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Gladiolus Plantlet Induction using In Vitro Benzyl Amino Purine (BAP) with Planting Material Derived from Cormel

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This study attempts to find out the best combination of explants and the concentration of 6 - *benzyl amino purine* (BAP) of gladiolus plant *(Gladiolus hibridus* L.*)* in Murashige and Skoog media. The experiment is carried out using a complete random environmental design (CRD) with 3 replications, and each treatment unit consisted of 50 bottles of plants. The percentage data undergo a sine transformation prior to analysis. The statistical analysis in various parameters is carried out according to the 5% level of Variant Analysis. Further tests are then carried out with 5% level of Duncan Multiple Range Test (DMRT). The results of this study show that the growth and development of in vitro gladiolus plantlets will grow maximally on the combination of explants originated from cormel shoots grown on MS medium plus 8 ppm BAP. The best plantlet induction is on the parameter of plantlet growth percentage, number and length of shoots, number of roots, wet weight, and dry weight of plantlets.

Keywords: Gladiolus Plantlet Induction, In Vitro Method, Benzyl Amino Purine

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INTRODUCTION

Gladiolus plant is widely known as ornamental plants because of the beauty of their flowers. There are about 180 species with more than 10,000 cultivars of gladiolus in various varieties. So far, gladiolus propagation has been carried out conventionally using corm and cormel (Badriah *et al*., 2000; Pragya *et al*., 2012). Under natural conditions, gladiolus plants can produce about 1-2 tubers depending on the genotype. In this way, it takes about 8-10 years to produce the planting material that is required for the commercialization of a new variety. The use of underground tubers as planting material often results in heavy pathogenic contamination, such as Fusarium wilt, rot Botrytis, etc. (Hashemabadi, 2012).

Plant tissue isolation method is one of the alternatives

of plant propagation that produces large amounts of plants. Widiastoety & Anggraeni (1994) stated that currently the plant tissue isolation method is one of the ways that is widely used in vegetative propagation. Plant propagation with plant tissue isolation method can obtain seeds that are free of pests and pathogens, have the same properties as the parent, and can be available in multiple numbers in a relatively short time (Wijayani & Srilestari, 2017). By adopting various in vitro methods, the time interval of 8-10 years to make gladiolus planting material can be shortened to 2 years. *Azka Agrihorti*, the variety under study, is a new genotype that has 8-20 flowers per stalk, with red and white stripe color of the top crown and yellow color on both sides of the strands, but it only produces 6-31 cormels per plant per year, and it has flower stalks that can accommodate (Balithi, 2018)

The success in plant tissue isolation method is obtained if the required conditions are met, one of which is the use of appropriate media. In addition, the use of growth regulators in in vitro culture media will affect the enlargement and division of cells, thus it can stimulate the growth of the explants. One of the most widely used growth regulators in plant tissue isolation method is Benzyl Amino Purine (BAP). According to George & Sherrington (1984), BAP is an active synthetic cytokinin with longer stimulating power because it is not easily broken down by enzymes in plants. Cytokinins also act as cytokinesis inducers, are involved in various biological processes, senescence, apical dominance, proliferation, and philotaxis. BAP has a high enough effectiveness for propagation, easy to obtain, and relatively cheaper than other kinetins. Another role of BAP is that it is more stable, resistant to oxidation, and has the potential to remove H2S from well water (Santos et al, 2020 ;Tzvi, Y. and Y. Paz, 2019)

Research on the *Dendrobium phalaenopsis* Deang Suree orchid proved that 1.5 ppm BAP treatment on the plant showed the best growth (Rosa, 2015). Wahyuni et al. (2014) examined that the fastest shoot emergence time was obtained in 0.4 mg/l BAP treatment, namely in the third week, and it could trigger the most shoot formation. Setyati et al. (1986) conducted a study using IAA 0.5 mg/l combined with BAP 5 mg/l, and the results showed an increase in the number of shoots and protocorm like bodies (plb) on the *Cattleya sp*. Likewise, the highest germination rate of *M. savatieri* seeds is 86% with the use of BAP 0.1 mg/l treatment (Li, et al., 2020)

MATERIALS AND METHOD

Source of Explants and Sterilization

Azka Aagrihorti gladiolus variety is used as the source of explants. Cormel is washed with Tween-20 for 30 minutes, and then the surface is sterilized with 0.1% Povidone iodine for 2 hours, followed by 0.1% HgCl2 for 10 minutes. Furthermore, the explants are washed with sterile distilled water.

Callus Induction and Cultivation Medium

The explants are derived from cormels that are cut for plantlet induction. The explants are then put into culture bottles containing Murashige and Skoog (MS) media with 2.5 ppm of NAA growth regulators and BAP according to the treatment (2, 5, 8, and 11 ppm). For growth and reproduction, the pH is adjusted to 5.8 before adding agar. The medium is then sterilized in an autoclave at a temperature of 121°C for 15 minutes, then tightly covered with aluminum voil, and then stored in an incubation room at 24° C.

Statistical Analysis

The experiment is carried out using a complete random environmental design (CRD) with 3 replications, and each treatment unit consisted of 50 bottles of plants. The percentage data underwent a sine transformation prior to analysis. The statistical analysis in various parameters is carried out according to the 5% level of Variant Analysis. Further tests are then carried out with 5% level of Duncan Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Shoot Regeneration

During the incubation period that has been done since the time the explants were planted to the last observation, various changes in the explants were seen. These changes include increasing explant size as well as the formation of shoots and roots in the planted explants. The initial response that occurs in most explants after planting is the swelling of the explant tissue. According to Herastuti and Setyaningrum (1997) the changes in these explants are related to the osmolality level of the media used due to the influence of nutritional factors and the addition of growth regulators. In Table 1, it can be seen that the regeneration of shoots on explant material in the form of cormel and BAP has a significant difference between treatments.

Table 1. The Influence of Explant Material and BAP toward Shoots Regeneration

No	Treatment	Growth Percentage (%)	Number of Shoots per explant	Length of Shoots (cm)
1	Shoots cormel on MS + 2 ppm BAP	47,33 bc	5,33 b	8,37 b
2	Shoots cormel on MS + 5 ppm BAP	59,34 b	8,82 a	9,23 b
3	Shoots cormel on MS + 8 ppm BAP	87,33 a	9,60 a	11,32 a
4	Shoots cormel on MS + 11 ppm BAP	40,25 c	4,69 b	10,89 a
5	No shoots cormel on MS + 2 ppm BAP	22,74 d	3,12 c	3,23 c
6	No shoots cormel on MS + 5 ppm BAP	40,74 c	2,87 cd	2,55 c
7	No shoots cormel on MS + 8 ppm BAP	48,45 bc	1,67 d	1,90 c
8	No shoots cormel on MS + 11 ppm BAP	20,17 d	1,67 d	1,97 c

Note: The average treatment followed by the same letter indicates that there is no significant difference in 5% level Duncan's Multiple Range Test (DMRT)

The highest percentage of plantlet life is the explants derived from cormel shoots grown on MS medium plus 8 ppm BAP (87.33%). On the other hand, the highest number of shoots is in the explants from cormel shoots grown on MS medium plus 8 pm BAPP (9.60 piece) and 5 ppm BAP (8.82 piece). The highest length of shoot is in the explants originated from cormel shoots grown on MS media plus 8 ppm BAP (11.32 cm) and 11 ppm BAP (10.89). The length and number of shoots are important variables in observing plant multiplication. Long shoots with many nodes will provide many explants as well. De Hertogh and Le Nard (1998) stated that the development of explants and organ formation in in vitro culture is caused by the nitrogen content in the basic media. Nitrogen is a component of proteins, nucleic acids, and other important substances required for the formation of protoplasm and functions to improve vegetative growth (Dantu & Bhojwani, 1992).

The growth of gladiolus explants begins with the fresh explants that occur at the initiation stage, indicating a reciprocal relationship between the types of explants and the media. The condition of the explants begins to swell because they absorb the nutrients present in the media. Physiologically, the growth of these explants undergoes physical and biochemical processes (Prasad & Gupta, 2006). The role of BAP in stimulating shoot growth is very large, especially for regulating cell division and morphogenesis (Wijayani *et al*., 2016). Cytokinins, either single factor or their combination with auxin in plant tissue isolation method play a role in inducing and multiplying shoots.

The addition of appropriate growth regulators to support the explants growth is one of the critical success factors in in vitro plant propagation. In Figure 1, it can be seen the development of explants into plantlets, as the callus still appears around the explants. The appropriate concentration of BAP in the medium will stimulate shoot growth, but the high amount of added BAP concentration will not necessarily make the growth better. An increase in BAP concentration will actually result in a decrease in the number of shoots formed. It is suspected that the addition of BAP with relatively high concentrations has become toxic, which result in stunted growth. Kumar *et al*. (2002) stated that the presence of auxin can be antagonistic to cytokinin activity, as the presence of external cytokinins cases the breakdown of endogenous cytokinins, and this breakdown is in line with the increase in auxin addition.

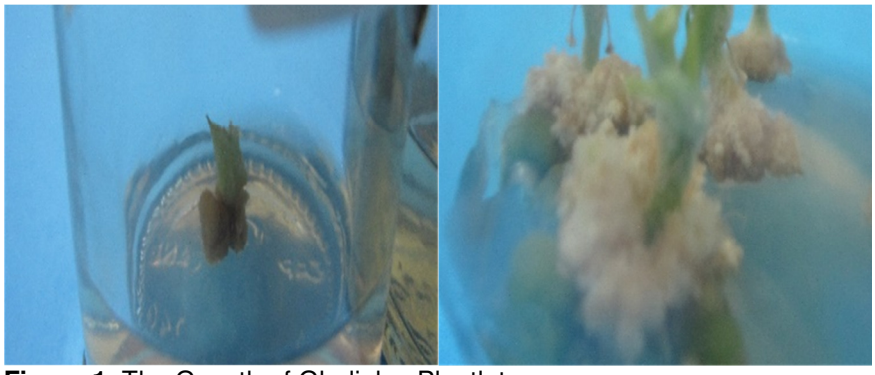

Figure 1. The Growth of Gladiolus Plantlet

Root Regeneration

In Table 2, it can be seen that there is a difference between treatments for root regeneration of the explant material in the form of cormel and BAP. The highest number of plantlet roots is in the explants originated from cormel shoot grown on MS medium plus 8 ppm BAP (9 pieces) and it is not significant with the explants derived from cormel shoot grown on MS medium plus 5 ppm BAP as well as the explants derived from cormel shoot grown on MS medium plus 2 ppm BAP. As for the length of plantlet roots is not significant in all of the treatments.

Table 2. The Influence of Explant Material and BAP on Root Regeneration, Wet Weight, and Dry Weight of Plantlets

No	Treatment	Number of Root	Length of Root (cm)	Weight Wet (g)	Dry Weight (g)
1	Shoots cormel on MS + 2 ppm BAP	8 ab	2,09 a	1,98 c	0,23 b
2	Shoots cormel on MS + 5 ppm BAP	8 ab	2,11 a	4,04 b	1,54 b
3	Shoots cormel on MS + 8 ppm BAP	9 a	2,45 a	8,72 a	4,59 a
4	Shoots cormel on MS + 11 ppm BAP	7 b	2,44 a	6,65 ab	4,57 a
5	No shoots cormel on MS + 2 ppm BAP	4 bc	1,98 a	3,22 b	1,49 b
6	No shoots cormel on MS + 5 ppm BAP	7 b	2,65 a	2,39 c	0,35 b
7	No shoots cormel on MS + 8 ppm BAP	4 bc	2,11 a	2,22 c	0,38 b
8	No shoots cormel on MS + 11 ppm BAP	3 c	2,33 a	1,72 c	0,29 b

Note: The average treatment followed by the same letter indicates that there is no significant difference in 5% level Duncan's Multiple Range Test (DMRT)

The number of and length of roots are important for in vitro explant growth. The number of large and long roots is good for the nutrients absorption from the media. The more and the longer the root, the wider the absorption of nutrients from the root media would be. From Table 2, it can be seen that cormel shoot explants grown on the medium with the addition of 2.5 ppm and 8 ppm of BAP produced more roots than the 11 ppm BAP concentration. It is stated by Wijayani and Srilestari (2017) that the BAP needed for root lengthening is only in small amounts, and there is a possibility that the need for cytokinins for root lengthening has been fulfilled from

endogenous cytokinins. In accordance with Kumar *et al*. (2002), the use of cytokinins in small amounts helps the formation of roots, in which the roots that have been formed will synthesize endogenous cytokinins, so that the administration of exogenous cytokinins often does not show its effect on root length. Likewise, cormel shoot explants have totipotential properties, because the merismatic cells in the end tissue are more actively dividing since the substances that stimulate growth are more present at the end than at the bottom (Widiastoety *et al*., 1991). This causes the growth becomes more significant than cormels without shoots.

Wet Weight and Dry Weight

In Table 2, it can be seen that the parameters of the wet weight and dry weight of the plantlets are significant between treatments. The largest wet weight and dry weight is the explants from shoot cormel grown on MS medium plus 8 ppm BAP, and it is not significant with the explants from shoot cormel grown on MS medium plus 11 ppm BAP. Kumar *et al.* (2002) and Pragya *et al.* (2012), stated that cultured explants undergo several stages, namely adaptation to culture media, proliferation, differentiation, and multiplication. The addition of appropriate growth regulators will stimulate explants for cell differentiation to form shoots, callus, and plantlets. The result of plant metabolism is plant growth indicated by the increase in the fresh and dry weight of the plant. This increase is due to the increase in cell size and number of cells. According to Setyati *et al*. (1986), the increase of cells occurs through several events, including changes in water, CO_2, and organic salts which are converted into living materials. These changes will be faster if it is supported by favorable environmental conditions.

Fresh weight is the accumulation of the weight of water contained in the results of cell metabolism. The higher the water content contained in the plant body, the higher the fresh weight of the plant would be. The amount of fresh weight affects the dry weight because the dry weight is the result of the wet weight minus water lost after drying. Dry weight is a description of the energy or photosynthetic produced by plants. The more energy that can be produced by plantlets, the greater the dry weight that would be obtained (Hashemabadi, 2012). The concentration and type of carbohydrates used during in vitro culture does not decrease the performance of photosynthesis. However, high concentrations lead to anatomical modifications, which indicate some level of stress suffered by plants, indicated by greater stomata density (Santos et al., 2020).

CONCLUSION

The growth and development of in vitro gladiolus plantlets will grow maximally on the combination of explants originated from cormel shoots grown on MS medium plus 8 ppm BAP. The best plantlet induction is on the parameter of plantlet growth percentage, number and length of shoots, number of roots, wet weight, and dry weight of plantlets.

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