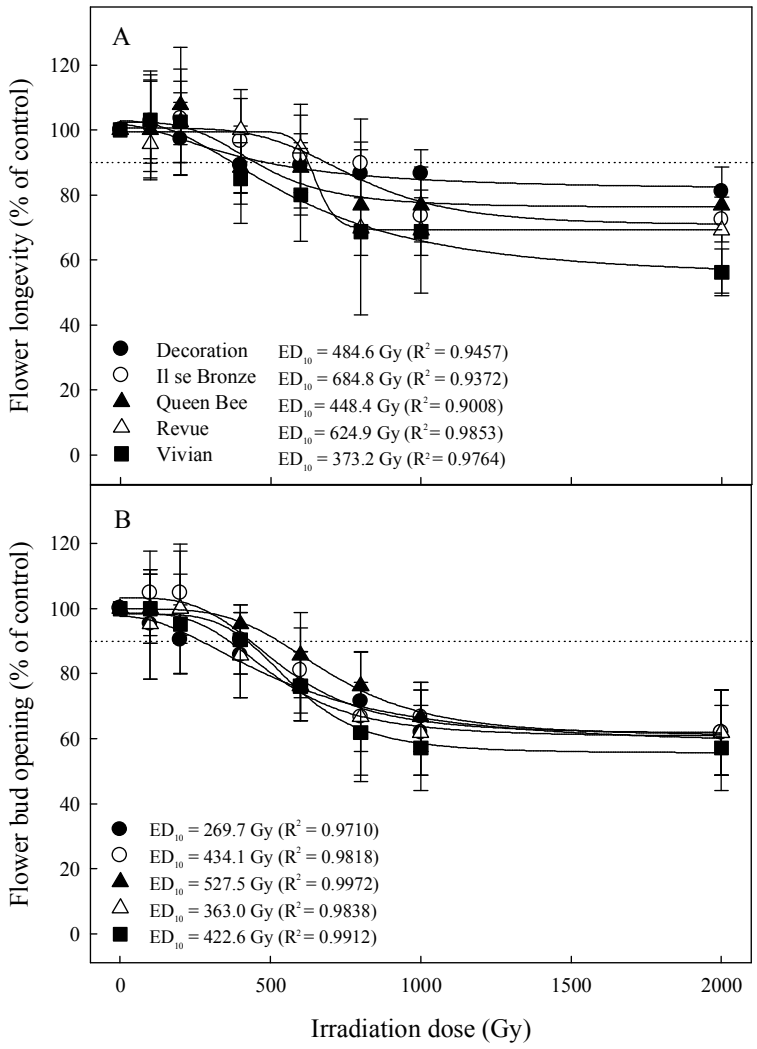

Fig. I-3. Dose response curves for electron beam irradiation effects on flower longevity (A) and flower bud opening (B) in five rose cultivars. Data are the means ± SD from five replications.

Chrysanthemum

The effect of electron beam irradiation on the postharvest quality of cut chrysanthemums were examined. The fresh weight decreased with increasing the irradiation dose (Fig. I-4). The decline in fresh weight coincided with the onset of flower wilting and desiccation. 'Baekma' cut chrysanthemums lost fresh weight rapidly when the irradiation dose was not less than 1,000 Gy, while 'Baekseon' and 'Leopard' showed a greater loss of fresh weight at 2,000 Gy dose. Irradiation at ≤ 400 Gy had no effects on flower longevity of 'Baekma' and 'Leopard' (Tables I-2, 3). Longevity of 'Baekseon' flowers irradiated up to 600 Gy was similar to that of non-irradiated control (Table I-4). However, the flower longevity sharply decreased in 'Baekma', 'Baekseon', and 'Leopard' chrysanthemums irradiated at ≥ 600, ≥ 800, and ≥ 600 Gy, respectively. In 'Baekma', chlorophyll content and visual quality of leaves remained high at ≤ 400 Gy, while in 'Leopard' those remained at ≤ 600 Gy. In 'Baekseon', the plants irradiated up to 1,000 Gy showed similar chlorophyll content and visual quality of leaves to control. With increasing the irradiation dose, flower diameter significantly decreased in both 'Baekma' and 'Baekseon' (Fig. I-5). Flower buds irradiated at ≥ 1,000 Gy did not develop, but wilted in a closed state. ED_{10} values were 431.4, 653.1, and 574.1 Gy in the 'Baekma', 'Baekseon', and 'Leopard', respectively (Fig. I-6). Therefore, 'Baekma', 'Baekseon', and 'Leopard' chrysanthemums could be tolerated to irradiation dose of 400 Gy.

Lily

The effect of electron beam irradiation on fresh weight loss of cut lilies were ex

amined (Fig. I-7).

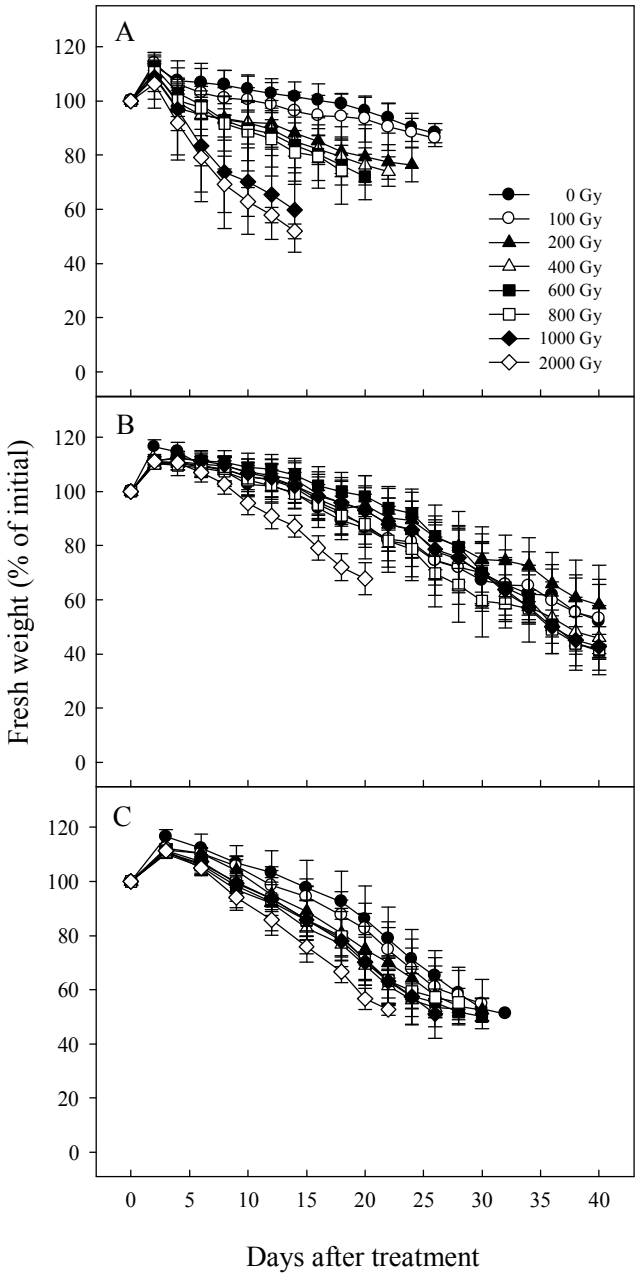

Fig. I-4. Effects of electron beam irradiation on fresh weight of 'Baekma' (A), 'Baekseon' (B), and 'Leopard' (C) chrysanthemums. Data are the means ± SD from five replications.

Table I-2. Effects of electron beam irradiation on the postharvest quality of 'Baekma' chrysanthemums.

Irradiation dose (Gy)	Flower longevity (day)	Flower diameter (mm)	Visual quality of leaves (1-5)[z]	Chlorophyll content (SPAD readout)[y]
0	25.2 a[x]	80.4 a	5.0 a	60.9 a
100	25.2 a	79.8 a	5.0 a	60.9 a
200	24.0 ab	76.5 a	5.0 a	57.8 ab
400	23.0 ab	73.2 a	5.0 a	56.2 ab
600	21.2 b	59.8 ab	4.2 b	54.2 b
800	17.2 c	57.3 ab	4.0 c	55.2 b
1000	12.0 d	27.6 c	1.6 c	47.9 c
2000	11.6 d	43.0 bc	1.2 c	41.5 d
Significance	****	**	****	****

[z]Based on a scale of 1 to 5, measured at 8 days after treatment. 1 = very poor quality (not acceptable, severe leaf necrosis or yellowing, not marketable); 2 = poor quality (not acceptable, large areas of necrosis or yellowing, poor form, not marketable); 3 = fair quality (marginally acceptable, somewhat desirable form and color); 4 = good quality (very acceptable, nice color without yellowing, good form, marketable); 5 = excellent.

[y]Leaf chlorophyll content at 6 days after treatment.

[x]Duncan's multiple range test within columns, $P = 0.05$.

**, ****Significant at $P = 0.01$ or 0.0001, respectively.

Table I-3. Effects of electron beam irradiation on the postharvest quality of 'Leopard' chrysanthemums.

Irradiation dose (Gy)	Flower longevity (day)	Chlorophyll content (SPAD readout)[z]	Visual quality of leaves (1-5)[y]
0	30.4 ab[x]	56.6 a	3.2 a
100	30.6 a	53.7 a	3.2 a
200	29.8 ab	54.1 a	3.2 a
400	29.2 bc	55.1 a	3.2 a
600	28.2 cd	51.1 ab	2.8 ab
800	27.0 d	45.7 bc	2.4 bc
1000	24.4 e	42.8 c	2.2 bc
2000	22.0 f	41.6 c	2.0 c
Significance	****	***	**

[z]Leaf chlorophyll content at 13 days after treatment.
[y]Based on a scale of 1 to 5, measured at 20 days after treatment. Refer to Table I-2.
[x]Duncan's multiple range test within columns, $P = 0.05$.
, *, ****Significant at $P = 0.01$, 0.001, or 0.0001, respectively.

Table I-4. Effects of electron beam irradiation on the postharvest quality of 'Baekseon' chrysanthemums.

Irradiation dose (Gy)	Flower longevity (day)	Flower diameter (mm)	Visual quality of flowers (1-5)[z]	Visual quality of leaves (1-5)[y]	Chlorophyll content (SPAD readout)[x]
0	40.4 a[w]	60.6 a	4.8 a	3.8 a	62.5 a
100	40.6 a	61.1 a	5.0 a	3.8 a	63.3 a
200	40.0 a	59.7 a	4.8 a	4.2 a	60.9 a
400	38.8 a	58.0 ab	5.0 a	4.0 a	61.7 a
600	38.4 ab	57.2 ab	5.0 a	4.2 a	61.7 a
800	34.4 bc	48.4 bc	4.8 a	3.6 a	60.5 a
1000	34.0 c	42.5 c	4.8 a	3.6 a	58.7 a
2000	20.0 d	21.9 d	2.0 b	2.2 b	44.6 b
Significance	****	****	****	****	***

[z]Based on a scale of 1 to 5, measured at 22 days after treatment. 1 = very poor quality (not acceptable, severe wilting or discoloration, not marketable); 2 = poor quality (not acceptable, large areas of necrosis or discoloration, poor form, not marketable); 3 = fair quality (marginally acceptable, somewhat desirable form and color); 4 = good quality (very acceptable, nice color without yellowing, good form, marketable); 5 = excellent.

[y]Based on a scale of 1 to 5, measured at 22 days after treatment. Refer to Table I-2.

[x]Leaf chlorophyll content at 22 days after treatment.

[w]Duncan's multiple range test within columns, $P = 0.05$.

***, ****Significant at $P = 0.001$ or 0.0001, respectively.

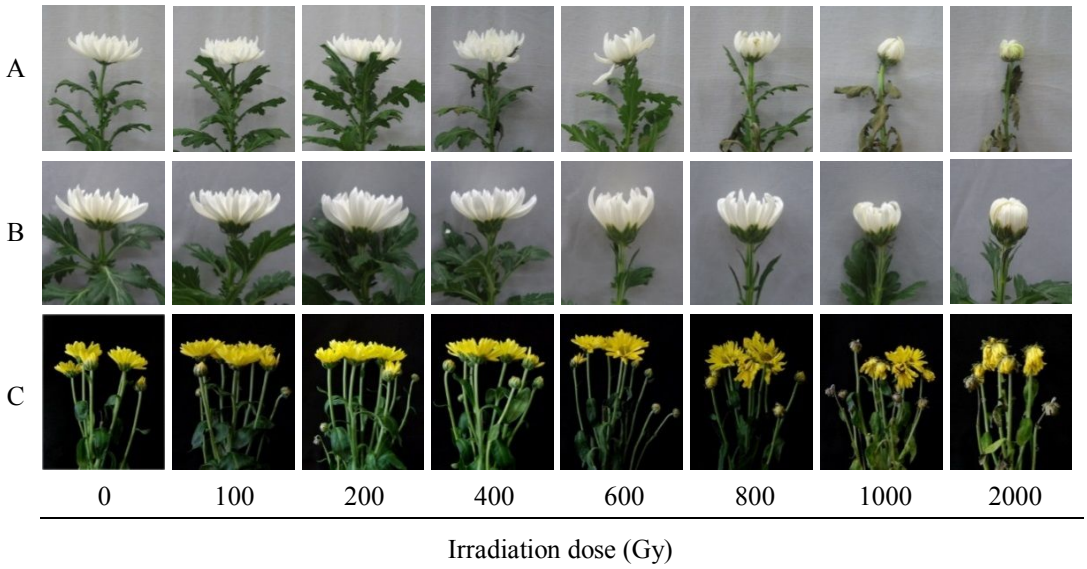

Fig. I-5. Appearance of ‘Baekma’ (A), ‘Baekseon’ (B), and ‘Leopard’ (C) chrysanthemum flowers at 14, 14, and 18 days after electron beam irradiation at various doses, respectively.

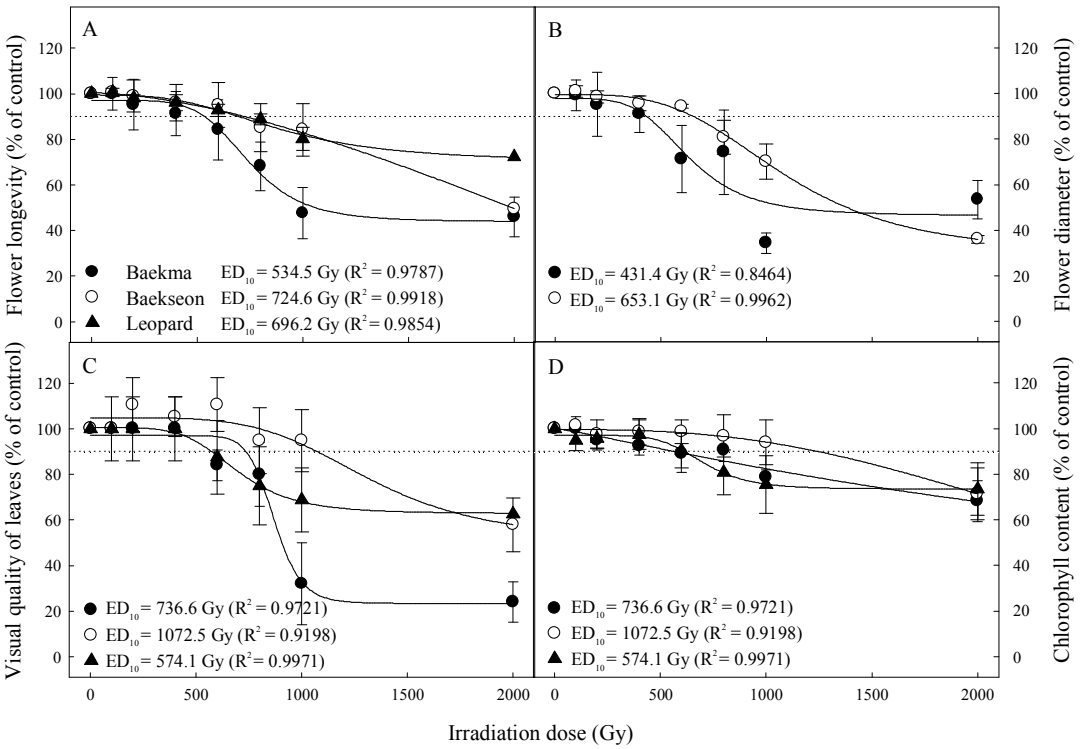

Fig. I-6. Dose response curves for electron beam irradiation effects on flower longevity (A), flower diameter (B), visual quality of leaves (C), and chlorophyll content (D) in three chrysanthemum cultivars. Data are the means ± SD from five replications.

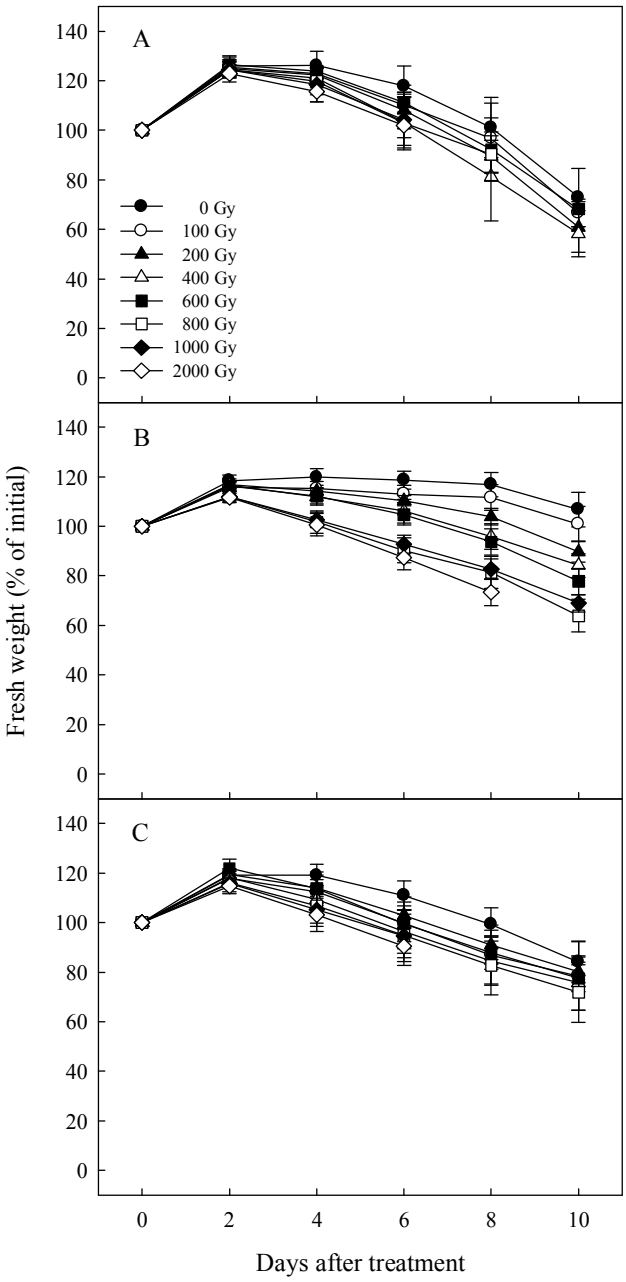

Fig. I-7. Effects of electron beam irradiation on fresh weight of 'Medusa' (A), 'Siberia' (B), and 'Augusta' (C) lilies. Data are the means ± SD from five replications.

‘Medusa’ and ‘Augusta’ lost their fresh weight faster than ‘Siberia’. However, ‘Siberia’ cut lilies lost fresh weight faster than other cultivars with increasing the irradiation dose. In ‘Medusa’ and ‘Augusta’, the fresh weight change of irradiated flowers showed similar pattern to that of non-irradiated controls. Flower longevity of ‘Medusa’ was maintained by irradiation up to 200 Gy (Table I-5). Flower longevities of ‘Siberia’ and ‘Augusta’ were not significantly different between the non-irradiated and irradiated ones at ≤ 400 Gy (Tables I-6, 7). With increasing the irradiation dose, flower diameter significantly decreased in the cut lilies. Corolla growth and opening was inhibited in ‘Medusa’, ‘Siberia’, and ‘Augusta’ lilies irradiated at ≥ 200, 600, and 400 Gy, respectively (Fig. I-8). In ‘Medusa’, irradiation up to 200 Gy did not affect visual quality of flowers and leaves. However, ‘Siberia’ and ‘Augusta’ remained high chlorophyll content and visual quality of leaves up to 400 Gy irradiation. ED_{10} values were 144.4, 306.3, and 243.4 Gy in ‘Medusa’, ‘Siberia, and ‘Augusta’, respectively (Fig. I-9). Therefore, ‘Medusa’, ‘Siberia, and ‘Augusta’ lilies could not be tolerated to irradiation dose of 400 Gy.

Carnation

The fresh weight change of ‘Montezuma’ flowers irradiated electron beam was similar to that of non-irradiated control (Fig. I-10). There were no significant differences in flower longevity, flower diameter, and visual quality of leaves between the non-irradiated and irradiated samples at ≤ 600 Gy (Table I-8). The results indicated that irradiation did not affect the flower opening of ‘Montezuma’

Table I-5. Effects of electron beam irradiation on the postharvest quality of 'Medusa' lilies.

Irradiation dose (Gy)	Flower longevity (day)	Flower diameter (mm)	Visual quality of flowers (1-5)[z]	Visual quality of leaves (1-5)[y]	Chlorophyll content (SPAD readout)[x]

carnations as flower diameter did not decrease with increasing the irradiation dose (Fig. I-11).

0	12.0 a[w]	140.3 a	4.2 a	5.0 a	57.9 a
100	11.8 a	128.2 a	4.6 a	5.0 a	56.7 a
200	11.4 a	109.7 b	4.2 a	4.8 a	56.5 a
400	10.0 b	90.9 c	3.4 b	4.0 b	52.7 ab
600	7.6 c	43.0 d	2.6 c	3.4 c	50.3 bc
800	6.4 cd	38.5 d	2.2 c	2.6 d	49.4 bc
1000	5.8 d	36.1 d	2.0 c	2.0 e	48.8 bc
2000	4.0 e	32.0 d	2.0 c	2.0 e	46.6 c
Significance	****	****	****	****	***

[z]Based on a scale of 1 to 5, measured at 6 days after treatment. Refer to Table I-3.
[y]Based on a scale of 1 to 5, measured at 6 days after treatment. Refer to Table I-2.
[x]Leaf chlorophyll content at 6 days after treatment.
[w]Duncan's multiple range test within columns, $P = 0.05$.
***, ****Significant at $P = 0.001$ or 0.0001, respectively.

Table I-6. Effects of electron beam irradiation on the postharvest quality of 'Siberia' lilies.

Irradiation dose (Gy)	Flower longevity (day)	Flower diameter (mm)	Visual quality of leaves (1-5)[z]	Chlorophyll content (SPAD readout)[y]
0	11.6 ab[x]	183.2 a	4.8 a	58.3 a
100	12.0 a	176.2 a	4.4 abc	57.2 a
200	11.0 ab	177.6 a	4.6 ab	55.0 a
400	10.6 b	158.6 a	4.0 abc	49.5 ab
600	8.8 c	120.1 b	3.8 bc	46.4 abc
800	8.0 cd	111.2 b	3.5 c	39.1 bc
1000	7.2 d	84.6 c	3.6 c	38.1 bc
2000	5.2 e	48.9 d	3.6 c	35.2 c
Significance	****	****	*	***

[z]Based on a scale of 1 to 5, measured at 4 days after treatment. Refer to Table I-2.
[y]Leaf chlorophyll content at 4 days after treatment.
[x]Duncan's multiple range test within columns, $P = 0.05$.
*, ***, ****Significant at $P = 0.05$ 0.001, or 0.0001, respectively.

Table I-7. Effects of electron beam irradiation on the postharvest quality of 'Augusta' lilies.

Irradiation dose (Gy)	Flower longevity (day)	Flower diameter (mm)	Visual quality of leaves (1-5)[z]	Chlorophyll content (SPAD readout)[y]
0	10.2 a[x]	105.7 a	5.0 a	58.1 a
100	9.0 ab	100.9 a	5.0 a	57.1 a
200	9.2 ab	101.4 a	4.6 a	54.9 ab
400	9.2 ab	72.3 b	4.4 ab	53.8 ab
600	8.8 b	49.3 c	3.8 b	49.2 bc
800	8.6 b	27.8 d	3.0 e	45.3 cd
1000	6.8 c	11.8 de	2.4 e	45.2 cd
2000	6.6 c	0.0 e	1.2 d	40.9 d
Significance	****	****	****	****

[z]Based on a scale of 1 to 5, measured at 8 days after treatment. Refer to Table I-2.
[y]Leaf chlorophyll content at 6 days after treatment.
[x]Duncan's multiple range test within columns, $P = 0.05$.
****Significant at $P = 0.0001$.

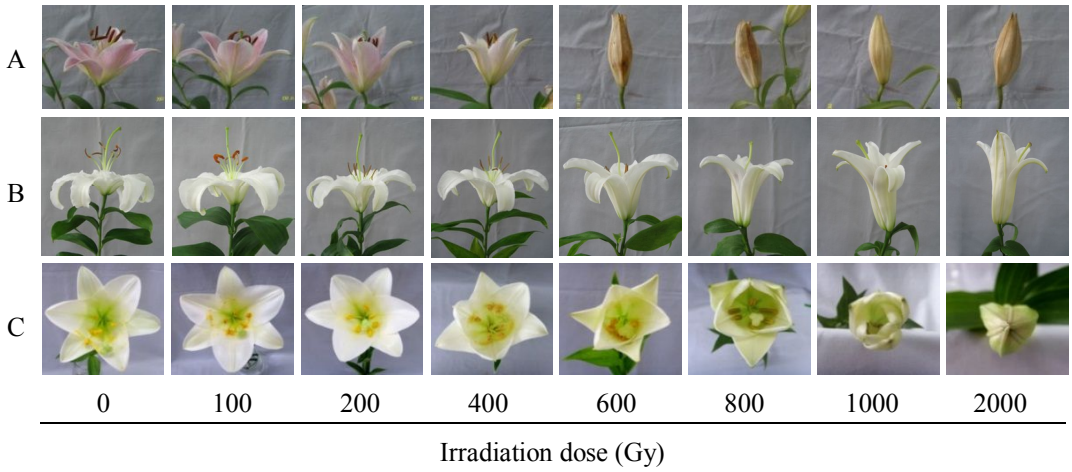

Fig. I-8. Appearance of ‘Medusa’ (A), ‘Siberia’ (B), and ‘Augusta’ (C) lily flowers at 6, 4, and 6 days after electron beam irradiation at various doses, respectively.

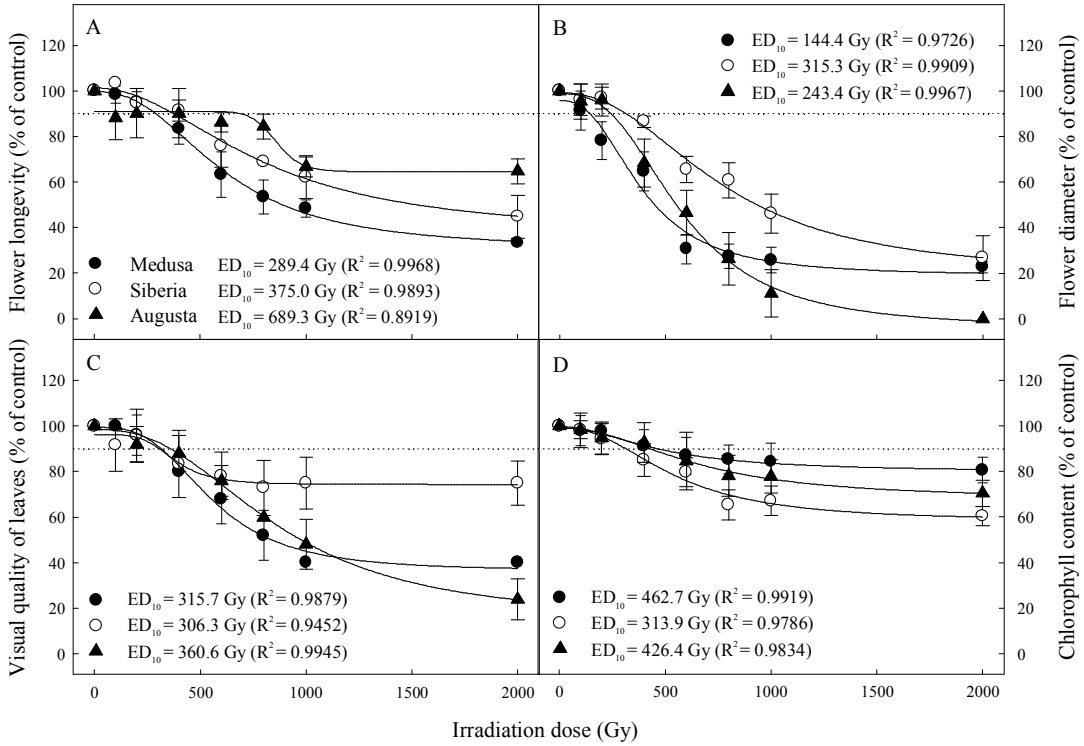

Fig. I-9. Dose response curves for electron beam irradiation effects on flower longevity (A), flower diameter (B), visual quality of leaves (C), and chlorophyll content (D) in three lily cultivars. Data are the means ± SD from five replications.

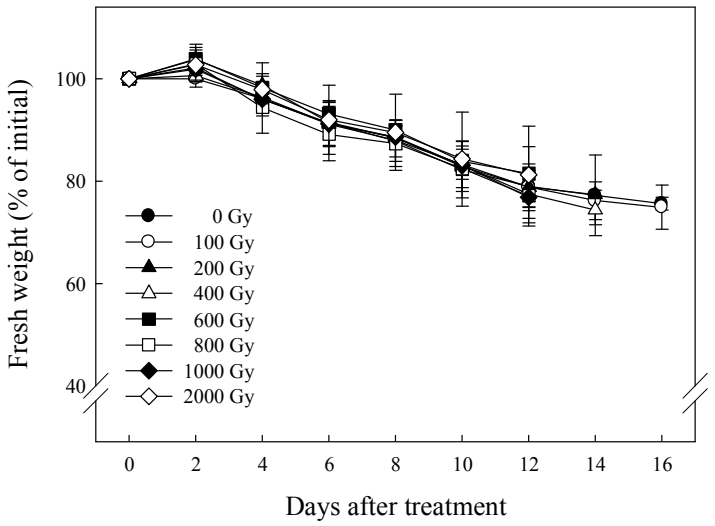

Fig. I-10. Effects of electron beam irradiation on fresh weight of 'Montezuma' carnations. Data are the means ± SD from five replications.

Table I-8. Effects of electron beam irradiation on the postharvest quality of 'Montezuma' carnations.

Irradiation dose (Gy)	Flower longevity (day)	Flower diameter (mm)	Visual quality of leaves (1-5)[z]
0	12.6 ab[y]	76.8 a	3.2 a
100	13.8 a	76.6 a	3.7 a
200	11.8 abc	75.2 ab	3.3 a
400	12.0 abc	77.4 a	3.0 ab
600	10.4 bc	74.3 abc	3.0 ab
800	9.6 c	70.5 bc	2.2 bc
1000	9.4 c	70.2 bc	2.0 c
2000	9.2 e	68.9 c	2.2 bc
Significance	*	*	**

[z]Based on a scale of 1 to 5, measured at 10 days after treatment. Refer to Table I-2.
[y]Duncan's multiple range test within columns, $P = 0.05$.
*, **Significant at $P = 0.05$ or 0.01, respectively.

0 100 200 400 600 800 1000 2000

Irradiation dose (Gy)

Fig. I-11. Appearance of ‘Montezuma’ carnation flowers at 10 days after electron beam irradiation at various doses.

Therefore, postharvest quality of ‘Montezuma’ carnation was maintained by electron beam irradiation up to 600 Gy. ED_{10} value was 451.6 Gy in the ‘Montezuma’ carnations (Fig. I-12). Therefore, ‘Montezuma’ carnations could be tolerated to irradiation dose of 400 Gy.

Eustoma

The fresh weight loss of ‘Rosina White’ eustomas was not different between the non-irradiated and irradiated samples (Fig. I-13). Flower longevity was maintained by irradiation up to 800 Gy, while no significant differences were observed in flower diameter and flowering rate when compared to control (Table I-9, Fig. I-14). Therefore, postharvest quality of ‘Rosina White’ eustomas was maintained by electron beam irradiation up to 800 Gy. ED_{10} value was 841.2 Gy in the ‘Rosina white’ eustomas (Fig. I-15). Therefore, ‘Rosina white’ eustomas could be tolerated to irradiation dose of 400 Gy.

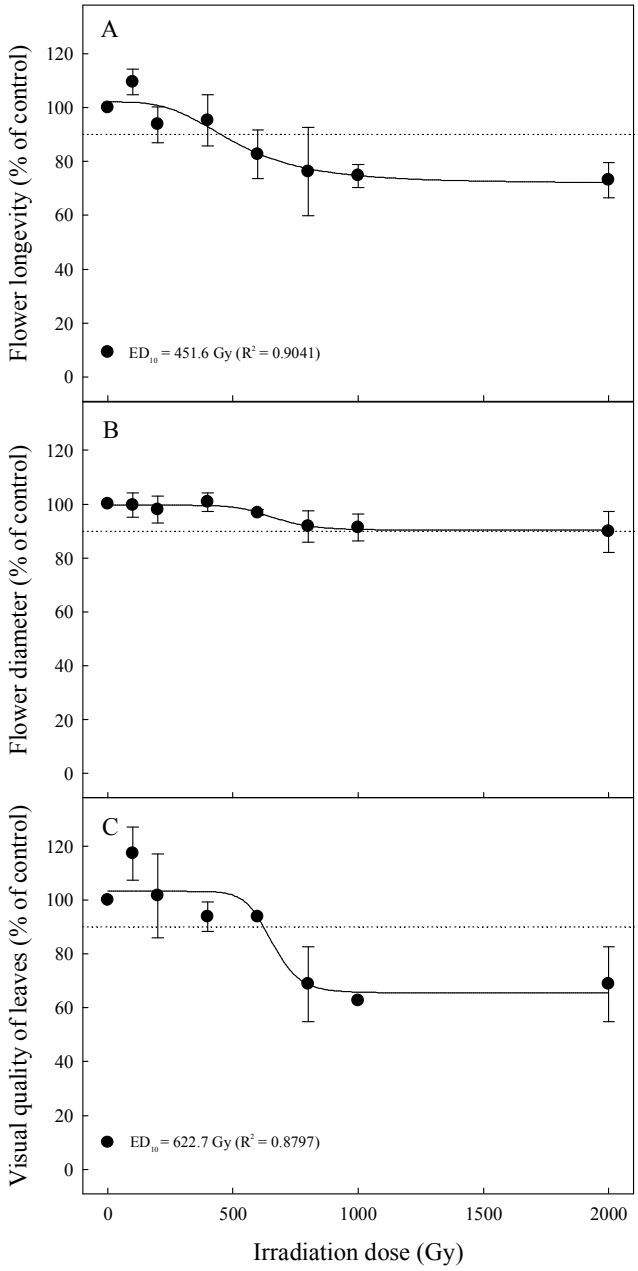

Fig. I-12. Dose response curves for electron beam irradiation effects on flower longevity (A), flower diameter (B), and visual quality of leaves (C) in 'Montezuma' carnations. Data are the means ± SD from five replications.

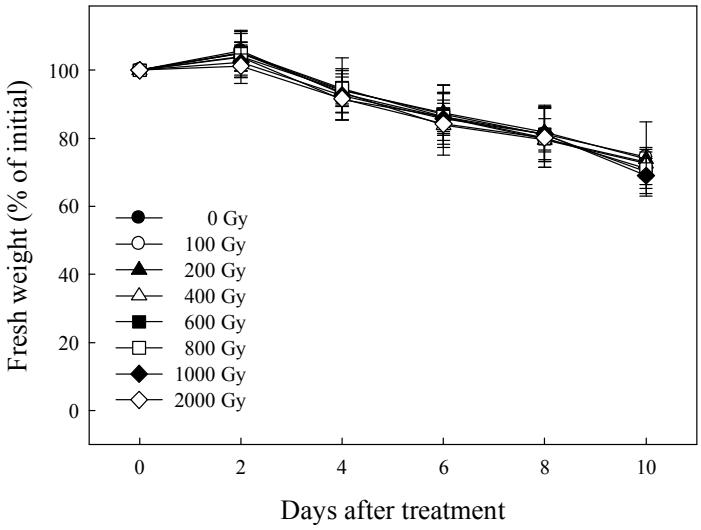

Fig. I-13. Effects of electron beam irradiation on fresh weight of 'Rosina White' eustomas. Data are the means ± SD from five replications.

Table I-9. Effects of electron beam irradiation on the postharvest quality of 'Rosina White' eustomas.

Irradiation dose (Gy)	Flower longevity (day)	Flower diameter (mm)	Flowering rate (%)[z]
0	9.0 a[y]	56.0 a	100.0 a
100	9.2 a	55.1 a	95.8 ab
200	9.2 a	56.2 a	95.8 ab
400	8.8a	56.9 a	95.8 ab
600	8.7 ab	55.8 a	95.8 ab
800	8.3 ab	56.2 a	91.7 ab
1000	7.7 b	53.9 a	87.5 ab
2000	7.7 b	53.4 a	84.2 b
Significance	*	NS	NS

[x]Ratio of fully open flowers to the total number of initial flower buds per bunch.
[y]Duncan's multiple range test within columns, P = 0.05.
[NS, *]Nonsignificant or significant at $P = 0.05$, respectively.

Fig. I-14. Appearance of ‘Rosina White’ eustoma flowers at 8 days after electron beam irradiation at various doses.

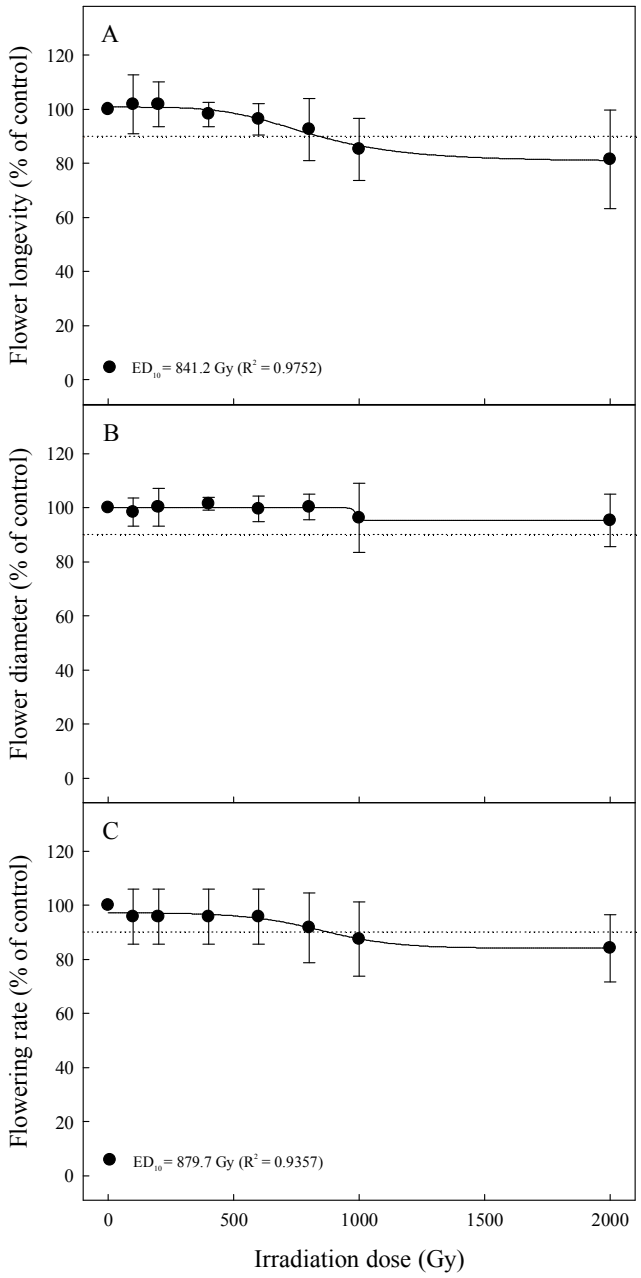

Fig. I-15. Dose response curves for electron beam irradiation effects on flower longevity (A), flower diameter (B), and flowering rate (C) in 'Rosina White' eustomas. Data are the means ± SD from five replications.

DISCUSSION

The objective of this study was to determine the tolerance of cut flowers to electron beam irradiation. Although the changes by irradiation are at the cellular level, the consequences of these changes vary with the organism. Effect of irradiation on plants depends on its radiation tolerance, adaptive response, and repair capabilities (Esnault et al., 2010). Whenever irradiation is used to fresh produce, a balance must be achieved between the effective dose for desired benefit such as disinfestation and decontamination and the tolerance level of the produce.

The present study described the response of several cut flowers to electron beam irradiation. Electron beam irradiation caused cut flowers to decrease fresh weight, flower diameter, chlorophyll content, and visual quality of flowers and leaves in a dose-dependent manner. Irradiated flowers lost fresh weight faster, and this loss decreased flower longevity (Table I-1, Fig. I-2). The results are in agreement with those of Chang et al. (1997), who found fresh weight loss of 'Royalty' roses irradiated with an electron beam at ≥ 500 Gy. Hasbullah et al. (2012) also reported that the fresh weight of gerbera callus was significantly declined by γ-irradiation, which may be caused by the lesser amount of endogenous hormones as well as inhibition of water intake to the cell.

This study demonstrated that irradiation tolerance of cut flowers vary according to cultivars as well as species. Chrysanthemum, carnation, and eustoma were tolerant when the dose of 400 Gy was considered as the minimum value for tolerance of the flowers (Figs. I-6, 12, 15). Our results are different from those of

Hayashi et al. (1998), who demonstrated that chrysanthemums were not tolerant to electron beam irradiation at 400 Gy. In this study, some cultivars of the rose and lily were considered as not tolerant because they were damaged by the irradiation at 400 Gy (Figs. I-3, 9). Sangwanangkul et al. (2008) also mentioned that tolerance varied with cultivars; for example, white dendrobiums 'UH306' were more sensitive than the pink variety 'UH232' to electron beam irradiation at 150 Gy. Although flower longevity was reduced by electron beam irradiation, chlorophyll content and visual quality of leaves remained high up to 600 Gy in the present study (Table I-3). These results were similar to that reported by Hayashi and Todoriki (1996), who exposed 'Seishu' chrysanthemums to γ-irradiation at 750 Gy and observed a decline in fresh weight as well as foliage yellowing. Flower buds irradiated at dose exceeding the maximum tolerance did not develop, but wilted closed in the present study (Fig. I-1). Flower opening inhibition by irradiation are applicable as a potential indicator of irradiation sensitivity. The results of the present study correspond well with those found in the earlier experimental studies in *Lilium speciosum* (Kikuchi, 2003) and 'Royalty' roses (Chang et al., 1997). Yang et al. (2002) also reported that fresh day-lily flowers irradiated with ≥ 1,000 Gy were damaged and the inhibition of the flower blooming caused injury on the epidermis of the flowers.

In conclusion, the present study suggests that tolerance of cut flowers to electron beam irradiation vary according to species and cultivars and the dose of 400 Gy may be a considerable as the minimum value for tolerance. Further studies are needed to understand the effect of electron beam irradiation on flower opening.

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CHAPTER II

Effect of Electron Beam Irradiation on Flower Opening

ABSTRACT

This study was conducted to determine the effects of electron beam irradiation on flower opening by monitoring the changes of osmolarity and sugar contents in tepals, as well as DNA damage. 'Siberia' and 'Medusa' lily flowers were irradiated at 100, 200, 400, 800, and 1,600 Gy with a 10 MeV linear electron beam accelerator. Changes in osmolarity and sugar contents were measured after irradiation in the tepals of 'Siberia' lilies. The radiation-induced DNA damage was determined by using a flow cytometry analysis in 'Siberia' and 'Medusa' lilies. In non-irradiated 'Siberia' lilies, concomitant with elevation of osmolarity, soluble sugar levels increased prior to the onset of flower opening. A trend toward increase in osmolarity was maintained up to day 6 in lily flowers irradiated at ≤ 200 Gy, while osmolarity was less increased or gradually decreased during bud development of flowers irradiated at ≥ 400 Gy. At first, higher levels of osmolarity were found in irradiated flowers than in non-irradiated controls. Total soluble sugars of the tepal irradiated at ≤ 200 Gy gradually increased with the developmental bud stage, and then decreased between day 4 and 8. In the tepal irradiated at ≥ 400 Gy, it increased at day 2, while followed by a decline between day 2 and 6. When subjected to electron beam irradiation, 'Siberia' and 'Medusa'

tepal cells showed broad CV of G0/G1 peaks, high percentage of Background Aggregates and Debris (BAD), and apoptotic peaks in a dose-dependent manner. By 0 days after irradiation, an apoptotic peak developed with increasing BAD after irradiation at ≥ 800 and ≥ 400 Gy in ‘Siberia’ and ‘Medusa’ lilies, respectively. By 2 days after irradiation, an apoptotic peaks were observed to be progressed in lower irradiation doses at ≥ 400 and ≥ 100 Gy in ‘Siberia’ and ‘Medusa’ lilies, respectively. Trypan blue staining also showed a radiation-induced cell death in tepals of ‘Siberia’ and ‘Medusa’ lilies irradiated at ≥ 400 and ≥ 100 Gy, respectively. As a result, ‘Medusa’ were more sensitive to irradiation than ‘Siberia’ lilies. In conclusion, the radiation-induced failure of flower opening may be due to insufficient osmotic pressure, discontinuation of the persistent sugar supply, DNA damage, and cell death.

Additional keywords: apoptotic peak, cell death, DNA damage, flow cytometry, osmolarity, sugar, trypan blue staining

INTRODUCTION

Flowering is a critical event in the life-cycle of angiosperms. Changes in carbohydrate metabolism and cell sap osmolarity are considered important driving forces in the flower opening (van Doorn and van Meeteren, 2003). During petal growth associated with flower opening, the petal cells divide and expand. Since cell division of the petals stops at a stage much earlier than flower opening, it mainly depends on cell expansion of the petals (Norikoshi et al., 2013). Rapid flower opening in many species, including roses (Evans and Reid, 1988) and daylily (Bieleski, 1993), was related to the hydrolysis of reserve carbohydrates. In lily flowers, young buds also contain adequate levels of starch being rapidly converted to glucose and fructose, which contribute to osmotic changes (van Doorn and van Meeteren, 2003). Therefore, flower opening are highly correlated with cell sap osmolarity changes, which regulate the direction of water movements, resulting in turgor changes and cell expansion (Bieleski, 1993; Ho and Nichols, 1977).

Ionizing radiation has been documented to cause the bud opening failure. Hayashi and Todoriki (1996) have indicated that γ-irradiation accelerated chrysanthemum bloom wilting and suppressed bud opening. Kikiuchi (2003) also reported that high doses of γ- and electron beam irradiation caused bud opening inhibition in many species, such as *Lilium speciosum* and *Lisianthus* spp. Although the mode of action of irradiation in bud opening inhibition has not yet been fully determined, it is well known that the radiation-induced DNA damage is responsible for inhibition of seed germination, plant growth, and reproduction.

The damaged DNA molecules can be detected in irradiated cells using microgel electrophoresis of single cells or nuclei (DNA comet assay) and terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL test). Another attempts have been made to detect radiation-induced changes in the DNA. Selvan and Thomas (1995) reported that flow cytometry analysis can be used for detecting radiation-induced changes in DNA of onion bulbs by determining CV of the G0/G1 peak and DNA index.

This study was conducted to determine the effects of electron beam irradiation on flower opening by monitoring the changes in osmolarity and sugar contents in tepals, as well as DNA damage. These results will provide an information for better understanding of the plant responses to electron beam irradiation, especially the possible involvement of carbohydrate redistribution, in the regulation of flower opening.

MATERIALS AND METHODS

Plant Materials

Two lily cultivars, *Lilium* Oriental hybrid 'Siberia' and 'Medusa', were used for experiments. 'Siberia' and 'Medusa' lilies were purchased from a local wholesaler in Seoul, Korea. Flowers were at the commercial harvest stage, with the largest bud showing color.

Electron Beam Irradiation

Electron beam irradiation was conducted in the EB-Tech Co., Ltd. (Daejeon, Korea) using a high energy linear accelerator (UEL V10-10S, 10 MeV). Cut lily flowers were treated with doses of 100, 200, 400, 800, and 1,600 Gy. Target doses were monitored by dosimetry with a radiochromic film dosimeter (GAF3002DS, GEX Corp., Centennial, CO, USA) (ISO/ASTM51275:2004(E)).

Stage of Flower Bud Opening

After irradiation, stems were recut to 30 cm and were placed in distilled water and then kept in an air-conditioned room with a 12 h light cycle at 23 ± 1^{o}C, a relative humidity of 60 ± 10%, and a leaf level photosynthetic photon flux density at 140 μmol·m^{-2}·s^{-1} provided by cool white fluorescent lamps. The flower bud opening was divided into five different stages till bloom and the characteristic changes are given in Table II-1.

Table II-1. Characteristics of flower bud opening stages in ‘Siberia’ lilies.

Stage	Characteristics of bud development
I	Very tight bud, no tepal separation
II	Locked bud, separation of tepals at the tip
III	Opening bud, separation of tepals along the half of the bud, bud cracking
IV	Tepals all fully separated and almost straight at approximately 45^{o}
V	Fully opened flower, maximum bloom diameter

Measurement of Osmolarity

For measurement of tepal osmolarity, tepals were detached separately from three flowers at intervals. Detached tepal was sealed individually in air-tight containers, frozen immediately in liquid nitrogen, and then stored at –20°C. The tepals were thawed by placing the sealed containers for 10 min in a stirred water bath at 60°C. Each tepal was removed and its sap expressed with a hand press. An aliquot of 10 μl of sap was collected on a filter paper disk and its osmolarity was determined using a vapor pressure osmometer (VAPRO 5600, Wescor Inc., Logan, UT, USA) that had been calibrated against NaCl solutions of known osmolarity.

Determination of Sugars

Tepals were detached separately from three flowers at intervals. Detached tepals were immersed in liquid nitrogen and then freeze-dried. Freeze-dried tepals were ground using a mill (Thomas Wiley® Mini Mill 3383-L10, Thomas Scientific, Swedesboro, NJ, USA) with 60-mesh sieve and stored as powder at –80°C until use. Soluble sugars were extracted using the method described by González-Rossia et al. (2008) with slight modifications. One hundred milligrams of ground powder were put in a 2 mL test tube containing 1 mL 80% ethanol and incubated at 85°C for 15 min. After centrifugation at 15,000 g for 5 min, the supernatants were collected and the pellets were re-extracted twice as above. The combined supernatants were evaporated using a N_2 evaporator (N-EVAPTM, Organomation Associates, Inc., West Berlin, MA, USA) at 60°C. The ethylic solution of sugar extracts was dissolved in 3 mL of distilled water and passed through 0.45 μm nylon filter (Acrodisc® 13 mm Syringe Filter, Pall Co.,

Washington, NY, USA) and C18 Sep-Pak cartridge (Waters Associates, Milford, MA, USA). High performance liquid chromatography (HPLC) chromatograms were recorded with a Waters 1525 Binary HPLC pump, using a Waters 2414 RI detector (Waters Associates). For HPLC, first grade solvents such as water and ACN (J.T. Baker, USA) were used as an elution solution, while all other reagents were analytical grade. Determination was achieved on a Waters carbohydrate analysis column (3.9 × 300 mm), and water : ACN (v/v, gradient solvent system) was employed as the mobile phase. The flow rate was kept constant at 2.0 mL·min^{-1}.

Flow Cytometry

For quantitative analysis of fragmented nuclei, 25 mm^2 portion of tepal was chopped with a sharp razor blade in nuclear extraction buffer, part of the Cystain UV Precise P (Partec GmbH, Münster, Germany). The extract was filtered through a 30 μm CellTrics® filter. A staining buffer containing the dye of 4,6-diamidino-2-phenylindole (DAPI), also part of the Cystain UV Precise P, was added. The DNA content of the isolated nuclei was analysed using a CyFlow® Cube 8 flow cytometry (Partec GmbH). Data were analyzed according to the software available with FCS Express 4, RUO edition (De Novo Software, Los Angeles, CA, USA). Apoptotic DNA fragments within the cell were quantitated by histogram analysis using a Multicycle AV for FCS Express when it is assumed that there is one extra peak to the left of the G0/G1 peak.

Trypan Blue Staining

To detect plant cell death, tepal discs were excised and stained with lactophenol-trypan blue solution (10 mL lactic acid, 10 mL glycerol, 9.3 mL phenol, 10 mL distilled water, and 20 mg trypan blue) diluted 1 : 2 in ethanol under vacuum as described (Yeom et al., 2012). The tepal discs were boiled in the lactophenol-trypan solution, incubated overnight in staining solution, and cleared with chloral hydrate (2.5 $g \cdot mL^{-1}$). The cleared tepal discs were mounted in 70% glycerol solution and observed using stereo microscopy (Dimis-M, Siwon Optical Technology Co., Ltd., Anyang, Korea).

Statistical Analysis

Statistical analysis was performed using SAS software, version 9.2 (SAS Inst., Cary, NC, USA). Differences among the group means were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test at the 5% level.

RESULTS

Osmotic Changes Associated with Flower Bud Opening

Lily flowers have two whorls of floral leaves, called tepals. The inner and outer whorls consisting of three tepals each are homologous to the petals and sepals, respectively. In stage I, the outer tepals, the sepals, entirely encased the inner tepals, petals, and their edge were tightly clasped in deep grooves beside the petal midribs (Table II-1). The sepal-petal lock became less marked with bud development (stage II), finally ceasing as the bud started to crack (stage III). After the cracking stage, unlocking the tepals allowed the bud to open significantly (stage IV) and then showed a rapid flower opening (stage V).

Effects of electron beam irradiation on flower bud opening of 'Siberia' lily were examined. The development and expansion of the tepal did not change with increasing the irradiation dose up to 200 Gy and the tepal taking normal course to the stage V (Fig. II-1). However, the flower buds irradiated at 400, 800, and 1,600 Gy was ceased to develop at stages IV, III, and II, respectively.

Non-irradiated tepals showed increased sap osmolarity between day 0 and 6, followed by a sharp decrease at day 8 (Fig. II-1). Tepals irradiated at $\leq$ 200 Gy also showed continuously increased sap osmolarity until day 6 and returned to fall at day 8. Irradiation at 400 Gy showed a sharp decline in osmolarity from day 4, while at 800 Gy osmolarity gradually decreased from day 0. Tepals irradiated at 1,600 Gy maintained their high osmolarity between day 0 and day 6, which was dramatically decreased at day 8. By regression analysis, osmolarity tended to increase in shape of Weibull peak with increasing the irradiation dose at day 0.

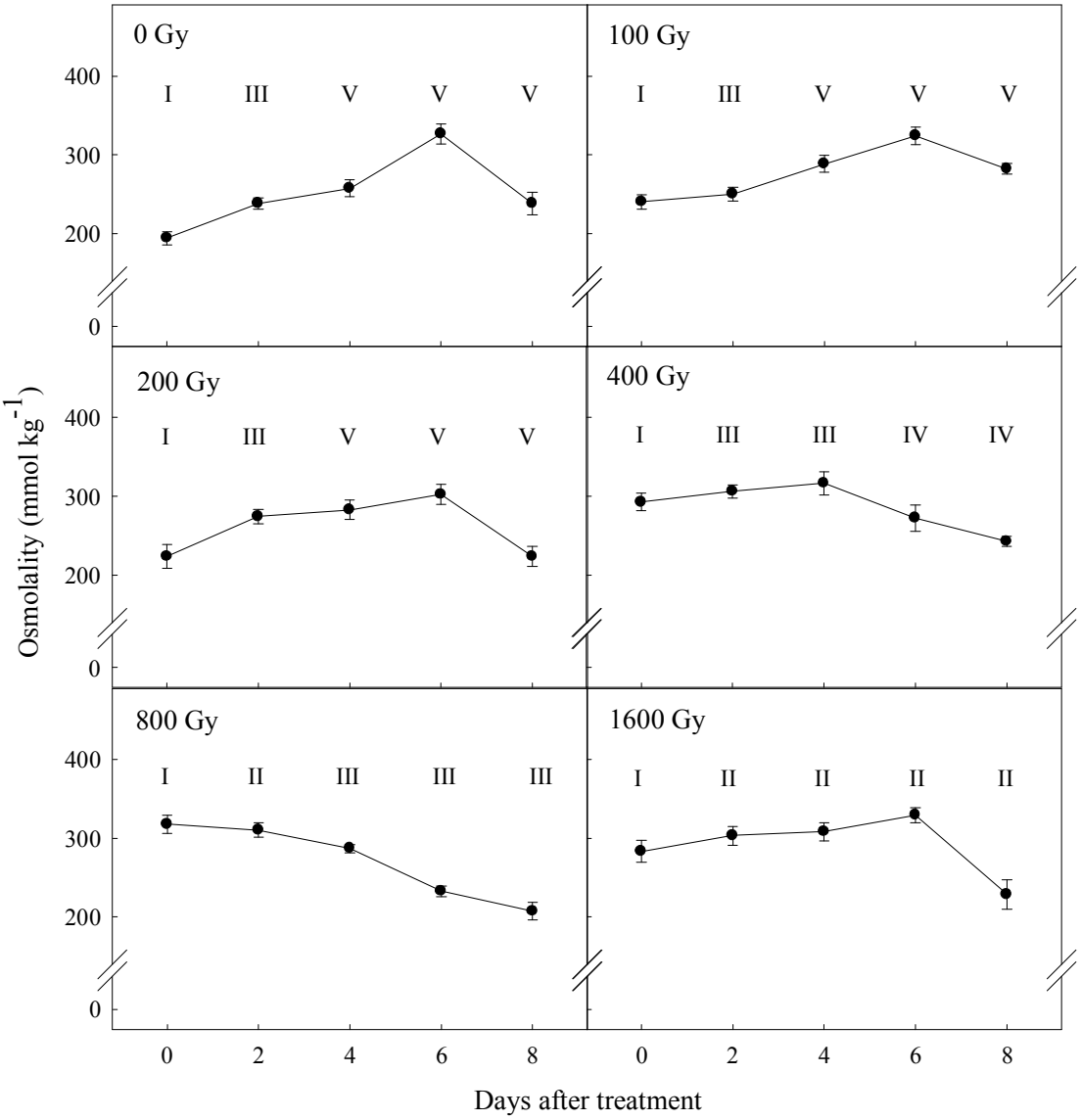

Fig. II-1. Effects of electron beam irradiation on osmolarity in tepals of 'Siberia' lilies during flower opening. Cut flowers were irradiated with dose of 0, 100, 200, 400, 800, and 1,600 Gy using a 10 MeV linear electron beam accelerator. Data are the means ± SD from three replications. Stages I to V indicate the flower bud opening. Refer to Table II-1.

Changes in Soluble Sugar Content

The contents of fructose, glucose, and sucrose were summed and expressed as total soluble sugar content during flower bud opening (Fig. II-2). Total soluble sugars gradually increased in the non-irradiated tepals with the bud developmental stage, and then decreased between day 4 and 8. Similar pattern was seen in the tepals irradiated at ≤ 200 Gy. Total soluble sugars of the tepal irradiated at ≥ 400 Gy increased at day 2, while followed by a decline between day 2 and 6. Moreover, doubling the total soluble sugar was shown at day 2 in the tepal irradiated at 1,600 Gy. Compare to bud developmental stage, the main soluble sugars of the tepal was glucose. Glucose levels increased during flower opening in the non-irradiated tepals. The major increase in glucose levels were observed between day 2 and 4 in the tepal irradiated at ≤ 200 Gy, consistently with their flower opening. Although glucose levels also elevated at day 2 in the tepal irradiated at ≥ 400 Gy, a trend toward reduction was observed after day 4.

Flow Cytometric Determination of DNA fluorescence after Irradiation

Flow cytometry of nuclei stained for DNA, with DAPI, shows the distribution of fluorescence signals. Quantitative DNA staining leads to a distribution histogram with the number of nuclei along the Y-axis and the DNA content of the nuclei along the X-axis. Flow cytometry of nuclei from ‘Siberia’ tepal cells at 0 days after irradiation showed a peak of nuclei at phase G0/G1 and no mitotic nuclei, G2/M (Fig. II-3). Because the measured fluorescence from G1 cells is a normally distributed Gaussian peak and variable observationally, the coefficient of variation (CV) of G0/G1 peaks is used to describe the width of the peak.

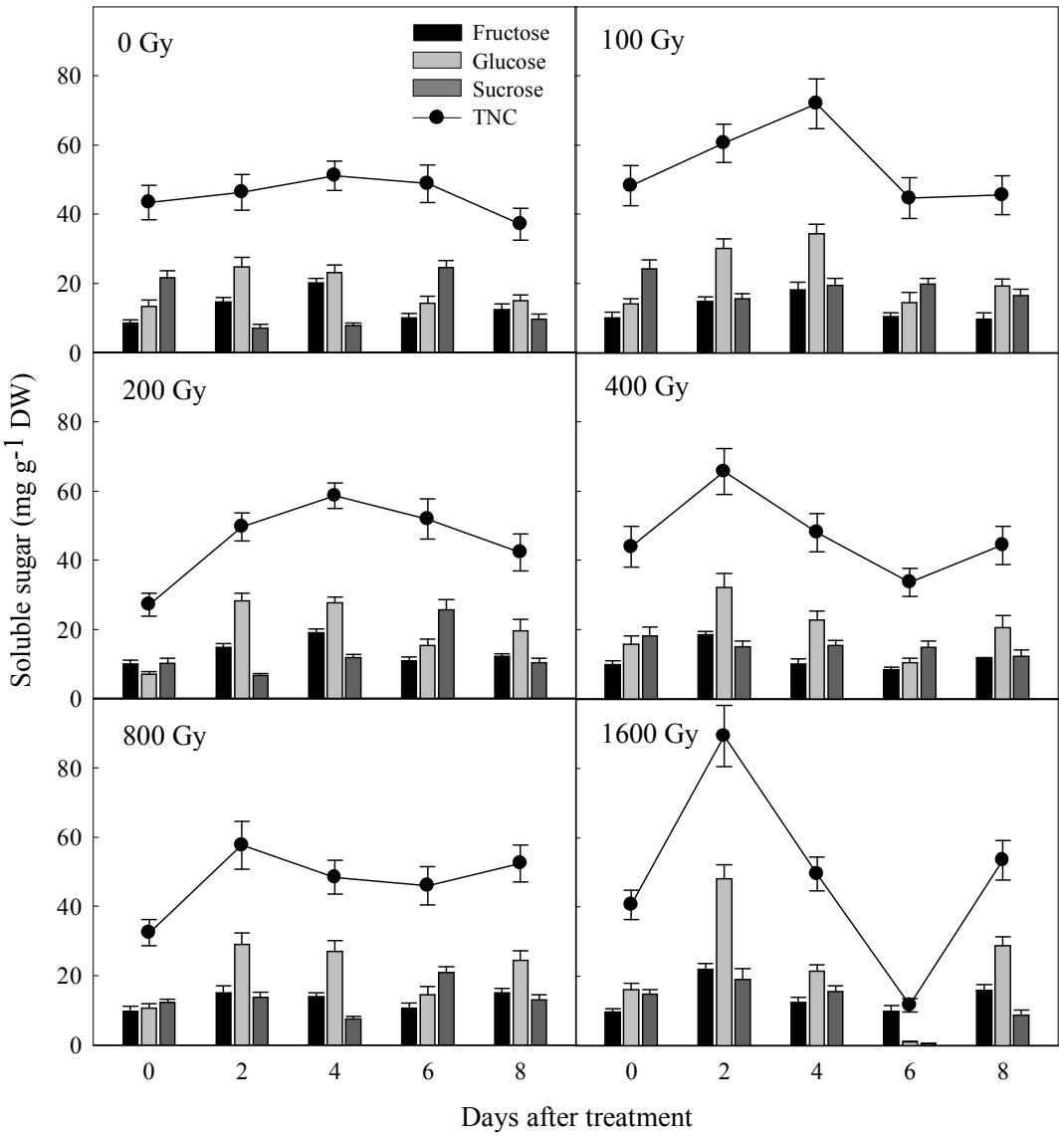

Fig. II-2. Effects of electron beam irradiation on soluble sugar contents in tepals of 'Siberia' lilies during flower opening. Cut flowers were irradiated with dose of 0, 100, 200, 400, 800, and 1,600 Gy using a 10 MeV linear electron beam accelerator. Data are the means ± SD from three replications.

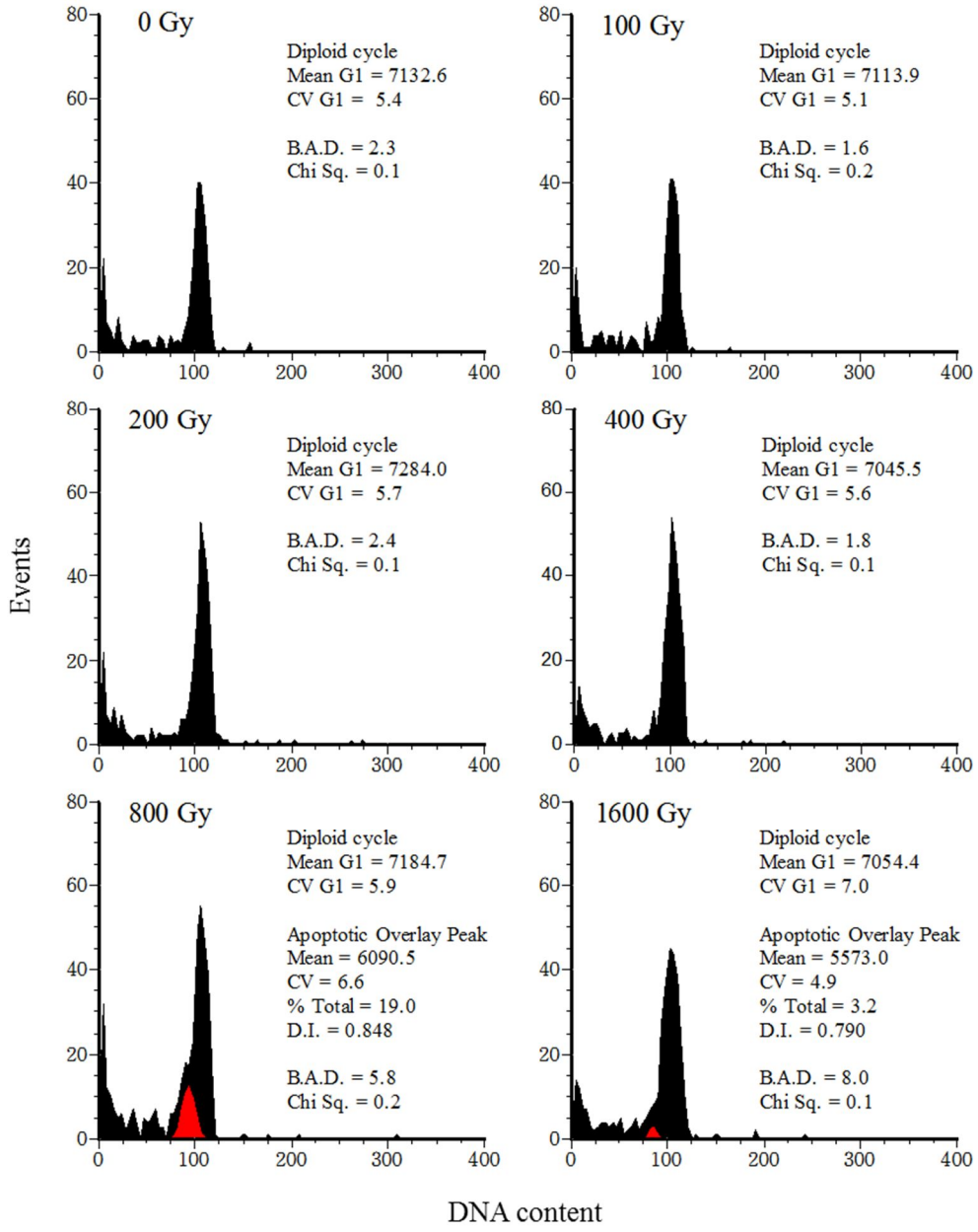

Fig. II-3. Flow cytometric determination of fluorescence by DAPI-stained DNA masses in tepals of 'Siberia' lilies at 0 days after electron beam irradiation. Data were analyzed with Multicycle AV and FCS Express. Red histogram portion, apoptotic DNA fragments.

The CV of the irradiated 'Siberia' tepal cells was increased in a dose-dependent manner. The broadest G1 peaks were observed in the flowers irradiated at 1,600 Gy. Flow cytometry revealed several nuclei with a fluorescence less than that of G0/G1 peak. The nuclei with such decreased DNA level were thought to be damaged or fragmented nuclei, debris. The percentage of Background Aggregates and Debris (BAD) is shown above the histograms and BAD increased sharply from 800 to 1,600 Gy. Moreover, the flowers irradiated at ≥ 800 Gy exhibited additional peaks with lower fluorescence values than that for G1. As a result of the histogram analysis using the "Overlapped Peak" fitting option, it was assumed that these sub-G1 peaks, so-called apoptotic peaks, are approximately Gaussian in shape and relevant to the DNA fragmentation.

By 2 days after irradiation, no change had occurred in the CV and BAD between the 'Siberia' flowers irradiated up to 200 Gy and control (Fig. II-4). The CV sharply increased in the flowers irradiated at ≥ 400 Gy. Moreover, an apoptotic peak developed with increasing BAD after irradiation at ≥ 400 Gy. The flowers irradiated at 1,600 Gy showed a great increase of BAD and the apoptotic peak represented 40.4% of cells. No clear shift in the G0/G1 peak of 'Siberia' petal cells was observed at 2 DAT when compared to 0 DAT.

In 'Medusa', the CV did not differ between irradiated flowers and control at 0 DAT, while the flowers irradiated at 400 Gy showed a sharply increased the CV (Fig. II-5). Apoptotic peak and BAD were rapidly progressed in the flowers irradiated at ≥ 400 Gy, indicating that response to irradiation was more sensitive in 'Medusa' than in 'Siberia'.

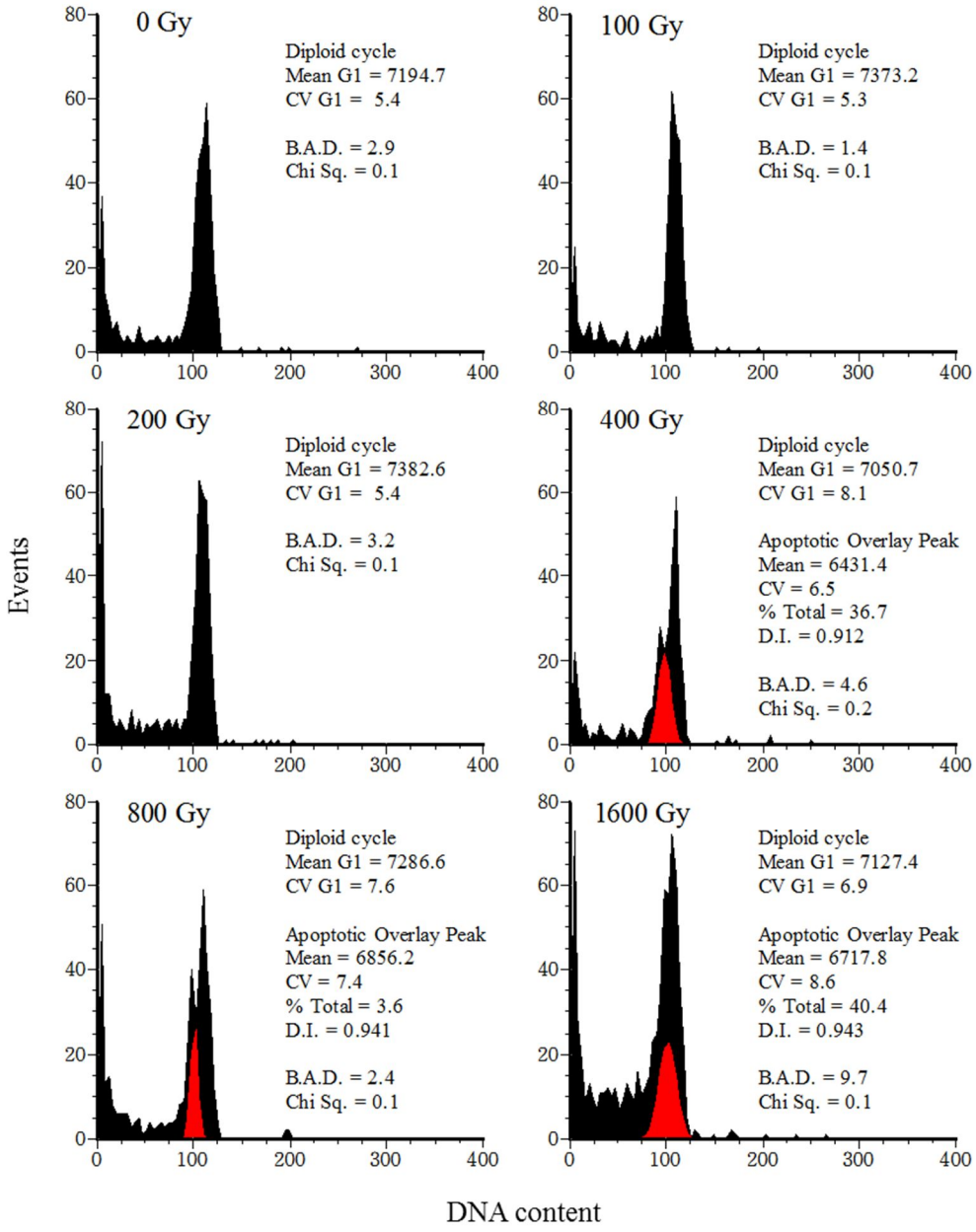

Fig. II-4. Flow cytometric determination of fluorescence by DAPI-stained DNA masses in tepals of 'Siberia' lilies at 2 days after electron beam irradiation. Data were analyzed with Multicycle AV and FCS Express. Red histogram portion, apoptotic DNA fragments.

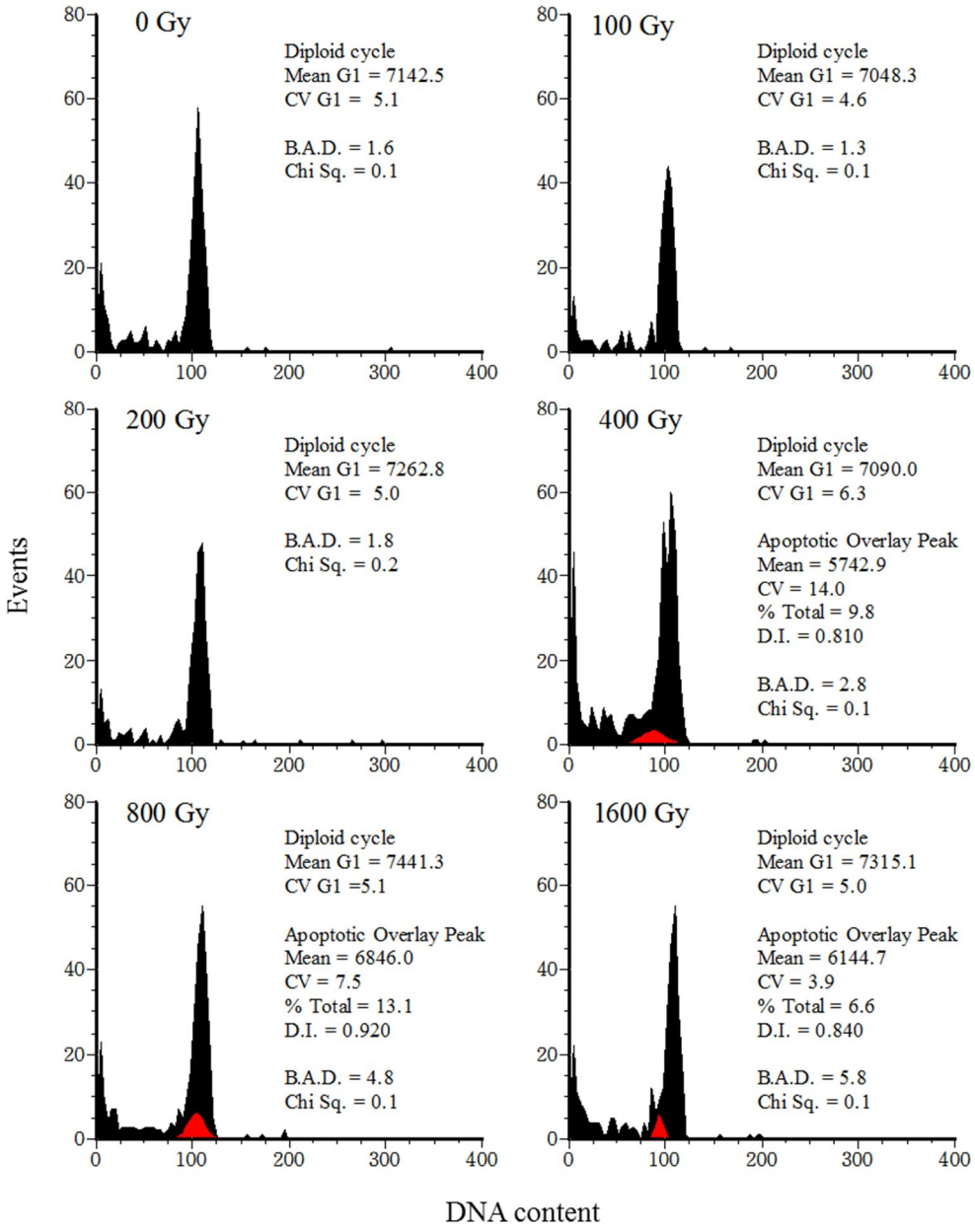

Fig. II-5. Flow cytometric determination of fluorescence by DAPI-stained DNA masses in tepals of 'Medusa' lilies at 0 days after electron beam irradiation. Data were analyzed with Multicycle AV and FCS Express. Red histogram portion, apoptotic DNA fragments.

By 2 days after irradiation, the CV of 'Medusa' tepal cells was elevated with increasing the irradiation dose (Fig. II-6). An apoptotic peak developed with great increasing BAD after irradiation at ≥ 100 Gy. The apoptotic peaks in the flowers irradiated at 100, 200, 400, and 800 Gy represented 26.3, 66.0, 68.9, and 60.6% of cells, respectively. The flowers irradiated at 1,600 Gy showed an apoptotic peak represented 25.3% of cells, while the peak had 79.0% the DNA staining intensity of diploid cells. These observations were attributed to DNA degradation and its subsequent leakage from the cell.

Electron Beam Irradiation-induced Cell Death

Cell death in tepals of 'Siberia' and 'Medusa' lilies was demonstrated by trypan blue staining. Electron beam irradiation did not cause cell death in the 'Siberia' and 'Medusa' lilies at 0 DAT (data not shown). By 2 days after irradiation, deep blue-stained dead cells were obvious in the 'Siberia' flowers irradiated at ≥ 400 Gy, while no cell death was observed in the flowers irradiated at ≤ 200 Gy (Fig. II-7). Cell death in the flowers irradiated at 400 Gy occurred in a small group of cells buried in a bulk of surrounding healthy cells. With increasing the irradiation dose, numerous dead cells were stained much darker.

By 2 days after irradiation, trypan blue staining also showed that numerous dead cells were present in tepals of 'Medusa' lilies irradiated at ≥ 100 Gy, but no dead cells were observed in the control (Fig. II-8). Staining intensity was greater in the flowers irradiated at ≥ 200 Gy than at 100 Gy.

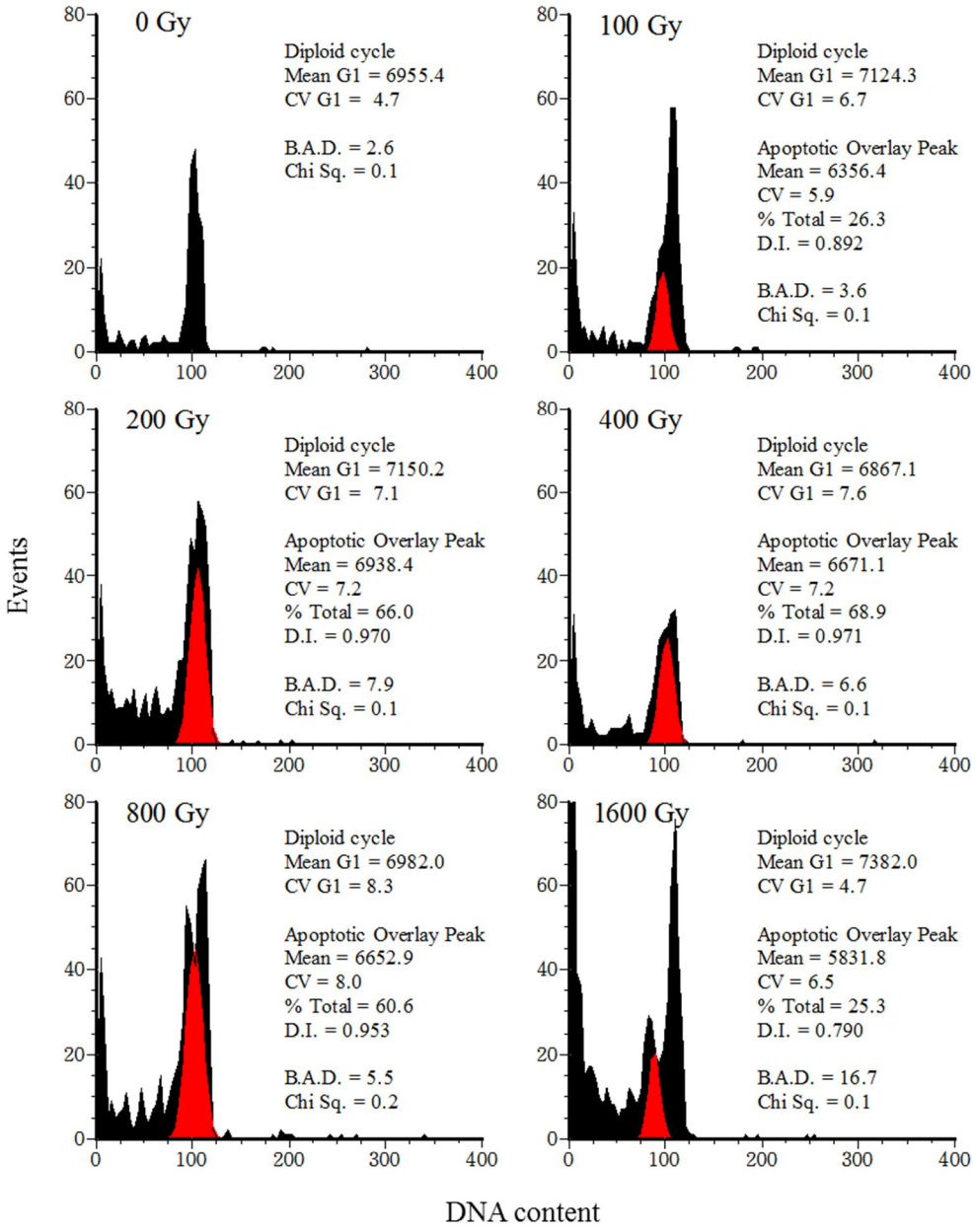

Fig. II-6. Flow cytometric determination of fluorescence by DAPI-stained DNA masses in tepals of 'Medusa' lilies at 2 days after electron beam irradiation. Data were analyzed with Multicycle AV and FCS Express. Red histogram portion, apoptotic DNA fragments.

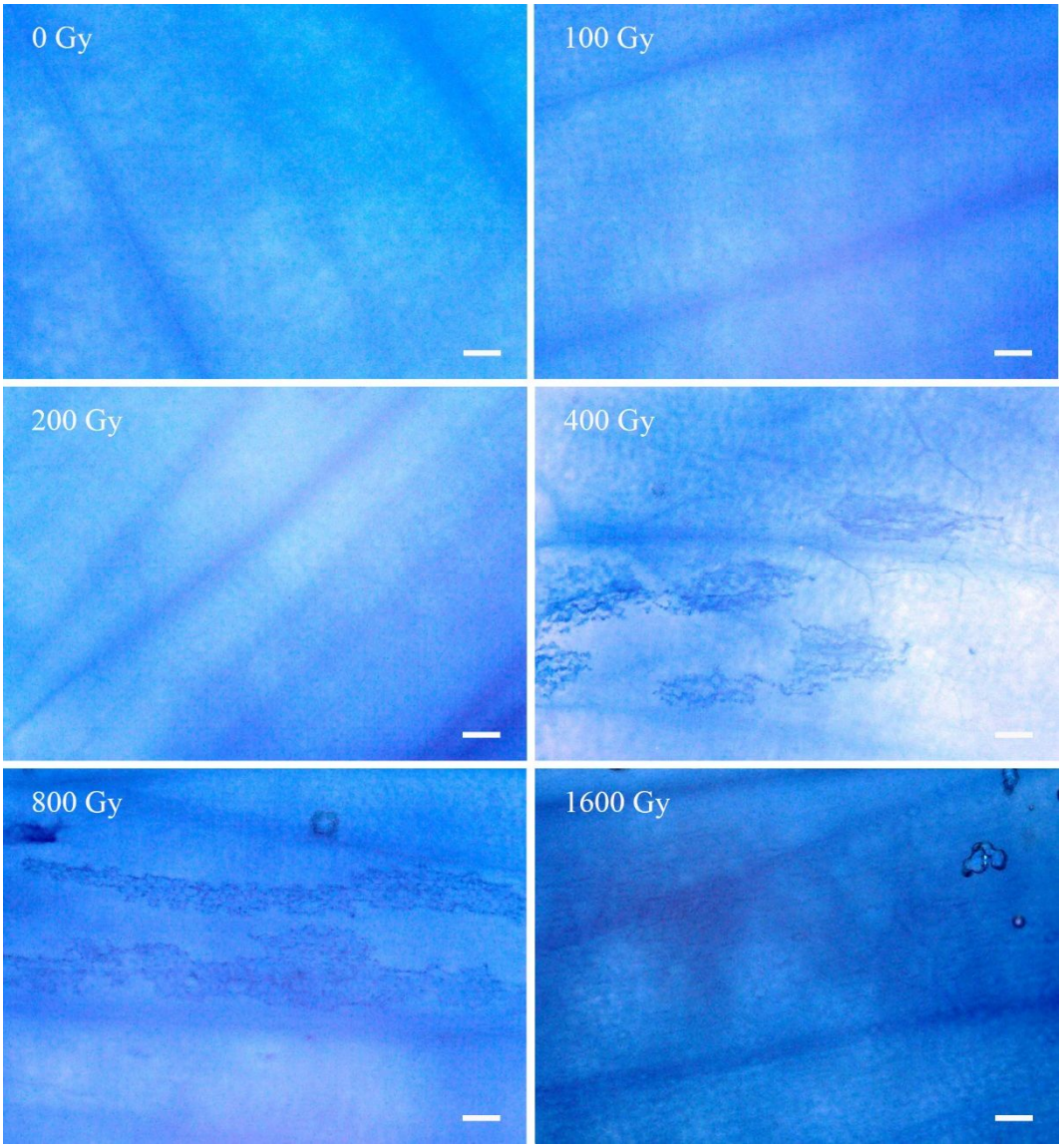

Fig. II-7. Trypan blue staining of epidermal cells in tepals of 'Siberia' lilies at 2 days after electron beam irradiation. Bars = 200 μm.

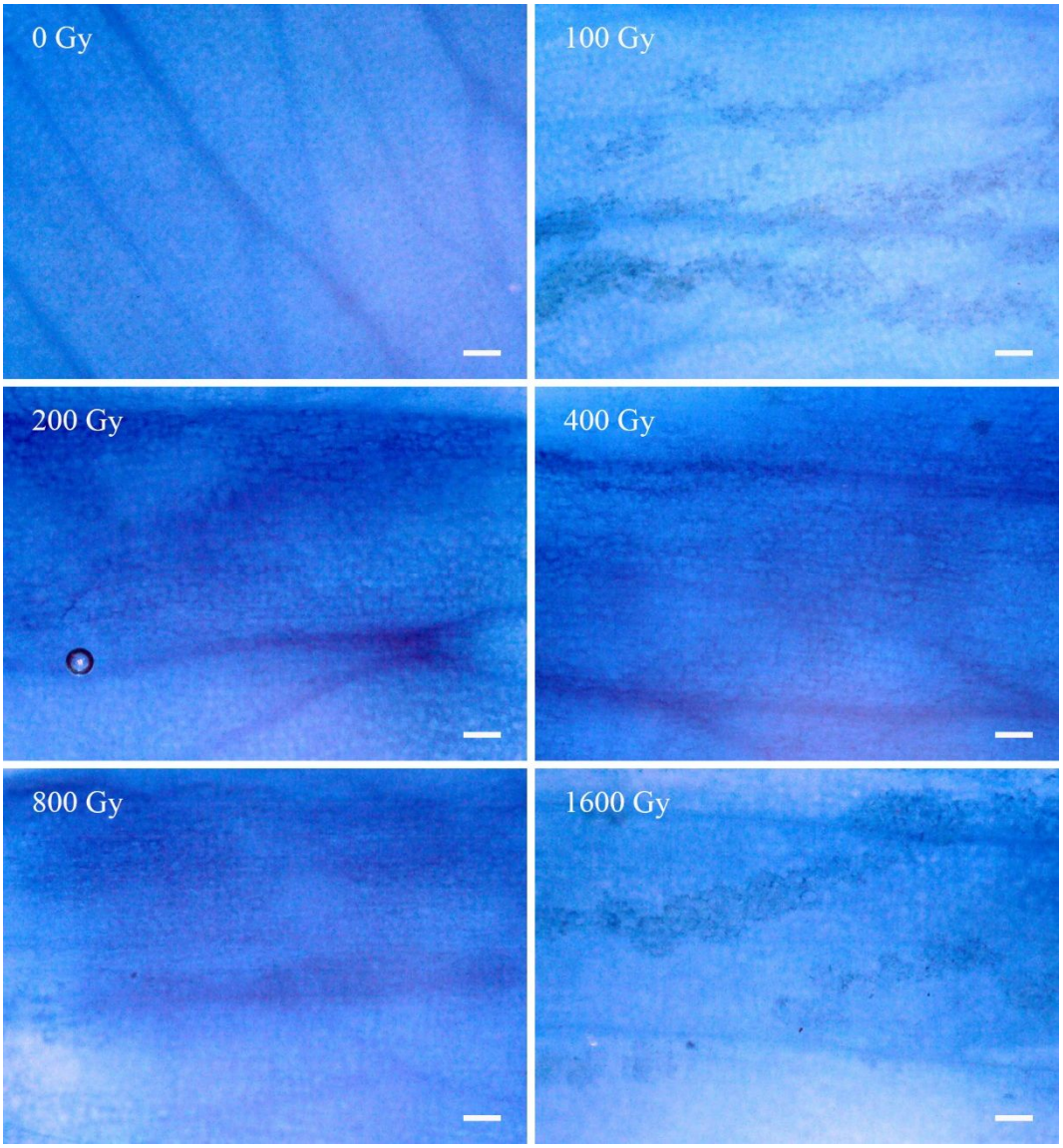

Fig. II-8 Trypan blue staining of epidermal cells in tepals of 'Medusa' lilies at 2 days after electron beam irradiation. Bars = 200 μm.

DISCUSSION

Tolerance of cut flowers to electron beam irradiation varied with species and cultivars. Dose response curves showed that lily flowers were highly sensitive to electron beam irradiation because their EC_{10} values were lower than other cut flowers from 144.4 to 306.3 Gy (Fig. I-9). Since failure of flower bud opening was the limiting factor for postharvest quality loss of irradiated cut lilies, the objective of this study was to determine the effects of electron beam irradiation on the major changes during flower bud opening, including changes in osmolarity and sugar contents, as well as DNA damage.

In non-irradiated lily flowers, concomitant with elevation of osmolarity, soluble sugar levels in tepals increased prior to the onset of flower opening (Figs. II-1, 2). A trend toward increase in osmolarity was maintained up to day 6, which resulted in a larger water potential gradient, possibly causing a great water influx into the expanding cells and subsequent turgor changes. Similar pattern was observed in lily flowers irradiated at $\leq$ 200 Gy, presumably a major force in driving flower opening. Electron beam irradiation caused the inhibition of flower bud opening at $\geq$ 400 Gy (Fig. II-1). These differences could be explained in several ways. First, the conversion of polysaccharides to monosaccharides in tepals has ceased. The total soluble sugars in tepals were highly accumulated at day 2, while followed by a rapid decline after day 2 (Fig. II-2). Second, early decline in osmotic pressure in bud stages might be another cause. Osmolarity was less increased or gradually decreased during bud development of flowers irradiated at $\geq$ 400 Gy (Fig. II-2). Rapid reduction in total soluble sugars followed

by gradual decline of osmolarity after day 2, which may be associated with lack of bud opening or partial opening of the flowers irradiated at ≥ 400 Gy.

Higher levels of osmolarity were found in irradiated flowers than in non-irradiated controls (Figs. II-1, 2). Compared to total soluble sugars, there was little or no change in cell sap osmolarity. The result suggest that the elevation of sugar concentrations did not contribute to the increase in osmolarity. Another osmoticum might be present in the irradiated tepals. The radiolytic products produced by irradiation could be considered an osmoticum, which generally act as oxidizing agents and can cause several changes in the molecular structure of organic matter. Many studies have summarized the effects of ionizing radiation on the primary components of plants, including the carbohydrates, lipids, and proteins. When subjected to ionizing radiation, the complex carbohydrates break down into the simpler sugars, while the monosaccharides break down into sugar acids and ketones. Proteins are also the splitting of large molecules into smaller units and lipids produce peroxides, alcohols, carbonyl compounds, hydroxy and keto acids, lactones and polymers by irradiation (Miller, 2005). Low radiation doses are known to have little effect on plant components, however, high radiation doses can weaken fibrous plant cell wall material leading to a deterioration of texture. In a study by Prakash et al. (2002), changes in the cell wall components, particularly the water-soluble pectin was observed in irradiated tomatoes. Irradiation can also induce functional impairments to the cellular membrane systems, therefore, damage symptoms such as water-soaked appearance, loss of turgor, and leakage of electrolytes are revealed. Fan and Sokorai (2005) found that

irradiation induced increase in electrolyte leakage in vegetables, and the radiosensitivity varied among the vegetables.

DNA is well known to be the major cellular target for ionizing radiation. Flowers can also be damaged by irradiation, depending on the tolerance of flowers to irradiation as well as the irradiation dose. The radiation-induced DNA damage could be analyzed by using a flow cytometry analysis. Selvan and Thomas (1995) showed that nuclei from irradiated (600 to 900 Gy) onions exhibited a broader DNA distribution profile appearing as a wide CV of the G0/G1 peak as compared to non-irradiated samples. The DNA index of the diploid cells in control onions was 1 as against 0.74 in irradiated samples thereby suggesting the presence of G0/G1, cells with abnormal DNA content in the meristem tissue of irradiated onions. This study also presents a flow cytometry analysis for detecting DNA damage. When subjected to electron beam irradiation, 'Siberia' and 'Medusa' tepal cells showed broad CV of Go/G1 peaks, high BAD, and apoptotic peaks in a dose-dependent manner (Figs. II-3, 4, 5, 6). By 0 days after irradiation, an apoptotic peak developed with increasing BAD after irradiation at ≥ 800 and ≥ 400 Gy in 'Siberia' and 'Medusa' lilies, respectively. By 2 days after irradiation, it was observed that an apoptotic peaks were progressed in lower irradiation doses at ≥ 400 and ≥ 100 Gy in 'Siberia' and 'Medusa' lilies, respectively. Therefore, 'Medusa' were more sensitive to irradiation than 'Siberia' lilies. These results are in agreement with those of previous studies determining dose effects for postharvest quality.

Senescence in flower petals (autophagic) and synchronized cell death by physical treatments such as irradiation (apoptotic-like) can be regarded as a form

of programmed cell death (PCD), which include cellular shrinkage, chromatin condensation, and DNA fragmentation, followed by nuclear disintegration and formation of apoptotic bodies (Wang et al., 1996). A variety of methods have been devised to detect apoptotic-like changes in plant cells. Yamada et al. (2003) reported that nuclear fragmentation correlated with cell death in gladiolus petals after full flower opening was confirmed using flow cytometry, concomitant with DNA fragmentation, chromatin condensation, and nuclear fragmentation. Battelli et al. (2011) showed that DNA fragmentation, confirmed by the TUNEL assay, was the first events in tepal senescence in *Lilium longiflorum*. Yamada et al. (2006) also reported that DNA degradation, determined by flow cytometry, occurred in the petals prior to visible wilting. Carballo et al. (2006) reported that root growth of onion was reduced by x-ray irradiation in a dose-dependent manner and activation of apoptosis was showed by flow cytometry plus TUNEL.

Histochemical measurements have been suggested for the identification of cell damage in irradiated plants (Thomas and Bramlage, 1986). Riov (1975) provided histochemical evidence for the cell death in irradiated citrus flavedo tissues. Trypan blue has been used to detect plant cell death. Although different modes of plant cell death exist in plants, one common characteristic of cell death is that the plasma membrane of dying cells ceases to function as a selective barrier (Lee et al., 2014; Pasqualini et al., 2003). This study also presents the cell damage in tepals of electron beam irradiated lilies (Figs. II-7, 8). The radiation-damaged cells were stained much darker with trypan blue than intact cells. The result of trypan blue staining is consistent with that of radiation-induced DNA damage by using a flow cytometry analysis.

In conclusion, failure of flower opening induced by electron beam irradiation may be due to insufficient osmotic pressure, discontinuation of the persistent sugar supply, DNA damage, and cell death. Dose-response DNA damage and cell death may also be the radiotolerance criteria of cut flowers and give sufficiently accurate dose estimation. Further studies will be required to promote flower opening and reduce the radiation-induced deterioration in cut flowers.

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CHAPTER III

Prevention of Electron Beam Irradiation-induced Deterioration of Cut Flowers

ABSTRACT

This study describes changes of postharvest quality of cut flowers that may have been damaged by electron beam irradiation and the effect of postharvest treatments known to minimize irradiation damage. The effects of electron beam irradiation on the postharvest quality of 'Siberia' lilies and 'Leopard' chrysanthemums were examined and it was determined whether radiation-induced deterioration of cut flowers can be prevented with postharvest treatment. In experiment 1, cut lily flowers were irradiated at 200 and 400 Gy with a 10 MeV linear electron beam accelerator and then placed in a preservative solution of 20 $g \cdot L^{-1}$ sucrose and 200 $mg \cdot L^{-1}$ hydroxyquinoline citrate (HQC). In experiment 2, cut lily flowers were pretreated with Chrysal SVB for 20 h and then irradiated at 100, 200, and 400 Gy. Preservative solution was used 1% Chrysal professional No. 3 (CP3). In experiment 3, cut chrysanthemum flowers were pretreated with 25 $mg \cdot L^{-1}$ benzyladenine (BA) and 100 $mg \cdot L^{-1}$ sodium hypochlorite (SH) for 4 h. Pretreated flowers were irradiated at 200 and 400 Gy and then placed in 1% CP3. Postharvest quality was determined by monitoring flower bud opening, flower diameter, fresh weight loss, flower longevity, visual quality of flowers and leaves,

flowering rate, and chlorophyll content. Sucrose treatment enabled more lily flowers to open fully. Pretreatment substances were less effective in extending flower longevity and in preventing fresh weight loss as the radiation dose increased in both lily and chrysanthemum flowers. In chrysanthemum flowers, BA pretreatment was effective in maintaining leaf quality and reducing irradiation damage to leaves. In all cut flowers, CP3 preservative solution significantly extended flower longevity, limited fresh weight loss, and increased the postharvest quality of flowers and leaves. In particular, flower longevity was more extended in the combination of CP3 and SH than CP3 and BA, regardless of irradiation dose in chrysanthemum flowers. In conclusion, preservative solution was more effective than pretreatment substances in preventing the detrimental effects of electron beam irradiation on cut flowers.

Additional keywords: benzyladenine, sodium hypochlorite, sucrose, postharvest quality, preservative solution

INTRODUCTION

It is well known that DNA is the major cellular target for ionizing radiation. Irradiation significantly affects physiological and biochemical processes in plants. The radiation-induced DNA damage in responsible for inhibition of seed germination, plant growth, and reproduction. Flowers can be damaged by irradiation, depending on the tolerance of flowers to irradiation as well as the irradiation dose; this damage includes reduced flower longevity, wilting, and inhibition of flower opening (Hayashi et al., 1998).

Many studies have shown that life span of individual flowers is limited by a decrease in available sugars in the cells. Exogenous sugars increase petal life span, possibly by preventing the early decrease in available sugars (Han, 2003). Pretreatments are short-term treatments implemented after harvest to increase the longevity of cut flowers, which include placing the stem in hot water or high sucrose solution, or exposing to an anti-ethylene compound. Preservative solution is used to increase water uptake and to extend flower longevity (Regan and Dole, 2010).

Recent surveys have attempted to reduce the radiation-induced deterioration of cut flowers. Hayashi and Todoriki (1996) also reported that sugars prevent the detrimental effects of γ-irradiation at 750 Gy on cut chrysanthemums. Sangwanangkul et al. (2008) also found that postharvest treatments such as 1-methylcyclopropene (1-MCP), hot water, benzyladenine (BA), and glucose are effective in extending the vase life of irradiated tropical ornamentals.

The present study was designed to investigate whether radiation-induced deterioration of cut flowers can be prevented with postharvest treatment.

MATERIALS AND METHODS

Plant Materials

In experiments 1 and 2, cut lily flowers (*Lilium* Oriental hybrid 'Siberia') were purchased from a local wholesaler in Seoul, Korea. Flowers were at the commercial harvest stage, with the largest bud showing color. In experiment 3, cut 'Leopard' flowers (*Dendranthema grandiflorum* 'Leopard'), a spray-type chrysanthemum, were obtained from Gumi Infrastructure Corporation (Gumi, Korea). Flowers were harvested at stage III, with 30-40% of flowers fully opened, according to the chronological stages of development suggested by Yoo and Roh (2012).

Electron Beam Irradiation

Electron beam irradiation was conducted in EB-Tech Co., Ltd. (Daejeon, Korea) using a high energy linear accelerator (UEL V10-10S, 10 MeV). In experiment 1 and 3, cut flowers were irradiated at 200 and 400 Gy. In experiment 2, cut flowers were irradiated at 100, 200, and 400 Gy. Target doses were monitored by dosimetry with a radiochromic film dosimeter (GAF3002DS, GEX Corp., Centennial, CO, USA) (ISO/ASTM51275:2004(E)).

Postharvest Treatments

Experiment 1. After irradiation, lily flower stems were recut to the same length (20 cm) and then placed in the two types of preservative solution. Control included 200 $mg \cdot L^{-1}$ of antimicrobial compound hydroxyquinoline citrate (HQC)

in the distilled water and treatment did 20 $g \cdot L^{-1}$ sucrose and 200 $mg \cdot L^{-1}$ HQC in the distilled water.

Experiment 2. The cut lily flowers were pretreated with distilled water and 1 tablet of Chrysal SVB (Chrysal International B.V., Naarden, The Netherlands) in 3 L of distilled water for 20 h. After irradiation, the flower stems were recut to the same length (30 cm) and then placed in distilled water and preservative solution of 1% Chrysal professional No. 3 (CP3, Chrysal International B.V).

Experiment 3. The cut chrysanthemum flowers were divided into three groups and then pretreated with distilled water, 25 $mg \cdot L^{-1}$ BA, and 100 $mg \cdot L^{-1}$ sodium hypochlorite (SH) for 4 h. After irradiation, the flower stems were recut to the same length (30 cm) and then placed in a distilled water and preservative solution of 1% CP3 (Chrysal International B.V).

Quality Evaluation

The flowers were maintained in an air-conditioned room with a 12 h light cycle at 23 ± 1°C, a relative humidity of 60 ± 10%, and a leaf level photosynthetic photon flux density at 140 $\mu mol \cdot m^{-2} \cdot s^{-1}$ provided by cool white fluorescent lamps. In experiment 1, flower bud opening was observed by the criteria as shown in Table II-1. In experiment 2, postharvest quality was determined by monitoring fresh weight loss, flower longevity, flower diameter, visual quality of flowers and leaves, and chlorophyll content. In experiment 3, postharvest quality was determined by monitoring fresh weight loss, flower longevity, flowering rate, and visual quality of flowers and leaves in the same manner. The experiments were replicated five times.

Statistical Analysis

Statistical analysis was performed using SAS software, version 9.2 (SAS Inst., Cary, NC, USA). Differences among the group means were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test at 5% level.

RESULTS

Experiment 1

The effects of sugar in the preservative solution on flower bud opening of 'Siberia' lilies after electron beam irradiation were examined. Addition of 20 $g \cdot L^{-1}$ sucrose and 200 $mg \cdot L^{-1}$ HQC into the preservative solution resulted in opening of more flower bud than controls regardless of electron beam irradiation (Fig. III-1). Sucrose treatment enabled more flowers to open fully after irradiation at ≤ 200 Gy, while flowers remained only partially open by sucrose treatment after 400 Gy irradiation. According to these results, treatment with 20 $g \cdot L^{-1}$ sucrose and 200 $mg \cdot L^{-1}$ HQC after 200 Gy irradiation might prevent irradiated-induced deterioration, lack of bud opening, whereas sucrose treatment was not yet adequate after 400 Gy irradiation. Studies are needed to prevent irradiation-induced deterioration and accelerate flower opening after 400 Gy irradiation.

Experiment 2

The effects of pretreatment substances, electron beam irradiation, and preservative solutions on the postharvest quality of cut 'Siberia' lilies were examined. SVB pretreated cut flowers lost their fresh weight faster than control with increasing the irradiation dose, while cut 'Siberia' lilies lost fresh weight slowly by using a preservative solution, CP3 regardless of the irradiation dose (Fig. III-2).

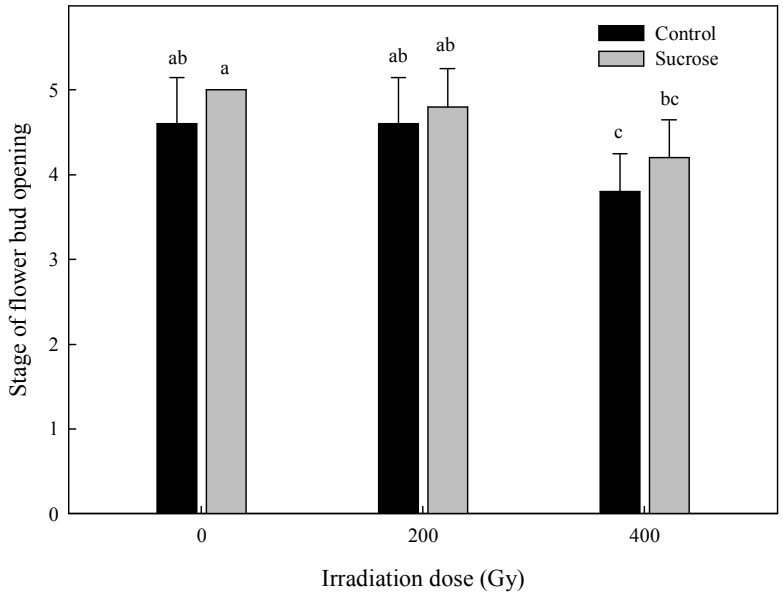

Fig. III-1. Effects of sugar in preservative solution on flower bud opening of 'Siberia' lilies after electron beam irradiation. Cut flowers were irradiated at 200 and 400 Gy with a 10 MeV linear electron beam accelerator. Control, solution containing 200 mg·L^{-1} HQC; Sucrose, solution containing 20 g·L^{-1} Sucrose and 200 mg·L^{-1} HQC. Data are the means ± SD from five replications.

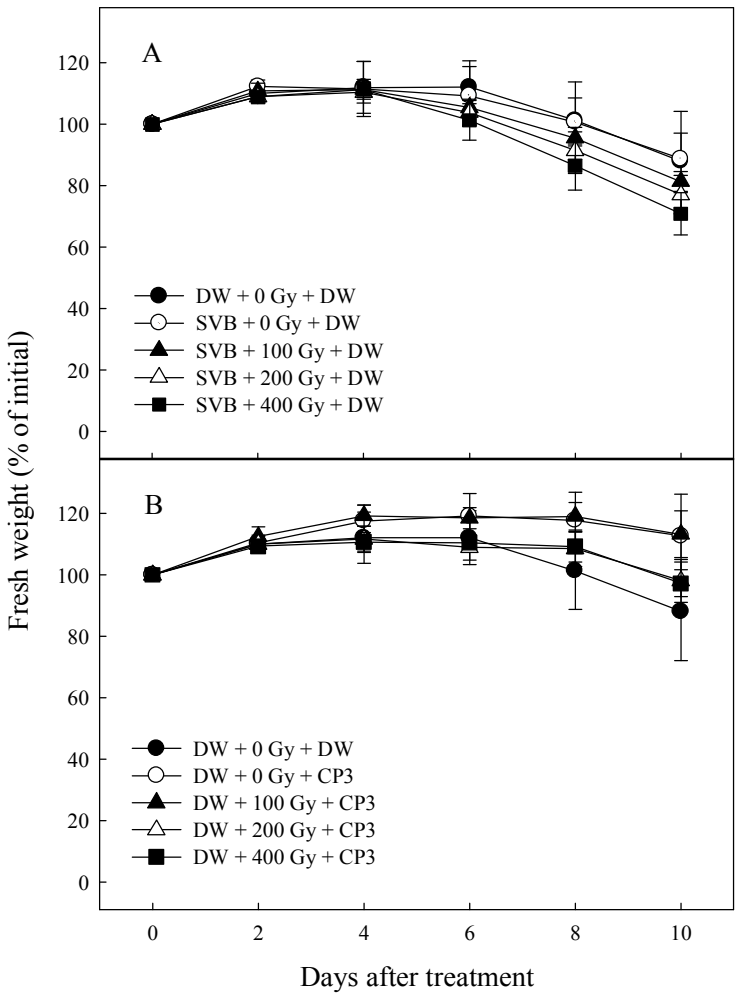

Fig. III-2. Effects of electron beam irradiation combined with pretreatment substances (A) and preservative solution (B) on fresh weight of 'Siberia' lilies. Cut flowers were irradiated at 100, 200, and 400 Gy with a 10 MeV linear electron beam accelerator. DW, distilled water; SVB, Chrysal SVB (1 tablet/3 L H_2O); CP3, 1% Chrysal professional No. 3. Data are the means ± SD from five replications.

Without regard to the electron beam irradiation, SVB pretreatment had no effect on flower longevity (Table III-1). However, CP3 preservative solution significantly extended the longevity irrespective of the irradiation dose. Especially, it was effective in extending flower longevity when the flowers were held in CP3 after irradiation with 100 Gy. SVB pretreatment did not prevent the inhibition of flower diameter under 400 Gy irradiation, whereas diameter of CP3 treated flowers was similar to that of control under same irradiation dose. The visual quality of flowers was significantly improved when the flowers were held in CP3 after irradiation with ≤ 200 Gy (Fig. III-3). The visual quality of leaves and chlorophyll content dropped under 400 Gy irradiation regardless of SVB or CP3.

Experiment 3

The effects of combination with pretreatment substances and preservative solutions on the fresh weight of non-irradiated 'Leopard' chrysanthemums were examined (Fig. III-4A). Pretreatment substances, BA and SH, had no effects on the fresh weight of non-irradiated flowers, while a conventional preservative solution of CP3 showed a significant increase in fresh weight. However, CP3 in combination with pretreatment substances resulted in a slower decline in fresh weight than distilled water alone. SH combined with CP3 was more effective in delaying the decrease of the fresh weight than BA combined with CP3.

Fig. III-4B shows the fresh weight of the flowers irradiated at 200 Gy. Both BA and SH did not prevent the decrease in fresh weight, whereas CP3 showed a significant increase in fresh weight under 200 Gy irradiation.

Table III-1. Effects of pretreatment substance, electron beam, and preservative solution on the postharvest quality of 'Siberia' lilies.

Pretreatment substance[z]	Dose (Gy)	Preservative solution[y]	Flower longevity (day)	Flower diameter (mm)	Visual quality of flowers (1-5)[x]	Visual quality of leaves (1-5)[w]	Chlorophyll content (SPAD readout)[v]
DW	0	DW	10.6 bc[u]	181.5 ab	4.0 cd	4.8 a	59.5 a
SVB	0	DW	10.6 bc	191.2 a	4.2 bcd	5.0 a	62.1 a
	100	DW	10.0 c	184.1 ab	4.0 cd	4.6 ab	62.7 a
	200	DW	10.0 c	184.6 ab	3.8 d	4.8 a	60.4 a
	400	DW	10.2 c	136.0 c	3.8 d	3.4 c	51.4 b
DW	0	CP3	11.4 ab	194.2 a	4.6 abc	4.0 bc	59.3 a
	100	CP3	12.2 a	193.1 a	5.0 a	4.8 a	62.1 a
	200	CP3	11.6 ab	191.7 a	4.8 ab	4.8 a	60.4 a
	400	CP3	11.0 bc	171.4 b	4.2 bcd	4.0 bc	52.7 b
Significance			***	****	**	***	***

[z]DW, SVB: distilled water and Chrysal SVB (1 tablet/3 L H_2O), respectively.
[y]DW, CP3: distilled water and 1% Chrysal professional No. 3, respectively.
[x]Based on a scale of 1 to 5, measured at 8 days after treatment. Refer to Table I-3.
[w]Based on a scale of 1 to 5, measured at 8 days after treatment. Refer to Table I-2.
[v]Leaf chlorophyll content at 8 days after treatment.
[u]Duncan's multiple range test within columns, $P = 0.05$.
, *, ****Significant at $P = 0.01$, 0.001, or 0.0001, respectively.

Fig. III-3. Appearance of ‘Siberia’ lily flowers at 8 days after electron beam irradiation. DW, distilled water; SVB, Chrysal SVB (1 tablet/3L H_2O); CP3, 1% Chrysal professional No. 3.

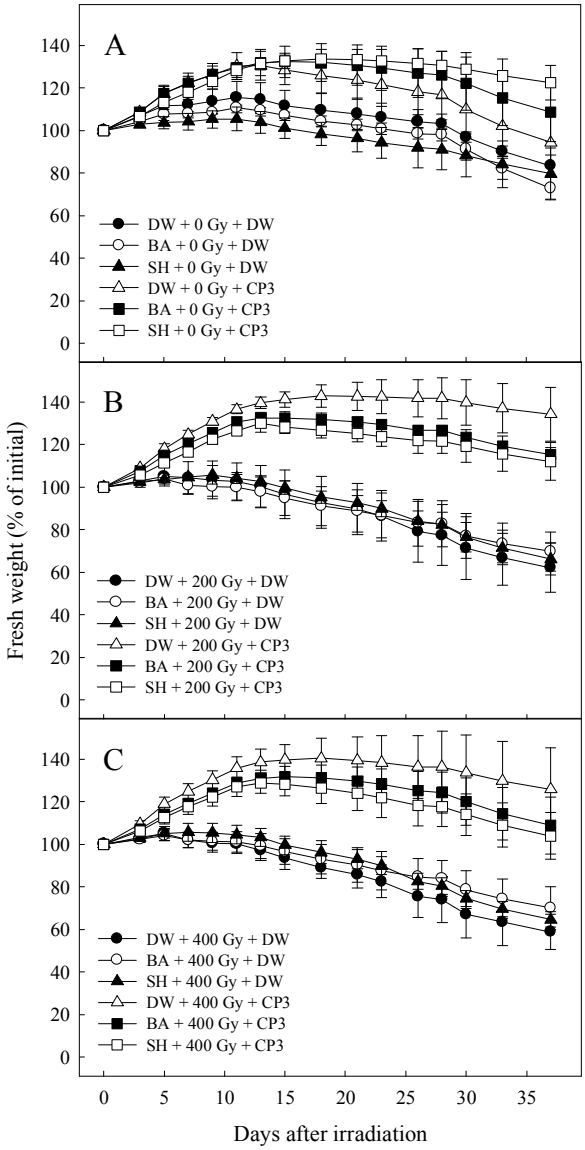

Fig. III-4. Effects of electron beam irradiation combined with pretreatment substances and preservative solution on fresh weight of 'Leopard' chrysanthemums. Cut flowers were irradiated at 0 (A), 200 (B), and 400 Gy (C) with a 10 MeV linear electron beam accelerator. DW, distilled water; CP3, 1% Chrysal professional No. 3; BA, 25 mg·L^{-1} benzyladenine; SH, 100 mg·L^{-1} sodium hypochlorite. Data are the means ± SD from five replications.

Under 200 Gy irradiation, CP3 alone was more effective than pretreatment substances combined with CP3 in delaying the decrease in fresh weight. Similar effects were observed for 400 Gy irradiation (Fig. III-4C). While pretreatment substances did not prevent a decrease in fresh weight when flowers were irradiated with 400 Gy, CP3 delayed this decrease, particularly in combination with distilled water.

SH pretreatment extended flower longevity more than BA pretreatment (Table III-2). However, pretreatment substances were less effective in extending flower longevity with increasing irradiation dose. CP3 preservative solution significantly extended the longevity of cut chrysanthemum flowers. The effect of CP3 on flower longevity was maintained, even if the irradiation dose increased. It was especially effective in extending flower longevity when the flowers were held in CP3 after irradiation with 200 Gy. CP3 in combination with SH extended flower longevity more than BA, regardless of the irradiation dose. In particular, SH + CP3-induced extension of flower longevity was more pronounced in irradiation up to 200 Gy.

A trend toward reduced flowering rate in non-pretreated flowers held in distilled water was observed, regardless of the irradiation dose (Table III-2). Pretreatment substances tended to increase flowering rate. As the irradiation dose increased, BA-pretreated flowers showed a reduction in flowering rate, while SH-pretreated samples retained a high flowering rate. Flowering rate increased in the flowers held in CP3 preservative solution. CP3-induced elevation of flowering rate was more pronounced after electron beam irradiation. Higher flowering rates

(≥ 98%) were significant in the combination of pretreatment substances and preservative solution.

Table III-2. Effects of pretreatment substance, electron beam, and preservative solution on the postharvest quality of 'Leopard' chrysanthemums.

Pretreatment substance[z]	Dose (Gy)	Preservative solution[y]	Flower longevity (day)	Flowering rate (%)[x]	Visual quality of flowers (1-5)[w]	Visual quality of leaves (1-5)[v]
DW	0	DW	33.0 d[u]	83.4 cd	3.0 c	3.2 c
		CP3	37.0 abc	92.0 abc	5.0 a	4.2 ab
	200	DW	37.4 abc	88.0 cd	3.8 b	3.8 bc
		CP3	40.4 a	100.0 a	5.0 a	4.8 a
	400	DW	35.4 cd	82.0 d	3.6 bc	3.8 bc
		CP3	39.4 ab	100.0 a	5.0 a	4.2 ab
BA	0	DW	37.8 abc	98.0 ab	3.6 bc	4.2 ab
		CP3	36.0 bcd	100.0 a	4.6 a	4.2 ab
	200	DW	38.8 abc	92.0 abc	3.8 b	4.2 ab
		CP3	38.8 abc	98.0 ab	4.8 a	4.2 ab
	400	DW	38.2 abc	88.0 cd	3.6 bc	4.0 b
		CP3	37.4 abc	100.0 a	5.0 a	4.2 ab
SH	0	DW	39.4 a	90.0 bcd	3.4 bc	3.8 bc
		CP3	40.4 a	100.0 a	4.8 a	4.2 ab
	200	DW	38.2 abc	98.0 ab	3.4 bc	4.0 b
		CP3	40.4 a	100.0 a	5.0 a	4.0 b
	400	DW	37.4 abc	98.0 ab	3.4 bc	4.0 b
		CP3	37.4 abc	98.0 ab	4.8 a	3.8 bc
Significance			**	****	****	*

[z]DW, BA, SH: distilled water, 25 mg·L^{-1} benzyladenine, and 100 mg·L^{-1} sodium hypochlorite, respectively.
[y]DW, CP3: distilled water and 1% Chrysal professional No. 3, respectively.
[x]Ratio of fully open flowers to the total number of initial flower buds per bunch.
[w]Based on a scale of 1 to 5, measured at 26 days after treatment. Refer to Table I-3.
[v]Based on a scale of 1 to 5, measured at 26 days after treatment. Refer to Table I-2.
[u]Duncan's multiple range test within columns, $P = 0.05$.
*, **, ****Significant at $P = 0.05$, 0.01, or 0.0001, respectively.

Postharvest quality was determined by rating the visual quality of flowers and leaves on a 5-point scale (Table III-2 and Fig. III-5). CP3 preservative solution was effective in increasing the visual quality of flowers in samples irradiated with up to400 Gy. On the other hand, pretreatment substances alone did not influence the visual quality of flowers. The visual quality of leaves was kept high by BA pretreatment. CP3 was also effective in increasing the visual quality of leaves.

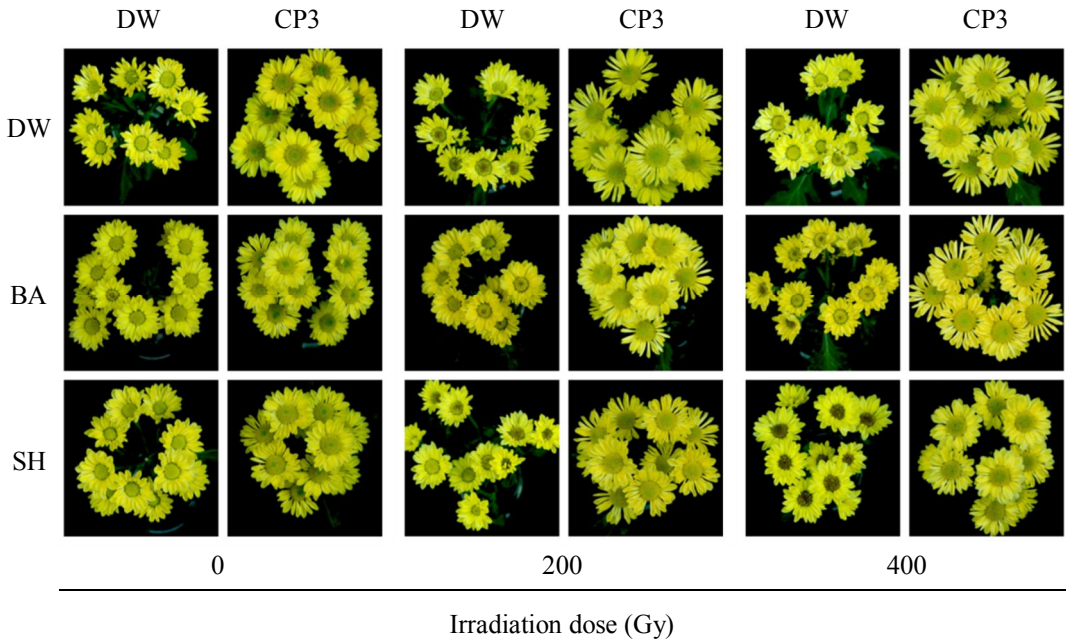

Fig. III-5. Appearance of 'Leopard' chrysanthemum flowers at 23 days after electron beam irradiation. DW, distilled water; CP3, 1% Chrysal professional No. 3; BA, 25 mg · L^{-1} benzyladenine; SH, 100 mg · L^{-1} sodium hypochlorite.

DISCUSSION

This study describes changes of postharvest quality of cut flowers that may have been damaged by electron beam irradiation and the effect of postharvest treatments known to minimize irradiation damage.

Sugars added to the preservative solution resulted in full opening of more flower buds in 'Siberia' lilies (Fig. III-1). Flower buds of cut inflorescences do not usually open fully, but the application of sucrose promotes flower opening. This suggests that soluble sugars are important in flower opening. In addition, the postharvest flower longevity of cut flowers is positively correlated with total sugar content of the tepals at the time of harvest (van Doorn and Han, 2011). In ethylene-sensitive flowers, exogenous sugars seem to delay petal senescence by reducing ethylene sensitivity (Hoeberichts et al., 2007). In ethylene-insensitive flowers, sugar might prevent a decline in osmotic pressure and delay cell death by providing an energy source (van Doorn, 2004; Yamada et al., 2003). Without regard to the electron beam irradiation, SVB pretreatment had no effect on flower longevity in 'Siberia' (Table III-1). The high water content of those flowers induced by 20 h pretreatment could increase their radiosensitivity.

SH, a strong oxidizer with broad-spectrum antimicrobial activity, has been used to extend the vase life of carnations (Edrisi et al., 2012) and gerbera (Macnish et al., 2008; van Meeteren, 1978). Lee and Lee (2013) also reported that SH pretreatment was highly effective in extending flower longevity of 'Leopard' chrysanthemums. Our results agree with previous studies indicating effects of SH on flower longevity (Table III-2).

BA application can improve water uptake, delay senescence, and enhance postharvest quality of cut flowers such as gerberas (Danaee et al., 2011), carnations (Mor et al., 1983), and roses (Gholami et al., 2011). The role of BA in delaying senescence of cut chrysanthemums is well established, and it has been used to prevent foliar chlorosis by retarding chlorophyll degradation in the leaves (Petridou et al., 2001). We also found that BA pretreatment is effective in maintaining leaf quality and reducing irradiation damage to leaves (Table III-2). These findings are consisted with those of Sangwanangkul et al. (2008), who reported effects of BA on prevention of irradiation-induced damage on red ginger inflorescence and Bird-of-Paradise leaves. However, our results are different from those of Hayashi and Todoriki (1996), who found that BA had little effect on 750 Gy γ-irradiated chrysanthemums.

Hayashi et al. (1998) reported that sugar treatments in the preservative solution after γ-irradiation at 750 Gy prevented radiation-induced damage to chrysanthemums, while sugar pretreatment before irradiation did not. This study also suggests that preservative solution is more effective than pretreatment substances for prevention of detrimental effects of electron beam irradiation on cut chrysanthemums, which is probably attributable to the importance of post-irradiation metabolism.

In conclusion, the present study documents an alleviating effect of postharvest treatments on irradiation-induced deterioration of cut flowers. This suggests that soluble sugar supply to the preservative solution may be more effective manner than pretreatment. Therefore, electron beam irradiation can be available as a quarantine treatment without detriment to postharvest quality of cut ‘Siberia’ lilies

and ‘Leopard’ chrysanthemums, considering that an irradiation dose of 400 Gy is established to be effective for quarantine security. Electron beam irradiation can be widely used by means of pretreatment substances and preservative solution.

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CHAPTER IV

Control of *Botrytis cinerea* by Electron Beam Irradiation

ABSTRACT

The present study was conducted to determine the effect of electron beam irradiation on control of *Botrytis cinerea,* troublesome to cut flowers during storage and shipment. Electron beam doses of 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 10, and 20 kGy were applied with a 10 MeV linear electron beam accelerator. Electron beams inhibited spore germination and mycelial growth of *B. cinerea* with increasing irradiation doses. Conidia of *B. cinerea* were more radiation resistant than mycelia: the effective irradiation doses for 50% inhibition (ED_{50}) of spore germination and mycelial growth were 2.02 and 0.89 kGy, respectively. Analysis of in vivo antifungal activity revealed that elevated irradiation doses exhibited increased control efficacy for tomato gray mold. In addition, electron beam irradiation was more effective in reducing mycelial growth of *B. cinerea* at 10^{o}C than at 20^{o}C. In conclusion, electron beam irradiation could control of *B. cinerea* in a dose-dependent manner and combined treatment of irradiation and low temperature may work synergistically to reduce *B. cinerea.*

Additional keywords: mycelial growth, spore germination, temperature, radiation tolerance

INTRODUCTION

Increasing worldwide demand for high quality floricultural produce requires due attention by industry to postharvest disease management (Dinh and Joyce, 2007). Although several diseases such as black spot (Reddy et al., 1992) and powdery mildew (Pasini et al., 1996) can be troublesome to cut flowers, the most severe economic damage to floriculture industry is caused by *Botrytis cinerea* (Elad, 1988).

B. cinerea is a widespread fungal pathogen that infects a variety of ornamentals such as rose, chrysanthemum, freesia, gerbera, and waxflower (Hammer, 1988). The damage can occur during various stages of plant growth and in postharvest during storage, transportation, and distribution (Elad, 1988). Disease symptoms are usually first appeared as water-soaked spots or flecks on the flower petals, then the lesions expand and coalesce, which causes the petals to turn brown and wither. Eventually, the entire flower may rot off at the receptacle (Hammer et al., 1990). Latent infections occur that are not visible at harvest but develop as flowers mature or under humid conditions during storage and shipment (Elad, 1988).

Control of *Botrytis* disease can be achieved by pre and/or post-harvest chemical applications. At present, many growers dip cut flowers in fungicides to prevent postharvest development of *B. cinerea* infections, but this practice leaves unsightly residues on the flowers and foliage (Hammer et al., 1990). Also, disease control with conventional fungicides carries the risk of development of fungicide resistant strains. Many cases of *B. cinerea* resistance to iprodione (Rovral) and

benomyl (Benlate) have been reported (Choi et al., 2009). Additionally, there is increasing public concern over fungicide usage in terms of human and environmental risk. Hence, there is a growing emphasis on environmentally friendly technologies in the flower industry, and the search for alternatives to chemical fungicides has received much attention.

In postharvest technology, several promising approaches such as irradiation, heat, antagonistic microorganisms, and methyl jasmonate are being actively pursued (Arabi et al., 2004; Darras et al., 2005; Elad and Volpin, 1991; Hang et al., 2005). Irradiation can be used for the purpose of reducing the attack of pathogens and maintaining the postharvest quality of fresh commodities. Irradiation allows the shelf life of fruits and vegetables to be extended throughout the inactivation or delay of the development of microorganisms which cause deterioration and quality loss during postharvest (Gomes et al., 2008). Many studies have shown that irradiation kills pathogens or markedly reduces pathogen counts (Kim et al., 2010; Takala et al., 2011). Electron beam irradiation has proven to be an effective treatment for inactivating pathogens such as *Phytophthora cinnamomi*, *P. citricola*, *Pythium ultimum*, and *Fusarium oxysporum* (Gryczka et al., 2010; Migdal et al., 2012; Orlikowski et al., 2011). The objectives of this research were to determine the effect of electron beam irradiation on control of *B. cinerea* and to evaluate the feasibility of irradiation as a postharvest disease management in cut flowers.

MATERIALS AND METHODS

Electron Beam Irradiation

Electron beam irradiation was conducted in the EB-Tech Co., Ltd. (Daejeon, Korea) using a high energy linear accelerator (UEL V10-10S, 10 MeV). Target doses of 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2 10, and 20 kGy were monitored by dosimetry with a radiochromic film dosimeter (GAF3002DS, GEX Corp., Centennial, CO, USA) (ISO/ASTM51275:2004(E))

Effect of Electron Beam Irradiation on Spore Germination

To evaluate the irradiation sensitivity of *B. cinerea*, spore germination by electron beam irradiation was measured. The fungal pathogen, *B. cinerea* was obtained from the Korea Research Institute of Chemical Technology. A spore suspension of *B. cinerea* at 1×10^5 spores·mL^{-1} was irradiated with doses of 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2 10, and 20 kGy, respectively. After irradiation, 80 μL of the spore suspension were placed on hole slide glass. The glasses were sealed in a moistened box, and incubated in the dark at 20°C for 12 h. The percentage of germinating spores were determined by the direct microscopic examination of 100 spores at ×300 magnification. Spores were considered germinated if the germ tube was equal to or greater than the diameter of the spore. There were three replicates for each treatment. Nonlinear regression was conducted to determine the electron beam irradiation dose and the inhibition of spore germination by Sigma Plot, version 10.0 (SPSS Science, Chicago, IL, USA). Data were fit to three-parameter sigmoidal curves using the following equation: $y = a/(1 + e^{(-(x - x0)/b)})$.

Effect of Electron Beam Irradiation on Mycelial Growth

To evaluate the irradiation sensitivity of *B. cinerea*, inhibition of mycelial growth by electron beam irradiation was measured. The fungal pathogen, *B. cinerea* was obtained from the Korea Research Institute of Chemical Technology. An agar plug (8 mm diameter) cut from the margin of actively growing cultures was placed to the potato dextrose agar (PDA) plates and then irradiated with doses of 0, 0.1, 0.2, 0.4, 0.6, 0.8, 1, 2, 10, and 20 kGy, respectively. The mycelial growth was examined by measuring the length of mycelium mat from the center of the plate after 4 days incubation in the dark at 20^{o}C. Inhibition of mycelial growth (%) was determined using the following equation: Inhibition of mycelial growth (%) = (1 – mycelial diameter of treatment/mycelial diameter of control) × 100. There were five replicates for each treatment. Nonlinear regression was conducted to determine the electron beam irradiation dose and the inhibition of mycelial growth by Sigma Plot, version 10.0 (SPSS Science). Data were fit to two-parameter exponential rise to maximum curves using the following equation: $y = a(1 - e^{-bx})$.

In Vivo Antifungal Activities of Electron Beam Irradiation

In vivo antifungal activities of electron beam irradiation against *B. cinerea* were tested. Tomato (*Lycopersicon esculentum* 'Seokwang') plants were grown in the plastic pots in a greenhouse at 25 ± 5°C for 3 weeks. A spore suspension of *B. cinerea* at 1×10^5 spores·mL^{-1} was irradiated with doses of 0, 0.2, 0.4, 1, 2, and 10 kGy, respectively. The plant seedlings of the 4th leaf stage were inoculated by spraying a spore suspension. The inoculated tomato plants were kept in the dark at

20°C and 95% RH. Disease severity was assessed 3 days after inoculation. There were five replicates for each treatment. Control efficacy of electron beam irradiation on tomato gray mold was determined using the following equation: Control value (%) = (1 – B/A) × 100, where A = the area of infection (%) on untreated leaves and B = the area of infection (%) on treated leaves.

Effect of Electron Beam Irradiation on Mycelial Growth at Different Incubation Temperature

To evaluate the irradiation resistance of *B. cinerea* at different incubation temperature, mycelial growth was measured. The fungal pathogen, *B. cinerea* was obtained from the Korea Research Institute of Chemical Technology. An agar plug (8 mm diameter) cut from the margin of actively growing cultures was placed to the PDA plates and then irradiated with doses of 0, 0.4, and 0.8 kGy, respectively. Irradiated PDA plates were incubated in the dark at 5, 10, 15, and 20°C, respectively. The mycelial growth was examined by measuring the length of mycelium mat from the center of the plate daily for 6 days. Inhibition of mycelial growth (%) at different incubation temperature was determined using the following equation: Inhibition of mycelial growth (%) = (1 – mycelial diameter of treatment/mycelial diameter of control) × 100. There were five replicates for each treatment. Nonlinear regression was conducted to determine the incubation temperature and the inhibition of mycelial growth after electron beam irradiation by Sigma Plot, version 10.0 (SPSS Science). Data were fit to three-parameter sigmoidal curves using the following equation: $y = a/(1 + e^{(-(x - x0)/b)})$.

Statistical Analysis

Statistical analysis was performed using SAS software, version 9.2 (SAS Inst., Cary, NC, USA). Differences among the group means were analyzed using analysis of variance (ANOVA) and Duncan's multiple range test at the 5% level.

RESULTS

Effect of Electron Beam Irradiation on Spore Germination

Electron beams inhibited spore germination of *B. cinerea* with increasing irradiation doses (Fig IV-1). Spore germination was inhibited 18.4 and 47.9% at irradiation doses of 1 and 2 kGy, respectively. The effective irradiation doses for 50% inhibition (ED_{50}) of spore germination was 2.02 kGy by regression analysis. Spore germination was completely inhibited at higher than or equal to 10 kGy.

Effect of Electron Beam Irradiation on Mycelial Growth

Fig. IV-2 shows the effect of electron beam irradiation on mycelial growth of *B. cinerea*. Electron beam irradiation significantly inhibited mycelial growth as the irradiation dose increased. Mycelial growth was particularly inhibited 70.1% at irradiation dose of 2 kGy. ED_{50} of mycelial growth was 0.89 kGy by regression analysis. Mycelial growth was completely inhibited at higher than or equal to 10 kGy.

In Vivo Antifungal Activities of Electron Beam Irradiation

Fig. IV-3 shows control efficacy of electron beam irradiation on tomato gray mold. Disease severity of tomato plants was decreased with increasing irradiation dose. Control efficacy for tomato gray mold increased to 14, 28, 47, and 52% as the electron beam irradiation dose increased to 0.2, 0.4, 1, and 2 kGy, respectively.

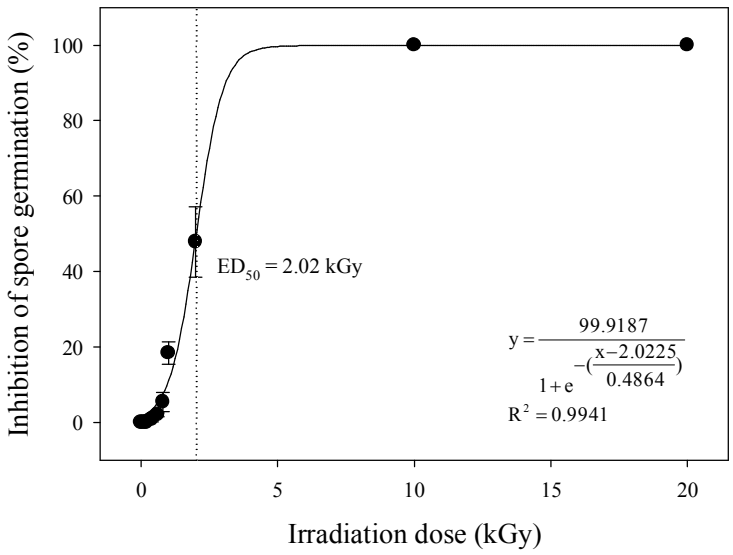

Fig. IV-1. Effects of electron beam irradiation on spore germination of *Botrytis cinerea.* Data are the means ± SD from three replications.

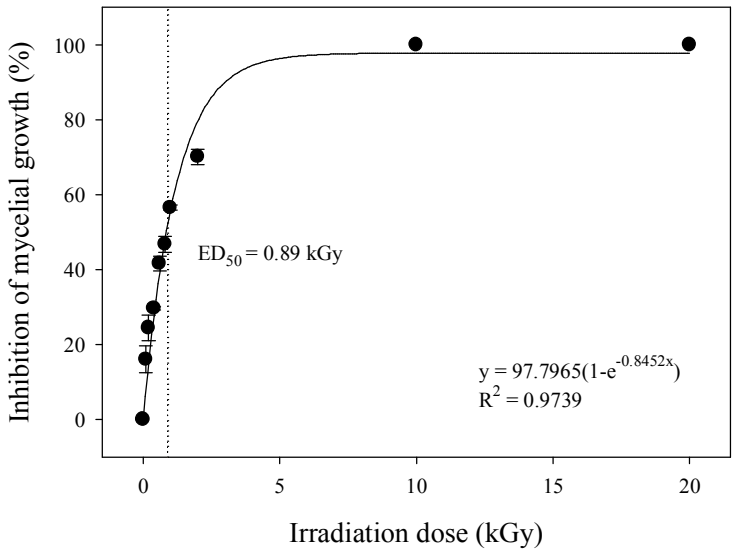

Fig. IV-2. Effects of electron beam irradiation on mycelial growth of *Botrytis cinerea*. Inhibition of mycelial growth (%) was determined using the following equation: Inhibition of mycelial growth (%) = (1 − mycelial diameter of treatment/mycelial diameter of control) × 100. Data are the means ± SD from five replications.

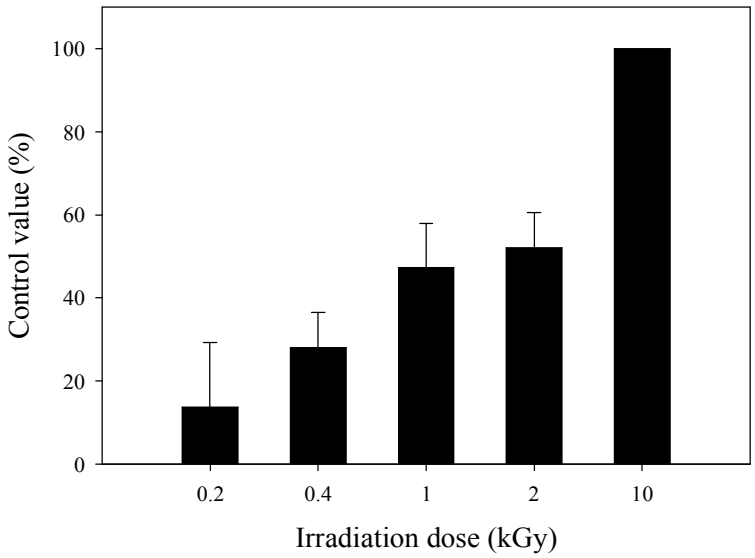

Fig. IV-3. Control efficacy of electron beam irradiation on tomato gray mold. The percentage of disease control was determined using the following equation: Control value (%) = (1 − B/A) × 100, where A = the area of infection (%) on untreated leaves and B = the area of infection (%) on treated leaves. Data are the means ± SD from five replications.

(

Effect of Electron Beam Irradiation on Mycelial Growth at Different Incubation Temperature

Effect of incubation temperature on mycelial growth of *B. cinerea* was determined (Fig. IV-4). Mycelial growth of *B. cinerea* was not observed at 5°C on day 6. Days to 50% mycelial growth were 4.1, 2.9, and 2.0 day at 10, 15, and 20°C, respectively. The growth rate of *B. cinerea* decreased when the incubation temperature was lowered.

Effect of electron beam irradiation on mycelial growth of *B. cinerea* according to temperature are shown in Fig. III-5. The efficacy of electron beam irradiation on inhibition of mycelium growth increased as the incubation temperature decreased. As the irradiation doses increased from 0.4 to 0.8 kGy, inhibition of mycelial growth increased at 10°C (Fig. IV-5A). In particular, mycelial growth was inhibited 100% at irradiation dose of 0.8 kGy on day 2. Days to 50% inhibition of mycelial growth were 4.6 and 6.2 day at 0.4 and 0.8 kGy by regression analysis.

At 15°C, the efficacy of electron beam on inhibition of mycelium growth maintained high and then decreased rapidly (Fig. IV-5B). The control efficacy decreased to 0% by day 5 in both 0.4 and 0.8 kGy. Days to 50% inhibition of mycelial growth were 3.0 and 3.7 day at 0.4 and 0.8 kGy by regression analysis.

At 20°C, the great reduction in the efficacy of electron beam on inhibition of mycelium growth was observed (Fig. IV-5C). Days to 50% inhibition of mycelial growth was 1.2 day at 0.8Gy by regression analysis. Electron beam irradiation had no effect in reducing mycelial growth of *B. cinerea* by day 4.

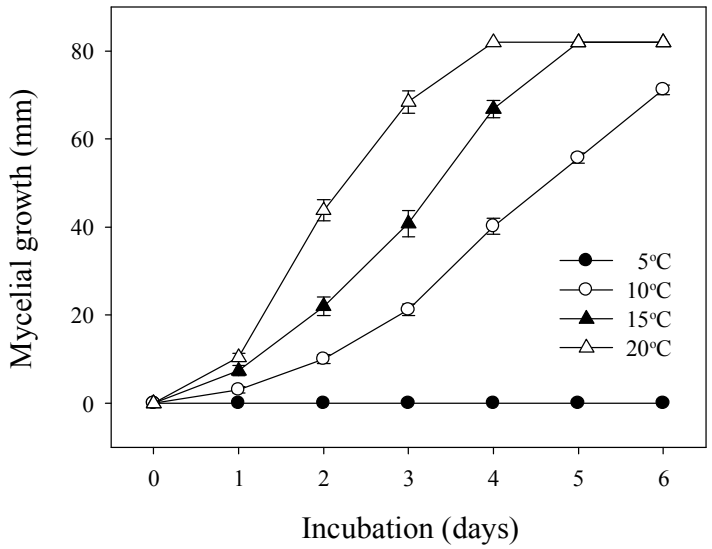

Fig. IV-4. Effects of incubation temperature on mycelial growth of *Botrytis cinerea*. Data are the means ± SD from five replications.

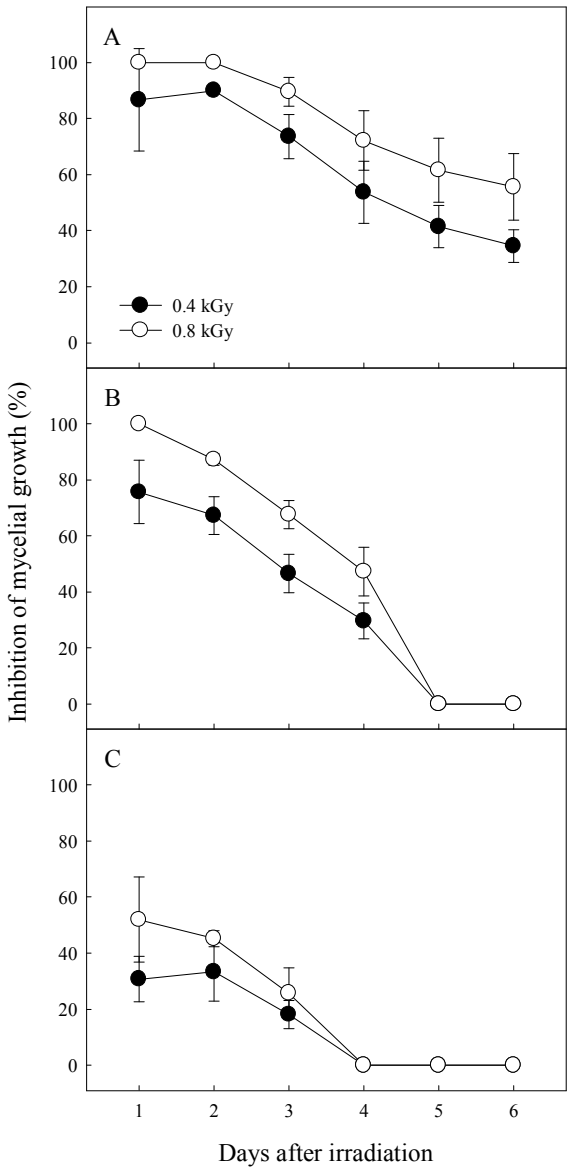

Fig. IV-5. Effects of electron beam irradiation on mycelial growth of *Botrytis cinerea* at different incubation temperatures. Mycelial growth of *B. cinerea* was observed at 10 (A), 15 (B), and 20°C (C). Electron beam was irradiated at doses of 0.4 and 0.8 kGy. Inhibition of mycelial growth (%) = (1 - mycelial diameter of treatment/mycelial diameter of control) × 100. Data are the means ± SD from five replications.

DISCUSSION

The present study described that spore germination and mycelial growth of *B. cinerea* was reduced by electron beam irradiation in a dose-dependent manner. Furthermore, conidia were more radiation resistant than mycelia (Fig. IV-1 and 2). Chang et al. (1997) found that spore germination of *B. cinerea* was inhibited 12, 69, and 98% at doses of 1, 2, and 4 kGy by 10 MeV electron beam irradiation, respectively. According to a study of Gryczka et al. (2010), spore germination of *F. oxysporum* f. sp. Dianthi was inhibited 40% at 1.5 kGy and 100% at ≥ 3 kGy by 9 MeV electron beam irradiation. Orilikowski et al. (2011) also reported that mycelial growth of *B. cinerea* was suppressed 62.8 and 93.1% in 3 and 6 kGy, respectively, when mycelial fragments was cultured for 4 days after 9 MeV electron beam irradiation. It shows that there is a difference in the sensitivity to the electron beam according to the type of microorganisms and physiological status. According to the result of Migdal et al. (2012), mycelial growth of *P. cinnamomi* was suppressed 50% at 1.5 kGy and 100% at 3 kGy. In particular, increasing irradiation dose to 3 kGy caused the decay of the pyphae. Thus, *B. cinerea* showed high resistance compared to *Phytophthora* species. The correlation of radiation sensitivity is roughly inversely proportional to the size a complexity of an organism. This correlation is related to genome size. Differences in radiation sensitivities within groups of similar organisms are related to differences in their chemical and physical structure and the ability to recover from radiation injury. In general, young mycelia and scleotia were more resistant and mature mycelia more sensitive than conidia (Sommer et al., 1972).

The present study demonstrated that the efficacy of electron beam irradiation on inhibition of mycelium growth increased as the incubation temperature decreased (Fig. IV-5). Numerous studies have attempted to determine the effect of irradiation combined with other treatments as a postharvest disease management such as *B. cinerea*. The effect of irradiation is more promising when applied in combination with hot water, modified atmosphere packaging, and low temperature (Barkai-Golan et al., 1993; Fan and Sokorai, 2011; Wani et al., 2008). The resistance of microorganisms to irradiation dis not a constant but dependent on various factors such as temperature, atmosphere, and water activity (Kim, 2006). Moreover, irradiated microorganisms could be more sensitive to post-irradiation conditions than the non-treated microorganisms (Aquino, 2012). Therefore, combined irradiation treatments would reduce the dose required to inactivate pathogen.

In conclusion, the present study indicates that electron beam irradiation could control of *B. cinerea* and combined treatment of irradiation and low temperature may work synergistically to reduce *B. cinerea.* Furthermore, these treatments can be easily applied to irradiation treatment of a wide variety of agricultural products.

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CONCLUSION

This study was focused the development reliable quarantine technology using electron beam irradiation that ensures the postharvest quality of cut flowers. Irradiation caused cut flowers to decrease fresh weight, flower diameter, chlorophyll content, and visual quality of flowers and leaves in a dose-dependent manner. This study suggests that the tolerance of cut flowers to electron beam irradiation vary according to cultivars and the dose of 400 Gy may be a considerable as the minimum value for tolerance. Because inhibition of flower opening was a constraint for application of irradiation to cut flowers, the effect of electron beam irradiation on flower opening needs to be investigated. Radiation-induced inhibition of flower opening was associated with insufficient osmotic pressure, which might be due to discontinuation of the persistent sugar supply, DNA damage, and cell death. Dose-response DNA damage and cell death may also be the radiotolerance criteria of cut flowers and give sufficiently accurate dose estimation. Soluble sugar supply to the preservative solution may be effective in alleviating radiation-induced deterioration of cut flowers, which could be more effective manner than pretreatment. Electron beam irradiation could control of *B. cinerea* and combined treatment of irradiation and low temperature may work synergistically to reduce *B. cinerea.* The results suggest that lowering the effective doses to inactivate *B. cinerea* throughout low temperature helps reduce radiation-induced damage of commodity. Therefore, electron beam irradiation can be available as a quarantine treatment without detriment to

postharvest quality of cut flowers, considering that an irradiation dose of 400 Gy is established to be effective for quarantine security.

ABSTRACT IN KOREAN

본 논문은 전자빔 조사를 통해 절화의 수확 후 품질을 확보할 수 있는 안정적인 검역 기술의 개발에 목적을 두었다. 전자빔 조사 선량이 증가함에 따라 급격한 생체중의 감소와 더불어 절화 수명, 봉오리 열림, 화경, 꽃과 잎의 관상가치, 엽록소 함량 등이 감소하여 절화의 수확 후 품질이 감소하였다. 절화별 전자빔 저항성을 알아보기 위해 전자빔 조사 후 품질이 10% 저해되는 선량 (ED_{10} 값)을 확인하였다. 그 결과, 장미의 ED_{10} 값은 269.7-448.4 Gy 수준이었으며, 국화는 431.4-653.1, 백합은 144.4-306.3 Gy, 카네이션은 451.6 Gy, 꽃도라지는 841.2 Gy 수준으로 확인되어 전자빔에 대한 절화의 저항성은 식물 종별, 품종별로 차이가 있음을 알 수 있었다. 전자빔 조사 선량이 증가함에 따라 'Siberia' 백합의 봉오리 열림이 심하게 저해되었는데, 이러한 증상에 대한 원인을 꽃잎 내부의 삼투압 및 탄수화물 변화와 DNA 피해를 통해 확인하였다. 꽃잎의 삼투압은 200 Gy 이하의 선량에서는 조사 후 6일까지 증가하는 경향이 관찰되었지만, 400 Gy 이상의 선량부터는 증가폭이 둔화되거나 감소하는 것으로 확인되었다. 꽃잎 내 탄수화물 함량 역시 200 Gy 이하의 선량에서는 봉오리 발달 단계에 따라 증가하다 조사 후 4일 이후부터 감소하였으나, 400 Gy 이상의 선량에서는 조사 후 2일째에 증가하였다가 곧바로 감소하는 것으로 확인되었다. 'Siberia'와 'Medusa' 백합을 대상으로 세포 주기 분석을 실시한 결과 전자빔 조사 선량이 증가함에 따라 G0/G1 피크의 CV 값과 Background Aggregates and Debris (BAD) 값이 증가되었고, apoptotic 피크를

확인하였다. ‘Medusa’ 백합에서 ‘Siberia’ 백합보다 DNA 피해가 심한 것을 확인하였으며, Trypan blue 염색을 통해 ‘Siberia’ 백합의 꽃잎은 400 Gy 이상의 선량에서, ‘Medusa’ 백합의 꽃잎은 100 Gy 이상의 선량에서 전자빔에 의한 세포사를 관찰하였다. 이를 통해 ‘Medusa’ 백합이 전자빔에 더욱 민감한 것으로 판단되었다. 절화 보존 용액 내의 탄수화물 첨가는 ‘Siberia’ 백합의 개화를 촉진하였으며, ‘Siberia’ 백합과 ‘Leopard’ 국화에서는 전처리제보다 보존 용액 처리를 통해 전자빔에 의한 수확 후 품질 저해를 경감시킬 수 있었다. 또한, ‘Leopard’ 국화는 전처리제와 보존 용액 복합 처리 역시 효과적이었다. 수출 및 저장 과정에서 절화의 수확 후 품질을 저해할 수 있는 잿빛곰팡이병원균(*Botrytis cinerea*) 균주를 대상으로 생육을 50% 저해하는 전자빔 선량(ED_{50} 값)을 확인하였다. 그 결과, 포자의 ED_{50} 값은 2.02 kGy 수준이었고, 균사의 ED_{50} 값은 0.89 kGy 수준으로 확인되어 포자가 균사에 비해 전자빔 저항성을 가지는 것으로 확인되었다. *B. cinerea* 균주에 전자빔을 조사한 후 저장 온도에 따른 균사 생육을 확인한 결과 10°C에서 20°C에서보다 균사 생육이 억제되는 것을 확인하였다. 따라서, 식물 위생 검역 요건에 맞게 전자빔을 조사하고 적절한 수확 후 처리와 함께 저온을 유지한다면 *B. cinerea* 균사 생육은 충분히 저해하면서 절화의 수확 후 품질을 안정적으로 유지할 수 있을 것으로 판단하였다.