

The Effects of Gamma Irradiation on the Growth and Propagation of *In-Vitro* Chrysanthemum Shoot Explants (cv. *Yellow Puma*)

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ABSTRACT

The study on the effect of gamma irradiation on *in-vitro* shoot growth of chrysanthemum cv. *Yellow Puma* has been carried out. The aim of the study was to observe genetic variability of shoot growth caused by gamma irradiation. Shoot explants with four leaves were irradiated by gamma with dose of 10, 15 and 20 Gy with 3 replications at each of dose. The irradiated shoot explants were then transferred into fresh MS solid medium and placed in a growth room. Observation was performed on number of leaves and branches on M1V0 generation, while plantlets height and number of branches were observed a M1V1 generation. Number of survival plantlets and multiplication rate on three subsequent subcultures were observed as well. Results showed that gamma rays with dose of 20 Gy inhibited growth of leaves as much as 50% compared to control (shoots without irradiation), and branches 73.7% in three weeks. Observation on multiplication rate at M1V1 generation showed that gamma irradiation with dose of 10 Gy promoted multiplication rate as much as 10% higher than control. It can be concluded that *in vitro* mutagenesis using gamma irradiation with dose of 10 to 15 Gy can be used for inducing genetic variability of chrysanthemum cv. *Yellow Puma*.

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INTRODUCTION

Chrysanthemum (*Dendranthema grandiflora*) is one of the most important floricultural (cut-flower) and ornamental (pot and garden) crops in the world [1]. Chrysanthemum either as cut flowers or potted flowers is very attractive and has high demand in Indonesia, especially for celebration ceremonies. Attempts to introduce new types to domestic market are important to maintain chrysanthemum grower business [2], as consumers requesting more attractive new colors and types. The market trend in the country suggests that in the future, new chrysanthemum varieties will be required for having exotic color, size and shape of flowers.

Increasing demands to new forms of chrysanthemum leads to research for obtaining new varieties; one of the methods is through mutation techniques. Mutation techniques are used because chrysanthemum is a hexaploid plant and vegetative propagated, which makes it difficult to conduct hybridization. Performing mutation on an

established cultivar also can be chosen as an alternative method to increase genetic variability [3]. Mutation breeding is already an established method which hold large role in developing many ornamental plant mutants in flower shapes and colors [4]. De Jong and Custer [5] used mutation induction method on *in-vitro* chrysanthemum using physical mutagen (gamma rays) and observed modification in inflorescence and production of cultivar "White Spider". Yamaguchi [6] reported that the registered cultivars originated from mutation induction had reached a total number of 272 new cultivars in ornamental plants, 106 of them are chrysanthemums.

In breeding of vegetative propagated crops, vegetative generation advancing from M1V1 to M1V3 or more is inevitable. To shorten the period, tissue culture method as a tool for obtaining chimera-free mutant tissue was proven to be useful [7]. Pogany and Lineberger [8] stated that *in-vitro* culture has become more and more important propagation tool for ornamental plants in the last fifteen years. *In-vitro* culture allows the production of large numbers of plants from small pieces of the stock plant in relatively short periods of time. Lineberger [9] reported that depending on the

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species in question, the original tissue piece may be taken from shoot tip, leaf, lateral bud, stem or root tissue. A lot of study in *in-vitro* culture of chrysanthemum has been conducted, the most recent were using callus culture from leaf explants [10] and stem thin cell layer [1].

The objective of this experiment is to study the effects of irradiation on vegetative growth and propagation of *in-vitro* culture of chrysanthemum cv. *Yellow Puma* shoot explants.

EXPERIMENTAL METHODS

Shoot induction

The material used in this experiment was chrysanthemum cv. *Yellow Puma*. Flower bud from the field was washed in running tap water for ten minutes, then was surface sterilized with 40% Chlorox solution added by two drops of Tween 20, shaken for 20 minutes, then washed three times with sterilized-distilled water. The sterilized florets were grown on Petri dish containing MS medium [11], nicotinic acid (0.5%), pyridoxine-HCl (0.5mg/l), myo-inositol (100 mg/l), sucrose (30 gr/l), kinetin (1 mg/l), and NAA (0.02 mg/l). After two weeks, the floret base was swollen and multi buds emerged which would regenerate into new shoots. The shoots obtained then were propagated by tissue culture techniques.

Explants preparation

Uniform-length shoot explants (4 leaves-shoots) derived from tissue culture propagation explained above was then cultured in MS-modified medium. Two days later, those shoots were exposed to gamma irradiation using ^{60}Co of gamma chamber 4000A with doses of 0, 10, 15 and 20 Gy, respectively. The result of Broetjes *et al.* [12] experiment indicated that optimal gamma irradiation doses for chrysanthemum explants growth ranged between 10 and 20 Gy. Meanwhile, higher irradiation dose above 25 Gy for chrysanthemum *in-vitro* culture is known to be lethal [13].

Three replications were applied to each dose, and each replication consisted of 10 explants. After irradiation, the explants were transferred into fresh agar medium in growth room. Four weeks later, first subculture was carried out by transferring new grown shoots (size of shoots around 1 cm) into fresh agar medium. The second subculture was done in the following 4 weeks, with the same size of explants. The third subculture was prepared for

acclimatization on process by transferring the cultures into rooting medium.

RESULTS AND DISCUSSION

After gamma irradiation treatment, explants were placed on growth room with controlled light and temperature. Observation for the growth of shoot explants was carried out every week till three weeks, and effect of gamma irradiation into number of leaves and branches can be seen in the Fig.1 and 2. On the fourth week, subculture process was carried out by transferring the shoots onto fresh medium.

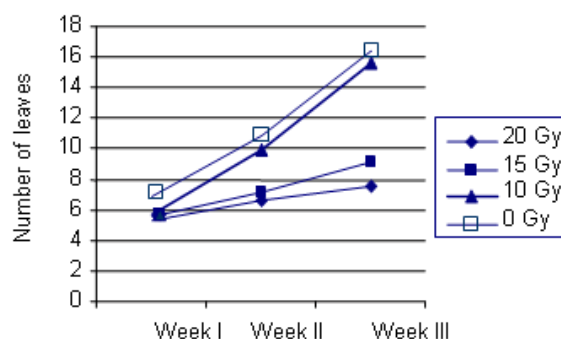


Fig. 1. Effects of gamma irradiation doses on number of leaves of chrysanthemum tissue culture.

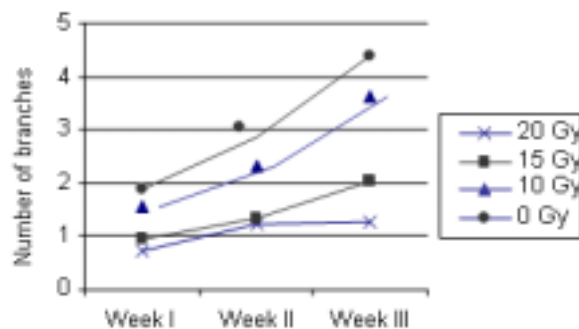


Fig. 2. Effects of gamma irradiation dose on number of branches of chrysanthemum tissue culture.

In the beginning of this experiment, every shoot explants had four leaves. Further development process, the number of leaves and branches increased continuously. In the first, second and third weeks, the leaf growths are varied among different doses (Fig 1), with average of 1.8 leaves, 2.8 leaves and 3.6 leaves per explants for the first, second and third respectively. The same trend happened in the number of branches, 1.3 branches in the first week, 1.9 branches in the second week and 2.8 branches in the third week. The number of branch from the first

week to the third week was raised 2 and 1.5 times respectively.

The first subculture was conducted at the fourth week past irradiation, it was noted that only one explant with 20 Gy doses changed the color into brown and died, while the rest of cultures were able to survive, and some of them occurred contamination. Number of shoot growth per explants was observed and result presented in Table 1.

Table 1. Observation on multiplication rate on the first sub culture

Irradiation dose (Gy)	Multiplication rate (shoots/explants)	Reduction of multiplication rate, (%)
0	6.89 ^a	-
10	3.91 ^b	43
15	3.79 ^b	45
20	1.76 ^c	74

Note: Similar letters in second column indicate that they were not significantly different from LSD₀₀₅ test.

The number of survival shoot irradiated explants with dose of 20 Gy decreased significantly (Table 1). The result on untreated explants was 6.89 shoots/explants. Statistical data analysis between control (untreated explants) and irradiated shoot was significantly different according to LSD test at level of 005. It was observed that on these two doses, many new shoots actually successfully regenerated, but later the shoot tips died or else failed to develop into perfect shoots. Instead they formed lumps of very small multi buds, which was difficult to be separated. Explants exposed by 20 Gy have the worst damage due to irradiation so that the number of shoots that successfully regenerated was very

low. On this dose, a small amount of shoots emerged died and some others formed lumps of very small shoots.

Twenty days after the first subculture, observation was carried out on shoot height and number of branches at M1V1 generation. The result of the observation was presented in Table 2.

Table 2. Observation on plantlet height and number of branches on M1V1 generation (20 days after subculture I)

Irradiation dose (Gy)	Plantlet height (cm)	Number of branches
0	1.61 ^a	2.04 ^{ab}
10	1.60 ^a	2.26 ^a
15	1.32 ^b	1.67 ^{ab}
20	1.17 ^b	1.20 ^b

Note: The same letters in the same column indicate that they were not significantly different by LSD₀₀₅ test.

The plant height derived from explants treated of M1V1 explants exposed by 10 Gy was not significantly different from that exposed by 0 Gy (non irradiated explant). The difference occurred on explants exposed by 15 and 20 Gy (Table 2). Meanwhile, 10 Gy dose explants showed the highest number of branches. Twenty (20) Gy of dose explants has the smallest number of branches (1.20), but the result still showed a significantly difference compared to that of untreated explants.

On the fourth week after first subculture, second subculture was done by transferring new shoots from M1V1 explants into fresh medium, which was the same as the medium used in the previous subculture. The third subculture, performed on the fourth week since the second subculture, was transferring new shoots into rooting medium. The data retrieved is shown in Table 3.

Table 3. Observation on the Second and Third Subculture of Chrysanthemum Shoot Explants

Irradiation dose (Gy)	Sub culture II (M1V2)			Sub culture III (M1V3)		
	Surviving plantlet (%)	Multiplication rate (shoots/explants)	Reduction of multiplication	Surviving plantlet (%)	Multiplication rate (shoots/explants)	Reduction of multiplication
0	93.89 ^a	4.42 ^{ab}	-	94.34 ^a	4.41 ^a	-
10	86.48 ^a	4.92 ^a	+13	78.00 ^b	4.91 ^{ab}	+13
15	55.50 ^b	3.71 ^{bc}	-16	66.96 ^b	3.83 ^{bc}	-14
20	32.12 ^c	3.03 ^c	-31	50.60 ^c	3.12 ^c	-29

Note: The same letters in the same column indicate that they were not significantly different by LSD₀₀₅ test

The percentage of surviving explants both in the second and the third subcultures decreased significantly along with the increase of irradiation dose (see Table 3). The multiplication rate of control and explants exposed by 10 Gy was the same as results reported by Carson and Leung [14] in experiment on *Leptinella nana* L. *in-vitro* culture, *i.e.* four to five explants per shoot multiplication rate, which was sub-cultured every 3 weeks on MS medium. The number of shoots produced on 10 Gy dose explants was a little higher (Fig. 3).



Fig. 3. The effect of irradiation doses on chrysanthemum explants growth (M1V0 generation) (left to right: 0, 10, 15 and 20 Gy).

Meanwhile on 15 and 20 Gy explants, multiplication rate was rather decreased, compared to control explants. Nevertheless, high dose explants (20 Gy) were able to regain the capability to produce shoots on the second and third subculture, eventhough not at the same rate as the untreated explants. On the first subculture (Table 1) multiplication rate on the dose of 20 Gy was only 1.76 shoots/explants, while on the second and third subcultures (Table 3) rose into 3.03 and 3.12 shoots/explants, respectively.

Growth of irradiated explants depressed when higher dose of gamma irradiation was treated. It was shown by stressed growth during the first cultures, both in number of leaves and number of branches produced. According to Carson and Leung [14], in average, number of leaves on the first shoot explants increased to 20 leaves after 17 days. The decrease of number of leaves and branches on chrysanthemum irradiated stem cuttings were also reported by Datta and Gupta [15], and Banerji and Datta [16]. It is assumed that the suppressed growth of explants tissue was caused by damage from irradiation treatment. It is noted that the number of leaves in irradiated plantlets was not reduced as much as number of branches, especially in low doses. This could be due to the development of buds which were more sensitive to the effect of gamma irradiation than the leaves development. The damage also intervene cell division and differentiation processes,

it made axillary's buds on leaves internodes which failed to develop. The higher doses given, the worse tissue damage will occurred. Normal growth was shown by control explants with no irradiation treatment.

The number of shoots in untreated explants higher than that of multiplication rate on the first subculture of *Leptinella nana* L. (Compositae family), which is five to six shoots every 3 weeks [14]. The difference is unusual because different species has different capability to produce shoots.

The observation result on plantlet height was similarly reported by Datta *et al.* [17], which concluded that plantlet height was decreased significantly on chrysanthemum plantlets given irradiation treatment according to the irradiation level function.

Otahola *et al.* [19] reported that dose of 20 Gy into explants, reduced the number of leaves growth per shoot around 50 % compared to untreated explants. In this experiment, 50% reduction was reached only in three weeks. This slightly different result might be due to various sensitivity of each cultivar to gamma irradiation. According to Tulmann [18], the difference in radiosensitivity varied among various part of plant, and greatly depends on physiological condition, irradiation time, and also pre- and post-irradiation condition.

On this M1V1 generation, it is assumed that physical damage from irradiation treatment has reduced, so that the phenotype alteration plantlet was due to mutation. During observation *viridis* and *albino* leaves were found from irradiated plant at dose of 10 Gy. The occurrence of *albino* and *viridis* showed that mutation in vegetative cells was caused by irradiation. [7].

During observation, it was observed that several leaves from irradiated chrysanthemum explants had morphological changes and become abnormal, blade-shape, round, dwarf, or shortened internodes rosette-like. These morphological changes were caused by mutation. Pogany and Lineberger [8] mentioned that the change or mutation occurred near the apical dome, the changes can be integrated into cell division sequence from that apical bud, but if mutation occurred in peripheral of meristem tissue where cell division rate was slow, the phenotype changed only on a sector of shoot, leaf, or part/segments of one leaf.

Datta *et al.* [17] reported that on chimerical plants, during continuous cell division, mutated cells competed with surrounding normal cells to survive. It is called diplontic selection. If those mutated cells successfully survived from selection, the change caused by mutation will be expressed on plants. The explants which were not able to survive would

eventually turn brown and died. The higher irradiation dose given, the rate of survival became lower (Table 3). It is assumed that the number of mutated cells were higher in high dose explants. Results of multiplication rate on 10 Gy explants were the highest, a little higher than control. The reason according to Otahola *et al.* [19] was because low dose irradiation has stimulator effect on regular natural growth.

The ability of chrysanthemum shoot explants to recover from cell damage caused by irradiation was regained after the second and third subcultures. In the first subculture, the plantlets were still carrying physiological damage from irradiation. But in the subsequent subculture, those effects were subsided and explants began to regain its viability. The physiological damage only happened to the "parent" explants which directly received the gamma irradiation treatment. Shoots derived from these explants were no longer bearing such damage and should be able to grow normally. This is shown by the chrysanthemum shoot explants which have the ability to recover from cell damage caused by irradiation in the subsequent subcultures. The abnormalities and lower survival rate observed in the M1V1 and other subsequent subcultures were originated from the occurred mutations.

Further research would be necessary to observe phenotype and performance of these mutant explants after acclimatization process in greenhouse.

CONCLUSIONS

The effect of gamma irradiation dose on chrysanthemum shoot explants in inducing genetic variability can be concluded that the first three weeks of post irradiation of explants occurred inhibition of shoot growth included leaves and branches more than 50%. While on subsequent generation (M1V1) showed that irradiated explants with dose of 10 Gy promoted multiplication rate of chrysanthemum compared to control. This situation was common phenomenon occurred on *in vitro* mutagenesis in plant. Gamma irradiation dose of 10 to 15 Gy was found to be the appropriate dose to obtain genetic variability for chrysanthemum because of low lethality of the cells.

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