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The Explants of Planlet Induction using Auxin and Cytokinin Shortly after the Gamma Ray Irradiation and the Gripped Poly Ethylene Glycol

Ari Wijayani and Rina Sri lestari
Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran",
Jl. SWK 104 Ringroad Utara Condongcatur, Yogyakarta, Indonesia

Abstract: Chrysanthemum is the one of popular houseplants commodityies from subtropical regions and it have already widespread in Indonesia, moreover it begins to develop from plateau untill lowland. One of the problems in the lowland is a slight decrased quality of the flower. Giving improvement of the chrysanthemum flower quality in the medium plain, it used the suitable seeds. During this time, there is no available special variety of chrysanthemum flowers for medium plain and lowland, even though chrysanthemum flower has developed in many lowlands in Indonesia. The technology which was used for PEG osmolitikum (Poly Ethylene Glycol) in tissue culture can be used for marking the flowers which could be growth either in high temperature or low temperature. The main purpose of this research is a quantitative research investigation for the variety of explants and the optimal compotition of matter to stimulate the plantlet regeneration post irradiation and PEG grapped. The method that was used for this research is examining ZPT dosis thought several explants which had already irridated and grapped by gamma ray an PEG. The result of this research showed that chrysanthemum plantlets was stimulated with ZPT IAA+1.5 ppm 0.5 ppm BA throught the corner explants to stimulate growth of shoot height, leaf, root number and root length.

Key words: Medium regeneration, chrysanthemum, regeneration plantlets, PEG, *in vitro*

INTRODUCTION

Chrysanthemum is origin plant from subtropical areas which it is developed in Indonesia, especially in the plateu areas. The areas under of 700 m below the plant began to developing this plant, one of them is Hargobinangun village, Pakem, Sleman. The area on the slopes of Mount Merapi with a high of 600-700 m above sea level has been designated as one of the centres of chrysanthemums by the Directorate General of Horticulture.

The approach of agro-climatic environment chrysanthemums modification needs to be processes, according to Wijayani and Muafi (2016) the day length of chrysanthemum each day which is not fixed. Based on the day length of chrysanthemum each day, chrysanthemum is the one of short day optional plant. The Critical Daylenght-CDL chrysanthemum is around 13, 5-16 h depening on genotip.

Chrysanthemums will still continue to grow throught vegetative when the length of the day that receives more than critical limits and will be inducted into the generative phase (flower initiation) when the length of day receives less than its critical limits. Regarding on the sensitive pattern of the length day chrysanthemum, the environmental modifications for instance the addition of light by using light at night needs to make on the cultivation of chrysanthemums piececs to obtain the expected plant height (vegetative phase) before flowering. The effect of length day on the physiology of flowering chrysanthemum interacts with daily temperatures frequently. On the other hand, in temperature conditions of lenght day around 22°C in the midday and 16°C in the night, the plants grow for plant and leaf optimally. The induction into the generative phase will occur when the temperature decreased during the day more than 18°C and the night temperature rose to less than 25°C (Harsanti and Mugiono, 2007; Fukai *et al*., 2002).

However, this condition is extremely rare in the medium land to the plateau in Indonesia. According to Wijayani and Srilestari (2016) chrysanthemums will grow optimally if it grows in aglokilmat above 900 m sea level and has a temperature below 25°C, besides, the higher temperatures will hamper the initiation process and the growth of flower seeds. High temperature will create effects to the colored a dull, pale and faded flower. However, with the intensity of radiation addition can reduce the risk of premature and apperance of knobs for brighter flower color (Sisworo *et al*., 2006). There are new

Corresponding Author: Ari Wijayani, Faculty of Agriculture, Universitas Pembangunan Nasional "Veteran" ,
Jl. SWK 104 Ringroad Utara Condongcatur, Yogyakarta, Indonesia

and different sollutions in this research, it is trying to improve the ammount of tolerant varieties thought high temperatures by using *in vitro* with PEG (Poly Ethylene Glycol). The writer suspected that farmers used the seeds derived from the origin generation of trees with poor quality and moreover, it reproduces the from the origin generation of trees repeatedly from the same plant. It will reduce the quality of the plant itself (Sisworo *et al*., 2006; Wijayani and Muafi, 2016; Sirohi and Behera, 2000; Medina *et al*., 2004).

As the matter of fact, the main problem which is being paramount of important in this research is a complete study of various aspects of the chrysanthemum plant regarding the plantation techniques that can improve growth conditions, especially for repairing the seedlings through the chrysanthemum production tolerant for high temperatures. If the assembled chrysanthemum using PEG is successful, the special seeds for medium land can be planted in the Hargobinangun medium land. This argument can be taken to considerate to the other medium land areas of chrysanthemum plant.

MATERIALS AND METHODS

This research was conducted in tissue culture laboratory of the faculty of Agriculture UPN "Veteran" Yogyakarta in two phases. The first phase was irradiated plantlets with 25 gray of gamma rays (Poerwanto and Wijayani, 2013) which was tested through the tolerance of PEG (Poly Ethylene Glycol). In the tolerance of chrysanthemum test with good gamma irradiation have a good growth planlet which is still green then moved into the media test. Media which was tested is adding ZPT auxin and cytokinin (0.5 ppm IAA+1.5 ppm BA, 1.0 ppm IAA+1.0 ppm BA and 1.5 ppm IAA+0.5 ppm BA) on various eksplan materials (tip, middle and base).

The implementation of this study begins with planting the plantlets from first phase results, thus, it was grown in the media with certain PEG supplemented treatments. Having four weeks after the subculture plantlets, it will show different conditions, the resistant plantlets will remain green while the plantlets that can not survive are black and die. Criteria of survival plantlets if it is >½ part of plantlets were treated are still alive or embryonic. The explants were put into culture bottles and then it sealed with aluminum foil and then it stored in room temperature incubation 24°C with irradiation of 16 h per day intensity. These treatments are processing until the plants of 8 weeks old. Furthermore, the plantlets which transferred to the media regeneration is the media ½ Murashige and Skoog (MS) which were added PGR auxin and cytokinin according to treatment and subsequently stored in room with temperature incubation 24°C and intensity irradiation of 16 h per day. These treatments are done until the plants are 8 weeks old. The results were analyzed with variability for 5% significance level, then it was tested further by Duncan's multiple range test at 5% level.

RESULTS AND DISCUSSION

According to Sirohi and Behera (2000), the growth and the morphogenesis of plants through *in vitro* was controlled by the interaction and the balance between growth regulator plant which is given into the media and the growth regulator plant produced endogenously by the cultured cells.

Table 1 shows the influence of auxin in the observed parameters, the high of cells (5.1 cm) and the number of leaves (20 pieces) but the lowest percentage of their lives is around 60%. Presumably, It is caused the composition of salt minerals that exist in the media are optimal enough for the growth of chrysanthemum plantlets. Nitrogen that plants needed in large quantities is stimulated the growth of the plants, medium MS reaching 840.6763 mg/L.

The exsisting of the organic carbon is suspected that it will increase the activity of cell division under the apical meristems which will be followed by the stage of enlargement cell and elongation cell. The additional size will improve the high of shoots. On the other sides, the growth regulator of auxin at 1.5 ppm will be combined with very high benzyl adenine 0.5 ppm, it will being atrigger role in the growth of shoots. The auxin in stimulating the growth of shoots is mainly for arranging cell division and morphogenesis (Harsanti and Mugiono, 2007). The auxin either single factor or combination with cytokines in tissue culture has playa role in inducing and stacked of shoots (Fig. 1). Fukai *et al*. (2002) thought that in the callus roots and shoots tissue can be formed completely on their own at once without a vascular connection between them.

Figure 1 shows the addition of 1.5 ppm IAA and 0.5 ppm benzyl adenine can improve the number of shoots. It means that the interaction and the balancing between plant growth regulator that is given in the media and is produced by cells endogenously determine the development of a culture (Sisworo *et al*., 2006; Wijayani and Muafi, 2016).

In Fig. 1 shows the addition of IAA 1.5 ppm and 0.5 ppm benzyl adenine that can increase the number of tunas. This pattern explained that the interaction and the balance between growth regulator plant in media and was produced by cells which determined the development of a culture (Sisworo *et al*., 2006; Wijayani and Muafi, 2016)

Table 1: The average percentage of live explants, the heigh of shoot and the number of leaves chrysanthemum plantlets which grown on medium regenation with PGR after the irradiated gamma ray and the PEG grapped

Treatment	Percentage of live explants (%)	Shoot height (cm)	Number of leaves
T1 = 0.5 ppm IAA+1.5 ppm BA/tip explants	96.59^{a}	2.5^{a}	5^{c}
T2 = 0.5 ppm IAA+1.5 ppm BA/middle explants	95.15^{a}	2.6^{a}	9^{b}
T3 = 0.5 ppm IAA+1.5 ppm BA/base explants	90.60^{a}	2.2^{b}	11^{b}
T4 = 1.0 ppm IAA+1.0 ppm BA/tip explants	95.85^{a}	1.8^{b}	6^{c}
T5 = 1.0 ppm IAA+1.0 ppm BA/middle explants	86.75^{b}	3.0^{a}	6^{c}
T6 = 1.0 ppm IAA+1.0 ppm BA/base explants	80.55^{b}	2.5^{a}	10^{b}
T7 = 1.5 ppm IAA+0.5 ppm BA/tip explants	70.23^{b}	2.0^{b}	9^{b}
T8 = 1.5 ppm IAA+0.5 ppm BA/middle explants	65.10^{c}	1.8^{b}	15^{a}
T9 = 1.5 ppm IAA+0.5 ppm BA/base explants	60.00^{c}	5.1^{a}	20^{a}

The average treatments followed by the same letter show no significant difference in the level of real UJBD 5%

Table 2: The average number of roots and the length of root callus resulted from gamma ray irradiation which growth on regeneration media

Treatment	Number of roots	Length of roots (cm)
T1 = 0.5 ppm IAA+1.5 ppm BA/tip explants	3.69^{c}	1.35^{c}
T2 = 0.5 ppm IAA+1.5 ppm BA/middle explants	5.89^{c}	2.50^{b}
T3 = 0.5 ppm IAA+1.5 ppm BA/base explants	15.33^{c}	3.33^{b}
T4 = 1.0 ppm IAA+1.0 ppm BA/tip explants	11.12^{c}	1.56^{c}
T5 = 1.0 ppm IAA+1.0 ppm BA/middle explants	8.99^{c}	2.33^{b}
T6 = 1.0 ppm IAA+1.0 ppm BA/base explants	22.11^{b}	4.68^{a}
T7 = 1.5 ppm IAA+0.5 ppm BA/tip explants	18.55^{b}	4.25^{a}
T8 = 1.5 ppm IAA+0.5 ppm BA/middle explants	21.60^{b}	4.65^{a}
T9 = 1.5 ppm IAA+0.5 ppm BA/base explants	42.21^{a}	4.20^{a}

The average treatment which was followed by the same letter explained that it has not significant difference in the level of UJBD 5%

Fig. 1: The development of the base plantlets on media regeneration T9 = 1.5 ppm IAA+0.5 ppm BA

endogenously. One of the auxin roles in the process of tissue culture is inducing adventitious roots on explants. The number of roots is paramount of imperative for the growth of explants *in vitro*. The higher total root and the longer then it will be great for the nutrients absorption from the media. This is because the higher root and the longer root will wider the nutrient absorption of in the root media (Sirohi and Behera, 2000). In the expreriment of M 3 E 3 = 1.5 ppm IAA+0.5 ppm BA with the explant on the base part was generated the highest number of roots of (42.21) compared with the other treatments. The roots of this research were formed directly on the base or derived from explants. At the beginning, the root was colored on the yellow-white roots and during the development, the color would change to green.

In Table 2, it can be seen that the higher giving the concentration 1AA, the longer and the more roots were formed. This was similirar with the opinion that auxin (IAA) was a play rules also it had rules on the lenght of roots in tissue culture. Instead the cytokinins needed in small amounts thus, perhaps, the requirement cytokinin purpose for the elongation of root elongation, it have been acquired by endogenous cytokines. This is same with the opinion by Fukai *et al.* (2002) that the used of cytokines in the little amount can help in the growth of roots, while the roots that have formed will synthesize with endogenous cytokines. The use of Kinetin and IAA as a growth chrysanthemum plant shoots and roots promoters from irradiated can save energy resources and the other natural resources because the endurant examining time is faster than the conventional way, growth tunas and cells regerneration. Presumably, the IAA (auxin) caused the wall cell sagged, then, the epidermal cells elongated rapidly and the subepidermis cells attached widely, finally the roots will grow longer. The longer root and the higher roots chrysanthemum seedlings will assest in the absorption of nutrients, thus it affected on the growth plant in the head section that will develop optimally too.

CONCLUSION

Chrysanthemum plantlets which were simulated with PGR (Plant Growth Regulator) explained their ability to life properly. Regeneration media regeneration ½ MS and 1.5 ppm IAA+0.5 ppm BA with the base explants (T9) will stimulate the hightgrowth of shoots, the number of leaves, the number of length roots and root.

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