INDUCED MUTATION OF CHRYSANT (*Dendranthema grandiflora* Tzvelev) THROUGH PLANTLET IRRADIATION

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Abstract

This research was conducted for the purpose of obtaining a novel variety of chrysant through the plantlet gamma irradiation. The treatment was given to seven local cultivars which had been released by Center of Research Institute for Ornamental Plants, Jakarta: cv. Saraswati, Sekartaji, Dewi Sartika, Kartini, Chandra Kirana, Larasati and Cut Nyak Dien. The gamma rays was twice irradiated to the plants : first irradiation used eight levels of gamma dose : 0, 10, 12.5, 20, 25, 30, 35 and 40 gray, and second irradiation used 15 gray for all plantlets that still survived in subcultured media. Gamma irradiation treatment inhibited or changed the growth and development of chrysant. These were shown by the mean of some vegetative and generative characters observed, compared to untreated plants. Each cultivar showed different responses to the irradiation, including variation of flower shape and colour of petals. Of eight irradiation doses, dose 10, 12.5, and 20 gray could induced the most change of flower shape and colour. This change indicated the genetic distortion caused by irradiation mutation. The LD_{50} *range for these chrysant cultivars is between 19.55 Gy to 23.55 Gy.*

Key words : Mutation, gamma irradiation, dose rate, LD_{50}

Introduction

Chrysant (*Dendranthema grandiflora* Tzvelev) is one of the most popular ornamental plant which is long time cultivated for commercial purpose : as cut flowers, potted plant, border plant or bedding plant. In Indonesia, the demand for chrysant tends to increase. Based on statistical data of Indonesian Horticulture, the demand for chrysant increase from 9 284 700 stems in 1996 to 12 220 800 stems in 1999 (Center of Research and Development for Horticulture, 1999).

A lot of obstacle faced in ornamentals cultivation. For chrysant, the most important problem is the scarcity of high quality seed/ seedling. Imported seed (or imported cutting) will become very expensive when the rupiah is depreciated. We therefore decided to create our own cultivar, which is originally bred by Indonesian breeder.

Mutations are the ultimate source of all variability in organisms. Variations caused by induced mutations is not essentially different from variability caused by spontaneous mutations during evolution. The direct use of mutation is a very valuable supplementary approach to plant breeding, particularly when it is desired to improve one or two easily identifiable characteristics in an otherwise well-adapted variety (Ibrahim, 2000).

Irradiation technique was chosen because this application enable us to create a new variety in relatively short time. Besides, since chrysant is vegetatively propagated crop, there is no anxiety due to genetic segregation when stabil mutant is obtained. Gamma irradiation was very useful to obtain novel variety in relatively short time. It has been reported that Nagatomi had succeeded to create 473 cultivars of chrysant through irradiation technique (Qosim *et al*., 2000).

The objectives of this study were (1) to observe the respons of chrysant cultivars to gamma irradiation and (2) to investigate LD_{50} for each chrysant cultivars

Materials and Methods

The research was conducted in tissue culture laboratory and green house of INLITHI, Cipanas, West Java (1100 m above sea level) and laboratory of Isotop and Radiation, BATAN, Jakarta, started from September 1999 untul September 2000.

Seven local cultivars of chrysant were used : cv. Saraswati, Sekartaji, Dewi Sartika, Kartini, Chandra Kirana, Larasati and Cut Nyak Dien. Gamma rays derived from Cobalt 60 through iradiator gamma chamber 4000A. Propagated media for tissue culture was Murashige and Skoog (MS) media, which was added by 0.1 mg/l IAA in subsequent subculture media. Media for acclimatisation utilized decayed bamboo leaf, whereas for potted media utilized soil : bamboo leaf : manure = 2 : 2 : 1.

The study used factorial randomized complete design, with two factors. The first factor is seven varieties of chrysant, and second factor is irradiation dose rate. Each experimental unit consist of 5 plants, so that all treatment covered 1440 plants.

Explant was taken from axillary bud which was sterilized by 70% alcohol, and then planted on MS media for 30 days. Gamma irradiation was applied twice. First irradiation was given for eight dose rates : 0, 10, 12.5, 20, 25, 20, 25, and 40 Gy. Second irradiation was given for only 15 gray, applied to all plantlets that still survived from first irradiation treatment. The survivors were those obtained 0, 10, 12.5 and 20 gray of gamma rays.

Irradiated plantlet were subcultures in MS media which contained 0.1 mg/l IAA (Indole-acetic acid). Each explant planted has one axillary bud only. All subculture plantlets were stored in tissue culture laboratory for eight weeks.

After that, all plantlets were acclimatized in green house for two weeks, utilizing decay bamboo leaf as media. Growing plantlets were replanted in 23 cm polybag, with the media of soil : bamboo leaf : manure = 2 : 2 : 1. All plants were maintained for 38 days in long day period, manipulated by artificial light given for 4 hours per day. NPK used for fertilizing plants with the dose 2 g/plant, given in 2, 4, and 8 week after planting. Pesticide used as required.

Observation was in both laboratory and green house for following characters :
(1) percentage of plant death after gamma irradiation, (2) plant height (cm), (3) total internodes, (4) leaf width, (5) total flower, (6) diameter of flower (cm), (7) diameter of petiole (mm), (8) frequency of mutation, and (9) Distortion of vegetative and or generative morphology.

Results and Discussion

Observation in Laboratory

Irradiated plantlet exhibited different respons, depend on cultivars. It was indicated by percentage of plantlet death (Table 1).

Table 1. Percentage of Plantlet Death of Several Chrysant Cultivars on Different Dose Rate of Gamma Irradiation

Dose rate (gray)	Cultivar						
	Saraswati	Sekartaji	Cut Nyak Dien	Dewi Sartika	Kartini	Chandra Kirana	Larasati
0	0	0	0	0	0	0	0
10	0	0	0	0	0	0	0
12.5	0	0	0	0	0	0	0
20	70	60	60	60	58.3	43.3	56
25	86.7	80	85	85	92.7	72.9	78.8
30	95.6	86.7	88.9	90	95	76	86.6
35	100	96.7	95	91.4	100	80	95
40	100	100	100	100	100	85	96

The growth of plantlet after irradiation of 0, 10 and 12.5 gray looked normal. It began to inhibit the plantlet growth on 20 to 40 gray, ended by the death of plantlet. It is proposed that the plantlet death was related to the cell/ tissue damage resulted by the increase of irradiation dose rate. Presumably, the high dose rate would destroy cells because the energy irradiated by gamma rays quite powerfull to create a high penetration capability. Another research done by Fereol (1996) showed that dose rate above 15 gray for *Alpinia purpurata* decrease the rate of survival.

Based on Table 1, the relation between irradiation dose rate and percentage of plant death for each cultivar follow equation of linear regression. From these data, LD_{50} (Lethal Dose 50, the dose when 50% of population is lethal) could be obtained (Table 2.)

Table 2. Equation of regression to Determine LD_{50} on Chrysant Cultivars

variety	Equation of regresion	Dose rate (gray)
Saraswati	$Y = 3.204x - 13.558$	19.55
Sekartaji	$Y = 3.151x – 15.033$	20.63
Cut Nyak Dien	$Y = 3.162x – 14.572$	20.42
Dewi Sartika	$Y = 3.132x – 14.232$	20.50
Kartini	$Y = 3.277x – 14.905$	19.80
Chandra Kirana	$Y = 2.694x – 13.436$	23.55
Larasati	$Y = 3.079x – 14.816$	21.05

Table 2. shows some differents values of LD_{50} for each cultivar. LD_{50} for these seven cultivars lay between 19.55 gray to 23.55 gray. Cv. 'Saraswati' and 'kartini' are the most intolerant cultivars to gamma rays.

Observation in the Green House

Compared to untreated plants, the growth and development of irradiated chrysant in green house found inhibited. The irradiation could cause some physiological disruption led to cells damage in plant tissues. Soeminto (1985) stated that irradiation causing damage through two ways : (1) direct radiation and (2) indirect radiation. Direct radiation resulted on destroyed cells become useless fragments, while the effect of indirect radiation on cell molecules resulted ionization and produced radicals (OH^-). These radicals will form peroxide (H_2O_2) which play a role in cell damages.

The interaction between cultivar and dose rate was significant for plant height, total internode and leaf width (vegetative parameters), total flower, diameter of flower, and diameter of petiole (generative parameters).

The height of irradiated plant tends to be lower than unirradiated plant. This phenomena gave evidence of the growth and development inhibition of apical meristematic cells. Wattimena (1992) proposed this inhibition was related to cellular damage on apical meristem. This damage is caused by the reduction of internal auxin (IAA) which was led to the plant height inhibition. But this inhibition was different from one cultivar to another. For instance, 10 gy gamma irradiation on cv 'Dewi Sartika' , 'Kartini', 'Larasati' and 'Chandra Kirana' reduced the plant height, but dose 12.5 gy even increase the height. Finally, 20 gy gamma irradiation would again decrease the plant height.

Total internode of cv. 'sekartaji', 'Dewi Sartika' and 'Kartini' decreased by increasing dose rate; Vice versa, total internode of cv. 'Saraswati', 'Cut Nyak Dien' and 'Larasati' tend to increase by increasing dose rate. There was also a tendency to reduce leaf width by increasing the dose rate.

Some of vegetative parameters (plant height, total internode, and leaf width) which were irradiated by gamma rays exhibited low value compared to unirradiated plants. This was presumably occurred by physiological disruption that obstructed the growth and development of plant. According to Kusumo (1989), ionized treatment from irradiation coud shortened roots and reduced total amount of roots. Therefore, the absorption of nutrition from the soil will be also reduced.

Irradiation treatment reduced the total amount of flower on almost all cultivars, except cv 'Dewi Sartika' and 'Kartini', whereas toward the diameter of petiole, it caused reduction in cv 'Kartini' only. It is presumably caused by the low accumulation of photosynthesis product during vegetative period, regarding to reduction of nutrition absorption. Marwoto (1999) stated that flowering time is related to the length of this accumulation period needed to alter physiological status from vegetative to generative phase. Flower induction need a quite high availability of energy, whereas each cultivar has their own ability to accumulate the photosynthesis product differently, which is genetically influenced.

Distortion on Vegetative and Generative Morphology of Chrysant

Some variegated leaves were found during observation (23%). Suppose, it was caused by chlorophyl deficiency on the leaves. According Wagner and Herscheel (1964) the deficiency on barley mutant was related to the activity of catalase enzyme in determining the ratio of plant pigment. Albino barley showed lower activity of catalase compared to normal barley. But the white colour would change to green colour again.

Ibrahim (2000) stated that irradiated cells will change in the unicelluler or multicelluler form to certain situation which is known as term “diplontic selection”. This phenomena explains the situation where mutated cells compete against normal cells in the vicinity. The mutated cells will establish a group, forming a tissue layer or even create a new organ. But, if the mutated cells are not survive in competition, they will be selected (lost) and the normal cell could be expressed again.

Change of flower colour or flower size take place in following cultivars :

(1) ‘Saraswati’ : light yellow to dark yellow, decreased in diameter at dose 12.5 gray
(2) ‘Kartini’ : dark red become yellowish red, decreased in diameter, and petal shape become ‘spoon-like’ petal, at dose 12.5 and 20 gray ,
(3) ‘Cut Nyak Dien’ : white to yellow at dose 12.5 gy
(4) ‘Chandra Kirana’ : white to yellow at dose 10 gy and 20 gy
(5) ‘Dewi Sartika’ : purple to white with pink spots, and reduced diameter at 12.5 gy

According to Davies and Kathy (*in* Geneve *et al*., 1997), the main pigment that controlled colour flower is flavonoid, carotenoid and betalains. The role of flavonoid is to produce pink, red, purple, blue and yellow colour. Carotenoid play role in producing yellow, dark pink and red colour, whereas betalains produce yellow, dark pink, red and purple. These three pigments influence the flower colour by the proportion of them in plant.

According to Ridwan *et al*. (1978) radiation causes molecular alteration, continued by biochemical damage and physiological effect, led to mutation in organisms. Physiological effect on flower usually happen to change the synthesis of flower pigment, that eventually causing the change on flower colour. Reducing anthocyanin in chrysant will change flower colour from pink to white. When the carotenoid increased, the flower colour will be easily change from white to yellow. The characteristic of flower colour is usually controlled by single dominant gene which give white and pink, while the recessive alel give yellow and dark pink colour. De Jong (1964, *in* Sanjaya, 1994) said that induced radiation could alter flower colour through this scheme :

$$\begin{tikzcd}
\text{Pink colour} \arrow[r] \arrow[d] & \text{red colour} \arrow[d] \\
\text{White colour} \arrow[r] & \text{yellow colour}
\end{tikzcd}$$

Based on the scheme above, the yellow colour of petals indicate the last colour, so that the opportunity to alter is limited.

Conclusion

Gamma irradiation inhibited and or altered the growth and development of chrysant. Each variety gave different respons to irradiation dose rate. LD_{50} for these seven cultivars lay between 19.55 gray to 23.55 gray. Amongst cultivars observed, cv. 'Saraswati' and cv. 'kartini' are the most intolerant cultivars to gamma rays.

Irradiation treatment created variability in colour and shape of flower. Of eight irradiation doses, dose 10, 12.5, and 20 gray could induced the most change of flower shape and colour. This change indicated the genetic distortion caused by irradiation mutation.

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