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EFFECTS OF ION BEAM IRRADIATION ON MORPHOLOGICAL AND FLOWERING CHARACTERISTICS OF CHRYSANTHEMUM

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ABSTRACT

Chrysanthemum morifolium is an important temperate cut flower for Malaysian floriculture industry and the lack of new local owned varieties led to this mutation breeding research. The objective of this study was to compare the effectiveness of ion beam irradiation in generating mutations on ray florets and nodal explants of Chrysanthemum morifolium cv. 'Reagan Red'. Ion beams has become an efficient physical mutagen for mutation breeding. The ray florets and nodal explants were irradiated with ion beams at doses 0, 0.5, 1.0, 2.0, 3.0, 5.0, 8.0, 10, 15, 20 and 30 Gy. The 50% of in vitro shoot regeneration (RD_{50}) for ray florets explants was 2.0 Gy and for nodal explants was 4.0 Gy. Thus, relative biological effectiveness (RBE) for ray florets was found 2.0 times higher than the nodal explants. The regenerated plantlets were planted in the greenhouse at MARDI, Cameron Highlands for morphological screening. Overall performance of survival plantlets derived from in vitro nodal and ray floret explants was recorded. The characters studied include plant morphology and flowering characteristic. The ray florets explants were found to be more sensitive to ion beam irradiation and generated more mutations as compared to nodal explants.

Keywords: Chrysanthemum, ion beams, mutations, regeneration, screening

INTRODUCTION

Chrysanthemum morifolium is an important temperate cut flower for Malaysian floriculture industry and one of the major cut flower exports for Malaysia. Chrysanthemum arises from multiple species and is allohexaploid in nature (2n=6x=54). Most cultivars derived from this species are vegetatively propagated (Anderson, 2007). The main problem for chrysanthemum industry in Malaysia is the lack of new local owned varieties and over-dependence on foreign mother plants. These scenarios led to this mutation breeding research. In chrysanthemum, the benefit of mutation breeding can be seen as alteration of flower colour, flower size, flower shape, length and firmness of flower stalk and increased uniformity (Ahloowalia, 1992; Jerzy, 1990; Tulmann Neto and Latado, 1996; Ueno et al., 2005). According to FAO/IAEA Mutant Variety Database (MVD), a total of 267 chrysanthemum varieties registered were derived from mutation induction by using physical mutagens, with 147 mutant varieties were from gamma irradiation, 106 from x-ray, 6 from chronic gamma and 2 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 (http://www-mvd.iaea.org).

In this study ion beams was used to irradiate ray florets (petals) and nodal explants of *Chrysanthemum morifolium* cv. 'Reagan Red'. The objective of this study was to compare the effectiveness of ion beam irradiation in generating mutations on ray florets and nodal explants of *Chrysanthemum morifolium* cv. 'Reagan Red'. The effectiveness of ion beams on mutation induction of ray florets and nodal explants was compared as relative biological effectiveness (RBE) which is defined as the ratio of an absorbed dose of radiation to absorbed dose of reference radiation required to produce an equivalent biological effect in a particular organism or tissue (Ukai and Yamashita, 2010).

MATERIALS AND METHODS

Radio sensitivity Test and Relative Biological Effectiveness (RBE) Explant Preparation

Ray florets explants

This study involved two types of explants which were ray florets and nodal explants. The ray florets are derived from fresh and fully opened flowers of *Chrysanthemum morifolium* cv. 'Reagan Red'. The flowers were washed under running tap water to remove all dust and dirt. Cleaned flowers were immersed in 1 ml/L in Teepol® for one hour and rinsed with reverse osmosis water. After that, the flower were immersed one hour in 1% of Benomyl® solution and followed by three times rinsing with reverse osmosis water. The subsequent procedures were carried out in a laminar air flow cabinet using sterilized glassware and apparatus. The flowers were immersed in 70% ethanol for one minute with occasionally shaking, followed by three times rinsing with sterilized reverse osmosis water. Surface sterilization was repeated by immersing in 0.26% sodium hypochlorite supplemented with a few drops of Tween-20 for 30 minutes. The flowers were then washed three times with sterile reverse osmosis water before being placed on sterile filter papers to blot dry excess water. Then the ray florets were peels off and cut into two sections. Only the lower part was placed horizontally on medium with the adaxial surface touching culture medium.

Nodal explants

Nodal explants were derived from *in vitro* nodes of chrysanthemum. The nodes were obtained from established *in vitro* cultures of *Chrysanthemum morifolium* cv. 'Reagan Red'. Terminal shoots and leaves were completely removed from the main stem. Then the stem was cut into single nodes (0.5 cm long). The nodes were cultured onto 4.5 cm^2 disposable petri dish containing 15 ml of half strength MS medium (Murashige and Skoog, 1962) without any growth regulator. Ray florets and nodal cultures were incubated at 25 ± 2ºC in 16 hours photoperiod provided by cool white fluorescent light before being irradiated with ion beam.

Irradiation

Ion beam irradiations were performed at Japan Atomic Energy Agency, Japan. The ion beam was generated by an azimuthally varying field (AVF) cyclotron from carbon-12 ($^{12}C^{6+}$) source with the energy of 320 MeV. Eleven doses at 0, 0.5, 1.0, 2.0, 3.0, 5.0, 8.0, 10.0, 15.0, 20.0 and 30.0 Gy were used in this study for irradiated both types of explants.

Sub-Culturing and *In Vitro* Data Collection

After irradiation, the ray florets explants were sub-cultured into fresh medium containing MS medium supplemented with 0.5 mg/L NAA and 2.0 mg/L BAP for shoot regeneration. For nodal explants, the nodes were sub-cultured into test tubes containing half strength of MS media without growth regulators. Data on the percentage of lethal dose and percentage of regeneration dose were observed after 5 and 8 weeks of irradiation, respectively. Based on the results, the relative biological effectiveness (RBE) was estimated. For the purpose of this study, the RBE was measured at 50% of shoot regenerations.

Statistical Analysis

The study was conducted in a Randomized Complete Block Design (RCBD). For ray floret explants, 5 replications were used and each replication was consisted of 10 explants. For nodal explants, 20 replications were used, where each node was considered as one replication. Statistical Analysis System software was used for analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) was used for comparison among treatment means at $P<0.05$.

Screening of Irradiated Plantlets Rooting, hardening and transplanting

Regenerated plantlets were used as planting materials. Healthy unrooted plantlets were selected and transplanted in square container containing Leca and Perlite in ratio of 1:2. Lids of the container were closed in order to maintain the moisture for root induction and hardening of the irradiated plantlets. The containers were placed in hardening room and the room temperature was controlled between 27 - 30ºC for 3 weeks under 16 hours photoperiod (12 hours of cool white and 4 hours of warm light). Once rooted, the irradiated plantlets were sown in 15 cm^2 pots containing soil and Perlite with the ratio of 1:2. The pots were placed in greenhouse at MARDI, Cameron Highland with an average daily temperature between 15 to 25^oC in order to investigate flower colour mutation.

Lighting and cultural practices

Chrysanthemum is a Short Day (SD) plant and they will initiate flower buds once exposed to 9.5 hours of continuously darkness. Since Malaysia has equal length of days and nights (12 hours of daylight and 12 hours of darkness) throughout the year, additional lights by using 100 watt of incandescent lamp per 10 m^2 unit areas were needed to prolong the day light until 14.5 hours every day and subsequently to promote vegetative growth. Immediately after transplanting, night interruption was provided up to 28 days. After 28 days, the lights were switched off for plants to initiate flower under natural photoperiod.

Cultural practices such as watering, fertilizing and controlling pest and diseases were applied according to the standard procedures for chrysanthemum pot plants production. The standard was established by Horticulture unit, MARDI. Watering was done twice a day by using sprinkler system. At the bud stage, the water was sprayed manually at the base of plants in order to produce high quality of flowers. Organic and compound fertilizer (12:12:17:2 + TE) was commencing a week after transplanting at the rate of 5 g/pots and continuously every two weeks until the bud stage. Pest and diseases were controlled only when the symptoms of the attack occur.

Data Collection

Data on plant height were measured from the base to the tip of the highest shoot and all the floral buds with diameter $\geq$ 3 mm were counted. Meanwhile, data on flower colours were investigated and colour descriptions were made based on RHS (Royal Horticulture Society) colour chart at full bloom stage in the greenhouse under full sunlight. Data on leaves shapes mutation were described based on technical guideline for Plant Variety Protection, schema produced by Department of

Agriculture (DOA), Malaysia. Any difference in leaf shape of the plants was considered as mutation. The mutation frequency was determined as the number of plants with flower colour or leaf mutation divided by the number of screened plants (Yamaguchi et al., 2009).

RESULTS AND DISCUSSION

Radio sensitivity Test and Relative Biological Effectiveness (RBE)

In this study, radiosensitivity test was carried out to investigate the most effective dose for mutation induction of *Chrysanthemum morifolium* cv. 'Reagan Red' irradiated with ion beam using ray florets and nodal explants. The effects of ion beam on shoots regeneration from both types of explants were evaluated. The data on radiosensitivity tests are important in mutation breeding work and used as preliminary evaluations to determine plant's sensitivity towards radiation. This is because the radiation effects were found to be varied depending on explants, radiation types and doses (Zhou et al., 2006).

After 8 weeks of irradiation, survival rates for both explants decreased as the doses increased. Green calli and shoot formation for ray florets explants occurred at doses below 8.0 Gy. Meanwhile, the nodes were still survived after 5 weeks of irradiation at doses up to 10.0 Gy. Shoot regeneration were followed after 8 weeks of irradiation for doses from 0 to 5.0 Gy; nodes irradiated at doses 8.0 to 10.0 Gy, turned to purple and their axillary buds were dormant; whilst those irradiated at doses above than 15.0 Gy, turned to brown and all axillary buds were died. Based on these results, the graphs for the percentage of lethal dose and regeneration dose after irradiation of (a) ray florets explants and (b) nodal explants with ion beam were plotted and illustrated in Figure 1.

The graph clearly indicated that increasing the doses of ion beam, resulted in the decrease in survival and shoot regeneration rates. It was observed that 50% lethal dose (LD_{50}) of ray florets explants was 15.5 Gy and 20% of lethal dose (LD_{20}) was at 11.5 Gy. Since the main focus was on the optimal dose for shoot regeneration and the highest percentage of shoot regeneration for ray florets explants was at 60%. Therefore, we considered 50% of regeneration dose (RD_{50}) as the optimal dose for shoot regenerations which is at 2.0 Gy. For nodal explants, the LD_{50} was at 6.5 Gy and LD_{20} was at 4.0 Gy. Meanwhile, RD_{50} was at 4.0 Gy. Therefore, the optimal dose for *in vitro* mutation induction of *Chrysanthemum morifolium* cv. 'Reagan Red' nodal explants using ion beam was estimated between 3.5 to 4.0 Gy. Considering RD_{50} for both explants in order to calculate the RBE (Relative Biological Effectiveness); the RBE obtained was 2.0. These data indicated that ray florets explants were approximately 2.0 times more sensitive to ion beam irradiation than nodal explants.

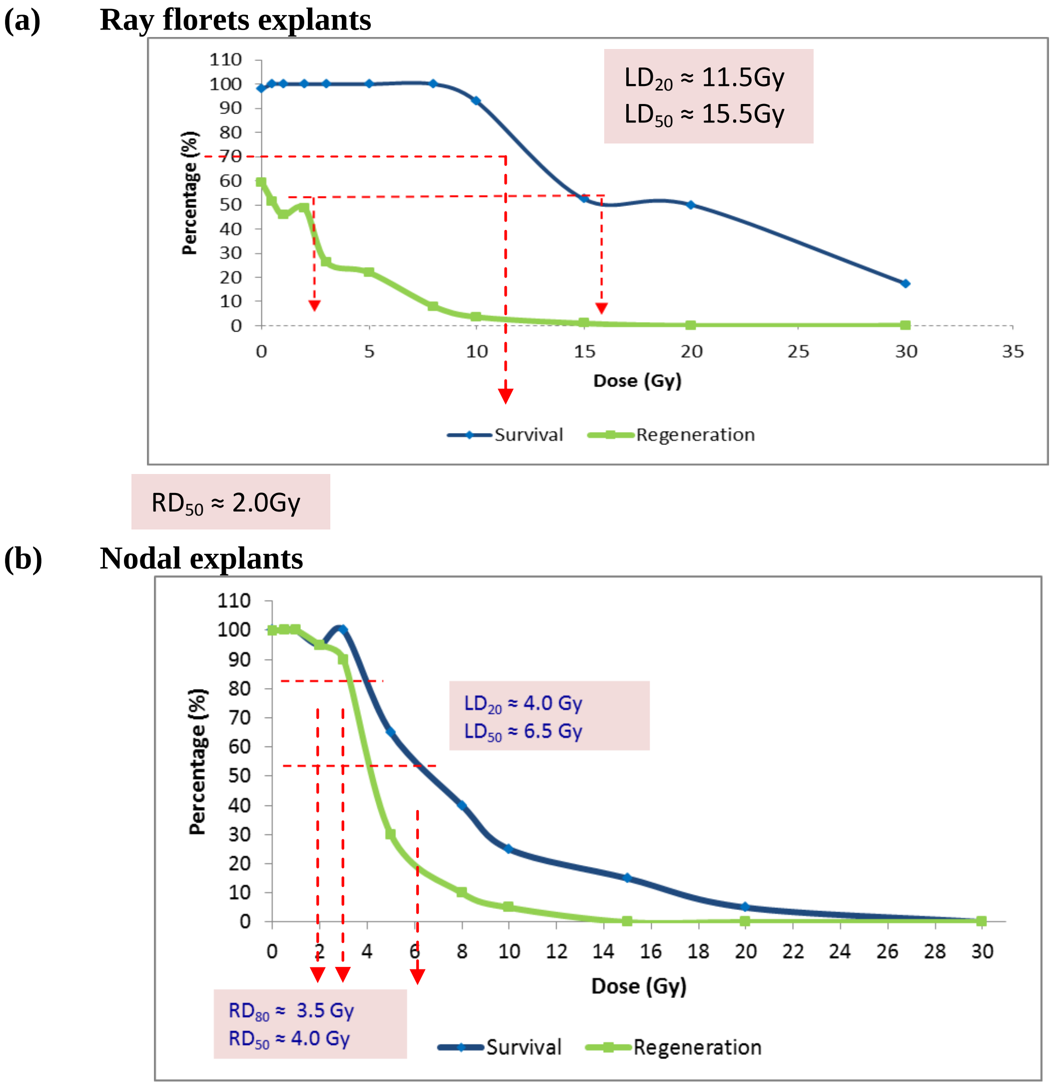

Figure 1: The percentage of survival and shoot regeneration for ray florets and nodal explants after 8 weeks of ion beam irradiation

Screening of Irradiated Plantlets

Plant height was measured two months after sowing the irradiated plantlet at MARDI, Cameron Highlands. Ion beam showed significant effect on plants that derived from irradiated ray florets explants and not significant for plants that derived from irradiated nodal explants (Figure 2). The height of plants that derived from irradiated ray florets explants was approximately two times higher than control at all ion beam doses (0.5, 1.0 and 2.0 Gy). Significant effect of ion beam in altering the plant height has also been reported in rice (Phanchaisri et al., 2007) and wheat (Chen et al., 2010). According to Chen et al. (2010), wheat mutants were taller than their control and suggested that ion beam could have altered the gibberellins' metabolism pathway by deactivating of GA 2-oxidases (GA2oxs) which is responsible for reducing the level of bioactive gibberellins in plants. Figure 3 exhibited number of flower bud produced for both type of irradiated explants. Ion beam significantly reduced number of flower buds produced for plants that derived from irradiated ray florets explants which is up to 71.8 % less at dose 0.5 Gy. Meanwhile for plant derived from irradiated nodal explants, the number of flower buds was increased up to 52.0 % at dose 3.0 Gy as compared to control plants.

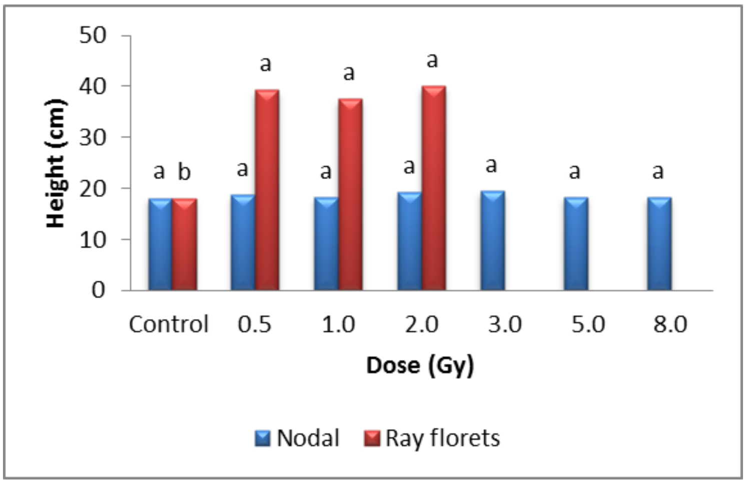

Figure 2: The height of plant derived from irradiated ray florets and nodal explants

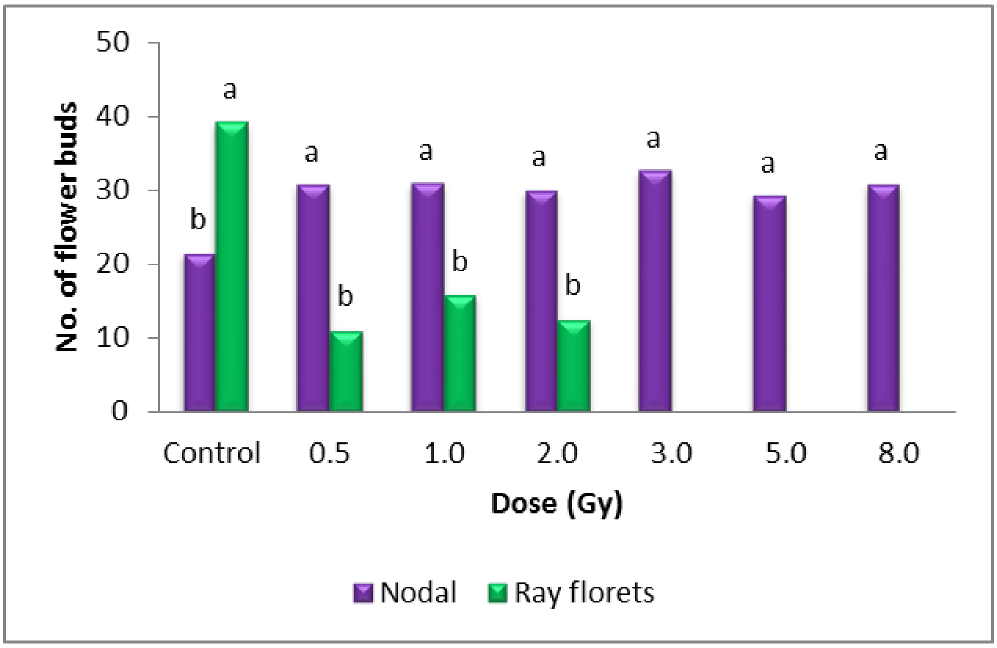

Figure 3: Number of flower bud produced for plant derived from irradiated ray florets and nodal explants

Referring to Figure 4, the frequencies of flower colour mutation for plant derived from irradiated ray florets explants were between 50 to 100 %. For irradiated nodal explants, the frequencies of flower colour mutation were between 38.3 to 58.8 %. This indicates that ray florets explants could generate higher flower colour mutation frequency than nodal explants. According to Nagatomi et al. (1995; 1997), the frequencies of flower colour and the multi colour mutation in chrysanthemum was increased several fold by culturing irradiated petals with ion beam rather than leaves, they also suggested that mutation rates of flower colour that has been induced by ion beam were approximately half of those induced by gamma ray in both floral petal and leaf. The success of flower colour mutation depends on various factors such as the right choice of physical mutagen, irradiation method, plants species and the explants types.

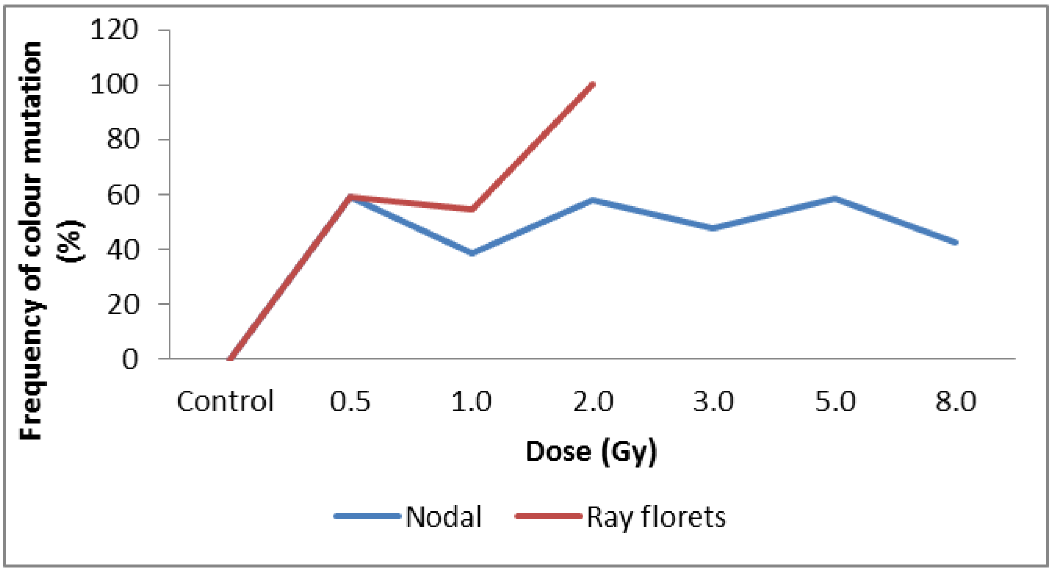

Figure 4: Flower colour mutation frequencies for plant derived from irradiated ray florets and nodal explants

CONCLUSIONS

In conclusion, the optimal doses for *in vitro* mutation induction of *Dendranthema grandiflora* cv. 'Red Reagan' using ray florets explants was at 2.0 Gy and for nodal explants was 4.0 Gy. The RBE (Relative Biological Effectiveness) for both explants types was estimated at 2.0; indicated that ray florets explants were approximately 2.0 times more sensitive to ion beam irradiation than nodal explants. The plant that derived from irradiated ray florets explants are taller than plant that derived from nodal explants. However, ion beam showed negative effect on number of flower buds for ray florets explants as compared to nodal explants. Ray florets explants also showed highest frequencies on flower colour mutation as compared to nodal explants. Thus, confirmed the results of radiosensitivity test study which is ray floret explants are more sensitive than nodal explants.

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