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IN VITRO REGENERATION OF CHRYSANTHEMUM CALLUS AFTER GAMMA RAY IRRADIATION FOR ITS RESISTANCE TO MEDIUM PLAINS

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Abstract

Development of chrysanthemum plants in medium plains is still facing obstacles due to unfavorable climate. Up until today, the availability of tolerant chrysanthemum seeds grown in medium plains is not maximized as a necessary effort in order to increase the genetic diversity available for genetic materials as the material selection to obtain tolerant chrysanthemums grown in medium plains. Researchers have been conducting studies at several stages in order to follow up the problems regarding the availability of tolerant chrysanthemums seeds grown in medium plains, one of which is the in vitro regeneration of chrysanthemum callus after gamma ray irradiation. Studies have been conducted in the tissue culture laboratory of the Faculty of Agriculture, Universitas Pembangunan Nasional"Veteran" Yogyakarta from February to June 2015. The regeneration media 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 that ½ of MS regeneration media with the addition of IAA 0.3 mg/l stimulated shoots growth with a percentage of 100%; with a growth time of 9,33 days, shoots height of 11,17 cm, and a total number of shoots of 15,11. The total number of roots grown given by the regeneration medium treatment with kinetin 2 mg/l + IAA 0.4 mg/l is 12,1.

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

1. Introduction

Chrysanthemum is one of the most important floriculture commodities in Indonesia and its production is still continued to be enhanced. One of the disadvantages of chrysanthemum cultivation in Indonesia is climate limitation. Chrysanthemum that comes from subtropical regions in Indonesia can live only in high plains [3]. Chrysanthemums can be planted in medium plains (500-800 m asl), but the flowers produced are low in quality. According [14], the one thing that limits chrysanthemums in medium plains is its seeds. All this time, farmers force to plant the seeds in high plains. As a result, plant growth is below par, vulnerable to pests and diseases, and flower quality is not good. The availability of tolerant chrysanthemum seeds in medium plains is only a little. Therefore it is necessary to attempt to increase genetic diversity to produce genetic materials as a selection to get tolerant chrysanthemums in medium plains [1]; [12].

To be able to cope with tolerant chrysanthemum seeds availability in medium plains, genetic improvement is necessary by using irradiation to obtain potential mutants to later be developed [8]. [12] mentioned that in vitro mutagenesis can be applied to a large number of plant materials and the required time to get new variants is faster than ex vitro mutagenesis. [6] stated that gamma ray

Table 1. Average of Throughout Shoots Growth (Day), Shoots Height (cm), and the total number of chrysanthemum shoots callus with gamma ray irradiation grown on regeneration medium.

Regeneration Media	Percentage of live explants (%)	Throughout Shoots Growth (Day)	Shoots Height (cm)	Total Shoots	Total Roots	Roots Length (cm)
T1: MS+Kin 2mg/l+IAA 0,1 mg/l	97,56 a	15,27 b	6,59 c	2,33 c	2,67 c	1,33 d
T2: MS+Kin 2mg/l+IAA 0,2 mg/l	98,11 a	15,67 b	7,44 b	2,82 bc	5,46 c	2,56 c
T3: MS+Kin 2mgl+/IAA 0,3 mg/l	100,00 a	9,33 c	11,17 a	15,11 a	8,99 b	3,87 b
T4: MS+Kin 2mg/l+IAA 0,4 mg/l	100,00 a	14,11 b	11,00 ab	8,67 b	12,11 a	4,33 b
T5: MS+Kin 2mgl+/IAA 0,5 mg/l	99,37 a	20,56 a	7,87 ab	2,54 c	10,00 b	4,67 a

Description: The average treatment followed by the same letters showed no significant difference in UJBD with a 5% significance level.

Table 1 shows the influence of auxin in 15.11 shoots and the fastest growing shoots (9.33 days). This is presumably because the composition of mineral salts that exist in the media is optimal for the growth of chrysanthemum plantlets. The nitrogen in large quantities needed by the plants stimulates its height. The presence of organic carbon is expected to increase the activity of cell division under the apical meristem, in which cell division will be followed by cell elongation and cell enlargement stages. The addition of these measures will increase the plant height. In addition, the growth regulator of kinetin at 2 mg/l had a very high role in triggering shoots growth. In stimulating shoots growth, kinetin had a very important role, especially for regulating cell division and morphogenesis [13]. Cytokines, both the single factor or its combination with auxin in tissue culture, had a role in shoots induction and multiplication. [10] stated that roots callus and shoots tissue can be formed completely on their own at the same time without a vascular connection between the two.

One of the roles of auxin in the process of tissue culture is inducing adventitious roots in explants [14]. The total number of roots is essential for the growth of in vitro explants. The bigger and longer the total number of roots, the better absorption of nutrients from the media. This is because with the bigger number of roots and the longer they are, the greater the root medium nutrient absorption field is [5]. T4 treatment (½ MS: Kinetin 2 mg/l + 1AA 0.4 mg/l) produced the highest number of roots (13;12) and T5 treatment (½ MS: Kinetin 2 mg/l + 1AA 0.5 mg/l) produced the longest roots (4.67 cm) compared to other treatments. The roots of this research are formed directly on the bottom, or in other words, derived from explants. At first, roots were yellowish-white in color and after experiencing growth, the color of roots changed green.

irradiation causes ionization that can penetrate materials, including living tissue through the cells and ionizing molecules within the cells, causing mutations. According to [9], the cells exposed to radiation will produce: normal cells, mutations, chromosomal damage, or cell death. Indonesia now produces better chrysanthemum varieties for high plains. Better chrysanthemum varieties in Indonesia, namely Puspita Nusantara, are widely cultivated in the both the high and medium plains. There is also Sakuntala - a standard chrysanthemum flower. Both types are forced to be planted in medium plains, producing low quality flowers, color fades, and flower diameter and plant height are not maximum [4]; [5];[11].

The regeneration media after gamma rays irradiation is an important step for seedlings development. Chrysanthemum propagation after in vitro gamma ray irradiation has several advantages compared to conventional propagation; it quickly produces plants in large quantities and does not depend on the seasons. This propagation is expected to produce a large number plants in a short amount of time [2]; [13].

2. Research Materials and Method

The material used as explants is chrysanthemum callus with a size of 0.5-1 cm. Bottles containing the explants were irradiated with gamma rays at various doses. The bottles were then planted in the media, followed by a test using PEG, and ended in the shoot regeneration phase. The shoot regeneration medium used were ½ Murashige and Skoog (MS) supplemented with a 2 mg/l hormone kinetin and an IAA auxin with appropriate treatment, i.e. 0.1 mg/l; 0.2 mg/l; 0.3 mg/l; 0.4 mg/l; and 0.5 mg/l. This study used laboratory experiments with a Completely Randomized Design (CRD), which was repeated three times with each treatment consisting of 10 bottles, and each bottle containing 2 explants. Explants were put into culture bottles, sealed with aluminum foil, and were then stored in the incubation room in a 24°C temperature with irradiation intensity of 16 hours per day. Maintenance were done until plants reached 12 weeks.

3. Results And Discussion

The most decisive stage of success of this study is in growing plantlets on regeneration media. The balance growth regulator, auxin and cytokinin in particular, contained in the media plays an important role in determining the direction of tissue culture [12];[10]. The equilibrium concentration of each growth regulator is determined by the type of explants used. This can be seen in Table 1; all treatments of explants' percentage lived long. Research result is in accordance with the opinion of [14], stating that the success of in vitro culture is influenced by explants and media compositions, namely the composition of auxin and cytokinin in the growth medium. Shoots or roots induction from callus generally requires a balanced composition between the two, allowing them to interact with each other [1];[5].

The use of Kinetin and IAA to spur the growth of irradiated chrysanthemum shoots and plant roots can save energy resources and natural resources due to the faster durability testing time compared to using the conventional way, as well as faster shoot growth and cell regeneration. It is also assumed that IAA (auxin) caused cell walls to sag, epidermal cells were extended rapidly, including the sub epidermis cells attached, making the roots to grow longer. The bigger and longer chrysanthemum plantlet roots will support nutrients absorption, which can affect plant growth in the apical meristems to develop optimally.

Conclusion

Regeneration media of ½ MS and 2 mg kinetin that was given an addition of 0.3 mg/l IAA auxin stimulated the total number of shoots grown, throughout the growing process, and the percentage of shoots produced. To stimulate root growth, the IAA level of regeneration media should be improved to 0.4 mg/l.

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