REGENERATION CALLUS OF *Chrysanthemum* AFTER IRRADIATED RAY GAMMA FOR THE RESILIENCE OF PLAIN MEDIUM

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Abstract

Development of chrysanthemum plants in the plains of the medium is still facing obstacles due to unfavorable climate. Until today the availability of seeds tolerant chrysanthemum grown in the plains of the medium is not maximized so that the necessary effort in order to increase the genetic diversity available genetic material as the material selection to obtain tolerant chrysanthemums grown in plain medium. Research in order to follow up the problems of availability of seeds tolerant chrysanthemums grown in the plains of the medium has been conducted by researchers at several stages, one of which is the induction of callus after gamma ray irradiation in vitro. Research has been conducted in tissue culture laboratory of the Faculty of Agriculture UPN "Veteran" Yogyakarta from February to June 2015. Media regeneration tested 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 ½MS regeneration media with the addition of IAA 0.3 mg / l spur shoots emerge percentage (100%); while growing shoots (8.67 days), high shoots (3:10 cm) and number of buds (7:00). Treatment regeneration medium 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 plains medium, in vitro selection, gamma ray irradiation,

INTRODUCTION

Chrysanthemum is one of the most important floriculture commodities in Indonesia and continues to be enhanced production. One disadvantage of chrysanthemum cultivation in Indonesia is climate limitations . Chrysanthemums are derived from subtropical regions can live in Indonesia only in the highlands alone . Medium plains (500-800 m asl) can be planted chrysanthemums , but low-quality flowers . It is becoming a limiting medium is the plain chrysanthemum seeds, forcing farmers during planting seeds to the highlands . As a result, plant growth less than the maximum , susceptible to pests and diseases and the quality of the flowers is not good . Availability of seeds tolerant chrysanthemum in plain medium that needs to be done has not been much effort to increase the genetic diversity that is

available genetic material as the material selection to get chrysanthemum tolerant in plain medium. (19 ; 5) .

To be able to cope with the availability of seeds tolerant chrysanthemum in plain medium , is the genetic improvement using irradiation 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 cause ionization of materials , including living tissue through the cells and ionize molecules within cells , causing mutations . According 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, studies of (11) to get the mutant rice with very good growth .

Indonesia now produces superior chrysanthemum varieties for highlands . Chrysanthemum varieties excel in Indonesia , namely Puspita Nusantara which is widely cultivated in the highlands and plains medium. There is also Sakuntala which is the standard of chrysanthemum flowers . Last IOCRI (Ornamental Plant Research Institute) released chrysanthemum Sasikirana , Kusumaswasti . All types are grown in the plains forced the medium so the quality is not good flower . Flower color fade , less than the maximum diameter of the flower and plant height was not optimal .

In order to overcome the problem of seeds in plain medium is irradiated with gamma rays . Some studies using gamma radiation is able to overcome the limitations of genetic variability , and lets get new varieties as expected . Research (21) generating genetic variability among populations of orchids in nature Ph amabilis (L.) Blume . Likewise (22) produces Sorghum genotypes resistant to dry land sour . Propagation chrysanthemum after diirasiasi gamma rays in vitro has several advantages when compared with conventional propagation is quickly produce in large quantities and do not depend on the season . With this multiplication is expected in a short time we will get the plants in large quantities .

MATERIALS AND METHODS

Materials used as explant is chrysanthemum callus having a size of 0.5-1 cm . The bottles contain gamma irradiated explants at various doses . Then planted in the media , followed by a test using PEG and shoot regeneration phase . Shoot regeneration medium used is ½ Murashige and Skoog medium (MS) supplemented hormone kinetin 2 mg / l and auxin IAA appropriate treatment , ie 0.1 mg / l ; 0.2 mg / l ; 0.3 mg / l ; 0.4 mg / l ; and 0.5 mg / l . Research 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 . Explant explants were put into culture bottles then sealed with aluminum foil and then stored in the incubation room temperature 24 C. with irradiation intensity of 16 hours per day . Maintenance performed until 12 weeks old plants .

RESULTS AND DISCUSSION

The most decisive stage of the 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 . This can be seen in table 1 in the percentage of live explants turns on all treatments all explants high. The same to (9) were declared successful in vitro culture of explants and composition is influenced by the media , namely the composition of auxin and cytokinin in the growth medium.Induction of shoots or the roots of callus generally requires a balance between the two 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 shoots that appear at most (100 %) .

Table 1. The mean percentage of live explants and callus percentage chrysanthemum buds appear gamma irradiated grown on regeneration medium

Media regeneration	The percentage of live explants (%)	Percentage emerging shoots (%)
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 is controlled by the interaction and balance between regulating substances given into the medium and regulating substances produced endogenously by the cells are cultured .

Table 2. Mean When Growing Tunas (day) , High Tunas (cm) and Total Tunas callus chrysanthemum result gamma ray irradiation grown on regeneration medium

Media regeneration	Growing moment shoot (days)	Plant hight (cm)	Total plant
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 shoots (7) and is currently the fastest growing shoots (9.33 days). This is presumably because the composition of mineral salts that exist in the media are optimal for the growth of banana plantlets yellow. 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. Besides the growth regulators kinetin at 5 mg / l is very high role in triggering the growth of shoots. Kinetin role in stimulating the growth of shoots very important role 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 shoots. (Figure 1). (2) found in the callus tissue formed roots and shoots 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

In Figure 1 also shows the addition 1AA 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 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 shoots decreased it can be said that at a certain point . Increasing concentrations of auxin would likely result in the decline in the number of shoots formed . Allegedly the addition of IAA with relatively high concentrations has become toxic , resulting in decrease in the number of shoots formed and impaired growth . (12) , stated that the presence of auxin can be antagonistic to the activity of cytokines , in which the presence of cytokines from outside the endogenous cytokines lead to the disintegration and decomposition is 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 roots growing 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 , yellow-white roots and after experiencing growth , the color will change to green .

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

Treatment	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 roots are formed will be more and more long. This is in accordance with the opinion (4) that auxin (1AA) 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, so there is the possibility of cytokinin requirement for the purposes of root elongation of endogenous cytokines have been met. 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 driver of growth of shoots and roots of plants irradiated chrysanthemum can save energy resources and natural resources for endurance testing time faster than secarak onvensional, time to sprout and regenerate cells faster. Allegedly also the IAA (auxin) causes the cell walls become loose, then quickly elongated epidermal cells, and cell subepidermis attached to it also extends, so that the roots will grow longer. Multiply and root length chrysanthemum seedlings will support in the absorption of nutrients, thus affecting the growth of the plant canopy will develop optimally anyway.

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 shoots , while growing shoots , and the percentage of emerging buds . To stimulate root growth , the regeneration medium should be increased IAAnya to 0.4 mg/l .

THANK-YOU NOTE

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