# Acclimatization of Chrysanthemum Plantlet after Gamma Ray Irradiation

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Abstract—One of the important stages of plants propagated by tissue culture before being moved to the field is acclimatization. Small plants must adapt to the outside environment in the acclimatization room. This research was aimed to figure out the composition of growing medium with the best concentration of auxin for the growth of chrysanthemum plant in the acclimatization room. This research was conducted from February until April 2017 in Hargobinangun Village, Pakem District, Sleman Regency, Special Region of Yogyakarta Province. The research was a field experiment with factorial Completely Randomized Design and was repeated five times. The growing medium tested was vermin compost (kascing fertilizer), manure and compost. Meanwhile, the concentrations of auxin being tested were 1.2 and 3 ppm. The results of the research show that the growing medium of compost made from bamboo leaves could increase the number of leaves, the height of the plant, the number of roots and the fresh weight of the plant. Meanwhile, the concentration of auxin of 2 ppm was able to increase the height of the plant, the number of roots, the length of roots, and the fresh weight of the plant.

*Index Terms*—acclimatization, chrysanthemum, growing medium, auxin, compost

# I. INTRODUCTION

The success of plants propagated by culture in the field is influenced by some factors such as the length of acclimatization period, the growing environment and the planting medium used. The length of time (period) of acclimatization significantly influences the growth of plants. The longer the acclimatization period, the higher the production cost. Conversely, the shorter the acclimatization period, the weaker the condition of the plant during the nursery. [1], [2]

In acclimatization, the right growing medium is needed. Loose growing medium with good aeration and sufficient nutrients will support the growth and development of plant roots [2]-[3]. In acclimatization technique, there are various growing media used, including soil, sand, compost, charcoal, husk, fern roots, vermin compost (kascing fertilizer) and manure. Some studies state that plant growth in the acclimatization stage depends on the variety of the plant. According to [4], the composition of media that is good and frequently used in acclimatization consists of sand and compost with a ratio of 1:1. This is because the media can be loose with good aeration and drainage. Sand consists of particles that are not adhesive and do not have plastic characteristic, so it can create a lot of macro porous space which allows water to percolate fast. Meanwhile, the addition of compost into sand medium that lacks nutrients will give a good yield, which means it can increase organic matters and create loose and fertile soil condition. [5]-[7]

Failure in acclimatization often occurs due to poor growth of plant roots. Chrysanthemum plantlet propagated by tissue culture to be acclimatized will be cut on its roots. To grow it, auxin type growth regulator is needed [8]-[10]. Auxin is an organic compound that also regulates and coordinates plant growth and development process.

## II. METHODOLOGY

This research was conducted in Pangerang Village, Hargobinangun District, Pakem, Special Region of Yogyakarta. The experiment method used was factorial Completely Randomized Design with two factors. The first factor was the types of acclimatization media (manure, compost and vermin compost (kascing)), while the second factor was auxin type growth regulator (1.2 and 3 ppm). The research was started by making acclimatization medium in the forms of a mixture of garden soil added with ameliorant in accordance with the treatment. The seeds produced by tissue culture were plucked and cleaned from the remaining gelatin. Then, the seeds were planted into acclimatization medium. Small plants were maintained in the acclimatization room in Hargobinangun, Pakem, Sleman. Auxin type growth regulator was sprayed five days after the seeds were planted. The variation of the results of the observation was analyzed at significant level of 5 percent, and then further tested using Duncan's multiple range test at the level of 5 percent [11].

## **III. RESULTS AND DISCUSSION**

# A. Plant Growth Test

The research results show that the growing medium made from compost was the best acclimatization medium. Compost increases the number and activities of microbes in the decomposition of organic matters in soil. In addition, compost also boosts the absorption and Cation

Manuscript received October 12, 2017; revised April 10, 2018.

Exchange Capacity (CEC), hence the required elements are available for plant growth [6], [12]. In plants cultivated on compost medium, the number of leaves could reach 16.13 and the plant height could reach 18.97 cm. It significantly differed from the manure medium, the plant cultivated on which only had 8.97 leaves and height of 12.45 cm. It is assumed that it is caused by the fact that compost has 1.7 percent N content, while manure only has 0.50 percent N content. Consequently, compost is better than manure in boosting the plant growth. [13], [14] suggests that the main role of N for plant is to induce the growth entirely, particularly for its stem, branches and leaves. When the growing medium has inadequate N, the growth of leaves will be inhibited. Another advantage of compost medium is its ability to retain/store water and oxygen. With this characteristic, compost made from bamboo leaves becomes the best medium for plant growing in the acclimatization room. One of the disadvantages of compost made from bamboo leaves is its potential to attract termites. Thus, the use of insecticide is highly recommended for the compost medium made from bamboo leaves, and it must be sterilized prior to its usage for plant growing medium [15], [16]. Nevertheless, the final result of plant dry weight does not show a significant difference.

 
 TABLE I.
 The Average Growth of Chrysanthemum Plant in the Acclimatization Room (CM)

Treatment	Number of Leaves	Height of Plant (cm)	Number of Roots	Length o Roots (cm)	Fresh Weight o Plant (g)	Dry Weight o Plant (g)
Vermin	9.85	15.25	23.12	11 56	17.83	3 34
Compost Manure Compost	b 8.97 b 16.13 a	13.25 ab 12.45 b 18.87 a	23.12 b 14.55 c 32.87 a	11.56 b 14.75 a 16.45 a	17.83 ab 15.38 b 25.05 a	3.34 a 3.05 a 4.56 a
NAA	11.05	12.62	4.56	6.50	18.95	3.67
I ppm	а	с	b	с	b	а
NAA 2 ppm	12.15 a	24.33 a	28.05 a	24.12 a	25.85 a	5.08 a
NAA 3 ppm	12.19 a	16.24 b	26.62 a	19.58 b	21.22 ab	4.33 a

Note: he average treatments followed by the same letter show insignificant difference in the DMRT test with significant level of 5 percent.

The results also show that plant roots grew better on the compost medium. It is because compost medium contains more P which stimulates plant roots formation. Compost contains 0.36 percent P, while Cow Manure only contains 0.20 percent P. Ganga *et al.* [6] say that compost is able to create a porous medium, thus the plant roots can grow better and flexibly absorb water and find air in the medium. Lack of organic matters in the growing medium will result in a small number of soil pores, which consequently will make it difficult for plant roots to grow and collect water and air for plant growth. According to the research conducted by Sun et al. [17], the composition that is good for plant growing medium for chrysanthemum seeds is compost. This is due to its advantages in providing micro nutrients for plant, fertilizing soil, improving soil structure and texture, increasing the porosity, aeration and composition of soil microorganisms, improving soil water holding capacity. boosting the growth of plant roots, holding water for a long period of time, preventing the dry layer of soil and preventing various root diseases [4], [7]. According to Nishihara et al. [16], compost usually contains excessive amount of sodium chloride compound, thus its usage in a big ratio will cause some damages in plant. Organic Monohydroxistearate matters produce and dihydroxistearate acids, which are sodium chloride compounds that are harmful for plants [17].

According to the results of the observation, the growth of chrysanthemum seeds with the addition of NAA (Naflatena Acetic Acid) type plant regulators were significant. Nearly all parameters of plant roots growth showed the best NAA concentration of 2 ppm. It also showed higher number of plant roots (28.05) and longer root (24.12) compared to other treatments. In this research, the plant roots were directly formed at the base of the plant. At first, the roots were yellowish white in color, and after experiencing some development, it turned into green.

In Table I, it can be seen that the higher NAA concentration given, the bigger the number and the length of the roots that were formed. This result is in accordance with the suggestion proposed by Dong et al. [8], that auxin affects the formation and lenghtening of plant roots. NAA was added until reaching a concentration of 3 ppm. The increased concentration of plant growth regulators given to the plant will not necessarily improve the growth of plants. At NAA concentration of 3 ppm, the plant height and length turned out to decrease. It can be said that at a particular point, an increase in the auxin concentration will decrease plant growth, instead. It is assumed that the addition of NAA in a relatively high concentration had turned it into toxic and caused stunted growth. [8], [12] says that the presence of auxin may have an antagonistic nature against the activity of other plant growth regulators such as cytokines. The existence of cytokines from the outside may result in the decomposition of endogenous cytokines, and this decomposition goes along with the increase in auxin.

## B. Colour Quality Test

According to the results of descriptive analysis on the leaf colour using Munsell colour chart for Plant tissues 7.5 GY (Fig. 1), there was a clear difference in the physical appearance of the plant. Most of the leaves radiated using gamma rays were green (value 5 and chroma 6-8) or dark green (value 4 and chroma 6) in color.

In Fig. 1 is visible that the color of the leaves with the highest growth was 5/6 and 5/8 - 7.5 GY (green),

amounting 80 percent and occurring in almost all combinations of treatments. Meanwhile, 4/6- 7.5 GY (dark green) amounted 10 percent. Yellowish green (7/6 and 7/8 - 7.5 GY) occurred in plants that were not radiated.



Figure 1. Chrysanthemum Leaves color (purple mark: radiated with gamma rays; red mark: not radiated))

Green color is closely related to the amount of chlorophyll contained in plant leaves. The greener the leaf, the higher the amount of chlorophyll. The chrysanthemum plants in the acclimatization room still required some shading. The amount of chlorophyll contained is higher, especially chlorophyll b (a type of green chlorophyll which constitutes the pigment in the tilakoid membranes), especially because each chloroplast has more grana compared to plant leaves which are directly exposed to the sun light [16], [17]. If a leaf contains much chlorophyll, the leaf color will be darker. But if it contains a little chlorophyll, the color will be lighter. The nitrogen existing in the growing medium is the main element that increases the plant production. The plant with less nitrogen element may be disturbed in its protein production and inhibited in its growth, causing chlorosis or lack of chlorophyll, which will determine the rate of photosynthesis and, and eventually decreases the plant production. The presence of magnesium (Mg) in compost will strongly support the formation of green color. Plants absorb Mg nutrient in the forms of ion Mg++, an important element for plants as the composer of chlorophyll, which is highly required to facilitate the photosynthesis process [5], [6]

The dark green color in the radiated chrysanthemum plants shows that there was a change in the color of the leaf which deviated from the normal color. This mutation was closely related to the dosage of gamma rays applied [11]. In another study conducted by Wijayani and Amiaji [9] that used colchicine, there was a change in the color into yellowish green, which is assumed to be caused by the application of too little concentration of colchicine. According to Chandler and Brugliera [12], colchicine will be effective in the concentration of 0.1 - 0.8 percent, with soaking time of 24-96 hours. The mutation in the color of the leaf still requires further testing for a longer period of time. Six months of testing is not enough. This is because the change in appearance after deviation will still continue, thus it will continuously result in various forms after being multiplied by vegetative propagation, and the deviating color is susceptible to turn back into the original color [7], [16], [17].

# IV. CONCLUSION

Compost growing medium which is made from bamboo leaves is the best growing medium for the acclimatization of chrysanthemum plants. Compost can significantly affect the parameters of number of leaves, plant height, number of roots, length of roots and plant fresh weight. Meanwhile, the addition of Auxin type growing regulators (NAA) is suitable for increasing the growth of plant roots. NAA concentration of 2 ppm will increase the plant height, and the number and length of chrysanthemum roots.

#### ACKNOWLEDGEMENT

Acknowledgements submitted to Ditlitabmas Kemenristekdikti, which has funded this research through Commodity Research Universities (PUPT) 2017. Also to LPPM UPN "Veteran" Yogyakarta that has facilitated this research activity can be settled.

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