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Report of Cooperative Research Programs in the Field of Ion-beam Breeding between Japan Atomic Energy Agency and Malaysian Nuclear Agency (Bilateral Cooperative Research)

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Biotechnology and Medical Application Division
Quantum Beam Science Center
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Report of Cooperative Research Programs in the Field of Ion-beam Breeding between Japan Atomic Energy Agency and Malaysian Nuclear Agency (Bilateral Cooperative Research)

(Eds.) Zaiton AHMAD[*1] and Yutaka OONO

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This report summarizes Bilateral Cooperative Research between Japan Atomic Energy Agency and Malaysian Nuclear Agency (a representative of the Government of Malaysia) implemented from 2002 to 2012 under "THE IMPLEMENTING ARRANGEMENT BETWEEN THE GOVERNMENT OF MALAYSIA AND THE JAPAN ATOMIC ENERGY AGENCY ON THE RESEARCH COOPERATION IN THE FIELD OF RADIATION PROCESSING". The research activities in two Cooperative Research Programs, "Mutation Induction of Orchid Plants by Ion Beams" and "Generating New Ornamental Plant Varieties Using Ion Beams" performed 2002-2007 and 2007-2012, respectively, are contained. The lists of steering committee meetings, irradiation experiments, and publications/presentations of each program are also attached in the Appendixes.

Keywords: *Dendrobium*, Orchid, Ion Beams, Mutation, Flower Colour, Insect Resistance, *Chrysanthemum morifolium*, Reagan Red, Pink, Flower Morphology

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日本原子力研究開発機構とマレーシア原子力庁による イオンビーム育種分野における研究協力プログラムに関する報告 （二国間研究協力）

日本原子力研究開発機構　原子力科学研究部門
量子ビーム応用研究センター
バイオ・医療応用研究ディビジョン

（編） Zaiton AHMAD*1, 大野　豊

（2015 年 12 月 17 日　受理）

本報告書は、「放射線加工処理の分野における研究協力に関するマレーシア政府と日本原子力研究開発機構との間の実施取決め」に基づいて 2002 年 12 月から 2012 年 12 月にかけて国立研究開発法人日本原子力研究開発機構とマレーシア政府下の研究実施機関であるマレーシア原子力庁により実施された二国間研究協力についてまとめたものである。この間に実施された２つのイオンビーム育種分野における研究プログラム「イオンビームによるランの突然変異誘発」（2002 年 12 月～2007 年 12 月）及び「イオンビームによる新規観賞植物品種の作出」（2007 年 12 月～2012 年 12 月）の研究活動に加え、付録としてそれぞれの研究プログラムに係わる運営員会の開催状況、イオンビーム照射実験の実施状況、論文・口頭発表リストについて収録した。

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1. Mutation Induction of Orchid Plants by Ion Beams

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Summary

Mutation induction using ionizing radiation provides an effective alternative for the improvement of orchids. Ion beams were used because they have much higher linear energy transfer (LET) than X-rays and gamma rays subsequently cause high mutation frequency and broad mutation spectrum. The protocorm-like bodies (PLBs) of two orchid species (*Dendrobium crumenatum* and *Dendrobium mirbellianum*) were irradiated with 320 MeV $^{12}C^{6+}$ ions accelerated by AVF cyclotron in Takasaki Ion Accelerators for Advanced Radiation Application (TIARA). Dose-dependent inhibition of regeneration frequency was observed in shoots from irradiated PLBs. Some morphological changes particularly on flower colour and morphology were observed in regenerated plants of *D. crumenatum*. Randomly selected regenerated *D. mirbellianum* plantlets from several doses were subjected to *in vitro* infestation with mites to analyze their resistance towards several pests. Potential insect resistant orchid mutants were then selected and subsequently planted in a glasshouse for secondary screenings at flowering stage. A total of 50 potential mite tolerant plantlets were identified at *in vitro* stage, and, of these, at least one plant was found tolerant to thrips when secondary screening was carried out at flowering stage (*in vivo*).

Keywords: *Dendrobium*, Orchid, Ion Beams, Mutation, Flower Morphology, Insect Resistance, Flower Colour

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1.1 Introduction

The technology for induction of mutations is a powerful tool for developing better varieties of food and industrial crops. Gamma irradiation and chemical mutagenesis are general methods for mutation induction and has been applied for plant breeding in many countries in the world. Whereas, establishing a new method has been expected to enhance more genetic diversity in available plant resources. Studies in JAEA have suggested that ion beams are characteristic of high relative biological effectiveness for survival and other endpoints, cause high mutation frequency, and are useful to isolate novel mutants in Arabidopsis[1),2),3)]. Mutation induction in chrysanthemums and carnations using ionizing radiation has also shown its reliability for the production of a wide range of variations with attractive combinations of spray length, bud number, flower color and form[4),5)]. The successful results in chrysanthemums and carnations prompt us to apply ion beams to other plant species to generate novel varieties that have never been obtained by previously available methods. In this study, possibility of the application of ion beams to produce novel orchid varieties is investigated under the cooperative research program between Nuclear Malaysia and JAEA.

Orchid industry is part of the global floriculture commerce valued at USD 9 billion. In spite of its small share of fresh flower market, the orchid industry in Malaysia is developing into a very viable and lucrative commercial enterprise. Orchid growing in Malaysia is a multi-million ringgit industry, most notably in the cut-flower trade[6)]. The value of Malaysia annual export and local markets is about RM (Malaysian Ringgit) 40 million and RM 20 million, both on flowers as well as plants[7)]. Approximately 24.3 million stalks of orchid cut-flowers were produced in year 2000 with *Dendrobium* topping the list at 13.1 million stalks. Its growth is expected to escalate since the Malaysian government has given top priority to agriculture and export-oriented high-value products like orchids.

A major issue faced by the orchid industry in Malaysia is associated with the lack of varieties. In order to keep up with the ever-changing tastes of consumers, there is an urgent need to create new and better varieties of orchids to sustain the floriculture industry. The use of conventional breeding methods to create variation in orchids is restricted by sexual incompatibility, sterility problems and long breeding time. Gamma irradiation has been successful in creating many *Dendrobium* 'Sonia' mutant varieties. In *Dendrobium* Ekapol and *Dendrobium* Sonia for examples, irradiation resulted in changes of flower pigmentation and size[8),9)]. More variations with attractive combinations of spray length, bud number, flower color and form are required to create commercially valuable varieties. Considering successful results in Arabidopsis, chrysanthemums, and carnations, ion beams are expected as tools to produce orchids with improved characteristic such as attractive flower colour and morphology, longer shelf life, and good flowering habit.

Insect infestation is another major problem for orchid industry and has caused a lot of losses to growers, as well as exporters due to strict quarantine regulations. There were cases when the whole orchid consignment had to be shipped back to the exporting countries due to the presence of insect pests. The common insects attacking orchids in Malaysia are thrips and mites, and as for mites, the most common is *Tenuipalpus pacificus* (false spider mite). This pest is found in the lower surface of leaves and sucks the sap of the leaf. The infested leaves become pale yellow with numerous small spots, which subsequently caused the infected leaves to fall and reduced the overall quality of the plant[10]. Orchid insect pests can be controlled by spraying with insecticides and miticides such as dicofol (Kelthane), hexakis (Orthonex III), and abamectin (Avid) (http://www.orchidplantcare.info/archives/orchid-plant-insects-overview). This practice, however, may lead to heavy use of chemicals. Studies have been conducted for post-harvest disinfestations of cut flower using irradiation. However, the post-harvest irradiation treatment has shown detrimental effect on the quality of the cut flowers. The strategy that can be adopted to overcome the problem is to breed for insect resistance in orchids. Insect resistant orchid hybrid may minimize the use of chemicals and overcome the strict quarantine requirements of importing countries. Mutagenesis is considered as an alternative approach to induce resistance as hybridization is limited by the unavailability of a resistant genotype and problem of sexual compatibility. Insect resistance has been successfully induced by using mutagenesis approach in varieties of plants such as mung bean and rice[11),12)]. Mutation induction by irradiation has effectively changed certain characteristics of these plants to be 'unattractive' to insects. Therefore, this sub-project, which was partially supported by FNCA (Forum for Nuclear Cooperation in Asia), was carried out with an aim to generate mite resistant mutant plants from a local orchid variety, *Dendrobium mirbellianum*. Preliminary evaluations for mite resistance were carried out on orchid tissue culture plantlets using an *in vitro* screening method to pre-select potential mutants and subsequent (secondary) screening was done at the flowering stage.

1.2 Materials and methodology

1.2.1 Study on irradiation condition for mutation induction

Plant materials

Two orchid species used in this project were *D. mirbellianum* (Figure 1) and *Dendrobium crumenatum* (Figure 2). *D. mirbellianum* is a robust and easy-to-grown spicies which produces long spray (up to 45 cm) with up to 30 flowers. It has good flowering habit and the flowers can last for about 4 weeks. *D. crumenatum* is known as Pigeon orchid and easily cultivated. It grows rapidly with white fragrant flowers that only last for a day. The flowering of *D. crumenatum* is triggered by sudden drop in temperature. The traits of interest in this project were flower colour, morphology and insect tolerance for *D. mirbellianum* and flower colour, morphology and longevity for *D. crumenatum*.

In vitro cultures of these species were established in Plant Biotechnology Laboratory at Malaysia Nuclear Agency. Mature seeds of these species were collected from self-pollinated flowers. The seed capsules were surface-sterilized by dipping them in ethanol followed by short flaming. They were cut and opened under a sterile condition and the seeds were germinated on half-strength Murashige and Skoog (½ MS) media[13] at 25±2 ^{o}C with 12-h photoperiod until protocorm-like bodies (PLBs) were formed. PLBs that were uniform in size with approximately 2 mm in diameter were chosen for ion beam irradiation experiment. Figure 3 shows the state of PLBs used in the irradiation.

Figure 1. Flowers of *D. mirbellianum*

Figure 2. Flowers of *D. crumenatum*

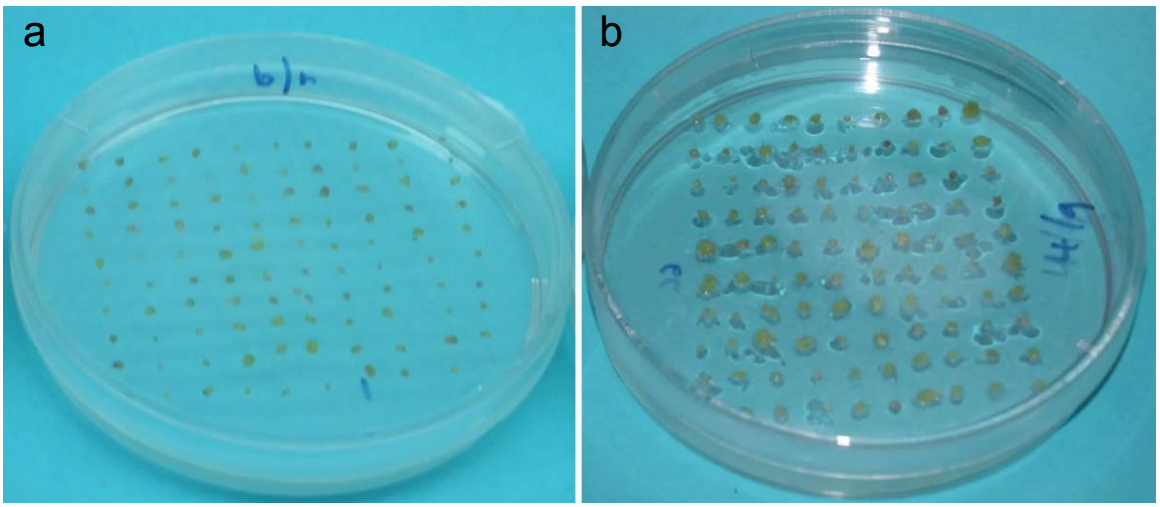

Figure 3. The state of PLBs used for irradiation. a: *D. crumenatum*, b: *D. mirbellianum*.

Ion beam irradiation

Irradiation was carried out at TIARA, JAEA. PLBs were placed on 6-cm sterile petri dishes containing ½ MS medium and covered with a sterile 8 μm-thick polyimide film (Kapton, Toray, Japan) (Figure 4). These PLBs were irradiated with 320 MeV $^{12}C^{6+}$ ions accelerated by an Azimuthally Varying Field (AVF) cyclotron (Figure 5). The irradiated PLBs were brought back to Nuclear Malaysia for *in vivo* propagation and screening.

In the preliminary stage of this project, because information on the effective dose for mutation induction in *Dendrobium* sp. had not yet been established, doses ranging up to 50 Gy were applied to the PLBs. It was found from this preliminary experiment that doses higher than 15 Gy severely inhibited growth. Hence, doses less than 15 Gy were used in the subsequent experiments.

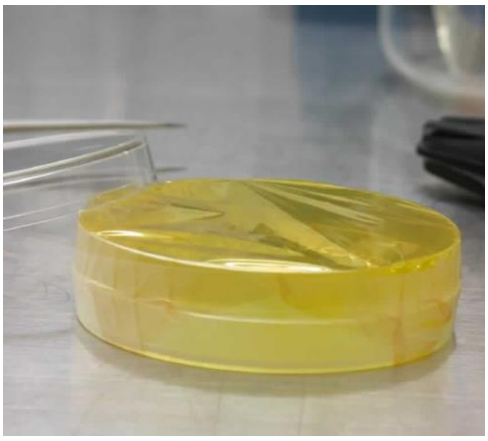

Figure 4. Kapton film covered petri dishes containing PLBs, which are ready for irradiation.

Figure 5. Irradiation with 320 MeV $^{12}C^{6+}$ ions accelerated by the AVF cyclotron at TIARA. Left: petri dishes containing PLBs are aligned on aluminium plates. Center: aluminium plates with petri dishes are inserted in the irradiation apparatus. Right: a petri dish is moved to underneath of ion-beam path automatically and irradiated one by one with a dose programmed in advance.

1.2.2 Mutant selection

Propagation of PLB

Propagation and screening of irradiated PLBs were carried out at Malaysian Nuclear Agency. The irradiated PLBs were transferred onto fresh ½ MS medium and incubated at 25±2 ^{o}C under 12-h photo period for proliferation. Subsequently, the cultures were transferred onto fresh media every four weeks for multiplication and regeneration. The number of PLB that regenerates shoots was recorded after 2 months. Plantlets were allowed to proliferate and multiply for several months before being hardened in a glasshouse.

In vitro *mite infestation and selection*

To pre-select candidate plantlets tolerant to mites for subsequent insect analysis at flowering stage, irradiated *D. mirbellianum* plantlets of about 4 cm in height and with 4 open leaves from doses of 0.4, 0.8, 1 and 2 Gy, were put in individual vials. Five adult mites (3 females and 2 males) were then put in each individual vials containing the plantlets. Observations on the pattern of infestation and the multiplication of mites were made every week according to Zhang (2001)[14] on all plantlets. For symptom analysis, each leaf was divided into five regions (Figure 6). Infestation was detected using a dissecting microscope. Scoring was done for the first 3 consecutive weeks and after 9 weeks, by estimating the area of infestation as shown in Figure 6. The infestation levels were scored as follow; 1: no infestation, 2: less than 5% infestation, 3: 5–10% infestation, 4: 10–25% infestation and 5: more than 25% infestation. In cases where the infestation occurred on different leaves, the areas of infestation were combined to get the total infestation area. After 3 months of infestation, surviving plants were transferred into small pots for hardening and left to grow in the greenhouse until flowering. Fertilizers were applied to the plants for growth, but not pesticides. Secondary screening at the flowering stage by infestation with the target insects were carried out on these potential mutants.

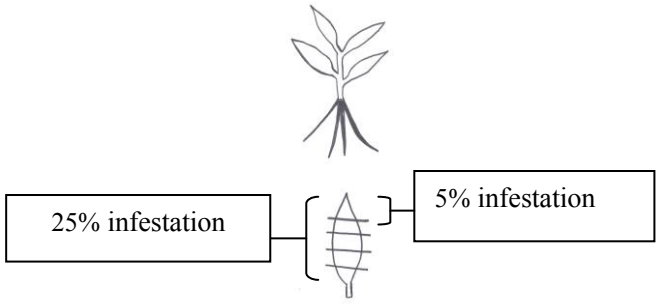

Figure 6. Illustrated scoring index for mite infestation on orchid leaf

1.3 Results

1.3.1 Irradiation condition

The size of *D. crumenatum* PLBs used for irradiation was found to be one of the important factors that influenced the survival of these PLBs after irradiation. In the initial experiment, PLBs less than 2 mm in size could not proliferate even in the controls and at lower doses. Therefore the optimum PLB size selected for subsequent (repeated) irradiations was 2–3 mm. Another significant factor for obtaining higher survival rates of PLBs after irradiation was pre-culturing these PLBs on ½ MS media before irradiation for a week to allow them to stabilize under the culture conditions.

For *D. mirbellianum*, the size of the PLBs was not a critical factor for survival as its individual PLB is naturally between 2–3 mm in size when it is established. Figure 7 shows examples of proliferating *D. mirbellianum* PLBs two months after irradiation.

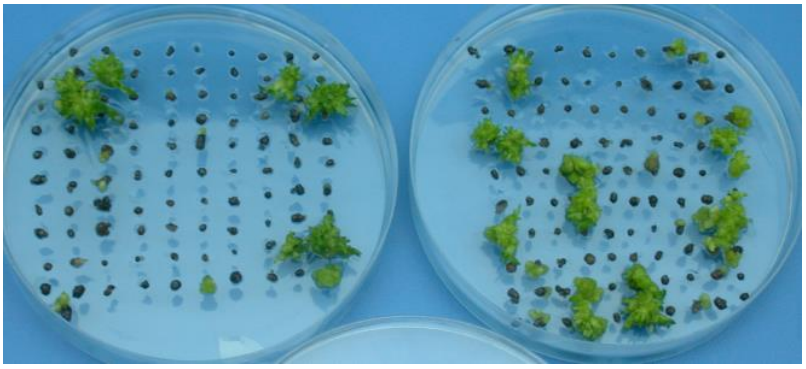

Figure 7. Proliferating *D. mirbellianum* PLBs after two months of irradiation at 2 Gy.

Figures 8 and 9 show the relationship between irradiation doses and percentage of regenerated shoots in *D. mirbellianum* and *D. crumenatum,* respectively, which are recorded at 8th week. In *D. mirbellianum* culture, we were able to obtain regenerated shoots from 72% of PLBs of control non-irradiated population. Effect of ion-beam irradiation was clearly observed as regeneration frequencies in the population irradiated at doses higher than 2 Gy were gradually reduced. Irradiation effect was reached to maximum at 6 Gy, where only 8% of PLBs were regenerated.

In *D. crumenatum* culture, regeneration frequency was 54% in the control population. Similar to *D. mirbellianum*, adverse effect of ion-beam irradiation was observed on PLBs irradiated at doses from 2 Gy onwards. In general, the regeneration frequency was inversely proportional with irradiation doses. However, unlike *D. mirbellianum*, regenerated shoots were observed in 34% and 22% of *D. crumenatum* PLBs irradiated at doses of 6 and 8 Gy, respectively, suggesting *D. crumenatum* was slightly more resistant to radiation than *D. mirbellianum*.

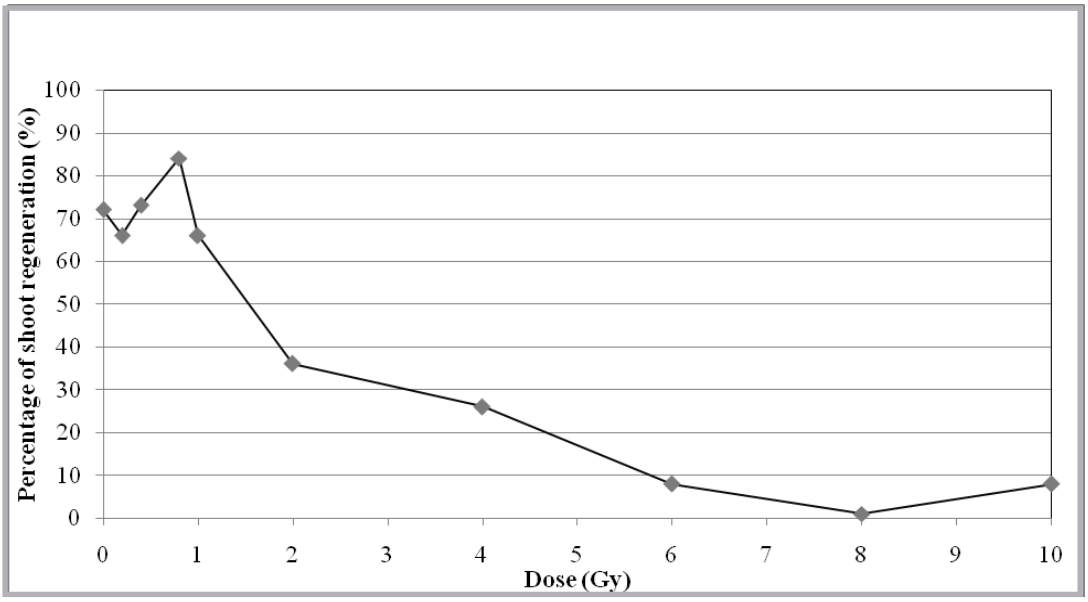

Figure 8. The dose response curve of shoot regeneration in *D. mirbellianum* PLBs irradiated with ion beams.

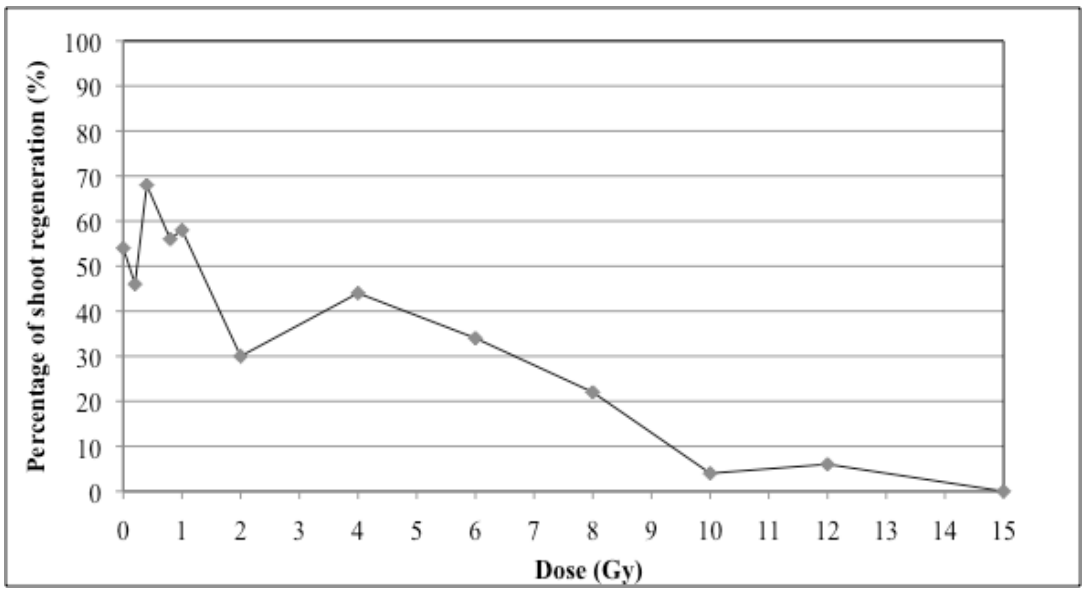

Figure 9. The dose response curve of shoot regeneration in *D. crumenatum* PLBs irradiated with ion beams.

1.3.2 *In vitro* observation

D. mirbellianum

Mutation effects of carbon ions could be observed at the tissue culture stage on some irradiated cultures. In *D. mirbellianum,* plants with chlorophyll mutation (variegated light yellow-green leaves) were generated from one PLB that was irradiated at 0.4 Gy (Figure 10). These plant cultures were mass-propagated and left to grow into complete rooted plantlets in order to increase the chance of survival during hardening process. However, they could not survive the glasshouse condition and died after two weeks of transfer.

Figure 10. Chlorophyll mutation observed in *D. mirbellianum* cultures generated from a PLB irradiated at 0.4 Gy.

D. crumenatum

Leaf morphological variations were also observed in a number of *D. crumenatum* cultures. Characteristics of leaf mutants are shown in Figures 11a to 11d, while the control is shown in Figure 11e. Figures 11a, 11b and 11c show shoot clumps regenerated from PLBs irradiated at 2 Gy, while Figure 11d shows shoot clumps irradiated at 0.2 Gy. In Figure 11a, variations could be observed in the shape of the leaves, whilst in Figure 11b, in the elongation of shoot stem. Majority of the cultures irradiated at 2 Gy demonstrated the same leaf pattern as in Figure 11c. Another radiation effect (slow growth) was observed in a small number of cultures irradiated at 0.2 Gy. The variations were not found in the control populations. Therefore, these could be considered as potential mutants caused by radiation and not somatic variations caused by tissue culture effect.

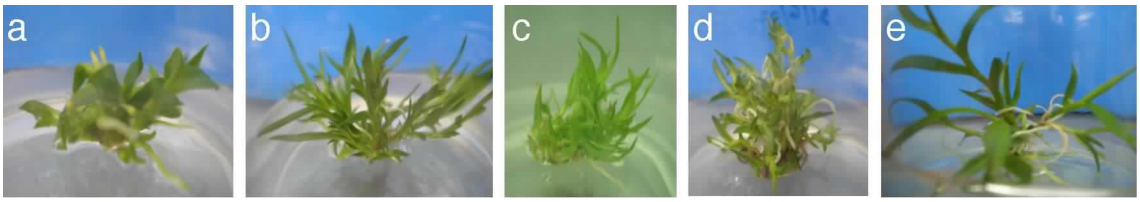

Figure 11. Regenerated shoots that show some abnormalities compare to the control (e). Shoots were regenerated from cultures of *D. crumenatum* irradiated with 2 Gy (a, b, and c) or 0.2 Gy (d) of ion beams.

1.3.3 Observation in glasshouse

D. mirbeliannum

In glasshouse, plantlets of *D. mirbellianum* were found to grow slower than plantlets of *D. crumenatum*. During nursery screening, the morphological characters observed were flower colours and morphology. However, none of the irradiated plants has shown different colour and morphological characters and were basically maintained the same traits as the controls. Figure 12 shows some pictures of irradiated plants during nursery screening.

Figure 12. Nursery screening of irradiated *D. mirbellianum*. a: plantlets 1 week after transplanting, b: 1 year old plants, c: flowering plants, d: close-up of flowering plants.

D. crumenatum

The characters observed for *D. crumenatum* during nursery screening were flower longevity, colours and morphology. In this study, no extension of blooming period was observed in irradiated plantlets as compared to the controls, which bloom for only one day. Details on morphological changes observed in flowering mutant plants are given in Table 1, whilst variations on the flower shapes and sizes are shown in Figure 13. One of the 6.0-Gy irradiated plantlets shows an increase in flower width. The flower measures 55 mm across compare to that of the contol, which was approximately 49 mm.

A plant in 0.2-Gy population exhibited a longer flower stalk which measures 31.2 cm compare to that of the control which is about 15 cm length. The flower stalk is shown in Figure 14.

Figure 13. Flowers of *D. crumenatum* regenerated from PLBs irradiated at 0 (a, control), 0.2 (b), 4 (c), 6 (d), and 8 (e) Gy. The bar indicates 10 mm. Flower width (mm) was 49.0 (a), 44.5 (b), 37.2 (c), 55.0 (d), and 48.0 (e).

Table 1. Morphological mutation of flowering *D. crumenatum* plants irradiated with ion beams.

Dose (Gy)	Number of flowering plants*	Number of mutants							% Mutant
		Flower morphology					Plant form	total	
		Large flower	Small flower	Long stalk	Different shape	Different orientation	Dwarf		
0	32							0	0
0.2	7		1	1				2	28.6
0.4	6							0	0
4	19					1		1	5.3
6	2				1			1	50.0
8	23		1					1	4.4
10	10	2						2	20.0
20	7						7	7	100.0

* including regenerated plants from PLBs irradiated in the preliminary experiments.

Figure 14. A longer flower stalk observed in a regenerated plant irradiated at 0.2 Gy.

1.3.4 *In vitro* screening for mite tolerance

Observations on *in vitro* insect screening experiments showed that after one week of challenge-infestation with mites, more than 80% of tested *D. mirbellianum* plants from all doses of irradiation, including the controls, have already showed very low level of infestation symptoms. However, plants irradiated at doses above 1 Gy in particular, only showed less than 5% infestation (Table 2). The severity of the infested leaves was gradually more visible in week 2 and 3, especially on those irradiated at doses below 0.8 Gy in which larger areas of the plant leaves were affected (Tables 3 and 4).

Table 2. Infestation scale on irradiated plantlets after 1 week of infestation with mites.

Dose (Gy)	Number of plants	Number of plantlets at different infestation scale* (Percentage, %)				
		1	2	3	4	5
0	52	43 (82.7)	4 (7.7)	5 (9.6)	0	0
0.4	50	44 (88.0)	5 (10.0)	1 (2.0)	0	0
0.8	57	51 (89.5)	4 (7.0)	2 (3.5)	0	0
1	26	26 (100)	0	0	0	0
2	10	9 (90.0)	1 (10.0)	0	0	0

*The infestation scales were scored as follow;
1 : no infestation, 2 : < 5% infested leaves, 3 : 5–10% infested leaves,
4 : 10–25% infested leaves, 5 : > 25% infested leaves

Table 3. Infestation scale on irradiated plantlets after 2 weeks of infestation with mites.

Dose (Gy)	Number of plants	Number of plantlets at different infestation scale* (Percentage, %)				
		1	2	3	4	5
0	52	33 (63.5)	6 (11.5)	7 (13.5)	5 (9.6)	1 (1.9)
0.4	50	38 (76.0)	1 (2.0)	7 (14.0)	4 (8.0)	0
0.8	57	47 (82.5)	3 (5.3)	2 (3.5)	5 (8.8)	0
1	26	21 (80.8)	4 (15.4)	1 (3.9)	0	0
2	10	7 (70.0)	2 (20.0)	1 (10.0)	0	0

*The infestation scales were scored as in Table 2.

Table 4. Infestation scale on irradiated plantlets after 3 weeks of infestation with mites

Dose (Gy)	Number of plants	Number of plantlets at different infestation scale* (Percentage, %)				
		1	2	3	4	5
0	52	33 (63.5)	4 (7.7)	5 (9.6)	8 (15.9)	2 (3.9)
0.4	50	37 (74.0)	2 (4.0)	3 (6.0)	3 (6.0)	5 (10.0)
0.8	57	45 (79.0)	3 (5.3)	3 (5.3)	4 (7.0)	2 (3.5)
1	26	21 (80.8)	0	4 (15.4)	1 (3.9)	0
2	10	7 (70.0)	1 (10.0)	2 (20.0)	0	0

* The infestation scales were scored as in Table 2.

The severity of infestation on the infected *D. mirbellianum* plantlets was monitored for 9 weeks, or until a complete life cycle of mites (Table 5). At this stage, all (100%) non-irradiated plants tested have showed infestation symptom on their leaves. Some of these control plantlets were also severely damaged and died. Irradiated plantlets did also exhibit infestation symptoms at various scales, ranging from very mild to heavily infested, but there were also plantlets that showed no sign of infestation and completely healthy. The percentages of non-infected plantlets were gradually increased with the increase in treated irradiation doses. The percentages of healthy plantlets after 9 weeks were 0%, 34%, 24.6%, 50%, and 60% for those irradiated at 0, 0.4, 0.8, 1, and 2 Gy, respectively. Figure 15 shows tested seedlings in individual vials as well as examples of healthy and infested ones.

Table 5. Infestation scale on irradiated plantlets after 9 weeks of infestation with mites.

Dose (Gy)	Number of plants	Number of plantlets at different infestation scale* (Percentage, %)				
		1	2	3	4	5
0	52	0 (0)	0 (0)	0 (0)	9 (17.3)	43 (82.7)
0.4	50	17 (34.0)	5 (10.0)	2 (4.0)	1 (2.0)	25 (50.0)
0.8	57	14 (24.6)	2 (3.5)	1 (1.8)	6 (10.5)	34 (59.7)
1	26	13 (50.0)	2 (7.7)	2 (7.7)	2 (7.7)	7 (26.9)
2	10	6 (60.0)	1 (10.0)	1 (10.0)	1 (10.0)	1 (10.0)

***** The infestation scales were scored as in Table 2.

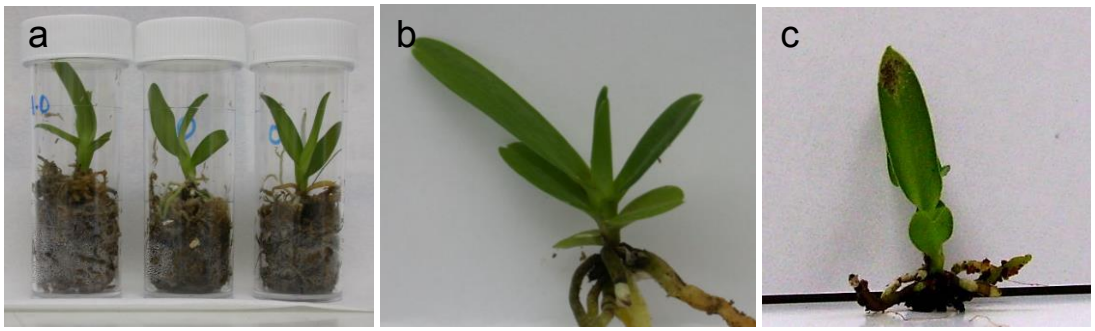

Figure 15. *In vitro* insect screening experiment. a: Plantlets in individual vials; b: A healthy plantlet; c: A mite-infested plantlet

After 3 months of infestation, healthy, non-infected and surviving plants were transferred into small pots for hardening. Subsequently they were allowed to grow under shade in the greenhouse. The plants were regularly sprayed with fertilizers for growth but were not sprayed with any pesticides. Ultimately, 50 potential *D. mirbellianum* mutants from ion-beam irradiated population have been successfully grown and survived in the glasshouse. Of these, 11 plants are from 0.4 Gy, 31 from 0.8 Gy, and 8 from 1.0 Gy. These plants were subsequently screened for insect tolerance at flowering stage.

1.3.5 Glasshouse screening for insect tolerance

In nature, expressions of disease or infestation symptoms in plants are influenced by a combination of factors such as susceptibility of the host, infective pathogen and favourable environmental conditions[15]. Therefore, these potential mutant plants will subsequently be screened at flowering stage by challenge-infestation with the target mite, to confirm the stability of the trait in natural growing conditions.

Mites and thrips are the main orchid pests in Malaysia. Mites are commonly found in the lower surface of leaves and suck the sap of the leaf. The leaves become pale yellow with numerous small spots, which reduce the quality of the leaves. Thrips normally infest young flower buds and newly expanded leaves. Blooms of infested plants may become prematurely brown, whilst the infested petals may either become spotted, streaked, silvery or discolored. Symptoms on leaves include chlorotic spots, wilting, and eventually dropping. Plant growth can also be stunted, and in a severe infestation case, the whole plant will die[16]. Common symptoms of mite and thrips infestation on orchids are as Figure 16.

Figure 16. A target insect and its infestation symptoms on orchid plants; mites (top), thrips (bottom).

Figure 17. *D. mirbellianum* mutant tolerant to both mites and thrips. Left: a control flower susceptible to thrips (a) and a mutant flower tolerant to insect infestation (b). Right: propagated mutant plants for field screening.

Based on the *in vivo* thrips screening at plant flowering stage, one potential mite tolerant plant was also found tolerant to thrips (Figure 17). All potential mite tolerant mutants selected at *in vitro* stage as well as the mite/thrips tolerant mutant selected at glasshouse stage were subsequently being propagated in the nursery, to achieve large number of clones. Several representatives of these mutants are now being tested at a commercial orchid nursery to observe their growth performance as well as tolerance to insects in actual conditions.

1.4 Conclusions and future plan

Inhibitory effect of ion-beam irradiation on shoot regeneration was observed on both *D. crumenatum* and *D. mirbellianum* PLBs irradiated at doses of 2 Gy and above. Several potential *D. crumenatum* mutants with different flower morphology/size and *D. mirbellianum* mutant tolerant to both mites and thrips were successfully generated. However, genetic inheritability of these mutations needs to be confirmed in the next few generations. At present, a large number of the irradiated plant population has not yet been completely examined. More data are also needed to confirm the relationship among irradiation dose, regeneration frequency, and mutation effects. Multiplication and propagation work for mutants is still continued at a glasshouse in Nuclear Malaysia with the aim to commercialize the mutants. At present, Nuclear Malaysia has signed a Non-Disclosure Agreement and Collaborative Agreement with a private collaborator (Hexagon Green Sdn Bhd) for a project on "Pre-commercialization of Mutant Orchids for Cut Flower Industry" which was financed by Ministry of Agriculture and Agro-Based Industry, Malaysia. The project will cover selected orchid mutants developed by Nuclear Malaysia, including our potential insect tolerant mutants. Finally, this 5-year bilateral project between JAEA and Nuclear Malaysia not only played a successful role on development of potential mutants of orchids but also contributed to accumulation of knowledge of ion beam mutagenesis techniques for orchid plants and other supportive work for international collaboration. The collaboration between JAEA and Nuclear Malaysia was renewed on December 2007 and continued as a new Cooperative Research Program on "Generating New Ornamental Plant Varieties using Ion Beams". In this new project, in addition to orchids, the possibility on the application of ion beams to improve chrysanthemum, one of the major cut flowers in Malaysia and internationally, is being explored. Through this new program, it is hoped that the technology for the production of new mutant varieties of chrysanthemum will be established. Besides generating new varieties of orchid and chrysanthemum, the efficiency of ion-beam and gamma-ray irradiations to induce mutation on chrysanthemums is being compared. The series of the programs is expected to facilitate the development and advancement of ion-beam technology for ornamental plant breeding specifically in orchids and chrysanthemums.

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This Bilateral Research Cooperation was supported by both agencies, JAEA and Nuclear Malaysia. The sub-project on insect resistance was a part of a multi-lateral FNCA Mutation Breeding Project entitled “Induction of Insect Resistance in Orchids” involving Malaysia, Thailand, Indonesia and Japan.

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Nuclear Malaysia:
Datuk Dr. Daud Mohamad (Director General)
Dr. Nahrul Khair Alang Md. Rashid (Deputy Director General, Research and technology Development)
Dr. Norimah Yusof (Director, Agrotechnology and Biosciences)
Dr. Ainul Hayati Hj. Daud (International Affairs Division)

JAEA:
Dr. Namba Hideki (Former Deputy Director General, Quantum Beam Science Directorate)
Dr. Tamikazu Kume (Former Director of Department of Ion-Beam-Applied Biology)

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Appendix 1.

List of steering committee meetings

1. The Sixteenth Steering Committee Meeting
(16 and 18 October 2002 at MINT[*1], Bangi)

MINT:

Dr. Nahrul Khair Alang Md. Rashid
Deputy Director General (Research and Technology Development)

Dr. Khairul Zaman Haji Mohd. Dahlan
Director, Radiation Processing Technology Division

Dr. Norimah Yusof
Director, Agrotechnology and Biosciences Division

JAERI[*2]:

Dr. Tamikazu Kume
Deputy Director, Department of Ion-Beam-Applied Biology,
Takasaki Radiation Chemistry Research Establishment

Dr. Atsushi Tanaka
Head, Plant Resources Laboratory,
Department of Ion-Beam-Applied Biology,
Takasaki Radiation Chemistry Research Establishment

Resource persons and observers:

Dr. Kamaruddin Hashim
Dr. Kamarudin Bahari
Ms. Norjanah Mohid
Dr. Ainul Hayati Hj.Daud
Dr. Mohd. Nazir Basiran
Ms. Sakinah Ariffin

2. The Seventeenth Steering Committee Meeting
(8-9 December 2003 at JAERI, Takasaki)

JAERI:

Dr. Tamikazu Kume
Director, Department of Ion-Beam-Applied Biology,
Takasaki Radiation Chemistry Research Establishment

Dr. Atsushi Tanaka
Group leader, Research Group for Plant Resource Application,
Takasaki Radiation Chemistry Research Establishment

MINT:

Dr. Nahrul Khair Alang Md. Rashid
Deputy Director General (Research and Technology Development)

Dr. Norimah Yusof
Director, Agrotechnology and Biosciences Division

Resource persons and observers:

Dr. Naoya Shikazono
Dr. Yutaka Oono
Dr. Yoshihiro Hase
Dr. Mohd. Nazir Basiran

3. The Eighteenth Steering Committee Meeting
(14-15 February 2005 at MINT, Bangi)

MINT:

Dr. Nahrul Khair Alang Md. Rashid
Deputy Director General (Research and Technology Development)

Dr. Mohd. Nazir Basiran
Manager, Ornamental Group,
Agrotechnology and Biosciences Division

JAERI:

Dr. Tamikazu Kume
Director, Department of Ion-Beam-Applied Biology,
Takasaki Radiation Chemistry Research Establishment

Dr. Atsushi Tanaka
Group leader, Research Group for Plant Resource Application,
Takasaki Radiation Chemistry Research Establishment

Resource persons and observers:

Dr. Norimah Yusof
Dr. Ainul Hayati Daud
Ms. Affrida Abu Hassan
Ms. Sakinah Ariffin
Ms. Shakinah Salleh

4. The Nineteenth Steering Committee Meeting
(6-7 February 2006 at JAEA, Takasaki)

JAEA:

Dr. Hideki Namba
Deputy Director General, Quantum Beam Science Directorate

Dr. Atsushi Tanaka
Unit Manager,
(Research Unit) Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Dr. Issay Narumi
Group Leader, Gene Resource Research Group,
Radiation-Applied Biology Division,
Quantum Beam Science Directorate

MINT:

Dr. Nahrul Khair Alang Md. Rashid
Deputy Director General (Research and Technology Development)

Dr. Norimah Yusof
Director, Agrotechnology and Biosciences Division

Dr. Mohd. Nazir Basiran
Manager, Ornamental Group,
Agrotechnology and Biosciences Division

Resource persons and observers:

Dr. Shinpei Matsuhashi
Dr. Yutaka Oono
Dr. Yoshihiro Hase
Dr. Satoshi Kitamura

5. The Twentieth Steering Committee Meeting
(22-23 January 2007 at Equatorial Hotel, Cameron Highlands)

Nuclear Malaysia:

Dr. Nahrul Khair Alang Md. Rashid
Deputy Director General (Research and Technology Development)

Dr. Norimah Yusof
Director, Agrotechnology and Biosciences Division

Ms. Affrida Abu Hassan
Manager, Ornamental Group,
Agrotechnology and Biosciences Division

JAEA:

Dr. Atsushi Tanaka
Unit Manager,
(Research Unit) Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Dr. Yutaka Oono
Assistant Principal Researcher, Gene Resource Research Group,
Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Resource persons and observers:

Dr. Zaiton Ahmad
Ms. Shakinah Salleh
Ms. Sakinah Ariffin
Mr. Shuhaimi Shamsudin

6. The Twenty First Steering Committee Meeting
(26-27 February 2008 at JAEA, Takasaki)

JAEA:

Dr. Hideki Namba
Deputy Director General, Quantum Beam Science Directorate

Dr. Atsushi Tanaka
Unit Manager,
(Research Unit) Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Dr. Yutaka Oono
Assistant Principal Researcher, Gene Resource Research Group,
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Nuclear Malaysia:

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Ms. Affrida Abu Hassan
Manager, Ornamental Group,
Agrotechnology and Biosciences Division

Resource persons and observers:

Dr. Masao Tamada
Dr. Ayako Sakamoto
Dr. Yoshihiro Hase
Dr. Tamikazu Kume
Dr. Ryouhei Yoshihara
Dr. Shigeki Nozawa

*1 Malaysian Institute of Nuclear Technology; the former organization of Nuclear Malaysia

*2 Japan Atomic Energy Research Institute; the former organization of JAEA

Appendix 2.

List of irradiation experiments

No.	Year	TIARA Project No.	Date	Researcher visited from MINT/Nuclear Malaysia	Date/ Time allocation of irradiation	Cooperative researchers in JAERI/JAEA	Report
1	FY 2003	31012	Aug 20-28, 2003	Mohd Nazir Basiran Sakinah Ariffin	Aug 25/ 2 hr	Naoya Shikazono Yoshihiro Hase	JAERI-Review 2004-025, 45-47
2			Dec 7-11, 2003	Mohd Nazir Basiran	Dec 8/ 2 hr		
3*			Mar 4-8, 2004	Mohd Nazir Basiran	Mar 5/ 2 hr		
4	FY 2004	41011	Jun 29-Jul 3, 2004	Affrida Abu Hassan Sakinah Ariffin	Jun 30/ 1.5 hr	Yutaka Oono Yoshihiro Hase	JAEA-Review 2005-001, 52-54
5			Mar 5-9, 2004	Mohd Nazir Basiran	Mar 7/ 1.5 hr		
6	FY 2005	51008	Jun 2-7, 2005	Affrida Abu Hassan	Jun 3/ 1.5 hr		JAEA-Review 2006-042, 79
7*			Feb 5-10, 2006	Mohd Nazir Basiran	Feb 9/ 2 hr		
8	FY 2006	63008	Nov 13-16 2006	Affrida Abu Hassan	Nov 14/ 2 hr		JAEA-Review 2007-060, 68
9			Mar 5-7, 2007	Sakinah Ariffin	Mar 5/ 2 hr		
10	FY 2007	71008	Nov 26-29, 2007	Affrida Abu Hassan	Nov 27/ 1.5 hr		JAEA-Review 2008-055, 61

*carried out concurrently with Steering Committee Meeting

Appendix 3.

List of publications and presentations

Publications

Mohd Nazir, B., Sakinah, A., Affrida, A.H., Zaiton, A., Tanaka, A., Shikazono, N., Oono, Y. and Hase Y.
Mutation induction in orchids using ion beam.
TIARA Annual Report 2003. JAERI-Review 2004-025: (2004) 45–47.

Sakinah, A., Affrida, A.H., Zaiton, A., Mohd Nazir, B., Tanaka, A., Shikazono, N., Oono, Y. and Hase Y.
Mutation induction in orchids using ion beam.
TIARA Annual Report 2004. JAEA-Review 2005-001: (2006) 52–54.

Affrida, A.H., Zaiton, A., Sakinah, A., Mohd Nazir, B., Tanaka, A., Shikazono, N., Oono, Y. and Hase Y.
Molecular analysis of ion beam-irradiated orchids. JAEA Takasaki Annual Report 2005. JAEA-Review 2006-042: (2007) 79.

Affrida, A.H., Sakinah, A., Zaiton, A., Mohd Nazir, B., Tanaka, A., Narumi, I., Oono, Y. and Hase Y.
Mutation induction in orchids using ion beams. JAEA Takasaki Annual Report 2006. JAEA-Review 2007-060: (2008) 68.

Affrida, A.H., Sakinah, A., Zaiton, A., Mohd Nazir, B., Tanaka, A., Narumi, I., Oono, Y. and Hase Y. Mutation induction in orchids using ion beams. JAEA Takasaki Annual Report 2007, JAEA-Review 2008-055: (2008) 61.

Presentations

Mohd Nazir, B., Zaiton, A., Sakinah, A. and Ros Anita, A.R.
Induction of insect resistance in Orchids.
FNCA 2005 Mutation Breeding Sub Project Review, 8 September 2005, Thailand.

Mohd Nazir, B., Zaiton, A., Sakinah, A. and Ros Anita, A.R.
Induction of insect resistance in Orchids.
FNCA 2006 Mutation Breeding Sub Project Review, 11–15 September 2006, Takasaki, Japan.

Zaiton, A., Affrida, A.H., Nurul Aliaa, I., Nurhanani, M.R. and Mohd Nazir, B.
Effect of ion beam irradiation on *Oncidium lanceanum*.
MINT R&D Seminar 2006, MINT, Bangi, Malaysia (Poster)

Affrida, A.H., Zaiton, A., Nurul Aliaa, I., Nurhanani, M.R., Norfazila, I., Mohd Nazir, B., Tanaka, A., Shikazono, N., Oono Y. and Hase, Y.
Detection of DNA polymorphism induced by ion beam irradiation on three Orchids species.
9th A-IMBN Conference and 16th MSMBB Scientific Meeting, 3–5 September 2006, Kuala Lumpur, Malaysia. (Poster)

Oono, Y., Hase, Y., Narumi, I., Tanaka, A., Affrida, A.H., Sakinah, A., Zaiton, A., Mohd Nazir, B.
Mutation induction of orchid plants by ion beams.
The 3rd Takasaki Advanced Radiation Research Symposium, 9–10 October 2008, Takasaki, Japan. (Poster)

2. Generating New Ornamental Plant Varieties Using Ion Beams

Zaiton AHMAD[1]*, Affrida ABU HASSAN[1], Shakinah SALLEH[1], Nurul Hidayah MAHMUD[1], Yutaka OONO[2]*, Yoshihiro HASE[2], Shigeki NOZAWA[2], Ryouhei YOSHIHARA[2,3], Issay NARUMI[2,4] and Atsushi TANAKA[2]

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Summary

Chrysanthemum is one of the most important ornamentals for both local and international market. One of the main problems for chrysanthemum in Malaysia is the lack of new varieties to meet the ever-changing customer demands. For this purpose, mutagenesis of chrysanthemum was carried out using a combination of ion-beam irradiation and *in vitro* organ cultures to produce new cultivars with novel traits and wider mutation spectrum. Ray floret and nodal cultures of chrysanthemum were irradiated at various doses with 320 MeV $^{12}C^{6+}$ ion beams. Eight mutants were selected based on their uniqueness and/or suitability for cut flower production. Among them, TIARA Red, Golden Eye, and Yellow Sun generated from Regan Red variety, and Majestic Pink from Pink variety have been filed for Plant Variety Protection (PVP) with Department of Agriculture (DOA) Malaysia.

KEYWORDS: *Chrysanthemum morifolium*, Reagan Red, Pink, Ion Beams, Mutation, Mutant, Flower Morphology, Flower Colour

*Corresponding authors

2.1 Introduction

2.1.1 Chrysanthemum - Nomenclature, taxonomy and characteristics

Chrysanthemum (Figure 1) is a dicotyledonous plant in the genus of *Chrysanthemum* L. It belongs to Asteraceae or Compositae family based on its flower characteristics. All plants in this genus are annuals or perennial herbs. Botanically this plant is currently known as *Dendranthema grandiflora* Tzvelev but was previously known as *Chrysanthemum morifolium* Ramat[1]. *Dendranthema grandiflora* is the most popular commercially grown species for ornamentals either as cut flower, potted plant or garden plant. This species is an allohexaploid (2n = 6x = 54) and has a single daisy flower type with colour of white, pink, lavender and yellow.

Figure 1. Some of chrysanthemum varieties with different flower colours and morphology.

2.1.2 Overview of chrysanthemum production in Malaysia

Chrysanthemum has been grown in Malaysia since 1970's, initially as a hobby. In mid-Eighties, the growers started to grow the plant commercially and since then, it has become major cut flowers besides orchids, roses and carnations. Japan is the main importer of Malaysian chrysanthemum. The main area for growing temperate cut flowers in Malaysia is Cameron Highlands, Pahang. Other areas include Gua Musang, Kelantan, and Kundasang, Sabah[2]. Floriculture industry in Malaysia has grown convincingly from only 773 ha of planting areas in 1984[3] to approximately 2213 ha in 2011[4]. This scenario occurs due to good market, lucrative returns, government support and campaigns[5]. According to data released by Statistics Unit,

Department of Agriculture[4], Malaysian floriculture export was valued at RM 354 million in 2011, and is expected to increase to approximately RM 857 million in 2020.

Most of the chrysanthemum cultivars cultivated in Malaysia are produced under rain shelters with controlled photoperiod. Since Malaysia has abundant rainfall (≈3,000 mm) annually, rain shelters are beneficial to prevent the plant and flower from being injured by heavy rainfall. Controlled photoperiods by using artificial lights are common practices for chrysanthemum production in tropical region including Malaysia as this region has almost equal day and night length (12 hours) throughout the year. Being a short day plant, the plant may flower pre-maturely in tropical region, if they are grown without any artificial light interruption. Night interruptions are started soon after planting and at least 4 hours is needed every day for 3 to 5 weeks or until the plant height is about 30 cm[6].

Since chrysanthemums are grown mainly for cut flower in Malaysia, the growers prefer to use non-bushy type either the spray or disbudded type. Some of the commercial varieties of chrysanthemum in Malaysia are 'Anastasia' (green, lilac, pink and white), 'Biarritz' (pink, purple and yellow), 'Chopin white', 'Deliflame orange', 'Delilah' (pink, yellow and white), 'Dinar pink', 'Reagan' (purple and sunny), 'Ping pong' (white and yellow), 'Monalisa' (dark pink, pink, rossy, white and yellow), 'Recyber' (pink, white and yellow), 'Vesuvio' (white and yellow), 'Sei Trinity' (orange and peach), and 'Remix red'[7].

2.1.3 Issues and problem of chrysanthemum industry in Malaysia

The main problems faced by chrysanthemum growers in Malaysia at the moment are two folds: high royalty cost for foreign mother plants and the lack of new local-owned varieties. Since the earlier planting days the growers at Cameron Highlands get the supply of planting materials (rooted or unrooted cuttings) from two foreign companies. These companies import mother plants from overseas, produce the cuttings locally and sell to local growers. As such, the growers have to pay high royalty cost for using the foreign mother plants which was calculated based on the number of cutting produced. Overall, these growers then have to pay between RM 0.09 to RM 0.13 per cutting as royalties. In 2005, approximately RM 8.3 to RM 12 million of royalties was paid to the foreign companies (Utusan Malaysia, 15 January 2007).

Besides, it is estimated that a total of 300 million chrysanthemum cuttings are required annually by growers in the Cameron Highlands and the both companies can only produce between 10–13% of the local needs[2]. Insufficient seedling cuttings may affect the development of floriculture industry in Malaysia. In order to overcome the insufficient supply, some of the growers have taken the initiative to do the conventional cutting. Somehow, this practice may lead to the tendency to generate or multiply virus infected seedling. At the moment, the application of biotechnology or specifically *in vitro* propagation technique is a good alternative for a large-scale production and also a proven technique for propagation of virus free clones[8, 9].

The development of local owned varieties could greatly help growers in which they do not have to heavily rely on imported cultivars as the mother plants in the production of cut flowers and potted plant for export and local market. If locally developed cultivars are made available, this would also reduce the amount of royalties, thus increasing grower incomes and reducing their overall planting expenditure costs and export royalties. Subsequently, this may also help the national revenue from floriculture industry in general.

2.1.4 Mutation induction of chrysanthemum

In chrysanthemum production, there is a continuous demand for new cultivars with variations in flower colour and shape coupled with other good traits such as flower uniformity, storability, response time, firmness of the stalk, growth vigour, leaf quality, and suitability for year-round production. To fulfill the demand in relatively short period of time, inducing mutation by using physical mutagen such as X-rays, gamma rays, or ion beams in combination with *in vitro* propagation is a very promising technique[10].

Previous studies showed that chrysanthemum is one of the most successful examples in mutation breeding work among the vegetatively propagated ornamentals. At present, among more than 2570 officially released mutant varieties worldwide, 625 are ornamental plants and of these, 267 varieties are the chrysanthemum species (http://www-mvd.iaea.org). One hundred forty-seven chrysanthemum species mutant varieties were derived from gamma irradiation, 106 from X-ray, six from chronic gamma-ray, and two from ion-beam irradiation. Among the physical mutagens, gamma rays have been commonly used effectively for mutation induction, followed by X-rays and ion beams.

2.1.5 Ion-beam irradiation of chrysanthemum

Ion beams have much higher linear energy transfer (LET) and relative biological effectiveness (RBE) than gamma rays or X-rays[11), 12), 13)]. Ion beams also have a potential to focus the high energy on a target site; as a consequence, ion beams can induce a high level of mutagenic effect[14)]. In contrast to radiation with low LET, ion beams produce more DNA damage and double-strand breaks[15)] or large DNA alterations such as inversions, translocations, and large deletions rather than point mutations[16)]. According to Tanaka[17)], half of the *Arabidopsis* mutants showed small mutations such as base changes and small deletions involving a few bases; the other half showed large DNA alterations such inversions, translocations and deletions. These results indicated that ion beam irradiations have broad spectrum and high frequency of mutations.

Study by Yamaguchi *et al.*[18)] showed that the numbers of chrysanthemum plant with reduced nuclear DNA content after irradiation were increased with increasing irradiation doses of 320 MeV carbon ions, 100 MeV helium ions, and gamma rays. In contrast, the nuclear DNA did not decrease with 220 MeV carbon ions, even when the doses were increased. They also found irradiation treatment with 220 and 320 MeV carbon ions and gamma rays showed a similar effect

on mutation induction, while the effect of 100 MeV helium ions was not as great. Thus, the effects of irradiation treatment on mutation induction and nuclear DNA content differed according to the type of ion beams. 220 MeV carbon ion beams seem to be the most appropriate among the three types of ion beams because 220 MeV carbon ion beams give a high mutation frequency with low damage of chromosomes.

Various ornamental mutant plants such as dahlia[19], carnation[20], rose[21, 22], *Osteospermum*[23], cyclamen[24], *Delphinium*[25], orchid[26], *Dianthus*[27], and chrysanthemum[28] have been successfully induced by ion beam irradiation. According to Nagatomi *et al*.[29], specific flower colour mutants of chrysanthemum that could not be obtained by gamma-ray irradiation could be generated by ion beam irradiation such as new mutant varieties with different flower colour[30] and mutants with less laterals buds[31].

In terms of flower colour mutation rates, Nagatomi *et al*.[32] found that the rates in plants regenerated from chrysanthemum floral petals were higher than those regenerated from leaves in both ion beams and gamma rays. Most of the mutants irradiated by gamma rays showed a single flower colour, whereas those by the ion beams exhibited complex and stripe types of flower colour. Further study by Nagatomi *et al*.[29] on different sources of ion-beam ($^{4}He^{2+}$, $^{12}C^{5+}$ and $^{20}Ne^{8+}$) irradiation showed a higher mutation rate and broader flower colour spectrum were induced from chrysanthemum floral petals than leaves using $^{4}He^{2+}$ and $^{12}C^{5+}$, but no difference in the explants was observed with $^{20}Ne^{8+}$.

2.1.6 Plant new variety protection in Malaysia

Malaysian government has also started to enforce the Protection of New Plant Varieties Regulation in 2008 under Protection of New Plant Varieties (PNPV) Act 2004 and the responsibility in implementing the PNPV Act 2004 has been entrusted to DOA, Malaysia. The objective of the Act is to provide for the protection of the rights of breeders of new plant varieties, and the recognition and protection of contribution made by farmers, local communities, and indigenous people towards the creation of new plant varieties; to encourage investment and development of the breeding of new plant varieties in both public and private sectors; and to provide for related matters. With the PVP legislation, Malaysian growers are in a better position to have access to new and improved varieties for commercial growing especially for temperate flower such as chrysanthemum. At present, 39 new varieties from various species have been registered under this act, and of these, 8 varieties are chrysanthemums (http://pvpbkkt.doa.gov.my).

2.1.7 Objectives of the project

The ultimate aim of this project was to produce new varieties of chrysanthemum through ion beam technologies in combination with *in vitro* culture techniques. Apart from producing new varieties, several other aspects of ion beam irradiation on chrysanthemum have also been studied

such radio-sensitivity and the effectiveness of ion beam irradiation in generating mutations in selected chrysanthemum varieties. The data obtained from this research would be very beneficial for further mutation breeding work on other chrysanthemum varieties or any similar plants. Specifically the objectives of the project were;

1) To optimise protocols for ion beam irradiation in chrysanthemum varieties
2) To study the effects of ion beam on morphological, flowering and growth characteristics of chrysanthemum mutants
3) To produce new varieties of chrysanthemum with improved characteristics such as new flower colour, forms, flowering habit, and/or suitability for cut flowers or other specific uses.

2.2 Materials and methodology

2.2.1 Plant materials

Chrysanthemum morifolium cv. 'Lameet' (Figure 2a) was the first selected variety for this project and its leaf and petal cultures were irradiated in 2007. However, we failed to generate any mutant mainly due to the difficulty in shoot regeneration after irradiation. Therefore, *Chrysanthemum morifolium* cv. 'Reagan Red' (Figure 2b) was subsequently selected and this variety was by far the most extensively studied throughout this five-year project. Our counterpart, the Malaysian Agricultural Research and Development Institute (MARDI) also suggested this variety as it is an old variety and free from royalty. The plants had red flowers with a yellow-green centre disk. The flowers are daisy-like with one or two layer ray florets and only ray florets are used as explants in this study. This spray type cultivar is suitable for cut flower production but it also can be grown as potted plants. Other varieties studied at the later stage of the project were the 'Pink' and 'Purple' varieties (Figure 2c and 2d). The purple variety did not perform well in our study and hence was not pursued. The pink variety has shown some impressive results and is discussed separately (from Reagan Red) towards the end of this report. All potted chrysanthemum plants were obtained from a local nursery in Sungai Buloh, Selangor and were maintained in the hardening room at 27 to 30 °C before use.

Figure 2. *Chrysanthemum morifolium* cv. 'Lameet' (a), 'Reagan Red' (b), 'Pink' (c) and 'Purple' (d) used in this project.

2.2.2 Surface sterilization

In this study, fresh and fully opened flowers were collected and used as explants. The explants were washed under running tap water to remove all dust and dirt. Then the explants were immersed in 1 ml/L dish detergent (Teepol) for 1 hour. After that the explants were rinsed three times with reverse osmosis (RO) water. Then the explants were immersed in 1% benomyl solution for 1 hour and followed by rinsing three times with RO water.

Subsequent procedures were carried out in a laminar air flow cabinet, using sterilized glassware and apparatus. The pre-sterilized explants were immersed in 70% ethanol for 1 minute with occasionally shaking, followed by rinsing three times with RO water. The explants were then again immersed in 0.26% sodium hypochlorite supplemented with a few drops of Tween-20 for 30 minutes and occasionally shaking. Finally, the flowers were rinsed three times with RO water before being placed on sterile filter papers to blot dry excess water.

2.2.3 Medium preparation

Murashige and Skoog medium[33] (MS Medium) was used as a basal medium. The media used in this study have been previously optimized containing α-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP)[34]. The pH of media was adjusted to 5.7 by acid (HCl) or alkali (NaOH) prior to addition of 30 g/L sucrose and 2.7 g/L agar (Gelrite). The mixtures were then autoclaved at 121 °C at 103 kPa for 15 minutes. The media (30 mL) were then poured into 9-cm^2 petri dish. The prepared media were stored in the culture room and used within 3-4 weeks.

2.2.4 Initiation of cultures

Ray florets

Ray florets were the primary choice of starting materials for irradiation as according to Nagatomi *et al.*[29], the induced frequencies of flower color and the multicolor mutation is generally increased several fold by culturing petals or ray florets as compared to other explants types. In these experiments, the ray florets were detached from the flowers (Figure 3a) and cut into two sections (Figure 3b). Only the lower parts of the floret (Figure 3c) were used in the study. The florets were placed horizontally on medium with the abaxial surface facing the optimized callus induction medium, MS supplemented with 0.5 mg/L NAA and 0.5 mg/L BAP. Cultures were incubated at 25 ± 2 °C in 16-hour day length provided by cool white fluorescent light. After 10 days of culture, the ray florets were irradiated with ion beams.

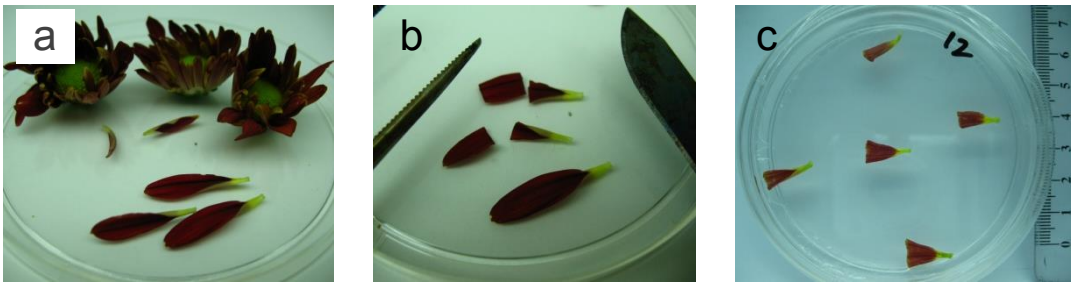


Figure 3. Preparation of florets for callus and shoot induction (a) florets were detached from the flowers, (b) ray florets were cut into two sections, (c) lowers parts of ray floret were placed on the media with the abaxial surface touching the media.

Nodals

For the nodal explants preparation; the shoots derived from ray florets cultures were cut into single node (between 0.5- and 1-cm long). Terminal shoot and all leaves were removed. The nodes were then cultured onto petri dishes containing half strength of MS medium without any growth regulators (Figure 4). After 4 days of culture, the nodes were irradiated with ion beams.

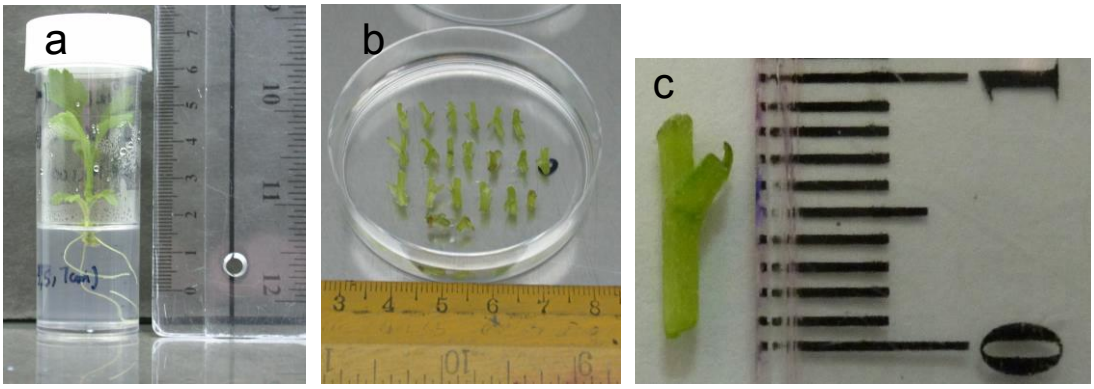


Figure 4. Preparation of nodal explants for irradiation. *in vitro* shoot as the source of nodal explants (a), single node was cut into ~0.5- to 1-cm long (b), close-up view of single node explant (c).

2.2.5 Ion-beam irradiation

Ion-beam irradiation was performed at JAEA, Takasaki, Japan. Details of doses used in each irradiation experiment are shown in Appendix 2 and Table 2. Ray floret and nodal explants were placed on 6-cm sterile petri dishes containing their respective medium and covered with a sterile 8 μm-thick polyimide film (Kapton, Toray, Japan). The carbon-12 ($^{12}C^{6+}$) ion beams with the total energy of 320 MeV were generated by an azimuthally varying field (AVF) cyclotron in the Takasaki Ion Accelerators for Advanced Radiation Application (TIARA). Twelve doses of ion beams at 0, 0.5, 0.8, 1, 2, 3, 5, 8, 10, 15, 20, and 30 Gy were used in the study. Figure 5 shows some of the processes during irradiation.

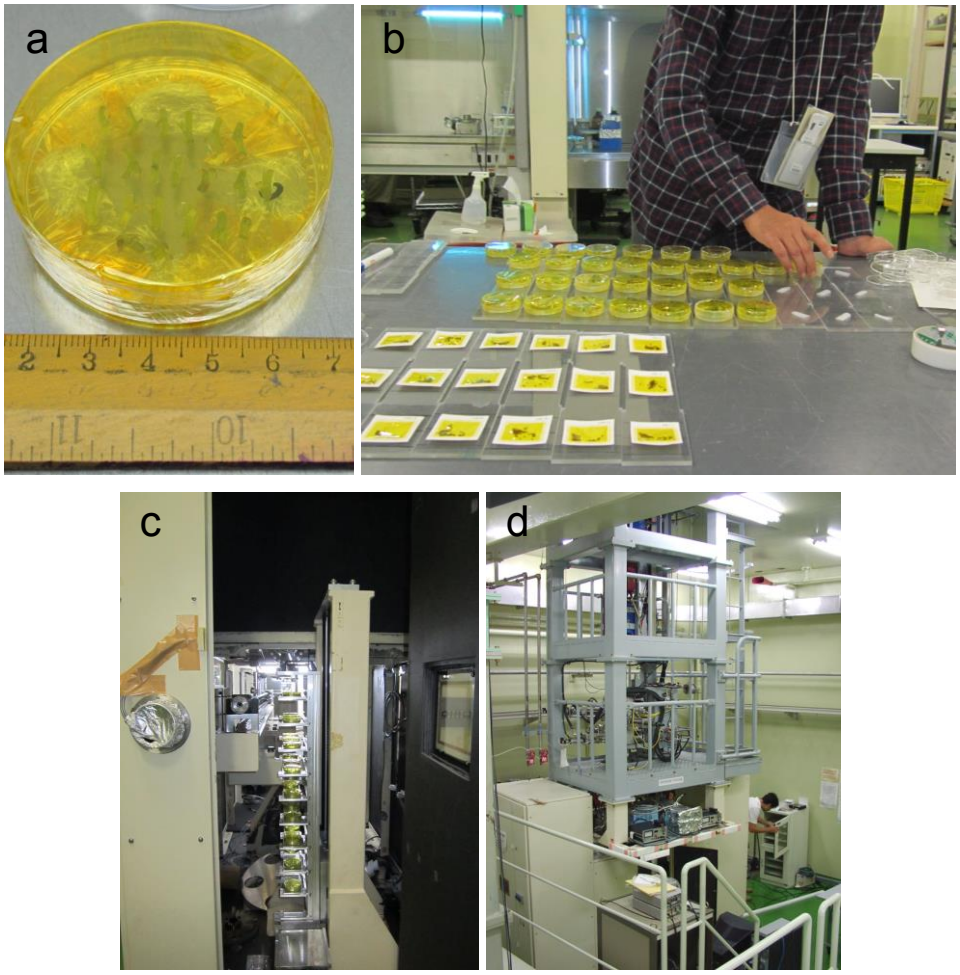


Figure 5. Some of the processes during ion-beam irradiation. Petri dish containing samples covered with Kapton film (a), samples were arranged according to dose treatments (b), samples were loaded into the irradiation apparatus (c), Full view of the irradiation apparatus connected with a beam line from AVF cyclotron (d).

2.2.6 Subculturing and data collection

Following ion-beam irradiations, the ray floret explants were transferred onto fresh media with the same formulation. Sub-culturing was carried out at two weeks interval for shoot regeneration. Data on the percentage of lethal dose and percentage of regeneration dose were observed after 8 weeks of irradiation.

The nodal explants were cultured individually into culture bottle containing 15 ml of half strength MS medium. The plants generated were sub-cultured monthly from M1V1 (mutation one, vegetative stage one) generation to M1V4 (mutation one, vegetative stage four) generation. Data on percentage of lethal dose and percentage of regeneration dose were recorded at 5 weeks after irradiation.

2.2.7 Experimental design and analysis

The radiosensitivity test for ray floret explants was conducted in a Randomized Complete Block Design (RCBD) with 5 replications for both irradiations. Each petri dish contained 10 explants and was considered as a replication. Fifty explants were used for each dose. Radiosensitivity test of nodal explants to ion beam, each dose of irradiation was replicated 20 times. Each node was considered as one replication.

Data on lethal dose and regeneration dose was determined by measuring *in vitro* survival and shoots regeneration for both chrysanthemums explants. The percentage of survival explants was calculated based on number of survival explants from the total number of explants as the following:

% of survival explants =

(Number of survival explants) / (Total number of irradiated explants) × 100

The percentage of regeneration explants was measured based on number of explants regenerated into shoot from the total number of explants, as below:

% of shoot regenerated =

(Number of regenerated explants) / (Total number of irradiated explants) × 100

2.2.8 Rooting, hardening, and transplanting

Regenerated plantlets derived from irradiated ray florets and nodal explants were used as planting materials. Healthy unrooted plantlets were selected and transplanted in square plastics containers containing soaked LECA (Lightweight expanded clay aggregate) and perlite in ratio 1:2. Lid of the container was closed in order to maintain the moisture for rooting and hardening the plantlets. The containers were placed in hardening room and the room temperature was controlled between 27 to 30 °C for 3 weeks under 16-hour photoperiod (12 hours of cool white and 4 hours of warm light)[35]. This light regime was practiced to promote vegetative growth and to prevent the plants from producing flower buds immaturely.

After rooting, the plantlets were transferred to MARDI Cameron Highland and sown individually in 15-cm pots containing top soil, compost, and perlite with the ratio of 1:1:2. The pots were placed in greenhouses at average temperature between 15 (night) to 25 °C (day) for subsequent screening process. Cultural practices such as watering, fertilizing, and controlling pest and diseases followed the standard procedures for chrysanthemum pot plants production, established by MARDI[36]. The screening study was conducted in a Randomized Complete Block Design (RCBD) with 12 replications for both irradiation types. Statistical Analysis System (SAS, version 9.2) software (SAS Institute, USA) was used to carry out the analysis of variance (ANOVA) and

Duncan's New Multiple Range Test (DNMRT) was used for comparison among treatment means at $p < 0.05$[37].

2.2.9 Screening of irradiated population

The main objective of screening the irradiated population was to identify potential mutants of chrysanthemum with unique morphological features for further stability tests. In addition to that, screenings were also carried out to evaluate the effects of individual doses of ion-beam irradiations on morphological and flowering characteristics of the irradiated plants. During this process, overall performance of regenerated plants derived from *in vitro* nodals and ray florets irradiated with ion beams were recorded. Morphological traits observed during screenings were;

1) Plant morphology (plant height, number of leaves, internodes length, and number of vegetative branches)
2) Flowering characteristic (days to bud break, numbers of flower buds, flower diameter, flower colour, and flower shape)
3) Leaf characteristic (blade length, shape, and leaf colour)

Leaf and flower colour are determined by the colour or the nearest possible colour according to The Royal Horticultural Society London (RHS) colour chart.

2.3 Experiments on *Chrysanthemum* cv. Reagan Red

2.3.1 Radiosensitivity of ray floret explants

The survival rate of ray floret cultures was recorded at 8 weeks after irradiation. As expected, it was found in these studies that survival rates decreased as the doses increased. Green calli and shoot formation only occurred on ray florets irradiated with considerably lower doses of ion beams (0, 0.5, 1, 2, 3, 5, and 8 Gy). Explants that failed to survive turned completely brown without any shoots. At higher doses above 8 Gy, callus formation was observed but these calli failed to develop further into complete shoots.

The graph as shown on Figure 6 was plotted to determine lethal and optimum regeneration doses for ion beam irradiations. From this graph, it was observed that the 50% lethal dose (LD_{50}) of ray florets explants irradiated with ion beams occurred at 15.5 Gy and 20% lethal dose (LD_{20}) was at 11.5 Gy. However, not all the survived explants were able to generate shoots even for non-irradiated explants. Generally, throughout this project, the percentage of non-irradiated explants of Reagan Red variety to successfully generate shoots was in the range of 60 to 80%. However, in this specific experiment, approximately 60% of control ray floret explants were able to regenerate further into shoots. For the irradiated cultures, the percentage was decreased with the increase in irradiation doses. The result obtained from this irradiation experiment was similar to those reported by Okamura *et al*.[20], who found that shoot regeneration frequency of carnation variety 'Vital' from leaf segments decreased with the increased in irradiation doses. From the plotted graph, 50% of shoots regeneration dose (SRD_{50}) was approximately fall at 2.8 Gy whilst 80% of shoots regeneration dose (SRD_{80}) was around 1.2 Gy. The terms SRD_{50} and SRD_{80} in this context refer to the dose that reduced shoot regeneration rate from irradiated explants to 50% and 80%, respectively.

The dose for mutation induction of *Chrysanthemum morifolium* cv. 'Reagan Red' was chosen based on the SRD_{50}, on which 5/6 relative regeneration rate of unirradiated control was observed, since the main objective of this mutagenesis work was to obtain irradiated shoots for further mutation screening. Therefore, the recommended dose for mutation induction of this cultivar using ion beams was in the range of 1–3 Gy.

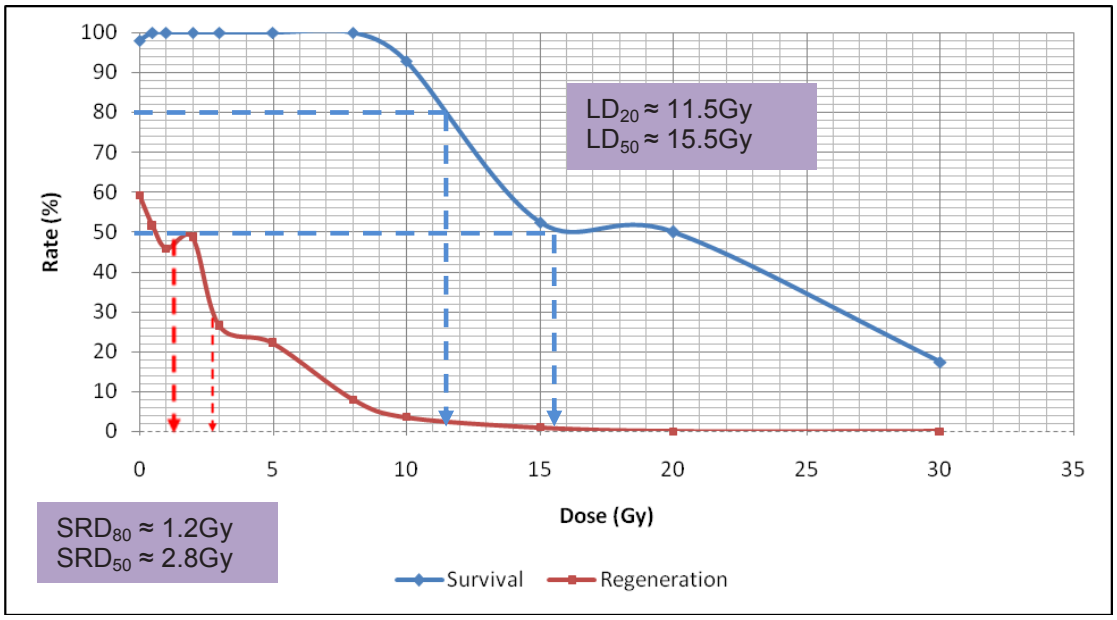


Figure 6. Survival (blue) or shoot regeneration (red) rate (%) of ray florets explants treated with different doses of ion beams at 8 weeks of culture.

Figure 7 shows pictures of irradiated ray florets at different doses at week 8 post-irradiation. Generally, increasing the dosage of ionizing radiation negatively affect callus growth and plant regeneration. This is because of ionizing radiations interact with atom or molecules and produce free radicals such as reactive oxygen species (ROS) in cells. ROS are constantly produced in cells, but when their concentrations are increased by ionizing radiation, they can lead to cell damages and lethality[38]. Besides, free radicals can also damage or modify important components of plant cells and have been reported to affect the morphology, anatomy, biochemistry and physiology of plants especially at higher doses[39].

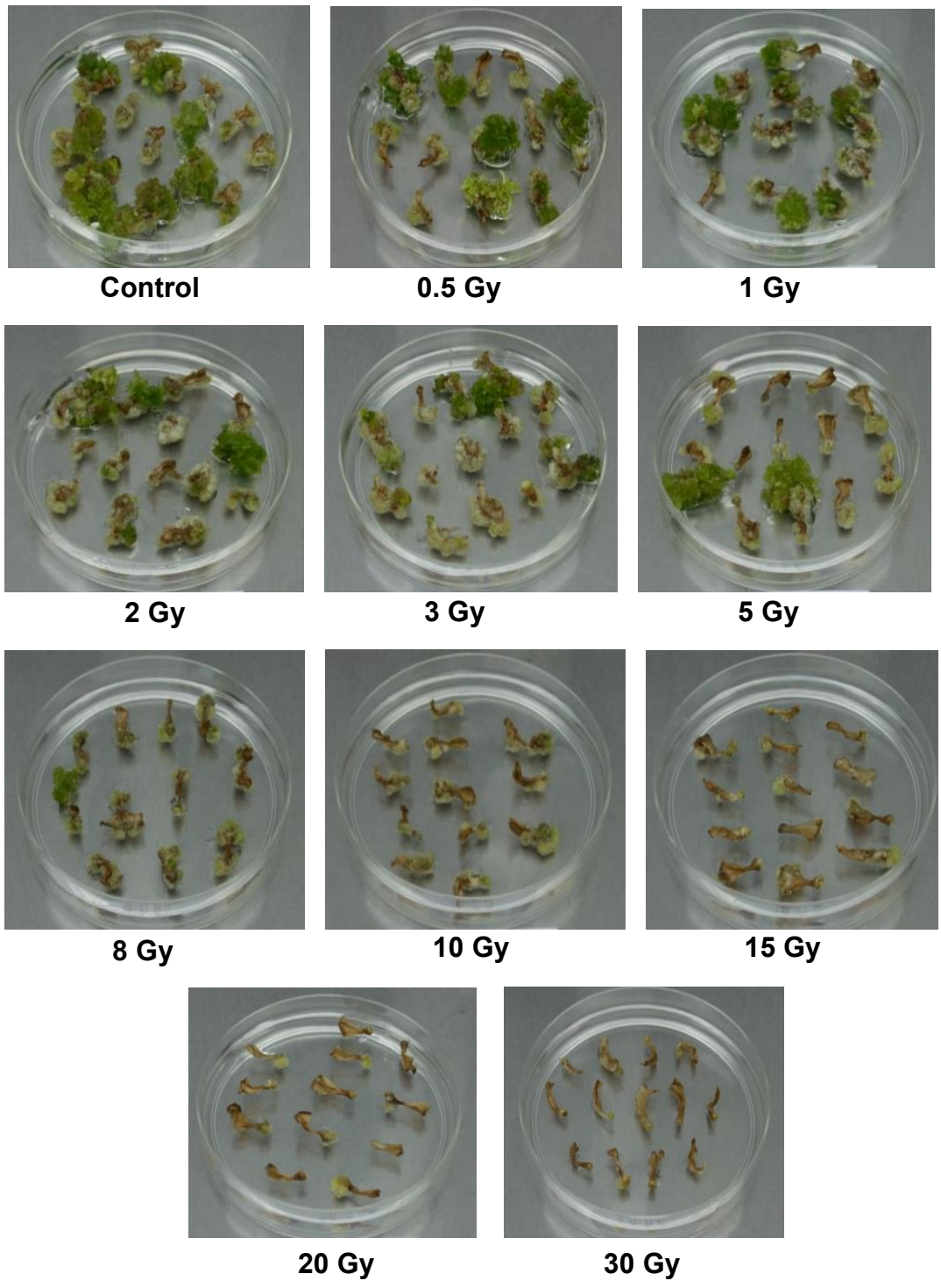


Figure 7. The response of ray floret explants to different doses of ion-beam irradiation after 8 weeks of cultures. Green tissues showed calli developing into adventitious shoots.

2.3.2 Radiosensitivity of nodal explants

Besides ray florets, sensitivity of nodal cultures to ion-beam irradiation was also evaluated. In this study, nodal explants were able to survive at doses from 0 to 10 Gy after 5 weeks of ion-beam irradiation, but shoot regeneration was only occurred at doses below 5 Gy. Nodes at doses 8 and 10 Gy mainly became purplish with dormant axillary buds. Nodal explants at higher doses (15 Gy and above) turned brown, without any axillary bud formation in majority of them. Even those with small bud formation did not manage to survive past 6 weeks (Figure 8).

Figure 8. Effect of ion-beam irradiation on survival and shoot generation of nodal explants after 5 weeks of irradiation at various doses.

The percentage of survival and shoot regeneration of nodal explants after irradiation by ion beams are shown in Figure 9. The LD_{50} value was estimated at 6.5 Gy whilst LD_{20} was approximately at 4 Gy. The SRD_{80} and SRD_{50} values were at 3.5 Gy and 4 Gy, respectively. These results showed that the estimated optimal dose for *in vitro* mutation induction of *Chrysanthemum morifolium* cv. 'Reagan Red' nodal explants using ion beam was in the range of 3.5 to 4 Gy.

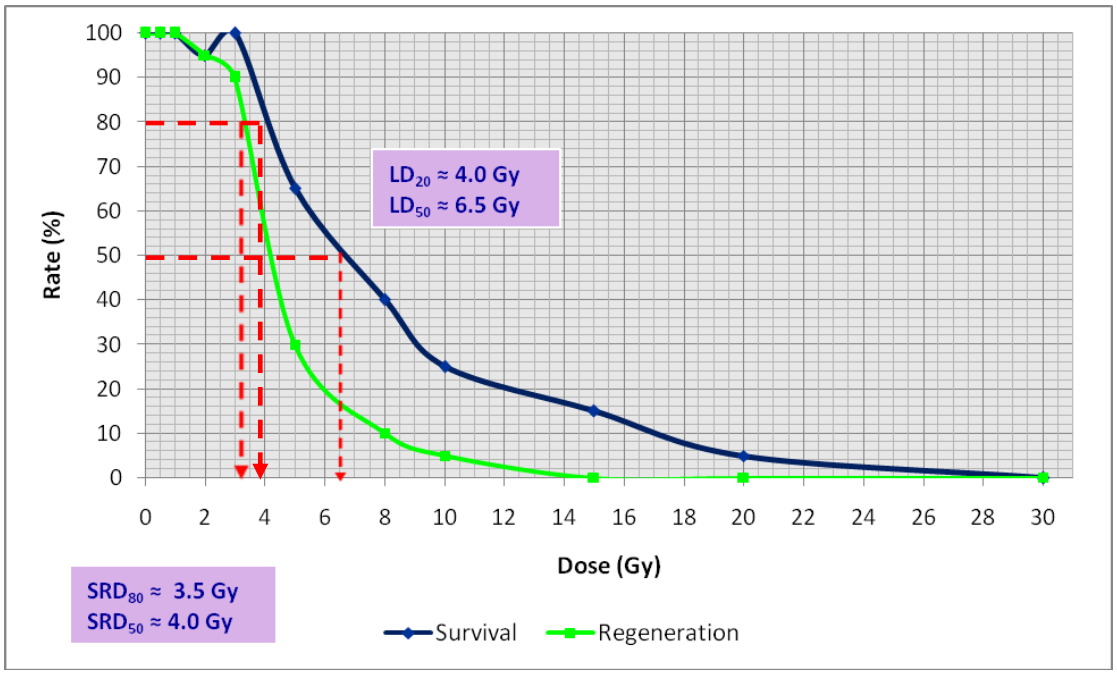


Figure 9. Survival (navy) or shoot regeneration (green) rate (%) of nodal explants treated with different doses of ion beam at five weeks of culture.

2.3.3 Overall characteristics of irradiated plants

Representatives of the irradiated plants derived from independent ray-floret and nodal explants were transplanted in the glasshouse at MARDI Cameron Highlands for morphological screenings. The initial numbers of transferred plants according to their doses are as in Table 1.

During the first transplanting of irradiated Reagan Red plants (around mid-March to April 2010), daytime temperature at Cameron Highlands was unusually much higher (28–29 °C) than usual (25 °C). As a result, some of the transferred seedlings were wilted and died within one week of transplanting. Therefore, morphological characters were measured from the survived populations only. The number of survived plants is detailed out as in Table 2.

The main bottleneck of any plant mutation breeding is the formation of chimeras[40]. This is especially the case when rooted cuttings are used as the starting explants for irradiation[41]. In this study, the mutations observed in the screening populations appeared to be solid. This might be due to the use of *in vitro* cultured ray florets and nodals as the starting explants in which the mutant shoots were directly regenerated from these explants. There is a general agreement that by using *in vitro* mutation induction technique, the problem associated with the generation of chimeric plants could be reduced. Broertjes *et al.*[42] found that *in vitro* irradiated cultures of chrysanthemum almost exclusively produced solid non-chimera mutants, as compared to their *in vivo* counterparts which generated a relatively high number of chimeras.

Table 1. Number on transplanted plants derived from independent (a) ray floret and (b) nodal explants.

a) Ray florets

Irradiation	Dose (Gy)	Number of independent ray floret-generated plants	Number of replicate per plant	Total number of transplanted plants
Control	0	12	1	12
Irradiated	0.5	12	3	36
	1	12	3	36
	2	12	3	36

b) Nodals

Irradiation	Dose (Gy)	Number of independent ray floret-generated plants	Number of replicate per plant	Total number of transplanted plants
Control	0	12	1	12
Irradiated	0.5	12	5	60
	1	12	5	60
	2	12	5	60
	3	12	5	60
	5	12	5	60
	8	12	3	36

Table 2. Number of initially transferred plants and those that survived after 1 week.

Explants	Dose (Gy)	Initial no. of transferred plants	No. of plants survived
Ray floret	0	12	8
	0.5	36	22
	1	36	22
	2	36	25
Nodal	0	12	11
	0.5	60	51
	1	60	47
	2	60	50
	3	60	44
	5	60	36
	8	60	26

Plants derived from ray florets

Plant morphological characters examined in this study were plant height, number of leaves, internode length and number of vegetative branch. Detailed characters observed on irradiated plants derived from ray florets are shown in Table 3. Significant effects of ion beam irradiation were seen on plants derived from ray florets irradiated with ion beams. It was found that the height of ion beam irradiated ray floret-generated plantlets from all doses (0.5, 1, and 2 Gy) were taller as compared to their controls. The same effect of ion beams in altering plant height has also been observed in rice[43] and wheat[44].

Table 3. Morphological characteristic of plants derived from *in vitro* irradiated ray florets explants.

Treatment	Dose (Gy)	Plant Height (cm)	No. of leaves	Internode length (cm)	No. of vegetative branches
Control	0	17.9^{b}	15.2^{c}	1.2^{c}	3.0^{a}
Ion beam	0.5	39.3^{a}	21.8^{b}	1.8^{a}	0.0^{b}
	1	37.6^{a}	22.1^{b}	1.7^{ab}	0.0^{b}
	2	40.1^{a}	26.6^{a}	1.5^{b}	0.0^{b}

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test ($p < 0.05$).

In terms of leaf number, there was a progressive increase in the number of leaves with the increase in irradiated doses. As for internode length, the figure increased by 0.6 cm at dose 0.5 Gy, but no significant difference was observed between doses. Interestingly, all the irradiated plants also did not produce any branch and continued to develop as a single stem. It is suggested that this result might also due to the interruptions of gibberellins' metabolism pathway. Gibberellins are the large class of endogenous plant hormones that influences several aspect of plant development such shoots growth, leaf expansion, stems elongation, and apical dominance[45]. Figure 10 shows examples of control and ion-beam mutants.

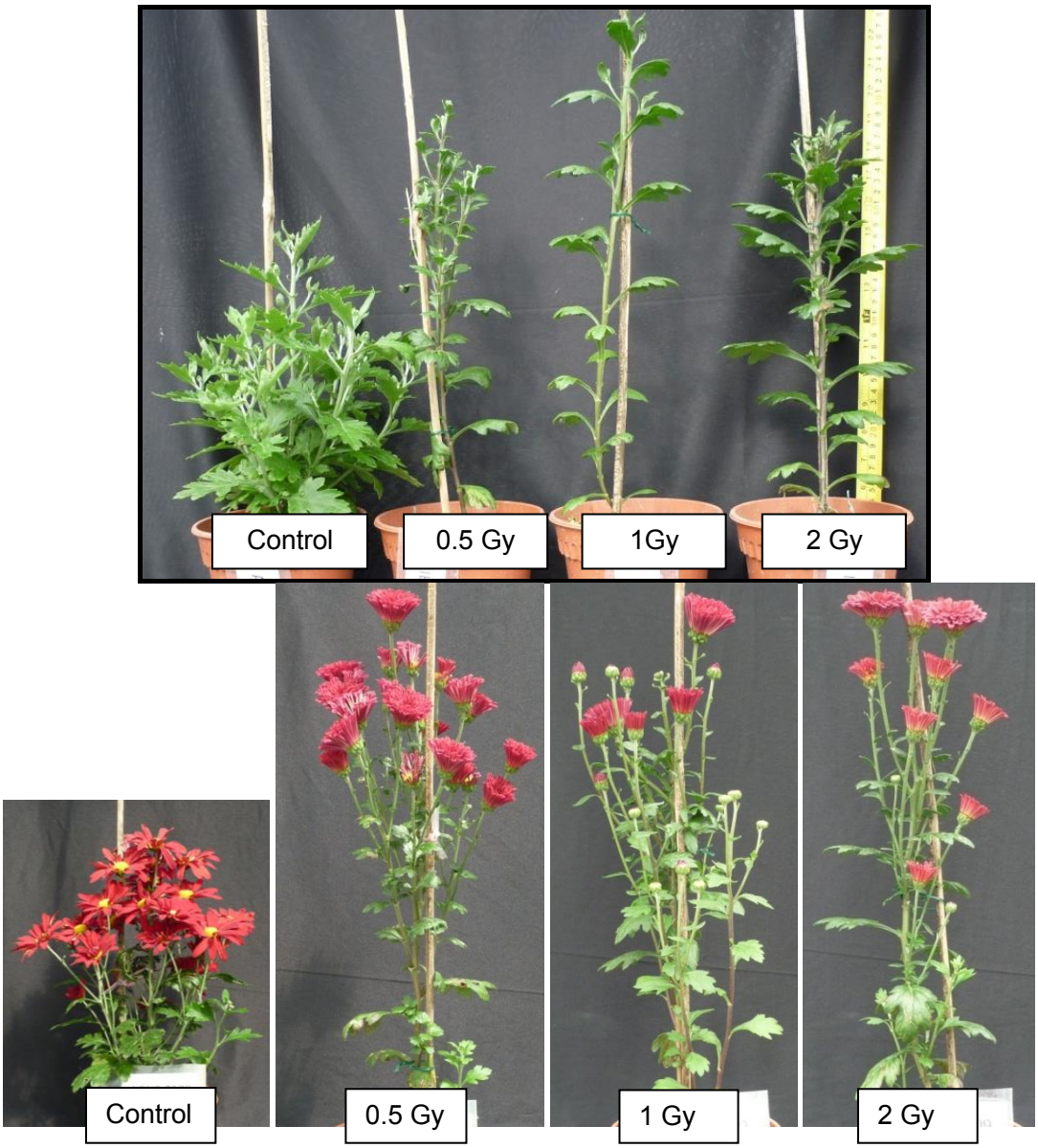


Figure 10. Morphology of vegetative and flowering plants derived from *in vitro* irradiated ray floret explants at different doses of ion-beam irradiation.

Plants derived from nodals

As in the ray floret-generated plants, the same characteristic were also evaluated for the nodal-derived plants. However, in this study, it was found that the plant height, number of leaves, internode length, and numbers of vegetative branches were not significantly affected by the applied doses (Table 4). It is assumed that the plant morphology of chrysanthemum cv. 'Reagan Red' could not be changed through the use of *in vitro* nodal explants in combination with ion-beam irradiation. The morphology of plants that derived from *in vitro* irradiated nodal explants using ion beams was shown in Figure 11.

Table 4. Morphological characteristic of plants derived from *in vitro* irradiated nodal explants.

Treatment	**Dose (Gy)**	**Plant Height (cm)**	**No. of leaves**	**Internode length (cm)**	**No. of vegetative branches**
Control	0	18.1^{a}	14.5^{ab}	1.3^{ab}	3.2^{ab}
Ion beam	0.5	18.8^{a}	13.8^{ab}	1.3^{ab}	4.5^{ab}
	1	18.3^{a}	14.1^{ab}	1.3^{ab}	4.4^{ab}
	2	19.3^{a}	14.3^{ab}	1.4^{a}	3.8^{ab}
	3	19.5^{a}	14.2^{ab}	1.4^{a}	4.9^{a}
	5	18.3^{a}	13.8^{ab}	1.3^{ab}	3.0^{b}
	8	18.3^{a}	14.5^{ab}	1.3^{ab}	3.5^{ab}

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test ($p < 0.05$).

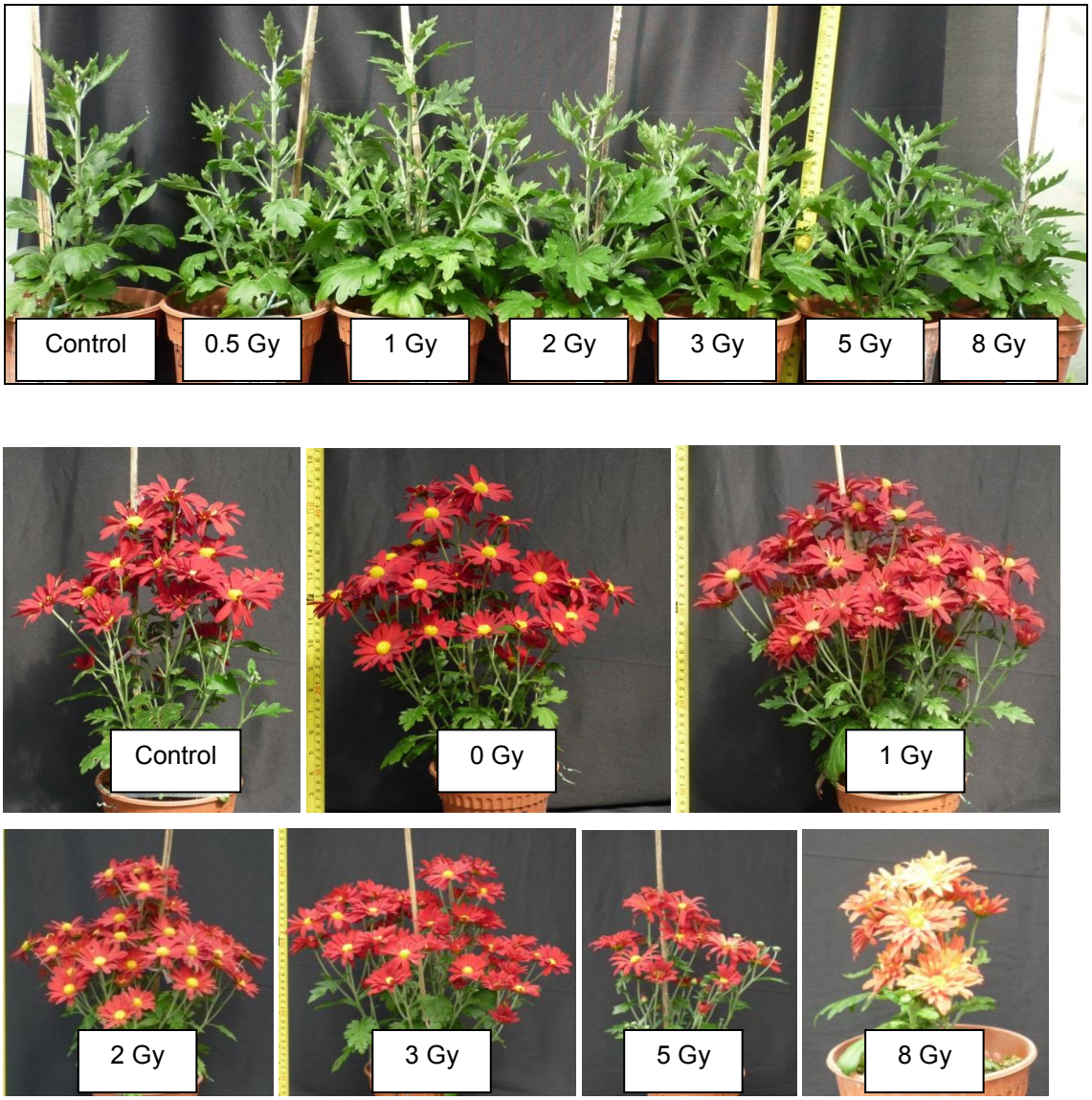


Figure 11. Morphology of vegetative and flowering plants derived from *in vitro* irradiated nodal explants at different doses of ion beams.

2.3.4 Flowering characteristics of irradiated plants

The success of flower mutations in chrysanthemum depends on various factors such as the right choice of physical mutagen, irradiation method, plant species, and explant types[46].

Ray floret-generated plants

Effects of ion beams in altering flowering characteristics of irradiated plants were evaluated. Based on the results shown in Table 5, ion beams seemed to delay bud breaking especially those irradiated at 0.5 and 1 Gy. As a comparison (in a concurrent study), the time to bud break appeared to gradually reduce with the increase in doses for plants irradiated with acute gamma rays and that the difference between ion beams and gamma rays on inducing bud breaking was significant (data not shown). The times taken to bud break indicate plant response time for most of the Reagan cultivars was approximately 7.5 weeks. Although both ion beams and gamma rays appeared to interrupt the response time, the change was considered as minor and still within the average response time of Reagan cultivars.

Ray floret explants were also appeared to be sensitive to ion-beam irradiation. The number of flower buds in mutant plants was reduced up to 71.8% at dose 0.5 Gy with ion beams. Ion-beam irradiation also affected the flower diameter in these screened population, in which the flower size was reduced up to 24% at dose 0.5 Gy. Increasing the dose of ion beams from 0.5 Gy to 2 Gy did not affect flower diameter.

Table 5. Flowering characteristics of plants derived from *in vitro* irradiated ray floret explants.

Irradiation	**Dose (Gy)**	**Time to bud break (days)**	**No. of flower buds**	**Flower diameter (cm)**
Control	0	54.3^{abc}	39.3^{a}	5.4^{a}
Ion beam	0.5	55.7^{a}	11.1^{c}	4.1^{b}
	1	55.6^{a}	15.9^{c}	4.4^{b}
	2	55.4^{ab}	12.6^{c}	4.2^{b}

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test ($p < 0.05$).

Other flowering characters observed in this screening were flower colour and flower shape. The frequency of flower colour mutants is shown in Figure 12. The mutant frequency was indeed 100% at dose 2 Gy and this indicates that ion beams had a significant effect on the frequency of flower colour mutants.

Flower colour mutation spectrum for irradiated ray floret-generated plants is shown in Figure 13. Based on RHS colour chart, 8 different colour types were observed on mutant plants (43A, 46A, 47A, 50A, 51A, 53A, 53B, and 53C). The colour code for the control flower was 45A. Five different colours were seen on flowers irradiated at 0.5 and 1 Gy, with the three most dominant colours in descending order were the original colour of the control (45A), 53A, and 46A. Interestingly, plants irradiated at 2 Gy exhibited maximum colour variations (6 types) which were totally different colour shades from the control (43A, 46A, 47A, 51A, 53A, and 53B). Based on this result, it is predicted that more than 6 types of colour could be produced if plants were irradiated at higher doses.

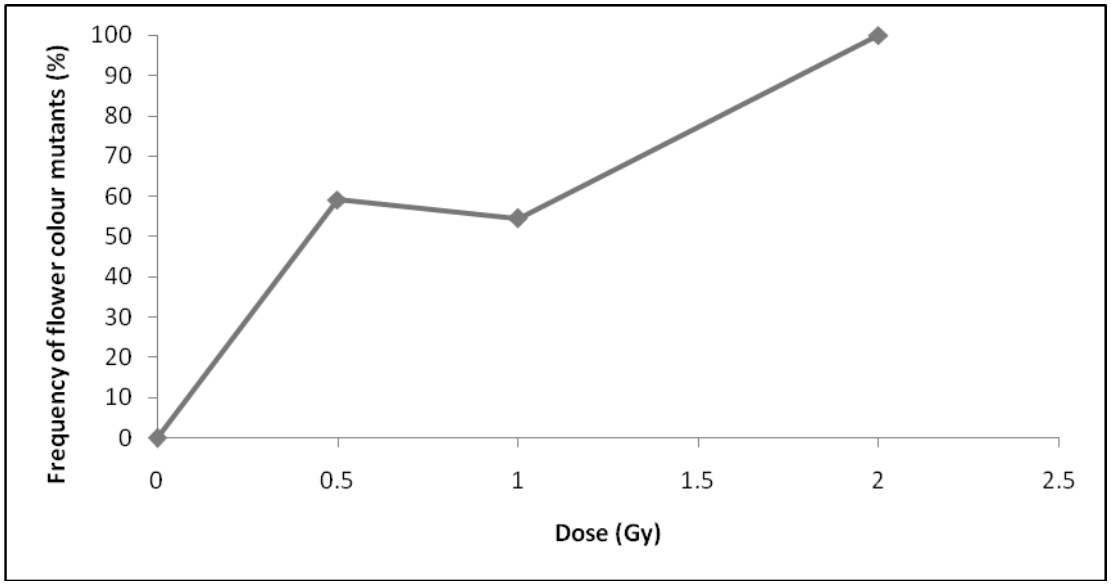
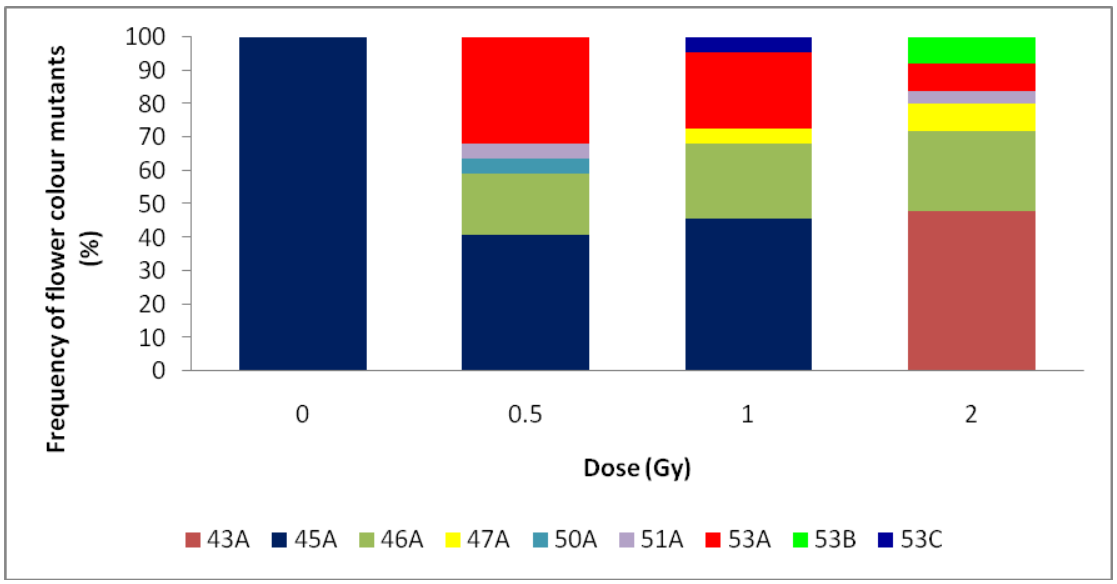


Figure 12. The frequency of flower colour mutants (%) in plants derived from *in vitro* irradiated ray florets by using ion-beams.

a



b

Figure 13. Flower colour spectrum of plant derived from *in vitro* irradiated ray florets based on RHS colour chart (a). Actual colours according to RHS colour chart (b).

In terms of flower morphology, the characters observed in this screening were the changes in their ray florets and flower head. Based on The Guidelines for the Conduct of Tests for Distinctness, Uniformity and Stability[47], the types for flower heads are divided into several groups; 'without ray florets', 'single', 'semi-double', 'daisy-eyed double', and 'double' (Table 6), whilst the shape of ray florets are divided into 5 types: 'ligulate', 'incurved', 'spatulate', 'quilled', and 'funnel' (Figure 14).

Table 6. Types of chrysanthemum flower head.

Types	**Description**	**Illustration**
1. Without ray florets	Flower heads consist of disc florets only.	
2. Single	Flower heads with one row of ray florets, and a clearly defined central disc which is always visible.	
3. Semi-double	Flower heads with more than one row of ray florets, and a clearly defined central disc which is always visible.	
4. Daisy-eyed double	Double flower heads where a disc is not visible in the early stages of flowering, but can be seen as the flower head opens fully. The disc is not always clearly defined.	
5. Double	Double flower heads where a disc is not visible at any stage of flowering.	

(Source: reference 47)

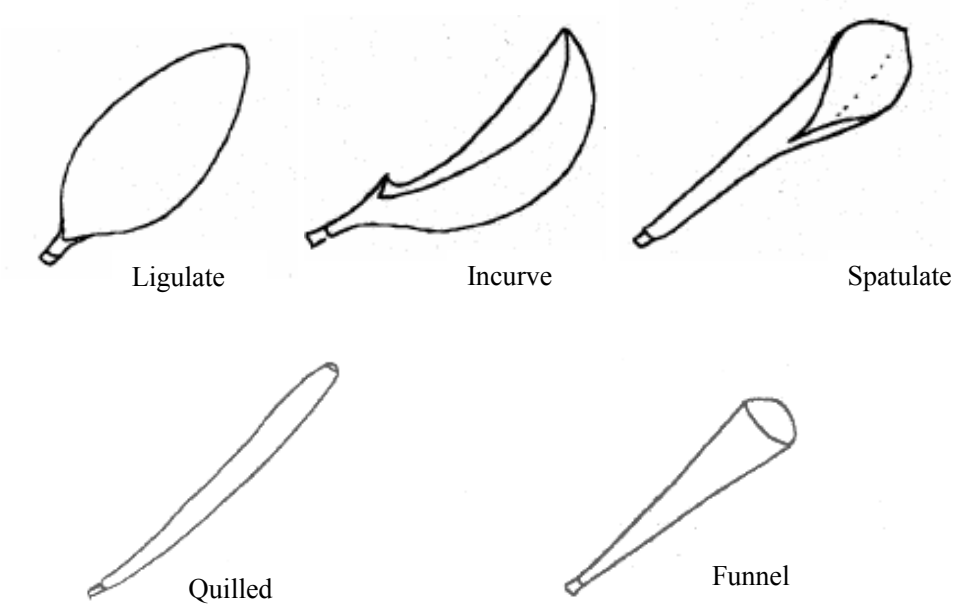


Figure 14. Various shapes of chrysanthemum ray florets (Source: reference 47)

Table 7 shows mutation frequencies of the ray floret-derived plants. In this study, it was found that all the screened plants at all doses (0.5, 1, and 2 Gy) have exhibited 100% morphological mutation on their ray florets and flower head characters. The ray floret was mutated from 'ligulate' to 'spatulate', whilst the flower head was changed from 'semi-double' to 'daisy-eyed double'.

Table 7. Flower shape mutants derived from *in vitro* irradiated ray floret explants.

Irradiation	**Dose (Gy)**	**Frequency of ray floret mutants (%)**			**Frequency of flower head mutant (%)**	
		Ligulate	**Incurved**	**Spatulate**	**Semi-double**	**Daisy-eyed double**
Control	0	100	0	0	100	0
Ion beams	0.5	0	0	100	0	100
	1	0	0	100	0	100
	2	0	0	100	0	100

Nodal-generated plants

Generally, all doses used did not significantly affect bud breaking period for plants derived from *in vitro* nodals except for those irradiated at 8 Gy which required a shorter period (53.5 days) to break bud, as compared to the control plants (55.3 days) (Table 8). However, ion-beam irradiations significantly increased the number of flower buds produced, with the highest figure (32.7 buds) was recorded at 3 Gy. There was also no significant difference observed in flower diameter between plants at different treatments.

Table 8. Flowering characteristic of plants derived from *in vitro* irradiated nodal explants.

Irradiation	**Dose (Gy)**	**Time to bud break (days)**	**No. of flower buds**	**Flower diameter (cm)**
Control	0	55.3^{a}	21.5^{b}	6.4^{a}
Ion beam	0.5	54.8^{ab}	30.8^{a}	6.5^{a}
	1	54.8^{ab}	31.0^{a}	6.3^{a}
	2	55.1^{ab}	30.1^{a}	6.4^{a}
	3	55.0^{ab}	32.7^{a}	6.3^{a}
	5	55.4^{a}	29.3^{a}	5.9^{ab}
	8	53.5^{b}	30.9^{a}	6.0^{a}

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test ($p < 0.05$).

In terms of flower colour, ion beams were also able to change the flower colour of the nodal-generated mutants, but at a slightly lower frequency that their ray floret counterparts. The frequencies of flower colour mutant were fluctuated in a range of 42.3 to 58.8% (Figure 15).

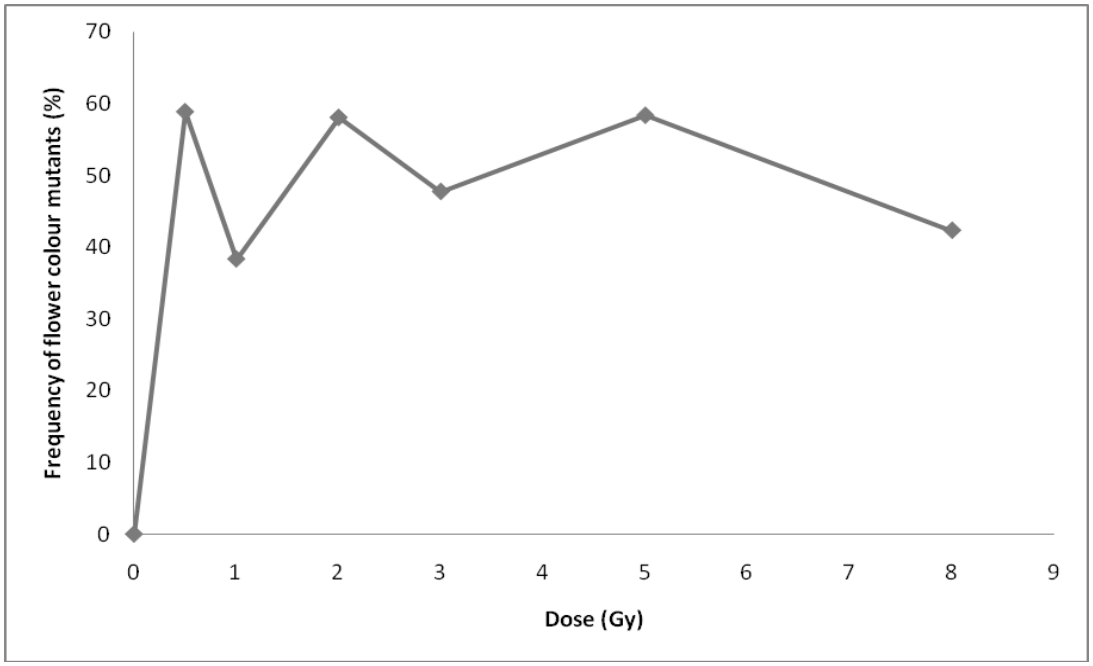
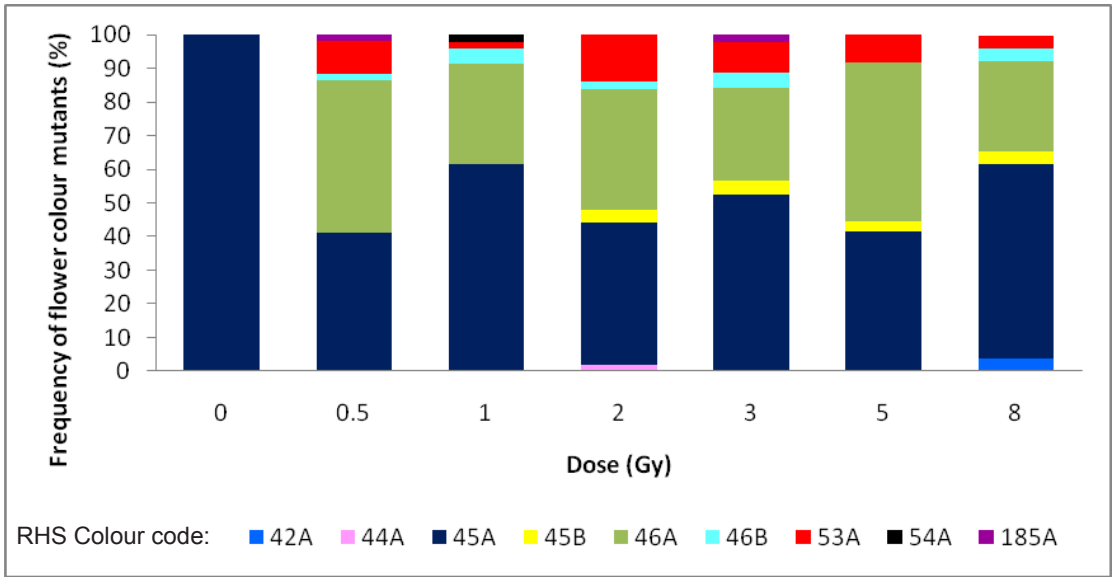


Figure 15. Flower colour mutant frequency for ion-beam irradiated nodal explants of chrysanthemum cv. 'Reagan Red'.

As for flower colour spectrum (Figure 16), 8 different types of colour were observed (42A, 44A, 45B, 46A, 46B, 53A, 54A, and 185A) in the irradiated population. Based on RHS colour chart, 7 out of 8 types of colour (42A, 44A, 45B, 46A, 46B, 53A, and 54A) were in red group and 1 of them (185A) was in greyed-purple group. All doses of ion-beam irradiation were able to induce different shades of flower colour and the variations varied between 4 to 5 types except for 5 Gy which only produced 3 types of colour.

a



b

Figure 16. Flower colour mutant spectrum for ion beam irradiated nodal explants of chrysanthemum cv. 'Reagan Red'. Colour codes are given based on RHS colour chart (a), and actual colours according to RHS colour chart (b).

In summary, flower colour mutations occurred at all doses but the distinct flower colour such as white, yellow, or violet did not occur. Majority of the plants produced flowers with intense or lighter colour in the red group, and a few with orange and greyed-purple colour. Yamaguchi *et al.*[22] also reported similar results when they irradiated a single node of *in vitro* cultured roses with ion beam. According to Nagatomi *et al.*[29), 32)], the frequencies of flower colour and the multicolor mutants in chrysanthemum could be increased by several folds if cultured petals were used as starting materials than other explants such as leaves.

Meanwhile ray floret mutants were only observed at doses 1, 5, and 8 Gy with the percentage of 21.3%, 16.7% and 42.3% respectively. At doses 1 Gy and 5 Gy, all the ray florets were mutated from 'ligulate' to 'spatulate' and from 'ligulate' to 'incurved' at dose 8 Gy. As for flower head characters, mutants were occurred only at higher doses (5 and 8 Gy) with the percentage of 16.7% and 15.4%, respectively, in which the flower head mutated from 'semi-double' to 'daisy-eyed double'. Table 9 shows details on flower shape mutants observed on irradiated nodal-derived plants.

Table 9. Flower shape mutants derived from *in vitro* irradiated nodal explants of chrysanthemum cv. 'Reagan Red'.

Irradiation	Dose (Gy)	Frequency of ray floret mutants (%)			Frequency of flower head mutants (%)	
		Ligulate	Incurved	Spatulate	Semi-double	Daisy-eyed double
Control	0	100	0	0	100	0
Ion beam	0.5	100	0	0	100	0
	1	78.7	0	21.3	100	0
	2	100	0	0	100	0
	3	100	0	0	100	0
	5	83.3	0	16.7	83.3	16.7
	8	57.7	42.3	0	84.6	15.4

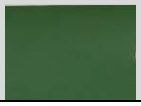
2.3.5 Leaf characteristics of irradiated plants

Plants derived from ray florets

Leaf characteristic such as shape, size, and colour are also the important features in ornamental plants. The effect of mutation induction on leaf characteristics has been reported earlier and in some cases has generated some improved genotypes of vegetatively propagated ornamental plants such as *Bougainvillea*[48), 49)], *Lantana*[50)] and *Kalanchoe*[51)] with attractive leaf characteristics. In this study, the leaf characteristics of chrysanthemum plants that were generated from ray floret explants treated with ion beams were evaluated and discussed.

For ray floret-generated mutants, 63.6% leaf colour variations occurred at 0.5 Gy and increased to 100% at doses 1 and 2 Gy. All the mutated plants had leaves with colour variation in group 137A which is lighter than control (Table 10). The lighter green colours of leaves could be associated to chlorophyll mutations. Several researchers also agreed that ion beams can cause chlorophyll mutation as observed in other crops such as *Arabidopsis thaliana*[52] and soybean[53].

Table 10. Leaf colour mutant frequencies and mutation spectrum for ion beam irradiated ray floret explants. Percent of frequencies is indicated in parentheses.

Dose	No. of tested plants	Leaf colour*			Total No. of plants with leaf colour mutation
		137A 	135A 	141A 	
0	8	0 (0)	8 (100)	0 (0)	0 (0)
0.5	22	14 (63.6)	8 (36.4)	0 (0)	14 (63.6)
1	22	22 (100)	0 (0)	0 (0)	22 (100)
2	25	25 (100)	0 (0)	0 (0)	25 (100)

*Colour codes based on RHS colour chart.

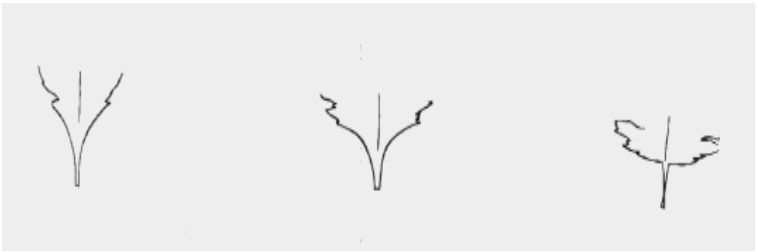
Beside leaf colour, the occurrence of mutation was also monitored on leaf blade length. Table 11 shows that the variations of leaf blade and total leaf length in ray floret-derived population. Even though ion beams could induce changes in leaf blade length in irradiated populations, there was no obvious correlation between doses and leaf blade length mutation. About 68.2% plants irradiated at 0.5 Gy have different leaf blade length that the controls, increased to 100% at 1 Gy, then reduced to 68.0% at 2 Gy. However, all mutations were in the form of leaves with the short (<5 cm) leaf blade. Leaf blade length is also directly correlated with the size of the leaves in which short leaf blade indicates the small leaves and vice versa. The variations in the leaf blade length and hence leaf size from the parent plants could be associated with the changes in genetics of the plants. Yamaguchi *et al*.[18] found that decreasing in nuclear DNA in chrysanthemum leaves had a significant positive correlation with size of the leaves. According to Tsukaya[54], final mature leave size depend on the final number and size of the cell within a leaf.

Table 11. Leaf blade length for irradiated chrysanthemum plant derived from ray florets explants. Percent of frequencies is indicated in parentheses.

Dose	No. of tested plants	Leaf blade length (cm)			Total No. of plants with leaf length mutation
		< 5	5 – 8	> 8	
0	8	0 (0)	8 (100)	0 (0)	0 (0)
0.5	22	15 (68.2)	7 (31.8)	0 (0)	15 (68.2)
1	22	22 (100)	0 (0)	0 (0)	22 (100)
2	25	17 (68.0)	8 (32.0)	0 (0)	17 (68.0)

Meanwhile, Table 12 shows detailed characteristic of leaves. Leaf shapes are described based on three common features which are the length of the terminal lobe relative to leaf length, the depth of lowest lateral sinus, and predominant shape of leaf base. Any changes in either one criterion will consider as a shape mutation. In this study, the highest leaf mutant frequency for ray floret-generated plants was 100% at dose 2 Gy (Figure 17). Therefore, it is suggested that ion-beam irradiation was very efficient in inducing leaf shape variation in chrysanthemum by using ray florets as the irradiated explants. Some of the leaf mutations that were observed on plants generated from *in vitro* irradiated ray florets using ion-beam irradiation are shown on Figure 18.

Table 12. Chrysanthemum leaf characteristics.

Types	Description
i) Length of terminal lobe relative to leaf length	 (a) Short (b) Medium (c) Long
ii) Depth of lowest lateral sinus	 (a) Shallow (b) Medium (c) Deep
iii) Predominant shape of base	 (a) Acute (b) Obtuse (c) Rounded  (d) Truncate (e) Cordate (f) Asymmetric

(Source: reference 47)

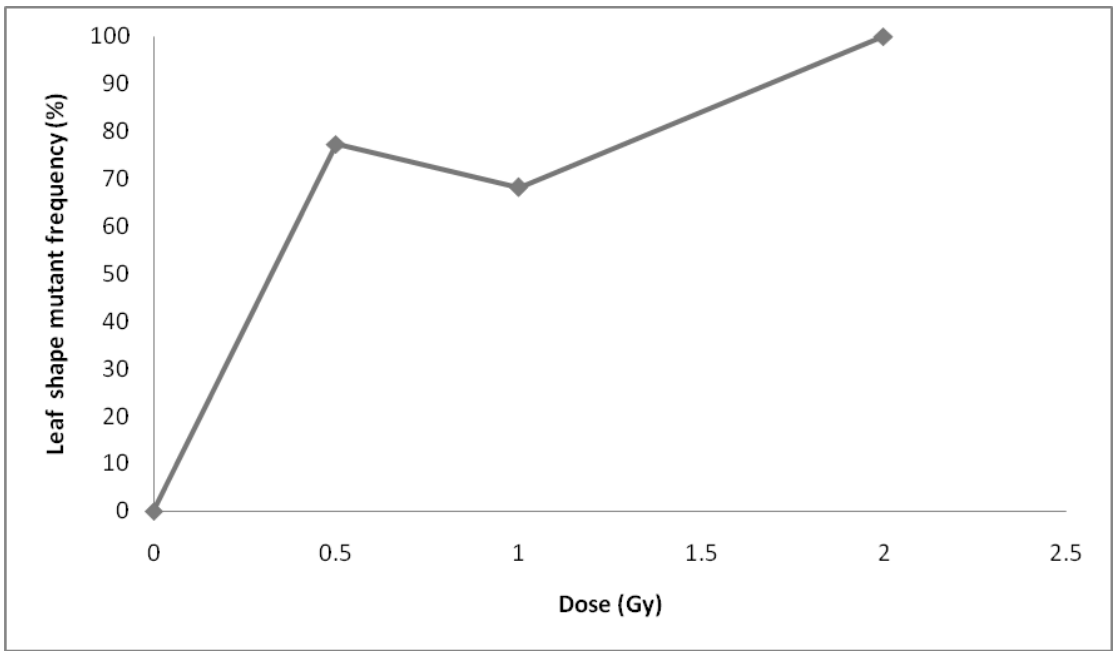


Figure 17. Leaf shape mutant frequency (%) for irradiated ray floret-derived plants.

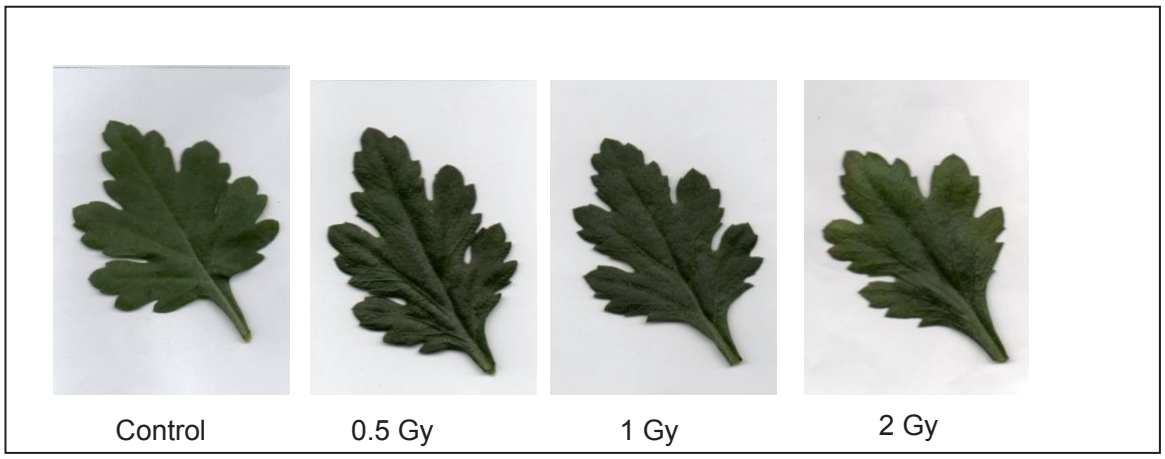


Figure 18. Leaves observed in the mutant plants generated from *in vitro* irradiated ray florets.

Plants derived from nodal explants

Frequencies of leaf colour variations for plants generated from ion-beam irradiated nodals were lower when compared to plants that derived from *in vitro* irradiated ray floret explants (Table 13). In this study, leaf colour variations for nodal-generated mutants were varied between 16.7 to 42.3% and not dependent on irradiated doses. The highest leaf colour mutation was recorded on plants irradiated at 8 Gy. Ion beam irradiated plants also produced leaves with chlorophyll mutations which were the lighter shades of green (137A and 141A) as compared to control plants. Therefore, it was found from this research that ion beam could be used for induction of leaf colour variations but the chances to get more colour variations were higher if irradiated ray florets explants were used as compared to nodal explants for chrysanthemum cv. 'Reagan Red'.

Table 13. Leaf colour mutant frequencies and mutation spectrum for plants generated from nodal explants. Percent of frequencies is indicated in parentheses.

Dose	**No. of tested plants**	**Leaf colors***			**Total No. of plants with leaf colour mutation**
		137A	**136A**	**141A**	
0	11	0 (0)	11 (100)	0 (0)	0 (0)
0.5	51	0 (0)	31 (60.8)	20 (39.2)	20 (39.2)
1	47	0 (0)	28 (59.6)	19 (40.4)	19 (40.4)
2	50	0 (0)	50 (100)	0 (0)	0 (0)
3	44	0 (0)	44 (100)	0 (0)	0 (0)
5	36	6 (16.7)	30 (83.3)	0 (0)	6 (16.7)
8	26	11 (42.3)	15 (57.7)	0 (0)	11 (42.3)

*Colour codes based on RHS colour chart.

Meanwhile, variations in leaf blade length were seen in all irradiated plants. Most of these variations involved shorter leaf blade (< 5 cm) or smaller leaves except for those irradiated at doses 0.5 and 2 Gy, which could also generated leaves with the blade length more than eight centimeter (> 8 cm). Frequencies of mutant of leaf blade length were varied between 33.3 to 100%. All plants irradiated at 0.5 Gy in particular showed abnormal leaf length, in which 60.8% produced short leaf blade and 39.2% with longer leaf blade. The detailed result is presented in Table 14.

Table 14. Leaf blade length for irradiated chrysanthemum plants derived from nodal explants. Percent of frequencies is indicated in parentheses.

Dose	No. of tested plants	Leaf blade length (cm)			Total No. of plants with abnormal leaves in length
		< 5	5 - 8	> 8	
0	11	0 (0)	11 (100)	0 (0)	0 (0)
0.5	51	31 (60.8)	0 (0)	20 (39.2)	51 (100)
1	47	28 (59.6)	19 (40.4)	0 (0)	28 (59.6)
2	50	31 (62.0)	11 (22.0)	8 (16.0)	39 (78.0)
3	44	21 (47.7)	23 (52.3)	0 (0)	21 (47.7)
5	36	12 (33.3)	24 (66.7)	0 (0)	12 (33.3)
8	26	11 (42.3)	15 (57.7)	0 (0)	11 (42.3)

The occurrence of leaf shape mutation in irradiated nodal-generated plants was also relatively high. Between 56.0 to 80.6% irradiated plants showed this mutation with the highest occurrence was observed in plants irradiated at 5 Gy (Figure 19). Mutant frequency did not directly correlate with irradiation doses as fluctuation in frequencies were seen throughout the doses. The differences in radiation response indicate that several physical and biological factors were involved in mutational process. Thus, it became extremely difficult to predict the occurrences of mutation in different varieties of crop plants[55]. Nevertheless, it can be concluded that ion beams can be used for inducing leaf variation in chrysanthemum cv. 'Reagan Red'. Some of the leaves variations that were obtained from *in vitro* irradiated nodal explants using ion-beam irradiation are shown in Figure 20.

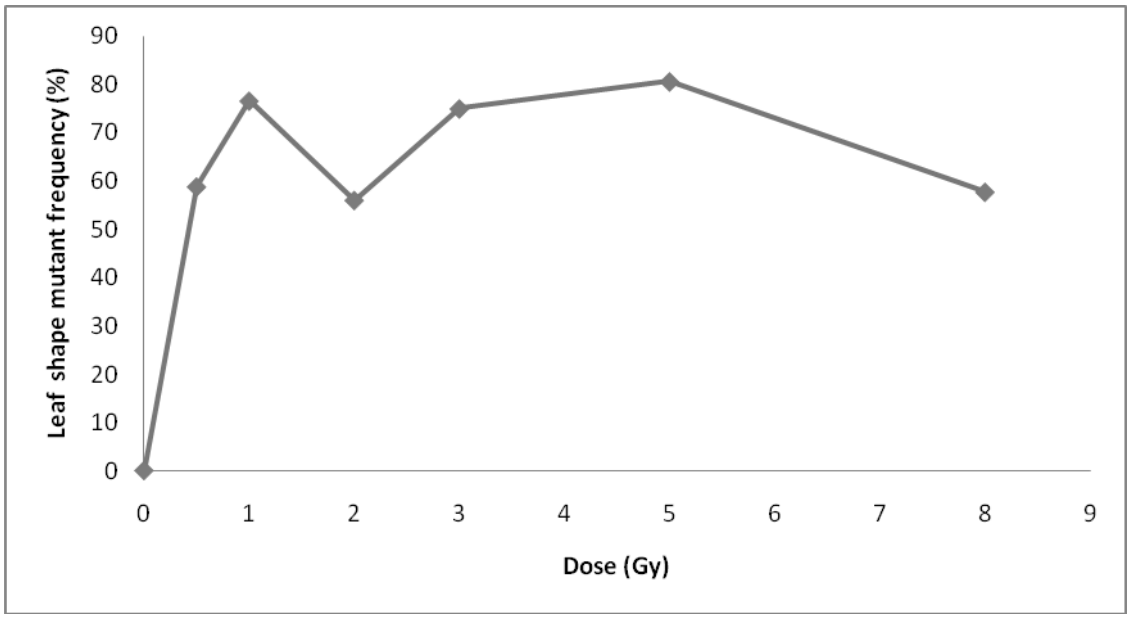


Figure 19. Leaf shape mutant frequency for ion beams irradiated nodal explants.

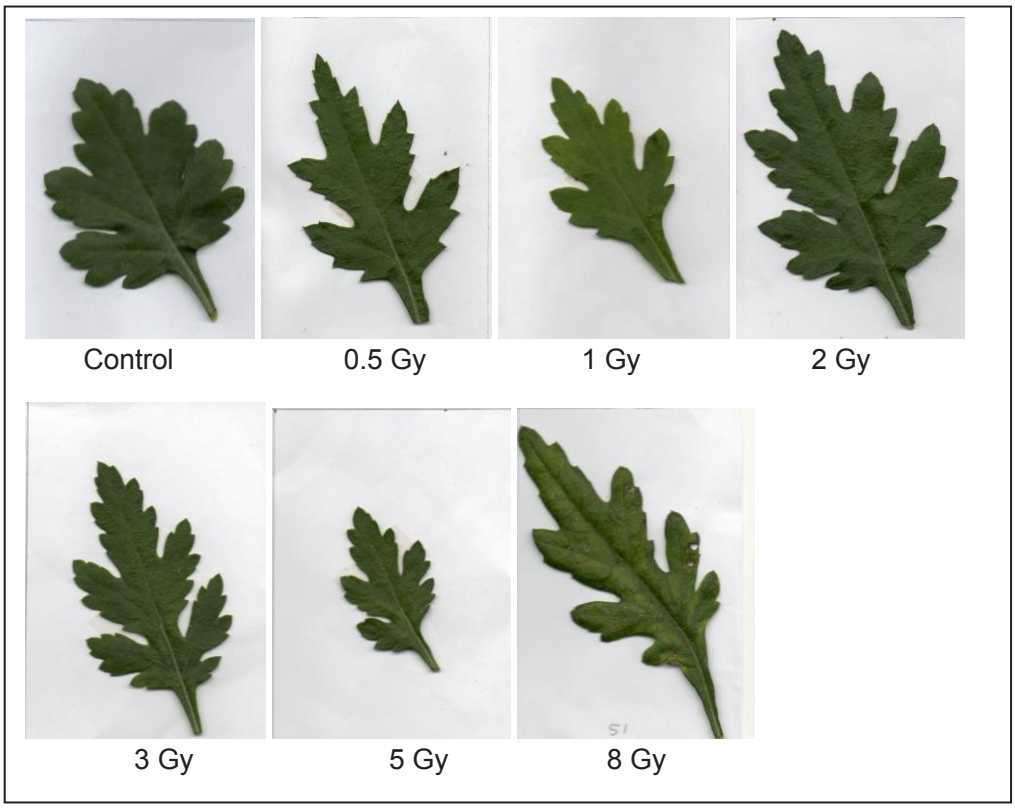


Figure 20. Leaves variations of plants derived from *in vitro* irradiated nodal explants.

2.3.6 Identification of Reagan Red mutants

A total of 13 Reagan Red stable mutants (Table 15) were successfully generated from this research. All these mutants were derived from ray floret explants. Finally, 4 mutants were selected based on their uniqueness and/or suitability for cut flower production, that were TIARA Red (irradiated at 2 Gy), Golden Eye (0.5 Gy), Yellow Sun (0.5 Gy), and an unnamed variety (IB0.5 28). Detailed characters of each mutant are listed in Table 16 and Table 17. Of these, TIARA Red, Golden Eye, and Yellow Sun have been filed for PVP with Department of Agriculture, Malaysia. The filing numbers are "PVBT 009/14", "PVBT 011/14", and "PVBT 013/14" for TIARA Red, Golden Eye, and Yellow Sun, respectively. Figure 21 shows various stages of activities involved torwards generating new mutant varieties.

Table 15. List of Reagan Red mutants and their characteristics.

No	Mutant name/code	Dose (Gy)	Description
1	TIARA Red	2	Purplish red, spatulate ray floret , tall plant
2	Golden Eye (IB0.5 14)	0.5	Small yellow/orangish red ray floret
3	Yellow Sun (IB0.5 2.2)	0.5	Small and sparse orangish red ray floret, button-like flower
4	IB0.5 28	0.5	Dark red, compact flower
5	IB0.5 40	0.5	Red, compact, short ray florect
6	IB1.0 123	1	Purplish red, incurved ray florets
7	IB0.5 38	0.5	Red, wider ray floret
8	IB0.5 7.2	0.5	Red, bigger flower
9	IB0.5 26	0.5	Small flower, red with orange stripe ray floret
10	IB0.5 803	0.5	Big disc floret, small red/orange ray florets
11	IB0.5 6.3	0.5	Red, bigger flower
12	IB0.5 508	0.5	Red, bigger flower
13	IB0.5 8.4	0.5	Red, small and incurved flower

Figure 21. Various stages of activities involved torwards generating new mutant varieties. (a): Chrysanthemum tissue culture plantletswere hardened in a transparent plastic container. (b): Shadehouse at MARDI Cameron Highland for mutant screening. (c): Pots containing planting medium. (d): Chrysanthemum plants after 1 week of transplanting. (e): Chrysanthemum plants during vagetative growth (1 months after transplanting). (f) Full bloom chrysanthemum mutants (3 months).

2.3.7 Detailed description of Reagan Red mutants

TIARA Red

TIARA Red was the first mutant generated from this project through irradiation of ray floret culture at 2 Gy. The plant is tall (approximately double the height of the control) but with a smaller flower (approximately half the size of the control). The unique features of TIARA Red are the shape of its flower head (semi double, "cone-like" shape) and ray floret (spatulate) as compared to semi double type and ligulate, respectively, for the control. The flower does not fully open even at full bloom stage. Besides, the flower colour is purplish red as compared to red in the control. Figure 22 shows some characters of the mutant.

Golden Eye (IB0.5 14)

Golden Eye (Figure 23), also coded as IB0.5 14, is generated through irradiation of ray floret culture at 0.5 Gy. The flower has smaller ray florets which are of incurved type. It also has red and yellow stripes on its petal (47A (main), 48B, 13B, according to RHS colour chart), as compared to solid red in control.

Yellow Sun (IB0.5 2.2)

Yellow Sun (coded as IB0.5 2.2) is also generated through irradiation of ray floret culture at 0.5 Gy. The flower head of this mutant is very small, approximately half the size of its control. In the early stage of flowering, only its bright yellow disc florets were clearly visible whilst its ray florets were almost non-existence. These ray florets were only visible during full bloom. This mutant is very suitable for stage decoration in combination with single or semi double flower to create visual impact (Figure 24).

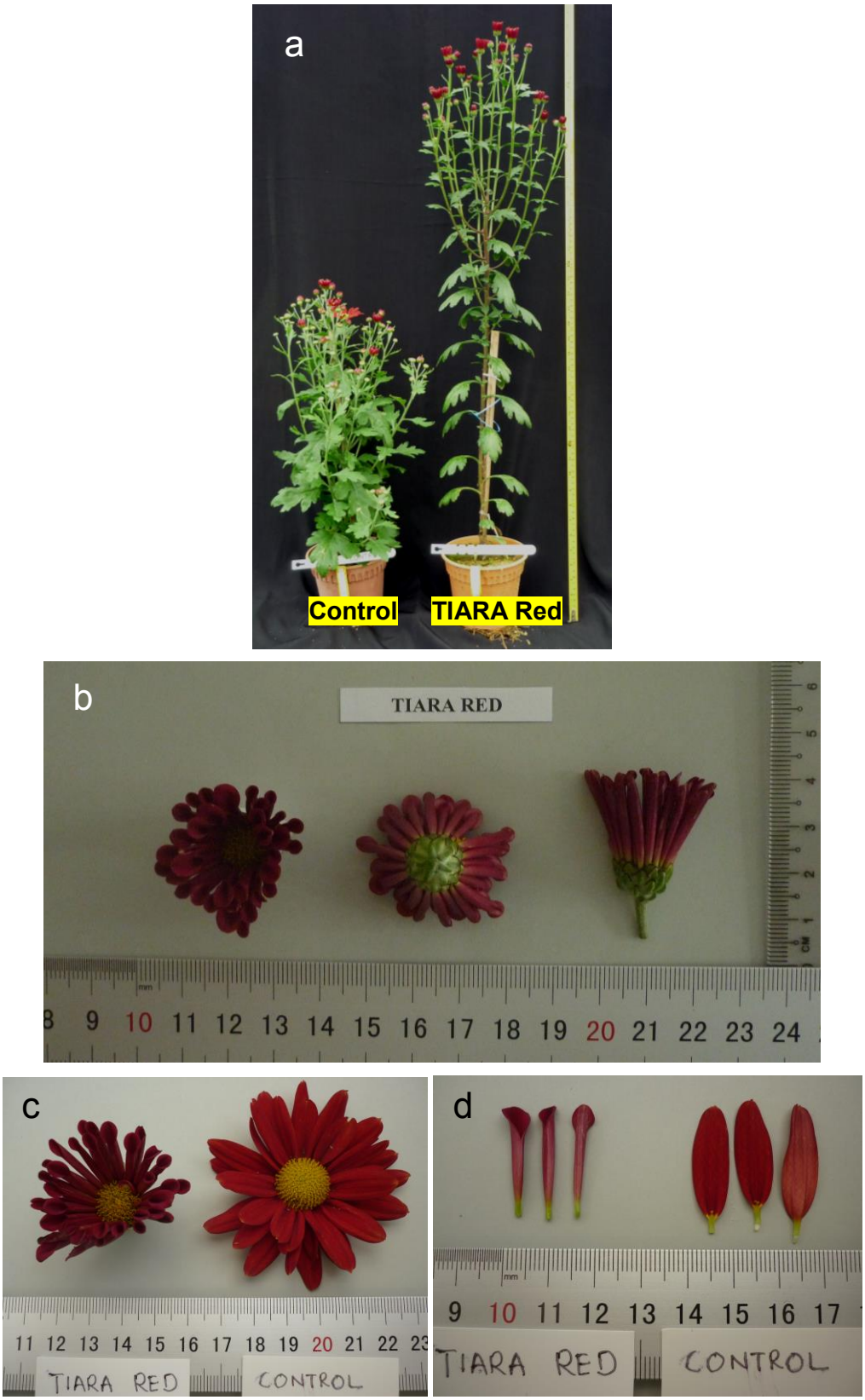


Figure 22. TIARA Red mutant. (a): TIARA Red plants against its control. (b): Top, bottom, and side views of TIARA Red flower heads (c): Comparison of TIARA Red flower head and its control (d): Spatulate type ray floret for TIARA Red as compared to ligulate type for control.

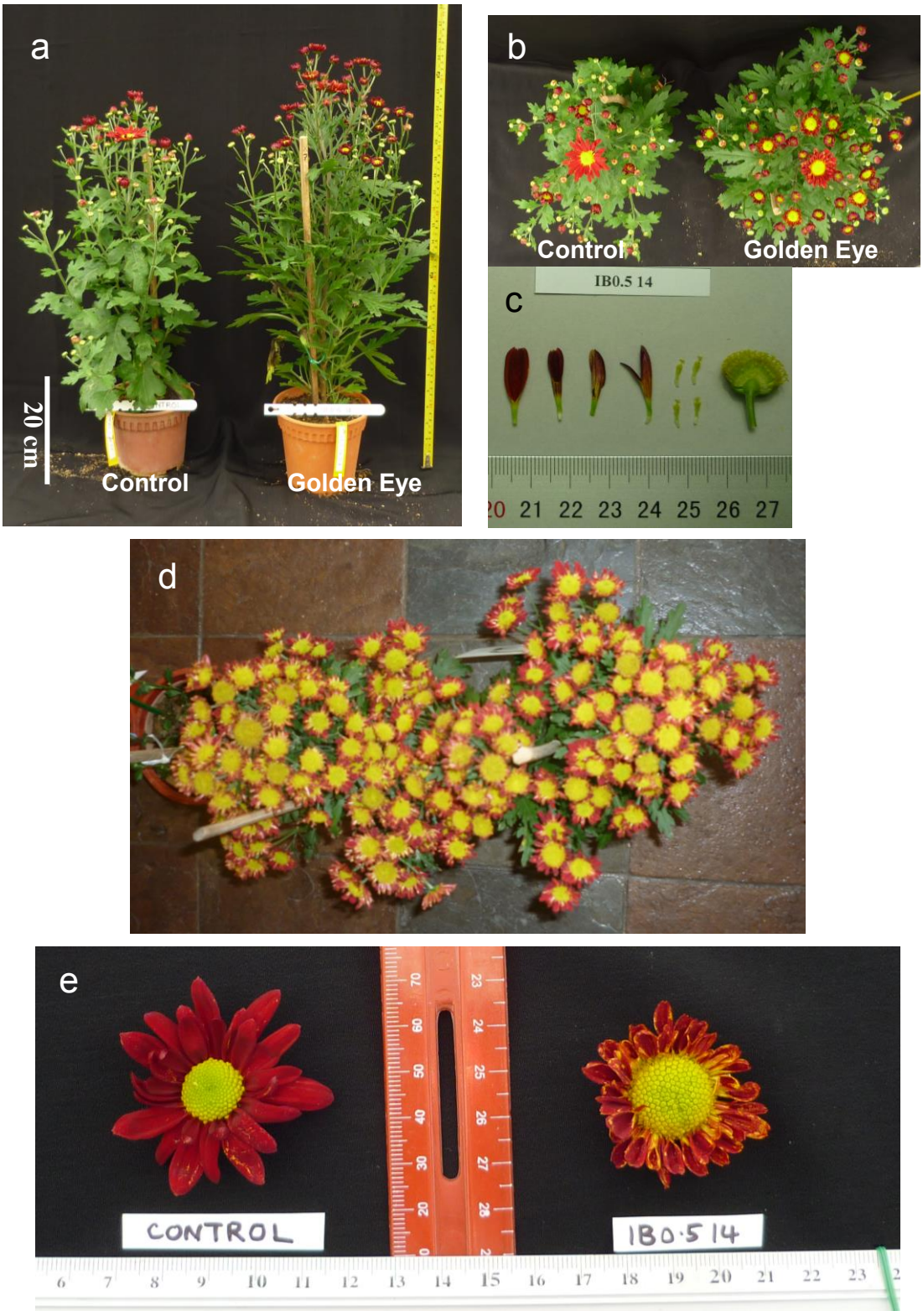


Figure 23. Golden Eye mutant. (a): Golden Eye mutant plant (right) and its control (left). (b): Top view of the mutant plant and its control during early flowering. (c): Ray florets, disc florets and a base of flower head of Golden Eye. (d): Close-up aerial view of the mutant. (e): Full bloom flower heads of control and the mutant. A unit of the ruler at the bottom of the panel e is in cm.

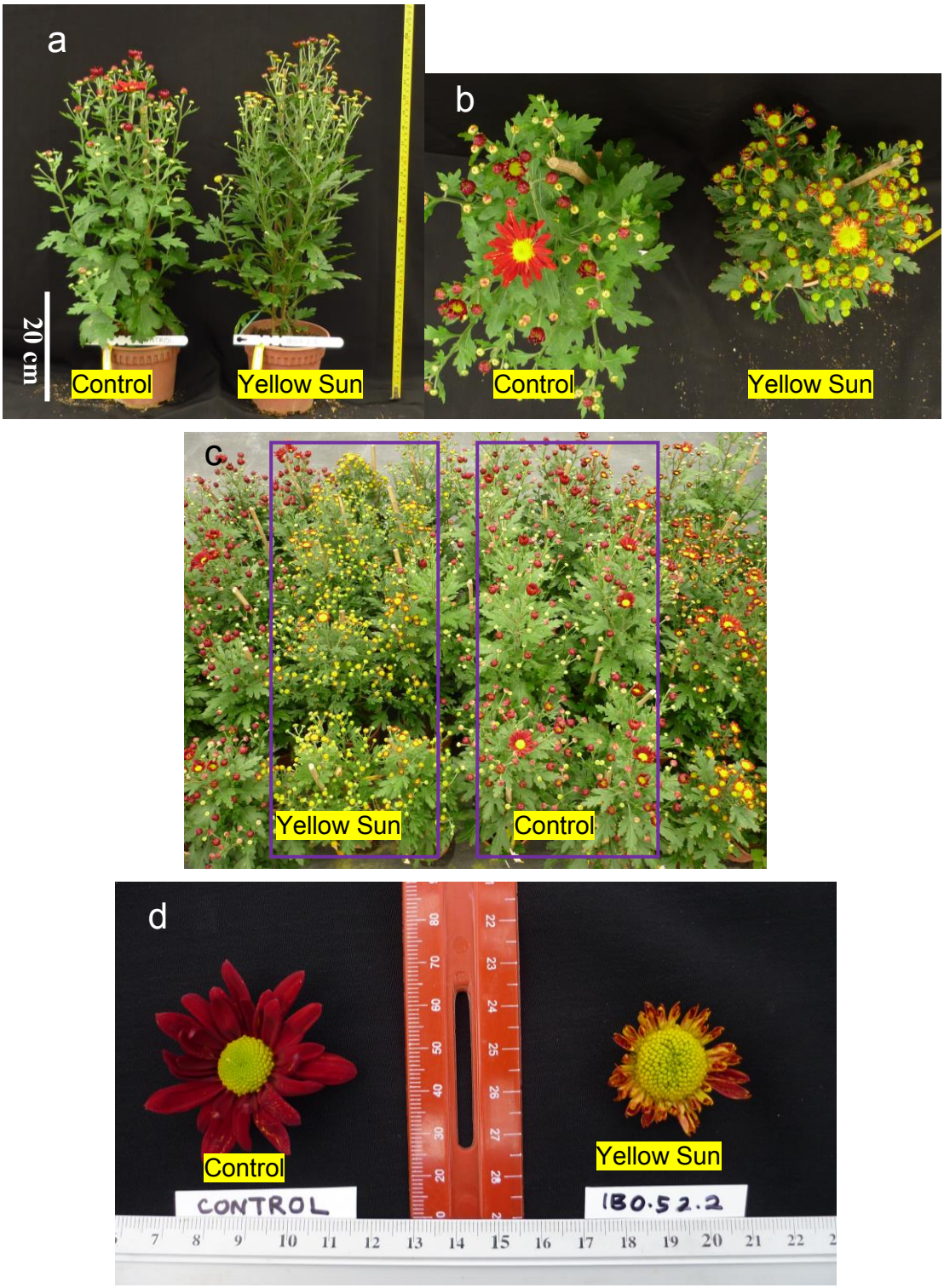


Figure 24. Yellow Sun mutant. (a): Yellow Sun mutant plant and its control. (b): Top view of Yellow Sun and the control. (c): Yellow Sun and the control during earlier flowering stage. (d): Full bloom Yellow Sun flower head against its control. A unit of the ruler at the bottom of the panel c is in cm.

IB0.5 28 Mutant

The IB0.5 28 mutant (Figure 25) was generated from irradiation of ray florets at 0.5 Gy. The mutant mainly maintained all characters of the control, except it has an additional layer of ray florets (3 rows) as compared to 2 rows in the control. As such, it also has more ray floret number per flower as compared to its control.

Figure 25. The flower heads of IB0.5 28 mutant as compared to the control. A unit of the ruler at the bottom is in cm.

Table 16. Detailed plant characteristics of Reagan Red control and mutants.

No.	Character	Control	Tiara Red (2 Gy)	Golden Eye (0.5 Gy)	Yellow Sun (0.5 Gy)	1B0.5 28 (0.5 Gy)
1	Average height (cm)	55.5	94.1	49.7	45	66
2	Plant type	Bushy	Non-bushy	Bushy	Bushy	Bushy
3	Plant growth habit	Upright	Semi upright	Upright	Upright	Upright
4	Density of branching	Compact	Sparse	Compact	Compact	Compact
5	Stem colour	Green	Green tinged with purple	Green	Green	Green
6	Stipule size	Medium	Absent or very small	Medium	Medium	Medium
7	Petiole attitude	Moderately upwards	horizontal	Moderately upwards	Moderately upwards	Moderately upwards
8	Average petiole relative length (cm)	3.5	2.22	4.1	3.8	4.2
9	Average leaf length + petiole (cm)	13.3	9.56	14.4	13.1	15.0
10	Average leaf width (cm)	6.5	4.4	6.3	6.0	6.7
11	Leaf ratio length/ width	Medium	Medium		Medium	Medium
12	Average length of terminal lobe (cm)	3.5	1.85	4.0	4.2	4.3
13	Average depth of lateral sinus (cm)	2.3	1.07	2.6	2.3	2.8
14	Lowest lateral sinus	Medium	Deep	Medium	Medium	Medium
15	Margin of lateral sinus	Diverging	Parallel	Parallel	Parallel	Diverging
16	Leaf base shape	Obtuse	Obtuse	Obtuse	Obtuse	Obtuse
17	Leaf upper color	Dark green	Dark green	Dark green	Dark green	Dark green
18	Number of leaf sinus	4	4	4	4	4

Table 17. Detailed flower characteristics of Reagan Red control and mutants.

No.	Flower Character	Control	Tiara Red (2 Gy)	Golden Eye (0.5 Gy)	Yellow Sun (0.5 Gy)	1B0.5 28 (0.5 Gy)
1	Inflorescence form	Type 5 (corymbiform)	Type 5 (corymbiform)	Type 5 (corymbiform)	Type 5 (corymbiform)	Type 5 (corymbiform)
2	Inflorescence angle between primary lateral shoot and stem	Not applicable for bushy type	Type 1 small	Not applicable for bushy type	Not applicable for bushy type	Not applicable for bushy type
3	Number of flower head	67	30.2	64	77	55
4	Average diameter: flower head (cm)	5.4	3.27	3.5	2.4	5.5
5	Average height: flower head (cm)	1.7	6.47	3.7	3.8	5.2
6	Type: flower head	Semi double	Semi double	Semi double	Semi double	Semi double
7	Bloom form	Semi double	Semi double /spoon	Semi double	Semi double	Semi double
8	Number of rows: ray floret	2	2.	2	1	3
9	Number of rows: Involucral bract	3	4	3	3	3
10	Longitudinal axis majority flower head	Type 1 upright	Type 1 upright	Type 1 upright	Type 1 upright	Type 1 upright
11	Longitudinal axis outer flower head	Type 1 upright	Type 1 upright	Type 1 upright	Type 1 upright	Type 1 upright
12	Attitude of lateral flower bud	Horizontal	Moderately ascending	Moderately ascending	Moderately ascending	Horizontal
13	Average length of corolla tube flower head (cm)	0.33	0.30	0.38	0.2	0.35
14	Cross section of ray floret	Weakly concave	Touching	Moderately concave	Moderately concave	Moderately concave
15	Average length of outer floret (cm)	2.5	2.50	1.8	1.4	2.8
16	Average width of outer floret (cm)	0.7	0.45	0.5	0.3	0.8
17	Ray floret type	Ligulate	Spatulate	Incurved	Incurved	Ligulate
18	Ray floret tips	Dentate	Rounded	Dentate	Fringed	Dentate
19	Average ray floret thickness (mm)	0.10	0.11	0.10	0.10	0.10

Table 17. Detailed flower characteristics of Reagan Red control and mutants (continued).

No.	Flower Character	Control	Tiara Red (2 Gy)	Golden Eye (0.5 Gy)	Yellow Sun (0.5 Gy)	1B0.5 28 (0.5 Gy)
20	Ray floret upper colour	45A	59A	47A (main), 48B, 13B	46A (main)	45A
22	Ray floret colour pattern	Solid	Solid	Diffuse stripes	Diffuse stripes	Solid
23	Average ray floret number	26	33.3	27	24	33
24	Ray floret texture surface	Keeled	Smooth	Keeled	Keeled	Keeled
25	Average disc diameter(cm)	1.47	1.22	1.43	1.4	1.53
26	Disc diameter relative to head diameter	Medium	Medium	Large	Large	Medium
27	Disc colour before dehiscence	7B	7B	7B	7B	7B
28	Disc colour after dehiscence	7A	7A	7A	7A	7A
29	Disc distribution type	Domed	Domed	Domed	Domed	Domed
30	Average disc floret overall length (cm)	0.5	0.60	0.61	0.55	0.60
31	Disc floret type	Petaloid	Quilled	Petaloid	Petaloid	Petaloid
32	Disc: profile in cross section		Slightly domed	Slightly domed	Slightly domed	Slightly domed
33	Disc: presence of dark spot at centre before anther dehiscence	Absent	Absent	Absent	Absent	Absent
34	Average receptacle diameter (cm)	0.5	0.51	0.47	0.37	0.52
35	Receptacle shape	Domed	Domed	Domed	Domed	Slightly domed
36	Response Group	7 weeks	7 weeks	7 weeks	7 weeks	7 weeks
37	Attitude of basal part (ray floret)	Horizontal	Ascending	Moderately ascending	Moderately ascending	Horizontal

2.3.8 Conclusion on Reagan Red studies

In this study, the 50% lethal dose (LD_{50}) of ray florets explants irradiated with ion beams occurred at 15.5 Gy and 20% of lethal dose (LD_{20}) was at 11.5 Gy. However, LD_{50} or LD_{20} were not used for determining optimum irradiation dose since not all of the survived explants were able to generate shoots. Thus, the most effective dose for ray florets explants was chosen based on optimal dose obtained at 50% of shoot regeneration (RD_{50}). RD_{50} for ray floret explants irradiated with ion beams was at 2 Gy. Therefore the recommended dose for mutation induction of *Chrysanthemum morifolium* cv. 'Reagan Red' using ray florets was less than 2 Gy.

For nodals, the LD_{50} and LD_{20} values were 6.5 Gy and 4 Gy, respectively. The optimal dose for shoot regeneration was estimated based on 80% and 50% of shoot regeneration (RD_{80} and RD_{50}), and the values recorded were 3.5 Gy and 4 Gy, respectively. Therefore, the optimal dose for *in vitro* mutation induction using nodals was less than 4 Gy.

From a series of morphological screenings, 13 Reagan Red mutants with various morphological characters have been identified. These mutants are being maintained at Nuclear Malaysia. Four of these mutants namely TIARA Red, Golden Eye, Yellow Sun and IB0.5 28 are being closely monitored as they have unique traits that may be of interest to end users. All of these mutants except IB0.5 28 have been filed for PVP with Department of Agriculture Malaysia.

2.4 Experiments on *Chrysanthemum* cv. 'Pink'

2.4.1 Irradiation of ray floret cultures

Selection of explants

The Pink variety was first irradiated on November 2010, and was repeated in May 2012. Ray floret cultures were used as the starting explants in both events of irradiations. The methods for tissue culture and ion-beam irradiation of Pink variety were similar to Reagan Red. Another chrysanthemum variety, Purple variety was irradiated along with the Pink variety. However, the Purple variety did not perform well, mainly due to very high contamination rates and its inability to generate high number of adventitious shoots after irradiation. However, a number of irradiated shoots from the Purple variety have been obtained and screened under our controlled environment glasshouse. At present, none of the screened plants have shown mutation in terms of flower colour and shapes for the Purple variety.

Statistical analysis and data collection

Data were analyzed using the SAS software program (version 9.2). Observation were made on the percentage of culture survival and shoot formation (first irradiation), callus formation and shoot regeneration (second irradiation).

Radiosensitivity of Pink ray floret cultures

In the first irradiation experiment, data on the number of surviving Pink cultures, were recorded at 4 and 8 weeks after the irradiation. It was observed that the percentage of survived cultures decreased at dose higher than 5 Gy, and dropped to 50% at 10 Gy as early as 4 weeks. At dose 20 Gy and higher, less than 10% of the petals could produce callus, and the growth was very slow and not vigorous. At week 8, cultures at doses above 8 Gy remained unchanged with almost no callus and shoot formed. The dose response curve is shown in Figure 26.

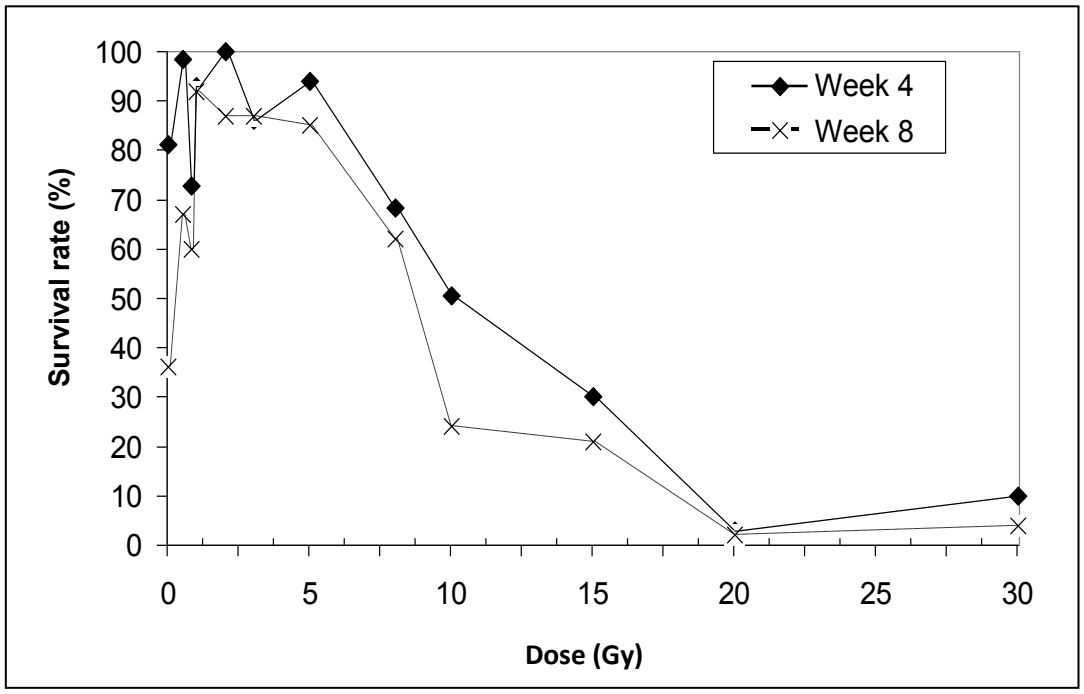


Figure 26. Survival rate (%) of cultures at week 4 and week 8 after irradiation.

Higher doses also affected shoot regeneration. The percentage of shoot formation was between 33 to 47.5% for doses below 2 Gy. The percentage dropped to lower than 5% at dose 5 Gy, whilst at doses higher than 8 Gy, no shoot formation was recorded (Figure 27).

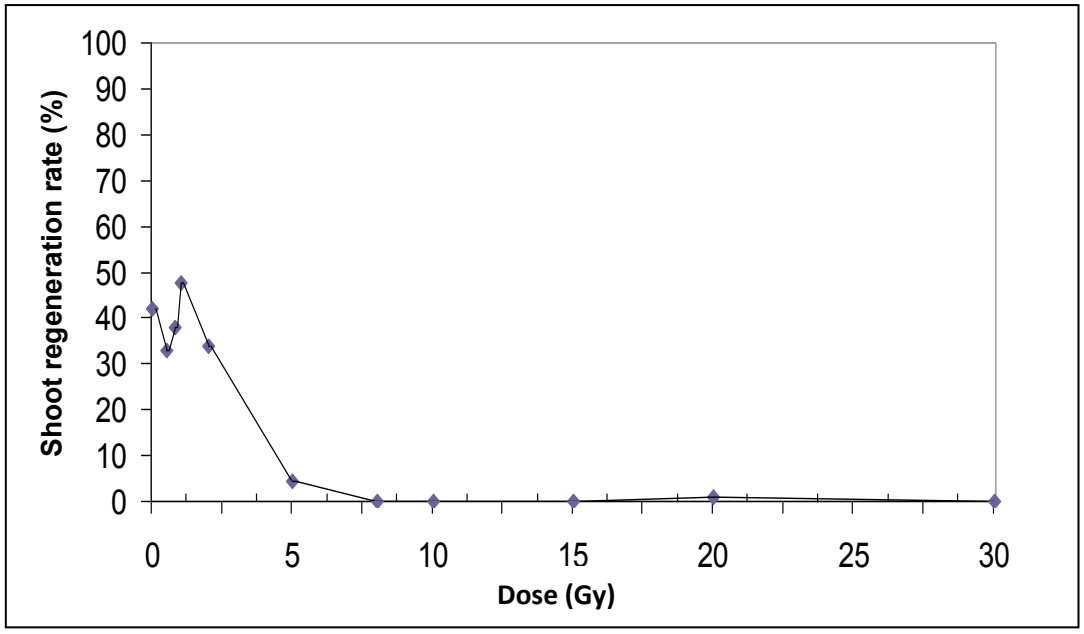


Figure 27. Shoot regeneration rate (%) from first batch of irradiation.

Irradiation experiment was repeated in 2012 to get more accurate dose response curve. In the second irradiation experiment on ray floret cultures, the efficiency of the cultures to form callus and shoot after irradiation treatment was monitored. The percentage of callus formation was recorded at week 6 post-irradiation, whilst shoot regeneration was taken at week 8 and 14. Table 18 shows the detailed data of callus formation after 6 weeks, whilst Figure 28 shows the plotted graph. Similar to the callus survival trend observed in the first irradiation experiment, there was also a sudden drop in the percentage callus formation after irradiation at doses higher than 5 Gy in the second irradiation.

Table 18. The percentage of callus formation 6 weeks after irradiation.

Dose (Gy)	Mean (%)	Dose (Gy)	Mean (%)
0	91.25^{ab}	8	52.5^{cd}
0.5	100^{a}	10	17.5^{ef}
0.8	100^{a}	15	8.75^{ef}
1	68.75^{bc}	20	3.75^{f}
3	93.75^{ab}	30	23.75^{def}
5	38.75^{de}		

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test ($p < 0.05$).

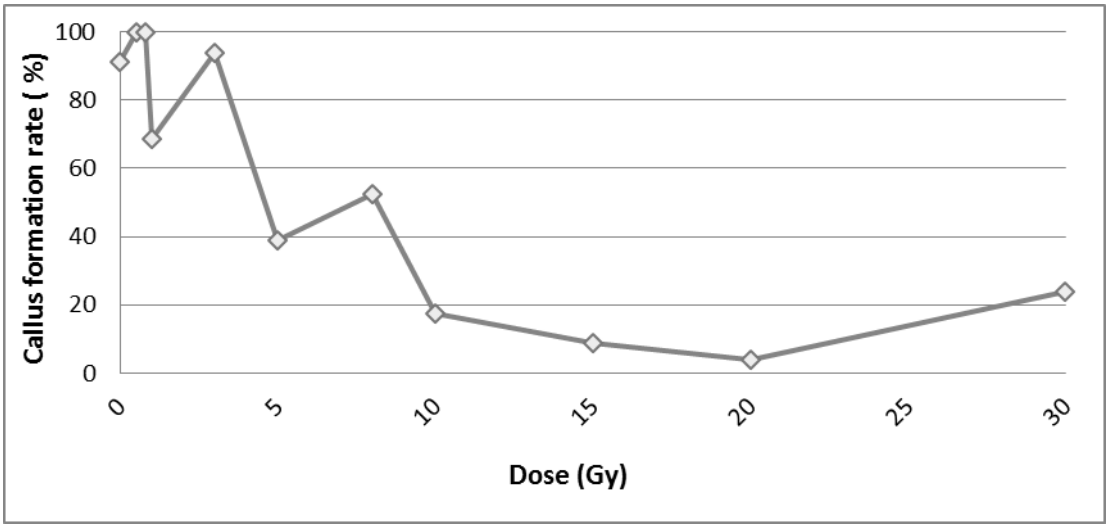


Figure 28. Callus formation rate (%) from irradiated Pink ray florets after 6 weeks.

Meanwhile, Table 19 and Table 20 show detailed figures on the percentage of adventitious shoot formation from irradiated ray floret explants after 8 and 14 weeks, respectively, whilst Figure 29 and Figure 30 show their respective curves. Interestingly, cultures irradiated at 0.5 Gy were able to generate higher percentage of shoots as compared to the controls.

Table 19. Percentage of shoot regeneration after week 8.

Dose (Gy)	Mean (%)	Dose (Gy)	Mean (%)
0	50^{b}	8	2.5^{c}
0.5	73.75^{a}	10	0^{c}
0.8	7.5^{c}	15	0^{c}
1	6.25^{c}	20	0^{c}
3	2.50^{c}	30	0^{c}
5	1.25^{c}		

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test ($p < 0.05$).

Table 20. Percentage of shoot regeneration from Pink ray florets after 14 weeks.

Dose (Gy)	Mean (%)	Dose (Gy)	Mean (%)
0	61.25^{b}	8	5^{c}
0.5	100^{a}	10	0^{c}
0.8	43.75^{bc}	15	6.25^{c}
1	20^{bc}	20	0^{c}
3	5^{c}	30	0^{c}
5	0^{c}		

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test ($p < 0.05$).

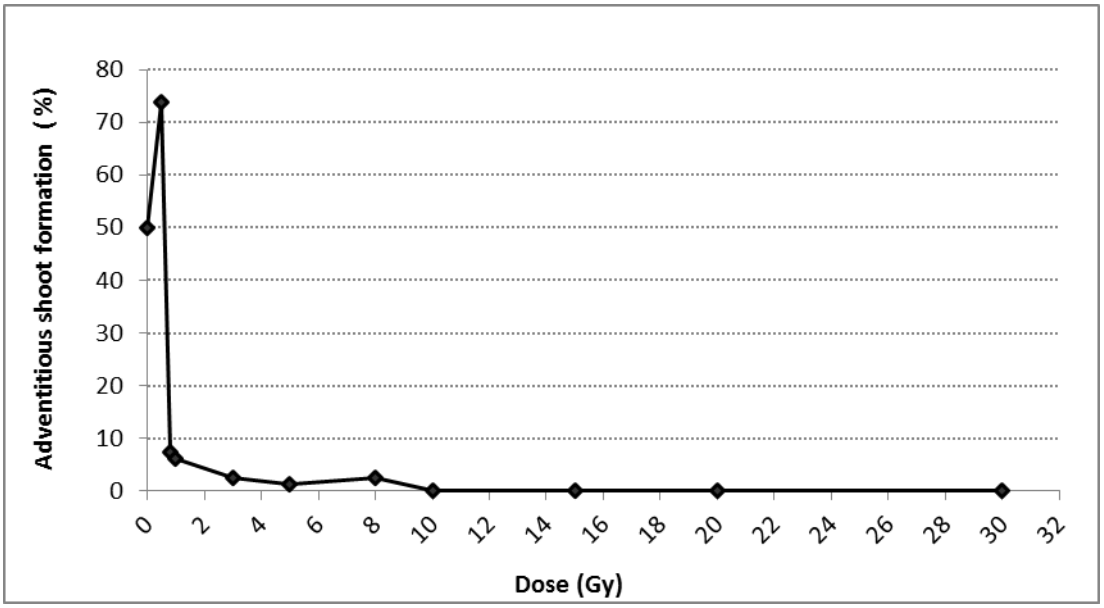


Figure 29. Adventitious shoot formation rate (%) from Pink ray florets irradiated at different doses after 8 weeks.

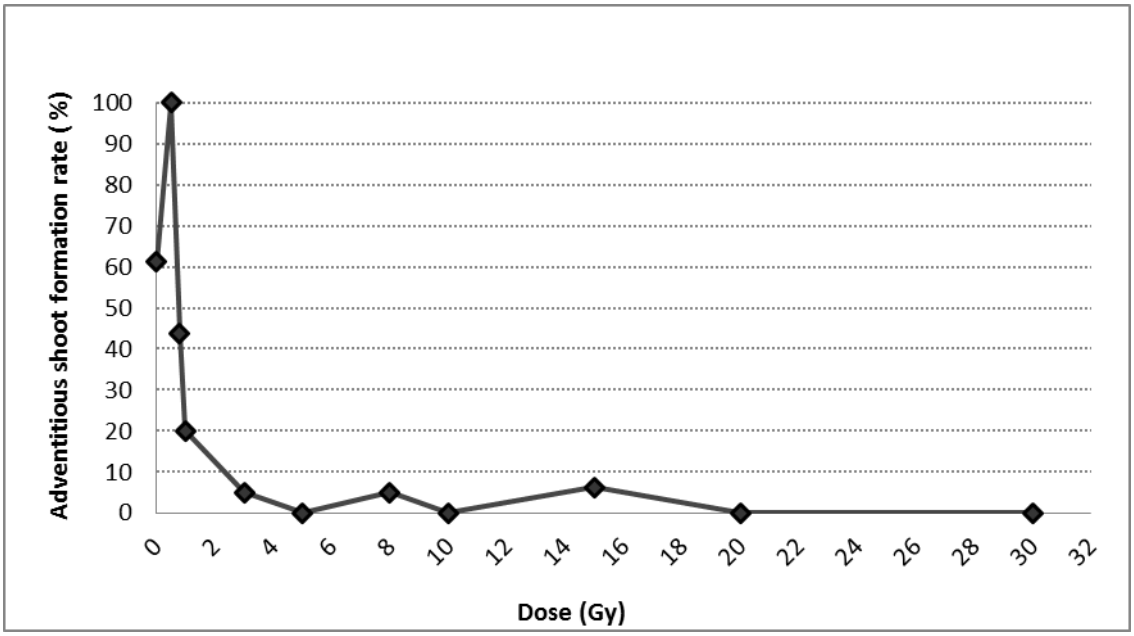


Figure 30. Adventitious shoot formation rate (%) from Pink ray florets irradiated at different doses after 14 weeks.

After 8 weeks, approximately 74% of those irradiated at 0.5 Gy have generated new shoots and by 14 weeks, all of them (100%) have been converted into shoots. In contrast, about 50% of the controls did generate shoots after 8 weeks and an increase of another 11% after 14 weeks. Based on regeneration data at week 14, irradiating the cultures above 3 Gy severely affect subsequent shoot regeneration. Pictures of callus formation on irradiated ray florets of pink variety are shown in Figure 31.

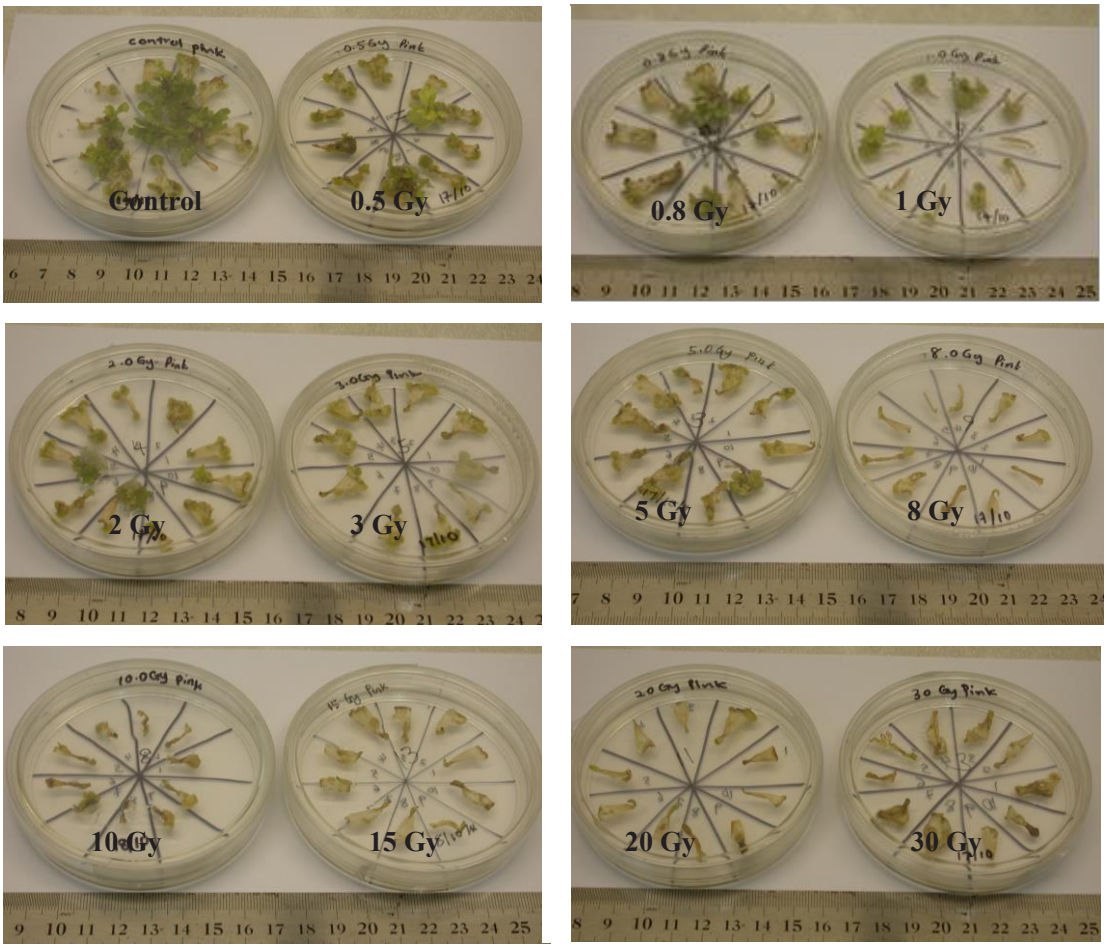


Figure 31. Callus formation on ray florets of the Pink variety after 14 weeks of irradiation at different doses.

2.4.2 Irradiation of nodal cultures

Explants preparation

In vitro stem of chrysanthemum pink was cut into single nodes (0.5 cm in length) and placed on petri dishes containing half strength of MS hormone-free medium. The cultures were incubated at 25 ± 2 °C under 16-hour photoperiod for approximately 5 days before irradiation. The processes involved were also similar to the ones used for Reagan Red variety.

Irradiation treatment

Ion-beam irradiation was carried out at doses of 0, 0.5, 0.8, 1, 2, 3, 5, 8, 10, 15, 20, and 30 Gy. Following irradiation treatment, the nodal segments were transferred onto fresh half strength of MS medium without any growth regulator and left to grow under the same conditions.

Statistical analysis and data collection

Data were analyzed using the SAS software program (version 9.2). Observation were made on the percentage of survived cultures, average height of regenerated shoots, number of leaves and roots at week 8 after irradiation.

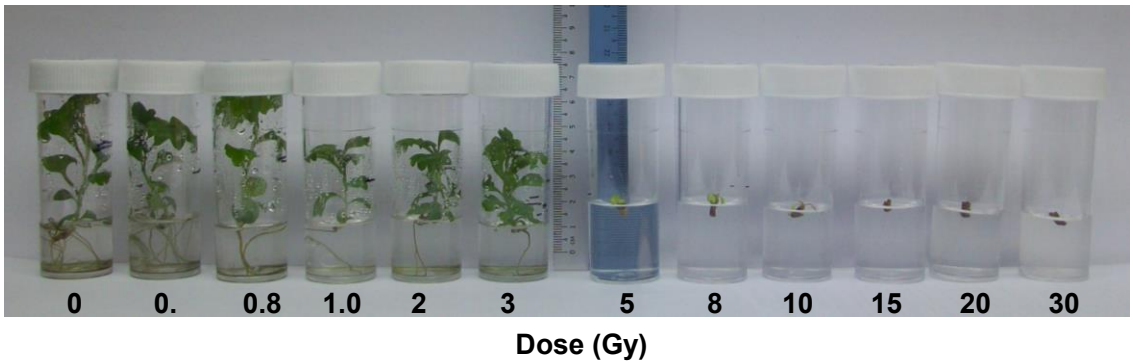


Figure 32. Chrysanthemum nodal cultures after 8 weeks of irradiation clearly shows growth reduction at doses 5 Gy and higher.

Radiosensitivity of nodal cultures to ion-beam irradiation

Observations on week 8 after irradiation have found no significant difference in terms of survival percentage of nodes irradiated at lower doses (2 Gy and below). These nodes showed a relatively high percentage of survival between 86–100%. However at 3 Gy, the survival percentage dropped drastically to 60% and further reduced to less than 50% at doses 5 Gy and higher. These indicate that the sensitivity of nodes to ion-beam irradiation is similar to that of their ray floret counterparts[56]. Figure 32 shows the condition of nodal cultures, whilst Table 21 and Figure 33 show detailed data on the percentage of survival and its growth curve, respectively, after 8 weeks of irradiation.

Table 21. Percentage of nodal survival 8 weeks after irradiation.

Dose (Gy)	Survival (%)	Dose (Gy)	Survival (%)
0	86. 7^{ab}	5	46.7^{cd}
0.5	100^{a}	8	33.3^{cde}
0.8	86.7^{ab}	10	40^{cde}
1	93.3^{a}	15	33.3^{cde}
2	100^{a}	20	13.3^{e}
3	60^{bc}	30	26.7^{de}

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test (p <0.05).

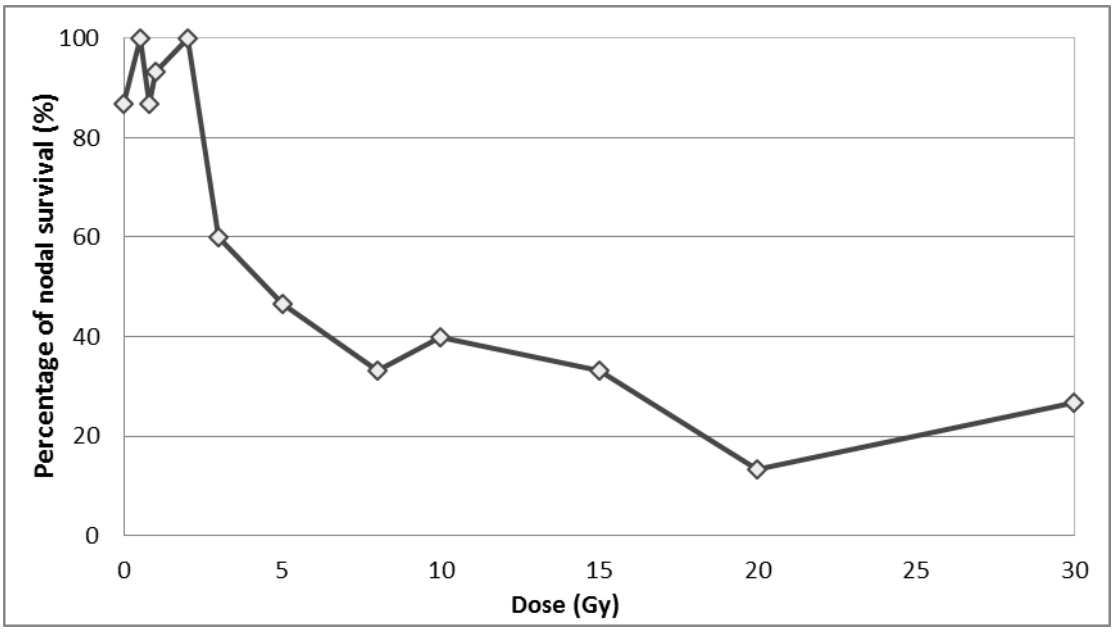


Figure 33. Growth response curve for irradiated nodes at different doses after 8 weeks.

In terms of mean shoot height, number of leaves and roots generated in survived nodal shoots (Table 22), it was also observed that 5 Gy was a cut-off dose value since the figures recorded at 5 Gy and higher showed a drastic decrease of more than 50% for all categories. Figure 34 to 36 show individual graph for average shoot height, leaf and root formation on irradiated samples after 8 weeks of culture. As expected, higher irradiation doses has an adverse effect on shoot height as well as the number of leaves and roots. However, we found that some of nodal shoots irradiated at higher doses (above 15 Gy) were still able to produce new leaves and roots, but were short and stunted, and eventually died.

Table 22. Average shoots height, number of leaf and root formation from irradiated nodal cultures at week 8.

Dose (Gy)	Shoot Height (cm)	Leaf Number	Root Number
0	2.11^{a}	9.93^{abc}	2.33^{ab}
0.5	2.22^{a}	12.06^{a}	2.53^{ab}
0.8	1.72^{ab}	12.07^{a}	2.82^{a}
1	1.47^{abc}	10.97^{ab}	2.02^{abc}
2	1.40^{abcd}	10.20^{abc}	1.47^{abc}
3	0.77^{bcde}	9.640^{abc}	0.61^{abc}
5	0.46^{cde}	7.00^{abcd}	0^{c}
8	0.34^{de}	4.17^{cd}	0.44^{bc}
10	0.13^{e}	1.22^{d}	0^{c}
15	0.31^{de}	1.56^{d}	0^{c}
20	0.27^{e}	4.33^{bcd}	0^{c}
30	0.89^{bcde}	4.78^{bcd}	1.78^{abc}

Means followed by the same letter in superscript are not significantly different, according to Duncan's New Multiple Range Test (p <0.05).

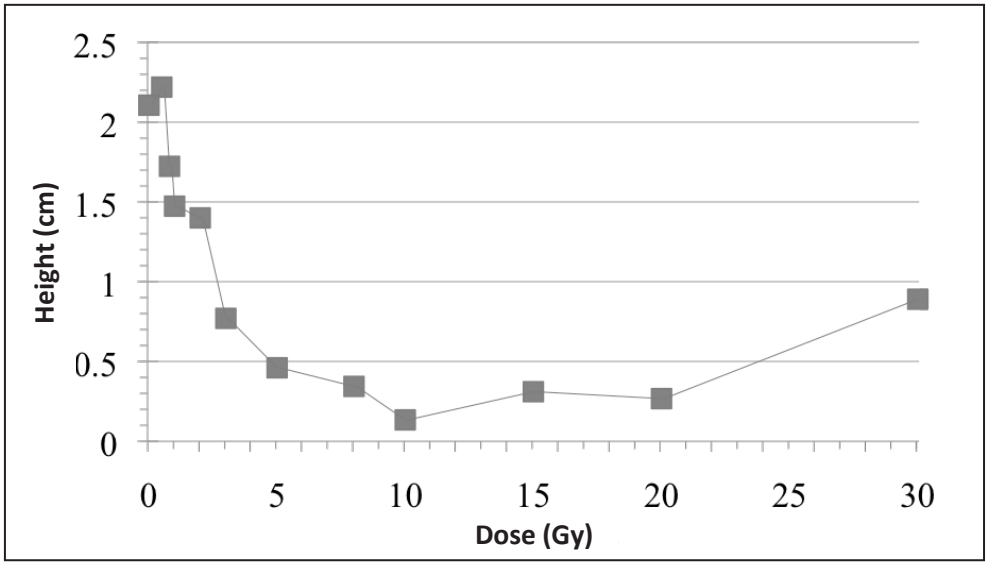


Figure 34. Average shoot height in irradiated nodes at week 8.

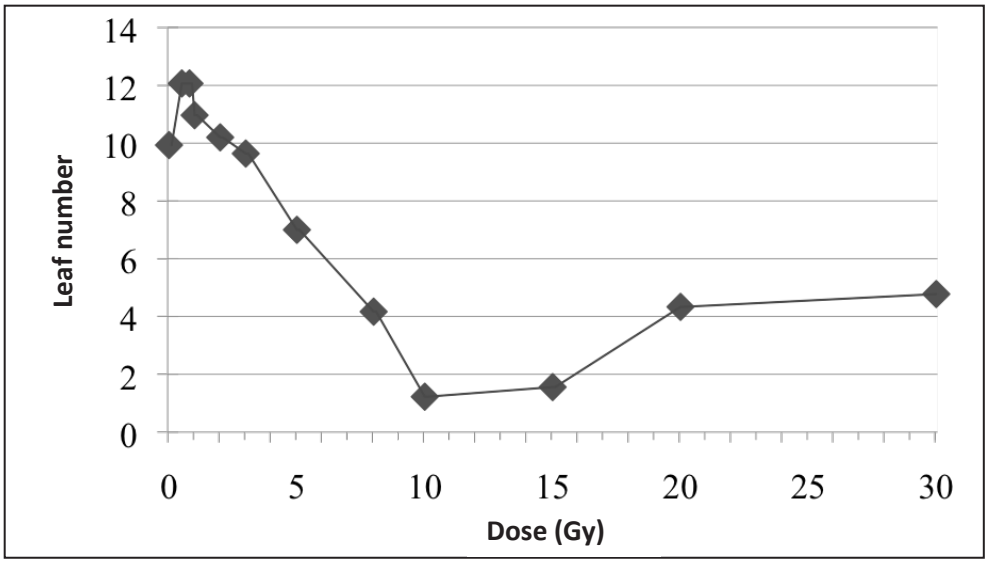


Figure 35. Average leaf number generated from irradiated nodes at week 8.

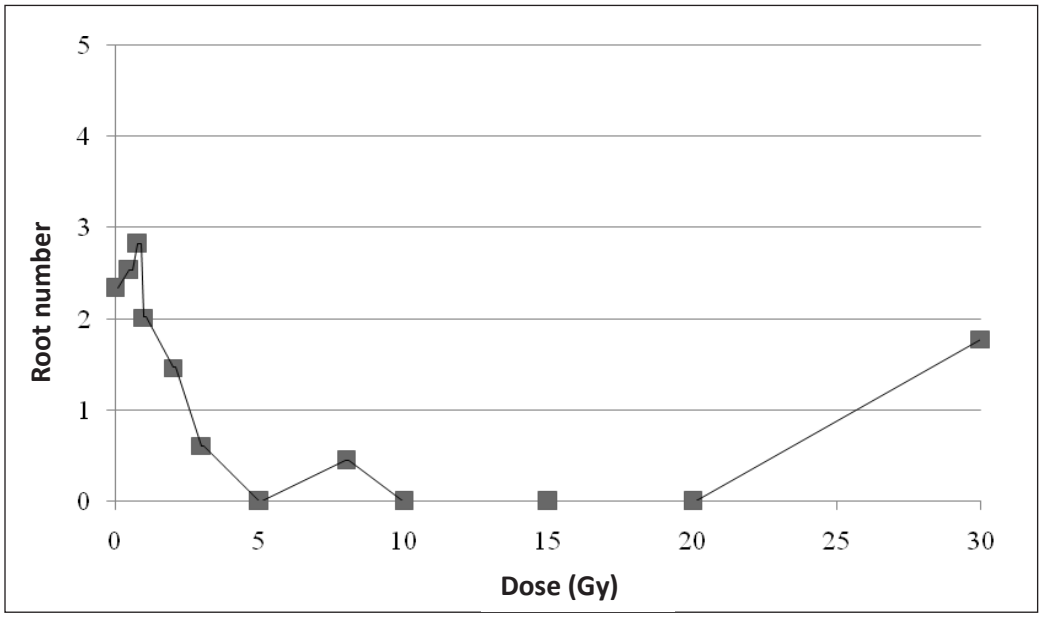


Figure 36. Average number of roots generated from irradiated nodes at week 8.

Overall, from these two irradiation experiments, it was recommended that the optimum dose for shoot regeneration from ray florets is in the range of 0.5 to 0.8 Gy. For nodals, the optimum dose is between 1 to 2 Gy.

2.4.3 Selection and identification of Pink mutants

For field screening, each independent mutant clone was represented by 30 replicate plants (10 replicate plants per block, 3 blocks). All these mutant clones were regenerated from ray floret culture. The screening work were carried out at both Nuclear Malaysia's glasshouse (Bio Design Facility) and MARDI Cameron Highlands. From a series of screenings, several mutants with unique features have been observed and selected. At present, four pink mutants were identified. These mutants were multiplied and maintained in tissue culture as well as in the glasshouse. One of the mutants, Majestic Pink (irradiated at 0.5 Gy and originally coded as P7E5 V0.5) has been filed for PVP and another three (P12E1 0.8Gy, P1E5 2.0Gy, and P11E3 3.0Gy) are yet to be named and registered. Some of the works involved in generating new mutant varieties of Pink are shown in Figure 37. Detailed characters of each mutant are described below and summarized in Table 23 and 24.

Majestic Pink

This mutant is generated from ray floret culture irradiated at 0.5 Gy. It has light pink flower and white/green leaf variegation. The plant is shorter than its control and fits well as a potted plant as compared to cut flower characteristic of the control. The flowers are also arranged in a compact manner (close to one another) and therefore are very suitable to be used in stage and table decoration as well as bouquet. Leaf variegation is clearly visible even at tissue culture stage. Figure 38 shows several characters of this mutant plant.

Figure 37. Some of the processes in developing new Pink mutants. Regenerated shoots from ray floret (a), hardening of irradiated shoots (b), transplanting in nursery (c), data collection on mutant plants (d), mutants in vegetative phase (e), and flowering mutants (f).

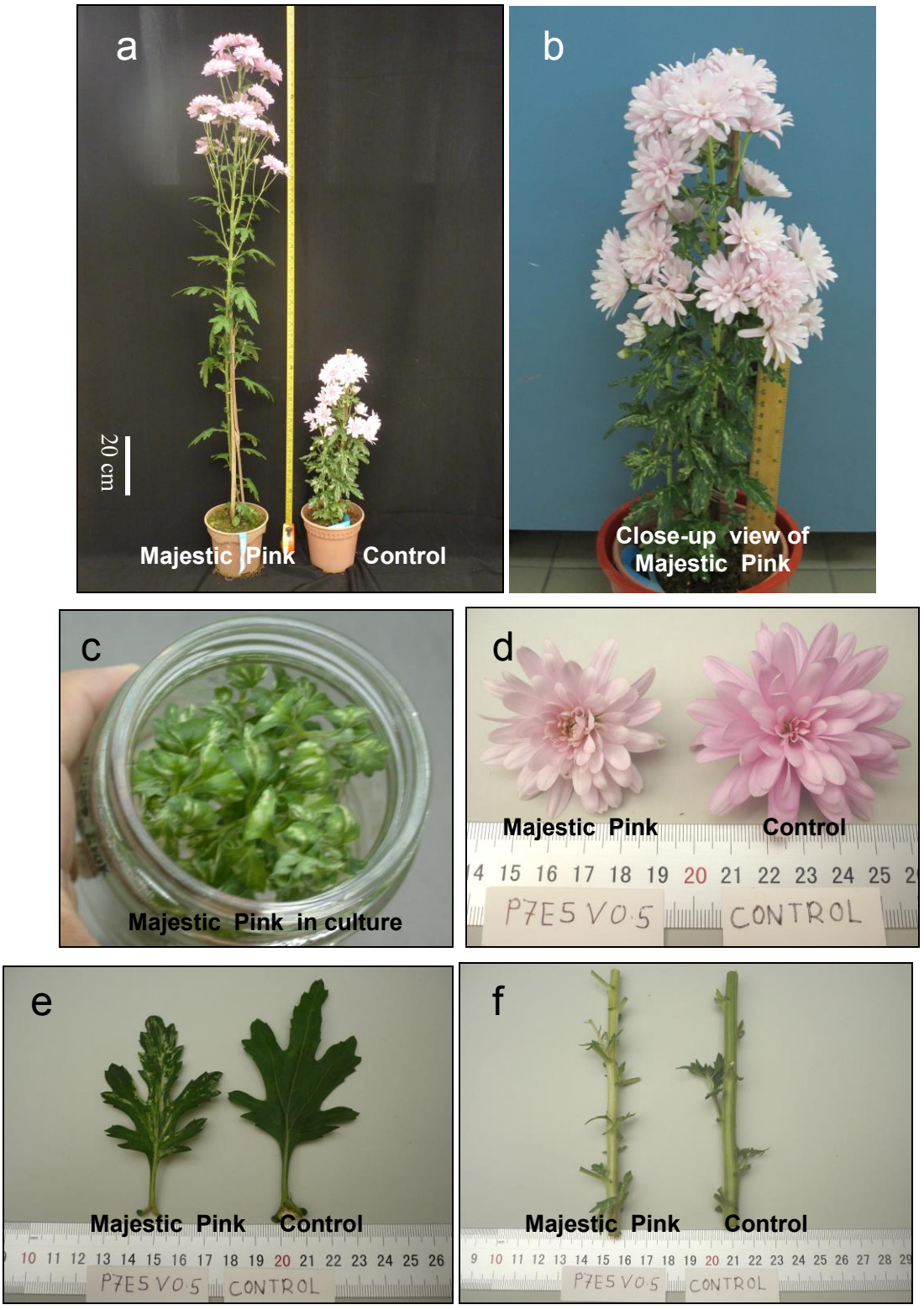


Figure 38. Majestic Pink mutant. The mutant plant as compared to its control (a), close-up view of Majestic Pink (b), the mutant in culture showing leaf variegation (c), Majestic Pink flower and its control (d), green/white variegation in Majestic Pink leaf as compared to solid green in control (e), and light green stem in Majestic Pink as compared to green in control (f).

Purple flower mutant (P12E1 0.8Gy)

This mutant (coded as P12E1 0.8Gy) was generated from irradiated petal culture at 0.8 Gy. The distinct features of this mutant is the purple colour of the flower as compared to pink in its control. The flower head is fall into semi double type as compared to daisy-eyed double for the control. Another distinct feature is the stem colour in which the mutant has green stem with traces of purple, whilst the control has solid green stem. Some of the features of this mutant are shown in Figure 39.

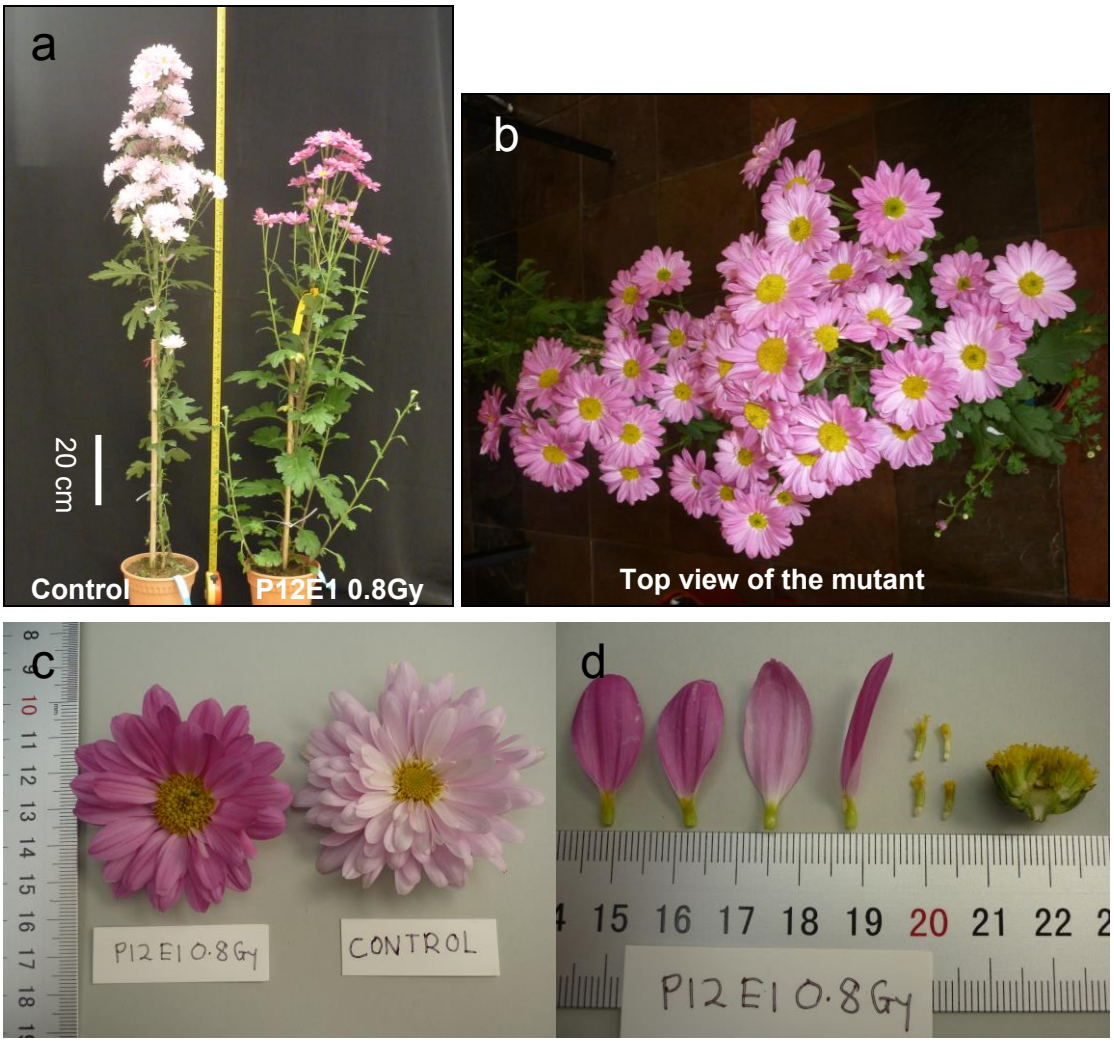


Figure 39. P12E1 0.8Gy mutant. The mutant plant as compared to the control (a) upper view of the mutant (b), the mutant flower and its control (c) and ray floret and disc floret of the mutant (d).

Light Pink mutant (P1E5 2.0Gy)

The light pink mutant, coded as P1E5 2.0Gy, was generated from ray floret culture irradiated at 2 Gy. The colour of the flower is pink but slightly lighter (69C, according to The Royal Horticultural Society (RHS) colour chart), as compared to 69B for the control. Although this mutant has lower average number of flower head per plant than its control, it has more ray florets per flower head (approximately 74) that are arranged in 6 layers as compared to an average of 51 ray florets in 4 layers for the control. Figure 40 shows the picture of this mutant.

Figure 40. The P1E5 2.0Gy mutant plant and its control (a), the mutant flowers (b), and its ray florets and disc florets (c).

P11E3 3.0Gy Mutant

This mutant, coded as P11E3 3.0Gy, also has light pink flowers (69C, RHS colour chart), as compared to 69B for the control, and was generated from ray floret culture irradiated at 3 Gy. This mutant has relatively similar average number of flower head per plant as its control, but has approximately 40 more ray florets per flower head than the control. Figure 41 shows the picture of this mutant.

Figure 41. P11E3 3.0Gy flower as compared with control flower. A unit of the ruler at the bottom is in cm.

Table 23. Detailed plant characteristics of Pink control and mutants.

No.	Character	Control	Majestic Pink (0.5 Gy)	P12E1 0.8Gy (0.8 Gy)	P1E5 2.0Gy (2 Gy)	P11E3 3.0Gy (3 Gy)
1	Average height (cm)	94.93	42.93	80.33	91.9	92.73
2	Plant type	Non bushy	Non bushy	Non bushy	Non bushy	non bushy
3	Plant growth habit	Upright	Upright	Semi upright	Upright	Upright
4	Density of branching	Medium	Sparse	Medium	Medium	Medium
5	Stem colour	Green	Light green	Green tinged with purple	Green	Green
6	Stipule size (cm)	0.35	0.15	0.51	0.26	0.33
7	Petiole attitude	Horizontal	Horizontal	Moderately upward	Horizontal	Horizontal
8	Average length of petioles (cm)	2	1.78	3.1467	1.87	2.19
9	Average leaf length + petiole (cm)	12.34	8.93	13.48	11.71	11.83
10	Average leaf width (cm)	6.24	5.46	7.607	5.55	5.39
11	Leaf ratio length/ width	Long	Medium	Long	Long	Long
12	Average length of terminal lobe (cm)	4.58	4.37	3.14	4.22	3.53
13	Average depth of lateral sinus (cm)	1.82	1.54	2.30	1.65	1.98
14	Lowest lateral sinus	Deep	Medium	Deep	Medium	Deep
15	Margin of lateral sinus	Parallel	Parallel	Parallel	Parallel	Parallel
16	Leaf base shape	Obtuse	Cordate	Obtuse	Obtuse	Obtuse
17	Leaf upper color	Dark green	Light green and variegated	Dark green	Dark green	Dark green
18	Number of leaf sinus	4	4	4	4	4

Table 24. Detailed flower characteristics of Pink control and mutants.

No.	Flower Character	Control	Majestic Pink (0.5 Gy)	P12E1 0.8Gy (0.8 Gy)	P1E5 2.0Gy (2 Gy)	P11E3 3.0Gy (3 Gy)
1	Inflorescence form	Corymbiform	Cylindrical	Corymbiform	Corymbiform	Corymbiform
2	Inflorescence angle between primary lateral shoot and stem	Medium	Small	Medium	Medium	Medium
3	Number of flower head	54.2	13.1	26.5	40.1	56.3
4	Average diameter: flower head (cm)	6.12	5.27	5.8	5.5	6.01
5	Average height: flower head (cm)	4.41	4.33	6.5	7.2	6.87
6	Type: flower head	Daisy-eyed double	Daisy-eyed double	Semi double	Daisy-eyed double	Daisy-eyed double
7	Bloom form	Daisy	Daisy	Semi double	Daisy	Daisy
8	Number of rows: ray floret	4	5 to 6 rows	3	6	6
9	Number of rows: involucral bract	3	3	3	3	3
10	Longitudinal axis majority flower head	Reflexing	Reflexing	Reflexing	Reflexing	Reflexing
11	Longitudinal axis outer flower head	Reflexing	Reflexing	Reflexing	Reflexing	Reflexing
12	Attitude of lateral flower bud	Upright	Upright	Upright	Upright	Upright
13	Average length of corolla tube flower head (cm)	0.29	0.27	0.49	0.30	0.30
14	Cross section of ray floret	Weakly convex	Weakly convex	Weakly convex	Weakly convex	Weakly convex
15	Average length of outer floret (cm)	3.01	2.90	2.85	2.98	3.09
16	Average width of outer floret (cm)	0.85	0.75	1.07	0.99	1.08
17	Ray floret type	Ligulate	Ligulate	Ligulate	Ligulate	Ligulate
18	Ray floret tips	Dentate	Dentate	Rounded	Dentate	Dentate
19	Average ray floret thickness (mm)	0.10	0.01	0.07	0.09	0.09

Table 24. Detailed flower characteristics of Pink control and mutants (continued).

No.	Flower Character	Control	Majestic Pink (0.5 Gy)	P12E1 0.8Gy (0.8 Gy)	P1E5 2.0Gy (2 Gy)	P11E3 3.0Gy (3 Gy)
20	Ray floret upper colour	69B	69C	74A	69C	69C
21	Ray floret lower colour	76C	75C	75A	69D	69D
22	Ray floret colour pattern	Solid	Solid	Solid	Solid	Solid
23	Average ray floret number	51.47	69.87	27.53	74.33	90.60
24	Ray floret texture surface	Keeled	Keeled	Keeled	Keeled	Keeled
25	Average disc diameter(cm)	0.96	0.63	1.57	0.85	0.81
26	Disc diameter relative to head diameter	Small	Small	Small	Small	Small
27	Disc colour before dehiscence	12B	None	154A	145A	1A
28	Disc colour after dehiscence	12A	1A	151B	153B	7A
29	Disc distribution type	Domed	Domed	Domed	Domed	Domed
30	Average disc floret overall length (cm)	0.54	0.51	0.59	0.50	0.40
31	Disc floret type	Quilled	Enlarged tubular	Petaloid	Quilled	Quilled
32	Disc: profile in cross section	Strongly domed	Strongly domed	Strongly domed	Strongly domed	Strongly domed
33	Disc: presence of dark spot at centre before anther dehiscence	None	Absent	None	None	None
34	Average receptacle diameter (cm)	0.55	0.51	0.51	0.47	0.49
35	Receptacle shape	Domed raised	domed	Domed raised	Domed raised	Domed raised
36	Response group	7 weeks	7 weeks	7 weeks	Strongly domed	Strongly domed
37	Attitude of basal part (ray floret)	Moderately ascending	Moderately ascending	Horizontal	Moderately ascending	Moderately ascending

2.5 Public preference and acceptance study on mutants

2.5.1 The aim and outline of the survey

A preliminary survey on public preference and acceptance of the mutants obtained in this work was conducted on 7 January 2014 at Agrotechnology and Bioscience Division, Malaysian Nuclear Agency. The main objective of this survey was to gather information from the public on overall appearance of these new varieties and their potential for commercialization. The same set of questions were used for both Reagan Red and Pink mutants. In order to gain fair feedback from the respondents, the plants in this survey were kept anonymous and only labeled with a letter from A to E for Pink variety and F to J for Reagan Red. The questionnaires (as below) were prepared in dual language (Malay and English). The respondents were asked to score the mutants according to scales from 1 (very attractive) to 5 (poor).

AM *GENERAL* (Bulatkan jawapan, *circle your answer*)

1. Umur anda (*Your age*)
   ≤ 20 tahun 21-35 tahun 35-50 tahun ≥ 50 tahun

2. Jantina (*Gender*)
   Lelaki (*Male*) Perempuan (*Female*)

3. Pekerjaan (*Occupation*)
   Pelajar (*Student*) Kerajaan (*Government*) Swasta (*Private*) Sendiri (*Own business)*

4. Adakah anda seorang…….. (*Are you a …….*)
   Penanam (*Grower*) Saintis (*Scientist*) Pengumpul (*Collector*) Pengemar (*Hobbyist*) Lain-lain (*Others*)

SPESIFIK : *SPECIFIC* (Tuliskan skala, *write your scale*)

1 = Sangat cantik (*very attractive*) 2 = Cantik (*attractive*) 3 = Sederhana (*satisfactory*)
4 = Kurang menarik (*less attractive*) 5 = Buruk (*poor*)

Berdasarkan skala di atas, nyatakan pendapat anda untuk soalan-soalan berikut? (*Using the scale above, how would you rank the following*?)

1. Bentuk bunga (*Flower shape*)

POKOK (*PLANT*)				
A	**B**	**C**	**D**	**E**
F	**G**	**H**	**I**	**J**

2. Warna bunga (*Flower colour*)

POKOK (*PLANT*)				
A	**B**	**C**	**D**	**E**
F	**G**	**H**	**I**	**J**

3. Keseluruhan rupa pokok (*Overall plant appearance*)

POKOK (*PLANT*)				
A	**B**	**C**	**D**	**E**
F	**G**	**H**	**I**	**J**

4. Jika ditawarkan, adakah anda berminat untuk membeli pokok ini
(*If offered, would you be interested to purchase this plant*)
Tandakan **(√)** jika YA dan **(X)** jika TIDAK. *Mark **(√)** if YES and **(X)** if NO*

POKOK (*PLANT*)				
A	**B**	**C**	**D**	**E**
F	**G**	**H**	**I**	**J**

5. Jika jawapan pada soalan 4 ialah YA, berikan cadangan harga (RM) untuk setiap SATU (1) pasu pokok ini
(*If your answer for question 4 is YES, please give a recommended price for ONE (1) pot of this plant*)

*Nota: harga semasa 1 pasu pokok kekwa RM 5.00 - 10.00
(*Note: current price for 1 potted chrysanthemum plant is RM 5.00 - 10.00*)

CADANGAN HARGA (RM) POKOK (*RECCOMMENDED PRICE (RM) PER PLANT*)				
A	**B**	**C**	**D**	**E**
F	**G**	**H**	**I**	**J**

End of survey

2.5.2 Background of the respondents

Approximately 60 participants were involved in this survey, which include staff of Nuclear Malaysia, university students, plant growers/collectors and hobbyists. Of these, 38% were male and 62% were female (Figure 42). Majority of these respondents (49%) were in the age of 35 to 50 years old; 28% were between 21 and 35 years old and 23% above 50 years old (Figure 43). Scientists made up the highest proportion of the respondents (46%), followed by hobbyists (31%), others (15%), growers (6%) and collectors (2%) (Figure 44). Others include students and general public.

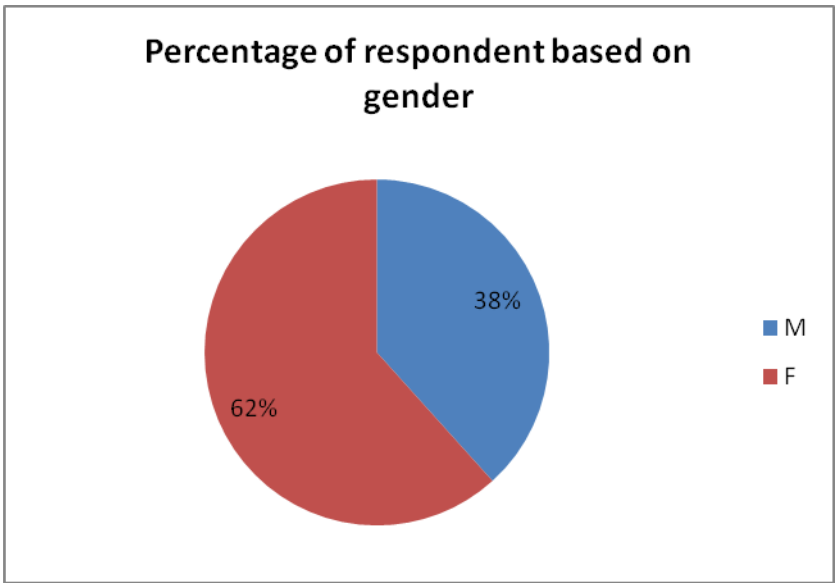


Figure 42. Percentage of respondents according to gender.

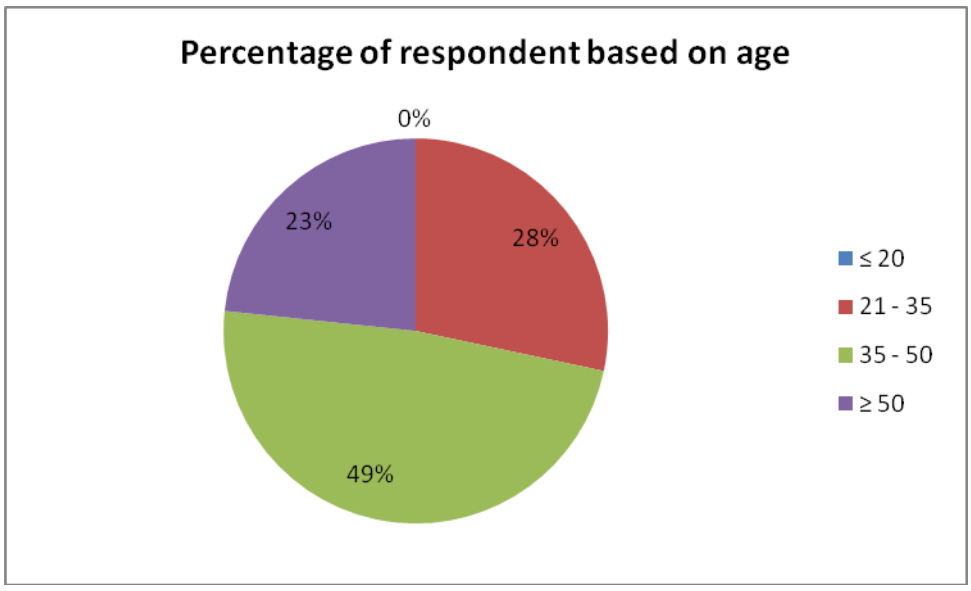


Figure 43. Percentage of respondents according to age.

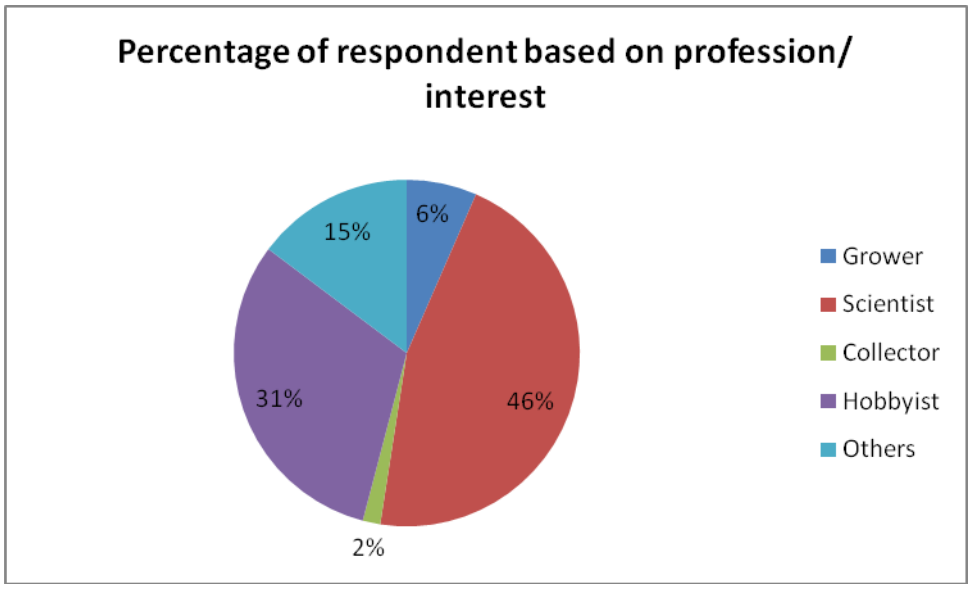


Figure 44. Percentage of respondents based on profession or interest.

2.5.3 Public acceptance on Reagan Red mutant

Reagan Red control plant and its four mutant varieties were used in this survey. Three of the mutants were generated from ion-beam irradiation (TIARA Red, Yellow Sun and Golden Eye) and one mutant (Cream Marble) from gamma irradiation (Figure 45a). Cream Marble has orangish red flower and yellowish cream/green variegated leaves (Figure 45b). It was generated through gamma irradiation of *in vitro* nodal culture of Reagan Red at 30 Gy. This mutant received a bronze medal at Nuclear Malaysia Innovation Award 2012.

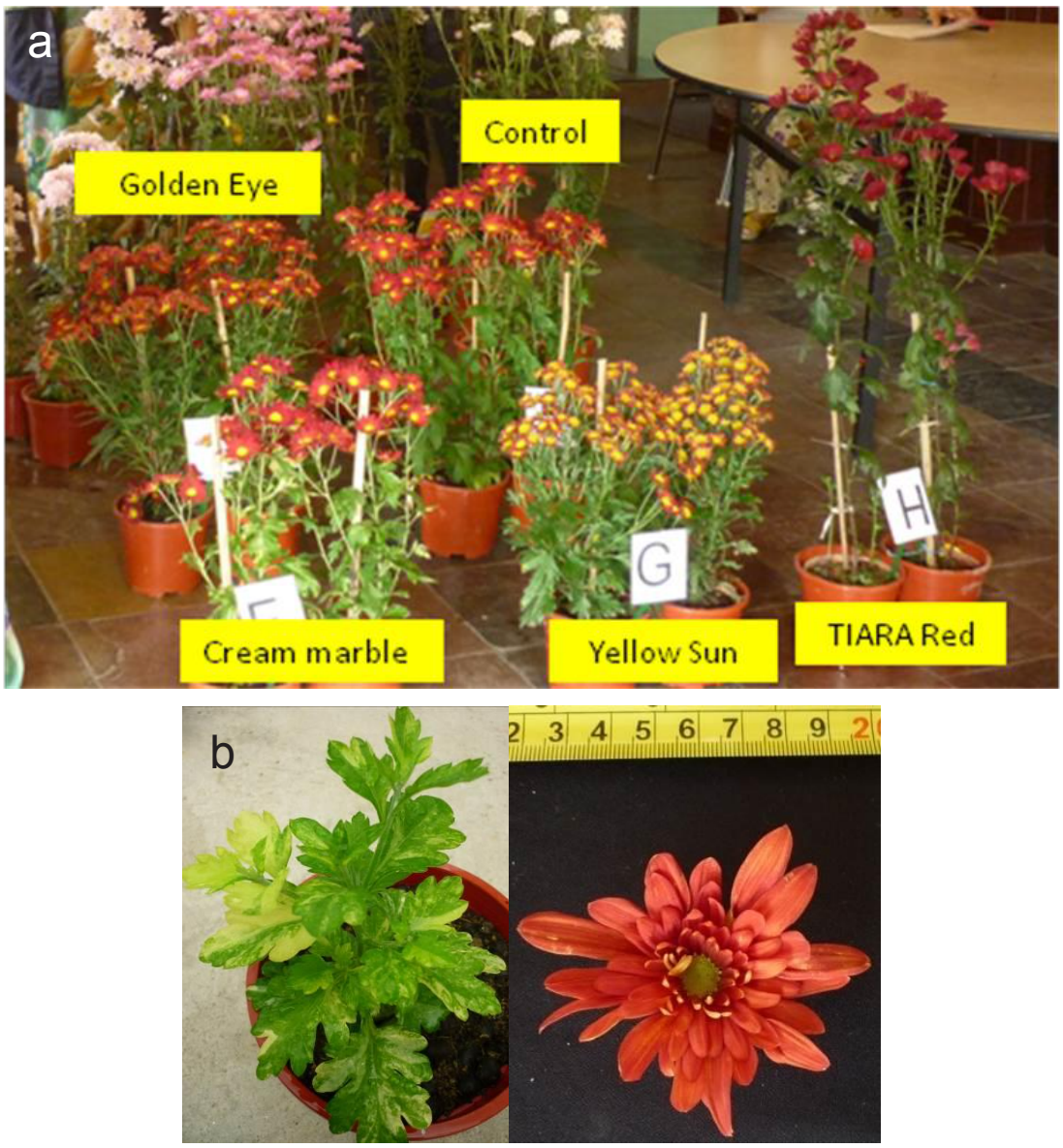


Figure 45. Photos of Chrysanthemum Reagan Red control and its mutants used in the survey (a) and close-up views of Cream Marble leaves and flower (b).

Based on the survey, the respondents gave gamma-irradiated Cream Marble as the most attractive among the mutants for all categories (flower shape, flower colour and overall plant appearance). As for ion beam irradiated mutants, TIARA Red received the most positive response from the respondents as compared to the other two mutants (Yellow Sun and Golden Eye), but generally was at par with the control for all characters. Table 25 shows detailed data on the average scales given to all mutants and the control by the respondents.

Table 25. Average scales given by respondents to several characters of Reagan Red mutants.

Plant Characters	Average Scale*				
	Cream Marble	Yellow Sun (IB0.52.)	TIARA Red	Golden Eye (IB0.514)	Control
Flower shape	1.72	3.1	2.07	2.32	2.07
Flower colour	1.58	2.78	1.78	2.22	1.8
Overall plant appearance	1.73	2.7	1.93	2.08	1.9

* Scale: **1** (very attractive); **2** (attractive); **3** (satisfactory); **4** (less attractive); **5** (poor)

In terms of public acceptance on the mutants (Table 26), majority of the respondents (78.3%) have shown interest to purchase Cream Marble, followed by control (66.7%), TIARA Red (56.7%), Golden Eye (53.3%) and Yellow Sun (33.3%). TIARA Red however edged out Cream Marble in term of price, being given a recommended price of RM 6.61 as compared to Cream Marble (RM 6.60).

Table 26. Public responses on willingness to purchase the mutants and the recommended price for each Reagan Red mutant

	Cream Marble	Yellow Sun (IB0.5 2.2)	TIARA Red	Golden Eye (IB0.5 14)	Control
Willingness to buy (%)	78.3	33.3	56.7	53.3	66.7
Suggested price per plant (RM)	6.60	4.36	6.61	5.71	6.48

2.5.4 Public acceptance on Pink mutants

As for Pink varieties, the four mutants (Figure 46) used in this survey were all from ion-beam irradiation (P1E5 2.0Gy, P11E3 3.0Gy, p12E1 0.8Gy, and Majestic Pink).

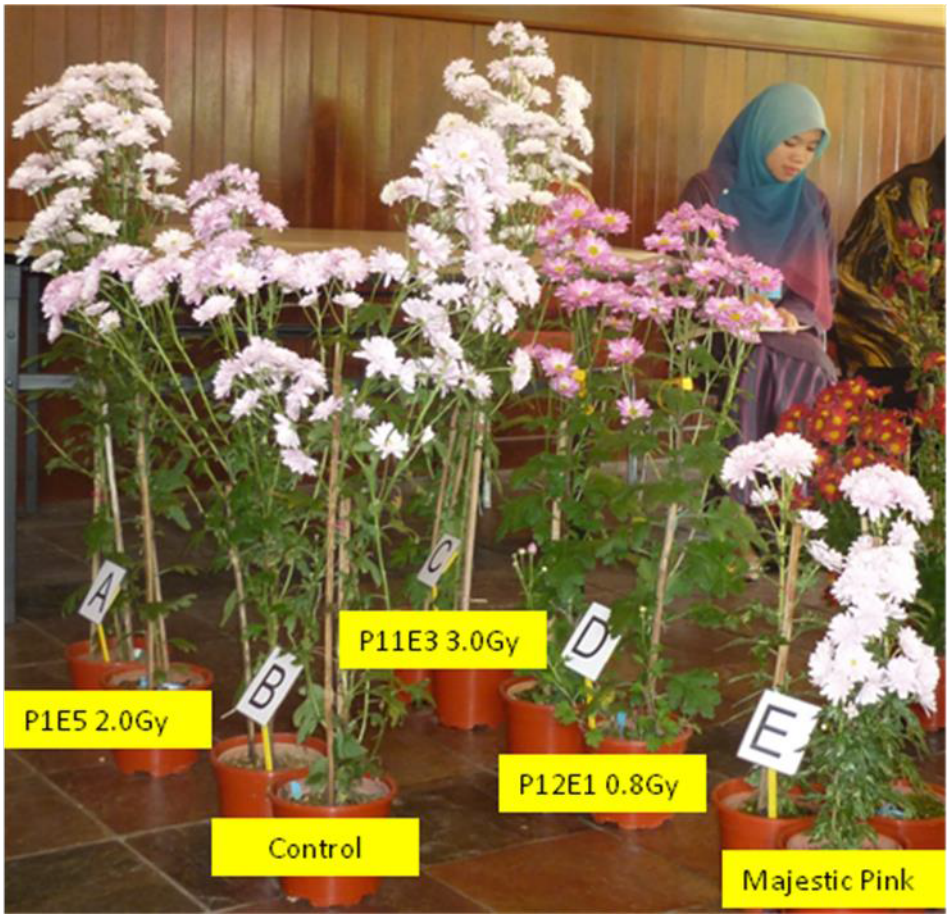


Figure 46. Photos of Chrysanthemum Pink mutants used in the survey.

In terms of preference, it appeared that P12E1 0.8Gy (purple flower, semi double mutant) and Majestic Pink (light pink flower, variegated leaves) were the favorites among respondents as they received average scales of below 2.0 for all categories. It means that these two mutants were ranked somewhere between attractive and very attractive by the respondents. P12E1 0.8Gy mutant received better review than Majestic Pink in terms of flower colour and overall plant appearance but came second behind Majestic Pink in terms of flower shape. The other mutants and the control fall into the attractive to satisfactory region for all these characters. Details on the scales given by respondents on all pink varieties are shown in Table 27.

Table 27. Average scales given by respondents on several characters of the Pink mutant plants.

Plant characters	**Average Scale***				
	P11E5 2.0Gy	**P11E3 3.0Gy**	**P12E1 0.8Gy**	**Majestic Pink**	**Control**
Flower shape	2.11	2.09	1.81	1.65	2.11
Flower colour	2.25	2.18	1.61	1.86	2.05
Overall plant appearance	2.02	2.02	1.7	1.72	2.04

* Scale: **1** (very attractive); **2** (attractive); **3** (satisfactory); **4** (less attractive); **5** (poor)

In terms of acceptance (Table 28), about 87.7% of the respondents were willing to buy Majestic Pink, followed by p12E1 0.8Gy (84.2%), control (73.7%), P11E3 3.0Gy (68.4%) and P1E5 2.0Gy (64.9%). The highest recommended price per plant was given to p12E1 0.8Gy mutant (RM 8.49), followed by Majestic Pink (RM 8.32), control (RM 6.89), P11E3 3.0Gy (RM 6.74 and P1E5 2.0Gy (RM 6.33).

Table 28. Public responses on willingness to purchase the mutants and the recommended price for each mutant.

	P11E5 2.0Gy	**P11E3 3.0Gy**	**P12E1 0.8Gy**	**Majestic Pink**	**Control**
Willingness to buy (%)	64.9	68.4	84.2	87.7	73.7
Suggested price per plant (RM)	6.33	6.74	8.49	8.32	6.89

Figure 47. Some of the participants during the survey on public preference and acceptance of chrysanthemum mutants.

2.6 Overall conclusions and future plan

This project has successfully delivered its objectives to optimize ion-beam irradiation protocols for chrysanthemum (two varieties; Reagan Red and Pink), to study ion-beam irradiation effects on the morphological aspects of the mutants and to generate new mutants with improved characteristics which might be of interest to end users.

The filing for PVP for 4 mutants (3 Reagan Red and 1 Pink) has been completed. Future plans include pre-commercialization studies (field performance, post harvest, flower quality, market survey etc.), molecular analysis (markers for traits of interest) and collaboration with interested industrial partners. These mutants, along with other irradiated populations, will also be screened for low land adaptability to select varieties that can adapt to low land temperature and conditions but still maintain the high flower quality as in highlands.

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   - Dr Muhd Noor Muhd Yunus (DDG, Research and Technology Development)
   - Dr Nahrul Khair Alang Md Rashid (Former DDG, Research and Technology Development)
   - Department of International Affair
   - Technology Commercialization Department
   - Human Resource Department

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   - Dr Khairuddin Abd Rahim (Director)
   - Dr Rusli Ibrahim
   - Ms Sakinah Ariffin
   - Ms Salahbiah Abd Majid
   - Technical support staff (Mr Shuhaimi Shamsudin, Mr Mohamed Najli Mohamed Yasin, Mr Salim Othman, Mr Othman Abu Sari)

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   - Dr. Namba Hideki (Former Deputy Director General, Quantum Beam Science Directorate)
   - Dr. HirokazuTsuji (Former Deputy Director General, Quantum Beam Science Directorate)
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   - Dr Thohirah Lee Abdullah

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   - Staff of MARDI Cameron Highlands (Dr. Wan Abdullah Wan Yusoff, Station Manager, Mr. Mohammad Abid Ahmad, Mr. Zulkifli Mohd Said)

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Appendix 1

List of steering committee meetings

1. The 21st Steering Committee Meeting
(26 and27 February 2008 at JAEA, Takasaki)

JAEA:

Dr. Hideki Namba
Deputy Director General, Quantum Beam Science Directorate

Dr. Atsushi Tanaka
Unit Manager,
(Research Unit) Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Dr. Yutaka Oono
Assistant Principal Researcher, Gene Resource Research Group,
Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Nuclear Malaysia:

Dr. NorimahYusof
Director, Agrotechnology and Biosciences Division

Ms. Affrida Abu Hassan
Manager, Ornamental Group,
Agrotechnology and Biosciences Division

Resource persons and observers:
Dr. Masao Tamada
Dr. Ayako Sakamoto
Dr. Yoshihiro Hase
Dr. Tamikazu Kume
Dr. Ryouhei Yoshihara
Dr. Shigeki Nozawa
Dr. Tamikazu Kume

2. The 22nd Steering Committee Meeting
(2 and 3 March 2009 at Nuclear Malaysia, Bangi)

Nuclear Malaysia:

Dr. Muhamad Lebai Juri
Deputy Director General (Research and Technology Development)

Dr. NorimahYusof
Director, Agrotechnology and Biosciences Division

Ms. Affrida Abu Hassan
Manager, Ornamental Group,
Agrotechnology and Biosciences Division

JAEA:

Dr. Atsushi Tanaka
Unit Manager,
(Research Unit) Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Dr. Yutaka Oono
Principal Researcher, Gene Resource Research Group,
Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Resource persons and observers:

Dr. Rusli Ibrahim
Dr. Azhar Mohamad
Dr. Zaiton Ahmad
Ms. Shakinah Salleh
Ms. Sakinah Ariffin
Mr. Shuhaimi Shamsudin
Ms. Saliza Jam

3. The 23rd Steering Committee Meeting
(8 and 9 March 2010 at JAEA, Takasaki)

JAEA:

Dr. Hideki Namba
Deputy Director General, Quantum Beam Science Directorate

Dr. Atsushi Tanaka
Unit Manager,
(Research Unit) Radiation-Applied Biology Division,
Quantum Beam Science Directorate

Nuclear Malaysia:

Dr. NorimahYusof
Director, Agrotechnology and Biosciences Division

Ms. Affrida Abu Hassan
Manager, Ornamental Group,
Agrotechnology and Biosciences Division

Resource persons and observers:

Dr. Issay Narumi
Dr. Yasuhiko Kobayashi
Dr. Yutaka Oono
Dr. Yoshihiro Hase
Mr. Masahiro Kikuchi
Dr. Shigeki Nozawa
Dr. Ryouhei Yoshihara

4. The 24th Steering Committee Meeting

(22 and 23 September 2011 at Nuclear Malaysia, Bangi)

Nuclear Malaysia:

Dr. Rusli Ibrahim
Acting Director
Agrotechnology and Biosciences Division

Dr. Zaiton Ahmad
Research Officer
Ornamental Group,
Agrotechnology and Biosciences Division

JAEA:

Dr. Atsushi Tanaka
Unit Manager,
Medical and Biotechnological Application Division
Quantum Beam Science Directorate

Dr. Yutaka Oono
Principal Researcher, Ion Beam Mutagenesis Research Group
Medical and Biotechnological Application Division
Quantum Beam Science Directorate

Resource persons and observers:

Dr. Norimah Yusof
Ms. Affrida Abu Hassan
Dr. Khairuddin Abdul Rahim
Ms. Shakinah Salleh
Ms. Sakinah Ariffin
Ms. Salahbiah Abdul Majid
Dr. Abdul Rahim Harun
Dr. Azhar Mohamad

Appendix 2.

Schedule of irradiation experiments

Table 1. Irradiation experiment performed in JAEA.

No.	Year	TIARA Project No.	Date	Researcher visited from Nuclear Malaysia	Date/ Time allocation of irradiation	Cooperative researchers in JAEA	Report
1	FY 2008	81007	Apr. 20-23, 2008	Sakinah Ariffin	Apr. 22/ 1 h	Yutaka Oono Yoshihiro Hase	JAEA-Review 2009-041: 70
2			Nov. 24-27, 2008	Affrida Abu Hassan	Nov. 25/ 1 h		
3	FY 2009	91007	May 26-29, 2009	Affrida Abu Hassan	May 27/ 1.5 hr	Yutaka Oono Ryohei Yoshihara	JAEA-Review 2010-065: 62
4			Nov. 10-13, 2009		Nov. 11/ 1.5 hr		
5	FY 2010	101010	May 16-19, 2010	Affrida Abu Hassan	May 17/ 2hr	Yutaka Oono Yoshihiro Hase Shigeki Nozawa	JAEA-Review 2011-043: 102
6			Nov. 10-13, 2010	Azhar Mohamad	Nov. 11/ 2 hr		
-	FY 2011	111009	(Canceled)*		May 16/ 2 hr		JAEA-Review 2012-046: 97
7			Oct. 12-15, 2011	Salahbiah Abdul Majid	Oct. 13/ 2 hr		
8	FY 2012	121008	May 16-18, 2012	Affrida Abu Hassan	May 16/ 2 hr		JAEA-Review 2013-059: 104
9			Nov. 28-Dec. 1, 2012	Zaiton Ahmad	Nov. 29/ 2hr		JAEA-Review 2014 -050: 108

*The irradiation experiment was canceled due to the effect of the electric power problem caused by Fukushima accident.

Table 2. Samples and irradiation dose examined.

No	Yr	Date (Researcher)	Samples (*Chrysanthemum morifolium*cv)		Dose (Gy)
0*		Nov 2007 (Ms. Affrida)	Lameet	Petal & Leaf	0, 0.2, 0.5, 1, 2, 5, 8, 10, 20, 30, 40
1	1 (FY2008)	Mac 2008 (Ms. Sakinah)	Reagan Red	Petal	0, 0.2, 0.5, 1, 2, 5, 8, 10, 20, 30, 40
2		Nov 2008 (Ms. Affrida)	Reagan Red	Petal & Shoot Node	0, 0.5, 1, 2, 5, 8, 10, 15, 20, 30
3	2 (FY2009)	May 2009 (Ms. Affrida)	Reagan Red	Petal	0, 0.5, 1, 2, 3, 5, 8, 10, 15, 20, 30
4		Nov 2009 (Ms. Affrida)	Reagan Red	Petal	0, 0.5, 1, 2, 3, 5, 8, 10, 15, 20, 30
5	3 (FY2010)	May 2010 (Ms. Affrida)	-*Unknown* -Reagan Red	Petal Shoot Node	0, 0.5, 1, 2, 3, 5, 8, 10, 15, 20, 30
6		Nov 2010 (Dr. Azhar)	(purple and pink varieties)	Petal	0, 0.5, 1, 2, 3, 5, 8, 10, 15, 20, 30
7	4 (FY2011)	Sept 2011 (Ms. Salahbiah)	Reagan Red	Petal	0, 0.5, 1, 2, 3, 5, 8, 10, 15, 20, 30
8	5 (FY2012)	May 16-18, 2012 (Ms. Affrida)	Pink and purple varieties	Petal	0, 0.5, 1, 2, 3, 5, 8, 10, 15, 20, 30
9		Nov 28-29, 2012 (Dr. Zaiton)	Pink variety	Nodes and leaves	0, 0.5, 0.8, 1, 2, 3, 5, 8, 10, 15, 20, 30

***Preliminary irradiation experiment was done in the previous bilateral collaboration project.**

Appendix 3

List of publications, presentations, and new varieties

Publications

Affrida A.H., Shakinah S., Zaiton A., Yoshihara, R., Narumi, I., Hase,Y. and Oono, Y. (2009). Generating new ornamental plant varieties using ion beams. JAEA Takasaki Annual Report 2008. JAEA-Review 2009-041**:** p.70.

Affrida A.H., Zaiton A., Salahbiah A.M., Shakinah S.,Yoshihara, R., Narumi, I., Hase, Y. and Oono, Y. (2010). Generating new ornamental plant varieties using ion beams. JAEA Takasaki Annual Report 2009. JAEA-Review 2010-065**:** p.62.

Shakinah S., Zaiton A., Affrida A.H., Nozawa, S., Narumi, I., Hase, Y. and Oono, Y. (2011). Characterization of ion beam irradiated chrysanthemum plants. JAEA Takasaki Annual Report 2010. JAEA-Review 2011-043: p.102.

Shakinah S., Zaiton A., Affrida A.H., Shuhaimi S., Yahya A., Ab. Kahar S. and Thohirah L.A. (2012). Studies on the effectiveness of acute gamma and ion beam irradiation in generating flower colour mutation for *Chrysanthemum morifolium*. *Jurnal Sains Nuklear Malaysia* 24: pp.59–70.

Zaiton A., Affrida A.H., Shakinah S., Nozawa, S., Narumi, I., Hase, Y. and Oono, Y. (2012). Generating new ornamental plant varieties using ion beams. JAEA Takasaki Annual Report 2011. JAEA-Review 2012-046: p.97.

Zaiton A., Affrida A.H., Shakinah S., Nurul Hidayah M., Nozawa, S., Narumi, I., Hase, Y. and Oono, Y. (2013). Generating new ornamental plant varieties using ion beams. JAEA Takasaki Annual Report 2012. JAEA-Review 2013-059: p.104.

Zaiton A., Affrida A.H., Shakinah S., Nurul Hidayah M., Hase, Y. and Oono, Y. (2014). Development of new *Chrysanthemum morifolium* Pink mutants through ion beam irradiation. JAEA Takasaki Annual Report 2013. JAEA-Review 2014-050: p.108.

Presentations

Shakinah S., Zaiton A., Affrida A.H., Shuhaimi S., Yahya A., Ab. Kahar S. and Thohirah L.A. Studies on the Effectiveness of Acute Gamma and Ion Beam Irradiation in Generating Flower Colour Mutation for *Chrysanthemum morifolium*. Nuclear Malaysia Technical Conference, 13–15 September 2011, Bangi Selangor, Malaysia.

Shakinah S., Yahya A., Thohirah L.A., Zaiton A. and Ab. Kahar S. Mutation induction of chrysanthemum using gamma and ion beam irradiations. International Agriculture Congress. 4–6 September 2012, Putrajaya, Malaysia.

Shakinah S., Zaiton A., Affrida A.H., Yahya A., Ab. Kahar S. and Thohirah L.A. Effect of ion beam irradiation on morphological and flowering characteristics of chrysanthemum. Nuclear Malaysia R&D Seminar. 26-28 September 2012, Bangi Selangor, Malaysia..

Shakinah S., Zaiton A., Affrida A.H., Yahya A., Nozawa, S., Narumi, I., Hase, Y. and Oono, Y. Development of *Chrysanthemum morifolium* cv Reagan Red Mutants Using Ion Beam Irradiation. The 7th Takasaki Advanced Radiation Research Symposium. 10–11 October 2012, Takasaki, Japan.

Zaiton A., Affrida A.H., Shakinah S., Nurul Hidayah M., Nozawa, S., Narumi, I., Hase, Y. and Oono, Y. Achievement of Nuclear Malaysia - JAEA Bilateral Project on Generating New Ornamental Plant Varieties using Ion Beams. The 8th Takasaki Advanced Radiation Research Symposium. 10–11 October 2013, Takasaki, Japan

Zaiton A., Affrida A.H., Shakinah S., Nurul Hidayah M., Nozawa, S., Narumi, I., Hase, Y. and Oono, Y. New Chrysanthemum Mutant Varieties Developed Through Ion Beam Irradiation Technology. The 9th Takasaki Advanced Radiation Research Symposium. 9–10 October 2014, Takasaki, Japan

Zaiton A., Affrida A.H., Shakinah S., Nurul Hidayah M., Shuhaimi S., Mohamed Najli M.Y. and Oono, Y. Development of new chrysanthemum mutants for Malaysian floriculture industry. Nuclear Malaysia R&D Seminar. 14–16 October 2014, Bangi Selangor, Malaysia.

Shakinah S., Zaiton A., Affrida A.H., Shuhaimi S., Nurul Hidayah M., Salim O. and Mohamed Najli M.Y. Customer acceptance survey on chrysanthemum mutants developed by Nuclear Malaysia. Nuclear Malaysia R&D Seminar. 14–16 October 2014, Bangi Selangor, Malaysia.

Affrida A.H., Zaiton A., Shakinah S., Oono, Y., Azhar M. and Wickneswari R. Characterization of Chrysanthemum CV. Reagan Red mutants. Nuclear Malaysia R&D Seminar. 14–16 October 2014, Bangi Selangor, Malaysia.

Shakinah S., Zaiton A., Affrida A. H., Shuhaimi S. and Oono, Y. Improvement of chrysanthemum varieties using radiation technology. Second International Conference on Crop Improvement (ICCI 2015). 2-3 December 2015. Universiti Putra Malaysia, Serdang, Selangor, Malaysia.
(Best Poster Award)

Zaiton A., Shakinah S., Affrida A. H., Nur Akmal M. R., Siti Mardhiah M. and Oono, Y. Mutagenic effects of ion beam irradiation on chrysanthemum flower colour trait. Second International Conference on Crop Improvement (ICCI 2015). 2-3 December 2015. Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

New varieties

1. Tiara Red (Filing no. PVBT 009/14. Filing date: 19 May 2014)
2. Golden Eye (Filing no. PVBT 011/14. Filing date: 19 May 2014)
3. Yellow Sun (Filing no. PVBT 013/14. Filing date: 19 May 2014)
4. Majestic Pink (Filing no. PVBT 010/14. Filing date: 19 May 2014)

PVBT: Perlindungan Varieti Baru Tumbuhan (New Plant Variety Protection), Department of Agriculture, Malaysia.

国際単位系（SI）

表１．SI 基本単位

基本量	SI 基本単位	
	名称	記号
長さ	メートル	m
質量	キログラム	kg
時間	秒	s
電流	アンペア	A
熱力学温度	ケルビン	K
物質量	モル	mol
光度	カンデラ	cd

表２．基本単位を用いて表されるSI組立単位の例

組立量	SI 組立単位	
	名称	記号
面積	平方メートル	m^2
体積	立方メートル	m^3
速さ，速度	メートル毎秒	m/s
加速度	メートル毎秒毎秒	m/s^2
波数	毎メートル	m^{-1}
密度，質量密度	キログラム毎立方メートル	kg/m^3
面積密度	キログラム毎平方メートル	kg/m^2
比体積	立方メートル毎キログラム	m^3/kg
電流密度	アンペア毎平方メートル	A/m^2
磁界の強さ	アンペア毎メートル	A/m
量濃度(a)，濃度	モル毎立方メートル	mol/m^3
質量濃度	キログラム毎立方メートル	kg/m^3
輝度	カンデラ毎平方メートル	cd/m^2
屈折率(b)	（数字の） 1	1
比透磁率(b)	（数字の） 1	1

(a) 量濃度（amount concentration）は臨床化学の分野では物質濃度（substance concentration）ともよばれる。
(b) これらは無次元量あるいは次元１をもつ量であるが、そのことを表す単位記号である数字の１は通常は表記しない。

表３．固有の名称と記号で表されるSI組立単位

組立量	SI 組立単位			
	名称	記号	他のSI単位による表し方	SI基本単位による表し方
平面角	ラジアン(b)	rad	1 (b)	m/m
立体角	ステラジアン(b)	sr(c)	1 (b)	m^2/m^2
周波数	ヘルツ(d)	Hz		s^{-1}
力	ニュートン	N		m kg s^{-2}
圧力，応力	パスカル	Pa	N/m^2	m^{-1} kg s^{-2}
エネルギー，仕事，熱量	ジュール	J	N m	m^2 kg s^{-2}
仕事率，工率，放射束	ワット	W	J/s	m^2 kg s^{-3}
電荷，電気量	クーロン	C		s A
電位差（電圧），起電力	ボルト	V	W/A	m^2 kg s^{-3} A^{-1}
静電容量	ファラド	F	C/V	m^{-2} kg^{-1} s^4 A^2
電気抵抗	オーム	Ω	V/A	m^2 kg s^{-3} A^{-2}
コンダクタンス	ジーメンス	S	A/V	m^{-2} kg^{-1} s^3 A^2
磁束	ウエーバ	Wb	Vs	m^2 kg s^{-2} A^{-1}
磁束密度	テスラ	T	Wb/m^2	kg s^{-2} A^{-1}
インダクタンス	ヘンリー	H	Wb/A	m^2 kg s^{-2} A^{-2}
セルシウス温度	セルシウス度(e)	℃		K
光束	ルーメン	lm	cd sr(c)	cd
照度	ルクス	lx	lm/m^2	m^{-2} cd
放射性核種の放射能(f)	ベクレル(d)	Bq		s^{-1}
吸収線量，比エネルギー分与，カーマ	グレイ	Gy	J/kg	m^2 s^{-2}
線量当量，周辺線量当量，方向性線量当量，個人線量当量	シーベルト(g)	Sv	J/kg	m^2 s^{-2}
酸素活性	カタール	kat		s^{-1} mol

(a)SI接頭語は固有の名称と記号を持つ組立単位と組み合わせても使用できる。しかし接頭語を付した単位はもはやコヒーレントではない。
(b)ラジアンとステラジアンは数字の１に対する単位の特別な名称で、量についての情報をつたえるために使われる。実際には、使用する時には記号radおよびsrが用いられるが、習慣として組立単位としての記号である数字の１は明示されない。
(c)測光学ではステラジアンという名称と記号srを単位の表し方の中に、そのまま維持している。
(d)ヘルツは周期現象についてのみ、ベクレルは放射性核種の統計的過程についてのみ使用される。
(e)セルシウス度はケルビンの特別な名称で、セルシウス温度を表すために使用される。セルシウス度とケルビンの単位の大きさは同一である。したがって、温度差や温度間隔を表す数値はどちらの単位で表しても同じである。
(f)放射性核種の放射能（activity referred to a radionuclide）は、しばしば誤った用語で"radioactivity"と記される。
(g)単位シーベルト（PV,2002,70,205）についてはCIPM勧告2（CI-2002）を参照。

表４．単位の中に固有の名称と記号を含むSI組立単位の例

組立量	SI 組立単位		
	名称	記号	SI 基本単位による表し方
粘度	パスカル秒	Pa s	m^{-1} kg s^{-1}
力のモーメント	ニュートンメートル	N m	m^2 kg s^{-2}
表面張力	ニュートン毎メートル	N/m	kg s^{-2}
角速度	ラジアン毎秒	rad/s	m m^{-1} s^{-1}=s^{-1}
角加速度	ラジアン毎秒毎秒	rad/s^2	m m^{-1} s^{-2}=s^{-2}
熱流密度，放射照度	ワット毎平方メートル	W/m^2	kg s^{-3}
熱容量，エントロピー	ジュール毎ケルビン	J/K	m^2 kg s^{-2} K^{-1}
比熱容量，比エントロピー	ジュール毎キログラム毎ケルビン	J/(kg K)	m^2 s^{-2} K^{-1}
比エネルギー	ジュール毎キログラム	J/kg	m^2 s^{-2}
熱伝導率	ワット毎メートル毎ケルビン	W/(m K)	m kg s^{-3} K^{-1}
体積エネルギー	ジュール毎立方メートル	J/m^3	m^{-1} kg s^{-2}
電界の強さ	ボルト毎メートル	V/m	m kg s^{-3} A^{-1}
電荷密度	クーロン毎立方メートル	C/m^3	m^{-3} s A
表面電荷	クーロン毎平方メートル	C/m^2	m^{-2} s A
電束密度，電気変位	クーロン毎平方メートル	C/m^2	m^{-2} s A
誘電率	ファラド毎メートル	F/m	m^{-3} kg^{-1} s^4 A^2
透磁率	ヘンリー毎メートル	H/m	m kg s^{-2} A^{-2}
モルエネルギー	ジュール毎モル	J/mol	m^2 kg s^{-2} mol^{-1}
モルエントロピー，モル熱容量	ジュール毎モル毎ケルビン	J/(mol K)	m^2 kg s^{-2} K^{-1} mol^{-1}
照射線量（X線及びγ線）	クーロン毎キログラム	C/kg	kg^{-1} s A
吸収線量率	グレイ毎秒	Gy/s	m^2 s^{-3}
放射強度	ワット毎ステラジアン	W/sr	m^4 m^{-2} kg s^{-3}=m^2 kg s^{-3}
放射輝度	ワット毎平方メートル毎ステラジアン	$W/(m^2$ sr)	m^2 m^{-2} kg s^{-3}=kg s^{-3}
酵素活性濃度	カタール毎立方メートル	kat/m^3	m^{-3} s^{-1} mol

表５．SI 接頭語

乗数	名称	記号	乗数	名称	記号
10^{24}	ヨタ	Y	10^{-1}	デシ	d
10^{21}	ゼタ	Z	10^{-2}	センチ	c
10^{18}	エクサ	E	10^{-3}	ミリ	m
10^{15}	ペタ	P	10^{-6}	マイクロ	μ
10^{12}	テラ	T	10^{-9}	ナノ	n
10^{9}	ギガ	G	10^{-12}	ピコ	p
10^{6}	メガ	M	10^{-15}	フェムト	f
10^{3}	キロ	k	10^{-18}	アト	a
10^{2}	ヘクト	h	10^{-21}	ゼプト	z
10^{1}	デカ	da	10^{-24}	ヨクト	y

表６．SIに属さないが、SIと併用される単位

名称	記号	SI 単位による値
分	min	1 min=60 s
時	h	1 h =60 min=3600 s
日	d	1 d=24 h=86 400 s
度	°	1°=(π/180) rad
分	’	1’=(1/60)°=(π/10 800) rad
秒	”	1”=(1/60)’=(π/648 000) rad
ヘクタール	ha	1 ha=1 hm^2=10^4m^2
リットル	L, l	1 L=1 l=1 dm^3=10^3cm^3=$10^{-3}m^3$
トン	t	1 t=10^3 kg

表７．SIに属さないが、SIと併用される単位で、SI単位で表される数値が実験的に得られるもの

名称	記号	SI 単位で表される数値
電子ボルト	eV	1 eV=1.602 176 53(14)×10^{-19}J
ダルトン	Da	1 Da=1.660 538 86(28)×10^{-27}kg
統一原子質量単位	u	1 u=1 Da
天文単位	ua	1 ua=1.495 978 706 91(6)×10^{11}m

表８．SIに属さないが、SIと併用されるその他の単位

名称	記号	SI 単位で表される数値
バール	bar	1 bar=0.1MPa=100 kPa=10^5Pa
水銀柱ミリメートル	mmHg	1 mmHg≈133.322Pa
オングストローム	Å	1 Å=0.1nm=100pm=10^{-10}m
海里	M	1 M=1852m
バーン	b	1 b=100fm^2=(10^{-12}cm)^2=$10^{-28}m^2$
ノット	kn	1 kn=(1852/3600)m/s
ネーパ	Np	SI単位との数値的な関係は、対数量の定義に依存。
ベル	B	
デシベル	dB	

表９．固有の名称をもつCGS組立単位

名称	記号	SI 単位で表される数値
エルグ	erg	1 erg=10^{-7} J
ダイン	dyn	1 dyn=10^{-5}N
ポアズ	P	1 P=1 dyn s cm^{-2}=0.1Pa s
ストークス	St	1 St =1cm^2 s^{-1}=$10^{-4}m^2$ s^{-1}
スチルブ	sb	1 sb =1cd cm^{-2}=10^4cd m^{-2}
フォト	ph	1 ph=1cd sr cm^{-2} =10^4lx
ガル	Gal	1 Gal =1cm s^{-2}=$10^{-2}ms^{-2}$
マクスウエル	Mx	1 Mx = 1G cm^2=10^{-8}Wb
ガウス	G	1 G =1Mx cm^{-2} =10^{-4}T
エルステッド(a)	Oe	1 Oe ≙ (10^3/4π)A m^{-1}

(a) ３元系のCGS単位系とSIでは直接比較できないため、等号「 ≙ 」は対応関係を示すものである。

表10．SIに属さないその他の単位の例

名称	記号	SI 単位で表される数値
キュリー	Ci	1 Ci=3.7×10^{10}Bq
レントゲン	R	1 R = 2.58×10^{-4}C/kg
ラド	rad	1 rad=1cGy=10^{-2}Gy
レム	rem	1 rem=1 cSv=10^{-2}Sv
ガンマ	γ	1 γ=1 nT=10^{-9}T
フェルミ		1 フェルミ=1 fm=10^{-15}m
メートル系カラット		1 メートル系カラット= 0.2 g = 2×10^{-4}kg
トル	Torr	1 Torr = (101 325/760) Pa
標準大気圧	atm	1 atm = 101 325 Pa
カロリー	cal	1 cal=4.1858J（「15℃」カロリー），4.1868J（「IT」カロリー），4.184J（「熱化学」カロリー）
ミクロン	μ	1 μ =1μm=10^{-6}m

（第8版，2006年）