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Callus induction of *Tacca integrifolia* Ker Gawl using stem nodal segment

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A study was conducted to determine the optimum concentration of plant growth regulators on *in vitro* callus induction of *Tacca integrifolia* using stem nodal segment. Fresh, dry weight and morphology of callus were evaluated and the results showed significant effects on callus induction when analyzed at a 5% level of significance. Among the treatments, Murashige and Skoog with only 6-Benzylaminopurine (1.0 mg/L) produced the highest result of fresh weight (1.2637 ± 0.14 g) and dry weight (0.1204 ± 0.01 g) and appeared the compact and green calli. Besides, the lowest results were 2,4-dichlorophenoxyacetic acid (2.5 mg/L) which only produced the fresh weight (0.2812 ± 0.04 g) and dry weight (0.0271 ± 0.00 g) and appeared friable and yellowish. The result of this study has revealed that the presence of 2,4-Dichlorophenoxyacetic acid or 1-Naphthaleneacetic acid in combination with 6-Benzylaminopurine does not giving much impact on callus induction based on mass production in addition, while 6-Benzylaminopurine alone, can produce more calli.

Key words: White bat flower, compact callus, Dioscoreaceae, 6-Benzylaminopurine (BAP).

INTRODUCTION

Tacca integrifolia, a white bat flower, is one of the tropical herbs belonging to the *Tacca* genus of the Taccaceae family, widely distributed in Southeast Asia (Zhang et al., 2006). It was classified under flowering plants in the yam family Dioscoreaceae after molecular research (Caddick et al., 2002). Nonetheless, both families still share a close taxonomic relationship (Borokini and Abiodun, 2012). *T. integrifolia* has several local names as 'Belimbing Tanah', 'Keladi Murai' and 'Janggut Adam' and this species very closely resembles *Tacca chantrieri* but differs only in petiolar-like sheath in the bract (Misrol et al., 2015; Baruah et al., 2015). The potential traditional medicine for this species was being practised by the Thai and Myanmar folk people to fight against the pest, control blood pressure, improve sexual function in humans, treatment for skin diseases and various kinds of cancers (Misrol et al., 2015; Hossen et al., 2016; Shwe et al., 2010).

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Tacca cristata Jack which is synonymized with *T. integrifolia* Ker Gawl due to their similar inflorescences (heteromorphic bracts: spathulate inner bracts and ovate outer bracts) believed to have antidiabetic properties in Malay folk medicine besides treatment of hypertension, haemorrhoids and heart failure (Nurraihana et al., 2016). Moreover, when the extracts from the tuber and roots were mixed together with *Goniothalamus malayanus* (Mempisang), it makes a good treatment for the kidney (Jamaludin and Mohamad, 2016). However, based on Jiang et al. (2014), *T. integrifolia* is mutagenic and its combined extracts from the medicinal plants are highly cytotoxic to the human cell lines, Hep2 and HFL1. In recent studies, Taccaceae are believed to produce specific secondary metabolites that have the potential for anti-cancer because they contain taccalonolide (Hapsari et al., 2018).

Seeing that this herbaceous plant is only grown explicitly in the wild forest and generally used in Southeast Asian countries for medicinal purposes, there is inadequate information on how to propagate this plant and bat flowers can be propagated from seeds and stem budding yet plants and seeds are rarely collected from the natural forest (Abd Razak et al., 2007). Nevertheless, some studies on *Tacca in-vitro* are limited; however, these initial studies confirmed the potential of Tacca plants' response to *in vitro* cultures and provide profitable data for further research. Hence, much more attention should be emphasized to *Tacca* species for further phytochemical, pharmacological and cultural studies. For the conservation aspect, overexploitation of the bioactive constituents can be minimized by practicing the *in vitro* techniques.

For all that, this research is an experiment to determine the optimum concentration of auxin and cytokinin on *in vitro* callus for *T. integrifolia*. Since *in vitro* callus is an alternative source to produce secondary metabolite production and generated callus culture could be utilized as an alternative and easy way for better screening, isolation and identification of secondary metabolites. In order to evaluate callus growth, the callus texture can act as one of the markers whereas the friable texture indicates the development of the somatic embryo and the solid compact texture also difficult to be released, will indicate growth into organogenesis.

MATERIALS AND METHODS

Explant selection

In the present experiment, the plantlet was grown in *in vitro* conditions and was maintained with periodic subculturing at an interval of four weeks at 25 ± 2°C, under a 16 h photoperiod. The sterilized stem nodal segments obtained from *in vitro* grown plants were cut approximately 0.5 to 1.0 cm in length and immediately inoculated aseptically in the Petri dish on the media supplemented with different sets concentrations and combinations of plant growth regulators.

Medium composition

The basal medium used for callus induction consisted of MS mineral salts with vitamin, 3% (W/V) sucrose, and 0.8% agar. The pH of the media was adjusted to 5.7 to 5.8. The plant growth regulators such as BAP, 2,4-D, NAA and Kinetin were added to the media before autoclaving for 20 min at 121°C and after being autoclaved, the medium was poured into several vials with an appropriate amount.

Effect of plant growth regulators on callus induction

Four hormones were involved in this experiment: 1-Naphthaleneacetic acid (NAA), 6-Benzylaminopurine (BAP), 2,4-Dichlorophenoxyacetic acid (2,4-D) and Kinetin, respectively. Different concentrations for each hormone are stated: NAA (0.1-2.0 mg/L), BAP (0.1-1.5 mg/L), 2,4-D (0.5-2.5 mg/L), BAP (0.1- 1.5 mg/L) with a constant concentration of 1.0 mg/L NAA, 2,4-D (0.5-2.5 mg/L) with a constant concentration of 1.5 mg/L Kn, BAP (0.1-1.5 mg/L) with a constant concentration of 0.5 mg/L 2,4-D and control (MS). The cultures were kept in dark conditions during the first month of culture before being kept under light (2000 lux) with a photoperiod of 16 h light at 25 ± 2°C. Callus induction was observed after 8 weeks when the fresh and dry weight was taken. The dry weight of the callus was obtained after drying to a constant weight at 45°C for 24 h. Besides, the morphology observation was recorded as the callus colour and texture. All experiments were repeated twice using 5 replicates (Petri dish) each containing three explants.

Statistical analysis

All the experiments were conducted using Randomized Completely Block Design (RCBD) which uses at least five replicates for each treatment. All the data was analyzed with one-way analysis of variance (ANOVA) using the SPSS V21 statistical program and the mean was compared by using Duncan's multiple range test at P=0.05. In all cases, a p-value of 0.05 or less was considered statistically significant.

RESULTS AND DISCUSSION

Callus induction for *T. integrifolia* was studied by using seven treatments of plant growth regulator (PGRs) including control (MS) media. Leaf as an explant to initiate the callus induction was reported as the best by other researchers (Khajuria et al., 2017; Pawar et al., 2018; Mahmuda et al., 2019) including a study on *T. chantrieri*. However, there is a lack of evidence showing that leaf is the best explant to induce the callus in Dioscoreaceae family. Hence, stem nodal segments were used as an explant source to induce the callus in the Petri dish. All the explants containing treatment except control successfully produced callus with the variable response (Table 1). The interactions among the different concentration for all treatments showed a significant *p*-value ≥ 0.05 which the null hypothesis (H_0) were rejected. There were significant differences in fresh and dry weight results.

In the present study, the callus induction began within one month in a dark condition before being transferred

Table 1. Mean of fresh weight, dry weight, and morphology observation on callus induction of *Tacca integrifolia*.

Treatment	Fresh Weight (g) (Mean ± SE)	Dry Weight (g) (Mean ± SE)	Morphology observation
Control (MS)	-	-	No callus
MS + BAP			
0.1	0.3728 ± 0.01^{b}	0.0391 ± 0.00^{b}	Green, friable
0.5	0.4434 ± 0.05^{b}	0.0412 ± 0.00^{b}	Green, friable shooty callus
1.0	1.2637 ± 0.14^{a}	0.1204 ± 0.01^{a}	Green, compact
1.5	0.3963 ± 0.03^{b}	0.0406 ± 0.00^{b}	Green, compact
MS + NAA			
0.1	0.6126 ± 0.03^{b}	0.0636 ± 0.00^{b}	Green, friable
1.0	0.8622 ± 0.04^{a}	0.0860 ± 0.00^{a}	Yellowish, friable shooty callus
1.5	0.5951 ± 0.05^{b}	0.0585 ± 0.00^{b}	Yellowish, friable shooty callus
2.0	0.3748 ± 0.05^{c}	0.0349 ± 0.00^{c}	Yellowish, friable
MS + 2,4-D			
0.5	0.5295 ± 0.10^{a}	0.0733 ± 0.01^{a}	Green, friable shooty callus
1.0	0.3052± 0.03^{b}	0.0303 ± 0.00^{b}	Yellowish, friable shooty callus
2.0	0.3562 ± 0.04^{ab}	0.0362 ± 0.00^{b}	Pale green , friable
2.5	0.2812 ± 0.04^{b}	0.0271 ± 0.00^{b}	White, friable
MS + BAP + NAA			
0.1 + 1.0	0.5102 ± 0.05^{b}	0.0458 ± 0.00^{b}	Green, friable, shooty callus
0.5 + 1.0	0.8210 ± 0.05^{a}	0.0837 ± 0.01^{a}	Yellowish, friable
1.0 + 1.0	0.7999 ± 0.05^{a}	0.0765 ± 0.01^{a}	Yellowish, friable
1.5 + 1.0	0.6332 ± 0.07^{b}	0.0689 ± 0.01^{a}	Yellowish, friable
MS+ BAP+2,4-D			
0.1 + 0.5	0.3211 ± 0.03^{b}	0.0328 ± 0.00^{b}	Green, friable
0.5 + 0.5	0.4324 ± 0.03^{b}	0.0451 ± 0.00^{b}	Green, friable
1.0 + 0.5	0.8503 ± 0.11^{a}	0.0779 ± 0.01^{a}	Green, friable
1.5 + 0.5	0.6853 ± 0.05^{a}	0.0682 ± 0.01^{a}	White, compact
MS+ 2,4-D + Kn			
0.5 + 1.5	0.2981 ± 0.03^{c}	0.0300 ± 0.00^{b}	Pale Green, friable, shooty callus
1.0 + 1.5	0.7574 ± 0.10^{a}	0.0708 ± 0.01^{a}	Yellowish, friable
2.0 + 1.5	0.5847 ± 0.03^{b}	0.0585 ± 0.00^{a}	Yellowish, friable
2.5 + 1.5	0.4464 ± 0.03^{bc}	0.0425 ± 0.00^{b}	Yellowish, friable

MS= Murashige and Skoog; FW=fresh weight, DW=dry weight, SE=standard error.
Source: Authors.

into a light condition. From all the single hormones used as treatment contained with Murashige and Skoog (MS), BAP produced a higher amount of callus at all concentrations between 0.1-1.5 mg/L. The highest fresh and dry weight that was produced at the concentration of 1.0 mg/L BAP is 1.2637 ± 0.14 and 0.1204 ± 0.01 g, respectively (Table 1). Besides, the morphology that appeared on the callus is compact and green in colour (Figure 1). A study on callus induction in the herbaceous plant, patchouli by Mayerni et al. (2020) reported that the callus successfully formed without any combination and produced best at a concentration of 1.0 mg/L BAP. In line with the results of their finding, it showed that the best range of BAP concentrations that can be used to form callus from leaf or node explants is between 1.0 and 2.0 mg/L BAP. It differs from several studies that had been reported on callus response of the same plant *Tacca* genus which is *T. chantrieri,* the very close species

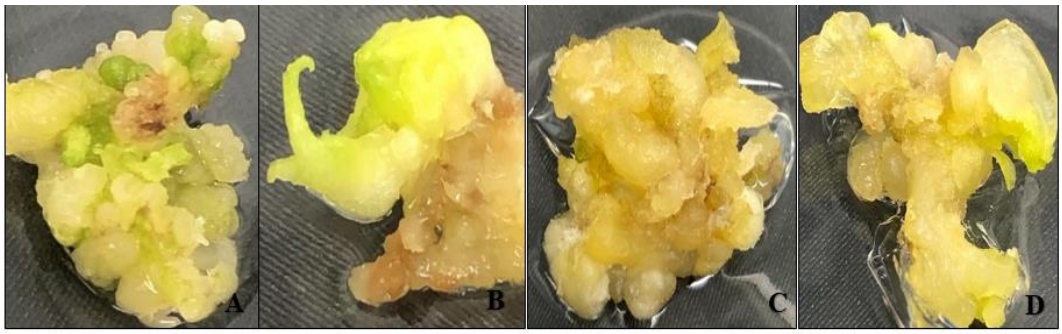

Figure 1. Callus induction from stem nodal segment explant on optimum treatment after 8 weeks of culture at 25 ± 2°C under light condition. a) 1.0 mg/L BAP. The calli formed greenish, compact and covered all the entire explants. b) The green and friable shooty callus appeared at 0.5 mg/L 2,4-D. c) The yellowish and friable callus appeared at MS+ 1.0 mg/L BAP + 0.5 mg/L 2,4-D. d) The pale green with friable shooty callus appeared at MS + 0.5 mg/L 2,4-D + 1.5 Kn.
Source: Authors.

related (Wei Ying et al., 2013; Yun Ping et al., 2012). However, in their research, they used leaf stalk and filament of flower explants with a trio combination MS + 2.0 mg/L BAP + 0.1 mg/L NAA + 0.1 mg/L 2,4-D. They found the best range of BAP concentration at 2.0 to 3.0 mg/L. Different responses might occur regarding the genotype variation and source of explants used for each species. Several researchers have found that the negative impact on callus formation occurred as increasing BAP to 2 mg/L while decreasing to 0.1 mg/L can reduce the rate of callus formation (Blinkov et al., 2022).

The following treatment, NAA with increasing concentration at 2.0 mg/L gave a lower amount of callus. Matsuoka and Hinata (1979) mentioned that when NAA at high concentration caused toxic, depressed callus growth also stimulated embryoid formation. This is proven by this study when the concentration increased; the value of fresh and dry weight gave a declined result. On the contrary, it differs from 1.0 mg/L NAA which is found as the best concentration and can produce more amount of callus weight with a compact callus on *Dioscorea rotundata* (Ezeibekwe et al., 2009). Nonetheless, the callus appears green and friable at low concentrations compared to the high concentration of NAA. Liu et al. (2018) have mentioned that the higher concentrations of NAA led to the formation of foamy, loose, and soft texture in calli. The green colour of the callus appeared due to the presence of light which promotes the synthesis of chlorophyll. Moreover, the decreased concentration of 2,4-D at 0.5 mg/L gave a higher amount of callus weight with green and friable shooty callus was observed. 2,4-D concentrations used between 0.5 and 2.5 mg/L were reported as the best treatments (Khajuria et al., 2017).

Since the callogenic response varies from hormonal concentration, they found that the lower concentration of 2,4-D alone or in combination with cytokinin is very promising for callus induction. Furthermore, the best media combinations that successfully produced the highest fresh and dry weight of callus were MS + 1.0 mg/L BAP + 0.5 mg/L 2,4-D with 0.9472 ± 0.17 g and 0.0789 ± 0.01 g, respectively while MS + 0.5 mg/L 2,4-D + 1.5 mg/L Kn gave the lowest weight of callus (Table 1). While in combination with Kinetin, 1.0 mg/L 2,4-D gave a higher weight of callus rather than 0.5 mg/L 2,4-D which only produced 0.2476±0.07 g fresh weight and 0.0326±0.01 g dry weight. This evidence is in line with the species study of *Barringtonia racemosa* (Nurul et al., 2013). BAP at a concentration between 0.5 and 1.5 mg/L is more suitable to be used when in combination with other PGRs such as NAA and 2,4-D. It was shown in this experiment that between that concentration, the callus weight produced higher when in combination with 0.5 mg/L 2,4-D or 1.0 mg/L NAA.

Conclusion

From the present study, it is revealed that the single hormone BAP at the concentration of 1.0 mg/L produce the highest amount of callus with 1.2637 ± 0.14 g fresh weight and 0.1204 ± 0.01 g dry weight with the morphology that appeared on callus being compact and green in colour. While MS + 0.5 mg/L 2,4-D + 1.5 mg/L Kn gave the lowest weight of callus which only produced 0.2476±0.07 g fresh weight and 0.0326±0.01 g dry weight. Plus, the callus appeared pale green in colour and friable shooty. In addition, the presence of 2,4-D or NAA in combination with BAP does not have much impact on the callus response. Further studies could be conducted since this finding was a starting point for enhancing the production of secondary metabolites. Besides, identifying novel approaches to utilize this plant in *in vitro* callus cultures for future use in pharmaceutical applications such as inducing the callus by using different

types of explant parts, different strengths of MS and other components.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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