

## Conference Paper

# Morphogenesis *in vitro* Flower Pedicel of *Lilium longiflorum* with NAA and BAP

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## Abstract

Lilies are one of the cut flowers most favored by consumers. Each lily plant can produce several flower stem, and each flower consists of many flower buds. Flower bud stems have the potential as explants *in vitro* culture. Propagation *in vitro* is a very promising technique for plant propagation. It has the advantage of being able to produce a lot of seedlings in a short time. Pedicel explants are reported to be the best explants after the petal to produce shoots and callus, but it is not yet known which pedicel age is best for explants. The aim of the study was to obtain information about the effect of NAA and BAP concentrations on Vacin & Went (VW) media and age of explant of flower stalks (pedicels) on morphogenesis of lilies *in vitro*. Method of this study is *in vitro* technique on VW media with complete randomized trial design. The results showed that explants of flower stems (pedicels) that were used *in vitro* culture were pedicel buds compared to pedicels with blooming flowers and withered flowers. Younger plants still have meristem tissue. The meristem tissue produces the auxin hormone which helps in cell division for faster bud growth. The best concentrations produced in this study were 1 ppm NAA and BAP in percentage of growth, time of callus initiation, number of shoots, number of browning and browning time and 0,5 ppm NAA + 1 ppm BAP for time of shoot initiation. The result of 1 ppm NAA and BAP showed the best response because the composition of the media by using a combination of growth regulators from a group of auxins and cytokinin in a balanced amount can initiate cell enlargement and induction callus.

**Keywords:** lily, pedicel, age, explant, stem

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Received: 1 February 2020

Accepted: 8 February 2020

Published: 16 February 2020

Publishing services provided by  
Knowledge E

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Selection and Peer-review under the responsibility of the IC-BIOLIS Conference Committee.

## 1. Introduction

The genus *Lilium*, which belongs to the family Liliaceae, is one of the most globally important cut flowers. It has been used as ornamental plants for centuries mainly because of their large flowers [1] Lily is spread throughout the world and favored by people for the attractive look of its flower. Most of lily's species are one season herbaceous plant propagated by tuber. Among many species cultivated, oriental lily is

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very popular in Japan, USA, and some European and Asian countries [2]. In Indonesia, lily is mostly cultivated in Cipanas-Cianjur, Bandung, Sukabumi, Malang, and Bali [3].

The conventional propagation of lily plant could be done by separating the tuber or clump. However, since the process which is considered very slow by the producers of commercial lily, vegetative propagation in such a manner could not be relied on to produce the seed massively if a certain clan of lily is need in huge amount. As a more promising alternative is propagation using tissue culture. This innovation is not only to provide the seeds massively, but also to eliminate viruses on lily [4]. Bud proliferation technique or embryogenesis callus induction is developed to provide good quality seed of lily. This technique is expected to fulfill the need of lily seeds, so that it could reduce the import volume from the Netherlands as what currently occurs, in vitro culture using various part of the plant is very advantageous in propagating the plant. In lily plant, the propagation through in vitro culture enables the development of agro-industry. Currently, most of decorative plan sold in international world is propagated using culture in vitro [5].

The propagation lily in vitro could be done using explant from various organ of a plant, some of them is tuber scale [6], anther [7], embryo [8], segment of branch and micro tuber [2], or [9]. Thin slice of explant of branch, leaves, roots or embryo is also used as the source of explant to initiate somatic embryogenesis and bud regeneration of some species of lily. Mericlone is in vitro cloning with bud meristem or pedicel meristem as the explant [10;11]. The use of flower stalk explant could induce the bud. Leaves from the bud could be used as the source of the next explant. Liu and Burger[12] reported the presence of different response of the formation of micro bud on the stalk explant of lily flower in the area of pedicle and receptacle. Many lily's micro buds are formed in the area of receptacle and pedicle. However, the optimal age of pedicle remains unknown from the flower used as culture explant.

In addition, the process of organ formation is not only dependent on explant and its age, but also by the growth of hormones, especially cytokinins and auxins. According to Wattimena et al.[13] auxin contribute in various aspects of growth and the plants' growth among other cell enlargement, inhibition of eye buds, cambium cell activity, and root growth. Synthetic auxins commonly used in in vitro culture include 2,4-dichlorophenoxy acetic acid (2,4-D), a-naphtalene acetic acid (NAA), indole acetic acid (IAA), 4-chlorophenoxy acetic acid (4-CPA), and picloram. According to George and Sherrington [14] the influence of cytokinins in tissue culture includes stimulating cell division, adventitious bud formation, axillary bud proliferation, and root formation. Cytokines commonly used are kinetin, Benzyl Amino Purine (BAP), and zeatin. So each plant has a

variety of different hormone needs. This makes it difficult to determine the concentration of hormones used [2]. Based on the results of research by Pramanik and Rachmawati [15] the combination of the different content of growth regulators give different effect to the initiation of callus, shoots, roots, and micro tubers in various oriental lily cv. Donau explants. Each type of media and type of explant induces specific morphogenesis growth. Therefore, the hormone needs to be determined empirically through observation of morphogenesis growth pattern in explant pedicels with 3 different types of flower ages.

## 2. Method

### 2.1. Time and Place of Research

The study was conducted in July 2018-January 2019 at the tissue culture laboratory at Crops Seed Center Hall, Luwus Tabanan Bali.

### 2.2. Explant Sterilization

After preparing explant dipped on liquid detergent in 10 min and rinsed with sterile water. Explants then were dipped 5% and 10% sodium hypochlorite with 3 drop tween-20 for 10 min. The sterilization process was continued in a laminar air flow cabinet by dipping the explants in 70% ethanol for 5 min and rinsed it with sterile water. After surface sterilization the explants were inoculated in cultur media. Pedicel explants were put into laminar air flow and the UV light was turned on for 10 minutes.

### 2.3. Preparation of explants

The materials used are *Lilium longiflorum* lilies of different ages, namely buds, blooms and withered. Pedicel explants were cut  $\pm$  3 cm above the petri dish with a scalpel and planted on the media in a vertical position. Make a small cut in the middle of the explant to stimulate plant morphogenesis initiation. Then the explants were planted in the callus initiation medium in an upside down position.

## 2.4. Culture Medium

The in vitro culture media used in this experiment is Vacin and Went media supplemented with 20 g L<sup>-1</sup> sucrose and 8 g L<sup>-1</sup> agarose. Various combinations of NAA and BAP were used as treatments, consisted of BAP 0 ppm+NAA 0 ppm, (2) B = VW+NAA 0,5 ppm + BAP 0,5 ppm, (3) C = VW+NAA 0,5 ppm+ BAP 1 ppm, dan (4) D = VW+NAA1 ppm+ BAP 0,5 ppm (5) VW+NAA 1ppm+ BAP 1 ppm. The pH of the media was adjusted at 5.6-5.8 before autoclaving at 121°C and 15 psi for 15 minutes. Inoculated explants were incubated in the culture room at 25°C and cool-white fluorescent lamps with 16 h photoperiod under.

## 2.5. Research design

The study used a completely randomized factorial design with two treatment factors and three replications. The first factor is pedicel explant from *Lilium longiflorum* flower of different ages, namely buds, blooms and withered. The second composition of the media with five levels, namely: (1) A = VW (Vacin and Went)+ BAP 0 ppm + NAA 0 ppm, (2) B = VW + NAA 0.5 ppm + BAP 0.5 ppm, (3) C = VW + NAA 0.5 ppm + BAP 1 ppm, and (4) D = VW + NAA1 ppm + BAP 0.5 ppm (5) VW + NAA 1ppm + BAP 1 ppm.

Observations were made from 1 week after incubation and were conducted periodically every week by observing the interaction between the types of explants and the media on the growth of explants. Observations were obtained by counting (1) frequency of the number of explants (in percent) that can initiate: (2) Time of callus initiation and (3) Time of shoot initiation (4) number of shoots (5) number of browning (6) Time of browning

## 2.6. Data analysis

The data obtained were analyzed by multivariate test with further tests to determine the difference in treatment mean using Duncan's Multiple Range Test (DMRT) at 5% test level.

## 3. Results

The results of observations for 16 weeks presented in the following table:

TABLE 1: Observation Results by Age of Flower.

Age of Flower	Percentage of growth	Time of callus initiation	Time of shoot initiation	number of shoots	number of browning	Time of browning
namely buds	70 <sup>c</sup>	17.20 <sup>a</sup>	40.20 <sup>a</sup>	1.50 <sup>a</sup>	2.00 <sup>a</sup>	15.00 <sup>b</sup>
Blooms	68 <sup>b</sup>	17.40 <sup>a</sup>	40.50 <sup>ab</sup>	1.40 <sup>a</sup>	2.20 <sup>a</sup>	13.20 <sup>a</sup>
Withered	20 <sup>a</sup>	19.50 <sup>b</sup>	41.50 <sup>b</sup>	1.00 <sup>a</sup>	2.40 <sup>a</sup>	13.00 <sup>a</sup>

Note: Values represent the mean followed by different letters are significantly different at p value=0.05 according to the least significant test

Table 1. shows the result the flower age factor shows the results of the age of flower namely buds on pedicel explants give the best results on variables percentage of growth, time of callus initiation and time of shoot initiation. the flower age factor has no effect on variable number of shoot, number of browning and time of browning (Figure 1).

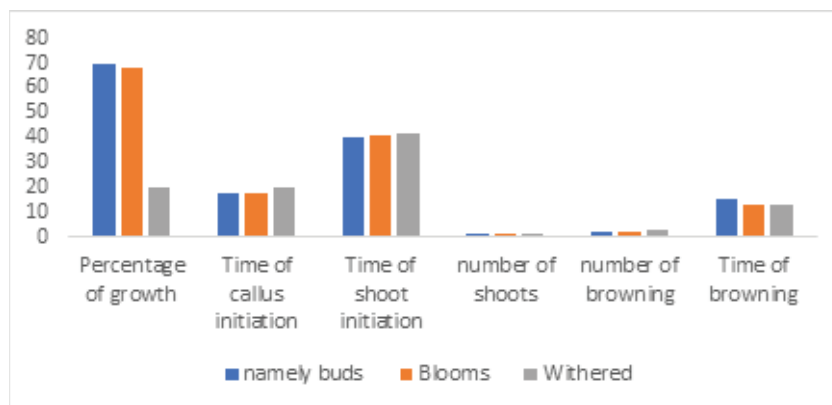


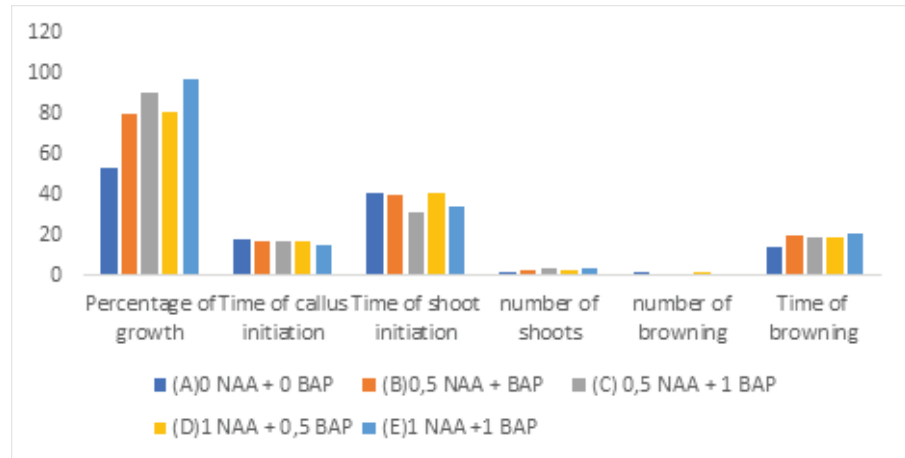
Figure 1: Graph of variables based on the age factor of lily flower in explant pedicels.

TABLE 2: Observation Results Based on Concentrations of Plant Growth Regulatory Substances.

Concentrations PGR (ppm)	Percentage of growth	Time of callus initiation	Time of shoot initiation	number of shoots	number of browning	Time of browning
(A)0 NAA + 0 BAP	52.67 <sup>a</sup>	18.03 <sup>a</sup>	40.73 <sup>a</sup>	1.97 <sup>a</sup>	1.50 <sup>c</sup>	13.73 <sup>a</sup>
(B)0,5 NAA + BAP	80.00 <sup>a</sup>	16.50 <sup>c</sup>	40.00 <sup>c</sup>	2.17 <sup>b</sup>	0.67 <sup>a</sup>	20.00 <sup>ab</sup>
(C) 0,5 NAA + 1 BAP	90.00 <sup>a</sup>	17.00 <sup>c</sup>	30.67 <sup>b</sup>	3.50 <sup>b</sup>	1.00 <sup>b</sup>	18.33 <sup>ab</sup>
(D)1 NAA + 0,5 BAP	80.67 <sup>b</sup>	16.67 <sup>c</sup>	40.33 <sup>c</sup>	2.33 <sup>b</sup>	1.33 <sup>ab</sup>	18.67 <sup>ab</sup>
(E)1 NAA +1 BAP	96.67 <sup>b</sup>	14.83 <sup>b</sup>	33.67 <sup>b</sup>	3.83 <sup>c</sup>	0.67 <sup>a</sup>	20.70 <sup>b</sup>

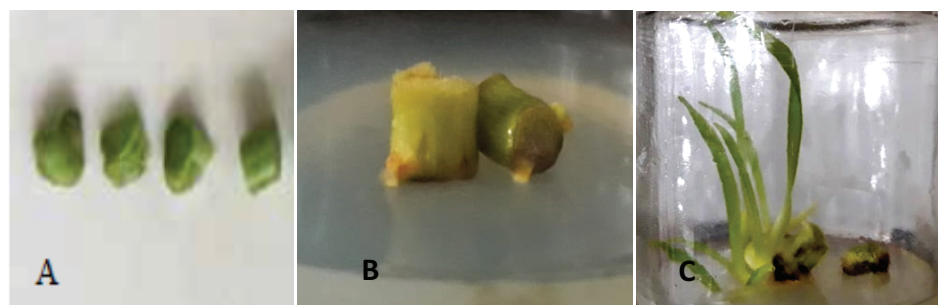
Note: Values represent the mean followed by different letters are significantly different at p value=0.05 according to the least significant tes

Table 2. shows the results of the growth of all variables measured and the best results in the treatment of PGR concentration of 1 ppm NAA + 1 ppm BAP for Percentage of growth, time of callus, number of shoot, number of browning and time of browning. The best result for time of shoot initiation of PGR concentration of 0,5 ppm NAA + 1 ppm BAP (Figure 2).



**Figure 2:** Graph of variables based on PGR NAA and BAP concentration factors.

Table 3. shows the results of the interaction of all variables measured and the best results in the treatment of namely bud flower age with the addition plant growth regulator (PGR) concentration of 1 ppm NAA + 1 ppm BAP (Figure 3).



**Figure 3:** Morphogenesis propagation of lily pedicels. Description: A) Pedicels are cut into several parts, B) Initiation of callus, C) Shoot growth from pedicel culture

## 4. Discussion

### 4.1. Percentage of Growth

Based on Table 1. there are various ages of flower conditions affecting the percentage of growth in pedicel explants. Age and conditions of bud flower have a higher percentage of growth compared to blooming and withering conditions. According to

TABLE 3: Interaction of Flowers' Age and PGR Concentration.

Age of Flower	Concentrations PGR (ppm)	Percentage of growth	Time of callus initiation	Time of shoot initiation	number of shoots	number of browning	Time of browning
namely buds	A	70.00 <sup>a</sup>	17.20 <sup>c</sup>	40.20 <sup>c</sup>	1.40 <sup>a</sup>	2.00 <sup>b</sup>	13.00 <sup>a</sup>
	B	100.00 <sup>b</sup>	15.00 <sup>a</sup>	39.00 <sup>b</sup>	2.50 <sup>b</sup>	1.00 <sup>ab</sup>	15.00 <sup>b</sup>
	C	100.00 <sup>b</sup>	15.80 <sup>ab</sup>	30.00 <sup>a</sup>	3.00 <sup>bc</sup>	0.67 <sup>a</sup>	15.00 <sup>b</sup>
	D	70.00 <sup>a</sup>	16.00 <sup>b</sup>	40.00 <sup>bc</sup>	1.50 <sup>a</sup>	1.00 <sup>ab</sup>	13.00 <sup>a</sup>
	E	100.00 <sup>b</sup>	14.00 <sup>a</sup>	30.20 <sup>a</sup>	4.00 <sup>c</sup>	0.47 <sup>a</sup>	20.00 <sup>c</sup>
Blooms	A	68.00 <sup>a</sup>	18.50 <sup>c</sup>	41.00 <sup>c</sup>	1.20 <sup>a</sup>	2.20 <sup>b</sup>	12.40 <sup>a</sup>
	B	100.00 <sup>c</sup>	16.50 <sup>bc</sup>	30.20 <sup>a</sup>	2.50 <sup>b</sup>	1.20 <sup>a</sup>	12.00 <sup>a</sup>
	C	78.00 <sup>b</sup>	15.00 <sup>ab</sup>	40.00 <sup>c</sup>	1.50 <sup>a</sup>	2.00 <sup>b</sup>	14.00 <sup>b</sup>
	D	90.00 <sup>c</sup>	16.00 <sup>bc</sup>	32.00 <sup>b</sup>	2.50 <sup>b</sup>	1.40 <sup>a</sup>	13.50 <sup>ab</sup>
	E	100.00 <sup>c</sup>	14.50 <sup>a</sup>	30.50 <sup>a</sup>	3.50 <sup>c</sup>	1.00 <sup>a</sup>	18.00 <sup>c</sup>
Withered	A	20.00 <sup>a</sup>	22.00 <sup>c</sup>	42.00 <sup>c</sup>	1.00 <sup>a</sup>	3.00 <sup>b</sup>	12.00 <sup>a</sup>
	B	30.00 <sup>ab</sup>	20.00 <sup>c</sup>	40.00 <sup>c</sup>	2.00 <sup>b</sup>	3.00 <sup>b</sup>	13.00 <sup>b</sup>
	C	30.00 <sup>ab</sup>	20.00 <sup>bc</sup>	30.20 <sup>a</sup>	1.20 <sup>a</sup>	2.20 <sup>a</sup>	13.00 <sup>b</sup>
	D	50.00 <sup>c</sup>	18.00 <sup>bc</sup>	39.00 <sup>bc</sup>	2.50 <sup>b</sup>	2.40 <sup>a</sup>	13.50 <sup>b</sup>
	E	50.00 <sup>c</sup>	15.50 <sup>a</sup>	33.50 <sup>ab</sup>	3.00 <sup>c</sup>	2.00 <sup>a</sup>	16.50 <sup>c</sup>

Note: Values represent the mean followed by different letters are significantly different at p value=0.05 according to the least significant test

Yusnita et al.,[16] age or age of explant tissue is a determining factor for organogenesis. Based on the research results explants of young leaves have a level of growth and organogenesis compared to the age of older explants. Van Tuyl et al.[17] reported that young lily anthers placed on a culture medium had a positive influence on the swelling of the ovules and ovary slice development.

Based on the treatment, the concentration of the treatment which gives the highest percentage of growth results is 1 ppm NAA +1 ppm BAP treatment. The combination of growth regulators added to the medium is the main factor determining the success of in vitro culture [18]. According to Davies[19], the interaction between auxin and cytokinin in an in vitro culture is able to make cells in plant tissue undergo a process of division and enlargement, whereas on media without the addition of BAP, auxin at this stage has begun to form plantlets.

The interaction between flower age and growth regulators significantly affected the age of flower buds that were given growth regulators 1 ppm NAA +1 ppm BAP. With younger plant tissues and the presence of exogenous growth regulators, it can increase

explant regeneration. Production of Arditti and Ernst also reported that the use of young leaf explants from mature plants did not produce PLBs at all. PLBs are only produced from leaf explants from young seedlings. And coupled with the addition of exogenous growth regulators will increase the regeneration power of a very young explant to grow.

## 4.2. Time of Callus Initiation

The parameter of callus initiation time on flowers' age explant factor significantly affected the small difference of average distance of initiation time that is 12.40-14.40 hst. The fastest time of initiation is at the age of the bud and bloom conditions. Callus initiation parameters related to the percentage of explant growth. Based on research by Yusnita et. al, [16] explants of leaf fragments from seedlings aged 10-12 months, the formation of protocorm-like bodies (PLBs) occurs first observed at age 8 weeks, whereas from younger explants namely seedling aged 3-4 month, the response was first observed in a 6 week old culture. PLBs from older explants do not continue with the development of adventitious shoots, whereas from younger explants, in its development they can become adventitious shoots.

The results showed that administration of growth regulators significantly affected callus initiation time, the fastest callus initiation time was at 1 ppm NAA + 1 ppm BAP treatment, which was 14.83 hst. A balanced comparison of auxin and cytokinin in explants can produce callus growth ([14]; [19]; [36]). According to Collin and Edward[20] auxin and cytokinin concentrations from 0 mg/L to 5 mg/L can produce callus growth optimally. Research by Sugiyarto and Paramita[21], that the use of 2,4-D growth regulators and BAP provides the highest callus growth response from explant of binahong leaves in MS medium with 1 ppm 2,4-D PGR and balanced IBA:BAP combination of 0.5 ppm which reaches 100%. The most optimal combination of PGR concentrations for callus color and texture is dark brown and compact colors including BAP concentrations of 1 ppm + 2,4-D 0.5 ppm; BAP 1 ppm + 2,4-D 1 ppm [18]

The interaction between flowers' age treatment and the administration of concentration of different growth regulators gave significantly different results with bud age with balanced NAA and BAP concentrations of 1 ppm NAA and BAP and 0.5 ppm NAA + BAP. Callus induction is closely related to endogenous and exogenous growth regulators. The most important growth regulators in callus induction are auxin and cytokinin. The use of auxin (2,4-D) and cytokines (BA or kinetin) will increase the callus induction process. Addition of auxin or cytokines to culture media can increase the concentration



of endogenous growth regulators in cells, so that it becomes a "trigger factor" in the process of growing and developing tissue [22].

### 4.3. Time of Shoots Initiation

The time of shoots initiation based on the age factor of flower in explant pedicel has a significantly different effect. Organogenesis begins with the formation of green globular structures called protocorm-like bodies (PLBs). PLBs that are formed initially are small (1-2 mm) globular in shape and light green in color. At a later stage of development, these PLBs grow into shoots-leafy Phalaenopsis shoots [16].

The best shoot initiation time in the treatment of plant growth regulators in the results of this study was 0.5 ppm NAA + 1 ppm BAP. Based on research by Anisah et., [23] in shoots of *Dendrobium* sp, a comparison between high cytokinins and auxin will encourage shoot formation, while the ratio of cytokinins and low auxin will encourage root formation, so that in addition to increasing the highest number of shoots, it can also increase the activity of cytokinins, which in turn will increase the effectiveness of cell division.

The interaction between flowers' age factors in pedicel explants and administration of concentrations of growth regulators gives significantly different results from the average time of shoots initiation. According to Fujimura and Komamine [24] for cultures derived from differentiated tissue, the addition of exogenous PGR, especially single auxins or auxins combined with cytokinins, is very important to induce organogenesis.

### 4.4. Number of Shoots

The age factor of growing flowers does not affect the parameters of the number of shoots. According to Yu et al.[25] in a manner of *in vitro*, plants have a wide potential for morphogenesis because they can evolve from plant vascular tissue. Nevertheless, the morphogenesis is influenced by internal and external factors. Internal factors due to age, explant size and type of explants while external factors due to environmental grow of the explants.

The highest number of shoots in the treatment of growth regulators gave the highest number of shoots at 1ppm NAA + BAP treatment, followed by 0.5 ppm NAA + 1 ppm BAP treatment. George and Sherrington [14] stated that BAP is a cytokinin that plays a role in the formation and multiplication of shoots and its effect is stronger than other cytokinins such as kinetin or 2-IP. forming adventitious buds needed cytokines,

for example benzyladenine (BA), kinetin, or thidiazuron (TDZ) or cytokinins at higher concentrations high combined with auxin in lower combinations [28;37].

The interaction of the number of shoots in the treatment of flowers' age and administration of different concentrations of growth regulators gave significantly different results. Regeneration ability of the number of shoots in younger explants and triggered again by the administration of exogenous regulating substances, resulting in the highest number of shoots. The highest concentration of shoots is 1 ppm NAA + BAP followed by 0.5 ppm NAA + 1 ppm concentration BAP. According to Hidayat [27] in young tissue there are more endogenous auxin hormones that can stimulate callus and shoot growth. And with the addition of an exogenous growth regulator drives the growth of the number of shoots that grow from the callus that is formed. The right combination of auxin and cytokinin will stimulate shoot growth due to the effect of synergism between these growth regulators [28].

#### 4.5. Number of Browning

The age factor of the flower does not affect the variable amount of browning on flower pedicel explants. Some explants that are not responsive turn brown and die, while others are still alive. Brownish and dead explants may have been poisoned by a blackish exudate which is thought to be a phenol compound. In responsive explants, organogenesis begins with the formation of callus which then undergoes organogenesis into micro leaf shoots. Based on research by **Arditti and Ernst (1992)**, that some explant leaf pieces that were cultured turned into black, then died. The black exudate was detected as a result of oxidation of phenolic compounds, which gradually blackened and proved toxic to cultured explants, thus affecting explant growth.

The administration of the concentration of growth regulators gives a significant effect, but still there are explants that experience browning in explants pedicel with the lowest amount of browning on the balanced cytokinin and auxin concentrations. Based on research by Dewi et al.[29] the percentage of success of inflorescent stalks explant forming a larger callus with higher cytokinin concentrations than auxin, which is about 53.94% compared with shoot tip and pith explants (34.76-42.85%), but along with the growth of callus formed experienced browning a lot due to the activity of phenol compounds and only 65% regenerated to form shoots.

The interaction of the number of browning on the age factor of flower in pedicel explants and concentration of growth regulators has a significantly different effect with the presence of very young cell tissues affecting the amount of browning and

administration of exogenous regulators which increasing the resistance of the growing callus to not experience browning. According to Yahmadi[30] also supports this that the low content of auxin in plant organs is associated with high levels of organ wilt.

#### 4.6. Browning Initiation Time

Browning initiation time affects the age factor of flowers in pedicel explants. In tissue culture explants often turn brown (blackening) or black (blackening) shortly after isolation which can further inhibit growth and ultimately cause tissue death. Phenol toxicity is probably caused by a reversible bond between hydrogen and protein. Irreversible growth inhibition occurs when phenol is oxidized to a high active quinon compound which then turns, polymerize and / or oxidizes protein into an increasingly melanate compound [32]. According to Vaughn and Duke [33] phenol oxidation compounds are not found in young, green and healthy organ tissues. In this study, the young tissue like pedicels of young flowers has a longer browning initiation time than the pedicel tissues of blooming and wilted flowers.

Based on the results of the study of browning initiation time significantly affected the treatment of auxin and cytokinin growth regulators concentration. According to Ahmad et al. [34] the time required for browning of *Pistacia vera* is influenced by the concentration and combination of growth regulators in the induction media. Based on the results of his research concentrations that can induce callus in media containing NAA (2 or 5 mg/l) or NAA (5 mg/l) and kinetin (2.5 mg/l) can maintain the callus remains greenish white for 10-13 weeks. In this study with a concentration of 1 ppm NAA + BAP can maintain explants up to 20 days for browning and has experienced callus growth.

The interaction between flowers' age and browning initiation time had a significantly different effect with the longest browning initiation time in the treatment of bud flower pedicels with a concentration of 1 ppm NAA + 1 ppm BAP. Wattimena [31] states that auxin can play a role in accelerating the hydrolysis rate of various forms of complex carbohydrates so that sugar accumulation occurs and the absorption and storage capacity of water from plant tissues will be stronger. In addition, auxin works and directly influences the transportation process, especially on horizontal movements (petal base).

### 5. Conclusions

The best age explant pedicel is namely buds flower *Lilum longiflorum* for percentage of growth, time of callus initiation, and time of shoot iniation. The best concentrations

produced in this study were 1 ppm NAA and BAP in percentage of growth, time of callus initiation, number of shoots, number of browning and browning time and 0,5 ppm NAA + 1 ppm BAP for time of shoot initiation. The interaction the age factor of flower in pedicel explants and concentration of plant growth regulators improve all variables in this study.

## Acknowledgements

The authors would like to thank The Ministry of Research, Technology and Higher Education of Republic of Indonesia for providing financial support, contract No. 016/UNDHIRA-LP2M/PN/2018.

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