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CALLUS REGENERATION OF *CHRYSANTHEMUM* AFTER GAMMA RAY IRRADIATION FOR THE RESILIENCE OF MEDIUM PLAIN

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Abstract

Development of chrysanthemum plants in the medium plains is still facing obstacles due to unfavorable climate. Until today, the availability of tolerant chrysanthemum seeds grown in the medium plains is not maximized so that the necessary effort in order to increase the genetic diversity as the material selection to obtain tolerant chrysanthemums grown in medium plain.

This research is being conducted in order to follow up the problems of availability of tolerant chrysanthemums seeds grown in the medium plains at several stages. Stage one is the induction of callus after gamma ray irradiation in vitro. This research has been conducted in tissue culture laboratory of the Faculty of Agriculture UPN "Veteran" Yogyakarta from February to June 2015. Tested regeneration media is ½ MS with the addition of IAA 0.1 mg/l; 0.2 mg/l; 0.3 mg/l; 0.4 mg/l and 0.5 mg/l.

The results showed that ½MS regeneration media with the addition of IAA 0.3 mg/l spur sprouts emerge percentage (100%); while growing sprouts (8.67 days), height are about sprouts (3:10 cm) and number of sprout (7:00). Regeneration medium Treatment with kinetin 2 mg/l + IAA 0.5 mg/l gives the number of root growth (13) and root length (4:07 cm)

Keywords: Chrysanthemum, tolerant medium plains, in vitro selection, gamma ray irradiation.

Introduction

Chrysanthemum is one of the most important floriculture commodities in Indonesia and continues to be an enhanced production. One disadvantage of chrysanthemum cultivation in Indonesia is climate limitations. Chrysanthemums are derived from subtropical regions and can live in Indonesia only in the highlands area.

Medium-heighted plains (500-800 m asl) can be planted chrysanthemums but having a low-quality flowers. It is becoming a limiting medium for the plain chrysanthemum seeds, forcing farmers during planting seeds to move onto the highlands. As a result, the plant growth less than its maximum, it merely susceptible to pests and diseases and also the quality of the flowers is not that good compared to the the plant grow in maximum level.

The availability of tolerant chrysanthemum seeds in medium plain that needs to be done has not been affected in increasing the genetic diversity that is available in genetic material as the material selection to get chrysanthemum tolerant in medium plain area. (19 ; 5) .

To be able to cope with the availability of tolerant chrysanthemum

seeds in medium plain, genetic improvement using irradiation is needed to be conducted to obtain mutants with the potential to be developed (6). Sisworo et al (20) stated that in vitro mutagenesis can be applied to a large number of plant material and time required to get new variants faster than ex vitro mutagenesis. (18) There are two types of mutagens that can be used for breeding mutations , ie radiation and chemical compounds. Irradiation with radiation include X-rays, gamma rays and ultraviolet light. While a chemical compound that can be used as mutagens include EMS (Ethyl Methyl Sulfanat), NMH (N - methyl - N - nitrosourea), NTG (Nitrosoguanidine) and colchicines.

Gamma rays can penetrate the ionization cause of materials, including living tissue through the cells and ionize molecules within cells, causing mutations . According to Broetjes and Van Harten cit (11), cells were exposed to radiation will produce: normal cells, cells that have mutations, chromosomal damage or cell death. Research conducted by Lestari (18 ; 9), the use of a dose of gamma rays 25 gy managed to lengthen the roots of potatoes. Likewise, the studies of (11) to get the mutant rice with very good growth rate.

Indonesia is now produces superior chrysanthemum varieties in its highland area. Chrysanthemum varieties excel in Indonesia, namely Puspita Nusantara which is widely cultivated in the highlands and medium plains. There is also Sakuntala which is the standard chrysanthemum flowers. The last IOCRI (Ornamental Plant Research Institute) issue released chrysanthemum named Sasikirana and Kusumaswasti All types of chrysanthemums are grown forced in the medium plains, so the quality of the flower is not good. The flower having a fade color, less in diameter and plant height was not optimal .

In order to overcome the problem of seeds in medium plain , the gamma rays irradiation seemed to be properly conducted. Some studies using gamma radiation is able to overcome the limitations of genetic variability, and somehow it can obatain a new varieties as expected. Research (21), generating genetic variability among populations of orchids in nature of *Phamabilis (L.) Blume*. Otherwise (22), produces Sorghum genotypes that resistant to dry land acidity. The propagation of the chrysanthemum after irradiated by gamma rays in vitro has several advantages when compared with conventional propagation. It is quickly produce in large quantities and not depend on the season. With this multiplication that expected in a short time, we will produce the plants in large quantities .

Materials and Method

Materials used as the explant is chrysanthemum callus having a size of 0.5-1 cm. The bottles contain gamma ray irradiated explants at various doses. Then planted in the media, followed by a test using PEG and sprout regeneration phase. Sprout regeneration medium used is ½ Murashige and Skoog medium (MS) supplemented with hormone kinetin 2 mg/l and auxin IAA appropriate treatment , ie 0.1 mg/lmg/l ; 0.2 mg/lmg/l ; 0.3 mg/lmg/l ; 0.4 mg/lmg/l ; and 0.5 mg/lmg/l.Research is conducted by using laboratory experiments with a completely randomized design (CRD), which was repeated three times with each treatment consisting of 10 bottles and each bottle contains 2 explants. Explantswere put into culture bottles then sealed with aluminum foil and then stored

in the incubation room with temperature of 24 degree centigrade with irradiation intensity of 16 hours per day. Maintenance performed until 12 weeks old plants.

Result and Discussion

The most decisive stage of success of this study is to grow plantlets on regeneration media. Balance growth regulator auxin and cytokinin in particular contained in the media plays an important role in determining the direction of a tissue culture (4 ; 13). Balance concentrations of each growth regulator is determined by the type of explants were used, and this can be seen in table 1 in the percentage of live explants. The same to (9) were declared successful in vitro culture of explants and composition is influenced by the media, the composition of auxin and cytokinin in the growth medium. Induction of sprouts or the roots of callus generally requires a balance between these two mediums so that they can interact with each other (3 , 5). In the treatment of Kinetin ½ MS + 2mg/l + 1AA 0.3 mg/l the percentage of sprouts that appear is perfect (100 %) .

Table 1. The mean percentage of live explants and sprout callus percentage appear after gamma ray irradiated grown on regeneration medium

Media regeneration	The percentage of live explants (%)	Percentage emerging sprouts (%)
T1: ½ MS+Kinetin 2mg/l+IAA 0,1 mg/l	97,56 a	50,67 **bc**
T2: ½ MS+Kinetin 2mg/l+IAA 0,2 mg/l	98,11 a	96,37 **b**
T3: ½ MS+Kinetin 2mg/l+IAA 0,3 mg/l	100,00 a	100,00 **a**
T4: ½ MS+Kinetin 2mg/l+IAA 0,4 mg/l	100,00 a	30,00 **c**
T5: ½ MS+Kinetin 2mg/l+IAA 0,5 mg/l	99,37 a	26,45 **c**

Description : The mean treatment followed by the same letter show no significant difference in UJBD the real level of 5 %

According to (4), plant growth and morphogenesis in vitro are controlled by the interaction and balance between regulating substances given into the medium and regulating substances produced endogenously by the cultured cells .

Table 2. Cultivation mean time of Growing Sprout (day) , height Sprout (cm) and Total Sprout of callus chrysanthemum resulted by gamma ray irradiation grown on regeneration medium

Media regeneration	Sprout growing moment	Plant height (cm)	Total plant

	(days)		
T1: MS+Kinetin 2mg/l+IAA 0,1 mg/l	15,27 b	6,59 c	2,33 c
T2: MS+Kinetin 2mg/l+IAA 0,2 mg/l	15,67 b	7,44 b	2,82 bc
T3: MS+Kinetin 2mgl+/IAA 0,3 mg/l	9,33 c	11,17 a	15,11 a
T4: MS+Kinetin 2mg/l+IAA 0,4 mg/l	14,11 b	11,00 ab	8,67 b
T5: MS+Kinetin 2mgl+/IAA 0,5 mg/l	20,56 a	7,87 ab	2,54 c

Description : The mean treatment followed by the same letter show no significant difference in UJBD the real level of 5 %

Table 2 shows the influence of auxin in the parameters observed, the number of sprouts (7) and is currently growing fast sprouts (9.33 days). This is presumably because the composition of salts mineral that exist in the media are optimal for the growth of yellow banana plantlets. Nitrogen is the element needed by plants in large amounts to stimulate the growth of plant height there in large numbers, media MS reached 840.6763 mg/l. Presence of organic carbon is expected to increase the activity of cell division under the apical meristems, which will be followed by the stage of cell division and cell elongation enlargement. The addition of these measures will increase the plant height. In the other hand, the growth regulators kinetin at 5 mg/l is playing a very high role in triggering the growth of sprouts. Kinetin role in stimulating the growth of sprouts is important especially for the regulation of cell division and morphogenesis (4).

Cytokinins either single factor or combination with auxin in tissue culture plays a role in inducing and multiplication of sprouts. (Figure 1). (2) found in the callus tissue formed roots and sprouts can complete on their own at a time together without a vascular connection between them

Figure 1. The development of plantlets on regeneration media T3: MS+Kinetin 2mgl+/IAA 0,3 mg/l

Figure 1 also shows the addition IAA up at a concentration of 0.3 mg/l in media containing kinetin can increase the number of planlet. This suggests that the

interaction and balance between growth regulator substances given in the media and which is produced by cells endogenously is determine the development of a culture(15). At the IAA concentration of 0.4 mg/l and 0.5 mg/l turns out the number of sprouts decreased and it can be said that at a certain point, increasing concentrations of auxin would likely result the decline in the number of sprouts formed. Allegedly, the addition of IAA with relatively high concentrations has become toxic, resulting in decrease in the number of sprouts formed and impaired growth. Research (12), stated that the presence of auxin can be antagonistic to the activity of cytokines, where the presence of cytokines from outside the endogenous cytokines lead to the disintegration and decomposition in line with the increase in the addition of auxin .

One role of auxin in the process of tissue culture is inducing adventitious roots in explant (24). Total root is essential for the growth of explants in vitro. Number of growing roots and the longer the better for the absorption of nutrients from the media. This is because the more and the longer the absorption of nutrients from the roots of the field, the greater the root medium (20). In the T4 treatment (½ MS : Kinetin 2 mg/l + 1AA 0.4 mg/l) it produces the highest number of roots (12.11) and T5 (½ MS : Kinetin 2 mg/l + 1AA 0.5 mg/l) longest root length (4.67 cm) compared to other treatments. The roots of this research are formed directly on the base or derived from explants. At first, it colored yellow-white roots and after experiencing growth the color will change to green .

Table 3. The mean number of chrysanthemum callus roots and root length after gamma ray irradiation results grown on regeneration medium

Treatments	Total Root	Root length (cm)
T1: MS+Kkinetin 2mg/l+IAA 0,1 mg/l	2,67 **c**	1,33 **d**
T2: MS+Kinetin 2mg/l+IAA 0,2 mg/l	5,46 **c**	2,56 **c**
T3: MS+Kinetin 2mg/l+IAA 0,3 mg/l	8,99 **b**	3,87 **b**
T4: MS+Kinetin 2mg/l+IAA 0,4 mg/l	12,11 a	4,33 **b**
T5: MS+Kinetin 2mg/l+IAA 0,5 mg/l	10,00 **b**	4,67 **a**

Description : The mean treatment followed by the same letter show no significant difference in UJBD the real level of 5 %

In table 3 it can be seen that the higher the concentration 1AA given then the longer roots will be formed. This is in accordance with the opinion (4) that auxin

(IAA) play a role in the formation of roots thus also play a role in root elongation in tissue culture. Instead cytokinins needed in small amounts, there is the possibility of cytokinin requirement for the purposes of root elongation of endogenous cytokines existed. This is in accordance with the opinion (7), that the use of cytokines in small amounts to help the formation of roots, while the roots that have formed will synthesize endogenous cytokines. The use of Kinetin and IAA as a catalyst of growth of sprouts and roots of irradiated chrysanthemum plants can save energy resources and natural resources for endurance testing time faster than the convensional way, time to sprout and regenerate cells faster. Allegedly, the IAA (auxin) also causes the cell walls become loose, then epidermal cells quickly become elongated, and cell subepidermis attached to it also extends, so that the roots will grow longer. Multiply and chrysanthemum seedlings root length will support in the absorption of nutrients, thus affecting the growth of the plant.

Conclusion

Media regeneration ½ MS and kinetin 2 mg by the addition of auxin IAA 0.3 mg/l spur growth in the number of sprouts, while growing sprouts, and the percentage of emerging sprout. To stimulate root growth, the regeneration medium should be increased IAAnya to 0.4 mg/l .

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